Assessment of Therapeutic Excellence of *Jasminum humile* (Linn.) and *Pleurospermum candollei* (DC.) C.B. Clarke Via *in vitro* and *in vivo* Assays

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In The Name of Allah
The Most Beneficent, the Most Gracious,
The Most Merciful

"And with Him are the keys of the unseen; none knows them except Him. And He knows what is on the land and in the sea. Not a leaf falls but that He knows it. And no grain is there within the darknesses of the earth and no moist or dry [thing] but that it is [written] in a clear record."

(Surah Al-An'am, 6:59)

Dedicated

To

My Parents

&

My Sister (Noshaba Kanwal)

Who taught me to trust in ALLAH, believe in my abilities, and believe that hard work really pays off.

Author's Declaration

I **Mehreen Fatima** hereby state that my PhD thesis, *titled* "**Assessment of Therapeutic Excellence of *Jasminum humile* (Linn.) and *Pleurospermum candollei* (DC.) C.B. Clarke Via *in Vitro* and *in Vivo* Assays**" is my own work and has not been submitted previously by me for taking any degree from

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No part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to the Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan in partial fulfillment of the requirements for the **Degree of Doctor of Philosophy** in the field of Biochemistry from Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan.

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Mehreen Fatima

Contents

Chapter 3……. *Pleurospermum candollei* methanolic extract ameliorates CCl_4-induced liver injury by modulating oxidative Stress, inflammatory, and apoptotic markers in rats

List of Publications

Peer-Reviewed Journal Publications

1. **Fatima, M.,** & Khan, M. R. (2024). Investigating the role of polyphenols from Pleurospermum candollei (DC.) extract against diabetic nephropathy through modulating inflammatory cytokines and renal gene expression in rats. Journal of Molecular Structure, 1305, 137832. (IF: 4.0) https://doi.org/10.1016/j.molstruc.2024.137832

2. **Fatima, M.,** Khan, M. R., Al-Keridis, L. A., Alshammari, N., Patel, M., Adnan, M., & Sahreen, S. (2023). Pleurospermum candollei Methanolic Extract Ameliorates CCl4-Induced Liver Injury by Modulating Oxidative Stress, Inflammatory, and Apoptotic Markers in Rats. ACS omega, 8(29), 25999-26011. (IF: 3.7) https://doi.org/10.1021/acsomega.3c02031

3. **Fatima, M.,** & Khan, M. R. (2023). Jasminum humile (Linn) ameliorates CCl4-induced oxidative stress by regulating ER stress, inflammatory, and fibrosis markers in rats. Inflammopharmacology, 31(3), 1405-1421. (IF:4.6) https://doi.org/10.1007/s10787-023-01230-z

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Chapter 1 INTRODUCTION

Chapter 2……..*Jasminum humile* (Linn) ameliorates CCl_4-induced oxidative stress by regulating ER stress, inflammatory, and fibrosis markers in rats

Chapter 3……. *Pleurospermum candollei* methanolic extract ameliorates CCl_4-induced liver injury by modulating oxidative Stress, inflammatory, and apoptotic markers in rats

Chapter 4…… Investigating the role of polyphenols from *Pleurospermum candollei* (DC.) extract against diabetic nephropathy through modulating inflammatory cytokines and renal gene expression in rats

Chapter 5……*Protective effects of Jasminum humile* extract against CCl_4-induced renal toxicity by inhibiting fibrosis, inflammation, apoptosis markers

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Chapter 3……. *Pleurospermum candollei* methanolic extract ameliorates CCl_4-induced liver injury by modulating oxidative Stress, inflammatory, and apoptotic markers in rats

Chapter 4…… Investigating the role of polyphenols from *Pleurospermum candollei* (DC.) extract against diabetic nephropathy through modulating inflammatory cytokines and renal gene expression in rats

Chapter 5……*Protective effects of Jasminum humile* extract against CCl_4-induced renal toxicity by inhibiting fibrosis, inflammation, apoptosis markers

List of Abbreviations

Abbreviation	**Full Form**
µg	Micro gram
µL	Micro liter
^0C	Centigrade
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALP	Alkaline phosphatase
CAT	Catalase
POD	Peroxidase
CHOP	C/EBP homologous protein
CCl_4	Carbon tetrachloride
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
EDTA	Ethylenediamine tetraacetic acid
ER	Endoplasmic Reticulum
G	Gram
GSH	Glutathione
GPx	Glutathione Peroxidase
GR	Glutathione Reductase
H_2O_2	Hydrogen peroxide

IL	Interleukin
i.e.	That is
i.p	Intraperitoneally
IC50	Inhibition concentration
JHM	*Jasminum humile* methanol
PCM	*Pleurospermum candollei* methanol
PCE	*Pleurospermum candollei* ethanol
STZ	Streptozotocin
NA	Nicotinamide
Mg	Milli gram
mL	Milli liter
mM	Milli molar
Nm	nanometer
O^{-2}	Superoxide
OD	Optical density
OH^{-}	Hydroxyl ion
$ONOO^{-}$	Peroxynitrite
P-450	Cytochrome P-450
NO	Nitric oxide
pH	Power of hydrogen
POD	Peroxidase
RNA	Ribonucleic acid

RNS	Reactive nitrogen species
ROO^-	Peroxy radical
ROS	Reactive oxygen species
RT-PCR	Real Time Polymerase Chain Reaction
SD	Standard deviation
SOD	Superoxide dismutation
TBA	Thiobarbituric acid
TBARS	Thiobarbituaric acid reactive substances
TNF	Tumor Necrosis Factor
TGF	Transforming Growth Factor
BCl-2	B-cell leukemia/lymphoma 2 protein
MCP	monocyte chemoattractant protein-1
Bax	BCL2 Associated X
Casp-3	Caspase 3
XBP1-s	*X-box binding protein 1 spliced*
XBP1-t	*X-box binding protein 1 total*
XBP1-u	*X-box binding protein 1 jointless*
TGF	Transforming growth factor
Smad	Small Mothers Against Decapentaplegic
LDL	Low density lipoprotein

BSA	Bovine Serum Albumin

Abstract

Medicinal plants contain several bioactive chemical constituents that provide significant therapeutic effects, either directly or indirectly, proving to be an effective source to deal with the current healthcare challenges including infectious disorders, inflammation, cardiovascular diseases, neurodegenerative diseases, cancer, diabetes and several other types of lethal ailments. On the basis of ethnopharmacological studies, two medicinally potent plants like *Jasminum humile* and *Pleurospermum candollei* were selected for this study. *J. humile* is one of potent therapeutic plants which is traditionally used as a remedy in treating diseases such as ringworm, chronic fistulas, hard lumps, anxiety, inflammatory disorders, and neurological problems. It is also used as an astringent, antiseptic, antispasmodic, and cardiac tonic. While *P. candollei* is a local vegetable which is traditionally employed to treat various serious diseases including digestive disorders, abdominal distress, respiratory issues, cardiac diseases, pain, diarrhea, and infertility complications. It is also used as a spice to introduce flavor in food. This research was conducted to investigate the pharmacological and therapeutic potential of selected plants through in-vitro antioxidant assays and in-vivo anti-inflammatory and anti-diabetic studies. The present study investigates the pharmacological potentials of *J. humile* and *P. candollei* against a few important clinical issues like oxidative stress, hepatotoxicity, and inflammation. Considering the aim of developing natural, plant-based therapeutic modes as effective alternatives for the prevention and treatment of such conditions.

Both samples were collected, dried and prepared dried powder was extracted with methanol, ethanol and water by shaking it continuously at room temperature. Prepared extract was dried through vacuum distillation and semi-solid extracts were obtained. Initial qualitative phytochemical studies were performed to assess the presence of various phytochemicals such as terpenoids, phalobatanins, triterpenoids, glycosides, oils, resins, alkaloids, carbohydrates, proteins, flavonoids, steroids, phytosteroids, betacyanin, anthocyanins, saponins, vitamins, sterols, quinones, and phenols. HPLC and FTIR analysis was conducted for further characterization of phytochemical antioxidants in relevant extracts. Based on qualitative and chromatographic analysis, quantitative assays were conducted. *In-vitro* antioxidant assays (FRAP, TAC. DPPH, hydroxyl ion, and metal

chelation) investigated the antioxidant properties of selected plant extracts. However, cytotoxic studies were performed to assess the toxicity of extracts in rats. Hepatoprotective potential of JHM and PCM was evaluated against oxidative damage mediated by carbon tetrachloride (CCl_4) in rats. While JHE was examined for therapeutic effects against renal toxicity instigated by CCl_4. Rats were divided into groups; CCl_4 was injected intraperitoneally, and various plant doses and reference drugs were administered orally. Blood and organs (i.e. liver and kidney) were extracted from various groups for enzymatic, free radicals and serum analysis. TRIzol method was used for RNA extraction and qRT-PCR analysis was performed for assessment of various inflammatory, apoptotic, fibrosis and renal markers. Similarly, induction of streptozotocin (STZ) along-with nicotinamide mediated type 2 diabetes in rats and diabetic nephropathy was induced. Blood glucose level was measured along with enzymatic analysis. qRT-PCR analysis was done for investigation of mRNA expression levels of diabetic genetic markers.

Flavonoids, tannins, and phenolic contents were quantified in significant quantity in *J. humile* methanol extract (JHM), *P. candollei* methanol extract (PCM), and *P. candollei* ethanol extract (PCE). HPLC analysis confirmed the presence of almost 10 polyphenolics in relevant extracts such as rutin, syringic acid, catechin, and vanillic acid found in JHM, emodin and catechin in PCM, cinnamic acid, rutin, and gallic acid in PCE. These extracts exhibited high antioxidant potential, least IC_{50} values against free radicals, strong correlations of TPC and TFC with all *in vitro* antioxidant assays conducted. High anti-diabetic activity was found in PCE against α-glucosidase enzyme at higher concentrations.

Cytotoxic studies of JHM, PCM, PCE on rats showed that extracts were non-toxic even at higher concentration (2000 and 3000 mg/kg bw) when administered orally. Enzymatic antioxidants and GSH were decreased in CCl_4 treated groups while plant extracts normalized the levels of these antioxidants and neutralized the higher production of free radicals due to CCl_4 induction. At molecular levels, CCl_4 increased the expression of ER stress genes (Xbp1s, Xbp1u, Xbp1t), inflammatory mediators (i.e. IL-6, MCP, TNF-α), apoptotic (Chop, Caspase 3) and fibrosis (Tgf-β, col1a1) markers. JHM and PCM reversed the toxic effects of CCl_4 on liver and kidney of rats and normalized the expression levels of these above-mentioned genes. PCE reduced the blood glucose levels and risk of obesity

in pre-induced diabetic rats. At molecular level, STZ increased inflammatory, fibrosis and apoptotic markers while PCE decreased the expression of these markers at normal level. Histological studies further confirmed the therapeutic effects of JHM, PCM and PCE on liver and kidney by showing normal physiology of hepatic and renal tissues.

The above results demonstrate strong evidence of using *J. humile* and *P. candollei* as promising medicinal agent against various diseases with no side effects. However, clinical trials will further elaborate the efficacy and potency of these plants to be introduced as a drug.

INTRODUCTION

1.1. Introduction to medicinal plants

Herbal and natural remedies have been developed over centuries through careful observation of their therapeutic benefits and potential risks, resulting in a thorough understanding of their properties and side effects. During experimentation, scientists extracted medicinal compounds from specific herbs in their laboratory to develop new medications that were often more potent than original herbs. This method eventually resulted in the development of various plant-based medicines and the synthesis of pharmaceuticals that contain similar active medicinal components of the original plants. Aspirin, codeine, digoxin, and other similar drugs are originally derived from plants and herbs (Yarnell, 2000). In many parts of the world, the use of plant-based medicines can generally be categorized into two main types: orally transmitted traditions that pass on generations and formal or “official” traditions that are based on scientific experimentations (Zevin *et al*., 1997). For therapeutic and pharmaceutical uses, around 35,000 plant species have been studied (Mustafa *et al*., 2017). According to the World Health Organization (WHO), the use of herbs as basic healthcare products by 4 to 4.7 billion people around the world, while traditional medicines developed from plant extract are known as "modern herbal medicines." (Pan *et al*., 2013).

1.2. Historical background of aromatic and medicinal plants

Herbs, medicinal and aromatic plants have been long used by both men and animals for shelter, treatment, flavors, and food. Medicinal plants are primarily utilized in pharmaceutical industry for traditional and formal medications while aromatic plants are extensively used as spices and flavoring agent in cooking and perfume industry. According to WHO, 'herbal medicines’ are products derived from plants that provide medicinal or other potential benefits to humans, and these can include raw or processed substances from various plants (WHO, 2001). The benefits of herbs and aromatic plants and their essential oils in treating various human diseases have been well documented in literature (Oliveira *et al*., 2018). These herbs were among the earliest therapeutic compounds utilized in ancient times for the treatment of diseases and other abnormalities and even today, they continue to be employed in folk and ethnomedicine practices (Christaki *et al*., 2012;

Giannenas *et al*., 2018). The medicinal properties of herbs and plants were recognized by nearly all ancient civilizations of the world. In those ancient civilizations, native populations utilized aromatic plants for treatment of both physical and mental disorders. During that era, diseases were often linked to supernatural or malevolent origins, thus, healers were respected by people as they were playing pivotal roles in societies (Kankara *et al*., 2015). In present-day societies residing in remote, rural, and mountainous regions with restricted access to formal healthcare facilities, such as in developing countries, many individuals continue to depend on a combination of physical and spiritual therapies, placing importance on the utilization of medicinal plants (Sen and Chakraborty, 2016; Solomou *et al*., 2016).

In the Nineteenth century, scientists started to study and research the chemical composition of medicinal plants. In 1833, atropine was the first isolated compound from medicinal plant, thus, it was evident that medicinal effects of natural plants are due to specific compounds that can be isolated and studied (Shikov *et al*., 2014). The Arabs played a role in advancement of pharmacotherapy by introducing many medicinal plants, mainly from India, with which they traded at that time. Many of these plants possessed true medicinal properties and are part of global pharmacopoeias till now (Srivastava, 2018).

Traditional therapists commonly practice traditional herbal medicines and these practitioners of Chinese, American, Tibetan, and Indian Ayurvedic culture are esteemed not only in their own countries but also in developed countries such as United Nations and Germany. These healers commonly employed a mixture of plant extracts, considering that these mixtures may synergistically enhance the overall efficiency of the medicinal herb, surpassing the combined effects of individual components. Furthermore, it is argued that use of whole traditional herb instead of isolated compounds can reduce its toxicity (Giannenas *et al*., 2020). The consumption of plant-based drugs in veterinary medicine is linked to traditional knowledge, based on practical experience, and passed down through generations, both verbally and in documentation (WHO, 2001). Since the 1990s, there has been a growing awareness that the inclusion of drugs, particularly antibiotics, as additives in animal feed, was contributing to the development of antibiotic resistance among microbial pathogens. This concern along with risk of potential toxicity of these drugs entering the food chain, has raised significant issues regarding health safety of ecosystem

(Dhama *et al*., 2015). The use of herbs, aromatic plants and their essential oils has tremendously increased as an alternative feed additive in animal food due to restrictions on antibiotic use (Stevanovic *et al*., 2018). Feed additives can be defined as products, used in animal feed to enhance the quality of food, health and growth rate of animals and their productivity (Huyghebaert *et al*., 2011).

1.3. Toxicity of plants and its impact on human and animal health

Studies have indicated the potential toxicity of medicinal plants and herbs from ancient times. Numerous plants thought to be medicinal have been shown to be potentially poisonous, mutagenic, and carcinogenic in recent scientific research, and serious adverse consequences following the administration of herbal products have been recorded (Fennel *et al*., 2004). There are only few plants known to be poisonous even if consumed in small amount (Anywar, 20020). Generally, most of the toxic herbs and plants induce poisonous effects under specific conditions such as consuming large amount of plant (Kankara *et al*., 2015). Toxicity reflects the presence of poisonous characteristics, manifesting as harmful effects from interactions between toxic substances and cellular structures. These interactions depend on the chemical attributes of the toxicants and the cellular membrane, and can take place on the cell's surface, inside the cell itself, or within the underlying tissues and the extracellular matrix. Toxic effects can manifest before toxicants bind to essential organs like the liver and kidneys (Asante-Duah, 2002). Therefore, assessing the toxic characteristics of a substance is essential for public health protection, as chemical exposure can pose serious risks and lead to detrimental health outcomes. Typically, this evaluation encompasses analyzing the acute, sub-chronic, chronic, carcinogenic, and reproductive impacts of the substance (Jothy *et al*., 2011)

1.4. Plant-based antioxidant phytochemicals

Plants and herbs synthesize a wide range of phytochemicals for their metabolic processes and defense activities against various diseases and predators. All plants produce primary metabolites such as fats and proteins, whereas most of them also produce secondary metabolites that have therapeutic properties. A few secondary metabolites serve as toxin to prevent predators while others act as pheromones to attract insects for pollination. Plants produce several major types of phytochemicals with medicinal nature, including alkaloids,

polyphenolics, terpenoids, and glycosides (Mosihuzzaman, 2012). Almost all types of fruits, vegetables, cereal grains, and medicinal plants are a good source of antioxidant phytochemicals (Deng *et al*., 2012a; Guo *et al*., 2012). Additionally, peel and seed of fruit also contain a large portion of antioxidants such as gallic acid, chlorogenic acid, catechin, epicatechin, and kaempferol (Deng *et al*., 2012a).

1.5. Phytochemical Screening: Identification of bioactive compounds in plants

Natural products have potential biological activities and are used for numerous purposes. Detailed phytochemistry and preclinical pharmacological investigation of compounds isolated from natural sources can confirm their activities as potent drugs (Sharifi-Rad *et al*., 2022).

1.5.1. Fourier transform infrared spectroscopy (FTIR)

FTIR is a modern spectroscopic method in which an interferogram is instantaneously transformed into spectrum by a computer, then a modernized software algorithm helps perform quantitative and qualitative analysis of organic samples revealing their chemical bonds and organic constituents (Ţucureanu *et al*., 2016). This analysis can be performed on a variety of samples, liquids, powders, gases, solutions, and pastes. FTIR has high accuracy and high sensitivity rates. Sample that needs to be characterized is illuminated with infrared (IR) radiation which triggers vibration of atoms present in sample. The spectroscopic method measures specific transmission (T%) or absorption (A) using these vibrations at atomic level. FTIR spectrum graphically represents 'T%' or 'A' against wavenumber (cm^{-1}). This spectrum is split into three wavenumber regions: near, mid and far. Although near and far contribute to analysis, mid region has the prime importance. (Nandiyanto *et al*., 2019).

1.5.2. High-performance liquid chromatography (HPLC)

HPLC is extensively utilized across various sectors such as pharmaceuticals, biotechnology, environmental science, polymers, and the food industry for separation and purification processes. HPLC can be used to identify, quantify, and resolve compounds. The technique involves injecting a tiny volume of liquid sample into a flowing stream of solvent, mobile phase, which then travels through stationary phase which is filled in a

column. The distribution of sample into its individual components relies on the varying levels of each component's retention within the column. HPLC represents one form of liquid chromatography, where the mobile phase consists of a liquid. The most prevalent variety is reversed-phase HPLC. In this method, the mobile phase is comparatively polar, while the stationary phase is relatively non-polar. HPLC systems are comprised of several key components: a solvent reservoir, pump, injector, column, detector, and either an integrator or a data acquisition and display system. At the core of the system is the column, where the actual separation takes place. The objective of the HPLC technique is to separate and quantify the primary drug, its reaction impurities, all synthetic intermediates present, and any degradation products (Ali, 2002; Gerber *et al*., 2004).

1.6. Oxidative stress and free radicals

In 1985, the term “oxidative stress” was introduced to explain an imbalance between prooxidants and antioxidants which leads to potential damage to cells (Sies, 1985). An antioxidant is a molecule that inhibits oxidation of other molecules by neutralizing free radicals (Kohen and Nyska, 2002). Extreme oxidative stress leads to lipid peroxidation, DNA damage, elevation in intracellular free calcium ion and iron levels, and destruction of protein transporters. Oxidative stress is caused by various processes including hypermetabolic conditions, carbon tetrachloride (CCl_4) toxicity, ischemia-reperfusion injury, and physical activities (Di Meo and Venditti, 2020).

Reactive oxygen species (ROS) refers to a class of oxidants that include both free radicals and molecular species with ability to produce free radicals. Primarily, superoxide radicals (O2 •−) and nitric oxide (NO•) radicals are produced within cells (Winterbourn, 2008). Normally, the body converts around 2% of the oxygen it consumes into O2 •− through various processes including phagocytosis and mitochondrial respiration. Some normal physiological processes such as gene expression, cellular development, and infection defense typically require low concentration of ROS. Additionally, these ROS also helps to stimulate some intracellular biochemical processes (Dröge, 2002). ROS influence cellular functions by reversibly oxidizing substrate sites of transcription factors like nuclear factor kappa B (NF-kB). This oxidation alters the function of these transcription factors, modulates gene expression, cell growth and proliferation. ROS can also indirectly activate

transcription factors by triggering various signal transduction pathways, such as activation of mitogen-activated protein kinases. ROS trigger NF-κB through phosphorylation of NF-κB p65/p50 subunits, which ultimately activates T cell proliferation, hence playing a role in regulating immune and inflammatory responses. Furthermore, ROS are also produced by neutrophils and macrophages to kill bacteria that they ingest through phagocytosis. Additionally, tumor necrosis factor (TNF-α) generates ROS and induces apoptosis to cause the cytotoxicity of tumor and virus-infected cells (Kunwar and Priyadarsini, 2011).

Different classes of free radicals such as O2 •− react with various biochemicals including proteins, lipids, carbohydrates, and DNA, generating various types of ROS, for example, sugar and base derived radicals, lipid radicals, amino acid radicals, and thiyl radicals. These radicals then react with oxygen and form peroxyl radicals which further activate chain reactions of biosystems (Winterbourn, 2008). However, the biological impact of these chain reactions is influenced by several factors, such as the location of ROS generation, the type of substrate involved, and the redox status (Sevanian and Ursini, 2000). For instance, lipid components of cellular membranes contain a high proportion of unsaturated fatty acids and make membranes prone to oxidation by ROS. ROS react with these fatty acids and initiate lipid peroxidation, which further results in protein crosslinking in membranes, lipid-protein and lipid-DNA adducts formation which may affect functioning ability of cells. Similarly, ROS reacts with protein causing direct and indirect impairments including cross-linking, fragmentation, damage to tertiary structures, and proteolytic degradation. Oxidation of protein through ROS produces aldehyde and ketone products. Although DNA is a well-protected molecule with high stability, ROS can still induce changes in DNA including damage to its structure, loss of purine, breaks in DNA strands, and DNA-protein crosslinking. For example, •OH radical form •OH radical adducts by interacting with DNA nitrogenous bases, and cause changes in these bases. DNA damage can lead to cell death, genetic mutation, carcinogenesis, and aging. Excessive production of ROS results in accumulation thus inducing intracellular oxidative stress. Cells have developed a defense system to protect against oxidative stress caused by ROS, including antioxidant defenses to inhibit uncontrolled production of ROS (Kunwar and Priyadarsini, 2011).

1.7. Carbon tetrachloride (CCl_4)

Inhaling vapours of CCl_4, a chlorinated hydrocarbon used as a refrigerant, and a drying agent, acts as a potent environmental hepatotoxin, cause deterioration in the hepatic and renal tissues and impairs nervous system activities. (Abraham *et al*., 1999). Food, skin, and respiratory tract are the main entering sites for the CCl_4 in the human body. However, inhalation is the primary mode of exposure to CCl_4 in humans, with pulmonary absorption estimated to be almost 60%. Diet has a significant impact on the fast rate of absorption from the gastrointestinal tract, one example is alcohol or fat boost intestinal absorption of CCl_4 (Weber *et al*, 2003). It is estimated that the average daily ingestion of CCl_4 through various routes for the common people is 0.1 mg. This poisonous substance spreads in the body through the blood, fat, muscle, liver, brain, and kidney to the maximum quantities when consumed, inhaled, or absorbed through the skin. Upon exposure to this toxic substance, it diffuses throughout the body, with the highest concentration typically found in the liver, brain, kidneys, muscles, fat, and blood (Makni *et al*., 2012; Srinivasan *et al*., 2005).

1.7.1. Biotransformation of CCl_4: Mechanism and pathway

In 1961, it was proposed that homolytic scission phenomenon of carbon-halogen bond of CCl_4 generates free radicals which result in hepatotoxicity activities (Butler, 1961). Similarly, in 1975, electron resonance analysis proved that CCl_4 generates free radical during its metabolism. Furthermore, studies demonstrated that the cytochrome P450, terminal oxidase in the hepatic oxidase system, catalyzes reductive dehalogenation of CCl_4 (Calligar and Vannini, 1975; Di Meo and Venditti, 2020). Cytochrome P450-mediated transfer, which involves moving one electron to the C-Cl bond, is the initial stage of tissue/cell damage caused by CCl_4. This results in the formation of trichloromethyl radical ($\bullet CCl_3$), an intermediate metabolite, which is subsequently transformed into the trichloromethyl peroxyl radical ($\bullet OOCCl_3$) in the presence of oxygen. These CCl_4 generated free radicals react with polyunsaturated fatty acids (PUFA) to initiate lipid peroxidation, breakage of cell membrane, leakage of microsomal enzymes and consequently cell damage (Noguchi *et al*., 1982). Because of their high reactivity and notable biological consequences, lipid peroxidation products can selectively modify cell

signaling, damage DNA and proteins, and induce cytotoxicity. CCl_4 is frequently used as a toxin in scientific studies to create experimental models for a variety of pathophysiological states (Abd-Elhakim *et al*., 2019; Kilany *et al*., 2020; Slama *et al*., 2020).

1.8. Antioxidant enzyme system of body

Antioxidants are known as compounds that possess the possibility of reducing or preventing the deleterious effects of free radicals and form a major portion of the defense system (YU, 1994). Antioxidants are available both in the natural and synthetic form besides being naturally present in the body (Brewer, 2011). The body contains some natural antioxidants including glutathione peroxidase (GP), catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST), and quinone reductase (QR) to naturally detoxify ROS. GP, CAT, and SOD are classified as primary enzymes, while GST and QR are considered secondary enzymes. While CAT and GPs both catalyze the removal of hydrogen peroxide (H_2O_2), SODs catalyze removal of harmful superoxide radicals (Wu and Cederbaum, 2003). As components of the antioxidant defense system, nicotinamide adenine dinucleotide phosphate (NADPH) and reduced glutathione (GSH) are non-enzymatic antioxidants. They play a significant part in eliminating ROS. More specifically, GSH eliminates ROS generated during the respiratory chain in mitochondria, while NADPH is involved in the GP system. Furthermore, ascorbic acid (vitamin C) and α-tocopherol (vitamin E), are examples of other nonenzymatic antioxidants (phytochemical substances) that are essential to the detoxification of ROS (Nimse and Pal, 2015).

1.9. An imbalance between free radicals and antioxidants

Under stress conditions, the biological systems generate ROS more than antioxidants of both types: enzymatic antioxidants, such as SOD, GPx, and CAT, and non-enzymatic antioxidants, such as flavonoids, glutathione vitamin C, carotenoids, and vitamin E. This imbalance leads to cellular damage and health issues (Pukalarasan and Kathiravan, 2017). A deficiency of antioxidants, which neutralize ROS, can contribute to onset of various degenerative disorders (Singh *et al*., 2019) like cancer and cardiovascular disorder, alzheimer's and neurodegenerative illness, inflammation. This imbalance can be addressed by supplementing the diet with natural antioxidants that are synthesized plants and herbs

(Akbari *et al*., 2022). Thus, these natural antioxidant compounds can be used as prophylactic medicines. Some previous studies have reported that a higher intake of an antioxidant-rich diet can reduce the risk of diseases in the human body (Sies, 1993). Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), two synthetically synthesized antioxidants that are frequently used in food items, considered as a reason of liver damage and cancer, so, there is now more interest in using natural antioxidants in the human diet (Krishnaiah *et al*., 2011).

1.10. Antioxidant capacity of medicinal plants: Heath implications

Excessive production of oxidants such as ROS and reactive nitrogen species (RNS) is responsible for pathogenesis of various chronic disorders in the human body, hence phytochemicals exhibiting protective roles found to be associated with their antioxidant property. Phytochemicals have been reported to possess antioxidant properties not only in vitro but in clinical studies. High intake of antioxidant phytochemicals rich vegetables and fruits results in higher antioxidant capacity of serum. For example, in elderly women, significant increase in the total antioxidant capacity of serum were observed after consuming red wine, strawberries, vitamin C, or spinach. Additionally, levels of vitamin C in plasma and levels of urate in serum were also high. However, the increased concentrations of vitamin C and urate could not entirely explain the enhanced total antioxidant capacity (Zhang *et al*., 2015).

Antioxidants, through both enzymatic and non-enzymatic mechanisms, protect the human body against oxidative stress. Non-enzymatic antioxidants are commonly included in foods to inhibit lipid oxidation. Under certain conditions, some lipid antioxidants can act as prooxidants, affecting other molecules in the body. Therefore, antioxidants intended for food and therapeutic use must be thoroughly characterized to ensure their safety and efficacy (Sultan, 2014). Medicinal plants and some natural food components including vegetables, grains, fruits, herbs, spices, mushrooms, and flowers are the primary source of exogenous antioxidant phytochemicals (Deng *et al*., 2013; Zhang, *et al*., 2016). However, the industries that process agricultural byproducts have the potential to be significant producers of natural antioxidants (Deng *et al*., 2012b). Natural plants and herbs mainly produce primary antioxidant compounds such as polyphenols, carotenoids, and vitamins.

Mainly, the biological effects of these naturally occurring antioxidants, polyphenols, and carotenoids, are diverse and include antioxidant, antibacterial, anti-cancer, and anti-inflammatory activities (Balmus *et al*., 2016; Xu *et al*., 2017; Zhou *et al*., 2016)

Antioxidants are substances that can inhibit oxidizing enzymes, or react with oxidizing agents before damaging other molecules, sequester metal ions, or even can repair body systems, for example, iron transporting proteins (Brewer, 2011). Medicinal plants have a diverse array of naturally occurring antioxidants that vary in their physical and chemical compositions and characteristics, and mode of action. However, phenolics and flavonoids are known to be potent antioxidants that have continuously provided protection by scavenging a wide range of ROS in different in vitro cellular models, such as hydroxyl radicals, peroxyl radicals, hypochlorous acids, superoxide anion, and peroxynitrite (Halliwell, 2007a). A lot of research has been done on the anti-inflammatory, hepatoprotective, anti-carcinogenic, antibacterial, and antiviral properties of polyphenols as well as their antioxidant activities (Serrano *et al*. 2009). While anthocyanin, an antioxidant found in plants, has potential against chemically induced tumors and proves to block the genes responsible for inflammation, proliferation, and angiogenesis (Karlsen *et al*. 2007). Additionally, they are claimed to be more powerful antioxidants than vitamin C or BHT since they have successfully prevented a critical stage in atherogenesis and inhibited LDL oxidation (Wang and Jiao 2000). Similarly, ascorbic acid is a strong antioxidant and known to reduce the oxidative stress produced during photosynthesis in plants as well as playing an important role in cell division and proliferation process. Ascorbic acid exhibits prooxidant and antioxidant properties and reduces transition metals during conversion process of ascorbate to dehydroascorbate, in vitro (McGregor and Biesalski 2006). Ascorbic acid is essential for treating and preventing scurvy. Additionally, numerous phytochemicals present in plants possess unique mechanisms of action for disease prevention and treatment. Medicinal plants are regarded as easily accessible and rich source of exogenous antioxidants as they are composed of various chemical components that can work independently or synergistically to improve health status and treat most diseases. A single plant may contain a wide variety of phytochemicals, such as tannins that act as natural antibiotics, bitter compounds that improve digestion, phenolic compounds with antioxidant and other pharmacological effects including antibacterial and

antifungal properties, alkaloids, and diuretic substances (Bhatt *et al*., 2012; Miguel 2010). Interestingly, the term 'drug' originates from the Swedish word 'druug,' meaning dried plant (Mosihuzzaman, 2012).

1.11. Inflammation: The body's protective response

Inflammation is pivotal to both maintaining homeostasis of cells and facilitating regenerative processes like wound healing. Additionally, it plays a role in acute phase reactions and immunological responses to infections. Normally inflammation is well-controlled in all these conditions. Whereas chronic inflammation and excessive production of cytokines are uncontrollable types of inflammatory response. Inflammation is a complex biological phenomenon characterized by coordinated interactions of various body cell types including B and T lymphocytes, epithelial, muscle and myeloid cells. These cells make a contact between them using soluble factors such as growth factors, cytokines, and chemokines. Among various cytokines, interleukin-6 (IL-6) contributes to cytokine storm, chronic inflammation disorders, autoimmune diseases, and cancer (García, 2020; Hirano and Murakami, 2020; Hirano, 2021; Hu *et al*., 2021; Rébé and Ghiringhelli, 2019). However, Anti-TNF-α biological products have shown clinical efficacy in treating inflammatory diseases because of their ability to effectively prevent TNF-α from binding to its cognate receptors, TNF receptor 1 and 2 (TNFR1 and TNFR2). From a long time, it was believed that this blockade lowers inflammatory response by inhibiting TNFR1 from initiating NF-κB pathway and MAPK pathways, which would otherwise result in the transcriptional upregulation of proinflammatory mediators that is responsible for inflammatory pathology. While this initial understanding remains valid, it is now known that TNF-α binding to its receptor also causes cell death, such as apoptosis, necroptosis, or pyroptosis, which exacerbates and indirectly increases inflammation (van Loo and Bertrand, 2023).

Acute inflammation is an immediate response to tissue injury and considered as fundamental part of innate immune system which acts as first line defense mechanism of body against pathogens and foreign invaders. When the body encounters a proper stimulus like microbial infection, pathogens, or external or internal irritants, then immune system responds immediately in the form of innate immunity. As the innate immune system is the

primary driver of inflammation, various immune cells i.e., neutrophils, and lymphocytes play crucial roles in the inflammatory response. Together these cells coordinate a rapid and effective inflammatory mechanism to protect the body from infection and promote healing (Akira *et al*., 2006). In addition to immune cells, other non-immune cells that are involved in inflammation processes include fibroblasts, endothelial cells, and epithelial cells. Target tissues and inflammatory pathways differ greatly depending on the stimulants type. Chronic inflammation is intricately connected to the development of inflammatory diseases, making it challenging to differentiate between cause and effect in these conditions. For instance, obesity can trigger inflammation, and in turn, chronic inflammation can contribute to the development of obesity-related diabetes, primarily due to insulin resistance. This bidirectional relationship underscores the complexity of chronic inflammatory diseases, where inflammation both arises from and exacerbates underlying health issues (Ahmed, 2011; Hotamisligil, 2006).

1.12. Apoptosis: Programmed cell death

Apoptosis is a clearly defined form of cell death intended to quickly remove cells that are unwanted or potentially dangerous. In addition, several human diseases such as cancer, AIDS, neurological disorders, and ischemic stroke, are linked to improper control of apoptosis. When a cell receives a signal to undergo apoptosis, caspases, initially synthesized as inactive zymogens or proenzymes, become functionally active protease enzymes (Song and Steller, 1999). Apoptosis is a homoeostatic mechanism that helps to maintain the balance and health of an organism by controlling cell numbers. During an organism's development, growth, and aging, cells can become damaged, redundant, or no longer necessary. It ensures that these cells are efficiently and systematically removed. Mitochondria play significant roles in the activation of apoptosis cascades because it provides a variety of pro-apoptotic signals. Because of the permeabilization of the mitochondrial membrane, mitochondria play a major role in the release of several key chemicals that induce apoptosis, such as cytochrome c, SMAC, apoptosis-inducing factor, or endonuclease G. Proapoptotic B cell lymphoma (Bcl)-2 family proteins cause permeabilization of mitochondrial membrane, however anti-apoptotic Bcl-2 family members maintain the integrity of membrane (Ferri and Kroemer, 2001; Kiraz *et al*., 2016; Ziegler and Groscurth, 2004). These medications are primarily caused by caspases, which

have the broad ability to cleave specific molecules from one or more precise location, degrading and inactivating the target protein. Furthermore, they have the ability to block the negative regulator domain of particular proteins, which activate the subjected molecules (Hengartner, 2000). One of their key roles is fragmentation of DNA (Kiraz *et al*., 2016).

Mitochondria play a crucial role in the regulation and amplification of the apoptotic cascade, primarily mediated by the BCl-2 protein family. The BCl-2 proteins are integral to the decision-making process of whether a cell will undergo apoptosis. These proteins can influence mitochondrial outer membrane permeability, a key event in the intrinsic pathway of apoptosis (Savitskaya and Onishchenko, 2015). By activating the inositol 1,4,5trisphosphate receptor on the ER membrane, CHOP triggers increased transcription of oxidase ERO1α (ER oxidase 1α), which produces ROS inside the ER and starts Ca2+ efflux into the cytosol. Increased Ca2+ levels trigger apoptosis by activating Ca2+/calmodulin independent protein kinase II. Additionally, CHOP activates proapoptotic proteins Bim, telomere repeat binding factor 3 (TRB3), and receptor death receptor 5 (DR5), while inhibiting the antiapoptotic protein BCl-2 (Savitskaya and Onishchenko, 2015).

1.13. Liver: The hub of metabolism

The liver plays a crucial role in nearly all biochemical processes related to growth, immunity, detoxification, energy production, and reproduction. One of its primary functions is the metabolism of carbohydrates, proteins, and fats (Kalra *et al*., 2021). Additionally, the liver is essential for storing glycogen, hormones, vitamins, iron, minerals, and various other substances. It is also the main location where toxins ingested from the digestive tract are processed, and it helps to break down and remove these toxins, which include drugs and various foreign substances (Okaiyeto *et al*., 2018). It detoxifies and converts numerous toxins, allowing them to be excreted through the kidneys via urine or into bile for elimination through the colon (Corsini and Bortolini, 2013). Enzymes in the liver change drugs into either inactive or active metabolites. The liver is the primary site of drug metabolism for a class of enzymes called cytochrome P-450 (Corsini and Bortolini, 2013; Okaiyeto *et al*., 2018; Venmathi Maran *et al*., 2022). It has the unique capacity to

regenerate after tissue injury and plays a crucial role in regulation of glucose and lipid levels in blood. These and numerous other functions demonstrate the liver's capacity to respond appropriately to the body's needs and maintain homeostasis. The unique physical position of liver, which allows continuous blood supply from the gastrointestinal tract and the hepatic arteries, emphasizes the liver's key role for homeostasis and inflammatory responses. Blood cells from the innate and adaptive immune systems travel through a network of sinusoids in the liver. This brings them into contact with various liver cell types, such as hepatocytes, endothelial cells, KCs, lymphocytes, HSCs, and other intrahepatic cell populations (Racanelli and Rehermann, 2006). Cytokines have a major role in the regulation of liver functions as well as communication between various cell types. Cytokines are low-molecular-weight signaling molecules secreted by cells to influence the behavior of other cells. They can act on the cell that secreted them (autocrine signaling), on nearby cells (paracrine signaling), or on cells in different organs (endocrine signaling) (Luedde *et al*., 2003). Resident or monocyte-derived KCs possess the ability to phagocytose and produce a wide range of cytokines that are crucial in determining the future responses of other immune cells and liver cells, in addition to the extent of organ damage (Bilzer, *et al*., 2006; Tacke *et al*., 2009). Hepatic illnesses lead to the deaths of thousands of individuals worldwide each year. Approximately 2 million people die annually from hepatic disorders (Asrani *et al*., 2013). No entirely effective pharmaceutical exists to support liver function, offer complete organ protection, or aid in hepatocyte regeneration, despite substantial advancements in modern medicine (Chattopadhyay, 2003). Numerous research investigations have documented the protective effects of herbs and their phytochemical components against a range of liver diseases (Amzar and Iqbal, 2017; Shah *et al*., 2017).

1.13.1. Liver’s role in glucose homeostasis

The role of liver in glucose uptake varies from one-third to 50-60%, making it crucial for post-meal glucose utilization. Peripheral tissues (skeletal muscle and brain) manage the rest of glucose elimination after food intake. Glucose entering hepatocytes is phosphorylated to glucose 6-phosphate by glucokinase for utilization. Glucokinase is inhibited by the glucokinase regulatory protein in the liver, acting as a competitive inhibitor of glucose binding (Agius, 2016). Numerous metabolic processes start with glucose 6-

phosphate, including those for the pentose phosphate pathway, glycogen production, oxidative methods, and the hexosamine pathway. In the liver, extra glucose is converted into fatty acids. Either through the production of glucose from precursors such as alanine, glycerol, and lactate (gluconeogenesis) or the breakdown of stored glycogen (glycogenolysis), the human liver also releases glucose into the bloodstream, alongside consumption of glucose. To survive periods of fasting, the human liver's special capacity to store and release glucose is vital (Adeva-andany *et al*., 2016; Sharabi *et al*., 2015). Glucose transporter-2 (GLUT2 or SLC2A2) is the primary hepatic glucose transporter in humans. It facilitates passive glucose transport into and out of cells. GLUT2 is expressed in human pancreatic cells, liver, small intestine (jejunum), and kidney. Hepatic glucose entry is primarily driven by hyperglycemia, not hyperinsulinemia, making insulin's impact on liver glucose uptake limited (Sharabi *et al*., 2015).

1.13.2. Hepatic inflammation induced by CCl4

The liver is a key organ responsible for various functions, its primary roles include processing and detoxification of both internal and external toxins, biotransformation of substances within body, metabolic homeostasis, and regulating various metabolic pathways (Nagata *et al*., 2007; Shin *et al*., 2013). Studies have reported that chronic liver disorders are caused by various drugs and toxic chemicals induced liver injuries (Chopra and Saxena, 2018). CCl_4 is classified as a hepatotoxin and requires metabolic activation to exert its effects. The metabolism of CCl_4 in hepatocytes generates free radicals to initiate lipid peroxidation process which cause structural damage, cell injury and death, and disruption of liver functions (Xu *et al*., 2019). The stages of liver damage instigated by CCl_4 are reductive dehalogenation, covalent binding of radicals, fat accumulation, loss of calcium homeostasis, inhibition of protein synthesis, fibrosis, and apoptosis (Boll, *et al*., 2001). Moreover, mechanisms which support CCl_4 instigated liver toxicity includes activation of Kupffer Cells (KCs), lipid peroxidation, hypomethylation of DNA, and release of proinflammatory cytokines (Koyama and Brenner, 2017).

Numerous proinflammatory cytokines, including leukotrienes, IL1β, monocyte chemoattractant protein-1 (MCP-1), and TNF-α, are released by KCs (Luedde *et al*., 2003). In both acute and chronic liver disorders, KCs and infiltrating monocyte-derived

macrophages are known to initiate inflammatory responses by releasing proinflammatory cytokines. This leads to various consequences, such as attracting T cells, inducing hepatocyte apoptosis, and activating fibrogenic HSCs (Bilzer *et al*., 2006; Schümann *et al*., 2000). However, experimental reports suggest that macrophages also play a crucial role in limiting the inflammatory response and degrading ECM proteins, which is essential to normalize liver fibrosis (Fallowfield *et al*., 2007). IL-6 attaches directly to hepatocytes by binding to an 80-kDa membrane glycoprotein (gp80), which forms a complex with a signal-transducing molecule called gp130 (Luedde and Trautwein, 2006). Serum and liver exhibit elevated levels of IL-6 in acute and chronic liver disorders. TNF-α and related cytokines play pivotal roles in liver homeostasis by activating both pro-apoptotic pathways, mainly involving caspases, and anti-apoptotic pathways, mainly mediated by NF-kappaB, within hepatocytes. Fas ligand (FasL) and TNF-α induce programmed cell death similarly by activating caspases (Tacke *et al*., 2008).

In addition to activating KCs, CCl_4 also plays role in activation of neutrophils, T-lymphocytes, and macrophages that are responsible for hepatic inflammation (Koyama and Brenner, 2017; Manibusan *et al*., 2007). CCl_4 is known as a hepatotoxic agent which is used to induce toxicity and to create models with hepatocellular carcinoma, hepatic fibrosis, liver diseases, and hepatitis. When exposed to high doses of CCl_4, liver cells lose their regenerative ability and this can lead to acute liver failure (Lee *et al*., 2019; Li *et al*., 2017; Manibusan *et al*., 2007). Induction of CCl_4 can lead to various severe pathological conditions in the liver including necrosis of hepatocytes, abnormal proliferation of bile duct cells, degeneration of liver cells, inflammation, vascular occlusion, central vasodilation, accumulation of collagen fibres, hepatic fibrosis, and cellular hypertrophy (Dutta *et al*., 2018; Huang *et al*., 2020). CCl_4 creates an imbalance between oxidant and antioxidant through formation of free radicals thus causing oxidative stress. This oxidative stress leads to liver diseases (Lee *et al*., 2019). CCl_4 also inhibits the activities of endogenous antioxidant enzymes (Hafez *et al*., 2015; Huang *et al*., 2020; Sahreen *et al*., 2013). CCl_4 exposure induces oxidative stress, resulting in increase in the levels of protein carbonyls, which are markers of protein oxidation, also causes an elevation in the levels of malondialdehyde (MDA), which is a byproduct of lipid peroxidation and serves as a biomarker for oxidative stress (Sun *et al*., 2018). Studies have shown that CCl_4 triggers an

inflammatory response mediated by KCs which further produce proinflammatory mediators such as TNF-α, MCP-1, IL-1beta, IL-6, and pro-fibrotic markers like transforming growth factor (TGF-β). CCl4 also proved to increase the mRNA levels of fibrosis markers like α-SMA and Col1a1 in liver. Furthermore, treatment with CCl_4 also upregulates the concentration of liver enzymes in serum. Upregulation of alanine transaminase (ALT), bilirubin, and aspartate transaminase (AST) indicates loss of functional integrity of liver cell membranes and liver cell disruptions, and these tests are important to evaluate liver functioning ability. Oral dosage of CCl4 changes status of hepatic enzymes, upregulates cholesterol levels, triglycerides, and LDL concentrations. Additionally, concentration of SREBP-1, transcription factor of lipogenesis also upregulates which interact with FAS enzyme to increase its activity. It is a primary factor contributing to oxidative stress, ER stress, inflammation, and liver injuries, all of which are induced by free radicals generated from CCl4. (Hafez *et al*., 2015; Lee *et al*., 2019; Scholten *et al*., 2015; Sun *et al*., 2018).

CCl_4 also causes endoplasmic reticulum (ER) stress through inducing glucose regulated protein of 78 kDa (GRP78), X-Box Binding Protein 1 total (XBP1-t), X-Box Binding Protein 1 added (XBP1-s), and X-Box Binding Protein 1 jointless (XBP1-u) (Zai *et al*., 2019). As a result, CCl_4 inhibits the proper functioning of mitochondria and ER by damaging the membrane of liver cells (Unsal *et al*., 2021).

1.13.3. Hepatic fibrosis induced by CCl_4

CCl_4 initiates lipid peroxidation, so it damages various structures of cells, including mitochondria and the endoplasmic reticulum. In addition, radicals disrupt various biological functions through interacting with proteins and nucleic acids. Additionally, CCl_3 radicals also react with DNA to initiate adduct formation (Hefnawy and Ramadan, 2013; Li *et al*., 2017; Scholten *et al*., 2015). After all these processes, excessive free radical production due to CCl_4 applications exceeds the antioxidant concentration in liver, resulting in severe tissue damage and oxidative damage to cell membranes. These tissue injuries play a major contribution in the development and progression of chronic liver disorders such as cirrhosis and fibrosis (Unsal *et al*., 2020). Mitochondria generates free radicals so higher numbers of mitochondrion in liver are a significant source of free

radicals. CCl_4 instigated liver toxicity reduces activities of mitochondrial complexes I and II, impairing ATP generation and increasing oxidative stress (Sun *et al*., 2018). However, CCl_4 generated free radicals disturb the stability and integrity of mitochondrial membrane and structure, leading to mitochondrial dysfunction. This mitochondrial dysfunction leads to the uncoupling of oxidative phosphorylation causing a decrease in energy production. Uncoupled electron transport chain leads to significant electron leaks, resulting in increased ROS production and oxidative stress. The outflow of the electrons to its final acceptor during electron transport process helps it to bind with oxygen and act as major source for ROS production. Higher production of ROS causes the malfunctioning of antioxidant system, initiate lipid peroxidation, dominate radical scavengers, and upregulates the oxidative stress levels in the body (Lin ShiYu *et al*., 2019). Higher levels of CCl_4 generated ROS is responsible for lipid peroxidation induced tissue damage, increased TIMP-1 expression, decreased Epidermal Growth Factor (EGF) expression, and accumulation of collagen which leads to liver fibrosis (Hafez *et al*., 2015)

A main pathological hallmark of liver fibrosis is overaccumulation of extra cellular matrix (ECM) components (Tacke and Trautwein, 2015). CCl_4 exposure upregulates the expression level of α-SMA-positive myofibroblast-like cells, which serve as an important marker of hepatic fibrosis. Moreover, it significantly raises the levels of collagen type III and IV, laminin, and hyaluronic acid. Recent studies revealed that CCl4 poisoning significantly raises the mRNA expression of STAT-3, TGF-β, and AKT while decreasing the Nrf-2 expression which is classified as transcription factor of antioxidant producing genes in the liver (Chopra and Saxena, 2018; Li *et al*., 2009; Lin *et al*., 2012).

1.13.4. Hepatic Apoptosis induced by CCl_4

CCl4 induces higher expression of proapoptotic genes like Bax whereas lowers the expression of anti-apoptotic genes like BCl-2. It also increases the mRNA expression of Fas/FasL along with increased activity level of cytochrome P450 2E1, caspase-3 and caspase-8, promoting apoptosis and hepatic injury. Activation of caspase-8 through formation of Fas associated death domain triggers further activation of caspase 3 and 9 (Hafez *et al*., 2015; Lin *et al*., 2012). However, antioxidants play a crucial role in reducing the risk of various CCl_4-induced liver conditions such as hepatic fibrosis, liver injuries,

hepatocellular carcinoma, and chemical hepatitis. Their significance lies in their ability to mitigate oxidative stress, mitochondrial stress, and ER stress while also inhibiting macromolecular oxidation and organelle dysfunction (Unsal *et al*., 2021).

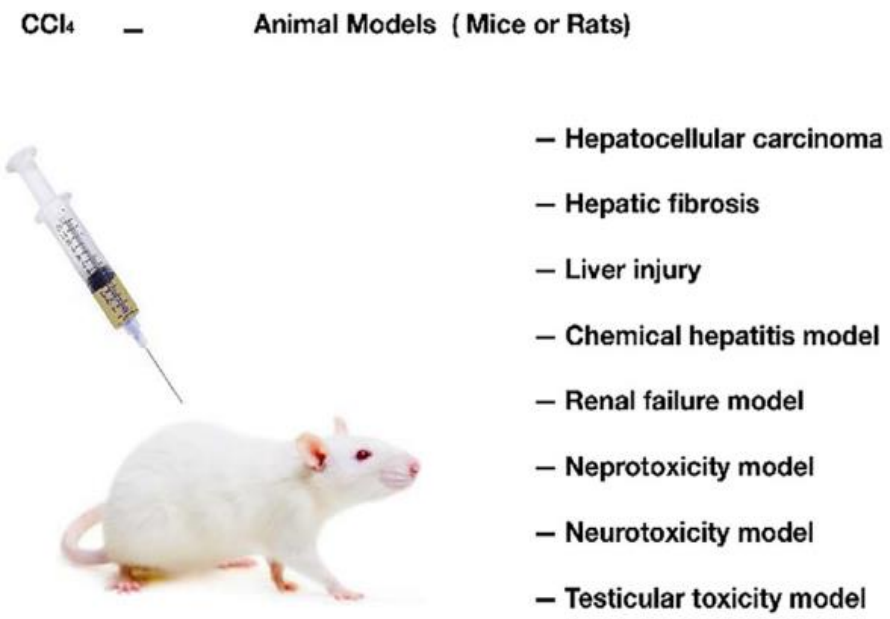

Figure 1.1: Mechanism of CCl_4 to induce hepatic and renal diseases in rat or mice (Unsal *et al*., 2021).

1.13.5. Increased apoptosis may lead to fibrosis

Apoptosis can happen as a result of genetic alterations, viral infections, exposure to any type of hepatocarcinogen, and excessive alcohol use. Liver has ability to maintain homeostasis and defend against infections which is increased by the high expression of cell-death associated receptors, particularly the Fas receptor, on its resident cells. Major liver cells, including hepatocytes, cholangiocytes, activated hepatic stellate cells (HSCs), and KCs, express Fas receptors (Faubion and Gores, 1999). The mitochondrial intrinsic pathways play a role in liver homeostasis, however Fas/FasL signaling has been primarily linked to hepatic pathophysiology. One of the BH-3 subfamily members, called Bid, is cleaved by caspase-8. The shortened version of Bid then moves to the mitochondria and triggers the intrinsic apoptosis pathway there. Studies have proved that animals lacking in bid are immune to the apoptosis of hepatic cells caused by Fas. Evidence suggests that HSCs engulf apoptotic bodies, known as phagocytosis, and may directly promote fibrinogenesis. While liver macrophages are generally considered the primary cells responsible for phagocytosis, studies have shown that endothelial cells and fibroblasts also play a role in clearing apoptotic bodies. The phosphatidyl serine receptor is expressed by both HSCs and KCs, indicating that both cell types have the capacity to absorb apoptotic bodies. The phagocytosis of apoptotic bodies by KCs leads to a significant increase in the expression of profibrogenic factors. Specifically, 24 hours after exposure to apoptotic bodies derived from hepatocytes, there is a marked upregulation of mRNA levels for TRAIL, TNF-α, FasL, and TGF-β1. Furthermore, hepatocyte apoptosis and liver damage were significantly reduced when KC was depleted from BDL animals, along with α-SMA and col1A1 mRNA expression at the same time. The elevated expression of TRAIL markers on KC could serve as effective biomarkers for the apoptosis of pathogenic hepatocytes. The prolongation of hepatocyte apoptosis triggers a cascade of inflammatory and fibrosis events. This implies that targeting KCs may be a useful therapeutic approach in cholestasis to decrease liver fibrosis and that engulfment of apoptotic bodies may extend the cycle of liver disease (Chakraborty *et al*., 2012). Blocking the engulfment of apoptotic bodies using Nocodazole negates the subsequent elevation in α-SMA and TGF-β1, suggesting that the fibrogenic activation of HSCs is specifically due to the engulfment of apoptotic bodies (Canbay *et al*., 2003).

1.14. Kidney: Organ for blood filtration

The kidneys perform a variety of functions that are combined to maintain life. The kidney regulates the balance of water and electrolytes by excreting excess when intake is excessive and conserving when there is insufficient intake or significant extrarenal loss. The kidneys actively preserve nutrients like glucose, protein, and amino acids, making them almost completely missing from normal urine. In order to maintain blood pH within extremely strict bounds, hydrogen ions are either conserved or eliminated. Urea, creatinine, and allantois are among the byproducts of nitrogen metabolism that are eliminated from the body through the urine, keeping blood levels low and stable. Apart from its regulatory functions, the kidney holds significant importance as an endocrine organ. In addition to producing erythropoietin and renin, it carries out a crucial hydroxylation needed for vitamin D function (Finco, 1980).

Kidney plays a critical role in maintaining overall body homeostasis by regulating the extracellular environment, including detoxification. However, CCl_4 treatment provides experimental models with induced renal failure, nephrotoxicity, and kidney disorders, allowing researchers to investigate various features of kidney pathology and therapeutic possibilities. Studies have reported that distribution of CCl_4 is higher in kidney as compared to liver and it has strong affinity for renal tissues (Bicalho *et al*., 2015; Makni *et al*., 2012). CCl_4 has deleterious effects on renal functions, causing various alterations in blood parameters indicative of renal dysfunction. Specifically, CCl_4 treatment is responsible for slowed kidney function, and increased creatine kinase (CK), lactate dehydrogenase (LDH), total protein, total bilirubin, albumin, blood urea nitrogen (BUN), white blood cells (WBCs), platelets, mean % of lymphocytes, granulocytes, monocytes, and decreased red blood cells (RBCs) levels in blood. Higher levels of all these parameters in blood is a sign of nephrotoxicity. While higher concentrations of urea and creatinine disrupt the structure of nephrons. These markers do not elevate until 50% of nephrons are already structurally damaged (Elsawy *et al*., 2019; Khan and Zehra, 2013; Safhi, 2018). Urine tests are important diagnostic tools for assessing kidney function. In CCl_4 treated rats, RBCs, WBCs, protein, urea, creatinine, albumin, and LDL levels are found to be high along with reduced urine pH, dehydration, chronic fibrosis, toxicity, renal necrosis, and glomerular damage (Khan and Zehra, 2013; Shehzadi *et al*., 2020). Proximal tubular cells

are highly vulnerable to CCl_4 instigated toxicity because they contain high levels of cytochrome P-450 enzymes. Metabolism of CCl_4 through this enzyme produces free radicals which cause renal injury and cell damage. Adverse effects of nephrotoxicity like inhibition of lysosomal hydrolase enzyme, failure of mitochondrial function, degradation of phospholipids, and high concentrations of intracellular calcium contribute to the development of proximal tubular toxicity (El-kholy *et al*., 2013; Unsal *et al*., 2021).

1.14.1. Renal inflammation

Since the body's reaction to disruptions in homeostasis is inflammation, it is well understood that any disease process that results in kidney damage could also trigger the inflammatory cascade at the same time. What might start as a typical inflammatory reaction can evolve into a maladaptive process, ultimately exacerbating the progression of disease. Regardless of the underlying cause of renal disease, there is compelling evidence that adults with end-stage kidney disease (ESKD) and CKD exhibit both acute and chronic pro-inflammatory states, and that inflammation increases morbidity and death rates. A significant contributing element to the development of diabetic neuropathy, retinopathy, vasculopathy, and vascular inflammation is advanced glycation end products (AGE). Atherosclerosis and inflammation are caused by interactions of these products with vascular endothelial cells (Silverstein, 2009).

Kidney failure and uraemia are significantly affected by oxidative stress. Deficiency of antioxidants helps in activation of ROS, P-53, MAPK, P-21 and ultimately causing cell death of renal tubules. ROS induces fibrosis in the kidney through direct oxidative damage to cells or indirectly by increasing inflammation. Inflammation and fibrosis create a self-perpetuating cycle that further induce ROS production and trigger release of growth factors and cytokines (JhaJay, *et al*., 2016). Free radicals generated by metabolic process of CCl_4 cause severe damage to cell membranes, protein, lipids, and DNA in renal tissues (Makni *et al*., 2012). In CCl_4 treated rats, kidneys of rats showed increased levels of lipid peroxidation products e.g. MDA, TBARS, LPO, highly induced DNA damage, and increased levels of protein oxidation products. Previous research has shown that CCl_4 administration drastically reduced concentration and activities of antioxidants, especially of glutathione system, in kidney tissues and cells. So, changes in antioxidant status due to

CCl_4 or free radicals can be a major cause of nephropathies. CCl_4 is also known to upregulate the release of inflammatory cytokines such as IL-1, IL-2, and TNF-α along with apoptotic markers such as caspase 9 and caspase 3 in renal tissues (Elsawy *et al*., 2019; Honda *et al*., 2019; Safhi, 2018). Renal tubular cells and leukocytes release cytokines which are linked to the inflammatory pathophysiology in acute renal injury. NF-kB is primarily responsible for inducing inflammatory processes by substantially modulating cytokine synthesis, which in turn raises the production of inflammatory cytokines (Safhi, 2018). CCl_4 induces significant histopathological modifications in renal tissues. CCl_4 treatments cause a range of histopathological alterations including loss of brush border, atrophy, thickening of glomerular membrane, interstitial tissue inflammation, interstitial inflammation, cellular invasion, pyknotic nucleus, medullary vascular congestion and glomerular necrosis, and epithelial cells separation in proximal and distal tubules in the kidneys of rats (Alm-Eldeen *et al*., 2016). Many research studies on the prevention and management of renal toxicity caused by CCl_4 have been carried out recently. These investigations have shown that antioxidants play a significant part in lowering or eliminating renal damage. High amounts of phenol have been linked to the protective properties of antioxidants against renal oxidative stress caused by CCl_4. According to studies, antioxidants can inhibit CCl_4-induced nephrotoxicity by elevating non-enzymatic antioxidant levels or antioxidant enzyme activity (Venkatanarayana *et al*., 2012).

1.14.2. Renal fibrosis

Renal fibrosis, especially tubulointerstitial fibrosis, is indeed important pathological feature of chronic kidney disease (CKD). Major cellular events in tubulointerstitial fibrosis include tubular shrinkage, fibroblast activation, peritubular capillary loss, and inflammatory cell infiltration. Mitochondria are essential for cellular energy production and they dysfunction is noted in individual with CKD (Huynh and Chai, 2019). The structural features of chronic kidney disease (CKD) may be influenced by the progressive loss of renal energy caused by mitochondrial dysfunction (Ducasa *et al*., 2019. Apoptosis of renal tubular epithelial cells is a significant characteristic of tubulointerstitial fibrosis (Li *et al*., 2019). This apoptosis leads to the development of CKD and renal fibrosis. The signaling cascade of the intrinsic apoptosis pathway is centred on mitochondria, and tubular apoptosis is exacerbated by mitochondrial dysfunction. The production of free radicals

causes oxidative stress and mitochondrial dysfunction aggravate this situation, leading to renal fibrosis. Therefore, an effective treatment to maintain normal mitochondrial function in renal tubular cells after injury could prevent oxidative stress and apoptosis, which have both been connected to renal fibrosis (Liu *et al*., 2020). Loss of kidney function is associated with the progression of renal fibrosis, which is the common pathway for almost all forms of kidney disease that lead to renal failure. Renal fibrosis is commonly driven by persistent fibroblasts and myofibroblasts, elevated levels of fibrogenic mediators such as TGF-β, PDGF, and IL-1β, and an imbalance between metalloproteinases and their inhibitors (Antar *et al*., 2022).

1.15. Cardiac inflammation

Inflammation, both systemic and localized, plays a crucial role in the development and advancement of cardiovascular disease (CVD), impacting various stages from endothelial dysfunction to the emergence of clinical symptoms (Libby, 2021). However, endothelial inflammatory response includes coordinated activity of innate immune system (e.g. macrophages) and adaptive immune system (e.g. B and T lymphocytes, dendritic cells). Once recruited monocytes reach subendothelial space, they transform themselves into macrophages. These macrophages then adapt themselves to various functional phenotypes based on their surrounding microenvironment (Bäck *et al*., 2015). T lymphocytes activate macrophages into either pro-inflammatory M1 macrophages or anti-inflammatory M2 macrophages. M1 produces pro-inflammatory cytokines, such as interleukin (IL)-1α, IL-4, IL-6, IL-12, IL-15, IL-18, and TNF-α, which contribute to the progression of atherosclerosis. In contrast, M2 macrophages secrete anti-inflammatory cytokines like IL-4, IL-10, IL-13, and TGF-β, which play a crucial role in inhibition of inflammation and promoting plaque healing (Vergallo and Crea, 2020). Main inflammatory biomarker of cardiovascular disease (CV risk), C-reactive protein (CRP), is produced in the liver by certain interleukins such as, IL-6, IL-12, and IL-1β (Ferencik *et al*., 2022; Henein *et al*., 2022).

1.15.1. Cardiac apoptosis

Apoptosis of cardiac cells has been observed in numerous cardiovascular conditions, spanning from atherosclerosis and myocardial ischemia to diabetic cardiomyopathy and

chronic heart failure. Studies conducted over the past ten years have revealed that apoptosis plays a substantial role in the loss of cardiac cells during reperfusion after MI. Previously, it was believed that loss of cardiac cells in response to ischemia/reperfusion (I/R) injury was caused by necrotic cell death (Olivetti *et al*., 1996). Apoptosis tends to occur mainly after ischemia is followed by reperfusion, whereas extended periods of ischemia result in necrosis. Additionally, there is proof that the acute and long-term loss of cardiac myocytes following a myocardial infarction is significantly influenced by apoptosis (Lee and Gustafsson, 2009).

1.15.2. Cardiac fibrosis

Fibrosis plays a significant role in the dysfunction of organs in numerous disorders, such as diabetic nephropathy, liver cirrhosis, progressive systemic sclerosis, and interstitial lung diseases (Ieronimakis wt al., 2013). Myocardial infarction (MI) is a common example of reparative fibrosis, which occurs when a significant number of cardiomyocytes suddenly die. This triggers inflammation, which in turn activates reparative myofibroblasts, forming a scar. In various cardiac conditions, fibrosis mainly occurs in the interstitial spaces and progresses slowly without significant cardiomyocyte loss (Frangogiannis, 2019). Cardiac fibrosis represents a significant pathological condition linked to numerous cardiovascular diseases (CVD). It is marked by accumulation of abundant ECM protein within the heart. The family of peptides known as TGF-β is extensively researched for its role as a key regulator in the fibrotic response, crucially contributing to the maladaptive remodelling of the heart after injury. The mRNA levels of TGF-β in myocardial tissue is significantly elevated in both animals experimentally induced with MI and patients with heart failure (HF). It has long been investigated that inhibition of the TGF-β signaling pathway could be used as a potential therapeutic approach to reduce fibrosis. Studying the TGF-β family poses a challenge due to the diverse effects that TGF-β peptides can induce across various cell types and under different conditions. It is well recognized that TGF-β is essential for controlling ECM accumulation and inflammation, two processes that are important components of the fibrotic response. TGF-β signaling inhibits inflammation and controls the production of pro-inflammatory cytokines including TNF-α. Mice lacking TGF-β1 have revealed elevated levels of autoimmunity, approving the significance of TGF-β in modulating the inflammatory response. However, it has been demonstrated that TGF-β

signaling causes fibroblasts to change into active myofibroblasts, which are the primary biological source of the ECM protein accumulation found in the fibrotic region. Because TGF-β signaling plays multiple roles, studies have shown that targeting this pathway effectively relies on precise timing and specificity for achieving desired outcomes (Park *et al*., 2019). Elevated levels of apoptosis have been observed in the cardiac tissues of both diabetic patients and diabetes induced animal models, with the loss of cardiac myocytes being linked to the onset of diabetic cardiomyopathy. Furthermore, it has been reported that the mortality rate from MI is more than twice as high in diabetic individuals as it is in non-diabetic individuals. Cardiac myocytes in the diabetic myocardium seem to be more prone to apoptosis, indicating that the increased vulnerability to MI is due to a greater loss of these cells through apoptosis in response to stress (Lee and Gustafsson, 2009).

1.16. Diabetes mellitus

Diabetes mellitus (DM) is a set of metabolic disorders associated with hyperglycemia, defective free radical scavenging enzymes, lipoprotein aberrations, elevated basal metabolic rate (BMR) and damage due to oxidative stress (Shah and Khan, 2014). A malfunction in the production of insulin, its action, or both, result in insulin insufficiency, which leads to persistent hyperglycemia with alterations in carbohydrate, lipid, and protein metabolism. (Doss *et al*., 2009).

When pancreatic beta cells are attacked by autoreactive T lymphocytes, it results in type 1 diabetes (T1D), which puts a person in peril to acquire hyperglycemia (Draznin *et al*., 2022). Although it only accounts for up to 10% of all instances of diabetes that have been documented globally, however, its prevalence is increasing (Mobasseri *et al*., 2020). Over 50 genetic loci have been reported for having moderate to small effect on inducing type 1 diabetes but the genetic alteration in the HLA region conferring the highest genetic risk factor associated with type 1 diabetes (Robertson & Rich, 2018). T2DM accounts for around 90% of diabetic patients. Inadequate tissue insulin resistance (IR), insufficient insulin secretory response from the pancreatic islet cells, and insufficient insulin production are the main features of T2DM. As the illness becomes more severe, the body's production of insulin is unable to keep the blood glucose level stable, which causes hyperglycemia (Galicia-Garcia *et al*., 2020; Yaribeygi *et al*., 2020).

1.16.1. Streptozotocin

Streptozotocin (STZ), is chemically a glucosamine nitrosourea compound with chemical formula $C_8H_{15}N_3O_7$, STZ appears as an off-white crystalline powder. It is a water loving compound. A soil bacterium called Streptomyces acromogenes provided the initial source of STZ, which exhibited bactericidal potency. It was primarily employed as an alkylating agent in chemotherapy for pancreatic cancer. STZ has diabetogenic potential and is preferable over several other chemicals available for inducing human diabetes in animals because the biochemical changes seen in STZ-induced diabetes closely resemble those exhibited in diabetic human beings. The induction of both type 1and 2 DM by STZ has been reported. The STZ dose and duration, however, are crucial in the development of the specific form of diabetes. STZ can be administered intravenous or intraperitoneally, however, it shows less toxicity intraperitoneally (Lenzen, 2008; Selvaraju *et al*., 2012). After administration it passes through three phases as below:

- In the first phase, two hours after injection the blood glucose level rises due to sudden glycogenolysis with a concomitant drop in insulin level.
- After about 6 hours of STZ injection, blood glucose level drops due to high level of insulin. This hypoglycemic state could be severe enough to cause death.
- In the third phase, hyperglycemia will remain permanently for a longer period. This will be observed after 12 to 48 hrs. of administration.

These alterations in the glucose and insulin contents in circulation reflect abnormalities in the functioning of beta cells of pancreas. STZ is taken up by GLUT2 transporters that are not only expressed in pancreas but also in the kidney hence causing damage to renal cells (Lenzen, 2008). Although streptozotocin is hydrophilic but the presence of nitrosourea moiety enables it to cross the cellular membranes and owing to glucose moiety in the structure of STZ allows it to be recognized by pancreatic beta cells GLUT2 transporters.

1.16.2. STZ: Mechanism of action

STZ has ability to methylate DNA at guanine nucleotide position because of nitrosourea moiety having DNA methylation property. It leads to DNA fragmentation which in turn activates PARP (poly ADP-ribose polymerase) for DNA repairing. PARP lowers cellular

energy by depleting NAD+ and ATP reserves. It also causes protein methylation and contributes to cell death (Rais *et al*., 2022).

1.16.2.1. Nitric Oxide (NO) production and nitrosative stress

STZ stimulates NO production which activates guanylyl cyclase enzyme to form cyclic guanosin-monophosphate (cGMP) that also contributes to DNA damage. As β-cells have low levels of antioxidant enzymes therefore more vulnerable to STZ toxicity by NO. Increased NO can hereby lead to nitrosative stress-a harmful condition characterized by the generation of RNS, including peroxynitrite. It is highly reactive molecule due to its action against cellular components, since it modifies proteins and nitrates them, disrupts membranes because of damage to lipids, and promotes DNA strand breaks-all compromising cellular integrity and function (Lenzen, 2008). This injury can provoke several pathophysiological processes such as inflammation and cell death among them. Among all of the cells in the body, pancreatic β-cells are uniquely sensitive to both oxidative and nitrosative stress due to an intrinsic low antioxidant defense. Indeed, insulin-producing β -cells have been demonstrated to express extremely low levels of general protective enzymes like CAT, SOD, POD to detoxify ROS and RNS. Therefore, β-cells are less well prepared to neutralize these active species whose high oxidative or nitrosative stress promotes readily their dysfunction and destruction. This is particularly important under conditions such as diabetes, where β-cell damage plays a part in the development of the disease (Lenzen, 2008). Antioxidant metabolites neutralize these RNS thus protecting β-cell from damage and inhibit progression of diabetes.

1.16.2.2. Oxidative stress

Oxidative stress also plays a role in the destruction of β-cells. As STZ causes glucotoxicity because of its elevated level, it results in several reactions including glucose autooxidation, polyol pathway, protein glycation or AGE's formation. These collectively results in an increase of ROS in cells and leads to oxidative stress. STZ is also known to lowers the level of antioxidant enzyme and elevates the pro-oxidant level which causes LPO to produce malondialdehyde, considered as a marker for oxidative stress. As STZ gets metabolized within cells, it causes ATP to degrade into uric acid in the presence of xanthine oxidase enzyme from hypoxanthine. During this conversion, OH- or superoxide radicals

are generated and contribute to the development of oxidative stress. Increased ROS production also reduces the activity of aconitase enzyme which is renowned for its protective role against mDNA damage (Nahdi *et al*., 2017).

1.16.2.3. ROS production

Furthermore, numerous studies have indicated that NO is not the sole molecule responsible for the cytotoxic effects of STZ. It has been discovered that STZ generates ROS, which also contributes to DNA fragmentation and other harmful cellular changes. Increased dephosphorylation of ATP enhances the availability of xanthine oxidase substrate, to which β-cells have a higher affinity, leading to an increased production of uric acid, the final degradation product of ATP. Xanthine oxidase catalyzes the reaction that produces superoxide anions. The formation of superoxide anions subsequently leads to the production of $H_2O_2$and hydroxyl radicals (Kumar *et al*., 2012a; Rais *et al*., 2022).

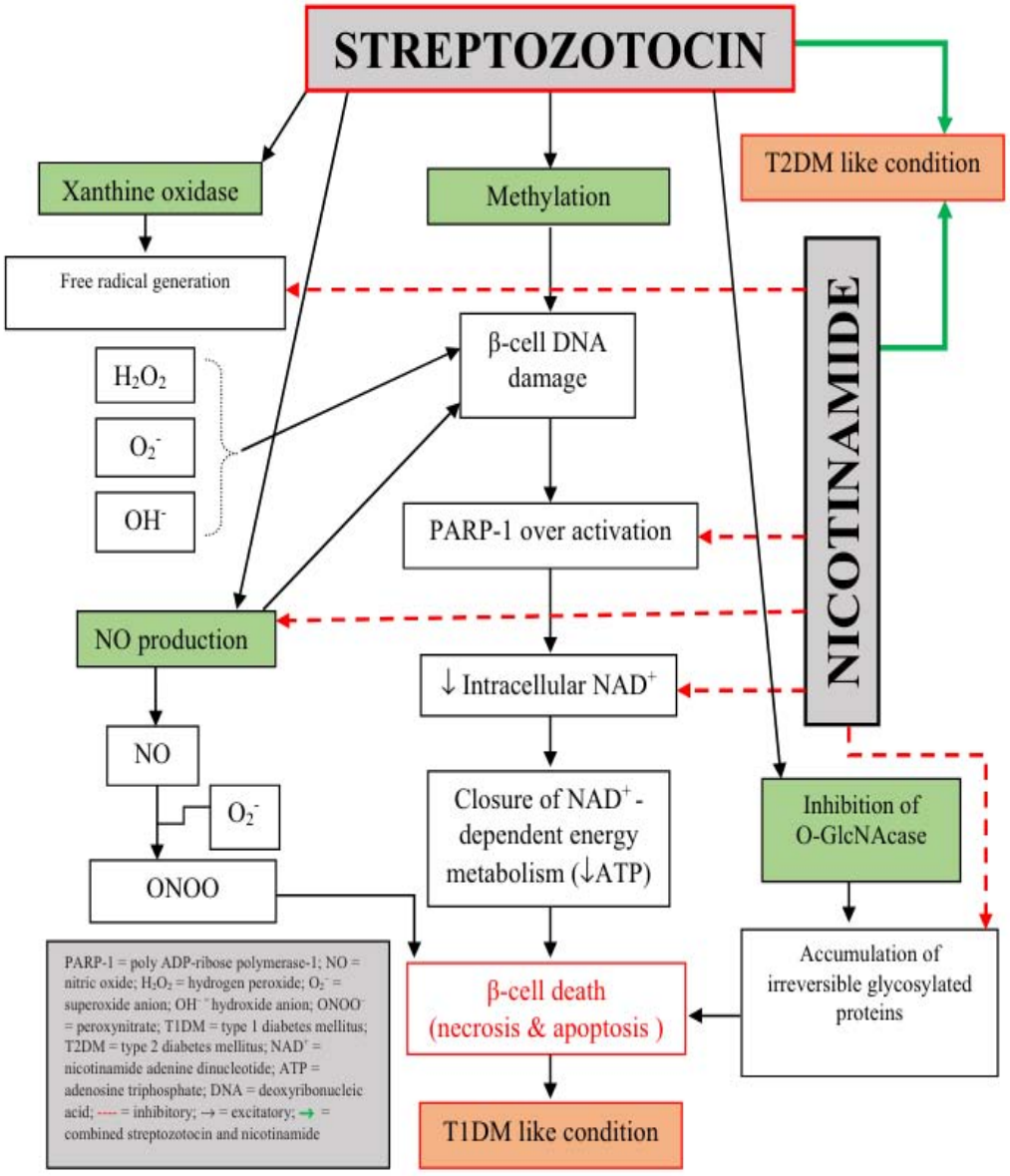

Figure 1.2: Mechanism of STZ action with partial protection provided by NA in pathogenesis of type-2 diabetes in-vivo model (Rias *et al*., 2022).

1.16.3. **Combined effects of streptozotocin and nicotinamide**

Nicotinamide (NA) is a derivative of niacin with sufficient antioxidative ability, which reduces the detrimental effects of cytotoxic agents like STZ. NA protects β-cells against STZ by several mechanisms. It has the ability to scrounge ROS and acts as a source of NAD^+. It augments the regeneration of beta islets and hinders apoptosis. NA also reduces DNA methylation effects of STZ by acting as an acceptor of methyl groups (Ghasemi *et al*., 2014). Hyperglycemia induced cellular damage is significantly influenced by oxidative stress. Free radical production is overstimulated during high levels of glucose while the body's defense system is weak enough to normalize the dominating oxidative damage. During this condition, consistent free radical generation seems to initiate the dys-functioning of β-cells. The insulin-sensitive tissues (e.g. muscular, hepatic and cardiac tissues) face high fatty acid fluctuation, while non-insulin sensitive tissues (e.g. renal and neural tissues) encounter both high glucose and high fatty acid levels. Each of these worst-case scenarios account for ROS induced diabetic complications (Yaribeygi *et al*., 2020). ROS formed due to oxidative stress trigger damaging inflammatory cascades (like hexosamine pathways) (Shah & Khan, 2014), generation of advanced glycation end products (AGEs) (Volpe *et al*., 2018) and apoptosis in beta cells (Kohnert *et al*., 2012). These processes cause severe molecular and cellular damage that provoke the disease. The pathways for development of oxidative stress in diabetic animals includes auto-oxidation of glucose, polyol pathway, and AGEs formation generating free radicals (Aboonabi *et al*., 2014). STZ diminishes the level of antioxidant enzymes, elevates pro-oxidants causing LPO to produce malondialdehyde, a marker for oxidative stress. STZ metabolism in cells forms uric acid from ATP by xanthine oxidase, liberates OH^- and superoxide radicals. Increased flush of ROS inactivates DNA protective enzyme, aconitase. Therefore, NO pathway and oxidative stress are somehow linked and cause β-cell destruction following STZ dose (Rias *et al*., 2020).

1.16.4. Oxidative stress and diabetic complications

Free radicals are highly reactive biomolecules produced physiologically during metabolic processes and by immune cells (Staveness *et al*., 2016). The majority of biologic cells have an internal defense system that includes enzymes like GSH, CAT, and SOD that protects

them from free radical damage. In addition to their direct harmful effects, free radicals can also cause indirect cell damage through the activation of a number of stress-sensitive intracellular signaling pathways, including sorbitol synthesis, AGE/RAGE interactions, and Nf-κb. Oxidative stress is central to the pathophysiology of various diabetic complications by causing lipid peroxidation, DNA damage, and mitochondrial dysfunction (Yaribeygi *et al*., 2020).

1.16.5. Diabetic inflammation

Inflammatory response is a fundamental molecular mechanism involved in the pathophysiology of insulin resistance, diabetes mellitus, and its related complications. Mounting evidence indicates that low-grade chronic inflammation is implicated in the pathophysiology of DM and insulin resistance (Yaribeygi *et al*., 2019). The relationship between inflammatory responses and DM is intricate and not completely understood (Keane *et al*., 2015). Cytokines have the ability to activate JNKs, which subsequently trigger serine phosphorylation of IRS-1, resulting in impaired insulin signaling transduction (IST). Similar effects have been observed with other inflammatory cytokines like TNF-α and NF-κB, which can influence serine phosphorylation of IRS-1 at different sites, thereby disrupting normal IST (Krause *et al*., 2012). Oxidative stress acts as an initiating event for inflammation by activating monocytes and macrophages and triggering inflammatory responses associated with insulin resistance and diabetes mellitus (Zhang *et al*., 2017). Moreover, it enhances the expression of pro-inflammatory cytokines, leading to an increase in inflammatory mediators at both the mRNA and protein levels (Elmarakby and Sullivan, 2012). Therefore, inflammation induced by free radicals represents another potential connection between oxidative stress and insulin resistance ((Keane *et al*., 2015).

1.16.6. Role of hyperglycemia in kidney disease

Hyperglycemia trigger glomerular hyperfiltration and hypertension, two hemodynamic mechanisms that have long been acknowledged as initiating and perpetuating kidney damage in diabetes (Kahn *et al*., 2014). High concentration of amino acids, such as those observed after excessive intake of protein or hormonal alterations linked to inadequate glycemic control, like elevated glucagon levels, worsen glomerular hyperfiltration (Yaribeygi *et al*., 2019) Furthermore, renin-angiotensin system activation is a major local

stimulus for glomerular hyperfiltration. Production of angiotensin II within the kidney leads to constriction of the efferent arteriole, thereby increasing glomerular pressure. Angiotensin II induces the release of proinflammatory and profibrotic mediators through both barotrauma and direct cellular effects (Kahn *et al*., 2014). Hyperglycemia initiates a cascade of intracellular events that contribute to renal injury by promoting inflammation and fibrosis (Staveness *et al*., 2016).

1.17. Role of medicinal plants in modern healthcare

Medicinal plants have been a fundamental part of human health and culture long before recorded history, with thousands of species used worldwide for their diverse therapeutic properties. These plants are rich in bioactive compounds that perform a variety of biological activities, i.e., antioxidant, and inhibitory effects against microbes and inflammation. In modern science, the study of these plants continues to inform pharmaceutical development, exemplified by drugs like aspirin, which was inspired by the properties of willow bark. Medicinal plants are rich sources of bioactive compounds, which offer a range of health benefits, including antioxidant, cytotoxic, nephroprotective, anti-fungal properties (Shah *et al*., 2018). These diverse effects make medicinal plants invaluable in traditional and contemporary medicine across the globe. Many countries such as Korea, Egypt, China, India, and Malaysia have developed unique formulations using various native plants and herbs, reflecting their rich medicinal heritage (Philip *et al*., 2009). The integration of traditional knowledge and botanical resources into modern pharmacology is evident, as nearly 25% of today's pharmaceuticals are derived from plants. Around 65% of patients in the USand Europe use herbal medicines to treat hepatic illness, attracted by their broad availability, diverse biochemical profiles, pharmacological benefits, and lower incidence of side effects compared to synthetic medications. Research has extensively explored phytochemicals from medicinal plants due to their role in disease prevention and health enhancement, investigating both their effectiveness and how they work. Findings suggest that these compounds can reduce the risk of coronary cardiac disease by minimizing the oxidation of low-density lipoprotein (LDL), cholesterol and improving arterial flexibility. Additionally, they contribute to the detoxification of cancer-causing toxins, the neutralization of ROS, the inhibition of enzymes that activate carcinogens, and the stimulation of enzymes that detoxify carcinogens. A study revealed

reveals that phenolics represent the most plentiful and structurally diverse group of phytochemicals in plants, extensively studied for their potential to combat oxidative damage linked to degenerative diseases which includes some conditions such as cancer, hepatotoxicity, inflammation, and cardiovascular disorders. The focus on phenolics stems from their effectiveness in addressing the oxidative stress that underpins many such health issues (Venmathi Maran *et al*., 2022).

1.18. Folk medicines: Traditional plant used against inflammation

Inflammation serves as a protective response by the immune system to tissue injury or the invasion of harmful foreign substances or pathogens (Pugin, 2012). Acute inflammation is characterized by the rapid healing and discarding of impaired cells at infection site in short time. Depending on the tissue type and stimuli, inflammation can be treated by a variety of mechanisms (Fullerton and Gilroy, 2016). Chronic inflammation is a key factor in the onset of numerous clinical conditions, such as lung diseases, rheumatoid arthritis, type II DM, obesity, CVD and cancer (Laveti *et al*., 2013; Kunnumakkara *et al*., 2018). According to the WHO (2005), chronic disorders are considered a major cause of death worldwide, with chronic inflammatory diseases accounting for 60% of these fatalities (Stuckler, 2008). Inflammatory and pain-related disorders continue to be significant global concerns, impacting people across all demographics. In addition, pain stands as the primary cause of disability around the world, severely affecting individuals' quality of life and productivity (Uritu *et al*., 2018). Despite the availability of several classes of medications for such diseases, non-steroidal anti-inflammatory drugs (NSAIDs) are the most prescribed. However, toxicity and various deleterious effects are associated with clinical use of NSAIDs (Sostres *et al*., 2010). Therefore, the pursuit of possible phytotherapies is essential. People throughout the world have been using medicinal plants for thousands of years and they serve as the basis for numerous allopathic drugs, functional food, dietary supplements, and medicines (Shakya, 2016). Secondary metabolites, which are bioactive components that are important in increasing the therapeutic impact of various diseases, are abundant in medicinal plants (Kumar *et al*., 2022). Medicinal plants can act as natural stimulators to help regulate immune responses (Devasagayam and Sainis, 2002). Recently, there has been an increase in interest in the medicinal efficiency of traditional medications because of increasing problems related to modern drugs and treatments like bacterial

resistance (Cheesman *et al*., 2018), diminishing efficacy of certain drugs, and adverse effects linked to many synthetic medications. Significantly, over half of the drugs utilized worldwide in advanced remedies are sourced from natural compounds. The usefulness of these plants as possible sources of pharmaceuticals is shown by the widespread commercialization of efficient and strong medications made from medicinal plants. Quinine, for example, is a well-known anti-malarial alkaloid isolated from the bark of the natural plant *Cinchona officinalis.* Chloroquine, a compound derived from quinine, has demonstrated the ability to modulate autoimmune disorders and has recently been identified as effective in antitumor treatments. Another notable example is the production of the anti-inflammatory drug acetylsalicylic acid (aspirin), which is synthesized from the natural compound salicin. It is widely believed that salicin is present in the bark of *Salix alba*. *Spiraea ulmaria* is a source of glycoside spiraein and this is the plant from which the name "Aspirin™" was derived. Since 400 BC, acetyl salicyclic acid-containing plant products have been utilized historically to alleviate fever and pain. Approximately 80% of people use natural compounds as pain-relieving or to inhibit nociception. The quest for possible anti-inflammatory treatments from natural products is not limited to southern Africa. For instance, researchers analyzed many Swedish herbal medicines which act as protective agents against prostaglandin synthase enzyme. Similarly, many medicinal plants in Asia are known to possess strong inhibitory activity against inflammation. The study reported eleven medicinal herbs, locally used as folk medicine in Turkey, contain anti-inflammatory and pain-relieving properties (Khumalo *et al*., 2022). 17 Australian natural plant species of Eucalyptus were examined for their properties i.e., anti-inflammatory, which are employed by the Dharawal locals. 70 Brazilian plant species were found to have anti-inflammatory potential. The growing interest in phytotherapies is also noticeable in developed countries, particularly across Europe and North America. *Harpagophytum procumbens*. and *Harpagophytum zeyheri* are well-known local plants in southern Africa, traditionally used for treating inflammation, autoimmune disease and as pain relievers (Khumalo *et al*., 2022). The Aloe genus is well-known for its powerful activities to treat inflammation and arthritis (Paul, 2021). Since 1761, *Aloe ferox Mill.* has been imported by Europe for commercialization purposes. The therapeutic and phytochemical characteristics of this plant have been thoroughly investigated (Sharma *et al*., 2014).

Notably, the anti-inflammatory potential of Aloe species impacted the activity of COX-1. Plant extracts with high concentrations of flavonoids and flavones demonstrated greater activity compared to other chemotypes (Sharma *et al*., 2014). Although numerous studies reported the therapeutic effectiveness of plant-based compounds for the treatment of inflammation, a significant challenge remains in validating their safety and efficacy through human trials. Several anti-inflammatory agents which are isolated from plants, such as curcumin, resveratrol, and quercetin, have been thoroughly studied and progressed to clinical trials. Curcumin, a key ingredient in turmeric spice, is obtained from *Curcuma longa* L. In Ayurvedic medicine, *C. longa* is a reme3dy for the cure of inflammatory ailments. Similarly, curcumin reduces the production of cytokines, blocks the expression of cell adhesion molecules and acts as an inhibitor of pro-inflammatory signaling pathways such as NFκB, MAPK, COX, and LOX. Although clinical trials of curcumin have been conducted, the available data is insufficient to meet the regulatory requirements for safety and efficacy necessary for commercialization (Gupta *et al*., 2013). Resveratrol, a stilbene constitute of wide range plant sources, has been thoroughly investigated against inflammation and found to inhibit the NFκB, AP-1, and COX-2 pathways (Fürst and Zündorf, 2014). Some NSAIDs, like indomethacin, are used in inhibiting prostaglandin transporter proteins. Prolonged use of NSAIDs has been linked to gastrointestinal toxicity due to the inhibition of PGI2 and PGE2, derived from COX-1. Additionally, efforts to create selective COX-2 inhibitors have led to unwanted cardiovascular risks (Khumalo *et al*., 2022). Polyphenols are known to possess antioxidant activity and many other physiological properties since they have potential to protect cardiovascular system of the body against oxidative stress, high blood pressure, and increased inflammation (Prahalathan *et al*., 2012). Stilbenoid, isolated from *Gnetum macrostachyum*, was reported to have an inhibitory capacity on adhesion and aggregation of human platelets because it has inhibition effects against inflammation. Consumption of plant extracts and fruits with high content of antioxidants exhibit anti-diabetic effects such as various citrus fruits exhibited promising inhibitory effects on α-glucosidase and pancreatic lipase in vitro, attributed to their rich content of flavanones. Furthermore, antioxidants found in *Vaccinium floribundum* (anthocyanins) and *Aristotelia chilensis* (proanthocyanidins) demonstrated the ability to attenuate adipogenesis and inflammation (Zhang *et al*., 2015).

1.19. Medicinal plants for diabetes treatment

Approximately 800 plants have been reported to possess antidiabetic potential. A large collection of active principles from plants, comprising various bioactive compounds, have shown potential for use in diabetes treatment. Some of the most commonly used and effective antidiabetic plants originating from India include neem (Azadirachta indica), onion (Allium cepa), eucalyptus (Eucalyptus globules), tulsi (Ocimum sanctum), jamun (Syzygium cumini), and sweet potato (Ipomoea batatas). All of these plants are rich in phytochemicals (Rizvi and Mishra, 2013).

1.20. Plant extracts: Healing properties

Plant crude extracts display a broad range of pharmacological capabilities i.e. anti-cancer, antimicrobial, antioxidant, anti-inflammatory, analgesic, antipyretic and hepatoprotective potential. Crude extracts are generally prepared by extracting the bioactive constituents from plant material with a suitable solvent. Different solvent systems can be used for extracting active components (antioxidants and polyphenols) from plants. The output of extraction varies depending on the nature of solvents and the extraction technique employed. Water, ethanol, acetone and methanol are the most commonly used solvents in plant extraction (Roby *et al*., 2013).

Plant crude extracts are mixtures of countless compounds which were screened previously for phytochemicals, anticancer, antioxidant, anti-inflammatory, analgesic, antipyretic and antimicrobial properties (Ali *et al*., 2017; Jan *et al*., 2016; Sahreen *et al*., 2017).

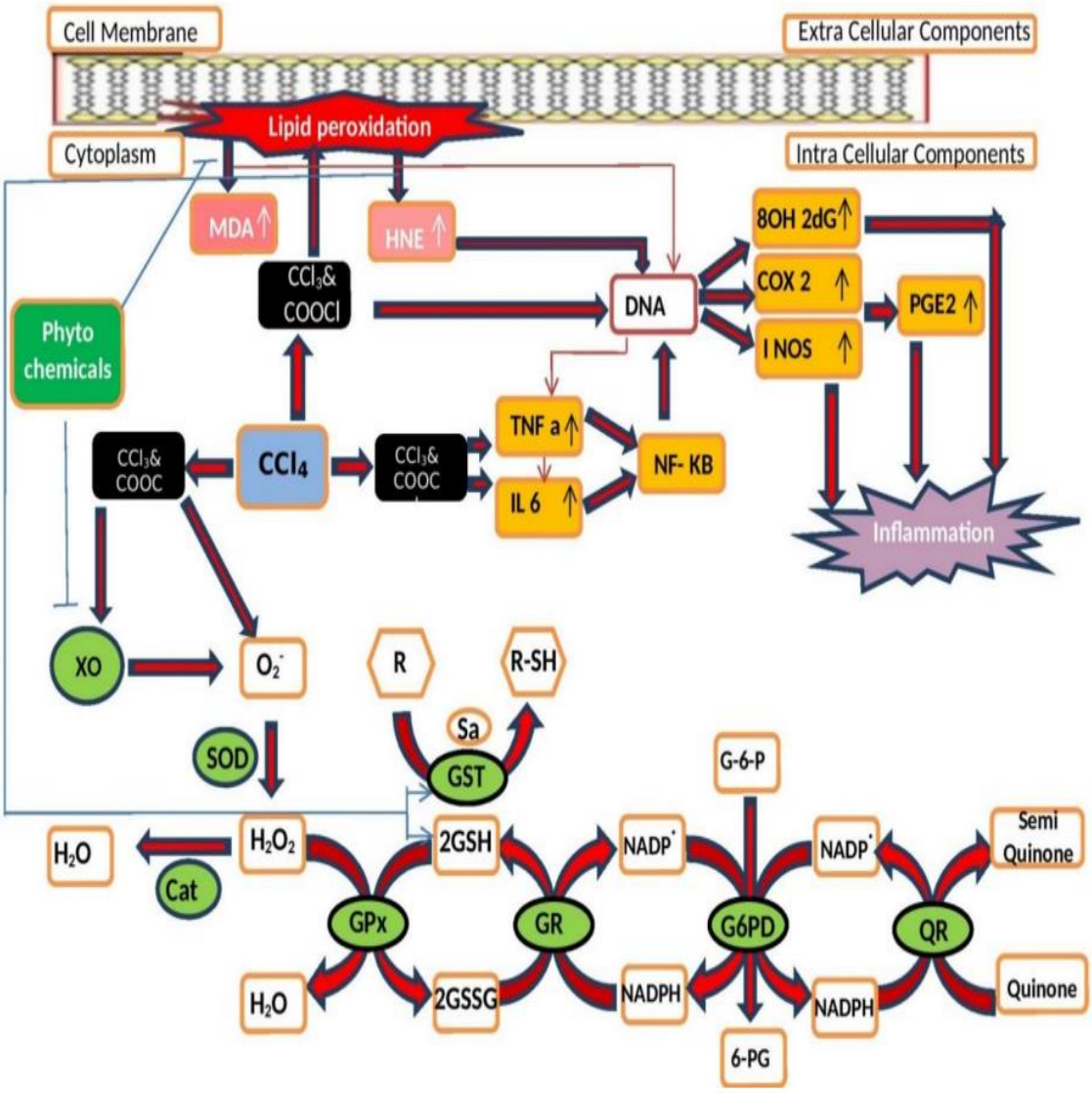

Figure 1.3: Effects of CCl_4-induced ROS on the defense system of enzymatic antioxidant and phytochemical antioxidant compounds. The enzymatic antioxidant along with phytochemicals can elevate the toxic effects of ROS through reduction in the levels of malondialdehyde (MDA) production and increasing the production levels of inflammatory cytokines and prostaglandin E2 (PGE2) (Venmathi Maran *et al*., 2022).

1.21. Oleaceae family

The Oleaceae family roughly contains 30 genera and over 600 species. *Jasminum L.* stands out as the largest genera of the family with 200 species. The family is renowned for its multiple uses including nutritional, ornamental & perfumery and horticulture as well. The species are mostly disseminated to the tropical region of the world whereas Southeast and Southwestern Asia forms the center of diversity for these species (Akhtar *et al*., 2021). The blooms are four-merous, hypogynous, and typically have two stamens, while some species have four stamens. The genera of the family also have economic importance such as olive is farmed for its fruit and oil, and *Fraxinus* species are grown for timber. Most importantly, the oleaceous family has got pharmacologically valued against treating various ailments such as the sprouts of *S. vulgaris* used against join pain, the bark of the manna ash is often used as antidiarrheal and lowering cholesterol. Similarly, both anticancer and antioxidant effects are mediated through olive oil (Khan *et al*., 2014) Phytochemical studies have shown that flavonoids, monoterpenoids, iridoids, secoiridoids, and phenylethanoid glycosides are the primary phytochemical compounds from this family (Huang *et al*., 2019).

1.21.1. Jasminum genus

Most of the flower bearing plants are categorized under Jasminum genus of the Oleaceae family. Jasmines come in a variety of genetic forms including seasonal or evergreen shrubs that may be erect or climbers with fragrant flowers of different colors (Akhtar *et al*., 2021). Quite a few volatile oils and essential oils are sourced from the flowers of such plants for applications in fragrances and therapeutics. Extracts from different species within the genus have demonstrated a range of medicinal properties, including anti-diabetic, anthelmintic, antibacterial, anticancer, antioxidant, cytotoxic, antiulcer, chemopreventive, anti-hepatitis B, antinociceptive, and anti-inflammatory effects (Khan *et al*., 2014).

Jasminum is the largest genera of Oleaceae family with ~200 species having major distribution in the tropical regions. Jasmines have vast genetic riches existing as wide and cultivated, with members as ever leaf, creeper or upright bushes with colored flowers (Akhtar *et al*., 2021). *J. grandiflorum*, one of the Jasminum species, may be found in moderate subtropical zone. There are several *J. humile* varieties scattered over the

temperate and subtropical regions from Abbottabad to the higher regions of Kohistan, Waziristan, and certain temperate sections of Baluchistan. A few species, such as *J. elongatum*, may be found in the Azad Jammu and Kashmir area of Pakistan. *J. mesnyi* is frequently observed in Islamabad, Pakistan, especially the area around the Margalla Hills. In Pakistan, *J. nitidum* and *J. sambac* are the most often grown plants (Huang *et al*., 2019).

1.21.2. Ethnobotanical use of Jasminum

Jasmines (*Jasminum* spp.) broadly planted flowering plant of the family Oleaceae. A vast majority of the species of this genus are traditionally being used in the medication against various ailments such as *J. sambac* is renowned for its use as a pain suppressant and disinfectant along with its application as a fragrancy odor cosmetic product. Additionally, oil extract was noted to possess antioxidative and antimicrobial effects (Abdoul-Latif *et al*., 2010).

Jasminum grandiflorum another species of this genus is had been utilized as folk medicine against treating ulcer, skin diseases, leprosy etc. (Sandeep *et al*., 2009). Several oleaceous plants have been investigated for their cytotoxic and anti-cancerous effects. Although some research has been done to dig the pharmacological potential of *Jasminum humile,* it had been in use traditionally as cardiac tonic, medicating against chronic fistulas and solid lumps. *J. humile* has also been reported as a therapeutic cure against ringworms. Several plants in the Oleaceae family, including Olea europaea and various Jasminum species, have been reported to possess potent cytotoxic and anticancer activities (Mansour *et al*., 2022).

1.21.3. *Jasminum humile*

Jasminum humile, originates from tropical regions of India, Burma and Himalayas, and is widely recognized for its use in the perfume industry. *J. humile* contains various antioxidant phytochemicals including indole alkaloids like gelsemine and gelsedine, in addition to iridoids, coumarins, and tannins (Nain *et al*., 2011). Previous literature, such as the Indian Materia Medica, demonstrates that this plant is used to cure various respiratory diseases (whooping cough, asthma), cardiac and bowel disorders, intestinal issues, and ringworm infections. This plant is well-known for its therapeutic activities including antispasmodic, antidepressant, CNS disorder treatment, anti-inflammatory, antiseptic, aphrodisiac, expectorant, and uterine tonic effects. The leaves are used to cure skin

conditions, they work similarly to contemporary topical anti-inflammatory drugs. The plant's milky liquid is also used to extract the diseased tissue that lines long-term sinuses and fistulas. There is still a dearth of thorough information regarding its biological characteristics, despite its outstanding applications. There hasn't been any substantial research done to date to support the traditional usage of *Jasminum humile* (Nain *et al*., 2011; Nain *et al*., 2018).

Figure 1.4: *Jasminum humile* (Linn.), Yellow Jasmine, Peeli Chameli

Figure 1.5: Herbarium Specimen of *Jasminum humile* (Linn)

1.22. Apiaceae family

Medicinal plants have garnered global attention from researchers due to their significant potential as raw materials for the food, flavouring, medicinal, and cosmetic manufacturing. Despite advancements in synthetic drug discovery, many of the chemical compounds in use today are still derived from plants, utilizing modern technologies to enhance traditional approaches. The Apiaceae family is a highly significant family of flowering plants, comprising 3,780 species across 434 genera. The Apiaceae family is distributed worldwide, primarily in northern temperate regions and at high altitudes in tropical areas. Apiaceae species share several key characteristics: they are floral and blossomy with characteristic fragrance, stems are hollow, bear small flowers, and their inflorescences are arranged in simple or compound umbels. Additionally, they produce indehiscent fruits or seeds that contain oil ducts. The secretory cavities in this family, which are found in the leaves, fruits, stems, and roots, are comprised of schizogenous oil ducts that contain mucilage, oil, or resin. This family is well recognized for its unique flavors.

1.22.1. Ethnobotanical use of Apiaceae

Apiaceae family offers numerous plants used for a variety of purposes, such as food, pharmaceuticals, beverages, seasoning, repellents, dyes, cosmetics, perfumes, and industrial applications. Many plants of this family are used to cure a variety of ailments affecting the reproductive, endocrine, digestive, and respiratory systems. This family has a high quantity of secondary metabolites and phytochemicals that could be used to make medications such as steroids, terpenoids, flavonoids, coumarins, polyacetylenes, and triterpenoid saponins. Moreover, this family has a number of species that are good sources of essential oils. The essential oils have been found to have over 760 distinct components from several chemical classes that have significant therapeutic value. The Apiaceae family is among the oldest groups of aromatic plants. Most of its species are native to Southwest Asia and the Mediterranean region, but they are introduced and grown all over the world for their use in food, medicine, cosmetics, and fragrance manufacturing. These plants, particularly cumin, caraway, and fennel, are indicated for gastritis and neurological disorders, vomiting, and diarrhea. They also exhibit potential therapeutic effects against muscle spasms, bacteria, abdominal pain, and ulcers. Apiaceae species are widely utilized

in daily nutrition, both as drinks and as food in a variety of preparations, particularly as spices (Christensen and Brandt, 2006; Sayed-Ahmad *et al*., 2017).

1.22.2. *Pleurospermum candollei* (DC.) C.B. Clarke

Pleurospermum candollei, known as Shoogroon in the reigon of Karakoram and Himalayan and a specie of family Apiaceae. This herb, which grows to a length of 30–40 cm, is consumed as a vegetable by local people. It is consumed by the residents of Karakoram region for its flavor and nutritional value. This species is available for commercial sale in certain areas and has also been used to treat various disorders. The whole plant is used to treat ailments of the stomach and abdomen. Additionally, it lowers blood pressure and cholesterol and relieves cardiac and gastric issues. To treat fever and headaches, one teaspoon of dry plant powder is taken with milk once a day for a week. It can also be cooked with leafy vegetables for the same purpose (Ahmed *et al*., 2022). It is used to treat respiratory conditions, and research indicates that it is also beneficial for treating pain, unconsciousness, and cerebral issues. The Himalayan people use this herb to treat a wide range of various illnesses. Various compounds of plant were extracted to investigate the anti-inflammatory properties (Ali *et al*., 2021). In the Gilgit-Baltistan region, the stem powder of *P. candollei* has been used to alleviate joint pain and back pain. Decoctions made from *P. candollei* have also been utilized to treat both male and female infertility. Additionally, it is also found beneficial against diarrhea in animals. The Apiaceae family and Pleurospermum genus have been demonstrated to be rich source of various phytochemical antioxidants and are used for their medicinal, pharmacological, nutraceutical, cosmetic, and food value due to the abundance of these antioxidants and their wide spectrum of biological activities. Although there is some evidence of local usage of *P. candollei* in healthcare, its phytochemical potential and biological activities have not been thoroughly investigated (Ahmed *et al*., 2022).

Figure 1.6: *Pleurospermum candollei* (DC.) C.B. Clarke

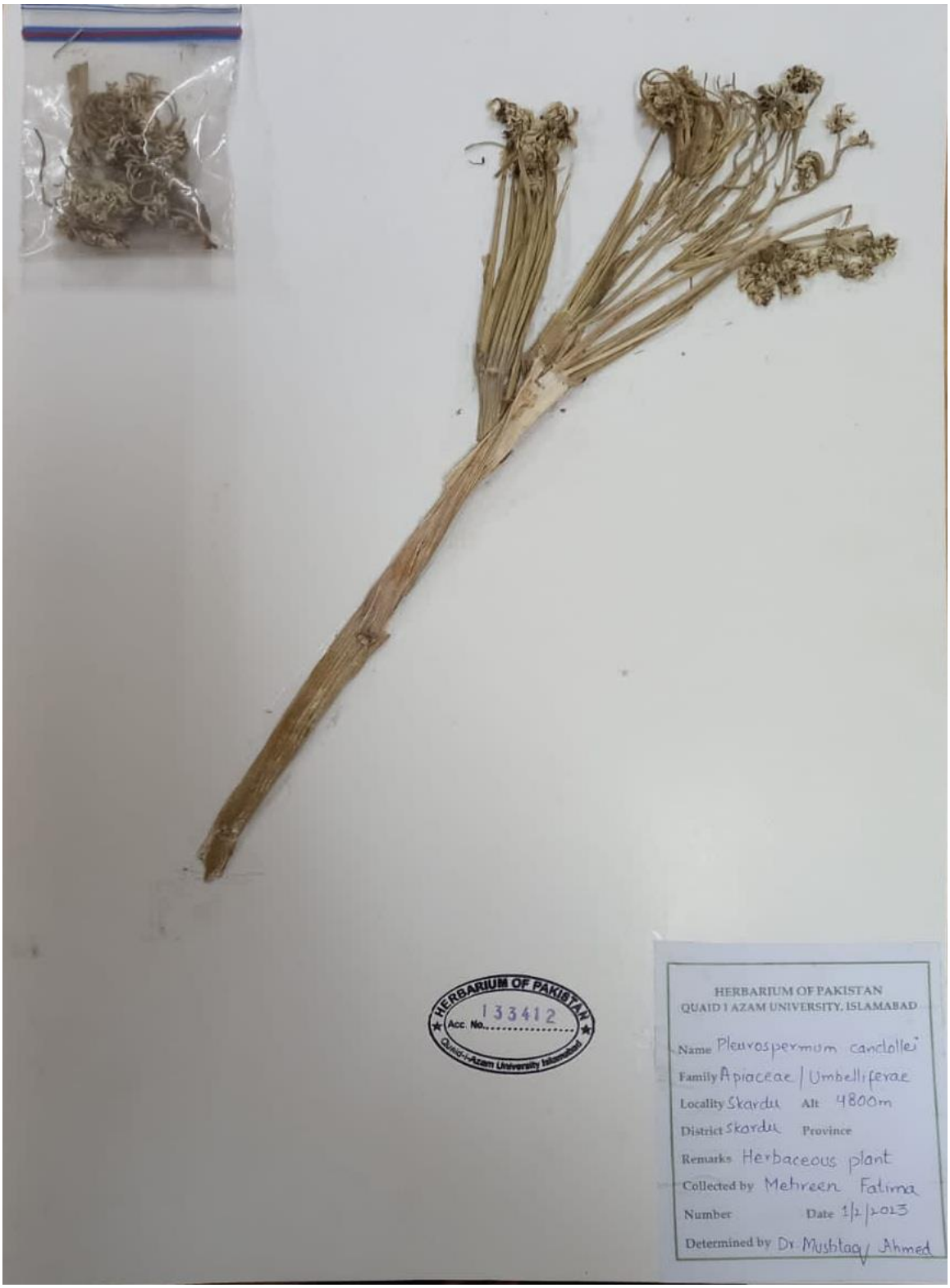

Figure 1.7: Herbarium specimen of *Pleurospermum candollei* (DC.) C.B. Calrke

Aims and objectives

This study was conducted to estimate the antioxidant and therapeutic potential of *J. humile and P. candollei* against toxicity in rats by following steps:

- Identification of plants and then preparation of crude extracts by soaking plants powder in solvents of different polarity to concentrate specific compounds of plants.
- Qualitative and quantitative phytochemical screening of extracts to estimate the groups of various compounds.
- Quantitative analysis of polyphenolic class of phytochemical through High Performance Liquid Chromatography.
- Functional group identification in plant extracts through FTIR spectroscopy.
- Evaluation of antioxidant potential of plant extracts by various *in-vitro* bioassays and its correlation with polyphenolic compounds.
- *In-vitro* anti-diabetic analysis of selected plant extracts against α-g;ucosideas enzyme activity.
- *In vivo* anti-inflammatory, analgesic, anti-diabetic screening of plant extracts through analyzing various serum and antioxidant parameters.
- Histological evaluation to detect the defensive potential of extracts opposite after tissue damage.
- Assessment of various genetic markers in relation with diabetes in kidney of rats after plant administration.
- Assessment of inflammation, fibrosis and apoptosis in hepatic and renal tissues of rats by down-regulation of multiple signal transduction pathways.

2. *Jasminum humile* (Linn) ameliorates CCl_4-induced oxidative stress by regulating ER stress, inflammatory, and fibrosis markers in rats

2.1. Introduction

The liver is a vital organ involved in many important functions of the human body. Many health issues in individuals are correlated with injuries and impairments to the liver. Excessive extracellular matrix (ECM) protein accumulation and viral hepatitis are major causes of liver fibrosis (Ali *et al*., 2017). Almost 184 countries are affected by liver fibrosis across the world due to its frequent recurrence (Akcora *et al*., 2018). Some inorganic chemicals and drugs are linked to chronic liver diseases such as severe infection, inflammation, cirrhosis, and many severe metabolic disorders. Iron, copper, arsenic, and phosphorus are the main compounds responsible for initiating hepatotoxicity in humans and animals. These toxic compounds accumulate in the liver and produce free radicals which disrupt the normal functioning capacity of the liver. ROS are produced by the process of oxidative stress in the body, these species react with cell membranes and thus lipid peroxidation or inflammation begins (Valko *et al*., 2016).

Carbon tetrachloride is a synthetic toxic compound that accelerates the process of lipid peroxidation. CCl_4 is converted to a tri-chloromethyl radical ($OCCl_3$) and a peroxy tri-chloromethyl radical. Lipid peroxides are then produced by the reaction of these ROS with polyunsaturated fatty acids (PUFAs). Additionally, inflammatory markers are elevated due to CCl_4-induced hepatotoxicity which is a source of severe hepatocyte inflammation (Hirschfield *et al*., 2018; Khedr and Khedr, 2014). Thus, cells inhibit the formation of unfolded-protein clusters by activating the unfolded-protein response (UPR) and directly reducing the stress. Triggered UPR leads to the over-expression of glucose-regulated protein (GRP-78), which is an intra-ER chaperone and enhances protein folding. Continued activation of UPR initiates the activity of IRE1-α an endonuclease enzyme that activates the conversion of X-box binding protein-1 (XBP-1u) into XBP-1s. This pathway is involved in the reduction of ER stress through activation of various proteins and ER-associated degradation. Over-expression of GRP-78 and XBP-1s markers may cause ER stress, as reported by researchers (Naz *et al*., 2020).

Damaged cells of the liver due to liver injury eventually produce ECMs including

collagen and inflammatory cytokines, and different growth factors are also released from these cells, hence activating Kupffer cells and hepatic stellate cells (HSCs) and provoking liver fibrosis (Bourebaba and Marycz, 2021). Damaged liver cells produce many inflammatory mediators such as prostaglandin (PGE2), different interleukins such as IL-6, IL-1, IL-18, IL-12, MCP- 1, and TNF-α, and several chemokines which ultimately induces further macrophages and neutrophils (Jiang *et al*., 2014; Labonte *et al*.. 2014). Therefore, the enhanced pro- duction of inflammatory mediators is a hallmark of liver cell damage and plays a crucial role in the pathogenesis of liver diseases.

Hepatic diseases are treated with several synthetic drugs which are available worldwide, but these are insufficient and possess some side effects. Thus, researchers are taking a keen interest in plants that are rich sources of phytochemicals and antioxidants. The antioxidants present in plants and their derived compounds are mainly directly related to the strength of the herb to quench the ROS by donating electrons to these species, thereby ultimately terminating the radical chain reactions. The body itself produces several antioxidants (e.g., peroxidase (POD) and glutathione), while others are ingested through diet. Many plants contain several antioxidants, such as alkaloids, flavonoids, tannins, glycosides, and vitamin C. Almost two-thirds of the world's plants are associated with some therapeutic properties and almost all of them contain enormous antioxidant aptitude. Therefore, medicinal plants and herbal products can be more effective than chemical drugs with fewer or no side effects. Researchers became curious about dietary plant antioxidants after extraction and isolation of ascorbic acid from various plants (Dzoyem and Eloff, 2015; Shukla *et al*., 2012). Silymarin is extracted from seeds of *Silybum Marianum* and acts as hepatoprotective agent. Silymarin is a bioactive component of milk thistle and highly valued for its high efficiency against inflammation, liver injuries, oxidative stress, and fibrosis. It acts as an antioxidant and stimulates the growth and regeneration of new liver cells (Mukhtar *et al*., 2021).

Jasminum humile (Linn) (also known as yellow jasmine or peeli chameli), which belongs to the Oleaceae family, is an erect much-branched shrub, growing to almost 1 m or taller, usually found in the Himalayan region. Researchers have reported that *Jasminum humile* has quenching power against free radicals and found it effective against bacterial diseases and it is used as a cardiac tonic and for treatment of chronic fistulas (Khan *et*

al., 2014; Malik *et al*., 2021) In India, a paste of the leaves of this plant is used to treat skin infections (Dhiman, 2004; Singh *et al*., 2021) and leaves are also used to reduce inflammation of the mouth (Chauhan, 1999). The juice of the roots is used to treat ringworm (Kala, 2005; Singh *et al*., 2021). As these conditions are related to infections and inflammation, we decided to test the extract of this plant against inflammatory markers. This study was designed to meet the following objectives: (a) identify the phytochemical profile of *J. humile,* (b) assess the antioxidant effects of *J. humile* against hepatotoxicity induced by CCl_4 in albino rats, and (c) evaluate the anti-inflammatory activity of JHM to ameliorate histopathological alteration in the liver.

2.2. Material and Methods

2.2.1. Chemicals

Methanol, carbon tetrachloride (CCl_4), acetic acid, rutin, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), ferrous chloride ($FeCl_2$), ethylenediaminetetraacetic acid (EDTA), Bovine serum albumin, and thiobarbituric acid reactive substances (TBARs) were purchased from Sigma-Aldrich (USA). Gallic acid and ammonium molybdate were purchased from BDH laboratories (England). Folin-Ciocalteu reagent was purchased from Biochem Chemopharma. Hydrogen peroxide and sodium nitrite were purchased from Merck (Germany). Silymarin was purchased from Abbott laboratories while TRIzol, cDNA kits, ultra-pure water, and SYBR Green were purchased from Thermo Fisher Scientific (USA). Formaldehyde and phenol red were purchased from Scharlau (Spain), while aluminum chloride ($AlCl_3$) was purchased from Duksan (South Korea). All the primers were provided by Eurofins Genomic (Germany).

2.2.2. Collection and identification of *Jasminum humile* plant

Fresh leaves of *Jasminum humile* were collected from Rawalpindi, in March 2021, and the plant was identified by Dr. Muhammad Zafar. Voucher specimen # 132015 was deposited at Pakistan Herbarium, Quaid-i-Azam University, Islamabad. The plant name was checked with the latest version of the plant list on 11-September-2022.

2.2.3. Extraction

Freshly collected leaves were dried in a non-humid, shaded place for almost three weeks

at room temperature, then con- verted into a very fine powder (1.5 kg) using a Wiley mill with an 80-mesh size. A standard ratio of 1:4 was used to prepare methanolic extract (JHM) by soaking in 95% methanol for almost 4 h. The material was continuously shaken (150 rpm) and then filtered. After filtration, the prepared extract was allowed to evaporate under low pressure in a rotary evaporator at (25 °C ± 2). *Jasminum humile* methanolic extract (JHM) was stored at 4 °C for further research.

2.2.4. Qualitative screening of phytochemicals

Phytochemical determination of proteins, phenols, terpenoids, alkaloids, tannins, carbohydrates, flavonoids, saponins, glycosides, and steroids was performed according to standard methods as previously described (Pawar *et al*., 2014; Yadav and Agarwala, 2011). Screening of phytosteroids, resins, vitamin C, phlobatannins, anthraquinone, oils and resins was carried out using the standard methods described in (Batool *et al*., 2019).

In short, powdered plant extract was dissolved in distilled water (1 mg/ml) and filtered. Few drops of Hager's reagent (picric acid solution) were added to 2 ml of prepared plant extract. Yellow colored precipitates indicated the presence of alkaloids. 1 ml of NaOH (2 N) was added to 1 ml of plant extract and yellow color of solution was observed for flavonoids. Foam formation confirmed the presence of saponins after vigorous shaking of plant extract with distilled water. 1 ml of plant extract was mixed with 2 ml of ferric chloride (5%), and greenish color indicated the manifestation of tannins. Salkowski test was performed for analysis of terpenoids. 2 ml of chloroform was mixed in 2 ml of plant extract, then 3 ml of sulphuric acid was added for layer formation. Brown or reddish precipitates at interface confirmed the presence of terpenoids, steroids, and phytosteroids. A mixture containing 2 ml of plant extract and ferric chloride was pre- pared. Bluish color confirmed the manifestation of phenols. Keller Killani test was performed by adding 2 ml of acetic acid (glacial) to 1 ml of plant extract, and then 2 ml of ferric chloride was added to the solution. Bluish solution indicated the presence of glycosides. Dilute hydrochloric acid (2%) was added to 1 ml of plant extract. The appearance of red color confirmed the occurrence of anthraquinone. Benedict's reagent (cupric-citrate complex alkaline solution) was used to confirm the presence of carbohydrates while ammonium solution was used to detect the presence of

phlobatannins.

The presence of vitamin C was confirmed by yellow precipitates after mixing of test sample with Dinitrophenyl hydrazine reagent. Few drops of nitric acid were added to the plant extract (1 ml) and appearance of yellow color confirmed the presence of proteins. The filter-paper method was applied to assess the presence of oils and resins in plant extract. The transparent appearance on filter paper indicted the presence of oils and resins in plant samples.

2.2.5. High-performance liquid chromatography (HPLC– DAD) analysis

HPLC–DAD was performed using an Agilent-1200 series (Germany) system for the analysis of JHM. For the process of compound separation, a Zorbaex Plus RSC-8 column (reverse-phased, Agilent, U.S.A) was used. Mobile phase A consisted of acetonitrile (5%), methanol (10%), water (85%), and acetic acid (1%), whereas mobile phase B contained acetonitrile (40%), methanol (60%), and acetic acid (1%), following the gradient program (Araujo *et al*. 2015). The solvent flow rate was maintained at 1.20 ml/min. HPLC-grade methanol (Sigma-Aldrich) was used to dissolve the sample (10 mg/ml) and the injection volume was maintained at 20 µl; 0.45 µm membrane filters were used for the filtration process. The retention time at the respective wavelengths of standard compounds was used to monitor and quantify the obtained peaks. The standards rutin and vanillic acid were eluted at 257 nm, gallic acid, catechin, syringic acid, and coumaric acid at 279 nm, caffeic acid, ferulic acid, cinnamic acid, and apigenin at 325 nm, myricetin, quercetin, and kaempferol at 368 nm.

2.2.6. Quantitative phytochemical analysis

The standardized $AlCl_3$ colorimetric method was followed to assess flavonoid content, while the Folin–Ciocalteu reagent method was adopted for the assessment of phenolic content (Kim *et al*., 2003; Park *et al*., 2008). Rutin and gallic acid were taken as standards, and the absorbance of solvent mixtures was measured at 490 nm and 750 nm, respectively. The calibration curve of gallic acid ($y = 0.0122 + 0.3092$, $R^2 = 0.9993$) was used for phenolics analysis, and the rutin curve ($y = 0.0017 + 0.1392$, $R^2 = 0.9995$) was used for flavonoids.

2.2.7. Antioxidant activities

Four different protocols were followed to assess the antioxidant potential of *Jasminum humile* including 2,2-diphe- nyl-1-picryl-hydrazyl (DPPH), phosphomolybdenum assay (TAC), hydroxyl ion, and ferric reducing antioxidant power (FRAP) assays as previously described (Gandhare *et al*., 2010). All readings were taken in triplicate. Percentage inhibition and IC_{50} values were also calculated as the plant extract showed high antioxidant activity. The calibration curve ($y = 0.0258 + 0.5023$, $R^2 = 0.9998$) and ($y = 0.00173 + 0.3119$, $R^2 = 0.9109$) were used to calculate IC_{50} values for TAC and FRAP assays, respectively.

2.2.8. Acute toxicity study

Standard procedure was used to perform the acute toxicity study of JHM as mentioned earlier (Zai *et al*., 2019). A 50 mg/kg body weight (bw) dose of the plant was administered to rats at the initial stage; signs of weight loss or deterioration were then investigated. The assay was continued at different concentrations of plant samples (100, 300, 1000, 1500, 2000, and 3000 mg/kg) to six female rats (for each respective dose), dose was given once, and left for almost 4 weeks for observation. As no mortality rate was observed, 600 mg/kg bw (1/5th), 300 mg/kg bw (1/10th), and 150 mg/kg bw (1/20th) of the highest dose were chosen to elucidate the hepatoprotective effect of JHM. For the hematological study, experimental rats were dissected to obtain blood through cardiac puncture after being treated with two high doses of JHM.

2.2.9. In vivo anti-inflammatory studies

Study approval was issued by the Ethics Committee (Protocol code: BCh-385) at Quaid-i-Azam University, Pakistan.

2.2.9.1. Experimental rats

Fifty-four (27 male and 27 female) Sprague–Dawley adult rats weighing 155–165 g were acquired from the primate facility at Quaid-i-Azam, University. Ambient temperature (25 °C) was maintained, with 12 h dark/light cycles and relative humidity (50% ± 3) for the rats and a standard diet and clean water were provided.

2.2.9.2. Experimental design

The fifty-four rats were assigned randomly to 9 groups (6 rats in each group; 3 male and 3 female rats) before the commencement of the experiment. Silymarin was used as reference agent and administered orally with 200 mg/kg bw as high dose of silymarin enhances the life span of rats with hepatotoxicity (Mukhtar *et al*., 2021). Doses were administered on alternate days for 6 weeks (18 doses).

- Group-I: Managed as normal, 0.9% saline water and a normal diet were given.
- Group-II: Treated as the negative control and injected with 1 ml/kg bw of CCl_4 (intra-peritoneally; dissolved in olive oil at a ratio of 3:7) for 14 days.
- Group-III: Administered with silymarin (200 mg/kg bw) and 1 ml/kg bw of CCl_4, treated as a positive control.
- Groups-IV, V, and VI: Administered with 150 mg/kg, 300 mg/kg, and 600 mg/kg of JHM, respectively.
- Groups-VII, VIII, and IX: Rats received an injection of CCl_4 and 150 mg/kg, 300 mg/kg or 600 mg/kg bw of JHM, respectively.

After completion of the experiment, the medication was discontinued for almost 24 h. Thereafter, the rats were anesthetized and killed; blood was collected through the cardiac puncture, and the rat livers were removed, washed with saline water, weighed, sectioned into different pieces for molecular and biochemical analysis, and stored at − 80 °C; one piece was preserved in formalin (10%) for histopathological study.

2.2.9.3. Analysis of liver and body weight

The rats were weighed before the commencement of experiment and at the end of experiment while the percentage of increase in body weight was calculated after dissection for each experimental rat. The absolute weight of the dissected liver was also taken after washing with saline water, and the relative weight of the liver was computed as liver weight/ body weight × 100.

2.2.9.4. Markers of serum

Serum was collected after centrifugation of the blood at 3000 rpm for 15 min.

Biochemical diagnostic kits (BIO- LATEST, USA) were used to estimate total proteins, globulin, bilirubin, and albumin. The levels of different serum markers including alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were also examined using these kits.

2.2.9.5. Tissue homogenate

Tissue homogenate of the frozen organ sample was prepared in 1 mM EDTA and (100 mM) phosphate buffer (pH = 7.4); centrifugation was performed at 1500 × g after incubation at 4 °C for 30 min. The supernatant (crude enzymes) was collected, and a total protein estimation test was performed by the protocol described in (Lowry *et al*., 1951). The absorbance of the reaction mixture was assessed at 595 nm. Total protein estimation was performed by drawing a standard curve for Bovine serum albumin.

2.2.9.6. Evaluation of antioxidant enzymes

In vivo antioxidant enzymes were assessed by following the standardized protocols. Catalase, peroxidase, superoxide dismutase, and reduced glutathione were estimated in the homogenate of the liver (Aebi, 1984; kakkar *et al*., 1984; Liu *et al*., 1998; Tan *et al*., 2014). The activity of CAT and POD was interpreted as a decrease in the value of absorbance at 0.01 units min^{-1}, while SOD and GSH activities were expressed in units/mg protein and μmol GSH/g tissue, respectively.

2.2.9.7. Evaluation of biochemical indices of liver

Standard protocols were followed to determine the levels of H_2O_2, thiobarbituric acid, and nitrites (Grisham *et al*., 1996; Lowry *et al*., 1951; Iqbal and wright, 1996; Pick and Keisari, 1981). Griess method was used for nitrite assessment. The absorbance of reaction mixtures was determined at 610 nm, 520 nm, and 540 nm. Phenol red and sodium nitrite were used as the standard curve to express H_2O_2 and nitrite concentrations, respectively. The amounts of TBARs and nitrite were expressed as TBARs/min/mg protein and nitrite/min/mg protein, while H_2O_2 was expressed as H_2O_2/min/mg tissue.

2.2.10. RNA extraction

The TRIzol method was used for the extraction of RNA from sample tissue and the process mentioned earlier (Chomczynski and Sacchi, 2006). Ethanol was used to wash the RNA

pellets, and 20 µl of Molecular Biology Grade Ultra-Pure water was used to suspend each pellet. Nanodrop quantification of samples was performed, and samples were reserved at − 80 °C.

2.2.11. cDNA and real-time PCR (RT-qPCR)

A cDNA Synthesis Kit (Revert Aid) was used to convert the RNA of the different samples to cDNA in a Thermal Gene Cycler (Biometra) according to manufacturer protocol. A 2X SYBR Green/ROX qPCR Master Mix (Applied Biosystems) was used with cDNA and 1 nM primers to produce the RT- PCR reaction mixture. The Real-time PCR program was set at 95 °C for 5 min, then repeated for 40 cycles at 95 °C for 15 s, followed by 55 °C for 10 s, and finally at 72 °C for 60 s. Melting curve analysis was used for authentication of primers by melting the PCR products at 60 °C to 95 °C, at a rate of 0.05 °C/s; beta-actin was the preferred reference gene. Table 2.1 lists the primer sequences and product sizes for each target gene. The $2^{-\Delta\Delta Ct}$ technique was used to accomplish relative quantification of the target genes, and the data were complied with Microsoft Excel.

Table 2.1: Primer sequences for real-time PCR

Gene		Primer sequence (5'-3')	Product size (bp)
GRP-78	F	GTAGCATATGGTGCCGCTGT	103
	R	GAGCAGGAGGGATTCCAGTC	
XBP-1s	F	CATGGATTCTGACGCTGTTG	110
	R	CTCTGGGAAGGACATTTGA	
XBP-1u	F	TGAAGCGCTGCGGAGGACA	114
	R	AGCTGGAGTTTCTGGTTCT	
XBP-1t	F	TGTCACCTCCCCAGAACATC	103
	R	ACAGGGTCCAACTTGTCCAG	
MCP-1	F	TGTTCACAGTTGCTGCCTGT	141
	R	CGACTCATTGGGATCATCT	
IL-6	F	GCCTGCAGAGAGATTCAATCA	140
	R	GTATCAGTGGGGGTCAGCAG	
TNF-α	F	GTCTGTGCCTCAGCCTCTTC	122
	R	GCCATGGAACTGATGAGAG	
TGF-ß	F	GCCTGCAGAGATTCAAGTCA	109
	R	GTATCAGTGGGGGTCAGCAG	
Smad-3	F	CCTCCTGGCTACCTGAGTGA	118
	R	GTTATTGTGTGCTGGGGACA	
Col1a1	F	GTCCCCGAGGAAACAATG	108
	R	ACCAGGCATTCCCTGAAGA	
Nrf-2	F	TCCAGACAGACACCAGTGGA	122
	R	GAATGTCTCTGCCAAAAGC	
Chop	F	CTAGGGATGCAGGGTCAAGA	132
	R	CCTGTCCTCAGATGAAATTGG	
GCLC	F	GAGAACATCAGGCTCTTTGC	106
	R	AGATGCACCTCCTTCCTCTG	
PDI	F	AGAACTCCAGGCGGTGTCT	150
	R	GCCATGGAACTGATGAGAG	
ß-actin	F	CCTCTATGCCAACACAGTGC	178
	R	CATCGTACTCCTGCTTGCTG	

2.2.12. Histopathology analysis

The stored organ from each rat was subjected to histopathological study. One section of each tissue was fixed in 10% formalin and a 4 μm thin section was then fixed into paraffin after being stained with hematoxylin and eosin. The prepared slides were well examined under a light microscope (DIALUX-20 EB) at 40X magnification

2.2.13. Statistical analysis

To illustrate the effects of the sample treatment, a one-way ANOVA was performed using Statistix 8.1 software; this was followed by Tukey's HSD to establish a relation between the significance level of the different treatment groups at a 0.05% level of probability. Graph prism 5.0 was used to graphically represent the data.

2.3. Results

2.3.1. Qualitative phytochemical profile of JHM

Different phytochemicals including alkaloids, glycosides, tannins, flavonoids, saponins, betacyanins, phenols, coumarins, steroids, oils and resins, carbohydrates, anthraquinones, and vitamin C were found in the plant extract. However, the analysis revealed negative results for some phytochemical classes such as anthocyanins, phlobatannins, steroids, terpenoids, proteins, and phytosteroids, as depicted in Table 2.2.

2.3.2. HPLC–DAD analysis of *Jasminum humile* extract

JHM was subjected to HPLC–DAD analysis to identify and quantify effective antioxidants. A comparison between retention duration and absorption spectra with reference standards provided identification of the compounds present in JHM. In Figure 2.1, the chromatographic form of the JHM HPLC–DAD profile is shown. The highest amount was for syringic acid (18.7 μg/mg JHM), followed by kaempferol (10.6 μg/mg JHM) and rutin (8.1 μg/mg JHM). Vanillic acid (6.1 μg/mg JHM), catechin (5.1 μg/mg JHM), coumaric acid (2.5 μg/mg JHM), caffeic acid (4.24 μg/mg JHM), ferulic acid (4.9 μg/mg of JHM), cinnamic acid (1.41 μg/mg of JHM), myricetin (2.1 μg/mg of JHM), apigenin (0.49 μg/mg of JHM), and quercetin (0.2 μg/mg of JHM) were also found.

Table 2.2: Phytochemical analysis of JHM

Alkaloids	+
Glycosides	+
Tannins	+
Terpenoids	−
Steroids	−
Phytosteroids	−
Flavonoids	+
Saponins	+
Proteins	−
Phenols	+
Betacyanin	+
Anthocyanins	−
Coumarins	+
Oils	+
Resins	+
Carbohydrates	+
Anthraquinones	−
Vitamin C	+
Phlobatnnins	−

JHM Jasminum humile, (+) constitute present, (−) constitute absent.

2.3.3. Acute toxicity and hematological profile

Table 2.4 illustrates the hematological profile of both normal and plant-treated rats. A significant increase was found in the levels of white blood cells (WBC) and platelets of rats medicated with JHM at 2000 mg/kg bw (13.3 ± 0.15); the results for rats administered with HM 3000 mg/kg bw (12.3 ± 0.42) were significantly ($p < 0.05$) higher than for the normal rats (6.88 ± 0.11). Non-significant results were observed for red blood cells (RBCs) in the rats treated with JHM 2000 mg/kg bw (7.31 ± 0.31) and JHM 3000 mg/kg bw (7.51 ± 0.17) as compared to control group (6.22 ± 0.30). Moreover, a significant ($p < 0.05$) increase was found in lymphocytes and mean corpuscular hemoglobin concentrations (MCHC) (2–3 folds) in rats that were treated with doses of 2000 and 3000 mg/kg bw compared with the control group; however, a non-significant increase was found in their hemoglobin (g/dl) level.

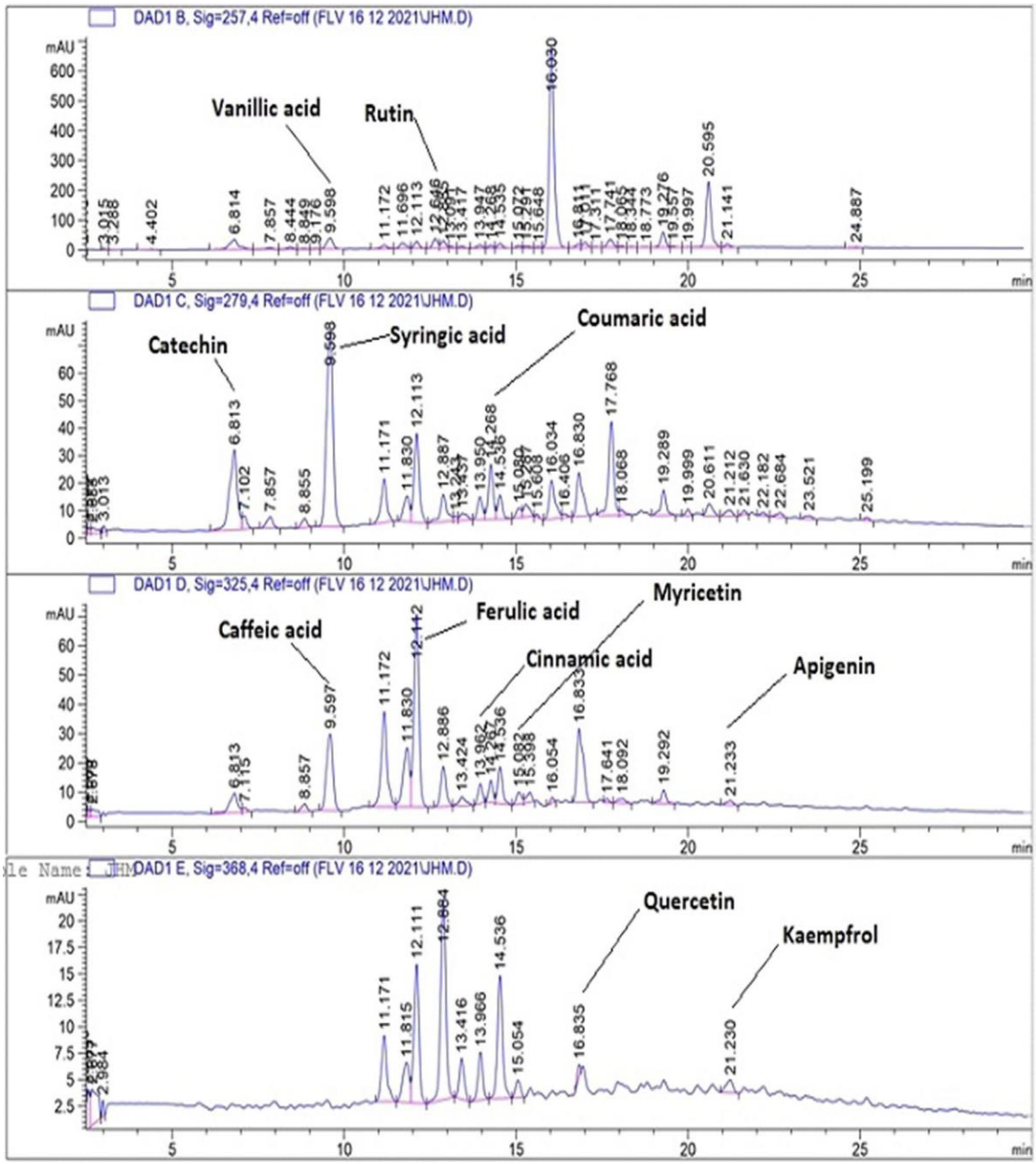

Figure 2.1: HPLC–DAD analysis of JHM (*Jasminum humile* methanol extract) at: Signal 1: 257 λ, Signal 2: 279 λ, Signal 3: 325 λ, Signal 4: 368 λ.

Table 2.3: Antioxidant potential of *Jasminum humile*

	DPPH % inhibition	Hydroxyl ion % inhibition	TAC IC_{50} (µg/ml)	FRAP IC_{50} (µg/ml)
JHM	77.83 ± 1.52^{b}	73.19 ± 2.01^{b}	1086 ± 2.84^{a}	1510 ± 3.42^{a}
Ascorbic acid	88.72 ± 1.01^{a}	78.79 ± 1.27^{a}	989 ± 2.65^{b}	
Gallic acid				1480 ± 2.01^{b}

Mean ± S.D. Mean values having different superscripts (a, b) show significance at $p < 0.05$. *JHM Jasminum humile* methanol extract

Table 2.4: Treatment effects of JHM on hematological profile

	WBC (10^3/ mm^3)	RBC (10^6/ mm^3)	Neutrophil (10^3/ mm^3)	HGB (g/dl)	MCV (fl)	PL (10^3/ ml)	LYM (10^3/m^3)	MCHC (g/dl)
Control	6.88 ± 0.11	6.26 ± 0.30	29.14 ± 0.67	13.24 ± 0.16	59.91 ± 0.43	190.32 ± 1.15	4.31 ± 0.13	8.19 ± 0.35
JHM (2000 mg/kg bw)	13.36 ± 0.15^{a}	7.31 ± 0.31	34.02 ± 0.21^{a}	14.32 ± 0.10	63.31 ± 0.49^{b}	234.45 ± 0.87^{b}	8.81 ± 0.11^{a}	6.05 ± 0.20^{b}
JHM (3000 mg/kg bw)	12.34 ± 0.42^{b}	7.51 ± 0.17	32.2 ± 0.67^{b}	14.73 ± 0.26	74.97 ± 0.72^{a}	74.97 ± 0.72^{a}	7.56 ± 0.23^{b}	12.21 ± 0.49^{a}

Mean ± S.D. (n = 6 female rats). Mean values having different superscripts (a, b) show non-identical groups at $p < 0.05$ using Tukey's post hoc test; ^{a,b} $p < 0.05$ vs. control rats. *JHM Jasminum humile* methanol extract, *HGB* hemoglobin, *WBC* white blood cells, *RBC* red blood cells, *MCV* mean corpuscular volume, *PL* Platelets, *LYM* lymphocytes, *MCHC* mean corpuscular hemoglobin concentration.

2.3.4. In vivo studies

2.3.4.1. Organ and body weight analysis

Rats intoxicated with CCl_4 showed a significant ($p < 0.05$) increase in the absolute and relative weights of the liver and a reduction in body weight. Silymarin neutralized the effect of CCl_4, with these rats' bodyweight close to that of the control rats. Each dose of JHM confirmed a significant ($p < 0.05$) restoration of body weight. Additionally, these groups showed a normal absolute and relative liver weights as compared to control group. Increased body weight was shown by all groups administered with JHM only (Table 2.5).

2.3.4.2. Rat serum analysis

Rats in which hepatotoxicity was induced by CCl_4 exhibited a remarkable ($p < 0.05$) increase in biochemical markers: AST, ALT, ALP, and total bilirubin. The levels of albumin, globulin, and total proteins were reduced by CCl_4 administration to rats. However, the administration of low, medium, and high doses of JHM significantly ($p < 0.05$) suppressed the production of serum enzymes, but an elevated production of albumin, total protein, and globulin was observed. Furthermore, the group treated with silymarin (600 mg/kg bw) indicated significant restoration in all the above-mentioned markers towards control group (Table 2.6).

Table 2.5: Treatment effects of JHM on body weight and liver weight of rats

	Initial body weight (g)	Final body weight (g)	% Increase in body weight	Absolute liver weight (g)	Relative liver weight (mg/g of body weight)
Control	157 ± 2.4	231 ± 1.7	47.22 ± 3.1	7.18 ± 0.07	31.07 ± 0.3
CCl_4 (1 ml/kg bw)	161 ± 1.5	200 ± 1.1	24.5 ± 1.4^{a}	9.90 ± 0.05^{a}	49.3 ± 0.1^{a}
CCl_4 + silymarin (200 mg/kg bw)	163 ± 2.4	236 ± 1.7	44.5 ± 2.4^{b}	7.30 ± 0.06^{b}	30.9 ± 0.2^{b}
CCl_4 + JHM (150 mg/kg bw)	159 ± 2.8	207 ± 1.9	29.9 ± 2.8^{ab}	9.14 ± 0.03^{ab}	44.1 ± 0.4^{ab}
CCl_4 + JHM (300 mg/kg bw)	162 ± 2.1	216 ± 1.2	33.2 ± 1.2^{ab}	8.90 ± 0.05^{ab}	41.1 ± 0.5^{ab}
CCl_4 + JHM (600 mg/kg bw)	162 ± 2.0	221 ± 1.4	37.1 ± 1.6^{ab}	8.58 ± 0.05^{ab}	38.8 ± 0.4^{ab}
JHM (150 mg/kg bw)	155 ± 2.7	227 ± 2.6	46.5 ± 2.1	7.86 ± 0.06	34.6 ± 0.7
JHM (300 mg/kg bw)	160 ± 1.7	237 ± 1.7	47.8 ± 1.9	7.62 ± 0.01	32.1 ± 0.2
JHM (600 mg/kg bw)	161 ± 1.5	242 ± 1.2	49.6 ± 0.9	7.56 ± 0.2	31.3 ± 0.7

Mean ± S.D. (n = 6 male rats). Means having different superscripts (a, b) show non-identical groups at $p < 0.05$ using Tukey's post hoc test; $^{a}p < 0.05$ vs. control rats; $^{b}p < 0.05$ vs. CCl_4-treated rats. *JHM Jasminum humile* methanol extract.

Table 2.6: Treatment effects of JHM on hepatic serum markers

	ALT (U/l)	AST (U/l)	ALP (U/l)	Albumin(mg/dl)	Bilirubin (mg/dl)
Control	46.25 ± 1.26	64.05 ± 0.98	60.28 ± 1.11	4.24 ± 0.06	0.70 ± 0.02
CCl_4 (1 ml/kg bw)	115.8 ± 0.69[a]	131.7 ± 1.06[a]	138.45 ± 0.79[a]	2.74 ± 0.10[a]	1.71 ± 0.02[a]
CCl_4 + silymarin (200 mg/kg bw)	59.33 ± 1.69[e]	72.81 ± 0.73[e]	74.43 ± 0.81[e]	3.85 ± 0.04[d]	0.79 ± 0.04[d]
CCl_4 + JHM (150 mg/kg bw)	92.63 ± 1.45[b]	86.53 ± 0.69[b]	104.31 ± 0.59[b]	2.90 ± 0.05[b]	1.12 ± 0.06[b]
CCl_4 + JHM (300 mg/kg bw)	79.98 ± 1.06[c]	81.45 ± 0.88[c]	91.98 ± 1.12[c]	3.11 ± 0.04[c]	0.93 ± 0.04[c]
CCl_4 + JHM (600 mg/kg bw)	71.9 ± 1.06[d]	77.85 ± 0.82[d]	81.9 ± 0.93[d]	3.42 ± 0.05[d]	0.83 ± 0.03[d]
JHM (150 mg/kg bw)	56.01 ± 0.91	78.8 ± 0.42[d]	72.56 ± 0.51	3.85 ± 0.07	0.75 ± 0.02
JHM (300 mg/kg bw)	52.88 ± 1.09	75.66 ± 0.55	65.75 ± 0.80	3.83 ± 0.06	0.74 ± 0.02
JHM (600 mg/kg bw)	47.35 ± 1.99	70.11 ± 0.64	62.56 ± 0.68	4.09 ± 0.07	0.72 ± 0.01

Mean ± S.D. (n = 6 male rats). Means having different superscripts (a-e) show non-identical groups at $p < 0.05$ using Tukey's post hoc test; $^{a}p < 0.05$ vs. control rats; $^{b-e}p <$ 0.05 vs. CCl4-treated rats. JHM: *Jasminum humile* methanol extract.

2.3.4.3. Antioxidant status in JHM-treated rats

The CCl_4-administered group indicated significantly lower levels of catalase, superoxide dismutase, peroxidase, and GSH, as well as proteins. CCl_4-induced toxicity was reduced by silymarin treatment as silymarin treatment remarkably restored the levels of antioxidant enzymes and total protein. Treatment with JHM, especially at medium and high doses in combination with CCl_4 significantly ($p < 0.05$) restored the levels of antioxidants towards the normal group. Anyhow, administration of either low, medium, or high doses of JHM only (150 mg/kg bw, 300 mg/kg bw, 600 mg/kg bw) revealed a slight reduction in enzymatic levels than in normal rats (Figure 2.2).

2.3.4.4. Liver profile in JHM-treated rats

The group administered with CCl_4 exhibited significantly ($P < 0.05$) excessive amounts of biochemical indices including TBARs, H_2O_2, and nitrites, while administration of silymarin (200 mg/kg bw) indicated restoration of these markers towards normal rats. A high dose of JHM (600 mg/kg bw) along with CCl_4 administration indicated higher restoration effects than low dose (300 mg/kg bw). Moreover, rats did not show any remarkable change in the concentration of these biomarkers when treated only with JHM doses (Table 2.7).

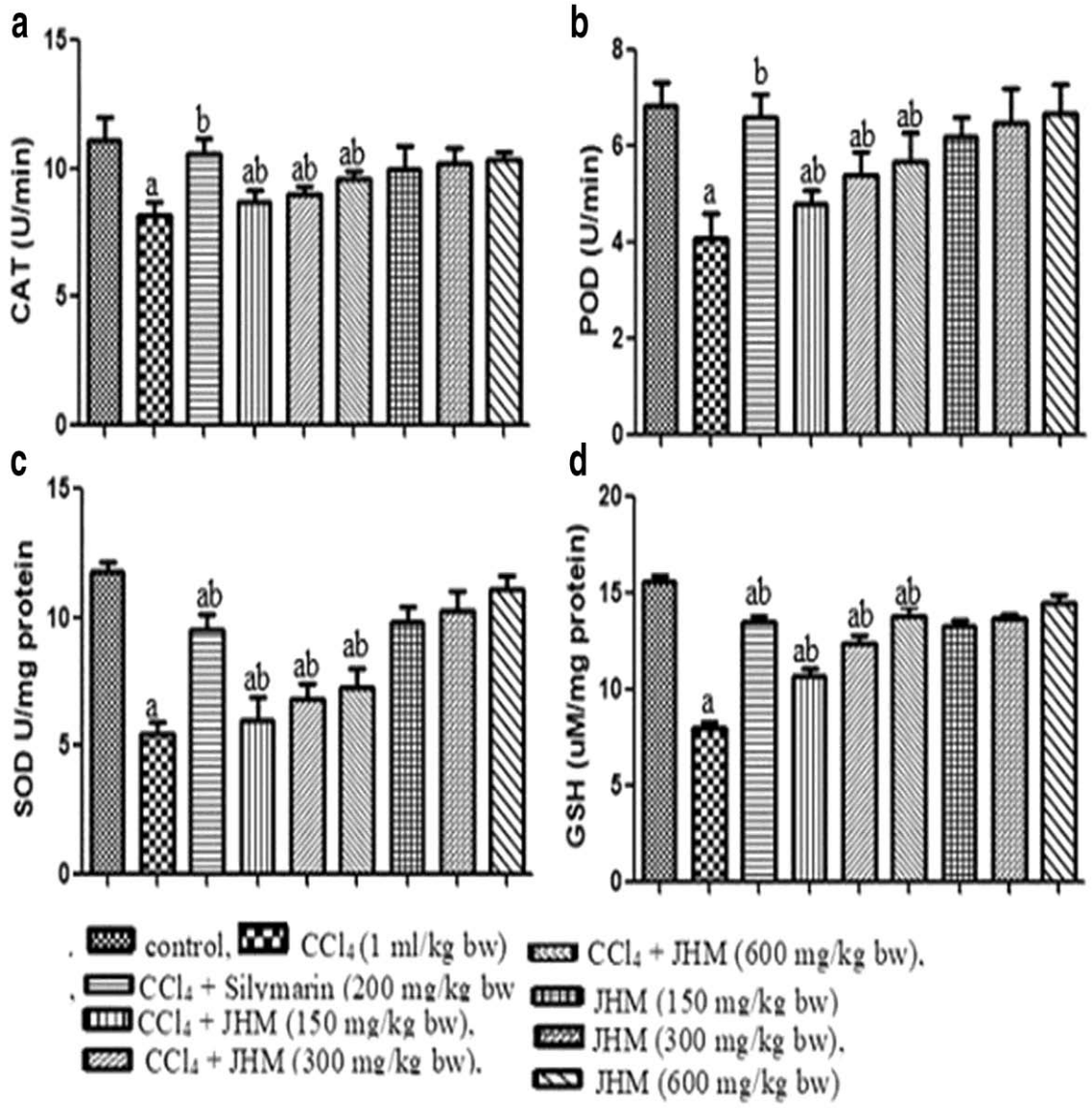

Figure 2.2: Effect of JHM on the antioxidant enzymes in rat liver. Different superscripts **a**, **b** shows non-identical groups at $p < 0.05$ using Tukey's post hoc test; $^{a}p < 0.05$ vs. control rats; $^{b}p < 0.05$ vs. CCl_4-treated rats **a** CAT: catalase, **b** POD: peroxidase, **c** SOD: superoxide, **d** *GSH:* glutathione. *JHM Jasminum humile* methanol extract.

Table 2.7: Treatment effects of JHM on hepatic biochemical markers

	Protein (µg/mg tissue)	TBARS (nM/min/mg protein)	H_2O_2 (nM/min/mg tissue)	Nitrite (µM/mg protein)
Control	12.17 ± 0.18	57.58 ± 0.9	6.39 ± 0.21	43.73 ± 1.36
CCl_4 (1 ml/kg bw)	6.68 ± 0.11[a]	106.03 ± 2.0[a]	10.03 ± 0.26[a]	62.50 ± 1.25[a]
CCl_4 + silymarin (200 mg/kg bw)	11.79 ± 0.11[e]	60.04 ± 0.5[d]	6.93 ± 0.34[e]	46.72 ± 1.11[e]
CCl_4 + JHM (150 mg/kg bw)	7.66 ± 0.10[b]	81.82 ± 0.8[b]	9.02 ± 0.34[b]	60.03 ± 1.49[b]
CCl_4 + JHM (300 mg/kg bw)	8.34 ± 0.11[c]	67.42 ± 0.9[c]	8.61 ± 0.32[c]	56.53 ± 1.56[c]
CCl_4 + JHM (600 mg/kg bw)	9.62 ± 0.11[d]	60.51 ± 0.9[d]	7.25 ± 0.25[d]	53.33 ± 1.31[d]
JHM (150 mg/kg bw)	10.25 ± 0.23	59.89 ± 1.3	6.56 ± 0.29	45.55 ± 1.36
JHM (300 mg/kg bw)	10.49 ± 0.14	58.67 ± 0.9	6.35 ± 0.27	45.22 ± 1.33
JHM (600 mg/kg bw)	11.17 ± 0.33	57.81 ± 1.4	6.14 ± 0.44	45.55 ± 1.36

Mean ± S.D. (n = 6 male rats). Means having different superscripts (a-e) show non-identical groups at $p < 0.05$ using Tukey's post hoc test; $^{a}p < 0.05$ vs. control rats; $^{b-e}p <$ 0.05 vs. CCl4-treated rats *JHM Jasminum humile* methanol extract, H_2O_2 Hydrogen peroxide

2.3.4.5. JHM effects on genetic markers

Different markers of stress and inflammation showed different expressions at the mRNA level, which is illustrated in Figure 2.3. However, compared with normal rats, XBP-1t, XBP- 1s, XBP-1u, GRP-78 and PDI were found to be significantly ($p < 0.05$) high in CCl_4-treated rats. Rats co-administered with silymarin showed restoration in mRNA levels of these markers against CCl_4-induced damage. A marked ($p < 0.05$) inhibition of stress-inducing markers was found after JHM treatment to stress-induced rats at low, medium, and high doses. Rats intoxicated with CCl_4 showed significant ($p < 0.05$) downregulation in the expression of antioxidant enzymes nuclear erythroid 2 p45-related factors 2 (Nrf-2), and γ-glutamyl cysteine ligase (GCLC). The expression level of these enzymes was, however, high in rats treated with silymarin and JHM at medium and high doses, along with CCl_4 and low in CCl_4-treated rats; in contrast with JHM treatment alone showed no significant ($p > 0.05$) results. Pro-inflammatory cytokines including IL-6 and TNF-α and chemokine MCP-1 showed significant ($p < 0.05$) up-regulated levels in the CCl_4-intoxicated rats. The expression of these genes was significantly reduced when JHM was co-administered with CCl_4 at medium (300 mg/kg bw) and high (600 mg/kg bw) doses. Co-administration of JHM at low dose (150 mg/kg) also showed reduction in stress and inflammatory markers but it was less effective than medium and high dose of JHM. Treatment with JHM extract alone, on the other hand, showed no signs of toxicity and stress at mRNA expression level of all of the genes.

2.3.4.6. Effect of JHM on hepatic fibrosis

CCl_4-mediated hepatotoxic rats showed marked ($p < 0.05$) fold change increase in Smad-3, TGF-ß, and Col1a1 when compared with levels for normal rats (Figure 2.3). A substantial reduction was observed in the expression of these genes when treated with silymarin and CCl_4 simultaneously. The expression level of fibrosis genes including Col1a1, Smad-3, and TGF-β was considerably lowered by the co-treatment of JHM at 300 and 600 mg/kg bw with CCl_4 proving that plant has protective effects against liver fibrosis. Rats treated with JHM at low, medium, and high doses alone showed non-significant ($P > 0.05$) change in expression level of these markers at mRNA level when compared with the normal rats.

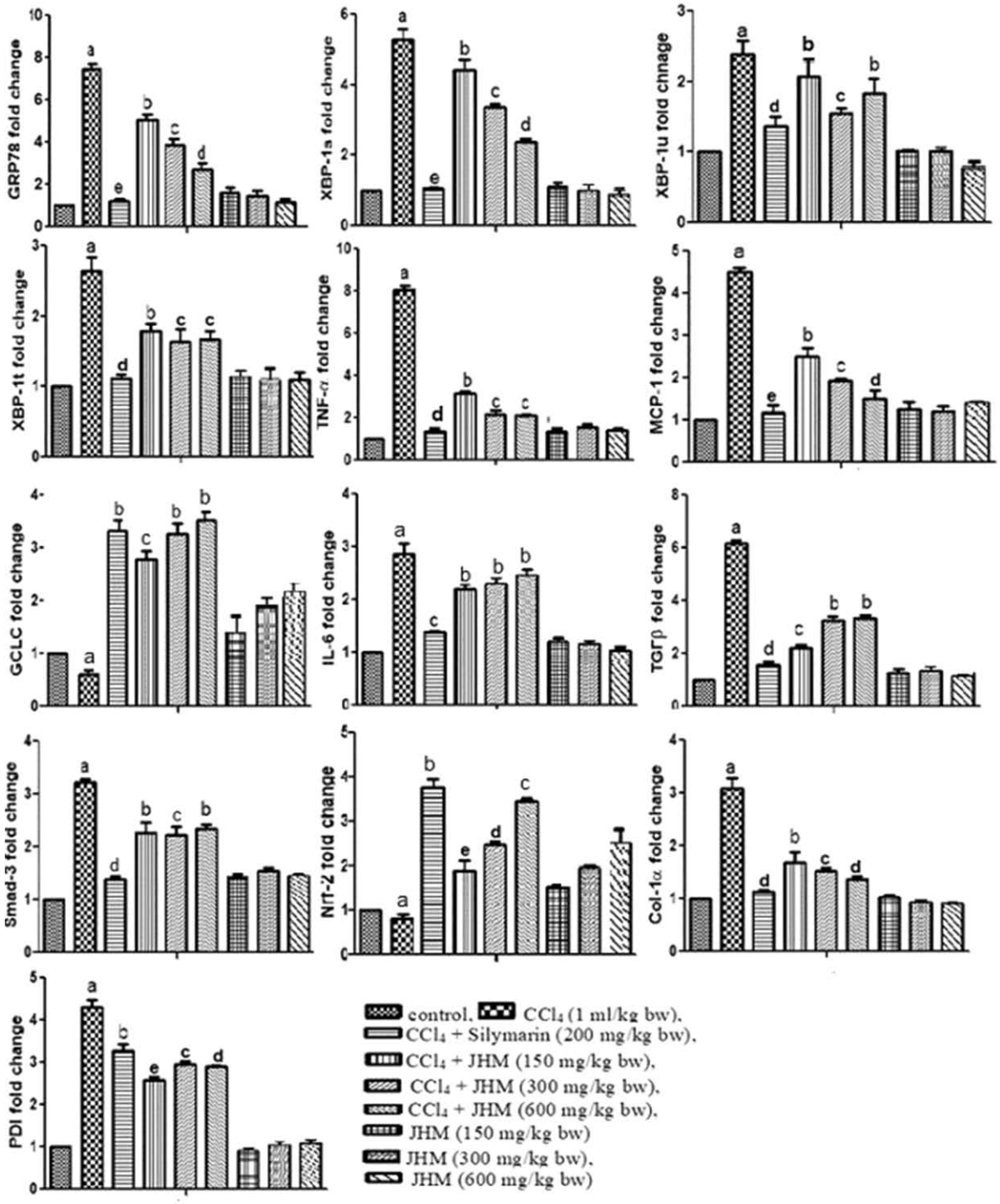

Figure 2.3: Graphical representation of JHM treatments on fold change of different genes mainly involved in various pathways: ER stress, inflammation, and fibrosis. Values: mean ± S.D. Various superscripts **a**–**e** show non-identical groups at $p < 0.05$ using Tukey's post hoc test; $^{a}p < 0.05$ vs. control rats; $^{b-e}p < 0.05$ vs. CCl4-treated rats

2.3.4.7. Histological anatomy

Histopathological testing revealed normal liver morphology in the control group (Figure 2.4a). CCl_4 administration to rats revealed sinusoidal obstruction, hypertrophy of cells, and neutrophil infiltration in the liver (Figure 2.4b). Decreased injuries and restored morphology were detected in silymarin-treated rats (Figure 2.4c). Simultaneous administration of JHM at 150, 300, and 600 mg/kg bw doses reduced hepatocellular damage, as illustrated in (Figure 2.4d–f). Rats on JHM treatment showed normal histological characteristics at each dose (Figure 2.4g–i). JHM plays a protective role against CCl_4-induced liver damage, as shown in (Figure 2.4j).

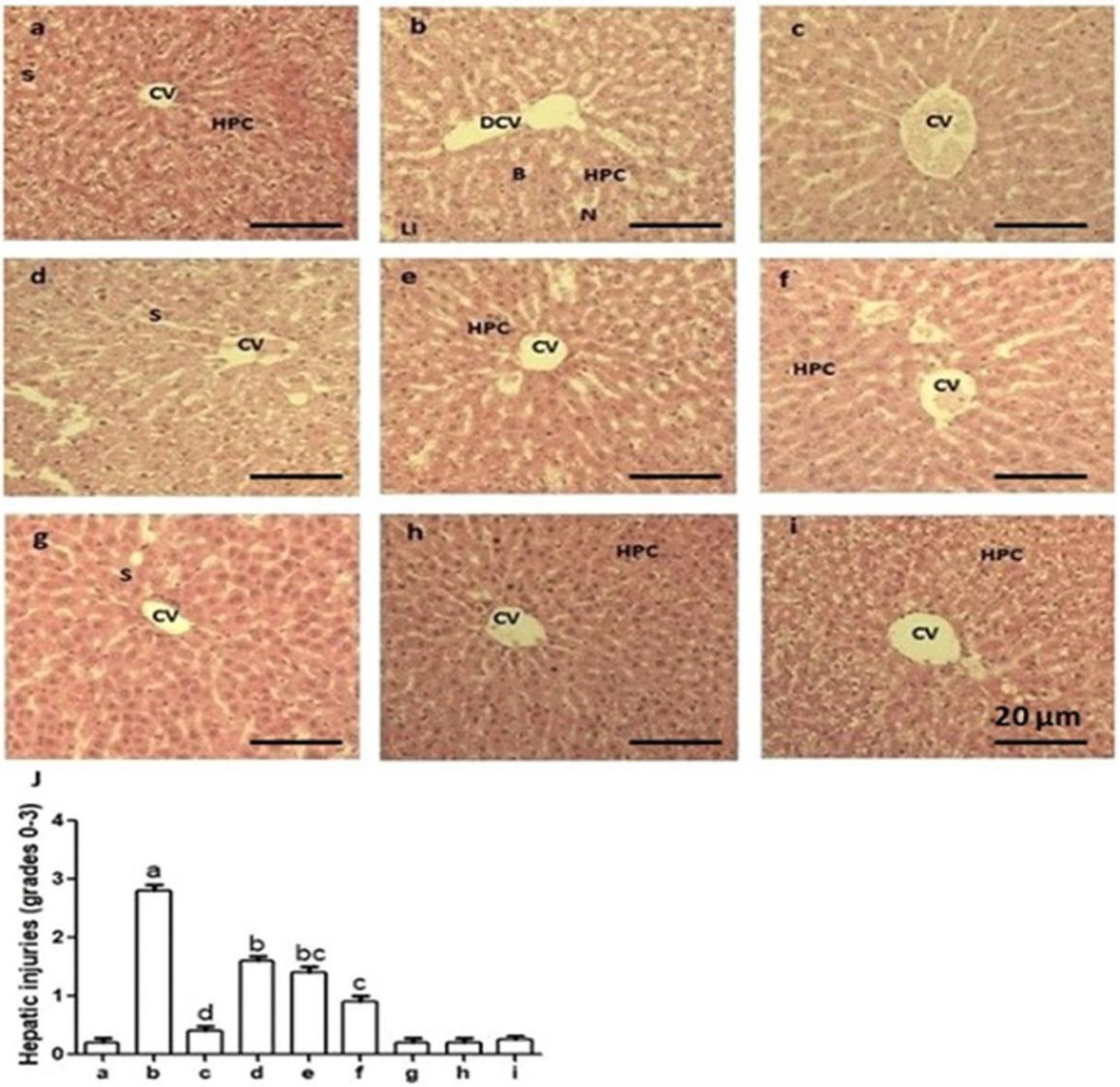

Figure 2.4: Images of liver with hematoxylin–eosin stain at 40 × magnification (Scale bar: 20 µm). Image depicts protective potency of JHM. **a** Control group; **b** CCl_4 (1 ml/kg bw); **c** CCl_4 + silymarin (200 mg/kg bw); **d** CCl_4 + JHM (150 mg/kg bw); **e** CCl_4 + JHM (300 mg/kg bw); **f** CCl_4 + JHM (600 mg/kg bw); **g** JHM (150 mg/kg bw); **h** JHM (300 mg/kg bw); **i** JHM (600 mg/kg bw); various superscripts **a**–**d** show non-identical groups at $p < 0.05$ using Tukey's post hoc test; $^{a}p < 0.05$ vs. control rats; $^{b–d}p < 0.05$ vs. CCl4-treated rats; superscript a shows severe injury while d shows minor injury; hepatic injuries range from 0 to 3. *HPC* hepatocytes, *N* necrosis, *LI* leukocyte infiltration, *CV* central vein, *DCV* damaged central vein, *S* sinusoids, *B* ballooning of hepatocytes, *JHM Jasminum humile* methanol extract.

2.4. Discussion

ER stress, inflammation, and liver fibrosis mainly result from hepatotoxicity which is induced by free radicals (Shin *et al*., 2009). Irreversible binding of these free radicals to several macromolecules such as nucleic acid, proteins, and lipids occurs by damaging their side chains, thereby triggering the phenomenon of lipid peroxidation, especially in polyunsaturated fatty acids (El-Kashef *et al*., 2022). The present study was conducted for 6 weeks on alternate days to analyze the hepatoprotective effects of methanolic extract of severe *Jasminum humile* against CCl_4-induced liver toxicity in rats. Commonly found plant products including phenolics and flavonoids were the main antioxidants, and their health benefits were already recognized in many studies (Apostolou, *et al*., 2013). Our qualitative analysis of phytochemicals has illustrated that alkaloid, phenols, flavonoids, glycosides, tannins, coumarins, and steroids were found in JHM. These phytochemicals collectively may act as antioxidants, thereby protecting the rat liver from CCl_4 toxicity.

The presence of some flavonoids and polyphenols in JHM, such as catechin, rutin, caffeic acid, myricetin, syringic acid, kaempferol, and apigenin, was confirmed by HPLC studies and further confirmed by quantitative analysis of TPC and TFC. Phenolic contents are an important field in the pharmaceutical industry as they reduce oxidative stress by quenching free radicals, thus having high antioxidant potential. Methanolic extract of *Jasminum humile* showed good antioxidant potential by quenching DPPH free radicals and hydroxyl ions. Plants rich in excellent antioxidant phytochemicals have long been utilized to combat ER stress, mitochondrial dysfunction, and stress-related impairments. Plants contain a generous amount of polyphenols that are powerful antioxidants and show therapeutic activities. Studies showed that rutin possesses antioxidant, antidiuretic, anti-inflammatory, anticoagulant, and hepatoprotective properties (Yang *et al*., 2008). Caffeic acid and catechin possess antioxidant potential and thus exhibited protective effects against apoptosis and inflammation (Majid *et al*., 2015). Pre-clinical studies have revealed the anticancer, antidiabetic, neurobiological, and analgesic properties of myricetin (Taheri *et al*., 2020). Vanillic acid was shown to possess antihyperglycemic and antihypertensive activities (Vinothiya and Ashokkumar, 2017). Syringic acid is an excellent antioxidant, antiendotoxic, and therapeutic compound (Huang *et al*., 2018). The abundance of these secondary metabolites in JHM confirmed its antioxidant potential and protective effect.

The current study concludes that, based on hematological and biochemical markers, JHM is non-toxic when administered to rats, and that it had no detrimental effect on the count of platelets, RBC, WBC, HB, MCV, or HCT at the highest dose of 3000 mg/kg. Furthermore, no evidence of mortality or any evident signs of abnormal physical behavior were found. Hence, previous research findings showed non-toxicity of methanolic extract of *Mentha spicata* which support our current study that methanol extracts of plant species are generally found non-toxic (Naidu *et al*., 2014).

Body weight and relative organ weight is a principal scale to assess the damaging and toxic effects of different xenobiotics. Our current study showed that CCl_4 treatment caused a decline in overall body weight, whereas an increase was observed in the relative and absolute weights of the liver. Similarly, studies showed that CCl_4 treatment induced decreased body weight and increased relative and absolute organ weights (Ben Hsouna *et al*., 2022). Toxicity of CCl_4 was prevented by JHM and restoration in percent increase of body weight was observed towards that of the normal rats. When JHM was administered to CCl_4-intoxicated rats, a decline was observed in the absolute and relative weight of the liver. These findings are similar to those of previous research showing that plant extracts can enhance body weight and reduce organ weight after CCl_4 intoxication (Lee *et al*., 2015). Hence, CCl_4 treatment in rats can cause an increase in organ weight probably due to metabolic changes which were restored by the plant extracts.

Lipid peroxidation and hepatic injury such as the destruction of membranes are stimulated by CCl_4 in the liver, thus activating the release of enzymes into the bloodstream. Assessment of ALP, AST, and ALT is widely used to determine the integrity of plasma membrane, providing the first sign of hepatic impairment (Yachi *et al*., 2010). Our research showed that, upon treatment with CCl_4, a significant rise in the levels of the above-mentioned enzymes was observed, indicating structural damage in hepatocytes due to oxidative stress. JHM doses in combination with CCl_4 protected the liver against oxidative stress, thus showing lower levels of markers in the serum. In the CCl_4-treated group, lower levels of albumin, but elevated levels of bilirubin, were observed, which is an indication of severe liver injury. Treatment of rats with high and medium doses of JHM showed restoration of all these proteins near to the normal group. The protective properties of this plant could be due to phenolics, and flavonoids found in JHM extract.

Our body can naturally produce various antioxidant enzymes which are the main source of defense against different toxic substances, xenobiotics, and free radicals. ROS are detoxified by these antioxidants and, thus, protecting our body from damage (Sutti *et al*., 2016). For example, SOD is involved in the process of the dismutation of superoxide radicals into oxygen and H_2O_2, hence protecting hepatic cells (Sarkhail *et al*., 2007). The conversion of H_2O_2 into water and oxygen then occurs by the action of CAT. Glutathione decreases the concentration of H_2O_2 by forming a disulfide bond with it. This study revealed reduced levels of tissue protein, as well as antioxidant enzymes, including SOD, CAT, and POD in rats that were already treated with CCl_4. GST activity may be reduced because of a reduction in GSH accessibility and an increase in lipid peroxidation. The toxicity of CCl_4 was significantly reduced, while the enzymatic activity of CAT, POD, and SOD was significantly increased when JHM doses were administered. De novo synthesis or regeneration of GSH might cause the increase in levels of GSH. The presence of ascorbic acid, phytosteroids, tannins, saponins, and flavonoids in JHM extract could be the reason for its protective benefits against CCl_4 damage.

Damage caused by CCl_4 can also be assessed through peroxidation of membrane lipids, which is related to increase in the production of TBARS. Polyunsaturated fatty acids (PUFAs) are oxidized by free radicals to produce peroxides and highly reactive aldehydes which cause impairment of the liver (Nowak, 2013) Higher production rates of NO leads to inflammation through the production of free radicals such as nitrites, thus, higher levels of NO are associated with oxidative stress. Inflammation and hepatic injuries caused by CCl_4 assist their production through endothelial cells, Kupffer cells, and hepatocytes (Al-Olayan *et al*., 2014). This study showed that treatment of CCl_4 caused increases in the levels of TBARS, H_2O_2, and nitrites; however, administration of JHM at low, medium, and high doses to CCl_4 toxicity-induced rats resulted in a decrease in these levels. The decrease in TBARS could be related to an increase in antioxidant enzymes, which help to detoxify these toxic substances. Other previous findings also obtained similar results (Breikaa *et al*., 2013).

ER stress conciliated by ROS disrupts the balance of ER hemostasis, resulting in the accumulation of unfolded proteins. As a result, ER induces protective feedback known as an unfolded-protein response (UPR) that aims to restore balance (Cheng, 2011). UPR

activates several signaling pathways to reduce the levels of abnormal proteins, these specific pathways inhibit the process of translation and stimulate the production of chaperones (associated with protein folding) (Batool *et al*., 2018). Recent studies have explained that XBP-1s transcription factor is generated through cleavage of XBP-1, which is stimulated by the IRE1-α branch of UPR during ER stress. Translated XBP-1s stimulates the transcription of chaperones such as protein disulfide isomerase (PDI) and ER proteins. When ROS caused irreparable damage and stress was prolonged, ER then activated various caspases to stimulate apoptotic and inflammatory pathways. GRP-78 binds to stress sensor at the lumen of ER and inactivates it, thus have been proven as regulator of ER stress response (Ron and Walter, 2007). CCl_4-induced ER stress thus affected the expression level of GRP-78, XBP-1s, and PDI. In contrast, the administration of JHM revealed the protective effect of the plant on the liver via activation of the IRE1-branched UPR pathway (Figure 2.3). Moreover, stimulation of the PKR-like ER protein kinase (PERK) branch of UPR phosphorylates –Nrf-2– a critical transcription factor which regulates various antioxidant enzymes, along with another antioxidant enzyme regulator GCLC which regulates synthesis of GSH, is implicated in this study (Cullinan *et al*., 2003). The CCl_4-intoxicated group showed remarkably lower levels of Nrf-2 and GCLC compared with the normal group. However, JHM treatment showed a promising restoration in the mRNA levels of these markers, thus indicating its protective effects against liver damage.

PERK acts as a key player in controlling stress by lowering the synthesis of proteins and arresting the cell cycle. Acute exposure to CCl_4 induces oxidative stress that might result in cell-injury associated diseases and activation of inflammatory responses by intracellularly accumulating misfolded proteins. Therefore, translational control that is regulated by PERK is a crucial factor to release pro-inflammatory cytokines IL-6 and TNF-α that mediates Nuclear Factor-ĸB (NF-ĸB) and c-jun N-terminal Kinase pathway (JNK). Hence, pro-inflammatory cytokines and chemokines are crucial contributors to the phenomenon of inflammation (Rutkowski *et al*. 2008). MCP-1 stimulates the recruitment of macrophages at the site of injury; thus, MCP-1 plays an important role in hepatic inflammation (Fu *et al*., 2008). Our findings demonstrate that an increase in the levels of these pro-inflammatory cytokines and chemokines was observed in the CCl_4-treated rats, indicating inflammation. Several studies reported their role in hepatic fibrosis, while higher

doses of JHM normalized their expression more effectively. However, the mechanisms behind the anti-inflammatory properties of JHM are not yet clearly understood. Polyphenols and other compounds in JHM can be considered the source of inhibition of IL-6 and TNF-α (Al-Rasheed *et al*., 2015). Other studies also declared similar results that natural flora (flavonoids) possess strong anti-inflammatory effects (Batool *et al*., 2018).

The protective effects of JHM against hepatic fibrosis were also analyzed in the current study. To induce liver fibrosis, increased expression levels of TGF-β, Smad-3, and Col1a1 were stimulated by the TGF-β/Smad signaling pathway (Xiao *et al*., 2014). As a result of impeding the TGB-/ Smad pathway, the current study discovered that JHM is significantly resistant to fibrosis. The non-toxic property of JHM was demonstrated by histopathological examination of liver from the normal rats, which showed normal hepatocyte architecture, whereas the histopathological analysis of the CCl_4-injected rats revealed liver injury with necrosis, sarcoidosis, and vacuolar deterioration (Chen *et al*., 2017); this indicates that the plant extract of *Jasminum humile* did not cause any changes in the structure of cells. A symmetrical arrangement of liver sinusoids, as well as normal hepatocyte morphology, was seen after doses of JHM (600 mg/kg) and silymarin. Similar results for silymarin were illustrated in previous study (Mukhtar *et al*., 2021). In addition, our study revealed a reduction in inflammation with normal cellular architecture, which is related to the rapid regenerating ability of JHM in a dose-dependent way. This study illustrated that a high dose of JHM has approximately the same efficiency as silymarin. Previous studies have shown that *Jasminum humile* fractions have cytotoxic effects against HepG-2, MCF-7, and THP-1 (Mansour *et al*., 2022) but a little data is available on phytochemical and antioxidant studies of *Jasminum humile.*

2.5. Conclusion

Plant extracts contain a mixture of various bioactive com- pounds, which act as antioxidants. In the present study, methanolic extract of *Jasminum humile* was evaluated with reference to its total flavonoid and phenolic content, antioxidant potential and medicinal effects. Presence of phenolic and flavonoids was indicated by various assays and HPLC analysis. Free radical scavenging activity and total antioxidant capacity was estimated by DPPH and TAC assays, was found to be high in plant extract. CCl_4 was used

to instigate hepatotoxicity in rats. Hence, levels of serum markers were increased while antioxidant enzymes were lowered in CCl_4-intoxicated rats. Co-administration of JHM with CCl_4 reduced the toxicity level by restoring the enzymatic level, stress markers (XBP-1 and PDI) and pro-inflammatory markers (IL-6 and TNF-α) at genetic levels. Finally, this study indicates that JHM contains high amounts of bioactive components, which are proven to be good antioxidants and anti-inflammatory agents.

2.6. Future aspects

First, clinical trials of *Jasminum humile* as liver protective agent will further confirm the safety and efficacy of plant sample. Furthermore, isolation of specific compounds from plant and investigation of that compound against oxidative stress could be an effective approach.

Second, *Jasminum humile* showed antioxidant effects thus this study could be expanded to investigate the therapeutic effects of *Jasminum humile* against other diseases which involve oxidative stress, such as diabetes, neurodegenerative disease, or cancer. Plant also showed protective effects against apoptotic genes so further studies on cancerous cell lines and apoptotic signaling pathway will be helpful.

Finally, the study could be expanded to evaluate the molecular mechanisms underlying the effects of *Jasminum humile* on oxidative stress. This could involve using techniques such as RNA sequencing, proteomics, or gene editing.

3. *Pleurospermum candollei* methanolic extract ameliorates CCl_4-induced liver injury by modulating oxidative stress, inflammatory, and apoptotic markers in rats

3.1. Introduction

Liver is involved in the metabolism of fatty acids and nutrients; synthesis of proteins; sugar storage; detoxification of chemicals, drugs, and xenobiotics; and other physiological functions (Jiang *et al*., 2023) Liver is susceptible to injury caused by various factors, such as drugs, viruses, xenobiotics, alcohol, and nutritional supplements, through various pathways triggered by toxic metabolites. These toxic substances and drugs are responsible for almost 50% of acute liver damages. It is well recognized that the combination of mechanisms including apoptosis, oxidative stress, and inflammation plays a role in the development of acute liver injury (Long *et al*., 2022; Sun *et al*., 2022). During aerobic respiration in the mitochondrial membrane through the electron transport chain, ROS are constantly generated as byproducts. Liver is very rich in mitochondria and has a high rate of oxygen consumption during metabolic processes, which increases its exposure to ROS (Wu *et al*., 2022).

Various xenobiotics including vinyl chloride, arsenic, and CCl_4 have been recognized as hepatotoxins (Devaraj *et al*., 2011; Ostapowicz *et al*., 2002). ROS and toxic metabolites may damage immune-mediated membrane or may cause disruption in intracellular processes to cause cell injury in the hepatocytes of both human and rats.[7] A cytochrome P-450 enzymatic system, such as cytochrome P-450 2E1 (CYP2E1), generates highly reactive species including the trichloromethyl radical (CCl_3) through the metabolic activation of CCl_4 in hepatocytes. This reactive intermediate interacts with molecular oxygen (O_2) to produce a highly reactive species, trichloromethyl peroxyl radical (CCl3OO*). This radical covalently binds to biological molecules, leading to the degradation of proteins and lipids oxidative stress, cellular damage, apoptosis, and necrosis of hepatocytes (Ben Hsouna *et al*., 2022).

Natural antioxidants (e.g., vitamin C, α-tocopherol, quercetin, curcumin, phenolics, flavonoid, and tannin) have been studied for their potency against liver diseases (Casas-Grajales and Muriel, 2015; Chávez *et al*., 2008; Yen *et al*., 2009), and certain plant-derived antioxidants have shown promising results in treating liver disorders in animal models (Mohammed *et al*., 2020). Nuclear factor kappa-B (NF-κB) regulates inflammatory responses, and the inhibition of the NF-κB pathway reduces the inflammation and severity of

liver injury. CCl_4 activates the NF-κB pathway, and this activation results in an increased expression of NF-κB p65 protein in liver, which in turns triggers the production and overexpression of inflammatory mediators and proinflammatory cytokines (such as TNF-α, MCP-1, and IL-6, leading to chronic inflammation. Therefore, several natural compounds have been investigated for their potential to target inflammation and oxidative stress as it can be an effective therapeutic approach for CCl4-induced liver injury (Sun *et al*., 2022).

Pleurospermum candollei (DC.) CB Clarke belongs to the family Apiaceae, commonly known as shabdun in the Himalayan and Karakoram zones. The herb is commercially available in this region and used as vegetable and for different ailments (Ghosh *et al*., 2015). The whole plant is beneficial for the treatment of stomach problems and abdominal issues. It also helps to reduce the cholesterol level and blood pressure in the body and shows a protective response to heart problems. Moreover, the stem powder of the plant is used to cure joint and back pain in Gilgit-Baltistan (Abbas *et al*., 2016; Abbas *et al*., 2019). *P. candollei* was found effective against unconsciousness and respiratory and cerebral disorders (Ali *et al*., 2021). The powder of this plant is also locally used to cure headache and fever (Shah *et al*., 2015). It is also used for the treatment of diarrhea in animals. A previous study has reported the isolation of bioactive compounds, which have anti-inflammatory activities, from *P. candollei*. The medicinal and pharmaceutical values of Pleurospermum genus is highly appreciated which is due to the presence of natural antioxidants and their biological proper- ties (Kawarty *et al*., 2020; Thiviya *et al*., 2021).

P. candollei, despite its traditional use against health issues, has not been thoroughly investigated for its biological and pharmacological activities. Hence, the current study was planned to explore the antioxidant and anti-inflammatory potential of plants against stress, inflammatory, apoptotic, and fibrotic markers for pharmaceutical applications.

3.2. Material and Methods

3.2.1. Plant collection

The collection of aerial parts of *P. candollei* was carried out from the region of Skardu, Gilgit- Baltistan. The collected plant was identified by Dr. Mushtaq Ahmed. A voucher specimen no. 132015 was deposited at Pakistan Herbarium, Quaid-i-Azam University, Pakistan.

3.2.2. Extraction

The plant material was shade dried in a non-humid area and ground in powder by a willy mill and extracted three times with 90% methanol by refluxing for 3 h in separatory funnel. The ratio of powdered plant material to methanol was 1:5 (w/v). After filtration, solvent was allowed to evaporate in a rotatory evaporator. The *P. candollei* methanol extract (PCM) was kept under 4 °C for further studies.

3.2.3. HPLC analysis

HPLC analysis of the *P. candollei* methanol extract was performed to assess flavonoids and polyphenols by using Agilent Technology-1200 Series, Germany. The HPLC column was reverse-phase Zorbex plus RSC80, with 25 mL of separation capacity and 5 μm particle size. The standards vanillic acid and rutin were eluted at 257 nm; gallic acid, emodin, catechin, and coumaric acid at 279 nm; caffeic acid, ferulic acid, cinnamic acid, and apigenin at 325 nm; and myricetin and kaempferol at 368 nm. The stock solutions of reference standards were prepared in methanol. The injection volume was 10 μL while a constant flow rate of 1 mL/min was maintained.

3.2.4. Recovery, precision, accuracy, and limit of detection

The accuracy of the assay was evaluated by adding standard analytes at four concentration levels (50, 100, 150, and 200 μg/mL) using the method of standard addition. Intra-day variability was evaluated by analyzing the samples within the same day, while inter-day precision was assessed by repeating the procedure on two nonconsecutive days.

3.2.5. Proximate analysis

The chemical profile of *P. candollei* was identified by the evaluation of the protein, moisture, fiber, carbohydrate, lipids, and ash content. The standard protocol of AOAC 1990 was followed for the analysis (Sambucetti and Zuleta, 1996).

3.2.6. Quantitative and antioxidant analysis of *P. candollei*

3.2.6.1. Total phenolic content (TPC) assay

The plant sample (500 μl), with a concentration of 1 mg/ml, was mixed with the Folin−Ciocalteu reagent. Following this, 200 μL of 10% sodium carbonate was added to the

mixture (Seifzadeh *et al*., 2019). It was allowed to incubate at room temperature for 30 min, and the absorbance was measured at 765 nm. The calibration curve of gallic acid ($y = 0.012x + 0.9883$: $R^2 = 0.9272$) was used to assess the phenolic content.

3.2.6.2. Vanillin−HCl assay

One milliliter of plant sample (1 mg/ml) was mixed with the same amount of vanillin solution (1% in methanol) and HCl (9 M). The mixture was allowed to stand for 20 min at 30 °C. The absorbance was measured at 500 nm (Herald *et al*., 2014). The calibration curve of catechin ($y = 0.031x + 0.328$: $R^2 = 0.997$) was used to determine vanillin.

3.2.6.3. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

One milliliter of plant extract (concentration, 0.1−1 mg/ml) was mixed with 1 mL of DPPH solution (0.3 mmol/L). The mixture was kept at room temperature for 10 min, and the absorbance was measured at 517 nm (Gökmen *et al*., 2009; Goupy *et al*., 2003). Ascorbic acid was used as a reference antioxidant. All of the experiments were run in triplicate. The percentage of inhibition was calculated by the following equation:

$$\% \text{ decolorization} = (1 - \text{sample/control}) \times 100$$

3.2.6.4. Reducing power (FRAP) assay

A reaction mixture was prepared by mixing 500 μL of the plant extract (concentration, 0.1−1 mg/ml) with 200 μL of $FeCl_3$ (0.1%). Then, 500 μL of 0.3 mol/L solution of 2,4,6-tris (2-pyridyl)-*s*-triazine (dissolved in 0.2 mol/L sodium phosphate buffer) was added to the mixture (Benzie and Strain, 1996). After incubation for 30 min at 40 °C, the absorbance was measured at 700 nm. Ascorbic acid was used as a reference antioxidant.

3.2.7. Ethical statement

The Ethical Committee of Quaid- i-Azam University approved the protocol of the current study. The research study was performed in accordance with the eighth edition of the NIH Guidelines for Laboratory Animal Care and Use.

3.2.8. Acute toxicity assay

Acute toxicity study was conducted by using a minimum dose of 50 mg/kg PCM at the earliest stage and was observed to notice any deterioration effects. This dose did not cause

any signs of toxicity. Subsequently, four female rats were given 100, 500, 1000, 2000, and the maximum dose of 3000 mg/kg PCM, and the rats were examined to observe any changes in their skin, weight, fur, behavior, or respiratory system (Naz *et al*., 2020). After 14 days, there were no signs of mortality, so blood was collected from the rats treated with higher doses of PCM (2000 and 3000 mg/kg), and eventually 600 mg/kg bw (1/5th), 300 mg/kg bw (1/10th), and 150 mg/kg bw (1/20th) of the highest dose were chosen to elucidate the hepatoprotective effect of PCM.

3.2.9. Animal treatment

A total of 54 Sprague male rats (weighing 150 ± 4.5 g) were used for the experiment. The rats were kept under laboratory standard conditions (12 h light- dark cycle) at 25 ± 2 °C. The rats were provided with clean water and a standard diet.

3.2.9.1. Experimental design

The experimental rats were exposed to a fasting period of 24 h before the commencement of the experiment. Rats were divided into nine groups (six rats in each) and treated with sample solutions on alternate days for 36 days (18 doses). Group I served as normal control and received 0.9% saline (1 mL/kg). Group II received CCl_4 intraperitoneally (i.p.) mixed with olive oil (3:7 v/v, 1 mL/ kg). Group III received i.p. injection of CCl_4 and silymarin (200 mg/kg) as a reference compound. Group IV, V, and VI were administered with i.p. injection of CCl_4 and 150, 300, and 600 mg/kg of PCM, respectively. Group VII, VIII, and IX were administered with 150, 300, and 600 mg/kg of PCM alone, respectively. The rationale behind selecting the specific dose was the high pharmacological activity and nontoxicity of the extract at a higher dose. Moreover, the oral route of administration results in lower bioavailability due to the metabolism and excretion of the extract. The plant extract was dissolved in distilled water. Rats of all nine groups were weighed on the first and last days of the experiment. Forty- eight hours after the last administration of test solutions, the rats were properly anesthetized, and blood was collected in centrifuge tubes. The rats were then sacrificed, and the liver was removed, weighed, and washed with saline water. A small section was stored in formalin for histological examination, while the remaining pieces were stored in liquid nitrogen and used for biochemical and molecular studies.

3.2.9.2. Biochemical parameters in serum

Centrifugation of the collected blood was carried out at 3000 rpm for 10 min to separate the serum for the evaluation of biochemical parameters. The level of alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, and albumin in serum were assessed with an AMP diagnostic kit (Austria).

3.2.9.3. Preparation of tissue homogenate

100 mg of liver tissue was homogenized in 50 mM potassium phosphate buffer with a glass homogenizer. The homogenate was incubated at 4 °C for 30 min, and the supernatant was collected after centrifugation at 12,000 × *g*.

3.2.9.4. Enzymatic antioxidants

Enzymatic antioxidants such as superoxide dismutase, peroxidase, and catalase were estimated according to the standard protocols described by Sun *et al*. (Sun *et al*., 2998) and Chance and Maehly (Chance and Maehly, 1955), respectively. CAT and POD activities were calculated as the absorbance change of 0.01 in 1 min of reaction, while all the activities were expressed as units per milligram of protein.

3.2.9.5. Non-enzymatic antioxidants molecules

Glutathione (GSH) activity of liver was determined spectrophotometrically following the protocol of Ellman (Ellman, 1959). The principle involved the oxidation of GSH to glutathione disulfide (GSSG) by 5,5′- dithio-bis (2-nitrobenzoic acid) (DTNB) to form a yellow solution. GSH activity was expressed as μM GSH/mg protein.

3.2.9.6. Nitrite assay

The Griess method was followed for the determination of the level of nitrite/nitrate in the liver homogenate (Green *et al*., 1982). 50 μL of sodium nitrite was mixed with each sample, and then 50 μL of the Griess reagent was added to the reaction mixtures. The mixture was incubated at room temperature for 10 min in the dark. Absorbance was measured at 512 nm. Using the standard curve of sodium nitrite, the nitrite concentration was calculated.

3.2.9.7. Hydrogen peroxide (H_2O_2) assay

The level of H_2O_2 production was assessed by the ability of horseradish peroxidase to oxidize

phenol red in the presence of H_2O_2. The concentration of H_2O_2 was expressed as nM H_2O_2/ min/mg tissue, and the phenol red curve was generated as a standard curve (Pick and Keisari, 1981).

3.2.9.8. Lipid peroxidation assay

The method of Iqbal and Wright was adapted for the assessment of lipid peroxidation in liver homogenate (Iqbal and Wright, 1996) and the levels of thiobarbituric acid were expressed as nM TBARS/min/mg tissue using the molar coefficient of $1.56 \times 105\ M^{-1}\ cm^{-1}$.

3.2.9.9. Protein estimation

The standard protocol of Lowry, Rosebrough was applied for the total protein estimation in the tissue homogenate (Lorwy *et al*., 1951). The absorbance was measured at 562 nm, and a standard curve of bovine serum albumin (BSA) was plotted to assess the total protein concentration.

3.2.9.10. RNA extraction

The total RNA from the tissue of rat was isolated using the TRIzol reagent (Chomczynski and Sacchi, 2006) 100 mg of tissue was homogenized by adding 1 mL of TRIzol and incubated for 5 min at room temperature. Chloroform was added to the solution, and the aqueous layer containing RNA was separated after centrifugation. Purification of isolated RNA pellets was done by the ethanol precipitation method. RNase-free DNase I was used to digest any residual genomic DNA, and 20 μL of RNase-free ultrapure water was used to dissolve each pellet. Nanodrop quantification of the samples was performed.

3.2.9.11. Quantitative RT-PCR analysis

First-stand complementary DNAs (cDNAs) were reverse-transcribed using a Script cDNA synthesis kit. The reaction mixture was prepared by adding the respective primers and Power SYBR Green (Invitrogen) to the cDNA samples, and qRT-PCR reaction was carried out on an Applied Biosystems 7500 Instrument. The cycling conditions for amplification were as follows: 95 °C for 60 s, 40 cycles at 94 °C for 15 s, and 60 °C for 90 s. Specific primers were purchased from Eurofins Genomics LLC (Kentucky, USA). Beta-actin was used as a housekeeping gene. Each reaction was carried out in duplicate to determine ΔCT. The primer sequences for beta-actin, IL-6. TNF-α, Chop, Casp-3, BCl-2, MCP-1, TGF-β, XBP

1s, XBP1u, XBP1t, Smad-3, GCLC, GRP-78, Nrf-2, and PDI are listed in Table 2.1.

3.2.9.12. Histological examination

The liver tissue was fixed in 10% formaldehyde, dehydrated in ethanol, and embedded in paraffin wax. Then, slides were prepared by the segmentation of 4−5 μm section of the tissue and stained with hematoxylin and eosin (H&E) dye. A light microscope (DIALUX 20EB) was used to examine the specimens with a 40× magnification lens, and images were captured.

3.2.10. Statistical analysis

Values are presented as mean ± standard deviation, and p values <0.05 were taken to be statistically significant. One-way analysis of variance (ANOVA) was performed on the data obtained from different assays. Tukey's post hoc test was applied to differentiate various groups.

3.3. Results

3.3.1. HPLC profile of *P. candollei*

Regression analysis, quantification, and retention time of standard polyphenolics for *P. candollei* are depicted in Table 3.1, while the HPLC chromatogram of *P. candollei* is illustrated in Figure 3.1. The HPLC-DAD profile indicated that the highest concentrations of emodin and catechin were found, followed by kaempferol, cinnamic acid, and catechin. Rutin, gallic acid, coumaric acid, caffeic acid, apigenin, and myricetin were the least abundant compounds.

3.3.2. Chemical profile of *P. candollei*

A 164 g portion of methanolic extract was obtained from 1000 g of the powdered weight of *P. candollei* (16.4% w/w). The proximate analysis of the plant extract revealed a high percentage of carbohydrate (30.05%) and crude fiber (22.71%). Moreover, significant amounts of protein (8.71%), lipids (4.11%), ash content (13.28%), and moisture (18.43%) were also present in the extract (Table 3.2).

3.3.3. Quantitative analysis of *P. candollei*

The quantitative phytochemical analysis of the *P. candollei* methanol extract revealed the

presence of phenols and condensed tannins as 72.72 ± 0.73 mg GAE/g and 34.65 ± 0.59 mg CE/g, respectively.

3.3.4. Antioxidant activities

The antioxidant activity of the *P. candollei* methanol extract was examined using DPPH and FRAP assays. Data indicated that the increased concentration of the plant showed a higher inhibition ability of the extract against free radicals. The DPPH assay showed an IC_{50} value for PCM (60.75 ± 2.01 μg/mg) which was significantly higher than the IC_{50} value of ascorbic acid (20.12 ± 0.48 μg/mg), while the FRAP assay showed an IC_{50} value for PCM (114.34 ± 1.16 μg/mg) against ascorbic acid (62.92 ± 0.93 μg/mg).

3.3.5. Acute toxicity assay

The clinical examination and observation of rats showed that the *P. candollei* methanol extract was nontoxic, and signs of stress or behavioral changes were not found in rats. The hematological study of rats showed significant ($p < 0.05$) changes in the count of platelets (PLT) and white blood cells (WBCs) at 2000 mg/kg bw and 3000 mg/kg bw (Table 3.3). In contrast, lymphocytes (LYM) and red blood cells (RBCs) did not show significant changes among groups. Furthermore, PCM significantly ($p < 0.05$) increased the concentration of neutrophils, hemoglobin, mean corpuscular volume (MCV), hemoglobin (HGB), and corpuscular hemoglobin concentration (MCHC) at 2000 and 3000 mg/kg.

3.3.6. Effect of *P. candollei* on the body and liver weight

A notable ($p < 0.05$) reduction in the body weight of rats was observed after CCl_4 treatment, while a remarkable ($p < 0.05$) increase in the absolute weight of liver was noted (Table 3.4). Administration of PCM (300 and 600 mg/kg) along with CCl_4 reduced the toxic effects of CCl_4 and significantly ($p < 0.05$) increased the body weight of rats. Furthermore, administration of PCM (300 and 600 mg/kg) in CCl_4-intoxicated rats also reduced the absolute liver weight of the respective rats. However, administration of PCM (150 mg/ kg) along with CCl_4 did not show significant change in the body and absolute liver weight. Indeed, the protective effect of PCM (600 mg/kg) was comparable to that of the silymarin-treated group.

3.3.7. Effect of *P. candollei* on serum markers

CCl_4 treatment significantly elevated the concentration of liver serum markers such as ALT, AST, ALP, and total bilirubin compared to the control rats (Table 3.5). However, a notable decrease was obvious in the levels of albumin after CCl_4 treatment. On the other hand, rats administered with *P. candollei* (150, 300, and 600 mg/kg) along with CCl_4 showed a significantly less pronounced elevation in the levels of serum function markers, while increased levels of albumin were recorded. The hepatoprotective effect of *P. candollei* (300 and 600 mg/kg) was found to be similar to that of silymarin. However, treatment with PCM (150, 300, and 600 mg/kg) alone showed no significant ($P > 0.05$) changes in the level of ALT, AST, ALP, albumin, and bilirubin as compared to the control group.

Table 3.1: Signal wavelength, regression analysis, and retention time of reference polyphenolics for the *P. candollei* methanol extract

reference polyphenolics	signal wavelength	retention time (min)	regression analysis (R^2) (μg/mg) of extracts		concentration
vanillic acid	257	8.367	$y = 7.311x + 19.83$	0.9959	2.23
Rutın	257	12.413	$y = 8.103x - 25.12$	0.9812	1.42
gallic acid	279	3.538	$y = 16.712x + 34.12$	0.9926	0.94
Catechın	279	6.489	$y = 22.003x + 7.364$	0.9915	8.07
coumaric acid	279	13.567	$y = 5.933x + 38.183$	0.9978	0.4
Emodın	279	27.289	$y = 8.384x + 19.32$	0.9829	12.12
caffeic acid	325	8.368	$y = 11.343x + 21.45$	0.9899	1.22
cinnamic acid	325	12.454	$y = 13.317x + 36.71$	0.9845	2.31
Apigenin	325	20.607	$y = 17.221x + 56.38$	0.9993	0.40
Myricetin	368	15.143	$y = 8.673x + 42.01$	0.9918	1.18
kaempferol	368	21.549	$y = 4.991x + 19.21$	0.9849	2.82

Table 3.2: Proximate analysis of the *P. candollei* methanol extract

	P. candollei methanol extract
moisture (%)	18.43 ± 0.42
ash content (%)	13.28 ± 0.28
crude protein (%)	8.71 ± 0.08
lipids (%)	4.11 ± 0.10
crude fibre (%)	22.71 ± 0.33
carbohydrates (%)	30.05 ± 0.36

[a]Values are represented as mean ± SEM.

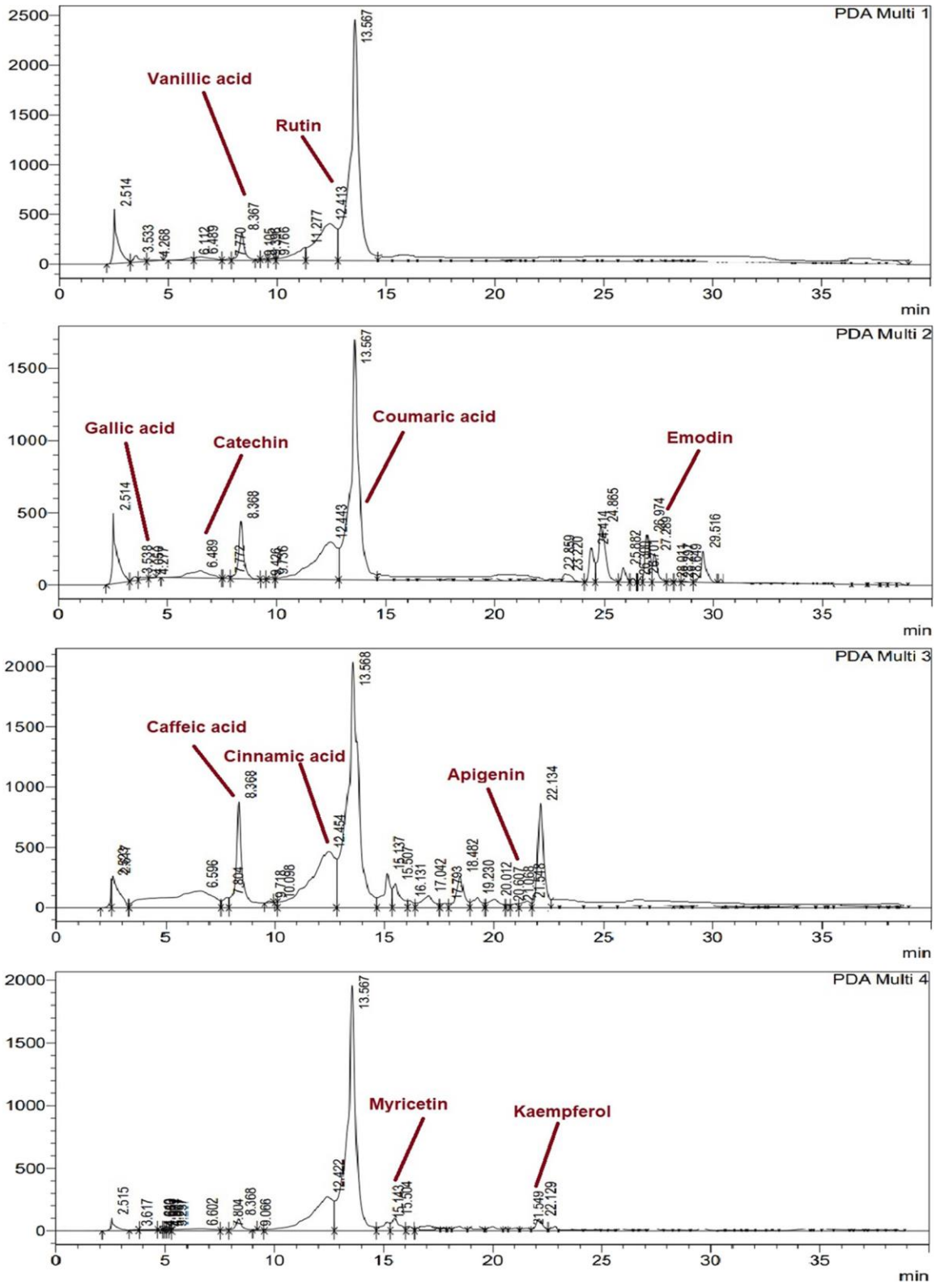

Figure 3.1: HPLC-DAD analysis of the *P. candollei* methanol extract at signal 1:257 λ, signal 2:279 λ, signal 3:325 λ, and signal 4:368 λ.

3.3.8. Effect of *P. candollei* on enzymatic and non- enzymatic antioxidants

To assess the antioxidant effect of *P. candollei*, enzymatic and nonenzymatic antioxidant molecules (CAT, SOD, POD, and GSH) were examined. CCl_4 treatment significantly ($p < 0.05$) inhibited the activities of antioxidant molecules such as CAT, SOD, POD, and GSH. However, *P. candollei* treatment significantly reversed the toxicity caused by CCl_4 and elevated the levels of antioxidants in the liver (Table 3.6). Silymarin administration along with CCl_4 also showed protective effects by increasing the activity levels of these antioxidants. Administration of *P. candollei* (150, 300, and 600 mg/kg) alone did not show any significant changes in antioxidants compared with normal rats.

3.3.9. Effect of *P. candollei* on the redox status and protein level in liver

Administration of CCl_4 significantly ($p < 0.05$) increased the concentration of nitrites, TBARS, and H_2O_2 but reduced the total soluble protein compared to the control group, thus causing a disturbance in the redox status of hepatocytes (Table 3.7). Coadministration of *P. candollei* (150, 300, and 600 mg/kg) restored the levels of biochemical parameters and the total protein toward the normal rats and reduced the disturbance in the redox status. Silymarin also revealed protective effects against oxidative stress by restoring the levels of free radicals and protein in the liver homogenate.

Table 3.3: Treatment effects of *P. candollei* on the hematological profile

	WBC (10^3/mm^3)	RBC (10^6/mm^3)	neutrophil (10^3/mm^3)	HGB (g/dL)	MCV (fl)	PLT (10^3/mL)	LYM (10^3/mm^3)	MCHC (g/dL)
Control	9.26 ± 0.12 ^{c}	7.01 ± 0.11^{a}	15.34 ± 0.17^{c}	11.13 ± 0.13^{c}	62.81 ± 0.34^{c}	221.46 ± 1.26^{c}	3.94 ± 0.11^{a}	34.17 ± 0.29^{b}
PCM (2000 mg/kg)	11.72 ± 0.09^{b}	6.91 ± 0.07^{a}	18.92 ± 0.15^{a}	12.86 ± 0.11^{b}	59.67 ± 0.25^{b}	261.61 ± 1.10^{b}	4.36 ± 0.13^{a}	31.74 ± 0.22^{c}
PCM (3000 mg/kg)	12.84 ± 0.17^{a}	7.23 ± 0.09^{a}	20.04 ± 0.25^{b}	13.74 ± 0.14^{a}	70.10 ± 0.23^{a}	283.22 ± 1.55^{a}	4.26 ± 0.10^{a}	39.29 ± 0.29^{a}

Mean ± SEM (n = 6 rats). Means having different superscripts show significance at $p < 0.05$. PCM, *Pleurospermum candollei* methanol extract.

Table 3.4: Treatment effects of *Pleurospermum candollei* on body and organ weight

Groups	Initial body weight (g)	Final body weight (g)	% Increase	Absolute liver weight (g)	Relative liver weight (mg/g)
Control	130 ± 0.53	213 ± 0.65	63.84 ± 2.3^{a}	7.42 ± 0.31^{f}	34.83 ± 1.21^{e}
CCl_4 (1 ml/kg)	136 ± 0.65	174 ± 0.86	27.94 ± 1.8^{f}	9.31 ± 0.42^{a}	53.51 ± 1.10^{a}
CCl_4 + Silymarin (200 mg/kg)	134 ± 0.49	208 ± 0.36	55.22 ± 0.65^{b}	7.81 ± 0.08^{d}	37.54 ± 0.33^{d}
CCl_4 + PCM (150 mg/kg)	133 ± 0.53	182 ± 0.53	36.84 ± 0.57^{e}	8.81 ± 0.15^{b}	48.41 ± 0.39^{b}
CCl_4 + PCM (300 mg/kg)	140 ± 0.61	201 ± 0.82	43.57 ± 0.90^{d}	8.82 ± 0.07^{b}	43.88 ± 0.21^{c}
CCl_4 + PCM (600 mg/kg)	138 ± 0.57	214 ± 0.32	55.07 ± 1.02^{b}	8.24 ± 0.11^{c}	38.50 ± 0.19^{d}
PCM (150 mg/kg)	143 ± 0.73	219 ± 0.77	53.14 ± 0.82^{c}	7.91 ± 0.06^{d}	36.11 ± 0.26^{d}
PCM (300 mg/kg)	137 ± 0.61	216 ± 0.45	57.66 ± 0.53^{b}	7.76 ± 0.14^{e}	35.93 ± 0.24^{de}
PCM (600 mg/kg)	144 ± 0.73	231 ± 0.82	60.41 ± 0.78^{ab}	7.88 ± 0.11^{d}	34.11 ± 0.30^{e}

Mean ± SEM (n = 6 rats). Various superscripts $^{(a–f)}$ in specify significance at $p < 0.05$. PCM; *Pleurospermum candollei* methanol extract. CCl_4; carbon tetrachloride.

Table 3.5: Treatment effects of *Pleurospermum candollei* on liver serum markers

	ALT (U/L)	ALP (U/L)	AST (U/L)	Albumin (mg/dl)	Bilirubin (mg/dl)
Control	46.11 ± 0.75^{f}	62.19 ± 1.11^{g}	53.05 ± 0.32^{g}	4.17 ± 0.06^{a}	0.51 ± 0.02^{g}
CCl_4 (1 ml/kg)	151.34 ± 0.83^{a}	142.30 ± 0.95^{a}	158.41 ± 0.49^{a}	1.63 ± 0.04^{g}	1.73 ± 0.03^{a}
CCl_4 + Silymarin (200 mg/kg)	56.71 ± 0.43^{d}	70.39 ± 0.70^{ef}	66.39 ± 0.54^{e}	3.82 ± 0.08^{c}	0.65 ± 0.04^{e}
CCl_4 + PCM (150 mg/kg)	115.51 ± 0.59^{b}	129.75 ± 0.79^{b}	134.65 ± 0.50^{b}	1.92 ± 0.07^{f}	1.42 ± 0.04^{b}
CCl_4 + PCM (300 mg/kg)	87.47 ± 0.57^{c}	101.43 ± 0.84^{c}	103.90 ± 0.68^{c}	2.67 ± 0.04^{e}	1.05 ± 0.02^{c}
CCl_4 + PCM (600 mg/kg)	74.23 ± 0.68^{cd}	81.37 ± 0.67^{d}	83.81 ± 0.60^{d}	3.59 ± 0.05^{d}	0.72 ± 0.03^{d}
PCM (150 mg/kg)	53.18 ± 0.56^{d}	72.18 ± 0.44^{e}	62.93 ± 0.38^{f}	3.89 ± 0.03^{c}	0.62 ± 0.02^{e}
PCM (300 mg/kg)	48.85 ± 0.86^{e}	68.26 ± 0.67^{f}	59.04 ± 0.15^{f}	3.96 ± 0.41^{b}	0.59 ± 0.01^{f}
PCM (600 mg/kg)	46.39 ± 0.60^{e}	63.18 ± 0.59^{g}	55.21 ± 0.47^{g}	4.11 ± 0.07^{a}	0.52 ± 0.02^{g}

Mean ± SEM (n = 6 rats). Various superscripts $^{(a–g)}$ in specify significance at $p < 0.05$. PCM; *Pleurospermum candollei* methanol extract. CCl_4; carbon tetrachloride.

Table 3.6: Treatment Effects of *P. candollei* on liver tissue antioxidants

	CAT (U/min)	POD (U/min)	SOD (U/mg protein)	GSH (μmol/mg)
control	11.90 ± 0.33^{a}	9.83 ± 0.20^{a}	6.78 ± 0.26^{a}	15.57 ± 0.35^{a}
CCl_4 (1 mL/kg)	2.18 ± 0.13^{g}	3.01 ± 0.22^{g}	1.79 ± 0.11^{e}	4.65 ± 0.34^{g}
CCl_4 + silymarin (200 mg/kg)	10.17 ± 0.38^{c}	8.94 ± 0.34^{d}	5.92 ± 0.13^{b}	$12.43 \pm 0.24^{b,c}$
CCl_4 + PCM (150 mg/kg)	4.39 ± 0.22^{f}	5.04 ± 0.25^{f}	2.40 ± 0.07^{d}	6.98 ± 0.44^{f}
CCl_4 + PCM (300 mg/kg)	6.61 ± 0.29^{e}	6.73 ± 0.15^{e}	3.66 ± 0.15^{c}	9.44 ± 0.26^{e}
CCl_4 + PCM (600 mg/kg)	9.83 ± 0.40^{d}	8.84 ± 0.28^{d}	5.80 ± 0.17^{b}	11.75 ± 0.32^{d}
PCM (150 mg/kg)	$10.47 \pm 0.35^{b,c}$	9.07 ± 0.16^{c}	$6.03 \pm 0.04^{a,b}$	13.71 ± 0.30^{c}
PCM (300 mg/kg)	$10.91 \pm 0.26_{b}$	$9.41 \pm 0.19^{b,c}$	6.36 ± 0.23^{a}	14.43 ± 0.39^{b}
PCM (600 mg/kg)	11.53 ± 0.36^{a}	9.86 ± 0.21^{a}	6.61 ± 0.19^{a}	15.40 ± 0.34^{a}

Mean ± SEM (n = 6 rats). The superscripts (a−g) specify significance at $p < 0.05$. PCM; *Pleurospermum candollei* methanol extract, CCl_4, carbon tetrachloride.

Table 3.7: Treatment effects of *Pleurospermum candollei* on redox status and protein of liver

	Protein (µg/mg tissue)	TBARS (nM/min/mg Protein)	H_2O_2 (nM/min/mg Tissue)	Nitrite Content (µM/mL)
Control	11.71 ± 0.75^{a}	51.30 ± 1.51^{g}	5.93 ± 0.21^{e}	53.37 ± 0.92^{g}
CCl_4 (1 ml/kg)	5.70 ± 0.42^{e}	113.16 ± 3.01^{a}	11.10 ± 0.48^{a}	82.05 ± 1.50^{a}
CCl_4 + Silymarin (200 mg/kg)	10.97 ± 0.69^{b}	63.49 ± 2.68^{e}	6.27 ± 0.36^{d}	58.28 ± 1.03^{e}
CCl_4 + PCM (150 mg/kg)	6.77 ± 0.35^{d}	98.12 ± 2.16^{b}	8.37 ± 0.24^{b}	72.60 ± 2.14^{b}
CCl_4 + PCM (300 mg/kg)	8.34 ± 0.40^{c}	82.17 ± 2.31^{c}	7.33 ± 0.40^{c}	67.33 ± 1.82^{c}
CCl_4 + PCM (600 mg/kg)	10.83 ± 0.51^{b}	70.73 ± 1.99^{d}	6.37 ± 0.27^{d}	60.31 ± 1.17^{d}
PCM (150 mg/kg)	11.18 ± 0.37^{ab}	61.24 ± 1.17^{e}	6.11 ± 0.15^{de}	56.83 ± 0.94^{f}
PCM (300 mg/kg)	11.46 ± 0.57^{ab}	55.67 ± 1.32^{f}	5.96 ± 0.11^{e}	53.64 ± 1.06^{g}
PCM (600 mg/kg)	11.81 ± 0.64^{a}	52.18 ± 1.98^{g}	5.81 ± 0.13^{f}	53.49 ± 1.24^{g}

Mean ± SD (n = 6 rats). Means with different superscripts $^{(a–g)}$ in specify significance at $p < 0.05$. PCM; *Pleurospermum candollei* methanol extract, CCl_4; carbon tetrachloride.

3.3.10. Effect of *P. candollei* on the ER stress marker

The study also examined the expression of ER stress markers, namely, GRP-78, XBP-1u, XBP-1t, and XBP-1s. CCl_4 treatment significantly ($P < 0.05$) induced an increase in the mRNA expression of these markers compared to the control group (Figure 3.2). In contrast, administration of *P. candollei* with CCl_4 reduced the CCl_4-induced cellular detoxification by suppressing the expression of ER stress markers. Similarly, silymarin treatment restored the levels of stress markers, thus protecting the hepatocytes from CCl_4-mediated toxicity.

3.3.11. Effect of *P. candollei* on inflammatory mediators

After CCl_4 administration to rats, inflammation was initiated and propagated through an elevation in the expression of inflammatory cytokines including IL-6, TNF-α, and chemokine MCP-1. Administration of *P. candollei* or silymarin to CCl_4-treated rats, however, inhibited the overexpression of proinflammatory mediators, suggesting that *P. candollei* exhibits an anti-inflammatory effect (Figure 3.2). However, treatment with *P. candollei* alone revealed a nonsignificant ($P > 0.05$) change in the expression of the abovementioned markers compared to that in normal rats.

3.3.12. Effect of *P. candollei* on apoptotic markers

In CCl_4-treated rats, the mRNA expression of apoptotic markers Chop, and caspase-3 was significantly ($P < 0.05$) enhanced as compared with normal rats, while that of the antiapoptotic marker, namely, BCl-2 was downregulated (Figure 3.2). However, treatment with *P. candollei* (150, 300, and 600 mg/ kg) along with CCl_4 significantly enhanced the levels of these antiapoptotic genes toward normal levels, indicating the protective effect of *P. candollei* against cell death. mRNA expression of Chop and caspase-3 was inhibited by the administration of *P. candollei* to CCl_4-intoxicated rats. Furthermore, treatment of rats with *P. candollei* (300 and 600 mg/kg) alone did not illustrate significant alterations in the levels of these markers compared with that of normal rats.

3.3.13. Effect of *P. candollei* on enzymatic antioxidants

In the CCl_4-intoxicated group, the mRNA expression of antioxidant markers GCLC and Nrf-2 was significantly reduced after 4 weeks of treatment as compared with the control group (Figure 3.2). On the contrary, treatment with *P. candollei* (150, 300, and 600 mg/kg)

along with CCl_4 significantly enhanced the levels of these antioxidant enzymes toward the normal group, indicating the protective effect of *P. candollei* on the hepatocytes. Silymarin also exhibited this protective effect, indicated by the increased levels of these enzymatic antioxidants in the relevant group. Furthermore, treatments of rats with *P. candollei* (300 and 600 mg/kg) alone also enhanced the levels of these antioxidant enzymes compared with that of normal rats.

3.3.14. Histopathological findings

The histological analysis of the sample tissues of the control group and rats treated with *P. candollei* (150, 300, and 600 mg/kg) alone revealed narrow sinusoids, normal hepatic cords radiating from the central vein, and prominent nuclei. On the contrary, the liver sections of CCl_4-treated groups showed hepatocyte ballooning, infiltration of inflammatory cells, and disruption in the architecture of hepatic lobules (Figure 3.3). However, the rats with the coadministration of *P. candollei* (300 and 600 mg/kg) with CCl_4 displayed less severe injuries than CCl_4- intoxicated rats, with minimal apoptotic hepatocytes. Rats treated with silymarin (200 mg/kg) showed much healthier liver tissues than the CCl_4-treated rats.

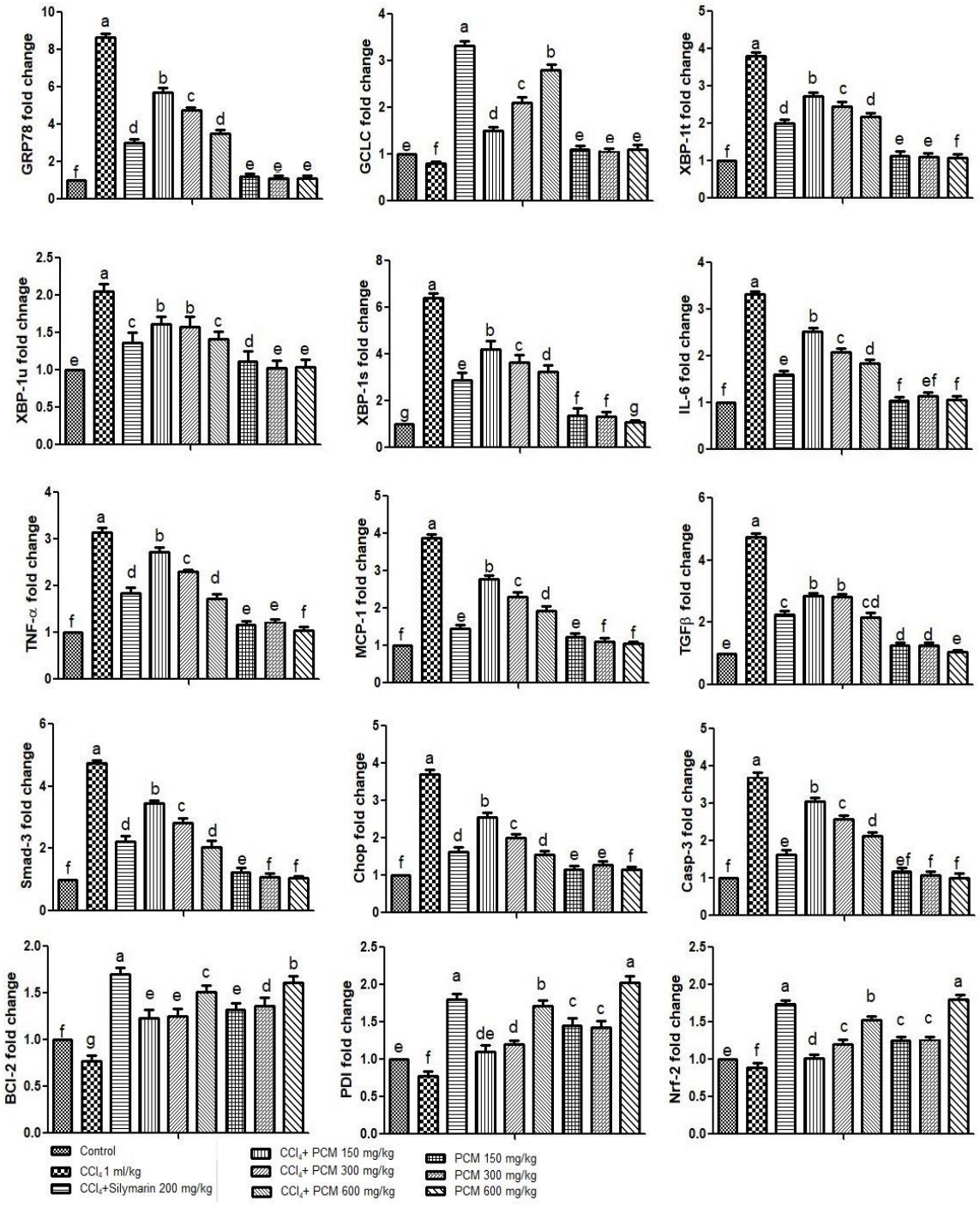

Figure 3.2: Graphical representation of treatment effects of *Pleurospermum candollei* on fold change of gene involved in ER stress, inflammation, apoptosis, and fibrosis. Various superscripts $^{(a\text{-}g)}$ specify significance at $p < 0.05$.

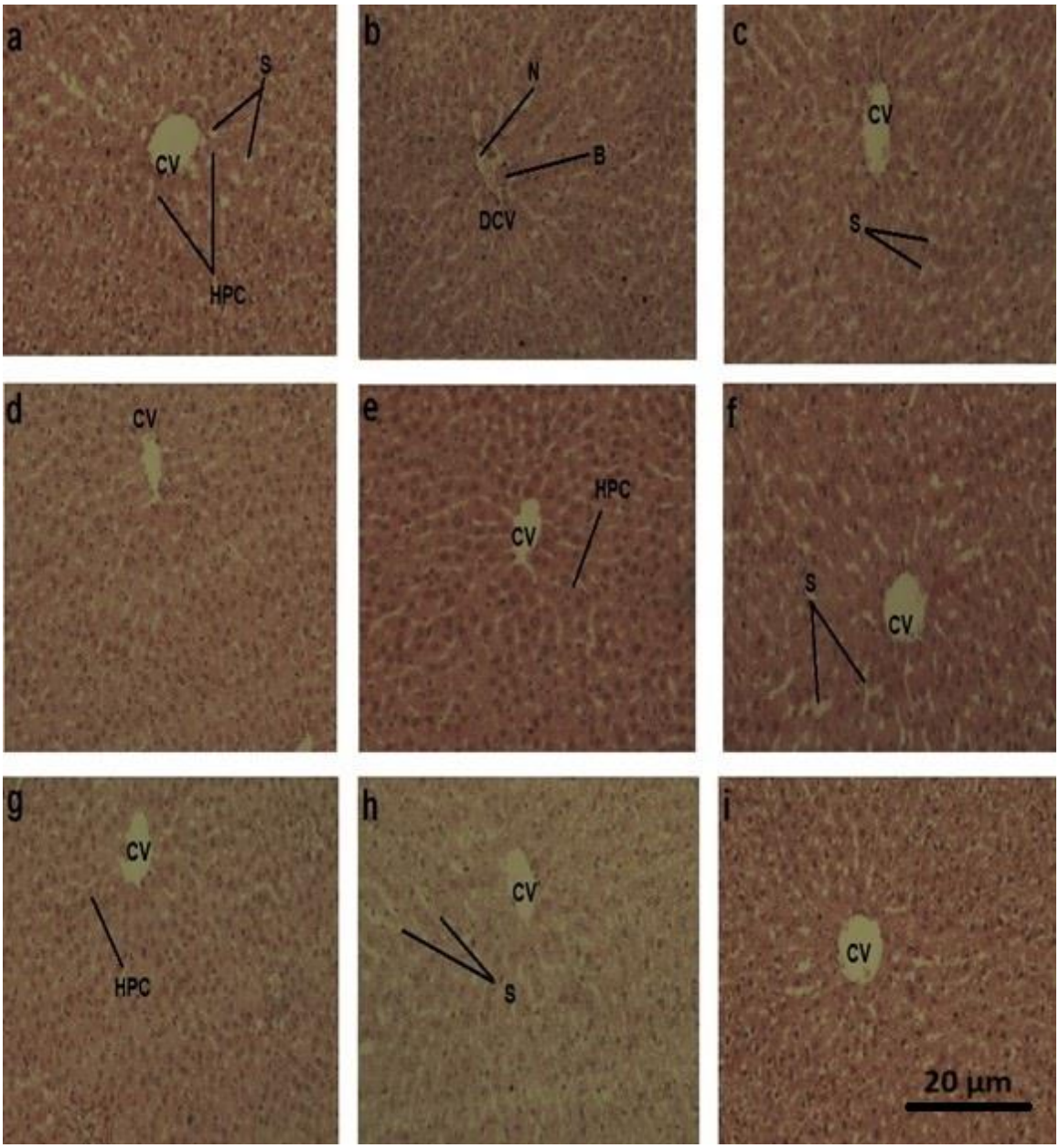

Figure 3.3: Protective effects of *Pleurospermum candollei* treatment on liver histopathology of rats (40× magnification with H&E stain). (a) Control group, (b) CCl_4 (1 ml/kg bw), (c) CCl_4 + silymarin (200 mg/kg), (d) CCl_4 + PCM (150 mg/kg), (e) CCl_4 + PCM (300 mg/kg), (f) CCl_4 + PCM (600 mg/kg), (g) PCM (150 mg/kg), (h) PCM (300 mg/kg), (i) PCM (600 mg/kg). CV; central vein. DCV; damaged central vein. HPC; hepatocytes. N; necrosis. B; ballooning of hepatocytes.

3.4. Discussion

CCl_4 belongs to the class of hepatotoxins that require metabolic activation to induce oxidative damage and hepatic injuries. When CCl_4 is metabolized by hepatocytes, it generates free radicals that can cause impairments to the hepatocyte cell membrane and organelles, such as mitochondria. Acute liver injury is related to the liver oxidative stress (Yang *et al*., 2020). This oxidative stress leads to peroxidation, which induces detrimental effects on liver, including inflammation (leukocyte infiltration), central vasodilation, hepatocellular fibrosis and necrosis, vascular degeneration, bile duct proliferation, vascular occlusion, cellular hypertrophy, and increased collage depositions (Dutta *et al*., 2018; Huang *et al*., 2020; Sahreen *et al*., 2013). The mechanism of hepatotoxicity due to oxidative stress involves the imbalance of oxidation and antioxidant systems; thus, liver exposure to CCl_4 increases the production of ROS and reduces the antioxidant capacity (Lee *et al*., 2019). The current study indicated that treatment with CCl_4 on alternate days for a period of 4 weeks led to severe hepatic injury and inflammation in rats. The findings of this study are similar to those of previous studies (Batool *et al*., 2018).

Natural products, which are derived from plants, animals, and microorganisms, have been studied for their potential to protect the liver from oxidative stress, reduce inflammation, and act as novel agents against injuries. Natural medicinal plants are valuable sources of bioactive compounds for the development of natural medicines such as vitexin, salvianolic acid, and polysaccharides, and Dendrobium nobile Lindl. alkaloids (DNLA) have been studied for their hepatoprotective effects (Sun *et al*., 2022). The plants of Apiaceae family and Pleurospermum genus have been recognized for their medicinal, nutraceutical, cosmeceutical, and traditional uses; furthermore, antioxidants found in these plants have the potential to prevent and treat diseases (Kawarty *et al*., 2020; Thiviya *et al*., 2021). *P. candollei* has been found to be used as a traditional medicine to treat various diseases; its antioxidant property and biological potential have not been thoroughly explored (Ahmed *et al*., 2022). Therefore, this study was designed to investigate the phytochemical profile through HPLC, antioxidant activities, anti-inflammatory activities in biological systems, and the effect on stress, fibrosis, and inflammatory markers through molecular studies to unfold the pharmaceutical applications of *P. candollei*.

Plants from the Apiaceae family are commonly used in everyday nutrition, particularly as spices. These plants are rich sources of dietary supplements due to their low-calorie content and high levels of protein, fixed oils, fibers, carbohydrates, and essential oils. Additionally, seeds of Apiaceae family contain a variety of water-soluble glycosides, aromatic compounds, phospholipids, flavonoids, terpenoids, sugars, lactones, qui- nones, and carotenoids (Sayed-Ahmad *et al*., 2017) In our study, the proximate analysis of the methanol extract of *P. candollei* revealed significant levels of proteins, carbohydrates, fibers, lipids, and moisture. Thus, this plant can also be a rich source of active compounds and nutrients.

Phenolic compounds have a functional hydroxy group attached to an aromatic ring that acts as an electron donor. This functional group transfers electrons to a free radical, neutralizing its activity and preventing it from damaging proteins and DNA. The presence of phenolic compounds in natural products is important because they can help to reduce the risk of various diseases associated with oxidative stress. The HPLC-DAD analysis confirmed high amounts of flavonoids and phenols in the methanol extract of *P. candollei*. Same results were reported by Al-Dalahmeh (Al-Dalahmeh *et al*., 2022) Reference polyphenols were selected on the basis of their medicinal properties; for example, rutin possesses antioxidant, antiviral, and hepatoprotective effects (Sajid *et al*., 2016), while gallic acid, caffeic acid, and catechin have antioxidant and anticancer properties (Roleira *et al*., 2015; Shahid *et al*., 2016). Myricetin is famous for its antioxidant and chelation properties against free radicals (Zhang *et al*., 2017). Apigenin is famous for having antioxidant, antiviral, anti-inflammatory, and anticancer properties (Jeong *et al*., 2009). Therefore, the presence of gallic acid, rutin, apigenin, myricetin, and catechin in *P. candollei* accounts for its antioxidant, antiapoptotic, anti-inflammatory, and protective properties. The presence of phytochemicals such as phenolics and vanillin in plant extracts is associated with their high antioxidant activities. Studies have found that plant extracts with high TPC and TFC levels have the potency to quench free radicals including DPPH and exhibit reducing power activities (Naz *et al*., 2022). Moreover, Apiaceae extracts typically contain polyphenolic compounds, including flavonoids, tannins, and phenolic acids. These phytochemicals exhibit anti-inflammatory activities, which is shown by the inhibition of the activation or action of proinflammatory mediators. As a result, they can be considered as a potential alternative to anti-inflammatory drugs. Essential oils extracted from these plants show stronger biological

activities due to the presence of high content of flavonoids and phenols (Unsal *et al*., 2021). In this study, TPC and HPLC-DAD analysis of *P. candollei* revealed the presence of polyphenols in the *P. candollei* extract, which provides a rationale for the anti-inflammatory and antioxidant properties of the *P. candollei* methanol extract.

Any injury to liver cells can lead to a process called degeneration, where cells lose their normal structure and begin to breakdown. ALT, ALP, and AST are found inside the cells, but when liver cells undergo degeneration, these enzymes leak out of the cells and enter the blood. Therefore, the levels of ALT, ALP, and AST in the serum can be used to evaluate the liver injury. In the current study, elevated levels of these enzymes and bilirubin in the serum after CCl_4 administration is a sign of liver disease. Same results were found in the previous research of Unsal (Unsal *et al*., 2021). However, our study confirmed that the *P. candollei* treatment resulted in the reduction in the level of these enzymes and bilirubin in serum. These findings suggested that the high polyphenolic content and antioxidant properties of *P. candollei* may be responsible for its significant protective effects against hepatic damage.

Antioxidant supplementation is an attractive strategy to reduce the risk of oxidative stress and various diseases induced by free radicals. Enzymatic defense systems of the body protect cells from degeneration and damage. SOD transfers highly reactive superoxide radicals into less reactive H_2O_2, while CAT and GSH neutralize H_2O_2 to protect the liver from ROS. POD also helps to maintain the hemostasis of cells by breaking down H_2O_2. In our study, CCl_4 exposure down-regulated the activity of antioxidants in the hepatocytes of rats, indicating a decline in the activity of the defense system (Cui *et al*., 2014; El-Aarag *et al*., 2019; Sun *et al*., 2013). In contrary, treatment of rats with the *P. candollei* methanol extract increased the levels of antioxidant enzymatic molecules, especially at a high dose of 600 mg/kg bw, suggesting that *P. candollei* is against oxidative stress by degrading the free radicals generated by CCl_4.

CCl_4 injection induced oxidative stress, which was validated by the increase in the concentration of lipid peroxidation, H_2O_2, and nitric oxide, additionally, with a reduction in GSH and total protein levels in the liver. Increased production of nitrites after CCl_4 treatment occurs through the activation of the inducible nitric oxide synthase (iNOS) enzyme. Depletion of GSH levels is associated with the weakened antioxidant defense

system of the body, while the reduction in total protein levels is likely due to the increased production of ROS, which inhibits protein synthesis and causes damage to the protein (Dkhil *et al*., 2019). In our current study, silymarin and *P. candollei* treatment mitigated the oxidative stress by inhibiting the production of ROS and normalizing the production of proteins and GSH levels. Thus, *P. candollei* can reduce oxidative stress in the liver by neutralizing the effects of ROS and activating the antioxidant system.

Studies have revealed that CCl_4 activates Kupffer cells, which upregulate TNF-α and IL-6. These upregulated proinflammatory cytokines can disrupt cellular signaling pathways, leading to tissue damage, inflammation, and apoptosis. ROS can trigger the NF-κB pathway by various mechanisms, including the degradation of the inhibitor of kappa B-alpha (IκB-α) and phosphorylation of NF-κB p65/p50 subunits, in response to the inflammatory cytokines. NF-κB stimulates the production of inflammatory cytokines, which in turn initiate inflammatory response, through a series of cascades (Sun *et al*., 2022). Our study also showed that the levels of these cytokines (TNF-α, IL-6, and MCP-1) were upregulated in CCl_4-intoxicated rats, while the expression of these markers was restored in PCM-treated rats, especially at a high dose of 600 mg/kg. This finding is similar to the previous study of Naz (Naz *et al*., 2022).

The TGF-β1/Smad signaling pathway is a key indicator of liver fibrosis. Overexpression of TGF-β1 is an attribute of damage to liver cells and liver fibrosis. TGF-β1 binds to the cell surface receptor of the Smad pathway and activates the Smad protein, which leads to cell apoptosis. In our current study, CCl_4 treatment increased the expression of TGF-β1 and Smad in liver tissues. Our results are consistent with that of previous study which also reported the overexpression of these fibrosis markers during liver injury (Niu *et al*., 2016). Inhibition of TGF-β1 expression can prevent liver fibrosis and can be employed as a therapeutic strategy. *P. candollei* treatment significantly downregulated the mRNA expression of these TGF-β1 and Smad.

Apoptosis is regulated by the activation of caspases, a family of cysteine proteases that cleave various proteins within the cells. One of the key regulators of apoptosis is BCl-2, an antiapoptotic protein, which inhibits the proapoptotic protein BAX (Lopez *et al*., 2015). The disturbance in balance between BCl-2 and BAX leads to various diseases, such as cancer.

In this study, CCl_4 treatment downregulated the expression of BCl-2 in comparison to the control group but upregulated the mRNA expression of caspase-3. Our studies are consistent with that of previous studies, reporting reduction in the activity of BCl-2 and elevation in the levels of caspase-3 as an indication of liver cell apoptosis (El-Aarag *et al*., 2019). On the contrary, *P. candollei* showed antiapoptotic effects against CCl_4 by restoring the normal levels of caspase-3 and BCl-2 in the liver of rats.

The Nrf-2 signaling pathway helps to modulate cellular redox homeostasis by activating the antioxidant genes. Numerous natural products, including phytochemicals, have the potency to activate the Nrf-2 pathway, thus providing protective effects. However, PDI is involved in the normal folding of newly synthesized proteins and preventing them from aggregation, thus regulating the ER stress response. Additionally, GCLC, an antioxidant enzyme regulator, plays a crucial role in the biosynthesis of the glutathione enzyme. Elevated expression of GCLC in cells is associated with increased glutathione levels in response to oxidative stress. In our current results, lower expression levels of Nrf-2, PDI, and GCLC were found in the liver homogenate after CCl_4 treatment, indicating liver injuries, ER stress, and hepatotoxicity. Our results are similar to those of previous studies (Dkhil *et al*., 2019; Naz *et al*., 2022). However, significant increases in the expression levels of these antioxidant markers were found after *P. candollei* administration. Furthermore, prior to clinical trials and dose selection for humans, the determination of safety and toxicity profile of extract in humans is mandatory. An equivalent human dose of extract can be calculated by the body surface area (BSA) scaling method. The following equation is used for this calculation:

$$\text{Human dose} = \text{Animal dose} \times (\text{Animal weight}/3)^{0.33} \times (\text{Human BSA}/\text{Animal BSA})$$

The value of 3.0 is the average weight of rats in toxicology studies. Once the equivalent human dose is calculated, it can be compared to typical human doses used in clinical studies or the estimated safe levels of exposure (Jacob *et al*., 2022).

3.5. Conclusion

The current study has found that *P. candollei* exhibits strong antioxidant, anti-inflammatory, and antiapoptotic properties. The findings illustrated the inhibition of ROS, which may be attributed to the presence of polyphenols in *P. candollei*. The results also revealed that natural

bioactive compounds provide amelioration effects against inflammation and fibrosis. Our results confirmed that this plant can restore the antioxidant enzymes of the biological system and can also normalize the mRNA expression of ER stress markers, apoptotic mediators, and inflammatory and fibrosis genes. Thus, *P. candollei* could be a promising anti-inflammatory and antifibrotic drug for the treatment of liver diseases.

4. Investigating the role of polyphenols from *Pleurospermum candollei* (DC.) extract against diabetic nephropathy through modulating inflammatory cytokines and renal gene expression in rats

4.1. Introduction

Diabetes Mellitus (DM) is known as an endocrine disorder characterized by hyperglycaemia which may result due to an impairment in insulin secretion, insulin action, or both (Chawla *et al*., 2016). The complications associated with diabetes can be categorized into two groups: microvascular complications which include neuropathy, nephropathy, and retinopathy, and macrovascular complications such as peripheral vascular disease, cardiovascular disorder, and stroke (Ahmed *et al*., 2022a). Sustained hyperglycemia is closely linked to the development of diabetic nephropathy (Mestry *et al*., 2017). This condition is the primary cause of end-stage renal disease and is associated with various factors such as hypertension, urinary tract infections, polyneuropathic bladder dysfunction, or macrovascular angioplasty (Poontawee *et al*., 2016). In diabetes mellitus, chronic hyperglycemia gives rise to a series of biochemical effects, and oxidative stress induced by diabetes may involve in the symptoms and development of the disease (Giugliano *et al*., 1996).

Enhanced production of free radicals resulting in the depletion of endogenous antioxidant components may result in disruption of cellular functions and damage to cellular membranes and may enhance susceptibility to lipid peroxidation (Baynes, 1991). Increase in free radical production in diabetes has been the subject of several studies. These hypotheses include several processes including the generation of advanced glycation end products (AGEs) by non-enzymatic glycation of proteins, increased glucose flux, and autoxidation of glucose (Rajasekaran *et al*., 2005; Tiwari and Rao, 2002). Elevated oxidative stress plays a significant role in activating the signaling pathway responsible for the enhanced expression of TGF-β in diabetic nephropathy (Han *et al*., 2017). Similarly, over- expression of a peptide known as Kidney injury molecule-1 (KIM-1) is reported in diabetic tubular injury. Erythropoietin (EPO) is involved in the inhibition of apoptosis, regulation of

inflammation, and formation of ROS (Mohamed *et al*., 2018).

Streptozotocin induces insulin-dependent diabetes mellitus by inhibiting insulin secretion. One of the key chemical properties of streptozotocin is DNA alkylation potency. Sulfonylureas such as glibenclamide are commonly employed as a standard drug in streptozotocin (STZ)-induced diabetic rats or mice to assess and compare the anti- diabetic properties of potential therapeutic compounds (Lenzen, 2008). Glibenclamide primarily acts by binding to specific sites on ATP-sensitive *K*+ (KATP) channels and inhibits their closure (Lv *et al*., 2022). This inhibition results in the depolarization of the plasma membrane and β-cells, modulation of voltage-gated Ca^{2+} channels, enhanced Ca^{2+} influx, and a rise in cytosolic (Ca^{2+}), and this may lead to insulin release. Despite the effectiveness of these Sulfonylureas in treating diabetes, their usage is hindered by certain factors such as their limited mechanism of action, pharmacokinetic properties, rates of secondary failure, and associated potential side effects (Islam, 2020). Existing antidiabetic drugs have injurious effects and can lead to severe complications within the human body, lack efficacy, and provide limited relief from symptoms. Therefore, there is a need for new effective drugs that have fewer side effects. The basic needs for diabetes regulation are oral hypoglycemic agents and routine insulin doses (Naik *et al*., 2022). However, none of the current antidiabetic medicine can maintain long-term glycemic control in the body without causing side effects. As a result, medicinal plants are extensively utilized in under-developed and developing countries as an alternative treatment against diabetes. These traditional medicinal plants exhibited effectiveness in improving plasma glucose levels while minimizing adverse reactions (Tran *et al*., 2020).

The use of plant-based bioactive compounds for the improvement of health and prevention of various diseases, specifically diabetes is rapidly growing (Putthapiban *et al*., 2017; Yeh *et al*., 2003). The use of dietary supplements along with therapy is a common practice for complex diseases such as diabetes (Durazzo *et al*., 2021; Thi Bui *et al*., 2021). While dietary supplements and plant-based raw materials do not provide a complete replacement for conventional therapy in the case of complex disorders like diabetes mellitus, they may have the potential to reduce the required

dosage or lower the number of medications used in the treatment to control glycemia (Mahdavi *et al*., 2021). Dietary modifications are the main approach to lower blood sugar levels, serving as the initial step in implementing a therapeutic protocol (Sip *et al*., 2022). Pleurospermum species are reported as strong analgesic, anti-inflammatory, and antimalarial agents (Ali *et al*., 2021). *Pleurospermum kamtschaticum*, an edible flower plant of family Apiaceae decreased glycogen levels enhanced plasma insulin levels in diabetic rats (Chung *et al*., 2015). *Pleurospermum lindleyanum* is widely used for prevention and treatment of hypertension and diabetes (Zhang *et al*., 2023). Similarly, *Pleurospermum benthamii* is reported as the potential source to treat diabetes and obesity (Pandey and Pradhan, 2020).

Pleurospermum candollei is a highly significant specie of family Apiaceae due to its medicinal properties, and it is found in Skardu, Pakistan (Khan *et al*., 2018). *P. candollei* is a 30-40 cm long herb and is employed for the treatment of various health disorders including diarrhea, heart dis- eases, gastric problems, blood pressure regulation, reduction in cholesterol levels, stomach, and abdominal problems (Khan *et al*., 2015a; Khan *et al*., 2015b). Moreover, the powder of stem is used to alleviate joint, back, and side pains and is found useful for the treatment of both male and female infertility (Ali *et al*., 2021). In the Himalayan region of Pakistan, *P. candollei* is additionally recog- nized for its therapeutic potential against respiratory diseases, cerebral disorders, unconsciousness, pains, and various other diseases. Due to its taste, it is also used as a vegetable by local tribes of the Karakorum area and is also commercially available in these regions (Abbas *et al*., 2019; Ahmed *et al*., 2022b).

4.2. Material and methods

4.2.1. Herbarium specimen collection

The aerial parts of *P. candollei* were collected in August 2022, from Skardu, Pakistan. The plant sample was authenticated by the Herbarium of Pakistan, Islamabad with a voucher number 133142.

4.2.2. Plant extraction

One kilogram of air-dried parts of *P. candollei* was ground into a fine powder in the Willey mill. The plant powder was immersed in 4 L of 90% ethyl-alcohol (C_2H_5OH; F.W. 46.07 g/mol; density: 0.789 g/ml) for 48 h at 50 ± 2 ˚C. The obtained liquid was filtered, and the filtrate was concentrated in a rotary evaporator under reduced pressure at 45 ˚C. Then, the obtained ethanol extract of *P. candollei* (PCE) was air-dried and weighed. Finally, 62 g of semi-solid, greenish material was obtained and stored at 4 ˚C for further analysis.

4.2.3. Qualitative analysis of phytochemicals

The various phytochemicals were identified using standard tests and reactions. Briefly, the ethanol extract of *P. candollei* was dissolved in distilled water (1 mg/ml) and filtered. Alkaloids were analyzed using Hager's test which produced yellowish precipitates with plant extract.

Flavonoids, phenols, and tannins were detected using tests such as ferric chloride ($FeCl_3$) or lead acetate solution. The yellow color indicated the presence of flavonoids, the greenish color confirmed the presence of tannins while blue precipitation indicated the presence of phenols (Pawar *et al*., 2014). Saponins were identified through foam formation tests, such as frothing, which resulted in stable foam formation. Anthocyanins were determined by observing color changes after changing the pH conditions of the plant solution. Glycosides were detected by hydrolyzing them and identifying the released aglycones using the Keller-Kiliani test. Vitamin C was analyzed by adding dinitrophenyl hydrazine reagent, and yellow precipitates confirmed the manifestation of Vitamin C. Oils and resins were assessed using the filter-paper method while the precipitation method was used for the detection of terpenoids and steroids (Batool *et al*., 2019; Yadav and Agarwala, 2011).

4.2.4. High-performance liquid chromatography (HPLC) analysis

To determine polyphenols in the ethanol extract of *P. candollei*, HPLC-DAD analysis was employed using an Agilent 1200 series system (Germany). For

compound separation, a reverse-phased (Agilent, USA) Zorbaex Plus RSC-8 column was utilized. The mobile phase-A comprised water (80%), methanol (10%), acetonitrile (5%), and acetic acid (1%). While mobile phase b comprised methanol (60%), acetonitrile (40%), and acetic acid (1%), following the gradient program (Khalil *et al*., 2020). The flow rate was adjusted at 1.20 mL/min, and plant extract was dissolved in HPLC-grade methanol (Sigma-Aldrich) at a concentration of 10 mg/ml. Filtration was carried out using 0.45 µm membrane filters while the injection volume was adjusted at 20 µl. For the quantification of obtained peaks, the retention time of the reference compounds was determined at specific wavelengths. Various peaks monitored at 257 nm, 279 nm, 325 nm, and 368 nm.

4.2.5. Quantification of bioactive phytochemicals

The total phenolic content (TPC) of plant extract was identified by the standard method of Folin-Ciocalteau (FC reagent) protocol (Phuyal *et al*., 2020). Readings were taken in triplicate at 765 nm and recorded as mg of gallic acid equivalents/ g of dried weight of extract (mg GAE/g wt.) using a standard curve of gallic acid ($y = 0.0125x + 0.9379$; $R^2 = 0.9991$) The total flavonoid content (TFC) of the plant sample was measured with an already-mentioned method with slight adaptations (Arvouet-Grand *et al*., 1994). Three readings were recorded at 420 nm, and TFC was recorded in mg of quercetin equivalent/ g of the dried weight of extract (mg QE/g wt.) using a standard curve of quercetin ($y = 0.0037 + 0.1881$; $R^2 = 0.9895$).

4.2.6. Antioxidant activities

4.2.6.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The standard method of DPPH assay was followed to estimate the antioxidant potency of *P. candollei* ethanol extract (Nithianantham *et al*., 2011). In brief, different concentrations of (15-1000 µg/ml) of *P. candollei* extract (50 µl) were added to 450 µl of DPPH solution (3 mg in 50 ml methanol). The mixture was incubated for 10 min at 25 ˚C. Absorbance was recorded at 517 nm. The percentage inhibition of DPPH free radical by plant extract was calculated by the following formula:

$$\% \text{ inhibition activity} = ((\text{Absorbance of control} - \text{Absorbance of sample / ascorbic acid})/\text{Absorbance of control}) \times 100$$

4.2.6.2. Metal chelating assay

A standard protocol was used to evaluate to assess the chelation property of *P. candollei* extract against ferrous ions (Ahmed *et al*., 2015). Different plant extract concentrations (15-1000 µg/ml) were mixed with 0.05 ml solution of $FeCl_2$ (1 mM). To initiate the reaction, 0.2 ml of ferrozine solution (1 mM) was added and the mixture was diluted to a final volume of 500 µl with methanol and allowed to incubate at 25 ˚C for 15 min. The absorbance of the mixture was recorded at 562 nm. The readings were taken in triplicate and EDTA was used as a reference. The inhibition of the formation of ferrozine-Fe^{2+} was calculated by the following formula:

$$\text{Metal chelating activity (\%)} = ((\text{Absorbance of control} - \text{Absorbance of sample / EDTA})/\text{Absorbance of control}) \times 100$$

4.2.6.3. α-Glucosidase inhibitory assay

The inhibitory action of *P. candollei* extract against α-glucosidase activity was determined using a spectrophotometric method with some modifications (Telagari and Hullatti, 2015). 50 µl of extract solution in various concentrations (15–1000 µg/mL), 200 µl of phosphate buffer (0.1 M, pH 6.8), and 30 µl α-glucosidase solution (1.0 U/mL) were mixed and pre-incubated at 37C for 10 min. Subsequently, 20 µl of p-nitrophenyl-α-D-glucopyranoside (pNPG, 5 mM prepared in phosphate buffer) solution was added and kept at 25 ˚C for 15 min. To terminate the reaction, 100 µl of sodium carbonate (0.2 M) was added to the mixture. The absorbance of the mixture was taken at 405 nm. All the readings were taken in triplicate at 405 nm. The rate of inhibition of enzyme activity was calculated by the following formula:

$$\text{\% inhibition activity} = ((\text{Absorbance of control} - \text{Absorbance of sample / acarbose})/\text{Absorbance of control}) \times 100$$

4.2.7. In-vivo anti-diabetic activity of *P. candollei* ethanol extract

The experiment was conducted on forty-nine Sprague Dawley male albino rats (from the Primate Facility of Quaid-I-Azam University), weighing 140 ± 5 g. The animals were kept under standard laboratory conditions (12 h light/dark cycle) at

an ambient temperature of 23 ± 2C with free access to tap water. All experiments were conducted ac- cording to the NIH and US Guidelines (2010/63/E.U.). Ethical approval was provided by the local ethics committee. For this study, normoglycemic rats with fasting blood glucose levels (FBGL) of 80 ± 5 mg/dl were selected.

4.2.8. Experimental design

To produce Streptozotocin-induced DM, animals received streptozotocin (STZ), 60 mg/kg, in 0.1 M sodium citrate buffer (pH 4.5). After a single dose of STZ, rats were provided with 5% glucose solution overnight to prevent drug-induced hypoglycemia. Blood was withdrawn from tail vein after 72 h of STZ injection to determine glucose level. Rats with FBGL over 250 mg/dl (>15 mmol/L) were considered diabetic. Forty-nine rats were divided into seven equal groups as follows:

1) Group I: received normal saline water intraperitoneally; Control group.
2) Group II: received intraperitoneal injection of STZ (60 mg/kg) only; diabetic group.
3) Group III: diabetic rats received glibenclamide (20 mg/kg) dissolved in water orally for 21 days once a day.
4) Group IV: diabetic rats received PCE (150 mg/kg dissolved in water) orally for 21 days.
5) Group V: diabetic rats received PCE (300 mg/kg dissolved in water) orally for 21 days.
6) Group VI: normal rats received PCE (150 mg/kg dissolved in water) orally for 21 days.
7) Group VII: normal rats received PCE (300 mg/kg dissolved in water) orally for 21 days

The dose and oral administration method of *P. candollei* extract was acquired based on significant therapeutic effects of plant extracts in a dose-dependent manner.

Fasting blood glucose level (FBGL) was measured by glucometer on the 14th day and 21st day of administration of the extract of *P. candollei*. The findings are presented in terms of mg/dl of blood (Ajiboye *et al*., 2020). Rats were killed by decapitation under chloroform anesthesia, and cardiac puncture allowed to collect blood and organ (kidney) was extracted from all the animals and stored at —20 ˚C and used for biochemical assay and molecular analysis.

4.2.8.1. Serum separation and analysis

The whole blood was kept at 4 ˚C for 40 min and then centrifuged for 15 min at 1500 ×*g* to separate serum. Serum creatinine, urea, uric acid, and alkaline phosphatase (ALP) were determined to assess indices of renal function, using Microlab 300 Semi Auto Biochemistry Analyzer (ELITechGroup, USA) in line with the manufacturer's guidelines.

4.2.8.2. Tissue homogenate preparation

The homogenization of the kidney was done with cold phosphate buffer (pH = 7.4) and kept at —4 ˚C for 20 min. A homogenized kidney was centrifuged at 1500 ×*g* for 30 min (Fatima and Khan, 2023). A clear solution was obtained and used to determine various oxidative stress biomarkers.

4.2.8.3. Determination of antioxidant enzymes

The activities of antioxidant enzymes including catalase, superoxide dismutase, peroxidase, and reduced glutathione were estimated (Chance and Maehly, 1955; Kakkar *et al*., 1984; Patterson and Lazarow, 1955). Characteristics reagents were added to the homogenate individually for each test. The activity of CAT and POD was determined by measuring the decrease in absorbance of the mixture at a rate of 0.01/min, while the activity of SOD and GSH was expressed as units per milligrams of protein and μM of GSH per gram of tissue, respectively.

4.2.8.4. Determination of biochemical indices

The levels of thiobarbituric acid, H_2O_2, and nitrites were assessed by using a standardized method (Grisham *et al*., 1996; Kakkar *et al*., 1997; Pick and Keisari, 1981). The absorbance of reaction mixtures was measured at 520 nm, 610 nm, and

540 nm, respectively. To quantify the concentration of H_2O_2 and nitrites, standard curves of phenol red and sodium nitrite were constructed. The levels of TBARs and nitrites in the sample solution were expressed as TBARs/min/mg protein and nitrites/min/mg protein, respectively.

4.2.8.5. Extraction of total RNA and cDNA conversion

100 mg of the kidney tissue was excised and homogenized with TRIzol buffer. 200 µl of chilled chloroform was added to the mixture and shaken. The aqueous layer was separated from the solution after centrifugation at 12,000 rpm/10 min. RNA from the separated layer was precipitated and washed with 75% ethanol. The pellet was dissolved in nuclease-free water (Ajiboye *et al*., 2019). To purify the RNA from DNA contamination, RNA was employed with DNAse I treatment. 2 µl solution containing 1000 ng DNA-free RNA was converted to cDNA using cDNA Reverse Transcriptase Kit in a thermal gene cycler (Biometra) following the guidelines of the manufacturer.

4.2.8.6. Real-time PCR (RT-qPCR) reaction

This was carried out using the 2 µl cDNA, 1 µl primer, 5 µl 2X SYBR Green/ROX qPCR Master Mix (Applied Biosystems), and 2 µl of nuclease-free water making a total volume of 10 µl reaction. The reaction mixture was subjected to the first denaturation step at 95 ˚C for 300s while amplification was performed by 40 cycles with following pro- gram: denaturation at 95 ˚C for 10 s, annealing at a specific temperature, and extension at 72 ˚C for 15 s. the amplification process was terminated by a final program at 72 ˚C for 60 s. In all experiments, beta-actin was employed as a reference gene. The primer sequences for each target gene are provided in Table 2.1 and 4.1. Relative quantification of genes was performed using the $2^{-\Delta\Delta Ct}$ method. The data obtained from the analysis were organized and processed using Microsoft Excel.

4.2.8.7. Histopathology of the kidney

The kidney samples from each rat group were collected and pre- served in 10% formalin. Afterward, the samples were rinsed overnight, dehydrated using various

concentrations of graded ethanol, and embedded in paraffin wax by following the standard procedure. The, 5 µm thick section was prepared from paraffin-embedded samples and these sections were stained with hematoxylin and eosin (H&E) for histological studies (Ajiboye *et al*., 2021). A light microscope (DIALUX-20 EB) at 40X magnification was employed to examine the prepared slides.

4.2.9. Statistical analysis

Graph prism 5.0 was used and all the results were expressed as a mean ± standard deviation (SD). And $p < 0.05$ was set to determine a significant difference by performing one-way ANOVA using Statistix 8.1 software.

Table 4.1: Primer sequences of target genes

Gene		**Primer sequence (5`-3`)**
TNF-α	F	GTCTGTGCCTCAGCCTCTTC
	R	GCCATGGAACTGATGAGAG
IL-6	F	GCCTGCAGAGAGATTCAATCA
	R	GTATCAGTGGGGGTCAGCAG
IL-1β	F	TTGAGTCTGCACAGTTCCCC
	R	TCCTGGGGAAGGCATTAGGA
TGF-β1	F	GCCTGCAGAGATTCAAGTCA
	R	GTATCAGTGGGGGTCAGCAG
EPO	F	AGGCGCGGAGATGGGGGTGC
	R	CCCCGGAGGAAGTTGGAGTAG
Caspase 6	F	CGGAGGCCTCAGGACGA
	R	TGGATCCAACACTTCCCTACTTC
KIM1	F	AAGCCGAGCAAACATTAGTGC
	R	TGAGCTAGAATTCAGCCACACA
Actin, beta (β-actin)	F	CCTCTATGCCAACACAGTGC
	R	CATCGTACTCCTGCTTGCTG

4.3. Results

4.3.1. Qualitative analysis of bioactive phytochemicals

Various bioactive phytochemicals were detected in *P. candollei* ethanol extract (PCE) including alkaloids, glycosides, flavonoids, ter- penoids, tannins, phenols, saponins, anthocyanins, Vitamin C, and Oils (Table 4.2). More intense results were seen for terpenoids, followed by alkaloids, steroids, tannins, glycosides, and saponins. However, resins and coumarins were not found in the sample extract.

4.3.2. HPLC-DAD quantitative identification of bioactive compounds

The chromatograms of HPLC-DAD analysis of ethanol extract of *P. candollei* are illustrated in Figure 4.1, while the quantification and retention time is presented in Table 3. Cinnamic acid (26.49 µg/mg), rutin (20.16 µg/mg) and gallic acid (15.96 µg/mg) were separated as major polyphenolics compound found in *P. candollei* ethanol extract at wavelength of 325 nm and 257 nm. A significant amount of gentisic acid, ferulic acid, kaempferol, ellagic acid, quercetin, and vanillic acid was also present in the sample. However, a minor quantity of syringic acid, emodin, and caffeic acid was also found in extract as depicted in Table 4.3.

4.3.3. Quantification of phytochemicals in *P. candollei* extract

The quantitative estimation of bioactive phytochemicals in *P. candollei* ethanol extract indicated the presence of significant quantities of phenols and flavonoids. The phenol content was found to be 36.38 ± 1.17 mg RE, while the flavonoid content was 44.38 ± 1.75 GAE/g. These results highlighted the rich presence of these bioactive compounds in the examined extract.

4.3.4. In vitro antioxidant activity

The antioxidant activity of *P. candollei* ethanol extract was examined by two methods including DPPH and metal chelating assay. The findings revealed that extract has high inhibition (%) activity against free radicals as DPPH assay depicted maximum percentage scavenging activity 59.89 ± 1.05 of plant extract at 1000 µg/ml while ascorbic acid revealed percentage inhibition of 74.29 ± 1.44. Similarly, it was observed that the chelating ability of the plant sample increased ($P < 0.05$) as

a function of concentration. *P. candollei* showed maximum chelating ability 61.68 ± 0.93 at 1000 µg/ml while EDTA showed 77.80 ± 2.01 at the same concentration. The percentage inhibition activity of plant extract at maximum concentration and IC_{50} values are presented in Table 4.4.

4.3.5. In vitro anti-diabetic activity

The enzyme inhibition potential of *P. candollei* extract was determined for its ability to inhibit α-glucosidase. The extracts showed significant ($P < 0.05$) inhibition potency against α-glucosidase (IC_{50} 49.12 ± 1.31 µg/ml) which is found more potent than standard acarbose drug (IC_{50} 765.13 ± 3.74 µg/ml).

Table 4.2: Qualitative phytochemical identification of *P. candollei* ethanol extract

Phytochemicals	***P. candollei* ethanol extract**
Alkaloids	+
Glycosides	+
Flavonoids	+
Terpenoids	+
Tannins	+
Phenols	+
Saponins	+
Anthocyanins	+
Coumarins	-
Vitamin C	+
Oils	+
Resins	-

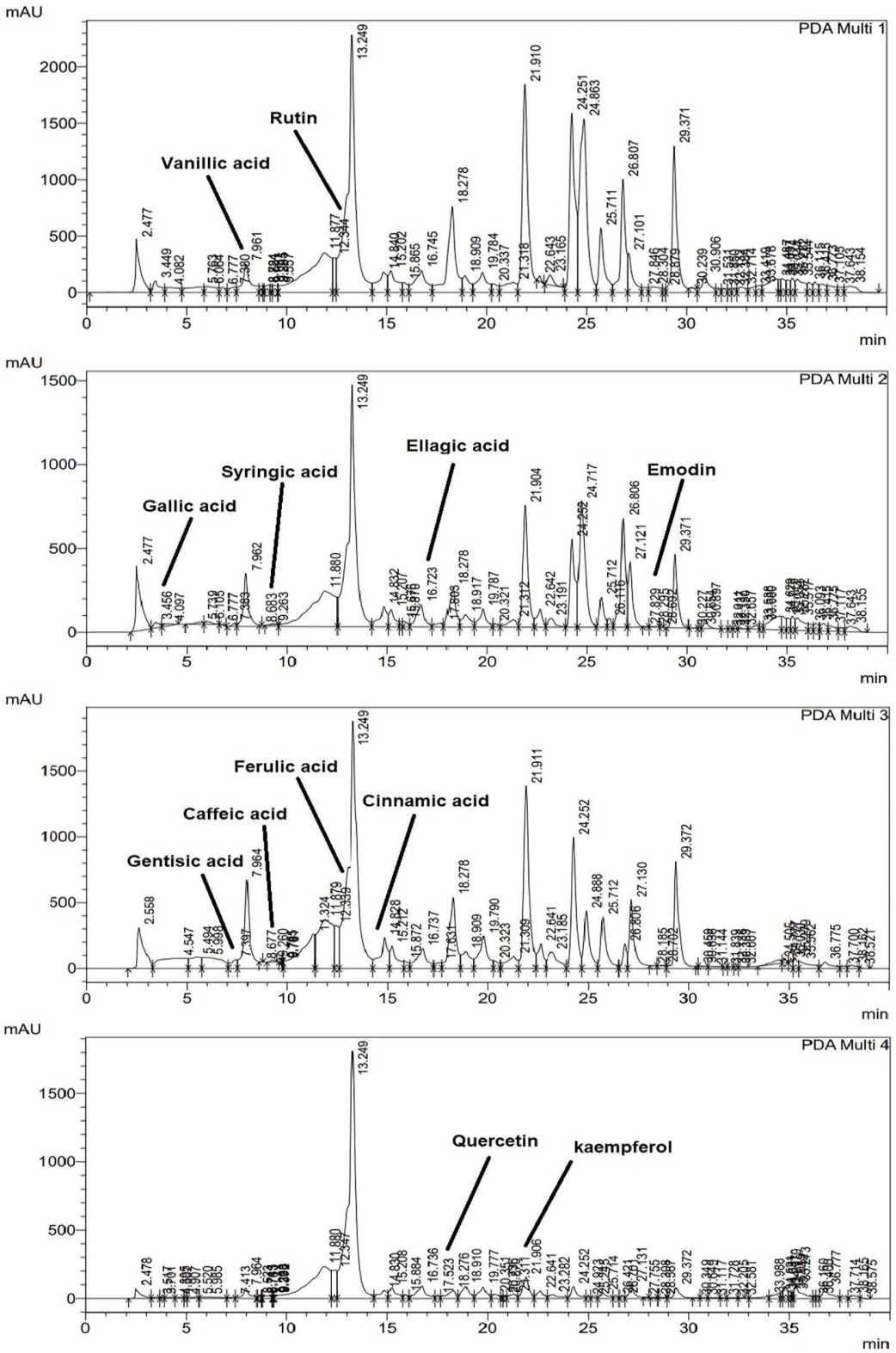

Figure 4.1: HPLC-DAD chromatograms of *P. candollei* ethanol extract at wavelengths of 257 nm, 279 nm, 325 nm, and 368 nm.

Table 4.3: Wavelength, retention time and quantification of phenolics and flavonoids for *P. candollei* ethanol extract

Polyphenolics	Wavelength (nm)	Retention time (min)	Quantity (µg/mg extract)
Vanillic acid	257	8.796	2.88
Rutin	257	12.122	20.16
Gallic acid	279	3.627	15.96
Catechin	279	6.427	Not detected
Syringic acid	279	8.673	0.06
Coumaric acid	279	13.725	Not detected
Ellagic acid	279	15.372	2.89
Emodin	279	27.901	1.24
Gentisic acid	325	7.184	8.21
Caffeic acid	325	8.665	0.32
Ferulic acid	325	12.257	6.66
Cinnamic acid	325	12.459	26.49
Apigenin	325	20.768	Not detected
Myricetin	368	14.391	Not detected
Quercetin	368	17.4	2.61
Kaempferol	368	20.142	5.66

Table 4.4: Antioxidant activity of *P. candollei* ethanol extract

	DPPH (% Inhibition)	Metal chelation (% Inhibition)	DPPH (IC_{50} (µg/ml)	Metal chelation IC_{50} (µg/ml)
P. candollei ethanol extract	59.89 ± 1.05^{b}	61.68 ± 0.93^{b}	390.1 ± 3.41^{a}	69.12 ± 1.37^{a}
Ascorbic acid	74.29 ± 1.44^{a}		83.12 ± 2.58^{b}	
EDTA		77.80 ± 2.01^{a}		50.55 ± 0.86^{b}

[a] significant difference vs control group; [b] Significant difference vs diabetic group at $p < 0.05$

4.3.6. Body weight analysis

There was a significant ($P < 0.05$) decline in body weight of diabetic group rats in comparison to control. However, after *P. candollei* extract administration at 150 mg/kg, the body weight restored notably but not to the control level (Table 4.5). In contrast, administration of extract at 300 mg/kg restored the body weight to the control level.

4.3.7. Fasting blood glucose level analysis

Before starting the experiment, FBGL of all experimental rats were within the normal range (Table 4.6). However, after 24 h of streptozotocin injection, the glucose levels showed a significant upregulation in com- parison to normal level. After administration of ethanol extract of *P. candollei*, blood glucose level was significantly different from diabetic group rats while insignificant difference when compared to control level (Table 4.6).

Table 4.5: Percentage increase in body weight of diabetic rats

	Initial body weight (g)	Final body weight (g)	Increase in body weight (%)
Control (Normal)	138 ± 1.8	204 ± 3.1	47.83 ± 3.37[b]
Diabetic rats; STZ (60 mg/kg)	140 ± 2.0	162 ± 2.2	15.71 ± 2.95[a]
STZ + Glibenclamide (20 mg/kg)	140 ± 1.5	190 ± 2.7	35.71 ± 3.46[a,b]
STZ + PCE (150 mg/kg)	137 ± 1.1	175 ± 1.9	27.73 ± 3.07[a,b]
STZ + PCE (300 mg/kg)	142 ± 2.1	188 ± 1.5	32.39 ± 4.12[a,b]
PCE (150 mg/kg)	141 ± 1.3	215 ± 1.7	52.48 ± 2.84[b]
PCE (300 mg/kg)	139 ± 1.8	216 ± 2.4	55.39 ± 4.28[b]

STZ: Streptozotocin; PCE: *P. candollei* ethanol extract; [a] significant difference vs control group; [b] Significant difference vs diabetic group at $p < 0.05$.

Table 4.6: Fasting blood glucose level (FBGL) (mg/dl) of treated groups

	Initial FBGL (0 day)	FBGL (after 24 h of injection)	FBGL (14th day)	FBGL (21st day)
Control (Normal)	82.3 ± 4.1	83.5 ± 5.2[b]	84.5 ± 4.7[b]	84.0 ± 3.7
Diabetic rats; STZ (60 mg/kg)	80.4 ± 4.7	328.2 ± 4.3[a]	323.9 ± 5.1[a]	318.4 ± 5.5[a]
STZ + Glibenclamide (20 mg/kg)	85.7 ± 3.9	320.5 ± 4.9[a]	93.3 ± 4.6[a,b]	87.6 ± 4.1[b]
STZ + PCE (150 mg/kg)	83.1 ± 5.0	316.8 ± 4.5[a]	96.1 ± 3.2[a,b]	90.3 ± 3.8[a,b]
STZ + PCE (300 mg/kg)	78.7 ± 3.8	310.5 ± 6.1[a]	84.7 ± 4.7[b]	81.6 ± 4.6[b]
PCE (150 mg/kg)	79.6 ± 4.3	82.3 ± 3.8[b]	81.7 ± 2.8[b]	81.5 ± 3.5[b]
PCE (300 mg/kg)	80.8 ± 2.7	79.4 ± 4.0[b]	82.2 ± 3.1[b]	80.1 ± 3.6[b]

Values expressed as mean ± S.D., n = 7 rats; PCE: *P. candollei* ethanol extract; [a] significant difference vs control group; [b] Significant difference vs diabetic group at $p < 0.05$.

4.3.8. Serum marker analysis

Rats administered with ethanol extract of *P. candollei* (150 and 300 mg/kg) alone, displayed no notable ($p > 0.05$) difference in urea, creatinine, uric acid, cholesterol, and alkaline phosphatase (ALP) level once compared with the normal control group but showed the significant ($p < 0.05$) difference when compared to diabetic rats. However, diabetic rats administered with extract showed significant ($p < 0.05$) restoration especially at (300 mg/kg) once matched to normal and diabetic control groups (Table 4.7).

4.3.9. Biochemical activities of stress marker in kidney

The biochemical examination of activities of CAT and SOD in kidney revealed that diabetic group rats administered with 150 mg/kg of extract exhibited non-significant ($p > 0.05$) difference once compared to diabetic rats. Whereas diabetic rats supplemented with on 300 mg/kg of extract revealed significant ($p < 0.05$) restoration to the normal control rats, thus, showed non-significant ($p > 0.05$) difference when compared to diabetic rats (Figure 4.2). There was a substantial difference found in activities POD and GSH of diabetic rats after supplementation with ethanol extract of *P. candollei* (150 and 300 mg/kg) when compared to diabetic control rats and showed significant ($p < 0.05$) restoration to normal levels (Table 7). However, administration of extract to normal rats showed levels of enzymatic activity as normal rats.

4.3.10. Biochemical indices of kidney

The diabetic rats supplemented with ethanol extract of *P. candollei* exhibited significantly ($P < 0.05$) low levels of free radicals including TBARs, H_2O_2, and nitrites when compared to normal rats, while administration of glibenclamide (20 mg/kg) also indicated restoration of these markers towards normal rats hence notable difference was found once matched to diabetic rats. The diabetic rats administered with high dose (300 mg/kg) of extract showed no substantial difference when compared to normal levels while a significant decline in free radicals was found in comparison to diabetic rats. How- ever, low dose (150 mg/kg) of extract showed non-significant result in comparison to normal rats while

a momentous change in values of biochemical indices was found once compared to diabetic rats (Table 4.8).

Table 4.7: Levels of renal serum markers of STZ-induced diabetic rats

	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)	Cholesterol (mg/dl)	ALP (U/I)
Control (Normal)	4.97 ± 0.51[b]	0.68 ± 0.01[b]	1.82 ± 0.33[b]	80.01 ± 2.98[b]	36.10 ± 3.01[b]
Diabetic rats; STZ (60 mg/kg)	15.62 ± 1.04[a]	5.82 ± 0.28[a]	5.41 ± 1.21[a]	276.27 ± 3.92[a]	115.67 ± 4.94[a]
STZ + Glibenclamide (20 mg/kg)	5.28 ± 0.83[b]	1.28 ± 0.82[a,b]	2.26 ± 1.06[a,b]	148.26 ± 1.99[a,b]	40.89 ± 2.98[b]
STZ + PCE (150 mg/kg)	9.15 ± 1.26[a,b]	2.73 ± 0.64[a,b]	3.94 ± 1.13[a,b]	201.63 ± 2.85[a,b]	68.83 ± 2.77[a,b]
STZ + PCE (300 mg/kg)	5.04 ± 0.72[b]	0.97 ± 0.39[a,b]	2.04 ± 0.83[b]	127.18 ± 2.18[b]	39.51 ± 3.05[b]
PCE (150 mg/kg)	4.91 ± 0.38[b]	0.74 ± 0.08[b]	1.94 ± 0.48[b]	78.18 ± 1.62[b]	38.91 ± 2.94[b]
PCE (300 mg/kg)	4.69 ± 0.63[b]	0.69 ± 0.07[b]	1.87 ± 0.26[b]	70.18 ± 1.41[b]	36.03 ± 2.47[b]

Values expressed as mean ± S.D., n = 7 rats; ALP: Alkaline phosphatase PCE: *P. candollei* ethanol extract; [a] significant difference vs control group; [b] Significant difference vs diabetic group at $p < 0.05$.

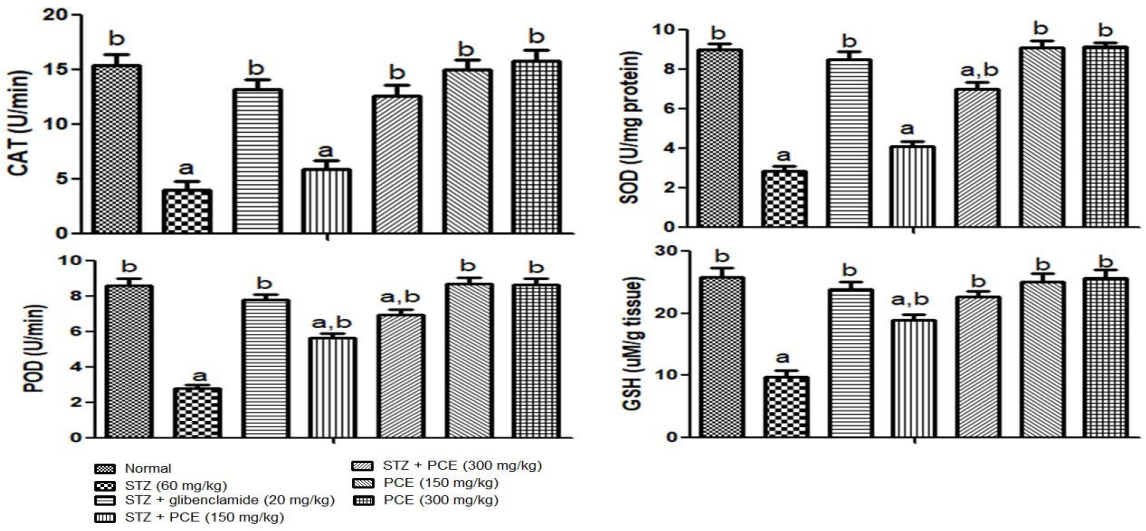

Figure 4.2: Antioxidant enzyme activity in kidney of diabetic rats treated with *P. candollei* ethanol extract; [a] significant difference vs control group; [b] Significant difference vs diabetic group at $p < 0.05$.

Table 4.8: Biochemical indices of kidney in diabetic rats

	TBARS (nM/min/mg protein)	H_2O_2 (nM/min/mg tissue)	Nitrite (µM/mg protein)
Control (Normal)	42.34 ± 2.59^{b}	4.93 ± 0.94^{b}	22.75 ± 1.35^{b}
Diabetic rats; STZ (60 mg/kg)	101.45 ± 3.82^{a}	11.28 ± 1.26^{a}	42.37 ± 2.48^{a}
STZ + Glibenclamide (20 mg/kg)	$53.71 \pm 2.67^{a,b}$	5.19 ± 0.83^{b}	24.17 ± 2.11^{b}
STZ + PCE (150 mg/kg)	$89.35 \pm 3.74^{a,b}$	$7.83 \pm 0.97^{a,b}$	$32.25 \pm 1.53^{a,b}$
STZ + PCE (300 mg/kg)	$50.05 \pm 4.01^{a,b}$	5.13 ± 0.28^{b}	23.62 ± 1.19^{b}
PCE (150 mg/kg)	44.19 ± 1.93^{b}	4.83 ± 0.77^{b}	21.72 ± 0.93^{b}
PCE (300 mg/kg)	44.38 ± 2.28^{b}	4.88 ± 0.63^{b}	$^{22.04}$ 2.03^{b}

Values expressed as mean ± S.D., n = 7 rats; PCE: *P. candollei* ethanol extract; [a] Significant difference vs control group; [b] Significant difference vs diabetic group at $p < 0.05$.

4.3.11. mRNA expression of genetic markers

Figure 4.3 illustrates the mRNA expressions of KIM-1, IL-1β, TNF-α, IL-6, TGF-β1, EPO, and Caspase 6 genes in the kidney of various group rats. IL- 1β, TNF-α, IL-6, and TGF-β1 expression levels in diabetic rats administered with ethanol extract of *P. candollei* (300 mg/kg) presented significant ($p < 0.05$) reduction when compared to diabetic group while insignificant ($p > 0.05$) results were found when compared to normal rats. Administration of glibenclamide also expressed restoration of IL-1β and TGF-β1 expression to normal levels.

The upregulation ($p < 0.05$) in expression levels of KIM-1 and Caspase 6 were found in diabetic rats administered *P. candollei* when linked to diabetic control, while there was no significant difference when compared to normal rats. Although, diabetic rats received glibenclamide (20 mg/kg) showed notable ($p < 0.05$) decrease when linked with diabetic rats.

The mRNA expression level of EPO demonstrated that diabetic rats received *P. candollei* extract (300 mg/kg) experienced elevation ($p > 0.05$) as compared to diabetic group while no difference was found in when compared to normal rats, while administration of *P. candollei* at 150 mg/kg revealed insignificant ($p > 0.05$) difference relative to diabetic rats. Similarly, administration of *P. candollei* extract to diabetic rats downregulated the expression level of KIM- when compared to the diabetic group, while no difference was found when compared to control group.

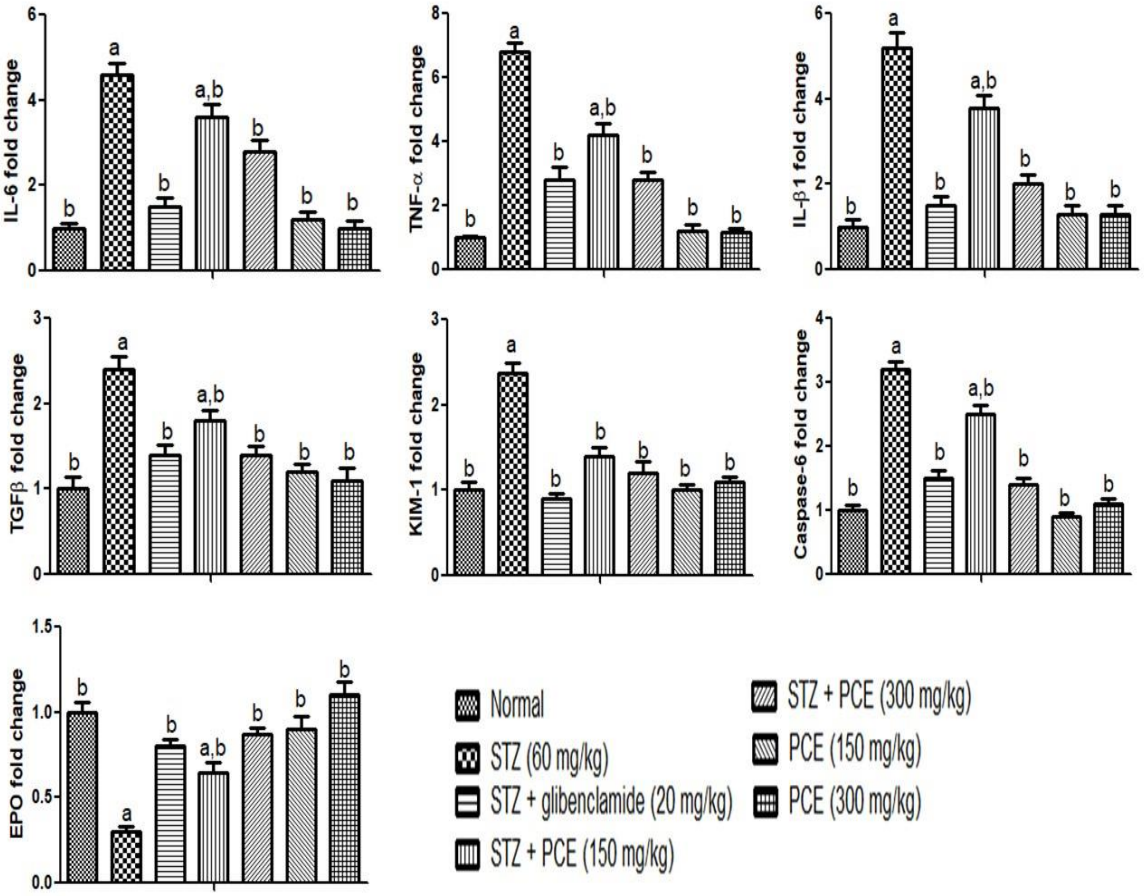

Figure 4.3: Graphical representation of mRNA expression of different genes involved in diabetic nephropathy. [a] significant difference vs control group; [b] Significant difference vs diabetic group at $p < 0.05$.

4.3.12. Histological studies

Figure 4.4 illustrates histological changes in the kidney of various groups of rats. The diabetic rats received 150 mg/kg of ethanol extract of *P. candollei* showed moderate degradation and normal necrosis while diabetic rats administered with 300 mg/kg of *P. candollei* and 20 mg/kg of glibenclamide showed mild degradation and necrosis in comparison to diabetic group. Furthermore, diabetic rats that received *P. candollei* displayed normal hemorrhage whereas diabetic rats administered glibenclamide showed mild hemorrhage when linked diabetic control. Also, the diabetic rat group administered with ethanol extract of *P. candollei* exhibited normal congestion. In contrast, diabetic rats placed on glibenclamide (20 mg/kg) showed moderate congestion relative to diabetic control group. While diabetic rats administered low and high doses of *P. candollei* and glibenclamide illustrated normal inflammation as compared to diabetic control group.

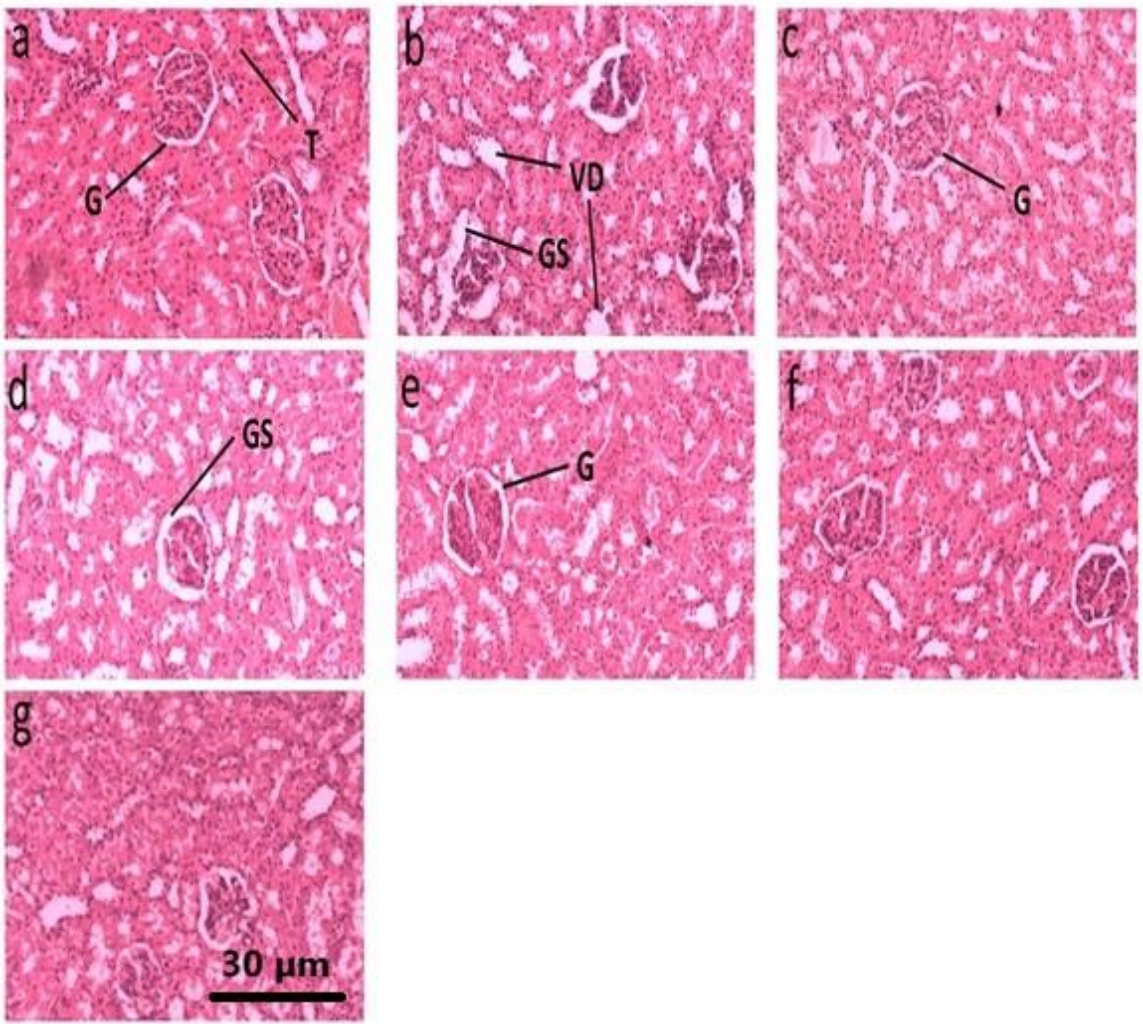

Figure 4.4: Histological representation of renal alterations in various treated group; (a) Control; (b) Diabetic group; (c) STZ + glibenclamide (20 mg/kg) (d) STZ + PCE (150 mg/kg); (e) STZ + PCE (300 mg/kg); (f) PCE (150 mg/kg); (g) PCE (300 mg/kg), VD: Vacuolar degeneration; GS: Glomerular space; G: Glomeruli.

4.4. Discussion

Diabetes mellitus causes chronic hyperglycemia and leads to various symptoms, including excessive increased hunger (polyphagia), thirst (polydipsia), frequent urination (polyuria), and sudden weight loss, etc (Mukhtar *et al*., 2020). Hyperglycemia leads to oxidative damage, thus causing diabetes-related diseases, particularly diabetic nephropathy which is considered the primary cause of end-stage renal failure in diabetic patients (Ajiboye *et al*., 2021). Hyperglycemia initiates various processes in the biological system including the higher production of AGEs, activation of polyol pathway, oxidative stress, and increased hexosamine flux. These factors collectively cause inflammation and damage to kidney tissues (Wolf and Ziyadeh, 2007). Subsequently, AGEs enhance the production of ECM proteins in the macrophages, mesangial cells, and endothelial cells of the kidney (Yonekura *et al*., 2005). In experimental animal models of diabetes, mesangial alterations such as damage to mesangial cells and the extracellular matrix of glomerulus were found to play a significant role in the decline of renal function, regardless of other structural and functional changes (Mason and Wahab, 2003). Antioxidants, such as flavonoids, can help prevent the accumulation of AGEs (Musabayane, 2012). Bioactive compounds derived from natural sources, especially from various plants, continue to be a subject of active research due to their high potential in providing new therapeutic strategies for various chronic diseases. The present study examines the anti-diabetic potential of *P. candollei* ethanol extract in a rat model of STZ-induced diabetes. Bioactive compounds of medicinal plants exhibit medicinal properties by scavenging free radicals and thus alleviating the pathologies associated with oxidative (Gupta *et al*., 2020). Synthetic antioxidants are effective, but they are toxic and carcinogenic to biological systems (Felter *et al*., 2021). Chemical analysis of *P. candollei* ethanol extract exhibited the presence of bioactive phytochemicals including alkaloids, saponins, steroids, flavonoids, tannins, carbohydrates and phenolics. Furthermore, HPLC studies confirmed that the presence of phenolics and flavonoids, namely vanillic acid, rutin, gallic acid, ellagic acid, caffeic acid, emodin, syringic acid, cinnamic acid, kaempferol, and quercetin were present in PCE. In the current study, it was

observed that *P. candollei* extract exhibited significant total phenolic content, while PCE extract was also rich in flavonoid content. The bioactive phytochemicals found in natural plants such as phenolics and flavonoids have been documented to possess various therapeutic properties such as antioxidant, antidiabetic, hepatoprotective and anti-inflammatory activities (Fatima and Khan, 2023; Gülcin, 2012).

To assess the antioxidant activity of *P. candollei* ethanol extract, the electron donation ability of the extract was determined by evaluating its scavenging potential against DPPH free radicals. The examined extract revealed concentration-dependent discoloration of the test solution in the DPPH assay, indicating its significant scavenging capacity against free radicals at higher concentrations. These results indicated that plant extract possesses the ability to donate electrons resulting in its antioxidant properties (Kumar and Pandey, 2015). The extract also revealed a notable ability to convert Fe^{3+} to Fe^{2+}, donating its capacity to donate hydrogen, which is crucial in breaking the free radical chain, a highlighted antioxidant property (Pandey *et al*., 2012). The higher DPPH radical scavenging activity and metal chelation property of plant extract may be attributed to its higher con- tent of phenolics and flavonoids (Kumar *et al*., 2012b). The polyphenols have shown enzyme inhibition ability against α-glucosidase (Kang *et al*., 2014; KWON *et al*., 2008). In previous studies, Flavonoids, which are known as a major group of polyphenols, have been reported to exhibit inhibitory effects against α-glucosidase (Tadera *et al*., 2006; Williamson, 2013). The present study showed the presence of a larger quantities of polyphenolics in *P. candollei* ethanol extract. Hence, the presence of these phenolic compounds and flavonoids in the PCE extract might be associated with the inhibition activity of the extract against α-glucosidase. In a previous study, the phytochemical profile, and various biological activities of this vegetable *P. candollei*, including antioxidant, antibacterial, thrombolytic, and enzyme inhibitory properties against tyrosinase and α-glucosidase were evaluated. The results demonstrated that the methanol and n-hexane extract of this plant have remarkable pharmacological activities

including enzyme inhibitory properties against α-glucosidase as similarly reported for *Pleurospermum benthamii* (Hassan *et al*., 2022; Khan *et al*., 2018)

Since the significant in vitro α-glucosidase inhibition action displayed by the plant extract, the objective of this study was to evaluate the potential effects of PCE extract to protect kidney tissues by deter- mining the key regulatory markers associated with hyperglycemia-mediated oxidative stress, pro-inflammatory cytokines, apoptotic markers, and histological alterations in STZ-induced diabetic rat models. Streptozotocin, is a compound known for causing DNA alkylation in the pancreas, leads to damage to β-cells, resulting in insulin deficiency and chronic hyperglycemia (Lenzen, 2008). Moreover, streptozotocin has been found to enhance the production of ROS such as nitric oxides, superoxide an- ions, hydroxyl radicals, and H_2O_2 (OKAWA and DOI, 1983), which are responsible for its deleterious effects in pancreas, liver, and kidney (Koenig, 1980). Diabetic rats revealed an increase in glucose, creatinine, urea, and uric acid which are primary indicators of diabetic nephropathy (Al-Awaida *et al*., 2020). During metabolism of protein, urea is released as a primary product. During diabetes, the process of protein glycation causes muscle thinning thereby provoking the release of purine. Uric acid is produced by degradation of purines through xanthine oxidase (Sivakumar *et al*., 2010). Creatinine is produced as a byproduct when creatine and phosphocreatine break- down on the muscles. Creatinine is naturally produced by the body and released into body fluids while its clearance is measured to determine the glomerulus filtration rate. The administration of PCE extract, the levels of blood urea, creatinine, and uric acid were reduced in rat models with STZ-induced diabetes. These findings indicated that *P. candollei* has protective effects on renal tissues. The antioxidant potential of *P. candollei* extract may be responsible for alleviating the oxidative stress caused by streptozotocin in the kidneys, thereby preventing further distortion of β-cells. Previous studies have demonstrated the effectiveness of D-pinitol, extracted from plant species, in controlling pathologies of diabetes (Sivakumar *et al*., 2010).

Previous studies have reported that alkaline phosphatase (ALP) levels are measured to identify kidney damage in diabetic patients (Vennela, 2023). The level

of enzyme activity is used to confirm the toxic effects of ingested compounds. ALP belongs to the membrane-bound enzyme family and is predominantly present in the kidney. The level of ALP increases when the cell membrane experiences permeability or ruptures completely. Hence, an increase in serum ALP levels serves as an indication of kidney cell damage due to the accumulation of free radicals and oxidative stress (Adesokan *et al*., 2009). The administration of PCE extract normalized this toxic effect of streptozotocin in diabetic rats and restored the levels of ALP to normal. Similar results were shown by *Sterculia tragacantha* aqueous extract (Ajiboye *et al*., 2021).

Enzymatic antioxidants such as CAT, SOD, POD, and GSH play a crucial role in protecting the cells from oxidative damage by regulating the levels of ROS within a normal physiological range (Pari and Latha, 2005). In our cur- rent study, impaired ion metabolism of trace elements was observed in the kidneys of diabetic rat models. GSH is a hydrophilic antioxidant that serves as an initial defense against oxidative stress (Meister, 1988). It acts as a direct radical scavenger as well as a co-substrate of GPx thereby functioning as a primary intracellular redox system. However, the levels of both enzymatic and non-enzymatic antioxidants were reduced in STZ-intoxicated diabetic rats. In our study, the administration of PCE extract to the diabetic group helped to recover the levels of these enzymatic antioxidants to the normal level as shown by Alnus nitida extract in previous study (Sajid *et al*., 2020). Chronic diabetes leads to higher production of TBARS, H_2O_2, and nitric oxide. These reactive species react with superoxide anion, resulting in the formation of peroxynitrite which further elevates the process of lipid peroxidation (Davi *et al*., 2005). The oral administration of PCE extract reduces the generation of lipid peroxides, H_2O_2 and nitrites in the diabetic rats, hence rendering the cells more protected against lipid peroxidation and peroxidative stress. These results illustrated the ability of extract to scavenge free radicals which explains its ability to act as antidiabetic agent as previously reported by *Alnus nitida* extract (Sajid *et al*., 2020).

Irreversible production of AGEs results from a reaction between sugars and free amino groups on proteins, lipids, and nuclei acids during chronic hyperglycemic

condition. Furthermore, AGEs activate several transcription factors including NF-κB which is involved in the pathogenesis of diabetic nephropathy (Forbes *et al*., 2003). In diabetic condition, activation of NF-κB pathway initiates a series of signaling pathways that ultimately contribute to renal disorder which is directly linked to enhanced levels of oxidative-nitrosative stress and inflammation. Furthermore, NF-κB induces the formation of nitric oxides which is a major contributor of diabetic nephropathy, especially, kidney tubular injury and cellular destruction (McLay *et al*., 1994). In hyperglycemic-mediated oxidative stress state, activation of NF-κB pathway directly triggers an increase of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6, identifying significant role in diabetic renal dysfunction (Ho and Bray, 1999).

TNF-α is produced by various cell types including glomerular, tubular, mesangial, dendritic, and renal tubular cells (Gülcin, 212) and cytotoxicity effects induced by TNF-α involve several mechanisms, including excessive generation of ROS, which subsequently cause damage to cellular components such as DNA, lipids, and proteins. The enhanced levels of TNF-α and TGF-β along with other inflammatory cytokines like IL-1β and IL-6 has been identified to develop and promote inflammation in diabetic renal dysfunction. Furthermore, IL-1β stimulates the production of prostaglandins and nitric oxides in mesangial cells (Kumar and Pandey, 2015). IL-6 promotes endothelial permeability, cell proliferation, and fibronectin expression in mesangial cells (Pandey *et al*., 2012). The increased expression levels of IL-6 and TNF-α are linked to a disturbance in glycemic control, increased insulin resistance, and dyslipidemia, which collectively contribute to the impairment of the metabolic status of diabetic patients (Kumar *et al*., 2012b). However, diabetic rats administered PCE extract exhibited a decline in the levels of inflammatory cytokines suggesting that plant extract may have anti-inflammatory activities against pro-inflammatory cytokines.

Metalloproteinase induces cleavage of KIM-1 which is associated with the shedding of its ectodomain and hence induces the over- expression of KIM-1 in the proximal tubules which is a sign of renal dysfunction in diabetic rats (Ahmed and Hamed, 2015). However, diabetic rats administered PCE extract showed a

reduction in mRNA expression of KIM-1, which approves the anti-hyperglycemic and anti-anemic properties of the extract. In contrast, the anemic state during diabetic nephropathy conditions might be associated with downregulated expression levels of EPO in diabetic control (Ibrahim *et al*., 2016). A decrease in EPO production is caused by tubulointerstitial damage which leads to the development of anemia (Ogata *et al*., 2023). Chronic hyperglycemia in diabetic control rats enhanced the expression of caspase 6 which modulates various apoptotic markers and contributes to improving inflammation (Galluzzi *et al*., 2016). In contrast, diabetic rats administered PCE extract showed a reduction in Caspase 6 expression level, these findings support the antioxidant potency of *Sterculia tragacantha* aqueous extract as shown by a previous study (Ajiboye *et al*., 2021).

Histological studies of diabetic rats demonstrated inflammation, necrosis, severe degradation, hemorrhage, and congestion indicating significant nephrotic alterations in structure. These alterations included interstitial infiltration of lymphocyte, glomerular hypertrophy, damage to collecting ducts as well as ascending and descending limbs (Zafar *et al*., 2009). However, these detrimental morphological alterations were mitigated by PCE extract administration to diabetic control. Similar results were reported by *Hibiscus sabdariffa* in a previous study (Ukoha *et al*., 2015). The ameliorative nature of *P. candollei* ethanol extract against anti-diabetic renal dysfunction may be due to the presence of flavonoids (kempferol) and phenols (gallic acid) as discussed by a previous study (Sadeer *et al*., 2019).

4.5. Conclusions

The in vitro and in vivo investigations in the current study showed that administration of PCE decreased the blood glucose level, ameliorated the oxidative stress by normalizing antioxidant status of kidney, normalized the serum biomarkers level along with restoration of inflammatory and renal molecular markers in STZ-diabetic rats. In short, PCE exhibits renal protective, antihyperglycemic, and anti-glycation properties. However, further research is required to assess the mechanism of action of PCE at clinical level.

4.6. Future aspects

Preparation of extracts and formulations of *P. candollei* with optimized bioavailability and stability could improve its therapeutic properties and facilitate clinical trials. Pharmacokinetic studies investigating the distribution, absorption, interaction with dietary and lifestyle fac- tors, metabolism and excretion of active compounds could improve formulation strategies for enhanced efficacy. Furthermore, clinical trials assessing the efficacy of *P. candollei* as therapeutic agent for diabetic nephropathy could provide insight into its safety and effectiveness in human systems.

5. *Protective effects of Jasminum humile* extract against CCl_4-induced renal toxicity by inhibiting fibrosis, inflammation, apoptosis markers

5.1. Introduction

Carbon tetrachloride is a non-polar colorless solvent widely used as a degreasing agent (Ebaid *et al*., 2021). CCl_4 promotes ROS production and creates a deficit of antioxidant enzymes and substrates, giving rise to redox imbalance in several body tissues (Halliwell, 2007b). These species react with polyunsaturated fatty acids (PUFAs) present in cell membranes, forming peroxides. The resulting lipid peroxides triggers an inflammatory response in the tissues. The release of inflammatory mediators such as IL-6 and TNF-α are observed in CCl_4-induced toxicity which is involved in initiating inflammation (Hirschfield *et al.*, 2018). Furthermore, CCl_4 is also known to increase the levels of TGF-β and Smad-3, thus causing fibrosis (Ma *et al.*, 2020). Organs such as the liver and kidneys are most susceptible to oxidative damage induced by free radicals. Different pathological conditions including acute and chronic renal disorders that resulted from cell membrane deterioration, caused by free radical-induced lipid peroxidation (Honda *et al.*, 2019). CCl_4 generated free radicals can lead to nephrotoxicity in addition to hepatic injuries in rats (Almundarij *et al.*, 2021; Lin *et al.*, 2008). The pathogenesis of CCl_4-induced kidney toxicity is heavily influenced by oxidative stress, inflammation, fibrosis, and apoptosis (Safhi, 2018; Hassan *et al*., 2020). Acute exposure to CCl_4 causes abnormal histological hallmarks such as cirrhosis, inflammatory leukocyte infiltration, fibrosis, necrosis, and even contributes to cancer progression (Qiu *et al*., 2005). CCl_4 can also cause blood diseases as well as liver, kidney, and testicular injuries (Balahoroğlu *et al*., 2008; Benjamin and Schneider, 2005; Wang *et al.*, 2023).

Apoptosis plays a vital role in various processes including tissue development and maintenance, and normal cells turnover without causing any damage to surrounding tissues. The process of apoptosis involves a series of molecular events that lead to characteristic changes in cell morphology such as DNA fragmentation, cell shrinkage, degradation of mRNA and ultimately result in cell death. One of the key mechanisms in apoptosis involves activation of caspase 9 (initiator caspase) which further activates caspase 3 (effector caspase). CCl_4 induces apoptosis in cells as activity of caspase 9 and caspase 3 increases in response to CCl_4 injections. Additionally, CCl_4 treatment affects the

balance of pro-apoptotic and anti-apoptotic proteins by promoting the expression of Bax and inhibiting the expression of BCl-2, respectively. Subsequently, triggered apoptotic process can lead to tissue damage and organ dysfunction (Safhi, 2018).

Living organisms possess enzyme-catalyzed and non-enzymatic antioxidant systems in their bodies that help in the prevention of damage caused by ROS (Chaudière and Ferrari-Iliou, 1999). These systems not only scavenge and detoxify ROS but also inhibit their synthesis by sequestering transition metals that produce free radicals. Exposure to CCl_4 changes antioxidant status in rats thus leading to nephropathies. Apart from naturally occurring antioxidants, other synthetic ROS scavengers have been predicted to lower the prevalence of diseases caused by free radicals. Antioxidants combat diseases by elevating endogenic levels of antioxidants and diminishing the phenomenon of lipid peroxidation. Such antioxidant properties can be utilized in developing non-toxic medicines targeted for degenerative diseases (Khan *et al.*, 2009).

All plants and vegetables contain a high number of antioxidants, mainly phenols, glycosides, tannins, flavonoids, and vitamin C, which can reduce the potential damage of free radicals in body (Afolayan and Jimoh, 2009). The free radical detoxification capacity of naturally occurring antioxidants depends upon their type and disease. Scientists are showing keen interest in using effective antioxidant components from natural plant products as a substitute for eliminating ROS. Owing to their natural origin and potent scavenging properties, phenolic compounds are particularly significant (Sahreen *et al.*, 2010). Previous research has demonstrated the scavenging propensity of polyphenols and flavonoids in both *in vitro* and *in vivo* analyses (Fatima and Khan, 2024).

J. humile (Italian Jasmine) belongs to the family Oleaceae, commonly found in the Himalayan region. Jasminum is a medicinally important genus as many species are used to treat mouth blisters and ringworms (Akhtar *et al.*, 2021). The essential oils (EOs) extracted from *Jasminum* species were employed in conventional aromatherapy for treating pyrexia, diarrhea, abdominal spasms, bronchial asthma, conjunctivitis, inflammatory skin conditions, and uterine hemorrhage (El-Baz *et al.*, 2021). The paste of leaves of *J. humile* is used against skin diseases while roots are traditionally used to cure ringworm (Singh *et al.*, 2021). Previous studies showed that different extracts of *J. humile* had a significant

concentration of phenolics and exhibited strong antioxidant, anti-inflammatory and non-toxic effects in rats (Fatima and Khan, 2023). Although *J. humile* possesses beneficial ethnopharmacological effects but its not been fully studied for its ability to ameliorate deterioration in kidney function against toxic effects of CCl_4. The current study reveals the nephroprotective potential of JHM against CCl_4-mediated oxidative stress in the kidney of experimental rats on relevant antioxidative enzymes, stress, inflammatory and apoptotic markers.

5.2. Materials and Methods

5.2.1. Sample Collection

Leaves of *J. humile* were collected from Rawalpindi in March 2021. A voucher specimen no. (132015) was preserved in Pakistan Herbarium, Quaid-i-Azam University, Islamabad.

5.2.2. Preparation of extract

In a non-humid, leaves were shade dried for 3-4 weeks at ambient temperature, milled to get fine powder (1 kg), and plant powder was extracted with 90% methanol (1:4) using a reflux bath at 67 ºC for 4 hours. Following filtration, the prepared extract was placed in a rotatory evaporator to vaporize the solvent under reduced pressure. The extract was stored at -4 °C in clean tubes for the designed study.

5.2.3. FTIR spectroscopy

The methanol extract of *J. humile* was grinded and mixed with potassium bromide in a mortar. The pellets were pressed at a pressure of 6 bars to prepare a KBr thin disc. After disc preparation, FTIR spectrum were obtained using infrared spectrometer (Bruker Germany Vertex 70 with accessories) in the frequency range of 400-4,000/cm was used to analyze and identify the chemical bonds. The bands at various wavelengths of FTIR were recorded.

5.2.4. Quantitative estimation of tannins

Total tannin content was determined by adding 0.1 M $FeCl_3$ prepared in 0.1 N HCl, followed by addition of 0.008 M potassium hexacyanoferrate prepared in distilled water were measured at 720 nm with a known gallic acid concentration as a reference and tannin content was expressed as mg of GAE/ g of extract (Adewolu *et al.*, 2021).

5.2.5. Quantitative estimation of alkaloids

1 ml of HCl along with 5 ml of bromocresol green solution were added to a stock of prepared extract (1 mg/ml). The mixture was shaken with 2 ml of chloroform (Tambe and Bhambar, 2014). The method utilized atropine as a reference agent and total alkaloid concentration was expressed as mg of AE/g of extract. The absorbance values were noted at 560 nm.

5.2.6. Nitric oxide radical scavenging assay

5mM Sodium nitroprusside solution was prepared in sodium phosphate buffer (pH 7.4). 300 µl of sodium nitroprusside solution was incorporated into 1000 µl of serial dilutions of plant extract and the solution was incubated for 5 hours at 25 °C. 500 µl of Griess reagent was added and the spectrophotometry was performed at 546 nm (Singh *et al.*, 2012).

5.2.7. DMSO radical scavenging activity

1 ml alkaline DMSO were added to 500 µl of serial dilutions of plant extract and 200 µl of NBT (100 mg in 20 ml of phosphate buffer) was mixed in solution (Furuno *et al.*, 2002), the absorbance values were noted at 560 nm.

5.2.8. Experimental design

Fifty-four adult male Sprague-Dawley rats, falling between the weight range of 155-165 g, were acquired from the animal facility in National Institute of Health, Islamabad. Rats were kept in normal cages with free access to food and water. Ambient temperature (25 °C) was maintained with daily 12-hour cycles of dark and light. A total of fifty-four rats were split up into nine groups (6 rats/group) before the commencement of the experiment. For 6 weeks, this assay was performed on alternating days (18 doses).

Group 1: Normal, provided with normal diet and 0.9% Saline water.

Group 2: Negative control, intoxicated with CCl_4 (1 ml/kg; i.p.) for 2 weeks.

Group 3: Positive control, medicated with silymarin (200 mg/kg) and CCl_4.

Group 4, 5, and 6: Provided with 150, 300, and 600 mg/kg of JHM, respectively.

Group 7, 8, and 9: Injected with CCl_4 along with 150, 300, and 600 mg/kg of JHM doses.

Rats were weighed and anatomized once the experiment was completed. Serum tubes were used to collect blood from the subjects. Both kidneys were separated, weighed, and rinsed with saline water. Each kidney was divided into two sections, one of which was embedded in formalin for histopathological study while the other half was kept at -70 °C for various molecular and biochemical investigations.

5.2.8.1. Markers of serum

To separate serum from blood, centrifugation was performed at 3000 rpm for 15 mins. Standard biochemical test kits (BIO-LATEST, USA) were employed to analyze urea, creatinine, protein, blood urea nitrogen (BUN), and albumin using clinical biochemistry analyzer.

5.2.8.2. Nephro-chemical studies:

Homogenization of renal tissue was done in 1 ml of phosphate buffer containing 1 mM EDTA (pH 7.4) and spun at a centrifugal speed of 12,000 xg for 30 min at 4°C. The supernatant was poured into another tube. The total protein concentration was estimated following the approach of Kashyap while using crystalline BSA as standard (Kashyap *et al.*,1980).

5.2.8.3. Evaluation of antioxidant enzymes

The concentration of different antioxidant enzymes, catalase, superoxide dismutase, peroxidase was estimated in the supernatant (Khan, 2012; Ma *et al*., 2010; Batool *et al*., 2017) by spectrophotometer (BIO-RAD, Germany). Reduced glutathione was determined by using 1,2-dithiol-bis nitro benzoic acid (DTNB) as substrate (Lamia *et al*., 2021).

5.2.8.4. Evaluation of biochemical indices of liver

The concentration of thiobarbituric acid-reactive substances, a key indicator of lipid peroxidation, were determined in sample tissues (Lamia *et al.*, 2021) and result was expressed as nm of MDA/mg protein. H_2O_2 was measured by H_2O_2-mediated horseradish peroxidase-dependent oxidation of phenol red; a method utilizing a standard curve of H_2O_2-oxidized phenol red (Pick and Keisari, 1981). Nitrite concentration was calculated using the Griess reagent method and plotted against the sodium nitrite standard curve (Giustarini *et al.*, 2008).

5.2.8.5. RNA extraction and cDNA conversion

RNA from sample tissue was extracted using TRIzol method (Chomczynski and Sacchi, 2006). Washing of the pellet of RNA was done by ethanol and 20 µl of milli-Q water was used for suspension of each pellet. cDNA synthesis kit (Bio-Rad, CA) was used to reverse transcribe the extracted RNA into cDNA.

5.2.8.6. Real Time qPCR (RT-qPCR)

qRT-PCR was applied on Biosystems 7500 instrument to ascertain the corresponding expression level of the targeted genes using SYBR Green. qRT-PCR analysis was done with relevant primers. β-actin was used as the reference gene. The $2^{-\Delta\Delta Ct}$ technique was employed to accomplish comparative quantification of the selected genes. Various genes Caspase 3, Bax, BCl-2, IL-6, TNF-α, TGF-β, Smad-3, and Col1a1 were assessed, and Beta-actin was used as reference gene. The primers sequences of genes are presented in Table 5.1.

Table 5.1: Primer Sequence of genes for qRT-PCR analysis

Gene		**Primer sequence (5'-3')**
TNF-α	F	GTCTGTGCCTCAGCCTCTTC
	R	GCCATGGAACTGATGAGAG
IL-6	F	GCCTGCAGAGAGATTCAATCA
	R	GTATCAGTGGGGGTCAGCAG
BCl-2	F	TATATGGCCCCAGCATGCGA
	R	GGGCAGGTTTGTCGACCTCA
Bax	F	CTGAGCTGACCTTGGAGC
	R	GACTCCAGCCACAAAGATG
Caspase 3	F	TACCCTGAAATGGGCTTGTGT
	R	GTTAACACGAGTGAGGATGTG
TGF-ß	F	GCCTCCGCATCCCACCTTTG
	R	GCGGGTGACTTCTTTGGCGT
Smad-3	F	CACCTCCTGGCTACCTGAGT
	R	GTTATTGTGTGCTGGGGACA
Col-1a1	F	TGTTCAGCTTTGTGGACCTC
	R	GACCCTTAGGCCATTGTGT
ß-actin	F	TCTACAATGAGCTGCGTGTG
	R	ACGTACATGGCTGGGGTGT

5.2.8.7. Histological analysis

Small pieces of kidney tissues were embedded in 40% formalin and subjected to histological analysis. Hematoxylin and eosin staining was used to stain 4-5 µm sections of each sample. Prepared slides sample were assessed under a light microscope (DIALUX 20 EB) at 10X and 40X.

5.2.9. Statistical analysis

Data was presented as mean ± standard deviation (S.D.) and the ANOVA test was conducted to evaluate the differences among treatments, with the least significance difference (LSD) set at 0.05. The statistical and graphical evaluations were performed using Graph prism 5.0, Statistix 8.1, and Microsoft Excel 2016.

5.3. Results

5.3.1. Quantitative phytochemical analysis

The methanol extract of *J. humile* contained 63.72 ± 1.27 GAE/g of tannin content and 46.2 ± 2.03 AE/g for alkaloid content.

5.3.2. FTIR spectroscopy

The compound assignments or functional groups found in JHM were evaluated using FTIR technique. The infrared absorption spectra were predicted to identify the functional groups and compounds. Figure 5.1 illustrates the FTIR spectrum of the JHM while Table 5.2 represents the elucidation of the chemical bonding. Broad and strong bands were recorded at 3316.04 cm^{-1}, 2925.51 cm^{-1} and 1068.71 cm^{-1} along with various other peaks. These results confirmed the presence of secondary amine groups, alkanes, carboxylic acids, alkyl halide compounds.

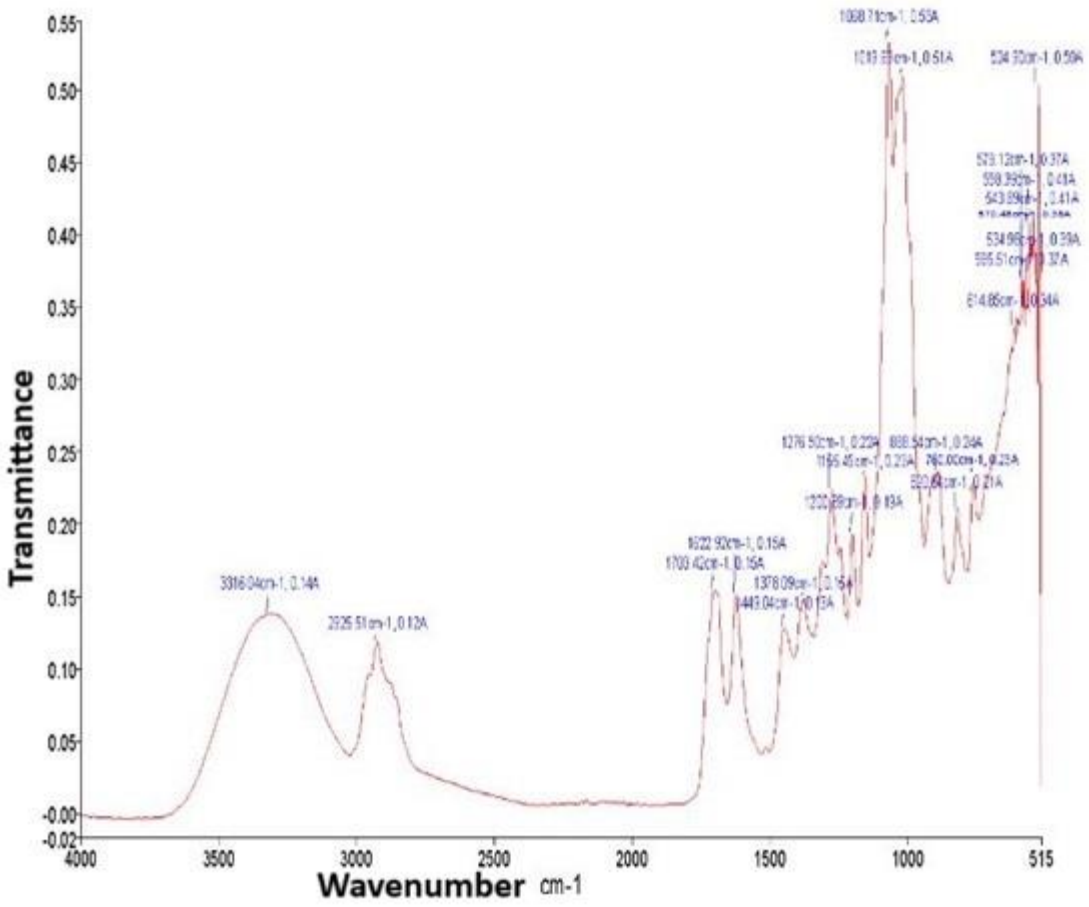

Figure 5.1: FTIR spectrum of *Jasminum humile* methanol extract.

Table 5.2: FTIR wave number and functional groups of *Jasminum humile* extract

Spectrum No.	Wave Number/cm	Wave Number/cm	Functional Group
1	3316.04	3400-3200	N-H stretching vibration
2	2925.51	3080-2760	C-H stretching
3	1703.42	1815-1650	C=O stretching vibration
4	1522.92	1500-1600	C=C stretching
5	1449.04	1400-1500	C-H bending or scissoring
6	1378.09	1400-1300	C-O stretch
7	1200.89	1200-1090	C-O stretch
8	1068.71	1080-1000	Aliphatic amines
9	820.80	900-700	C-H out-of-plane bending (oop)
10	579.12	500-700	Alkyl halides

5.3.3. Antioxidant activities

The superoxide radical scavenging activity of JHM was evaluated using the alkaline DMSO approach. The production of superoxide radicals was highly suppressed in the presence of all plant fractions. The percent inhibition of JHM against superoxide generation was 87.03 ± 1.02 %. In the nitric oxide radical scavenging assay, the JHM was found to be strongly potent when compared to quercetin with percent inhibition of 84.44 ± 2.41%. The IC_{50} values of plant extract and standard compounds are represented in Table 5.3.

5.3.4. Body weight and kidney weight of rats

Table 5.4 displays the impact of different JHM concentrations on the rat body and kidney weight. Rats exposed to CCl_4 toxicity had significantly ($P < 0.05$) lower absolute and percent increases in body weight and increased renal and relative kidney weight. Rats receiving varying doses of JHM in comparison to rats provided with CCl_4 treatment recovered their body weight, % increase in body weight, as well as absolute kidney and relative kidney weight. When JHM was administered in higher doses (600 mg/kg body weight), more pronounced effects were observed.

3.5. Serum profile of rats

The serum levels of creatinine, albumin, protein, blood urea nitrogen, and urea were used as markers to detect kidney damage and functioning. Table 5.5 shows that the administration of CCl_4 affected these biochemical markers. When compared to the normal group, the serum levels of creatinine and urea were considerably ($P < 0.05$) elevated after CCl_4 intoxication while the levels of albumin, and protein were decreased. JHM doses helped alleviate the CCl_4 toxicity and restored the levels of serum creatinine, albumin, protein, blood urea nitrogen and urea to normal.

Table 5.3: The antioxidant potency of *Jasminum humile* extract

	Nitric Oxide Scavenging IC_{50}	Superoxide Scavenging IC_{50} (µg/ml)
JHM (1 mg/ml)	250.31 ± 1.25^{a}	196.06 ± 1.9^{a}
Quercetin (1 mg/ml)	95.63 ± 2.54^{b}	
Ascorbic acid (1 mg/ml)		105.33 ± 1.96^{b}

Mean ± S.D. (n = 3 replicates). Means with varying superscripts indicate significance at $P < 0.05$. JHM; methanol extract of *J. humile*.

Table 5.4: Effects of *Jasminum humile* extract on kidney and body weight of rats

	Initial body weight (g)	Final body weight (g)	% Increase in body weight	Absolute kidney weight (g)	Relative kidney weight (mg/g of body weight)
Control	157 ± 2.4	231 ± 1.7	47.22 ± 3.1^{a}	0.80 ± 0.03^{a}	3.74 ± 0.15^{a}
CCl_4 (1 ml/kg)	161 ± 1.5	200 ± 1.1	24.5 ± 1.4^{b}	1.30 ± 0.03^{b}	6.68 ± 0.16^{b}
CCl_4 + Silymarin (200 mg/kg)	163 ± 2.4	236 ± 1.7	44.5 ± 2.4^{a}	0.69 ± 0.02^{a}	2.93 ± 0.11^{a}
CCl_4 + JHM (150 mg/kg)	159 ± 2.8	207 ± 1.9	29.9 ± 2.8^{b}	1.04 ± 0.03^{ab}	5.05 ± 0.17^{ab}
CCl_4 + JHM (300 mg/kg)	162 ± 2.1	216 ± 1.2	33.2 ± 1.2^{ab}	1.08 ± 0.06^{ab}	5.03 ± 0.25^{ab}
CCl_4 + JHM (600 mg/kg)	162 ± 2.0	221 ± 1.4	37.1 ± 1.6^{ab}	0.85 ± 0.03^{a}	3.84 ± 0.11^{a}
JHM (150 mg/kg)	155 ± 2.7	227 ± 2.6	46.5 ± 2.1^{a}	0.90 ± 0.03^{a}	3.96 ± 0.15^{a}
JHM (300 mg/kg)	160 ± 1.7	237 ± 1.7	47.8 ± 1.9^{a}	0.78 ± 0.06^{a}	3.30 ± 0.27^{a}
JHM (600 mg/kg)	161 ± 1.5	242 ± 1.2	49.6 ± 0.9^{a}	0.82 ± 0.07^{a}	3.41 ± 0.30^{a}

Mean ± S.D. (n = 6 male rats). [a] Significance difference vs CCl_4 group; [b] Significance difference vs Control group at $P < 0.05$; JHM: methanol extract of *J. humile*.

Table 5.5: Effects of *Jasminum humile* extract on renal serum markers

	Urea (mg/dl)	Creatinine (mg/dl)	Blood urea nitrogen (mg/dl)	Total Protein (mg/dl)	Albumin (mg/dl)
Control	42.65 ± 1.26[a]	64.05 ± 0.98[a]	60.28 ± 1.11[a]	45.24 ± 0.06[a]	10.70 ± 0.02[a]
CCl_4 (1 ml/kg)	115.8 ± 0.69[b]	131.7 ± 1.06[b]	138.45 ± 0.79[b]	28.74 ± 0.10[b]	5.71 ± 0.02[b]
CCl_4 + Silymarin (200 mg/kg)	59.33 ± 1.69[ab]	72.81 ± 0.73[a]	72.43 ± 0.81[a]	39.85 ± 0.04[ab]	12.75 ± 0.04[a]
CCl_4 + JHM (150 mg/kg)	92.63 ± 1.45[ab]	86.53 ± 0.69[ab]	124.31 ± 0.59[b]	30.90 ± 0.05[b]	16.12 ± 0.06[ab]
CCl_4 + JHM (300 mg/kg)	79.98 ± 1.06[ab]	81.45 ± 0.88[ab]	91.98 ± 1.12[ab]	35.11 ± 0.04[ab]	14.93 ± 0.04[ab]
CCl_4 + JHM (600 mg/kg)	71.9 ± 1.06[ab]	77.85 ± 0.82[ab]	81.9 ± 0.93[ab]	41.42 ± 0.05[a]	12.83 ± 0.03[a]
JHM (150 mg/kg)	46.01 ± 0.91[a]	68.8 ± 0.42[a]	74.56 ± 0.51[a]	43.55 ± 0.07[a]	10.01 ± 0.02[a]
JHM (300 mg/kg)	52.88 ± 1.09[a]	70.66 ± 0.55[a]	65.75 ± 0.80[a]	43.83 ± 0.06[a]	10.04 ± 0.02[a]
JHM (600 mg/kg)	47.35 ±1.99[a]	70.11 ± 0.64[a]	62.56 ± 0.68[a]	44.09 ± 0.07[a]	09.72 ± 0.01[a]

Mean ± S.D. (n = 6 male rats). [a] Significance difference vs CCl_4 group; [b] Significance difference vs Control group at $P < 0.05$; JHM: methanol extract of *J. humile*.

5.3.6. Antioxidant profile of kidney

Rats exposed to CCl_4 experienced a significant ($P < 0.05$) reduction in activities of antioxidant enzymes such as GSH, peroxidase, SOD, and CAT (Table 6). Rats provided with JHM had higher activity of these enzymes than rats with CCl_4 treatment. Rats treated with JHM alone did not show considerable alterations in antioxidant defense system in comparison to normal rats.

5.3.7. Biochemical indices of kidney

Table 7 displays the impact of CCl_4 and JHM on renal TBARs, protein, nitrite, and H_2O_2 levels. Rats intoxicated with CCl_4 exhibited significantly ($P < 0.05$) higher levels of TBARs, nitrite and H_2O_2 levels in kidney tissue, while a significant decline in renal protein content. Treatment with silymarin and plant extract in a dose-dependent manner increased the level of renal proteins, decreased MDA, H_2O_2, and nitrites in comparison to CCl_4 group, hence protecting the renal tissues of rats from CCl_4-induced oxidative stress.

5.3.8. mRNA expression of apoptotic and inflammatory markers

CCl_4 treatment to rats exhibited considerable ($P < 0.05$) increase in levels of Caspase-3 and Bax and reduction in BCl-2 levels in comparison to normal rats (Figure 2). After treatment with JHM to toxicity-induced rats, apoptotic indicators were significantly ($P < 0.05$) suppressed. Similarly, CCl_4 treatment displayed increased IL-6 and TNF-α levels in tissue samples. While co-administration of JHM with CCl_4 at 300 mg/kg and 600 mg/kg doses caused restoration in expression of these genes towards normal levels. On the contrary, JHM treatment alone led to non-significant ($P > 0.05$) changes in the mRNA expression of all of cytokines when compared to normal rats.

5.3.9. mRNA expression of fibrosis markers

CCl_4 treatment to rats had shown substantial ($P < 0.05$) elevation in fold change of TGF-ß, Smad-3, and Col1a1 in comparison to levels of normal rats (Figure 5.2). Simultaneous treatment with silymarin and JHM (300 and 600 mg/kg) resulted in downregulated expression of these genes. Rats treated with JHM alone showed a non-significant reduction mRNA expression level of these markers in contrast to the normal rats, indicating that JHM has beneficial effects against fibrosis.

Table 5.6: Effects of *Jasminum humile* extract on kidney antioxidant profile

	CAT (U/min)	SOD (U/min/mg of protein)	POD (U/min)	GSH (μM/g tissue)
Control	7.50 ± 0.53^{a}	7.40 ± 0.48^{a}	9.56 ± 0.35^{a}	7.72 ± 0.09^{a}
CCl_4 (1 ml/kg)	4.78 ± 0.77^{b}	4.02 ± 0.13	4.98 ± 0.60^{b}	4.51 ± 0.17^{b}
CCl_4 + Silymarin (200 mg/kg)	6.74 ± 0.57^{ab}	7.05 ± 0.78^{ab}	9.18 ± 0.45^{a}	7.00 ± 0.17^{a}
CCl_4 + JHM (150 mg/kg)	5.62 ± 0.82^{ab}	4.54 ± 0.80^{b}	6.80 ± 0.73^{ab}	6.10 ± 0.19^{ab}
CCl_4 + JHM (300 mg/kg)	6.24 ± 0.63^{ab}	5.20 ± 0.85^{ab}	7.35 ± 1.35^{ab}	6.55 ± 0.12^{ab}
CCl_4 + JHM (600 mg/kg)	6.84 ± 0.92^{ab}	5.37 ± 0.42^{ab}	8.06 ± 0.85^{ab}	6.96 ± 0.32^{ab}
JHM (150 mg/kg)	7.31 ± 0.75^{ab}	6.96 ± 0.66^{a}	8.95 ± 0.97^{a}	7.39 ± 0.14^{a}
JHM (300 mg/kg)	7.41 ± 0.72^{ab}	7.17 ± 0.85^{a}	9.02 ± 1.17^{a}	7.41 ± 0.15^{a}
JHM (600 mg/kg)	8.04 ± 0.74^{ab}	7.71 ± 0.33^{a}	9.11 ± 0.59^{a}	7.58 ± 0.17^{a}

Mean ± S.D. (n = 6 male rats). [a] Significance difference vs CCl_4 group; [b] Significance difference vs Control group at $P < 0.05$; JHM: methanol extract of *J. humile.*

Table 5.7: Effects of *Jasminum humile* extract on renal biochemical markers

	Protein (µg/mg tissue)	MDA (nM/mg protein)	H_2O_2 (nM/min/mg tissue)	Nitrite (µM/mg protein)
Control	8.92 ± 0.05^{a}	7.85 ± 0.04^{a}	4.77 ± 0.23^{a}	36.67 ± 0.76^{a}
CCl_4 (1 ml/kg)	4.55 ± 0.19^{b}	16.96 ± 0.69^{b}	10.0 ± 0.21^{b}	56.64 ± 1.35^{b}
CCl_4 + Silymarin (200 mg/kg)	8.34 ± 0.09^{a}	8.48 ± 0.09^{ab}	5.46 ± 0.19^{ab}	41.84 ± 0.92^{ab}
CCl_4 + JHM (150 mg/kg)	6.25 ± 0.11^{ab}	12.23 ± 0.27^{ab}	9.02 ± 0.34^{b}	$49.20 \pm 1.^{44ab}$
CCl_4 + JHM (300 mg/kg)	7.07 ± 0.09^{ab}	10.8 ± 0.17^{ab}	7.17 ± 0.21^{ab}	45.17 ± 1.38^{ab}
CCl_4 + JHM (600 mg/kg)	7.69 ± 0.09^{ab}	8.38 ± 0.12^{ab}	5.03 ± 0.22^{a}	40.12 ± 0.76^{ab}
JHM (150 mg/kg)	8.60 ± 0.11^{a}	7.93 ± 0.18^{a}	4.86 ± 0.19^{a}	38.64 ± 1.45^{a}
JHM (300 mg/kg)	8.81 ± 0.11^{a}	7.46 ± 0.98^{a}	4.83 ± 0.27^{a}	38.33 ± 0.88^{a}
JHM (600 mg/kg)	8.91 ± 0.08^{a}	7.69 ± 0.07^{a}	4.61 ± 0.16^{a}	34.71 ± 0.91^{a}

Mean ± S.D. (n = 6 male rats). [a] Significance difference vs CCl_4 group; [b] Significance difference vs Control group at $P < 0.05$; JHM: methanol extract of *J. humile*; H_2O_2: Hydrogen peroxide.

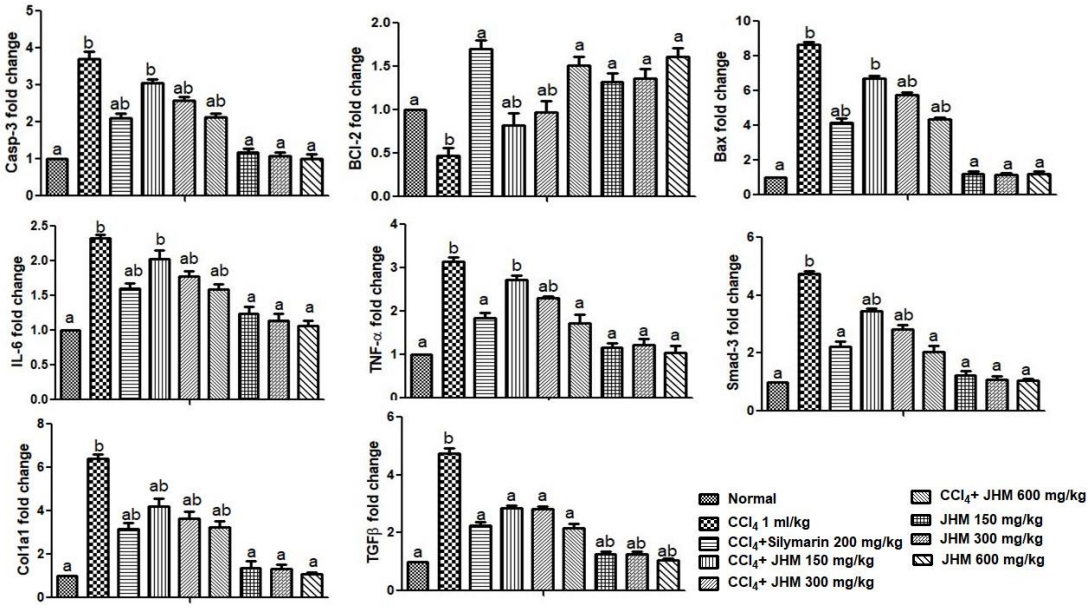

Figure 5.2: mRNA expression of inflammatory, apoptotic and fibrosis genes (fold change). [a] Significance difference vs CCl_4 group; [b] Significance difference vs Control group at $P < 0.05$.

5.3.10. Histological anatomy

Rats administered with CCl_4 showed deteriorated renal tubules with debris in lumen, glomerulus hypertrophy, and neutrophil infiltration into intratubular spaces, and cellular apoptosis (Figure 5.3b). The untreated and silymarin treated rats showed normal renal tubules, less glomerulus space, and improved morphology (Figure 5.3a, c). these pathological alterations were significantly restored after JHM administration (Figure 5.3d, e, & f). Rats administered JHM alone displayed normal characteristics of histology in renal tissues (Figure 5.3g, h, & i).

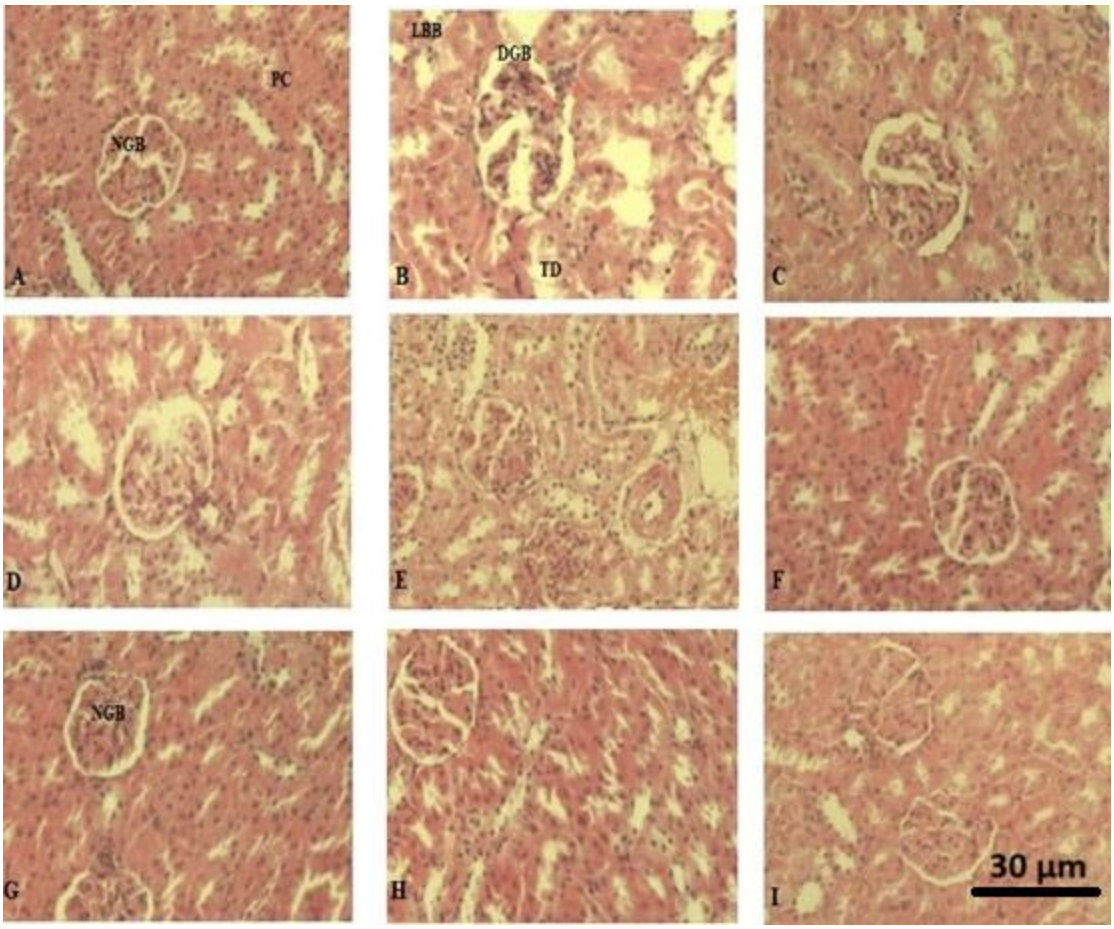

Figure 5.3: Ameliorative potential of *Jasminum humile* extract on the renal histopathology against CCl_4. (a) Normal group, (b) CCl_4 (1 mL/kg bw), (c) CCl_4 + silymarin (200 mg/kg), (d) CCl_4 + JHM (150 mg/kg), (e) CCl_4 + JHM (300 mg/kg), (f) CCl_4 + JHM (600 mg/kg), (g, h ,i) JHM (150, 300, and 600 mg/kg). ; NGB, normal glomerulus and bowman capsule; TD, tissue damages; ; PC, proximal convoluted tubule; DGB, damaged glomerulus; LBB, loss of brush border and bowman capsule.

5.4: Discussion

Therapeutic plants are a valuable reservoir of secondary metabolites that have significant pharmaceutical potential (Yeshi *et al.,* 2022). These secondary metabolites are not explicitly involved in growth of plants but exhibit various therapeutic properties and have long been utilized in folk medicine (Twilley *et al*., 2020). The phytochemical diversity of natural plants is vast and encompasses a wide range of compounds including terpenoids, tannins, alkaloids, saponins, steroids, oils, glycosides, reducing sugars, and polyphenolics (Sofowora, 1993). In a previous study, the phytochemical assessment of JHM leaves demonstrated positive results for tannins, phenols, carbohydrates, alkaloids, steroids and saponins (Fatima and Khan, 2023). These bioactive compounds possess exclusive medicinal and physiological activities (Sofowora, 1993). In this study, quantitative analysis of JHM demonstrated the presence of tannins and alkaloids (Table 2). Tannins were traditionally utilized to treat diarrhea, hemorrhage, and detoxification (Abdul-Awal *et al.*, 2016). Alkaloids, composed of a substantial group of nitrogenous compounds are extensively employed for cancer chemotherapy (Pandrangi *et al*., 2022). Alkaloids and saponins are natural compounds known to possesses pharmacological effects including analgesic and antispasmodic properties. These compounds had a history of traditional used for ameliorating chest pain and arthritis (Abdul-Awal *et al.*, 2016The presence of alkaloids in leaf extracts may can attribute to their traditional use as remedies for pain relief. Phenolic compounds, alkaloids, flavonoids, tannins, saponins and glycosides exhibited antioxidant activities and helped to neutralize the free radicals and oxidative stress associated pathologies (Wanjala *et al.*, 2023).

The FTIR analysis indicated the presence of C-H out-of-plane bending (oop bend) vibration in the substituted benzene ring, affirming the presence of flavonoids and phenolics in JHM. The Flavonoids are class pf polyphenols with two benzene rings attached through a linear carbon chain (Pharmawati and Wrasiati, 2020). The identification of benzenoid groups via FTIR studies supported results of a previous phytochemical and HPLC analysis of JHM, which showed the presence of polyphenolic compounds in extract (Fatima and Khan, 2023). The bioactive compounds containing major functional groups of amines, imines, alkanes and phenols were identified in JHM, similar to previous study (Pratheeba *et al.*, 2015). The peak at 1522 cm^{-1} is due to C=C stretching affiliated with the

aromatic skeleton of the JHM. While the peak at 3316.04 cm^{-1} is due to N-H stretching vibration and associated with amine function groups of bioactive compounds.

The breakdown of amino acids such as arginine in phagocytes and endothelial cells generate nitric oxides and reaction of this free radical with oxygen generates peroxy nitrite and their toxicity doubles. The higher production of nitric oxides is linked to higher risk of illness (Ialenti *et al.*, 1993; Kanchana *et al.*, 2023). Nitrite ions generated by incubation of sodium nitroprusside solution was decreased by action of antioxidants in plant extract. This can be associated with the capability of antioxidants presented in extract to prevent oxygen from reacting with nitric oxide and, therefore, impeding the production of nitrite. Furthermore, DMSO reacts with a strong base in the vicinity of oxygen molecules that could generate superoxide anions by univalent reduction of oxygen molecule (Yao *et al.*, 2021). However, concerning the capability to scavenge superoxide ions, plant extract has showed high scavenging abilities in comparison to ascorbic acid. The high antioxidant activity of *J. humile* extract was attributed to its high total tannin and alkaloid content. The vital role of phytonutrients as free radical scavenging agents was described in some previous studies (Jit *et al*., 2022).

To elucidate the protective potential of JHM against CCl_4 mediated oxidative damage to kidney, we determined the oxidative stress profile and levels of serum biochemical markers. It is well established that CCl_4 induces production of ROS and creates deficit of antioxidant enzymes and substrates (Khan *et al.*, 2010b). In this study, treatment with CCl_4 caused elevated urea and creatinine levels in serum, which indicates significant nephrotoxicity and damage to the kidneys (Fristiohady *et al.*, 2020). The structural integrity of nephrons was compromised because of high serum creatinine status. In contrast, levels of protein and albumin were reduced in serum, indicating possible kidney dysfunction, ultimately causing proteinuria. However, the co-treatment of JHM improved the levels of serum biomarkers and alleviated toxicity associated with CCl_4. Comparable results were observed when Silymarin was used in rats in conjunction with JHM. The findings of this investigation were consistent with a prior study addressing the nephroprotective role of medicinal plants in mitigating CCl_4-induced toxicity in rats (Khan and Siddique, 2012).

CAT and SOD catalyze the transition of harmful superoxide radicals to H_2O_2 and its further breakdown into dioxygen and H_2O, correspondingly (Oyeleke and Owoyele, 2022). The accumulation of super-oxides and H_2O_2 in renal tissues was due to CCl_4 incited redox imbalance. The prooxidant-antioxidant balance in tissues can be evaluated indirectly by measuring the status of certain antioxidant enzymes. The increased lipid peroxidation resulted in the reduction of these enzymes simultaneously with a drop in GSH levels. GSH is one of the non-enzymatic antioxidants which performs its tasks in both intracellular and extracellular environments in tandem with other enzyme-based antioxidants that help in the detoxification of H_2O_2 and hydro-peroxides (Sachdev *et al*., 2023). CCl_4 induction in rats led to a substantial reduction of components of the GSH enzyme system in renal tissues. These findings demonstrated that CCl_4-induced renal injury was the most studied mechanism of xenobiotic-instigated renal diseases, as it resulted in profound oxidative injury in renal tissues. During this analysis, the changed concentrations of the antioxidant enzymes in CCl_4-induced oxidative tissue damage were restored significantly by *J. humile* extract, indicating its nephroprotective benefits (Khan *et al.*, 2010b).

The reduced enzymatic activities correspond to weak antioxidant defense and lead to higher formation of H_2O_2 and other free radicals (Ofoedu *et al*., 2021)). CCl_4 treatment is also related to increased production of TBARs which is an indication of high lipid peroxidation in tissues (Khan *et al.*, 2010b). In this study, higher levels of free radicals after CCl_4 administration indicated a weak defense system in tissues. However, the co-treatment of rats with JHM normalized the levels of these renal function biomarkers thus exhibiting the antioxidant and nephroprotective activities.

Elevated NO levels produce nitrites, which trigger oxidative damage that causes inflammation and contributes to cellular oxidative stress (Wood et al., 2003). In the current investigation, rats receiving CCl4 treatment exhibited increased nitrite levels. This increase was significantly reduced following JHM administration, indicating the nephroprotective properties of the plant extract.

Chemicals and drugs alter normal redox metabolism and regulation of inflammatory cytokines, thus causing acute kidney damage. The current research is focused on a thorough examination of molecular markers expressed in renal tissues during inflammation (IL-6

and TNF-α) and apoptosis (Caspase 3). The inflammatory cytokines are produced more frequently when oxidative damage is present, or vice versa. This inverse relation between inflammatory mediators and redox imbalance has been documented in several studies (Sofowora, 1993). The overproduction of inflammatory cytokines is also stimulated by hazardous trichloromethyl ($CCl_3^{\bullet}$) and trichloromethyl-peroxyl ($CCl_3O_2^{\bullet}$) radicals generated by CCl_4. These cytokines are related with the pathogenic inflammation in acute kidney injury and are secreted by tubular cells and leukocytes. The inflammatory responses are primarily triggered by NF-κB, which regulates cytokine synthesis, thus increasing inflammatory cytokine output (Li *et al.,* 2021). TNF*α* is a key player in inflammation-induced tissue impairment and nephron toxicity. It maintains the immune system's homeostasis by acting as an immunological regulator. The results showed that CCl_4 and toxic radicals ($CCl_3^{\bullet}$ and $CCl_3O_2^{\bullet}$) generated from it not only augmented the inflammatory genes but also instigated activation of NF-κB signaling pathway. Similarly, administration of Zingerone restored the high expression of IL-6 and TNFα back to normal (Wang *et al.*, 2015).

The excessive instigation of apoptosis triggered by phosphorylation of caspases may worsen nephrotoxicity by destroying the tubular cells (Safhi, 2018). Caspases are the primary effectors of apoptosis and have been divided into distinct categories based on how they work, namely initiator caspases and executioner caspases. The executioner caspase is activated by initiator caspase which further coordinates its actions to an important downstream structural protein Bax/Bcl-2 to complete apoptosis (Yang *et al.*, 2002). CCl_4 injections increased the release of caspase 3 which facilitated apoptosis by raising the concentration of apoptosis promoting Bax and deregulating the apoptosis inhibiting Bcl-2 proteins. Caspase 3 activity significantly decreased after administration of JHM as supported by previous study as supported by a previous study (Yang *et al*., 2002).

TGF-β1 is a signaling protein that controls multiple cellular processes and its canonical signaling pathway involves phosphorylation od receptor activated Smad-2 and Smad-3 (Huang *et al*., 2015). Smad-7 acts as negative feedback regular of TGF-β signaling. It antagonizes the activity of receptor-regulator Smad, which ultimately terminates the signaling of TGF-β. The TGF-β/Smad3 pathway regulates the production of ECM and profibrotic genes transcription. The CCl_4 exposed experimental animals experienced

kidney inflammation and fibrosis due to increased expression of TGF-β1 and p-Smad-3 (Ma *et al.*, 2021). However, the administration of JHM along with CCl_4 restored the renal TGF-β, Col1a1, and Smad-3 expression to normal levels. Our study demonstrated that *J. humile* possess treatment properties against oxidative stress instigated by CCl_4 in kidney tissues as reported by previous study that plant extract had therapeutic potential against nephrotoxicity (Ma *et al.*, 2021).

The microanatomy of CCl_4 treated kidneys suggested severe oxidative damage in this group considering the loss of brush border and tubular damage (Fig. 3). These injuries were less severe in groups that received plant extract, demonstrating the protective properties of *J. humile* in attenuating CCl_4-induced pathological alterations in tissues. Likewise, the renal tissues of CCl_4 treated rats in a previous study disclosed abnormalities in tissue structure (Khan *et al.*, 2010a) A wide range of chemicals, particularly oxidizing agents, and food preservatives, pose detrimental health effects in both humans as well as experimental subjects as demonstrated in several investigations (de Diego-Otero *et al.*, 2009). These chemicals damage the structural and functional integrity of vital organs i.e., kidney, liver, intestine, and heart (Kohn *et al.*, 2005). One such chemical is CCl_4, an atypical hepatotoxin, also responsible for oxidative stress mediated renal toxicity (Yao *et al.*, 2021). It has been speculated that the antioxidant efficacy of *J. humile* extract against CCl_4-induced renal impairment may be pertinent to human health because a similar P_{450} enzymatic system engages in the biotransformation of a variety of toxic compounds. This investigation validated the ethnopharmacological benefits of JHM against renal disorders as claimed by a previous report for plant extract (Khan and Zehra, 2013).

5.5. Conclusion

This study illustrated that JHM had antioxidative properties. Plant extract contains antioxidant phytochemicals including alkaloids, tannins, and polyphenolics. Alkaloids were detected in significant amount referring to the antioxidant and therapeutic nature of JHM. The *in vitro* studies revealed high inhibition property of JHM against various free radical systems. Furthermore, JHM treatment suppressed the production of H_2O_2 and nitrites in kidney tissues while revived the status of antioxidants in rats. mRNA expression of smad-3, TGF-β, Col1a1, IL-6, TNF-α, BCl-2, Bax, and caspase 3 was restored towards

normal group rats after JHM extract administration. Histological damages were less obvious and restored in JHM treated rats. However, clinical studies on JHM may reveal potential of extract as nephroprotective agent in humans.

6.1. Conclusion

In conclusion, *J. humile* and *P. candollei* are most beneficial traditional therapeutic plants against various oxidative stress related disorders. Now a days, researchers are interested in pharmacological and antioxidant activities of selected plants elucidated in this study. *In vitro* phytochemical and antioxidant analyses showed that examined plants are comprised of polyphenolics and have high inhibition activity against free radicals. STZ and CCl_4 mediated hepatic and renal inflammation and diabetes in rats, respectively. This study showed that polyphenols of selected plants ameliorated oxidative stress and normalized inflammatory, apoptotic and fibrosis markers to reduce the hepatic and renal damages. These extracts and their constituents can be used as natural anti-inflammatory and anti-fibrotic drugs due to its effectiveness in protecting liver and kidney against toxins and chemical drugs.

6.2. Future Perspectives

The current study confirmed *J. humile* and *P. candollei* as a potent therapeutic source against xenobiotics like CCl_4 induced hepatic intoxications and STZ mediated diabetes. Therefore, it should be considered in the development of natural product-oriented medications capable of combatting many life-threatening ailments prevalent in developing and third-world countries. Advanced analytical techniques including metabolomics and proteomics could help unravel the complex interactions between these compounds and cellular pathways involved in glucose homeostasis and reduction of oxidative stress. Similarly, further extensive and effective clinical experimentation is required to identify efficacy and toxicity of *J. humile* and *P. candollei* extracts. The key findings of this study will serve as the foundation for designing trials at a massive scale for determining the medicinal potency of these plants or plant parts usage involving different ailments. Further research based on the findings on the pharmacological effects of *J. humile* and *P. candollei* may include the isolation and characterization of particular bioactive compounds responsible for the observed therapeutic activities. Furthermore, research on the possibility of developing standardized plant-based formulations for clinical applications may offer new prospects as alternatives or adjuncts in the management of liver toxicity, diabetes, and related metabolic disorders. Future studies may also be directed at long-term safety and efficacy testing of these natural extracts in human clinical trials, including the combined synergistic effect in combination with conventional

treatments.

In fact, an incremental number of studies on their possible effects against other pathologies related to oxidative and nitrosative stress, such as neurodegenerative disorders, might widen the perspective of their application. Finally, new drug delivery systems would improve the actual bioavailability and/or specific action of these plant natural products, widening their therapeutic potential and opening up a completely new prospect in the treatment of chronic diseases.

References

1. Abbas, Q., Hussain, A., Khan, S. W., Hussain, A., Shinwari, S., Hussain, A., ... & Ali, K. (2019). Floristic Diversity, Ethnobotany and Traditional Recipes of Medicinal Plants of Maruk Nallah, Haramosh Valley, District Gilgit, Gilgit Baltistan: Traditional recipes of Maruk Nallah, Haramosh Valley, District Gilgit. *Proceedings of the Pakistan Academy of Sciences: B. Life and Environmental Sciences*, *56*(3), 97-112.
2. Abbas, Z., Khan, S. M., Abbasi, A. M., Pieroni, A., Ullah, Z., Iqbal, M., & Ahmad, Z. (2016). Ethnobotany of the balti community, tormik valley, karakorum range, baltistan, pakistan. *Journal of ethnobiology and ethnomedicine*, *12*, 1-16.
3. Abd-Elhakim, Y. M., Ghoneim, M. H., Khairy, M. H., & Eissa, S. A. (2020). Single or combined protective and therapeutic impact of taurine and hesperidin on carbon tetrachloride-induced acute hepatic injury in rat. *Environmental Science and Pollution Research*, *27*, 13180-13193.
4. Abdoul-Latif, F., Edou, P., Eba, F., Mohamed, N., Ali, A., Djama, S., ... & Dicko, M. (2010). Antimicrobial and antioxidant activities of essential oil and methanol extract of Jasminum sambac from Djibouti. *African Journal of Plant Science*, *4*(3), 38-43.
5. Abdul-Awal, S. M., Nazmir, S., Nasrin, S., Nurunnabi, T. R., & Uddin, S. J. (2016). Evaluation of pharmacological activity of Hibiscus tiliaceus. *Springerplus*, *5*, 1-6.
6. Aboonabi, A., Rahmat, A., & Othman, F. (2014). Antioxidant effect of pomegranate against streptozotocin-nicotinamide generated oxidative stress induced diabetic rats. *Toxicology Reports*, *1*, 915-922.
7. Abraham, P., Wilfred, G., & Ramakrishna, B. (1999). Decreased activity of hepatic alkaline protease in rats with carbon tetrachloride-induced liver cirrhosis.
8. Adesokan, A. A., Oyewole, O. I., & Turay, B. M. S. (2009). Kidney and liver function parameters in alloxan-induced diabetic rats treated with aloe barbadensis juice extract. *Sierra leone journal of biomedical research*, *1*(1), 33-37.
9. Adeva-Andany, M. M., González-Lucán, M., Donapetry-García, C., Fernández-Fernández, C., & Ameneiros-Rodríguez, E. (2016). Glycogen metabolism in humans. *BBA clinical*, *5*, 85-100.

10. Adewolu, A., Adenekan, A. S., Uzamat, O. F., & Ajayi, O. O. (2021). Ameliorative Effects of Ethanolic Leaf Extract of Physalis angulata (Ewe Koropo) on Diabetic-Induced Wistar Rats in South West Nigeria. *Open Journal of Medicinal Chemistry*, *11*(8).
11. Aebi, H. (1984). [13] Catalase in vitro. In *Methods in enzymology* (Vol. 105, pp. 121-126). Academic press.
12. Afolayan, A. J., & Jimoh, F. O. (2009). Nutritional quality of some wild leafy vegetables in South Africa. *International journal of food sciences and nutrition*, *60*(5), 424-431.
13. Agius, L. (2016). Hormonal and metabolite regulation of hepatic glucokinase. *Annual review of nutrition*, *36*, 389-415.
14. Ahmed, A. U. (2011). An overview of inflammation: mechanism and consequences. *Frontiers in Biology*, *6*(4), 274-281.
15. Ahmed, A., Sattar, N., & Yaghootkar, H. (2022a). Advancing a causal role of type 2 diabetes and its components in developing macro-and microvascular complications via genetic studies. *Diabetic Medicine*, *39*(12), e14982.
16. Ahmed, D., Khan, M. M., & Saeed, R. (2015). Comparative analysis of phenolics, flavonoids, and antioxidant and antibacterial potential of methanolic, hexanic and aqueous extracts from Adiantum caudatum leaves. *Antioxidants*, *4*(2), 394-409.
17. Ahmed, M., Khan, K. U. R., Ahmad, S., Aati, H. Y., Ovatlarnporn, C., Rehman, M. S. U., ... & Anwar, M. (2022b). Comprehensive phytochemical profiling, biological activities, and molecular docking studies of Pleurospermum candollei: An insight into potential for natural products development. *Molecules*, *27*(13), 4113.
18. Ahmed, S. A., & Hamed, M. A. (2015). Kidney injury molecule-1 as a predicting factor for inflamed kidney, diabetic and diabetic nephropathy Egyptian patients. *Journal of diabetes & metabolic disorders*, *14*, 1-6.
19. Ajiboye, B. O., Oyinloye, B. E., Agboinghale, P. E., Onikanni, S. A., Asogwa, E., & Kappo, A. P. (2019). Antihyperglycaemia and related gene expressions of aqueous extract of Gongronema latifolium leaf in alloxan-induced diabetic rats. *Pharmaceutical biology*, *57*(1), 604-611.
20. Ajiboye, B. O., Oloyede, H. O. B., & Salawu, M. O. (2020). Antidiabetic Activity of Triticum aestivum Seed–Based Diet on Alloxan-Induced Diabetic Rats. *Journal of dietary supplements*, *17*(2), 133-149.

21. Ajiboye, B. O., Oyinloye, B. E., Essien, P. E., Onikanni, S. A., Ojo, O. A., & Kappo, A. P. (2021). Ameliorative potential of Sterculia tragacantha aqueous extract on renal gene expression and biochemical parameters in streptozotocin-induced diabetic rats. *Journal of Pharmaceutical Investigation*, *51*, 103-113.
22. Akbari, B., Baghaei-Yazdi, N., Bahmaie, M., & Mahdavi Abhari, F. (2022). The role of plant-derived natural antioxidants in reduction of oxidative stress. *BioFactors*, *48*(3), 611-633.
23. Akcora, B. Ö., Storm, G., & Bansal, R. (2018). Inhibition of canonical WNT signaling pathway by β-catenin/CBP inhibitor ICG-001 ameliorates liver fibrosis in vivo through suppression of stromal CXCL12. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1864*(3), 804-818.
24. Akhtar, N., Hafiz, I. A., Hayat, M. Q., Potter, D., Abbasi, N. A., Habib, U., ... & Malik, S. I. (2021). ISSR-Based Genetic Diversity Assessment of Genus J asminum L.(Oleaceae) from Pakistan. *Plants*, *10*(7), 1270.
25. Akira, S., Uematsu, S., & Takeuchi, O. (2006). Pathogen recognition and innate immunity. *Cell*, *124*(4), 783-801.
26. Al-Awaida, W. J., Sharab, A. S., Al-Ameer, H. J., & Ayoub, N. Y. (2020). Effect of simulated microgravity on the antidiabetic properties of wheatgrass (Triticum aestivum) in streptozotocin-induced diabetic rats. *npj Microgravity*, *6*(1), 6.
27. Al-Dalahmeh, Y., Al-Bataineh, N., Al-Balawi, S. S., Lahham, J. N., Al-Momani, I. F., Al-Sheraideh, M. S., ... & Al-Qudah, M. A. (2022). LC-MS/MS screening, total phenolic, flavonoid and antioxidant contents of crude extracts from three Asclepiadaceae species growing in Jordan. *Molecules*, *27*(3), 859.
28. Al-Olayan, E. M., El-Khadragy, M. F., Aref, A. M., Othman, M. S., Kassab, R. B., & Abdel Moneim, A. E. (2014). The potential protective effect of Physalis peruviana L. against carbon tetrachloride-induced hepatotoxicity in rats is mediated by suppression of oxidative stress and downregulation of MMP-9 expression. *Oxidative medicine and cellular longevity*, *2014*(1), 381413.
29. Al-Rasheed, N. M., Attia, H. A., Mohamad, R. A., Al-Rasheed, N. M., Al-Amin, M. A., & Al-Onazi, A. (2015). Aqueous Date Flesh or Pits Extract Attenuates Liver Fibrosis via Suppression of Hepatic Stellate Cell Activation and Reduction of Inflammatory Cytokines,

Transforming Growth Factor-β1 and Angiogenic Markers in Carbon Tetrachloride-Intoxicated Rats. *Evidence-Based Complementary and Alternative Medicine*, *2015*(1), 247357.

30. Ali, A. H. (2022). High-performance liquid chromatography (HPLC): a review. *Ann. Adv. Chem*, *6*, 010-020.
31. Ali, I., Mu, Y., Atif, M., Hussain, H., Li, J., Li, D., ... & Wang, X. (2021). Separation and anti-inflammatory evaluation of phytochemical constituents from Pleurospermum candollei (Apiaceae) by high-speed countercurrent chromatography with continuous sample load. *Journal of Separation Science*, *44*(13), 2663-2673.
32. Ali, S., Khan, M. R., & Sajid, M. (2017). Protective potential of Parrotiopsis jacquemontiana (Decne) Rehder on carbon tetrachloride induced hepatotoxicity in experimental rats. *Biomedicine & Pharmacotherapy*, *95*, 1853-1867.
33. Alm-Eldeen, A. A., El-Naggar, S. A., El-Boray, K. F., Elgebaly, H. A., & Osman, I. H. (2016). Protective role of Commiphora molmol extract against liver and kidney toxicity induced by carbon tetrachloride in mice. *Tropical Journal of Pharmaceutical Research*, *15*(1), 65-72.
34. Almundarij, T. I., Alharbi, Y. M., Abdel-Rahman, H. A., & Barakat, H. (2021). Antioxidant activity, phenolic profile, and nephroprotective potential of Anastatica hierochuntica ethanolic and aqueous extracts against ccl4-induced nephrotoxicity in rats. *Nutrients*, *13*(9), 2973.
35. Amzar, N., & Iqbal, M. (2017). The Hepatoprotective Effect of Clidemia hirta against Carbon Tetrachloride (CCl 4)− Induced Oxidative Stress and Hepatic Damage in Mice. *Journal of Environmental Pathology, Toxicology and Oncology*, *36*(4).
36. Antar, S. A., Saleh, M. A., & Al-Karmalawy, A. A. (2022). Investigating the possible mechanisms of pirfenidone to be targeted as a promising anti-inflammatory, anti-fibrotic, anti-oxidant, anti-apoptotic, anti-tumor, and/or anti-SARS-CoV-2. *Life Sciences*, *309*, 121048.
37. Anywar, G. (2020). Historical use of toxic plants. *Poisonous Plants and Phytochemicals in Drug Discovery*, 1-17.
38. Apostolou, A., Stagos, D., Galitsiou, E., Spyrou, A., Haroutounian, S., Portesis, N., ... & Kouretas, D. (2013). Assessment of polyphenolic content, antioxidant activity, protection

against ROS-induced DNA damage and anticancer activity of Vitis vinifera stem extracts. *Food and chemical toxicology*, *61*, 60-68.

39. Araujo Leon, J. A., Ruiz Ciau, D. V., Coral Martinez, T. I., & Cantillo Ciau, Z. O. (2015). Comparative fingerprint analyses of extracts from the root bark of wild Hippocratea excelsa and "Cancerina" by high-performance liquid chromatography. *Journal of separation science*, *38*(22), 3870-3875.
40. Arvouet-Grand, A., Vennat, B., Pourrat, A., & Legret, P. (1994). Standardization of propolis extract and identification of principal constituents. *Journal de pharmacie de Belgique*, *49*(6), 462-468.
41. Asante-Duah, D. K. (2002). *Public health risk assessment for human exposure to chemicals* (Vol. 6). London: Kluwer Academic.
42. Asrani, S. K., Larson, J. J., Yawn, B., Therneau, T. M., & Kim, W. R. (2013). Underestimation of liver-related mortality in the United States. *Gastroenterology*, *145*(2), 375-382.
43. Bäck, M., Weber, C., & Lutgens, E. (2015). Regulation of atherosclerotic plaque inflammation. *Journal of internal medicine*, *278*(5), 462-482.
44. Balahoroğlu, R., Dülger, H., Özbek, H., Bayram, İ., & Şekeroğlu, M. R. (2008). Protective effects of antioxidants on the experimental liver and kidney toxicity in mice. *European Journal of General Medicine*, *5*(3), 157-164.
45. Balmus, I. M., Ciobica, A., Trifan, A., & Stanciu, C. (2016). The implications of oxidative stress and antioxidant therapies in Inflammatory Bowel Disease: Clinical aspects and animal models. *Saudi journal of gastroenterology*, *22*(1), 3-17.
46. Batool, R., Khan, M. R., & Majid, M. (2017). Euphorbia dracunculoides L. abrogates carbon tetrachloride induced liver and DNA damage in rats. *BMC complementary and alternative medicine*, *17*, 1-11.
47. Batool, R., Khan, M. R., Zai, J. A., Ali, S., Maryam, S., Naz, I., & Bibi, S. (2018). Brachychiton populneus (Schott & Endl.) R. Br. ameliorate carbon tetrachloride induced oxidative stress through regulation of endoplasmic reticulum stress markers and inflammatory mediators in Sprague-Dawley male rats. *Biomedicine & Pharmacotherapy*, *107*, 1601-1610.

48. Batool, R., Khan, M. R., Sajid, M., Ali, S., & Zahra, Z. (2019). Estimation of phytochemical constituents and in vitro antioxidant potencies of Brachychiton populneus (Schott & Endl.) R. Br. *BMC chemistry*, *13*, 1-15.
49. Baynes, J. W. (1991). Role of oxidative stress in development of complications in diabetes. *Diabetes*, *40*(4), 405-412.
50. Ben Hsouna, A., Hfaiedh, M., Ben Slima, S., Romdhane, W. B., Akacha, B. B., Bouterra, M. T., ... & Ben Salah, R. (2022). Antioxidant and hepatoprotective effects of novel heteropolysaccharide isolated from Lobularia maritima on CCl4-induced liver injury in rats. *Food Science & Nutrition*, *10*(7), 2271-2284.
51. Benjamin, I. J., & Schneider, M. D. (2005). Learning from failure: congestive heart failure in the postgenomic age. *The Journal of clinical investigation*, *115*(3), 495-499.
52. Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, *239*(1), 70-76.
53. Bhatt, I. D., Rawat, S., & Rawal, R. S. (2012). Antioxidants in medicinal plants. In *Biotechnology for Medicinal Plants: Micropropagation and Improvement* (pp. 295-326). Berlin, Heidelberg: Springer Berlin Heidelberg.
54. Bicalho, M. D., Soares, D. B., Botoni, F. A., Reis, A. M. M., & Martins, M. A. P. (2015). Drug-induced nephrotoxicity and dose adjustment recommendations: agreement among four drug information sources. *International journal of environmental research and public health*, *12*(9), 11227-11240.
55. Bilzer, M., Roggel, F., & Gerbes, A. L. (2006). Role of Kupffer cells in host defense and liver disease. *Liver International*, *26*(10), 1175-1186.
56. Boll, M., Lutz, W. D., Becker, E., & Stampfl, A. (2001). Mechanism of carbon tetrachloride-induced hepatotoxicity. Hepatocellular damage by reactive carbon tetrachloride metabolites. *Zeitschrift für Naturforschung C*, *56*(7-8), 649-659.
57. Bourebaba, N., & Marycz, K. (2021). Hepatic stellate cells role in the course of metabolic disorders development–A molecular overview. *Pharmacological Research*, *170*, 105739.
58. Breikaa, R. M., Algandaby, M. M., El-Demerdash, E., & Abdel-Naim, A. B. (2013). Multimechanistic antifibrotic effect of biochanin a in rats: implications of proinflammatory and profibrogenic mediators. *PLoS One*, *8*(7), e69276.

59. Brewer, M. S. (2011). Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Comprehensive reviews in food science and food safety*, *10*(4), 221-247.
60. Butler, T. C. (1961). Reduction of carbon tetrachloride in vivo and reduction of carbon tetrachloride and chloroform in vitro by tissues and tissue constituents. *Journal of Pharmacology and Experimental Therapeutics*, *134*(3), 311-319.
61. Calligar, A., & Vannini, V. (1975). Electron spin resonance study of homolytic cleavage of carbon tetrachloride in rat liver: trichloromethyl free radicals. *Pharmacological Research Communications*, *7*(4), 323-329.
62. Canbay, A., Taimr, P., Torok, N., Higuchi, H., Friedman, S., & Gores, G. J. (2003). Apoptotic body engulfment by a human stellate cell line is profibrogenic. *Laboratory investigation*, *83*(5), 655-663.
63. Casas-Grajales, S., & Muriel, P. (2015). Antioxidants in liver health. *World journal of gastrointestinal pharmacology and therapeutics*, *6*(3), 59.
64. Chakraborty, J. B., Oakley, F., & Walsh, M. J. (2012). Mechanisms and biomarkers of apoptosis in liver disease and fibrosis. *International journal of hepatology*, *2012*(1), 648915.
65. Chance, B., & Maehly, A. C. (1955). [136] Assay of catalases and peroxidases.
66. Chattopadhyay, R. (2003). Possible mechanism of hepatoprotective activity of Azadirachta indica leaf extract: part II. *Journal of ethnopharmacology*, *89*(2-3), 217-219.
67. Chaudière, J., & Ferrari-Iliou, R. (1999). Intracellular antioxidants: from chemical to biochemical mechanisms. *Food and chemical toxicology*, *37*(9-10), 949-962.
68. Chauhan, N. S. (1999). *Medicinal and aromatic plants of Himachal Pradesh*. Indus publishing.
69. Chávez, E., Reyes-Gordillo, K., Segovia, J., Shibayama, M., Tsutsumi, V., Vergara, P., ... & Muriel, P. (2008). Resveratrol prevents fibrosis, NF-κB activation and TGF-β increases induced by chronic CCl4 treatment in rats. *Journal of Applied Toxicology: An International Journal*, *28*(1), 35-43.
70. Chawla, A., Chawla, R., & Jaggi, S. (2016). Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum?. *Indian journal of endocrinology and metabolism*, *20*(4), 546-551.

71. Cheesman, M. J., Ilanko, A., Blonk, B., & Cock, I. E. (2017). Developing new antimicrobial therapies: are synergistic combinations of plant extracts/compounds with conventional antibiotics the solution?. *Pharmacognosy reviews*, *11*(22), 57.
72. Chen, Q., Zhan, Q., Li, Y., Sun, S., Zhao, L., Zhang, H., & Zhang, G. (2017). Schisandra lignan extract protects against carbon tetrachloride-induced liver injury in mice by inhibiting oxidative stress and regulating the NF-κB and JNK signaling pathways. *Evidence-Based Complementary and Alternative Medicine*, *2017*(1), 5140297.
73. Cheng, Y., & Yang, J. M. (2011). Survival and death of endoplasmic-reticulum-stressed cells: Role of autophagy. *World Journal of Biological Chemistry*, *2*(10), 226.
74. Chomczynski, P., & Sacchi, N. (2006). The single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction: twenty-something years on. *Nature protocols*, *1*(2), 581-585.
75. Chopra, S., & Saxena, R. (2018). Drug-induced liver injury—perspectives from pathology. *Current Pharmacology Reports*, *4*, 182-192.
76. Christaki, E., Bonos, E., Giannenas, I., & Florou-Paneri, P. (2012). Aromatic plants as a source of bioactive compounds. *Agriculture*, *2*(3), 228-243.
77. Christensen, L. P., & Brandt, K. (2006). Bioactive polyacetylenes in food plants of the Apiaceae family: occurrence, bioactivity and analysis. *Journal of pharmaceutical and biomedical analysis*, *41*(3), 683-693.
78. Chung, M. J., Park, Y. I., & Kwon, K. H. (2015). Neuroprotective effects of cirsium setidens, pleurospermum kamtschaticumin, and allium victorials based on antioxidant and p38 phosphorylation inhibitory activities in SK-N-SH neuronal cells. *Journal of the Korean Society of Food Science and Nutrition*, *44*(3), 347-355.
79. Corsini, A., & Bortolini, M. (2013). Drug-induced liver injury: The role of drug metabolism and transport. *The journal of clinical pharmacology*, *53*(5), 463-474.
80. Cui, Y., Yang, X., Lu, X., Chen, J., & Zhao, Y. (2014). Protective effects of polyphenols-enriched extract from Huangshan Maofeng green tea against CCl4-induced liver injury in mice. *Chemico-biological interactions*, *220*, 75-83.
81. Cullinan, S. B., Zhang, D., Hannink, M., Arvisais, E., Kaufman, R. J., & Diehl, J. A. (2003). Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. *Molecular and cellular biology*, *23*(20), 7198-7209.

82. Davì, G., Falco, A., & Patrono, C. (2005). Lipid peroxidation in diabetes mellitus. *Antioxidants & redox signaling*, *7*(1-2), 256-268.
83. de Diego-Otero, Y., Romero-Zerbo, Y., Bekay, R. E., Decara, J., Sanchez, L., Fonseca, F. R. D., & Arco-Herrera, I. D. (2009). α-tocopherol protects against oxidative stress in the fragile X knockout mouse: an experimental therapeutic approach for the Fmr1 deficiency. *Neuropsychopharmacology*, *34*(4), 1011-1026.
84. de Oliveira, M. S., Almeida, M. M., Salazar, M. L. A. R., Pires, F. C. S., Bezerra, F. W. F., Cunha, V. M. B., ... & de Carvalho Junior, R. N. (2018). Potential of medicinal use of essential oils from aromatic plants. *Potential of essential oils*, 1-21.
85. Deng, G. F., Xu, X. R., Guo, Y. J., Xia, E. Q., Li, S., Wu, S., ... & Li, H. B. (2012a). Determination of antioxidant property and their lipophilic and hydrophilic phenolic contents in cereal grains. *Journal of Functional Foods*, *4*(4), 906-914.
86. Deng, G. F., Shen, C., Xu, X. R., Kuang, R. D., Guo, Y. J., Zeng, L. S., ... & Li, H. B. (2012b). Potential of fruit wastes as natural resources of bioactive compounds. *International journal of molecular sciences*, *13*(7), 8308-8323.
87. Deng, G. F., Lin, X., Xu, X. R., Gao, L. L., Xie, J. F., & Li, H. B. (2013). Antioxidant capacities and total phenolic contents of 56 vegetables. *Journal of functional foods*, *5*(1), 260-266.
88. Devaraj, V. C., Krishna, B. G., Viswanatha, G. L., Kamath, J. V., & Kumar, S. (2011). Hepatoprotective activity of Hepax–A polyherbal formulation. *Asian Pacific journal of tropical biomedicine*, *1*(2), 142-146.
89. Devasagayam, T. P. A., & Sainis, K. B. (2002). Immune system and antioxidants, especially those derived from Indian medicinal plants.
90. Dhama, K., Latheef, S. K., Mani, S., Samad, H. A., Karthik, K., Tiwari, R., ... & Tufarelli, V. (2015). Multiple beneficial applications and modes of action of herbs in poultry health and production-a review. *International Journal of pharmacology*, *11*(3), 152-176.
91. Dhiman, A. K. (2004). *Medicinal plants of Uttaranchal state* (Vol. 2). Chowkhamba Sanskrit Series Office.
92. Di Meo, S., & Venditti, P. (2020). Evolution of the knowledge of free radicals and other oxidants. *Oxidative medicine and cellular longevity*, *2020*(1), 9829176.

93. Dkhil, M. A., Abdel Moneim, A. E., Hafez, T. A., Mubaraki, M. A., Mohamed, W. F., Thagfan, F. A., & Al-Quraishy, S. (2019). Myristica fragrans kernels prevent paracetamol-induced hepatotoxicity by inducing anti-apoptotic genes and Nrf2/HO-1 pathway. *International journal of molecular sciences*, *20*(4), 993.
94. Doss, A., & Anand, S. P. (2014). Antihyperglycemic activity of methanol and aqueous extracts of Pergularia daemia Linn. *African Journal of Biotechnology*, *13*(1), 170.
95. Draznin, B., Aroda, V. R., Bakris, G., Benson, G., Brown, F. M., Freeman, R., ... & American Diabetes Association Professional Practice Committee. (2022). 16. Diabetes Care in the Hospital: Standards of Medical Care in Diabetes-2022. *Diabetes Care*, *45*.
96. Dröge, W. (2002). Free radicals in the physiological control of cell function. *Physiological reviews*.
97. Ducasa, G. M., Mitrofanova, A., & Fornoni, A. (2019). Crosstalk between lipids and mitochondria in diabetic kidney disease. *Current diabetes reports*, *19*, 1-12
98. Dufey, E., Sepúlveda, D., Rojas-Rivera, D., & Hetz, C. (2014). Cellular mechanisms of endoplasmic reticulum stress signaling in health and disease. 1. An overview. *American Journal of Physiology-Cell Physiology*, *307*(7), C582-C594.
99. Durazzo, A., Lucarini, M., & Santini, A. (2021). Plants and diabetes: Description, role, comprehension and exploitation. *International journal of molecular sciences*, *22*(8), 3938.
100. Dutta, S., Chakraborty, A. K., Dey, P., Kar, P., Guha, P., Sen, S., ... & Chaudhuri, T. K. (2018). Amelioration of CCl4 induced liver injury in swiss albino mice by antioxidant rich leaf extract of Croton bonplandianus Baill. *PloS one*, *13*(4), e0196411.
101. Dzoyem, J. P., & Eloff, J. N. (2015). Anti-inflammatory, anticholinesterase and antioxidant activity of leaf extracts of twelve plants used traditionally to alleviate pain and inflammation in South Africa. *Journal of Ethnopharmacology*, *160*, 194-201.
102. Ebaid, H., Al-Tamimi, J., Habila, M., Hassan, I., Rady, A., & Alhazza, I. M. (2021). Potential therapeutic effect of synthesized AgNP using curcumin extract on CCl4-induced nephrotoxicity in male mice. *Journal of King Saud University-Science*, *33*(2), 101356.
103. El-Aarag, B., Khairy, A., Khalifa, S. A., & El-Seedi, H. R. (2019). Protective effects of flavone from Tamarix aphylla against CCl4-induced liver injury in mice mediated by suppression of oxidative stress, apoptosis and angiogenesis. *International journal of molecular sciences*, *20*(20), 5215.

104. El-Baz, A. M., Mosbah, R. A., Goda, R. M., Mansour, B., Sultana, T., Dahms, T. E., & El-Ganiny, A. M. (2021). Back to nature: Combating Candida albicans biofilm, phospholipase and hemolysin using plant essential oils. *Antibiotics*, *10*(1), 81.

105. El-Kashef, D. H., & Zaghloul, R. A. (2022). Ameliorative effect of montelukast against carbon tetrachloride-induced hepatotoxicity: Targeting NLRP3 inflammasome pathway. *Life Sciences*, *304*, 120707.

106. El-kholy, T. A., Hassanen, N. H. M., & Abbas, H. Y. (2013). Protection of the mushroom (shiitake" lentinus-edodes) against carbon-tetrachloride-induced renal injury in rats. *Life Sci J*, *10*(1), 1701-8.

107. Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of biochemistry and biophysics*, *82*(1), 70-77.

108. Elmarakby, A. A., & Sullivan, J. C. (2012). Relationship between oxidative stress and inflammatory cytokines in diabetic nephropathy. *Cardiovascular therapeutics*, *30*(1), 49-59.

109. Elsawy, H., Badr, G. M., Sedky, A., Abdallah, B. M., Alzahrani, A. M., & Abdel-Moneim, A. M. (2019). Rutin ameliorates carbon tetrachloride (CCl4)-induced hepatorenal toxicity and hypogonadism in male rats. *PeerJ*, *7*, e7011.

110. Fallowfield, J. A., Mizuno, M., Kendall, T. J., Constandinou, C. M., Benyon, R. C., Duffield, J. S., & Iredale, J. P. (2007). Scar-associated macrophages are a major source of hepatic matrix metalloproteinase-13 and facilitate the resolution of murine hepatic fibrosis. *The Journal of Immunology*, *178*(8), 5288-5295.

111. Fatima, M., & Khan, M. R. (2023). Jasminum humile (Linn) ameliorates CCl4-induced oxidative stress by regulating ER stress, inflammatory, and fibrosis markers in rats. *Inflammopharmacology*, *31*(3), 1405-1421.

112. Fatima, M., & Khan, M. R. (2024). Investigating the role of Polyphenols from Pleurospermum candollei (DC.) extract against diabetic nephropathy through modulating inflammatory cytokines and renal gene expression in rats. *Journal of Molecular Structure*, 137832.

113. Faubion, W. A., & Gores, G. J. (1999). Death receptors in liver biology and pathobiology. *Hepatology*, *29*(1), 1-4.

114. Felter, S. P., Zhang, X., & Thompson, C. (2021). Butylated hydroxyanisole: Carcinogenic food additive to be avoided or harmless antioxidant important to protect food supply?. *Regulatory Toxicology and Pharmacology*, *121*, 104887.

115. Fennell, C. W., Lindsey, K. L., McGaw, L. J., Sparg, S. G., Stafford, G. I., Elgorashi, E. E., ... & Van Staden, J. (2004). Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. *Journal of ethnopharmacology*, *94*(2-3), 205-217.

116. Ferencik, M., Mayrhofer, T., Lu, M. T., Bittner, D. O., Emami, H., Puchner, S. B., ... & Hoffmann, U. (2022). Coronary atherosclerosis, cardiac troponin, and interleukin-6 in patients with chest pain: the PROMISE trial results. *Cardiovascular Imaging*, *15*(8), 1427-1438.

117. Ferri, K. F., & Kroemer, G. (2001). Organelle-specific initiation of cell death pathways. *Nature cell biology*, *3*(11), E255-E263.

118. FINCO, D. R. (1980). Kidney function. In *Clinical biochemistry of domestic animals* (pp. 337-400). Academic Press.

119. Forbes, J. M., Cooper, M. E., Oldfield, M. D., & Thomas, M. C. (2003). Role of advanced glycation end products in diabetic nephropathy. *Journal of the American Society of Nephrology*, *14*(suppl_3), S254-S258.

120. Frangogiannis, N. G. (2019). Cardiac fibrosis: Cell biological mechanisms, molecular pathways and therapeutic opportunities. *Molecular aspects of medicine*, *65*, 70-99.

121. Fristiohady, A., Wahyuni, W., Bafadal, M., Sangadji, F., Malaka, M. H., Malik, F., ... & Sahidin, I. (2020). Nephroprotective effect of extract etlingera elatior (jack) RM Smith on CCl4-induced nephrotoxicity in rats. *Current Research on Biosciences and Biotechnology*, *1*(2), 62-65.

122. Fu, Y., Zheng, S., Lin, J., Ryerse, J., & Chen, A. (2008). Curcumin protects the rat liver from CCl4-caused injury and fibrogenesis by attenuating oxidative stress and suppressing inflammation. *Molecular pharmacology*, *73*(2), 399-409.

123. Fullerton, J. N., & Gilroy, D. W. (2016). Resolution of inflammation: a new therapeutic frontier. *Nature reviews Drug discovery*, *15*(8), 551-567.

124. Fürst, R., & Zündorf, I. (2014). Plant-derived anti-inflammatory compounds: Hopes and disappointments regarding the translation of preclinical knowledge into clinical progress. *Mediators of inflammation*, *2014*(1), 146832.

125. Furuno, K., Akasako, T., & Sugihara, N. (2002). The contribution of the pyrogallol moiety to the superoxide radical scavenging activity of flavonoids. *Biological and Pharmaceutical Bulletin*, *25*(1), 19-23.

126. Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., ... & Martín, C. (2020). Pathophysiology of type 2 diabetes mellitus. *International journal of molecular sciences*, *21*(17), 6275.

127. Galluzzi, L., López-Soto, A., Kumar, S., & Kroemer, G. (2016). Caspases connect cell-death signaling to organismal homeostasis. *Immunity*, *44*(2), 221-231.

128. Gandhare, B., Soni, N., & Dhongade, H. J. (2010). In vitro antioxidant activity of Bombax ceiba. *Int. J. Biomed. Res*, *1*(2), 31-36.

129. García, L. F. (2020). Immune response, inflammation, and the clinical spectrum of COVID-19. *Frontiers in immunology*, *11*, 556786.

130. Gerber, F., Krummen, M., Potgeter, H., Roth, A., Siffrin, C., & Spoendlin, C. (2004). Practical aspects of fast reversed-phase high-performance liquid chromatography using 3 μm particle packed columns and monolithic columns in pharmaceutical development and production working under current good manufacturing practice. *Journal of Chromatography A*, *1036*(2), 127-133.

131. Ghasemi, A., Khalifi, S., & Jedi, S. (2014). Streptozotocin-nicotinamide-induced rat model of type 2 diabetes. *Acta Physiologica Hungarica*, *101*(4), 408-420.

132. Ghosh, S. K., & Sur, P. K. (2015). Sanjeevani and Bishalyakarani plants-myth or real. *Int. J. Curr. Res*, *7*, 19964-19969.

133. Giannenas, I., Bonos, E., Christaki, E., & Florou-Paneri, P. (2018). Oregano: A feed additive with functional properties. In *Therapeutic Foods* (pp. 179-208). Academic Press.

134. Giannenas, I., Sidiropoulou, E., Bonos, E., Christaki, E., & Florou-Paneri, P. (2020). The history of herbs, medicinal and aromatic plants, and their extracts: Past, current situation and future perspectives. In *Feed additives*, 1-18.

135. Giugliano, D., Ceriello, A., & Paolisso, G. (1996). Oxidative stress and diabetic vascular complications. *Diabetes care*, *19*(3), 257-267.

136. Giustarini, D., Rossi, R., Milzani, A., & Dalle-Donne, I. (2008). Nitrite and nitrate measurement by Griess reagent in human plasma: evaluation of interferences and standardization. *Methods in enzymology*, *440*, 361-380.

137. Gökmen, V., Serpen, A., & Fogliano, V. (2009). Direct measurement of the total antioxidant capacity of foods: the 'QUENCHER'approach. *Trends in Food Science & Technology*, *20*(6-7), 278-288.

138. Goupy, P., Dufour, C., Loonis, M., & Dangles, O. (2003). Quantitative kinetic analysis of hydrogen transfer reactions from dietary polyphenols to the DPPH radical. *Journal of Agricultural and Food Chemistry*, *51*(3), 615-622.

139. Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S., & Tannenbaum, S. R. (1982). Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Analytical biochemistry*, *126*(1), 131-138.

140. Grisham, M. B., Johnson, G. G., & Lancaster Jr, J. R. (1996). Quantitation of nitrate and nitrite in extracellular fluids. In *Methods in enzymology*, 268, 237-246.

141. Gülcin, I. (2012). Antioxidant activity of food constituents: an overview. *Archives of toxicology*, *86*, 345-391.

142. Guo, Y. J., Deng, G. F., Xu, X. R., Wu, S., Li, S., Xia, E. Q., ... & Li, H. B. (2012). Antioxidant capacities, phenolic compounds and polysaccharide contents of 49 edible macro-fungi. *Food & Function*, *3*(11), 1195-1205.

143. Gupta, A., Kumar, R., & Pandey, A. K. (2020). Antioxidant and antidiabetic activities of Terminalia bellirica fruit in alloxan induced diabetic rats. *South African journal of botany*, *130*, 308-315.

144. Gupta, S. C., Patchva, S., & Aggarwal, B. B. (2013). Therapeutic roles of curcumin: lessons learned from clinical trials. *The AAPS journal*, *15*, 195-218.

145. Hafez, M. M., Al-Harbi, N. O., Al-Hoshani, A. R., Al-Hosaini, K. A., Al Shrari, S. D., Al Rejaie, S. S., ... & Al-Shabanah, O. A. (2015). Hepato-protective effect of rutin via IL-6/STAT3 pathway in CCl 4-induced hepatotoxicity in rats. *Biological research*, *48*, 1-10.

146. Halliwell, B. (2007a). Flavonoids: a Re-Run of the Carotenoids Story?. In *Dietary Supplements and Health: Novartis Foundation Symposium,* 282, 93-104.

147. Halliwell B (2007b). Cellular responses to oxidative stress: adaptation, damage, repair, senescence and death. Free radi. bio. med. 187-267.

148. Han, H., Cao, A., Wang, L., Guo, H., Zang, Y., Li, Z., ... & Peng, W. (2017). Huangqi decoction ameliorates streptozotocin-induced rat diabetic nephropathy through antioxidant and regulation of the TGF-β/MAPK/PPAR-γ signaling. *Cellular Physiology and Biochemistry*, *42*(5), 1934-1944.

149. Hassan, I., Ebaid, H., Alhazza, I. M., & Al-Tamimi, J. (2020). The Alleviative Effect of Vitamin B2 on Potassium Bromate-Induced Hepatotoxicity in Male Rats. *BioMed Research International*, *2020*(1), 8274261.

150. Hassan, S. S. U., Abdel-Daim, M. M., Behl, T., & Bungau, S. (2022). Natural products for chronic diseases: A ray of hope. *Molecules*, *27*(17), 5573.

151. Hefnawy, H. T. M., & Ramadan, M. F. (2013). Protective effects of Lactuca sativa ethanolic extract on carbon tetrachloride induced oxidative damage in rats. *Asian Pacific Journal of Tropical Disease*, *3*(4), 277-285.

152. Henein, M. Y., Vancheri, S., Longo, G., & Vancheri, F. (2022). The role of inflammation in cardiovascular disease. *International journal of molecular sciences*, *23*(21), 12906.

153. Hengartner, M. O. (2000). The biochemistry of apoptosis. *Nature*, *407*(6805), 770-776.

154. Herald, T. J., Gadgil, P., Perumal, R., Bean, S. R., & Wilson, J. D. (2014). High-throughput micro-plate HCl–vanillin assay for screening tannin content in sorghum grain. *Journal of the Science of Food and Agriculture*, *94*(10), 2133-2136.

155. Hirano, T., & Murakami, M. (2020). COVID-19: a new virus, but a familiar receptor and cytokine release syndrome. *Immunity*, *52*(5), 731-733.

156. Hirano, T. (2021). IL-6 in inflammation, autoimmunity and cancer. *International immunology*, *33*(3), 127-148.

157. Hirschfield, G. M., Dyson, J. K., Alexander, G. J., Chapman, M. H., Collier, J., Hübscher, S., ... & Jones, D. E. (2018). The British Society of Gastroenterology/UK-PBC primary biliary cholangitis treatment and management guidelines. *Gut*, *67*(9), 1568-1594.

158. Ho, E., & Bray, T. M. (1999). Antioxidants, NFκB activation, and diabetogenesis. *Proceedings of the Society for Experimental Biology and Medicine*, *222*(3), 205-213.

159. Honda, T., Hirakawa, Y., & Nangaku, M. (2019). The role of oxidative stress and hypoxia in renal disease. *Kidney Research and Clinical Practice*, *38*(4), 414.

160. Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. *Nature*, *444*(7121), 860-867.

161. Hu, B., Huang, S., & Yin, L. (2021). The cytokine storm and COVID-19. *Journal of medical virology*, *93*(1), 250-256.

162. Huang, H., Lei, H., Yang, F., Fan, X., Dang, Q., & Li, Y. (2018). Activation of the bile acid receptor GPBAR1 (TGR5) ameliorates interleukin-1β (IL-1β)-induced chondrocytes senescence. *Biomedicine & Pharmacotherapy*, *106*, 1713-1719.

163. Huang, W., Li, L., Tian, X., Yan, J., Yang, X., Wang, X., ... & Qiu, G. (2015). Astragalus and Paeoniae radix rubra extract inhibits liver fibrosis by modulating the transforming growth factor-β/Smad pathway in rats. *Molecular medicine reports*, *11*(2), 805-814.

164. Huang, X., Wang, L., Meng, M., Zhang, S., Pham, T. T. H., Jiang, L., ... & Huang, R. (2020). Extract of Averrhoacarambola L.(Oxalidaceae) roots ameliorates carbon tetrachloride-induced hepatic fibrosis in rats. *Biomedicine & Pharmacotherapy*, *121*, 109516.

165. Huang, Y. L., Oppong, M. B., Guo, Y., Wang, L. Z., Fang, S. M., Deng, Y. R., & Gao, X. M. (2019). The Oleaceae family: A source of secoiridoids with multiple biological activities. *Fitoterapia*, *136*, 104155.

166. Huyghebaert, G., Ducatelle, R., & Van Immerseel, F. (2011). An update on alternatives to antimicrobial growth promoters for broilers. *The Veterinary Journal*, *187*(2), 182-188.

167. Huynh, P., & Chai, Z. (2019). Transforming growth factor β (TGFβ) and related molecules in chronic kidney disease (CKD). *Clinical Science*, *133*(2), 287-313.

168. Ialenti, A., Moncada, S., & Di Rosa, M. (1993). Modulation of adjuvant arthritis by endogenous nitric oxide. *British journal of pharmacology*, *110*(2), 701-706.

169. Ibrahim, Z. S., Alkafafy, M. E., Ahmed, M. M., & Soliman, M. M. (2016). Renoprotective effect of curcumin against the combined oxidative stress of diabetes and nicotine in rats. *Molecular medicine reports*, *13*(4), 3017-3026.

170. Ieronimakis, N., Hays, A. L., Janebodin, K., Mahoney Jr, W. M., Duffield, J. S., Majesky, M. W., & Reyes, M. (2013). Coronary adventitial cells are linked to perivascular cardiac fibrosis via TGFβ1 signaling in the mdx mouse model of Duchenne muscular dystrophy. *Journal of molecular and cellular cardiology*, *63*, 122-134.

171. Iqbal, M., & Wright, D. J. (1996). Host resistance to insecticides can confer protection to endo-larval parasitoids. *Bulletin of Entomological Research*, *86*(6), 721-723.

172. Iqbal, M., Sharma, S. D., Rezazadeh, H., Hasan, N., Abdulla, M., & Athar, M. J. R. R. (1996). Glutathione metabolizing enzymes and oxidative stress in ferric nitrilotriacetate mediated hepatic injury. *Redox Report*, *2*(6), 385-391.

173. Islam, M. S. (2020). Stimulus-secretion coupling in beta-cells: from basic to bedside. *Calcium Signaling*, 943-963.

174. Jacob, S., Nair, A. B., & Morsy, M. A. (2022). Dose conversion between animals and humans: A practical solution. *Indian J. Pharm. Educ. Res*, *56*, 600-607.

175. Jan, S., & Khan, M. R. (2016). Antipyretic, analgesic and anti-inflammatory effects of Kickxia ramosissima. *Journal of ethnopharmacology*, *182*, 90-100.

176. Jeong, G. S., Lee, S. H., Jeong, S. N., Kim, Y. C., & Kim, E. C. (2009). Anti-inflammatory effects of apigenin on nicotine-and lipopolysaccharide-stimulated human periodontal ligament cells via heme oxygenase-1. *International Immunopharmacology*, *9*(12), 1374-1380.

177. Jiang, Y., Wang, C., Li, Y. Y., Wang, X. C., An, J. D., Wang, Y. J., & Wang, X. J. (2014). Mistletoe alkaloid fractions alleviates carbon tetrachloride-induced liver fibrosis through inhibition of hepatic stellate cell activation via TGF-β/Smad interference. *Journal of ethnopharmacology*, *158*, 230-238.

178. Jiang, M., Huang, C., Wu, Q., Su, Y., Wang, X., Xuan, Z., ... & Ge, C. (2023). Sini San ameliorates CCl4-induced liver fibrosis in mice by inhibiting AKT-mediated hepatocyte apoptosis. *Journal of Ethnopharmacology*, *303*, 115965.

179. Jothy, S. L., Zakaria, Z., Chen, Y., Lau, Y. L., Latha, L. Y., & Sasidharan, S. (2011). Acute oral toxicity of methanolic seed extract of Cassia fistula in mice. *Molecules*, *16*(6), 5268-5282

180. JhaJay, C., ChowBryna, S. M., & CooperMark, E. (2016). Diabetes and kidney disease: role of oxidative stress. *Antioxidants & redox signaling*.

181. Jit, B. P., Pattnaik, S., Arya, R., Dash, R., Sahoo, S. S., Pradhan, B., ... & Behera, R. K. (2022). Phytochemicals: A potential next generation agent for radioprotection. *Phytomedicine*, *106*, 154188.

182. Kahn, S. E., Cooper, M. E., & Del Prato, S. (2014). Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. *The Lancet*, *383*(9922), 1068-1083.

183. Kakkar, P., Das, B., & Viswanathan, P. N. (1984). A modified spectrophotometric assay of superoxide dismutase.

184. Kakkar, R., Mantha, S. V., Radhi, J., Prasad, K., & Kalra, J. (1997). Antioxidant defense system in diabetic kidney: a time course study. *Life sciences*, *60*(9), 667-679.

185. Kala, C. P. (2005). Ethnomedicinal botany of the Apatani in the Eastern Himalayan region of India. *Journal of ethnobiology and Ethnomedicine*, *1*, 1-8.

186. Kalra, A.; Yetiskul, E.; Wehrle, C.J.; Tuma, F. Physiology, Liver; StatPearls Publishing: Petersburg, VA, USA, 2021.

187. Kanchana, S. P., VJ, U. P., & Horta, S. S. (2023). In vitro antioxidant activity and electro analytic approach on Indigofera tirunelvelica Sanjappa. *Materials Today: Proceedings*, *92*, 1283-1288.

188. Kang, B. H., Racicot, K., Pilkenton, S. J., & Apostolidis, E. (2014). Evaluation of the in vitro anti-hyperglycemic effect of Cinnamomum cassia derived phenolic phytochemicals, via carbohydrate hydrolyzing enzyme inhibition. *Plant foods for human nutrition*, *69*, 155-160.

189. Kankara, S. S., Ibrahim, M. H., Mustafa, M., & Go, R. (2015). Ethnobotanical survey of medicinal plants used for traditional maternal healthcare in Katsina state, Nigeria. *South African journal of botany*, *97*, 165-175.

190. Karlsen, A., Retterstøl, L., Laake, P., Paur, I., Kjølsrud-Bøhn, S., Sandvik, L., & Blomhoff, R. (2007). Anthocyanins Inhibit Nuclear Factor-κB Activation in Monocytes and Reduce Plasma Concentrations of Pro-Inflammatory Mediators in Healthy Adults, 3. *The Journal of nutrition*, *137*(8), 1951-1954.

191. Kashyap, M. L., Hynd, B. A., & Robinson, K. (1980). A rapid and simple method for measurement of total protein in very low density lipoproteins by the Lowry assay. *Journal of Lipid Research*, *21*(4), 491-495.

192. Kawarty, A. M. A., Behçet, L., & Çakilcioğlu, U. (2020). An ethnobotanical survey of medicinal plants in Ballakayati (Erbil, North Iraq). *Turkish Journal of Botany*, *44*(3), 345-357.

193. Keane, K. N., Cruzat, V. F., Carlessi, R., de Bittencourt Jr, P. I. H., & Newsholme, P. (2015). Molecular events linking oxidative stress and inflammation to insulin resistance and β-cell dysfunction. *Oxidative medicine and cellular longevity*, *2015*(1), 181643.

194. Khalil, R., Ali, Q., Hafeez, M. M., & Malik, A. (2020). Phenolic acid profiling by RP-HPLC: evaluation of antibacterial and anticancer activities of Conocarpus erectus plant extracts. *Biological and Clinical Sciences Research Journal*, *2020*(1).

195. Khan, A., Farooq, U., Ullah, F., Iqbal, J., Khan, A. F., Zaib, S., ... & Azarpira, A. (2014). Determination of biological activities and total phenolic contents of flowers of Jasminum humile and roots of Dorema aucheri. *J Chem Soc Pak*, *36*(2), 291-5.

196. Khan, K. U., Shah, M., Ahmad, H., Ashraf, M., Rahman, I. U., Iqbal, Z., ... & Majid, A. (2015a). Investigation of traditional veterinary phytomedicines used in Deosai Plateau, Pakistan. *Global Vet*, *15*(4), 381-388.

197. Khan, M. R., & Siddique, F. (2012). Antioxidant effects of Citharexylum spinosum in CCl4 induced nephrotoxicity in rat. *Experimental and toxicologic pathology*, *64*(4), 349-355.

198. Khan, M. R., & Zehra, H. (2013). Amelioration of CCl4-induced nephrotoxicity by Oxalis corniculata in rat. *Experimental and toxicologic pathology*, *65*(3), 327-334.

199. Khan, M. R., Rizvi, W., Khan, G. N., Khan, R. A., & Shaheen, S. (2009). Carbon tetrachloride-induced nephrotoxicity in rats: Protective role of Digera muricata. *Journal of ethnopharmacology*, *122*(1), 91-99.

200. Khan, R. A., Khan, M. R., & Sahreen, S. (2010a). Evaluation of Launaea procumbens use in renal disorders: A rat model. *Journal of ethnopharmacology*, *128*(2), 452-461.

201. Khan, R. A., Khan, M. R., Sahreen, S., & Bokhari, J. (2010b). Prevention of CCl4-induced nephrotoxicity with Sonchus asper in rat. *Food and Chemical Toxicology*, *48*(8-9), 2469-2476.

202. Khan, R. A. (2012). Protective effects of Sonchus asper (L.) Hill,(Asteraceae) against CCl 4-induced oxidative stress in the thyroid tissue of rats. *BMC complementary and alternative medicine*, *12*, 1-8.

203. Khan, S. W., Abbas, Q., Hassan, S. N., Khan, H., & Hussain, A. (2015b). Medicinal Plants of Turmic Valley (Central Karakoram National Park), Gilgit-Baltistan, Pakistan. *Journal of Bioresource Management*, *2*(2), 11.

204. Khan, S. W., Khatoon, S., Hussain, A., Abbas, H., & Ali, H. (2018). Inventorying and monitoring of alpine specific genera and species of Haramosh and Bagraote valleys.,(Karakoram range) Gilgit-Baltistan, Pakistan. *Pakistan Journal of Agricultural Sciences*, *55*(3).

205. Khedr, N. F., & Khedr, E. G. (2014). Antioxidant and anti-inflammatory effects of curcumin on CCl4–induced liver fibrosis in rats. *Am. J. Biomed. Sci*, *6*(3), 191-200.

206. Khumalo, G. P., Van Wyk, B. E., Feng, Y., & Cock, I. E. (2022). A review of the traditional use of southern African medicinal plants for the treatment of inflammation and inflammatory pain. *Journal of Ethnopharmacology*, *283*, 114436.

207. Kilany, O. E., El-Beltagy, M. A., & El-Sherbeeny, N. A. (2020). Tribulus terrestris ameliorates carbon tetrachloride-induced hepatotoxicity in male rats through suppression of oxidative stress and inflammation. *Environmental Science and Pollution Research*, *27*, 24967-24981.

208. Kim, D. O., Jeong, S. W., & Lee, C. Y. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food chemistry*, *81*(3), 321-326.

209. Kiraz, Y., Adan, A., Kartal Yandim, M., & Baran, Y. (2016). Major apoptotic mechanisms and genes involved in apoptosis. *Tumor Biology*, *37*, 8471-8486.

210. Koenig, R. J. (1980). Hemoglobin AIc and diabetes mellitus. *Annual review of medicine*, *31*(1), 29-34.

211. Kohen, R., & Nyska, A. (2002). Invited review: oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicologic pathology*, *30*(6), 620-650.

212. Kohn, S., Fradis, M., Robinson, E., & Iancu, T. C. (2005). Hepatotoxicity of combined treatment with cisplatin and gentamicin in the guinea pig. *Ultrastructural pathology*, *29*(2), 129-137.

213. Koyama, Y., & Brenner, D. A. (2017). Liver inflammation and fibrosis. *The Journal of clinical investigation*, *127*(1), 55-64.

214. Krishnaiah, D., Sarbatly, R., & Nithyanandam, R. (2011). A review of the antioxidant potential of medicinal plant species. *Food and bioproducts processing*, *89*(3), 217-233.

215. Krause, M., Rodrigues-Krause, J., O'Hagan, C., De Vito, G., Boreham, C., Susta, D., ... & Murphy, C. (2012). Differential nitric oxide levels in the blood and skeletal muscle of type

2 diabetic subjects may be consequence of adiposity: a preliminary study. *Metabolism*, *61*(11), 1528-1537.

216. Kumar, S., Singh, R., Vasudeva, N., & Sharma, S. (2012a). Acute and chronic animal models for the evaluation of anti-diabetic agents. *Cardiovascular diabetology*, *11*, 1-13.

217. Kumar, S., Sharma, U. K., Sharma, A. K., & Pandey, A. K. (2012b). Protective efficacy of Solanum xanthocarpum root extracts against free radical damage: phytochemical analysis and antioxidant effect. *Cellular and Molecular Biology*, *58*(1), 174-181.

218. Kumar, S., & Pandey, A. (2015). Free radicals: health implications and their mitigation by herbals. *British Journal of Medicine and Medical Research*, *7*(6), 438-457.

219. Kumar, S., Saini, R., Suthar, P., Kumar, V., & Sharma, R. (2022). Plant secondary metabolites: Their food and therapeutic importance. In *Plant secondary metabolites: Physico-chemical properties and therapeutic applications*, 371-413.

220. Kunnumakkara, A. B., Sailo, B. L., Banik, K., Harsha, C., Prasad, S., Gupta, S. C., ... & Aggarwal, B. B. (2018). Chronic diseases, inflammation, and spices: how are they linked?. *Journal of translational medicine*, *16*, 1-25.

221. Kunwar, A., & Priyadarsini, K. I. (2011). Free radicals, oxidative stress and importance of antioxidants in human health. *J Med Allied Sci*, *1*(2), 53-60.

222. KWON, Y. I., Apostolidis, E., & Shetty, K. (2008). Inhibitory potential of wine and tea against α-amylase and α-glucosidase for management of hyperglycemia linked to type 2 diabetes. *Journal of Food Biochemistry*, *32*(1), 15-31.

223. Labonte, A. C., Tosello-Trampont, A. C., & Hahn, Y. S. (2014). The role of macrophage polarization in infectious and inflammatory diseases. *Molecules and cells*, *37*(4), 275-285.

224. Lamia, S. S., Emran, T., Rikta, J. K., Chowdhury, N. I., Sarker, M., Jain, P., ... & Reza, H. M. (2021). Coenzyme Q10 and silymarin reduce CCl4-induced oxidative stress and liver and kidney injury in ovariectomized rats—implications for protective therapy in chronic liver and kidney diseases. *Pathophysiology*, *28*(1), 50-63.

225. Laveti, D., Kumar, M., Hemalatha, R., Sistla, R., Gm Naidu, V., Talla, V., ... & Nagpal, R. (2013). Anti-inflammatory treatments for chronic diseases: a review. *Inflammation & Allergy-Drug Targets (Formerly Current Drug Targets-Inflammation & Allergy)(Discontinued)*, *12*(5), 349-361.

226. Lee, D., Park, J., Kim, M., Lee, Y., Seo, J., Ha, N., & Kim, K. (2015). Hepatoprotective effect of Bifidobacterium adolescentis SPM0212 on carbon tetrachloride induced hepatotoxicity. *Korean Journal of Microbiology*, *51*(3), 280-287.

227. Lee, H. Y., Lee, G. H., Yoon, Y., & Chae, H. J. (2019). R. verniciflua and E. ulmoides extract (ILF-RE) protects against chronic CCl4-induced liver damage by enhancing antioxidation. *Nutrients*, *11*(2), 382.

228. Lee, W. M. (1993). Review article: drug-induced hepatotoxicity. *Aliment Pharmacol Ther*, *7*(5), 477-485.

229. Lee, Y., & Gustafsson, Å. B. (2009). Role of apoptosis in cardiovascular disease. *Apoptosis*, *14*, 536-548.

230. Lenzen, S. (2008). The mechanisms of alloxan-and streptozotocin-induced diabetes. *Diabetologia*, *51*(2), 216-226.

231. Li, A. N., Li, S., Li, H. B., Xu, D. P., Xu, X. R., & Chen, F. (2014). Total phenolic contents and antioxidant capacities of 51 edible and wild flowers. *Journal of functional foods*, *6*, 319-330.

232. Li, G., Han, C., Xu, L., Lim, K., Isse, K., & Wu, T. (2009). Cyclooxygenase-2 prevents fas-induced liver injury through up-regulation of epidermal growth factor receptor. *Hepatology*, *50*(3), 834-843.

233. Li, H., Xu, Y., Zhang, Q., Xu, H., Xu, Y., & Ling, K. (2019). Microvesicles containing miR-34a induce apoptosis of proximal tubular epithelial cells and participate in renal interstitial fibrosis. *Experimental and Therapeutic Medicine*, *17*(3), 2310-2316.

234. Li, R., Yang, W., Yin, Y., Ma, X., Zhang, P., & Tao, K. (2021). 4-OI attenuates carbon tetrachloride-induced hepatic injury via regulating oxidative stress and the inflammatory response. *Frontiers in pharmacology*, *12*, 651444.

235. Li, T., Meng, X., Wu, C., Fan, G., Yang, J., & Pan, W. (2019). Anticancer activity of a novel glycoprotein from Camellia oleifera Abel seeds against hepatic carcinoma in vitro and in vivo. *International journal of biological macromolecules*, *136*, 284-295.

236. Li, X., Wang, L., & Chen, C. (2017). Effects of exogenous thymosin β4 on carbon tetrachloride-induced liver injury and fibrosis. *Scientific reports*, *7*(1), 5872.

237. Li, Y., Zhang, J. J., Xu, D. P., Zhou, T., Zhou, Y., Li, S., & Li, H. B. (2016). Bioactivities and health benefits of wild fruits. *International journal of molecular sciences*, *17*(8), 1258

238. Li, S., Li, S. K., Gan, R. Y., Song, F. L., Kuang, L., & Li, H. B. (2013). Antioxidant capacities and total phenolic contents of infusions from 223 medicinal plants. *Industrial Crops and Products*, *51*, 289-298.

239. Libby, P. (2021). Inflammation during the life cycle of the atherosclerotic plaque. *Cardiovascular Research*, *117*(13), 2525-2536.

240. Lin, H. M., Tseng, H. C., Wang, C. J., Lin, J. J., Lo, C. W., & Chou, F. P. (2008). Hepatoprotective effects of Solanum nigrum Linn extract against CCl4-iduced oxidative damage in rats. *Chemico-Biological Interactions*, *171*(3), 283-293.

241. Lin, S. Y., Xu, D., Du, X. X., Ran, C. L., Xu, L., Ren, S. J., ... & Shu, G. (2019). Protective effects of salidroside against carbon tetrachloride (CCl4)-induced liver injury by initiating mitochondria to resist oxidative stress in mice. *International journal of molecular sciences*, *20*(13), 3187.

242. Lin, X., Huang, R., Zhang, S., Zheng, L., Wei, L., He, M., ... & Huang, Q. (2012). Methyl helicterate protects against CCl4-induced liver injury in rats by inhibiting oxidative stress, NF-κB activation, Fas/FasL pathway and cytochrome P4502E1 level. *Food and Chemical Toxicology*, *50*(10), 3413-3420.

243. Liu, J. Q., Gao, S. J., Luo, G. M., Yan, G. L., & Shen, J. C. (1998). Artificial imitation of glutathione peroxidase with 6-selenium-bridged β-cyclodextrin. *Biochemical and biophysical research communications*, *247*(2), 397-400.

244. Long, X., Wang, P., Zhou, Y., Wang, Q., Ren, L., Li, Q., & Zhao, X. (2022). Preventive effect of Lactobacillus plantarum HFY15 on carbon tetrachloride (CCl4)-induced acute liver injury in mice. *Journal of Food Science*, *87*(6), 2626-2639.

245. Lopez, J., & Tait, S. W. G. (2015). Mitochondrial apoptosis: killing cancer using the enemy within. *British journal of cancer*, *112*(6), 957-962.

246. Lorwy, O. H., Rosenbrough, N. J., Farr, A. L., & Randall, T. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem*, *193*, 265-275.

247. Liu, T., Yang, Q., Zhang, X., Qin, R., Shan, W., Zhang, H., & Chen, X. (2020). Quercetin alleviates kidney fibrosis by reducing renal tubular epithelial cell senescence through the SIRT1/PINK1/mitophagy axis. *Life Sciences*, *257*, 118116.

248. Luedde, T., Liedtke, C., Manns, M. P., & Trautwein, C. (2003). Losing balance: cytokine signaling and cell death in the context of hepatocyte injury and hepatic failure. *European cytokine network*, *13*(4), 377-83.

249. Luedde, T., & Trautwein, C. (2006). Intracellular survival pathways in the liver. *Liver international*, *26*(10), 1163-1174.

250. Lv, J., Xiao, X., Bi, M., Tang, T., Kong, D., Diao, M., ... & Jiang, H. (2022). ATP-sensitive potassium channels: A double-edged sword in neurodegenerative diseases. *Ageing Research Reviews*, *80*, 101676.

251. Ma, J. Q., Sun, Y. Z., Ming, Q. L., Tian, Z. K., Zhang, Y. J., & Liu, C. M. (2020). Effects of gastrodin against carbon tetrachloride induced kidney inflammation and fibrosis in mice associated with the AMPK/Nrf2/HMGB1 pathway. *Food & function*, *11*(5), 4615-4624.

252. Ma, J. Q., Zhang, Y. J., Tian, Z. K., & Liu, C. M. (2021). Bixin attenuates carbon tetrachloride induced oxidative stress, inflammation and fibrosis in kidney by regulating the Nrf2/TLR4/MyD88 and PPAR-γ/TGF-β1/Smad3 pathway. *International immunopharmacology*, *90*, 107117.

253. Ma, L., Liu, J., Li, N., Wang, J., Duan, Y., Yan, J., ... & Hong, F. (2010). Oxidative stress in the brain of mice caused by translocated nanoparticulate TiO2 delivered to the abdominal cavity. *Biomaterials*, *31*(1), 99-105.

254. Mahdavi, A., Bagherniya, M., Mirenayat, M. S., Atkin, S. L., & Sahebkar, A. (2021). Medicinal plants and phytochemicals regulating insulin resistance and glucose homeostasis in type 2 diabetic patients: a clinical review. *Pharmacological Properties of Plant-Derived Natural Products and Implications for Human Health*, 161-183.

255. Majid, M., Khan, M. R., Shah, N. A., Haq, I. U., Farooq, M. A., Ullah, S., ... & Sajid, M. (2015). Studies on phytochemical, antioxidant, anti-inflammatory and analgesic activities of Euphorbia dracunculoides. *BMC complementary and alternative medicine*, *15*, 1-15.

256. Makni, M., Chtourou, Y., Garoui, E. M., Boudawara, T., & Fetoui, H. (2012). Carbon tetrachloride-induced nephrotoxicity and DNA damage in rats: protective role of vanillin. *Human & experimental toxicology*, *31*(8), 844-852.

257. Malik, K., Ahmad, M., Öztürk, M., Altay, V., Zafar, M., & Sultana, S. (2021). *Herbals of Asia: prevalent diseases and their treatments*. Springer.

258. Manibusan, M. K., Odin, M., & Eastmond, D. A. (2007). Postulated carbon tetrachloride mode of action: a review. *Journal of Environmental Science and Health Part C*, *25*(3), 185-209.

259. Mansour, K. A., Elbermawi, A., Al-Karmalawy, A. A., Lahloub, M. F., & El-Neketi, M. (2022). Cytotoxic effects of extracts obtained from plants of the Oleaceae family: bio-guided isolation and molecular docking of new secoiridoids from Jasminum humile. *Pharmaceutical Biology*, *60*(1), 1374-1383.

260. Mason, R. M., & Wahab, N. A. (2003). Extracellular matrix metabolism in diabetic nephropathy. *Journal of the American Society of Nephrology*, *14*(5), 1358-1373.

261. McGregor, G. P., & Biesalski, H. K. (2006). Rationale and impact of vitamin C in clinical nutrition. *Current Opinion in Clinical Nutrition & Metabolic Care*, *9*(6), 697-703.

262. McLay, J. S., Chatterjee, P., Nicolson, A. G., Jardine, A. G., McKay, N. G., Ralston, S. H., ... & Hawksworth, G. M. (1994). Nitric oxide production by human proximal tubular cells: a novel immunomodulatory mechanism?. *Kidney international*, *46*(4), 1043-1049.

263. Meister, A. (1988). Glutathione metabolism and its selective modification. *Journal of biological chemistry*, *263*(33), 17205-17208.

264. Mestry, S. N., Dhodi, J. B., Kumbhar, S. B., & Juvekar, A. R. (2017). Attenuation of diabetic nephropathy in streptozotocin-induced diabetic rats by Punica granatum Linn. leaves extract. *Journal of traditional and complementary medicine*, *7*(3), 273-280.

265. Miguel, M. G. (2010). Antioxidant activity of medicinal and aromatic plants. A review. *Flavour and Fragrance Journal*, *25*(5), 291-312.

266. Mobasseri, M., Shirmohammadi, M., Amiri, T., Vahed, N., Fard, H. H., & Ghojazadeh, M. (2020). Prevalence and incidence of type 1 diabetes in the world: a systematic review and meta-analysis. *Health promotion perspectives*, *10*(2), 98.

267. Mohamed, N. A., Shehata, M. I., El-Sawaf, A. L., Hussein, H. K., & Michel, T. N. (2018). The Ameliorating Effect of Erythropoietin on Diabetic Neurodegeneration by Modulating the Antioxidant-Oxidant Imbalance and Apoptosis in Diabetic Male Rats. *Jordan Journal of Biological Sciences*, *11*(3).

268. Mohammed, S. A., Khan, R. A., El-Readi, M. Z., Emwas, A. H., Sioud, S., Poulson, B. G., ... & Mohammed, H. A. (2020). Suaeda vermiculata aqueous-ethanolic extract-based

mitigation of ccl4-induced hepatotoxicity in rats, and hepg-2 and hepg-2/adr cell-lines-based cytotoxicity evaluations. *Plants*, *9*(10), 1291.

269. Mosihuzzaman, M. (2012). Herbal medicine in healthcare-an overview. *Natural product communications*, *7*(6).

270. Mukhtar, S., Xiaoxiong, Z., Qamer, S., Saad, M., Mubarik, M. S., Mahmoud, A. H., & Mohammed, O. B. (2021). Hepatoprotective activity of silymarin encapsulation against hepatic damage in albino rats. *Saudi Journal of Biological Sciences*, *28*(1), 717-723.

271. Mukhtar, Y., Galalain, A., & Yunusa, U. (2020). A modern overview on diabetes mellitus: a chronic endocrine disorder. *European Journal of Biology*, *5*(2), 1-14.

272. Musabayane, C. T. (2012). The effects of medicinal plants on renal function and blood pressure in diabetes mellitus. *South African Journal of Diabetes and Vascular Disease*, *9*(3), 114-119.

273. Mustafa, G., Arif, R., Atta, A., Sharif, S., & Jamil, A. (2017). Bioactive compounds from medicinal plants and their importance in drug discovery in Pakistan. *Matrix Sci. Pharma*, *1*(1), 17-26.

274. Nagata, K., Suzuki, H., & Sakaguchi, S. (2007). Common pathogenic mechanism in development progression of liver injury caused by non-alcoholic or alcoholic steatohepatitis. *The Journal of toxicological sciences*, *32*(5), 453-468.

275. Nahdi, A. M. A., John, A., & Raza, H. (2017). Elucidation of molecular mechanisms of streptozotocin-induced oxidative stress, apoptosis, and mitochondrial dysfunction in Rin-5F pancreatic β-cells. *Oxidative medicine and cellular longevity*, *2017*(1), 7054272.

276. Naik, A., Adeyemi, S. B., Vyas, B., & Krishnamurthy, R. (2022). Effect of co-administration of metformin and extracts of Costus pictus D. Don leaves on alloxan-induced diabetes in rats. *Journal of Traditional and Complementary Medicine*, *12*(3), 269-280.

277. Naidu, J. R., Ismail, R., & Sasidharan, S. (2014). Acute oral toxicity and brine shrimp lethality of methanol extract of Mentha Spicata L (Lamiaceae). *Tropical Journal of Pharmaceutical Research*, *13*(1), 101-107.

278. Nain, P., Kumar, A., Sharma, S., & Nain, J. (2011). In vitro evaluation of antimicrobial and antioxidant activities of methanolic extract of Jasminum humile leaves. *Asian Pacific Journal of Tropical Medicine*, *4*(10), 804-807.

279. Nain, P., Bhatia, M., & Kaur, J. (2018). Methanolic Extract of Jasminum humile L. Leaves Possess Anxiolytic Activity Mediated via the GABAergic System in the Central Nervous System of Mice. *Ancient Science of Life*, *38*(2), 45-52.

280. Nandiyanto, A. B. D., Oktiani, R., & Ragadhita, R. (2019). How to read and interpret FTIR spectroscope of organic material. *Indonesian Journal of Science and Technology*, *4*(1), 97-118.

281. Naz, I., Khan, M. R., Zai, J. A., Batool, R., Zahra, Z., & Tahir, A. (2020). Pilea umbrosa ameliorate CCl 4 induced hepatic injuries by regulating endoplasmic reticulum stress, pro-inflammatory and fibrosis genes in rat. *Environmental Health and Preventive Medicine*, *25*, 1-15.

282. Naz, I., Khan, M. R., Zai, J. A., Batool, R., Maryam, S., & Majid, M. (2022). Indigofera linifolia ameliorated CCl4 induced endoplasmic reticulum stress in liver of rat. *Journal of Ethnopharmacology*, *285*, 114826.

283. Nimse, S. B., & Pal, D. (2015). Free radicals, natural antioxidants, and their reaction mechanisms. *RSC advances*, *5*(35), 27986-28006.

284. Nithianantham, K., Shyamala, M., Chen, Y., Latha, L. Y., Jothy, S. L., & Sasidharan, S. (2011). Hepatoprotective potential of Clitoria ternatea leaf extract against paracetamol induced damage in mice. *Molecules*, *16*(12), 10134-10145.

285. Niu, L., Cui, X., Qi, Y., Xie, D., Wu, Q., Chen, X., ... & Liu, Z. (2016). Involvement of TGF-β1/Smad3 signaling in carbon tetrachloride-induced acute liver injury in mice. *PLoS One*, *11*(5), e0156090.

286. Noguchi, T., Fong, K. L., Lai, E. K., Alexander, S. S., King, M. M., Olson, L., ... & McCay, P. B. (1982). Specificity of a phenobarbital-induced cytochrome P-450 for metabolism of carbon tetrachloride to the trichloromethyl radical. *Biochemical pharmacology*, *31*(5), 615-624.

287. Nowak, J. Z. (2013). Oxidative stress, polyunsaturated fatty acids-derived oxidation products and bisretinoids as potential inducers of CNS diseases: focus on age-related macular degeneration. *Pharmacological reports*, *65*(2), 288-304.

288. Ofoedu, C. E., You, L., Osuji, C. M., Iwouno, J. O., Kabuo, N. O., Ojukwu, M., ... & Korzeniowska, M. (2021). Hydrogen peroxide effects on natural-sourced polysacchrides:

free radical formation/production, degradation process, and reaction mechanism—a critical synopsis. *Foods*, *10*(4), 699.

289. Ogata, M., Fujii, H., Sumi, H., Ushimaru, S., Fujishima, R., & Tominaga, N. (2023). Renal anemia with a relatively normal renal function in vasculitis-induced acute tubulointerstitial damage. *Renal Failure*, *45*(1), 2173490.

290. Okaiyeto, K., Nwodo, U., Mabinya, L., & Okoh, A. (2018). A review on some medicinal plants with hepatoprotective effects. *Pharmacognosy Reviews*, *12*(24), 186-199.

291. OKAWA, H., & DOI, K. (1983). Neoplastic lesions in streptozotocin-treated rats. *Experimental Animals*, *32*(2), 77-84.

292. Olivetti, G., Quaini, F., Sala, R., Lagrasta, C., Corradi, D., Bonacina, E., ... & Anversa, P. (1996). Acute myocardial infarction in humans is associated with activation of programmed myocyte cell death in the surviving portion of the heart. *Journal of molecular and cellular cardiology*, *28*(9), 2005-2016.

293. Ostapowicz, G., Fontana, R. J., Schiødt, F. V., Larson, A., Davern, T. J., Han, S. H., ... & US Acute Liver Failure Study Group*. (2002). Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Annals of internal medicine*, *137*(12), 947-954.

294. Oyeleke, M. B., & Owoyele, B. V. (2022). Saponins and flavonoids from Bacopa floribunda plant extract exhibit antioxidant and anti-inflammatory effects on amyloid beta 1-42-induced Alzheimer's disease in BALB/c mice. *Journal of Ethnopharmacology*, *288*, 114997.

295. Pan, Y., Birdsey, R. A., Phillips, O. L., & Jackson, R. B. (2013). The structure, distribution, and biomass of the world's forests. *Annual Review of Ecology, Evolution, and Systematics*, *44*, 593-622.

296. Pandey, A. K., Mishra, A. K., & Mishra, A. (2012). Antifungal and antioxidative potential of oil and extracts derived from leaves of Indian spice plant Cinnamomum tamala. *Cellular and Molecular Biology*, *58*(1), 142-147.

297. Pandey, B. P., & Pradhan, S. P. (2020). Chemical composition, in vitro antioxidant, and enzymes inhibitory potential of three medicinally important plants from Nepal (Lepisorus mehrae, Pleurospermum benthamii, and Roscoea auriculata). *Advances in Traditional Medicine*, 1-16.

298. Pandrangi, S. L., Chalumuri, S. S., & Garimella, S. (2022). Emerging therapeutic efficacy of alkaloids as anticancer agents. *Annals of the Romanian Society for Cell Biology*, *26*(01), 64-74.

299. Park, S., Nguyen, N. B., Pezhouman, A., & Ardehali, R. (2019). Cardiac fibrosis: potential therapeutic targets. *Translational Research*, *209*, 121-137.

300. Park, Y. S., Jung, S. T., Kang, S. G., Heo, B. G., Arancibia-Avila, P., Toledo, F., ... & Gorinstein, S. (2008). Antioxidants and proteins in ethylene-treated kiwifruits. *Food Chemistry*, *107*(2), 640-648.

301. Pari, L., & Latha, M. (2005). Effect on lipid peroxidation in streptozotocin diabetes. *Gen Physiol Biophys*, *24*(1), 13-26.

302. Patterson, J. W., & Lazarow, A. (1955). Determination of glutathione. *Methods of biochemical analysis*, 259-278.

303. Paul, S. (2021). *Exploration of Anti-inflammatory and Anti-arthritic propertics of Aloe Vera (L.) Burm. f. from the Sub-Himalayan West Bengal, India* (Doctoral dissertation, University of North Bengal).

304. Pawar, H., Karde, M., Mundle, N., Jadhav, P., & Mehra, K. J. M. C. (2014). Phytochemical evaluation and curcumin content determination of turmeric rhizomes collected from Bhandara District of Maharashtra (India). *Med. Chem*, *4*(8), 588-591.

305. Pharmawati, M., & Wrasiati, L. P. (2020). Phytochemical screening and FTIR spectroscopy on crude extract from Enhalus acoroides leaves. *Malaysian journal of analytical sciences*, *24*(1), 70-77.

306. Philip, K., Malek, S. N., Sani, W., Shin, S. K., Kumar, S., Lai, H. S., ... & Rahman, S. N. (2009). Antimicrobial activity of some medicinal plants from Malaysia. *American journal of applied sciences*, *6*(8), 1613.

307. Phuyal, N., Jha, P. K., Raturi, P. P., & Rajbhandary, S. (2020). Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of Zanthoxylum armatum DC. *The Scientific World Journal*, *2020*(1), 8780704.

308. Pick, E., & Keisari, Y. (1981). Superoxide anion and hydrogen peroxide production by chemically elicited peritoneal macrophages—induction by multiple nonphagocytic stimuli. *Cellular immunology*, *59*(2), 301-318.

309. Poontawee, W., Natakankitkul, S., & Wongmekiat, O. (2016). Protective effect of Cleistocalyx nervosum var. paniala fruit extract against oxidative renal damage caused by cadmium. *Molecules*, *21*(2), 133.

310. Prahalathan, P., Saravanakumar, M., & Raja, B. (2012). The flavonoid morin restores blood pressure and lipid metabolism in DOCA-salt hypertensive rats. *Redox Report*, *17*(4), 167-175.

311. Pratheeba, T., Ragavendran, C., & Natarajan, D. (2015). Larvicidal, pupicidal and adulticidal potential of Ocimum gratissimum plant leaf extracts against filariasis inducing vector. *International journal of mosquito research*, *2*(2), 1-8.

312. Pugin, J. (2012). How tissue injury alarms the immune system and causes a systemic inflammatory response syndrome. *Annals of intensive care*, *2*, 1-6.

313. Pukalarasan, G., & Kathiravan, C. (2017). World Journal of Science and Research. *World*, *1*(2), 37-42.

314. Putthapiban, P., Sukhumthammarat, W., & Sriphrapradang, C. (2017). Concealed use of herbal and dietary supplements among Thai patients with type 2 diabetes mellitus. *Journal of Diabetes & Metabolic Disorders*, *16*, 1-7.

315. Qiu, D. K., Hua, J., Li, J. Q., & Li, E. L. (2005). CD14 expression on Kupffer cells during the course of carbon tetrachloride-mediated liver injury. *Chinese Journal of Digestive Diseases*, *6*(3), 137-141.

316. Racanelli, V., & Rehermann, B. (2006). The liver as an immunological organ. *Hepatology*, *43*(S1), S54-S62.

317. Rais, N., Ved, A., Ahmad, R., Parveen, K., Gautam, G. K., Bari, D. G., ... & Singh, A. P. (2022). Model of streptozotocin-nicotinamide Induced Type 2 diabetes: a comparative review. *Current diabetes reviews*, *18*(8), 58-69.

318. Rajasekaran, S., Sivagnanam, K., & Subramanian, S. (2005). Antioxidant effect of Aloe vera gel extract in streptozotocin-induced diabetes in rats. *Pharmacol Rep*, *57*(1), 90-96.

319. Rébé, C., & Ghiringhelli, F. (2019). STAT3, a master regulator of anti-tumor immune response. *Cancers*, *11*(9), 1280.

320. Rizvi, S. I., & Mishra, N. (2013). Traditional Indian medicines used for the management of diabetes mellitus. *Journal of diabetes research*, *2013*(1), 712092.

321. Roby, M. H. H., Sarhan, M. A., Selim, K. A. H., & Khalel, K. I. (2013). Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (Thymus vulgaris L.), sage (Salvia officinalis L.), and marjoram (Origanum majorana L.) extracts. *Industrial Crops and Products*, *43*, 827-831.

322. Roleira, F. M., Tavares-da-Silva, E. J., Varela, C. L., Costa, S. C., Silva, T., Garrido, J., & Borges, F. (2015). Plant derived and dietary phenolic antioxidants: Anticancer properties. *Food Chemistry*, *183*, 235-258.

323. Ron, D., & Walter, P. (2007). Signal integration in the endoplasmic reticulum unfolded protein response. *Nature reviews Molecular cell biology*, *8*(7), 519-529.

324. Rutkowski, D. T., Wu, J., Back, S. H., Callaghan, M. U., Ferris, S. P., Iqbal, J., ... & Kaufman, R. J. (2008). UPR pathways combine to prevent hepatic steatosis caused by ER stress-mediated suppression of transcriptional master regulators. *Developmental cell*, *15*(6), 829-840.

325. Sachdev, S., Ansari, S. A., & Ansari, M. I. (2023). Antioxidant defensive mechanisms to regulate cellular redox homeostatic balance. In *Reactive Oxygen Species in Plants: The Right Balance*,. 143-172.

326. Sadeer, N. B., Llorent-Martínez, E. J., Bene, K., Mahomoodally, M. F., Mollica, A., Sinan, K. I., ... & Zengin, G. (2019). Chemical profiling, antioxidant, enzyme inhibitory and molecular modelling studies on the leaves and stem bark extracts of three African medicinal plants. *Journal of Pharmaceutical and Biomedical Analysis*, *174*, 19-33.

327. Safhi, M. M. (2018). Nephroprotective effect of zingerone against CCl4-induced renal toxicity in Swiss albino mice: Molecular mechanism. *Oxidative medicine and cellular longevity*, *2018*(1), 2474831.

328. Sahreen, S., Khan, M. R., & Khan, R. A. (2010). Evaluation of antioxidant activities of various solvent extracts of Carissa opaca fruits. *Food chemistry*, *122*(4), 1205-1211.

329. Sahreen, S., Khan, M. R., & Khan, R. A. (2013). Ameliorating effect of various fractions of Rumex hastatus roots against hepato-and testicular toxicity caused by CCl4. *Oxidative medicine and cellular longevity*, *2013*(1), 325406.

330. Sahreen, S., Khan, M. R., & Khan, R. A. (2017). Evaluation of antioxidant profile of various solvent extracts of Carissa opaca leaves: an edible plant. *Chemistry Central Journal*, *11*, 1-7.

331. Sajid, M., Khan, M. R., Shah, N. A., Shah, S. A., Ismail, H., Younis, T., & Zahra, Z. (2016). Phytochemical, antioxidant and hepatoprotective effects of Alnus nitida bark in carbon tetrachloride challenged Sprague Dawley rats. *BMC complementary and alternative medicine*, *16*, 1-17.

332. Sambucetti, M. E., & Zuleta, A. (1996). Resistant starch in dietary fiber values measured by the AOAC method in different cereals. *Cereal Chemistry*, *73*(6), 759-761.

333. Sajid, M., Khan, M. R., Ismail, H., Latif, S., Rahim, A. A., Mehboob, R., & Shah, S. A. (2020). Antidiabetic and antioxidant potential of Alnus nitida leaves in alloxan induced diabetic rats. *Journal of ethnopharmacology*, *251*, 112544.

334. Sarkhail, P., Rahmanipour, S., Fadyevatan, S., Mohammadirad, A., Dehghan, G., Amin, G., ... & Abdollahi, M. (2007). Antidiabetic effect of Phlomis anisodonta: effects on hepatic cells lipid peroxidation and antioxidant enzymes in experimental diabetes. *Pharmacological Research*, *56*(3), 261-266.

335. Savitskaya, M. A., & Onishchenko, G. E. (2015). Mechanisms of apoptosis. *Biochemistry (Moscow)*, *80*, 1393-1405.

336. Sayed-Ahmad, B., Talou, T., Saad, Z., Hijazi, A., & Merah, O. (2017). The Apiaceae: Ethnomedicinal family as source for industrial uses. *Industrial crops and products*, *109*, 661-671.

337. Scholten, D., Trebicka, J., Liedtke, C., & Weiskirchen, R. (2015). The carbon tetrachloride model in mice. *Laboratory animals*, *49*(1_suppl), 4-11.

338. Schümann, J., Wolf, D., Pahl, A., Brune, K., Papadopoulos, T., van Rooijen, N., & Tiegs, G. (2000). Importance of Kupffer cells for T-cell-dependent liver injury in mice. *The American journal of pathology*, *157*(5), 1671-1683.

339. Seifzadeh, N., Sahari, M. A., Barzegar, M., Gavlighi, H. A., Calani, L., Del Rio, D., & Galaverna, G. (2019). Evaluation of polyphenolic compounds in membrane concentrated pistachio hull extract. *Food chemistry*, *277*, 398-406.

340. Selvaraju, V., Joshi, M., Suresh, S., Sanchez, J. A., Maulik, N., & Maulik, G. (2012). Diabetes, oxidative stress, molecular mechanism, and cardiovascular disease–an overview. *Toxicology mechanisms and methods*, *22*(5), 330-335.

341. Sen, S., & Chakraborty, R. (2017). Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future. *Journal of traditional and complementary medicine*, *7*(2), 234-244.

342. Serrano, J., Puupponen-Pimiä, R., Dauer, A., Aura, A. M., & Saura-Calixto, F. (2009). Tannins: current knowledge of food sources, intake, bioavailability and biological effects. *Molecular nutrition & food research*, *53*(S2), S310-S329.

343. Sevanian, A., & Ursini, F. (2000). Lipid peroxidation in membranes and low-density lipoproteins: similarities and differences. *Free Radical Biology and Medicine*, *29*(3-4), 306-311.

344. Shah, A., Bharati, K. A., Ahmad, J., & Sharma, M. P. (2015). New ethnomedicinal claims from Gujjar and Bakerwals tribes of Rajouri and Poonch districts of Jammu and Kashmir, India. *Journal of ethnopharmacology*, *166*, 119-128.

345. Shah, M. D., D'souza, U. J., & Iqbal, M. (2017). The potential protective effect of Commelina nudiflora L. against carbon tetrachloride (CCl 4)-induced hepatotoxicity in rats, mediated by suppression of oxidative stress and inflammation. *Environmental health and preventive medicine*, *22*, 1-19.

346. Shah, M. D., & Iqbal, M. O. H. A. M. M. A. D. (2018). Antioxidant activity, phytochemical analysis and total polyphenolics content of essential oil, methanol extract and methanol fractions from Commelinanudiflora. *Int. J. Pharm. Pharm. Sci*, *10*(8), 36-43.

347. Shah, N. A., & Khan, M. R. (2014). Antidiabetic effect of Sida cordata in alloxan induced diabetic rats. *BioMed research international*, *2014*(1), 671294.

348. Shahid, A., Ali, R., Ali, N., Hasan, S. K., Bernwal, P., Afzal, S. M., ... & Sultana, S. (2016). Modulatory effects of catechin hydrate against genotoxicity, oxidative stress, inflammation and apoptosis induced by benzo (a) pyrene in mice. *Food and Chemical Toxicology*, *92*, 64-74.

349. Shakya, A. K. (2016). Medicinal plants: Future source of new drugs. *International journal of herbal medicine*, *4*(4), 59-64.

350. Sharabi, K., Tavares, C. D., Rines, A. K., & Puigserver, P. (2015). Molecular pathophysiology of hepatic glucose production. *Molecular aspects of medicine*, *46*, 21-33.

351. Sharifi-Rad, J., Herrera-Bravo, J., Semwal, P., Painuli, S., Badoni, H., Ezzat, S. M., ... & Cho, W. C. (2022). Artemisia spp.: an update on its chemical composition, pharmacological and toxicological profiles. *Oxidative Medicine and Cellular Longevity*, *2022*(1), 5628601.

352. Sharma, P., Kharkwal, A. C., Kharkwal, H., Abdin, M. Z., & Varma, A. (2014). A review on pharmacological properties of Aloe vera. *Int J Pharm Sci Rev Res*, *29*(2), 31-37.

353. Shehzadi, I., Shah, N. A., Khan, M. R., Shuaib, M., Shah, M., Khan, A., ... & Shaheen, G. (2020). In vivo Antioxidant Potential of Raphanus sativus Seeds in Rat Kidney Against CCl 4-Induced Toxicity. *Polish Journal of Environmental Studies*, *29*(1).

354. Shikov, A. N., Pozharitskaya, O. N., Makarov, V. G., Wagner, H., Verpoorte, R., & Heinrich, M. (2014). Medicinal plants of the Russian Pharmacopoeia; their history and applications. *Journal of ethnopharmacology*, *154*(3), 481-536.

355. Shin, S. M., Cho, I. J., & Kim, S. G. (2009). Resveratrol protects mitochondria against oxidative stress through AMP-activated protein kinase-mediated glycogen synthase kinase-3β inhibition downstream of poly (ADP-ribose) polymerase-LKB1 pathway. *Molecular pharmacology*, *76*(4), 884-895.

356. Shin, S. M., Yang, J. H., & Ki, S. H. (2013). Role of the Nrf2-ARE pathway in liver diseases. *Oxidative medicine and cellular longevity*, *2013*(1), 763257.

357. Shukla, S., Mehta, A., Mehta, P., & Bajpai, V. K. (2012). Antioxidant ability and total phenolic content of aqueous leaf extract of Stevia rebaudiana Bert. *Experimental and Toxicologic Pathology*, *64*(7-8), 807-811.

358. Sies, H. (1985). Introductory remarks in oxidative stress.

359. Sies, H. (1993). Strategies of antioxidant defense. *European journal of biochemistry*, *215*(2), 213-219.

360. Silverstein, D. M. (2009). Inflammation in chronic kidney disease: role in the progression of renal and cardiovascular disease. *Pediatric nephrology*, *24*(8), 1445-1452.

361. Singh, A., Kukreti, R., Saso, L., & Kukreti, S. (2019). Oxidative stress: a key modulator in neurodegenerative diseases. *Molecules*, *24*(8), 1583.

362. Singh, B., Singh, S., Kishor, A., & Singh, B. (2021). Traditional usage of medicinal plants in humans and animals health care and their chemical constituents from hills and valleys of Jammu province, Western Himalaya. Journal of Natural Products Resources, 12, 84–100.

363. Singh, D., Mishra, M., Gupta, M., Singh, P., Gupta, A., & Nema, R. (2012). Nitric oxide radical scavenging assay of bioactive compounds present in methanol extract of Centella asiatica. *International Journal of Pharmacy and Pharmaceutical Science Research*, *2*(3), 42-44.

364. Sip, S., Szymanowska, D., Chanaj-Kaczmarek, J., Skalicka-Woźniak, K., Budzyńska, B., Wronikowska-Denysiuk, O., ... & Cielecka-Piontek, J. (2022). Potential for prebiotic stabilized Cornus mas L. lyophilized extract in the prophylaxis of diabetes mellitus in streptozotocin diabetic rats. *Antioxidants*, *11*(2), 380.

365. Sivakumar, S., Palsamy, P., & Subramanian, S. P. (2010). Impact of D-pinitol on the attenuation of proinflammatory cytokines, hyperglycemia-mediated oxidative stress and protection of kidney tissue ultrastructure in streptozotocin-induced diabetic rats. *Chemico-biological interactions*, *188*(1), 237-245.

366. Slama, K., Boumendjel, M., Taibi, F., Boumendjel, A., & Messarah, M. (2020). Atriplex halimus aqueous extract abrogates carbon tetrachloride-induced hepatotoxicity by modulating biochemical and histological changes in rats. *Archives of physiology and biochemistry*, *126*(1), 49-60.

367. Sofowora, A. (1993). Recent trends in research into African medicinal plants. *Journal of ethnopharmacology*, *38*(2-3), 197-208.

368. Solomou, A. D., Martinos, K., Skoufogianni, E., & Danalatos, N. G. (2016). Medicinal and aromatic plants diversity in Greece and their future prospects: A review. *Agricultural Science*, *4*(1), 9-21.

369. Song, Z., & Steller, H. (1999). Death by design: mechanism and control of apoptosis. *Trends in Biochemical Sciences*, *24*(12), M49-M52.

370. Sostres, C., Gargallo, C. J., Arroyo, M. T., & Lanas, A. (2010). Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. *Best practice & research Clinical gastroenterology*, *24*(2), 121-132.

371. Srinivasan, M., Rukkumani, R., Ram Sudheer, A., & Menon, V. P. (2005). Ferulic acid, a natural protector against carbon tetrachloride-induced toxicity. *Fundamental & clinical pharmacology*, *19*(4), 491-496

372. Srivastava, A. K. (2018). Significance of medicinal plants in human life. In *Synthesis of Medicinal Agents from Plants* (pp. 1-24). Elsevier.

373. Staveness, D., Bosque, I., & Stephenson, C. R. (2016). Free radical chemistry enabled by visible light-induced electron transfer. *Accounts of chemical research*, *49*(10), 2295-2306

374. Stevanović, Z. D., Bošnjak-Neumüller, J., Pajić-Lijaković, I., Raj, J., & Vasiljević, M. (2018). Essential oils as feed additives—Future perspectives. *Molecules*, *23*(7), 1717.

375. Stuckler, D. (2008). Population causes and consequences of leading chronic diseases: a comparative analysis of prevailing explanations. *The Milbank Quarterly*, *86*(2), 273-326.

376. Sultan, S. (2014). Reviewing the protective role of antioxidants in oxidative stress caused by free radicals. *Asian Pacific Journal of Health Science*, *1*(4), 401-406.

377. Sun, J., Wu, Y., Long, C., He, P., Gu, J., Yang, L., et al. (2018) Anthocyanins isolated from blueberry ameliorates CCl4 induced liver fibrosis by modulation of oxidative stress, inflammation and stellate cell activation in mice. *Food Chemistry Toxicology*, 120, 491–9.

378. Sun, L., Zhang, Y., Wen, S., Li, Q., Chen, R., Lai, X., ... & Sun, S. (2022). Extract of Jasminum grandiflorum L. alleviates CCl4-induced liver injury by decreasing inflammation, oxidative stress and hepatic CYP2E1 expression in mice. *Biomedicine & Pharmacotherapy*, *152*, 113255.

379. Sun, Y. I., Oberley, L. W., & Li, Y. (1988). A simple method for clinical assay of superoxide dismutase. *Clinical chemistry*, *34*(3), 497-500.

380. Sun, Y., Yang, X., Lu, X., Wang, D., & Zhao, Y. (2013). Protective effects of Keemun black tea polysaccharides on acute carbon tetrachloride-caused oxidative hepatotoxicity in mice. *Food and Chemical Toxicology*, *58*, 184-192.

381. Sutti, S., Bruzzì, S., & Albano, E. (2016). The role of immune mechanisms in alcoholic and nonalcoholic steatohepatitis: a 2015 update. *Expert Review of Gastroenterology & Hepatology*, *10*(2), 243-253.

382. Tacke, F., Luedde, T., & Trautwein, C. (2009). Inflammatory pathways in liver homeostasis and liver injury. *Clinical reviews in allergy & immunology*, *36*, 4-12.

383. Tacke, F., & Trautwein, C. (2015). Mechanisms of liver fibrosis resolution. *Journal of hepatology*, *63*(4), 1038-1039.

384. Tadera, K., Minami, Y., Takamatsu, K., & Matsuoka, T. (2006). Inhibition of α-glucosidase and α-amylase by flavonoids. *Journal of nutritional science and vitaminology*, *52*(2), 149-153.

385. Taheri, Y., Suleria, H. A. R., Martins, N., Sytar, O., Beyatli, A., Yeskaliyeva, B., ... & Sharifi-Rad, J. (2020). Myricetin bioactive effects: Moving from preclinical evidence to potential clinical applications. *BMC complementary medicine and therapies*, *20*, 1-14.

386. Tambe, V. D., & Bhambar, R. S. (2014). Estimation of total phenol, tannin, alkaloid and flavonoid in Hibiscus tiliaceus Linn. wood extracts. *Journal of pharmacognosy and phytochemistry*, *2*(4), 41-47.

387. Tan, T. C., Cheng, L. H., Bhat, R., Rusul, G., & Easa, A. M. (2014). Composition, physicochemical properties and thermal inactivation kinetics of polyphenol oxidase and peroxidase from coconut (Cocos nucifera) water obtained from immature, mature and overly-mature coconut. *Food Chemistry*, *142*, 121-128.

388. Telagari, M., & Hullatti, K. (2015). In-vitro α-amylase and α-glucosidase inhibitory activity of Adiantum caudatum Linn. and Celosia argentea Linn. extracts and fractions. *Indian journal of pharmacology*, *47*(4), 425-429.

389. Thi Bui, D. H., Nguyen, B. X., Truong, D. C., Meyrowitsch, D. W., Søndergaard, J., Gammeltoft, T., ... & Jannie, N. (2021). Polypharmacy among people living with type 2 diabetes mellitus in rural communes in Vietnam. *Plos one*, *16*(4), e0249849.

390. Thiviya, P., Gamage, A., Piumali, D., Merah, O., & Madhujith, T. (2021). Apiaceae as an important source of antioxidants and their applications. *Cosmetics*, *8*(4), 111.

391. Tiwari, A. K., & Rao, J. M. (2002). Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Current science*, 30-38.

392. Tran, N., Pham, B., & Le, L. (2020). Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discovery. *Biology*, *9*(9), 252.

393. Ţucureanu, V., Matei, A., & Avram, A. M. (2016). FTIR spectroscopy for carbon family study. *Critical reviews in analytical chemistry*, *46*(6), 502-520.

394. Twilley, D., Rademan, S., & Lall, N. (2020). A review on traditionally used South African medicinal plants, their secondary metabolites and their potential development into anticancer agents. *Journal of ethnopharmacology*, *261*, 113101.

395. Ukoha, U. U., Mbagwu, S. I., Ndukwe, G. U., & Obiagboso, C. (2015). Histological and biochemical evaluation of the kidney following chronic consumption of Hibiscus sabdariffa. *Advances in Biology*, *2015*(1), 486510.

396. Unsal, V., Dalkıran, T., Çiçek, M., & Kölükçü, E. (2020). The role of natural antioxidants against reactive oxygen species produced by cadmium toxicity: a review. *Advanced pharmaceutical bulletin*, *10*(2), 184.

397. Unsal, V., Cicek, M., & Sabancilar, İ. (2021). Toxicity of carbon tetrachloride, free radicals and role of antioxidants. *Reviews on environmental health*, *36*(2), 279-295.

398. Uritu, C. M., Mihai, C. T., Stanciu, G. D., Dodi, G., Alexa-Stratulat, T., Luca, A., ... & Tamba, B. I. (2018). Medicinal plants of the family Lamiaceae in pain therapy: A review. *Pain Research and Management*, *2018*(1), 7801543.

399. Valko, M., Jomova, K., Rhodes, C. J., Kuča, K., & Musílek, K. (2016). Redox-and non-redox-metal-induced formation of free radicals and their role in human disease. *Archives of toxicology*, *90*, 1-37.

400. van Loo, G., & Bertrand, M. J. (2023). Death by TNF: a road to inflammation. *Nature Reviews Immunology*, *23*(5), 289-303.

401. Venkatanarayana, G., Sudhakara, G., Sivajyothi, P., & Indira, P. (2012). Protective effects of curcumin and vitamin E on carbon tetrachloride-induced nephrotoxicity in rats. *EXCLI journal*, *11*, 641

402. Venmathi Maran, B. A., Iqbal, M., Gangadaran, P., Ahn, B. C., Rao, P. V., & Shah, M. D. (2022). Hepatoprotective potential of Malaysian medicinal plants: a review on phytochemicals, oxidative stress, and antioxidant mechanisms. *Molecules*, *27*(5), 1533.

403. Vennela, P. (2023). Study of C-reactive protein and alkaline phosphatase among type 2 diabetes mellitus patients. *European Journal of Molecular & Clinical Medicine*, *10*(04).

404. Vergallo, R., & Crea, F. (2020). Atherosclerotic plaque healing. *New England journal of medicine*, *383*(9), 846-857.

405. Vinothiya, K., & Ashokkumar, N. (2017). Modulatory effect of vanillic acid on antioxidant status in high fat diet-induced changes in diabetic hypertensive rats. *Biomedicine & Pharmacotherapy*, *87*, 640-652.

406. Xiao, J., Ho, C. T., Liong, E. C., Nanji, A. A., Leung, T. M., Lau, T. Y. H., ... & Tipoe, G. L. (2014). Epigallocatechin gallate attenuates fibrosis, oxidative stress, and inflammation in non-alcoholic fatty liver disease rat model through TGF/SMAD, PI3 K/Akt/FoxO1, and NF-kappa B pathways. *European journal of nutrition*, *53*, 187-199.

407. Xu, D. P., Li, Y., Meng, X., Zhou, T., Zhou, Y., Zheng, J., ... & Li, H. B. (2017). Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. *International journal of molecular sciences*, *18*(1), 96.

408. Xu, P., Yao, J., Ji, J., Shi, H., Jiao, Y., Hao, S., ... & Shi, H. (2019). Deficiency of apoptosis-stimulating protein 2 of p53 protects mice from acute hepatic injury induced by CCl4 via autophagy. *Toxicology Letters*, *316*, 85-93.

409. Wang, S. Y., & Jiao, H. (2000). Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. *Journal of agricultural and food chemistry*, *48*(11), 5677-5684.

410. Weber, L. W., Boll, M., & Stampfl, A. (2003). Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Critical reviews in toxicology*, *33*(2), 105-136.

411. Wang, Z., Xia, T., & Liu, S. (2015). Mechanisms of nanosilver-induced toxicological effects: more attention should be paid to its sublethal effects. *Nanoscale*, *7*(17), 7470-7481.

412. Wanjala Wafula, K., Kiambi Mworia, J., & Piero Ngugi, M. (2023). Phytochemical Screening and In Vitro Evaluation of the Antioxidant Potential of Dichloromethane Extracts of Strychnos henningsii Gilg. and Ficus sycomorus L. *The Scientific World Journal*, *2023*(1), 8494176.

413. Williamson, G. (2013). Possible effects of dietary polyphenols on sugar absorption and digestion. *Molecular nutrition & food research*, *57*(1), 48-57.

414. Winterbourn, C. C. (2008). Reconciling the chemistry and biology of reactive oxygen species. *Nature chemical biology*, *4*(5), 278-286.

415. Wolf, G., & Ziyadeh, F. N. (2007). Cellular and molecular mechanisms of proteinuria in diabetic nephropathy. *Nephron Physiology*, *106*(2), p26-p31.

416. Wood, L. G., Gibson, P. G., & Garg, M. L. (2003). Biomarkers of lipid peroxidation, airway inflammation and asthma. *European Respiratory Journal*, *21*(1), 177-186.

417. World Health Organization. (2001). *Legal status of traditional medicine and complementary* (No. WHO/EDM/TRM/2001.2). World Health Organization.

418. Wu, D., & Cederbaum, A. I. (2003). Alcohol, oxidative stress, and free radical damage. *Alcohol research & health*, *27*(4), 277.

419. Wu, Z., Sun, L., Chen, R., Wen, S., Li, Q., Lai, X., ... & Sun, S. (2022). Chinese tea alleviates CCl4-induced liver injury through the NF-κBorNrf2Signaling pathway in C57BL-6J mice. *Nutrients*, *14*(5), 972.

420. Yachi, R., Igarashi, O., & Kiyose, C. (2010). Protective effects of vitamin E analogs against carbon tetrachloride-induced fatty liver in rats. *Journal of Clinical Biochemistry and Nutrition*, *47*(2), 148-154.

421. Yadav, R. N. S., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of phytology*, *3*(12).

422. Yang, B., Johnson, T. S., Thomas, G. L., Watson, P. F., Wagner, B., Furness, P. N., & El Nahas, A. M. (2002). A shift in the Bax/Bcl-2 balance may activate caspase-3 and modulate apoptosis in experimental glomerulonephritis. *Kidney international*, *62*(4), 1301-1313.

423. Yang, J., Guo, J., & Yuan, J. (2008). In vitro antioxidant properties of rutin. LWT Food Sci Technol 41: 1060–1066.

424. Yang, K., Zou, Z., Wu, Y., & Hu, G. (2020). MiR-195 suppression alleviates apoptosis and oxidative stress in CCl4-induced ALI in mice by targeting Pim-1. *Experimental and Molecular Pathology*, *115*, 104438.

425. Yao, Y., Chen, S., & Li, H. (2021). An Improved System to Evaluate Superoxide-Scavenging Effects of Bioflavonoids. *ChemistryOpen*, *10*(4), 503-514.

426. Yaribeygi, H., Katsiki, N., Butler, A. E., & Sahebkar, A. (2019). Effects of antidiabetic drugs on NLRP3 inflammasome activity, with a focus on diabetic kidneys. *Drug discovery today*, *24*(1), 256-262.

427. Yaribeygi, H., Sathyapalan, T., Atkin, S. L., & Sahebkar, A. (2020). Molecular mechanisms linking oxidative stress and diabetes mellitus. *Oxidative medicine and cellular longevity*, *2020*(1), 8609213.

428. Yarnell, E. (2000). The botanical roots of pharmaceutical discovery. *Alternative and Complementary Therapies*, *6*(3), 125-128.

429. Yeh, G. Y., Eisenberg, D. M., Kaptchuk, T. J., & Phillips, R. S. (2003). Systematic review of herbs and dietary supplements for glycemic control in diabetes. *Diabetes care*, *26*(4), 1277-1294.

430. Yen, F. L., Wu, T. H., Lin, L. T., Cham, T. M., & Lin, C. C. (2009). Naringenin-loaded nanoparticles improve the physicochemical properties and the hepatoprotective effects of

naringenin in orally-administered rats with CCl 4-induced acute liver failure. *Pharmaceutical research*, *26*, 893-902.

431. Yeshi, K., Crayn, D., Ritmejerytė, E., & Wangchuk, P. (2022). Plant secondary metabolites produced in response to abiotic stresses has potential application in pharmaceutical product development. *Molecules*, *27*(1), 313.

432. Yonekura, H., Yamamoto, Y., Sakurai, S., Watanabe, T., & Yamamoto, H. (2005). Roles of the receptor for advanced glycation endproducts in diabetes-induced vascular injury. *Journal of pharmacological sciences*, *97*(3), 305-311.

433. Yu, B. P. (1994). Cellular defenses against damage from reactive oxygen species. *Physiological reviews*, *74*(1), 139-162.

434. Zafar, M., Naqvi, S. N. U. H., Ahmed, M., & Kaimkhani, Z. A. (2009). Altered Liver Morphology and Enzymes in Streptozotocin Induced Diabetic Rats. *International Journal of Morphology*, *27*(3).

435. Zai, J. A., Khan, M. R., Mughal, Z. U. N., Batool, R., Naz, I., Maryam, S., & Zahra, Z. (2019). Methanol extract of Iphiona aucheri ameliorates CCl4 induced hepatic injuries by regulation of genes in rats. *Toxicology Research*, *8*(6), 815-832.

436. Zevin, I. Altman, N. Zevin, L. (1997). A Russian herbal: traditional remedies for health and healing. Healing Arts Press, Vermont, 250.

437. Zhang, B., Shen, Q., Chen, Y., Pan, R., Kuang, S., Liu, G., ... & Sun, X. (2017). Myricitrin alleviates oxidative stress-induced inflammation and apoptosis and protects mice against diabetic cardiomyopathy. *Scientific reports*, *7*(1), 44239.

438. Zhang, C., Zhang, G., Liao, Y., & Gong, D. (2017). Myricetin inhibits the generation of superoxide anion by reduced form of xanthine oxidase. *Food chemistry*, *221*, 1569-1577.

439. Zhang, J. J., Li, Y., Zhou, T., Xu, D. P., Zhang, P., Li, S., & Li, H. B. (2016). Bioactivities and health benefits of mushrooms mainly from China. *Molecules*, *21*(7), 938.

440. Zhang, P., Li, D., Zhu, J., & Hu, J. (2023). Antihypertensive effects of Pleurospermum lindleyanum aqueous extract in spontaneously hypertensive rats. *Journal of Ethnopharmacology*, *308*, 116261.

441. Zhou, Y., Zheng, J., Li, Y., Xu, D. P., Li, S., Chen, Y. M., & Li, H. B. (2016). Natural polyphenols for prevention and treatment of cancer. *Nutrients*, *8*(8), 515.

442. Zhang, Y. J., Gan, R. Y., Li, S., Zhou, Y., Li, A. N., Xu, D. P., & Li, H. B. (2015). Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Molecules*, *20*(12), 21138-21156.

443. Ziegler, U., & Groscurth, P. (2004). Morphological features of cell death. *Physiology*, *19*(3), 124-128.

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Inflammopharmacology

ORIGINAL ARTICLE

Jasminum humile (Linn) ameliorates CCl_4-induced oxidative stress by regulating ER stress, inflammatory, and fibrosis markers in rats

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Abstract

Jasminum humile (Linn) is highly valued for its medicinal properties. The pulp and decoction made from its leaves are effective for skin diseases. Juice prepared from roots is used against ringworm illness. Our current study aims to illustrate the non-toxicity and protective potential of methanol extract of *Jasminum humile* (JHM) against CCl_4-induced oxidative stress in the liver of rats. Qualitative phytochemical screening, total flavonoids (TFC), and total phenolic content (TPC) assays were performed with JHM. The toxicity of the plant was estimated by treating female rats at different JHM doses while to assess anti-inflammatory potential of plant nine groups of male rats (six rats/group) received different treatments such as: CCl_4 only (1 ml/kg mixed with olive oil in a ratio of 3:7), silymarin (200 mg/kg)+CCl_4, different doses of JHM alone at a ratio of 1:2:4, and JHM (at a ratio of 1:2:4)+CCl_4, and were examined for different antioxidant enzymes, serum markers, and histological changes, while mRNA expression of stress, inflammatory and fibrosis markers were assessed by real-time polymerase chain reaction analysis. Different phytochemicals were found in JHM. A high amount of total phenolic and flavonoid content was found (89.71 ± 2.79 mg RE/g and 124.77 ± 2.41 mg GAE/g) in the methanolic extract of the plant. Non-toxicity of JHM was revealed even at higher doses of JHM. Normal levels of serum markers in blood serum and antioxidant enzymes in tissue homogenates were found after co-administration of JHM along with CCl_4. However, CCl_4 treatment caused oxidative stress in the liver by enhancing the levels of stress and inflammatory markers and reducing antioxidant enzyme levels, while JHM treatment showed significant ($P < 0.05$) downregulation was in mRNA expression of those markers. Investigation of mechanism of specific signaling pathways related to apoptosis and clinical trials to assess safety and efficacy of optimal dosage of *Jasminum humile* will be helpful to develop FDA-approved drug.

Keywords *Jasminum humile* · Oxidative stress · Antioxidants · Phenolics · Flavonoids · Anti-inflammatory

Introduction

The liver is a vital organ involved in many important functions of the human body. Many health issues in individuals are correlated with injuries and impairments to the liver. Excessive extracellular matrix (ECM) protein accumulation and viral hepatitis are major causes of liver fibrosis (Ali et al. 2017). Almost 184 countries are affected by liver fibrosis across the world due to its frequent recurrence (Akcora et al. 2018). Some inorganic chemicals and drugs are linked to chronic liver diseases such as severe infection, inflammation, cirrhosis, and many severe metabolic disorders. Iron, copper, arsenic, and phosphorus are the main compounds responsible for initiating hepatotoxicity in humans and animals. These toxic compounds accumulate in the liver and produce free radicals which disrupt the normal functioning capacity of the liver. Reactive oxygen species (ROS) are produced by the process of oxidative stress in the body; these species react with cell membranes and thus lipid peroxidation or inflammation begins (Valko et al. 2016).

Carbon tetrachloride (CCl_4) is a synthetic toxic compound that accelerates the process of lipid peroxidation. CCl_4 is converted to a tri-chloromethyl radical ($OCCl_3$) and a peroxy tri-chloromethyl radical. Lipid peroxides are then produced by the reaction of these ROS with polyunsaturated fatty acids (PUFAs). Additionally, pro-inflammatory markers including tumor necrosis factor (TNF-α), interleukin

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Article

Pleurospermum candollei Methanolic Extract Ameliorates CCl_4-Induced Liver Injury by Modulating Oxidative Stress, Inflammatory, and Apoptotic Markers in Rats

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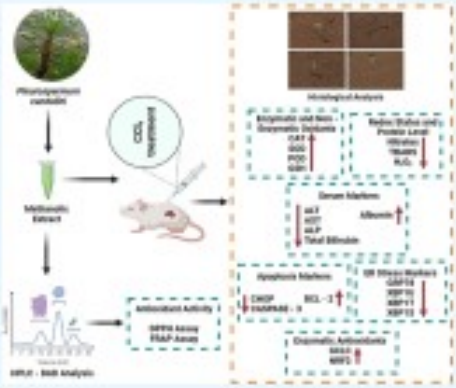
ABSTRACT: The main objective of this study was to investigate the hepatoprotective potency of the *Pleurospermum candollei* methanol extract against CCl_4-induced liver damage in rats. HPLC technique was used to estimate the presence of polyphenols in the methanol extract of *P. candollei* (PCM), while proximate analysis revealed the presence of carbohydrates, lipids, and moisture in the extract. The antioxidant potential of PCM was evaluated by 2,2-diphenylpicrylhydrazyl (DPPH) and reducing power assay, which showed a high percentage of inhibition against free radicals. Hepatotoxicity was induced by carbon tetrachloride (CCl_4). CCl_4 administration reduced the activity of endogenous antioxidants, whereas it increased the production of nitrites and hydrogen peroxide (H_2O_2) in rats. Furthermore, the level of hepatic markers in serum was also elevated after CCl_4 administration. Moreover, the expression of stress-related markers, proinflammatory mediators, and apoptotic genes was enhanced in CCl_4-treated rats. Coadministration of PCM along with CCl_4 in rats reduced the levels of free radicals and the above genes to normal levels. CCl_4 administration caused histopathological alterations in liver tissues, while cotreatment with PCM mitigated liver injuries. These findings suggest that the methanol extract of *P. candollei* possesses antioxidant and anti-inflammatory properties and can prevent liver injury. Further pharmacological research will be helpful in determining the effectiveness of *P. candollei* in humans. Development of FDA-approved plant-based anti-inflammatory drugs can help treat patients and reduce the chances of toxicity.



1. INTRODUCTION

Liver is involved in the metabolism of fatty acids and nutrients; synthesis of proteins; sugar storage; detoxification of chemicals, drugs, and xenobiotics; and other physiological functions.[1] Liver is susceptible to injury caused by various factors, such as drugs, viruses, xenobiotics, alcohol, and nutritional supplements, through various pathways triggered by toxic metabolites. These toxic substances and drugs are responsible for almost 50% of acute liver damages. It is well recognized that the combination of mechanisms including apoptosis, oxidative stress, and inflammation plays a role in the development of acute liver injury.[2,3] During aerobic respiration in the mitochondrial membrane through the electron transport chain, reactive oxygen species (ROS) are constantly generated as byproducts. Liver is very rich in mitochondria and has a high rate of oxygen consumption during metabolic processes, which increases its exposure to ROS.[4]

Various xenobiotics including vinyl chloride, arsenic, and carbon tetrachloride (CCl_4) have been recognized as hepatotoxins.[5,6] ROS and toxic metabolites may damage immune-mediated membrane or may cause disruption in intracellular processes to cause cell injury in the hepatocytes of both human and rats.[7] A cytochrome P-450 enzymatic system, such as cytochrome P-450 2E1 (CYP2E1), generates highly reactive species including the trichloromethyl radical (CCl_3) through the metabolic activation of CCl_4 in hepatocytes. This reactive intermediate interacts with molecular oxygen (O_2) to produce a highly reactive species, trichloromethyl peroxyl radical (CCl3OO*). This radical covalently binds to biological molecules, leading to the degradation of proteins and lipids, oxidative stress, cellular damage, apoptosis, and necrosis of hepatocytes.[8]

Natural antioxidants (e.g., vitamin C, α-tocopherol, quercetin, curcumin, phenolics, flavonoid, and tannin) have been studied for their potency against liver diseases,[9−11] and certain plant-derived antioxidants have shown promising

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Investigating the role of polyphenols from *Pleurospermum candollei* (DC.) extract against diabetic nephropathy through modulating inflammatory cytokines and renal gene expression in rats

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ABSTRACT

Pleurospermum candollei is a local vegetable that is traditionally used to cure abdominal problems, pain, and infertility. The purpose of this study was to evaluate the therapeutic potential of *P. candollei* ethanolic extract against T2DM through modulation of renal genetic markers. Biochemical composition of extract was investigated through high performance liquid chromatography and quantitative phytochemical analyses. In rats, T2DM was induced by injecting streptozotocin (STZ), and *P. candollei* ethanol extract was administered for 21 days. Antioxidant enzymes and renal biomarkers were assessed in homogenized kidney samples of treated groups. qRT-PCR analysis of isolated RNA was performed for estimation of inflammatory and apoptotic markers. These biochemical analyses revealed that plant extract significantly normalized the levels of catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH) after 21 days of treatment. However, high dose of plant extract (300 mg/kg) exhibited more prominent results in restoring the inflammatory (IL-6, IL-β1, and TNF-α) and apoptotic markers than low dose (150 mg/kg). Similarly, histological studies also revealed that there was minor inflammation and necrosis in kidney of plant treated rats. Generally, the present study showed that administration of *P. candollei* extract exhibits renal protective, anti-hyperglycemic, and anti-glycation properties. However, further research is required to assess the mechanism of action of PCE at clinical level.

1. Introduction

Diabetes Mellitus (DM) is known as an endocrine disorder characterized by hyperglycaemia which may result due to an impairment in insulin secretion, insulin action, or both [1]. The complications associated with diabetes can be categorized into two groups: microvascular complications which include neuropathy, nephropathy, and retinopathy, and macrovascular complications such as peripheral vascular disease, cardiovascular disorder, and stroke [2]. Sustained hyperglycemia is closely linked to the development of diabetic nephropathy [3]. This condition is the primary cause of end-stage renal disease and is associated with various factors such as hypertension, urinary tract infections, polyneuropathic bladder dysfunction, or macrovascular angioplasty [4]. In diabetes mellitus, chronic hyperglycemia gives rise to a series of biochemical effects, and oxidative stress induced by diabetes may involve in the symptoms and development of the disease [5]. Enhanced production of free radicals resulting in the depletion of endogenous antioxidant components may result in disruption of cellular functions and damage to cellular membranes and may enhance susceptibility to lipid peroxidation [6]. Increase in free radical production in diabetes has been the subject of several studies. These hypotheses include several processes including the generation of advanced glycation end products (AGEs) by non-enzymatic glycation of proteins, increased glucose flux, and autoxidation of glucose [7,8]. Elevated oxidative stress plays a significant role in activating the signaling pathway responsible for the enhanced expression of transforming growth factor-β (TGF-β) in diabetic nephropathy [9]. Similarly, overexpression of a peptide known as Kidney injury molecule-1 (KIM-1) is reported in diabetic tubular injury. Erythropoietin (EPO) is involved in the inhibition of apoptosis, regulation of inflammation, and formation of reactive oxygen species (ROS) [10].

Streptozotocin induces insulin-dependent diabetes mellitus by

Abbreviations: CAT, Catalase; SOD, Superoxide dismutase; POD, Peroxidase; GSH, reduced glutathione; TBARS, thiobarbituric acid reactive species; ALT, Alanine transaminase; PCE, *Pleurospermum candollei* ethanol extract.

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