Jasminum humile Attenuates Hyperglycemia, Fibrosis and Dyslipidemia in Streptozotocin and Alloxan Treated Rats

By

Aiman Siddique

Department of Biochemistry

Faculty of Biological Sciences

Quaid-i-Azam University

Islamabad, Pakistan

2023

Jasminum humile Attenuates Hyperglycemia, Fibrosis and Dyslipidemia in Streptozotocin and Alloxan Treated Rats

A thesis submitted in fulfillment of requirement for the

Degree of Master of Philosophy

In

Biochemistry

By

Aiman Siddique

Department of Biochemistry

Faculty of Biological Sciences

Quaid-i-Azam University

Islamabad, Pakistan

2023

In The Name of Allah

The Most Beneficent, The Most Gracious,

The Most Merciful

"Recite: In the name of thy Lord who created man from a clot. Recite: And thy Lord is the Most Generous Who taught by the pen, taught man that which he knew not."

(Quran, 96:1-5)

Quaid-i-Azam University, Islamabad, PAKISTAN
Department of Biochemistry
Faculty of Biological Sciences

CERTIFICATE

This thesis, submitted by **Ms. Aiman Siddique** to the Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan, is accepted in its present form as satisfying the thesis requirement for the Degree of Master of Philosophy in Biochemistry/Molecular Biology.

Examination Committee:

4. **External Examiner:** Signature: _____
   Dr. Sumaira Sahreen
   Associate Curator
   Botanical Sciences Division
   Pakistan Museum of Natural History, Islamabad

5. **Supervisor:** Signature: _____
   Prof. Dr. M. Rashid Khan

6. **Chairperson:** Signature: _____
   Prof. Dr. Iram Murtaza

Dated: **October 17, 2023**

DECLARATION

I, Aiman Siddique, hereby declare that my M.Phil. thesis, titled **"*Jasminum humile* Attenuates Hyperglycemia, Fibrosis and Dyslipidemia in Streptozotocin and Alloxan Treated Rats"** is my effort, and all work was carried out under the regulations set by Quaid-i-Azam University. No part of this thesis has been previously presented for any other degree. If found anything contrary, I shall be held responsible.

Aiman Siddique

DEDICATION

Humbly dedicated to my parents, whose boundless love and unwavering support have been my driving force to pursue knowledge and excel in my academic pursuits.

ACKNOWLEDGMENTS

All praise to the creator of the universe, **Allah Almighty**, the Merciful, the Benevolent, the Lord of the Lords. It is He who gave me more opportunities to explore what He has created. I offer my salutations to the **Holy Prophet Muhammad (PBUH)**, who has always been my greatest source of inspiration throughout my life. I am honored to be a part of his Ummah. I am deeply thankful to him for the moral lessons he taught us.

I owe my gratitude to all those beautiful people out there who have made this thesis possible. I have always been supported and supervised by many people to whom I would like to express my deepest gratitude. Thank you for making my research work memorable, these memories would remain with me for a very long time.

My deepest gratitude is to my supervisor, **Dr. Muhammad Rashid Khan**, Professor, Department of Biochemistry, Faculty of Biological sciences, Quaid-i-Azam University, Islamabad, Pakistan, whose expertise, understanding, and patience, added substantially to my research experience. I am very obliged to him for his reassurance, and counseling, especially for generating and maintaining a friendly environment throughout my study.

I have great pleasure in acknowledging my gratitude to my senior **Mehreen Fatima**, for her discerning comments and constructive criticisms at various stages of my research and for always being there to guide me. I am deeply thankful to her for the long discussions that helped me solve the technical errors in my work and for holding me to a high research standard and enforcing strict validations for each result, and thus teaching me how to perform a standard research. I am very thankful to her for her cooperation and support in my research work.

Many friends have helped me stay stable throughout this time. Their support and care helped me to overcome setbacks and stay focused on my research. I greatly value their friendship and I deeply appreciate their belief in me. I want to specially mention my close friends including **Areeba Ansar**, **Sheeba Sadiq** & **Zainab Shahzor**. I am so lucky to have such great and supporting people in my life.

I sincerely thank my lab fellows and colleagues especially **Maria Masood**, **Pakeeza Arshad** & **Memoona Noor** for their invaluable help during the research work and for filling my days with unforgettable moments, laughter and camaraderie. Your friendship and support have made this academic journey truly enriching and joyful.

I would like to thank all of the staff of the Department of Biochemistry specially **Mr. Farooq**, **Mr. Masood**, **Mr. Rasheed**, **Mr. Tariq**, **Mr. Fayyaz** & **Mr. Shehzad** for their cooperation during the conduct of this research work.

Most importantly, none of this would have been possible without love and patience of my family. I would like to express my heart-felt gratitude to my supportive Father, **Muhammad Siddique**, and my dearest Mother, **Nazia Siddique**, for their unflagging love and unconditional support throughout my life and my studies. You have given me the most unique, magical and care free childhood that has made me who I am now! I have to mention special thanks to my siblings **Muhammad Bilal Siddique** & **Abubakar Siddique** for making every moment more meaningful and cherished. Finally, I would like to thank, whoever has helped me in one way or the other in completion of this thesis, as well as expressing my apology that I could not mention their names.

Aiman Siddique

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Abbreviation	Full name
µg	Micro gram
µl	Micro liter
µM	Micro molar
Abs.	Absorbance
ACC	Acetyl-CoA carboxylase
AGEs	Advanced glycation end products
ALP	Alanine phosphatase
ALT	Alanine aminotransferase
ALX	Alloxan
AMPK	AMP-activated protein kinase
AST	Aspartate transaminase
BHA	Butylated hydroxyl anisole
BHT	Butylated hydroxytoluene
CAT	Catalase
cDNA	Complementary DNA
COL1A1	Collagen type 1 alpha 1
Conc.	Concentration
$CuSO_4$	Copper sulphate
DM	Diabetes mellitus
DMSO	Dimethyl sulfoxide
DNL	De novo lipid synthesis
DTNB	5,5'-Dithio-bis-(2-nitrobenzoic acid)

ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic Acid
ETC	Electron transport chain
FAS/FASN	Fatty acid synthase
FC	Folin-Ciocalteu phenol reagent
Fe^{+2}	Ferrous ions
$FeCl_3$	Ferric chloride
FFA	Free fatty acids
FTIR	Fourier transform infrared spectroscopy
g	Gram
GAE	Gallic acid equivalent
GDM	Gestational diabetes mellitus
GLI	Glibenclamide
GLUT2	Glucose transporter 2
GPx	Glutathione peroxidases
GR	Glutathione reductases
GSH	Glutathione
GSIS	Glucose-stimulated insulin secretion
GSTs	Glutathione-S-transferases
H&E	Hematoxylin & Eosin staining
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
H_3PO_4	Phosphoric acid
HCl	Hydrochloric acid
HDL	High density lipoprotein

HRP	Horse radish peroxidase
i.e.	That is
IC_{50}	Half maximal inhibitory concentration
IR	Infrared
J. humile	*Jasminum humile*
JHA	*Jasminum humile* aqueous fraction
JHB	*Jasminum humile* butanol fraction
JHC	*Jasminum humile* chloroform fraction
JHE	*Jasminum humile* ethanol extract
JHEA	*Jasminum humile* ethyl-acetate fraction
JHH	*Jasminum humile* hexane fraction
kg	Kilogram
LDL	Low density lipoprotein
M	Molar
mg	Milli gram
min	Minutes
ml	Milli liter
mM	Milli molar
MODY	Maturity onset diabetes of the young
mRNA	Messenger RNA
N	Normality
NAFLD	Non-alcoholic fatty liver disease
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide
NASH	Non-alcoholic steatohepatitis

NIH	National Institute of Health
nm	Nano meter
O^{-2}	Super oxide anion
^{o}C	Degree Celsius
OD	Optical density
OG	Octyl gallate
P_{450}	Cytochrome P-450
PBS	Phosphate buffer saline
PG	Propyl gallate
pH	Power of hydrogen ions
PMS	Phenazine methosulphate
POD	Peroxidase
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
rpm	Revolutions per minute
RT-PCR	Reverse transcription polymerase chain reaction
SD	Standard deviation
SMAD3	SMAD family member 3
SOD	Superoxide dismutase
SREBP-1	Sterol regulatory element-binding transcription factor 1
STZ	Streptozotocin
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TBARS	Thio-barbituric acid reactive substances
TCA cycle	Tricarboxylic acid cycle

TG	Triglycerides
TGFβ1	Transforming growth factor beta 1
TPC	Total phenolic content
UV	Ultraviolet
WHO	World Health Organization
$ZnSO_4$	Zinc sulfate

ABSTRACT

Diabetes mellitus (DM) is a multi-factorial disease associated with metabolic dysfunction, insulin impairments and increased oxidative stress. Hyperglycemia, the most prominent hallmark of DM, gives rise to other diabetic complications such as dyslipidemia and fibrosis. Despite advances in management of DM, the results are still far from perfect. However, there is a substantial degree of optimism regarding the potential efficacy of phytomedicine in this context. *Jasminum humile* possesses remarkable antioxidative and anti-inflammatory properties; however, the effects of this plant in maintaining diabetes are not well-documented. In accordance with this, the plant was examined for its therapeutic effects against streptozotocin-induced and alloxan-induced diabetes, with focus on selected aspects of hyperglycemia, dyslipidemia and fibrosis. The butanol fraction of *J. humile* was subjected to *in vitro* testing to comprehend its phytochemistry, antioxidant and antidiabetic aptitude. For *in vivo* study, JHB was administered orally to diabetic rats, followed by several biochemical, histological, and molecular analyses. Glycemic levels, serum markers, antioxidant enzymes, radical species and liver histological specimens were examined under varying circumstances. A series of versatile regulators were selected to analyze pathways involved in hyperglycemia, fibrosis and dyslipidemia. *In vitro* study revealed the presence of polyphenols along with significant radical scavenging and α-amylase inhibitory activity of JHB. *In vivo* investigations unveiled a distinctive trend: JHB exhibited superior outcomes against alloxan diabetes compared to STZ diabetes. The rationale ought to be reduction of oxidative stress, created by cytotoxic action of alloxan, owing to plant's polyphenolic content. JHB alleviated alloxan-induced toxicity by re-instating euglycemia, normal serum profile and oxidant/antioxidant balance. RT-PCR analysis exhibited restoration of mRNA expression of genes linked to glucose metabolism, lipid synthesis, and fibrosis. In conclusion, the outcomes of this study prove multi-faceted therapeutic effects of *J. humile* against diabetes and its complications.

Key words: Alloxan, diabetes, dyslipidemia, fibrosis, hyperglycemia, oxidative stress.

1. INTRODUCTION

1.1. 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 & 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 (Bastaki, 2005; Doss *et al*., 2009). The two primary types which account for the major burden of diabetes are; type 1, in which T-cell mediated immune response destroys insulin producing β-cells followed by absence of insulin synthesis and action, and type 2, characterized by insulin insensitivity and insufficient insulin production.

Diabetes is becoming more widespread after cancer and cardiovascular diseases (El-Beih *et al*., 2019). In the year 2015, 415 million adults were identified with diabetes and its growing incidence will rise to 642 million by the year 2040 globally (Ogurtsova *et al*., 2017; Mansoori *et al*., 2023). Studies have shown correlation of DM with a number of liver pathologies including unusual elevation of liver enzymes, fibrosis, non-alcoholic fatty liver disease (NAFLD), hepatitis and hepatocellular carcinomas.

1.2. Types of Diabetes

Diabetes is a group of chronic illnesses and it has four major types; type 1, type 2, gestational and other specific types of diabetes which may be genetic (controlled by a single gene such as maturity onset diabetes of the young) or secondary to drugs, pancreatic impairments or other illnesses. Out of all types, type-1 DM and type-2 DM account for the majority of diabetes cases (Forouhi & Wareham, 2019).

1.2.1. Type-1 Diabetes Mellitus (T1DM)

Type 1 diabetes occurs when body's immune system attacks pancreatic β-cells and destroys them, resulting in absolute insulin deficiency and hyperglycemia (Egan & Dinneeen, 2019). In contrast to type-2 DM, where both insulin insensitivity and decreased insulin secretion by the beta cells play a synergistic role, T1DM has a different pathogenesis (Paschou *et al*., 2013). T1DM pathogenesis has been reported to be a gradual process that

begins with the detection of auto-antibodies, progresses to beta cell damage, and, eventually, symptoms associated with hyperglycemia (Katsarou *et al*., 2017). If overlooked, insulin insufficiency causes a progressive metabolic disorder that promotes hyperglycemia, ketoacidosis and mortality (Gregory *et al*., 2013).

1.2.2. Type-2 Diabetes Mellitus (T2DM)

This type of diabetes is associated with β-cell dysfunction that leads to relative insulin deficit and insulin resistance in targeted organs (Chatterjee *et al*., 2017). Diminished insulin levels cause a decreased glucose uptake by liver, adipocytes and muscle cells, and subsequent increase in blood sugar levels (Olokoba *et al*., 2012). It occurs at later stages in life and increases with age (Masharani & Karam, 2001). It is diagnosed after about fourth decade of life. Approximately 90% of diabetes accounts for this type. This type of diabetes is subdivided into obese and non-obese diabetes (Kaul *et al*., 2013). In the former type, diabetic patients develop insulin resistance due to changes in cell receptors by deposition of abdominal fat, while in the later one, insulin resistance gets developed due to reduced synthesis and release of insulin. In addition to that, diet and lifestyle also contribute to the development of T2DM.

1.2.3. Gestational Diabetes (GD)

Gestational diabetes is a common complication of pregnancy first recognized at any time during gestation period (Zhang *et al*., 2023). Increased levels of reproductive hormones, particularly estrogens and progestogens, result in reduced fasting glucose levels and fat deposition. However, as pregnancy progresses, blood glucose levels after a meal slowly rise as tissue response to insulin declines. To maintain normal glucose levels throughout pregnancy, the maternal pancreatic beta cells must secrete enough insulin to compensate for the associated insulin insensitivity by target tissues. Women who develop gestational diabetes during pregnancy are presumably unable to boost their insulin secretion to compensate for their increased insulin resistance (Reece *et al*., 2009).

1.2.4. Maturity Onset Diabetes of the Young (MODY)

MODY is a rare form of diabetes, inherited in autosomal dominant pattern and caused by heterozygous mutations in multiple transcription regulators involved in pancreatic cell growth and maturation. Furthermore, mutations in enzymes involved in cell glucose sensing have been linked to early-onset diabetes. MODY is distinguished by autosomal pattern of inheritance, early development of diabetes (often before the age of 25), absence of symptoms associated with the autoimmune process or insulin resistance, and the maintenance of endogenous insulin production. (Anık *et al*., 2015).

1.3. Epidemiology

Epidemiology of DM is influenced by both genetic and environmental factors (Chatterjee *et al*., 2017). Although it accounts for only 10% of all occurrences of diabetes globally, T1DM manifests itself substantially earlier in life (Paschou *et al*., 2013). The incidence of T1DM is high in Europe and North America, intermediate in Africa and low in Asia. Genetic predisposition is crucial but insufficient in the development of T1DM. Environmental factors like viral infections, vaccinations, drugs, toxins, dietary and nutritional factors also play role in progression of disease (Forouhi & Wareham, 2019).

Surveys conducted in multicultural populations indicate that inhabitants of South Asia are probably predisposed to develop T2DM and insulin resistance upon exposure to unfavorable conditions (Carulli *et al*., 2005). Biological effects of insulin sensitivity may be reduced significantly due to multiple mutations in genes associated with it (Carulli *et al*., 2005). Environmental factors that cause T2DM include obesity, sedentary lifestyle, alcohol consumption, excessive sugar consumption, uncontrolled fat intake, cigarette smoking etc. (Chatterjee *et al*., 2017).

1.4. Oxidative Stress Mediated Damage in Diabetes

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 cancel out the dominating oxidative damage. Although a certain proportion of reactive oxygen species (ROS) is needed for playing key metabolic roles, unrestrained production is deleterious as it can mediate insulin impairments and lead

to diabetes mellitus. In diabetes, β-cell function is lost due to high influx of nutrients and resulting ROS generation. The peripheral target tissues of insulin (muscular, hepatic and cardiac tissues) face greater fatty acid (FA) fluctuation, whilst non-target tissues including the (renal and neural tissues) encounter both high glucose and FA levels. Each of these worst-case scenarios account for ROS induced diabetic complications. The 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 apoptotic death in beta cells (Kohnert *et al*., 2012). These processes cause severe molecular and cellular damage that provoke the disease.

1.5. DNA Damage in Diabetes

Diabetes mellitus is a multi-factorial disease. Although insulin insufficiency and insulin insensitivity are considered the main causes of DM, its development is also influenced by environmental factors and epigenetics (Alam *et al*., 2016). There is evidence that alterations in DNA methylation may contribute to the increased prevalence of both type 1 and type 2 diabetes. The transfer of methyl group causes damage to DNA and results in its fragmentation. To repair this damage, nuclear enzymes like Poly-ADP ribose polymerase (PARP) are hyper-stimulated which cause depletion of NAD^+ and ATP reserves. Cellular energy resources do not comply with the requirements and cells undergo necrotic death ((Lenzen, 2008). Several factors are known to alter methylation patterns which cause progression of diabetes. With increasing age, error rate of methylation also increases which can predispose diabetes related genes to silencing. Toxins like arsenic and a diet deficient in sulfur also increase the risk of DM; arsenic affects expression of genes related to glucose homeostasis, while sulfur deficiency causes depletion of S-adenosyl methionine (SAM), a universal methyl donor (Alam *et al*., 2016).

1.6. Diabetes – Correlation With Dyslipidemia and Fibrosis

Hyperlipidemia refers to high levels of lipids in blood, particularly triglycerides and cholesterol. The link between hyperglycemia and hyperlipidemia is multifactorial and involves several mechanisms:

- Hyperglycemia triggers the release of free fatty acids from energy reserves of adipose tissue. These released fatty acids are then transported to the liver, where they are converted into triglycerides, contributing to elevated blood lipid levels.
- High blood glucose levels can stimulate the liver to synthesize more lipids, including triglycerides and cholesterol. This occurs via various pathways, including the upregulation of enzymes involved in lipid synthesis.
- Prolonged hyperglycemia can lead to oxidative load and chronic inflammation, which also contribute to dyslipidemia (abnormal lipid levels). Inflammation can affect lipid metabolism and induce the release of pro-inflammatory cytokines that further worsen lipid profiles.

Diabetes is strongly linked with the development and progression of liver fibrosis through various mechanisms:

- Diabetes is contemporary with abnormal redox status and inflammation in liver. Inflammation damages hepatocytes and prompts the production of fibrous tissue, leading to fibrosis.
- Elevated blood glucose levels in diabetes can instigate the formation of AGEs, which trigger inflammation and contribute to liver fibrosis.

Diabetes is closely linked to NAFLD, a condition characterized by the build-up of fat in the liver. NAFLD can progress from simple steatosis (fatty liver) to non-alcoholic steatohepatitis (NASH), a more severe form of inflammatory liver disease, which eventually triggers fibrosis of liver (Mohamed *et al*., 2016).

1.7. Conventional Diabetes Treatment

At present, modifications in lifestyle to control contributary factors like hyperlipidemia, prescription of oral anti-hyperglycemic medications and insulin shots are among primary approaches to cope with hyperglycemia. However, these approaches are costly with detrimental side effects and reduced efficacy. These limitations make it challenging to manage diabetes (Chaudhury *et al*., 2017; Omale *et al*., 2023).

Table 1.1. Commonly used oral anti-hyperglycemic medications (Omale *et al*., 2023).

Class	Drug
Sulfonylureas	Glibenclamide, Chlorpropamide
Biguanides	Metformin
Insulin Sensitizers	Thiazolidinediones
α-Glucosidase Inhibitors	Acarbose

1.8. Phytomedicine

The global worsening of morbidity and mortality from diabetes justifies the need for more diversified research for new therapies. For the prevention and treatment of human diseases, medicinal plants have been utilized across the span of human history. A practice that began with error and trial by our forefathers has undergone numerous alterations and improvements leading to its current position. Earth is home to approximately 4.2 million plant species, but the lack of awareness about them and their various uses demands to be explored. In consolidation with various healthcare fields, phytomedicine has certainly reconstituted and underwired the fundamentals of the current healthcare system and conquered major shares in the industry. Reports collected transnationally indicated that nearly 35,000 plant species are utilized in formulation of ayurvedic medicines. However, only a few percent of the total advances to the biological screening stage. Many of them are still in the process of their advantageous properties exploration, resulting in the unearthing of novel plant-based medications. However, countless medicinal plants still need some investigation by modern bits of knowledge. Therefore, the future of plant-based medications seems to have remarkable scopes for uncovering some new and innovative therapeutic approaches (Khan, 2015).

1.9. Significance of Phytomedicine

Plants contribute a central part to human pharmaceutical services. As estimated by the World Health Organization (WHO), phytomedicine is the most reliable approach after primary healthcare for around 70% to 95% of the population residing across the globe, and

a significant portion of folk medicine involves plant extract-derived medicines and decoctions, better known as 'modern herbal medicine' (Pan *et al*., 2013). The defense system of plants owing to diversified secondary metabolites, which makes the phytoconstituents therapeutically active, makes them used as medicines (Patel & Rajput, 2013). The synergistic effect of phytochemicals due to different functional groups helps them in treating various ailments. They have antioxidative, anti-inflammatory, anti-allergic, anticarcinogenic and hepatoprotective properties (Halliwell & Gutteridge, 1990). Besides, they can scavenge the reactive species that cause oxidative damage to cells and strengthen the biological environment with minimal side effects (Krishnaiah *et al*., 2011).

1.10. Screening of Plant Phytochemicals

Plant secondary metabolites are identified in thousands, but it is far anticipated that other lots are to be discovered. Since these secondary metabolites are isolated from natural assets and have been elaborated within living systems, therefore they are perceived to exhibit more drug like properties and are biologically friendly compared to synthetic molecules. This makes them worthy candidates for advance drug development (Vitali *et al*., 2016).

Phytochemicals are non-nutritive chemical compounds with diverse protective properties. Though plants contain thousands of metabolites, yet its qualitative screening may provide a narrow spectrum of constituents present within it. The examination of chemical components in plant reveals only those constituents accumulated in specific organ sites whereas the absence and presence of these constituents depend largely upon extent of accumulation, quantity of plant material being used, and type of analytical technique employed (Yusuf *et al*., 2014).

The assays used for screening have to be simple, reproducible, in expensive and rapid in addition to being sensitive enough for reliable detection of bioactive constituents in crude extracts, where they are usually present in low concentration. Its selectivity should be able to reduce the chance of false positives and at the same time prove insensitive to possible interferences of other plant metabolites. Owing to the poor solubility of plant extracts or fractions under test conditions, false positive and false negative results become a serious problem when compared to pure compounds. However, a number of assays have emerged

and are used by scientists in phytochemical laboratories routinely (Hamburger and Hostettmann, 1991).

1.11. Antioxidants

Antioxidants are potent compounds that can hinder or restrain the cycle of oxidative responses by scavenging free radicals and reactive oxygen species (ROS) and retarding the progression of many chronic disorders (Gulcin, 2020). Free radicals have a natural tendency to perform chain reactions. Antioxidants are substances that prevent cell damage by evading these chain reactions (Neha *et al*., 2019). They remove free radicals and block other oxidation reactions by being oxidized themselves. They act as reducing agents most of the time.

An extensive range of natural and synthetic antioxidants have been discovered over the years. Natural antioxidants react with radicals and form stable products. This breaks the cycle of oxidative reactions. They include antioxidant minerals, vitamins and phytochemicals. Minerals e.g., selenium, copper, iron, zinc and manganese, act as co-factors for enzymes involved in antioxidation. Vitamin B, C and E are potent antioxidants and needed for vital metabolic functions. Phytochemicals are also bio-active compounds and include flavonoids, anthocyanins, lignans, stilbenes, carotenoids and other polyphenols (Hamid *et al*., 2010).

Antioxidants act as a defense system as they inhibit formation of free radicals inside body. They protect biological system by preventing the production of new radicals, terminate chain reactions initiated by these radicals and restore damage inflicted by them. Based on their activity, they are classified as enzymatic and non-enzymatic compounds. Inside body, enzymes like catalase, glutathione peroxidase, superoxide dismutase and glutathione reductase work in harmony to protect body tissues from free radical damage. These enzyme based antioxidants need mineral ions such as zinc, copper, selenium, manganese as co-factors. Non-enzyme based antioxidants include vitamins, proteases, lipases, flavonoids, carotenoids, catechins & flavones (Zulaikhah, 2017; Neha *et al*., 2019).

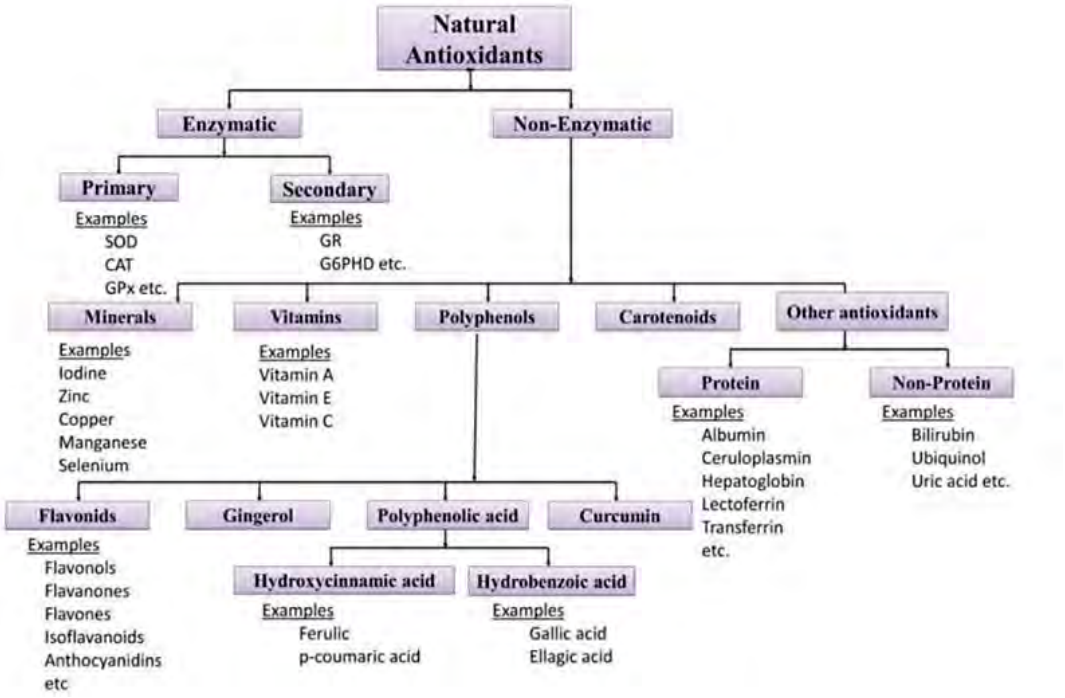


Figure 1.1. Classification of antioxidants found in natural sources (Anwar *et al*., 2018).

1.12. Pharmacological Properties of Phyto-Antioxidants

Phyto-antioxidants play protective roles by restraining free radical propagation hence proving advantageous in alleviating oxidative stress related diseases. Polyphenols are in the limelight nowadays among researchers owing to their potent antioxidant properties. Plant polyphenols play key roles in glucose metabolism by decreasing intestinal glucose uptake, inhibiting carbohydrate hydrolyzing enzymes (such as α-amylase), and increasing insulin secretion from β-islets. Flavonoids and tannins reduce the expression of glucose transporters on liver and inhibit gluconeogenesis (Shahwan *et al*., 2022). By virtue of their harmless nature, plant derived antioxidants and other bioactive compounds are the primary focus of contemporary scientific research.

1.13. Oleaceae – The Olive Family

The Oleaceae family roughly contains 28 genera and over 900 species (Akhtar *et al*., 2021). It is well-recognised for its multi-faceted benefits in nutrition, medicine and horticulture. In eastern Europe, the buds of *Syringa vulgaris* L. are fermented into wine and used to

relieve joint pain. In Meridione, the outer bark of *Fraxinus ornus* L. is used to combat diarrhea and hypercholesteremia. The fruit of the *Ligustrum lucidum* W.T. Aiton possesses great hepatoprotective and nephroprotective benefits. In Greece, the leaves of *Olea europaea* L. are used to alleviate hypertension (Mansour *et al*., 2022). In Oman, oil isolated from *O. europaea* is used as a purgative and has great antioxidant, anti-inflammatory & anti-cancer properties. (El Haouari *et al*., 2020). The seeds of the *F. excelsior* L. are used as a natural remedy for diabetes because of their anti-hyperglycemic effect (Huang *et al*., 2019)

1.14. Jasminum – A Major Genus of Flowering Plants

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 number of volatile oils and essential oils are sourced from the flowers of such plants for applications in fragrances and therapeutics. Various plant species from this genus have been screened for their curative properties against ulcers (Nilesh *et al*., 2009), cancer (Asirvatham *et al*., 2012) and diarrhea (Jia *et al*., 2008). Jasminum species also exhibit antioxidant, antimicrobial and wound healing activities (El-Hawary *et al*., 2021). This genus is widely distributed in the tropical and sub-tropical lands of Asia as well as in the group of islands surrounded by Pacific Ocean (Quang *et al*., 2021).

1.15. *Jasminum humile*

Jasminum humile is an evergreen shrub widely cultivated as an ornamental plant due to its fragrant and attractive flowers. In addition to its ornamental value, *J. humile* has been traditionally used for medicinal purposes by various indigenous communities. Researchers have demonstrated therapeutic powers of *J. humile* against inflammatory and infectious conditions of skin and mouth. The plant is used to prepare tonics to boost cardiac health and to cure ringworm disease. It possesses great radical-scavenging and antibacterial activity and is used for treating chronic fistulas (Fatima & Khan, 2023).

1.15.1. Morphology

Evergreen erect shrub growing 1-3 m tall, stem is much-branched, green in color, angular in shape, pubescent or puberulent. Leaves are compound or simple with 0.5-2 cm long petiole. Leaves are mostly alternate, varying in size, 2-10 cm long. Leaf and leaf blades are papery, 3-9 leaflets, dark green above, pale green beneath, elliptical and ovate. Flowers are yellow in color, fragrant, 1- 10 in cymes terminal with pedicel length of 0.2-3 cm. Calyx tube is about 3 mm long while yellow corolla tube has a length of 1-2.5 cm; lobes are ovate or round, 5 in number. Fruit is in the form of berries, simple or didymous, globular-ellipsoid in shape, 4-6 mm long, purple-black when ripe and full of crimson juice. Flowering season is April-June, fruit is observed in the months of September-December. It is planted in homes and institutions as an ornamental plant (Flora of Pakistan, Flora of China).

Figure 1.2. *J. humile*, an evergreen shrub with multi-branched green stems, compound leaves and yellow flowers.

1.15.2. Geological Distribution of *J. humile*

J. humile is grown all over the world for its decorative, pharmacological, and cultural aspects. The plant is native to the Himalayas, Pakistan, Tajikistan, western part of China, Burma and Nepal. It is also cultivated in the Mediterranean area, Iran, Italy and in islands enclosed by Pacific Ocean. It grows naturally in dry and moist climates, as observed in tropical and sub-alpine regions (Kunwar *et al*., 2021). It is widely spread at an altitude of 1000-3000 m (Flora of Pakistan). In Pakistan, *J. humile* variants are dispersed in the temperate and subtropical localities of Islamabad, Abbottabad, Baluchistan and aerial parts of Kohistan (Akhtar *et al*., 2013).

1.15.3. Taxonomical Ranking

Table 1.2. Taxonomical classification of *J. humile*.

Kingdom	Plantae
Superphylum	Embryophyte
Phylum	Tracheophyta
Class	Spermatopsida
Subclass	Magnoliidae
Superorder	Asteranae
Order	Lamiales
Family	Oleaceae
Genus	Jasminum
Specie	*Jasminum humile*

1.15.4. Synonyms

Chrysojasminum humile

Jasminum chrysanthemum Roxb.

Jasminum farreri Gilmour

Jasminum wallichii Jacques

Jasminum italicum Dippel

1.15.5. Local Names

English: Italian jasmine, yellow Jasmine

Spanish: Jazmín de Italia

Chinese: Ai tan chun

Hindi: Peeli Chameli

Malayalam: Ponmallika

Bengali: Svarnajui

Tamil: Semmallingai

Jammu: Sanairad, Jard Siyoon

Pashto: Zyar rambil chambil

Sanskrit: Svarnajuthica

1.16. Ethnobotany of Jasminum Species

Apart from ornamental aspect, Jasminum species have revealed many biological capabilities. *Jasminum grandiflorum* is used against ulcers, skin diseases, leprosy and dysmenorrhea. The plant exhibited great anti-hypertensive potential in vitro (El-Shiekh et al., 2020) and potent anti-inflammatory activities against bowel disease and arthritis (El-Shiekh et al., 2021). Essential oils from *Jasminum sambac* L. are incorporated in skin care items due to their aroma and antioxidant effects (Mansour et al., 2022). The plant also possesses cytotoxic and anti-cancer properties (Olatunde et al., 2023).

J. humile is quite beneficial for treating infections caused by parasitic worms (Helminths). Roots of the plant are used as diuretic to balance out water and salts in body and maintain a healthy blood pressure (Akhtar *et al*., 2013). Root decoctions are also used to treat ringworm disease and sinusitis (Rashid *et al*., 2015). Flower decoctions are used for purifying blood, treating jaundice (Jan *et al*., 2009) and getting rid of kidney stones (Irfan *et al*., 2018). Leaves are used for making tea (Kunwar *et al*., 2012), relieving throat pain and ENT problems (Shrestha & Dhillion, 2003).

Figure 1.3. *J. humile*. Herbarium specimen deposited in Herbarium of Pakistan, QAU, Islamabad.

1.17. Research Goals and Objectives

This study is focused on ascertaining the antioxidant and antidiabetic properties of *J. humile* in streptozotocin and alloxan treated rats, by modulating mRNA expression of molecular markers associated with hyperglycemia, fibrosis and dyslipidemia.

1.18. Work Outline

- Collection, identification and pulverization of leaves of *J. humile*.
- Formulation of crude extract followed by fractionation with organic solvents to leech out bio-active constituents.
- Qualitative and quantitative phytochemical assessment to validate different classes of phyto-chemicals.
- Antioxidant and anti-diabetic appraisal by various in vitro bio-assays.
- In vivo assessment of *J. humile* against alloxan and streptozotocin-induced diabetes in rats.
- Histological study to detect the defensive potential of extract on damaged tissues.
- Serum analysis of hepatic bio-markers (AST, ALT, ALP, triglycerides, cholesterol, albumin) and tissue analysis for investigating activity of antioxidant enzymes (CAT, POD, SOD, GSH), activity of free radical species (H_2O_2, nitrite, TBARS) and total protein present in tissue.
- RNA extraction from hepatic tissues of rat.
- Quantitative assessment of extracted.
- RNA synthesis of cDNA from RNA.
- RT-PCR to study the mRNA expression of genes associated with diabetes, dyslipidemia & liver fibrosis.

2. LITERATURE REVIEW

2.1. Diabetes and Hepatic Dysfunction

Diabetes is a set of heterogeneous disorders with serious clinical consequences. It has been long recognized that the underlying pathogenic mechanism for diabetes is hepatic dysfunction. Liver is the hub of multitude of metabolic processes that are crucial for the human body. It has been long recognized that liver is essential for carbohydrate homeostasis, primarily through glucose breakdown, synthesis, and accumulation. It is also responsible for regulating blood glucose levels due to its capacity to utilize, store, produce, and release glucose (Corless & Middleton, 1983). Apart from carbohydrate metabolism, it performs multiple vital functions which include systematic uptake of lipids, amino acids, cholesterol and bile acids in order to store or metabolize them and later on releases them in blood or bile.

2.2. Blood Glucose Regulation by Liver

In human body, all the physiological processes occur in a regulated manner to maintain a stable state said to be homeostasis. The level of glucose in blood is regulated by negative feedback mechanism depending upon release of insulin and glucagon from pancreatic islets. Glucagon is released by α-cells while β-cells release insulin under different circumstances. When glucose levels decrease, there is predominant release of glucagon. In the opposite situation, insulin; the antagonist of glucagon, is released.

During hyperglycemia, insulin secreted by β-cells binds with tyrosine kinase insulin receptor present in membrane of hepatocytes. This binding activates auto-phosphorylation of β-subunit of insulin receptor and modulates signals in hepatocytes for conversion of excess glucose into glycogen. Insulin also directs other target cells (adipose tissue and skeletal muscles) to facilitate glucose uptake by cells via incorporating more GLUT4 in plasma membrane. This whole process promotes glycogen synthesis from glucose and normal glucose level is restored.

During hypoglycemia, α-cells promote the release of glucose by signaling hepatocytes to switch to glycogenolysis (converting glycogen to glucose). Glucose is then released into

the bloodstream. This phenomenon also promotes gluconeogenesis in the liver (Kaul *et al*., 2013).

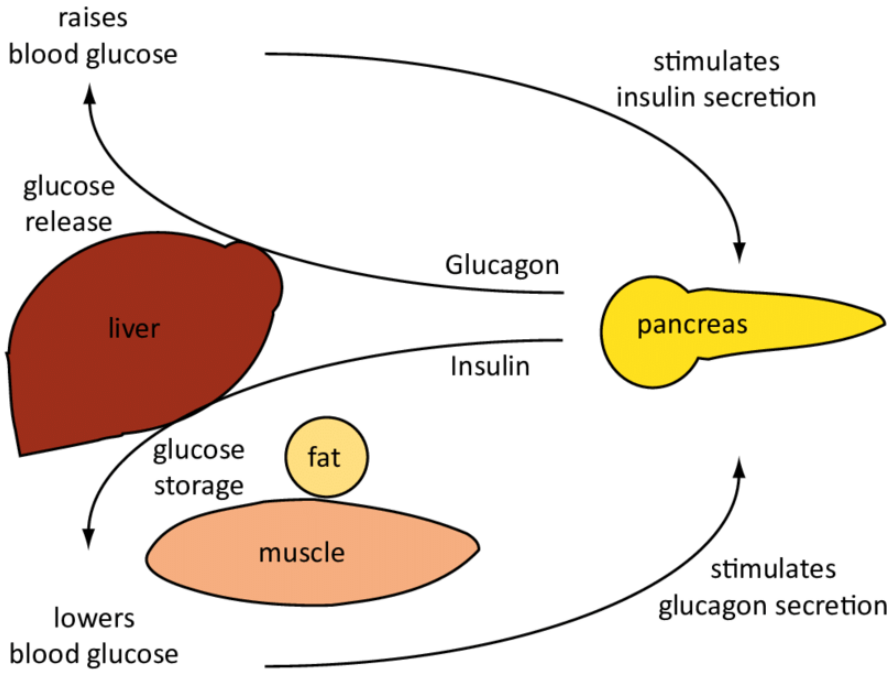


Figure 2.1. Blood glucose regulation by antagonistic actions of insulin and glucagon (Steinbusch *et al*., 2011).

2.3. Diabetes – Complications

When body suffers from hyperglycemia, liver becomes unable to recognize pre-existent glucose and keeps on producing more with the help of enzymes for glucose metabolism. Prolonged hyperglycemia can lead to both microvascular complications (including damage to eyes, kidneys, nerves) and macrovascular complications (damaged blood vessels, myocardial infarction, limb amputation, stroke) (Defronzo *et al*., 2015; Sharma *et al*., 2016; Mishra *et al*., 2022).

Aside from these traditional complications, studies have reported correlation of diabetes with a number of cancers, liver disease, cognitive and functional impairments, mental and sleep disorders, and infections (Tomic *et al*., 2022). DM is strongly linked to NAFLD which is caused by uncontrolled deposition of lipids in liver (Cernea & Raz, 2021; Targher

et al., 2021) and if the condition worsens, it can transform into non-alcoholic steatohepatitis (NASH) (Friedman *et al*., 2018).

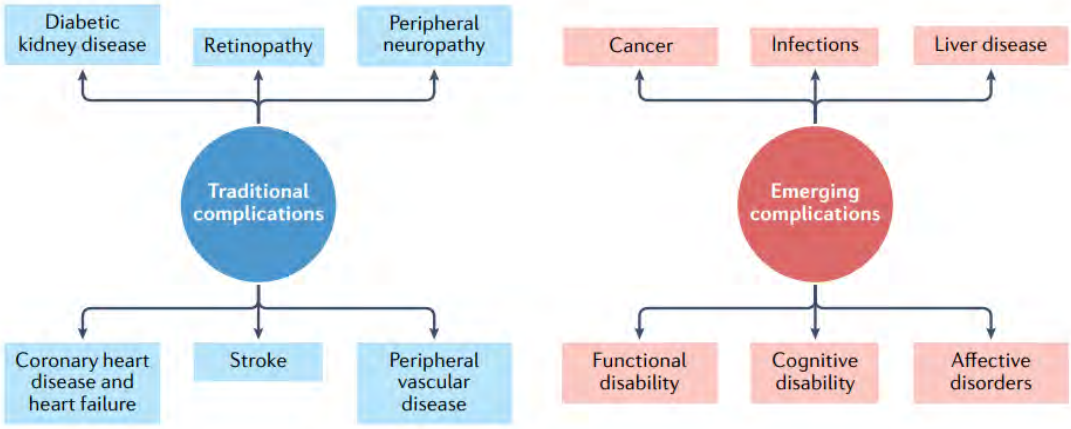


Figure 2.2. Major traditional and emerging complications of diabetes (Friedman *et al*., 2018).

2.4. Hepatic Serum Markers – Association With Diabetes

Individuals suffering from DM are highly vulnerable to liver function test abnormalities as compared to healthy or non-diabetic individuals. Fluctuations in levels of serum markers are highly involved with insulin resistance and risk of DM (Islam *et al*., 2020).

Alanine transaminase is primarily a hepatic enzyme localized in hepatic cell cytoplasm. It is concentrated in liver as compared to other organs like kidney, muscle or heart where it catalyses important transamination reaction in cytoplasm. Any type of minor hepatic cell damage can increase ALT levels in blood referring to hepatic disorders.

AST is also a hepatic enzyme, localized in mitochondria, that helps in metabolism of amino acids. Usually AST levels are low but remarkable increase in AST levels refer to hepatic inflammation, or hepatic disorder.

ALP is hepatic enzyme crucial for protein disintegration. It is also present in bones. Higher than normal ALP levels indicate hepatic injury like blocked bile duct or bone diseases.

Serum albumin is a crucial plasma protein synthesized by liver. It is a strong prognostic marker of many disease states. It can scavenge a variety of oxygen free radicals and also binds unconjugated bilirubin (Levitt & Levitt, 2016).

During diabetic state, lipoprotein profile of affected individuals undergoes drastic changes due to underutilization of glucose. Accumulation of triglycerides in serum is the key feature of diabetic dyslipidemia (Shishehbor *et al*., 2008).

People with diabetes are more prone to having high cholesterol. Total serum cholesterol includes both good (HDL) and bad (LDL) cholesterol as well as other lipid components.

2.5. Free Radicals – The Pro-Oxidants

Free radicals are the species that can exist alone and have one or more than one unpaired electron in their valance or outermost shell. They are short lived and unstable but highly reactive towards vital biological entities (Yaribeygi *et al*., 2019). Some free radicals are of much significance in biochemistry because of their crucial roles in various physiological circumstances. Others are well known for their practical implications in a wide range of ailments. Generally free radicals exist in two forms, RNS (reactive nitrogen species) and ROS (reactive oxygen species) (Phaniendra *et al*., 2015). Dual role is played by ROS/RNS as both of them are important and harmful to the living cells. At medium or low concentration, ROS/RNS engage in physiological tasks like immune protection, mediators of signaling pathways and regulators of redox reactions. Both ROS and RNS mediate oxidative and nitrosative stress at elevated levels leading to plausible devastation to biomolecules.

Oxidants are generally sub-divided into radicals and non-radicals. The former are unstable and potentially hazardous, while the latter are stable in nature but are easy targets for radicals (Qazi & Molvi, 2018). Some of the radicals and non-radicals are mentioned in Table 2.1.

Table 2.1. Classification of oxidants into radicals and non-radicals (Qazi & Molvi, 2018).

Radicals	Non-Radicals
Superoxide ($O_2^{-\cdot}$), hydroxyl ($OH^{\cdot}$), alkoxyl ($RO^{\cdot}$) & peroxyl radicals ($ROO^{\cdot}$).	Hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), ozone (O_3).
Nitrogen monoxide ($NO^{\cdot}$), nitrogen dioxide ($NO_2^{\cdot}$).	Peroxynitrite, nitroxyl anion (ONOOH), nitronium cation (NO_2^+).

2.6. Generation of Free Radicals in the Body

The production of free radicals in the body is a ceaseless and inevitable phenomenon. They are generated during both intrinsic and extrinsic reactions. Intrinsically ROS is formed during the physiological phenomenon related with mitochondrial respiration, assimilation of NADPH oxidase in lysosomes, xanthine oxidase activity, phagocytosis, cytochrome P_{450} enzyme system associated with endoplasmic reticulum and reactions catalyzed by peroxisomal enzymes. Extrinsically ROS formation can be due to gamma irradiation, sunlight, cigarette smoke, xenobiotics and anthracycline containing drugs (Santo *et al*., 2016; Yaribeygi *et al*., 2019). As per Sena and Chandel (2012), mitochondrial respiratory chain remains number one in producing the highest supply of free radicals.

2.7. Impact of Oxidative Stress on Key Biomolecules

Lipids are the foremost victims of degradation by ROS. As a result of peroxidation of lipids, aldehydes with great reactivity are produced, malonaldehyde (MDA) being one of them. Higher concentration of MDA correlates with greater peroxidative damage suggesting a deficit of antioxidants. Consequently, the oxidative imbalance and glycemic levels elevate. Oxidation of proteins results in the production of carbonyls, advanced oxidation protein products (AOPPs) and advanced glycation end products (AGEs). Carbonyls are puissant oxidative stress indicators. AOPPs are responsible for endothelial deterioration. AGEs trigger inflammatory cytokine production via intracellular modifications of proteins.

Glutathione is a small tripeptide with great efficacy as antioxidant. It can successfully detoxify radicals, maintain reduced state of SH functional groups of proteins and assist in catalysis as a co-enzyme. In DM, reduced GSH to oxidized GSH (GSSG) ratio is altered which leads to beta cell deterioration and diabetic complications. A deficit in precursor amino acids can bring about reduced GSH and correspondingly greater oxidative stress. Deficiency of catalase and superoxide dismutase are also among the risk factors for DM. Catalase protects beta islets from hydrogen peroxide indicted damage while SOD prevents generation of hydrogen peroxide from superoxide anions (Tiwari *et al*., 2013).

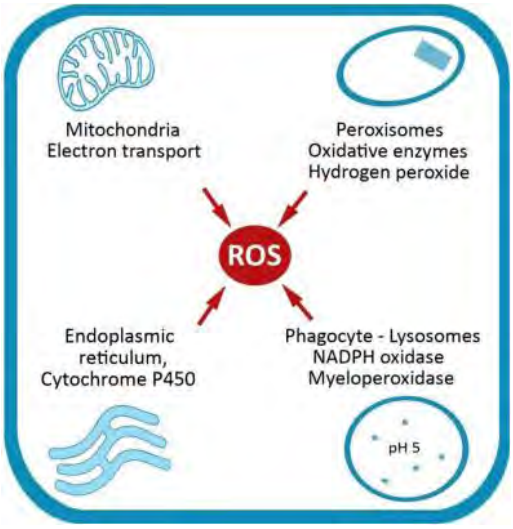


Figure 2.3. Organelles with high demands for oxygen produce ROS (Santo *et al*., 2016).

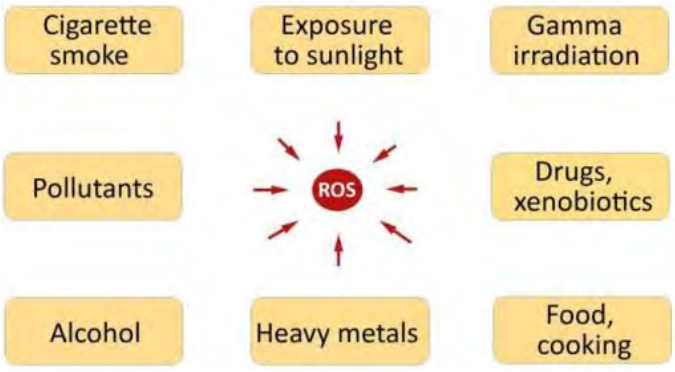


Figure 2.4. Exogenous sources of ROS include a number of environmental factors (Santo *et al*., 2016).

2.8. Antioxidative Potency of Crude Herbal Extracts

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 (Durai *et al*., 2016), anticancer (Batool *et al*., 2017), antioxidant (Sahreen *et al*., 2017), anti-inflammatory (Achakzai *et al*., 2019), analgesic (Majid *et al*., 2015), antipyretic (Afsar *et al*., 2015) and antimicrobial properties (Ali *et al*., 2018).

2.9. Antidiabetic Perspective of Therapeutic Plants

Despite the availability of anti-diabetic treatment regimens in the market, treating diabetes using medicinal plants is usually beneficial. Around the globe, significant therapeutic choices for the management of this disorder include herbal medications and plant components without adverse effects. A number of studies have demonstrated the favorable effects of medicinal plants in the management of diabetes, owing to their hypoglycemic properties. Flavonoids, tannins, phenolic acids, and alkaloids are the most often utilised herbal active components in the treatment of diabetes. The significance of anti-diabetic capabilities of these plants is implied by the presence of these chemicals. For instance, tannins boost insulin release and enhance pancreatic β-cell activity. In addition to averting lipid peroxidation and metal ion chelation, quercetin works through a number of processes associated with the elimination of oxygen radicals. Hypoglycemic plants actually work by raising insulin secretion and inhibiting the synthesis of glucose by liver cells which are mostly responsible for the alleviation or elimination of diabetes complications (Kooti *et al*., 2016).

2.10. Potential Therapeutic Targets of Diabetes and Its Complications

GLUT2 transporters mediate uptake of glucose by hepatocytes. In the course of hyperglycemia, glucose uptake by liver cells increases where it is fed into glycolysis, converted into glycogen for storage purposes, or used for synthesis of lipids from scratch. High glucose concentration in liver accelerates carbohydrate metabolism and inflicts hepatotoxicity. Additionally, the already elevated levels of triglycerides (TG) and free fatty acids (FFA) are exacerbated by *de novo* synthesis of lipids. This overload of glucose and FFA is oxidized via TCA cycle. On top of that, the defective mitochondrial electron transport chain produces ROS that leads to inflammation and fibrosis of liver (Kumar *et al*., 2021). These pathways show potential as therapeutic targets for diabetes.

2.10.1. Hyperglycemia

Hyperglycemia, as name indicates, refers to elevated blood sugar levels. It is closely linked with glucose transporters (GLUTs) in the context of glucose homeostasis and metabolism. GLUTs are major glycoproteins that reside within the lipid bilayer and provide metabolic energy via facilitative diffusion of sugars. The members of this family exhibit various patterns of expression and changes in metabolic states can fluctuate their abundance. In humans and rodents, loss of expression of GLUT in pancreatic β-cells is related to elevated glycemic levels and reduced glucose-stimulated insulin secretion (GSIS). Both hyperglycemia and GSIS are early indicators of diabetes that promote the onset of insulin impairments (Ohtsubo *et al*., 2005). GLUT2 or SLC2A2 (Solute carrier 2A2) is predominantly expressed in sinusoidal membrane of hepatocytes and beta cells of the pancreas. It is a high-capacity, low-affinity glucose transporter, that mediates uptake of glucose in liver and pancreatic β-cells to reinstate normoglycemia (Møller *et al*., 2001). GLUT2 expressed in pancreatic beta cells can detect hyperglycemia due to its high affinity for glucose and stimulate insulin secretion from these cells (Almutairi *et al*., 2019). When blood sugar level is high, liver GLUT2 mediates uptake of glucose, where it is converted to glucose 6-phosphate for further metabolic processing. The inactivation or decreased expression of GLUT2 expressed in liver during fasting or starvation can alter the fat and energy metabolism in the body.

2.10.2. Liver Fibrosis

Diabetes and liver fibrosis are interrelated and promote each other. Fibrosis is characterized by an excessive buildup of ECM, mostly made of Collagen type-I. During liver fibrosis, synthesis of extracellular matrix (ECM) exceeds degradation of ECM. Excessive accumulation of ECM damages the hepatic architecture and disrupts normal function of the liver (Xu *et al*., 2016). The activation of quiescent hepatic stellate cells (HSCs) triggers fibrogenesis. Upon activation in response to liver injury, HSCs transform into proliferative fibrogenic cells that release collagens especially collagen type I. Damaged liver cells secrete various fibrogenic stimulants and cytokines, notably transforming growth factor β1 (TGF-β1), the most potent of all (Liu *et al*., 2012). Upon activation, TGF-β1 binds to respective transmembrane receptors of cells at site of injury. These receptor domains phosphorylate Smad2/3 which translocate to nucleus and induce expression of fibrogenic genes such as procollagen type-1 alpha 1 (COL1A1). COL1A1 is responsible for the production of ECM for damage repair. Thus, inhibiting TGF-β1 signaling in HSCs is a key target of therapy for restricting the onset and progression of hepatic fibrosis (Yang *et al*., 2015; Xiang *et al*.,2020).

2.10.3. *De Novo* Lipid Synthesis

Uncontrolled fat deposition in liver can exacerbate diabetic condition. Within the context of lipid metabolism, AMPK acts as the protagonist which monitors the deposition of fats in liver. AMPK impedes the biogenesis of lipids by phosphorylating acetyl-CoA carboxylase (ACC). The dismissal of ACC results in reduced concentration of malonyl CoA and liver switches towards oxidation of fatty acids. AMPK also inhibits certain transcription factors needed for the expression of lipogenic genes such as fatty acid synthase (FASN). One such transcription factor is sterol-regulatory-element-binding protein-1c (SREBP-1c), which is reportedly downregulated by AMPK along with its target genes (ACC & FASN). Upregulation of AMPK is a promising curative approach for treating diabetic dyslipidemia as it alleviates insulin unresponsiveness by target cells and balances plasma glucose levels. Upregulation of AMPK can effectively modulate altered expression of hepatic ACC, FASN and SREBP-1c (Ben Djoudi Ouadda *et al*., 2009).

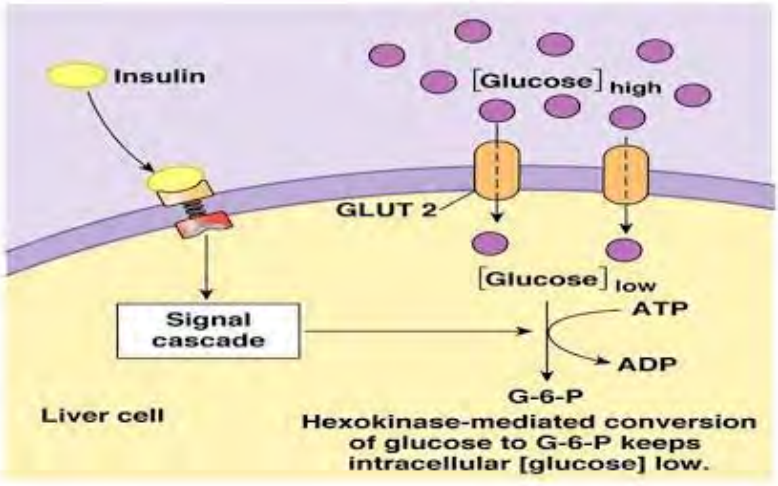


Figure 2.5. GLUT2 mediating uptake of glucose in liver cells during starvation or fasting.

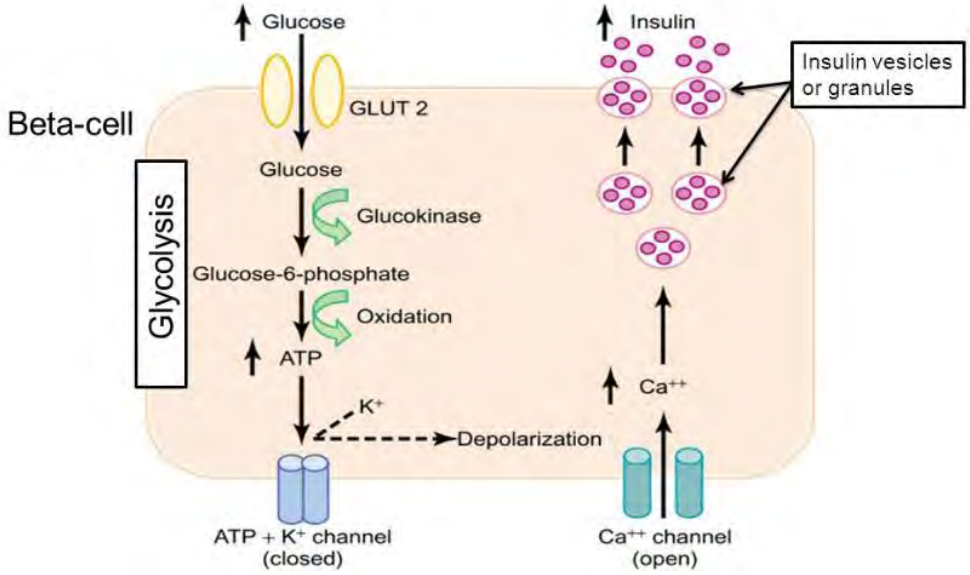


Figure 2.6. GLUT2 expressed in pancreatic beta islets stimulate insulin secretion from these cells (Kraeima, 2016).

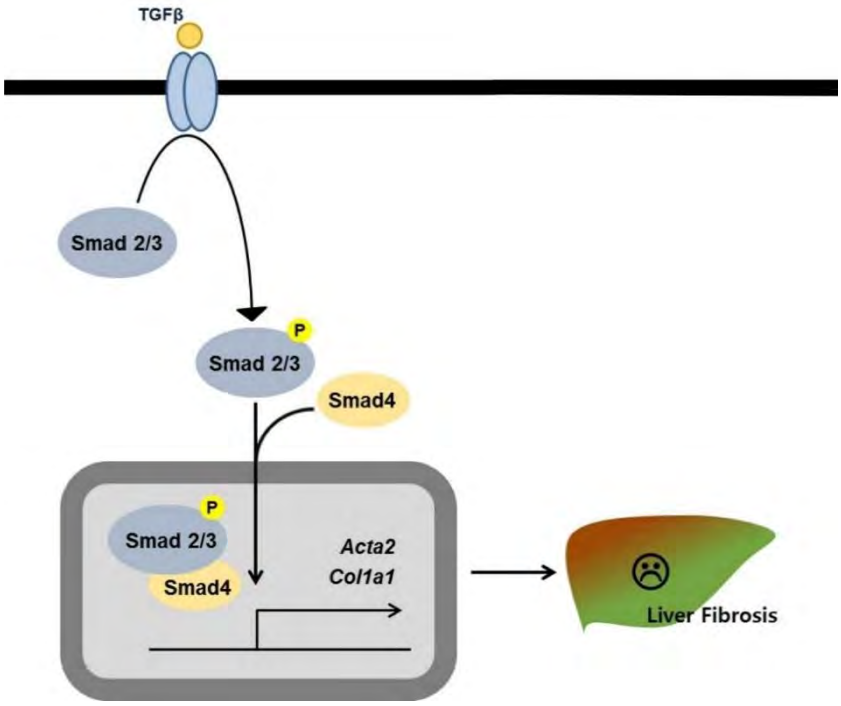


Figure 2.7. TGF*β* overexpression induces transcription of fibrogenic genes which causes increased synthesis of ECM and accelerate liver fibrosis (Lee *et al*., 2019).

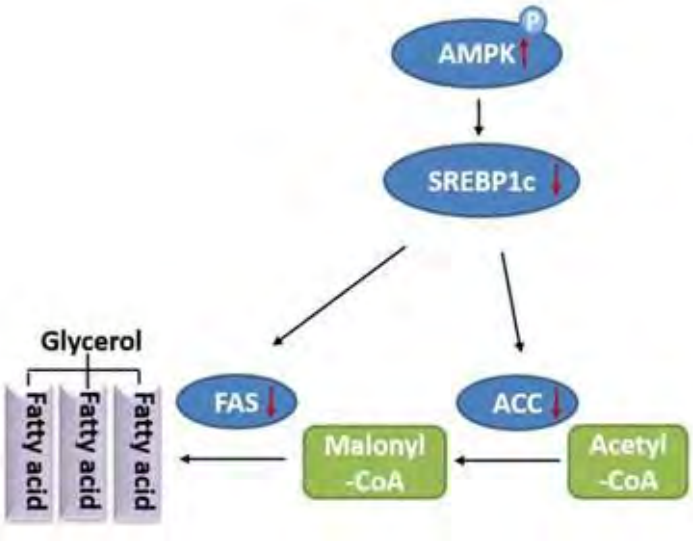


Figure 2.8. Activation of AMPK reduces the expression of downstream regulators and inhibits synthesis of lipids (Guo *et al*., 2018).

2.11. Chemical Induction of Diabetes – Experimental Insights

2.11.1. Alloxan Diabetes

Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil) is an oxygenated pyrimidine derivative (Rohilla & Ali, 2012) that has hydrophilic and unstable nature. At neutral pH and a temperature of 37 °C, it has a shelf-life of 1.5 min and stability tends to increase at lower temperatures (Szkudelski, 2001). Alloxan was reported to induce diabetes in animal models in 1943 by Dunn and McLetchie. This experimental state of diabetes, also called alloxan diabetes, occurs due to selective necrosis of pancreatic β-cells (Peschke *et al*., 2000) and leads to severe insulinopenia (Lenzen, 2008). The minimum amount of dose required to induce alloxan diabetes is 125 mg/kg.

2.11.1.1. Phases of Diabetes Induction

Effective doses of alloxan can be administered parentally i.e., intraperitoneally, subcutaneously and intravenously for experimental diabetes induction in rats. When injected in animal model, alloxan produces multiple phases of alternating blood glucose levels that correlate inversely with plasma levels of insulin. The first phase involves transient hyperinsulinemia and corresponding hypoglycemia due to inhibition of glucokinase. Apparently, the inhibition of enzyme glucokinase inhibits glucose phosphorylation which leads to elevated concentration of ATP. This is followed by second phase of hyperglycemia when alloxan reaches β-islets of pancreas. The third phase is again hypoglycemic due to alloxan-induced membrane rupture of insulin secreting cells and subsequent insulin flooding. The last phase is the final permanent phase of hyperglycemia in which beta cells lose their integrity (Rohilla & Ali, 2012).

2.11.1.2. Alloxan – Mechanism of Action

Upon reaching the insulin producing beta islets of pancreas, alloxan penetrates the permeated lipid bilayer of these cells by attracting GLUT2 glucose transporters (Longkumer *et al*., 2021). Alloxan is first reduced to dialuric acid (Lenzen, 2008) and then re-oxidized back to alloxan, thus creating a redox cycle for the production of reactive oxygen species (ROS) (Szkudelski, 2001). These free radicals impose DNA damage in β-islets and cause cell death. Alongside this, alloxan reacts with SH functional groups of

glutathione (GSH) compounds (Lenzen, 2008) and proteins, mainly glucokinase, thus promoting necrotic death (Maqbool *et al*., 2019).

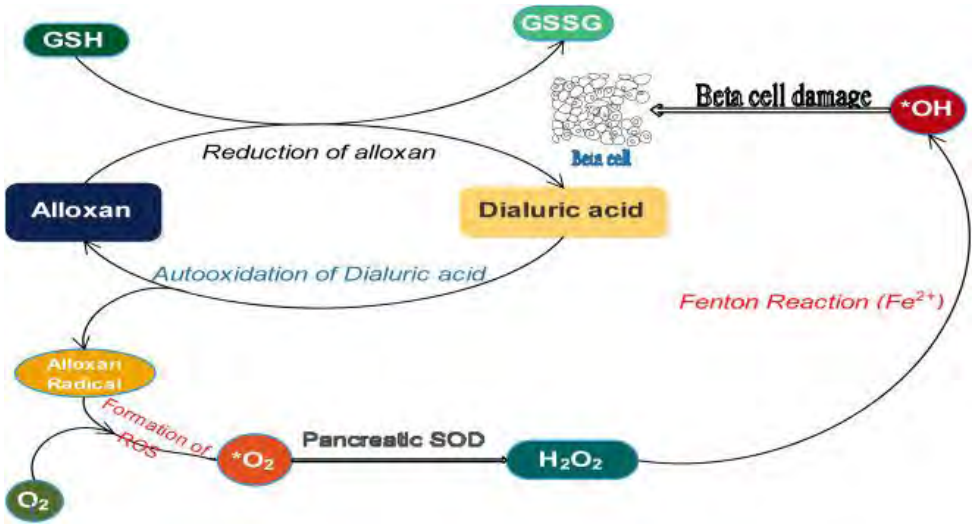


Figure 2.9. Redox cycling between alloxan and dialuric acid generate ROS that damage beta cells (Macdonald Ighodaro *et al*., 2017).

2.11.2. Streptozotocin Diabetes

Streptozotocin (STZ), 2-deoxy-2-(3-methyl-3-nitrosourea) 1-D glucopyranose, is a naturally occruing alkylating agent that is especially harmful to insulin secreting beta islets of pancreas. It is used in medicine to treat certain cancers of pancreatic islets (Brentjens & Saltz, 2001). It is used in medical research to create an experimental model for hyperglycemia (Costa *et al*., 2016). It was discovered in the strain of actinobacteria *Streptomyces achromogenes* (Ng *et al*., 2019). Although it is utilized as an antitumor agent but has various deleterious effects such as nausea, vomiting, diarrhea, dizziness, liver inflammation, kidney damage, and alterations in blood glucose levels.

2.11.2.1. STZ-Induced Hyperglycemia

Streptozotocin causes necrosis in pancreatic beta cells which leads to hypoinsulinemia and hyperglycemia (Lenzen, 2008). Just like alloxan, STZ is also a cytotoxic analogue of glucose. It was reported as a diabetogenic agent by Rakieten and coworkers in the year 1963. Depending on the dose, STZ can cause diabetes in two ways. The β-cells are specific

for preferentially accumulating chemicals in cell following entry across the GLUT2 receptors; the structural similarity of STZ to glucose allows it to interact with this receptor. When its level gets elevated, it attacks β-cells using its alkylating properties. STZ induces an immune and inflammatory response at multiple doses (Kottaisamy *et al*., 2021). The demolition of β-cells and elicitation of hyperglycemia, in this condition, is coupled with inflammatory mediators in the pancreatic β-cells. STZ also damages other organs expressing GLUT2 transporter, especially liver. Multiple low doses of 40-60 mg/kg can successfully induce streptozotocin diabetes within 1-2 days in almost all species (Ventura-Sobrevilla *et al*., 2011). As seen in alloxan diabetes, the blood glucose level (BGL) shows the same multiphasic response while maintaining an inverse relationship with insulin levels in plasma (Maqbool *et al*., 2019). After drug administration, blood glucose rises due to sudden glycogenolysis within a short interval of time (2 hrs). In the second phase, hypoglycemia occurs (after 6 hrs) which is followed by final phase of permanent hyperglycemia (after 12-48 hrs) (Lee *et al*., 2010).

2.11.2.2. STZ – Mechanism of Action

Streptozotocin is a nitrosourea molecule containing glucose moiety. Nitrosourea molecule is lipophilic in nature and allow it to cross the cellular membranes while glucose moiety allows STZ to be recognized by pancreatic cells. It is administered intraperitoneally to minimize its toxic effects. Owing to this glucose group, STZ enter beta cells via GLUT2 transporters present in membranes of cell. Pancreatic beta cells undergo cellular death by three major pathways: DNA methylation, NO production and oxidative stress.

The nitrosourea moiety in STZ can methylate DNA at guanine nucleotide (Murata *et al*., 1999). This results in activation of poly ADP ribose polymerase (PARP), a crucial enzyme for DNA repair. PARP also contributes to cell death by triggering DNA fragmentation (Wang *et al*., 2019).

STZ stimulates NO production which activates guanylyl cyclase enzyme to form cGMP which also takes part in DNA damage. As β-cells have inherently low concentrations of antioxidant enzymes, therefore more vulnerable to STZ toxicity by NO (Friederich *et al*., 2009).

Oxidative stress also plays a prominent role in the destruction of β-cells. As STZ causes glucotoxicity, it results in several reactions including glucose auto-oxidation, polyol pathway, protein glycation or AGE formation. These collectively result in increased production of ROS in cells and lead to oxidative stress. STZ lowers levels of antioxidant enzymes and elevates those of pro-oxidants which cause lipid peroxidation (LPO) to produce malondialdehyde (MDA), 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, hydroxyl or superoxide radicals are generated ultimately causing oxidative stress. Hydrogen peroxide also plays a role in free radical mediated oxidative stress. Increased ROS production also reduces activity of aconitase enzyme which is renowned for its protective role against mitochondrial DNA (Vergani *et al*., 2004).

2.11.2.3. STZ – Nicotinamide Model of Diabetes

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).

The STZ-NA experimental model of diabetes is predicated on NA's ability to counteract the cytotoxic effects of STZ on beta islets. According to reports, this model is appropriate for both biochemical and pharmaceutical studies on antidiabetic effects of commercial and organic compounds (Ghasemi *et al*., 2014).

Table 2.2. The STZ-NA rat model showcases advantageous characteristics that render it suitable for experimental investigations (Ghasemi *et al*., 2014).

Advantages of STZ-NA Model of Diabetes
Stable hyperglycemia, 150–180 mg/ dL and glucose intolerance
Insulin responsiveness to glucose and sulfonylureas
Suitable for assessing effectiveness of new potential anti-diabetic agents
No need of exogenous insulin for survival of experimental subjects
Reduction of β-cells (- 40%) and reduced pancreatic insulin stores by 60%

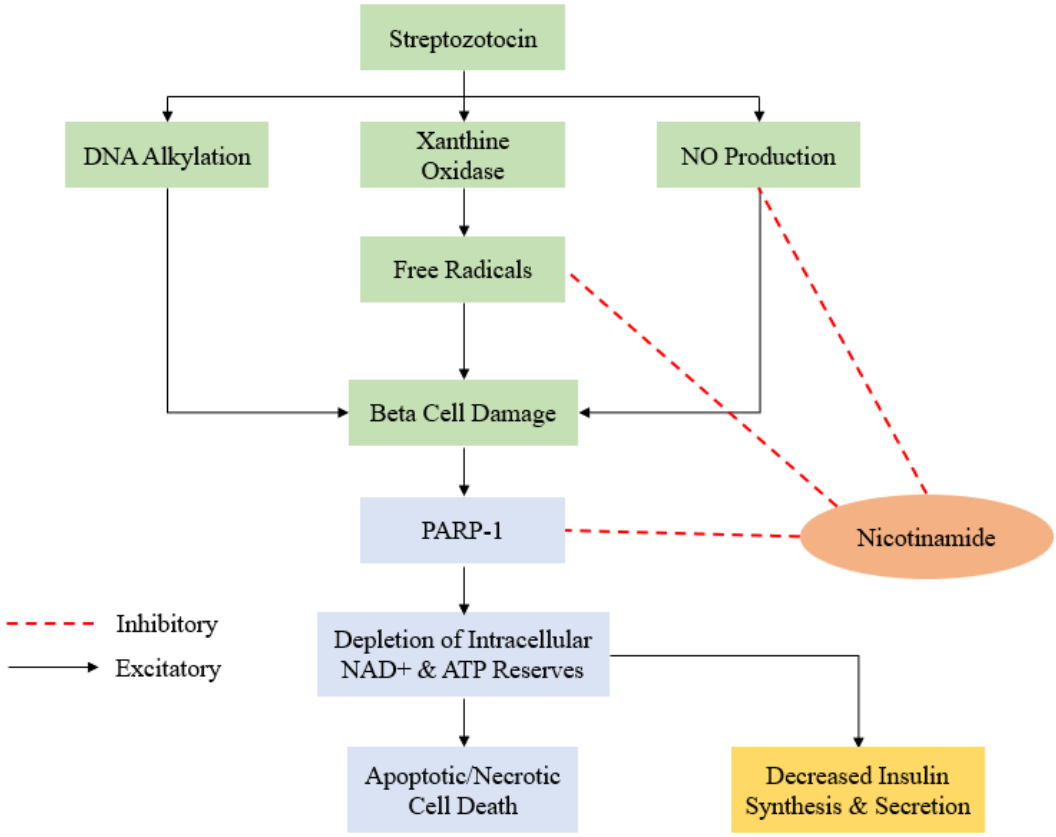


Figure 2.10. Illustrative depiction detailing the diverse mechanisms through which STZ induces β-cell damage. Nicotinamide co-administration in the presence of STZ induces partial reduction of beta cells (Ghasemi *et al*., 2014).

2.12. Glibenclamide – Standard Hypoglycemic Drug

Glibenclamide (GLI), also known as glyburide, is a hypoglycemic agent that stabilizes glucose levels by affecting insulin secretion (Lebovitz *et al*., 1977). It is a second-generation sulfonyl urea drug (Lucio *et al*., 2017) that is used to treat non-insulin-dependant diabetes (Bolen *et al*., 2007). It was created in 1996 by Boehringer and Hoechst (Marble, 1971). Because of its low solubility in physiological media, it is administered orally. It lowers blood glucose levels by increasing insulin release as it blocks potassium-ATP channels and boosts calcium influx (Guagnano *et al*., 2001).

2.12.1. Mode of Action of Glibenclamide

GLI is the most frequently used sulfonylurea drug for the treatment of diabetes. It inhibits ATP sensitive potassium channels embedded in lipid bilayer of pancreatic β-cells, which causes depolarization of voltage gated Ca^{+2} channels and their transition into open state. This causes an increased intracellular calcium in beta cells and stimulates insulin release. GLI also contributes to the reduction of pro-inflammatory cytokines production by the neutrophils of diabetic patients in response to infection (Kewcharoenwong *et al*., 2013). In STZ-induced diabetic rodents, GLI acts as an anti-inflammatory agent by reducing IL-1β secretion and bacterial spread to distant organs (Koh *et al*., 2013). Since 1996, it has been one of the most widely used therapies of DM, and possesses both antioxidant and anti-inflammatory properties.

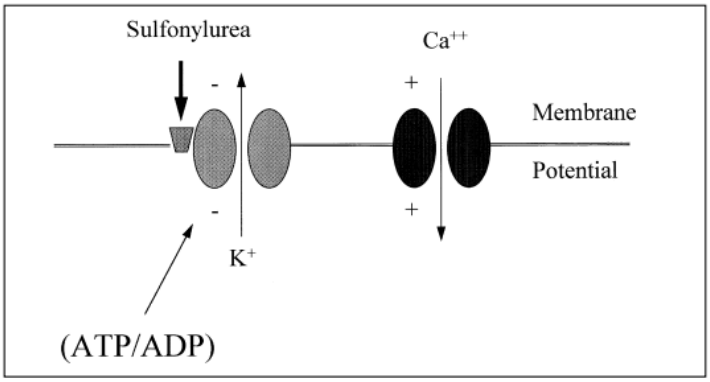


Figure 2.11. Mode of action of glibenclamide, a sulfonylurea drug (Luzi & Pozza,1997).

2.13. Histopathology

Histopathology is the microscopic examination of physiologically damaged tissues. It is an important diagnostic technique that is utilized for anatomical and histological research of human and animal models via microscope. It can be done by analysing a very thin slice of tissue under light microscopes. This expertise is composed of many different procedures which are used to see the structural fragments of abnormal tissue and cellular entities microscopically and distinguish definite alteration in the shape within certain diseases. The tissues under examination are fixed via formalin, embedded in paraffin, and then sliced with the aid of microtome. Then paraffin wax is washed from slices and they are dehydrated. The slices are then passed through a process of staining using hematoxylin and eosin (H&E). Histology of liver can be done by dipping liver chunks in fixative and further dipping in paraffin for thick sustenance. Then 3-4 μm of flat slices are cut down to stain with dyes like eosin and hematoxylin for visualization in light microscope.

3. MATERIALS AND METHODS

3.1. Plant Collection

Fresh leaves of *J. humile* were collected from district of Rawalpindi, Pakistan, in October, 2022. The plant was identified from the Department of Plant Sciences, Faculty of Biological Sciences, QAU, Islamabad by Dr. Muhammad Zafar. A voucher specimen No. 133452 was deposited at Herbarium of Pakistan, QAU, Islamabad.

3.2. Preparation of Crude Extract

The leaves were cleansed thoroughly to eradicate dust and other debris. For four weeks, they were stored under shade, so that the plant dried was completely dehydrated and dried. Electric grinder was used to get fine powder of the dried plant. For production of same sized particles, plant powder was passed through 60-mesh topology Willy mill. Extraction of solvents was the next phase, in which powdered material was dipped in pure ethanol for 72 hours for isolation of phytocomponents. To obtain refined *J. humile* ethanolic leaf extract (JHE), Whatman filter paper No. 1 was used for filtration, that results in the formation of filtrate which was then dried via rotatory vacuum evaporator and then solvent was totally vaporized.

3.3. Fractionation

After ethanolic extraction, fractionation was the next step. It was done in order to isolate compounds according to the ascending order of the polar nature from crude extract. An amount of 40 g of JHE was mixed and dissolved in 250 ml of distilled water. Then liquid-liquid partition was performed and fractions were prepared by addition of solvents in below mentioned order: n-hexane (JHH), chloroform (JHC), ethyl acetate (JHE), butanol (JHB). The filtrate left behind after fractionation was also obtained and dehydrated to form aqueous fraction (JHA). For the evaluation of phytochemical content and *in vitro* biochemical analysis, water content was removed from every fraction, they were weighed and stored at 4^{o}C.

3.4. Qualitative Phytochemical Testing

All fractions of *J. humile* leaf extract were screened qualitatively for different phytochemical classes using several standardized assays. Test samples were prepared in distilled water (1 mg/ml).

3.4.1. Alkaloids Detection (Hager's Test)

In each plant sample (2 ml), 2 ml HCl was added dropwise along with few drops of Hager's reagent (1g picric acid in 100 ml distilled water). Alkaloids were detected by presence of yellow precipitates (Archana *et al*., 2012).

3.4.2. Alkaloids Detection (Wagner's Test)

Each test sample (1 ml) was treated with 1 ml Wagner's reagent (2g iodine + 6g potassium iodide + 100 ml water + few drops of HCl). Alkaloids were detected by presence of reddish brown precipitates (Akkiraju *et al*., 2016).

3.4.3. Glycosides Detection (Keller Kiliani Test)

Each test sample (1 ml) was treated with glial acetic acid (1 ml) and cooled down. 3-4 drops of $FeCl_3$ followed by few drops of concentrated H_2SO_4 were added along test tube walls carefully. Glycosides were indicated by formation of a brownish-red colored ring at junction of the two layers (Archana *et al*., 2012).

3.4.4. Glycosides Detection (Concentrated H_2SO_4 Test)

Each test sample (1 ml) was treated with concentrated H_2SO_4 (1 ml) and left for 2 minutes. Glycosides were indicated by presence of reddish precipitates (Khan *et al*., 2012).

3.4.5. Flavonoids Detection (Alkaline Reagent Test)

For flavonoids examination, a revised protocol by Awoyinka et al. (2007) was practiced. Each test sample (1 ml) was treated with 2N NaOH (1 ml). Flavonoids were indicated by yellow color formation in test samples.

3.4.6. Flavonoids Detection ($FeCl_3$ Test)

1 ml of each test sample was reacted with few drops of $FeCl_3$. Flavonoids were detected by production of black-red precipitates (Al-Birawee & Nasser, 2019).

3.4.7. Tannins Detection (Alkaline Reagent Test)

Each test sample (2 ml) was treated with 1N NaOH (2 ml). Tannins were indicated by yellow-red color in test samples (Archana *et al*., 2012).

3.4.8. Tannins Detection ($FeCl_3$ Test)

Each test sample (1 ml) was treated with 5% $FeCl_3$ (2 ml). Tannins were indicated by greenish-black or dark blue color in test samples (Fahmy *et al*., 2017).

3.4.9. Saponins Detection

Each test sample (2 ml) was added to distilled water (2 ml) and shaken vigorously for 10-15 minutes in graduated cylinder. Saponins were indicated by foam layer formation in test samples (Damodaran & Manoher, 2012).

3.4.10. Anthocyanin & Betacyanin Detection

Each test sample (2 ml) was treated with 2N NaOH (1 ml) and heated at 100^oC for 5 minutes. Anthocyanins in test sample were indicated by greenish-blue color whereas betacyanin by yellow color (Fahmy *et al*., 2017).

3.4.11. Phenols Detection ($FeCl_3$ Test)

Each test sample (2 ml) was treated with 10% $FeCl_3$ (1 ml). Phenols were indicated with intense color (Sonam *et al*., 2017).

3.4.12. Phenols Detection (Ellagic Acid Test)

In 1 ml extract, 5% glacial acetic acid was added drop wise. Then 5% $NaNO_2$ was incorporated. Muddy brown color formation indicated the presence of phenols (Hashmi *et al*., 2021).

3.4.13. Sterols Detection (Salkowski Test)

Each test sample (1 ml) was treated with chloroform (5 ml) followed by adding about 1 ml concentrated H_2SO_4 along test tube walls carefully. Sterols were indicated by reddish brown color appearance in lower area of test sample (Harborne, 1998).

3.4.14. Steroids Detection

Each sample weighing 1 mg was dissolved in 1 ml chloroform. Upon adding a few drops of concentrated H_2SO_4, a brown colored ring is formed which would indicate presence of steroids (Pathan *et al*., 2012).

3.4.15. Quinones Detection

Each test sample (1 ml) was treated with concentrated H_2SO_4 (1 ml). Quinones were indicated by red color in test samples (Damodaran & Manoher, 2012).

3.4.16. Phlobatannins Detection

To 1 ml of leaf extract, few drops of 10% ammonia were added. Formation of pink colored precipitates would indicate the presence of Phlobatannins (Ali *et al*., 2018).

3.4.17. Proteins Detection (Xanthoproteic Test)

In this test, 2 ml plant sample was treated with few drops of concentrated HNO_3. Presence of proteins would result in the production of yellow color (Ali *et al*., 2018).

3.4.18. Vitamin C Detection (DNPH Test)

A dinitrophenyl hydrazine (DNPH) suspension prepared in concentrated H_2SO_4 was added to 1 ml of leaf extract. Formation of yellow colored precipitates would confirm the existence of vitamin C (Ali *et al*., 2018).

3.4.19. Anthraquinones Detection

2 ml of dilute HCl was incorporated to 1 mg of all test samples. The advent of red color indicated the presence of anthraquinones (Harborne, 1998).

3.4.20. Coumarins Detection

Each test sample was treated with 10% NaOH solution (1 ml). Coumarins were indicated by bright yellow color in test samples (Harborne, 1998).

3.5. Quantitative Phytochemical Analysis

3.5.1. Total Phenolic Content (TPC)

The total phenolic content was estimated by standardized Folin Ciocalteu method. To 80 µl of plant extract, 360 µl of undiluted Folin Ciocalteu reagent was added. The mixture was allowed to sit for 10 mins at room temperature. After that, 360 µl of 6% Na_2CO_3 was added following another incubation of 90 mins at 25°C. Gallic acid was used as the standard and absorbance was taken at 750 nm using a spectrophotometer (BIORAD, Germany) (Park *et al*., 2008).

3.6. *In Vitro* Antioxidant Assays

3.6.1. Superoxide Radical Scavenging Assay

For this assay, 1 ml DMSO and 0.2 ml of NBT (20mM) were added to 500 µl of plant samples. Optical density was recorded at 560 nm (Kunchandy & Rao, 1990). Ascorbic acid as positive control was also used for comparing antioxidant activities. The whole assay was performed twice and the data averaged. Superoxide scavenging potential was determined via following equation:

$$\text{Superoxide scavenging (\%)} = A_0\text{-}A_1/A_0 \times 100$$

Where A_0 = Absorbance of control, A_1 = Absorbance of sample.

3.6.2. Hydrogen Peroxide Scavenging Assay

The ability of different extracts to scavenge hydrogen peroxide (H_2O_2) was explored following a standardized method (Okoko & Ere, 2012). In a flask, 7 ml of potassium iodide (1.8M), 10 ml of sulphuric acid (2M), 1 ml of sample, and 3 ml of ammonium molybdate (3%) were delivered. This was titrated against sodium thiosulphate (5.09M) until the yellow colour disappears. This was presumed as the end point of the reaction. Absorbance was measured at 560 nm. The ability of the extracts to scavenge the H_2O_2 was calculated using the following equation:

$$\text{Hydrogen peroxide scavenging (\%)} = A_0\text{-}A_1/A_0 \times 100$$

Where A_0 = Absorbance of control, A_1 = Absorbance of sample.

3.6.3. Reducing Power Assay (FRAP)

The reducing power of all fractions were assayed by taking 100 µl sample and mixing it with 250 µl of 0.2M phosphate buffer (pH 6.6) and 250 µl of 0.1% potassium ferricyanide (w/v). The mixture was incubated at 50ºC for 30 min and then 250 µl 10% trichloroacetic acid solution (TCA, w/v) was added. After centrifugation, the supernatant (250 µl) was mixed with 250 µl of 0.1% $FeCl_3$ (w/v), and the absorbance was recorded at 700 nm. Rutin was used as the positive standard (Yin *et al*., 2018).

3.7. Antidiabetic Assay

3.7.1. Alpha-Amylase Inhibition Assay

This assay for evaluating antidiabetic potential of plant was reported by Keerthana *et al*. (2013). In this assay, we add 30 µl potassium phosphate buffer (0.05M), 10 µl α-amylase enzyme (0.12 U), 20 µl plant samples and 40 µl starch (2 g/L, prepared in phosphate buffer upon slight heating) in wells of 96 microwells-plate. Incubate it for 60 min at 50°C. After half an hour, 20 µl of HCl was added to inhibit the activity of enzyme. Following that, 100 µl of iodine reagent was added to loaded wells with enzyme containing reaction mixture. Absorbance was recorded at 540 nm. Blank containing starch solution and iodine reagent without enzyme was also loaded into wells. Acarbose was used as positive control (Sajid *et al*., 2020).

3.8. *In Vivo* Study

To elucidate the functional aspects including antioxidant, antidiabetic as well as hepatoprotective activity of butanol fraction of *J. humile*, *in vivo* assays were required. *In vivo* study was conducted on hepatic tissues of streptozotocin and alloxan-induced diabetic rats. For this study, hepatic tissues were scrutinized on biochemical (enzymatic as well as biomarkers), molecular and histological basis.

3.8.1. Study Design

For *in vivo* study, 30 Sprague Dawley male rats were employed. Their weight range was observed between 180-220 g, at the beginning of experiment. We had to follow all the rules and regulations suggested by NIH (National Institute of Health) and strategical plan

proposed by Bioethical Committee of QAU, Islamabad, for any sort of *in vivo* study. These rats under consideration were provided with standard conditions of living i.e., 25℃ temperature, 12 hours of dark/light period & proper care of diet as well.

3.8.2. Drug Usage

DM was induced in rats used as animal model by giving them intraperitoneal injections of streptozotocin and alloxan. An intraperitoneal injection of nicotinamide (210 mg/kg) was given 15-20 mins prior to administration of streptozotocin (55 mg/kg). Concerning alloxan, intraperitoneal injections were given (125 mg/kg). For induction of DM multiple low doses of STZ and alloxan (2-3) were administered. Glibenclamide (GLI), used as reference agent, was also administered intraperitoneally (20 mg/kg). An injection of 1 ml/100g was given to rats depending upon their body weights. Streptozotocin, alloxan and nicotinamide were dissolved in 0.9% saline for the purpose of *in vivo* study and then given to overnight fasted rats. High fat diet and 20% glucose solution was fed to rats to achieve hyperglycemia. After about 48 hours, their glucose was checked by using glucometer. When their glucose level elevated to 200 mg/dL, rats were said to be affected by diabetes (Rashid *et al*., 2019; Sajid *et al*., 2020).

3.8.3. Plant Doses

When rats were affected and attained irreversible hyperglycemic state, plant doses of butanol fraction were given to rats for about 3 weeks consecutively. Plant dose was prepared by adding calculated amount of plant extract in distilled water and kept overnight in order to make it fairly soluble. Dosing was also carried out according to the body weight of rats via pigeon feeding tubes. While carrying out dosing of rats, glucose level was examined on alternative days.

3.8.4. Grouping of Rats

For this study, rats were categorized in 8 different groups in triplicate. Proper care of feed and water was carried out for rats under experiment. At the beginning of experiment, before giving STZ and alloxan injections, rats were allowed to fast overnight. For *in vivo* study, proper strategic plan was followed which is demonstrated below in Table 3.1. When plant dosing of rats was achieved, rats were dissected and blood and tissues under study were

preserved for further study of *in vivo* parameters. For dissection, rats were fainted by keeping them in closed jar containing chloroform. Then they were dissected on ventral side. The blood was collected from heart immediately after dissection and poured into anticoagulant tubes for serum analysis. The tissue under study i.e., liver was separated and washed with 1% saline water. A portion of liver was preserved in liquid nitrogen for investigating biochemical and molecular parameters. The remaining organ was preserved in 10% formalin for histological examination.

3.8.5. Body and Organ Weight

The rats were weighed initially (at the start of experiment) and finally (at end of plant dosing; before dissection) to evaluate the percentage of varying body weight. While dissection, isolated liver was also weighed after washing by saline water.

Table 3.1. Grouping of rats according to administration of doses.

Group	Treatment
Group 1 (Control)	Normal treatment
Group 2 (STZ)	55 mg/kg (5.5 mg/ml in 0.9% saline)
Group 3 (ALX)	125 mg/kg (12.5 mg/ml in 0.9% saline)
Group 4 (STZ + GLI)	STZ dose + 20 mg/kg (2 mg/ml in d.H_2O)
Group 5 (ALX + GLI)	Alloxan dose + 20 mg/kg (2 mg/ml in d.H_2O)
Group 6 (STZ + JHB)	STZ dose + 300 mg/kg of JHB
Group 7 (ALX + JHB)	Alloxan dose + 300 mg/kg of JHB
Group 8 (JHB)	300 mg/kg

ALX; Alloxan, STZ; Streptozotocin, GLI; Glibenclamide, JHB; *J. humile* butanol fraction.

3.9. *In Vivo* Assays

Certain assays were carried out for scrutinizing biochemical parameters by using butanol fraction of *J. humile* against STZ and alloxan-induced DM affected hepatic tissues.

- Serum analysis
- Tissue analysis

3.9.1. Serum Analysis

Serum analysis was carried out by ascertaining serum markers quantitatively for assessing liver function. Alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), served as biomarkers for physiology of liver. Biochemical diagnostic kits were used to estimate triglycerides, total cholesterol and albumin.

3.9.1.1. Alanine Transaminase (ALT)

ALT levels in serum are measured by following the protocol of provided kits and reagents via spectrophotometric method. Protocol involves two reagents, Reagent 1 and Reagent 2.

Reagent 1 contains 110mM/L Tris buffer (pH 7.50) and 550mM/L L-alanine.

Reagent 2 contains 1200U/L LDH, 16mM/L α-ketoglutarate and 0.20mM/L NADH.

For reaction to occur, 100 µl of sample serum was mixed with 800 µl of reagent 1 and 200 µl of reagent 2 followed by incubation at 37℃ for 2 minutes. Mixture of two reagents with distilled water was taken as blank. After two minutes, absorbance of the mixture was observed thrice at 340 nm, each after 1 minute. Mean of the three obtained values was calculated and used in equation to calculate ALT value.

$$\text{ALT (Unit/Litre)} = \Delta A/\text{min} \times 1.10 \times 103/6.22 \times 0.10 \times 1$$

3.9.1.2. Aspartate Aminotransferase (AST)

To measure AST levels, 100 µl of sample serum was mixed with 1 ml of reagent containing 240mM/L L-aspartic acid, 12mM/L α-ketoglutarate, 80mM/L Tris buffer, 0.18mM/L NADH, 0.01% sodium azide and 800U/L MDH. Reaction amalgamation was then incubated at 37℃ for 1 minute followed by absorbance measurement thrice at 340 nm within next 3 minutes. Distilled water with reagents was taken as blank. Mean of the three absorbance values was calculated to obtain the levels of AST.

$$\text{AST (Unit/Litre)} = \Delta A/\text{min} \times 1.10 \times 103/6.22 \times 0.10 \times 1$$

3.9.1.3. Alkaline Phosphatase (ALP)

To measure ALP levels, 2 reagents were required.

Reagent 1 was comprised of 1.2mol/L Diethanolamine buffer (pH 9.8) and 0.6mmol/L Magnesium chloride.

Reagent 2 was comprised of 50mmol/L p-Nitrophenyl phosphate.

20 µl of serum sample was amalgamated with 800 µl of Reagent 1 and 200 µl of Reagent 2. Mixture was then incubated for 1 minute at 37℃ followed by measurement of absorbance at 340 nm. 3 readings were taken within 3 minutes and mean value was used to calculate ALP levels.

$$\text{ALP (Unit/Litre)} = \Delta A/\text{min} \times 1.10 \times 103/6.22 \times 0.10 \times 1$$

3.9.1.4. Albumin

Serum albumin levels were assessed by colorimetric method using bromocresol green. 1 ml of reagent containing 7.35 ml/L Brij35, 0.2mmol/L bromocresol green, and 87mmol/L Succinate buffer at pH 4.20 was mixed with 10 µl of sample serum. Distilled water with reagent was taken as blank whereas albumin was taken as standard. Reaction mixture and blank were then incubated at room temperature for 5 minutes and absorbance was measured at 620 nm. Formula used for albumin quantification is written below:

$$\text{Albumin (mg/dl)} = \text{Abs. of sample/Abs. of standard} \times n$$

Where n = concentration of standard (mg/dl).

3.9.1.5. Triglycerides

For quantification of triglyceride (TG) levels, a ready-to-use reagent comprising of sodium azide (15 Mm/L), glycerol phosphate oxidase (4000 U/I), peroxidase (800 U/I), lipoprotein lipase (350 KU/I), glycerol kinase (40 U/I), 4-aminophenazone (0.8 µM/L), ATP (1 Mm/L), magnesium ions (0.6 Mm/L), ethyl sulphopropyl toluidine (2 Mm/L), and PIPES buffer (20 Mm/L) was added to sample serum (10 µl). The working mixture was then incubated at 37ºC for 10 minutes followed by measuring absorbance at 555 nm.

$$\text{Triglycerides (mg/dl)} = \text{Abs. of sample/Abs. of blank x } n$$

Where, n = concentration of standard (mg/dl).

3.9.1.6. Total Cholesterol

In this assay, ready-to-use reagent (1 mM/L sodium cholate, 0.33 mM/L 4-amino antipyrine, 1 KU/L peroxidase, 4 mM/L phenol, buffer pH 7.4, biocides 4 g/L, 240 U/L cholesterol oxidase & 250 U/L cholesterol esterase) was mixed with 10 µl of each serum and incubated at 37^{o}C for 10 mins. Then, absorbance was recorded at 500 nm against water combined with the reagent as blank. Cholesterol levels were quantified by employing the formula:

Total cholesterol (mg/dl) = Abs. of sample/Abs. of standard x n

Where n = concentration of standard (mg/dl).

3.9.2. Tissue Analysis

In order to determine the activity of antioxidant enzymes, protein content and oxidative species present in hepatic tissues under normal or diseased circumstances, we had to perform certain spectrophotometry based assays including: CAT, POD, SOD, Nitrite, TBARS, H_2O_2, Protein estimation and GSH assay. For carrying out these assays, homogenate of liver tissue was required.

3.9.2.1. Tissue Homogenate

For preparing tissue homogenate, cut a small piece of liver and weigh it to about 100mg. This small piece of tissue was then crushed with the help of pestle and mortar in the presence of liquid nitrogen. 1ml of phosphate buffer (0.1M) containing EDTA (0.001M) was added to crushed tissues for further homogenization. After homogenizing tissue, the homogenate obtained was then allowed to undergo centrifugation for half an hour at 10,000 rpm at a temperature of 4°C. Later on, the upper layer i.e., supernatant was collected with the help of pipette and stored at 4°C for further assays, while the pellet was to be discarded.

3.9.2.2. Catalase Assay

Catalase enzyme is known for its activity against hydrogen peroxide. It has reduction potential for reducing oxidative specie, hydrogen peroxide, into oxygen and water and plays its part in overcoming oxidative stress. As it reduces hydrogen peroxide, therefore, decreasing conc. of H_2O_2 is associated with increasing catalase activity. For evaluating

catalase activity in tissue under observation, a reaction cocktail was prepared consisting of 500 µl phosphate buffer (50mM, pH 6.6), 80 µl H_2O_2 (5.9mM) and 20 µl tissue homogenate (Bonaventura *et al*., 1972). After preparing this reaction mixture, we had to measure their absorbance spectrophotometrically at 240 nm after an interval of 1 min as initial and final value.

3.9.2.3. Peroxidase Assay

For evaluating peroxidase activity, we would take 40 µl tissue homogenate and 700 µl phosphate buffer, 120 µl hydrogen peroxide and lastly 60 µl guaiacol was added to it. Then we would measure absorbance at 470 nm after an interval of 1 min (Chance & Maehly, 1955).

3.9.2.4. Superoxide Dismutase Assay

Superoxide dismutase enzyme is renowned for its catalytic activity against superoxide free radicals. Super-oxide radicals are generated in PMS-NADH system. SOD enzyme activity was investigated on the basis of its activity against superoxide free radicals by scavenging them. For quantifying SOD activity, we would add 50 µl PMS (188µM) and 350 µl sodium pyrophosphate buffer to 75 µl tissue homogenate and mixed to prepare reaction mixture. Then it was allowed to undergo centrifugation at 2500xg and 10,000xg for 20 min. After centrifugation was achieved, 100 µl NADH was incorporated for initiating the reaction. The aliquot was kept aside for half an hour. After completing incubation period, 500 µl glacial acetic was added to terminate the reaction. At last, optical density was recorded at the wavelength of about 560 nm (Kakkar *et al*., 1984).

3.9.2.5. Lipid Peroxidation Assay

In this assay, 2 µl $FeCl_3$ (0.1M), 20 µl ascorbic acid (0.1M) and 60 µl phosphate buffer (0.1M) was added to 20 µl tissue homogenate and was incubated for 60 min on shaking/water bath at 37℃. Then, 100 µl of TCA (10%) and equal volume of TBA (0.67%) was added and again these samples were placed in water bath for 20 min at boiling temperature. After removing from water bath, cool them by placing on ice. After cooling, centrifugation was carried out for 10 min at speed of 2500xg. OD was then measured at 535 nm (Fraga *et al*., 1988).

3.9.2.6. Hydrogen Peroxide Assay

In this assay, we had to evaluate the antioxidant activity against hydrogen peroxide present in tissue homogenate under consideration. For this purpose, we prepared reaction mixture containing 400 µl tissue homogenate, 200 µl phenol red (0.28mM), 1.7 units of HRP enzyme, 50 µl dextrose (5.5mM), 100 µl phosphate buffer and then this reaction mixture was incubated at 37℃ for 60 min. After 1 hour, 2 µl of NaOH was added to it and centrifuged at 800xg for 5 min. Absorbance measured at 610nm (Pick & Keisari, 1981).

3.9.2.7. Nitrite Assay

For estimating nitrite concentration, a piece of tissue of about 100 mg was taken and 100 µl of NaOH (0.3M), 100 µl $ZnSO_4$ (5%) was added to it. Centrifuge this mixture for 20 min at 6400xg. After centrifugation, pellet was discarded while 20 µl supernatant was collected. Following this, we added 1 ml Griess reagent to it and its absorbance was measured at 540 nm (Green *et al*., 1982).

3.9.2.8. Reduced Glutathione Assay

In this assay, GSH level was quantified in test tissue homogenate. GSH forms colored complex upon reacting with DTNB and oxidized to GSSG. In the presence of NADPH and GR enzyme, oxidized GSSG was reverted back to reduced form GSH. Because of oxidative stress, the level of reduced glutathione GSH would be decreased. In this case, we would add 100 µl tissue homogenate to equal volume of sulfosalicylic acid (4%) and incubated them at 4℃ for 60 min. After incubation period, centrifugation was carried out at 4℃ for 20 min at speed of 1200xg. When centrifugation was done, 10 µl of upper layer of supernatant was collected and 270 µl of phosphate buffer (0.1M) and 20 µl DTNB (0.1M) was added to it. Then, its absorbance was recorded at 412 nm (Mohandas *et al*., 1984; Iqbal *et al*., 1996).

3.9.2.9. Total Protein Estimation

For measuring total protein content in tissue sample, we weighed 80 mg of tissue and crushed or homogenized it in phosphate buffer (0.1M). It was then centrifuged for 20 min at 10,000 rpm at temperature of 4℃. After centrifugation, 10 min incubation was required. We collected upper layer of supernatant of about 200 µl and added equal volume of alkaline

solution and FC reagent. These reagents were mixed thoroughly with the help of vortex and again incubated for half an hour. Now, we had to measured its absorbance at 595 nm (Lowry *et al*., 1951).

3.10. Histopathological Assessment of Hepatic Tissues

For histological study of liver, after surgical removal from rat, the organ was preserved in 10% formalin. The liver tissues were then fixed in paraffin wax and they were sliced into fine thin sections with the help of microtome. These thin sections were then washed with ethanol having serially graded concentration (50%, 70%, 90%, 100%). After washing, these tissue sections were subjected to complete dehydration. These tissue sections were then stained with eosin and hematoxylin stains. Slides were imaged under microscope DIALUX 20EB and images were captured by HDCE 50B automated system (Sajid *et al*., 2016).

3.11. Molecular Assessment

To observe the effectiveness of JHB against alloxan diabetes and streptozotocin diabetes at molecular level, liver tissues that were stored at -70^{o}C were subjected to RNA extraction followed by RT-PCR.

3.11.1. RNA Extraction

RNA extraction was carried out using TRIzol reagent-based procedure by Chomczynski & Mackey (1995). The stepwise adopted methodology was:

1. Liver tissue preserved in liquid nitrogen was sliced into a small piece of about 100 mg. Then 1 ml chilled TRIzol reagent was taken in glass homogenizer and tissue is homogenized till cell lysis occurs.
2. The homogenized tissue in TRIzol reagent was then transferred from homogenizer into pre-chilled eppendorf and incubated for 5 minutes at 25℃.
3. After 5 minutes, 200 µl chloroform was added to homogenate and shaken slightly by turning eppendorf upside down for 5 minutes or about 15 times.
4. It was then allowed to centrifuge for 15 minutes, at 4℃ at the speed of 12000 rpm. After centrifugation, two layers are formed. Separate the upper layer of supernatant

carefully without any mixing with other layer and pour into another pre-chilled eppendorf while lower layer was discarded.

5. Now, 200 µl isopropanol was added to separated layer of supernatant gently and slight precipitation was observed. Keep it aside for 10 minutes without shaking.
6. It was then centrifuged for 10 minutes at the speed of 12,000 rpm and temperature 4℃ in microcentrifuge. After centrifugation, clear white colored pellet will be observed. In this case, supernatant was discarded.
7. The pellet obtained was then washed with ethanol 75% for 5 minutes at speed of 7500 rpm and supernatant obtained was discarded. Again, pellet formed as a result of first washing step was again subjected to washing step but now with 100% ethanol and centrifuge for 5 min at 7500 rpm.
8. At this step, after discarding supernatant, pellet alone was again centrifuged for 3 min at 7500 rpm for removal of any sort of ethanol still retain behind. After centrifugation, eppendorf containing RNA pellet was tapped on tissue paper carefully by inverting it upside down.
9. The RNA pellet now obtained was dissolved in ultrapure RNAase free distilled water and stored at -80℃ temperature for later use.

3.11.2. Quantification of RNA

The obtained RNA was then quantitatively scrutinized by performing Nanodrop by putting 2 µl of dissolved RNA on Nanodrop machine.

3.11.3. cDNA Synthesis

The extracted RNA was then reverse transcribed into cDNA by using Thermo-fisher Scientific Revert Aid kit for further analysis at molecular level. For cDNA synthesis, firstly master mix was prepared by using reagents demonstrated in Table 3.2. Before using these reagents, these were thawed slightly and briefly centrifuged for few seconds. After being synthesized, cDNA was diluted 10X by double-PCR water and stored at -80℃ before further processing (Meis & Khanna, 2009).

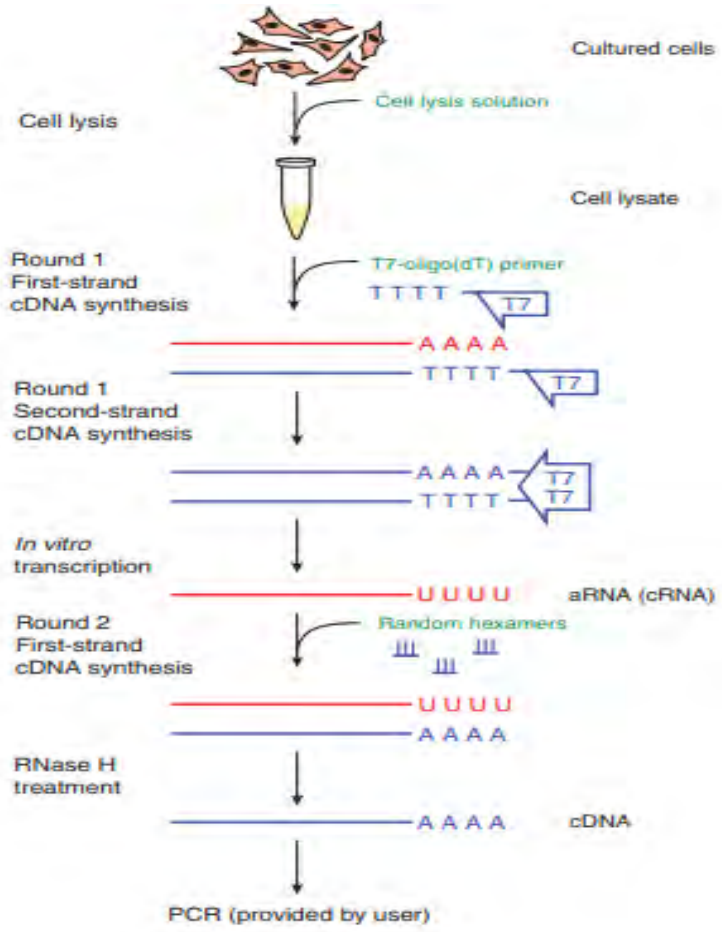


Figure 3.1. Sequential steps of cDNA synthesis from extracted RNA for RT-PCR analysis.

Table 3.2. Reagents required for cDNA synthesis.

Reagents	**Volume (Per Sample)**
RT buffer	4 µl
Oligo dT	1 µl
Random primer (hexamer)	1 µl
dNTP's	4 µl
Ribo-lock (RNAase inhibitor)	2 µl
Revert Aid (Reverse Transcriptase)	2 µl
Total Volume	14 µl

3.11.4. RT-PCR

The cDNA synthesized by afore-mentioned procedure was used for elucidating the changes occurring in the expression of target genes under study because of diabetes. For this molecular approach, RT-PCR assessment had to be required which was carried out by using equipment My-Go RT-PCR. It amplifies the specific target gene in cDNA. For doing RT-PCR, following reagents illustrated below in Table 3.3 were mixed in a specific proportion into PCR tubes and tubes were then loaded in My-Go PCR. While preparing reaction mixture, cDNA and primers were used in diluted form. In this reaction for molecular analysis, GAPDH was used as loading control in addition to target genes (SLC2A2, TGF-β1, SMAD3, COL1A1, AMPK, ACC, FASN) under consideration. Primers sequences for control as well as target genes are mentioned in Table 3.4. For RT-PCR, temperature profile was also adjusted according to optimized requirements shown in Table 3.5.

Table 3.3. Reagents required for RT-PCR reaction.

Reagents	Volume (Per Sample)
cDNA	1 µl
Forward Primer	0.5 µl
Reverse Primer	0.5 µl
PCR water	8 µl
Syber Green Dye	10 µl
Total Volume	20 µl

Table 3.4. Primer sequences of genes under investigation.

Genes		Primer Sequences (5′-3′)
GAPDH	Forward	ACGGCCACTCAGAAGACAGT
GAPDH	Reverse	CATAGGTCCCTTGGCTGCT
SLC2A2	Forward	GCCATCTTCCTCTTCGTCAG
SLC2A2	Reverse	GCCGAACCACTCTTCTTCC
TGFβ1	Forward	GCTACCATGCCAACTTCTGT
TGFβ1	Reverse	CGGGTTGTGTTGGTTGTAGA
SMAD3	Forward	CCTCCTGGCTACCTGAGTGA
SMAD3	Reverse	GTTATTGTGTGCTGGGGACA
COL1A1	Forward	GTCCCCGAGAGGAAACAATG
COL1A1	Reverse	ACCACGCATTCCCTGAAGA
AMPK	Forward	ACCTGAAAACGTCCTGCTTG
AMPK	Reverse	CAGCTCGTTCTTAAAAATTCACC
ACC	Forward	GGTAGCTGTGCAGTTTGCTG
ACC	Reverse	GCCTCAGTCGCCAGTAGAAG
FASN	Forward	CCACCTACGGTCTGCAGTG
FASN	Reverse	GCACCTGCTTGATGCAATC

Table 3.5. Optimized conditions for RT-PCR.

Program		**Temperature (°C)**	**Ramp (°C/s)**	**Hold (s)**
Hold		95	4	600
3-Step Amplification	Denaturation	95	5	15
	Annealing	60	4	30
	Extension	72	5	30
Pre-Melt Hold		95	5	10
High Resolution Melting	Initial Stage	60	4	60
	Final Stage	97	0.05	15

3.11.5. Quantification of Gene Expression

The expression of target genes was then calculated by relative ΔCT method. Then, fold changes for gene expression evaluation was calculated by using formula mentioned below (Schmittgen & Zakrajsek, 2000):

$$\Delta CT_{(Treated)} = CT_{(Treated\ gene)} - CT_{(Internal\ control)}$$

$$\Delta CT_{(Reference)} = CT_{(Ref.\ gene)} - CT_{(Internal\ control)}$$

$$\Delta\Delta CT = \Delta CT_{(Treated)} - \Delta CT_{(Reference)}$$

$$\text{Fold Change} = (2)^{\wedge -\Delta\Delta CT} \ (\text{At } p < 0.05)$$

3.12. Statistical Analysis

After performing in vivo assays, the results obtained were statistically analysed by using different software and tools. Graphs were plotted on Graph Pad Prism 10 and ANOVA was also applied. Statistics was applied on data by using Statistix 10 software for assessing the significance of data calculated. Results were documented in the form of Mean ± S.D.

4. RESULTS

4.1. Yield of *J. humile* Fractions

The obtained yield of crude ethanolic extract from 1 kg powdered leaves was 82.7 g. About 40 g of ethanolic extract (JHE) was utilized for liquid-liquid extraction with organic solvents of varying polarity. Upon drying, 13.4 g of JHA, 8.4 g of JHB, 6.2 g of JHC, 4.3 g of JHH, and 2.2 g of JHEA were extracted (Figure 4.1). The obtained yields were in the order of JHA > JHB > JHC > JHH > JHEA.

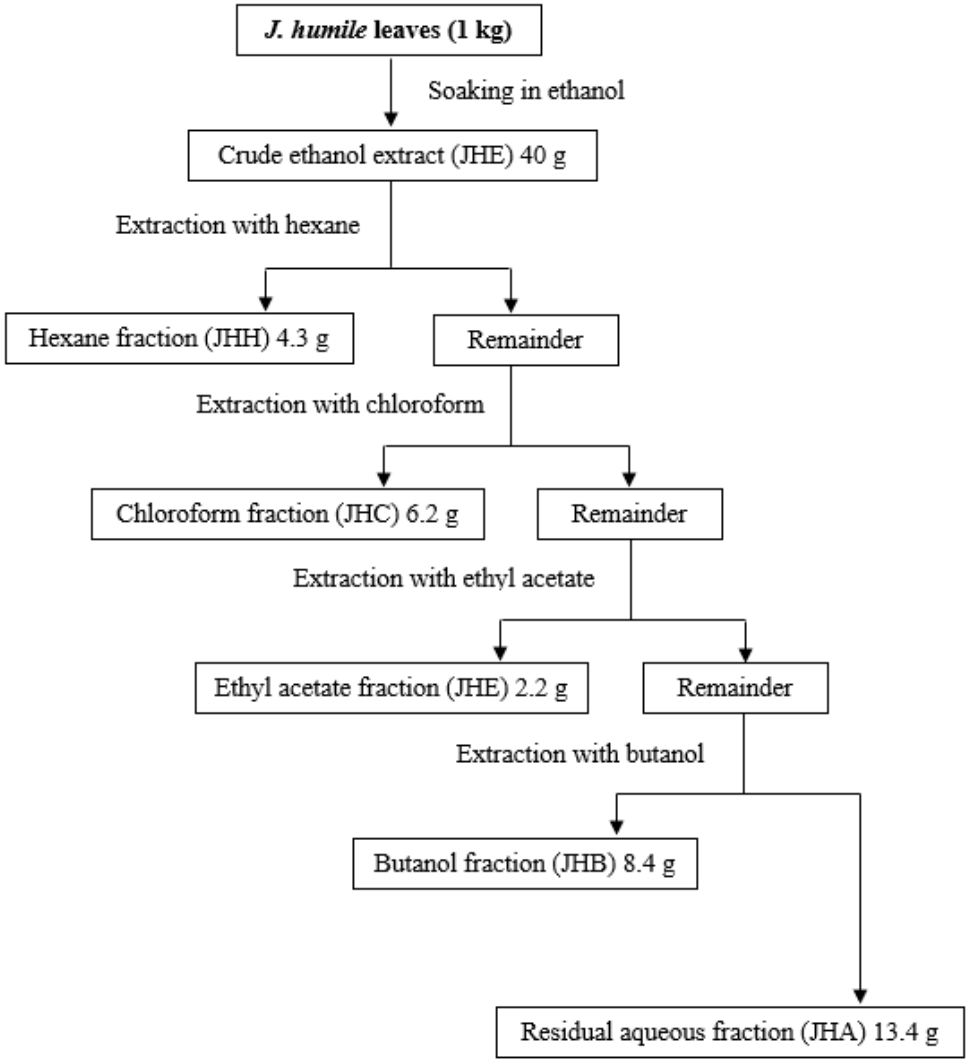


Figure 4.1. Schematic representation of *J. humile* fractionation and yields of extracted fractions.

4.2. Phytochemical Profiling

4.2.1. Qualitative Phytochemical Analysis

Phytochemicals, the chemical constituents of plants, are present in varying amounts in different fractions depending on their polarity index. Therefore, the fractions of *J. humile* were qualitatively analyzed for numerous phytochemicals such as glycosides, alkaloids, phenols, flavonoids, tannins, saponins, sterols, phlobatannins, proteins, vitamin C, quinones, anthraquinones, anthocyanin, betacyanin and steroids. The outcomes are listed in Table 4.1. The results showed the presence of phenols, glycosides, alkaloids, quinones, flavonoids and saponins in all fractions. Anthraquinones and phlobatannins were guaranteed absent in every fraction. The rest of the phytochemicals were present or absent at varying notches in different fractions.

4.2.2. Quantitative Phytochemical Analysis

4.2.2.1. Total Phenolic Content (TPC)

For determining the TPC of *J. humile* fractions, the equivalents of standard such as mg of gallic acid equivalent/g of dry extract (mg GAE/g dry sample) was computed via the standard regression line of gallic acid (Table 4.2). The maximum quantity of TPC was expressed by JHB (188.39 ± 0.33^{a}) followed by JHE, JHA, JHC, JHEA and JHH in descending trend as shown in Table 4.2.

4.3. *In Vitro* Antioxidant Assays

4.3.1. Superoxide Radical Scavenging Assay

The half-maximal inhibitory concentration (IC_{50}) for superoxide scavenging potency was calculated for *J. humile* and its fractions with ascorbic acid as standard (Table 4.3). JHB displayed the minimum IC_{50} of all samples, whereas the maximum was shown by JHH. The observed trend of IC_{50} values of the test samples was JHB > JHE > JHC > JHA > JHEA > JHH. The percentage inhibition outline is in Figure 4.2.

4.3.2. Hydrogen Peroxide Scavenging Assay

Based on the IC_{50} values for hydrogen peroxide scavenging activity computed in Table 4.3, JHB showed the best IC_{50} value followed by JHA, JHC and JHE when compared to the

standard (Ascorbic acid). Other than that, JHH and JHEA displayed IC_{50} values greater than 500. The scrutinized concentration-dependent radical foraging potentials are shown in Figure 4.3.

4.3.3. Reducing Power Assay (FRAP)

The reducing potential of test samples, calculated at a concentration of 500 µg/ml of fractions, was expressed as mg of gallic acid/g of dry sample (mg GAE/ g sample), computed in Table 4.3. JHB presented the highest reducing power (873.41 ± 1.26^{a}), JHC comes afterwards (842.7 ± 2.47^{b}), followed by JHE, JHA, JHEA and JHH. However, the fractions displayed variable frequencies of reducing potential at different concentrations as shown in Figure 4.4.

4.4. Antidiabetic Activity

4.4.1 Alpha-Amylase Inhibition Assay

The antidiabetic activity of plant extract is elucidated on the basis of its inhibitory activity against α-amylase enzyme that is renowned for its activity of starch breakdown to glucose. In hyperglycemic state, the activity of α-amylase enzyme increases and maintains high blood glucose level. The plant extract that inhibits α-amylase activity against starch breakdown possess antidiabetic activity and is expressed in IC_{50} value. In this assay, antidiabetic drug Acarbose (metformin) is used as positive control possessing highest α-amylase inhibitory activity. Among plant fractions, JHB has highest α-amylase inhibitory activity while JHEA has lowest α-amylase inhibitory activity. The α-amylase inhibitory activity of all plant fractions is listed in Table 4.4 in terms of their IC_{50} values. The plant fractions having different percentage of inhibition of α-amylase activity at different concentrations is presented graphically in Figure 4.5.

Table 4.1. Qualitative phytochemical appraisal of JHE and its derived fractions.

Phytochemicals		**JHE**	**JHH**	**JHEA**	**JHC**	**JHB**	**JHA**
Alkaloids	Hager's test	+	-	+	+	++	++
	Wagner's test	++	-	+	++	++	++
Glycosides	Keller Kiliani test	++	+	+	+++	+++	++
	Conc. H_2SO_4 test	++	-	+	++	+++	++
Flavonoids	Alkaline reagent test	++	-	-	+	++	++
	$FeCl_3$ test	++	-	+++	++	+++	+++
Tannin	Alkaline reagent test	+	+	+	+	++	++
	$FeCl_3$ test	-	++	-	-	-	-
Saponin		+	-	+++	+++	+++	+
Anthocyanin/Betacyanin		+	+	+	+	+	+
Phenol	$FeCl_3$ test	++	+	+++	+++	+++	++
	Ellagic acid test	+	++	+	+	+	+
Sterol		-	+	+	-	++	++
Steroids		-	+	+	+	+	+
Quinones		-	++	+++	+++	+++	-
Phlobatannins		-	+	-	-	-	++
Protein		-	+	+	+	+	+
Vitamin C		+++	++	+	+	+	++
Anthraquinone		-	+	+	-	-	-
Coumarins		+	++	+	+	+	++

The signs indicate: (+) component present, (++) moderate concentration of component, (+++) highest concentration of component, (-) component absent.

Table 4.2. Total phenolic content of *J. humile* ethanol extract and its derived fractions.

Fraction	TPC Expressed as mg GAE/g of Extract
JHE	168.31 ± 0.46^{b}
JHH	68.02 ± 0.44^{f}
JHEA	76.61 ± 0.55^{e}
JHC	106.17 ± 0.46^{d}
JHB	188.39 ± 0.33^{a}
JHA	125.65 ± 0.55^{c}

The values are represented as Mean ± SD (n=3). The different superscripts (a-f) in the rows depict that means are significantly ($p < 0.05$) different from one another.

Table 4.3. IC_{50} values of different antioxidative activities of JHE and its derived fractions.

Fraction	Superoxide Radical Scavenging Assay	Hydrogen Peroxide Scavenging Assay	Reducing Power Assay (FRAP)
	IC_{50} (µg/ml)	IC_{50} (µg/ml)	mg GAE/g sample
JHE	278.5 ± 1.91^{e}	363.16 ± 2.2^{c}	763.03 ± 1.3^{c}
JHH	865.7 ± 1.73^{a}	607.28 ± 1.18^{b}	628.3 ± 1.84^{f}
JHEA	568.5 ± 0.98^{b}	802.2 ± 1.76^{a}	652.44 ± 1.38^{e}
JHC	354.09 ± 1.55^{d}	292.07 ± 1.52^{d}	842.7 ± 2.47^{b}
JHB	132.65 ± 1.61^{f}	172.01 ± 1.03^{f}	873.4 ± 1.26^{a}
JHA	438.4 ± 1.88^{c}	255.46 ± 1.36^{e}	709.5 ± 2.39^{d}
AA	61.74 ± 2.06^{g}	67.78 ± 0.87^{g}	-

Each value is expressed as Mean ± SD (n=3). The means in the rows are different from one another at a significant level ($p < 0.05$) represented with different superscripts (a-g).

Table 4.4. IC_{50} values of antidiabetic activity of JHE and its derived fractions via α-amylase inhibition assay.

Fraction	**Alpha-Amylase Inhibition Assay**
	IC_{50} (µg/ml)
JHE	307.24 ± 0.98^{d}
JHH	571.26 ± 1.04^{b}
JHEA	790.61 ± 2.75^{a}
JHC	306.85 ± 0.74^{d}
JHB	263.04 ± 1.1^{e}
JHA	446.47 ± 1.36^{c}
Acarbose	110.69 ± 0.95^{f}

Each value is expressed as Mean ± SD (n=3). All means are different from one another at a significant level ($p < 0.05$) represented with different superscripts (a-f).

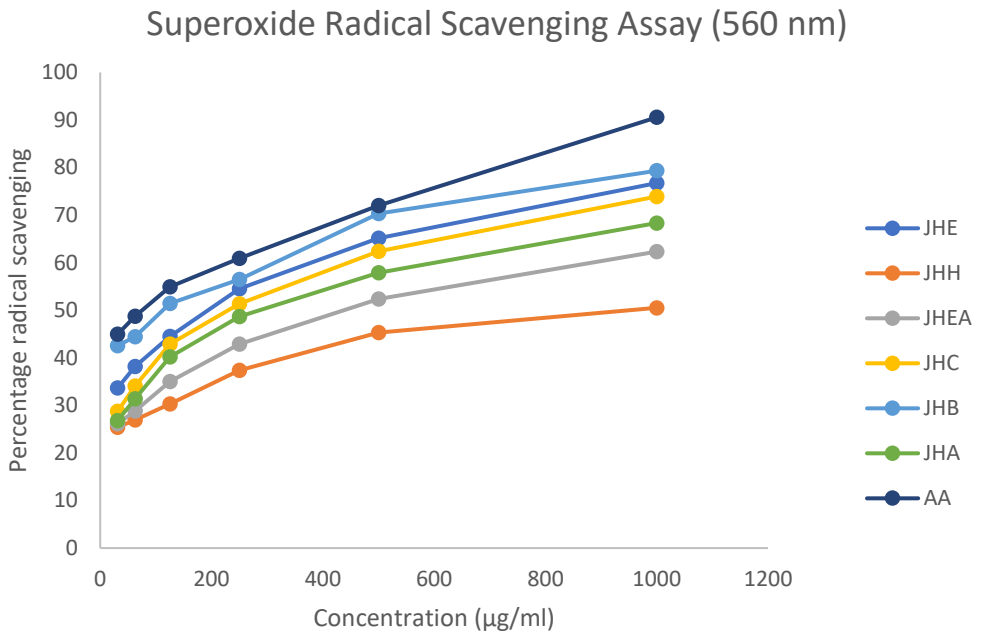


Figure 4.2. Superoxide scavenging effect of JHE and its fractions at various concentrations.

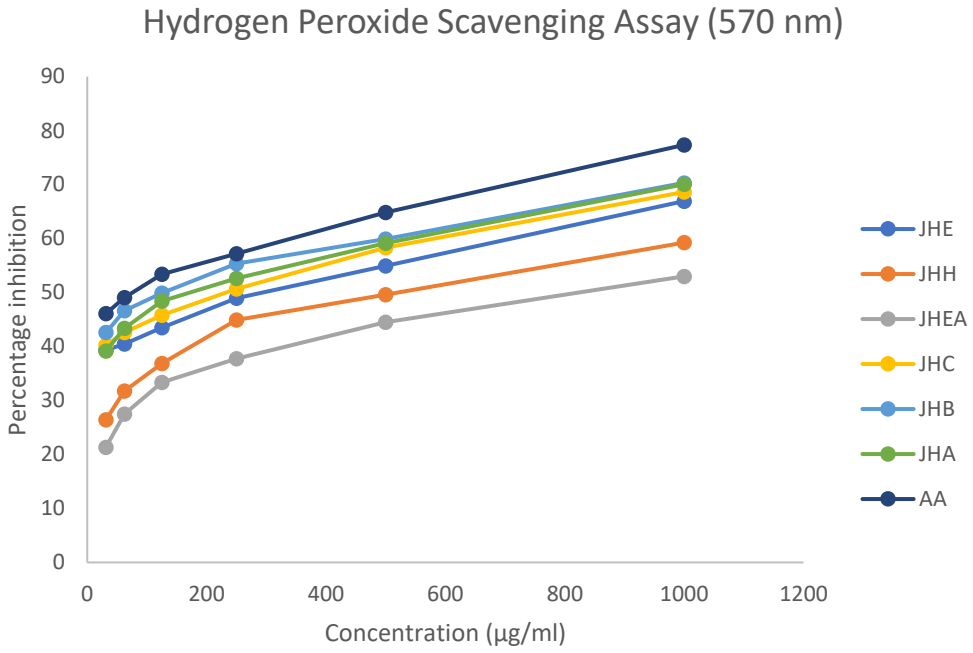


Figure 4.3. H_2O_2 foraging activity of JHE and its fractions at various concentrations.

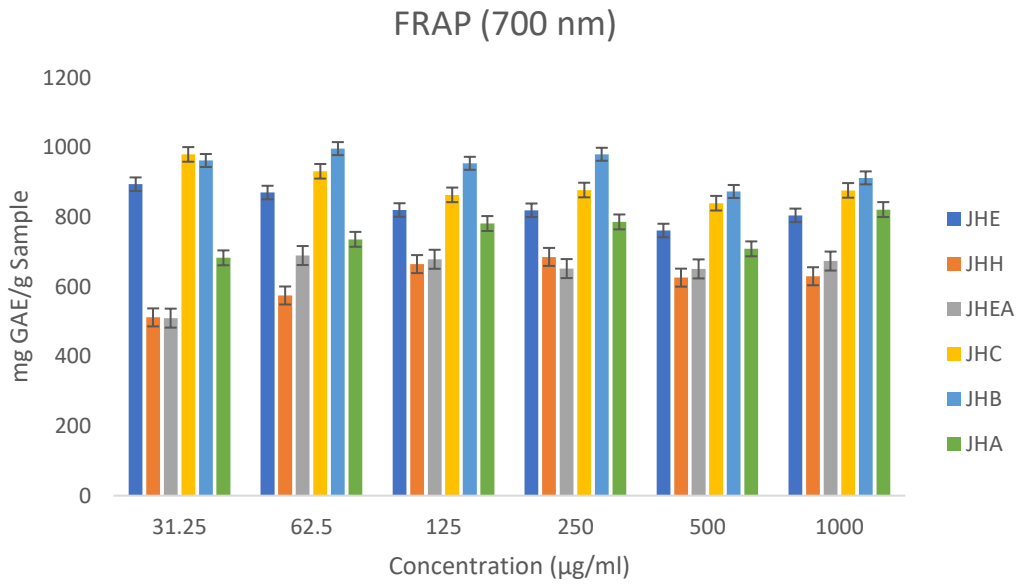


Figure 4.4. Reducing power effect of JHE and derived fractions at different concentrations expressed as mg of gallic acid equivalent/g of dry sample (mg GAE/g of sample).

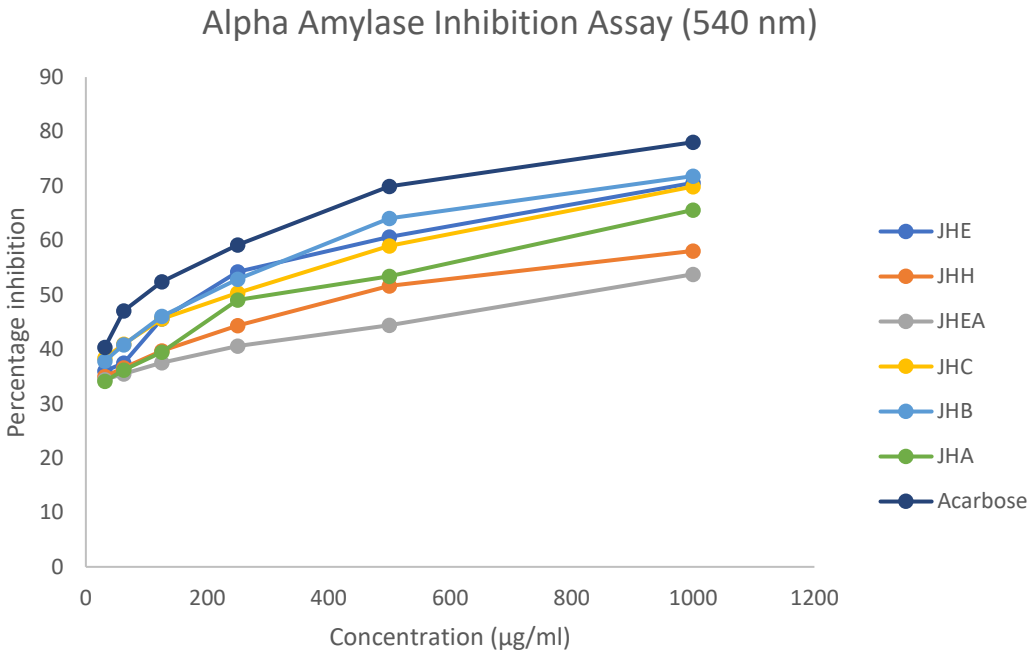


Figure 4.5. Alpha-amylase inhibition activity of JHE and its fractions at various concentrations.

4.5. Hepatoprotective Effect of *J. humile* Against Alloxan and STZ Diabetes

4.5.1. Effect of JHB on Weight of Body and Organ

The significance of JHB administration, against alloxan-induced diabetes and streptozotocin-induced diabetes, on the percent increase in body weight, absolute liver weight and relative liver weight of rats is depicted in Table 4.5. Rats treated with alloxan and STZ remarkably repressed the % increase in body weight, whereas it raised the absolute and relative liver weight compared to untreated rats. Among diabetic groups co-administered with JHB, alloxan rats showed a greater % increase in body weight and significant repression of the absolute and relative weight of liver, while streptozotocin rats exhibited only a slight % increase in body weight and considerable drop in absolute and relative liver weight. The diabetic rats restored body and organ weight towards the untreated control upon administration of glibenclamide. However, no such notable variation was observed in the rats treated with JHB doses alone.

4.5.2. Effect of JHB on Blood Glucose Levels

In control rats, there was euglycemic state ranging from 80 mg/dL to 120 mg/dL. In rats intraperitonially administrated with alloxan and STZ injections, the glucose levels were elevated to 280 mg/dL indicating diabetes. Rats administered orally with plant doses and glibenclamide (standard hypoglycemic agent) depicted significant decrease in glucose levels. JHB doses showed remarkable effects in lowering blood glucose levels in both alloxan and STZ rats. The plant doses alone had little to no effect on the glycemic levels. The alterations occurring in the levels of blood glucose are represented graphically in Figure 4.6.

4.5.3. Effect of JHB on Serum Biomarkers

Hepatic markers level in serum extracted from all rat groups under treatment is given in Table 4.6 and Table 4.7. The intensity of these markers in the blood plays a significant role in determining the health of hepatic tissues as they are associated with liver functionality. A noteworthy increased level of ALP, AST, ALT, triglycerides & cholesterol was seen in rats intoxicated with alloxan and STZ. Co-administration of JHB doses to alloxan rats restored the level of these biomarkers towards the untreated group. However, JHB doses

given to streptozotocin rats could not restore normal levels of these markers at a significant level. The toxicity prompted by alloxan and STZ was amended by glibenclamide treatment to rats resulting in decreased level of hepatic markers. Whereas, no significant ($p < 0.05$) variation in the levels of these biomarkers was shown by the groups treated with JHB doses only (Figures 4.7.a, b & c and Figure 4.8.a, b).

4.5.4. Effect of JHB on Hepatic Proteins

Serum protein profiling is one of the parameters to determine liver damage. A decrease in albumin protein was observed in alloxan and STZ intoxicated rats. The diabetic groups co-administered with glibenclamide showed restored levels of albumin towards control group. Alloxan rats upon treatment with JHB rehabilitated the levels of albumin in serum considerably, while streptozotocin rats exhibited moderate restoration of the levels of this plasma protein. The level of serum proteins in group administered with JHB alone manifested no significant changes. The serum albumin profile is demonstrated in Table 4.7 along with graphical representation in Figure 4.9.

4.5.5. Effect of JHB on Antioxidant Enzymes in Liver

The tissue homogenate was assessed to illustrate the shielding effect of JHB against experimental diabetes induced by alloxan and STZ. Table 4.8 presents the variations observed in the levels of antioxidant enzymes (CAT, SOD & POD). Being crucial scavengers of noxious species, any alteration in their levels significantly affects liver physiology. The levels of CAT, SOD & POD were considerably ($p < 0.05$) downregulated in tissue samples of diabetic rats, whereas, marked increase in levels of these antioxidant enzymes were shown in diabetic rats co-administered with glibenclamide when compared to control group. Plant doses given to streptozotocin rats were unable to upregulate levels of above-mentioned antioxidant enzymes eminently. However, alloxan rats co-treated with JHB displayed superlative performance in upregulating levels of these enzymes.

4.5.6. Effect of JHB on Total Protein and GSH Content

The resultant effects of JHB on GSH levels and total protein content are listed in Table 4.8 & 4.9 respectively. The levels of protein and GSH were expressively reduced in rats suffering from diabetes as compared to the untreated ones. Like glibenclamide, the

treatment of JHB remarkably upregulated the levels of GSH as well as total protein content in alloxan rats. The outcome of JHB treatment to streptozotocin rats was again, less satisfactory. Both the levels of GSH and total protein were slightly deviated from control group.

4.5.7. Effect of JHB on TBARS, H_2O_2 & Nitrite

In hepatic tissues of rats, alloxan and STZ intoxication remarkably ($p < 0.05$) elevated the levels of TBARS, H_2O_2 & Nitrite. Dose-dependent administration of JHB to alloxan rats meticulously restored the elevated levels of TBARS, H_2O_2 & Nitrite towards control group. Plant doses provided to the other diabetic group i.e. STZ, exhibited moderate restoration of levels of these harmful substances. The only doses of JHB depicted non-significant ($p < 0.05$) difference compared to the untreated group. The levels of these parameters in glibenclamide treated groups also showed a notable drop. The results are computed in Table 4.9.

Table 4.5. Outcomes of JHB treatment on % increase in body and organ weight of rats.

Treatment Groups	Initial Body Weight	Final Body Weight	% Increase in Body Weight	Absolute Liver Weight	Relative Liver Weight
	(g)	(g)		(g)	(mg/g)
Control	203 ± 2.4	300 ± 3.5	47.3 ± 1.39^{ab}	8.34 ± 0.03^{g}	27.8 ± 0.27^{f}
ALX	194 ± 1.3	221 ± 2.2	13.7 ± 1.63^{e}	10.7 ± 0.03^{a}	48.41 ± 0.39^{a}
STZ	242 ± 2.4	280 ± 3.5	15.6 ± 1.82^{e}	10.4 ± 0.02^{b}	37.32 ± 0.52^{b}
ALX + GLI	192 ± 2.1	277 ± 2.7	44.4 ± 1.77^{b}	9.03 ± 0.01^{f}	32.52 ± 0.33^{cd}
STZ + GLI	212 ± 2.8	288 ± 2.5	43.1 ± 1.01^{c}	9.16 ± 0.02^{e}	30.2 ± 0.45^{d}
JHB	199 ± 1.3	294 ± 3.2	47.7 ± 2.6^{a}	8.38 ± 0.02^{g}	28.48 ± 0.39^{e}
ALX + JHB	191 ± 1.7	274 ± 2.1	43.8 ± 1.16^{c}	9.22 ± 0.01^{d}	33.57 ± 0.22^{c}
STZ + JHB	219 ± 2.1	281 ± 2.4	28 ± 2.13^{d}	9.45 ± 0.02^{c}	33.61 ± 0.37^{c}

The values are expressed as Mean ± SD (n=6). The superscripts (a-g) above each value represent the significant difference ($p < 0.05$) among means. ALX; Alloxan, STZ; Streptozotocin, GLI; Glibenclamide, JHB; *J. humile* butanol fraction.

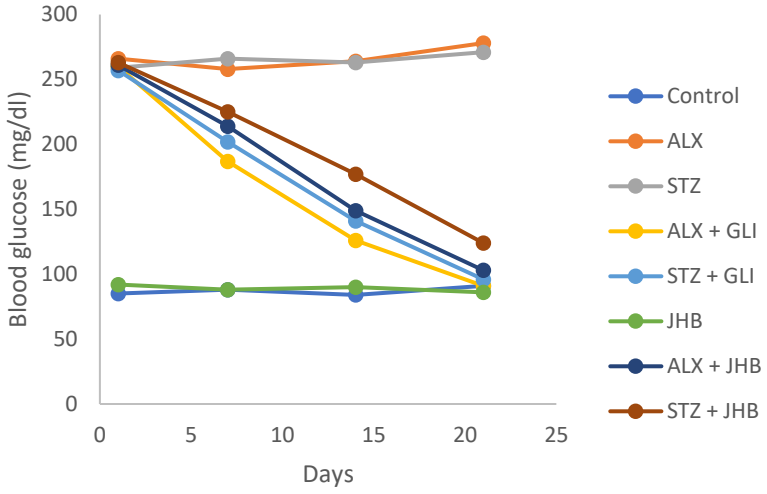
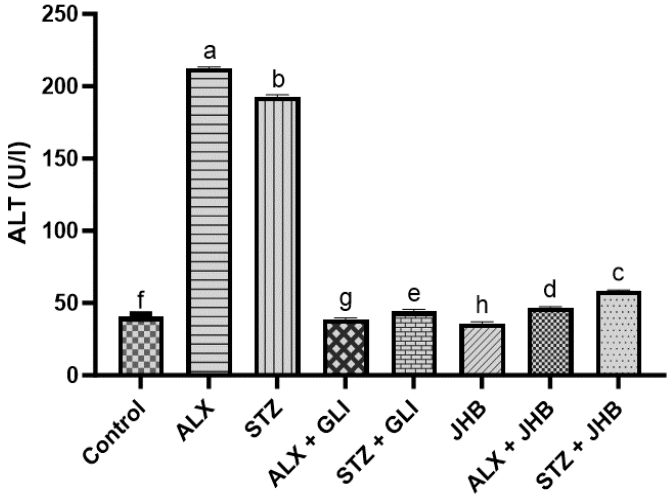


Figure 4.6. Effect of JHB on blood glucose levels of experimental subjects over the course of time.

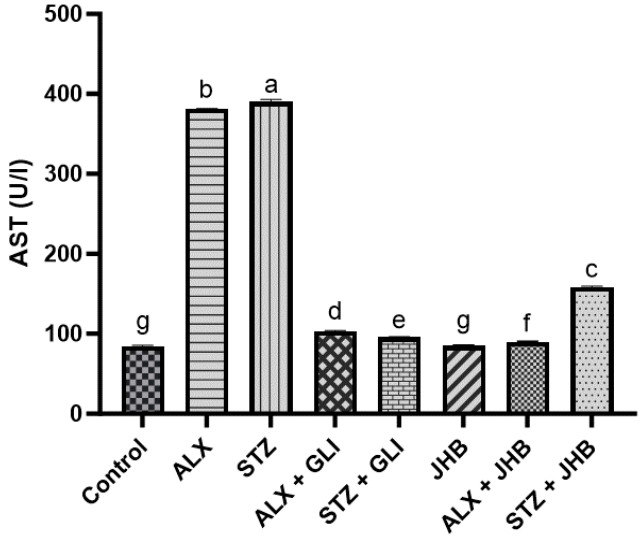
Table 4.6. Effect of JHB treatment of hepatic serum markers.

Groups	ALT	AST	ALP
	(U/I)	(U/I)	(U/I)
Control	40.64 ± 0.94^{f}	84.67 ± 0.88^{g}	51.46 ± 1.21^{f}
ALX	212.58 ± 0.76^{a}	380.98 ± 0.87^{b}	302.23 ± 0.89^{a}
STZ	192.65 ± 1.35^{b}	390.31 ± 2.72^{a}	278.99 ± 0.96^{b}
ALX + GLI	38.4 ± 1.19^{g}	103.02 ± 0.91^{d}	67.95 ± 0.75^{e}
STZ + GLI	44.27 ± 1.45^{e}	95.73 ± 0.6^{e}	70.1 ± 1.13^{d}
JHB	35.96 ± 1.11^{h}	85.03 ± 1.11^{g}	47.17 ± 0.55^{g}
ALX + JHB	46.61 ± 0.88^{d}	90.3 ± 0.81^{f}	67.2 ± 1.45^{e}
STZ + JHB	58.45 ± 0.54^{c}	158.5 ± 0.95^{c}	74.94 ± 1.03^{c}

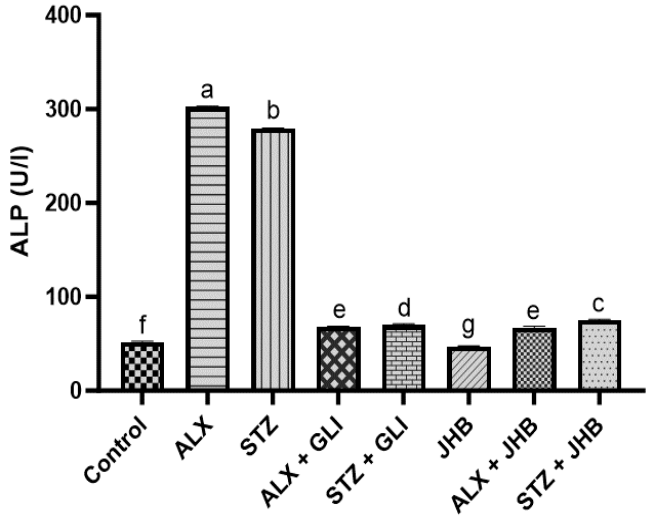
The outcomes are presented as Mean ± SD (n=6). The superscripts (a-h) above each value represent the significant difference ($p < 0.05$) among means. ALX; Alloxan, STZ; Streptozotocin, GLI; Glibenclamide, JHB; *J. humile* butanol fraction.



(a)



(b)



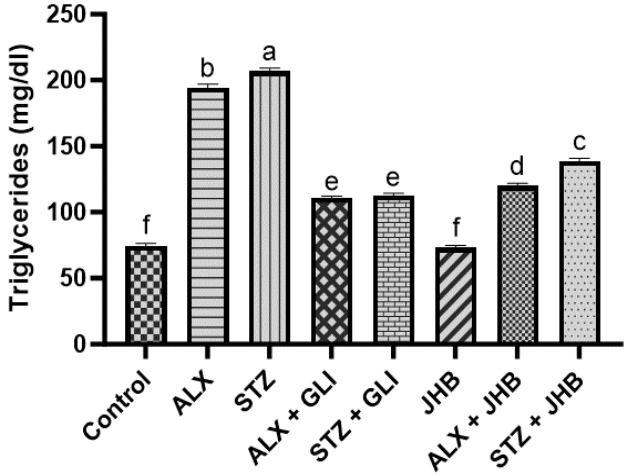
(c)

Figure 4.7. Graphical representation of effect of JHB on levels of hepatic serum markers: (a) ALT; alanine transaminase, (b) AST; aspartate aminotransferase, (c) ALP; alkaline phosphatase. ALX; Alloxan, STZ; Streptozotocin, GLI; Glibenclamide, JHB; *J. humile* butanol fraction.

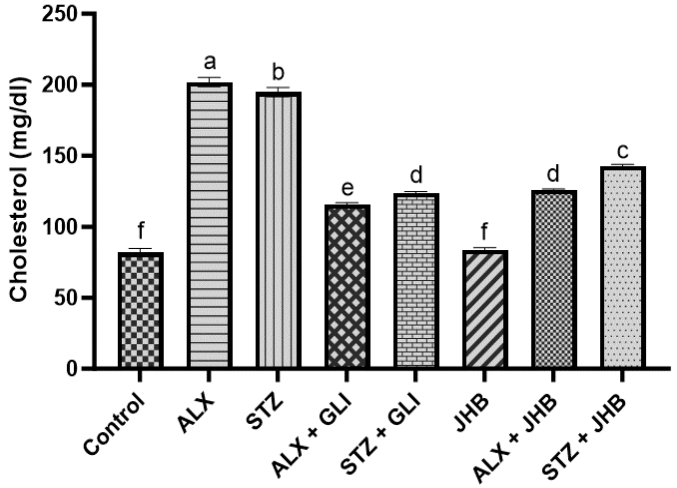
Table 4.7. Effect of JHB on levels of triglycerides, cholesterol and albumin in serum.

Groups	Triglycerides (mg/dl)	Cholesterol (mg/dl)	Albumin (mg/dl)
Control	74.5 ± 1.8^{f}	81.84 ± 2.9^{f}	4.05 ± 0.02^{a}
ALX	194.07 ± 2.8^{b}	201.91 ± 3.27^{a}	2.82 ± 0.03^{g}
STZ	206.83 ± 2.53^{a}	194.82 ± 3.26^{b}	3.17 ± 0.03^{f}
ALX + GLI	110.64 ± 1.56^{e}	115.81 ± 1.14^{e}	3.61 ± 0.04^{c}
STZ + GLI	112.84 ± 1.48^{e}	123.67 ± 1.22^{d}	3.48 ± 0.03^{d}
JHB	73.03 ± 1.94^{f}	83.7 ± 1.53^{f}	4.02 ± 0.03^{a}
ALX + JHB	120.04 ± 1.87^{c}	125.7 ± 0.85^{d}	3.73 ± 0.04^{b}
STZ + JHB	138.68 ± 2.14^{d}	142.52 ± 1.44^{c}	3.39 ± 0.05^{e}

The outcomes are presented as Mean ± SD (n=6). The superscripts (a-g) above each value represent the significant difference ($p < 0.05$) among means. ALX; Alloxan, STZ; Streptozotocin, GLI; Glibenclamide, JHB; *J. humile* butanol fraction.



(a)



(b)

Figure 4.8. Effect of JHB on levels of (a) Triglycerides & (b) Cholesterol. ALX; Alloxan, STZ; Streptozotocin, GLI; Glibenclamide, JHB; *J. humile* butanol fraction.

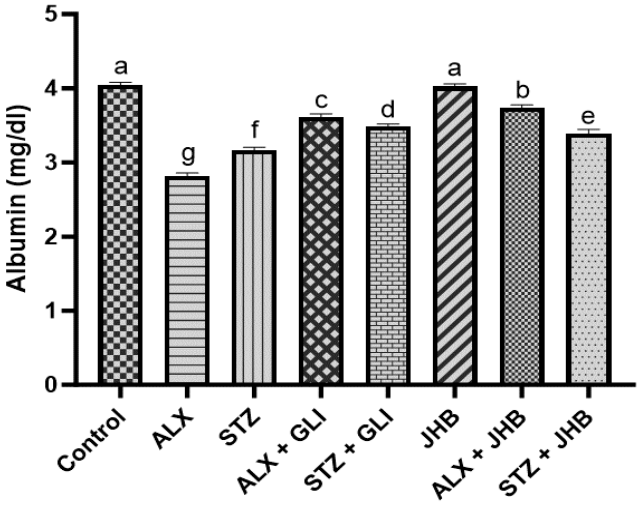


Figure 4.9. Effect of JHB on serum albumin levels. ALX; Alloxan, STZ; Streptozotocin, GLI; Glibenclamide, JHB; *J. humile* butanol fraction.

Table 4.8. Hepatoprotective effect of JHB treatment on antioxidant enzymes.

Groups	CAT	POD	SOD	GSH
	(U/min)	(U/min)	(U/mg of protein)	(µM/mg of protein)
Control	18.62 ± 0.55^{a}	25.33 ± 0.32^{bc}	23.86 ± 0.65^{a}	18.05 ± 0.25^{b}
ALX	5.46 ± 1.27^{e}	6.15 ± 1.68^{e}	8.68 ± 0.59^{e}	4.38 ± 0.22^{f}
STZ	5.77 ± 1.01^{e}	4.86 ± 1.51^{e}	9.25 ± 0.93^{e}	5.01 ± 0.22^{f}
ALX + GLI	16.48 ± 0.66^{b}	26.39 ± 1.44^{b}	22.76 ± 0.54^{a}	17.83 ± 0.77^{b}
STZ + GLI	15.05 ± 0.49^{c}	23.18 ± 1.17^{c}	19.66 ± 0.33^{b}	16.96 ± 0.42^{c}
JHB	19.67 ± 0.37^{a}	29.2 ± 0.46^{a}	20.49 ± 0.4^{b}	19.18 ± 0.24^{a}
ALX + JHB	14.41 ± 0.64^{c}	25.0 ± 1.27^{bc}	18.13 ± 1.12^{c}	15.04 ± 0.59^{d}
STZ + JHB	10.0 ± 0.67^{d}	19.23 ± 1.26^{d}	12.43 ± 0.59^{d}	9.45 ± 0.4^{e}

The outcomes are presented as Mean ± SD (n=6). The superscripts (a-f) above each value represent the significant difference ($p < 0.05$) among means.

Table 4.9. Hepatoprotective effect of JHB on total protein, TBARS, H_2O_2 & Nitrite.

Groups	Total Protein	TBARS	H_2O_2	Nitrite
	(µg/mg Tissue)	(nM/min/mg of Protein)	(µM/min/mg of Protein)	(µM/mg of Protein)
Control	8.23 ± 0.13^{b}	8.9 ± 0.11^{e}	4.03 ± 0.09^{e}	28.66 ± 1.16^{f}
ALX	4.05 ± 0.18^{f}	19.5 ± 0.63^{a}	13.51 ± 0.14^{a}	77.19 ± 0.63^{b}
STZ	4.53 ± 0.25^{e}	18.2 ± 0.94^{b}	11.84 ± 0.1^{b}	79.38 ± 0.52^{a}
ALX + GLI	6.58 ± 0.22^{c}	11.6 ± 0.4^{d}	3.56 ± 0.08^{f}	27.79 ± 0.42^{f}
STZ + GLI	6.59 ± 0.18^{c}	11.63 ± 0.32^{d}	4.08 ± 0.08^{e}	35.15 ± 0.84^{e}
JHB	9.09 ± 0.02^{a}	9.03 ± 0.04^{e}	4.07 ± 0.08^{e}	26.24 ± 0.65^{g}
ALX + JHB	6.39 ± 0.24^{c}	11.66 ± 0.47^{d}	5.73 ± 0.13^{d}	40.02 ± 0.32^{d}
STZ + JHB	5.75 ± 0.23^{d}	16.55 ± 0.66^{c}	8.43 ± 0.1^{c}	57.21 ± 0.72^{c}

The outcomes are presented as Mean ± SD (n=6). The superscripts (a-g) above each value represent the significant difference ($p < 0.05$) among means.

4.5.8. Effect of JHB on Histology of Liver

A normal hepatocyte morphology was displayed by control group rats; a prominent central vein from which cords of hepatocytes spread outward (Figure 4.10.a). Cellular degeneration, interrupted non-radiating sinusoids that tend to be wider, ruptured central vein and necrosis were observed in alloxan and STZ-induced diabetic rats (Figure 4.10.b & c). Reduced cellular disruption and close to control liver morphology was displayed by the groups receiving glibenclamide (Figure 4.10.d & e). Co-administered doses of JHB alleviated the hepatocellular injury effectively in alloxan rats and moderately in STZ rats (Figure 4.10.f & g). The treated groups showed well-radiated hepatocytes, less dilated sinusoids and fairly undistorted central vein. However, JHB treatment alone displayed the typical level morphology without any significant changes (Figure 4.10.h).

4.5.9. Hepatoprotective Effect of JHB on mRNA Expression of Genes

The altered regulation of different genes due to alloxan and STZ-induced diabetes was monitored by RT-PCR analysis. The expression of SLC2A2, the gene that encodes for GLUT2, was significantly ($p < 0.05$) upregulated during diabetic state. Administration of standard drug glibenclamide to rats treated with diabetogenic toxins regularized the fold change of SLC2A2 as compared to diabetic controls. Among plant-treated diabetic groups, co-administration of JHB to alloxan rats remarkably ($p < 0.05$) repressed the mRNA expression level of SLC2A2 in comparison to that of diabetic groups. STZ group, however, showed moderate restoration of SLC2A2 expression. Administration of JHB alone showed no significant difference in fold change when compared to untreated control (Figure 4.11).

The expression level of TGF-β1, SMAD3 and COL1A1 was markedly ($p < 0.05$) upregulated in diseased condition. Co-administration of glibenclamide to both diabetic groups, and co-administration of JHB to alloxan group, suppressed the over-expression of these fibrosis markers effectively. Plant doses provided to STZ diabetic group did not elicit considerable de-regulation of these markers (Figure 4.12, 4.13 & 4.14).

In both types of experimental diabetes, there was a significant ($p < 0.05$) decrease in expression of AMPK while its downstream targets ACC and FASN exhibited over-expression. Like glibenclamide, plant doses showed restoration of AMPK levels towards

normal and a subsequent decrease in levels of ACC and FASN, when administered to alloxan rats only. In case of STZ diabetes, moderate restoration of AMPK was noted along with slight decrease in ACC and FASN expression upon comparison with diabetic control (Figure 4.15, 4.16 & 4.17).

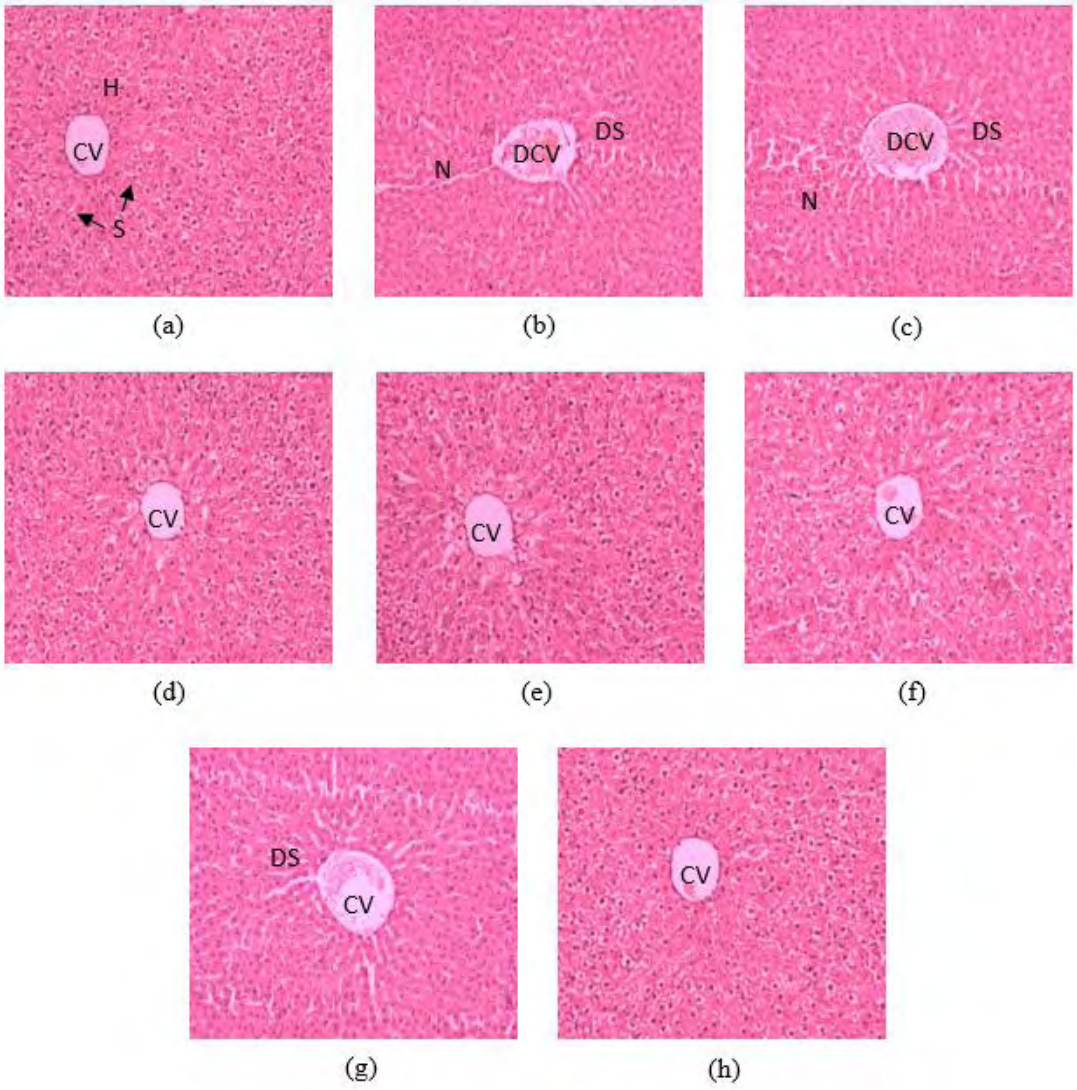


Figure 4.10. Effect of JHB on liver histology (40X magnification with H&E staining) (a) Untreated Control, (b) ALX control, (c) STZ control, (d) ALX + GLI, (e) STZ + GLI, (f) ALX + JHB, (g) STZ + JHB, (h) JHB only. CV; central vein, HPC; normal hepatocytes, S; sinusoids, DCV; damaged central vein, DS; dilated sinusoids, N; necrosis, ALX; Alloxan, STZ; Streptozotocin, GLI; Glibenclamide, JHB; *J. humile* butanol fraction.

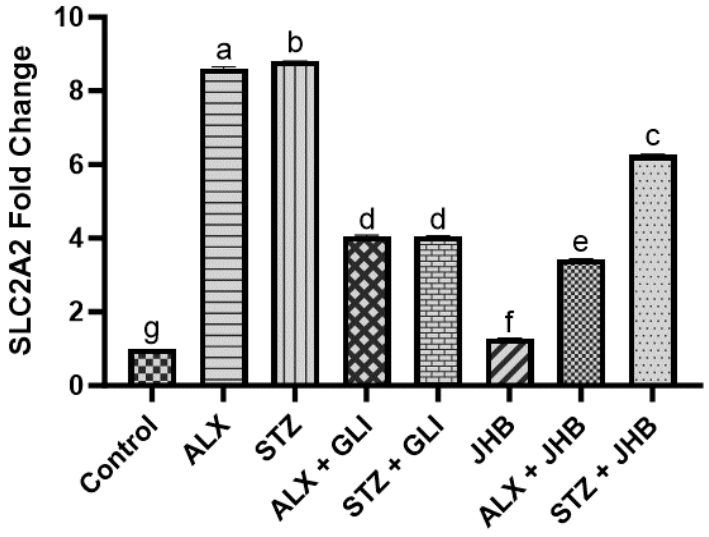


Figure 4.11. Hepatoprotective effect of JHB on SLC2A2 expression. ALX; Alloxan, STZ; Streptozotocin, GLI; Glibenclamide, JHB; *J. humile* butanol fraction.

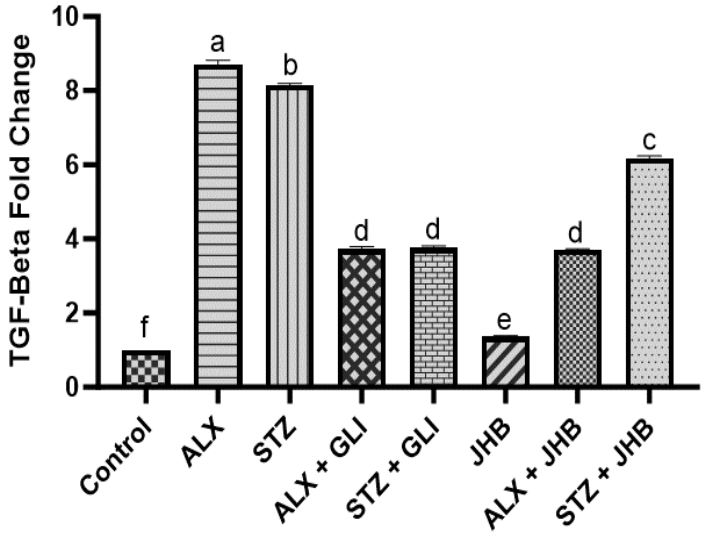


Figure 4.12. Hepatoprotective effect of JHB on TGF-β1 expression. ALX; Alloxan, STZ; Streptozotocin, GLI; Glibenclamide, JHB; *J. humile* butanol fraction.

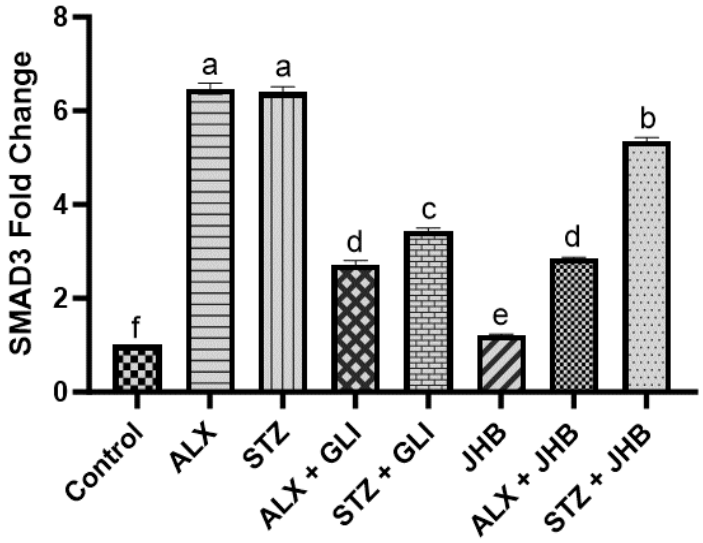


Figure 4.13. Hepatoprotective effect of JHB on SMAD3 expression. ALX; Alloxan, STZ; Streptozotocin, GLI; Glibenclamide, JHB; *J. humile* butanol fraction.

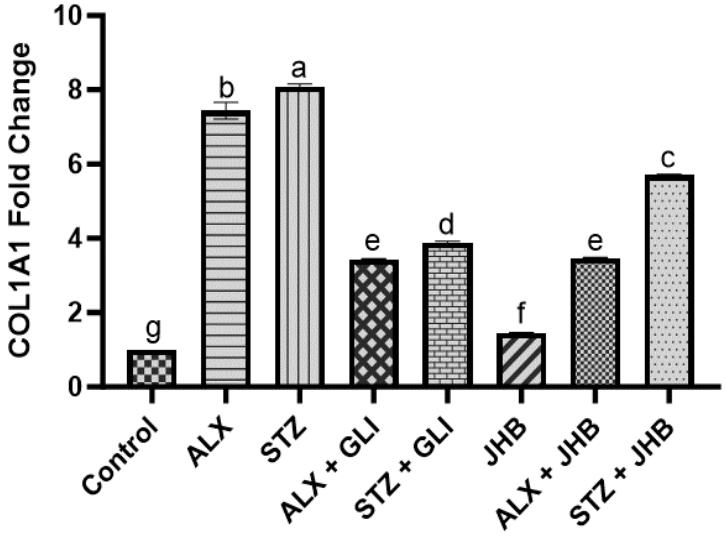


Figure 4.14. Hepatoprotective effect of JHB on COL1A1 expression. ALX; Alloxan, STZ; Streptozotocin, GLI; Glibenclamide, JHB; *J. humile* butanol fraction.

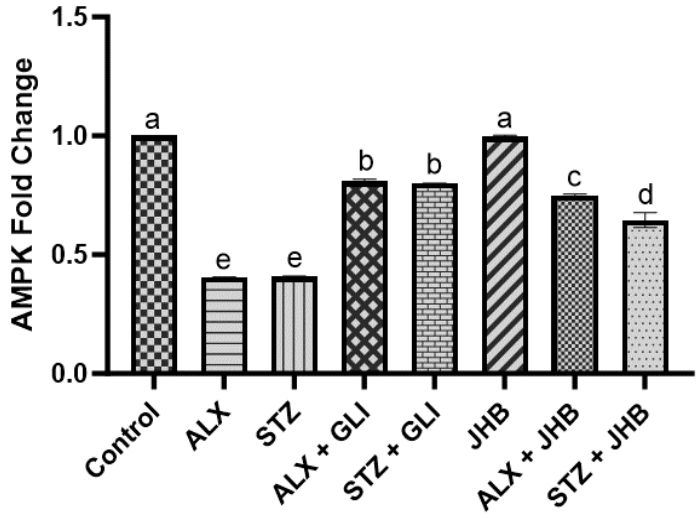


Figure 4.15. Hepatoprotective effect of JHB on AMPK expression. ALX; Alloxan, STZ; Streptozotocin, GLI; Glibenclamide, JHB; *J. humile* butanol fraction.

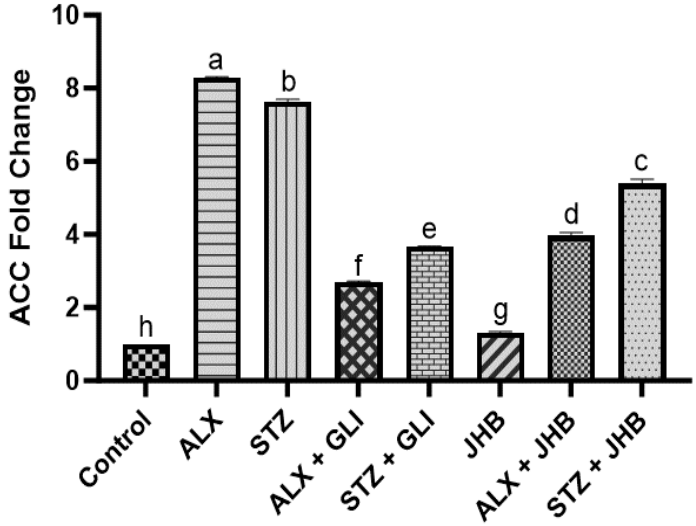


Figure 4.16. Hepatoprotective effect of JHB on ACC expression. ALX; Alloxan, STZ; Streptozotocin, GLI; Glibenclamide, JHB; *J. humile* butanol fraction.

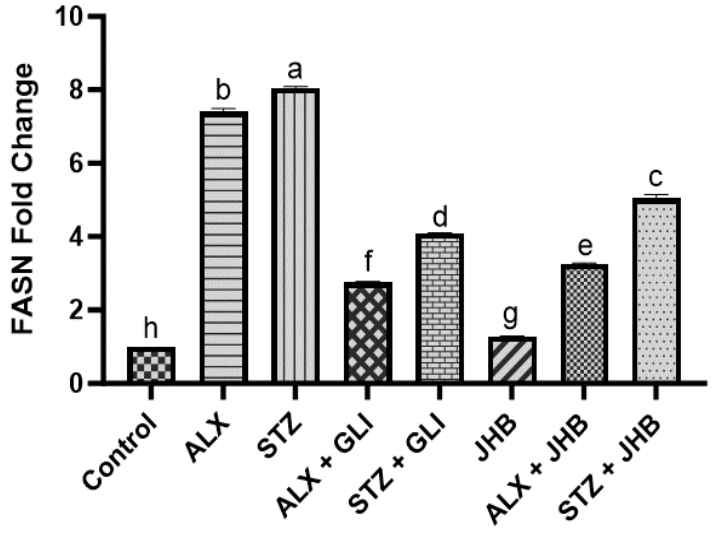


Figure 4.17. Hepatoprotective effect of JHB on FASN expression. ALX; Alloxan, STZ; Streptozotocin, GLI; Glibenclamide, JHB; *J. humile* butanol fraction.

5. DISCUSSION

Diabetes is a set of metabolic illnesses characterized by altered metabolism of carbohydrates, fats and proteins. Individuals with diabetes are unable to maintain euglycemia either due to lack of insulin action or inadequate secretion of insulin. Over the last 20 years, the global diabetes prevalence has nearly doubled (Zimmet *et al*., 2014). Diabetes, if left unchecked, can lead to a number of micro- and macro-vascular complications in many organs, especially liver. Diabetes can now be managed with a variety of therapies, including shots of insulin, oral medications, and dietary counselling. There are a number of anti-hyperglycemic drugs that exert strong anti-diabetic effect. However, such treatments possess multiple drawbacks, such as drug resistance, adverse effects, and even toxicity. Sulfonylureas, for example, lose their efficacy after prolonged use in diabetic individuals. Moreover, these drugs are also unable to combat diabetic dyslipidemia. Such limitations highlight the need for novel medications that are not only safe but also cost-effective. Plant-based medicines fulfil this criteria and can replace synthetic drugs owing to their curative properties. The ROS-mediated damage in diabetes can be corrected by using medicinal plants which are well equipped with bioactive components such as flavonoids, polyphenols, alkaloids and tannins. These compounds restore normal liver and pancreas physiology, thus improving the diabetic condition (Kooti *et al*., 2016).

5.1. Phytochemical Profiling

Phytochemicals are bio-active compounds that are abundantly present in several food sources; from legumes, vegetables, fruits, grains to seeds, nuts, tea and spices. However, only a few of these compounds have been isolated and characterized (Singh & Chaudhuri, 2018). Several compounds from plant sources including flavonoids, alkaloids, glycosides and saponins have been reported as potent anti-hyperglycemic agents. Saponins, are bioactive constituents present in many medicinal plants and known to possess potent hypoglycemic activity (Toma *et al*., 2012). Flavonoids also possess remarkable antioxidative and antidiabetic activities (Suresh & Abraham, 2020). Screening of chemical composition of a plant species is important for unveiling novel bioactive compounds capable of curing certain ailments. In the current study, the outcomes of qualitative

screening of *J. humile* validated the presence of phenols, glycosides, alkaloids, quinones, flavonoids, saponins, tannins, vitamin C and coumarins in different solvents according to their polarities. All fractions revealed the absence of phlobatannins and anthraquinones. The phytochemical composition reported in this study was found to be in accordance with previous study conducted on solvent extracts of many other plant species (Mohan *et al*., 2021). The existence of adequate quantity of phenols in different fractions of *J. humile* was further excavated by quantitative assessment. Countless medicinal attributes to the plants are believed to be conferred by these composites (Batool *et al*., 2019). The quantitative findings showed the maximum phenolic content (188.39 ± 0.33^{a}) in JHB. As per previous studies, the butanol fractions of *Monotheca buxifolia* fruit, and *Thymus vulgaris* also presented maximum total phenolic content (Hossain *et al*., 2013; Jan *et al*., 2013). The deep penetration capability of polar solvents like butanol, facilitates in mining natural antioxidants like flavonoids and phenolic compounds.

5.2. *In Vitro* Bio-Assays

Plant antioxidants display a distinctive free radical binding ability. In the present study, *in vitro* antioxidant assays were conducted to scrutinize the antioxidant profile of *J. humile*. Superoxide radicals are oxygen derived free radicals generated as a result of metabolic reactions taking place in body. Superoxide anions are low to moderately reactive; however, they can react with hydrogen peroxide in cells and generate hydroxyl radicals which are far more reactive than them (Benov, 2001). These anions can damage DNA, kill granulocytes and disintegrate erythrocytes (Fridovich, 1978). Thus, despite its moderate chemical reactivity, superoxide can cause significant cell damage. To evaluate the superoxide radical scavenging ability of *J. humile*, alkaline DMSO method was employed. The reduction of nitro-blue tetrazolium (NBT) was determined in the presence of plant samples. This assessment revealed JHB as the best superoxide scavenger of all fractions with significantly lower IC_{50} (132.65 ± 1.61^{f}). This scavenging capacity can be credited to various bioactive compounds present in it.

Like superoxide anion, hydrogen peroxide also has moderate chemical reactivity and it reacts with many bio-molecules at low rates. However, it can also generate the short-lived but toxic hydroxyl radicals in the presence of metal ion complexes such as Fe^{+2} and Cu^{+2}.

H_2O_2 can penetrate plasma membrane easily where it reacts with chromatin bound iron, which produces hydroxyl radicals that induce DNA damage and cellular death (Mello Filho, 1984). Despite being a weak oxidizing agent, H_2O_2 can inactivate some enzymes (Sharma & Gupta, 2008). For the estimation of hydrogen peroxide scavenging activity, replacement titration method was used. Among *J. humile* fractions, JHB showed the most potent hydrogen peroxide scavenging activity based on IC_{50} (172.01 ± 1.03^{f}). Our results are similar to past research conducted on *Panax notoginseng*, a traditional Chinese herb, which exhibited a high H_2O_2 scavenging rate (Zhao *et al*., 2006).

Reducing power assay, also known as potassium ferricyanide reduction assay, is a quantitative method employed for the assessment of reducing power of plant extracts. The principle of this assay is based on reduction of ferric ion (Fe^{+3}) to ferrous ion (Fe^{+2}) by antioxidant with a color shift from yellow to green (Chen *et al*., 2010). The potent the antioxidant, the more intense will be the color of the solution. It is disclosed in our results that JHB (873.41 ± 1.26^{a}) possesses remarkable reducing power owing to the presence of phenolic content. A comparative report on extracts of different medicinal plants displayed similar outcomes for antioxidant and reducing power (Stanković *et al*., 2016).

Alpha amylase is a key enzyme involved in breakdown of long chain carbohydrates. Higher eukaryotes produce inhibitor of this enzyme in order to regulate its activity. Such inhibitors can decrease postprandial glucose levels by slowing down the rate of breakdown of carbohydrates into simple sugars. In diabetics, where absence of insulin prevents clearance of glucose from blood, α-amylase inhibitors can be of help. These inhibitors are potential therapeutic targets for diabetes as low levels of α-amylase can keep blood sugar under control. Plant sources rich in polyphenols mimic the actions of insulin and act as potent inhibitors of α-amylase (Nair *et al*., 2013). Kifle & Enyew (2020) have previously reported the α-amylase inhibitory potential of leaf crude extract of *Bersama abyssinica*, a traditional medicinal plant with glucose lowering activity. To ascertain the anti-diabetic activity of *J. humile*, alpha amylase inhibition assay was performed using the DNSA (3,5-dinitrosalicylic acid) method. The highest α-amylase inhibitory activity was expressed by JHB (263.04 ± 1.1^{e}) in comparison to other fractions.

5.3. *In Vivo* Assessment of *J. humile* Butanol (JHB) Fraction

Alloxan and streptozotocin (STZ) are the most eminent diabetogenic agents. Both toxins have steric similarity to glucose and use GLUT2 to penetrate lipid bilayer of beta cells; however, the mechanisms with which they pose liver toxicity are different. Alloxan monohydrate is first reduced to dialuric acid and then re-oxidized back to alloxan, thus creating a redox cycle for the production of ROS that results in selective necrosis of beta cells. It also desensitizes the glucose sensors of beta cells and inhibits GSIS. STZ, on the other hand, induces indirect oxidative damage. This hydrophilic toxin has not only DNA alkylating properties but also stimulates NO production in beta cells. It also stimulates the generation of hydroxyl or superoxide radicals via dismutation of H_2O_2 (Lenzen, 2008). Our study demonstrates the development of alloxan diabetes and STZ-NA diabetes in rats. Besides, we tried to evaluate the hepatoprotective proclivity of *J. humile* butanol fraction (JHB) against both types of experimental diabetes, as all our findings from *in vitro* antioxidant and antidiabetic assessments proved strong antioxidative and hypoglycemic potency of this fraction. Experimental groups were provided with JHB doses and glibenclamide (standard anti-hyperglycemic drug) for 21 days. Our study is focused on selected aspects of diabetes, diabetic dyslipidemia and hepatic fibrosis.

In the current study, a reduction in percent increase of body weight and elevation in absolute and relative liver weight was instigated by alloxan and STZ. This alteration occurring in body and organ weight due to alloxan diabetes and STZ diabetes in rats has already been reported by Ezejiofor *et al*. (2017) and Rashid & Khan (2021) respectively. Co-treatment with JHB doses alleviated alloxan diabetes effectively and retained the percent rise in bodyweight close to untreated group. JHB doses also proved efficacious in cutting back the absolute and relative liver weight in alloxan rats. However, co-administration of JHB to STZ rats could not restore the drop in body weight and raise in organ weights effectively. In diabetic controls, decrease in body weight occurs due to hyper-glycemia and hypo-insulinemia. Due to decreased insulin production, cellular uptake and metabolism of glucose decreases and cellular energy requirements remain unfulfilled. In such circumstances, to meet the energy demands, fats breakdown and glycogenolysis occurs in muscles which results in proteolytic degradation of proteins and

ultimately decreased body weight. It has also been proposed that the increase in organ weight might be due to altered metabolic processes in liver suffering from oxidative damage. In diabetes, hypoinsulinemia induced high fatty acid influx causes triglyceride accumulation in liver, as a result of which its weight increases (Zafar & Naqvi, 2010). As in succinct, the retainment of % rise in body weight and reversion in organ weight towards normal by JHB therapy supports the notion that the plant fraction has hepatoprotective properties. In the present study, diabetic groups treated with glibenclamide as well as JHB showed reinstatement of normal sugar levels. This outcome justifies the hypoglycemic potential of *J. humile* butanol fraction.

The activities of ALT, AST & ALP levels (liver function markers) were measured to elucidate the toxic effects of diabetogenic alloxan and STZ. ALT & AST are considered as the most definitive markers of liver injury (Shibabaw *et al*., 2019). Elevated ALP activity, which indicates greater oxidative stress, might possibly serve as an early sign of chronic illness (Tabatabaee *et al*., 2021). Previous studies conducted on rats with experimental diabetes have reported that activities of hepatic enzymes i.e. ALT, AST, ALP, were considerably higher relative to their normal levels in both alloxan diabetic rats (Mansour *et al*., 2002) and STZ diabetic rats (Mori *et al*., 2003). Our results presented an elevated level of these enzymes in diabetic controls suggesting significant oxidative injury. Decreased levels of these hepatic markers were observed upon JHB treatment to alloxan rats. While STZ rats, showed partial restoration of these serum markers. Furthermore, this study assessed the serum lipid profile of rats as part of examining complications arising from diabetic hyperglycemia, with a focus on dyslipidemia. The outcomes reflected substantial escalation in levels of triglycerides and cholesterol in serums of diabetic rats. A measurement of serum albumin levels of diabetic controls showed reduction in albumin concentration whose normal levels are crucial to impeccable liver physiology. The oral administration of JHB to alloxan rats has shown more encouraging results, in maintaining the lipid profile and serum protein concentration close to untreated group, than the STZ diabetic controls. The presence of active phytoconstituents in JHB can be credited for its therapeutic potential. A past study conducted by Ullah *et al*. (2022) has reported restoration

of hepatic markers to normal levels in alloxan treated rats, upon treatment with *Viscum album*, a traditional plant with hypoglycemic effects.

Nature has bestowed living organisms with the innate antioxidant defense system that comprises both the enzymatic (CAT, SOD, POD, GPx) and non-enzymatic (Vitamin C & E) antioxidants. These potent antioxidants protect the biological system from oxidative injury via scavenging reactive species. For instant, SOD, the metalloenzyme dismutase, can transform superoxide anions into less toxic H_2O_2, which is neutralized by CAT enzyme. The enzyme GPx can reduce organic peroxidation by 70% and is capable of reducing approximately 90% of H_2O_2 content (Zulaikhah, 2017). Insufficient levels of antioxidant enzymes act as an indicator of overabundance of free radicals in the body. The reduction of thiol functional groups present in these enzyme complexes develops antioxidant insufficiency which creates a deficit of reduced form of glutathione i.e. GSH. During liver diseases, GSH levels fall due to oxidative stress, increased use and decreased synthesis (Lu, 2020). In the current study, diabetes induction by alloxan and STZ in rats resulted in rigorous depletion of intracellular GSH and levels of other antioxidant enzymes (CAT, SOD, POD) by production of ROS, which inflicted severe hepatocellular damage. The oral administration of JHB in a dose-dependent manner eventually restored the altered levels of GSH and other endogenous antioxidants towards the untreated control effectively in alloxan treated rats and moderately in STZ treated group. Our findings are concurrent with the work of Iwalewa (2019), who reported the anti-hyperglycemic and anti-hyperlipidemic effects of *Peristrophe bicalyculata* leaf extract against chemically induced diabetes.

Lipids are the foremost victims of degradation by ROS. As a result of peroxidation of lipids, aldehydes with great reactivity are produced. One such aldehyde radical is TBARS, whose elevated levels act as a benchmark for assessing hepatic injury mediated by diabetogenic toxins. These diabetogenic glucose analogues impose failure of antioxidant defense mechanisms and lift the embargo on free radicals production (Unuvar *et al*., 2021). At low levels, H_2O_2 acts as a mediator of insulin-induced and growth factor-induced signaling cascades but its accumulation inside cells leads to cellular dysfunction and cytotoxicity (Coyle *et al*., 2006; Sies, 2014). The cytotoxic actions of alloxan and STZ also

induce production of nitric oxides from kupffer cells and hepatocytes. An elevated level of nitric oxide builds up oxidative stress via production of nitrite radicals. As per our study, the levels of nitrite radicals, H_2O_2 and TBARS were remarkably upregulated in both diabetic controls, while total protein content regressed significantly ($p < 0.05$). Concurrent JHB treatment doses resulted in decreased nitrites, H_2O_2 and TBARS content and upraised the total protein with more promising outcomes against alloxan diabetes. The reduction in these reactive species might be attributed to plant treatment which restored activity of antioxidant enzymes. Similar outcomes were previously reported in a study by Sajid *et al*. (2020), where *Alnus nitida* leaves showed promising antioxidant and antidiabetic potency in alloxan-induced diabetic rats.

Histological analysis provides a convenient and visual method for evaluating the protective efficacy of plant extract against hepatic injuries. In our histological findings, alloxan and STZ exhibited extreme disruption of hepatic cellular morphology, sinusoidal dilation and necrosis. However, JHB co-treatment displayed a marked reduction in these pathological symptoms with more obvious changes being noticed against alloxan-induced hepatotoxicity.

At molecular level, the effects of JHB on mRNA expression profiles of several genes, involved in carbohydrate metabolism, *de novo* lipid synthesis and fibrosis, were studied via RT-PCR analysis. Impaired carbohydrate metabolism is a common characteristic of diabetes. The hepatic glucose output is strongly influenced by overexpression of GLUT2 in diabetes. David-Silva *et al*. (2013) reported overexpression of SLC2A2 gene and GLUT2 protein in diabetic rats, which decreased significantly upon treatment with insulin. Likewise, our findings revealed many folds enhanced expression of SLC2A2 in diabetic controls. This alteration was normalized significantly ($p < 0.05$) by JHB treatment in case of alloxan diabetes.

Diabetes induced oxidative stress and lipid peroxidation poses severe damage to liver cells. Injured hepatocytes produce more aldehyde radicals that exacerbate liver injury and lay foundation of hepatic fibrogenesis by over-production of TGF-β1 (Mustafa, 2016). TGF-β1, one of the most potent fibrogenic cytokines, is considered a key player in the process of fibrosis. Alloxan and STZ inflicted redox imbalance in liver increases TGF-β1 induced

activation of HSCs, which move to the site of injury and initiate transcription of COL1A1 via a series of downstream regulators, SMAD3 being one of them (Aboulmagd *et al*., 2020). Our study on anti-fibrotic effects of JHB revealed downregulation of TGF-β1 and its downstream targets, i.e. SMAD3 and COL1A1, significantly in alloxan-induced diabetic rats. Treatment of STZ diabetic rats with JHB displayed unfavorable outcomes related to expression of these fibrosis markers. Our findings regarding the anti-fibrotic activity of plant against alloxan diabetes are coherent with the study of Arafat *et al*. (2016).

Hyperglycemia is strongly associated with increased lipid levels. AMPK is an upstream kinase that not only regulates blood glucose levels by inhibiting gluconeogenesis, but also maintains lipid profile by inhibiting *de novo* synthesis of fatty acids and promoting β-oxidation of lipids (Vinayagam *et al*., 2018; Rafailovska *et al*., 2023). AMPK activation causes downregulation of lipogenic genes i.e. ACC and FASN, via a series of downstream regulators. ACC is a key enzyme in fatty acid synthesis as it controls the rate-limiting step for the production of precursor malonyl Co-A. De-regulation of ACC by AMPK results in decreased FA synthesis and subsequent reduction in storage of TG (Viollet *et al*., 2009). FASN is another lipogenic enzyme that catalyzes the production of palmitate from acetyl Co-A and malonyl Co-A (Li *et al*., 2023). Overexpression of FASN increases FA yield and contributes to progression of diabetic dyslipidemia (Bedi *et al*., 2021). As per RT-PCR analysis, diabetic controls of our study showed de-regulated expression of AMPK, while that of ACC and FASN was upregulated considerably ($p < 0.05$). JHB co-treatment produced different lipogenic gene expression profiles in alloxan and STZ groups. Alloxan rats exhibited normalized expression of genes of interest. Our findings are concomitant with the work of Zhong *et al*. (2022).

In the course of this study, the effects of JHB on experimentally induced diabetes were meticulously examined. This investigation encompassed the utilization of two distinct diabetogenic agents, alloxan and streptozotocin (STZ), known for their divergent mechanisms of action in diabetes induction. Alloxan operates through direct provocation of ROS infliction, thereby inducing oxidative damage. On the contrary, STZ primarily exerts its influence by instigating DNA alkylation, consequently eliciting a cascade of events that culminate in heightened oxidative stress. The outcomes of this study unveiled

a noteworthy disparity in the efficacy of the JHB against the two induction methodologies. JHB exhibited a discernible effectiveness against alloxan-induced diabetes, potentially attributed to its inherent antioxidant properties that could counteract ROS-mediated cellular damage. However, the observed lack of efficacy against STZ-induced diabetes warrants further exploration, possibly suggesting a nuanced interplay between the fraction's constituents and the intricate molecular processes initiated by STZ. These findings not only underscore the complex nature of diabetes etiology but also accentuate the potential of *J. humile* as a prospective therapeutic approach, particularly in contexts where ROS driven oxidative stress contributes to the pathogenesis of diabetes.

Conclusion

Our study has offered valuable insights into the therapeutic potential of *J. humile* in mitigating the intricate array of challenges posed by hyperglycemia and diabetes induced oxidative stress. The presence of diverse range of phytochemicals within all plant fractions displayed potent antioxidant and antidiabetic activities *in vitro*; however, butanol fraction particularly stood out for its pronounced effects. The *in vivo* findings unveiled a distinctive trend; the butanol fraction exhibited superior outcomes against alloxan diabetes compared to STZ diabetes. JHB alleviated alloxan-induced hepatotoxicity by restoration of glycemic levels, serum markers, antioxidant enzymes and reactive species towards normal. JHB therapy significantly normalized the mRNA expression of target genes involved in glucose metabolism, lipid synthesis and fibrotic modulation. As contemporary medical approaches continue to grapple with the complex interplay of hyperglycemia, dyslipidemia and fibrosis, the antioxidant-rich constituents of *J. humile* present a promising avenue for therapeutic exploration.

Future Perspectives

The current study has confirmed that *J. humile* possesses significant therapeutic potential against alloxan-induced hepatotoxicity. These initial findings lay a foundation for future investigations, urging a more in-depth pharmacological and biochemical assessment of phytoconstituents and their interactions with molecular targets related to diabetes, diabetic dyslipidemia and fibrosis. Another noteworthy avenue is the exploration of combination therapy involving this plant. Studies designed to investigate the synergistic effects that could arise from integrating the active constituents of *J. humile* with existing antidiabetic medications can enhance the overall therapeutic outcomes while minimizing possible side effects associated with conventional treatments. By systematically evaluating the interactions between phytoconstituents from *J. humile* and established antidiabetic drugs, this area of research could contribute to optimizing diabetes treatment regimens and lending substantial support to the traditional use of this plant in management of diabetes and complications associated with it.

6. REFERENCES

Aboulmagd, Y. M., El-Bahy, A. A., Menze, E. T., Azab, S. S., & El-Demerdash, E. (2020). Role of linagliptin in preventing the pathological progression of hepatic fibrosis in high fat diet and streptozotocin-induced diabetic obese rats. *European Journal of Pharmacology*, *881*, 173224.

Achakzai, J. K., Anwar Panezai, M., Kakar, M. A., Kakar, A. M., Kakar, S., Khan, J., ... & Tareen, A. K. (2019). In vitro anticancer MCF-7, anti-inflammatory, and brine shrimp lethality assay (BSLA) and GC-MS analysis of whole plant butanol fraction of Rheum ribes (WBFRR). *BioMed research international*, *2019*.

Afsar, T., Khan, M. R., Razak, S., Ullah, S., & Mirza, B. (2015). Antipyretic, anti-inflammatory and analgesic activity of Acacia hydaspica R. Parker and its phytochemical analysis. *BMC complementary and alternative medicine*, *15*(1), 1-12.

Akhtar, N., Hayat, M. Q., Hafiz, I. A., Abbasi, N. A., Malik, S. I., Habib, U., ... & Potter, D. (2021). Comparative palynology and taxonomic implication of Jasminum L.(Oleaceae) species from Pakistan on the bases of scanning electron microscopy. *Microscopy Research and Technique*, *84*(10), 2325-2336.

Akhtar, N., Rashid, A., Murad, W., & Bergmeier, E. (2013). Diversity and use of ethno-medicinal plants in the region of Swat, North Pakistan. *Journal of ethnobiology and ethnomedicine*, *9*, 1-14.

Akkiraju, P. C., Suryawanshi, D. D., Jawakekar, A. J., Tambe, H. S., & Mamillapalli, S. (2016). Phytochemical analysis and HPLC study of vitamin-C from Punica granatum L. Aarakta variety of India. *J Med Plant Stud*, *4*(6), 09-12.

Alam, F., Islam, A., Hua Gan, S., Mohamed, M., & Haryo Sasongko, T. (2016). DNA methylation: an epigenetic insight into type 2 diabetes mellitus. *Current pharmaceutical design*, *22*(28), 4398-4419.

Al-Birawee, A. R., & Nasser, A. K. (2019). Gel extraction from caper fruits (Capparies spinosa L.) and assess its effectiveness as antioxidants. *Basrah Journal of Agricultural Sciences*, *32*(2), 74-84.

Ali, S., Khan, M. R., Irfanullah, Sajid, M., & Zahra, Z. (2018). Phytochemical investigation and antimicrobial appraisal of Parrotiopsis jacquemontiana (Decne) Rehder. *BMC complementary and alternative medicine*, *18*, 1-15.

Almutairi, F. M., Mir, R., Abu-Duhier, F., Khan, R., Harby, K., & Elfaki, I. (2019). Slc2a2 gene (glucose transporter 2) variation is associated with an increased risk of developing t2d in an ethnic population of saudi arabia. Indian J. Public Health Res. Dev, 10, 600-605.

Anık, A., Çatlı, G., Abacı, A., & Böber, E. (2015). Maturity-onset diabetes of the young (MODY): an update. *Journal of pediatric endocrinology and metabolism*, *28*(3-4), 251-263.

Anwar, H., Hussain, G., & Mustafa, I. (2018). Antioxidants from natural sources. *Antioxidants in foods and its applications*, *3*.

Arafat, S. Y., Nayeem, M., Jahan, S., Karim, Z., Reza, H. M., Hossain, M. H., ... & Alam, M. A. (2016). Ellagic acid rich Momordica charantia fruit pulp supplementation prevented oxidative stress, fibrosis and inflammation in liver of alloxan induced diabetic rats. *Oriental Pharmacy and Experimental Medicine*, *16*, 267-278.

Archana, P., Samatha, T., Mahitha, B., & Chamundeswari, N. R. (2012). Preliminary phytochemical screening from leaf and seed extracts of Senna alata L. Roxb-an ethno medicinal plant. *Int J Pharm Biol Res*, *3*(3), 82-89.

Asirvatham, R., Antonysamy, A., Rajkumar, P., & Narayanan, L. (2012). Anticancer activity of Jasminum angustifolium Linn against Ehrlich ascites carcinoma cells bearing mice. *Journal of Experimental & Integrative Medicine*, *2*(3).

Awoyinka, O. A., Balogun, I. O., & Ogunnowo, A. A. (2007). Phytochemical screening and in vitro bioactivity of Cnidoscolus aconitifolius (Euphorbiaceae). *Journal of Medicinal Plants Research*, *1*(3), 63-65.

Bastaki, S. (2005). Diabetes mellitus and its treatment. *Dubai Diabetes And Endocrinology Journal*, *13*(3), 111-134.

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.

Batool, R., Salahuddin, H., Mahmood, T., & Ismail, M. (2017). Study of anticancer and antibacterial activities of Foeniculum vulgare, Justicia adhatoda and Urtica dioica as natural curatives. *Cellular and Molecular Biology*, *63*(9), 109-114.

Bedi, O., Srivastava, N., Parsad, D., & Krishan, P. (2021). Fatty acid synthase inhibition ameliorates diabetes induced liver injury in rodent experimental model. *European Journal of Pharmacology*, *901*, 174078.

Ben Djoudi Ouadda, A., Levy, E., Ziv, E., Lalonde, G., Sané, A. T., Delvin, E., & Elchebly, M. (2009). Increased hepatic lipogenesis in insulin resistance and Type 2 diabetes is associated with AMPK signalling pathway up-regulation in Psammomys obesus. *Bioscience reports*, *29*(5), 283-292.

Benov, L. (2001). How superoxide radical damages the cell. *Protoplasma*, *217*, 33-36.

Bolen, S., Feldman, L., Vassy, J., Wilson, L., Yeh, H. C., Marinopoulos, S., ... & Brancati, F. L. (2007). Systematic review: comparative effectiveness and safety of oral medications for type 2 diabetes mellitus. *Annals of internal medicine*, *147*(6), 386-399.

Bonaventura, J., Schroeder, W. A., & Fang, S. (1972). Human erythrocyte catalase: an improved method of isolation and a re-evaluation of reported properties. *Archives of biochemistry and biophysics*, *150*(2), 606-617.

Brentjens, R., & Saltz, L. (2001). Islet cell tumors of the pancreas: the medical oncologist's perspective. *Surgical Clinics of North America*, *81*(3), 527-542.

Carulli, L., Rondinella, S., Lombardini, S., Canedi, I., Loria, P., & Carulli, N. (2005). Diabetes, genetics and ethnicity. *Alimentary pharmacology & therapeutics*, *22*, 16-19.

Cernea, S., & Raz, I. (2021). NAFLD in type 2 diabetes mellitus: still many challenging questions. *Diabetes/Metabolism Research and Reviews*, *37*(2), e3386.

Chance, B., & Maehly, A. C. (1955). [136] Assay of catalases and peroxidases.

Chatterjee, S., Khunti, K., & Davies, M. J. (2017). Type 2 diabetes. *The lancet*, *389*(10085), 2239-2251.

Chen, T. S., Liou, S. Y., Wu, H. C., Tsai, F. J., Tsai, C. H., Huang, C. Y., & Chang, Y. L. (2010). New analytical method for investigating the antioxidant power of food extracts on the basis of their electron-donating ability: comparison to the ferric reducing/antioxidant power (FRAP) assay. *Journal of Agricultural and Food Chemistry*, *58*(15), 8477-8480.

Chomczynski, P., & Mackey, K. (1995). Short technical reports. Modification of the TRI reagent procedure for isolation of RNA from polysaccharide-and proteoglycan-rich sources. *Biotechniques*, *19*(6), 942-945.

Corless, J. K., & Middleton, H. M. (1983). Normal liver function: A basis for understanding hepatic disease. *Archives of internal medicine*, *143*(12), 2291-2294.

Costa, M., Bernardi, J., Fiuza, T., Costa, L., Brandão, R., & Pereira, M. E. (2016). N-acetylcysteine protects memory decline induced by streptozotocin in mice. *Chemico-biological interactions*, *253*, 10-17.

Coyle, C. H., Martinez, L. J., Coleman, M. C., Spitz, D. R., Weintraub, N. L., & Kader, K. N. (2006). Mechanisms of H2O2-induced oxidative stress in endothelial cells. *Free Radical Biology and Medicine*, *40*(12), 2206-2213.

Damodaran, A., & Manohar, S. (2012). Qualitative screening for phytochemicals of various solvent extracts of Cassia alata Linn. leaves. *Herbal Technological Industry*, 11-13.

David-Silva, A., Freitas, H. S., Okamoto, M. M., Sabino-Silva, R., Schaan, B. D., & Machado, U. F. (2013). Hepatocyte nuclear factors 1α/4α and forkhead box A2 regulate the solute carrier 2A2 (Slc2a2) gene expression in the liver and kidney of diabetic rats. *Life sciences*, *93*(22), 805-813.

DeFronzo, R. A., Ferrannini, E., Groop, L., Henry, R. R., Herman, W. H., Holst, J. J., ... & Weiss, R. (2015). Type 2 diabetes mellitus. *Nature reviews Disease primers*, *1*(1), 1-22.

Doss, A., Palaniswamy, M., Angayarkanni, J., & Dhanabalan, R. (2009). Antidiabetic activity of water extract of Solanum trilobatum (Linn.) in alloxan-induced diabetes in rats. *African Journal of Biotechnology*, *8*(20).

Dunn, J. S., & McLetchie, N. G. B. (1943). Experimental alloxan diabetes in the rat. *The Lancet*, *242*(6265), 384-387.

Durai, M. V., Balamuniappan, G., Anandalakshmi, R., Geetha, S., & Kumar, N. S. (2016). Qualitative and quantitative analysis of phytochemicals in crude extract of big-leaf mahogany (Swietenia macrophylla King). *International Journal of Herbal Medicine*, *4*(6), 88-91.

Egan, A. M., & Dinneen, S. F. (2019). What is diabetes?. *Medicine*, *47*(1), 1-4.

El Haouari, M., Quintero, J. E., & Rosado, J. A. (2020). Anticancer molecular mechanisms of oleocanthal. *Phytotherapy Research*, *34*(11), 2820-2834.

El-Beih, N. M., Ramadan, G., El-Husseiny, E. A., & Hussein, A. M. (2019). Effects of pomegranate aril juice and its punicalagin on some key regulators of insulin resistance and oxidative liver injury in streptozotocin-nicotinamide type 2 diabetic rats. *Molecular biology reports*, *46*(4), 3701-3711.

El-Hawary, S. S., El-Hefnawy, H. M., Osman, S. M., El-Raey, M. A., & Mokhtar Ali, F. A. (2021). Phenolic profiling of different Jasminum species cultivated in Egypt and their antioxidant activity. *Natural Product Research*, *35*(22), 4663-4668.

El-Shiekh, R. A., Hussein, D., Atta, A. H., Mounier, S. M., Mousa, M. R., & Abdel-Sattar, E. (2021). Anti-inflammatory activity of Jasminum grandiflorum L. subsp. floribundum (Oleaceae) in inflammatory bowel disease and arthritis models. *Biomedicine & Pharmacotherapy*, *140*, 111770.

El-Shiekh, R. A., Saber, F. R., & Abdel-Sattar, E. A. (2020). In vitro anti-hypertensive activity of Jasminum grandiflorum subsp. floribundum (Oleaceae) in relation to its

metabolite profile as revealed via UPLC-HRMS analysis. *Journal of Chromatography B*, *1158*, 122334.

Ezejiofor, A. N., Igweze, Z. N., Udowelle, N. A., & Orisakwe, O. E. (2017). Histopathological and biochemical assessments of Costus afer stem on alloxan-induced diabetic rats. *Journal of Basic and Clinical Physiology and Pharmacology*, *28*(4), 383-391.

Fahmy, N. M., Al-Sayed, E., & Singab, A. N. B. (2017). Phytochemical screening and antioxidant activity of Terminalia muelleri benth. leaf extract. *Archives of Pharmaceutical Sciences Ain Shams University*, *1*(1), 1-4.

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*, 1-17.

Forouhi, N. G., & Wareham, N. J. (2019). Epidemiology of diabetes. *Medicine*, *47*(1), 22-27.

Fraga, C. G., Leibovitz, B. E., & Tappel, A. L. (1988). Lipid peroxidation measured as thiobarbituric acid-reactive substances in tissue slices: characterization and comparison with homogenates and microsomes. *Free Radical Biology and Medicine*, *4*(3), 155-161.

Fridovich, I. (1978). Superoxide radicals, superoxide dismutases and the aerobic lifestyle. *Photochemistry and photobiology*, *28*(4-5), 733-741.

Friederich, M., Hansell, P., & Palm, F. (2009). Diabetes, oxidative stress, nitric oxide and mitochondria function. *Current diabetes reviews*, *5*(2), 120-144.

Friedman, S. L., Neuschwander-Tetri, B. A., Rinella, M., & Sanyal, A. J. (2018). Mechanisms of NAFLD development and therapeutic strategies. *Nature medicine*, *24*(7), 908-922.

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

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.

Gregory, J. M., Moore, D. J., & Simmons, J. H. (2013). Type 1 diabetes mellitus. *Pediatrics in review*, *34*(5), 203-215.

Guagnano, M. T., Pace-Palitti, V., Manigrasso, M. R., Merlitti, D., Parra, H. S., & Sensi, S. (2001). Non insulin-dependent diabetes mellitus (type 2) secondary failure. Metformin-glibenclamide treatment. *International Journal of Immunopathology and Pharmacology*, *14*(1), 31-43.

Gulcin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. *Archives of toxicology*, *94*(3), 651-715.

Guo, W. W., Wang, X., Chen, X. Q., Ba, Y. Y., Zhang, N., Xu, R. R., ... & Wu, X. (2018). Flavonones from Penthorum chinense ameliorate hepatic steatosis by activating the SIRT1/AMPK pathway in HepG2 cells. *International Journal of Molecular Sciences*, *19*(9), 2555.

Hamburger, M., & Hostettmann, K. (1991). 7. Bioactivity in plants: the link between phytochemistry and medicine. *Phytochemistry*, *30*(12), 3864-3874.

Hamid, A. A., Aiyelaagbe, O. O., Usman, L. A., Ameen, O. M., & Lawal, A. (2010). Antioxidants: Its medicinal and pharmacological applications. *African Journal of pure and applied chemistry*, *4*(8), 142-151.

Harborne, A. J. (1998). Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media.

Hashmi, H. F., Bibi, S., Anwar, M., & Khan, M. R. (2021). Qualitative and Quantitative analysis of phytochemicals in Lepidium Pinnatifidum Ledeb. *Sch Int J Tradit Complement Med*, *4*(5), 67-75.

Hossain, M. A., AL-Raqmi, K. A. S., Al-Mijizy, Z. H., Weli, A. M., & Al-Riyami, Q. (2013). Study of total phenol, flavonoids contents and phytochemical screening of

various leaves crude extracts of locally grown Thymus vulgaris. *Asian Pacific journal of tropical biomedicine*, *3*(9), 705-710.

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.

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.

Irfan, M., Nabeela, I. K., Kamil, M., Ullah, S., Khan, S., Shah, M., ... & Murad, W. (2018). Ethnobotanical survey of the flora of tehsil Balakot, district Mansehra, Khyber Pakhtunkhwa, Pakistan. *Journal of Applied Environmental and Biological Sciences*, *8*(8), 1-13.

Islam, S., Rahman, S., Haque, T., Sumon, A. H., Ahmed, A. M., & Ali, N. (2020). Prevalence of elevated liver enzymes and its association with type 2 diabetes: A cross-sectional study in Bangladeshi adults. *Endocrinology, diabetes & metabolism*, *3*(2), e00116.

Iwalewa, E. O. (2019). Antidiabetic activity of Peristrophe bicalyculata Leaf Extract in Streptozotocin and Alloxan-induced diabetic rats. *Archives of Basic and Applied Medicine*, *7*(2), 95-100.

Jan, G., Khan, M. A., & Gul, F. (2009). Ethnomedicinal plants used against jaundice in Dir Kohistan valleys (NWFP), Pakistan. *Ethnobotanical leaflets*, *2009*(8), 7.

Jan, S., Khan, M. R., Rashid, U., & Bokhari, J. (2013). Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of Monotheca buxifolia fruit. *Osong public health and research perspectives*, *4*(5), 246-254.

Jia, Q., Su, W., Peng, W., Li, P., & Wang, Y. (2008). Anti-diarrhoea and analgesic activities of the methanol extract and its fractions of Jasminum amplexicaule Buch.-Ham.(Oleaceae). *Journal of ethnopharmacology*, *119*(2), 299-304.

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

Katsarou, A., Gudbjörnsdottir, S., Rawshani, A., Dabelea, D., Bonifacio, E., Anderson, B. J., ... & Lernmark, Å. (2017). Type 1 diabetes mellitus. *Nature reviews Disease primers*, *3*(1), 1-17.

Kaul, K., Tarr, J. M., Ahmad, S. I., Kohner, E. M., & Chibber, R. (2013). Introduction to diabetes mellitus. *Diabetes: an old disease, a new insight*, 1-11.

Keerthana, G., Kalaivani, M. K., & Sumathy, A. (2013). In-vitro alpha amylase inhibitory and anti-oxidant activities of ethanolic leaf extract of Croton bonplandianum. *Asian J Pharm Clin Res*, *6*(4), 32-36.

Kewcharoenwong, C., Rinchai, D., Utispan, K., Suwannasaen, D., Bancroft, G. J., Ato, M., & Lertmemongkolchai, G. (2013). Glibenclamide reduces pro-inflammatory cytokine production by neutrophils of diabetes patients in response to bacterial infection. *Scientific reports*, *3*(1), 3363.

Khan Pathan, R., Gali, P. R., Pathan, P., Gowtham, T., & Pasupuleti, S. (2012). In vitro antimicrobial activity of Citrus aurantifolia and its phytochemical screening. *Asian Pacific Journal of Tropical Disease*, *2*, S328-S331.

Khan, B. A., & Akhtar, N. (2012). Phytochemical analysis and acute toxicity tests of two medicinal plant extracts. *J Med Plants Res*, *6*(19), 3545-8.

Khan, H. (2015). Brilliant future of phytomedicines in the light of latest technological developments. *J. Phytopharm*, *4*, 58-60.

Khan, R. A., Khan, M. R., & Sahreen, S. (2012). Protective effect of Sonchus asper extracts against experimentally induced lung injuries in rats: a novel study. *Experimental and toxicologic pathology*, *64*(7-8), 725-731.

Kifle, Z. D., & Enyew, E. F. (2020). Evaluation of in vivo antidiabetic, in vitro α-amylase inhibitory, and in vitro antioxidant activity of leaves crude extract and solvent fractions of Bersama abyssinica fresen (melianthaceae). *Journal of Evidence-Based Integrative Medicine*, *25*, 2515690X20935827.

Koh, G. C., Weehuizen, T. A., Breitbach, K., Krause, K., de Jong, H. K., Kager, L. M., ... & Wiersinga, W. J. (2013). Glyburide reduces bacterial dissemination in a mouse model of melioidosis. *PLoS neglected tropical diseases*, *7*(10), e2500.

Kohnert, K. D., Freyse, E. J., & Salzsieder, E. (2012). Glycaemic Variability and Pancreatic ß-cell Dysfunction. *Current Diabetes Reviews*, *8*(5), 345-354.

Kooti, W., Farokhipour, M., Asadzadeh, Z., Ashtary-Larky, D., & Asadi-Samani, M. (2016). The role of medicinal plants in the treatment of diabetes: a systematic review. *Electronic physician*, *8*(1), 1832.

Kottaisamy, C. P. D., Raj, D. S., Prasanth Kumar, V., & Sankaran, U. (2021). Experimental animal models for diabetes and its related complications—a review. *Laboratory animal research*, *37*(1), 1-14.

Kraeima, B. R. A. M. (2016). *Measuring insulin resistance in clinical practice* (Doctoral dissertation, Faculty of Science and Engineering).

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.

Kumar, V., Xin, X., Ma, J., Tan, C., Osna, N., & Mahato, R. I. (2021). Therapeutic targets, novel drugs, and delivery systems for diabetes associated NAFLD and liver fibrosis. *Advanced Drug Delivery Reviews*, *176*, 113888.

Kunchandy, E., & Rao, M. N. A. (1990). Generation of superoxide anion and hydrogen peroxide by (+)-cyanidanol-3. *International journal of pharmaceutics*, *65*(3), 261-263.

Kunwar, R. M., Mahat, L., Sharma, L. N., Shrestha, K. P., Kominee, H., & Bussmann, R. W. (2012). Underutilized plant species in far west Nepal. *Journal of Mountain Science*, *9*, 589-600.

Kunwar, R. M., Sher, H., & Bussmann, R. W. (Eds.). (2021). *Ethnobotany of the Himalayas*. Springer Nature.

Lebovitz, H. E., Feinglos, M. N., Bucholtz, H. K., & Lebovitz, F. L. (1977). Potentiation of insulin action: a probable mechanism for the anti-diabetic action of sulfonylurea drugs. *The Journal of Clinical Endocrinology & Metabolism*, *45*(3), 601-604.

Lee, H., Yu, D. M., Um, M. J., Yoon, S. Y., Kim, K. T., Kwon, Y. J., ... & Ko, Y. G. (2019). Hepatocytic Prominin-1 protects against liver fibrosis by stabilizing the SMAD7 protein. *BioRxiv*, 846493.

Lee, J. H., Yang, S. H., Oh, J. M., & Lee, M. G. (2010). Pharmacokinetics of drugs in rats with diabetes mellitus induced by alloxan or streptozocin: comparison with those in patients with type I diabetes mellitus. *Journal of Pharmacy and Pharmacology*, *62*(1), 1-23.

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

Levitt, D. G., & Levitt, M. D. (2016). Human serum albumin homeostasis: a new look at the roles of synthesis, catabolism, renal and gastrointestinal excretion, and the clinical value of serum albumin measurements. *International journal of general medicine*, 229-255.

Li, S. Y., Li, Y., Wu, Z. H., Zhou, Z. J., Li, C. Y., Wu, T. T., ... & Zhong, Y. (2023). Study on the mechanism of action of effective monomeric, berberine of Xianglian Pill in inhibiting human colon cancer cells based on fatty acid synthase target. *Journal of Traditional and Complementary Medicine*.

Liu, T., Wang, X., Karsdal, M. A., Leeming, D. J., & Genovese, F. (2012). Molecular serum markers of liver fibrosis. *Biomarker insights*, *7*, BMI-S10009.

Longkumer, S., Jamir, A., Kechu, M., Ezung, S., & Pankaj, P. P. (2021). Alloxan monohydrate induced diabetes: A comprehensive review. *Sciences*, *1*(1).

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of biological chemistry*, *193*, 265-275.

Lu, S. C. (2020). Dysregulation of glutathione synthesis in liver disease. *Liver Research*, *4*(2), 64-73.

Lucio, D., Irache, J. M., Font, M., & Martínez-Ohárriz, M. C. (2017). Supramolecular structure of glibenclamide and β-cyclodextrins complexes. *International Journal of Pharmaceutics*, *530*(1-2), 377-386.

Luzi, L., & Pozza, G. (1997). Glibenclamide: an old drug with a novel mechanism of action?. *Acta diabetologica*, *34*(4), 239-244.

Macdonald Ighodaro, O., Mohammed Adeosun, A., & Adeboye Akinloye, O. (2017). Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *Medicina*, *53*(6), 365-374.

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), 1-15.

Mansoori, A., Sahranavard, T., Hosseini, Z. S., Soflaei, S. S., Emrani, N., Nazar, E., ... & Mobarhan, M. G. (2023). Prediction of type 2 diabetes mellitus using hematological factors based on machine learning approaches: a cohort study analysis. *Scientific Reports*, *13*(1), 1-11.

Mansour, H. A., Newairy, A. S. A., Yousef, M. I., & Sheweita, S. A. (2002). Biochemical study on the effects of some Egyptian herbs in alloxan-induced diabetic rats. *Toxicology*, *170*(3), 221-228.

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.

Maqbool, M., Dar, M. A., Gani, I., & Mir, S. A. (2019). Animal models in diabetes mellitus: an overview. *Journal of Drug Delivery and Therapeutics*, *9*(1-s), 472-475.

Marble, A. (1971). Glibenclamide, a new sulphonylurea: whither oral hypoglycaemic agents?. *Drugs*, *1*(2), 109-115.

Masharani, U., & Karam, J. H. Diabetes mellitus and Hypoglycemia. Current Medical Diagnosis and Treatment 2001.

Meis, J. E., & Khanna, A. (2009). RNA amplification and cDNA synthesis for qRT-PCR directly from a single cell.

Mello Filho, A. C., Hoffmann, M. E., & Meneghini, R. (1984). Cell killing and DNA damage by hydrogen peroxide are mediated by intracellular iron. *Biochemical Journal*, *218*(1), 273.

Mishra, A. K., Chahal, P., Singh, H., Kumar, A., & Mishra, A. (2022). Exploration of hepatoprotective and antidiabetic potential of Senna occidentalis L. and isolation of bioactive compound by column chromatography. *South African Journal of Botany*, *148*, 144-153.

Mohamed, J., Nafizah, A. N., Zariyantey, A. H., & Budin, S. (2016). Mechanisms of diabetes-induced liver damage: the role of oxidative stress and inflammation. *Sultan Qaboos University Medical Journal*, *16*(2), e132.

Mohan, E., Suriya, S., Shanmugam, S., & Rajendran, K. (2021). Qualitative phytochemical screening of selected medicinal plants. *Journal of Drug Delivery and Therapeutics*, *11*(2), 141-144.

Mohandas, J., Marshall, J. J., Duggin, G. G., Horvath, J. S., & Tiller, D. J. (1984). Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney: possible implications in analgesic nephropathy. *Biochemical pharmacology*, *33*(11), 1801-1807.

Møller, A. M., Jensen, N. M., Pildal, J., Drivsholm, T., Borch-Johnsen, K., Urhammer, S. A., ... & Pedersen, O. (2001). Studies of genetic variability of the glucose transporter 2 promoter in patients with type 2 diabetes mellitus. *The Journal of Clinical Endocrinology & Metabolism*, *86*(5), 2181-2186.

Mori, D. M., Baviera, A. M., de Oliveira Ramalho, L. T., Vendramini, R. C., Brunetti, I. L., & Pepato, M. T. (2003). Temporal response pattern of biochemical analytes in experimental diabetes. *Biotechnology and applied biochemistry*, *38*(2), 183-191.

Murata, M., Takahashi, A., Saito, I., & Kawanishi, S. (1999). Site-specific DNA methylation and apoptosis: induction by diabetogenic streptozotocin. *Biochemical pharmacology*, *57*(8), 881-887.

Mustafa, H. N. (2016). The role of curcumin in streptozotocin-induced hepatic damage and the trans-differentiation of hepatic stellate cells. *Tissue and Cell*, *48*(2), 81-88.

Nair, S. S., Kavrekar, V., & Mishra, A. (2013). In vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. *European journal of experimental biology*, *3*(1), 128-132.

Neha, K., Haider, M. R., Pathak, A., & Yar, M. S. (2019). Medicinal prospects of antioxidants: A review. *European journal of medicinal chemistry*, *178*, 687-704.

Ng, T. L., Rohac, R., Mitchell, A. J., Boal, A. K., & Balskus, E. P. (2019). An N-nitrosating metalloenzyme constructs the pharmacophore of streptozotocin. *Nature*, *566*(7742), 94-99.

Nilesh, M., Dinesh, S., & Dhirendra, S. (2009). Evaluation of anti-ulcer potential of leaves of Jasminum grandiflorum L. *Int J Ph Sci*, *1*(2), 247-9.

Ogurtsova, K., da Rocha Fernandes, J. D., Huang, Y., Linnenkamp, U., Guariguata, L., Cho, N. H., ... & Makaroff, L. E. (2017). IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes research and clinical practice*, *128*, 40-50.

Ohtsubo, K., Takamatsu, S., Minowa, M. T., Yoshida, A., Takeuchi, M., & Marth, J. D. (2005). Dietary and genetic control of glucose transporter 2 glycosylation promotes insulin secretion in suppressing diabetes. *Cell*, *123*(7), 1307-1321.

Okoko, T., & Ere, D. (2012). Reduction of hydrogen peroxide–induced erythrocyte damage by Carica papaya leaf extract. *Asian Pacific journal of tropical biomedicine*, *2*(6), 449-453.

Olatunde, O. Z., Yong, J., & Lu, C. (2023). Isolation, Structural Elucidation of a New Triterpenoid from the Roots of Jasminum sambac (L.) Ait. with Potent Cytotoxicity against MCF-7 Cell Lines. *ACS omega*.

Olokoba, A. B., Obateru, O. A., & Olokoba, L. B. (2012). Type 2 diabetes mellitus: a review of current trends. *Oman medical journal*, *27*(4), 269.

Omale, S., Amagon, K. I., Johnson, T. O., Bremner, S. K., & Gould, G. W. (2023). A systematic analysis of anti-diabetic medicinal plants from cells to clinical trials. *PeerJ*, *11*, e14639.

Pan, S. Y., Zhou, S. F., Gao, S. H., Yu, Z. L., Zhang, S. F., Tang, M. K., ... & Ko, K. M. (2013). New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. *Evidence-Based Complementary and Alternative Medicine*, *2013*.

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.

Paschou, S. A., Papadopoulou-Marketou, N., Chrousos, G. P., & Kanaka-Gantenbein, C. (2018). On type 1 diabetes mellitus pathogenesis. *Endocrine connections*, *7*(1), R38-R46.

Patel, M. K., & Rajput, A. P. (2013). Therapeutic significance of Abutilon indicum: An overview. *Am. J. Pharm. Tech. Res*, *4*, 20-35.

Peschke E, Ebelt H, Brömme HJ, Peschke D (2000) ‘Classical’ and ‘new’ diabetogens—comparison of their effects on isolated rat pancreatic islets in vitro. Cell Mol Life Sci 57:158–164

Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian journal of clinical biochemistry*, *30*, 11-26.

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.

Qazi, M. A., & Molvi, K. I. (2018). Free radicals and their management. *American Journal of Pharmacy and Health Research*, *6*(4), 1-10.

Quang, B. H., Choudhary, R. K., & Lee, J. (2021). Nomenclatural notes and typification of nine names related to Jasminum (Oleaceae). *PhytoKeys*, *183*, 55.

Rafailovska, E., Tushevski, O., Shijakova, K., Simic, S. G., Kjovkarovska, S. D., & Miova, B. (2023). Hypericum perforatum L. extract exerts insulinotropic effects and inhibits gluconeogenesis in diabetic rats by regulating AMPK expression and PKCε concentration. *Journal of Ethnopharmacology*, *302*, 115899.

Rakieten, N. (1963). Studies on the diabetogenic action of STZ (NSC 37917). *Cancer Chemotherapy Rept*, *29*, 91-98.

Rashid, S., Ahmad, M., Zafar, M., Sultana, S., Ayub, M., Khan, M. A., & Yaseen, G. (2015). Ethnobotanical survey of medicinally important shrubs and trees of Himalayan region of Azad Jammu and Kashmir, Pakistan. *Journal of Ethnopharmacology*, *166*, 340-351.

Rashid, U., & Khan, M. R. (2021). Phytochemicals of Periploca aphylla Dcne. ameliorated streptozotocin-induced diabetes in rat. *Environmental health and preventive medicine*, *26*(1), 1-14.

Rashid, U., Khan, M. R., & Sajid, M. (2019). Antioxidant, anti-inflammatory and hypoglycemic effects of Fagonia olivieri DC on STZ-nicotinamide induced diabetic rats-in vivo and in vitro study. *Journal of ethnopharmacology*, *242*, 112038.

Reece, E. A., Leguizamón, G., & Wiznitzer, A. (2009). Gestational diabetes: the need for a common ground. *The Lancet*, *373*(9677), 1789-1797.

Rohilla, A., & Ali, S. (2012). Alloxan induced diabetes: mechanisms and effects. *International journal of research in pharmaceutical and biomedical sciences*, *3*(2), 819-823.

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), 1-7.

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.

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), 1-17.

Saltiel, A. R., & Kahn, C. R. (2001). Insulin signalling and the regulation of glucose and lipid metabolism. *Nature*, *414*(6865), 799-806.

Santo, A., Zhu, H., & Li, Y. R. (2016). Free radicals: From health to disease. *React. Oxyg. Species*, *2*, 245-263.

Santo, A., Zhu, H., & Li, Y. R. (2016). Free radicals: From health to disease. *React. Oxyg. Species*, *2*, 245-263.

Schmittgen, T. D., & Zakrajsek, B. A. (2000). Effect of experimental treatment on housekeeping gene expression: validation by real-time, quantitative RT-PCR. *Journal of biochemical and biophysical methods*, *46*(1-2), 69-81.

Sena, L. A., & Chandel, N. S. (2012). Physiological roles of mitochondrial reactive oxygen species. *Molecular cell*, *48*(2), 158-167.

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

Shahwan, M., Alhumaydhi, F., Ashraf, G. M., Hasan, P. M., & Shamsi, A. (2022). Role of polyphenols in combating Type 2 Diabetes and insulin resistance. *International Journal of Biological Macromolecules*, *206*, 567-579.

Sharifi-Rad, J., Quispe, C., Bouyahya, A., El Menyiy, N., El Omari, N., Shahinozzaman, M., ... & Calina, D. (2022). Ethnobotany, phytochemistry, biological activities, and health-promoting effects of the genus Bulbophyllum. *Evidence-Based Complementary and Alternative Medicine*, *2022*.

Sharma, P., Garg, A., Garg, S., & Singh, V. (2016). Animal model used for experimental study of Diabetes Mellitus: an overview. *Asian J Biomat Res*, *2*(4), 99-110.

Sharma, S., & Gupta, V. (2008). In vitro antioxidant studies of Ficus racemosa Linn. root. *Pharmacognosy Magazine*, *4*(13), 70.

Shibabaw, T., Dessie, G., Molla, M. D., Zerihun, M. F., & Ayelign, B. (2019). Assessment of liver marker enzymes and its association with type 2 diabetes mellitus in Northwest Ethiopia. *BMC research notes*, *12*, 1-5.

Shishehbor, F., Mansoori, A., Sarkaki, A. R., Jalali, M. T., & Latifi, S. M. (2008). Apple cider vinegar attenuates lipid profile in normal and diabetic rats. *Pakistan journal of biological sciences: PJBS*, *11*(23), 2634-2638.

Shrestha, P. M., & Dhillion, S. S. (2003). Medicinal plant diversity and use in the highlands of Dolakha district, Nepal. *Journal of ethnopharmacology*, *86*(1), 81-96.

Sies, H. (2014). Role of metabolic H_2O_2 generation: redox signaling and oxidative stress. *Journal of Biological Chemistry*, *289*(13), 8735-8741.

Singh, D., & Chaudhuri, P. K. (2018). A review on phytochemical and pharmacological properties of Holy basil (Ocimum sanctum L.). *Industrial Crops and Products*, *118*, 367-382.

Sonam, M., Singh, R. P., & Pooja, S. (2017). Phytochemical screening and TLC profiling of various extracts of Reinwardtia indica. *International Journal of Pharmacognosy and Phytochemical Research*, *9*(4), 523-527.

Stanković, N., Mihajilov-Krstev, T., Zlatković, B., Stankov-Jovanović, V., Mitić, V., Jović, J., ... & Bernstein, N. (2016). Antibacterial and antioxidant activity of traditional medicinal plants from the Balkan Peninsula. *NJAS*-Wageningen *Journal of Life Sciences*, *78*, 21-28.

Steinbusch, L. K., Luiken, J. J., Vlasblom, R., Chabowski, A., Hoebers, N. T., Coumans, W. A., ... & Diamant, M. (2011). Absence of fatty acid transporter CD36 protects against Western-type diet-related cardiac dysfunction following pressure overload in mice. *American journal of physiology-endocrinology and metabolism*, *301*(4), E618-E627.

Suresh, A., & Abraham, J. (2020). Phytochemicals and their role in pharmaceuticals. *Advances in Pharmaceutical Biotechnology: Recent Progress and Future Applications*, 193-218.

Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological research*, *50*(6), 537-546.

Tabatabaee, N., Heidarpour, M., & Khoramian, B. (2021). Milk metabolites, proteins and oxidative stress markers in dairy cows suffering from Staphylococcus aureus subclinical mastitis with or without spontaneous cure. *Journal of Dairy Research*, *88*(3), 326-329.

Targher, G., Corey, K. E., Byrne, C. D., & Roden, M. (2021). The complex link between NAFLD and type 2 diabetes mellitus—mechanisms and treatments. *Nature reviews Gastroenterology & hepatology*, *18*(9), 599-612.

Tiwari, B. K., Pandey, K. B., Abidi, A. B., & Rizvi, S. I. (2013). Markers of oxidative stress during diabetes mellitus. *Journal of biomarkers*, *2013*.

Toma, A., Makonnen, E., Debella, A., & Tesfaye, B. (2012). Antihyperglycemic effect on chronic administration of butanol fraction of ethanol extract of Moringa stenopetala leaves in alloxan induced diabetic mice. *Asian* Pacific *Journal of Tropical Biomedicine*, *2*(3), S1606-S1610.

Tomic, D., Shaw, J. E., & Magliano, D. J. (2022). The burden and risks of emerging complications of diabetes mellitus. *Nature Reviews Endocrinology*, *18*(9), 525-539.

Ţucureanu, V., Matei, A., & Avram, A. M. (2016). FTIR spectroscopy for carbon family study. *Critical reviews in analytical chemistry*, *46*(6), 502-520.

Ullah, M., Mehmood, S., Khan, R. A., Ali, M., Fozia, F., Waqas, M., ... & Sultan, M. A. (2022). Assessment of antidiabetic potential and phytochemical profiling of Viscum album, a traditional antidiabetic plant. *Journal of Food Quality*, *2022*, 1-9.

Ünüvar, S. O. N. G. Ü. L., Gürsoy, Ş., Berk, A., Kaymaz, B., İlhan, N., & Aktay, G. Ö. K. N. U. R. (2021). Antioxidant Effect of a Dihydropyridine Calcium Antagonist Nitrendipine in Streptozotocin-Induced Diabetes. *Journal of Evolutionary Biochemistry and Physiology*, *57*, 126-133.

Ventura-Sobrevilla, J., Boone-Villa, V. D., Aguilar, C. N., Román-Ramos, R., Vega-Avila, E., Campos-Sepúlveda, E., & Alarcón-Aguilar, F. (2011, January). Effect of varying dose and administration of streptozotocin on blood sugar in male CD1 mice. In *Proc West Pharmacol Soc* (Vol. 54, No. 5, p. 9).

Vergani, L., Floreani, M., Russell, A., Ceccon, M., Napoli, E., Cabrelle, A., ... & Dabbeni-Sala, F. (2004). Antioxidant defences and homeostasis of reactive oxygen species in different human mitochondrial DNA-depleted cell lines. *European journal of biochemistry*, *271*(18), 3646-3656.

Vinayagam, R., Jayachandran, M., Chung, S. S. M., & Xu, B. (2018). Guava leaf inhibits hepatic gluconeogenesis and increases glycogen synthesis via AMPK/ACC signaling pathways in streptozotocin-induced diabetic rats. *Biomedicine & Pharmacotherapy*, *103*, 1012-1017.

Viollet, B., Lantier, L., Devin-Leclerc, J., Hébrard, S., Amouyal, C., Mounier, R., ... & Andreelli, F. (2009). Targeting the AMPK pathway for the treatment of Type 2 diabetes. *Frontiers in bioscience (Landmark edition)*, *14*, 3380.

Vitali, L. A., Beghelli, D., Nya, P. C. B., Bistoni, O., Cappellacci, L., Damiano, S., ... & Bramucci, M. (2016). Diverse biological effects of the essential oil from Iranian Trachyspermum ammi. *Arabian Journal of Chemistry*, *9*(6), 775-786.

Volpe, C. M. O., Villar-Delfino, P. H., Dos Anjos, P. M. F., & Nogueira-Machado, J. A. (2018). Cellular death, reactive oxygen species (ROS) and diabetic complications. *Cell death & disease*, *9*(2), 1-9.

Wang, Y., Luo, W., & Wang, Y. (2019). PARP-1 and its associated nucleases in DNA damage response. *DNA repair*, *81*, 102651.

Xiang, D., Zou, J., Zhu, X., Chen, X., Luo, J., Kong, L., & Zhang, H. (2020). Physalin D attenuates hepatic stellate cell activation and liver fibrosis by blocking TGF-β/Smad and YAP signaling. *Phytomedicine*, *78*, 153294.

Xu, F., Liu, C., Zhou, D., & Zhang, L. (2016). TGF-β/SMAD pathway and its regulation in hepatic fibrosis. *Journal of Histochemistry & Cytochemistry*, *64*(3), 157-167.

Yang, Y., Kim, B., Park, Y. K., Koo, S. I., & Lee, J. Y. (2015). Astaxanthin prevents TGFβ1-induced pro-fibrogenic gene expression by inhibiting Smad3 activation in hepatic stellate cells. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1850*(1), 178-185.

Yaribeygi, H., Atkin, S. L., & Sahebkar, A. (2019). A review of the molecular mechanisms of hyperglycemia-induced free radical generation leading to oxidative stress. *Journal of cellular physiology*, *234*(2), 1300-1312.

Yin, C., Fan, X., Fan, Z., Shi, D., & Gao, H. (2018). Optimization of enzymes-microwave-ultrasound assisted extraction of Lentinus edodes polysaccharides and determination of its antioxidant activity. *International journal of biological macromolecules*, *111*, 446-454.

Yusuf, A. Z., Zakir, A., Shemau, Z., Abdullahi, M., & Halima, S. A. (2014). Phytochemical analysis of the methanol leaves extract of Paullinia pinnata linn. *Journal of Pharmacognosy and Phytotherapy*, *6*(2), 10-16.

Zafar, M., & Naqvi, S. N. U. H. (2010). Effects of STZ-Induced Diabetes on the Relative Weights of Kidney, Liver and Pancreas in Albino Rats: A Comparative Study. *International Journal of Morphology*, *28*(1).

Zhang, X., Wang, Y., Xiao, W., Jiang, D., Zhou, J., Ye, X., ... & Song, P. (2023). Hyperglycaemia in pregnancy and offspring blood pressure: a systematic review and meta-analysis. *Diabetology & Metabolic Syndrome*, *15*(1), 1-16.

Zhao, G. R., Xiang, Z. J., Ye, T. X., Yuan, Y. J., & Guo, Z. X. (2006). Antioxidant activities of Salvia miltiorrhiza and Panax notoginseng. *Food chemistry*, *99*(4), 767-774.

Zhong, Y., Li, Z., Jin, R., Yao, Y., He, S., Lei, M., ... & Peng, X. (2022). Diosgenin ameliorated type II diabetes-associated nonalcoholic fatty liver disease through inhibiting de novo lipogenesis and improving fatty acid oxidation and mitochondrial function in rats. *Nutrients*, *14*(23), 4994.

Zimmet, P. Z., Magliano, D. J., Herman, W. H., & Shaw, J. E. (2014). Diabetes: a 21st century challenge. *The lancet Diabetes & endocrinology*, *2*(1), 56-64.

Zulaikhah, S. T. (2017). The role of antioxidant to prevent free radicals in the body. *Sains Medika*, *8*(1), 39-45.