Nephroprotective Effects of Magnolol against Cisplatin-Induced Nephrotoxicity in *In Vivo* Model

M.Phil Thesis

by

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Nephroprotective Effects of Magnolol against Cisplatin-Induced Nephrotoxicity in *In Vivo* Model

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I, Ariba Sattar, here by state that my M. Phil thesis title **"Nephroprotective Effects of Magnolol against Cisplatin-Induced Nephrotoxicity in *In Vivo* Model"** submitted to the Department of Pharmacy, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, Pakistan for the award of the degree of Master of Philosophy in **Pharmacy (Pharmacology)** is the result of research work carried out by me under the supervision of Dr. Salman Khan during the period of 2021-2023. I further declare that the results presented in this thesis have not been submitted for the award of any other degree from this University or anywhere else in the country/world and the University has the right to withdraw my M.Phil degree, if my statement is found incorrect at any time, even after my graduation.

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DEDICATION

To my family for your unwavering support and belief in my abilities that have been a constant source of inspiration. To my father, ***Dr. Abdul Sattar Mirani****, for your boundless wisdom, strength, and unconditional love have guided me through every step of my journey. This thesis stands as a testament to the values you have instilled in me—diligence, perseverance, and a thirst for knowledge. Your tireless dedication to my education has been the foundation upon which I have built my academic pursuits.*
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Acknowledgments

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Ariba Sattar

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List of Abbreviations

Abbreviations	Description
AKI	Acute Kidney Injury
BUN	Blood Urea Nitrogen
COX	Cycloxygenase
CKD	Chronic Kidney Disease
CP	Cisplatin
EPO	Eosinophil Peroxidase
GFR	Glomerular Filtration Rate
IKB	Inhibitor of KB
MPO	Myeloperoxidase
NSAID	Nonsteriod Anti-Inflammatory Drugs
NT	Nephrotoxicity
OCT	Organic Cation Transporter
PBS	Phosphate Buffer Saline
SCr	Serum Creatinine
TBA	Thiobarbituric Acid
TCA	Trichloroacetic Acid
TLRs	Toll-Like Receptors
TNBS	Trinitrobenzenesulfonic Acid
TNF-α	Tumor Necrosis Factor-Alpha

Abstract

Nephrotoxicity refers to the harmful effects that certain substances or drugs can have on the kidneys, causing damage to renal tissue and impairing the organ's ability to filter and eliminate waste products. This can result in acute kidney injury or chronic kidney disease, which are major contributors to kidney damage and failure. Magnolol, a natural compound found in the bark and cones of the Magnolia tree, has demonstrated anti-inflammatory properties by blocking the production of pro-inflammatory molecules. It also exhibits potent antioxidant effects, which can help protect cells from oxidative damage caused by free radicals.

Studies have shown that magnolol induces programmed cell death in cancer cells and inhibits tumor growth in animal models. Additionally, magnolol has anxiolytic properties, acting on the brain's GABAergic system to reduce anxiety. It also possesses antimicrobial activity, particularly against gram-positive bacteria, making it a potential candidate for new antibiotics. Magnolol has shown cardiovascular protective effects by lowering blood pressure, improving blood flow, and preventing blood clot formation. Overall, magnolol shows promise as a natural compound with potential therapeutic applications in various health conditions, although further research is required to establish its safety and efficacy in humans.

The current study aimed to investigate the pharmacological effects of Magnolol in a model of cisplatin-induced nephrotoxicity. Kidney function biomarkers such as serum creatinine, BUN, urea, and uric acid were measured to assess the impact of Magnolol on kidney function. Magnolol administration resulted in a satisfactory decrease in the levels of these biomarkers.

Hematological data revealed a significant increase in various blood cell counts, indicating the induction of toxicity, which were subsequently normalized within the normal range by Magnolol treatment. The kidney, as a major organ of the excretory system, plays a crucial role in maintaining serum electrolyte balance.

The study evaluated the pharmacological effect of Magnolol on antioxidant indices in renal tissues by measuring lipid peroxidation, myeloperoxidase, nitric oxide, and erythropoietin. Magnolol exhibited significant protective effects against cisplatin-induced disruptions in the antioxidant system.

Spontaneous pain was significantly reduced, and the structural integrity of kidney tissue cells, as assessed by hematoxylin and eosin staining, was restored by Magnolol

treatment. Magnolol also decreased abnormal nitric oxide production in kidney tissues. In summary, Magnolol effectively mitigated cisplatin-induced nephrotoxicity, alleviating spontaneous pain and inflammatory responses in this study.

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

1.1. Background

The abdomen's rear wall is where the two kidneys, which resemble beans, are located retroperitoneally. The renal hilum, which is located on the medial side of the kidney, allows the ureters, lymphatic vessels, nerves, and renal blood vessels to enter and exit the kidneys (Ray and Reddy, 2023). The outer cortex and inner medulla of the kidneys, which are encased in an outer capsule, are further separated into renal pyramids with cone-like shapes. The papilla, which changes into the renal pelvis, is located at the base of each pyramid. Major calyces that extend downward as minor calyces gather urine from each papilla along the pelvic exterior line. Urine eventually moves from the calices, pelvis, and ureter to the bladder due to their contractile walls (Ray and Reddy, 2023).

The kidney plays a number of significant regulatory and homeostatic functions in the human body. The majority of the body's vital functions, including the removal of endogenous waste products, reabsorption of nutrients, and elimination of xenobiotics and/or their metabolites, are carried out by this important filtrating organ of the excretory system (Radi, 2019). In addition to filtering, it also manages a number of basic physiological processes, including blood cell creation, blood pressure regulation, and acid-base balance. As well as producing numerous critical hormones like calcitriol, erythropoietin, and renin, it is essential for the re-absorption of amino acids, water, and glucose (Radi, 2019).

In adult human being the average weight of each kidney is between 125 and 170 grams and about 11 centimeters (4.3 inches) in length (Glassock and Rule, 2012). 99depression called hilum through which nerves and renal artery can enter the kidney. It also acts as an outlet for the ureter and renal vein. Lymphatic vessel drainage is also taking place in the hilum (Sampaio, 2000). Nephrons are the basic structural and functional unit of kidney and their number in a normal adult human kidney can vary from 200,000 to over 1.8 million. Tubular portion of nephron is composed of bowman capsule (BC), proximal (PCT) and distal convoluted tubule (DCT) and finally descending as well as ascending loop of Henle (Chen *et al.*, 2016).

Due to the abundant blood flow they receive, kidneys have the ability to observe and adjust numerous functions of different organ systems. Consequently, renal

malfunction may arise from or contribute to different pathological states (Bagshaw *et al.*, 2007). Kidney diseases have a significant influence on other organs, leading to the development of multiple health conditions like cardiovascular diseases, hypertension, and diabetes mellitus. Consequently, the impact of kidney disease on quality of life of a patient is substantial (Bagshaw *et al.*, 2007)

1.2. Kidney: An Overview of Acute Nephrotoxicity

Nephrotoxicity is the term used to show the toxic effects of any substance on the kidney. Liver is generally considered as the main target organ for several materials that produce toxic or lethal effects. Nephrotoxicity arises when the specialized

processes in the kidneys responsible for detoxification and elimination are impaired, leading to dysfunction or harm caused by toxic substances originating from either external sources or internal processes.

The kidneys, which play a crucial role in maintaining the body's balance, are particularly vulnerable to the harmful effects of drugs and other foreign substances, making them highly susceptible to damage (Kim and Moon, 2012). The kidney's susceptibility to harm from harmful substances is primarily attributed to factors such as its blood supply, location, and role within the body. Despite comprising only 0.5% of the total body weight, the kidney receives approximately 20-25% of the blood pumped by the heart, making it more vulnerable to a relatively higher concentration of toxic substances in the bloodstream (Aliu, 2015).

1.3. Types of Kidney Diseases

Renal diseases are commonly classified as either acute or chronic. Acute renal failure (ARF) often occurs in connection with bacterial or sepsis-induced infections, as well as ischemic reperfusion. These conditions can also progress to chronic kidney disease (Bonventre and Weinberg, 2003). Chronic kidney disease usually originate from high blood pressure, complications associated with diabetes, obesity and autoimmune diseases (Dalrymple and Go, 2008). The initial causes of kidney disorders can vary, but acute kidney injury (AKI) can deteriorate further into chronic kidney disease (CKD), and if not managed, both AKI and CKD can lead to end-stage renal disease (ESRD) (Locatelli *et al.*, 2003).

1.4. Acute Kidney Injury

Acute kidney injury or acute renal failure is a sudden loss of kidney function and their ability to eliminate extra fluids and waste materials from the blood (Kellum *et al.*, 2013). It is characterized by a sudden or rapid increment in the levels of serum creatinine (SCr) and blood urea nitrogen (BUN) in a few hours to number of days or within weeks (Kellum *et al.*, 2013). It takes place in various conditions, kidney's ability of removal of waste material from the body are abruptly compromised (Kellum *et al.*, 2013). AKI is not limited to a single occurrence; it results in dysfunction of other major body organs such as brain, lungs, intestine and heart. This dysfunction is brought about by a pro-inflammatory mechanism that involves the migration of neutrophil cells, expression of cytokines, and increased oxidative stress (Grams and Rabb, 2012). In ARF, clinical symptoms range from a small increase of creatinine in serum to entire kidney failure (Lassnigg *et al.*, 2004).

Latest studies in epidemiology have demonstrated that acute kidney failure is evident across a wide range of causes and risk factors, leading to significant and severe outcomes. (Dalrymple, 2008). Mortality rate is increased due to this disease in patients needing dialysis along with CKD development and it further leads to dialysis dependency (Metnitz *et al.*, 2002). Over the past few decades, considerable attention has been directed towards extensive clinical and fundamental research concerning acute kidney failure. This emphasis arises from numerous research discoveries indicating that even minor fluctuations in serum creatinine levels can result in increased mortality rates among hospitalized patients (Selby *et al.*, 2012). Despite that, there is a considerable limitation of lack of standard definition for acute renal failure, significant progress has been achieved in understanding the fundamental knowledge of biology and mechanism related to acute kidney failure in various animal models (Devarajan, 2006).

1.4.1. Cisplatin-induced nephrotoxicity

Nephrotoxicity refers to a specific characteristic affecting the kidneys, where the process of eliminating waste substances or medications is hindered due to the presence of toxic chemicals (Finn and Porter, 2003). Around 20% of cases of kidney damage are caused by medications, but the occurrence of nephrotoxicity rises to 66% in the elderly population as life expectancy grows. The effectiveness of chemotherapy

or anticancer drugs has been restricted due to their tendency to cause kidney damage (Kohli *et al.*, 2000). The identification of nephrotoxicity can be accomplished using a straightforward blood test. The assessment of nephrotoxicity through blood tests involves examining various factors such as blood urea nitrogen (BUN), serum creatinine levels, glomerular filtration rate, and creatinine clearance. It is important to note that these evaluations for nephrotoxicity are feasible only when a significant portion of kidney function has been impaired (Kirtane *et al.*, 2005).

1.4.2. Nephrotoxicity

Cisplatin is a common chemotherapy drug used to treat solid tumors in many different organs, including the head and neck, testicles, ovaries, lung, and bladder. However, clinical studies have shown that using cisplatin is associated with serious side effects, such as nephrotoxicity, ototoxicity, and neurotoxicity (Lippert, 1999).

Cisplatin is a highly effective anti-cancer drug commonly used for treatment of wide variety of solid tumors (Tayem *et al.*, 2006). Nevertheless, the primary negative consequence in cisplatin treatment is nephrotoxicity, which acts as a limiting factor for the dosage. Numerous findings strongly support the involvement of reactive oxygen species (ROS) and lipid peroxidation in the renal harm that occurs as a result of cisplatin administration (Arany and Safirstein, 2003). Exposure to chemotherapy drugs can cause nephrotoxicity, which may result in the early dysfunction of the renal tubules, damage to the glomeruli, and the development of acute or chronic kidney injury (Perazella and Moeckel, 2010).

In individuals who are in good health and of a young age, the rate at which the glomerulus filters blood, known as the glomerular filtration rate (GFR), is approximately 120 milliliters per minute. The kidneys are capable of maintaining a consistent filtration rate and ensuring the proper flow of urine by regulating the blood flow in both the afferent and efferent arteries, which helps adjust or sustain the pressure within the glomerulus. The circulation of prostaglandin is utilized to widen the afferent arteries, promoting increased blood flow (Naughton, 2008). Nonsteroidal anti-inflammatory drugs (NSAIDs) and medications with anti-angiotensin properties, such as angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs), have been demonstrated to cause kidney damage specifically in the glomerulus, leading to elevated blood pressure (Olyaei *et al.*, 1999).

Drug toxicity significantly affects renal tubules, particularly the cells of the proximal tubule, as they come into contact with drugs during the concentration and reabsorption process through the glomerulus (Perazella and Moeckel, 2010). Cytotoxicity arises from the presence of dysfunctional mitochondria in tubules, disruption of the tubular transport system, and the elevation of oxidative stress caused by the production of free radicals (Perazella and Moeckel, 2010).

1.4.3. Mechanism of cisplatin-induced nephrotoxicity

Cisplatin toxicity's underlying mechanisms can be classified into four types of harm. It can cause tubular toxicity, resulting in imbalances in water and electrolytes, along with acute renal failure due to tubular necrosis. Additionally, it can induce damage to small and medium-sized arteries, glomerular impairment, and interstitial damage due to prolonged cisplatin exposure, which might evolve into chronic kidney disease (Sánchez-González *et al.*, 2011).

The epithelial lining of the proximal tubule, which is responsible for facilitating substance transport into its cells, is compromised. This triggers the commencement of urine formation, resulting in higher levels of potential toxins in the tubular fluids. This, in turn, facilitates the passive diffusion of toxins into the tubular cells (Sánchez-González *et al.*, 2011).

Cisplatin, an uncharged compound with low molecular weight, easily passes through the glomerular filtration and is entirely excreted in urine. During this journey, cisplatin enters the tubular cells, accumulating in significant amounts within the proximal tubular cells of the renal cortex and medulla. In the collecting duct and distal tubule, injury occurs in a manner dependent on the dosage (Taguchi *et al.*, 2005).

Through passive diffusion or facilitated diffusion cisplatin enters into the renal tubular cells via basolateral organic cation transporter (OCT) (Ciarimboli *et al.*, 2010). Cisplatin when administered intravenously is rapidly diffused into the tissues and binds to plasma proteins. It forms reaction of amino acid thiol groups which is strong (Cepeda *et al.*, 2007). Platinum ions with a positive charge exhibit greater toxicity in kidney cells compared to the parenteral system. They interact with DNA, RNA, and proteins, resulting in their binding. This binding forms connections between DNA and cisplatin, which hinder the processes of replication and transcription.

Consequently, cell cycle progression is halted, and apoptosis, or programmed cell death, occurs (Cepeda *et al.*, 2007).

Cisplatin exhibits cytotoxicity by creating connections within and between the DNA chains of kidney cells. The extent of this platination process is often linked to the entry of cisplatin into the nuclei of cells, which occurs as a result of drug accumulation. The bond between platinum and DNA gives rise to adducts or novel compounds that trigger diverse cellular reactions, such as signaling of DNA damage, activation of cell cycle checkpoints, initiation of DNA repair mechanisms, and induction of cell death (Cepeda *et al.*, 2007).

Cisplatin-induced kidney toxicity is characterized by impaired function of renal proximal tubular cells. Cisplatin disrupts the transport of water and essential nutrients in these tubular cells. The transportation process relies on sodium pumps such as Na/K/ATPase, Na-K-2Cl cotransporter, and type III Na/H exchanger. Additionally, water-permeable channels, including aquaporins 1, 2, and 3, are involved in this transport. Cisplatin hampers the function of these transporters located on the brush border, both in in vivo and in vitro models. The damage inflicted by cisplatin can also impact the structural integrity and polarity of the cell, leading to changes in the levels of hydrogen, potassium, magnesium, and calcium ions (dos Santos *et al.*, 2012).

These alterations contribute to decreased rates of ion reabsorption in the proximal and distal tubules, leading to higher excretion of these ions in urine. The disturbance of the tubular epithelial cell barrier and/or the connections between healthy cells within the tubular injury caused by cisplatin can trigger a reversal of the glomerular filtrate, sending it back into the bloodstream. As a result, the apparent glomerular filtration rate (GFR) appears to decrease (Lajer *et al.*, 2005).

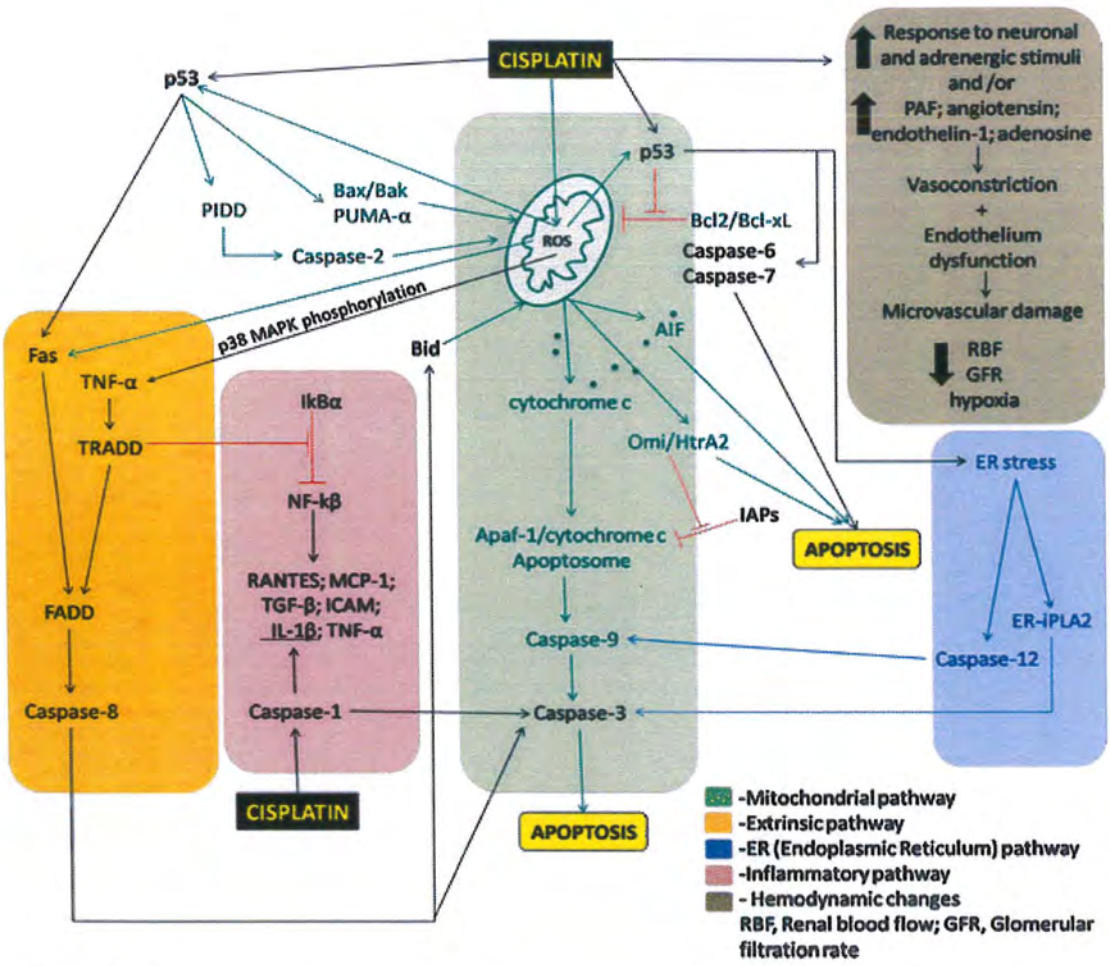


Figure 1.1. Mechanism of cisplatin-induced nephrotoxicity (dos Santos *et al.*, 2012).

1.5. Aim and Objectives

1.5.1. Aim

The aim of the study was to examine the protective effects of natural compound (Magnolol) in Cisplatin-induced nephrotoxicity model on kidney.

1.5.2. Objectives

The main objectives of the study included:

- To check the pharmacological impact of Magnolol on spontaneous pain during cisplatin-induced nephrotoxicity.
- To check the pharmacological impact of Magnolol on renal functional tests during CP-induced Nephrotoxicity.
- To check the pharmacological impact of Magnolol on oxidative stress markers during CP-induced Nephrotoxicity.
- To check the pharmacological impact of Magnolol on histopathological changes occurring during Cisplatin-induced Nephrotoxicity.

CHAPTER 2

MATERIALS AND METHODS

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant Materials

The bark of plant *magnolia officinalis* was collected and identified in South Korea whereas, the purification and isolation of Magnolol was done elsewhere (Kim and Kang, 1998).

2.1.2. Chemicals and reagents

The chemicals and medications employed in the present investigation were of recognized analytical grade and were procured from reputable commercial vendors. These substances encompass magnolol, dexamethasone, and cisplatin. All the mentioned chemicals and drugs were recently prepared and put to use.

2.1.3. Equipment

Measuring flasks, Petri dishes, aluminum foil, beakers, homogenizer (D-91126, Heidolph, Germany), Cages, water bottles, pipette, micropipette, micropipette tips, Eppendorff (1ml, 2ml), magnetic stirrer, clean microscopic slide, gloves, face mask, tissue papers, slide rack, falcon tubes (15ml, 50ml), weighing balance (RADWAG Wagi Elektroniczne, AS 220.R2) syringes, spatula, glass stirrer, EDTA tubes, xMark microplate spectrophotometer (BIO-RAD, USA), glass slide, coverslip, vortex mixer (scientific industries.com Model SI-T 256), water distillation apparatus (Irmeco, GmbH IM50, Germany), 96 well plates, dissection apparatus, shaker and water bath (SW22, Julabo, Allentown, PA, USA), spatula, glass stirrer.

2.2. Methods

2.2.1. Animals

Albino mice, aged approximately 3-4 weeks and weighing between 30-33 grams, were acquired from NIH, Islamabad. Grant was obtained from Quaid-i-Azam University under Approval No. #BEC-FBS-QAU2022-410 for the use of animals in experiments evaluating drug activities. The mice were housed in plastic cages with bedding of wood, which was altered every other day. They were kept in a laboratory environment free from pathogens, with regulated room temperature and humidity. The mice were provided with a high-quality laboratory diet and water. All experiments conducted on the animals followed the guidelines and principles set forth.by NIH for the ethical treatment of experimental animals. The behavioral and biochemical procedures adhered to the precautions outlined by both NIH and the bioethical committee of Quaid-i-Azam University. Animals were humanely euthanized using a standardized procedure to minimize any prolonged suffering. All animal-related

activities took place during daytime hours, specifically from 8:00 am to 5:00 pm. Each experimental animal was used only once, and every effort was made to minimize the number of mice used in the experiments.

2.2.2. Dosing and grouping of animals

Cisplatin dose of 10mg/kg was prepared by dissolving measured quantity of cisplatin in autoclaved double distilled water. Intended amount of magnolol was dissolved in 2% DMSO solution and different doses of magnolol (0.1 mg/kg, 1 mg/kg and 10 mg/kg) were prepared by dilution with normal saline of 0.9%. Animals were categorized into six groups each consisted eight numbers of mice. Group I was vehicle group and given the vehicle (PBS) intraperitonially daily; group II was negative control in which cisplatin 10 mg/kg was administered intraperitoneally; group III was positive control which was given dexamethasone 10 mg/kg intraperitonially and three groups of treatment received magnolol in doses of (0.1 mg/kg, 1 mg/kg and 10 mg/kg). All the drugs used were dissolved in normal saline with 2% DMSO solution. Post treatment was given by administering drug through intraperitoneal route after 1 hour of cisplatin.

2.3. Assessment of Variation in Body Weight

For assessing loss or gain in weight of mice over time with treatments and other groups, the initial (at 0 hour), after 25 hour and final body weights of mice (at 72 hour) were obtained by means of digital weighing balance (Ohaus corporation, USA).

2.4. Assessment of Renal Index

After dissection, all the kidneys were collected from mice of different groups and weighed accordingly, by using digital weighing balance (Ohaus corporation, USA). Body weights of mice were determined one hour before sacrifice and renal tissues were collected from different groups after dissection and weighed accordingly after washing with normal saline. Renal index (RW/BW) ration was determined by arithmetic simple calculation in which the weight of kidney was divided by weight of mice (Park *et al.*, 2009) .

2.5. Assessment of Spontaneous Pain

Spontaneous pain was observed in all groups of mice after administration of cisplatin. Licking, writhing and rearing were measured in all experimental groups which were considered as essential behaviors of pain in rodents (Yin *et al.*, 2016). In any closed chamber, mice were observed for above mentioned behaviors of pain through video recording by a video camera for 30 minutes (Yin *et al.*, 2016)

2.6. Serum Electrolyte Determination

Serum electrolytes play an important role in conduction of electrical impulses throughout the body and helps various organ in performing fundamental activities. To determine effect of magnolol on serum electrolytes level in nephrotoxicity, the test responsible for detection and quantification of sodium, potassium and bicarbonate (mEq/L) were performed by ELISA kits, because these electrolytes are of core importance in kidney functions. After 24 hours of experiment, all the animals were euthanized and collection of blood and plasma for method developed by (Terker *et al.*, 2015). Blood collection was done through heart puncturing of mice. For serum separation blood were centrifuged at 4°C for 10 minutes at centrifugation speed of 5000 x g. Na+, K+ and HCO3 concentration in serum were determined enzymatically through use of standardized kits such as automated chemical analyzer of audit Tecno 786, Ireland.

2.7. Hematological Data

For observation of homological variation in complete blood profile the pharmacology effect of magnolol on various blood cells count was checked (OA *et al.*, 2002). Roughly 1 mL of blood was obtained from every mouse using a heparin EDTA tube. The collected blood was then utilized for determination of hemoglobin level in blood with the help of commercially available automated hematology analyzer of Abbott diagnostic (OA *et al.*, 2002).

2.8. Serum Biochemistry Analysis

Serum biochemistry was carried out to assess the concentrations of different serum biomarkers, including serum creatinine, BUN, urea, and uric acid. Once blood samples were collected from various animal groups, they underwent centrifugation at 2000 x g for 10 minutes to separate the serum. The isolated serum was deproteinized by adding 3% trichloroacetic acid at a 1:3 ratio. After a 10-minute interval, the treated serum was centrifuged again at 2000 x g for another 10 minutes. A segment of the resultant protein-free supernatant was employed to measure serum creatinine. Meanwhile, the levels of BUN, urea, and uric acid were directly assessed in the serum samples using the method established by Fawcett and Scott in 1960 (Fawcett and Scott, 1960).

2.9. Oxidative Stress Markers

The level of MPO, EPO, LPO and NO which are oxidative stress markers were used to measure the oxidative stress marker. To process the above assays, tissue homogenates of different organ were prepared, and supernatant were collected for analysis.

2.9.1. Myeloperoxidase (MPO) assay

The myeloperoxidase (MPO) enzyme is commonly utilized to evaluate the presence of neutrophilic infiltration at the site of inflammation. In accordance with previous reports, the MPO assay was conducted using Hexadecyltrimethylammonium bromide (HTAB) and o-dianisidine (Fernández *et al.*, 2002). The tissue samples were treated and homogenized in HTAB within a 50 mM phosphate buffer to disrupt the cells and release the MPO. After subjecting the HTAB-treated tissue to three cycles of freezing and thawing, it underwent centrifugation. The resulting supernatant from the HTAB-treated tissue was mixed with o-dianisidine and H2O2. The absorbance at wavelength 460 nm was measured, and the assay was performed in triplicate (Brown *et al.*, 2001).

2.9.2. Eosinophil peroxidase (EPO) assay

The EPO assay, previously described (Forbes *et al.*, 2004), was utilized to measure the level of eosinophil peroxidase in the kidney tissue. Briefly, the tissue from the incised paw was collected and homogenized. The resulting homogenized tissue underwent centrifugation, and the supernatant was obtained in Eppendorf tubes. To assess the impact of magnolol on the eosinophil peroxidase level, PO substrate solution (75 µL) was included to an equal volume of the supernatant and incubated at room temperature for 20 minutes. The reaction was halted using sulfuric acid, and the absorbance at 490 mn was measured.

2.9.3. Nitric oxide (NO) assay

At the conclusion of the experiment, animals were euthanized, and their blood was collected in EDTA tubes. The collected blood was then centrifuged at a speed of 500×g for 10 minutes. The resulting serum was subjected to NO determination using the Griess reaction method. In summary, 50 µl of blood plasma and 50 µl of normal saline were mixed with an equal volume of Griess reagent (consisting of 1% sulfanilamide, 0.1% naphtylethylenediamine dihydrochloride, and 5% phosphoric acid in distilled water). The mixture was then analyzed for absorbance using a microplate reader at 540 nm, and the absorbance coefficient was calibrated using a standard sodium nitrite solution (Ali *et al.*, 2019).

2.9.4. Lipid peroxidation (LPO) assay

Rate of lipid peroxidation was measured by quantification of malonaldehyde concentration (MDA) in renal tissue homogenate because MDA is considered as a final biproduct of unsaturated fatty acids peroxidation. Method previously described by (Farombi *et al.*, 2007) was used in quantification of MDA level in kidney. In this method, 0.25 ml of tissue homogenate was to prepared in 10% of phosphate solution of 7.4 pH. It was then incubated for one hour at temperature in water bath.

After incubation for one hour, 0.25 ml of 5% TCA (trichloroacetic acid) and 0.5 ml of 0.67% TBA (thiobarbituric acid) included into homogenate mixture and finally centrifugation for 10 mins at 3000 g x g was performed. Clear supernatant separated from above mentioned solution was again put on water bath for next 10 mins. After proper cooling, the color intensity of this mixture was determined at wavelength of 535 nm. Finally for estimation of MDA concentration given formula $\sum 1.56$ x 105 M/cm determined by (Farombi *et al.*, 2007) was used.

2.10. Histopathological Analysis

For histopathological examination of kidney in cisplatin-induced nephrotoxicity model, one kidney from each mouse was cut longitudinally and were fixed in formalin solution of 10% strength (Forsthoefel *et al,* 2018). The dehydration of tissues was occurred by treating with ascending order of alcohol concentration from 50% to 90%. For tissue fixation, paraffin wax was used in which the renal tissue was imbedded (Yen *et al.*, 2011). Rotatory microtome (Germany, micro) was used for cutting. tissues into small pieces. For visualization of renal tissues under light microscope (Germany) H & E staining was done (Forsthoefel *et al.*, 2020).

2.11. Immunohistochemistry

Immunohistochemistry analyses were conducted to look at the expression of several inflammatory markers. Small sections of the colon sample were cut into pieces, mounted to the adhesive slide at room temperature, and baked for an hour at 65°C. Deparaffinizing the paraffin-coated slides was the first step after preparation. On the first day, slides were first immersed in xylene for five minutes, followed by 5 minutes each in 100%, 90%, 80%, and 70% alcohol, distilled water, and PBS. Slides were dipped in PBS for an additional 5 minutes after a drop of Proteinase K was added. 35% H_2O_2 solution was inserted for 10 minutes, then dipped in PBS for 5 minutes. After adding 10 µl of NGS (normal goat serum), the slides were placed in the refrigerator for 90 minutes (Park *et al.*, 2015).

In the next step, a primary antibody was added, and slides were placed in the refrigerator for an overnight period. On the next day, washed the slide by PBS for 10 minutes. Then 10 µl of secondary antibody was used for two hours. Then the slides were refrigerated for 75 minutes. After that washed for 5 minutes with PBS and ABC (avidin-biotin complex) was applied for 75 minutes. Again, washed the slide with PBS, 100% alcohol, and with xylene. Slides were stained with DAP dye then air dried. A drop of mounted media was used, and cover slip was kept on it. Slide were observed on 10X and 40X under the microscope (Park *et al.*, 2015).

2.12. Fourier Transform Infrared (FTIR) Analysis

Prior to FT-IR analysis, kidney tissues were subjected to a 12-hour lyophilization process (Alpha 1–2 LD plus, Martin Christ freeze dryer) to eliminate moisture content. Subsequently, the dried samples were examined using an FT-IR spectrometer (IR tracer, Shimadzu, Japan) to evaluate protein and lipid content, as well as their secondary derivatives. The absorbance spectra of kidney tissue samples were captured within the 4000–500 cm^{-1} range, with a spectral resolution of 4 cm^{-1} at a temperature of around 25 ± 1°C. To ensure accuracy, sections from the same anatomical region of each mouse were repeated three times. Absorption bands corresponding to proteins and lipids were evident in all samples. To delve deeper into the secondary structure of proteins and lipids, secondary derivatives of the FT-IR spectra were determined by refining the resolution of the obtained spectra. Uniform conditions were applied to process all samples, and the average FT-IR spectra were generated for distinct kidney tissue regions. The software Origin 8.5 was employed for the analysis of the spectra Azu *et al.*, 2010).

2.13. Evans Blue Assay

To assess the harm caused to the small blood vessels in the kidneys due to decreased blood supply, the scientists investigated the leakage of Evans blue dye combined with albumin from the kidney tissue. Before euthanizing the mice, a solution containing Evans blue dye at a concentration of 1% in normal saline was injected into the right jugular vein while the animals were lightly anesthetized with ether. Immediately after putting the mice down with an overdose of pentobarbital, the researchers cleared the circulatory system by flushing the heart with 10 ml of saline containing heparin. Subsequently, the kidneys were taken out, dried for 3 days at 60°C, and weighed.

These dried kidneys were then blended with a mixture of formamide and water at a ratio of 1:20 (weight/volume). After being incubated overnight at 60°C, the resulting mixtures were subjected to centrifugation at 14,000 revolutions per minute and 4°C for 30 minutes to remove any suspended particles. The quantity of Evans blue dye extracted in the liquid above the sediment was calculated by measuring the absorbance at a specific wavelength and adjusted for the extraction volume. To convert the absorbance readings into micrograms of Evans blue per gram of dried kidney tissue, a standard curve was prepared using Evans blue dye in a formamide solution without kidney tissue. (Carattino *et al.*, 1999).

2.14. Statistical Analysis

The mean of each group was compared with the mean of negative control group with the help of one-way ANOVA followed by Dunnett's test. The results shown were expressed as mean ± standard deviation. The significant value for each group was ($p < 0.05$).

CHAPTER 3

RESULTS

3. RESULTS

3.1. Body Weight Assessment in Cisplatin-Induced Nephrotoxicity

In cisplatin-induced nephrotoxicity model, there was seen a profound decrease in mean weights of animals in negative control and treatment group of magnolol 0.1 mg/kg when compared with the mean weight of animals in normal group. The decrease in mean weight of dexamethasone and treatment groups (magnolol 1 mg/kg and 10 mg/kg) was much less when compared with normal and was significantly dissimilar when compared with negative control group as shown in Figure 3.1(A).

3.2. Spontaneous Pain Assessment

Spontaneous pain scoring was increased in cisplatin group and was found significantly dissimilar when compared with Dexamethasone and treatment groups magnolol 0.1 mg/kg, magnolol 1 mg/kg and magnolol 10 mg/kg and was found extremely significant in comparison with normal control group as shown in Figure 3.1(B).

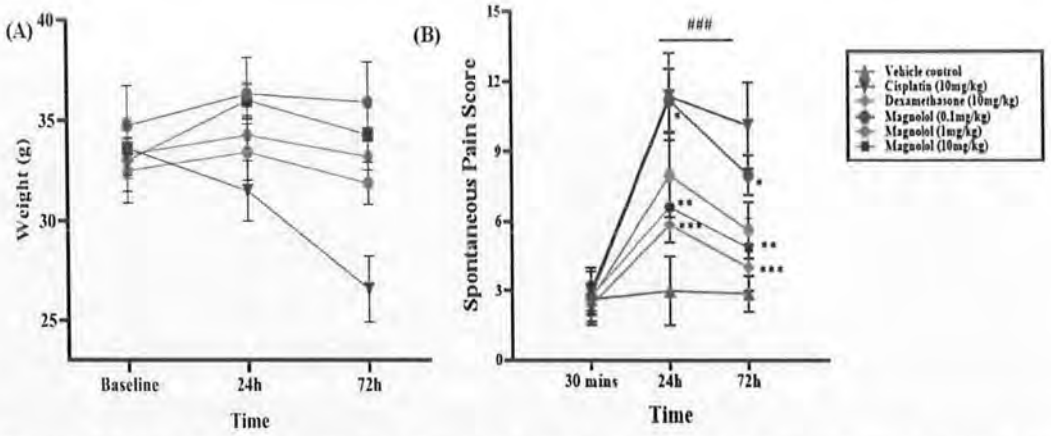


Figure 3.1. (A) Effect of magnolol on body weight following cisplatin-induced Nephrotoxicity model. (B) Effect of magnolol on the cisplatin induced spontaneous pain. ***Note:*** *All the values were explained as mean ($n = 8$) ± SD. ###$p < 0.001$ compared to control group; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared to cisplatin-treated group.*

3.3. Renal Index Assessment in Cisplatin-Induced Nephrotoxicity

A significant increase in renal index of negative control group has been observed when compared with normal control group. There is a decrease in the renal index in positive control group when compared to the negative control group. Similarly, treatment with magnolol in different doses has been significantly attenuated renal indices against negative control group as shown in Table 3.1.

Table 3.1. Effect of magnolol on renal index in cisplatin-induced nephrotoxicity model. Weights of all groups are taken in grams.

Sample	Body weight (g)	Kidney weight (g)	Renal index (g/g)
Vehicle control	35.912 ± 1.99	0.205 ± 0.02	0.571 ± 0.048
Cisplatin (10mg/kg)	26.375 ± 1.657	0.325 ± 0.02	1.227 ± 0.106 ###
Dexamethasone (10mg/kg)	34.293 ± 1.744	0.236 ± 0.01	0.690 ± 0.053 ***
Magnolol (0.1 mg/kg)	31.887 ± 1.045	0.312 ± 0.07	0.978 ± 0.215 *
Magnolol (1 mg/kg)	33.212 ± 1.458	0.265 ± 0.01	0.874 ± 0.225 **
Magnolol (10 mg/kg)	34.293 ± 0.744	0.205 ± 0.03	0.598 ± 0.088 ***

*Note: All the values were expressed as mean ± SD, n = 8, *P < 0.05, **P < 0.01, ***P < 0.001 as compared to disease control group.*

3.4. Effect of Magnolol on Serum Creatinine, BUN and Uric acid

Serum creatinine and BUN are the two main essential biomarkers usually assessed for checking kidney functions. In this study there was noticed a prominent increase in both creatinine and BUN level in serum of mice of negative control group in comparison with serum creatinine and BUN level determined in normal control group. Administration of dexamethasone in positive control and magnolol treatment group showed a dose dependent decrease in both creatinine and BUN level in serum nearly bringing their levels to normal ranges. Administration of cisplatin into mice produced a noticeable increase in level of urea in serum of negative control group 70 ± 2 mg/dL) as compared to level of urea in serum of normal control mice (24.4 ± 2.7 mg/dL) within 24 hours. The level of serum urea was significantly decreased to normal value following Dexamethasone injection (28 ± 1.9 mg/dL, $P < 0.001$). similarly, treatment of mice with cisplatin in doses of 0.1 mg/kg (62.4 ± 3.2 mg/dL, $P < 0.05$), 1 mg/kg (54.8 ± 5.7 mg/dL, $P < 0.001$) and 10 mg/kg (35 ± 3.3 mg/dL, $P < 0.001$) showed a significant declination in level of urea in serum of treatment control group in a dose dependent manner as shown in Figure 3.2.

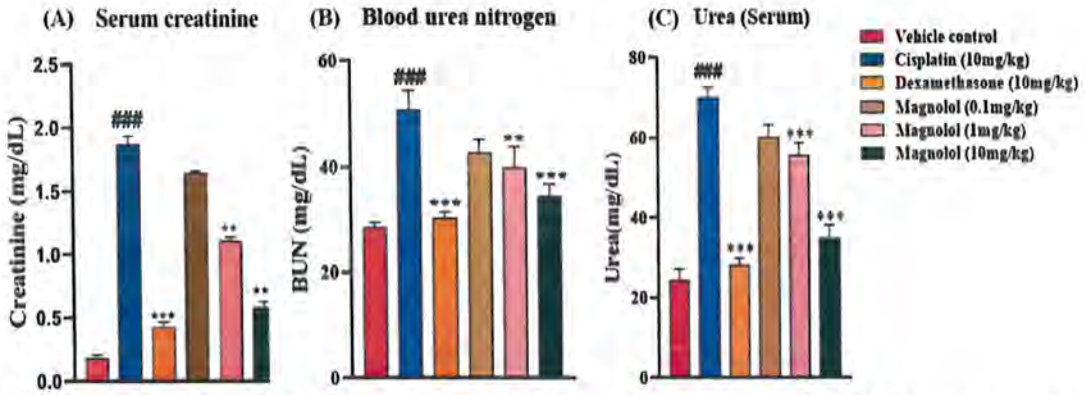


Figure 3.2. Effect of magnolol on Cisplatin induced changes in serum creatinine, blood urea nitrogen and serum urea.

*Note: All the values were expressed as mean ± SD, n = 8, *P < 0.05, **P < 0.01, ***P < 0.001 as compared to disease control group.*

3.5. Hematological Variations in Cisplatin-Induced Nephrotoxicity

A portion of collected blood from cisplatin, dexamethasone and magnolol treated mice were subjected for hematological assay. There was a prominent increase in the white blood cells, neutrophils and lymphocytes in mice treated with cisplatin when compared with mice in normal group and have been significantly decreased to normal range in dexamethasone and magnolol treated group in dose dependent manner. There was also seen a noticeable variation in other blood cells count such as platelets (PLT), red blood cells (RBC) and hemoglobin (HB) in magnolol treatment group as compared to the normal and disease group whose results has been shown in the following Table 3.2.

Table 3.2. Effect of magnolol on blood profile in cisplatin-induced nephrotoxicity model.

Sample	White blood cells (10^9/l)	Lymphocytes (10^9/l)	Neutrophils (10^9/l)	Red blood cells (10^{12}/l)	Platelets (10^9/l)	Hemoglobin (g/dl)
Vehicle control	4.40 ± 0.29	3.88 ± 1.10	2.11 ± 0.39	4.91 ± 0.43	191.3 ± 7	14.3 ± 0.50
Cisplatin (10 mg/kg)	2.47 ±0.5 ###	1.57 ± 0.26 ###	4.27 ± 0.64 ###	2.83± 0.23 ###	141.1±9 ###	12.3 ± 0.77 ###
Dexamethasone (10 mg/kg)	4.58 ± 0.35 ***	4.47 ± 0.74 ***	2.82 ± 0.26 ***	4.78 ± 0.33 ***	187.5 ± 9 ***	14.4 ± 0.5***
Magnolol (0.1 mg/kg)	2.18 ± 0.45	2.18 ± 0.45	3.95 ± 1.03 *	4.07 ± 0.59	150.6 ± 4	13.8 ± 1.03
Magnolol (1 mg/kg)	2.20 ± 0.55 *	2.28 ± 0.47**	3.49 ± 0.48 **	4.55 ± 0.72 **	164.3 ± 17 **	14.1 ± 0.46**
Magnolol (10 mg/kg)	4.87 ± 0.70 ***	4.73 ± 0.57***	2.64 ± 0.42 ***	4.73 ± 0.27 ***	170.2 ±10 ***	14.2 ± 0.31***

*Note: All the values were expressed as mean ± SD, n = 8, *P < 0.05, **P < 0.01, ***P < 0.001 as compared to disease control group.*

3.6. Effect of Magnolol on Serum Electrolytes in Cisplatin-induced Nephrotoxicity

The administration of cisplatin had a notable impact on the levels of sodium, potassium, and bicarbonate in the bloodstream. Sodium ions were significantly reduced, while potassium and carbonate ions showed increased retention in the groups treated with cisplatin. However, the administration of manolol effectively restored the balance of serum electrolytes, as observed in comparison to the negative control as indicated in Table 3.3.

Table 3.3 Effect of magnolol on serum electrolytes in cisplatin-induced nephrotoxicity model.

Sample	Sodium	Potassium	Bicarbonate
Vehicle control	141.6 ± 1.2	7.21 ± 0.7	25.7 ± 1.3
Cisplatin (10mg/kg)	125.7 ± 2.1 ###	9.82 ± 0.6 ###	41.2 ± 2.9 ###
Dexamethasone (10mg/kg)	140.0 ± 1.9 **	8.00 ± 0.5 **	25.7 ± 2.1 **
Magnolol (0.1mg/kg)	132.9 ± 3.1 *	9.03 ± 0.7	32.2 ± 1.9
Magnolol (1mg/kg)	132.1 ± 2.6 **	8.51 ± 0.6 **	31.1 ± 2.9 *
Magnolol (10mg/kg)	138.8 ± 2.9 **	8.13 ± 0.4 **	37 ± 2.4 ***

*Note: All the values were expressed as mean ± SD, n = 8, *P < 0.05, **P < 0.01, ***P < 0.001 as compared to disease control group.*

3.7. Effect of Magnolol on NO Production in Cisplatin-Induced Nephrotoxicity Model

Excessive production of NO is considered as main characteristic of sepsis and it plays an important role in oxidative stress associated kidney injury. There was a massive increment in NO production in renal tissue of negative control as compared with renal tissue of normal mice while its concentration in renal tissues homogenate of Dexamethasone and treatment groups were decreased in significant way in cisplatin-induced nephrotoxicity. Treatment with magnolol effectively reduced the cisplatin induced production of nitric oxide, hence it confirmed the biochemical anti-inflammatory impact of magnolol in kidney damage as shown in Figure 3.3.

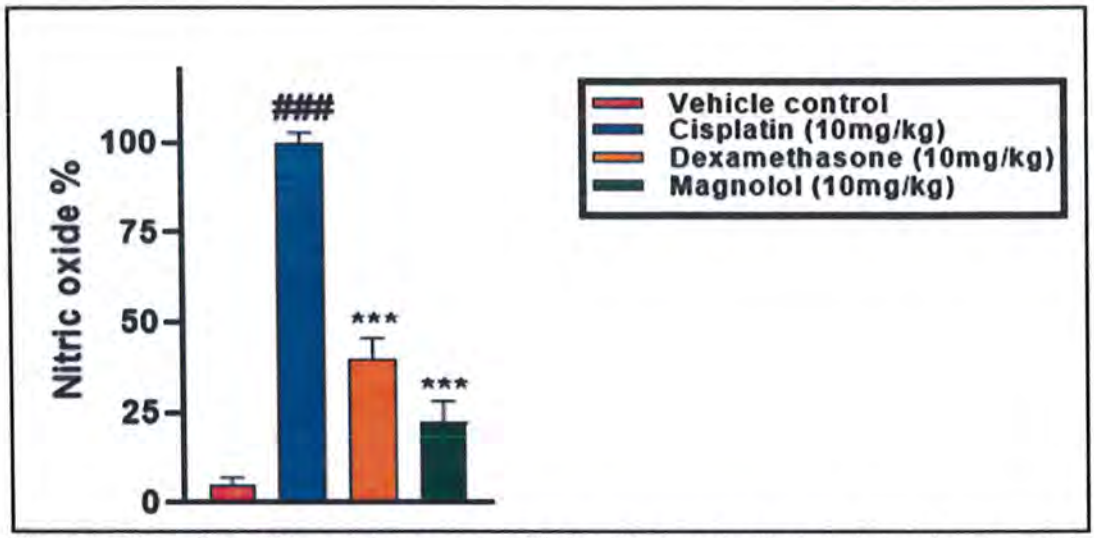


Figure 3.3 The effect of Magnolol treatment on Nitric oxide assay in Cisplatin-induced nephrotoxicity model.
*Note: All values were expressed as mean ± SD, n = 8, *P < 0.05, **P < 0.05, ***P <0.001.*

3.8. Effect of Oxidative Stress Markers on Magnolol Treatment in Cisplatin-Induced Nephrotoxicity

The cisplatin administered groups observed showed remarkedly increase in the LPO, EPO and MPO activity. This enhanced activity results due to increased inflammation and infiltration of neutrophils. Whereas, in magnolol treated group and positive group significant decrease in activities was observed as shown in Figure 3.4.

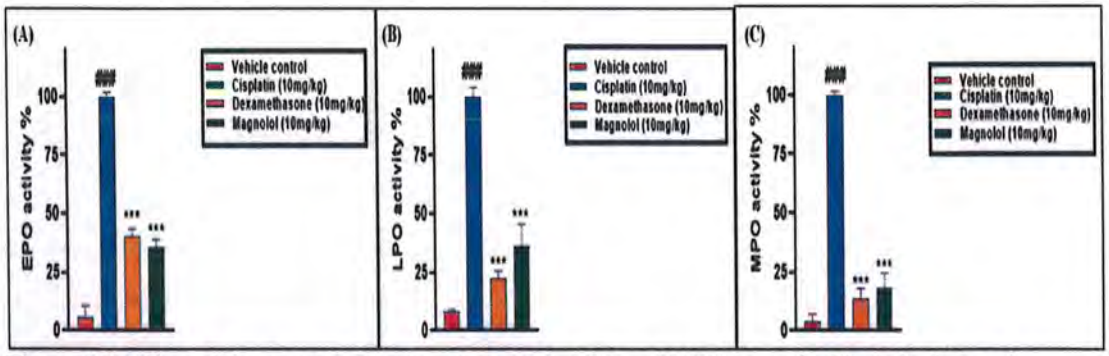


Figure 3.4 Effect of the magnolol treatment on LPO, EPO and MPO assay in Cisplatin-induced nephrotoxicity model.
*Note: All values were expressed as mean ± SD, n = 8, *P < 0.05, **P < 0.05, ***P <0.001.*

3.9. Immunohistochemistry Analysis

Immunohistochemistry results indicate increment in the anti-inflammatory markers i.e. iNOS, TLR-4, COX2 and NFκB in Cisplatin administered groups and decreased in magnolol treatment group. Whereas, inflammatory marker IκB decreased in Cisplatin administered group and significantly increased in magnolol treatment group.

The histological images for expressions iNOS, TLR4, NFκB, IκB and COX2 are shown in Figure 3.5, 3.6, 3.7, 3.8 and 3.9 respectively alongwith its scoring.

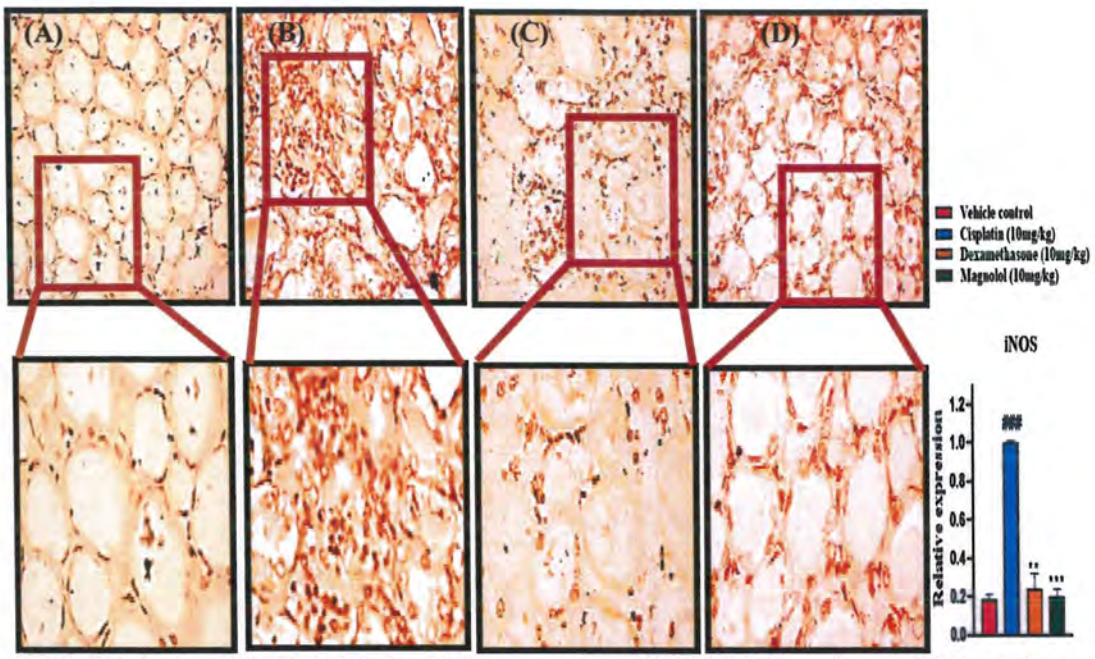


Figure 3.5. Effect of magnolol on the relative expression of iNOS in kidney tissues (A) Normal control (B) Cisplatin (10mg/kg) (C) Dexamethasone (10mg/kg) (D) Magnolol (10mg/kg). *Note: All values are expressed as mean ± SD. (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$ shows significant difference compared with cisplatin group. (###) denotes comparison of Normal control and cisplatin group with Magnolol group.*

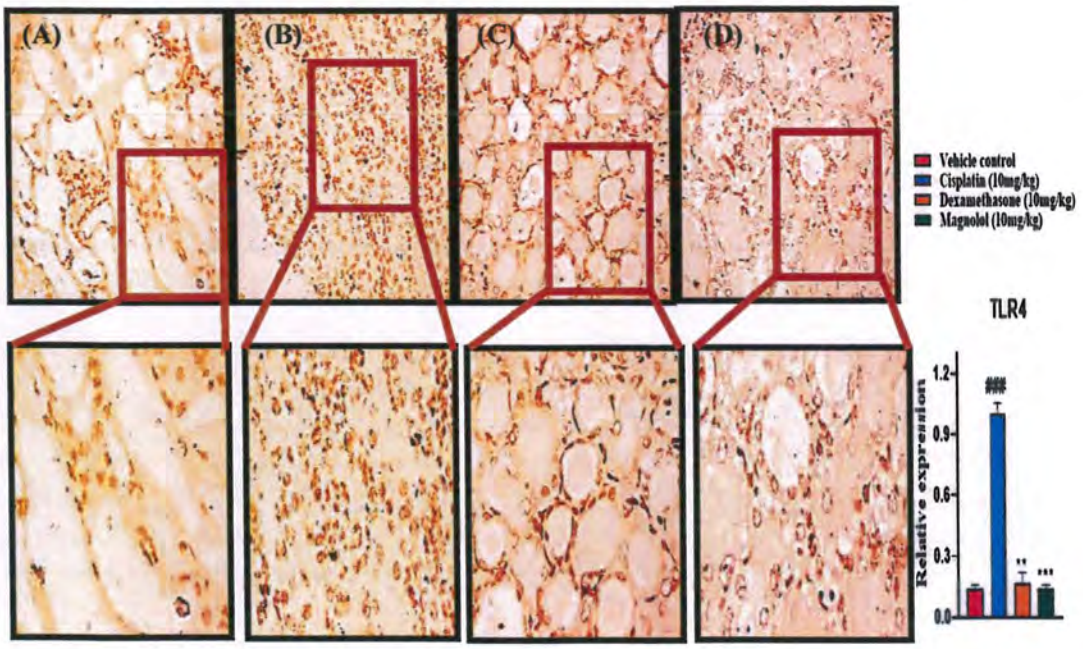


Figure 3.6. Effect of magnolol on the relative expression of TLR4 in kidney tissues (A) Normal control (B) Cisplatin (10mg/kg) (C) Dexamethasone (10mg/kg) (D) Magnolol (10mg/kg). *Note: All values are expressed as mean ± SD. (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$ shows significant difference compared with cisplatin group. (###) denotes comparison of Normal control and cisplatin group with Magnolol group.*

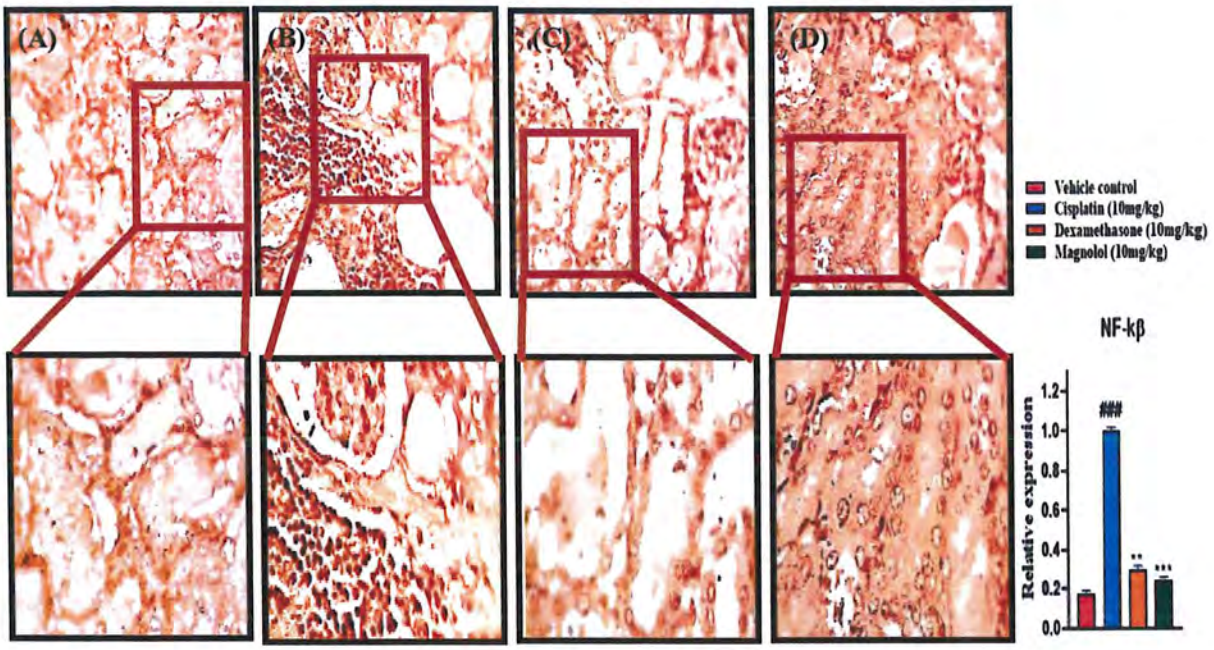


Figure 3.7. Effect of magnolol on the relative expression of NF-kB in kidney tissues (A) Normal control (B) Cisplatin (10mg/kg) (C) Dexamethasone (10mg/kg) (D) Magnolol (10mg/kg). *Note: All values are expressed as mean ± SD. (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$ shows significant difference compared with cisplatin group. (###) denotes comparison of Normal control and cisplatin group with Magnolol group.*

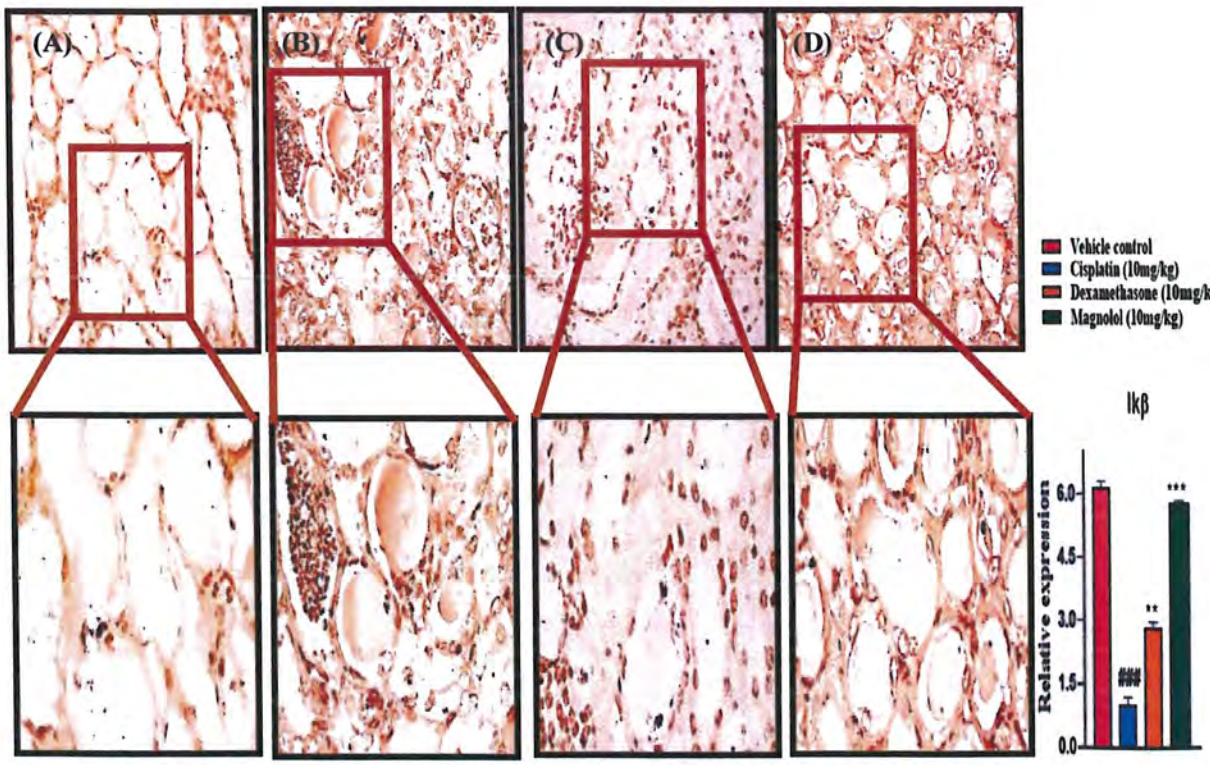


Figure 3.8. Effect of magnolol on the relative expression of IkB in kidney tissues (A) Normal control (B) Cisplatin (10mg/kg) (C) Dexamethasone (10mg/kg) (D) Magnolol (10mg/kg). *Note: All values are expressed as mean ± SD. (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$ shows significant difference compared with cisplatin group. (###) denotes comparison of Normal control and cisplatin group with Magnolol group.*

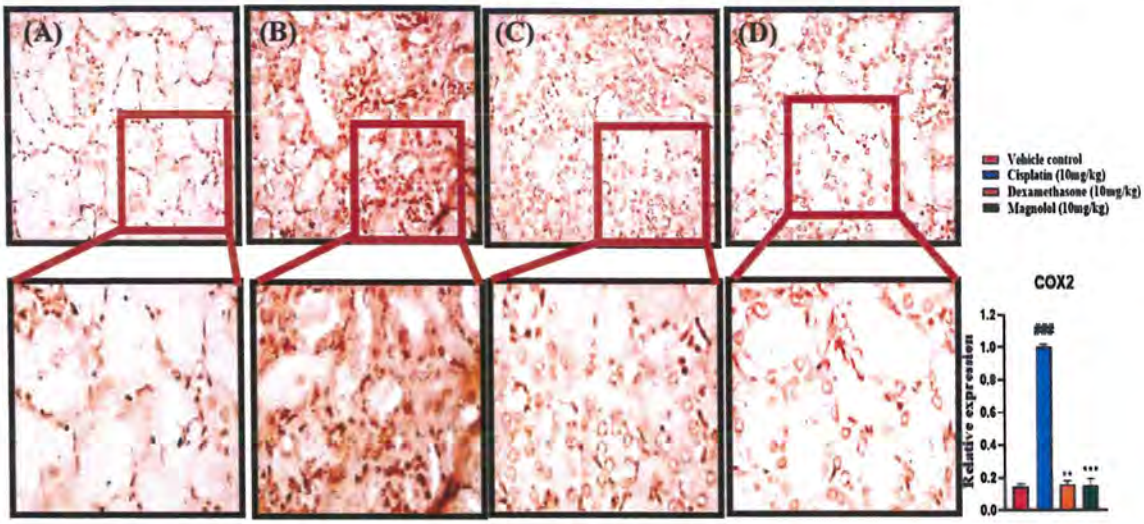


Figure 3.9. Effect of magnolol on the relative expression of COX2 in kidney tissues (A) Normal control (B) Cisplatin (10mg/kg) (C) Dexamethasone (10mg/kg) (D) Magnolol (10mg/kg). *Note: All values are expressed as mean ± SD. (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$ shows significant difference compared with cisplatin group. (###) denotes comparison of Normal control and cisplatin group with Magnolol group.*

3.10. Trichrome Staining

In the control group of mice, collagen fibers were observed to be organized in a typical manner within the capsular wall, peritubular area, and around the blood vessels in the kidney cortex. In the other three groups, there were no noticeable structural differences in the quantity and organization of collagen fibers when compared to the mice in the control group.

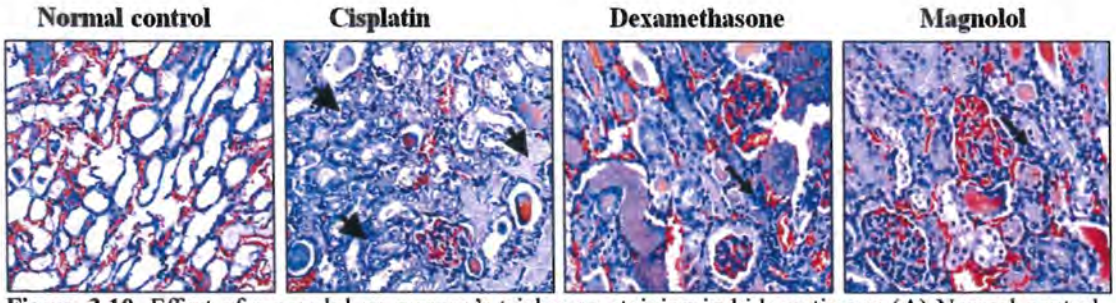


Figure 3.10. Effect of magnolol on masson's trichrome staining in kidney tissues (A) Normal control (B) Cisplatin (10mg/kg) (C) Dexamethasone (10mg/kg) (D) Magnolol (10mg/kg). *Note: All values are expressed as mean ± SD. (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$ shows significant difference compared with cisplatin group. (###) denotes comparison of Normal control and cisplatin group with Magnolol group.*

3.11. H and E Staining Analysis

Examination of kidney tissues from regular mice through light microscopy unveiled the typical structure of tubules, without any signs of inflammation, cellular decay, or the formation of casts. In the set of subjects administered with cisplatin injections, noticeable deteriorative alterations were evident primarily in the proximal tubules,

and to a lesser degree in the distal tubules and renal corpuscles. Within the deep cortical area, the proximal tubules displayed degeneration, necrosis, and apoptotic nuclear modifications in the epithelial cell lining. There was also evidence of luminal dilation, the accumulation of a solid material (cast), and loss of hair-like projections (apical microvilli). The distal convoluted tubules displayed dilatation of their luminal space with disproportionate cast accumulation.

The renal corpuscles demonstrated an expanded space surrounding the capsule with a condensed cluster of blood vessels (glomerular capillary tuft). Additionally, inflammatory cells infiltrated the peritubular region, there was vascular congestion, and localized bleeding was observed within the renal cortex. In the subset that received a low Magnolol dosage (0.1 mg/kg), notable harm to the tubular structures and the infiltration of inflammatory cells were evident.

Conversely, at the elevated Magnolol dosage (1 mg/kg), the tubules displayed distinct histological normalcy, with the absence of inflammation and cast formation observed in kidney tissue shown in Figure 3.11.

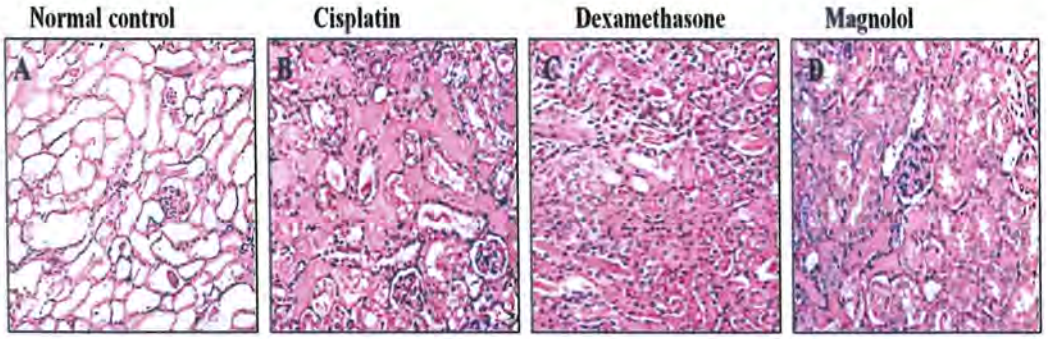
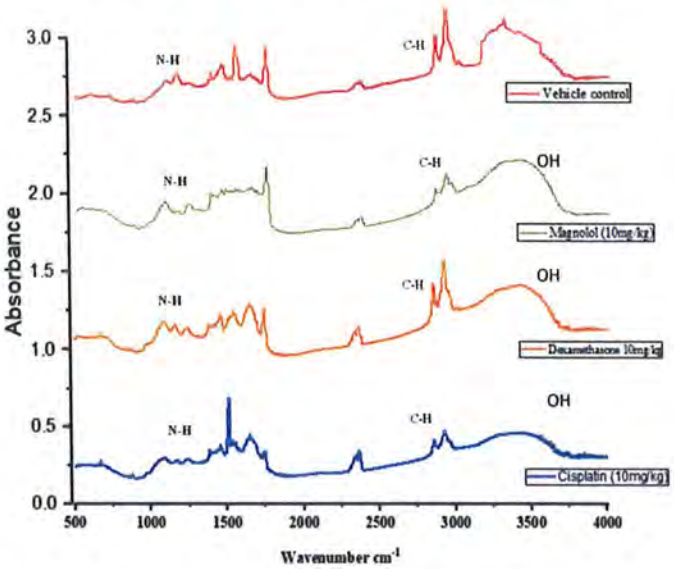


Figure 3.11. Effect of magnolol on histopathological changes in kidney tissues (A) Normal control (B) Cisplatin (10mg/kg) (C) Dexamethasone (10mg/kg) (D) Magnolol (10mg/kg). *Note: All values are expressed as mean ± SD. (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$ shows significant difference compared with cisplatin group. (###) denotes comparison of Normal control and cisplatin group with Magnolol group.*

3.12. Fourier Transform Infrared (FTIR) Analysis

FTIR examination offers distinct proof of Magnolol undergoing enolization. In contrast, CP leads to the creation of diammonium platinum oxide. Upon light exposure, the π-bond connecting platinum (Pt) and oxygen within diammoniumplatinum oxide can be heterolytically fractured, resulting in the formation of an electron-deficient entity. This exceedingly precarious species possesses the potential to engage with the unshared electron pairs situated on nitrogen

atoms within the alloxazine ring. Results show that treatment group magnolol showed intact bonding in structure of proteins, lipids and amine groups whereas damaged in case of cisplatin treated group as shown in Figure 3.12.



Frequency range	Definition of the Spectral Assignments
3020-2819 cm-1	Lipids
1720-1480 cm-1	Proteins
1750-1725 cm-1	Lipids
1200-900 cm-1	Carbohydrates and nucleic acids (DNA/RNA)
1301-1229 cm-1	Proteins

Figure 3.12. Effect of Magnolol treatment on FTIR analysis. The cisplatin group reflected marked renal deformities. Cisplatin treatment group showing suppressed secondary protein structure alterations. And magnolol treated group preserved normal structure of proteins.

3.13. Evans Blue Assessment

The results show increased permeation of evans blue dye in Cisplatin administered group and minimum permeation in magnolol treatment group as in Figure 3.13.

Figure 3.13. Effect of Magnolol on evans blue in kidney tissues (A) Normal control (B) Cisplatin (10mg/kg) (C) Dexamethasone (10mg/kg) (D) Magnolol (10mg/kg).
Note: All values are expressed as mean ± SD. (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$ shows significant difference compared with cisplatin group. (###) denotes comparison of Normal control and Cisplatin group with Magnolol group.

magnolol treatment significantly reduced the permeation into the kidney tissues compared to Cisplatin group. In Cisplatin administered group permeation increases marginally at the site of inflammation.

CHAPTER 4

DISCUSSION

4. DISCUSSION

Cisplatin is one of the most used and effective anticancer agents, though its therapeutic uses are limited due to its adverse effects. The exact underlying mechanism of cisplatin-induced nephrotoxicity is not well known (Jung *et al.*, 2009). However, one of the main mechanisms of cisplatin induced nephrotoxicity is the oxidative stress (Mansour *et al.*, 2002).

In this study, it was observed that mice injected with cisplatin (CP) showed a notable decrease in body weight, a decrease in kidney weight, and an increase in the ratio of kidney weight to body weight compared to the normal group. These results align with previous studies conducted by other researchers, which also reported a nephrotoxic effect of cisplatin characterized by a significant reduction in body weight and an increase in the relative weight of the kidneys (Azu *et al.*, 2010). The decrease in body weight observed in mice treated with CP may be attributed, at least partially, to gastrointestinal toxicity. This toxicity can lead to a loss of appetite in the animals, resulting in reduced food intake (Atessahin *et al.*, 2005). Alternatively, the excessive loss of water, salts, and proteins due to renal injury may also contribute to dehydration and subsequent weight loss (Azu *et al.*, 2010). The alterations in the ratio of kidney weight to body weight observed in mice treated with CP may be attributed to tissue damage and a decrease in kidney functions (Park *et al.*, 2009).

The findings of this study demonstrated a significant increase in the levels of creatinine and BUN in the cisplatin group when compared to the normal group. Consistent with our findings, previous studies conducted by (Mansour *et al.*, 2002) and (Santos *et al.*, 2008) also reported a notable rise in creatinine and BUN levels as evidence of the nephrotoxic effects of CP. The elevated creatinine levels observed in CP-treated mice may be attributed to glomerular damage caused by the generation of reactive oxygen species (ROS), as reported by (Mansour *et al.*, 2002). ROS can impair the activity of antioxidant enzymes in renal tissues, leading to increased lipid peroxidation and subsequent nephrotoxicity, as described by (An *et al.*, 2011). Maintaining hematology values within the standard range is crucial for assessing one's wellbeing (Alabi *et al.*, 2017). A reduction in red blood cells could reveal an imbalance between its production and depletion (Sirag *et al.*, 2009). The studies show that red blood cells and hemoglobin count were significantly decreased after cisplatin injection. These findings are consistent with the studies conducted by (Wood and

Hrushesky, 1995), (Marković *et al.*, 2011) and (Longchar and Prasad *et al.*, 2016), which reported a decline in RBC count, HCT, and Hb levels following cisplatin administration in both humans and mice models. The observed decrease in RBC count and lower Hb concentrations strongly suggest that cisplatin treatment may have led to the destruction of RBCs, reduced tissue iron levels, and/or disrupted Hb biosynthesis. Nonetheless, the hematological parameters of the mice subjected to magnolol treatment displayed notable improvement in comparison to the mice treated solely with cisplatin. This indicates that magnolol possesses the capability to enhance hemoglobin levels by increasing or restoring the count of red blood cells (RBCs). As a result, it confirms the combined effect of these drugs in countering the hematotoxicity induced by Cisplatin. The mice treated with cisplatin exhibited a noteworthy reduction in their white blood cell (WBC) counts compared to the control group. This decline in WBC counts may indicate changes in the immune functions of the cisplatin-treated mice. However, the administration of magnolol resulted in significant improvement in mitigating the decrease in WBC counts in the mice treated with cisplatin. This implies that this drug has beneficial effects on the white blood cells, enhancing their levels in cisplatin-treated mice. A decline in the number of lymphocytes was noted, potentially attributable to the immunosuppressive impact of cisplatin on the bone marrow, achieved by inhibiting T-cell function (Li and Schluesener *et al.*, 2006). Magnolol treatment resulted in the recovery of lymphocyte count towards normal levels, which can be credited to its ability to boost the host's immune response.

The cisplatin-injected group showed a decrease in serum sodium concentration and an increase in the potassium concentration as compared to the control group. This decrease was linked to tubular cell damage and dysfunction of cell membrane pumps, specifically sodium-potassium pumps. As a consequence, the reabsorption of sodium was reduced, leading to higher urinary concentrations (Khan *et al.*, 2007), (Kim *et al.*, 1995) and (Kishimoto *et al.*, 2006). These findings are consistent with a previous study that also reported similar results (El Daly *et al.*, 1996). In magnolol administered groups, sodium and potassium concentrations seem to restore to their normal concentrations.

In our study, histopathology of cisplatin administered group showed abnormal characteristics as compared to vehicle group. A significant tubular degeneration, necrosis, and shedding of the tubular epithelial cells, along with the formation of

cysts, infiltration of cells in the interstitial spaces, widening of the capsular space, and congestion of glomerular capillary tufts was observed in cisplatin injected group. We also observed morphological changes in the kidneys of cisplatin-treated mice, including damage to both glomerular and tubular structures. These morphological alterations may explain the disruptions in both glomerular and tubular functions, which could be the underlying cause of reduced body weight, increased relative kidney weight, and elevated levels of creatinine and BUN. Whereas magnolol administered group showed less abnormalities as compared to cisplatin group. It showed results similar to vehicle group. In line with the findings of our study, (Abdelmeguid *et al.*, 2010; Azu *et al.*, 2010) and Ozer reported similar results in their studies regarding the renal sections of the cisplatin group observed under light microscopy (Ozer *et al.*, 2011). These authors further emphasized that oxidative stress played a major role in cisplatin-induced nephrotoxicity. The oxidative stress inhibits antioxidant enzymes and generates reactive oxygen species (ROS), which cause damage to lipids, proteins, and DNA components of the cells, leading to enzymatic inactivation and dysfunction of mitochondria.

Moreover, essential players in cellular inflammation are inflammatory cytokines like TNFα and IL1β, which have a significant link to nephrotoxicity's pathophysiology. Studies in the past have indicated that cisplatin induces phosphorylation, leading to the transportation of the NF-kB transcription factor into the nucleus along with the degradation of the inhibitory IkBα protein (Humanes *et al.*, 2017). In our research, we observed a notable rise in the expression of TNFa and IL1B. However, the administration of magnolol resulted in a reduction of the heightened expression of these inflammatory mediators. A previous study supports the anti-inflammatory properties of magnolol, as it inhibits the expression of TNFa, IL-6, and IL1-B by interfering with TLR4-mediated NF-kB and MAPKs signaling pathways (Fu *et al.*, 2013).

CONCLUSIONS

The results of the research showed that Magnolol can shield the kidneys against the harmful impacts caused by cisplatin. The investigation illustrated that Magnolol's potential to safeguard the kidneys might stem from its ability to hinder the creation of inflammatory compounds, uphold a stable oxidative state, and quell the initiation of NF-κβ signaling through reactive oxygen species (ROS). Magnolol successfully diminished oxidative stress and heightened antioxidant levels in mice. Nevertheless, more comprehensive research is needed to identify the exact target and underlying mechanism responsible for Magnolol's safeguarding benefits.

FUTURE PROSPECTIVES

The extensive molecular mechanism of action of Magnolol exerting nephroprotective effect is yet to be known. PCR and western blot techniques may be conducted to further elaborate the comprehensive physiology of Magnolol against cisplatin-induced nephrotoxicity.

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Annexure I: Approval from Bioethics Committee

قائداعظم یونیورسٹی

QUAID-I-AZAM UNIVERSITY

Faculty of Biological Sciences
Bioethics Committee

No. #BEC-FBS-QAU2022-410 Dated: 28-07-2022

Miss Ariba Sattar
C/O Dr. Salman Khan
Department of Pharmacy,
Faculty of Biological Sciences,
Quaid-i-Azam University, Islamabad
45320, Pakistan

Subject: - **"Potential protective effect of Magnolol in cisplatin-induced nephrotoxicity and associated depression with it."**

Dear Miss Ariba Sattar,

We wish to inform you that your subject research study has been reviewed and is hereby granted approval for implementation by Bio-Ethical Committee (BEC) of Quaid-i-Azam University, Your study has been assigned protocol **#BEC-FBS-QAU2022-410.**

While the study is in progress, please inform us of any adverse events or new, relevant information about risks associated with the research. In case changes have to be made to the study procedure, the informed consent from and or informed consent process, the BEC must review and approve any of these changes prior to implementation.

Sincerely,

Prof. Dr. Sarwat Jahan
Department of Zoology

cc:
Dean, F.B.S

Annexure II: Turnitin Similarity Index Report

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23/10/2023

ASSOCIATE PROFESSOR
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