#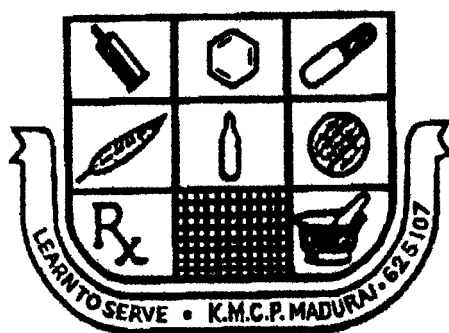 PROTECTIVE EFFECTS OF HYDROALCOHOLIC EXTRACT OF *Boerhaavia diffusa* Linn AGAINST CISPLATIN INDUCED NEPHROTOXICITY IN RATS

Dissertation submitted in partial fulfillment of the Requirement for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY, CHENNAI

DEPARTMENT OF PHARMACOLOGY

K.M.COLLEGE OF PHARMACY

UTHANGUDI

MADURAI-625107

APRIL-2014

CERTIFICATE

This is to certify that the dissertation entitled **"Protective Effects of Hydro alcoholic Extract of *Boerhaavia diffusa* linn against Cisplatin Induced Nephrotoxicity in Rats",** submitted by **Mr.B.MANOHARAN., Reg.No 261225056** in partial fulfillment for the degree of **"Master of Pharmacy** in **Pharmacology"** under The Tamilnadu Dr. M.G.R Medical University Chennai, at K.M.College of pharmacy, Madurai–107, is a bonafide work carried out by him under my guidance and supervision during the academic year of **2013 – 2014**. This dissertation partially or fully has not been submitted for any other degree or diploma of this university.

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This is to certify that the dissertation entitled **"Protective Effects of Hydroalcoholic Extract of *Boerhaavia diffusa* linn against Cisplatin Induced Nephrotoxicity in Rats",** is a bonafide work done by **Mr.B. MANOHARAN., Reg.No: 261225056** at K.M.College of pharmacy, Uthangudi, Madurai – 107, in partial fulfillment of the university rules and regulations for the award of **Master of Pharmacy in Pharmacology** under my guidance and supervision during the academic year of 2013 – 2014. This dissertation partially or fully has not been submitted for any other degree or diploma of this university.

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Abbreviations:

AIF   Apoptosis induced factor

Akt/PKB Serine/threonine kinase/protein kinase B

ATP   Adenosine triphosphate

Bid   Bcl-2 interacting domain

BIP   Bax inhibiting peptide

Cdk2   Cyclin-dependent kinase 2

c-FLIP   FLICE-like inhibitory protein

DIABLO Direct IAP binding protein with low pI

DR 4/5   Death receptor 4/5

EGF   Epidermal growth factor

EGFR   Epidermal growth factor receptor

ER   Endoplasmic reticulum

ERK   Extracellular signal-regulated kinase

ESRD   End-stage renal disease

FasL   Fas ligand

FLICE   Fas-associated-death domain like

IL   1b-converting enzyme

GADD   Growth arrest and DNA damage-inductible

GAPDH Glyceraldehyde-3-phosphate dehydrogenase

G-CSF   Granulocyte colony-stimulating factor

HEK   Human embryo kidney cells

HGF   Hepatocyte growth factor

HK-2   Human proximal tubular epithelial cell line

HSP70   Heat shock protein 70

IAP   Inhibitors of apoptosis proteins

IGF-   1 Insulin-like growth factor 1

IRE-   1a Inositol-requiring enzyme 1alpha

JNKs   Janus kinases

MCT   Murine cortical tubular cells

MDCK   Madin-darby canine kidney cells

MDR   Multi drug resistance

MAPKs   Mitogen-activated protein kinases

MEK   MAPK kinase

NF-jB   Nuclear factor jB

NRK-   52E Normal rat kidney epithelia

OAT   Organic anion transporter

OCT   Organic cation transporter

HtrA2   High temperature requirement protein A2

PgP   P-glycoprotein

PI3K   Phosphatidylinositol-3-kinase

PIDD   p53-induced death domain

PLA2   Phospholipase A2

RAP   Receptor associated protein

RMIC   Renal medullary interstitial cells

ROS   Reactive oxygen species

RPT   Rat proximal tubular cells

RPTC   Rabbit proximal tubular cells

siRNA   Small interfering RNA

Smac   Second mitochondria-derived activator of caspase

TGF   Transforming growth factor

TNF   Tumor necrosis factor

TNFR   Tumor necrosis factor receptor

TRAIL   TNF-related apoptosis inducing ligand

TUNEL   Terminal deoxynucleotidyl transferase

     (TdT)-mediated dUTP-biotin nick-end labeling

VEGF   Vascular endothelial growth factor

INTRODUCTION

The kidneys are a pair of organs that are primarily responsible for filtering metabolites and minerals from the circulatory system. These secretions are passed to the bladder and out of the body as the urine. [1]

Each kidney is enclosed in a fibrous capsule and is composed of a cortex and an inner medulla.

The functional units of the kidney called nephrons, with in the cortex and medulla, filters the blood under pressure and then reabsorb water and selected substance back in to the blood. Approximately 1.0 – 1.5 million nephrons were packed in the average human kidney.

The urine thus formed is conducted from the nephrons via the renal tubules into the renal pelvis and from there to ureter, which leads to the bladder. Two basic types of nephrons can be distinguished,

1. Cortical nephrons   -   85%

2. Juxtamedullary nephrons -   15%
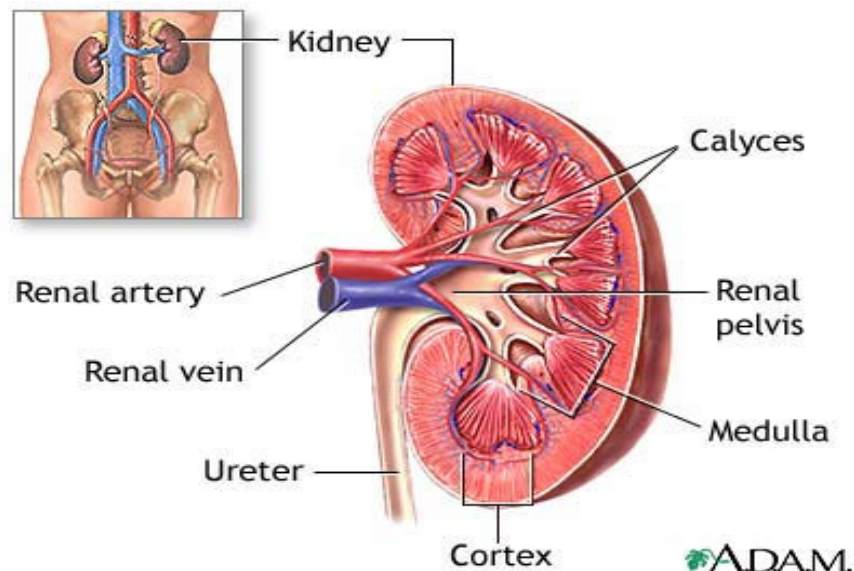
Figure. No: 1 Anatomy of kidney

The sole function of the urinary tract is the transmission and storage of urine. The urinary tract consists of the minor and major calyces, renal pelvis, ureters, urinary bladder and urethra.

RENAL DISEASES

Renal diseases are classified into the six different physiological categories.

1. Acute Renal Failure (ARF)

A sudden decline in kidney function occurs in patients with pre-existing renal impairment is called Acute Renal Failure. ARF can be diagnostically classified into prerenal, intrarenal and postrenal failure. [2, 3]

2. Chronic Renal Failure (CRF) [4]

CRF is due a number of processes leading to permanent loss of kidney, function. Its primary causes are high blood pressure and diabetes, but it can also be due to urinary tract obstruction and kidney abnormalities, like polycystic kidney disease.

Causes of CRF includes: 1) Chronic glomerulonephritis

2) Hypertension

3) Chronic pyleonephritis

4) Urinary obstruction

5) Congenital abnormalities

6) Metabolic abnormalities

3. Hypertensive Kidney Disease

Patients with severe hypertension cause renal lesions that diminish the blood flow or diminished glomerular filteration per nephron.

4. The Nephrotic syndrome

Nephrotic syndrome is a disease, which is characterized especially by loss of large quantities of plasma proteins through the urine. Protein loss is due to increased permeability of glomerular membrane.

5. Specific Tubular Disorders

They can cause abnormal reabsorption or lack of reabsorption of certain substances by the tubules. If any required gene is absent or abnormal, then tubules might be deficient of one particular enzyme or carrier. This leads to different tubular disorder.

6. Drug Induced Renal Disease

Drug - induced kidney failure is a major adverse event associated with multiple medication classes. The kidney is particularly vulnerable to drugs and other agents that cause renal damage (nephrolithiasis). Medicatins as diverse as OTC analgesics (ibuprofen, acetaminophen), antibiotics and chemotherapeutics agents can cause kidney damage.

NEPHROTOXICITY

Nephrotoxicity can be defined as renal disease or dysfunction that arises as a direct or indirect result of exposure to medicines, and industrial or environmental chemicals. Drug nephrotoxicity is therefore any renal dysfunction attributable to drugs. . Nephrotoxicity may also be referred to as renal toxicity Nephrotoxicity is one of the most common kidney problems and occurs when your body is exposed to a drug or toxin that causes damage to your kidneys. When kidney damage occurs, it's unable to rid body of excess urine, and wastes. Blood electrolytes (such as potassium and magnesium) will all become elevated. It produces a temporary elevation of Blood urea Nitrogen (BUN) and/or creatinine levels. Early diagnosis is critical; therefore, physicians must be aware of the nephrotoxic potential of the medications they prescribe and the risk status of their patients. [5,6]

Proximal convoluted tubule
(S1/S2 segments)
Aminoglycosides
Cephaloridine
Cadmium chloride
Potassium dichromate

Glomeruli
Interferon—α
Gold
Penicillamine

Renal vessels
NSAIDs
ACE inhibitors
Cyclosporin A

Proximal straight tubule
(S3 segment)
Cisplatin
Mercuric chloride
Dichlorovinyl—L—cysteine

Papillae
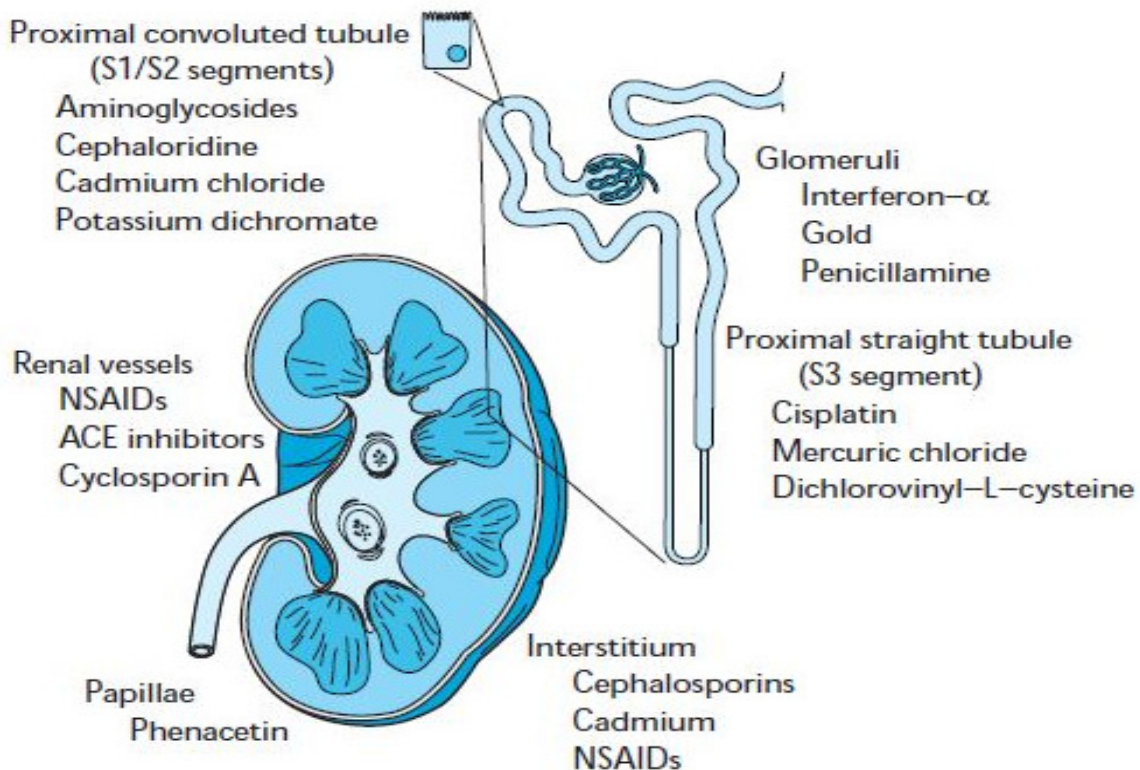Phenacetin

Interstitium
Cephalosporins
Cadmium
NSAIDs

Figure. No: 2 Drug Induced Nephrotoxicity

Nephrotoxicants may act at different sites in the kidney, resulting in altered renal function. The sites of injury by selected nephrotoxicants are shown.

➢ Nonsteroidal anti-inflammatory drugs (NSAIDs), angiotensin-converting enzyme (ACE) inhibitors, cyclosporin A, and radiographic contrast media cause vasoconstriction.

➢ Gold, interferon-alpha, and penicillamine can alter glomerular function and result in proteinuria and decreased renal function. Many nephrotoxicants damage tubular epithelial cells directly.

➢ Aminoglycosides, cephaloridine, cadmium chloride, and potassium dichromate affect the S1 and S2 segments of the proximal tubule, whereas cisplatin, mercuric chloride, and dichlorovinyl-L-cysteine affect the S3 segment of the proximal tubule.

➢ Cephalosporins, cadmium chloride, and NSAIDs cause interstitial nephritis whereas phenacetin causes renal papillary necrosis.

Brief Histology and Physiology:

The nephron is the functional unit of the kidney and consists of a continuous tube of highly specialized heterogeneous cells, which show sub-specialization along the length of nephrons and between them. It is the major organ of excretion and homeostasis for water-soluble molecules; because it is a metabolically active organ, it can concentrate certain substances actively. In addition, its cells have the potential to bio convert chemicals and metabolically activate a variety of compounds.

Since the kidney excretes many drugs, it is routinely exposed to high concentrations of these drugs or their metabolites or both. Furthermore, the kidney has several features that allow nephrotoxins to accumulate. It is highly vascular, receiving about 25% of the resting cardiac output. The proximal renal tubule presents a large area for nephrotoxin

binding and transport into the renal epithelium. Reabsorption of the glomerular filtrate progressively increases intraluminal nephrotoxin concentrations, while specific transport pathways in the kidney may engender site-specific toxicity.

Nephrotoxicity Induced Oxidative Stress:

Nephrotoxicants are generally thought to produce cell injury and death through one of two mechanisms, either alone or in combination. In some cases the toxicant may have a high affinity for a specific macromolecule or class of macromolecules that result in altered activity (increase or decrease) of these molecules, resulting in cell injury. Alternatively, the parent nephrotoxicant may not be toxic until it is bio transformed into a reactive intermediate that binds covalently to macromolecules and in turn alters their activity, resulting in cell injury. Finally, the toxicant may increase reactive oxygen species in the cells directly, after being bio transformed into a reactive intermediate or through redox cycling. The resulting increase in reactive oxygen species results in oxidative damage and cell injury. [7,8]

Chart. No.1: Nephrotoxicity Induced Oxidative Stress**.**
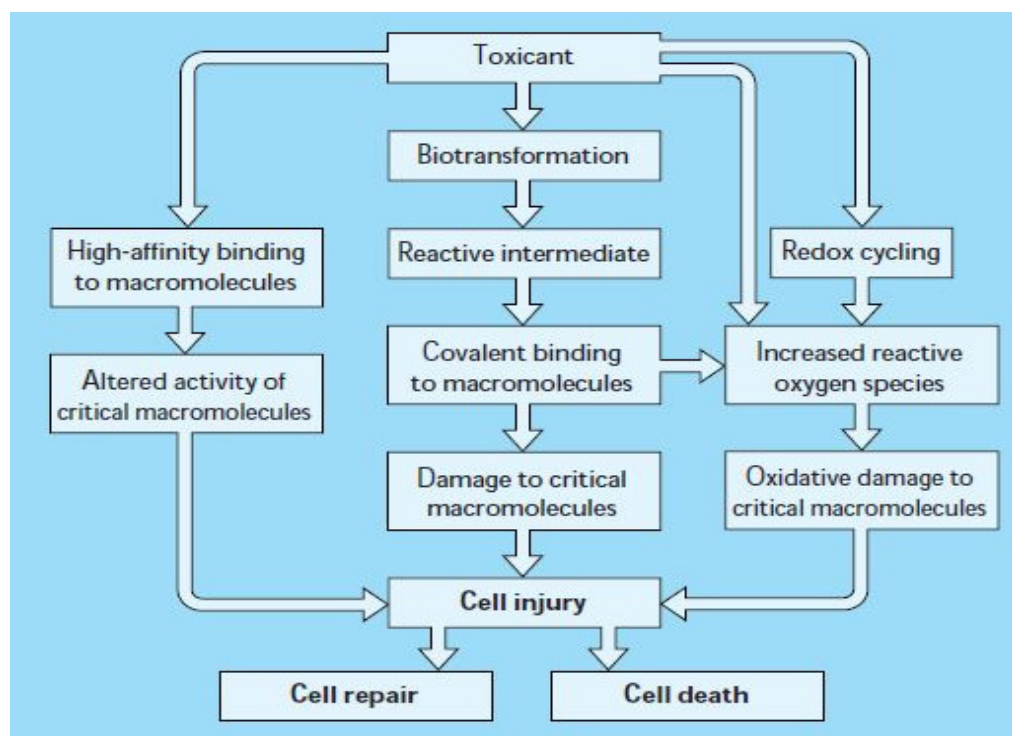
MECHANISMS OF TOXICITY:

Many drugs can injure the kidney but they cause renal injury via only a few mechanism. Renal injury can be in the form of **acute renal failure**, **nephrotic syndrome, chronic renal failure** & **renal tubular toxicity**.[9]

I. **ACUTE RENAL FAILURE**

Drugs can cause acute renal failure by three mechanisms:

1. **Pre-renal**
2. **Intrinsic**
3. **Obstructive**.

1. Pre-Renal:

Some drugs can cause acute renal failure by reducing the volume or pressure or both of blood delivered to the kidney; the resulting renal failure is therefore termed "prerenal." Patients at risk are those who already have compromised renal blood flow such as with bilateral renal artery stenosis, or with decreased effective circulatory volume as with cirrhosis, nephrotic syndrome, or congestive heart failure. Because of hypovolemia, urine volume and sodium excretion are low while osmolality is high. The urine sediment is usually without casts, red blood cells and white blood cells.

2. Intrinsic/renal:

Three types of intrinsic acute renal failure exist:

A. Acute tubular necrosis
B. Acute interstitial nephritis
C. Thrombotic microangiopathy

A. Acute Tubular Necrosis:

Mechanisms of injury are multiple but may overlap, including direct tubular toxicity, deranged cellular energy production, free radical injury heme tubular toxicity, abnormal phospholipid metabolism, and intracellular calcium toxicity. [10]

B. **Acute interstitial nephritis:**

Acute interstitial nephritis presents with systemic manifestations of a hypersensitivity reaction such as fever, rash, and arthralgias. Although penicillins and cephalosporins are well-recognized culprits, almost any antibiotic can cause it occasionally. The fluoroquinolone ciprofloxacin is now a well-recognized cause of allergic interstitial nephritis.[11, 12]

Urinary findings include white blood cells, red blood cells, and white cell casts. The major histologic findings are interstitial edema and variable cellular infiltration by eosinophils, plasma cells, T lymphocytes, monocytes, and neutrophils. In rare cases, granulomas may be seen on kidney biopsy; patients with granulomas may also present with uveitis. In most cases, acute interstitial nephritis is reversible when the offending agent is stopped.

C. **Thrombotic microangiopathy:**

Thrombotic microangiopathy can cause severe acute renal failure. In general, the pathologic hallmark of thrombotic microangiopathy is hyaline thrombi in the microvasculature of many organs. Changes in the kidney include afferent arteriolar and glomerular thrombosis and thickening of the glomerular capillary wall on electron microscopy due to the deposition of fibrin-like materials. Thrombotic microangiopathy may manifest with fever, hemolytic anemia, thrombocytopenia, renal dysfunction, and central nervous system disease —thrombocytopenic purpura (TTP) most frequently seen in adults. Urinalysis shows microscopic hematuria, subnephrotic proteinuria, hyaline, and few granular casts. The reticulocyte count is elevated, haptoglobin levels are low, schistocytes are present in the peripheral blood smear, and the lactate dehydrogenase level is high. Overall mortality is high, though

many patients may survive on chronic dialysis. Steroids are of no proven benefit in this syndrome.

2. Obstructive:

Drug-associated obstruction of urine outflow can occur at several sites: within the tubules or the ureters (due to crystal formation), and outside the ureters (due to retroperitoneal fibrosis caused by agents such as methysergide).[11] Risk factors for crystal-induced acute renal failure include severe volume depletion underlying renal insufficiency, bolus drug administration, and metabolic disorders such as metabolic acidosis or alkalosis. The urine sediment may contain red cells, white cells, and crystals.

II. Nephrotic Syndrome:

The nephrotic syndrome is due to glomerular dysfunction and marked by heavy proteinuria. Drugs implicated include gold, NSAIDs, penicillamine, interferon, and captopril. Patients may present with edema, proteinuria, and hypoalbuminemia. Membranous nephropathy is the most common form reported, though minimal change nephropathy has also been seen with NSAIDs, as discussed below.

III. Chronic Renal Insufficiency :

Chronic renal insufficiency caused by drugs generally presents as tubulointerstitial disease. It is important to note that for some drugs (e.g., cyclosporine, lithium), the mechanism of acute renal toxicity may be different from that of chronic renal injury. Patients may present with slowly progressive elevation of creatinine, with or without renal tubular dysfunction syndromes. These syndromes may manifest as renal tubular acidosis, renal potassium wasting, concentration defects, and tubular proteinuria. These syndromes may also occur without renal failure. In some cases, the renal damage is reversible when the offending drug is

stopped, but in other cases it is irreversible. Frequently reversible forms include those due to 5- aminosalicylic acid, 6 mesalamine, and ifosfamide, while lithium and cyclosporine cause irreversible injury.

IV. **Analgesic nephropathy:**

Analgesic nephropathy, simply, is renal dysfunction caused by analgesics. It may be a consequence of the excessive consumption of mixed analgesics or single analgesic use. . A variety of analgesics and NSAIDs have been shown to have the potential to cause RPN and interstitial nephritis.[13,14] The resultant lesions can be recognized by radiological examinations or ultrasonography and consist of calcifications along the line line of Hodson, shrinking of the kidneys resulting in irregular contours, and decreased length of both kidneys. Necrotic papillae may be voided in the urine and this is occasionally observed.

V. **Tubular Cell Toxicity**

Renal tubular cells, in particular proximal tubule cells, are vulnerable to the toxic effects of drugs because their role in concentrating and reabsorbing glomerular filtrate exposes them to high levels of circulating toxins.[15] Drugs that cause tubular cell toxicity do so by impairing mitochondrial function, interfering with tubular transport, increasing oxidative stress, or forming free radicals.[16,17] Drugs associated with this pathogenic mechanism of injury include aminoglycosides, amphotericin B cisplatin, foscarnet and zoledronate.

Other Causes Are:

- Inflammation

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10

- Crystal nephropathy

- Rhabdomyolysis
- Altered intraglomerular Hemodynamics
- Thrombotic micro angiopathy

❖ Inflammation:

Drugs can cause inflammatory changes in the glomerulus, renal tubular cells, and the surrounding interstitium, leading to fibrosis and renal scarring. Glomerulonephritis is an inflammatory condition caused primarily by immune mechanisms and is often associated with proteinuria in the nephrotic range.[15] Medications such as gold therapy, hydralazine,lithium, NSAIDs, propylthiouracil, and pamidronate have been reported as causative agents.[15,16,17]

Acute interstitial nephritis, which can result from an allergic response to a suspected drug, develops in an idiosyncratic, non–dose-dependent fashion. Medications that cause acute interstitial nephritis are thought to bind to antigens in the kidney or act as antigens that are then deposited into the interstitium, inducing an immune reaction. However, classic symptoms of a hypersensitivity reaction (i.e., fever, rash, and eosinophilia) are not always observed.[16,18] Numerous drugs have been implicated, including allopurinol Beta lactams antibiotics, quinolones, rifampin ,sulfonamides, and vancomycin, acyclovir and loop diuretics thiazides, NSAID omeprazole ,pantoprazole, and lansoprazole.

❖ Crystal Nephropathy:

Renal impairment may result from the use of drugs that produce crystals that are insoluble in human urine. The crystals precipitate, usually within the distal tubular lumen, obstructing urine flow and eliciting an interstitial reaction.[13] The likelihood of crystal precipitation depends on the concentration of the drug in the urine and the urinary pH.[19] Patients most at risk of crystal nephropathy are those with volume depletion and underlying renal insufficiency. Commonly prescribed drugs associated

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11

with production of crystals include antibiotics, ampicillin, ciprofloxacin sulfonamides antivirals acyclovir, foscarnet, ganciclovir methotrexate; and triamterene . Chemotherapy for lymphoproliferative disease, leading to tumor lysis syndrome with uric acid and calcium phosphate crystal deposition, has also been associated with renal failure.[20]

❖ Rhabdomyolysis:

Rhabdomyolysis is a syndrome in which skeletal muscle injury leads to lysis of the myocyte, releasing intracellular contents including myoglobin and creatine kinase into the plasma. Myoglobin induces renal injury secondary to direct toxicity, tubular obstruction, and alterations in GFR. Drugs may induce rhabdomyolysis directly secondary to a toxic effect on myocyte function, or indirectly by predisposing the myocyte to injury. Clinical manifestations of rhabdomyolysis include weakness, myalgia, and tea-colored urine.Statins are the most recognizable agents associated with rhabdomyolysis.Many drugs of abuse, such as cocaine, heroin, ketamine ,methadone, and methamphetamine, have been reported to cause rhabdomyolysis. Drugs and alcohol are causative factors in up to 81 percent of cases of rhabdomyolysis, and up to 50 percent of patients subsequently develop acute renal failure. [21-23]

❖ Thrombotic Microangiopathy:

In thrombotic microangiopathy, organ damage is caused by platelet thrombi in the microcirculation, as in thrombotic thrombocytopenic purpura. Mechanisms of renal injury secondary to drug-induced thrombotic microangiopathy include an immune-mediated reaction or direct endothelial toxicity.[24] Drugs most often associated with this pathogenic mechanism of nephrotoxicity include antiplatelet agents clopidogrel ,ticlopidine cyclosporine, mitomycin-C and quinine .

Most drugs found to cause nephrotoxicity exert toxic effects by one or more common pathogenic mechanisms:

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12

Drug class/drug(s)	Pathophysiologic mechanism of renal
Acyclovir (Zovirax)	Acute interstitial nephritis, crystal nephropathy
Nonsteroidal anti-inflammatory drugs	Acute interstitial nephritis, altered intraglomerular hemodynamics, chronic interstitial nephritis, glomerulonephritis
Antihistamines	
Diphenhydramine (Benadryl), doxylamine (Unisom)	Rhabdomyolysis
Aminoglycosides	Tubular cell toxicity

Ganciclovir (Cytovene)	Crystal nephropathy
Rifampin (Rifadin)	Acute interstitial nephritis
Beta lactams (penicillins, cephalosporins)	Acute interstitial nephritis, glomerulonephritis (ampicillin, penicillin)
Sulfonamides	Acute interstitial nephritis, crystal nephropathy
Vancomycin (Vancocin)	Acute interstitial nephritis
Cisplatin (Platinol)	Chronic interstitial nephritis, tubular cell toxicity

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14

Methotrexate   Crystal nephropathy

Mitomycin-C (Mutamycin)   Thrombotic
                                                          microangiopathy

Triamterene (Dyrenium)   Crystal nephropathy

Cocaine, heroin, ketamine ,methadone, methamphetamine   Rhabdomyolysis

Proton pump inhibitors

Lansoprazole ,omeprazole, pantoprazole   Acute interstitial

nephritis

Diuretics

Loops, thiazides

<u>CISPLATIN</u>

Cisplatin is one of the most widely used and most potent chemotherapy drugs. Cisplatin (*cis*-diamminedichloroplatinum(II), CDDP) is an antineoplastic drug used in the treatment of many solid-organ cancers, including those of the head, neck, lung, testis, ovary, and breast. While toxicities include ototoxicity, gastro toxicity, myelosuppression, and allergic reactions [25,26] the main dose-limiting side effect of cisplatin is nephrotoxicity.[27,28,29] 20% of patients receiving high-dose cisplatin have severe renal dysfunction.

Cisplatin-DNA crosslinks cause cytotoxic lesions in tumors and other dividing cells . In rapidly dividing cells, such as those in cancers, cross-linking can further induce DNA damage. Mildly damaged DNA can be repaired, whereas extensive DNA damage leads to irreversible injury and cell death. Cisplatin has multiple intracellular effects, including regulating genes, causing direct cytotoxicity with reactive oxygen species, activating mitogen-activated protein kinases, inducing apoptosis, and stimulating inflammation and fibro genesis. These events cause tubular damage and tubular dysfunction with sodium, potassium, and magnesium wasting. Most patients have a reversible decrease in glomerular filtration, but some have an irreversible decrease in glomerular filtration.

Renal manifestations of cisplatin treatment:

Cisplatin nephrotoxicity can present in a number of ways. However, the most serious and one of the more common presentations is acute kidney injury (AKI) which occurs in 20–30% of patients.

- Acute kidney injury (20–30%)
- Hypomagnesemia (40–100%)
- Fanconi-like syndrome
- Distal renal tubular acidosis
- Hypocalcemia
- Renal salt wasting
- Renal concentrating defect
- Hyperuricemia
- Transient proteinuria
- Erythropoietin deficiency
- Thrombotic microangiopathy
- Chronic renal failure

Cisplatin Uptake into Renal Cells:

Uptake of cisplatin is mainly through the organic transporter pathway. Transporter mediated uptake is likely the major pathway in renal cells.[30] The organic cation transporter (OCT 2) is the critical transporter for cisplatin uptake in proximal tubules in both animals and humans. Transport mediated by these membrane proteins is polyspecific, electrogenic, voltage-dependent, bi-directional, pH-independent, and Na_-independent.Three isoforms of OCT have been identified in humans. OCT2 is the main OCT in the kidney, OCT1 is the main isoform of the liver, and OCT3 is widely expressed, especially in the placenta. Cisplatin is not transported through human OCT1, which may help explain its organ-specific toxicity. In addition to a transporter-mediated process, cisplatin enters the cell through passive diffusion. In addition, the organic cation transporter OCT2 (SLC22A2) transports cisplatin. Cisplatin was shown to inhibit the uptake of other OCT2 substrates, consistent with the view that these substrates share a common transport pathway. Two recent observations point to an important role for OCT2 in mediating renal cisplatin uptake and toxicity. First, knockout of theOCT2 gene significantly reduced urinary cisplatin excretion and nephrotoxicity. Second, a nonsynonymous single-nucleotide polymorphism (SNP) in the OCT2 gene (rs316019) was associated with reduced cisplatin-induced nephrotoxicity in patients.[35,36]
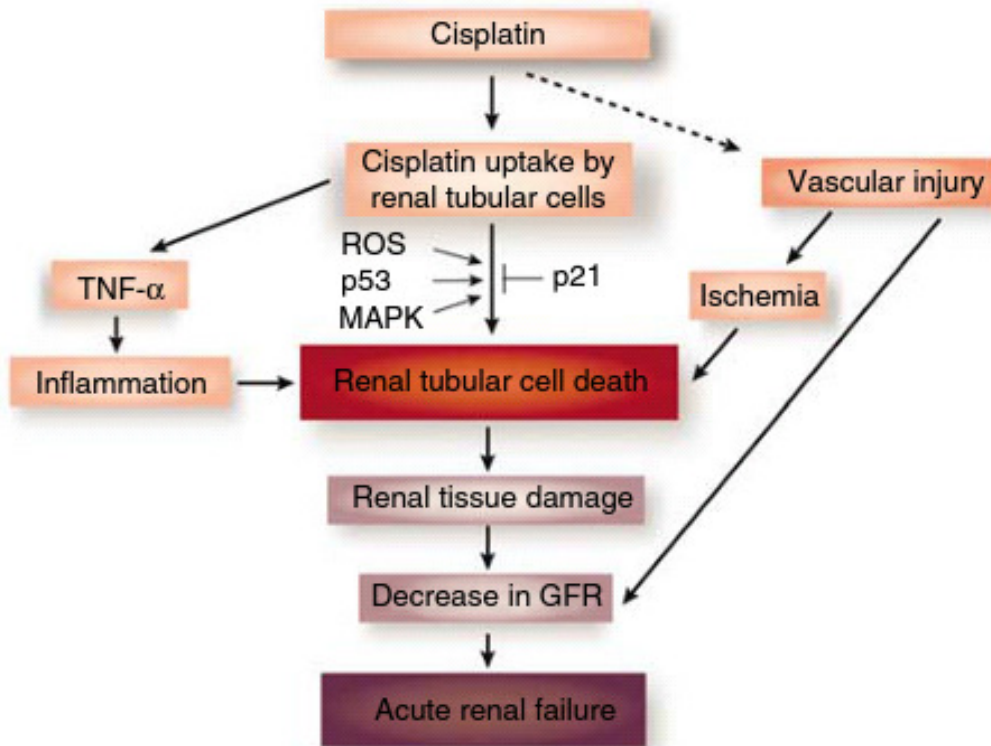
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18

Figure. No: 3 Cisplatin Uptake into Renal Cells

Renal accumulation of cisplatin:

The kidney accumulates cisplatin to a greater degree than other organs and is the major route for its excretion. The cisplatin concentration in proximal tubular epithelial cells is about 5 times the serum concentration. The disproportionate accumulation of cisplatin in kidney tissue contributes to cisplatin-induced nephrotoxicity.[37,38] Cisplatin is accumulated by peritubular uptake in both the proximal and distal nephrons.[39,40] The S3 segment of the proximal tubule accumulates the highest concentration of cisplatin, followed by the distal collecting tubule and the S1 segment in the proximal tubule. Cisplatin concentrations within the kidney exceed those in blood suggesting an active accumulation of drug by renal parenchymal cells.

RISK FACTORS FOR CISPLATIN NEPHROTOXICITY.

Increased risk

- Dose
- Frequency
- Cumulative dose
- Older age
- Female sex
- Smoking
- Hypoalbuminemia
- Pre-existing renal insufficiency (limited data in humans)

Decreased risk

- Diabetes (uncertain in humans)
- OCT2 polymorphisms[41,42]

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20

Patient-related factors:

Age, sex, race Previous renal insufficiency Specific diseases (diabetes mellitus, hypertension, sickle cell disease, multiple myeloma, proteinuric disease, SLE) Sodium-retaining states (cirrhosis, heart failure, nephrosis) Dehydration and volume depletion Acidosis, potassium and magnesium depletion Hyperuricemia, hyperuricosuria Sepsis, shock Renal transplantation.

Drug-related factors:

Inherent nephrotoxic potential Dose Duration, frequency, and form of administration Repeated exposure.

Drug interactions:

Combined or closely associated use of diagnostic or therapeutic agents with added or synergistic nephrotoxic potential (e.g., radiocontrast agents, aminoglycosides, nonsteroidal anti-inflammatory drugs, cisplatin, angiotensin-converting enzyme inhibitors)

Cisplatin Metabolism:

Conversion of cisplatin to nephrotoxic molecules in the proximal tubule cells is required for cell injury. The highest concentration of cisplatin is found in cytosol, mitochondria, nuclei, and microsomes. Studies in rats and mice indicate that cisplatin undergoes metabolic activation in the kidney to a more potent toxin. This process begins with the formation of glutathione conjugates in the circulation, perhaps mediated by glutathione-S-transferase. As the glutathione-conjugates pass through the kidney, they are cleaved to cysteinyl-glycine-conjugates by gamma glutamyl transpeptidase (GGT) expressed on the surface of the proximal tubule cells. The cysteinyl-glycine-conjugates are further metabolized to cysteine-conjugates by aminodipeptidases, also expressed on the surface of the proximal

tubule cells. The cysteine-conjugates are transported into the proximal tubule cells, where they are further metabolized by cysteine-S-conjugate beta-lyase to highly reactive thiols.[41-45]
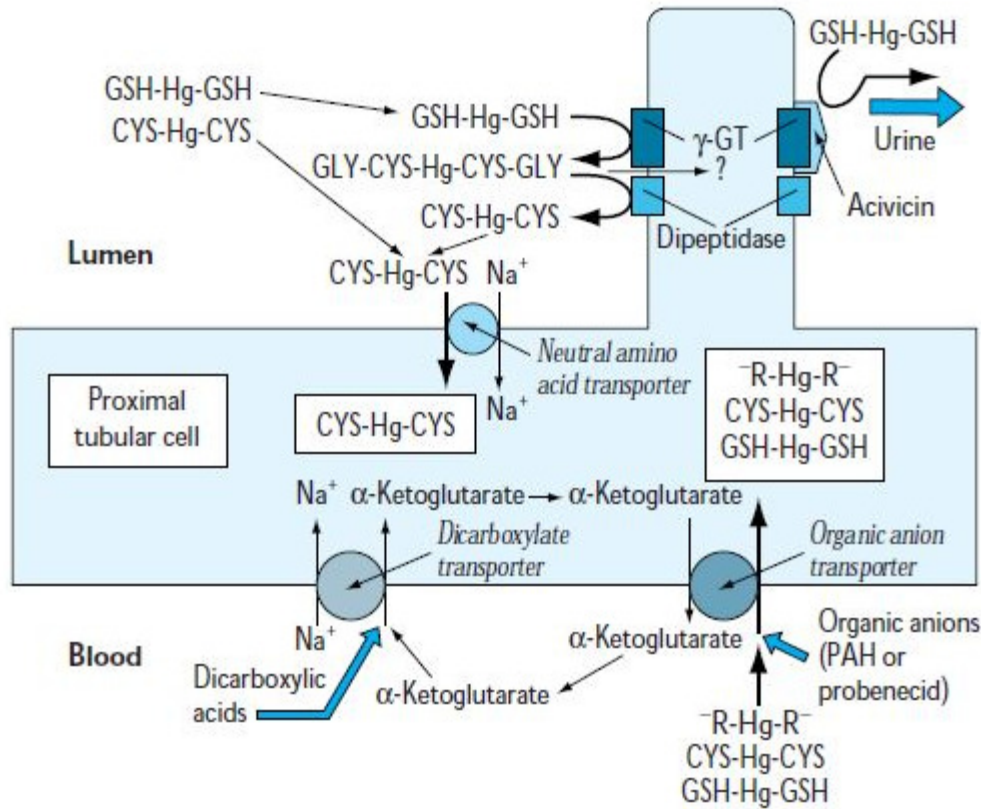

Figure.No: 4 Cisplatin Metabolism.

The importance of cellular transport in mediating toxicity. Proximal tubular uptake of inorganic mercury is thought to be the result of the transport of mercuric conjugates (*eg*, diglutathione mercury conjugate [GSH-Hg-GSH], dicysteine mercuric conjugate [CYS-Hg-CYS]). At the luminal membrane, GSH-Hg-GSH appears to be metabolized by (-glutamyl transferase ((-GT) and a dipeptidase to form CYS-Hg-CYS. The CYS-Hg-CYS may be taken up by an amino acid transporter. At the basolateral membrane, mercuric conjugates appear to be transported by the organic anion transporter. (-Ketoglutarate and the dicarboxylate transporter seem to play important roles in basolateral membrane uptake of mercuric conjugates. Uptake of mercuric-protein conjugates by endocytosis may play a minor role in the uptake of inorganic mercury transport. PAH—*para*-aminohippurate.[46,47]
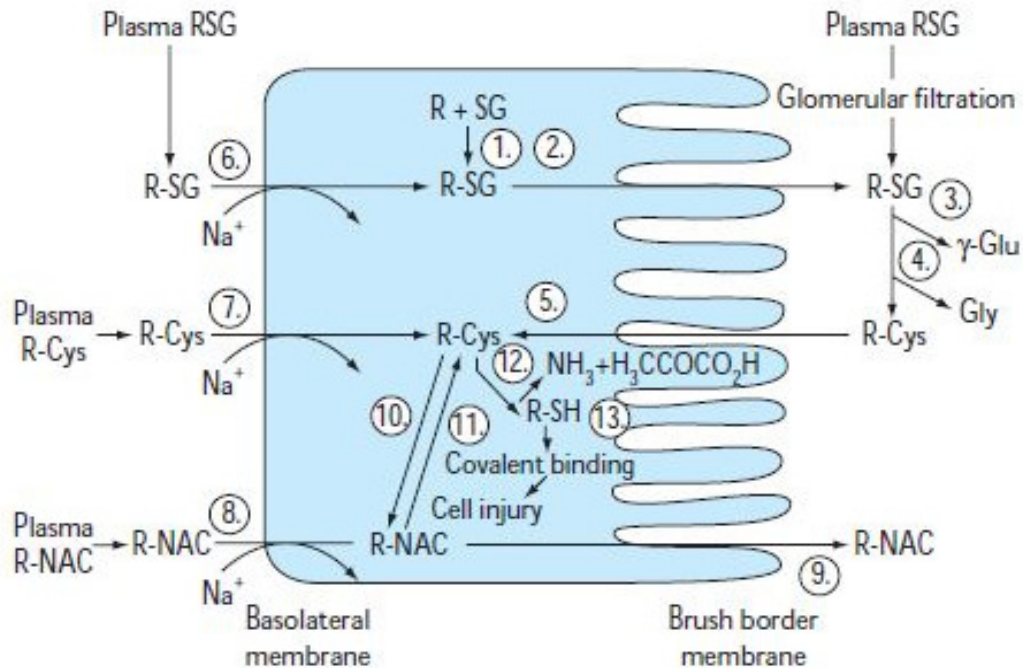
Figure. No: 5 glutathione and cysteine conjugates cisplatin induced nephrotoxity.

This figure illustrates the renal proximal tubular uptake, biotransformation, and toxicity of glutathione and cysteine conjugates and mercapturic acids of haloalkanes and haloalkenes.[48-50]

1. Formation of a glutathione conjugates within the renal cell (R-SG).

2. Secretion of the R-SG into the lumen.

3. Removal of the _-glutamyl residue (_-Glu) by _-glutamyl transferase.

4. Removal of the glycinyl residue (Gly) by a dipeptidase.

5. Luminal uptake of the cysteine conjugate (R-Cys). Basolateral membrane uptake of R-SG

6. R-Cys (7), and a mercapturic acid (*N*-acetyl cysteine conjugate; R-NAC)

8,9 Secretion of R-NAC into the lumen.

10. Acetylation of R-Cys to form R-NAC.

11. Deacetylation of R-NAC to form R-Cys.

12. Biotransformation of the penultimate nephrotoxic species (R-Cys) by cysteine conjugate -lyase to a reactive intermediate (R-SH), ammonia, and pyruvate.

13. Binding of the reactive thiol to cellular macromolecules (*eg*, lipids, proteins) and initiation of cell injury.

Cisplatin can form monohydrated complexes by hydrolytic reactions. The monohydrated complex is more toxic to the renal cells than cisplatin but it is not kidney specific. The normal low intracellular chloride concentrations promote its formation. Using hypertonic saline to reconstitute cisplatin can decrease the amount of monohydrated complex formed. This approach attenuates nephrotoxicity but may also compromise its antitumor activity.

Cellular Targets of Cisplatin:

Platinum compounds are believed to mediate their cytotoxic effects through their interaction with DNA (Figure 6). In an aqueous environment, the chloride ligands of cisplatin are replaced by water molecules generating a positively charged electrophile. This electrophile reacts with nucleophilic sites on intracellular macromolecules to form DNA, RNA, and protein adducts. Cisplatin binds to DNA leading to the formation of inter- and intra-strand cross-links, thereby arresting DNA synthesis and replication in rapidly proliferating cells. The finding that cells deficient in DNA repair are more sensitive to cisplatin-induced cell death supports the concept that cisplatin mediates its anti-tumor effects through DNA damage. However, the primacy of nuclear DNA damage as the cause of cisplatin-induced cell death has been challenged. In fact, only a small amount of cellular platinum (<1%) is bound to nuclear DNA and there is a poor correlation between the sensitivity of cells to cisplatin-induced cell death and the extent of DNA platination Cisplatin is hydrolyzed to generate a positively charged metabolite which preferentially accumulates within the negatively charged mitochondria. Thus, the sensitivity of cells to cisplatin appears to correlate with both the density of mitochondria and the mitochondrial membrane potential. This observation may explain the particular sensitivity of the renal proximal tubule to cisplatin toxicity, as this segment exhibits one of the highest densities of mitochondria in the kidney.[51,52]

Moreover, depletion of mitochondrial DNA by growth of cells in ethidium bromide rendered cells highly resistant to cisplatin. Finally, mitochondrial DNA may be more susceptible than nuclear DNA to cisplatin-induced damage, due to less efficient DNA repair

Protective effects of hydro alcoholic extract of Boerhaavia diffusa linn against cisplatin induced nephrotoxicity in rats.

24

mechanisms. Taken together, these observations point to mitochondrial DNA as an important target in cisplatin toxicity.
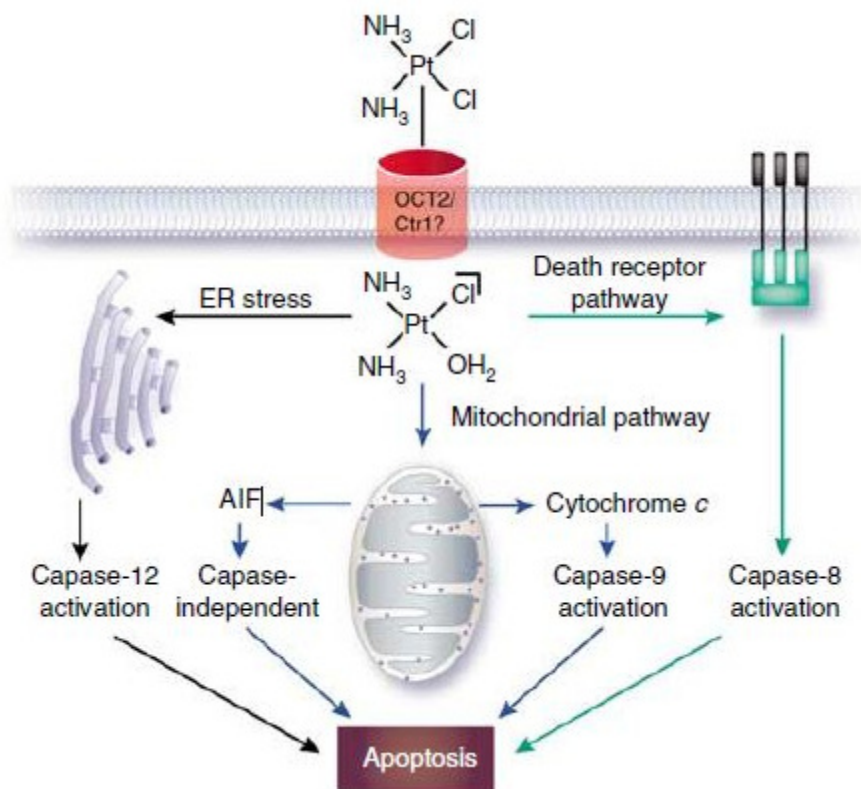

Figure. No: 6 Pathways of cisplatin-induced epithelial cell death. Cisplatin enters renal epithelial cells via the OCT2 and, to a lesser extent, Ctr1 transporters. Cisplatin causes damage to nuclear and mitochondrial DNA and production of reactive oxygen species (ROS) which lead to activation of both mitochondrial and non-mitochondrial pathways of apoptosis and necrosis.[53-57]

Mitochondrial energetics is also disrupted by cisplatin and may contribute to nephrotoxicity. Fatty acids are the major source of energy for the proximal tubule, the primary site of cisplatin kidney injury. Cisplatin inhibits fatty acid oxidation in mouse kidney and in proximal tubule cells in culture through a reduction in PPAR-α mediated expression of genes involved in cellular fatty acid utilization. Cisplatin also affects mitochondrial respiratory complexes and function. Exposure of cultured proximal tubule cells to cisplatin *in vitro*

inhibited mitochondrial complexes I to IV of the respiratory chain and, as a result, decreased intracellular ATP levels. Cisplatin treatment *in vivo* also resulted in mitochondrial dysfunction as evidenced by a decline in membrane electrochemical potential, a substantial decrease in mitochondrial calcium uptake and depletion of mitochondrial antioxidant defense systems.

Biochemical changes in the renal cell:

Cisplatin induces specific gene changes. Cisplatin-induced nephrotoxicity is mediated by mitogen-activated protein kinase (MAPK) intracellular signaling pathways. The MAPK pathways are a series of parallel cascades of serine/threonine kinases that are activated by diverse extracellular physical and chemical stresses. They regulate cell proliferation, differentiation, and survival.[58,59] The 3major MAPK pathways terminate in the extracellular regulated kinase (ERK), p38, and Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) enzymes. The ERK pathway is typically activated by extracellular growth factors and has been linked to both cell survival and cell death. The p38 and JNK/SAPK pathways are activated by a variety of stresses, for example, oxidants, UV irradiation, hyperosmolality, and inflammatory cytokines; they have been linked to cell death. ERK pathway contributes to proximal tubule injury is not clear, but certain responses in the distal nephron could induce adjacent proximal tubule injury through autocrine and paracrine processes.[60-62] P38 activation mediates proximal tubule cells injury. Stimulation of p38 is mediated by hydroxyl radicals, which are induced by cisplatin. The JNK/SAPK pathway in the cisplatin-induced nephrotoxicity has not been well studied. Intracellular Events that Damage Renal Cells The in vivo mechanisms of cisplatin nephrotoxicity are complex and involve oxidative stress, apoptosis, inflammation, and fibrogenesis. High concentrations of cisplatin induce necrosis in proximal tubule cells, whereas lower concentrations induce apoptosis through a caspase-9–dependent pathway.[63,64]

Protective effects of hydro alcoholic extract of Boerhaavia diffusa linn against cisplatin induced nephrotoxicity in rats.

26

Apoptotic pathways in cisplatin nephrotoxicity:

Apoptosis of renal tubular cells has been a focus of mechanistic investigation of cisplatin nephrotoxicity. Several pathways of apoptosis have been implicated, including the extrinsic pathway mediated by death receptors, the pathway centered on mitochondria, and the endoplasmic reticulum (ER)-stress pathway (Figure 7). In the extrinsic pathway, binding of the death receptors by ligands at the plasma membrane leads to the recruitment and activation of caspase-8, which further activate downstream caspases to induce apoptosis.[65] Major death receptors include Fas, tumor-necrosis factor-a (TNF-a) receptor (TNFR) 1 and importantly, pharmacological and genetic inhibition of TNF-a attenuated the production of various cytokines and chemokines, which was accompanied by the amelioration of cisplatin nephrotoxicity. TNF-a in the inflammatory response and cisplatin nephrotoxicity, it is suggested that TNFR2, and not TNFR1, is mainly responsible for the pathogenic signaling of TNF-a TNF-a and its receptors may induce renal injury primarily by mounting a disastrous inflammatory response rather than by directly activating the extrinsic pathway of apoptosis. [66,67] On the other hand, the intrinsic or mitochondrial pathway has emerged as the major apoptotic pathway in cisplatin nephrotoxicity. In the intrinsic pathway, cellular stress leads to the activation of the proapoptotic Bcl-2 family proteins Bax and Bak, which form porous defects on the outer membrane of mitochondria, resulting in the release of apoptogenic factors from the organelles. The apoptogenic factors released from mitochondria include cytochrome c, AIF (apoptosis-inducing factor), Smac/DIABLO, endonuclease G, and others. Cytochrome c, after being released into the cytosol, binds to and induces conformational changes in the adaptor protein Apaf-1, leading to the recruitment and activation of caspase-9, which in turn after proteolytic processing activates downstream caspases for caspase-dependent apoptosis. Smac, after being released into cytosol, can bind and antagonize the caspase inhibitor proteins, IAPs (inhibitor of apoptosis proteins) to further augment caspase activation.
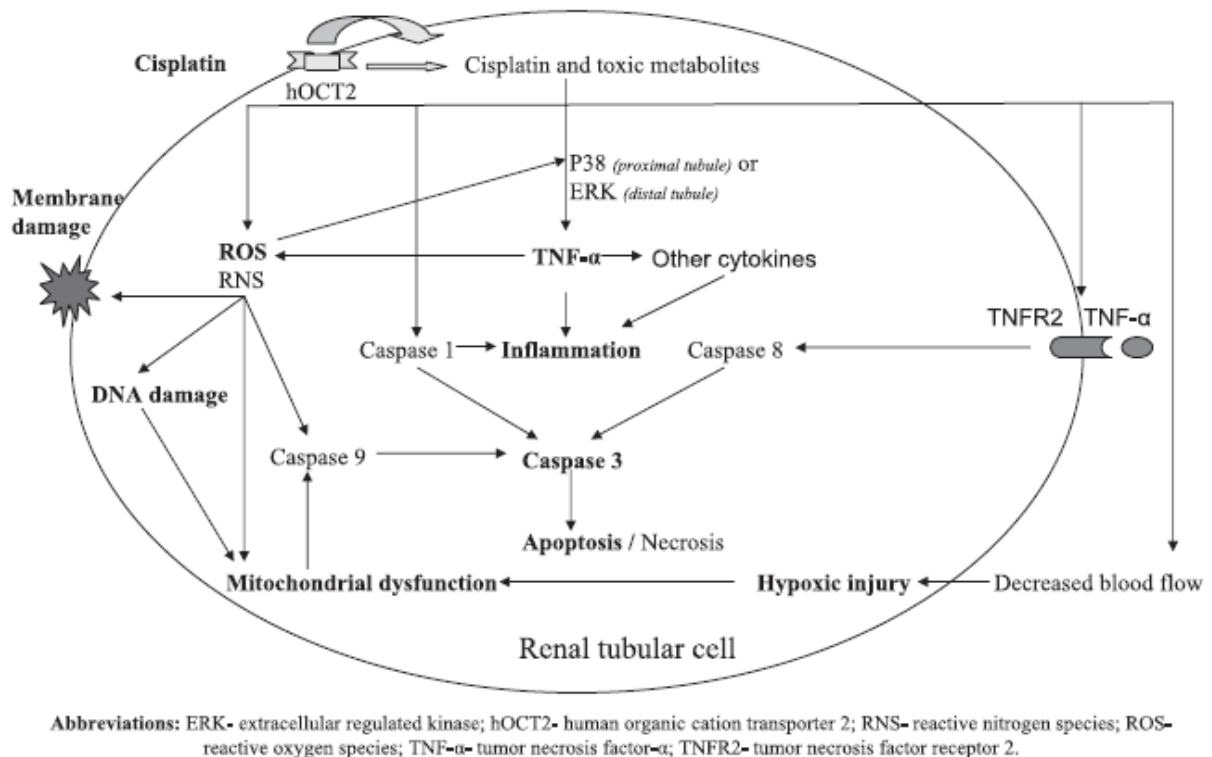
Abbreviations: ERK- extracellular regulated kinase; hOCT2- human organic cation transporter 2; RNS- reactive nitrogen species; ROS- reactive oxygen species; TNF-α- tumor necrosis factor-α; TNFR2- tumor necrosis factor receptor 2.

Figure. No: 7 Apoptotic Pathways in Cisplatin Nephrotoxicity.

Oxidative stress injury is actively involved in the pathogenesis of cisplatin-induced acute kidney injury.

Reactive oxygen species (ROS) directly act on cell components, including lipids, proteins, and DNA, and destroy their structure. ROS are produced via the xanthine-xanthine oxidase system, mitochondria, and NADPH oxidase in cells. In the presence of cisplatin, ROS are produced through all these pathways and are implicated in the pathogenesis of acute cisplatin-induced renal injury. First, once in a cell, cisplatin is aquated into a highly reactive form, which can rapidly react with thiol-containing molecules including glutathinone, a well-recognized cellular antioxidant.[68] Depletion or inactivation of glutathione and related antioxidants by cisplatin is expected to shift the cellular redox status, leading to the accumulation of endogenous ROS and oxidative stress within the cells. Second, cisplatin may induce mitochondrial dysfunction and increase ROS production via the disrupted respiratory chain. Resulting in ROS generation. Paradoxically, ROS formation under this situation was dependent on residual electron flow through the mitochondrial respiratory chain, because

complete inhibition of the respiration complexes blocked ROS accumulation. Finally, cisplatin may induce ROS formation in the microsomes via the cytochrome P450 (CYP) system. Cisplatin induces glucose-6-phosphate dehydrogenase and hexokinase activity, which increase free radical production and decrease antioxidant production.[69,70]

GUIDELINES TO PREVENTING DRUG-INDUCED NEPHROTOXICITY

In brief, the best clinical approach to drug-induced nephrotoxicity is prevention, which starts with the recognition that drug-induced renal injury occurs and is seen predominantly in patients at risk. The following steps are necessary:

● Anticipate. Be aware of nephrotoxic potential of specific drugs.

● Identify patients at risk.(those with renal insufficiency, dehydration, salt-retaining states, diabetes, and multiple myeloma).

● Be aware of increased risk in elderly patients.

● Carefully assess the benefits of prescribed drugs against potential risk.

● Whenever possible, select diagnostic procedures or therapeutic measures without nephrotoxic potential.

● Avoid dehydration mandatorily in high-risk patients. Pretreatment hydration is very important.

● Limit total daily dosage and duration of treatment with certain drugs.

● Adjust the daily dosage to ongoing alterations in the GFR.

● Avoid a combination of potentially nephrotoxic drugs.

● Urinary alkalinization to prevent renal failure from sulphonamides, methotrexate, triamterene, etc.

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29

EXPERIMENTAL STRATEGIES TO PREVENT CISPLATIN NEPHROTOXICITY

Reduced renal cisplatin accumulation or activation:

- OCT2 inhibitors, e.g., cimetidine or metformin.
- Ctr1 inhibitors, e.g., copper.
- Micellar/liposomal cisplatin.
- Gamma-glutamyl transpeptidase inhibitors.
- Glutathione transferase inhibitors.

Anti-oxidants:

- Amifostine.
- BNP7787.
- *N*-acetyl cysteine.
- Superoxide dismutase Catalase.
- Selenium and Vitamin E.
- Heme oxygenase-1 induction.
- Iron chelators, e.g., Desferoximine .
- Allopurinol plus ebselen .
- Milk thistle extracts (silymarin).
- Cannabidiol.
- Lycopene.

Anti-apoptosis:

- p53 inhibitors, e.g., pifithrin.
- HDAC inhibitors.
- Caspase inhibitors.
- p21agonists/CDK2 inhibitors.

Anti-inflammation:

- TNF-α antagonist.
- TLR4 antagonists.
- p38 inhibitors.
- JNK inhibitors Salicylates.
- PPAR-α ligands, e.g., fibrates.
- PPAR-γ ligands, e.g. rosiglitazone.
- Alpha lipoic acid.
- IL-10.
-

Treatment of cisplatin nephrotoxicity:

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30

There is no specific treatment for cisplatin-induced renal dysfunction or injury. These patients need careful attention to hydration and electrolyte treatment. They frequently need magnesium and potassium replacement. Cisplatin and magnesium affect the same sodium and water channels in the outer medulla. Cisplatin induces magnesium depletion, and magnesium deficiency itself may enhance cisplatin nephrotoxicity. Cisplatin treatment often produces extensive gastrointestinal side effects, which might lead to more magnesium depletion through anorexia and diarrhea. Therefore, magnesium repletion may attenuate cisplatin-induced nephrotoxicity.

Signal Transduction:

Serum thymic factor is a non apeptide thymic hormone isolated from the thymus and is involved in functional activation and differentiation of T cells. Serum thymic factor significantly ameliorates sustained ERK activation and induces the increased level of heat shock protein 70, which prevents cisplatin- induced renal damage in the rat model. Other selective MAPK/ERK inhibitors have also been shown to attenuate cisplatin-induced renal injury by decreasing inflammation and apoptosis. However, because cisplatin-induced apoptosis in human tumor cells is mediated by MAPK/ERK activation, inhibition of this pathway may disturb the anti-tumor activity of cisplatin. [71,72]

Antioxidant Drugs:

The combination of allopurinol and ebselen reduces cisplatin-induced nephrotoxicity and ototoxicity in a rat model. Allopurinol is a xanthine oxidase inhibitor with the potential to reduce ROS generation. Ebselen, a glutathione peroxidase mimic, is an excellent scavenger of peroxynitrite and can protect against lipid peroxidation in the presence of glutathione or other thiols. Ebselen has excellent oral availability and has been evaluated in human clinical testing for the treatment of acute ischemic stroke.. Erdosteine increases glucose-6-phosphate dehydrogenase activity, which helps maintain the proper intracellular redox state and protects against oxidant stress. Edaravone and *N*-acetylcysteine can replete intracellular stores of reduced glutathione. Other compounds with antioxidant property such as silymarin, naringernin, vitamin C, and vitamin E have also been found to have renoprotective function in animal studies. [73]

Amifostine:

Amifostine, an organic triphosphate, may diminish cisplatin-induced toxicity by donating a protective thiol group, an effect that is highly selective for normal but not malignant tissue. Amifostine is the only FDA-approved agent for the reduction of cumulative renal toxicity in advanced ovarian and non–small-cell lung cancer patients receiving cisplatin. This drug limits toxicity by binding free radicals. It may also bind and detoxify platinum agents by reduction of platinum-DNA adduct formation. [74]

Anti-inflammatory Drugs:

However, high doses of salicylates can stabilize I_B and reduce NF-_B transcription activity, and these effects attenuate TNF-_ production and reduce renal inflammation in cisplatin toxicity models. This may be explained by the observation that cisplatin nephrotoxicity is mediated via TNFR2, whereas the anti-tumor effect of TNF is mediated by TNFR1. Moreover, inhibition of NF-_B, a cell survival factor, by salicylate might increase the effectiveness of chemotherapy. [75]

A. Sreedevi, k., et al., (2011) Examined the effect of petroleum ether, ethyl acetate and alcoholic extracts of aerial parts of *Vernonia cinerea* (500 mg/kg, p.o.) on cisplatin-induced nephrotoxicity (6mg/kg, i.p.) in albino rats. Prophylactic and curative effect was assessed by estimating blood urea nitrogen, serum creatinine, serum total proteins, urinary protein, creatinine clearance and urine to serum creatinine ratio. Among the three extracts, alcoholic extract showed pronounced curative activity, ethyl acetate extract exhibited good prophylactic activity and petroleum ether extract showed moderate protection in both curative and prophylactic models against cisplatin-induced toxicity.[76]

Sahar Khalil Abdel-Gawad, et al., (2010) Investigated whether silymarin administration protects against cisplatin-induced nephrotoxicity in adult male albino rats. This study was performed on 30 adult male albino rats; Biopsies were processed for light microscopic studies. Immunohistochemical expression of Bak protein was investigated. In observation there was a decrease in the PAS +ve material at brush borders of the PCT and a positive cytoplasmic reaction of Bak protein in renal tubular cells. It concluded Silymarin pretreatment prevented the histopathological changes caused by cisplatin& also proved Silymarin exert protecting effective against cisplatin-induced nephrotoxicity. [77]

El-Sayed el-sayed, et al., (2008) Investigated captopril, an angiotensin-converting enzyme (ACE) inhibitor containing sulfhydryl (-SH) group showed protecting effect against cisplatin-induced nephrotoxicity in rats. In cisplatin induced toxicity, a significant increase in blood urea nitrogen (BUN) and creatinine levels and marked elevation in lipid peroxides measured as malondialdehyde (MDA), accompanied by a significant decrease in reduced glutathione (GSH) as compared to control group. In addition marked increase in kidney tissue content of nitric oxide (NO) and plasma endothelin-1(ET-1). Result indicated captopril, an ACEI, has a protective effect against cisplatin-induced damage to kidney. That it may relate to its free radicals scavenging and antioxidant effects which are sulfhydryl dependent. [78]

SarawootPalipoch, et al., (2013) Investigated the possible protective role of curcumin and α-tocopherol against cisplatin induced nephrotoxicity in rat. Serum blood urea nitrogen (BUN), creatinine and malondialdehyde (MDA) levels, superoxide dismutase (SOD) and catalase activities, kidney histopathological study and gene expressions of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and p38 mitogen activated protein kinase (p38-MAPK) were investigated Pre-treatment with combined curcumin and α-tocopherol exhibited significantly reduced MDA levels and enhanced activities of SOD and catalase compared with cisplatin-treated group ($p < 0.05$). It also improved BUN as well as creatinine levels and kidney histopathology. Moreover, gene expressions of NADPH oxidase were decreased, whereas p38-MAPK gene expressions were not significant compared with cisplatin-treated group. In conclusion combined curcumin and α-tocopherol are able to reduce cisplatin-induced nephrotoxicity via possible inhibition of NADPH oxidase, resulting in improvement of kidney function and histology.[79]

GholamrezaKarimi, et al.,(2009) Demonstrated protective effect of aqueous and ethanolic extracts of *portulacaoleracea* against cisplatin induced nephrotoxicity in rats. Blood urea nitrogen (BUN) and serum creatinine (Scr) concentration were determined. Morphological renal damage was evaluated. Results indicated that treatment with aqueous and ethanolic extracts of *P. oleracea in* cisplatin induced toxicity reduced BUN and Scr. Tubular necrotic damage was not observed. It concluded P. *oleracea* extract may protect against cisplatin-induced renal toxicity and might serve as a novel combination agent with cisplan to limit renal injury.[80]

Annie shirwaikar, et al., (2003) Studied protective role of Ethanolic extract of flowers of *pongamiapinnata* against cisplatin and gentamicine induced renal injury in rats. Body weight, blood urea and serum creatinine was observed. Extract at the dose of 600mg/kg normalized the raised blood urea and serum creatinine levels in histopathological examination reversal of cisplatin and gentamicine renal cell damage as induced tubular necrosis, marked congestion of the glomeruli with glomerular atrophy, degeneration of tubular epithelial cells with casts in the in the tubular lumen and infiltration of inflammatory

cells in the interstitium were confirmed. In addition ethanolic extract of flowers had a marked nitric oxide free radical scavenging suggesting an anti-oxidative property.[81]

Salma Khanametal, et al., (2011) Investigated the importance of *Tinospora cordifolia* stem extract for its possible curative effect in male wistar rats against the cisplatin induced nephrotoxicity. There was an increase in serum creatinine, blood urea nitrogen and alkaline phosphatase in rats treated with Cisplatin. Administration of Cisplatin followed by alcoholic extract of *Tinospora cordifolia* decreased the increased levels of serum creatinine, blood urea nitrogen and alkaline phosphatase in rats. These biochemical observations were supplemented by histopathological examination of kidney section. Results of this study revealed that the alcoholic stem extract of *Tinospora cordifolia* has curative action against Cisplatin induced nephrotoxicity.[82]

Amr A. Fouadetal, et al., (2010) Investigated nephroprotective effect of coenzyme Q10 in mice with acute renal injury induced by a single i.p. injection of cisplatin. Coenzyme Q10 treatment was applied for 6 consecutive days, 1 day before cisplatin administration. Coenzyme Q10 significantly reduced glutathione level and superoxide dismutase activity, suppressed lipid peroxidation, decreased the elevations of tumor necrosis factor nitric oxideand platinumion concentration, and attenuated there ductions of selenium and zinc ions in renal tissue resulted from cisplatin administration. Histopathological renal tissue damage mediated by cisplatin was ameliorated by coenzymeQ10 treatment. Immunohistochemical analysis revealed that coenzymeQ10 significantly decreased the cisplatin-induced overexpression of inducible nitric oxide synthase, nuclear factor- B, caspase-3andp53inrenaltissue It was concluded that coenzyme Q10 represents a potential therapeutic option to protect against acute cisplatin nephrotoxicity in clinical practice.[83]

N.sheena, etal,. (2003) Evaluated the preventive effect of the methanolic extract of *G.Lucidum* to ameliorate cisplatin-induced nephrotoxicity in mice. Nephrotoxicity was assessed   by determining the serum creatinine and urea levels and renal antioxidant status in mice after cisplatin administration Methanolic extract of *G.lucidum given* orally before cisplatin injection. The extract significantly reduced the elevated serum creatinine and urea

levels. Renal antioxidant defense systems, such as superoxide dismutase, catalase, glutathione peroxidase activities and reduced glutathione level, depleted by cisplatin therapy were restored to normal by treatment with the extract. Cisplatin-induced lipid peroxidation was found markedly reduced by treatment with the extract. The results indicated that methanolic extract of *G. lucidum* rendered significant preventive effect against cisplatin-induced nephrotoxicity. The findings suggest the potential therapeutic use of South Indian *G. lucidum* in cancer chemotherapy. [84]

Kundan, G.,inganle, et al., (2013) Evaluated the nephroprotective effective of methanolic extract of *hygrophila spinosa* (HSME) in cisplatin induced acute renal failure in rats .HSME were a dministered orally to male wistar rats, the parameters glutathione (GSH), catalase (CAT), superoxide dismutase (SOD)and GSH peroxidase activities . histopathological examination was carried out. The result revealed that HSME pretreatment significantly reduced blood urea and also attenuated cisplatin induced increase in MDA and decrease in reduced GSH and CAT and SOD and GSH peroxidase activites in renal cortical homogenates. Histopathological examination showed that HSME marked ameliorated cisplatin induced renal tubular necrosis.It concluded that the aerial parts of *H.spinosa* are endowed with nephroprotective activity.[85]

Salman khan M, et al., (2013) Investigated chemotherapeutic potential of *Boerhaavia diffusa* linked from ancient time to the present with the scope in future. Furthermore a recent update on mechanistic approaches of *B. diffusa* has also been discussed. Based on antioxidant & antdiabetic characteristic it is hypothesized that *Boerhaavia diffusa* might exhibit antiglycating properties as well.[86]

Kanjoormana Aryan Manu, et al., (2009) Evaluated Immunomodulatory activities of punarnavine, an alkaloid from *B. diffusa* using Balb/C mice. Punarnavine enhanced proliferation of splenocytes, thymocytes and bone marrow cells both in presence and absence of specific mitogens in vitro and in vivo. More over administration of Punarnavine significantly reduced the LPS induced elevated levels of pro inflammatory cytokines such as TNF-α, IL-1β and IL-6 in mice. [87]

Protective effects of hydro alcoholic extract of Boerhaavia diffusa linn against cisplatin induced nephrotoxicity in rats

35

Mandeepkaur, et al., (2009) Investigated the methanolic extract of *Boerhaavia diffusa* roots & its different fraction including liriodendrin rich fraction for exploring the possible role of liriodendrin rich in its anticonvulsant activity. These finding concluded that observed anticonvulsant activity was due to calcium channel antagonistic action as this activity was retained only in liodendrin rich fraction which posse's significant anticonvulsant activity of liriodendrin in BAY K-8644 induced seizures.[88]

Sandhya.k, et al., (2010) Had conducted a comparative study of hydro alcoholic extract and poly herbal formulation of *Boerhaavia diffusa* for their anti-stress activity using cold restrainst stress model. Due to cold restrain stress there was imbalance in the level of biochemical parameter like glucose, triglycerides, cholesterol, SGOT, SGPT which were near normalized following the administration of HAEBD & PHF-09. HAEBD and PHF-09 were found to have comparable anti stress activity.[89]

Surendar. k. Pareta, et al., (2011) Found out the effects of pretreatment of aqueous extract of *Boerhaavia diffusa* root (200-400 mg/kg /day) in repeated dose acetaminophen nephrotoxic rats for 14 days. Acetaminophen administration characterized by significant increase in Blood urea nitrogen (BUN), serum creatinine and increased level of kidney malondialdehyde proleinthiol, along with depletion of SOD, CAT, GPX and GSH. .Histopathological changes showed significant structural damage to kidney. The result suggest that *Boerhaavia diffusa* has the potential in preventing the acetaminophen induced Nephrotoxicity.[90]

Ramachandran. y. L, et al., (2011) Evaluated the hepatoprotective properties of petroleum ether extract, Methanolic extract and isolated compound of *B. diffusa* & A. lanata against carbon tetra chloride induced hepatic damage in rats. This study reveals that different dose of plant extract offer significant protection of serum test and liver histology.[91]

Shisode. k.s, et al., (2011) Had studied that different extract of roots of *Boerhaavia diffusa* for invitro antioxidant activities & phytochemical screening. Among these there extract, ethanolic extract had shown better antioxidant activity & phytochemical screening revealed the presence of carbohydrate saponins, proteins, flavonoids, steroids, fats & alkaloid. [92]

Gopal. T.k, et al., (2010) Evaluated invitro antioxidant activities of chloroform, ethanol & ethyl acetate fraction of *B. diffusa.L* which might have improved it' hepatoprotective action. The extract found to have significant nitric oxide and DPPH radical scavenging activity. The result suggest that roots of *Boerhaavia diffusa* were found to reveal antioxidant potential which support the use of plant in traditional medicine.[93]

ApurbasarkerApu. et al., (2012) Investigated the bioactivities of crude n-hexane, ethyl acetate and methanolic extract of aerial parts of the *Boerhaavia diffusa* linn and its phytochemical analysis. Methanolic extracts showed higher antioxidant, thrombolytic activity and less cytotoxic activity than that of n-hexane & ethyl acetate extract of *Boerhaavia diffusa*. All the extract showed significant inhibitory activity against *candida albicans* at a concentration of 1000µg/disc. These findings suggest that plant could be important source of medicinally important natural compound.[94]

ShuklaAnamik, Gupta Rakeshkumar, (2011) Studied the effects of aqueous extract of *Boerhaavia diffusa* roots and leaves on blood sugar level is alloxan induced diabetic rats. These studies conclude that aqueous extract of *B. diffusa* have shown hypoglycemic effect may be due to presence of glycosides, flavonoids, tannins and saponin in the extract. [95]

Suralkas A.A et al., (2012) Investigated antihistamine activity of ethanolic extract of *Boerhaavia diffusa* linn roots using isolated goat tracheal chain and histamine induced bronchoconstriction in Guinea pig *Boerhaavia diffusa* significantly inhibited dose dependent contraction of goat tracheal chain produced by histamine and also showed significant protection by prolonging pre convulsion dyspnoea time in guinea pigs. Thus *Boerhaavia diffusa* showed antihistaminic and broncho dilating activity against histamine and hence posses potential role in treatment of asthma.[96]

Surendran. k, et al., (2010) Evaluated anti urolithiatic activity of *Boerhaavia diffusa linn* root aqueous extract and rationalize its use in treating renal stone. The lithogenic treatment causes weight loss, hyperoxalurea and impairment of renal function. *Boerhaavia diffusa* linn causes diuresis and hasten the process of dissolving crystals and helps in mechanical expulsion of stones and improve the renal function by removing the waste product and decrease oxalate excretion by interfering with metabolism. Results of this study indicate

Boerhaavia diffusa lin aposses antiurolithiatic that possibly mediated through diuretic and hypo-oxaluric effects.[97]

Mahesh, A.R, et al., (2012) Had conducted a detailed study on *Boerhaavia diffusa* for it is medicinal importance. Various phytochemical, pharmacological, experimental and clinical investigations are done on *Boerhaavia diffusa*. This include evidence based over view of pharmacological, phytochemical properties of aerial parts & the roots of *Boerhaavia diffusa*, which may be helpful to establish a standard natural drug for further studies.[98]

Meena, A.K, et al., (2010) Investigated the standardized and phytochemically evaluated aqueous and hydroalcoholic extracts of *Boerhaavia diffusa*. It involves pharmacognostical examination of morphological and microscopical characters and phytochemical investigation of *Boerhaavia diffusa* including determination of loss on drying, ash values, TLC and extractive values. The qualitative chemical examination revealed the presence of various phytoconstituents like carbohydrate, saponins, phenolic compound and mucilage in the extract .[99]

Babita Aqrawal,et al., (2011) Have Investigated a review on it is phytochemical and pharmacological profile Phytochemical studies had shown the presence of rich source of alkaloids, steroids and flavones. Pharmacological research explains hepatoprotective, diuretic, anti-inflammatory, anti-stress and immunomodulation anti fertility, antimicrobial, antiviral, and insecticidal activities. In conclusion *Boerhaavia diffusa* contain biologically active compounds that may serve as candidate for new drugs in the treatment and prevention of human livestock diseases.[100]

Goyal.B.M et al., (2010) Analyzed an overview of pharmacological potential of *Boerhaavia diffusa*. It covers various physiology, pathology of disease and their therapies. This article includes evidence based information regarding pharmacological activity of this plant. It has many ethanol botanical users and is medicinally used in the traditional Ayurvedic system.[101]

Bhavin, A, et al., (2013) Investigated the effect of hydro alcoholic extract of roots of *Boerhaavia diffusa* in experimental Beningn prostatic hyperplasia in rats. Body weight, prostate weight, bladder weight and serum testosterone were measured and histological

studies were carried out. The result suggested that treatment with *Boerhaavia diffusa* may improve symptoms of disease and inhibit the increased prostrate sign. Invitro study implies that herbal extract had a beneficial effect on prostatic smooth muscles which relieve the urinary symptom and disease. [102]

Krishna murti, et al., (2001) evaluated antidiabetic activity of ethanolic extract of roots of *Boerhaavia diffusa* against streptozocin induced experimental rats. Blood glucose level were determined on 0, 7th, 14, & 21st day after oral administration. The effect of ethanolic extract of *B.diffusa* on serum lipid profile like total cholesterol, triglycerides, LDL, VLDL, HDL were also measured in diabetic and non-diabetic rats. The ethanolic extract of *Boerhaavia diffusa* was found to reduce blood sugar level in streptozocin induced diabetic rats. There was significant reduction in total cholesterol, LDL, VLDL, & improvement in HDL cholesterol in diabetic rats. The results indicated that *Boerhaavia diffusa* possess hypoglycemic & anti-hyperlipidemic effect. [103]

Ajmire. P.V, etal., (2011) Had conducted study of alcoholic & aqueous extract of whole plant of *Boerhaavia diffusa* against DMNO induced liver cirrhosis in rat's model. The activity was assessed using ILS, histopathological studies of liver, biochemical &heamatological studies. EEBD & AEBD shows significant increase in survival time, a decrease in cirrhotic nodules. The biochemical &heamatological parameter were also corrected by EEBD & AEBD in DMN induced rats. However out of these two extract, EEBD shows maximum anti cirrhotic effect than AEBD. [104]

Venkatesh. P, et al., (2010) Evaluated analgesic & antipyretic activity of various doses of alcoholic extracts of stem & leaves of *Boerhaavia diffusa* & leaves of *Anisochiluscarnosus*. Tail immersion method & Hot plate in mice were studied for analgesic activity. Alcoholic extract of *Boerhaavia diffusa* had shown significant analgesic & antipyretic activity. [105]

Mohammed Khalid, et al., (2012) Had studied pharmacological evaluation and qualitative analysis of *Boerhaavia diffusa L.* root. Various parameters like macroscopic, microscopy, fluorescence analysis as well as extraction value and qualitative phytochemical screening of

different extraction were studied. The major components of extractions like total phenolic, total flavonoids were also estimated. [106]

Venkatesh. P, et al., (2012) evaluated a study on alcoholic extract of stem and leaves of *Boerhaavia diffusa* and leaves of *Anisochiluscarnosus* on CCl$_4$ induced hepatotoxicity in rats. Different dose levels administered. Biochemical parameters of liver like SGOT, SGPT, serum alkaline phosphatase, total and direct serum bilirubin were determined. It was concluded that the alcoholic extract of AEBD and AEAC possess hepato protective activity against ccl4 induced hepatotoxicity in rats. [107]

AIM OF THE STUDY:

The present study was undertaken to find out the effect of the potential nephroprotective effect of *Boherhavia diffusa* and their effects on cisplatin induced nephrotoxicity in wistar rats.

Plants have played a weighty role in maintaining human health and improving the quality of human life for thousands of years and have several precious components of medicines, seasonings, beverages, cosmetics and dyes. Herbal medicines are based on the premise that plants contain natural substances that can promote health and alleviate illness. In recent times, focus on plant research has increased all over the world and large evidence has collected to show immense potential of medicinal plants used in various traditional systems.

Today we are witnessing a great deal of public interest in the use of herbal remedies. Many western drugs had their origin in plant extract. There are many herbs, which are preponderantly used to treat cardiovascular problems, liver disorders, central nervous system, digestive and metabolic disorders. Given their potential to generate significant therapeutic effect, they can be useful as drug or supplement in the treatment, management of many diseases. Herbal drug or medicinal plants, their extracts and isolated compounds have demonstrated spectrum of biological activities. Such have been used and continued to be used as medicine in folk–fore or food supplement for various disorders.

In our country, so many plant preparations are used for the treatment of disease. Only limited number of institutions carries out these research studies. Still there are a number of plants that have not been screened and mostly used by tribals.

The present investigation was undertaken to study the effect of *Boherhavia diffusa* on changes in kidney parameters such as Blood urea nitrogen, serum creatinine, urea and anti-oxidant enzymes in cisplatin-induced nephrotoxicity in rats. The effects produced by this plant extract on different parameters were compared with silymarin.

PLAN OF WORK

- Collection of plant material (*Boherhaavia diffusa*) and preparation of plant extracts.

- Evaluation of Nephroprotective activity *Boherhaavia diffusa* and their effects on Kidney, anti-oxidant enzymes.

- Selection, grouping and acclimatization of the animals.

- Induction of nephrotoxicity in rats by cisplatin.

- Treatment protocol.

- Evaluation of serum parameters and kidney enzymes.

- Histopathological examinations of kidney rats.

Dr. D. Stephen,   **The American College,**
Lecturer,   **Madurai-2**
Department of Botany.

<u>CERTIFICATE</u>

This is to certify that the plant specimen brought to me by

Mr.MANOHARAN .B, II year M**. Pharm (Pharmacology**)**; Student of**

K.M.College of pharmacy, Madurai has been identified as whole plant

of *Boerhaavia diffusa* belonging to the family **Nyctaginaceae.**

Dr.D.Stephen.

Date ; 22/08/2013

Madurai

Tamil nadu

PLANT PROFILE

Figure. No.8: *Boerhaavia diffusa* linn

Figure. No.9: *Boerhaavia diffusa* linn

PLANT PROFILE

Scientific Name **:** *Boerhaavia diffusa* linn. Syn. *B. repens; B. repens* var. *diffusa.*

Family **:** Nyctaginaceae.

Family Name **:** Hog weed, Horse Purslane.

Taxonomical classification:

Kingdom **:** Plantae.

Family **:** Nyctaginaceae.

Division **:** Magnoliophyta.

Class **:** Magnoliopsida.

Order **:** Caryophyllales.

Genus **:** *Boerhaavia.*

Species **:** B.diffusa.

Common Names:

Raktapunarnava, Shothaghni, Kathillaka, Kshudra, Varshabhu, Raktapushpa, Varshaketu, Shilatika.

Vernacular Names: [108]

Bengali   :   Raktapunarnava.

English   :   Horse Purslene, Hog Weed.

Hindi   :   Gadapurna, Lalpunarnava.

Kannada   :   Sanadika, Kommeberu, Komma.

Malayalam   :   ChuvannaTazhutawa.

Tamil   :   Mukurattai (Shihappu).

Telugu   :   Atikamamidi, Erragalijeru.

GEOGRAPHICAL DISTRIBUTION:

Boerhaavia diffusa is also indigenous to India; it is found throughout the warmer parts of the country up to an altitude of 2000 m in the Himalayan region. The genus *Boerhaavia* has several species, and is distributed in the tropical, subtropical, and temperate regions of the world. [109] It is found in Australia, China, Pakistan, Sudan, Sri Lanka, Egypt, South Africa, USA and in several countries of the Middle East. Out of the 40 species of this genus, 6 species are found in India – *B. diffusa, B. chinensis, B. erecta, B. repens, B.rependa*, and *B. rubicund.*

ORIGIN AND HABITAT:

Boerhaavia diffusa is a perennial creeping weed, prostrate or ascending herb, up to 1 m long or more, having this is found throughout India. It grows up to an altitude of 70 centimeters especially during the rainy season. It has a large root system and produces yellow and white flowers. It can be found in many tropical and warm-climate countries. [110]

USEFUL PARTS:

Root, leaves & seeds, stem, flowers, fruits.

DESCRIPTION:

Boerhaavia diffusa is a perennial creeping weed, prostrate or ascending herb, up to 1 m long or more, having spreading branches.

The roots are very variable diffusely branched low spreading or creeping herbaceous perennial with an elongated fusiform or tapering tap root. The roots are stout and fusiform with a woody.

The stems are numerous; 1-2 m long & the stem is prostrate, woody or succulent, cylindrical, often purplish, hairy, and thickened at the nodes.

Leaves are simple, thick, fleshy and hairy arranged in unequal pairs, green and glabrous. The shape of the leaves varies considerably ovate oblong, round or subcordate at the base and smooth above. The leaves are simple, opposite, short petiolate, exstipulate, unequal in each pair, 2.5-5 cm long by 1-4.5 cm wide, oblong or suborbicular, acute, obtuse or rounded at apex, cordate rounded or truncate at base, entire or wavy along the margin, subfleshy, glabrous or sparingly hairy above, silvery white beneath, petioles 0.7-3 cm long, slender, deeply grooved above.

The flowers are small, regular, sessile or subsessile, pale rose to pink, in irregular clusters of 4-10, small umbels on extra axillary peduncles.

The fruits are very small, one seeded and enclosed in persistent lower half the perianth. The perianth is covered with sticky glandular hairs.

Table.No:1

Part	*Boerhaavia diffusa*
Plant	A perennial herb from a fusiform root
Leaves	Opposite or sub-opposite, two of a node unequal, broadly ovate or sub-orbicular, obtuse to rounded or sub-cordate at the base.
Stem	Prostrate, decumbent or ascending, 4-10 cm long, rather slender, divaricately branched
Flowers	In pendunculate, glomerulate clusters arranged in slender, long stalked, axillary or terminal corymbs
Fruit	Ovoid or sub-ellipsoid, rounded above, slightly cuneate, below, broadly and bluntly 5-ribbed, very glandular throughout
Flowering and Fruiting	Throughout the year in Indian conditions

Phytochemicals:

      Boerhaavia diffusa contains a large number of phytoconstituents, namely flavonoids (Pandey et al., 2005), alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates proteins and glycoproteins (Surange and Pandse1972).

- Plant also includes a series of rotenoids boeravinones from roots of the plant viz., Boeravinone (A-F) [111-118]
- Punarnavoside, a phenolic glycoside, is reportedly present in roots.[119-121] C-methyl flavone also has been isolated from *Boerhaavia diffusa* roots.
- Two known lignans viz.,liriodendrin and syringaresinol mono-β-D-glycoside isolated.[122]
- Presence of a purine nucleoside hypoxanthine 9-L-arabinose ,dihydroisofuroxanthone-borhavine , phytosterols have been isolated from the plant.

- It contains about 0.04 % of alkaloids known as punarnavine and punernavoside an antifibrinolytic agent.

- It also contains about 6 % of potassium nitrate an oily substance and ursolic acid.[123]

- The seeds of this plant contain fatty acids and allantoin and the roots contain alkaloids.[124] The green stalk of the plant has also been reported to contain boerhavin and boerhavic acid.

Pharmacological and Clinical properties of *B. diffusa*

Various parts of *B. diffusa* are used for the treatment of numerous disorders in different parts of India. The root, leaves, aerial parts or the whole plant of *B. diffusa* have been employed for the treatment of various disorders in the Ayurvedic herbal medicine. The pharmacological studies have demonstrated that the roots of *B. diffusa* exhibit a wide range of properties such as hepatoprotectant, anticonvulsant activity, immunomodulatory activity, It is clinically proved as a useful and safe drug in the patient of nephritic syndrome & cancer chemopreventive property against papillomagenesis. potent antibacterial activity, diuretic, anti-inflammatory, antifibrinolytic, antibacterial, antistress agent, antihepatotoxic, anthelmintic febrifuge, antileprosy, anti-asthmatic, antiscabies, and anti-urethritis, and antinematodal activity .
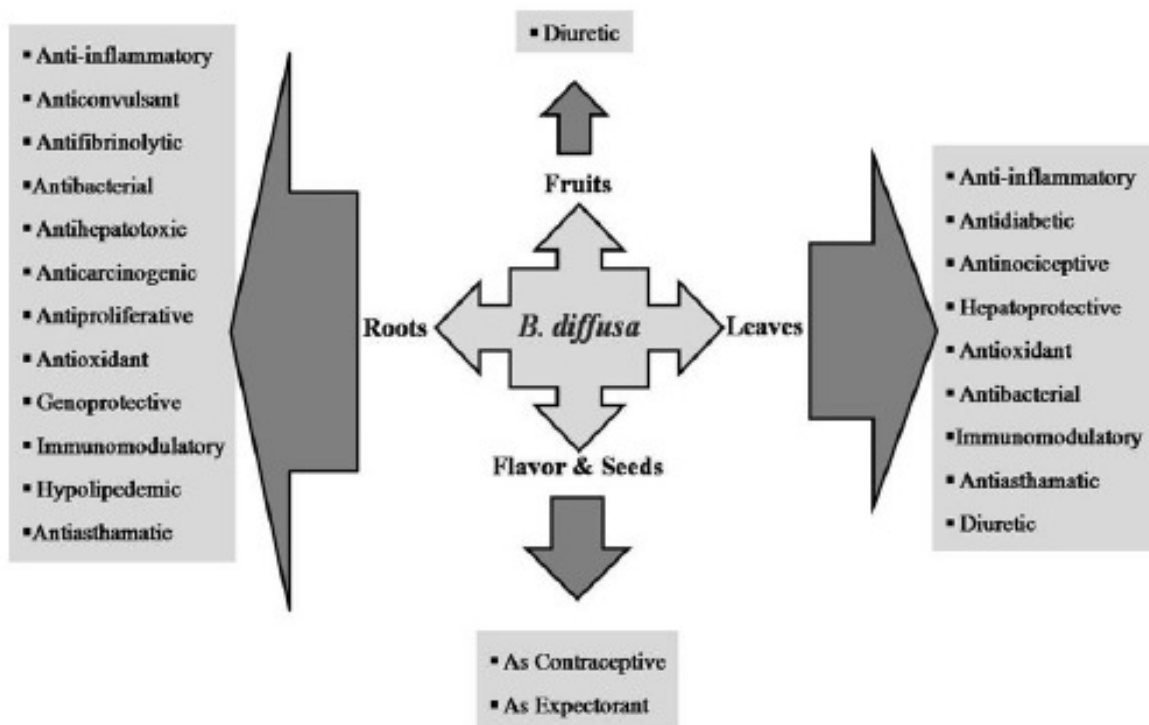
- Diuretic

Fruits

Roots ← **B. diffusa** → **Leaves**

Flavor & Seeds

Roots:
- Anti-inflammatory
- Anticonvulsant
- Antifibrinolytic
- Antibacterial
- Antihepatotoxic
- Anticarcinogenic
- Antiproliferative
- Antioxidant
- Genoprotective
- Immunomodulatory
- Hypolipedemic
- Antiasthamatic

Leaves:
- Anti-inflammatory
- Antidiabetic
- Antinociceptive
- Hepatoprotective
- Antioxidant
- Antibacterial
- Immunomodulatory
- Antiasthamatic
- Diuretic

Flavor & Seeds:
- As Contraceptive
- As Expectorant

Chart. No:2 pharmacological and clinical properties of *Boerhaavia diffusa.*

PHYTOCHEMICAL AND QUALITATIVE ANALYSIS

EXTRACTION METHODS:

Whole plant of *Boerhaavia diffusa* were collected from Tamilnadu, kerala forest, shed dried for a week in a shadow and blended to coarse powder.

About 500gm of dried fine powder of *Boerhaavia diffusa* were soaked in the extractor and macerated for 30 hrs with petroleum ether .There it is reflexed successfully with chloroform, after that it is extracted with alcohol and water by continuous hot percolation method using soxhlet apparatus for 40 hrs separately. Hydro alcoholic extracted was filtered and concentrated in vacuum using rotary flask evaporator under reduced pressure .After concentration hydro alcoholic extract of *Boerhaavia diffusa* given brownish residue stored in air tight container were subjected to qualitative test for identification of various plant constituents.

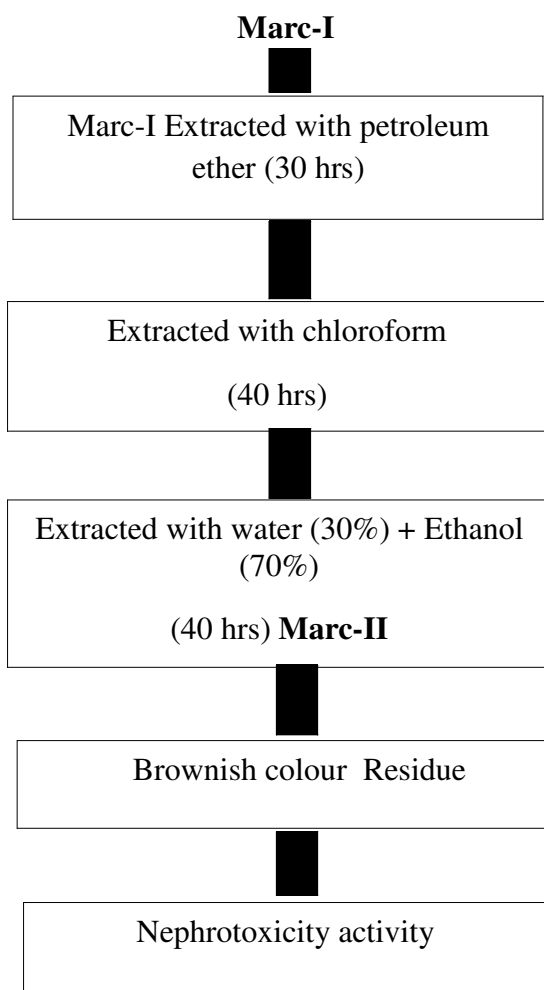**Dried whole plant of *Boerhaavia diffusa* material extracted by soxhlet method**

Marc-I

Marc-I Extracted with petroleum ether (30 hrs)

Extracted with chloroform (40 hrs)

Extracted with water (30%) + Ethanol (70%) (40 hrs) **Marc-II**

Brownish colour Residue

Nephrotoxicity activity

Chart. No: 3 Extraction procedure.

PHYTOCHEMICAL INVESTIGATION OF EXTRACTS OF

Boerhaavia diffusa Linn.

All the extracts of leaf of *Boerhaavia diffusa* linn were subjected to various tests for identification of constituents.

1) <u>Detection of Carbohydrates</u>:

Small quantities of ethanolic and aqueous extract were dissolved in distilled water separately and filtered. The filtrates were taken for the various tests to detect the absence of carbohydrates. Absence of carbohydrate in *Boerhaavia diffusa.*

A. Molisch's Test:

The filtrates were treated with 2-3 drops of 1% alcoholic - napthol and 2 ml. of concentrated sulphuric acid was added along the sides of the test tube. A brown ring was observed. Ethanolic *Boerhaavia diffusa* plant extract showed the absence of sugar.

B. Fehling's Test:

Small portion of the filtrates were treated with equal volume of Fehling's solution A and B and then heated. A brick red precipitate formed in alcoholic plant extract of *Boerhaavia diffusa* show absence of reducing sugar.

C. Benedict's Test:

Small portion of the filtrates were treated with equal volume of Benedict's reagent. A yellow precipitate was formed in alcoholic extract *Boerhaavia diffusa* indicating the absence of reducing sugar

D. Barfoed's Test:

Small portion of the both the plant extract was treated with Barfoed's reagent. Red precipitate was not formed in the ethanolic plant extract *Boerhaavia diffusa*.

E. Test for Starch:

A small amount of the *Boerhaavia diffusa* ethanolic plant extract was treated with dilute iodine solution. No bluish black colour was observed in the both the plant extracts showing the absence of starch**.**

2) Tests for Gums and Mucilage's:

Alcoholic precipitation and Molisch's test.

The, *Boerhaavia diffusa* plant extract was treated with absolute alcohol, stirred and filtered. The filtrate was dried and examined for its swelling properties. The extracts were answered for the presence of gums and mucilage.

3) Test for Proteins and Amino Acids:

Small quantities of alcoholic extract was dissolved in few ml of distilled water and subjected to Ninhydrin test, Xanthoprotein test, test with tannic acid and heavy metals.

A. Ninhydrin Test:

Ethanolic extract of the both the plants were treated with ninhydrin reagent (0.1% solution) and boiled. Purple colour was observed indicating the presence of protein.

B. Biuret Test:

To a portion of the above prepared extracts, equal volumes of 5% w/v sodium hydroxide and 4 drops of 1% w/v copper sulphate solution were added. Violet colour was formed, indicating the presence of protein in the extract.

C. Millon's test of cole's mercuric nitrite test**:**

To the above-prepared extracts, millon's reagent was added. White precipitate was formed, showing the presence of protein in the extract.

D. Xanthoprotein Test:

To 3 ml of the above-prepared extracts, 1 ml of the concentrated nitric acid was added, boiled for one minute, cooled and concentrated ammonia was added till alkaline. An orange colour was not formed, showing the presence of protein in the extracts.

4) <u>Test for Fixed Oils and Fats:</u>

A. Spot Test:

A small quantity of various extracts was pressed between two filter papers. Oil stains were observed with the extracts indicating the presence of fixed oils and fats.

B. Saponification Test:

Few drops of 0.5 N alcoholic potassium hydroxide was added various extracts along with of few drops of phenolphthalein. The mixture was heated on a water bath for one hour. Soap was formed with the extracts indicating the presence of fixed oils and fats.

5) <u>Test for Alkaloids</u>:

Small amount of the solvent free ethanolic and aqueous extracts were separately stirred with a few ml of dilute HCl and filtered. The filtrates were tested with various alcoholic reagents.

A. Mayer's test :

To the small quantities of the extracts, Mayer's reagent was added. Presence of cream-colored precipitate indicates the presence of alkaloids in both the extracts.

B. Dragendorff's Test:

To small quantity of extracts, Dragendorff's reagent was added. Presence of orange brown precipitate indicates the presence of alkaloids in both plant extracts.

C. Wagner's Test:

To small quantity of the extracts, Wagner's reagent was added. Presence of reddish brown precipitate, indicate the presence of alkaloids in *Boerhaavia diffusa* extract

D. Hager's Test:

To small quantity of the extracts, Hager's reagent was added. Presence of yellow precipitate, indicate the presence of alkaloids in *Boerhaavia diffusa* extract.

6) Tests for Glycosides:

A small amount of the different extracts were dissolved separately in 5 ml of distilled water and filtered. Another portion of the extracts were hydrolyzed with hydrochloric acid for one hour on a water bath and hydrolyzate was subjected to Legal's, Baljet's, Borntrager's, Keller-Killani's tests and for the presence of cyanogenetic glycosides.

A. Legal's Test:

To the hydrolyzate, 1 ml of pyridine and a few drops of sodium nitroprusside solution was added and then made alkaline with sodium hydroxide solution. Pink colour was observed in *Boerhaavia diffusa* extract.

B. Baljet's Test:

To a section of plant extract, sodium picrate solution was added. Yellowish orange colour was observed in *Boerhaavia diffusa* extract.

C. Borntrager's Test:

Hydrolyzed was treated with chloroform and the chloroform layer was separated. To this, equal quantity of dilute ammonia solution was added. Pink colour was not observed in the ammonical layer of chloroform and both the extracts showed the absence of glycosides.

D. Test for Deoxy Sugar (Keller-Killani Test):

To the different extracts 10 ml of 70% alcohol were added, boiled on a water bath, filtered. The filtrates were diluted with 1 ml of distilled water; 1ml of strong lead acetate solution was added and filtered. The filtrates were extracted with an equal volume of chloroform. The chloroform layer was pipetted out and evaporated to dryness. The residue obtained was dissolved in 3 ml of 3.5% of ferric chloride in glacial acetic acid, left for one minute and then transferred to a test tube. To the side of the test tube, 1.5 ml of sulphuric acid was added carefully, which formed a separate layer at the bottom and kept for few minutes.

Blue colour at the interface and pale green colour in the upper layer was not observed in any of the extracts indicating the absence of cardiac glycoside.

7) <u>Test for Phytosterols:</u>

Small quantities of the various extract were dissolved in the 5 ml of chloroform separately. Then these chloroform solutions were subjected to Libermann's test, Libermann-Burchard's test, Salkowski's test.

A. Libermann-Burchard's Test:

The residue was dissolved in chloroform. To this Libermann-Burchard's reagent was added. Green colour was produced in both the extract indicating the presence of phytosterols.

B. Salkowski's test:

A few drops of concentrated sulphuric acid were added to chloroform solution. The lower layer of the solution turned brownish red colour with both the extracts indicating the presence of phytosterols.

8) <u>Test for Flavanoids:</u>

The different extracts were separately dissolved in ethanol and then subjected to the following tests.

A. Ferric chloride Tests:

To a small quantity of the ethanolic extract, few drops of neutral ferric chloride were added. Blackish red colour was observed in *Boerhaavia diffusa* extract indicating the presence of flavonoids.

B. Shinoda's test:

A small quantity of the extract was dissolved in alcohol and to this magnesium metal followed by concentrated hydrochloric acid, was added drop wise and heated. A magenta colour was produced in *Boerhaavia diffusa* extract indicating the presence of flavonoids.

C. Flavones:

1. With sodium hydroxide solution, the extract gave yellow colour.
2. Ethanolic extract gave orange colour with concentrated sulphuric acid.
.

9) <u>TEST FOR TANNINS:</u>

The extracts were dissolved in water and filtered. The filtrates were treated with various reagents.

A. Ferric chloride test:

Few ml of the filtrates were treated with 5% ferric chloride solution. A bluish black colour was observed indicating the presence of tannins in both the extracts.

B. Reaction with lead acetate:

Few ml of the filtrates were treated with lead acetate solution. White precipitates were produced in *Boerhaavia diffusa* extract indicating the presence of tannins.

C. Gelatin Test:

The extract were dissolved separately in minimum amount of water and filtered. To the filtrate, add 1 ml of 1 % solution of gelatin. Both the extract did not produce any white precipitate.

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QUALITATIVE CHEMICAL ANALYSIS OF ETHANOLIC EXTRACT OF

Boerhaavia diffusa Linn.

Table.No:2

SI.NO	Test for plant constituents	*Boerhaavia diffusa Linn.*
1	*Test for alkaloids*	
	a. Mayer's Test	+
	b. Dragendorff's Test	+
	c. Wagner's Test	+
	d. Hager's Test	+
2	*Test for Glycosides*	
	a. Legal's Test	+
	b. Baljet's Test	+
	c. Borntrager's Test	+
	d. Keller-Killani's Test	+
3	*Test for Flavanoids*	
	a. Ferric chloride Test	+
	b. Shinoda's Test	+
	c. Fluorescence Test	+
	d. Reaction with alkali and acid	+
4	*Test for Tannins and Phenolic Compounds*	
	a. 5% Ferric chloride solution test	+
	b. Reaction with lead acetate	+
	c. Gelatin test	_
6	*Test for Proteins and amino acids*	
	a. Ninhydrin Test	+
	b. Biuret Test	+
	c. Millon's test or Cole's Mercuric Nitrate test	+
	d. Xanthoprotein test	+
7	**Test for Carbohydrates**	
	a. Molisch's test	-
	b. Fehling's test	-
	c. Benedict's test	-
	d. Barfoed's test	-
	e. Test for Starch	-

8	**Test for Gums and Mucilage**	
	Alcoholic precipitation and Molisch's test	+

EXPERIMENTAL MODELS

For the study of Nephroprotective and antioxidant activity an animal model was added that would satisfy the following conditions.

- ❖ The animal should develop nephrotoxicity rapidly and reproducibly.
- ❖ Pathological changes in the site of induction should result from kidney damage.
- ❖ The symptoms should be ameliorated or prevented by a drug treatment effective in human beings .
- ❖ The drug tested should be administered orally.
- ❖ Drug dosage approximate the optimum therapeutic range for human, scaled the test animal weight.

LABORATORY ANIMAL MODELS

Induction of nephro toxicity and free radicals in animal model:

The *invivo* mechanisms of cisplatin nephrotoxicity are complex and involve oxidative stress, inflammation and apoptosis.[125] Apoptotic markers include proteins from both pro-apoptotic and anti-apoptotic elements. Bak is a pro-apoptotic member of the Bcl-2 family of apoptotic proteins. The Bcl-2 related proteins interact with one another through the formation of homo and heterodimers.

The susceptibility of cells to apoptotic stimuli is thought to be controlled by the relative ratios of the different Bcl-2 family proteins. This protein localizes to mitochondria and functions to induce apoptosis. It interacts with and accelerates the opening of the mitochondrial voltage-dependent anion channel, which leads to a loss in membrane potential and the release of cytochrome c.[126]

MATERIALS AND METHODS:

Animals

In-house laboratory bred 6 week old wistar rats were selected for the study. Animals were maintained under controlled temperature at 20 ± 2^0c and relative humidity of 50-60% with an alternating 12hr light/ dark cycle. Food and water provided ad libitum. The research work was approved by Institutional animal Ethical Committee.(IAEC/KMCP/61.REG NO:261225056 M.PHARMA/2012-2014.)

Experimental protocol

Rats were divided into 5 groups of 6 each.

Group 1: Served as Normal Control, which received 10ml/kg of normal saline.

Group 2: Served as Toxic control, which received Cisplatin (7.5mg/kg b.w, i.p) on day one.

Group 3: Served as positive control which received Cisplatin (5mg/kg b.w, i.p) on day one followed by silymarin (50mg/kg b.w, orally) for 10 days.

Group 4: Served as Treatment group, which received Cisplatin (5mg/kg b.w,i.p) single dose on the day one followed by HAEBD at a dose of 200mg/kg orally for 10 days.

Group 5: Served as Treatment group, which received Cisplatin (5mg/kg b.w,i.p) single dose on the day one followed by HAEBD at a dose of 400mg/kg orally for 10 days.

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BIOCHEMICAL ASSAY

On day 11 cisplatin injection was administered to all groups except normal control. After 72 hrs of cisplatin injection animals were sacrificed using ether anesthesia; blood samples were collected by retro orbital puncture for measuring BUN,Scr,Alp and urea. Kidneys were quickly removed and washed with ice –cold normal saline and homogenates (10%w/v) were prepared in PBS.A part of the homogenate was used for the estimation of glutathione (GSH) and lipid per oxidation. The remaining homogenate was centrifuged at 5000 rpm for 10 min at 4^0C; after removal of the cell debris, the supernatant was used for the assay of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX).

Serum creatinine was assayed according to Jaffe's kinetic method,[127] Blood urea nitrogen and urea was assayed according to Berthelot end point assay [128] and Alkaline phosphatase according to pNPP- AMP (IFCC), kinetic assay [129] using Autospan kits.

The GSH level was measured colorimetrically using 5, 5'- Dithio.bis (2 - nitrobenzoic acid) (DTNB) as the substrate. The concentrations of malondialdehyde (MDA) as indices of lipid peroxidation were assessed. The SOD activity was determined by the Nitro blue tetrazolium (NBT) reduction method. The GPx activity was determined by the method. The CAT activity was determined from the rate of decomposition of H_2O_2, method.

Histopathological analysis:

Rats were euthanized under light anaesthesia with ether and the kidneys were dissected out. Kidneys were perfused with buffered saline to remove blood and then the left kidney was fixed in 10% neutralized buffered formalin for histopathology studies. Histopathological studies were done at Apollo diagnostics, Madurai.

Statistical analysis:

The results were expressed as Mean± S.E.M and analyzed with one way analysis of variance between the two groups and followed by newmann keuls multiple range tests. Probability values p≤ 0.05 were considered significant.

Effects of ethanolic extract of *Boerhaavia diffusa* on renal malondialdehyde, glutathione, catalase, and superoxidedismutase, glutathione peroxidase (gp$_x$) activities in treated rats.

TABLE NO: 3

Group No.	TREATMENT DOSE (mg/Kg)	MDAu/ mg Protein	GSH u/mg Protein	CAT u/mg protein	SODu/m g Protein	GPX u/mg Protein
I	Normal control 10ml/kg normal saline	2.11±0.18	15.15±0.40	55.30±1.40	15.30±0.40	23.20±0.90
II	Toxic control 7.5mg/kg Cisplatin induced	4.90±0.42[*a]	4.40±0.20[*a]	10.45±0.35[*a]	6.15±0.16[*a]	10.10±0.32[*a]
III	Positive control 50mg/kg Silymarin	2.85±0.24[*b]	13.40±0.36[*b]	40.90±0.75[*b]	13.05±0.32[*b]	20.15±0.56[*b]
IV	Treatment control HAEBD 200 mg/kg	3.65±0.32[*b]	10.52±0.26[*b]	29.45±0.45[*b]	10.90±0.25[*b]	16.40±0.62[*b]
V	Treatment control HAEBD 400mg/kg	3.05±0.26[*b]	11.25±0.30[*b]	34.50±0.68[*b]	12.60±0.28[*b]	18.25±0.68[*b]

➢ Values are expressed as Mean ± SEM.
➢ Values are found out by using one way ANOVA followed by Newmann kevl's multiple range tests.
➢ *a – values are significantly different from Normal control at $P < 0.01$.
➢ *b – values are significantly different from Toxic control (G2) at $p < 0.01$.

Effect of HAEBD on serum creatinine, blood urea nitrogen &alkaline phosphatase

Table :4

Group. No.	TREATMENT DOSE (mg/Kg)	Serum creatinine	Blood urea nitrogen mg/dl	Alkaline phosphate mg/dl	Urea mg/dl
I	Normal control 10ml/kg normal saline	0.05±0.02	18.55±0.58	155.60±0.75	17.2±0.40
II	Toxic control 7.5mg/kg Cisplatin induced	1.310±0.05[*a]	104.30±5.55[*a]	345.20±1.58[*a]	78.6±1.65[*a]
III	Positive control 50mg/kg Silymarin	0.796±0.03[*b]	38.75±1.28[*b]	186.75±0.90[*b]	24.5±0.48[*b]
IV	Treatment control HAEBD 200 mg/kg	0.870±0.04[*b]	56.10±2.05[*b]	248.60±1.05[*b]	46.4±0.56[*b]
V	Treatment control HAEBD 400mg/kg	0.805±0.03[*b]	44.25±1.60[*b]	210.45±0.96[*b]	38.2±0.50[*b]

➢ Values are expressed as Mean ± SEM.
➢ Values are found out by using one way ANOVA followed by Newmannkeul's multiple range

  tests.
➢ *a – values are significantly different from Normal control at $P < 0.01$.
➢ *b – values are significantly different from Toxic control (G2) at $p < 0.01$.

Figure.No: 10

SERUM LEVELS OF MDA U/G PROTEIN VALUES OF MEANS OF 6 RATS IN EACH GROUP.

Chart Title
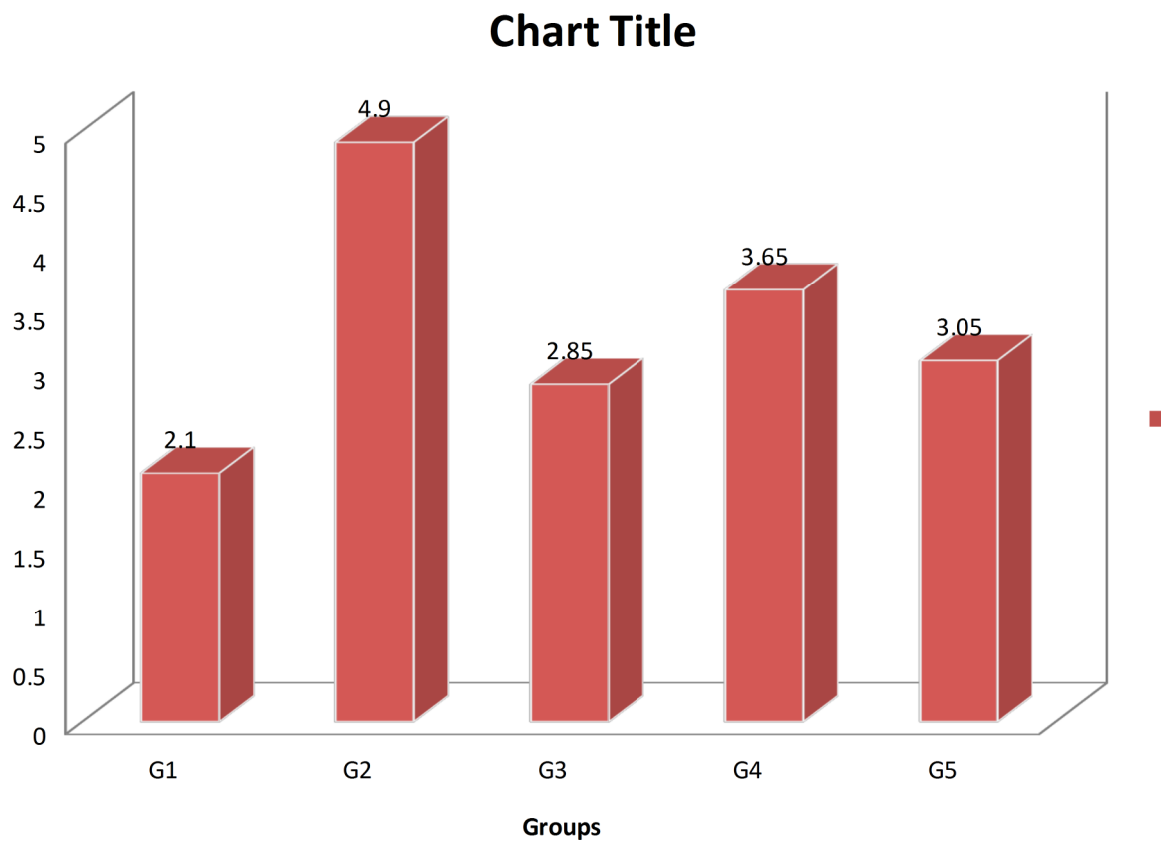
Figure. No: 11

SERUM LEVELS OF GSH U/G VALUES OF MEANS OF 6 RATS IN EACH GROUP.

Chart Title
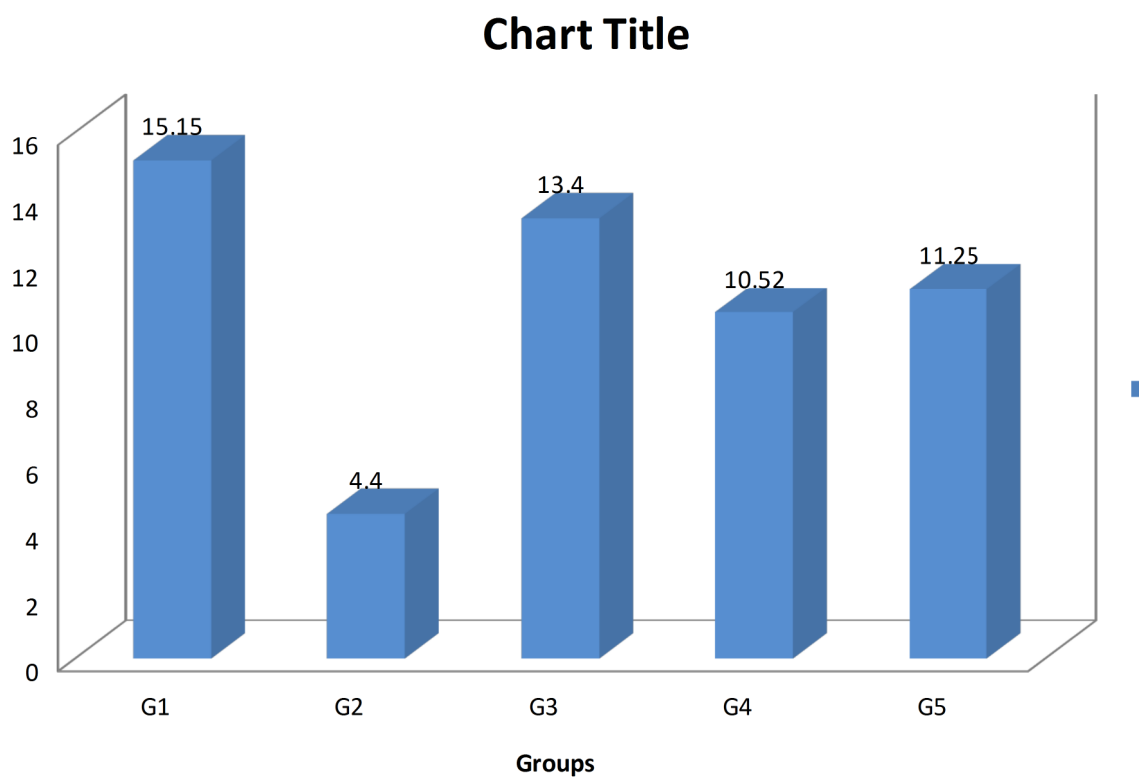
Figure. No: 12

SERUM LEVELS OF CAT U/G VALUES OF MEANS OF 6 RATS IN EACH GROUP.

Chart Title
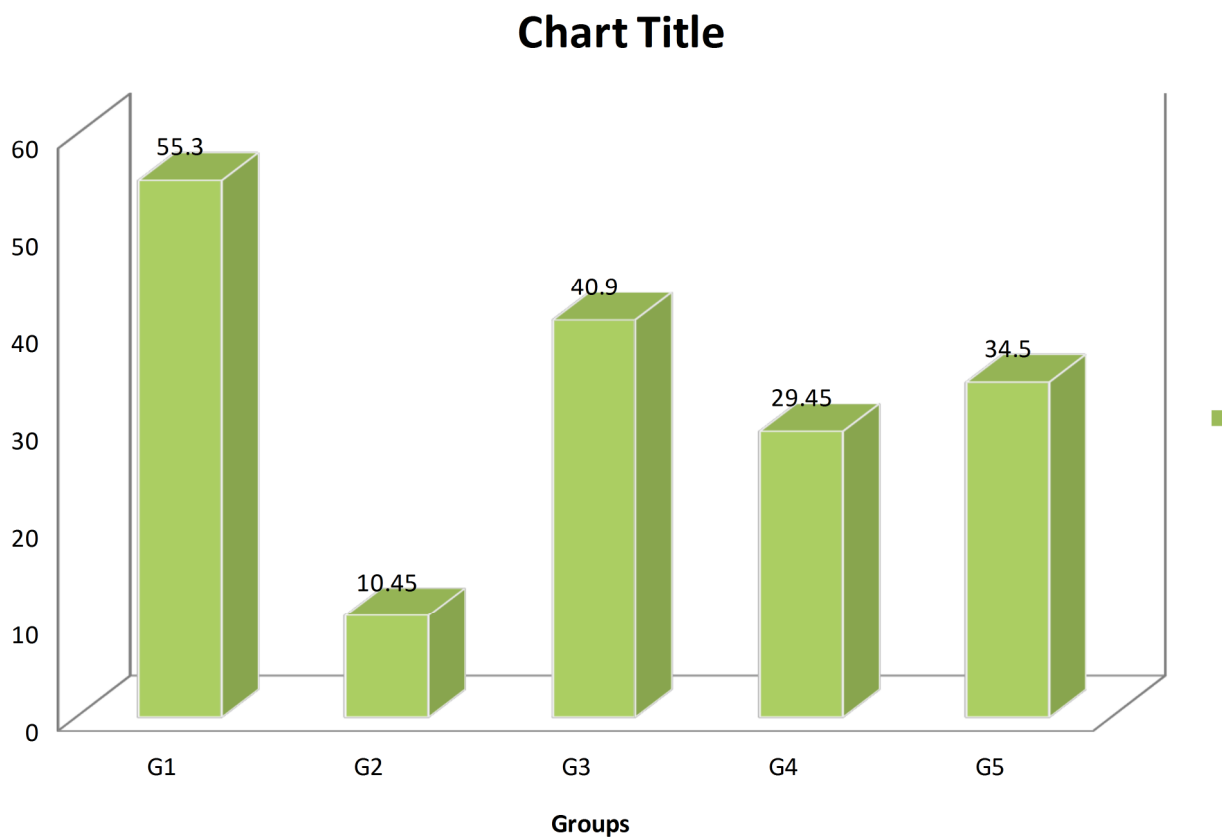
Figure. No: 13

SERUM LEVELS OF SOD U/G VALUES OF MEANS OF 6 RATS IN EACH GROUP

Chart Title
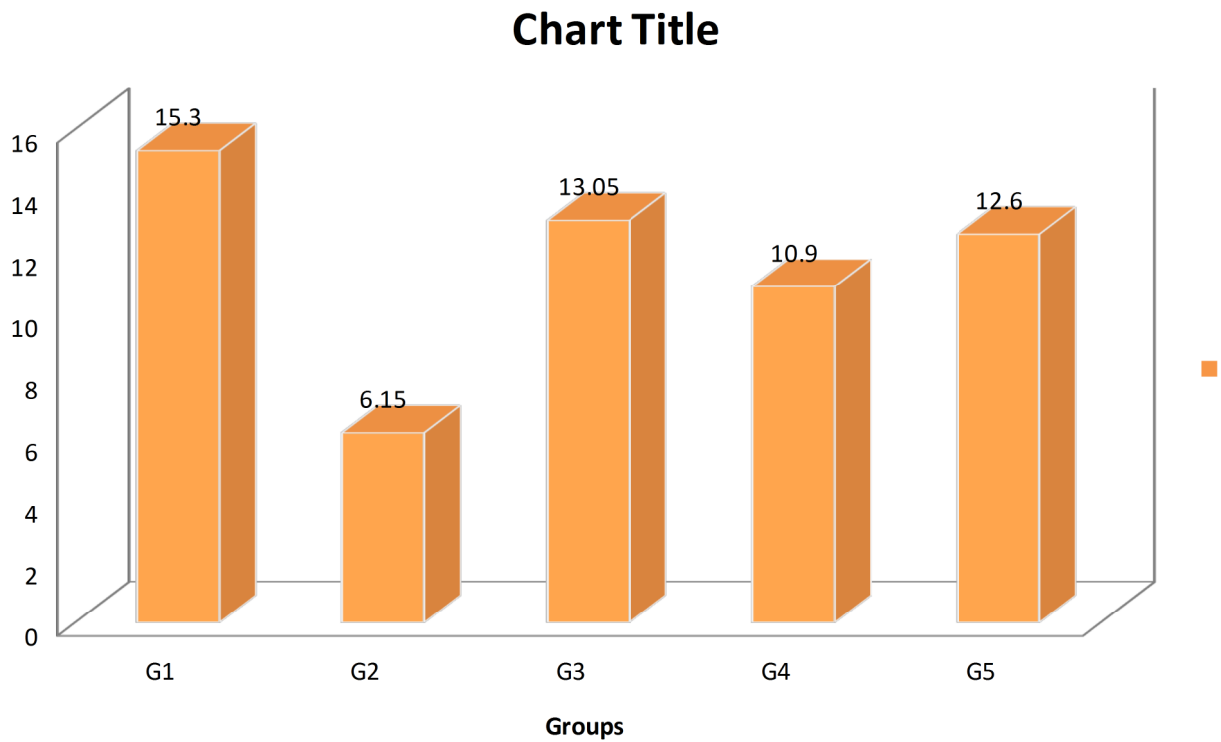
Figure. No: 14

SERUM LEVELS OF SOD U/G VALUES OF MEANS OF 5 RATS IN EACH GROUP.

Chart Title
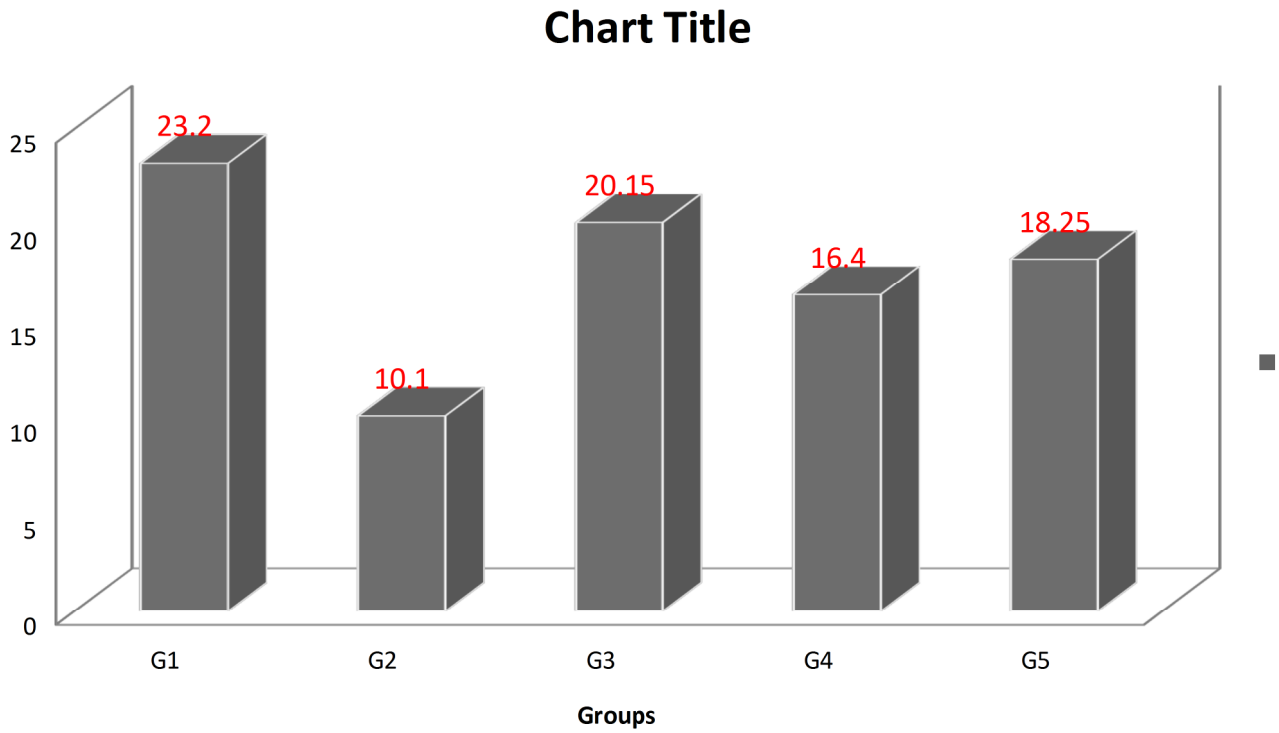
EFFECT OF HAEBD ON SERUM CREATININE ,BLOOD UREA NITROGEN &ALKALINE PHOSPHATASE

Figure. No: 15

SERUM CREATININEVALUES OF MEANS OF 6 RATS IN EACH GROUP.

Chart Title
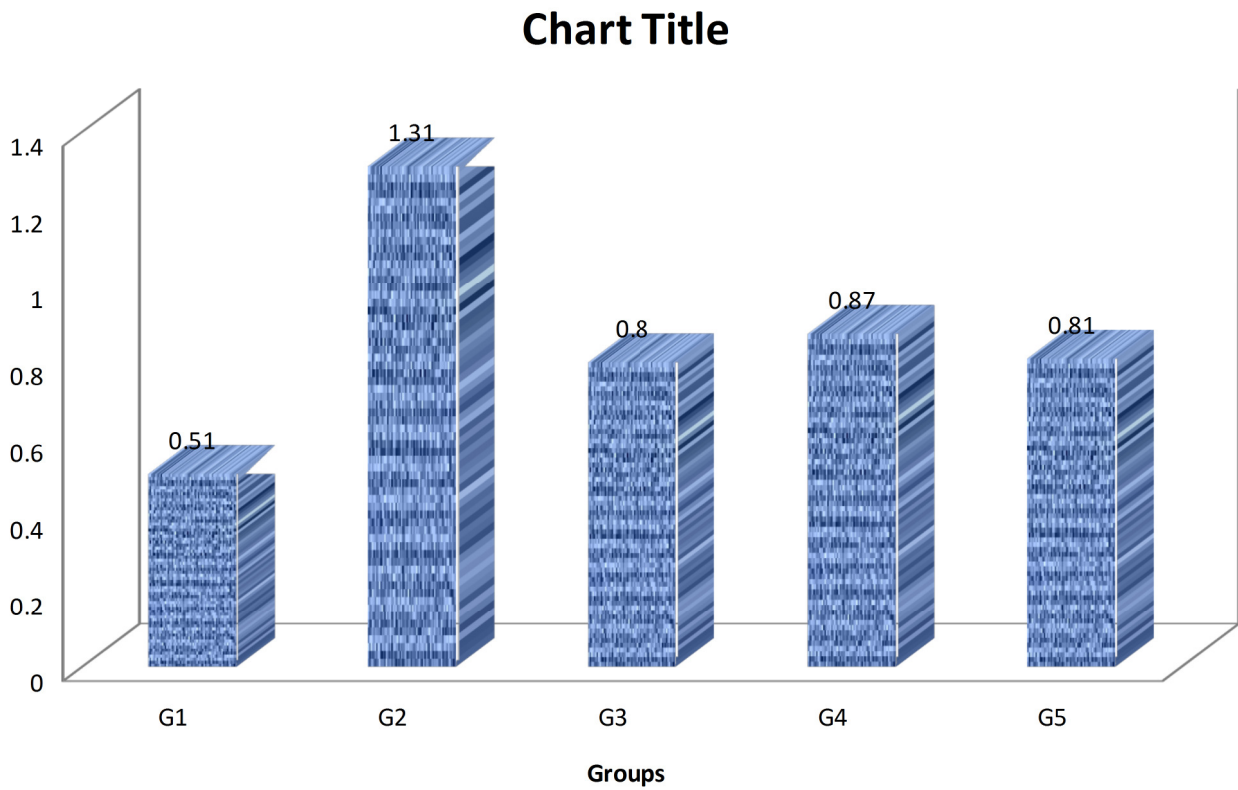
Figure. No: 16

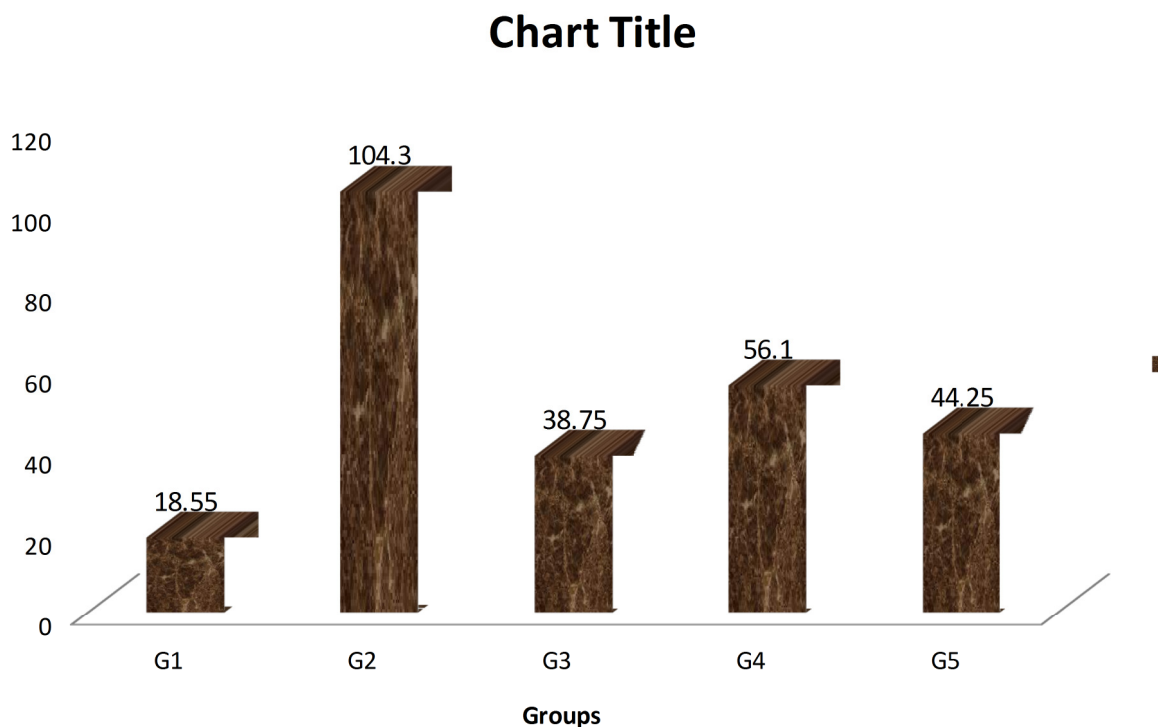**BLOOD UREA NITROGEN VALUES OF MEANS OF 6 RATS IN EACH GROUP.**

Chart Title

Figure no: 17

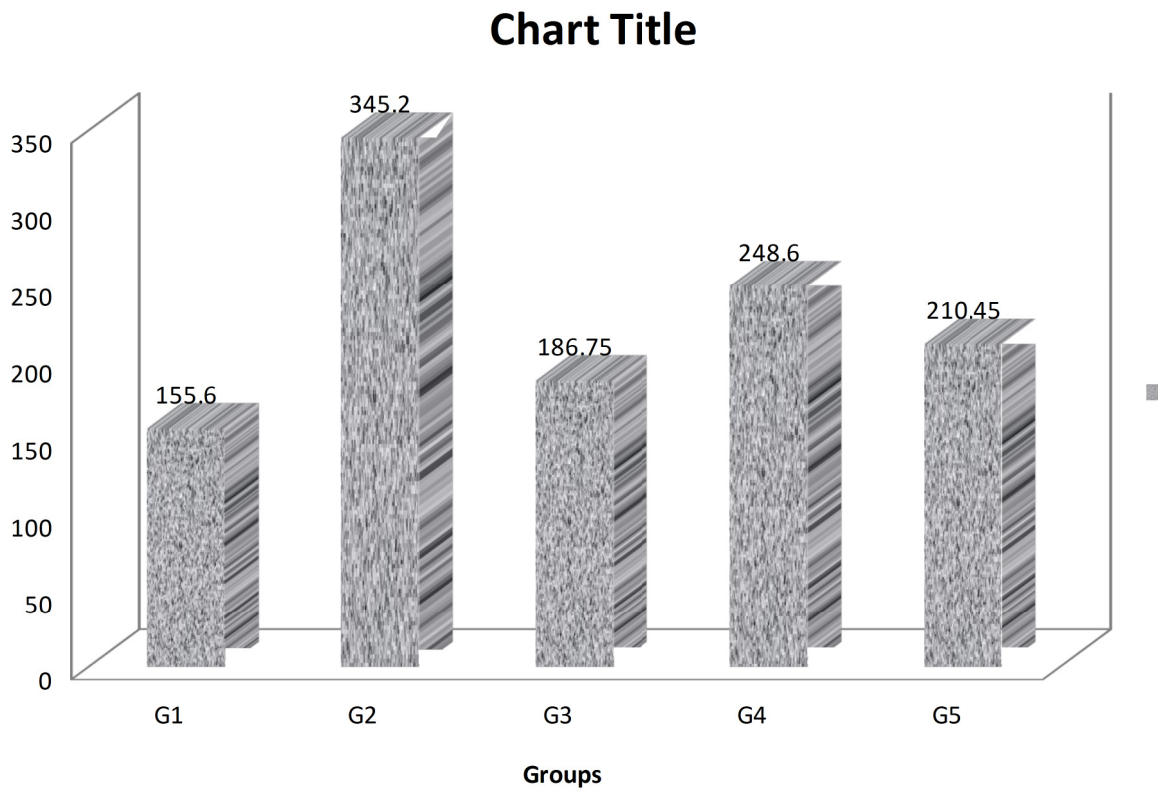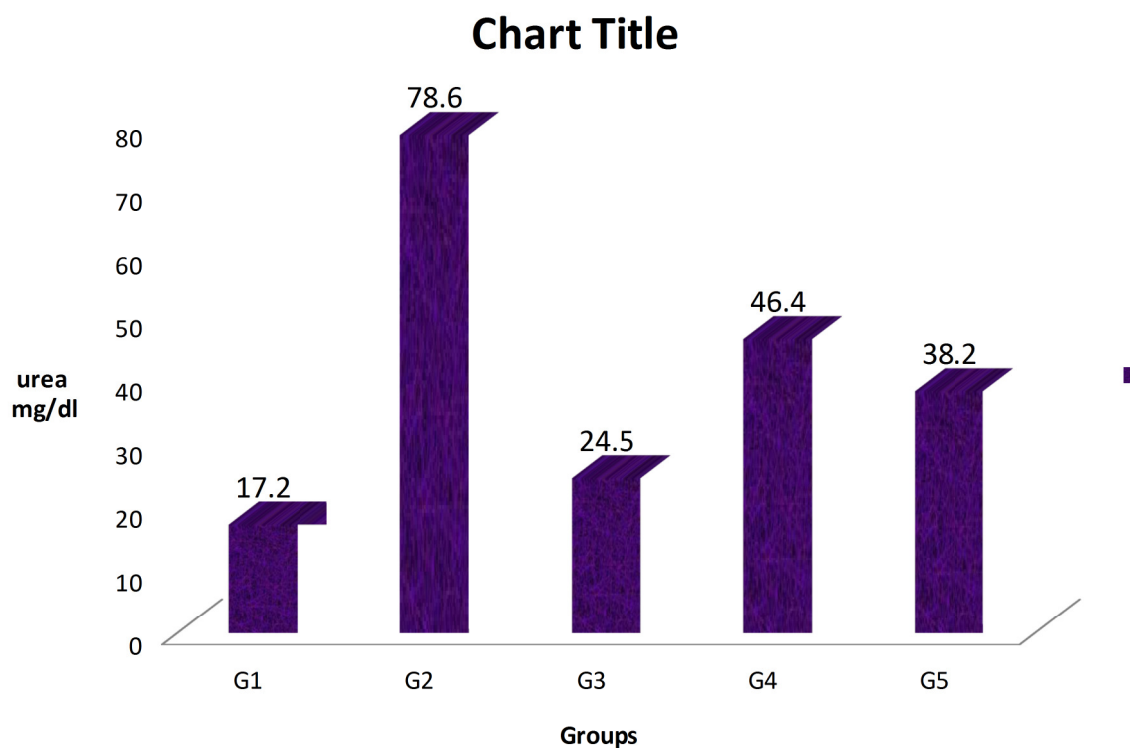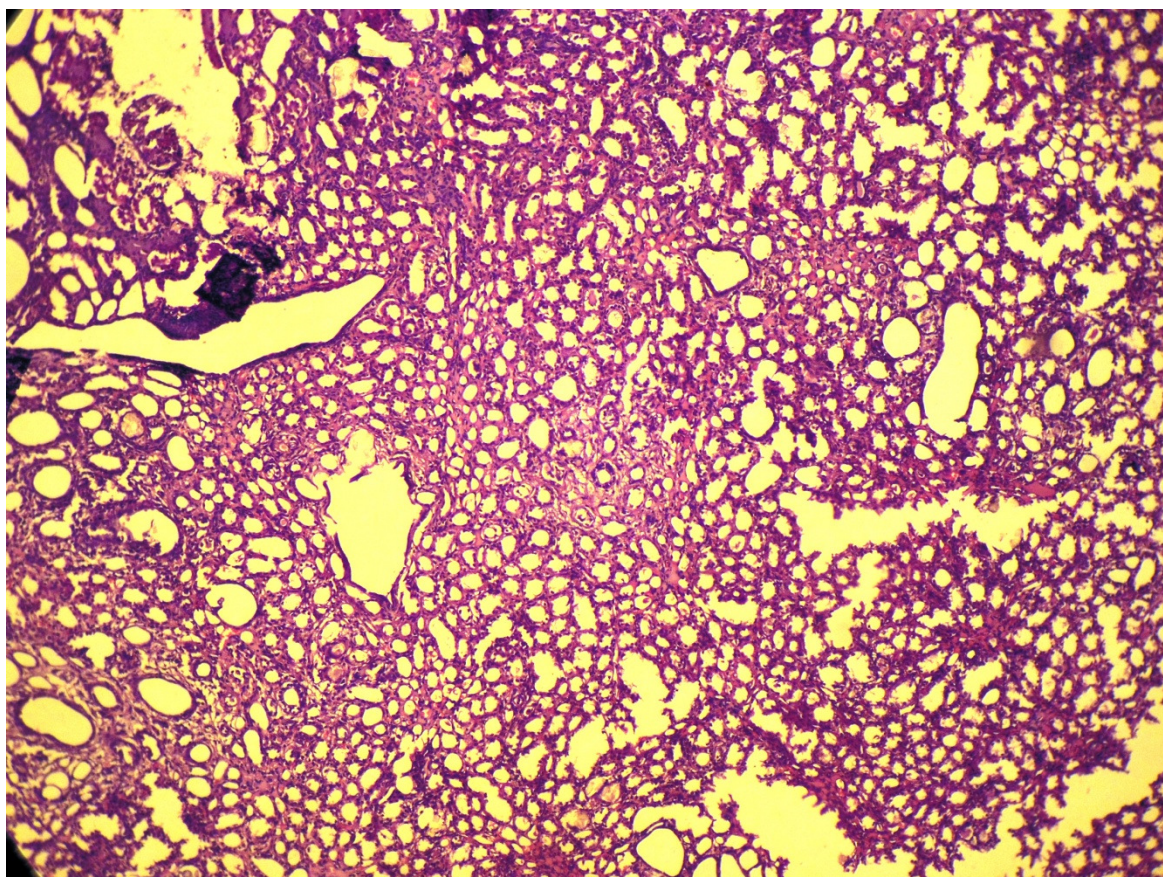**ALKALINE PHOSPHATE VALUES OF MEANS OF 6 RATS IN EACH GROUP.**

Chart Title

Figure. No: 18

UREA VALUES OF MEANS OF 6 RATS IN EACH GROUP.

Chart Title

Protective effects of hydro alcoholic extract of Boerhaavia diffusa linn against cisplatin induced nephrotoxicity in rats
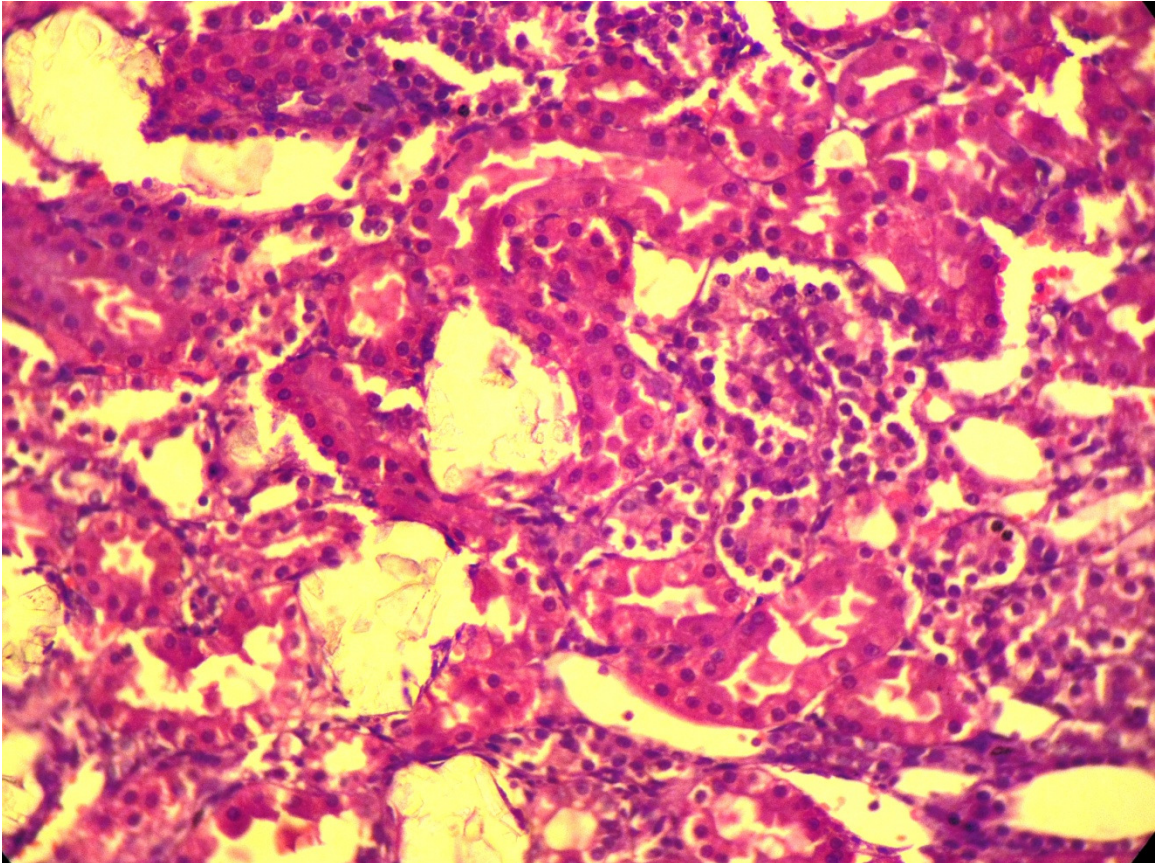
73

HISTOPATHOLOGICAL STUDIES
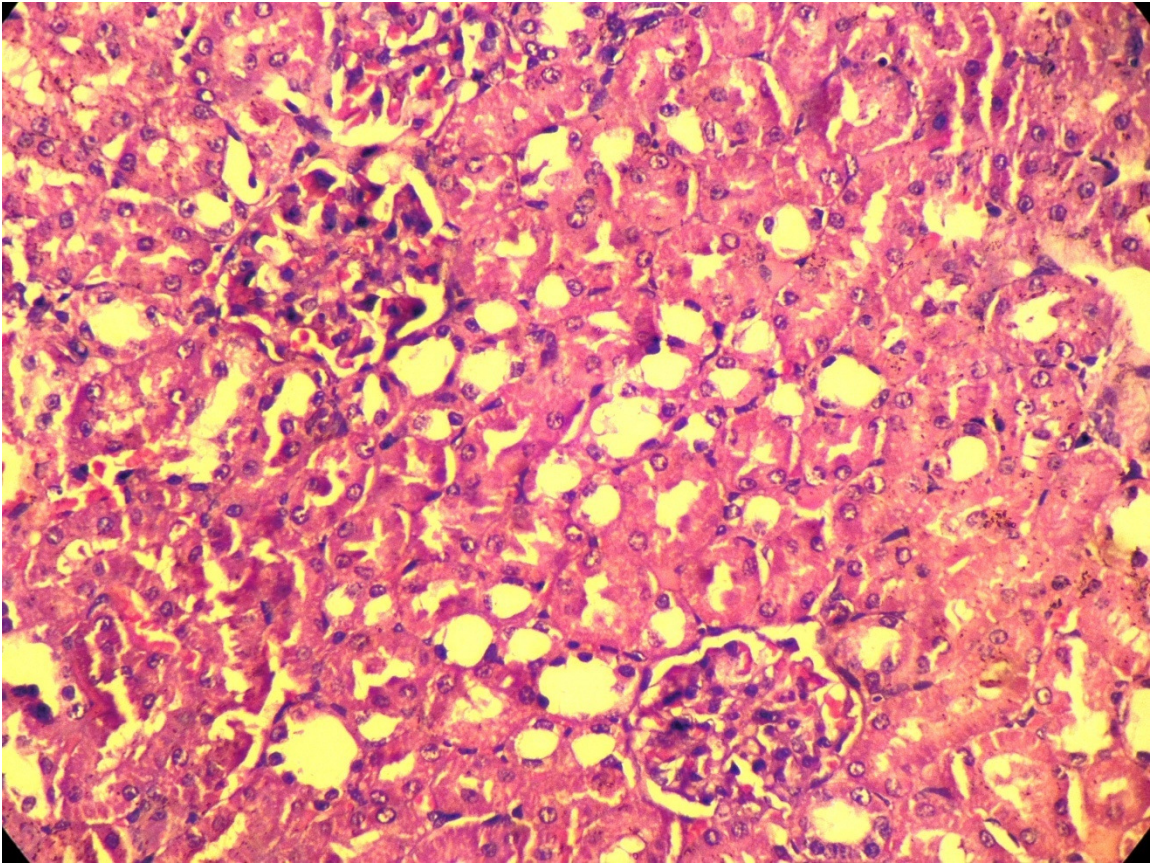
Figure. No: 19

Kidney Section of GP₁ (Normal Control) Rats

There was no glomerular congestion with any evidence of edema, heamorrhage, inflammation or tubular necrosis.

Protective effects of hydro alcoholic extract of Boerhaavia diffusa linn against cisplatin induced nephrotoxicity in rats

74

Figure. No: 20

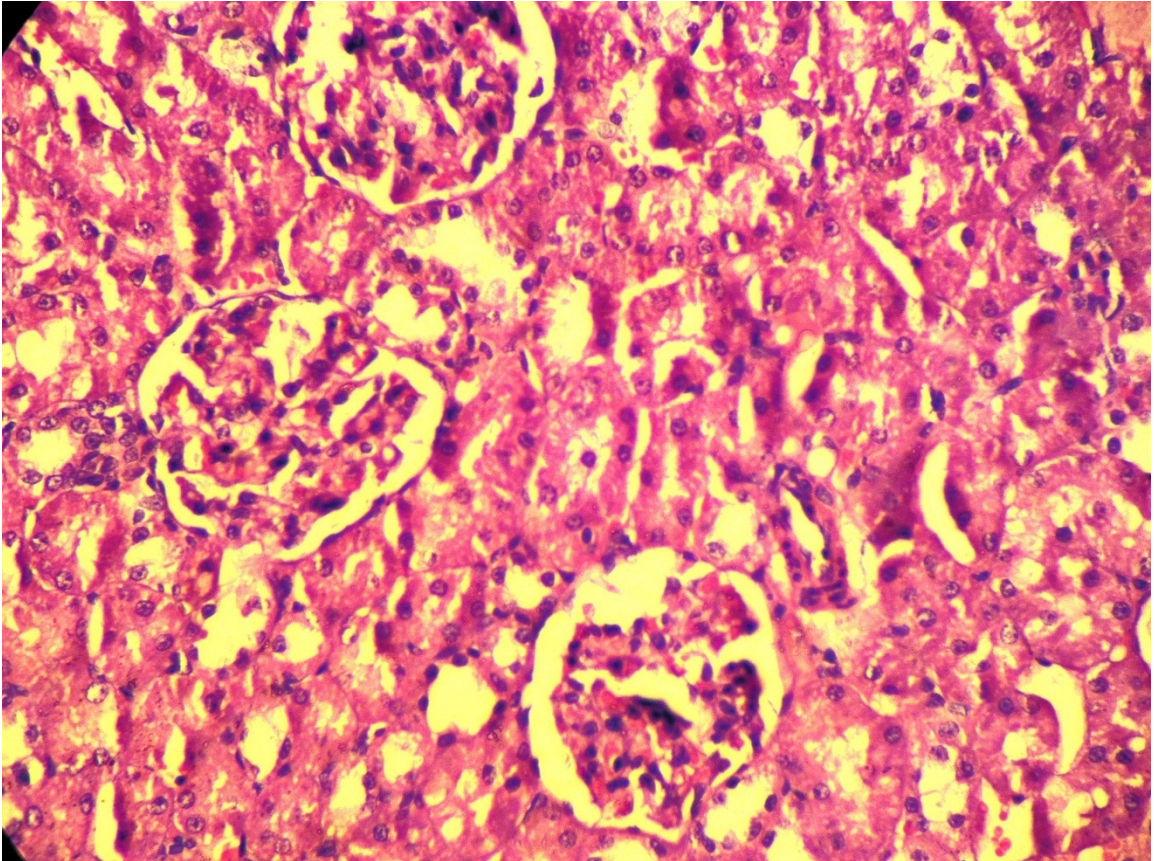**Kidney Section of GP₂ (cisplatin induced toxic Control) Rats**

Kidney Section of GP₃(silymarin 50mg/kg/rat)

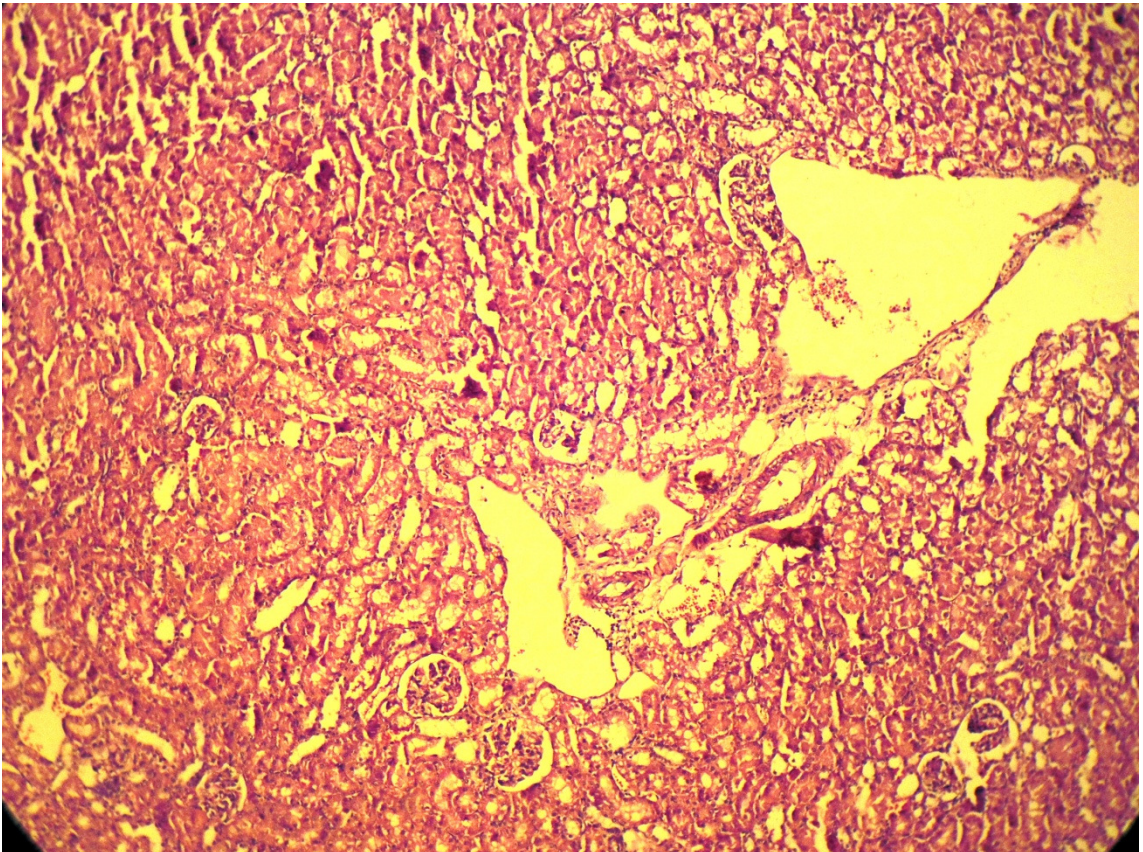Shows mild renal tissue damage .No evidence of congestion.

Figure. No: 22

Kidney Section of GP₄(*Boerhaavia diffusa* extract 200mg/kg/rat)

Shows mild renal tissue damage.No evidence of heamorrhage,glomerular atrophy

Figure. No: 23

Kidney Section of GP$_5$ (Boerhaavia diffusa 400mg/kg)

Shows mild renal tissue damage.No evidence of heamorrhage,glomerular atrophy

Protective effects of hydro alcoholic extract of Boerhaavia diffusa linn against cisplatin induced nephrotoxicity in rats

78

RESULTS AND DISCUSSION

Result:

In acute oral toxicity study the extract was found to be safe up to a dose of 2000 mg/kg.

In the present studies, the cisplatin treated animals showed a significant increase in blood urea, blood urea nitrogen and serum creatinine. As shown in [Table no:4] serum creatinine, Blood urea nitrogen (BUN), and blood urea levels were significantly higher after 72 h respectively, after administration of single dose of cisplatin when compared to the control group. The pre-treatment with Hydro alcoholic extract of *Boerhaavia diffusa* (HAEBD) p.o. significantly ($P < 0.01$) lowered the elevated serum urea creatinine, and Blood urea nitrogen (BUN). When compared to the cisplatin group. The pre-treatment with silymarin showed a marked decrease in concentrations of blood urea, serum creatinine, Blood urea nitrogen and alkaline phosphatase.

Effects of HAEBD on renal oxidant/antioxidant status:

In cisplatin treated group the activities of antioxidant enzymes like SOD, CAT and GPx and levels of GSH were found to be significantly decreased with marked increase in MDA as compared with control ($P < 0.01$). The pre-treatment of HAEBD and silymarin was found to significantly elevate the decreased activities of SOD, CAT and GPx ($P < 0.01$). The activities of renal SOD, CAT and GPx in the cisplatin treated, cisplatin plus HAEBD and cisplatin plus silymarin administered groups were given in [Table no:3]. Administration of HAEBD and silymarin also inhibited the cisplatin-induced elevation in the MDA. The decrease in the GSH levels in renal tissues induced by cisplatin was prevented by the administration of HAEBD and silymarin ($P < 0.01$) [Table no:3]

Effects of HAEBD on kidney histology:

Treatment with cisplatin caused a marked necrosis in proximal tubules and degeneration of the tubular epithelial cells [Figure.No:23] The pre-treatment with HAEBD and silymarin decreased the cisplatin induced tubular necrosis when compared with cisplatin treated group.

Discussion:

The impairment of kidney function by cisplatin is recognized as the main side effect and he most important dose limiting factor associated with its clinical use. Several investigators [130,131] reported that the alterations induced by cisplatin in the kidney functions were characterized by signs of injury, such as increase of products of lipid peroxidation (LPO) and changes in GSH levels in kidney tissue, creatinine and urea levels in plasma.

The renal antioxidant status, such as SOD, CAT, GPx activities, and reduced GSH concentration are significantly decreased in the cisplatin treated group of animals compared to the control group. The declined antioxidant status partially explains the mechanism of nephrotoxicity induced by cisplatin. The renal accumulation of platinum and covalent binding of renal protein may also play a role in the nephrotoxicity.[132,133] In the present study, increased serum creatinine and urea were observed in cisplatin treated rats may be due to reduction in glomerular filtration rate. The impairment in kidney function was accompanied by an increase in MDA concentrations in kidney tissue. The above findings were well-correlated with the renal histological results. These observations indicated that cisplatin induced nephrotoxicity and the results are in accordance with previous findings. The pre-treatment of HAEBD provides a significant protection against cisplatin-induced nephrotoxicity with lowering the level of plasma creatinine and blood urea in cisplatin treated animals.

Decreased concentration of GSH increases the sensitivity of organs to oxidative and chemical injury. The role of GSH, non-protein thiols in the cells, in the formation of conjugates with electrophilic drug metabolites, most often formed by cytochrome P-450-linked monooxygenase, is well-established. [134] Studies with a number of models show that the metabolism of xenobiotics often produced GSH depletion. Reduced renal GSH can markedly increase the toxicity of cisplatin. The depletion of GSH also seems to be a prime factor that permits lipid peroxidation in the cisplatin-treated group. Moreover, the protection of GSH is also by forming the substrate for the GPx activity that can react directly with various aldehydes produced from the peroxidation of membrane lipids. The enhanced GPx activity could partially explain the protection of bio membranes from oxidative attack.

Decreased SOD activity could cause the initiation and propagation of lipid peroxidation in the cisplatin treated group. This may be either due to loss of copper and zinc, essential for the activity of enzyme or due to ROS induced inactivation of enzyme proteins. The decrease in activities of CAT and GPx could enhance the lipid peroxidation. Thus, the levels of MDA, as a result of lipid peroxidation, were increased in the cisplatin-treated animals. Although, the exact mechanism of cisplatin-induced nephrotoxicity is not well-understood, several investigators have shown that cisplatin nephrotoxicity is associated with LPO in renal tissue. LPO is ascribed to a free radical-mediated chain reaction that damages cell membranes, and inhibition of this process by HAEBD is mainly attributed to the ability of scavenger free radicals.[135] In the present investigation, pre-treatment with HAEBD inhibited the increase in LPO induced by cisplatin in renal tissue, indicating antioxidant activity of HAEBD.[136]

The histopathological evaluation of the kidney preparations in treatment group also revealed a decreased cisplatin-induced tubular necrosis. Cisplatin-induced renal damage is associated with increased renal vascular resistance and histopathological damage to proximal tubular cells. On the other hand, an increase in GSH levels in the renal tissue indicates that pretreatment with HAEBD was due to oxidative stress. The effects of HAEBD on cellular GSH may be due to antioxidant effects. The treatment with HAEBD prevented the lipid peroxidation by enhancing the renal CAT, SOD and GPx activities.

Conclusion

In conclusion, it was shown that cisplatin treatment induced renal damage and pretreatment with hydro alcoholic extract of *Boerhaavia diffusa* (HAEBD) provided protective effect against this cisplatin-induced nephrotoxicity. However, before concluding a potential usefulness of hydro alcoholic extract of *Boerhaavia diffusa* (HAEBD) as adjunct to the cisplatin therapy, further clinical investigation is needed.

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