Chapter-1 INTRODUCTION

1.1 Nephrotoxicity:

The kidneys are paired and bean shaped organs, located on either sides of the vertebral column. The kidney forms about 0.5% of the total mass. Through renal artery kidney receives 20-25% of the total arterial blood. Each kidney consists of about ten lakh nephrons. It is the unit of the kidney. Nephron forms urine by filtering the blood. The small ions, water molecules and other small molecules get reabsorbed back into the peritubular capillaries by reabsorption. The waste molecules, ions remain in the urine and they get eliminated through urine.

Acute kidney injury is mainly due to drugs. Most of the patients are subjected to the diagnostic and clinical procedures and they have more co morbidities with the potential kidney failure. The drug induced nephrotoxicity is more common in patients receiving certain medication and undergoing certain specific clinical conditions. The preventive

![Figure 1 Location of kidneys](image)
measures include alternative use of safer drugs wherever possible; and by correcting the risk factors of nephrotoxicity, by adjusting the dosage of drugs, by monitoring the renal function by conducting the renal function test during the therapy, by monitoring the vital body function, renal function and by observing vital signs during therapy; and by avoiding nephrotoxic drug combination(Cynthia A, et al., 2008).

The nephrotoxicity is due to poisonous effect of some toxic chemicals and certain type of drugs on the kidneys. Nephrotoxins are the agents displaying nephrotoxicity. The nephrotoxic effect develops more in patients who are already suffering from the renal impairment. The chronic administration of certain drugs causes nephrotoxicity. If it occurs, then the kidneys fails to remove excess urine, and waste. The kidneys are important organs as they involved in the excretion of waste products, toxic substances in the body. In kidney failure, the toxic metabolites retain in the body leading severe complications like edema, ascites, hepatomegaly, etc.

The glomerular filtration rate is 125ml/min and blood flow to kidney is about 625ml/min. Hence, the blood flow to kidney is about four times more when compared to other organs. The glomerular filter present in the Bowman’s capsule of the nephron filters about 125ml/min, thus per day about 180Lt of blood get filtered by the kidneys. Hence the kidneys are exposed to the toxic substances present in the blood and are susceptible for the damaging effects of theses toxic substances. The nephron is the structural and functional unit of the kidney. This consists of renal corpuscles and renal tubules.

The renal corpuscles consist of glomerulus and Bowman’s capsule. The renal glomerular capillaries are made up of squamous epithelial cells with a basement membrane. The Bowman’s capsule consists of two layers-parietal and visceral layers. The parietal layer is made up of squamous epithelium consisting squamous epithelial cells and the visceral layer is made up of specialized epithelial cells known as podocytes. These cells have foot like projections called pedicels, which act as filter. When blood enters Bowman’s capsule through afferent arteriole, the small ions, water, glucose, urea, etc get filtered by the glomerular filter and the filtrate enter the glomerular space. Certain forces are responsible for the glomerular filtration. These include glomerular blood hydrostatic pressure, the pressure due the fluid of the capsular space called capsular hydrostatic pressure, and the pressure due to the plasma proteins like albumin, globulin and fibrinogen- these expresses the blood colloidal osmotic
pressure. The normal GBHP is 55mm HG, which favors the filtration. The normal CHP is 15mmHG and the normal BCOP is 30mmHG, both of these pressures opposes the filtration. Hence the net filtration pressure (NFP), 10mmHG is responsible for the filtration. The solutes which are required for the body needs are also filtered, but then these solutes get reabsorbed back into the peritubular capillaries.

![Normal and diseased kidney](image)

**Figure- 2 Normal and diseased kidney**

Various nephrotoxicants causes nephrotoxicity like acute and chronic renal failure. The use of various drugs also leads to the development of various types of kidney disorders like acute and chronic renal failure. The nephrotoxic effect is usually more in patients who have the history of renal impairments. Nephrotoxicity occurs when the renal blood is exposed to a nephrotoxic drug or toxin that causes damage to the kidneys. When kidney damage occurs, the kidney fails to remove excess urine, and waste. The kidney filters the metabolic toxic waste substances from the blood and helps in excreting these substances from the body in the form of urine.
The blood is filtered by the glomerular capillaries to form filtrate. The normal glomerular filtration rate is 125ml/min. The filtrate moves through the Bowman’s capsule into the tubules. About 99% of the filtrate gets reabsorbed into the peritubular capillaries by a process called tubular reabsorption. The unabsorbed fraction is eliminated after passing into the urinary bladder. If the blood contains toxic substance, because of accidental or intention consumption of poison, the kidneys gets exposed to these agents and it causes damaging effects on the kidneys by number of ways. Some toxic substances may cause damaging effects on glomerulus or the tubules by causing necrosis of cells of the glomerular capillaries or the tubules. Some toxic substance may lead to the formation of metabolites or conditions that may result in the necrosis of cells of the kidney.

The cells of ascending loop of Henley are of squamous epithelial type, hence this part is considered as thin section of loop of Henley, whereas the descending loop of Henley consists of cuboidal epithelium. This part of nephron is important as they regulate the homeostasis of the extracellular fluid. As this part of the nephron receives less blood and oxygen supply, there is hypertonic environment. The thick ascending limb of loop of Henley cells contains highest number of mitochondria; they play an important role in oxidative biotransformation, by producing more ATPs required for the reabsorption of various ions through symporters expressed on the tubular cells. This helps for the normal reabsorption of electrolyte like sodium, chloride, potassium and other anions and cations.
Furosemide is one of the diuretics. It produces its effect by targeting the transporter present on the tubular cells. The nephrotoxic drugs like tacrolimus and cyclosporine also acts on these medullary tubular cells of the nephron causing nephrotoxicity with altered functions of these cells. The medullary tubular cells also produce a specific glycoprotein called as Tamm-Horsfall leading to the nephrotoxicity. The appearance of these glycoproteins in the urine is an indicative of tubular damage.

Figure 4  Reabsorption of various electrolytes at the thick ascending loop of Henley cells

The part of thick ascending loop of Henley with the renal medullary tubular cells expresses various transporter proteins which are involved in the reuptake of various ions present in the glomerular filtrate. The symporters like Na\(^+\)/K\(^+\)/2Cl\(^-\) are expressed on the apical surfacesof the thick ascending loop of Henley. These transporters are involved in the reabsorption of sodium and chloride ions across the membrane. From the tubular cells the sodium is pushedout by an active transport system by sodium–potassium ATPase pumps expressed on the basolateral membrane. The cations are also reabsorbed through the paracellular route. The potassium ions get secreted into the lumen of the tubule. (Fredrik Palm et al., 2005).
In PCT, $\text{Na}^+$/H$^+$ antiporters move H$^+$ ions directly into the lumen of the tubule. Here H$^+$ ions combine with HCO$_3^-$ ions present in the filtrate to form CO$_2$ and H$_2$O. The CO$_2$ present in the filtrate, cytosol of the cells of the PCT and interstitial fluid combine with H$_2$CO$_3$. The carbonic acid gets dissociated by carbonic unhydrase enzyme and dissociated to proton and bicarbonate ions. The protons are transported by active transport into the lumen of the tubule and the bicarbonate ions is passively diffused into the peritubular capillaries. The sodium ions are transported by active transport by using the sodium-potassium ATPase pumps of the tubular cells into the interstitial spaces. From there the sodium is returned to the peritubular capillaries by passive diffusion. The H$^+$ATPase pumps expressed on the apical surfaces of the distal convoluted cells actively transport protons from the tubular cells into the lumen of the tubules. In the distal convoluted tubules and collecting ducts, the proton ATPase pumps H$^+$ ions by active transport into the lumen of the tubules, then these H$^+$ ions passes through the urine after combining with ammonia by forming ammonium ions. The DCT plays only a minor role in adjustment of blood/filtrate pH.

**Figure 5 Reabsorption at PCT**

[Diagram showing reabsorption at PCT with metabolic reactions and transport processes labeled.]
The nephrotoxic substances cause nephrotoxicity. This may lead to acute kidney failure. In this condition, the kidney function deteriorates and may lead to chronic kidney failure. If unchecked, kidney failure may lead to death. Number of nephron toxicants is responsible for the kidney failure. Some of the nephrotoxic drugs are antibiotics (aminoglycosides, sulphonamides, amphotericin-B, neomycin, polymyxin, chlorotetracyclines), rifampicin, bacitracin, trimethoprim, cephaloridine, methicillin, aminosalicylic acid, oxy- and chlor-tetracyclines), analgesics (NSAIDs, ibuprofen, acetaminophen), contrast agents (sodium iodide), heavy metals (lead, mercury, uranium and arsenic), anti-cancer drugs like cyclosporine, cisplatin and cyclophosphamide, methanoglobin forming agents, solvents and fuels like carbon tetrachloride, methanol, amylalcohol, glycol, herbicides, pesticides, and diseases that cause the overproduction of uric acid.
The symptoms of renal failure are of different types, in most of cases the symptoms depends on the nature of nephrotoxicants. The nephrotoxicity symptoms include azotemia (excess urea in the blood) because the nephrotoxic kidneys fail to excrete normal quantities of urea from the blood. This leads to increased serum concentration of urea. The nephrotoxicants are responsible for the injury and blood loss through the urine. This leads to anemia due to decreased RBC count. The damaged proton transporters result in disturbed transport of protons from the blood. This causes acidosis. The appearance of blood proteins also takes place. This condition is called hematuria. Albuminuria is another symptom of kidney failure due to the excretion of proteins in the urine. Pus may also appear in the urine. If unchecked, some more serious symptoms are observed and may lead to seizure and coma.

The treatment of the nephrotoxic injury caused by the nephrotoxic drugs includes the removal of toxins from the patient’s body by maintaining the normal kidney functions. By
administering chelating agents the toxins can be removed from the patients’ blood. With the administration of diuretics, the toxic substances can also be removed from the body. In clinical emergencies, the nephrotoxic substances are removed from the blood via hemodialysis. The maintenance of vital body organ functions is necessary during the treatment of nephrotoxicity. This prevents the further damage to the other body organs.

As the kidneys receive the blood flow of 525ml/min, and they are mainly involved in the filtration and concentration of various metabolites, toxicants and chemicals. Through renal arteries these substances reach nephrons of the kidneys. As their concentration increases in these areas, the cells of the nephrons are susceptible to the injury. The morphological examination of the kidney biopsy has shown the alterations in the normal morphological structures of the nephron indicating the nephrotoxicity. The damage to the visceral layer of the Bowman’s capsule-podocytes may cause a disorder known as nephritic syndrome. This may also lead to the hematuria as well as albuminuria or proteinuria. If the damaging effects are more, it leads to the severe form of the disorder known as glomerulonephritis.

The vascular injury deals with the extra glomerular vasculature. The primary abnormalities include smooth muscle and damage to the myocytes and epithelium; finally it may lead to nodular hyalinosis. The most common cause of tubule interstitial nephritis is drug related. Antibiotics and analgesics are widely available drugs all over the world. As these drugs causes nephrotoxicity, more people in the world are susceptible for the nephrotoxicity caused by these agents.

Lithium in the form of lithium chloride is usually indicated in psychotic disturbances like bipolar disorder. Usually lithium administration causes the development of nephrogenic diabetes insipidus slowly. This development is due to the decreased expression of aquaporin-2 channels on the apical surfaces of the PCT cells. The prolonged administration of lithium containing drugs over the years may also lead to the development of chronic tubulointerstitial nephritis and tubular microcysts.

The urolithial carcinoma caused by the ‘Chinese Herb’ was first identified by Belgium women while taking this for the weight reduction. This disorder is a form of interstitial nephritis. Later aristolochic acid was identified as one of the ingredients of this herbal preparation and it was isolated from Aristolochia fangchi. Most of the analgesics are
available in combination dosage forms. The large cumulative doses of analgesics may lead to
a more serious form of nephrotoxicity known as analgesic nephropathy.

The nephrotoxic drugs induce renal injury due to the formation of crystal like substances in
the kidney. This leads to the development of tubular injury, interstitial inflammation and
obstruction. Some drugs like sulfonamides and metabolites of some drugs cause the
deposition of crystals in the kidney. The decrease in the blood volume, difficulty in
micturition are some of the conditions which follow the depositions of crystals in the kidney.
The excess concentration of vitamin D is also one of the causative factors for the deposition
of crystals in the kidney. The excess concentration of vitamin C also leads to the crystal urea,
as this vitamin metabolizes to oxalic acid. The hyperuricemia is also due to the administration
of chemotherapeutic agents. This may trigger a tumor lysis syndrome, causing uric acid
nephropathy.

The shape of these crystals is needle-shaped and they get dissolved in the tissue forming
amorphous aggregates surrounded by special tubular cells. The acute nephropathy is due to
the deposition of excess of uric acid in the kidney. The linear yellow striations are deposited
in the renal medulla and papillae of the kidney. The crystals are also getting deposited in the
collecting ducts. (Alwin HL Loh, et al., 2009).

1.2 Gentamicin induced nephrotoxicity:

Aminoglycoside antibiotics have been used widely for the treatment of infections caused by
gram-negative organisms. Their use in clinical practice is restricted because of
nephrotoxicity and ototoxicity. The order of their toxicities is indicated as in the order,
neomycin > gentamicin > tobramycin. The gentamicin induced nephrotoxicity occurs in
some patients who are receiving these drugs. The developed kidney failure is characterized
by transient increase in the levels of serum creatinine levels and hypoosmolar urinary output.

Gentamicin gets filtered in the glomeruli, are reabsorbed actively via lysosomes in the brush
border membrane of proximal tubular cells and thereafter produces phospholipidosis. The
gentamicin drug molecules enter the tubular cells by absorptive/receptor mediated
endocytosis after binding to acidic phospholipids and megalin receptors. Rodents
administered with less and therapeutic doses of gentamicin show both accumulation of lipid
substances in the tubular cells like phospholipidosis and apoptosis in proximal tubular cells (Rajkumar V.Shete et al., 2011).

The knowledge of causative factor is essential before the treatment of nephrotoxic patients, as the degree of damage varies from one drug to another. The aminoglycoside induced nephrotoxicity causes a slow rise in the serum creatinine levels associated with nonoliguria with an increased concentration of serum creatinine levels. This also causes hypoosmolar urinary output. The symptoms of nephrotoxicity are observed if the period of aminoglycoside administration exceeds 10 days. The aminoglycoside drug induced toxicity is mainly due to gentamicin administration. Some efforts made to minimize the nephrotoxicity of the available agents have reached the practical clinical practices. As most of the ICU patients receive aminoglycosides, acute kidney failure is a common problem among them with a high rate of mortality.

The aminoglycoside-associated nephrotoxicity is independent of other risk factors like diabetes, hypotension, use of other drugs causing acute and chronic kidney failure including iodinated radio contrast substances (Joao FP et al., 2009).
The usual dose of aminoglycoside antibiotic like gentamicin is less than 80 to 100mg/kg for about 5 days. If the therapeutic doses of gentamicin are administered over several days, it leads to induction of structural architecture changes of lysosomes of PCT with the deposition of abnormal tissue masses in the tubular cells. These alterations in the PCT cells are followed by the appearance of signs of tubular dysfunctions or tubular damage such as the release of lysosomal enzymes from the PCT and slowed absorption of small proteins and peptides. The appearance of potassium, magnesium and glucose wasting, the development of phospholipidurea and signs of cast in the urine - the appearance of these signs is followed by the appearance of renal failure with a fall in creatinine clearance (Marie-Paule, et.al., 1999).

The infrequent progression of renal failure with decreased renal output upon drug discontinuation is most often observed. It may lead to a syndrome known as Fanconi’s syndrome or a Bartter’s like syndrome. The appearance of focal necrosis and apoptosis in the tubular epithelium with tubular alterations are noticed in animals with nephrotoxicity.

The histopathological examinations of the kidney tissue provide the evidence for the tubular necrosis caused by the gentamicin. This supports the first hypothesis that the aminoglycoside exerts its toxicity due to its concentration in the tissues. Because of its local accumulation, the
lysosomal alterations take place. It supports second hypothesis that the aminoglycosides become toxic once if they are released from the lysosomes.

Various changes are observed in the metabolism within the tubular cells due to the release of excess aminoglycosides from the lysosomes. These metabolic alterations are capable of causing cell death. The decreased rate of mitochondrial respiration and calcium transport or lipid peroxidation are few examples. Both lead to the development of irreversible cell damage.

![Diagram of Retention of the GM by the proximal tubular cells](image)

**Figure 9 Gentamicin induced kidney injury**

The aminoglycosides like gentamicin released from lysosomes of the tubular cells act indirectly as nephrotoxic. The released gentamicin chelates with iron ion of the mitochondria forming oxidant molecule ferric-gentamicin complex. The death of tubular hair cells in the PCT is mainly due to this complex. When the gentamicin is present within the lysosomes, usually it is nontoxic. But once it comes out of the lysosomes of the tubular cells, it becomes toxic and produces toxic effects locally.
The alterations in the sodium-glucose symporters and sodium-hydrogen antiporters in the PCT cells of the nephrons is mainly due to fluidity of the membrane caused by the gentamicin. Gentamicin also causes the direct inhibition of Na⁺/K⁺ ATPase of PCT cells. It causes the release of lactate dehydrogenase resulting in cellular death (Marie-Paule et al., 1999).

**Figure- 10 Pathway of gentamicin induced nephrotoxicity**

The chronic kidney failure is mainly due to the activation of various inflammatory mediators. The kinase inhibitors display tubular toxicity by binding to the gentamicin molecules. Aromatic nitrogen atoms present in the aromatic nitrogen ring are able to bind with this linker. These drug-lysozyme conjugate complexes are filtered in the glomerulus, they enter tubular fluid and then they are reabsorbed via endocytic pathway. This leads to the clearance of low-molecular weight proteins.
1.3 Cisplatin induced nephrotoxicity:

Chemically it is cis-diammine-dichloro-platinum. It is used in the treatment of cancers like breast cancer, head cancer, neck cancer, testes and ovarian cancers, etc. The adverse reaction of cisplatin includes ototoxicity, gastrototoxicity, myelosupression, allergic reaction and nephrotoxicity. It occurs in about 20-30% of patients taking cisplatin. Through several studies, it is documented that the acute kidney injury caused by cisplatin is an inflammatory disease associated adoptosis and cellular death (Amala Rajasundari et al 2011).

The almost universal nephrotoxicity associated with CP results in significant morbidity and complications and often limits tolerable dosage. It is reported that the hydration and intravenous mannitol decreases the appearance of cisplatin induced nephrotoxicity. It acts by decreasing the uptake of cisplatin into the renal tubular cells. Currently, despite routine dehydration and frequent use of mannitol before CP administration, there is still significant incidence of renal insufficiency.
The prophylaxis of CP nephrotoxicity is important as it reduces the morbidity and complications, reduces the hospitalization costs, and helps to administer higher dosage of this effective anti-tumor drug with added therapeutically potential.

Once cisplatin is filtered by the glomerular filter of the Bowman’s capsule, the cisplatin enter the tubular lumen of the nephron. Then the cisplatin molecules enter the tubular cells by either passive process or facilitated transport mechanism. Various signaling pathways (like MAPK, p53, ROS and so on) of the tubular cells are activated by the cisplatin. It also induces TNF-alpha formation in the tubular cells. This facilitates the inflammatory response, leading to tubular cell injury and necrotic death of the tubular cells. The renal vasculature is also affected by the cisplatin as it causes vasoconstriction of the afferent arterioles, leading to the decreased blood flow to the Bowman’s capsule. This decreases GFR. These pathological events may precipitate the acute kidney failure.

**Figure 12 Cisplatin induced nephrotoxicity**
The intrinsic mitochondrial and extrinsic death receptor may also be induced. Activation of these pathways leads to caspase-dependent or independent apoptosis. The cisplatin administration leads to the induction of inhibitory protein p21 in the tubular cells and the activation of cdk 2 protein in the renal tubular cells. The activated cdk2 proteins result in the
necrosis of cells of the kidney. The disturbed equilibrium between the proteins p21 and cdk2 is an important causative factor of cell apoptosis and acute renal failure in cisplatin nephrotoxicity. The injury of DNA caused by the cisplatin in the tubular cells leads to stimulation of ATR which leads to phosphorylation and stimulation of inhibitory protein p53. Transcription of apoptosis genes is induced by these p53 proteins in the neuroncells. The apoptosis genes include PUMA-alpha and PIDD.

The capspase-2 is activated by PIDD via formation of PIDDosome. It leads to the secretion of AIF from the mitochondria of the tubular cells and causes caspase independent apoptosis. The PUMA-alpha translocates to the mitochondria, where it interacts with cellular components and neutralizes Bcl-XL. Due to this they form oligomer pores on the outer surface of the cellular power house and causes release of cytochrome C into the cytoplasm; this stimulates the caspases leading to caspase-dependent apoptosis (PrabhaN et al., 2008).

![Figure-15 Cisplatin induced nephrotoxicity](image)

**1.4 Cyclosporine induced nephrotoxicity:**
Cyclosporine is a cyclic polypeptide drug. This acts by causing calcium-dependent, reversible inhibition of T lymphocytes. They inhibit activation and maturation of various cell types involved in cell mediated immunity. This leads to inhibition of production of a range of various cytokines. Cyclosporine has immunosuppressant properties, hence it is used in the first line therapy in the prophylaxis and treatment of various transplant rejection. Oral cyclosporine is an effective therapy in various ocular disorders, skin disorders, pelvic inflammation, uveitis, psoriasis, rheumatic arthritis and active Crohn’s disease. It can be considered as first line therapy in patients with moderate or severe anaemias. (Faulds et al., 1993).

The immunosuppression induced by the cyclosporine is caused by the formation of a complex substance with cyclophilin. This complex decreases protein phosphatase 2B calcineurin activity in the cells. The cyclosporine is also called as calcineurin inhibitor. As cyclosporine is responsible for nephrotoxicity, hypertension and increased cardiovascular risks, its therapeutic use are restricted. The nephrotoxicity is the most severe complication of CsA and it is reported in both transplant and nontransplant settings. Acute and chronic kidney failures are the two forms of CsA induced nephrotoxicity.

The vasoconstriction of the renal blood vessels caused by the CsA leads to decrease in regular renal function. This type of nephrotoxicity is a reversible form of acute kidney failure if the treatment with CsA is discontinued. But the chronic administration of CsA not only leads to the development of renal vasoconstriction, but it is also responsible for the damage of the nephrons in the kidney. The chronic renal failure caused by cyclosporine is also characterized by the arteriolopathy and tubulointerstitial fibroses. These effects are irreversible and in the end it may lead to the end stage renal disease. (Norma A et al., 2007).
Figure 16  CsA induced nephrotoxicity

1.5 Lithium induced nephrogenic diabetes insipidus:

The patients who are suffering from the psychiatric problem receive lithium for the treatment. Most of these patients (20-54%) suffer from the urine concentrating defects during and after the lithium treatment. This may lead to the development of diabetes insipidus if the use of lithium containing drugs is continued over a prolonged period. About 50% of individuals on chronic lithium toxicities are reversible and irreversible in 20% of the cases. The prolonged administration of lithium slows down AQP$_2$ gene expression.

The NDI is a disorder of the kidney in which kidneys fail concentrate urine in response to AVP. It is caused by alterations of the gene that expresses AQP$_2$ water channel. The water and osmotic balance are maintained by the kidneys. The mammalian kidney forms 180 litres of urine per 24h. Most of this gets reabsorbed. The reabsorption of water takes place mainly through AQP$_1$ and AQP$_2$ water channels. AQP$_1$ is expressed on the apical and basolateral
membrane of proximal tubules and descending limbs of Henley, and are required for water reabsorption.

**Figure -17 Locations of aquaporin channels**

The ADH stimulates its receptors in the principal cells of collecting duct, concentrate pro-
urine via AQP2 in the plasma membrane and through aquaporin receptors like AQP3 and
AQP4 in the basolateral plasma membrane. Binding of ADH to the V2 receptors increases
intracellular cAMP levels and causes the phosphorylation of other proteins. After this the
AQV2 located in the intracellular vesicles get fused with the apical plasma membrane,
making the cell wall permeable.

**Figure -18 Location of V2 receptor**
After the dissociation of ADH from the receptors, the AQP2 are internalized by endocytosis and restores the water-impermeable state of the cell. In NDI the urine concentration capacity of the kidney decreases leading to the increased elimination of diluted urine. NDI is due to alterations of the genes that expresses the vasopressin receptors on the cells of PCT and the aquaporin 2 channels expressed on the apical surfaces of the cells of PCT. (Erik-Jan Kamsteeg et al., 1999).

In psychiatric diseases lithium containing drugs are used. The numbers of lithium salts are also used as mood stabilizers mainly for the treatment of bipolar illness. The lithium compounds are important drugs for the treatment of both acute and long term CNS disorders depression and mania. They are more effective in treating mania than depression. The lithium containing drugs also reduces the suicidal tendencies in certain bipolar patients.

Lithium is available in carbonate, chloride, and citrate forms, mainly in tablet dosage forms. In the early 20th century lithium was used as a substitute in soft drink 7Up. Chemically lithium is a univalent cation and it is related to ions like sodium and potassium. If it is administered it gets absorbed by the GIT. The lithium containing drugs usually do not bind
to plasma proteins and are completely filtered in the glomerular capillaries of the Bowman’s capsule.

The most of the solutes and the water present in the glomerular filtrate gets reabsorbed by the apical surfaces of the PCT cells and a small percentage of these are also reabsorbed by the cells of the loop of Henley and DCT and collecting ducts of the renal tubule. The renal function is affected by the lithium by several ways.

Anatriuretic effect is produced due to acute or chronic administration of lithium containing drugs. This effect is usually associated with a disturbed regulation of expression of the epithelial sodium channels in the collecting tubules. Lithium also inhibits the ability of aldosterone to increase apical membrane sodium channel expression, resulting in inappropriate sodium losses. Lithium impairs the stimulatory effects of ADH on adenylate cyclase. cAMP level decreases and leads to nephrogenic diabetes insipidus (Eleanor et al., 2012).

1.6 Importance of herbs in nephroprotection:

The chronic administration of nephrotoxic drugs causes nephrotoxicity in patients who are receiving these drugs. In nephrotoxicity, the kidney fails to excrete the metabolic wastes from the body leading to their accumulation and disturbance in the ionic and electrolyte balance. The drugs like gentamicin, cisplatin, cyclosporine, analgesics (NSAIDS) lead to kidney failure if they are used over a prolonged time. These nephrotoxicity include acute kidney failure, chronic kidney failure, and interstitial nephritis.

These complications are more in patients who are using these drugs. The other drugs, like heavy metals- mercury, lead, arsenic and cadmium, glycol, carbon tetrachloride, etc. also induces nephrotoxicity characterized by the above symptoms of renal failure. Globally in recent years more attention is given to the research on medicinal plants. Even in India, a large number of drugs obtained from plants are studied for their therapeutic applications. Now the researchers are studying at molecular levels to understand their mechanism of action by isolating the plant constituents. Various kidney protective herbs are prescribed for the nephrotoxicity by the ancient literature.
To identify the group of those plants, the term ‘Prashanabeda’ has been indicated in various literature in indigenous system of medicine. These medicines are used to dissolve urinary stones or urinary calculi. It is reported that the milk thistle (*Silbum marianum*) seeds have the potential antioxidant parameters like flavonolignans. These are collectively called silmarin. The silmarin have hepatic and renal protective effects when tested in animal models.

The scavenging effects produced by these antioxidant parameters are essential for the protection against the damaging effects caused by oxygen free radicals. It is also reported that the antioxidant parameters present in these plant seeds not only scavenges the oxygen free radicals, but also activates the RNA and protein synthesis. This exerts an important effect on the repair of renal and hepatic cells. Another finding reports that the flavonolignans are responsible for the kidney protection, when it is tested in paracetamol, cisplatin and vincristine induced nephrotoxic renal culture cells.

The silibinin protects the renal tubular cells from oxidative damage caused by cisplatin. Silibinin also protects experimental cyclosporine nephrotoxicity (*M.Afzal et al.*, 2004). Jawarish Zorooni S is the Unani preparation having nearly about 15 ingredients, majority of them having diuretic and kidney protection effect. Kidney is one of the vital organs of the body as it maintains the normal homeostasis of the body fluids.

The kidney is the main excretory organ of the body and it has important roles. It maintains the normal fluid balance by eliminating excess fluid by filtration and excretion. Kidney removes the waste substances like urea, electrolytes from the blood and nitrogenous waste materials along with water as water. The kidney cells recognize the varied composition of ions such as sodium, potassium, hydrogen and amino acids, creatinine, bicarbonates, and glucose. These are the important factors regulating BP, glucose metabolism and erythropoiesis.

Kidney disease not only has a significant morbidity, but a high mortality as well. Besides, because of the high cost and complexity of the treatments, few patients are able to get adequate treatment for kidney disorders. Nephrotoxicity is an important adverse effect of various antibiotics, anticancer drugs and other synthetic molecules. Extracts of various natural products and dietary antioxidants have been reported to show protective effects against nephrotoxicity (*Rasikh Javaid et al.*, 2012).
### Table-1 No Plants containing protective activity against gentamicin induced nephrotoxicity (Ramya Pydi et al., 2011)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the plant</th>
<th>Family</th>
<th>Parts used</th>
<th>Chemicals constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aegle marmelos</td>
<td>Rutaceae family</td>
<td>Leaf</td>
<td>β-D-glucoside, Mamesnine, Lupeol, Tannins, Aegeline, Ageline, Rutin, Sterol Flavonoids, Umbelliferone, Quercetin and volatile oils.</td>
</tr>
<tr>
<td>2</td>
<td>Aerva lanata</td>
<td>Amaranthaceae</td>
<td>Whole plant</td>
<td>Botulin, β-sitosterol, Amyrin, Hentriacontane, Stigmasterol, Kaempferol, Propionic acid, β-carboline-I, Aervoside and Aervolanine.</td>
</tr>
<tr>
<td>3</td>
<td>Cassia auriculata</td>
<td>Fabaceae</td>
<td>Root</td>
<td>Tannins, Di-(2-ethyl) hexyl phthalate, Alkaloids, Resins, Ca&lt;sup&gt;2+&lt;/sup&gt; and phosphorus.</td>
</tr>
<tr>
<td>4</td>
<td>Crataeva nurvula</td>
<td>Capparidaceae</td>
<td>Fruit</td>
<td>Kaemferol-3-O-a-D-glucoside, Quercitin-3-O-a-D-lucoside, Flavonoid, Glucosinolates, Steroids, Lupeol and Tannins.</td>
</tr>
<tr>
<td>5</td>
<td>Emblica officinalis</td>
<td>Euphobiaceae</td>
<td>Fruit</td>
<td>Vitamin-C, Carotene, Nicotinic acid, Riboflavin, D-glucose, D-fructose, Myoinositol, D-galacturonic acid, D-arabinosyl, D-manosyl, D-galactosyl, Embicol, Tannins, Ellagic acid, L-rhamnosyl, G-glycosyl, etc.</td>
</tr>
<tr>
<td>6</td>
<td>Glycyrrhiza glabra</td>
<td>Fabaceae</td>
<td>Rhizome</td>
<td>Glycyrrhizin, Glycyrrhetinic acid, Glycosides, Steroids, Glucose, Sucrose, Resin, Starch and Essential oil.</td>
</tr>
<tr>
<td>7</td>
<td>Hygrophila spinosas T</td>
<td>Acanthaceae</td>
<td>Whole</td>
<td>B-sitosterol, Lupeol, Minerals like Na, K, Ca, P and polyphenols.</td>
</tr>
<tr>
<td>8</td>
<td>Morindacitifolia L</td>
<td>Rubiaceae</td>
<td>Fruit</td>
<td>Americanol A, Morindolin and Isoprinceptin.</td>
</tr>
<tr>
<td>9</td>
<td>Kalanchoepinnata Pars</td>
<td>Rubiaceae</td>
<td>Fruit</td>
<td>Alkanes, Tricontane alpha and beta, Malic acid, Citric acid, Quercetin, Kaempferol, Calcium Sulphate and calcium Oxalate.</td>
</tr>
<tr>
<td>10</td>
<td>Nigella sativa</td>
<td>Ranunculaceae</td>
<td>Whole plant</td>
<td>Alanine, Arabic acid, Carvone, Aspartic acid, Cystein, Cholesterol, Glutamic acid, Linonie acid, etc.</td>
</tr>
<tr>
<td></td>
<td><strong>Plant</strong></td>
<td><strong>Family</strong></td>
<td><strong>Part</strong></td>
<td><strong>Active Constituents</strong></td>
</tr>
<tr>
<td>---</td>
<td>----------------</td>
<td>------------</td>
<td>---------</td>
<td>-----------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>11</td>
<td><em>Ocimum sanctum</em></td>
<td>Lamiaceae</td>
<td>Leaf</td>
<td>Eugenol, Euginol methyl ether, Carvacrol, Ursolic acid, Glycosides, Saponins and Tannins.</td>
</tr>
<tr>
<td>12</td>
<td><em>Orthosiphon stamineus</em></td>
<td>Laminaceae</td>
<td>Whole plant</td>
<td>Flavonoids, Phenols, Carbohydrates, Steroids, Tannins, Terpenes and Saponins.</td>
</tr>
<tr>
<td>13</td>
<td><em>Rhazya stricta</em></td>
<td>Apocynaceae</td>
<td>Leaf</td>
<td>Condyloacarpine and Vincamine.</td>
</tr>
<tr>
<td>14</td>
<td><em>Solanum nigrum</em></td>
<td>Solanaceae</td>
<td>Whole plant</td>
<td>Alkaloids, Saponins, Tamatidenol, Solamargine, Trigogenine, etc.</td>
</tr>
<tr>
<td>15</td>
<td><em>Tribulus potatorum</em></td>
<td>Loganiaceae</td>
<td>Seed</td>
<td>Flavonoids, Phenols, Saponins, Alkaloids, Steroids, Tannins, Glycosides and Lignins.</td>
</tr>
<tr>
<td>16</td>
<td><em>Tribulus sativus</em></td>
<td>Zygophyllaceae</td>
<td>Fruit</td>
<td>Alkaloids, Harmine, Harman, Saponins, Steroidal Sapogenins, Flavonoids, Kaemferol, etc.</td>
</tr>
</tbody>
</table>
Table No 2 Plants containing protective activity against cisplatin induced nephrotoxicity (Ramya Pydi et al., 2011).

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the plant</th>
<th>Family</th>
<th>Parts used</th>
<th>Chemicals constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aerva javanica</td>
<td>Amaranthaceae</td>
<td>Fresh Roots</td>
<td>Isoquercetin, 5 methylmellein, Kaempferol, etc.</td>
</tr>
<tr>
<td>2</td>
<td>Aerva lanata</td>
<td>Amaranthaceae</td>
<td>Whole plant</td>
<td>Botulin, β-sitosterol, Amyrin, Campesterol, Kaempferol, etc.</td>
</tr>
<tr>
<td>3</td>
<td>Cassia auriculata</td>
<td>Fabaceae</td>
<td>Root</td>
<td>Tannins, Alkaloids, Resins, etc.</td>
</tr>
<tr>
<td>4</td>
<td>Carica Papaya</td>
<td>Caricaceae</td>
<td>Seed</td>
<td>Flavonoids, Phenols, Alkaloids, Sterols, Terpenoids, Tannins, Saponins, etc.</td>
</tr>
<tr>
<td>5</td>
<td>Cururbita pepo</td>
<td>Cucubitaceae</td>
<td>Seed</td>
<td>Flavonoids, Phenols, Alkaloids, Tannins, Terpines and Saponins.</td>
</tr>
<tr>
<td>6</td>
<td>Dichrostachyscinera</td>
<td>Mimosaceae</td>
<td>Root</td>
<td>Fixed oils, Steroids, Flavonoids, etc</td>
</tr>
<tr>
<td>7</td>
<td>Kigelia africana</td>
<td>Bignoniaceae</td>
<td>Matured fruits</td>
<td>Iridoids, Flavonoids, Tannins, Terpenes, Steroids, Saponins and caffeic acid.</td>
</tr>
<tr>
<td>8</td>
<td>Ficus religiosa L</td>
<td>Moraceae</td>
<td>Latex</td>
<td>Flavonoids, tannins and Amino acids.</td>
</tr>
<tr>
<td>9</td>
<td>Lepidium sativum L</td>
<td>Brassicaceae</td>
<td>Seed</td>
<td>Volatile essential aromatic oils, Fatty oils, Proteins, Fatty acid, Riboflavin, flavonoids, Glycosides, etc.</td>
</tr>
<tr>
<td>11</td>
<td>Pedialium mures L</td>
<td>Pedaliaceae</td>
<td>Dried fruits</td>
<td>Flavonoids, Flavones, Alkaloids, Glycosides and saponins.</td>
</tr>
<tr>
<td>12</td>
<td>Picrorhizakurroa Royle</td>
<td>Scrophulariaceae.</td>
<td>Rhizome</td>
<td>Tannins.</td>
</tr>
<tr>
<td>13</td>
<td>Pongamia pinnata</td>
<td>Papilionaceae</td>
<td>Flowers</td>
<td>Pongamol, Protein, Alkaloids, Tannins, Sugar, Resin and Fatty oil.</td>
</tr>
<tr>
<td>14</td>
<td>Salviae radix</td>
<td>Lamiaceae</td>
<td>Whole plant</td>
<td>Lithospermic acid, Isoferulic acid, Salvianolic acid, Rosmarinic acid, etc.</td>
</tr>
<tr>
<td>15</td>
<td>Vernonia cinerea</td>
<td>Compositae</td>
<td>Aerial parts</td>
<td>Triterpenoids like α-amyrin, β-amyrin and lupeol.</td>
</tr>
</tbody>
</table>
1.7 Importance of the proposed work

The plant derived traditional medicaments are important in relieving various disorders. This helps in reducing the occurrence of life threatening disorders in the human beings. Due to the increased influence of modern drugs, modern science and recent advances in the health care system, and production of synthetic drugs, the traditional drugs have taken back stage. Number of naturally available plant derived medicines are used to treat number of disorders in many parts of the world. It is reported that number of plant derived extracts are used by the native practitioners to prevent the nephrotoxicity. In this context the folk medicine Ficus racemosa bark extracts were studied for its nephron protective activity.

It is reported that furanocoumarins ingredients are present in several species of Ficus. This ingredient is one of the important phototoxin. It is also reported that Moraceae family contains various plant constituents like flavonoids, flavonoids with isoprenoid substituents and stibenes. It is also reported that various antioxidants separated from figs protect the lipoproteins present in the blood plasma from oxidation. This also produces antioxidant activity. (Duenas M et al., 2008).

Pathologic disturbances that inhibit the ability of ADH to stimulate water reabsorption at the nephron can result in diabetes insipidus (DI). A distinction is made between two types of DI. Neurogenic DI results from the inability of hypothalamic neurons to synthesize or secrete ADH. Nephrogenic DI results from an inability of the nephrons to respond to ADH. This is usually caused by an abnormal gene which expresses the V2 receptor. Because of this, ADH can no longer bind or stimulate V2 receptor signalling. No specific pharmacological treatment exists for nephrogenic DI; patients are usually treated by restricting fluid intake or given diuretics to inhibit excessive dilution of the urine. A hypothetical pharmacological therapy for nephrogenic DI might consist of a compound that directly stimulates expression of nephron collecting duct water channels, thus bypassing the nonfunctional V2 receptor (David E. Golan, et al., 2005).

If kidney excretes excessively diluted urine due to the inactivity of ADH receptors in the basolateral surfaces of the DCT and collecting ducts, it leads to severe dehydration. This results in the hyperosmolar concentration of blood plasma. This is due to the loss of more fluid from the body than fluid intake. This imbalances the proper blood supply to the brain. If this is left untreated for a long time, permanent brain damage can occur. The severe dehydration also causes structural changes in the ureters and the urinary bladder, leading to
potential problems. Very severe dehydration may also lead to the death. The psychiatric problems like bipolar psychosis, mania, etc. is treated with lithium compounds. Currently lithium is also indicated as a drug for treating alcoholism, Alzheimer’s disorder, schizophrenia, headache and AIDS.

Many patients who receive lithium compounds as medication for the treatment of psychiatric problems, develop NDI. This may be caused by the impairment of ADH receptors expressed on the PCT cells and cells of the collecting duct. The inactivity of these receptors cause dehydration. The aquaporin channels are not expressed properly on the apical tubular cells. The reabsorption of water decreases; this also decreases the reabsorption of sodium ions. The urine output increases leading to dehydration. This results in increase in blood concentration leading to severe consequences. The exact mechanism involved in the induction of nephrogenic diabetes insipidus by the lithium is still unclear (Yuedan Li et al., 2006).