1.8 Review of literature:

1.8.1 Nephron: Most of the ions and solute transported across the tubular cells is by active transport by utilizing energy in the form of ATPs. The release of energy takes place in the tubular cells. Various ions and molecules are also transported by passive diffusion. The electrochemical gradient is essential for the transportation by passive diffusion. Most of the ions and the molecules are transported from the interstitial spaces into the peritubular capillaries by passive diffusion. During the active transport the molecules are transported against electrochemical gradient and the energy required for the process is derived from the consumption of ATP. The anions and cations are also transport across the trans-cellular and para-cellular route in the tubular cells of the nephron.

The glomerular capillaries are enclosed in a double layered structure known as Bowman’s capsule. The outer layer is the parietal layer and the inner one is the visceral layer. The parietal layer consists of a simple pavement or squamous epithelium, while the visceral layer consists of podocytes wrapped around glomerular capillaries. The blood filtered by the glomerular filter is the glomerular filtrate. This filtered fluid then enters into the renal tubule. The tubule consists of the proximal convoluted tubule, loop of Henley, the distal convoluted tubule and the collecting duct. The collecting duct ends at the papilla and the primary calyx.

The thick ascending loop of Henley leads to the PCT. It is the longest tubule of the nephron and it is most coiled part of the tubule. The PCT consists of cuboidal epithelium with microvilli on their apical surfaces. The nephron is a U-shaped nephron loop. The initial part of the loop is the descending loop, and then the ascending limb. The squamous epithelium is present in the descending loop and along a thin section of ascending loop. The cuboidal epithelium is present in the thick section of the nephron. It is the lower part of the ascending limb. The distal convoluted tubule is the smaller and less twisted than proximal convoluted tubule. The PCT consists of cuboidal epithelium with micro villi on their epithelial cells.
Figure 20 Reabsorption at nephron

The PCT is finally connected to the collecting duct of the nephron. Several nephrons drain the formed urine into the duct called collecting duct. The collecting duct then opens into the papillary duct which then opens into the renal papillae and then into the minor calyx. Near to the thick ascending limb of loop of Henley and the afferent arteriole, juxtaglomerular apparatus is located. This apparatus consists of juxtaglomerular cells which secrete renin when the renal blood flow decreases. Through renin-angiotensin-aldosterone pathway they look after the changes in the BP in the kidneys.

In a drug induced kidney failure, in kidney infections or in trauma, the glomerular filtration membrane may get damaged and structural alterations take place resulting in the filtration of larger molecules like albumin or blood cells leading to albumin urea and hematuria. Urine mainly consists of water (95%) and solutes (5%). The solute part of the urine mainly consists of urea, sodium, chloride, potassium ions, creatinine and the uric acid.

Bilirubin, derived from the breakdown of hemoglobin, is present in urine in traces. The kidney toxicity is detected by observing the abnormal content of glucose, free haemoglobin, albumin, ketones, or more than a trace of bile pigments in the urine. Nephron injury in the
study of protective effects of stem bark extracts of ficus racemosa in drug induced nephrogenic diabetes insipidus and nephrotoxicity in animal models

Kidney is irreversible. If some of the nephrons get injured, the remaining nephrons become active and are involved in their function in place of damaged nephrons.

When 75% of the nephrons are lost in a kidney, urine output may be as low as 30ml/h when compared to the normal of 50 to 60ml/h. The disturbed homeostatic is accompanied by azotemia and acidosis. When there is 90% loss of kidney function, leads to uremia. The insufficiency of kidney is also susceptible to cause anemia because of the decreased formation of erythropoietin, the hormone that stimulates red blood formation.

1.8.2 Nephrotoxicity by nephrotoxins-

The nephrotoxic drugs are classified into the following groups. antimicrobial agents (aminoglycosides antibiotics), antifungal antibiotics (amphotericin B, nystatin), antibiotics that cause acute interstitial nephritis, antiviral agents, radio contrast agents, NSAIDs, ACE inhibitors and angiotensin II receptor blockers, volume expanders and miscellaneous.

The incidence of aminoglycoside nephrotoxicity depends on the number of patients and duration of the drug therapy. Aminoglycoside antibiotics exhibit rapid concentration, bactericidal activity and persistent post therapy pharmacological effects. Age, presence of kidney disease, volume depletion, hepato-biliary disorder, diabetes mellitus, and endotoxemia and electrolyte imbalances are the factors that increase nephrotoxicity from aminoglycosides.

Among the aminoglycosides gentamicin exhibit increased incidence of nephrotoxicity than amikacin, tobramycin or nitilmicin. Gentamicin increases the nephrotoxicity with increased cumulative dosing, duration more than one week and simultaneous use of other nephrotoxins, such as vancomycin, cephalosporins, NSAIDs, penicillin, amphotericin B, ACE inhibitors, cisplatin, frusemide, etc.

Aminoglycoside antibiotics are cation containing molecules. The protein binding capacity of these molecules is less and is easily filtered by the glomerular filtration. Most of the filtered drug is excreted through urine. Some of these drug molecules are pinocytosis by proximal tubular cells; within these tubular cells they get fused with lysosomes to form myeloid bodies. The continuous administration of aminoglycoside antibiotics for 7-10 days, results in the damage of proximal tubular cells and a noticeable decline in GFR, and this leads to a...
subsequent increase in serum creatinine. Nephrotoxicity in most of cases is reversible with discontinuation of the aminoglycoside antibiotics. The administration of resveratrol (a natural ingredient) alone or with diphenylene iodonium (an antioxidant) with gentamicin has shown protective effect against gentamicin induced kidney failure.

It is reported that the aminoglycoside- the less purified vancomycin has shown higher incidence of the development of acute kidney failure. But the study also reveals that decreased incidence of AKI with purified compound. Simultaneous and prolonged administration of vancomycin with nephrotoxic drugs such as cyclosporine, amphotericin B, cephalosporin, aminoglycoside antibiotics and loop diuretics. Higher doses of vancomycin (IV and intra-peritoneal) showed proximal tubular cell damage in animal models and at very high doses, the animal model have shown tubular epithelial necrosis.

Some antifungal drugs like amphotericin-B is indicated in the treatment of systemic infections in neurotropic patients and in bone marrow and solid organ transplant recipients. The glomerular filtration is decreased to 40% in patients receiving antifungal antibiotics within 2 weeks of initial treatment. As the dose increases, the extent of irreversible renal damage increases. As the dose increases, the incidence of nephrotoxicity also increases. Nephrotoxicity is developed in the laboratory animals with a dose dependent administration of amphotericin B.

The fungi destroying activity of amphotericin B is mediated by its capacity to enter into the fungal walls. The amphotericin B also causes the damaging effects in renal epithelial cells, mostly the cells of the Juxta-glomerular cells of the nephron. It is concluded that the damage in the epithelial cell increases sodium influx across the tubular walls. The constriction of the renal blood vessels decrease in GFR can also due to their direct effect on renal blood vessels. The deoxycholate portion of amphotericin B causes vasoconstriction and precipitation of tubular toxicity.

The various antibiotics such as penicillin, cephalosporin, sulfonamide, quinolone, macrolide, are also causes acute renal failure. Acute renal failure symptoms are recognized in 10 to 15% of renal biopsies in patients with unexplained renal failure. The serum creatinine level is increased in patients within 2 to 44 days of drug exposure, and earlier with subsequent re-exposure to the same drug (Ghousia Wadjida et al., 2008).
The therapy with antiviral drugs gained importance in recent years. These drugs cause the development of AKI in immune-compromised patients, solid organ and bone marrow transplant recipients, and HIV patients are taking antiretroviral treatment. Drugs used in the treatment of infection caused by cytomegalovirus. The patients receiving drugs such as ganciclovir, foscarnet, and cidofovir and posttransplant patients develop reversible-dose dependent nephrotoxicity. The simultaneous treatment with other nephrotoxic drugs, such as aminoglycosides, NSAIDs, amphotericin B and pentamidine, also leads to the development of acute renal failure. The anticytomegaloviral drugs are eliminated without any modification by the kidneys and they get concentrated in the renal cortex, where they induce tubular toxicity. The antiviral drug like ganciclovir gets precipitated in the distal tubular lumen, causing renal injury.

The use of intravascular contrast media in radiographic and cardiovascular procedures is one or the commonest causes of AKI in ICU patients. The nephrotoxicity caused by radio contrast media is the third most common cause of AKI in hospitalized patients. The etiology and mechanisms of contrast-mediated nephropathy is complex and not fully understood.

Contrast agents are iodinated drugs available as anions at physiologic pH, showing poor plasma protein binding, decreased fat solubility, and poor membrane penetrating efficacy. The intravenous administration of radio contrast media is associated with vasodilation followed by prolonged vasoconstriction. This accounts a low excreted portion of filtered sodium seen in early hours following the administration of contrast media. The radio contrast drugs are freely filtered by the glomerulus. The reabsorption of water and other electrolytes increases the concentration of contrast agents in the tubular lumen. This leads to the development of tubular toxicity, which is mediated by free oxygen radicals, interfering with cell membranes and oxidative metabolism.

The use of some analgesics leads to the development of AKI within few days of initiation of treatment. The danger of acute renal failure is increased if the diuretics are taken along with some analgesics and some calcium channel blockers. NSAIDs act by decreasing the secretion of prostaglandins by inhibiting cyclooxygenase enzymes. This enzyme is responsible for the conversion of arachidonic acid to prostaglandins and thromboxanes, attenuating prostaglandin-mediated vasodilation effect. The NSAIDs induced vasoconstrictor effect leads to ischemia and results in a decline in GFR and the development of AKI. The immune-mediated phenomenon of NSAIDs leads to the development.
The use of antihypertensive drugs also leads to a decline in GFR. The drugs like ACE inhibitors and ARBs inhibit the angiotensin-II. This causes a more decline in GFR. This effect is more in critically ill patients and patients with volume depletion, and in the combination of NSAIDs with diuretics.

The IV administration of drugs like sucrose, mannitol, and complex carbohydrates like dextran and hydroxyl ethyl starch are associated with the development of AKI. Serum creatinine concentration is significantly lower in the gelatin administered group than in the HES-gelatin group after 10 days after transplantation. The induced AKI in most cases get corrected when the discontinuation of the drug and correction of electrolyte abnormalities takes place (Ghousia Wadjida et al., 2008).

Acute tubular necrosis is usually developed by ischemia, and the administration of nephrotoxicants. Some diagnostic tests like obstruction of perirenal azotemia are indicated in ATN. The second major cause of ATN is the nephrotoxic injury. The drugs like gentamicin, cisplatin, amphotericin-B, chemotherapeutical agents, and radio contrast drug, hemoglobin and myeloma leads to the development of ATN. Acute kidney failure is mainly due to aminoglycoside antibiotics and radio contrast media.

The exact mode of action of nephrotoxicity caused by the administration of aminoglycoside antibiotics is not clearly understood. Usually acute renal failure is generally develops in 8 days after the antibiotic treatment is commenced. It is evident that the aminoglycoside induced nephrotoxicity reduces the glomerular filtration rate. This reduction is mainly due to hemodynamic abnormalities, obstruction in the tubules, and leakage in the glomerular filter, changes in the glomerular blood hydrostatic, capsular hydrostatic and blood colloidal osmotic pressures.
Table No- 3 Drugs showing nephrotoxicity

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Cause of renal injury</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analgesics</strong></td>
<td></td>
</tr>
<tr>
<td>Acetaminophen, aspirin</td>
<td>Interstitial nephritis</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>Acute interstitial nephritis changed intra-glomerular hemodynamics, glomerulonephritis.</td>
</tr>
<tr>
<td><strong>Antidepressants</strong></td>
<td></td>
</tr>
<tr>
<td>Amitriptyline, fluoxetine</td>
<td>Rhabdomyolysis</td>
</tr>
<tr>
<td>Lithium salts</td>
<td>Glomerulonephritis, rhabdomyolysis.</td>
</tr>
<tr>
<td><strong>Antihistamines</strong></td>
<td></td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>Rhabdomyolysis</td>
</tr>
<tr>
<td><strong>Antimicrobial agents</strong></td>
<td></td>
</tr>
<tr>
<td>Acyclovir</td>
<td>Acute interstitial nephritis, nephropathy.</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Nephrotoxicity</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>Nephrotoxicity</td>
</tr>
<tr>
<td><strong>Beta lactam antibiotics</strong></td>
<td></td>
</tr>
<tr>
<td>Penicillin and cephalosporin</td>
<td>Acute interstitial nephritis, nephrotoxicity</td>
</tr>
<tr>
<td>Foscarnet</td>
<td>Nephrotoxicity</td>
</tr>
<tr>
<td>Ganciclovir</td>
<td>Acute kidney injury</td>
</tr>
<tr>
<td>Pentamidine</td>
<td>Nephrotoxicity</td>
</tr>
<tr>
<td>Quinolones</td>
<td>Acute interstitial nephritis, crystal nephropathy</td>
</tr>
<tr>
<td>Rifampin</td>
<td>Acute interstitial nephritis</td>
</tr>
<tr>
<td>Sulfonamides</td>
<td>Acute interstitial nephritis, crystal nephropathy</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Acute interstitial nephritis</td>
</tr>
<tr>
<td><strong>Antiretroviral agents</strong></td>
<td></td>
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<tr>
<td>Adefovir</td>
<td>Tubular cell toxicity</td>
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<tr>
<td>Indinavir</td>
<td>Acute interstitial nephritis, crystal nephropathy</td>
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<tr>
<td>Benzodiazepines</td>
<td>Rhabdomyolysis</td>
</tr>
<tr>
<td><strong>Calcineurin inhibitors</strong></td>
<td></td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>Altered intra-glomerular hemodynamics, interstitial nephritis, micro-angiography</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>Altered intra-glomerular hemodynamics</td>
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<tr>
<td><strong>Cardiovascular agents</strong></td>
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<tr>
<td>ACEI, angiotensin receptor blockers</td>
<td>Altered intra-glomerular hemodynamics</td>
</tr>
<tr>
<td>Clopidogrel, ticlopidine</td>
<td>Thrombic microangiopathy</td>
</tr>
<tr>
<td>Statins</td>
<td>Rhabdomyolysis</td>
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</tbody>
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<table>
<thead>
<tr>
<th><strong>Chemotherapeutic agents</strong></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Carmustine, semustine</td>
<td>Chronic interstitial nephritis</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>Interstitial nephritis, tubular cell nephritis.</td>
</tr>
<tr>
<td>Interferon- alpha</td>
<td>Glomerulonephritis</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Crystal nephropathy</td>
</tr>
<tr>
<td>Mitomycin-C</td>
<td>Thrombotic micro-angiopathy</td>
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<tr>
<td>Contrast dye</td>
<td>Tubular cell toxicity</td>
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<table>
<thead>
<tr>
<th><strong>Diuretics</strong></th>
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<tbody>
<tr>
<td>Loop diuretics, thiazides</td>
<td>Acute interstitial nephritis</td>
</tr>
<tr>
<td>Triamterene</td>
<td>Crystal nephropathy</td>
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<tr>
<th><strong>Drugs of abuse</strong></th>
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<tbody>
<tr>
<td>Cocaine, heroin, ketamine</td>
<td>Rhabdomyolysis</td>
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<tr>
<th><strong>Natural drugs</strong></th>
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<tbody>
<tr>
<td>Natural drugs with aristocholic acid</td>
<td>Prolonged kidney failure with inflammation of interstitial fluid</td>
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<tr>
<th><strong>Proton pump inhibitors</strong></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Lansoprazole, omeprazole, pantaprazole</td>
<td>Acute interstitial nephritis.</td>
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<table>
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<tr>
<th><strong>Others</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Allopurinol</td>
<td>Acute kidney failure with interstitial nephritis</td>
</tr>
<tr>
<td>Gold therapy</td>
<td>Glomerulonephritis</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>Rhabdomyolysis</td>
</tr>
<tr>
<td>Pamidronate</td>
<td>Glomerulonephritis</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Acute interstitial nephritis</td>
</tr>
<tr>
<td>Quinine</td>
<td>Thrombotic microangiopathy</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>Acute interstitial nephritis</td>
</tr>
<tr>
<td>Zoledronate</td>
<td>Tubular cell toxicity</td>
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The administration of nephrotoxic drugs involved in the development of acute renal injury. Today patients have more co-morbidities, and are exposed to more number of therapeutic agents, diagnostic agents, therapeutic procedures, which are usually have the risk of developing toxicities to the kidneys. The drug-induced nephrotoxicities are more common in patients undergoing treatment with various drugs. There is a need of protection from these adverse effects produced by number of drugs including aminoglycosides, cisplatin, cyclosporine, etc. (Cynthia A Naughton, 2008).

Nephrotoxicity is frequently seen with many medications in use today. Most of the blood ejected out from the ventricles of the heart reach the kidneys through blood circulation. The nephrotoxic drug present in the blood reaches the large surface area of the kidney producing the adverse drug reactions. As the kidney consists of numerous enzymes, tubular cell transporters, the drug molecules are exposed to the tubular cells, renal glomerular cells and interstitial cells of the nephrons leading to the toxicity. The metabolites of the drug also formed in the kidneys because of high rate of Metabolisms. These metabolites are also exposed to the kidney cells. Some drugs and their metabolites produce direct effect on the renal blood vessels by causing vasoconstriction. This alters the vasoregulatory mechanism of the kidney.

The kidney damage is usually recognized when filtration is affected, characterized by an increased concentration of BUN and creatinine level in the blood, because there are yet no good serologic markers to determine the subtle insults to the kidneys. The true incidence of drug-induced nephropathy is difficult to establish because the clinicopathologic presentation can be quite varied. Medications can cause AFR by varieties mechanisms, which include decreased renal perfusion, renal blood vessel damage or vasoconstriction, damage to the cells of the tubules, allergic interstitial injury. The nephrotoxic drugs administration also responsible for the kidney failure along with damaging effect to the vasculature of the renal blood vessels, renal tubular cells, damage to the interstitial cells and papillary structures. These damaging effects are usually due to electrolyte imbalances, acid-base imbalances, abnormalities in the water balance, etc. This alterations may lead to the progression of morbidities. (Devasmita Choudhury et al., 2009).

Programmed cell death play a vital play in different human kidney disorders and the development of kidneytoxicity. The programmed death pathway in the tubular cells are
initiated by number of nephrotoxic drugs leading to the kidney failure. There are two phases in apoptosis pathways. The internal or intrinsic pathway involves organelles of the cell. The another pathway is the extrinsic pathway. The above pathways cause the stimulation of specific proteases called the caspase-3 and caspase-7, this causes the formation of characteristic morphologic symptoms of apoptosis that include membrane blebbing, shrinkage of the cell, and fragmentation of the DNA in the cell (Servais *et al.*, 2007).

**1.8.3 Molecular mechanisms of aminoglycoside induced nephrotoxicity:**

Aminoglycosides is one of the important groups of antibiotics and still it is indicated in various infections of Gram-ve bacteria. But its disadvantage is the development of ototoxicity and nephrotoxicity. Aminoglycoside antibiotics administration over prolonged period leads to the formation of acute kidney failure. In this condition the creatinine elimination decreases, as the injury makes the kidney to excrete diminished levels of creatinine. This leads to the increased levels of serum creatinine levels. This can be measured from the blood and urine samples using auto analyzer.

The creatinine estimation is usually performed together with urea estimation to assess the damaging effect produced by the aminoglycoside therapy. The injured kidney fails to excrete normal concentrations of urea from the blood. Hence serum urea level increases, suggesting the renal injury by the aminoglycoside therapy. The aminoglycosides if administered, in their free from they get filtered in the glomerular capillaries to enter the lumen of the tubules. As these molecules having cationic properties, they facilitate binding to the epithelial membrane of the cells of PCT. They get transported into the tubular cells, by virtue of their cationic properties and get accumulated in the tubular cells to produce damage in the tubular cell.
The aminoglycoside concentration in the tubular cells of the nephrons may disturb the normal physiology and normal function of the cells. Usually these get accumulated in the lysosomal organelles of the tubular cells. The regular cellular functions like synthesis of proteins, mitochondrial functions get disrupted. These changes may lead to programmed cell death because of its activation. The aminoglycoside antibiotics like gentamicin activates the calcium receptor expressed on the tubular cells, which causes cell signaling and cell death. Elevated cytosolic calcium is yet another attractive and associated with sepsis, immune-mediated disease, and glomerular diseases (Neesh Pannu et al., 2008).

Aminoglycoside antibiotic molecules are not toxic as such; they require some conditions like oxidation of metal ion to become destructive. Thus, an aminoglycoside chelates with a metallic ion and chelated metal complexes are generated which are potential to release reactive oxygen species (ROS) including free radicals. Some reports suggest that at least some of the damage by aminoglycosides involves ROS and reactive nitrogen species (RNS).

The interaction of aminoglycoside molecules with the cellular components of the target tissues causes the release of oxygen free radicals. The interaction between radicals with cellular components is responsible for the development of ototoxicity. In this condition the target tissues expresses cytosolic superoxide dismutase in excess, these antioxidants metabolizes the superoxide anion radical (O$_2^-$) and these radicals are converted to less toxic non-radical form such as hydrogen peroxide, when it is given along with aminoglycosides like kanamycin, when it is compared to the SOD activity in normal animals.

The cochlear hair cells are located on the basilar membrane of the cochlear duct initiates the auditory impulses. The JNK is the signaling pathway responsible for the normal activity of
the receptor hair cells. The administration of aminoglycoside antibiotics are generated oxygen free radicals, these activates this pathway. If this pathway is inhibited then the damage to the hair cell by the administration of aminoglycoside antibiotic like neomycin is prevented. Hence the role of JNK pathway is very important for the protection of receptor cells located in the cochlear duct of the cochlea from the aminoglycoside antibiotic administration. Apart from this pathway the role of transcription kappa-beta also important for its protective effect hair cell by the kanamycin administration.

The outer cell layer present in the organ of corti gets destroyed by the treatment of kanamycin administration also leads to the loss of inhibitory cellular proteins in these cells. The role of metal chelates is also important in the protection of hair cells. The aminoglycosides produce toxic effects by reacting with transition metals like iron leads to the release of destructive reactive species. Hence chelation of metal ions like iron is important in preventing the cytotoxicity produced by a kanamycin.

. The PCT consists of brush border cuboidal epithelial cells. The apical surfaces of these cells express various transporters like sodium/glucose symporters, sodium/proton antiporters. The aminoglycoside antibiotics mainly produce injurious effect on these cells and causes tubular necrosis. If gentamicin is administered it get accumulated in these cells. The megalin and cubulin receptors are mainly expressed on the cuboidal epithelial cells of the PCT. These receptors transport cation drugs like gentamicin into the tubular cells. This leads to the accumulation of gentamicin. These receptors also transport some protein molecules into the tubular cells. Within the tubular cells of the PCT, the gentamicin molecules get accumulated in the endoplasmic reticulum and Golgi complex. The accumulated molecule then gets concentrated in the cell organelles like mitochondria, where they induce the intrinsic apoptosis pathway. (Yaremi Quiros et al., 2010).
Figure 22 Aminoglycoside induced apoptosis

Nephrotoxicity induced by aminoglycoside manifests clinically as oliguric acute kidney failure. There is transient increase in the serum creatinine level. The hypoosmolar urinary output is developed after the administration of aminoglycoside antibiotics for several days.

The chronic administration of aminoglycoside antibiotic like gentamicin causes nephrotoxicity because small quantity gets remained in the epithelial cells of the PCT after glomerular filtration. The aminoglycosides get concentrated in the lysosomal vacuoles of cells. They are also localized with the Golgi complex.

Animals administrated with less doses of aminoglycosides shows phospholipidosis and apoptosis in the PCT. The alteration in the lysosomal membrane is caused by the gentamicin leads to the release of various lysosomal constituents like calcium and proteases. Proximal tubule injury leading to cell necrosis underlines aminoglycoside nephrotoxicity. The gentamicin is one of the aminoglycoside antibiotic consisting of cationic moiety have the ability to join to the acidic phosphoinositide of the PCT.
After uptake into PCT cells, the numbers of intracellular processes are disrupted by the presence of aminoglycoside (Sandhu JS et al., 2007). Aminoglycosides like gentamicin get accumulated in the PCT cells of the kidney tubules. The levels of these molecules are several times more than their concentrations in the blood.

The aminoglycoside antibiotic like gentamicin gets bind to the cellular components the PCT cells could be demonstrated. Various transporters expressed on the apical surfaces of the PCT cells helps in the transportation of the gentamicin into the cells. This process is known as endocytosis. Within the cells these molecules exert inhibitory activities on lysosomal phospholipases and sphingomyelinase. The tubular cell membranes are rich in phospholipids, the accumulation of gentamicin leads to the inhibition of degradation of phospholipids. This causes the accumulation of phospholipids in the tubular cell membrane of the PCT cells. The development of myeloid bodies in the tubular cells of the PCT is due to the deposition of lysosomes. The increased concentration of phospholipid is due to the effect of gentamicin molecules changes the structural integrity and strength of lysosomal membrane, leading the leakage of lysosomal enzymes into the cytosol of the tubular cells.

This gives the information about the pathological changes in the tubular cells by the aminoglycosides like gentamicin. The apoptosis pathway alteration is also caused by the gentamicin in the cells of PCT. The normal structure of the PCT cells gets altered by the adverse effects of gentamicin on the cellular components of the PCT cells. The various transported on the apical surfaces also get affected by the aminoglycoside antibiotics. (Ozaki N et al., 2009).

Gentamicin molecules reach the PCT cells through the multigrade receptor megalin and clathrin- treated openings. Gentamicin transported in to the tubular cells through these receptors into the lysosomes, Golgi complex, rough ER through retrograde manner. The aminoglycoside antibiotic like gentamicin chelates with iron ions forming soluble complexes. These complexes catalyze the formation of ROS. The accumulated gentamicin molecules release cathepsins, which are responsible for apoptosis.
Study of protective effects of stem bark extracts of Ficus racemosa in drug induced nephrogenic diabetes insipidus and nephrotoxicity in animal models

Figure 23 Apoptosis pathway

The gentamicin stimulates mitochondria to release cytochrome C into the renal tubular cells. This is the important step in the apoptosis pathway. The reactive oxidative species so released increases the formation of stress causing genes like chaperones and oxido-reductive enzymes. Sometimes gentamicin may also causes intracellular adverse effects without entering into the cells. The gentamicin molecules are responsible for the influx of Ca$^{2+}$ ions through the voltage gated calcium channels formed on the apical surfaces of the PCT cells. This takes place by the membrane Ca$^{2+}$ receptor CaR (Andrew Prayle et al., 2009).

Clinical nephrotoxicity induced by the gentamicin manifests itself clinically as non-oliguric renal damage. After several days of the gentamicin administration, several changes take place in the anatomical parameters as well as the functional aspects leading to the kidney failure. This disturbs the normal functioning of the kidney resulting in wasting of essential anions as well as cations. The glomerular filtration, some portion of gentamicin molecules is retained in the epithelial cells of the proximal tubules.
The gentamicin induced nephrotoxicity is developed by the entry of drug molecules into the tubular cells by receptor mediated endocytosis. This takes place when the drug molecules bind to the phospholipids and megalin receptors found on the membranes of the lysosomes. The animals treated with aminoglycoside antibiotics like gentamicin display lysosomal phospholipidosis in the cells of PCT.

The lysosomal membrane is raptured by the gentamicin, which could result in release of the drug to the cytosol and lysosomal constituents such as cathepsins. If the gentamicin is introduced directly into the cytosol by electroporation technique also induces apoptosis even at lower concentration. This indicates that only a small fraction of the amount of gentamicin stored in lysosomes needs to be released into the cytosol to trigger apoptosis.

When gentamicin is present in lysosomes, it is involved in protective mechanism but once the drug moves out of lysosomes, it leads to the generation of the nephrotoxic adverse effects. The next steps involve mitochondrial activation, release of cytochrome C and the expression of Bcl-2 due to the activation of caspase-3. Gentamicin located at cytoplasm may act on mitochondria. Gentamicin stimulates the formation of ROS in vitro in the presence of polyunsaturated lipids, which could also participate in this process (Servais H et al., 2008).

**Figure- 24 Drug induced apoptosis pathway**

**1.8.4 Effects of clinical and high doses aminoglycosides (AGs) in animals:**
Amino glycoside antibiotics like gentamicin when administered for few days of clinically doses to animals (10-20 mg/kg), AGs produce a characteristic alterations in lysosomes of PCT with the accumulation of polar lipids (myeloid bodies). These changes lead to the development of signs of symptoms tubular alterations or dysfunctions like destruction of brush-border and enzymes of the lysosomes, destruction of various transporters like sodium/glucose symporters and sodium/proton antiporters, decreased absorption of various electrolytes like K⁺, Mg²⁺, Ca²⁺ and glucose, etc. phospholipidurea and cast excretion).

![Figure 25 The mechanism of GM induced nephrotoxicity](image)

The tubular alterations have clearly associated with the development of focal necrosis and apoptosis in the tubular epithelium, without an apparent change in kidney function. The severe form of acute kidney failure is due to the administration of high doses (80mg/kg for gentamicin). This develops extended cortical necrosis and renal dysfunction. Most of the components of the tubular cells get affected by the aminoglycoside antibiotic drugs. The anatomical feature, metabolic components and functional alterations also take place in the tubular cells, leading to the necrosis of the tubular cells.

The apical membranes of the PCT cells get altered by the nephrotoxic drugs. The inhibition of protein synthesis required for the normal functioning of the cell also takes place. The other possible effects on the tubular cells include mitochondrial alteration, modulation of gene expression. The enzymes located on the cytosolic side of the peritubular membrane involve
uptake and intracellular distribution of the drug to the corresponding targets (David E. Golan, et al., 2005).

**Fig No-26**  Cellular changes in GM induced nephrotoxicity-A-Normal, B-Therapeutic dose, C-High dose.

### 1.8.5 Molecular mechanisms of cisplatin induced nephrotoxicity:

Platinating agents, including cisplatin, carboplatin, and oxaplatin, have been used clinically for nearly 39 y. These drugs are used for the treatment of certain types of cancers including cervical, lung, ovarian, colorectal and lymphomas.

Chemically cisplatin is cis-diamminedichloroplatinum and it is one of the important chemotherapeutic agents employed in the treatment of various cancers including lung cancers, ovarian cancers, germ cell cancers, etc. It is a major antineoplastic drug used in the relief of cancers. But its main adverse reaction is the development of nephrotoxicity; 20% of patients receiving high-dose of cisplatin have severe renal dysfunction. The cisplatin is used to induce nephrotoxicity in rodents like rats.

**Figure 27**  Structure of cisplatin and related drugs

### 1.8.6 Cisplatin uptake into renal cells:

Uptake of cisplatin is mainly through organic transporter pathway. Kidney is the major route for its excretion. The cisplatin concentration in PCT is about 5 times the serum concentration.
In the rat, its excretion occurs predominantly by glomerular filtration and to a lesser extent by tubular secretion. Tubular reabsorption is not takes place. The S₃ segment of the proximal tubule accumulates the highest concentration of this, followed by the distal convoluted tubule and the S₁ segment of the proximal tubule.

In addition to a transporter-mediated process, it enters the cell through passive diffusion. The transporter (OCT₂) is the critical transporter for its uptake in proximal tubules in both animals and humans. Three isoforms of OCT have been identified in cells of the human. The important type is OCT₂ are expressed in the cells of the kidney. The other type OCT1 is expressed on the cells of the liver, and OCT₃ is widely expressed, especially in the placenta (Xin Yao et al., 2007).

1.8.7 Intracellular events that damage the renal cells:

The *invivo* mechanisms of cisplatin nephrotoxicity are complex and involve programmed cell death pathway, formation of fibers, and inflammation. High concentrations of this induce necrosis in proximal tubular cells, whereas low concentrations of this induce apoptosis through a caspase-9-dependent pathway. Oxidative stress injury is actively involved in the pathogenesis of cisplatin-induced acute kidney injury. Reactive oxygen species (ROS) directly act cell organelles and constituents including lipids, proteins, and DNA, and destroy their structure. ROS are produced by various stress processes takes place in the cell.

The administered cisplatin enter the kidney without undergoing metabolism and these molecules enter the renal cells either passive diffusion or facilitated diffusion. Various pathways leading to the programmed renal cell death are activated by the cisplatin. These include MAPK, p53, ROS etc. It also stimulates the inflammatory response in the renal cells by stimulating tissue necrotic factor alpha production. This is also one of the causes for the renal cell injury and death. The vascular diameter of the afferent arterioles and the glomerular capillaries also get constricted by this molecules and this leads to ischemic tubular cell death of the nephron, because of the decreased blood supply. This ischemia also decreases the glomerular filtration and glomerular filtration rate. Hence this leads to the development of decreased urine formation. Altogether leads to the development of acute renal failure.
Cisplatin activates both intrinsic mitochondrial pathway and extrinsic death receptor pathway of cell death apoptosis. The endoplasmic reticulum also gets affected. Activation of these pathways leads to caspase-dependent or independent apoptosis. The p21 is induced and cdk\(_2\) is activated during cisplatin administration. The inhibitory protein p21 play a role in the protection of the tubular cells by inhibiting the signaling pathways leading to the death of the renal cells. Hence there is a crucial balance between p21 and cdk2 and this get disturbed leading to the tubular cell apoptosis and tubular cell injury because of the chronic administration of the cisplatin. The DNA damage is induced by the cisplatin and this activates phosphorylation because of the stimulation of ATR. This also results in the activation of p53. The activated p53 is also responsible for the transcription of apoptotic genes including PUMA-alpha and PIDD. PIDD activates caspase-2.

Cisplatin is a potent antitumor drug. High dose therapy with cisplatin is limited by its cumulative nephrotoxicity and neurotoxicity. It is transported into cells by the copper transporter (Ctr 1) expressed on the apical surfaces of the tubular cells. Once this enters the tubular cell, the chloride ions of the cisplatin get releases due to the lowered chloride concentration within the cells. The cations form of the platnumbinds cellular nucleophiles in DNA, RNA and proteins. The experimental result gives the evidences the complexes of platinum-DNA are lethal to the dividing cells. The GSH consists of thiols and these replace...
Study of protective effects of stem bark extracts of Ficus racemosa in drug induced nephrogenic diabetes insipidus and nephrotoxicity in animal models

the chloride from the platinum after they bind to them. Hence the GSH prevents the binding of platinum molecules to the other cellular components like nucleophiles. Several reports of research articles explain the role of the enzyme gamma-glutamyl-transpeptidase in cisplatin toxicity. If this enzyme expresses in other tissues then there is increased resistance to the toxic effects of cisplatin. But in the kidney the over expression of this enzyme leading to the increased chances of cisplatin toxicities as this enzyme separates the glutamic acid and cystenyl-glycin from the GSH. This reduces the scavenging or antioxidant activity of GSH leading to the nephrotoxicity. Cystenyl-glycine is cleaved into cystein and glycine by diaminopeptidase N.

Several scientists reported that the cisplatin not shown any nephrotoxicity in inbreed mice without the GGT gene or in GGT knockout mice. This suggested the role of GGT in the development of cisplatin induced nephrotoxicity. There is compelling evidence from both in studies on various models of animals and cell cultures that the cisplatin get bio transformed into a toxic form through a GSH- conjugate intermediate as are the halogenated alkenes. Platinum-GSH conjugates may be formed in either the liver or the kidney. (Marie H.Hanigan, et al., 2003)

It is also reported that copper transporters may mediate cisplatin uptake in both yeast and mammals. It is interesting to note that Ctrl is also highly expressed in proximal tubular cells. The organic cation transporters (OCTs) are also involved in the uptake of cisplatin. The cisplatin induced tubular injury may be related to basolateral OCTs. It is reported that the H$_2$ blockers like cimetidine act as OCTs inhibitor and it is thought that this drug prevents the cisplatin induced nephrotoxicity to some extent. Several OCTs are expressed in various tissues, but the OCT$_2$ is expressed on the apical surfaces of the tubular cells and is responsible for the uptake of this into the tubular cells and is responsible for the nephrotoxicity. The cisplatin uptake is increased by OCT$_2$ over expression in the tubular cells leading to the increased nephrotoxicity by the cisplatin. (Pabla N et.al., 2008).

Cisplatin induces cumulative and dose dependent kidney toxicity, this decreases the utilization of increased doses minimize the adverse effects. A sizable number of patients (about one third) receiving cisplatin showing the symptoms of nephrotoxicity after the treatment with cisplatin. The administered cisplatin after exerting its effect reaches the kidney through renal arteries. The cisplatin gets transported into the tubular cells via transporter expressed on the apical membrane of the cells. Highest concentration of cisplatin is present in
the PCT cells. Hence these parts of the tubule get affected severely. This causes the necrosis of some PCT cells and death of the cells due to the stimulation of apoptosis pathway in the cells.

![Renal Tubular Epithelial Cell](image)

**Figure 29 Pathway of cisplatin induced nephrotoxicity**

The acute renal injury caused by the cisplatin is due to number of complexes, these include DNA damage, activation of apoptosis pathway. This results in the dysfunction of mitochondria of the cell and release of various reactive oxygen species and nitrogen species. This leads to the development of cellular inflammation.

Based on the recent studies, it is suggested that the pathogenesis of cisplatin induced nephrotoxicity is due to the recruitment of macrophages and leucocytes and other inflammatory cells. This is responsible for the damage of kidney tissue due to the administration of cisplatin.

The experimental evidences suggested that the variety of pro-inflammatory cytokines and chemokines like tumor interleukin (IL)-1β and necrotic factor-alpha are expressed on the renal tubular cells are responsible for the nephrotoxicity induced by the cisplatin administration. Cisplatin-induced kidney injury largely depends on interleukin (IL)-1β and necrotic factor-resistant to cisplatin-induced kidney damage as reported previously. (Hao Pan et al., 2008).

The anticancer effect of cisplatin is mainly due to its binding ability to the DNA components of the cancer cells. This leads to the arrest the proliferation of cell cycle and cell division. The cisplatin resistance is by both acquired and intrinsic ways. The resistance may be caused by a
number of factors including cellular adaptation occur in the cancer cells like inhibition of
cisplatin uptake mechanism in the cancer cells, inactivation of cisplatin molecules by the
antioxidants like glutathione and other antioxidants. The resistance is also due to the
facilitated DNA repairing process in the cell cycle of the cancer cells or DNA tolerance.
(Cara A Rabik et al., 2007).

![Figure 30 Cisplatin induced nephrotoxicity](image)

1.8.8 Apoptotic pathways pathways activated by cisplatin in renal tubular cells. –

The apoptosis stimulatory activity of the cisplatin is the causative factor for its
nephrotoxicity. The cisplatin molecules bind with death receptors expressed on the renal
tubular cells activates the caspase-8. The activated caspase-8 further stimulates the other
caspases in the tubular cells of the nephron to induce apoptosis pathway. The death receptors
expressed on the target renal tubular cells include Fas, TNF-alpha 1 and 2. It was reported
that cisplatin is responsible for the up-regulation of Fas and Fas ligand human cultured cells
and this is associated with apoptosis pathway.

The major apoptosis pathway developed in cisplatin induced nephrotoxicity is the intrinsic or
mitochondrial pathway. In the second type of cisplatin induced apoptosis pathway include the
activation of various components of the cell like Bcl-2 proteins Bax and Bak. This belongs to
intrinsic apoptosis pathway which occurs in the tubular cells due to the administration of
cisplatin. In this pathway due to the expression of apoptosis protein like Bax and Bak leads
to the formation of defected pores in the outer mitochondrial membrane of the tubular cells and causes the release of apoptotic factors from organelles- cytochrome-c, apoptotic inducing factor (AIF), endonuclease G and others. Cytochrom-C release into the cytosol causes the conformational changes in the adapter protein Apaf-1 leading to the recruitment and activation of capase-9, which in turn after proteolytic processing activates downstream caspases. (Pabla N et.al., 2008).

Solid type of cancers is treated by the administration of cisplatin. Testicular cancer is one of the solid tumors well treated with the administration of this drug. The use of cisplatin in the treatment of solid tumors is restricted due to the development of resistance produced by cisplatin. Two types of resistances are produced by the cisplatin, intrinsic and acquired resistance.

Translation of these preclinical findings to the clinics is emerging, but still scarce (Beate Koberle et al., 2010). DNA damage signaling, stress related signals, mitochondrial pathway, ERK pathway and other pathways are the important apoptosis pathways in cisplatin induced nephrotoxicity. Some scientists are developed dosage forms like liposomal encapsulation of cisplatin, that can be applied into tumor targeted 110-nm in diameter nanoparticles.

![Figure 31 Apoptosis pathway](image-url)
Several attempts are made to minimize the nephrotoxicity of cisplatin. By knowing the molecular mechanisms of apoptosis pathway and DNA damage an effort is made to use lipoplatin instead of cisplatin to avoid the nephrotoxicity. The lipoplatin has more advantages than cisplatin, as its target is the primary tumors and metastases. The lipoprotein causes the damaging effect to the vasculature of the tumor. This destroys the cancer tissue without affecting the host tissue. This effect of lipoprotein has been demonstrated in human clinical trials (Teni Boulikas et al., 2007).

1.8.9 Role of oxidative stress in cisplatin induced nephrotoxicity-

The oxidative stress is an important factor that contributes to cisplatin nephrotoxicity. It is reported that there is increased levels of ROS during this treatment in cultured renal tubular cells, kidney slices and in whole animals. The cisplatin can rapidly react with thiol-containing molecules including glutathione.

![Figure 32 Cisplatin induced kidney damage](image)
1.8.10 Cyclosporine induced nephrotoxicity:

Cyclosporine is a lipophilic drug belongs to cyclic polypeptide. This produces interleukin-2 and cytokins, by a type of helper lymphocytes. This decreases the formation of various types of cytokins, inhibiting the activation and development of different types of cells including the cells participating in cell mediated immunity. Hence the cyclosporine has immunosuppressant activities. It acts by inhibiting calcineurin in the cells thereby suppressing the immunity during solid organ transplantation. But its use is restricted by its nephrotoxic properties.

1.8.11 Molecular mechanisms of CsA induced acute nephrotoxicity:

Administration of CsA induces a marked afferent arteriolar vasoconstriction resulting in decreased renal blood flow and GFR. The renal sympathetic nervous system has been implicated in the renal functional effects of CsA because the alpha-adrenergic antagonists’ phenoxybenzamine and prazosin prevent a CsA-induced fall in renal blood flow and GFR. Hypovolemia could contribute to renal vasoconstriction with CsA therapy because CsA-treated rats have reduced plasma volume and saline expansion reverses the deficits in renal blood flow and GFR. Studies with furosemide, mannitol and chronic sodium depletion have demonstrated that hypovolemia potentiates CsA nephrotoxicity.

In animal models, CsA consistently increases the generation of thromboxane A\(_2\), a potent renal vasoconstrictor. It is reported that TxA\(_2\) receptor antagonists also attenuated chronic cyclosporine nephrotoxicity in rats. CsA also activates platelet activating factor (PAF), a vasoconstrictor. Chronic cyclosporine induced nephrotoxicity is also attenuated in rats treated with the PAF antagonists. CsA treatment has been shown to stimulate endothelin production. Endothelin appears to mediate CsA-induced renal vasoconstriction in the rats. The resulting reduced single-nephron GFR and glomerular plasma flow rate, as well as the decreased glomerular capillary pressure, was attenuated by an antiendothelin antibody.

Similarly, the endothelin receptor antagonist has the potential to prevent hypofilidate to explain the CsA-induced vasoconstriction. This has been demonstrated in cultured rat mesangial cells as well as in vascular smooth muscle cells. The augmented transmembrane Ca\(^{2+}\) influx and intracellular Ca\(^{2+}\) mobilization could account for the protective effects of calcium channel antagonists in acute as well as chronic cyclosporine nephrotoxicity. (Robert W.Schrier, et al., 2006).
The cyclosporine in the dose of 15 to 50 mg/kg body weight if administered to the rodents like rats subcutaneously for a period of 7-28 days, leads to the development of acute nephrotoxicity. The CsA administration is associated with afferent and efferent arteriolar vasoconstriction, with predominant preglomerular vasoconstriction that results in a significant reduction of renal plasma flow.

A reduction of the ultrafiltration coefficient has also been observed. The cyclosporine administration causes this observation. The reason for the vascular dysfunction caused by the cyclosporine includes the elevated release of vasoconstrictors factors like endothelin, angiotensin II and thromboxane. The reduction in the levels of vasodilator factors such as prostacyclin and nitric oxide also takes place because of cyclosporine. The role of the several other factors in the development of structural injury caused by the administration of CsA also important to understand the mode of action involved in the development of nephrotoxicity. These include –activation of renin induced renin-angiotensin-aldosterone system, renal hypoxia. The renin release stimulates the formation of angiotension I from the inactive form i.e, angiotensinogen. The Angiotension converting enzyme (ACE) present in the lungs
converts angiotension I to angiotension II. The binding of angiotension II on AT1 receptors located on the vascular smooth vessels result in the vasoconstriction. The angiotension induced renal vasoconstriction leads to the development of renal hypoxia- insufficient blood supply to the kidney leads to kidney injury.

The nephrotoxicity is induced by CsA leading to the formation of ROS, that cause cellular injury and promotes cellular death by apoptosis, and 3) up-regulation of transforming growth factors-β (TGF-β), which promotes renal fibrosis by increasing the production and decreasing the degradation of extracellular matrix proteins (Norma A et al., 2007). It has been shown that CsA is able to generate oxygen species and lipid peroxidation, which are directly involved in this nephrotoxicity. It has been reported that, CsA treatment also leads to the formation of heat shock proteins, heme oxygenase-1 (HO-1), in rat kidney. These data have led to studies examining whether antioxidants can neutralize the adverse effects of CsA (Patrizia Galletti et al., 2005).

The appearance of acute renal failure and morphologic evidence of proximal tubule pathology following administration of cyclosporine to patients suggests that the drug might be a proximal tubule toxin. Indeed, tubular toxicity may be seen when cyclosporine levels are elevated above 1500-200ng/ml in whole blood. Under these conditions, the proximal tubule may show giant mitochondria and isometric vacuolization, so named because the epithelial cell cytoplasm contains many clear vacuoles of similar size. However, at therapeutic blood levels of cyclosporine, proximal tubule function is not depressed by cyclosporine. The first recognition of renal vascular injury with cyclosporine was reported as glomerular thrombosis in the setting of bone marrow transplantation.

Cyclosporine-associated arteriolopathy of afferent arterioles and small arteries was reported. At the ultrastructural level, the most striking pathology lies in the media: necrosis of smooth muscle cells, accumulation of matrix between cells, and loss of continuity of the basal lamina. The endothelial cells also show evidence of toxicity, with cell swelling and loss of tight junctions but rarely with necrosis. The cyclosporine induced interstitial fibrosis has been reported by several scientists.
Initially, the fibrosis is often occurs in a characteristic striped distribution, which extends perpendicularly from the cortico-medullary junction and suggests a vascular etiology. A sparse mononuclear cell infiltrates may be present in the interstices. Glomerular changes include a segmental or global expansion of the mesangial matrix, best organized at the ultrastructure level.

In late stages, glomeruli within the fibrotic regions show evidence of sclerosis and collapse. (Jeffrey B.Kopp et al., 1990). The chronic nephrotoxicity is due to the excess release of free radical formation. This leads to inadequate renal perfusion and injury because of hypoxia. The injury due to hypoxia is essential for nephrotoxicity, as it inhibits the calcineurin. The nephrotoxicity caused by the cyclosporine is mainly due to vasoconstriction of tubular arteries. The cyclosporine induced vasoconstriction leads to the damage including arteriopathy and fibrosis of tubulointerstitial cells of the nephrons. Usually these effects of cyclosporine are not observed in many patients who are taking cyclosporine. The vasoconstriction effects of this drug are mainly due to acute unequivocal consequences. The cyclosporine induced vasoconstriction is also responsible for the hypertension in patients with kidney disorder.
The pathogenesis of chronic kidney failure in patients with cyclosporine therapy is also due to the activation of renin-angiotensin-aldosterone pathway, as there is decreased vasculature of the renal blood vessels. The cyclosporine-induced vasoconstriction leads to the decreased supply of blood to the glomerular capillaries through afferent arterioles. This decreases the blood flow to the kidneys. This is sensed by the juxta-glomerular apparatus and releases the rennin into the blood. The released renin activates the rennin-aldosterone pathway. This increases the blood pressure by inhibiting the tubular reabsorption of salt and water. The hypertension is also due to the direct vasopressor effect of angiotensin II (Sang Pil Yoon et al., 2012).

The administration of cyclosporine increases the morbidity rate in patients with long-term kidney transplant. The cyclosporine is indicated in solid organ transplantation and many diseases. But its use is restricted due to the nephrotoxicity. The irreversible injury to the renal membranes, glomerular capillaries, arterioles and tubule-interstitial injury are the symptoms of nephrotoxicity caused by the chronic administration of cyclosporine. (Maarten Naesens et al., 2009). The calcineurin inhibition shown decreased ability for angiogenesis or a decrease in the animal’s ability to grow and develop new circulatory vessels (Patrick L. Gentry et al., 2006).

**1.8.12 Pathophysiology of nephrogenic diabetes insipidus:**

The aquaporin channels are concerned with the reabsorption of water along with sodium ions into the peritubular capillaries. This process helps to reabsorb the pro-urine into blood and maintain water-salt balance. The excess water is taken back by the blood. The water re-absorption of the cells of collecting ducts of the nephrons is mainly regulated by ADH receptors. When ADH binds to these receptors, triggers the release of cAMP in the tubular cells. This causes the release of aquaporin channels from the storage vesicles. The released channels get inserted into the apical membrane of the tubular cells.

In NDI developed by birth, the abnormal gene expresses abnormal type of ADH receptors and these become unresponsive to the ADH due to the abnormal expression of defected ADH receptors on the apical surfaces of the tubular cells. The insertion of aquaporin channels decreases. This fails to reabsorb water in the urine resulting in polyuria. This disorder is the NDI. It is caused by the changes in the gene that expresses the ADH receptors. This leads to the development of recessive and other forms of NDI (Patrik D et al., 1999).
Figure 35 Absorption of water through aquaporin channels

In the PCT and loop of Henley cells the water channels are expressed on both the surfaces (apical and basal) involved in the reabsorption of water from the glomerular filtrate.

The water reabsorption is mainly regulated by the arginine vasopressin or ADH receptors. When a ligand of this receptor activates the G proteins linked to the receptors. The activated adenyl cyclase stimulates the formation of cAMP within the tubular cells. Through signaling cascades, cAMP activates PKC. This phosphorylates the aquaporin-2 channels leading to the reabsorption of water.

When the ADH is not binding with their receptors, leads to a decrease in the level of cAMP and the expression of aquaporin channels also decreases. Hence more amounts of water are eliminated from the kidney (F.de Mattia et al., 2004). The water reabsorption also important in plants as some regulatory mechanism is involved in plants that maintain the water balance in the plants.
1.8.13 Molecular mode of action of lithium induced NDI.

The administration of lithium containing drugs not undergo metabolism in the liver, the free form of the drug enter the glomerulus of the Bowman’s capsule, where it freely filtered into the lumen of the tubule of the kidney. Most of the filtered lithium is absorbed back into the blood and this takes place in the cells of PCT. A small amount of lithium reabsorption also takes place in the loop of Henley and collecting duct. The reabsorption of lithium in the PCT is similar to the reabsorption of sodium ions. It takes place by the symporters like glucose sodium symporters which are expressed on the apical surfaces of PCT. In certain pathological conditions like polyurea, diarrhea, congestive heart failure there is increase in the sodium in the PCT, this follows the increased reabsorption of lithium resulting in increased serum cation levels.

The sodium-potassium-chloride symporters are expressed on the ascending limb of loop of Henley normally reabsorbs sodium ions as well as anions like chloride ions. These
symporters also reabsorbs the lithium ions. The sodium free channels expressed on the apical surfaces in the collecting duct cells also reabsorb lithium ions.

The important type of lithium-induced kidney injury is the NDI. The signs and symptoms like impaired urine concentration ability, dehydration, etc were detected about 8 weeks after lithium initiation. The mutated gene expresses the abnormal types of ADH receptors on the nephrons. In presence of abnormal types of ADH receptors, the tubular cells resist the actions of ADH leading to NDI. The water diuresis and natriuresis is due suppression of the adenyl cyclase enzyme results in decreased cAMP (Jobson Iopes de O et al., 2004).

**Outer and inner medullary collecting duct**

![Diagram of outer and inner medullary collecting duct](image)

**Figure-37 Activation of V2 receptor**

The important type of kidney injury because of the administration of lithium containing drugs is the disturbed urine concentrating capacity of the kidney. Two important functions are the basic reasons for the kidney urinary concentrating ability.

The primary event is the formation of a hypertonic environment in the interstitial fluid and the second reason is the decreased insertion of aquaporin channels because of decreased levels of cAMP in the tubular cells. The decreased expression of various aquaporin like AQP2, AQP3 and AQP4. This leads to the inhibition of reabsorption of water molecules and causes the pressure gradient in the membranes of the collecting duct and DCT. The osmotic
pressure within the interstitial fluid increases leading to the toxicity in the nearby structures. Because of the increased loss of water from the body, the osmolality of the serum also increases. This condition stimulates the posterior pituitary gland to secrete ADH. These bind with the available receptors available on the basolateral membranes of the DCT and CT. This stimulates the intracellular events that increase the insertion of AQP₂ molecules in the apical membrane, which is facing the tubular lumen.

The mode of action involved in the lithium induced NDI is not well established. The possible mechanism for this is the inhibition of the membrane bound adenyl cyclase activity on the PCT and CT. This leads to reduced expression of AQP₂. It is reported that the administration of lithium for 25 days induced a decrease in AQP₂ expression. Once the lithium filtered by the glomerulus, it reaches the lumen of the collecting duct. Then enter the tubular cells via sodium transporters, as these transporters have more affinity for the lithium than sodium ions. Na ions from the tubular cells expelled into the interstitial spaces utilizing energy by the pump Na/K ATPase active transport. But lithium ions are not expelled out like sodium ions and they get trapped in the tubular cells and their concentration increases leading to their toxicity.

The glycogen synthase kinase is the key enzyme that regulates movement of water molecules and sodium ions. This enzyme activity is inhibited by the lithium ions. Because of this cell loses the ability to respond to the effects of aldosterone and AVP. The inhibited glycogen kinase synthase 3 also inhibit the cyclooxygenase enzyme in the medulla of the kidney. The lithium can also inhibit the cyclooxygenase-2 (COX-2) expression in the kidney medulla. Both of these enzymes perform an important role in lithium-induced polyurea. The pathogenic mechanisms by which lithium causes tubule interstitial nephritis and glomerular injury are not clearly known.

The inositol monophosphate pathway in the tubular cells is altered by the lithium ions. This decreases the inositol levels in the cells and causes inhibition of cell cycle. Hence the accumulated lithium ions in the DCT or collecting duct cells via sodium transporters causes acute tubular injury and result in chronic kidney failure. (Jobson Lopes de Oliveira et al., 2010).
The apical surface of the tubular cells expresses the epithelial sodium channels (symporters or antiporters). Through these sodium transporters the filtered lithium enters the tubular cells.

The aldosterone hormone increases the reabsorption of lithium ions as these hormones increases the expression of sodium channels in the nephrons. The drugs like amloride decrease the tubular reabsorption of lithium, as it inhibits the sodium channels. Different molecular mechanisms are involved in the NDI caused by the lithium compounds. The permeability for the water molecule gets altered by the lithium ions. (Jennifer J. Bedford et al., 2008).

Lithium compounds are used in various psychotic disorders. Several studies suggested that the treatment with lithium chloride in rats, exhibit increased elimination of prostaglandins (PGE2). It is also reported that the lithium administration increases the expression of COX2 in cultured cells of the kidney. This inhibition occurs via inhibition of glycogen synthase kinase-3 (Reena Rao et al., 2005).

### 1.8.14 Biomarkers and nephrotoxicity:

The AKI result in the elimination of various metabolites through the urine. This is due to the impact of nephrotoxic drugs on the vasculature and tubular cells. The elimination of various metabolites is due to altered permeability due to the damage of glomerular capillaries, altered reabsorption of various metabolites in the tubular cells, altered function of various symporters and antiporters in the nephrons. This causes changes in the excretion levels of creatinine, urea and other metabolites. The serum levels of such metabolites are also altered. Some new biomarkers also produced due to kidney injury. These include urinary KIM-1, macroglobulin, cystatin, clusterin, trefoil factor, etc. The detection of such metabolites helps for the detection of kidney injury.

### 1.8.15 Plant profile of Ficus racemosa:

Name: Ficus racemosa
Botanical name: Ficus racemosa Linn.
Family: Moraceae
Sanskrit synonyms
Udambara, Krimiphala, Jantuphala, Sevya

Plant names in different languages
English: Country fig, Cluster fig,
1.8.16 Distribution and descriptions of the plant:

Braby et al., (2010) were reported that the tree grows to about 10-16 meters height and it is medium tall tree. The color of the bark of this tree is reddish grey and its leaves having dark green color. The length of the leaf is about 7.5 to 10 cm long, the shape is ovate or elliptic. This tree maintains the green color throughout the year. It grows in various parts in India and also in villages for its edible fruit.

Arunachalam A, et.al (2010) reported that the stem portion of the plant *Ficus racemosa* have highest percentage of phenols (about 8%) when compared to the phenolic content in other parts like leaf, root, etc. They estimated the phenol content by a method known as Folin-Denis method. The study also reveals the highest content of tannins (5.95%) in the stem portion of the plant when compared to the leaf and roots. The phytochemical investigation of this plant reveals that this plant possesses glycosides, phenols and flavonoids.

Padmaa M Paarakh (2009) was reported the traditional use of *Ficus racemosa* - The various parts of this plant possess a number of therapeutic applications. It can be indicated in both internal and external use for the treatment of different disorders including edema, pain and inflammation, etc. The bark extraction of this plant is also indicated in the treatment of stomatitis and mouth disorder like sore throat. The external dosage form is prepared from its latex part and alleviation is observed if it is applied on the skin with adenitis, parotitis,
swelling and toothache. The liquid dosage form prepared from this plant can be taken internally for the relief of diarrhea and dysentery. The decoction prepared from its bark can also be used for the treatment of diabetes and as anti-diuretic. Anorexient can be treated by the administration of its powder. The bark and leaves of this plant can be used for the preparation of infusion and this dosage form can be used for the cleaning of spongy gums, menorrhage and in dysentery.

1.8.17 Reported activities of *Ficus racemosa* Linn –

Chandrashekhar CH et al., (2008) were evaluated the bark extract of *Ficus racemosa* for the anthelmintic activity. They were used earth worms for reporting the anthelmnetnic activity. The standard drug used for this study is piperazin citrate.

Abu Hassan *et al.*, (2011) were reported the hypoglycemic and in-vitro antioxidant activities of *Ficus racemosa* ethanol extracts. They tested the antidiabetic activity in alloxan induced diabetic Swiss albino mice. They reported the activity by comparing the results with standard antidiabetic drug treated group.

Abu Hassanaat, *et al.*, (2011): were reported the hypolipidemic and antioxidant properties of *Ficus racemosain* diabetic rats. They used glibenclamide as the standard antidiabetic drug in their studies.

Faiyaz Ahmed, *et al.*, (2010) were reported the anticholinesterase activities of the various extracts of *Ficus racemosa* stem bark by studying the effect on rat brain anticholinesterase activity.

Ahmed F et al., (2009) were reported the hypolipidemic, hepato protective and glucose lowering actions of this plant. They studied the antidiebetic activity of this plant extracts on rats. Ahmed F et al., (2010) were studied and reported the antibacterial activities of *Ficus racemosa* stem bark extracts. They tested the extracts on various microorganisms and compared the obtained results in presence of standard antibacterial agent. Bhaskhar Rao B et al., (2002) were evaluated and reported the antidiebetic activity of this plant extract. They tested the efficacy of the plant extract by comparing the results with diabetic rats induced by alloxan.

Jaykaran *et al.*, (2009) reported the acute toxicity results of *Ficus racemosa* aqueous extracts of stem bark in albino mice. They tested the acute toxicity in four groups of animal each
consisting of six. Jaykaran et al., (2009) reported that the *Ficus racemosa* bark possesses dietary fibers, minerals and phenolic compounds. Veerapur V.P. et al., (2007) reported the antioxidant properties of this plant. Ahmed F. et al., (2010) studied and reported liver protective effect of this plant. The carbon tetrachloride was used for the induction of hepatotoxicity in rats. They studied various biomarkers of the liver tissue of the rats. Ranasooriya WD et al., (2003) reported the antidiuretic effect of this plant.

1.8.18 Natural kidney protective agents in the prophylaxis of drug induced nephrotoxicity:

Marjan Ajami et al., (2010) were studied and reported the nephroprotective effect of *Crocus sativus*. They tested effect in gentamicin induced nephrototoxic rats.

Lakshmi BVS et al., (2009) were studied and reported the efficacy of *Bauhinia purpurea* in acute renal failure rats induced by gentamicin.

Niraj M Bhattet al., (2011) were reported the effectiveness of *Enicosstemma littoral* blame extract nephroprotection on gentamicin induced nephrotoxicity in rats.

Mathew JE et al., (2011) were tested and reported the nephroprotective efficacy of the entire plant of *Spharanthus indicus*. They tested the effects in cisplatin induced nephrototoxic rats.

Kim YH et al., (2006) were shown the effectiveness of nephroprotection of extract prepared from the ethyl alcohol and the roots of *Brassica rapa* (EBR) in cisplatin (7mg/kg ip) induced nephrototoxic rats.

Wonqmekiat O et al., (2008) were evaluated the nephroprotective effect of garlic in rats treated with cyclosporine for the development of kidney toxicity.

Wonqmekiat O et al., (2008) were reported the kidney toxicity prevention effect of *Allium ascalonicum* L in cyclosporine induced nephrototoxic rats.

Jennifer J et al., (2008) were evaluated and reported the nephroprotection effect NDI rats administered with lithium compounds.

Shai Efrati et al., (2005) were studied and reported the nephroprotective effect in lithium induced NDI rats.
Vijaimohan K etal., (2010) were studied the protective role of *Solanum triobatum* in lithium induced multiple organ toxicity including nephrotoxicity in rats.

Jakob Nielsen et al., (2008) were evaluated and reported the nephroprotective effect in lithium induced NDI rats.

Shu-Huei Kaol et al., (2008) were studied oxidative stress effect of lithium chloride in rats. They reported that the chronic administration of drugs containing lithium causes the accumulation of reactive oxygen species in the target tubular cells. They also reported the significance of N-acetylcysteine in decreasing the intracellular production of reactive oxygen species as a result of decreased HO-I expression in C6 glioma cells.

Wan-Loy Chu et al., (2010) were evaluated and reported the protective efficacy of spirulina extract in rats.

Ihsan Yamanet al., (2010) were investigated and reported the protective efficacy of *Nigella sativa* in gentamicin treated nephrotoxic rats.

Bibu KJ et al., (2009) were investigated and reported the therapeutic efficacy of in nephrotoxic rats induced by gentamicin.

Eslami SH et al., (2011) were studied and reported the kidney protective role of *Erygnium caucasicum* in gentamicin induced kidney injury in rats.

Sandeep Det al., (2010) were evaluated and reported the kidney protection effect of *Hemidesmus indicus* L and *Acorus calamus* L in nephrotoxic rats induced by cisplatin.

Yapar Ket al., (2009) were established the significant role of royal jelly and green tea in protecting the nephrotoxicity caused by cisplatin administration.

Gholamreza Karimiet al., (2010) were investigated the protective role of *Portulaca oleracea* L. in nephrotoxic rats induced by the cisplatin.

Naveen Tirkeyet al., (2005) were studied and reported the protective role of CMN on kidney against kidney injury caused by cyclosporine in rats.

Ahmet Gokce et al., (2009) were investigated and reported the nephroprotective role of CAPE in nephrotoxic rats caused by the administration of cyclosporine.
Sabahattin Ocak et al., (2007) were studied the efficacy of nephroprotective role the constituents like caffeic acid phenyl ether ester, vitamins-C and E and N-ethylcysteine in kidney injury caused by aminoglycosides like vancomycin.

1.8.19 Objectives of the study:
The renal glands are vital organs of the body which perform the vital functions like removal various metabolites, wastes, drugs,. Kidney also secrete several hormone which regulates the body vital functions like blood pressure, water balance, homeostasis. The kidney also produces cacitriol-, a form of vitamin D, is required for the formation of strong and healthy bones. They also control the production of RBCs.

The various complex constituents present in the medicinal plants helps for the protection and treatment of various disorders. The literature in the Ancient books also indicated the importance of herbs in the treatment of several kidney disorders. The administration of medicinal natural herbs together with the conventional drugs helps for the

. Co-administration of various medicinal plants possessing nephroprotective activity along with different nephrotoxicity agents may attenuate its toxicity. Keeping all these facts is view the present study is aimed at giving a scientific basis for the native claims. The decrease of urine output activity of Ficus racemosa was reported. Desmopressin is the specific drug, its structure and function is similar to the endogenous ADH, used in the treatment of nephrogenic diabetes insipidus. It was reported that this plant is having antidiuretic activity. This is the basis of this study. The desmopressin is useful in neurogenic diabetes insipidus, as it is due to the deficiency of ADH. The objective of this study was to evaluate the kidney protective activity of ethanol and aqueous bark extracts of Ficus racemosa on gentamicin, cisplatin and nephrotoxicity in experimental animals and also to find the protective role in NDI rats induced by the administration of lithium compounds.

1.8.20 Need for the study:
Kidney is one of the vital organs of the body. Because of introduction of more number of drugs possessing nephrotoxicity, increasing number of patients are suffering from drug induced nephrotoxicity. The trend of using herbal medicines for various disorders is increasing in most of the countries of the world.

In recent years most of the individuals are more prone to the development of diabetes mellitus and cardiovascular diseases and they will get a chance to take more medication as
well as for the diagnosis of these disorders when compared to such type of patients 30 years ago. This type of multiple medications, diagnostic procedures make the patients to develop kidney disorders. But in most of these conditions the developed disorders are usually reversible if such medication is discontinued. The drug induced nephrotoxicity is usually exerted by various mechanisms in clinical situations. Therefore successful prevention is required while administering these potential drugs (Cynthia A Naughton, 2008).

It is the known fact and also due to the results of various studies supports the importance of antioxidants in protecting the drug induced nephrotoxicity. Based on the recent experimental reports, now it is clear that the mechanism involved in the kidney protection is not only due to the modulation of oxidative stress, and due to the mechanism beyond this. Rasikh Javaid et al., 2012 reported that various polyphenolic compounds are responsible for the nephron protection. The herbal drugs are gaining importance in treating number of ailments; some herbal drugs are also exploited without providing any experimental evidence.

Due to the development of modern medicine science, there is a need to study the herbal drugs in a systematic way. Its detailed study, preliminary phytochemical investigations, isolation of active principles, development of monographs, standards for the evaluation of their efficacy, preclinical investigations, safety pharmacological studies, etc are required for the establishment of their reported activities.

In keeping these views an effort is made for the scientific study of preclinical studies for claiming their use for preventing the drug induced nephrotoxicity of *Ficus racemosa* bark extracts, the study was conducted to check the possible protective role of this plant in drug induced renal disorders, an in drug induced nephrotoxicity and lithium induced nephrogenic diabetes insipidus in animal models. *Ficus racemosa* is a well-known plant grows in India. In Ayurveda *Ficus racemosa* is used in the treatment of number of disorders like diabetes, liver problems, diarrhea, and inflammatory bowel diseases. It is also used hemorrhoids, respiratory and urinary disorders.

In India and other countries the incidences of drug induced nephrotoxicity are increasing due to easy availability of OTC drugs in medical stores. These drugs include various antibiotics like gentamicin, cyclosporine, cisplatin, ACE inhibitors, and radio contrast agents.. They are the major drugs contributory to kidney damage. Drug-induced acute renal failure (AFR) for
20% of all AFR in an Indian study, of which aminoglycosides accounted for 40% of total cases.

*Ficus racemosa* bark is a drug that has been traditionally for treating renal disease. Its antioxidant potential and anti-apoptotic effects make it an interesting herbal medicine.

As there are no effective modern nephroprotective drugs and there are no drugs for the treatment of NDI. Hence this work was selected for this study.

**1.8.21 Aim of the present research work:**

The aim of the present is the study of the efficacy of *Ficus racemosa* bark extracts for its protective role against gentamicin, cisplatin and cyclosporine induced nephrotoxic animal models. This study was also aimed to develop herbal formulation for the prevention of nephrogenic diabetes insipidus, as there are no primary drugs for the protection or treatment of nephrogenic diabetes insipidus. The NDI was induced in the rats with the administration of lithium for a period of four weeks.