ABSTRACT

Nephrotoxicity is one of the most common kidney problems and occurs when the body is exposed to a drug or toxin that causes damage to the kidneys. When kidney damage occurs, we are unable to rid our body of excess urine, and wastes. Your blood electrolytes (such as potassium, and magnesium) all becomes elevated. Nephrotoxicity can be temporary with a temporary elevation of lab values (BUN and/or creatinine). If these levels are elevated, these may be due to a temporary condition such as dehydration or may be developing renal (kidney failure). If the cause of the increased BUN and/or creatinine levels is determined early, and healthcare provider implements the appropriate intervention, permanent kidney problems may be avoided. Nephrotoxicity may also be referred to as renal toxicity. Drugs are a common source of acute kidney injury. Compared with 30 years ago, the average patient today is older, has more co morbidities, and is exposed to more diagnostic and therapeutic 0-procedures with the potential to harm kidney function. Drugs shown to cause nephrotoxicity exert their toxic effects by one or more common pathogenic mechanisms. Drug-induced nephrotoxicity tends to be more common among certain patients and in specific clinical situations. Therefore, successful prevention requires knowledge of pathogenic mechanisms of renal injury, patient related risk factors, drug-related risk factors, and pre-emptive measures, coupled with vigilance and early intervention. Some patient-related risk factors for drug-induced nephrotoxicity are age older than 60 years, underlying renal insufficiency (e.g., glomerular filtration rate of less than 60 ml per minute per 1.73 m²), volume depletion, diabetes, heart failure, and sepsis. General preventive measures include using alternative non-nephrotoxic drugs whenever possible; correcting risk factors, if possible; assessing baseline
renal function before initiation of therapy, followed by adjusting the dosage; monitoring renal function and vital signs during therapy; and avoiding nephrotoxic drug combinations. Drug-induced nephrotoxicity (DIN) occurs in all settings in which drugs are ingested or administered. It is a significant source of morbidity and mortality in the acute care hospital setting. DIN accounts for nearly 7% of all drug toxicity and from 18% to 27% of all cases of acute renal failure in hospitals. Overall, in-hospital drug use may contribute to 35% of all cases of acute tubular necrosis (ATN), most cases of allergic interstitial nephritis (AIN), as well as to nephropathy due to alterations in renal hemodynamics and post renal obstruction. The incidence and characteristics of outpatient or community acquired DIN are less well understood since mild toxicity is often unrecognized. However, the pharmacoepidemiology of these effects have become more important as care increasingly shifts to the outpatient setting. Although as many as 3% to 6% of hospital admissions have been attributed to adverse drug effects during outpatient therapy, 20% of hospital admissions due to acute renal failure have been attributed specifically to community-acquired DIN.\(^{(1)}\)

Manifestations of drug-induced nephrotoxicity include acid-base abnormalities, electrolyte imbalances, urine sediment abnormalities, proteinuria, pyuria, hematuria and most commonly, a decline in the glomerular filtration rate. The mechanisms of drug-induced nephrotoxicity may differ between various drugs or drug classes, and they are generally categorized based on the histological component of the kidney that is affected. Aminoglycoside antibiotics, radiocontrast media, conventional nonselective nonsteroidal anti-inflammatory drugs, and selective cyclooxygenase-2 inhibitors, amphotericin B, and angiotensin-converting enzyme inhibitors have been frequently implicated. For several thousands of years, natural products have played an important role in treating and preventing human diseases. Natural product medicines have come from various sources including terrestrial plants, terrestrial microorganisms, marine organisms, terrestrial vertebrates and invertebrate. The importance of natural products in modern medicine has been discussed in many recent reviews and reports. The sophistication of herbal remedies used around the world varies with the technological advancement of countries that produce and use them. These remedies range from medicinal teas and crude tablets used in traditional medicine to concentrated standardized extracts produced in modern pharmaceutical facilities and used in modern medical systems under a physician's supervision. Many drugs, including strychnine, vincristine, taxol, curare, ergot etc., are of herbal origin evolved as a result of ethnomedical research. About one-quarter of the prescription drugs dispensed by community pharmacies in the United States contain at least one active ingredient derived from plant material.
Some known herbal plants used as nephroprotective drugs are Harungana madagascariensis, Acorus calamus, Ficus religiosa L, Indigofera barberi L., Achyranthes aspera L., Kigelia africana. Many herbal plants have proved to be nephroprotective agent by many preclinical and clinical studies and are widely used in renal diseases including nephrotoxicity. One another plant is said to be used as nephroprotective agent, known as Trichosanthes dioica. It contains many chemical constituents which exhibits protective actions against nephrotoxicity. This plant also contains anti-inflammatory, antioxidant and immunomodulator activity which are helpful to exhibit its nephroprotective activity. But it does not contain any scientific evidence about its safety and efficacy. So the present study was undertaken to evaluate nephroprotective activity of Trichosanthes dioica on gentamicin induced nephrotoxicity.

**Keywords:** *Trichosanthes dioica*, Gentamicin, Serum creatinine, Blood Urea Nitrogen, Phospholipids

**INTRODUCTION**

1. Nephrotoxicity
Kidneys have some delicate tasks, especially when they have to deal with unwanted substances, which they have to clear from the system, especially toxins. On top of this they play an important part in the maintenance of our endocrine and acid-base balance, blood pressure, erythropoiesis (reaction of new red blood cells) etc. Therefore it becomes critical when kidney functions decline, induced by diseases which seem to have no direct relation to renal pathophysiology. Nephrotoxicity (from Greek: nephros, "kidney") is a poisonous effect of some substances, both toxic chemicals and medication, on the kidneys. There are various forms of toxicity. Nephrotoxicity should not be confused with the fact that some medications have a predominantly renal excretion and need their dose adjusted for the decreased renal function. (1)

Nephrotoxins are chemicals displaying nephrotoxicity. Several drugs are nephrotoxic (table 1). (1) Reaction to drugs and other compounds are relatively common and have been described for many substances. They are commonly associated with renal dysfunction although the actual incidence of drug-induced renal failure has not been reported, since incidence is complicated by the complexity of the causes of ARF in seriously ill patients. Nephrotoxicity arises through several mechanisms, including general and local vascular effects, direct effect on renal tubules, tubular obstruction and acute interstitial nephritis. Acute glomerulonephritis can also occur although this is less common. (1)
Table 1: Drugs which can cause renal failure and their mechanism of toxic effect

<table>
<thead>
<tr>
<th>Mechanism of toxic effect</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular</td>
<td>Diuretics, β blockers, vasodilator agents</td>
</tr>
<tr>
<td>Local</td>
<td>ACE inhibitors, cyclosporine A</td>
</tr>
<tr>
<td>Direct tubular effect</td>
<td>Proximal tubule</td>
</tr>
<tr>
<td></td>
<td>Aminoglycosides, amphotericin B, cisplatin, radiocontrast media, immune-globulin, mannitol</td>
</tr>
<tr>
<td>Direct tubular effect</td>
<td>Distal tubule</td>
</tr>
<tr>
<td></td>
<td>NSAIDs, ACE inhibitors, cyclosporine A, lithium, cyclophosphamide, amphotericin B</td>
</tr>
<tr>
<td>Tubular obstruction</td>
<td>Sulphonamides, acyclovir, poly ethylene glycol</td>
</tr>
<tr>
<td>Acute interstitial nephritis</td>
<td>β-lactams, vancomycin, rifampicin, sulphonamides, ciprofloxacin, NSAIDs, ranitidine, cimetidine, furosemide, thiazides, phenytoin</td>
</tr>
<tr>
<td>Acute glomerulonephritis</td>
<td>Penicillamine</td>
</tr>
</tbody>
</table>

Drugs cause approximately 20 percent of community and hospital acquired episodes of acute renal failure. Among older adults, the incidence of drug-induced nephrotoxicity may be as high as 66 percent. Compared with 30 years ago, patients today are older, have a higher incidence of diabetes and cardiovascular disease, take multiple medications, and are exposed to more diagnostic and therapeutic procedures with the potential to harm kidney function. Although renal impairment is often reversible if the offending drug is discontinued, the condition can be costly and may require multiple interventions, including hospitalization.

Most drugs found to cause nephrotoxicity exert toxic effects by one or more common pathogenic mechanisms. These include altered intraglomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy. Knowledge of offending drugs and their particular pathogenic mechanisms of renal injury is critical to recognizing and preventing drug-induced renal impairment.

**Altered Intra Glomerular Hemodynamics**

In healthy young adult, approximately 120 ml of plasma is filtered under pressure through the glomerulus per minute, which corresponds to the glomerular filtration rate (GFR). The kidney maintains or auto regulates intraglomerular pressure by modulating the afferent and efferent
arterial tone to preserve GFR and urine output. For instance, in patients with volume depletion, renal perfusion depends on circulating prostaglandins to vasodilate the afferent arterioles, allowing more blood flow through the glomerulus. At the same time, intraglomerular pressure is sustained by the action of angiotensin-II mediated vasoconstriction of the efferent arteriole. Drugs with antiprostaglandin activity (e.g., nonsteroidal anti-inflammatory drugs [NSAIDs]) or those with antiangiotensin-II activity (e.g., angiotensin-converting enzyme inhibitors, angiotensin receptor blockers) can interfere with the kidneys’ ability to auto regulate glomerular pressure and decrease GFR.\(^\text{12, 13}\) Other drugs, such as calcineurin inhibitors (e.g., cyclosporine, tacrolimus), cause dose-dependent vasoconstriction of the afferent arterioles, leading to renal impairment in at-risk patients.\(^\text{14}\)

**Tubular Cell Toxicity includes**

Renal tubular cells, in particular proximal tubule cells, are vulnerable to the toxic effects of drugs because their role in concentrating and reabsorbing glomerular filtrate exposes them to high levels of circulating toxins.\(^\text{15}\) Drugs that cause tubular cell toxicity do so by impairing mitochondrial function, interfering with tubular transport, increasing oxidative stress, or forming free radicals.\(^\text{10, 16}\) Drugs associated with this pathogenic mechanism of injury include aminoglycosides, amphotericin B, antiretrovirals, cidofovir, tenofovir, cisplatin, contrast dye, foscarnet (Foscavir), and zoledronate (Zometa).\(^\text{15-17}\)

**Inflammation**

Drugs can cause inflammatory changes in the glomerulus, renal tubular cells, and the surrounding interstitium, leading to fibrosis and renal scarring. Glomerulonephritis is an inflammatory condition caused primarily by immune mechanisms and is often associated with proteinuria in the nephritic range.\(^\text{15}\) Medications such as gold therapy, hydralazine, interferon-alfa (Intron A), lithium, NSAIDs, propylthiouracil, and pamidronate have been reported as causative agents.\(^\text{15, 16, 18}\) Acute interstitial nephritis, which can result from an allergic response to a suspected drug, develops in an idiosyncratic, non–dose-dependent fashion.\(^\text{19}\)

Medications that cause acute interstitial nephritis are thought to bind to antigens in the kidney or act as antigens that are then deposited into the interstitium, inducing an immune reaction.\(^\text{19}\) However, classic symptoms of a hypersensitivity reaction (i.e., fever, rash, and eosinophilia) are not always observed.\(^\text{16, 19}\) Numerous drugs have been implicated, including allopurinol; antibiotics (especially β-lactams, quinolones, rifampin, sulfonamides, and
vancomycin); antivirals (especially acyclovir and indinavir); diuretics (loops, thiazides); NSAIDs; phenytoin; proton pump inhibitors (especially omeprazole, pantoprazole, and lansoprazole); and ranitidine.\textsuperscript{(16, 19-22)} Chronic interstitial nephritis is less likely than acute interstitial nephritis to be drug induced; it is also insidious in onset, and signs of hypersensitivity are often lacking.\textsuperscript{(23)} Drugs associated with this mechanism of nephrotoxicity include calcineurin inhibitors (e.g., cyclosporine, tacrolimus), certain chemotherapy agents, Chinese herbals containing aristocholic acid, and lithium.\textsuperscript{(14, 23, 24)} Chronic interstitial nephritis has been reported with analgesics such as acetaminophen, aspirin, and NSAIDs when used chronically in high dosages.\textsuperscript{(8)}

**Crystal Nephropathy**

Renal impairment may result from the use of drugs that produce crystals that are insoluble in human urine. The crystals precipitate, usually within the distal tubular lumen, obstructing urine flow and eliciting an interstitial reaction.\textsuperscript{(16)} Commonly prescribed drugs associated with production of crystals include antibiotics (e.g., ampicillin, ciprofloxacin, sulfonamides); antivirals (e.g., acyclovir, foscarnet, ganciclovir); indinavir; methotrexate and triamterene.\textsuperscript{(15, 16, 25)} The likelihood of crystal precipitation depends on the concentration of the drug in the urine and the urinary pH.\textsuperscript{(25)} Patients mostly at risk of crystal nephropathy are those with volume depletion and underlying renal insufficiency.\textsuperscript{(25)} Chemotherapy for lymphoproliferative disease, leading to tumor lysis syndrome with uric acid and calcium phosphate crystal deposition, has also been associated with renal failure. Patient-related risk factors for drug-induced nephrotoxicity are Absolute or effective intravascular volume depletion, Age older than 60 years, Diabetes, Exposure to multiple nephrotoxins, Heart failure, Sepsis and Underlying renal insufficiency.\textsuperscript{(26)} The incidence of nephrotoxicity from aminoglycosides has increased from 2 to 3% in 1969 to 20% in the past decade. Despite nephrotoxicity and ototoxicity, the aminoglycosides are continuously being used in clinical practice because of their bactericidal efficacy, synergism with ß-lactam agents, low cost, limited bacterial resistance, and a post-antibiotic effect.\textsuperscript{(27)}

Nephrotoxicity has been recognized as a major complication of aminoglycoside antibiotics for many years. During the past 6 to 8 years, this problem has attracted the attention and interest of a number of investigators, resulting in the generation of a large body of experimental data that has greatly expanded our understanding of the pathogenesis of this disorder.\textsuperscript{(28)}
The human beings are exposed to environmental, occupational and xenobiotic challenges due to modern life style. Enormous free radicals are generated during the exposure to such stressful challenges. In addition the process of metabolism and excretion of xenobiotics may also generate free radicals. These free radicals bind covalently with the tissue macromolecules leading to the cell necrosis.  

2. Gentamicin induced nephrotoxicity

Gentamicin is an antibiotic that exhibits a broad spectrum of activity and is particularly valuable in severe sepsis. Its use is, however, restricted because of the development of ototoxicity and nephrotoxicity. At physiologic pH, the drug is highly charged and water soluble, and therefore it is practically unable to diffuse through biologic membranes.

Nephrotoxicity has been related to a selective accumulation of gentamicin in the renal cortex. Morphologic lesions of proximal tubules have been documented in optic microscopy. At the ultra-structural level, the earliest lesions observed concern the lysosomes, which show an accumulation of myeloid bodies.

Symptoms include decreased consciousness, coma, delirium or confusion, drowsiness, lethargy, hard to arouse, decreased urine output or no urine output, general swelling, fluid retention, nausea and vomiting.

Signs and tests include are indicates acute kidney failure. There may be signs of fluid overload, including abnormal sounds on listening to the heart and lungs with a stethoscope. Other signs include, BUN and serum creatinine levels may increase, Fractional excretion of sodium and of urea may be relatively high, Kidney biopsy may show acute tubular necrosis, Urinalysis may show casts, kidney tubular cells, and red blood cells, Urine sodium may be high, Urine specific gravity and osmolarity of urine indicate dilute urine.
2.1 Pathophysiology
Oxidant mechanisms in gentamicin nephrotoxicity.

Acute renal failure is a major complication of aminoglycoside antibiotics, which are widely used in the treatment of gram-negative infections. Sequential reduction of oxygen along the univalent pathway leads to the generation of superoxide anion, hydrogen peroxide, hydroxyl radical, and water. A large body of in-vitro and in vivo evidence indicates that these partially reduced oxygen metabolites are important mediators of gentamicin nephrotoxicity. Gentamicin has been shown to enhance the generation of superoxide anion and hydrogen peroxide by renal cortical mitochondria. The interaction between superoxide anion and hydrogen peroxide in the presence of metal catalyst can lead to the generation of hydroxyl radical. Gentamicin has been shown to release iron from renal cortical mitochondria and to enhance generation of hydroxyl radical. These in vitro observations have been supported by in-vivo studies in which scavengers of reactive oxygen metabolites and iron chelators have shown to be protective in gentamicin induced acute renal failure. These studies may have broader implication in being relevant to other aminoglycosides including streptomycin and being applicable to other major toxicity of aminoglycoside such as ototoxicity.\(^{(39)}\)

![Fig.1 Pathological mechanisms pertaining to gentamicin-induced nephrotoxicity.\(^{(40)}\)](image)

MAPK, mitogen activated protein kinase; TNF-α, tumor necrosis factor-alpha; PARS, poly (ADP-ribose) synthetase or poly (ADP-ribose) polymerase; NFkB, nuclear factor kappa B; TGF-β, transforming growth factor β.
Molecular parameters involved in aminoglycoside nephrotoxicity.

Aminoglycoside antibiotics are hydrophilic molecules consisting of an animated cyclitol associated with amino sugar. They bind in vivo as well as in vitro to negatively charged membranes. Their use as chemotherapeutic agents is unfortunately accompanied by oto- and nephrotoxic reactions, and the purpose of this review is to examine the role of the molecular interactions between aminoglycosides and membranes in the development of nephrotoxicity.

31P Nuclear magnetic resonance (NMR) and fluorescence depolarization have been used to characterize the effect of aminoglycosides on phosphate heads and fatty acyl chains of phospholipids. 15N NMR has been used to obtain interesting information on regioselective interactions of amino groups of antibiotics with phospholipids. The binding of aminoglycosides with negatively charged membranes is associated with impairment of phospholipid catabolism, change in membrane permeability, and membrane aggregation. Biochemical analysis and 1H NMR spectroscopy have brought information on the molecular mechanism involved in the impairment of phospholipids catabolism. Nephrotoxic aminoglycosides could induce sequestration of phosphatidylinositol and therefore reduce the amount of negative charge available for optimal lysosomal phospholipase activity toward phosphatidylcholine included in liposomes that also contain cholesterol and sphingomyelin.

Fig.2 Pathological role of reactive oxygen species in the induction of gentamicin-nephrotoxicity.\(^{(40)}\) ICAM-1, intercellular cellular adhesion molecule-1; MCP-1, monocyte chemoattractant protein-1; \(\text{O}_2^\cdot\), superoxide radical; \(\text{OH}^\cdot\), hydroxyl radical; \(\text{H}_2\text{O}_2\), hydrogen peroxide.
Conformational analysis shows that aminoglycosides, which have a high potency to inhibit lysosomal phospholipase activity, adopt an orientation parallel to the lipid/water interface. This orientation of the aminoglycoside molecule at the interface is also critical to explain the marked increase of membrane permeability induced by less nephrotoxic aminoglycosides such as isepamicin and amikacin. This effect is indeed only observed with aminoglycosides oriented perpendicular to this interface, probably related to the creation of a local condition of disorder. The impairment of phospholipid catabolism, which is considered to be an early and significant step in the development of aminoglycoside toxicity, is therefore not related to the change in membrane permeability. However, the role of this latter phenomenon and of membrane aggregation for aminoglycoside nephrotoxicity could be further investigated. (41)

**General features of aminoglycoside nephrotoxicity**

Nephrotoxicity induced by aminoglycosides manifests clinically as non-oliguric renal failure, with a slow rise in serum creatinine and a hypoosmolar urinary output developing after several days of treatment. Aminoglycosides are nephrotoxic because a small but sizable proportion of the administered dose (5%) is retained in the epithelial cells lining the S1 and S2 segments of the proximal tubules after glomerular filtration. Aminoglycosides accumulated by these cells are mainly localized with endosomal and lysosomal vacuoles but are also localized with the Golgi complex. They elicit an array of morphological and functional alterations of increasing severity. (46)

**Tubular changes in the pathophysiology of ischemic acute tubular necrosis**

After ischemia and reperfusion, morphological changes occur in the proximal tubules, including loss of polarity, loss of the brush border, and redistribution of integrins and sodium/potassium ATPase to the apical surface. Calcium and reactive oxygen species also have roles in these morphological changes, in addition to subsequent cell death resulting from necrosis and apoptosis. Both viable and non-viable cells are shed into the tubular lumen, resulting in the formation of casts and luminal obstruction and contributing to the reduction in the GFR. (47)
3. Effects of clinical doses in animals and humans

After only a few days of administration of clinical doses to humans or of low multiples of the human therapeutic dose to animals (typically 10 to 20 mg/kg of bodyweight for a laboratory rat), aminoglycosides induce conspicuous and characteristic changes in lysosomes of proximal tubular cells consistent with the accumulation of polar lipids (myeloid bodies). These changes are preceded and accompanied by signs of tubular dysfunctions or alterations (release of brush border and lysosomal enzymes; decreased reabsorption of filtered proteins; wasting of K⁺, Mg²⁺, Ca⁺, and glucose; phospholipiduria; and cast excretion). In humans, the occurrence of these signs may be followed by the development of overt renal failure.
characterized mainly by a nonoliguric and even often polyuric hypoosmotic fall in creatinine clearance.\(^{(52)}\) Progression to oliguric or anuric renal failure is infrequent, and recovery upon drug discontinuation is most often observed. Occasionally, a Fanconi’s syndrome or a Bartter’s-like syndrome has been observed.\(^{(53, 54)}\) A correlation between the development of these clinical signs and the severity or rate of progression of the subclinical alterations remains difficult to establish mainly because of large interpatient variations. Consequently, the usefulness of monitoring the sub clinical changes to detect individuals at risk has remained questionable. In animals, tubular alterations have clearly been associated with the development of focal necroses and apoptosis in the tubular epithelium, together with an extensive tubular and peri tubular cell proliferation without an apparent change in kidney function.\(^{(56, 56)}\)

**Effects of high dose in animals**

High doses (40 mg/kg or more for gentamicin) are necessary in animals to rapidly induce extended cortical necrosis and overt renal dysfunction.\(^{(50, 57)}\) At this stage, a large number of structural, metabolic, and functional alterations are observed in tubular cells and several of these alterations have been claimed to be responsible for cell death or dysfunction. Many of the changes observed at the level of the apical membrane could, however, be merely mediated by a direct effect of the drug on this structure during its initial stages of uptake in proximal tubular cells.\(^{(56-62)}\) Conversely, other effects, such as inhibition of protein synthesis and modulation of gene expression, mitochondrial alterations, or inhibition of enzymes located on the cytosolic side of the pericellular membrane, must involve uptake and intracellular distribution of the drug to the corresponding targets.

4. **Main alterations elicited by aminoglycosides in kidney cortex**

4.1 **At low doses**

**A. Early alterations**

- Accumulation of phospholipids in lysosomes and enlargement of these organelles.\(^{(50, 51)}\)
- Inhibition of the activities of lysosomal phospholipases and sphingomyelinase.\(^{(63)}\)
- Decreased reabsorption and/or intracellular lysosomal sequestration and digestion of filtrated, low-molecular-weight proteins (e.g., lysozyme, alpha-2- macroglobulin, β-2-microglobulin.\(^{(46, 52)}\)
- Shedding of brush-border enzymes (e.g., alanyl amino peptidase) and release of lysosomal enzymes (e.g., N-acetyl-β-glucosaminidase).\(^{(46)}\)
B. Later alterations

**B.1 Degenerative alterations**
- Course granulation of epithelial cells.\(^{(46)}\)
- Focal necroses, apoptosis.\(^{(53)}\)
- Increased phospholipids excretion and cast in urine.\(^{(53)}\)
- Proteinuria, hypoosmotic polyuria, (in humans only).\(^{(52)}\)
- Decreased glomerular filtration and increase in blood urea nitrogen and creatinine, without immediate signs of glomerular damage, (in humans only).\(^{(46)}\)

**B.2 Regenerative lesions**
- Tubular cell proliferation and dedifferentiation.\(^{(54)}\)
- Tubular dilatation.\(^{(46)}\)
- Interstitial proliferation (fibroblastic cells) and focal infiltration by inflammatory cells.\(^{(46)}\)

**4.2 At high doses**

**A. Brush-border and apical membranes**
- Wasting of K\(^+\), Mg\(^{2+}\), and Ca\(^{2+}\).\(^{(52, 62)}\)
- Decreased reabsorption of water, HCO\(^{-3}\), glucose.\(^{(64)}\)
- Decreased of Na\(^+\) and P\(^+\) cotransport and Na\(^+\) and H\(^+\) exchange.\(^{(65)}\)
- Inhibition of phosphatidylinositol phospholipids C
- Decrease of carrier-mediated dipeptide transport.\(^{(61)}\)

**B. Basolateral membrane**
- Impairment of organic acid and bases transport.\(^{(66)}\)
- Inhibition of Na\(^+\)/K\(^+\) ATPase.\(^{(66, 67)}\)
- Reduction of the electrogenic Na\(^+\) transport.\(^{(68)}\)

**C. Mitochondria**
- Impairment of respiration and caption transport; swelling.\(^{(52)}\)
- Impairment of the activities of key mitochondrial enzymes in gluconeogenesis, ammoniogenesis and tricarboxylic acid oxidation pathways.\(^{(66, 69)}\)

**D. Protein synthesis and related phenomena**
- Inhibition of protein synthesis and dilatation of endoplasmic reticulum.\(^{(50)}\)
- Suppression of gene expression for the Na+ and Ca^{2+} exchanger, Na+- dependent D-glucose transporter, and alpha, subunit of Na+/K+ ATPase.\(^{(70, 71)}\)

- Expression and move of heat shock proteins from nucleus to lysosomes.\(^{(72)}\)

5. Renal handling of aminoglycosides

Aminoglycosides are polycationic, a property that is responsible for their poor oral absorption, a poor penetration into CSF, and a rapid renal clearance. The poly cationic charge also appears to contribute to nephrotoxicity. Aminoglycosides have molecular weight of approximately 500 Dalton and are water-soluble and minimally protein bound. The primary route of elimination from the body is glomerular filtration, which is nearly equal to inulin clearance. A small percentage (approximately 5) of the filtered aminoglycoside gets actively reabsorbed in the proximal tubule. The serum half-life of aminoglycosides is a few hours as compared to 4 to 5 days in proximal tubule cells.\(^{(73-76)}\)

6. Clinical features of aminoglycoside induced nephrotoxicity

The most common clinical presentation is non-oliguric acute renal failure. The earliest urinary manifestations are an increase in urine output and the appearance of enzymuria. Enzymuria represents the elimination in the urine of fragments of brush border membrane or lysosomal enzymes. Measuring enzymuria as an early marker of tubular damage is of unproven efficacy and is impractical. The onset of renal failure is usually slower and the daily rise of serum creatinine tends to be lower than other causes of acute renal failure. Serum creatinine and blood urea nitrogen characteristically increase 7 to 10 days after initiation of aminoglycoside therapy. In more than half of the cases with nephrotoxicity, the decline in renal function occurs only after the therapy has been completed. Recovery from aminoglycoside nephrotoxicity is usually slow, often taking 4-6 weeks’ time, particularly in elderly individuals. Although the vast majority of patients do recover, the presence of several risk factors may alter the clinical presentation or the course, resulting in the early appearance of acute renal failure as well as a protracted course. In patients with underlying chronic kidney disease, recovery in renal function may be incomplete insome.\(^{(77)}\) In addition, various tubular dysfunction and electrolyte abnormalities may also occur.\(^{(78, 79)}\) Nephrotoxicity is a common complication of aminoglycoside antibiotic therapy in man.\(^{(80)}\) Early signs of nephrotoxicity include increased urinary excretion of proximal tubular cell brush-border membrane enzymes such as alanine aminopeptidase, proteins of relative low molecular mass such as lysozyme and β2-microglobulin, and granular casts.\(^{(81)}\) A urine-concentrating defect
is usually evident and may explain the non-oliguric acute renal failure typically observed in these patients. Less common manifestations of tubular dysfunction include potassium, magnesium, calcium, and glucose loss in the urine. Azotaemia and elevation of the serum creatinine concentration are relatively late manifestations of nephrotoxicity and reflect depression of glomerular filtration rate consequent to extensive proximal tubular cell necrosis. Patients receiving standard doses of amino-glycoside antibiotics usually do not manifest depression of glomerular filtration until after seven or more days of drug therapy. However, pathological changes confined to the proximal tubule can be seen in renal biopsy material obtained before this time.\textsuperscript{(81, 82)} At the light microscope level, these changes range from loss of brush-border membrane, apical blebbing, and prominent vacuoles to cloudy swelling, patchy cell necrosis, and ploughing of necrotic cells with cast formation in the lumen. Electron microscopy reveals the presence of multicentric multilamellar membrane structures known as myeloid bodies within distended lysosomes.\textsuperscript{(83)} These lysosomal lesions can be seen within 1-2 days of drug treatment and they increase in size and number as therapy is prolonged.

The nephrotoxicity potential of aminoglycosides has been ranked as neomycin > gentamicin > sisomicin = kanamycin > tobramycin > netilmicin > streptomycin.\textsuperscript{(84)} The situation with amikacin has been somewhat controversial, but recent studies have suggested that it is less nephrotoxic, even in experimental animals, than the other clinically available aminoglycosides, except for streptomycin. Clear-cut therapeutic advantages of any particular aminoglycoside are not readily apparent in patients because of the serious nature of their underlying illness and concurrent therapy with multiple drugs. Furthermore, the relative nephrotoxicity is usually assessed by insensitive techniques, such as blood urea nitrogen, serum creatinine, and enzymuria, that do not give a quantitative representation of the extent of renal injury. In humans, few would argue that neomycin and gentamicin are much more nephrotoxic in therapeutic use than streptomycin, but there are also other important risk factors that relate to the clinical condition of the patient:

- Dehydration, volume depletion, diuretic-induced volume depletion;
- Advanced age;
- Pre-existing renal disease;
- Electrolyte imbalance (acidosis, hypomagnesaemia, hypokalaemia, hypocalcaemia);
- Hypotension/renal ischaemia;
Extra renal target organ disease such as cirrhosis of the liver;
- Exposure to multiple nephrotoxins;
- Frequent dose regimens as opposed to larger doses given less frequently;
- Elevated aminoglycoside trough concentrations.
- Current understanding of the pathogenesis of amino-glycoside nephrotoxicity has been derived primarily from studies in rats, which exhibit a pattern of renal injury indistinguishable from that observed in man.\(^66, 85, 86\) The drug dose, in relation to body weight, required to induce injury in the rat is considerably larger than that required in man, whereas the dose is approximately the same when expressed in relation to body surface area. From such studies has emerged unequivocal evidence that aminoglycoside nephrotoxicity is causally linked to the transport and accumulation of drugs by renal proximal tubular cells. Following parenteral administration, aminoglycosides are eliminated unchanged in the urine by glomerular filtration. A small fraction of the filtered drug is taken up by the renal proximal tubular cells via a low affinity, high capacity transport mechanism that exhibits saturation kinetics.\(^87, 88\) The first step in this transport process involves binding of the cationic aminoglycoside to apical membrane receptors, thought to be anionic phospholipids such as phosphatidylinositol.\(^89\) This is followed by uptake into the cell by adsorptive endocytosis with subsequent translocation and sequestration of the drug in high concentration within lysosomes.\(^90-92\) In addition a small quantity of drug appears to gain access into the cell across the basolateral membrane.\(^93\) Following uptake into proximal tubular cells, aminoglycosides express their nephrotoxicity potential by disrupting one or more critical intracellular metabolic pathways.

Although these drugs have been shown to effect a variety of biochemical processes at several sites within proximal tubular cells, it remains to be established which if any of these actions are causally linked to the cascade that eventuates in cell injury and necrosis.\(^94\) Prominent among the biochemical derangements is a disturbance of phospholipid metabolism reflected by an increase in renal cortical phospholipid enriched in phosphatidylinositol.\(^94\) The phospholipidosis has been shown to be due primarily to the accumulation of lysosomal myeloid bodies\(^92\), which form as a consequence of the inhibition of lysosomal phospholipases by the high concentration of drug within the lysosomal compartment.\(^95-97\) The mechanism of inhibition is thought to be related to an electrostatic interaction between the cationic aminoglycoside and anionic phospholipid. Another example of an adverse interaction between aminoglycosides and phospholipid is the observation that gentamicin
inhibits agonist activation of the phosphatidylinositol cascade, an effect that localizes the site of interaction at the cytoplasmic surface of the plasma membrane and most likely reflects binding of the polycationic gentamicin to the polyanionic phospholipid, phosphatidylinositol-4,5-bis-phosphate. This effect may also explain the observation that aminoglycosides inhibit phosphatidylinositol- specific phospholipase C in renal brush-border membranes. Alterations of other biochemical processes associated with plasma membranes have been described, including depressions of Na\(^+\)-K\(^+\)- ATPase, adenylate cyclase, alkaline phosphatase, and calcium binding. Impaired mitochondrial respiration and decreased incorporation of leucine into microsomal protein have also been observed prior to the onset of obvious irreversible cell injury. These findings emphasize that multiple sites serve as targets for drug-cell interaction. However, it remains uncertain which of these biochemical abnormalities are proximal events causally linked to toxicity.

One theory that attempts to integrate these diverse observations focuses on the lysosomal accumulation of aminoglycosides, with induction of a lysosomal phospholipidosis as the critical first step. If the injury threshold concentration of aminoglycoside is not reached, the lysosomal phospholipidosis regresses without any biochemical or morphological evidence of cellular necrosis and regeneration. However, if the injury threshold concentration is exceeded, the lysosomal phospholipidosis progresses and the overloaded lysosomes swell, resulting in the loss of integrity of the lysosomal membrane and the release of lysosomal enzymes, toxins, and large quantities of aminoglycosides into the cytosol. The extralysosomal aminoglycoside interacts with and disrupts the functional integrity of other subcellular membranes, thereby initiating the injury cascade that eventuates in cell death. It should be emphasized that aminoglycoside-induced proximal tubular cell necrosis is accompanied by a conspicuous regenerative response. Thus, the clinical threshold for nephrotoxicity is determined by the balance between the rate of necrosis and the rate of regeneration of proximal tubular cells. If necrosis dominates, overt renal failure ensues.

Aminoglycoside nephrotoxicity is accompanied by increased generation of free radicals. Furthermore, nephrotoxicity is blocked with free radical scavengers/antioxidants such as dimethylthiourea, dimethyl sulfoxide, sodium benzoate, or deferoxamine. However other studies have demonstrated that antioxidants such as vitamin E do not protect against aminoglycoside-induced injury. The reasons for this apparent discrepancy are not
known, and the exact role of lipid peroxidation in gentamicin nephrotoxicity therefore remains unclear.

**Table 2: Currently used drugs to prevent/treat drug induced nephrotoxicity.**

<table>
<thead>
<tr>
<th>Pharmacological class</th>
<th>Pharmacological interventions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics</td>
<td>Fosfomycin, Fleroxacin</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>Nifedipine, Amlodipine</td>
</tr>
<tr>
<td>β-blocker</td>
<td>Carvedilol</td>
</tr>
<tr>
<td>Cytoprotective antianginal</td>
<td>Trimetazine</td>
</tr>
<tr>
<td>iNOS inhibitor</td>
<td>L-NIL</td>
</tr>
<tr>
<td>NO precursor</td>
<td>L-arginine</td>
</tr>
<tr>
<td>Hormones</td>
<td>Melatonin, Thyroxine</td>
</tr>
<tr>
<td>TNF-α synthesis inhibitor</td>
<td>Pentoxifylline</td>
</tr>
<tr>
<td>Free radical scavengers</td>
<td>S-allylcysteine, Diallly sulphide, Caffeic acid Phenethyl ester, S-allylmercaptopcysteine,</td>
</tr>
<tr>
<td>Antioxidants</td>
<td>Probucole, Aminoguanidine, L-carnitine, Ebselen, N-acetylcysteine, Lycopene, Curcumin, Thymoquinone, Fish oil, Vitamin E, Vitamin C, Sesame oil, Halofuginone, Resveratrol, Quercetin.</td>
</tr>
<tr>
<td>Antiplatelet agent</td>
<td>Trapidil</td>
</tr>
<tr>
<td>Statins</td>
<td>Atorvastatin</td>
</tr>
<tr>
<td>PPAR-λ agonist</td>
<td>Rosiglitazone</td>
</tr>
<tr>
<td>TNF-α synthesis inhibitor</td>
<td>Pentoxifylline</td>
</tr>
<tr>
<td>Biguanides</td>
<td>Metformin</td>
</tr>
<tr>
<td>Anti-oxidant enzyme</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>Superoxide dismutase mimetic</td>
<td>M40403</td>
</tr>
<tr>
<td>Herbal extracts</td>
<td><em>Rhazya stricta, Garlic, Cassia auriculata, Soyabean, Phylanthus amarus, Morchella esculenta, Green tea, Nigella sativa.</em></td>
</tr>
</tbody>
</table>

**7. Role of herbs in nephrotoxicity**

In The recent years many researchers have examined the effects of plants used traditionally by indigenous healers and herbalists to support kidney function and treat diseases of the
kidney. In most cases, research has confirmed traditional experience and wisdom by discovering the mechanism and mode of action of these plants as well as reaffirming the therapeutic effectiveness of certain plants or plant extracts in clinical studies. Several hundred plants have been examined for use in a wide variety of kidney disorders. Just a handful has been fairly well researched. The aim of the research is to find out new nephroprotective drugs from indigenous plants which are potent and non-toxic agents. Normally herbal plants are free from side effects adverse effects and they are low cost medicines, which will be beneficial for the people.

8. Plant profile

*Trichosanthes*, a genus of family Cucurbitaceae is an annual or perennial herb distributed in tropical Asia and Australia. Pointed gourd (*Trichosanthes dioica* Roxb.) is known by a common name of *parwal* and cultivated mainly as a vegetable. Juice of leaves of *T. dioica* is used as tonic, febrifuge, in oedema, alopecia and in subacute cases of enlargement of liver. In Charaka Samhita leaves and fruits find mention for treating alcoholism and jaundice. A lot of pharmacological work has been scientifically carried out on various parts of *T. dioica* but some other traditionally important therapeutical uses are also remaining to proof till now scientifically. According to ayurveda leaves of the plants are used as antipyretic, diuretic, cardiotonic, laxative, antiulcer, etc. It is also used in skin disorder by some communities of Asia traditionally.\(^{(109)}\)

**B.S.: Trichosanthes dioica**

- **Common name:** Pointed gourd (patol)
- **Sanskrit name:** Patola; Kulak; Karkashchchhad; Rajiphal; Beejagarbha; Parval
- **Family:** Cucurbitaceae
- **Genus:** Trichosanthes
- **Species:** *Trichosanthes dioica*
- **Part used:** Fruits

![Fig 4. Shows whole plant of *T. dioica* Roxb.](image1)

![Fig 5. Fruits of *T. dioica* Roxb.](image2)
Chemical Constituents

Earlier chemical study reveals that in addition to a number of tetra & pentacyclic triterpenes, the toxic bitter principles cucurbitacins (a group of often highly oxygenated tetracyclic compounds with a unique carbon skeleton & almost a carbonyl group in ring C) may be considered as a taxonomic character of Cucurbitaceae.

Cucurbitacin-A

Molecular formula-C_{30}H_{42}O_{7}

Cucurbitacin-B

Molecular formula-C_{32}H_{46}O_{8}

Cucurbitacin-D

Molecular formula-C_{30}H_{44}O_{7}

Cucurbitacin-E

Molecular formula-C_{32}H_{44}O_{8}

Pointed gourd is rich in vitamins and contain 9.0 mg Mg^{2+}, 2.6 mg Na+, 83.0 mg K+, 1.1 mg Cu^{2+}, and 17.0 mg S per 100 g edible part. The seeds of *T. dioica* contain a large amount of peptides. The seed peptides have the unique property of being resistant to the action of silver nitrate, a sensitive reagent commonly used to stain proteins. The various chemical constituents present in *T. dioica* are vitamin A, vitamin C, tannins, saponin. Phytochemical evaluations of Aqueous and Ethanolic extracts have showed the presence of saponins & tannins. The seed extract of *T. dioica* contains 7-oxidohydrokarounidol-3-benzoate as the most predominant component in the highly polar fraction of then on saponifiable lipid. Two main phytosterols present in *T. dioica* are namely, 24α-ethylcholest-7-enol & 24β-ethylcholest-7-enol. Seeds of *T. dioica* also contain lectin, acarbohydrate (specifically galactose) binding protein which is homologous to Type-II ribosome inhibitory proteins (Type-II RIP).
Anti-oxidant, Anti Diabetic, Anti toxic, Anti-oxidant and hepatoprotective, Anti Convulsant, Cytotoxic, Cholesterol lowering, Wound Healing, Anti-oxidant and anti-inflammatory, Laxative.\(^{110}\)

10. Phytochemical Study
The coarse powder was extracted with 1–1.5 liters of methanol by continuous hot Soxhlet apparatus. After completion of extraction, extract was dried under rotary evaporator. The dried extract was stored in a desiccator.

Preliminary phytochemical studies.\(^{111–114}\)

Methanolic extract of the fruits of *Trichosanthes dioica* were subjected to chemical tests for the identification of their active constituents.

1. Tests for carbohydrates and glycosides

A small quantity of the extract was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to Molisch’s test to detect the presence of carbohydrates.

**A. Molisch’s Test**

Filtrate was treated with 2–3 drops of 1% alcoholic α-naphthol solution and 2ml of con. H2SO4 was added along the sides of the test tube. Appearance of violet coloured ring at the junction of two liquids shows the presence of carbohydrates. Another portion of the extract was hydrolyzed with HCl for few hours on a water bath and the hydrolysate was subjected to Legal’s and Borntrager’s test to detect the presence of different glycosides.

**B. Legal’s Test**

To the hydrolysate, 1ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

**C. Borntrager’s Test**

Hydrolysate was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammoniacal layer acquires pink colour showing the presence of glycosides.

2. Tests for alkaloids

A small portion of the methanol extract was stirred separately with few drops of dil. HCl and
filtered. The filtrate was treated with various reagents as shown for the presence of alkaloids. Mayer’s reagent – Creamy precipitate Dragendorff’s reagent – Orange brown precipitate Hager’s reagent – Yellow precipitate Wagner’s reagent – Reddish brown precipitate.

3. Tests for phytosterol

The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol.

**Libermann Burchard Test**

The residue was dissolved in few drops of acetic acid, 3 drops of acetic anhydride was added followed by few drops of con. H₂SO₄. Appearance of bluish green colour shows the presence of phytosterols.

4. Tests for fixed oils

**Spot test**

Small quantity of extract was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil. Few drops of 0.5N alcoholic potassium hydroxide were added to a small quantity of extract along with a drop of phenolphthalein. The mixture was heated on a water bath for 1–2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

5. Tests for gums and mucilages

Small quantity of the extract was added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties for the presence of gums and mucilages.

6. Tests for saponins

The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam shows the presence of saponins.

7. Tests for proteins and free amino acids

Small quantity of the extract was dissolved in few ml of water and treated with following reagents.

A. Millon’s reagent – Appearance of red colour shows the presence of protein and free amino acids.
B. Ninhydrin reagent – Appearance of purple colour shows the presence of proteins and free amino acids.
C. Biuret test – Equal volumes of 5% NaOH solution and 1% copper sulphate solution were added. Appearance of pink or purple colour shows the presence of proteins and free amino acids.

8. Tests for phenolic compounds and tannins: Small quantity of the extract was taken separately in water and tested for the presence of phenolic compounds and tannins using following reagents.
A. Dil. FeCl₃ solution (5%) – violet colour
B. 1% solution of gelatin containing 10% NaCl – white precipitate
C. 10% lead acetate solution – white precipitate.

9. Tests for flavonoids
A. With aqueous Sodium hydroxide solution: Blue to violet colour (anthocyanins), yellow colour (flavones), yellow to orange (flavonones)
B. With Con. H₂SO₄: Yellow orange colour (anthocyanins), yellow to orange colour (flavones), orange to crimson (flavonones)
C. Shinoda’s test
Small quantity of the extract was dissolved in alcohol and to that a piece of magnesium followed by Con. HCl drop wise was added and heated. Appearance of magenta colour shows the presence of flavonoids.

Table 3: Data showing preliminary phytochemical screening of the methanolic extract of *Trichosanthes dioica* Roxb.

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Methanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>++</td>
</tr>
<tr>
<td>Glycosides</td>
<td>++</td>
</tr>
<tr>
<td>Fixed oils and fats</td>
<td>+</td>
</tr>
<tr>
<td>Gums and mucilage</td>
<td>–</td>
</tr>
<tr>
<td>Proteins and amino acids</td>
<td>–</td>
</tr>
<tr>
<td>Saponins</td>
<td>–</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
</tbody>
</table>

+/++ : Present    – : Absent
CONCLUSION
Many herbal plants have proved to be nephroprotective agent by many preclinical and clinical studies and are widely used in renal diseases including nephrotoxicity. One another plant is said to be used as nephroprotective agent, known as *Trichosanthes dioica*. It contains many chemical constituents which exhibits protective actions against nephrotoxicity. This plant also contains anti-inflammatory, antioxidant and immunomodulator activity which are helpful to exhibit its nephroprotective activity. But it does not contain any scientific evidence about its safety and efficacy. So the present study was undertaken to evaluate nephroprotective activity of *Trichosanthes dioica* on gentamicin induced nephrotoxicity.

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