wjpmr, 2025, 11(3), 101-110

SJIF Impact Factor: 6.842

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

NEPHROPROTECTIVE ACTIVITY OF HYDROALCOHOLIC AERIAL PLANT EXTRACT OF *AMARANTHUS ROXBURGHIANUS* NEVSKI IN GENTAMICIN-INDUCED NEPHROTOXICITY IN WISTAR RATS

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Article Received on 10/01/2025 **Article Revised on 31/01/2025** **Article Accepted on 20/02/2025**

ABSTRACT

Aim: *Amaranthus roxburghianus* Nevski Hydroalcoholic aerial plant extract was taken to treat Gentamicin induced nephrotoxicity in Wistar rats. **Methodology:** 30 male wistar rats were taken and randomly divided into 5 groups containing 6 animals. Group-I was considered as control treated with normal saline (10ml/kg p.o), Group-II was treated with Gentamicin (100mg/kg i.p), Group-III was treated with Gentamicin (100mg/kg i.p) and Vitamin-E (250mg/kg p.o), Group-IV and Group-V were treated with Gentamicin (100mg/kg i.p) and Hydroalcoholic Extract of *Amaranthus roxburghianus* Nevski (200mg/kg p.o and 400mg/kg p.o respectively) for 7 days. At the end of the treatment i.e. on 8th day the animals were sacrificed and biochemical changes in creatinine, urea, uric acid and total protein were estimated in blood. Histopathological evaluation of kidney was done. The statistical analysis was done. **Results:** The plant extract revealed Alkaloids, Flavonoids, Phenolic compounds, Tannins, Glycosides, Amino acids, Terpenoids, Steroids, Lipids, Saponins. In the treatment groups, the hydroalcoholic extract of plant had significantly reduced the increased levels of serum creatinine, urea, and uric acid levels and improved serum total protein levels. It also normalized the changes in the anti-oxidant parameters. Histological examination of the kidneys revealed that treatment with the extracts restored normal kidney structures in the animals treated with HARE 200mg/kg & 400mg/kg along with GEN as compared to GEN treated group.

KEYWORDS: Gentamicin, nephrotoxicity, nephroprotective, hydroalcoholic extract of *Amaranthus roxburghianus* Nevski, Flavonoids, phenolic compounds.

INTRODUCTION

Kidney is the main organ in the human body that performs various important functions that include detoxification, regulation of extracellular fluids, homeostasis, and excretion of toxic metabolites.[1] Nephrotoxicity is defined as rapid deterioration in the kidney function which can be as a result of hemodynamic changes, direct injury to the cells and tissue, inflammatory tissue injury, and/or obstruction of renal excretion due to toxic effect of mediations and other chemical substances. The nephrotoxic effect of most of the medication drugs is usually found in the patients whose kidney functions are compromised. About 20% of nephrotoxic incidents are often induced due to medication and this percentage is augmented in elderly patients due to age factors and poly-medications.[2]

Amaranthus roxburghianus Nevski belongs to the family *Amaranthaceae* which is a wild herb and used as common leafy vegetable. It is commonly known as 'chiri kura' in Telugu language. It is a cosmopolitan genus of annual or short-lived perennial plants collectively known as amaranths. *Amaranthus roxburghianus* is used as an abortifacient by tribes of Chittoor district, Andhra Pradesh state of India. Used as iron tonic, its extracts or herbal formulations are rich in alkaloids, used in traditional Chinese and Ayurvedic medicine. Its root extract in combination with piperine is used in the effective treatment of inflammatory bowel disease. Further consumption of its leaves is reported for the treatment of sunstroke and urinary disorders.[4]

MATERIALS AND METHODS

Plant Collection and Preparation of Extract

Amaranthus roxburghianus Nevski plant was acquired from the Hyderabad's local marketplace and authenticated by Dr. K. Madava Chetty, Plant Taxonomist, Asst. Professor, Dept. of Botany, Sri Venkateswara University, Tirupati, A.P., India, authenticated the plant. There is a voucher specimen of the plant (Ref. No. 0891), dated 08/03/2022, that has been archived for future reference. The hydroalcoholic extract of the plant was prepared by soxhlet apparatus.

Thirty male Wistar rats weighing 200-250g were procured and the animals were placed in polypropylene cages housed at 24°±2°C on a 12-hour light/dark cycle in animal house.

Acute toxicity studies on HARE (Hydroalcoholic Amaranthus roxburghianus Extract) were not performed since its safety up to 2000 mg/kg had been established in an earlier study. The two doses of 200 and 400 mg/kg were selected based on prior research that demonstrated superior response at doses above 400 mg/kg in terms of analgesic and nephroprotective actions.

1. Experimental Design

A total of 30 male and female Wistar rats weighing 200g–250 g were selected for the study and were allocated to five groups of six rats in each group (n=6).

2. Experimental Procedure

- *Group1* (Control): treated with normal saline orally once daily and continued till day 7.
- *Group 2* (Gentamicin): treated with daily dose of (100 mg/kg/ i.p) till day 7.
- *Group3* (Vitamin E+ gentamicin): treated with daily oral dose of vitamin E (250 mg/kg p.o) dissolved in coconut oil administered by gavage, following the administration of Gentamicin (100mg/kg/ i.p) till day 7.
- *Group 4* (Hydroalcoholic extract of *Amaranthus roxburghianus* (HARE)+ Gentamicin): treated with daily oral dose of HARE (200mg/kg p.o) dissolved in Tween-80 and administered by gavage, followed by administration of Gentamicin (100mg/kg i.p) till day 7.
- *Group 5* (Hydroalcoholic extract of *Amaranthus roxburghianus* (HARE)+ Gentamicin): treated with daily oral dose of HARE (400 mg/kg p.o) dissolved in Tween-80 and administered by gavage, followed by administration of Gentamicin (400mg/kg i.p) till day 7.

On 8th day rats were euthanized with carbon dioxide inhalation to obtain blood samples for the estimation of various biochemical parameters like total protein, creatinine, urea and uric acid. Kidneys were isolated for histopathological analysis and measurement of Malondialdehyde(MDA), Catalase (CAT), Reduced Glutathione (GSH), Glutathione Reductase (GR).

Statistical Analysis

The results were expressed as mean ± SEM. The data was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. A value of $P < 0.05$ was considered as statistically significant.

RESULTS

1. PRELIMINARY PHYTOCHEMICAL ANALYSIS OF HYDROALCOHOLIC AERIAL PLANT EXTRACT OF *A. ROXBURGHIANUS* NEVSKI

The hydroalcoholic aerial plant extract of *A. Roxburghianus* Nevski underwent a preliminary phytochemical analysis, which found the presence of a number of phytochemicals including alkaloids, flavonoids, phenolic compounds, tannins, and glycosides.

2. ANALYIS OF SERUM BIOCHEMICAL PARAMETERS

Table no 1: Effect of Vit-E and HARE on Serum Parameters in GEN induced Nephrotoxic rats.

S.NO	GROUP	Serum CRE (mg/dl)	Serum URE (mg/dl)	Serum UAC (mg/dl)	TSP (gm/dl)
1	Normal saline (10ml/kg p.o)	0.77 ± 0.05	29.82 ± 1.17	1.22 ± 0.18	12.02 ± 0.10
2	GEN-induced (100mg/kg i.p)	1.98 ± 0.04$^{###}$	61.17 ± 1.62$^{###}$	2.77 ± 0.16$^{###}$	6.92 ± 0.17$^{###}$
3	GEN (100mg/kg i.p) + Vit-E (250mg/kg p.o)	0.89 ± 0.03***	47 ± 2.26***	1.28 ± 0.06***	9.28 ± 0.16***
4	GEN (100mg/kg i.p) + HARE (200mg/kg p.o)	1.80 ± 0.06 ^{ns}	51.17 ± 1.07**	2.60 ± 0.18^{ns}	7.20 ± 0.18^{ns}
5	GEN (100mg/kg i.p) + HARE (400mg/kg p.o)	1.69 ± 0.08**	43.17 ± 2.07***	2.02 ± 0.11**	8.01 ± 0.27**

n=6, values are Mean ± SEM, one way ANOVA followed by Dunnett's post hock test. Significant at, $^{##}P<0.01$ as compared to normal control group, **P<0.01, ***P<0.001 as compared to GEN group. Vit-E- vitamin-E, GEN-Gentamicin, HARE- Hydroalcoholic *Amaranthus roxburghianus* Extract.

2. ESTIAMTION OF ANTI-OXIDANT PARAMETERS:

Table no 1: Effect of Vit-E and HARE on Anti-oxidant parameters in GEN induced Nephrotoxic rats.

S.NO	GROUP	MDA	CAT	GSH	GR
1	Normal saline (10ml/kg p.o)	7.69 ± 0.12	182 ± 4.28	32.15 ± 1.56	20.02 ± 0.88
2	GEN-induced (100mg/kg i.p)	15.07 ± 0.27$^{###}$	101.9 ± 5.49$^{###}$	18.2 ± 0.76$^{###}$	42.04 ± 1.54$^{###}$
3	GEN (100mg/kg i.p) + Vit-E (250mg/kg p.o)	7.97 ± 0.16***	158 ± 2.92***	26.41 ± 1.03***	24.04 ± 0.93***
4	GEN (100mg/kg i.p) + HARE (200mg/kg p.o)	8.80 ± 0.31***	120 ± 8.58^{ns}	22.31 ± 1.39^{ns}	36.46 ± 1.02**
5	GEN (100mg/kg i.p) + HARE (400mg/kg p.o)	8.06 ± 0.25***	146 ± 2.62***	24.62 ± 1.12**	29.02 ± 0.75***

n=6, values are Mean ± SEM, one way ANOVA followed by Dunnett's post hock test. Significant at, $^{###}$P<0.001 as compared to normal control group, **P<0.01, ***P<0.001 as compared to GEN group. Vit-E- vitamin-E, GEN-Gentamicin, HARE- Hydroalcoholic *Amaranthus roxburghianus* Extract.

HISTOPATHOLOGICAL STUDIES

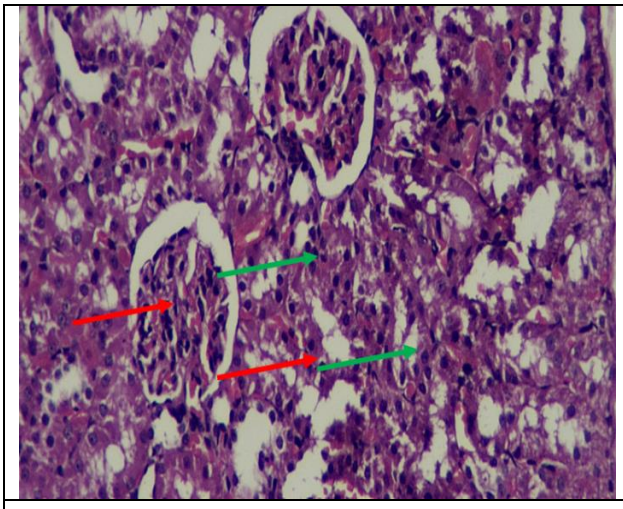

Fig no:1 Transverse section of a normal kidney showing normal orientation of nephrons with adequate glomeruli and well-spaced tubules.
Red arrows- Normal morphology of glomerulus
Green arrows- Tubules of kidneys in cortex region

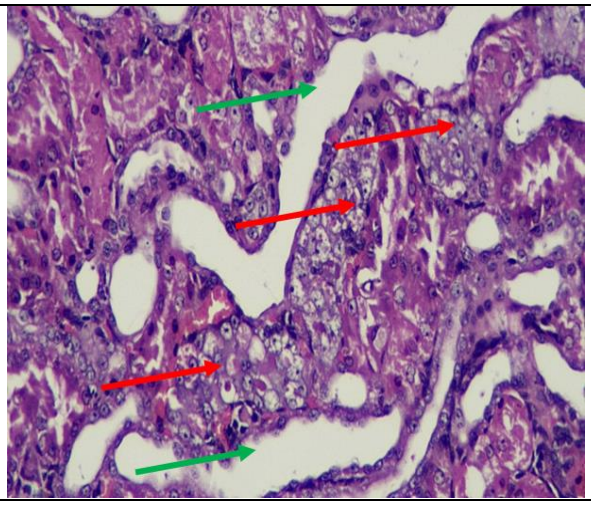

Fig no:2 Transverse section of nephrotoxic rat kidney (treated with 100mg/kg i.p GEN) showing renal cell necrosis, tubular dilatation and cystic degeneration of tubules.
Red arrow- Severe renal cell necrosis in proximatetubules of kidney in cortex region
Green arrow- Severe tubular dilatation and cystic degeneration of tubules was observed cortex region of kidney.

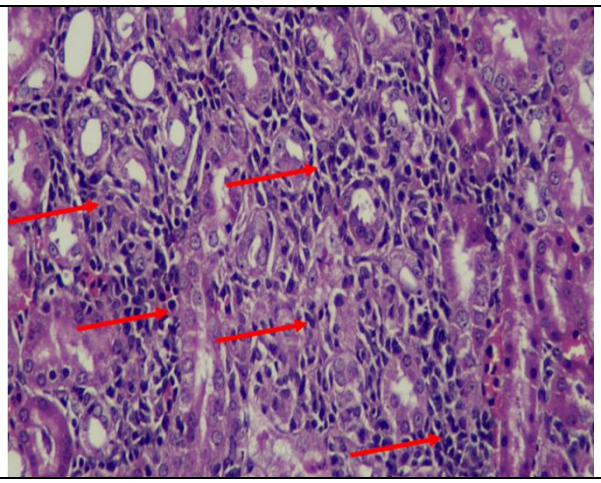

Fig no:3 Transverse section of nephrotoxic rat kidney (treated with 100mg/kg i.p GEN + 250mg/kg p.o Vit-E) showing moderate renal cell necrosis and

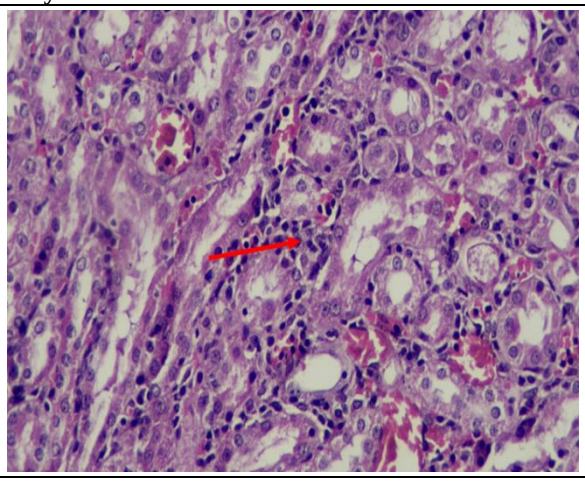

Fig no:4 Transverse section of nephrotoxic rat kidney (treated with 100mg/kg i.p GEN + 200mg/kg p.o HARE) showing foci of regenerating tubules

tubular inflammation. **Red arrow- Moderate renal cell necrosis and tubular inflammation with infiltration of inflammatory cells was observed**	**Red arrow- Most of tubules are appeared normal, Foci of regenerating tubules with infiltration of inflammatory cells were observed in between regenerated tubules**

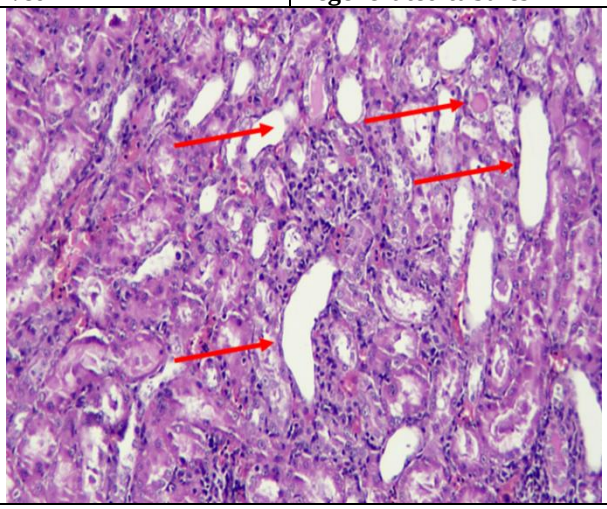

Fig no:5 Transverse section of nephrotoxic rat kidney (treated with 100mg/kg i.p GEN + 400mg/kg p.o HARE) showing mild tubular dilatation and mild cystic degeneration of tubules.
Red arrow- Mild tubular dilatation and mild cystic degeneration of tubules was observed cortex region of kidney

DISCUSSION

Nephrotoxicity is defined as the rapid deterioration in the kidney function due to toxic effect of medications and chemicals.[8] Nephrotoxicity is also defined as the adverse effects of substances on renal function.[9] Some medications may directly or indirectly cause renal failure and this condition can be termed as drug-induced renal toxicity/ drug-induced renal disorders. Medications that cause renal damage include Anti-microbials, NSAIDs, immunosuppressants, chemo-therapeutic agents, among others. The drug-induced renal toxicity is a serious cause of morbidity and mortality and about 18-27% of all acute renal toxicities in US hospitals are associated with drug-induced nephrotoxicity.[10] Animal and experimental studies as well as clinical case reports have suggested that there are numerous environmental and occupational nephrotoxins that cause damage to kidneys due to acute and chronic exposures.[11]

Aminoglycosides (e.g., Gentamicin) are a class of antibiotics that cause nephrotoxicity by apoptotic.[12-14] as well as necrotic[15] cell death of tubular epithelial cells of kidney. Hence, a Gentamicin-induced nephrotoxic rat model can be used to investigate the mechanisms involved in producing drug-induced renal failure and also for screening new agents with Nephroprotective activity. Gentamicin-induced renal toxicity affects many components of the nephron structure such as the glomerulus, tubules (mainly proximal tubules), and renal microvasculature. Pathogenic mechanisms may include altered glomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy (TMA). Moreover, renal damage cause delayed drug excretion and metabolic and systemic toxicity.[16]

Nephrotoxicity is generally managed by maintaining the fluid volume in body, undergoing dialysis or by adjusting the dose of the drug. In mild to moderate conditions, it can be managed by discontinuing the drug that is causing the nephrotoxicity or by altering its dose. Steroid therapy (mostly Corticosteroids) can be given. Diuretics such as Furosemide can be used to treat renal failure. Other medications include, and are not limited to Phosphate binders, erythropoietin-stimulating agents (ESA), intravenous (IV) iron agents, B complex vitamins, and immunosuppressants.[17,18]

Biomarkers of Renal Failure

The most common biomarkers of nephrotoxicity and renal dysfunction are serum urea (URE), Uric acid (UAC) and serum creatinine (CRE) which are specific but have low sensitivity to detect earlier renal damage. Thus, new biomarkers which are more sensitive and highly specific are required to discover the initial renal injury.[19] Serum total protein is regarded as a potential biomarker to detect acute and chronic stage renal damage that is induced by nephrotoxic drugs. The levels of serum total protein, transferrin, albumin, and prealbumin commonly indicate nutrition reserves in visceral protein stores. When the patients are suffering with CKD (chronic kidney disease) of any stage, these parameters are questionable for nutritional status because of the metabolic and fluid derangements associated with the uremic state. All serum protein levels are affected by hydration status. Of these three serum proteins, albumin is most often used to assess visceral stores, mostly reflecting the association between albumin and clinical outcomes.[20]

The estimation of levels of serum creatinine may be used to estimate Glomerular filtration rate (how quickly the kidneys filter blood). GFR may indicate kidney functioning, because of variability in serum creatinine from one person to another.[21] Serum urea is more powerful predictor of survival than eGFR (estimated Glomerular Filtration rate) in patients with HF (Heart failure). This may be due to urea's relation to key biological parameters including renal, hemodynamic, and neurohormonal parameters pertaining to the overall clinical status of the patient with chronic HF.[22] The elevated levels of serum urea and creatinine indicates that kidneys are not functioning efficiently (often referred to as Renal failure). Although urea is filtered into the urine by the kidney, some of the filtered urea will get reabsorbed and reused by the body.[21] The estimation of levels of serum Uric Acid is generally used as a diagnostic tool for screening purine metabolic disorders.[23] High concentrations of serum Uric Acid is indicated as a risk factor for causing CKD and also is recognized as cardio metabolic risk factor (CRF).[24] Serum proteins act as carriers of lipids, hormones, vitamins, and minerals in the circulatory system, and they are involved in the regulation of cellular activity and immune system.[25] Some blood proteins play important roles as enzymes, complement components, or protease inhibitors. Total serum protein (TSP) levels are measured to assess nutritional, liver, and kidney disorders.[26] High levels of protein in blood indicates chronic infection or inflammation that might lead to renal failure.[27]

Since renal failure can be triggered by some of the medications such as aminoglycoside antibiotics it is beneficial to select the herbal plants which are having nephroprotective activity along with anti-oxidant properties. The present study was carried out to screen Nephroprotective activity of Hydroalcoholic aerial plant extract of *Amaranthus roxburghianus* in Gentamicin-induced nephrotoxic rats. The study comprised of evaluation of serum CRE, URE, UAC and TSP, kidney weight, urine volume, urine PH and histopathology of kidney Gentamicin-induced nephrotoxic rats. Phytochemical investigation of plant extract was done using preliminary tests as per the standard methods mentioned in C.K. Kokate.

The Preliminary Phytochemical Analysis Of Hydroalcoholic Aerial Plant Extract Of *A.Roxburghianus* Nevski have revealed the presence of various phytochemicals such as Alkaloids, Flavonoids, Phenolic compounds, Tannins, Glycosides, Amino acids, Terpenoids, Steroids, Lipids, Saponins. Previous studies revealed that the presence of active constituents such as alkaloids, benzoquinones, catechols, carotenoids, flavonoids, glycosides, flavonol glycosides, steroid glycosides, glycoalkaloids, terpenoids, monoterpenoids, diterpenoids, triterpene saponins, sterols and polyphenols show nephroprotective activity and that have been proven in various experimental animal models.[28]

By the end of treatment period the levels of serum CRE, URE and UAC were significantly increased and TSP decreased in Gentamicin-induced nephrotoxic rats. The plant extract showed dose-dependent improvement in the levels of serum CRE, URE, UAC and TSP. The kidney weights were increased as a consequence of accumulation of Urine volume due to renal obstruction. The animal body weights were normalized with the treatment of extract which is further supported by histopathology and Statistical analysis of the results.

Nephroprotective activity of active constituents

Flavonoids

In several animal studies that were carried out to assess the safety of chemotherapeutic agents, Flavonoids were found to attenuate cisplatin induced renal damage in rats and mice by showing histopathological alterations and reducing the increased levels of serum creatinine and blood urea nitrogen. The mechanisms involve several pathways of the inflammatory cascade and oxidative perturbations including: the downregulation of activated NF-κB p65 protein expression and its downstream effectors (e.g., iNOS and TNF-α), with restoration of the anti-inflammatory IL-10; and reductions in phospho-NF-κB p65 and phospho-P38 MAPK activation and Nrf2 expression in cisplatin-induced renal injury. Flavonoids were also found to downregulate the expression of the apoptotic marker caspase-3, inhibiting cisplatin-induced apoptosis and thereby favouring renal cell survival.[29]

Alkaloids

Alkaloids are known to produce nephroprotective activity. Several studies revealed that alkaloids can be used as a healthy alternative for treating oxidative-stress related conditions and therefore can be used for treating kidney related disorders.[30] The presence of alkaloids has been reported to enhance the free radical scavenging mechanism, reducing lipid peroxidation and renal damage, thereby exhibiting nephroprotective activities.[31,32]

Phenolic Compounds

Most researchers suggested that phenolic compounds may be useful in reducing the cytotoxic effect on normal cells caused by chemotherapeutic agents.[33] These are the secondary metabolic phytoconstituents that participate in the protection against oxidation process, thereby possess anti-oxidant activity. Phenolic compounds show different therapeutic activities including antimicrobial, anti-inflammatory, reno protective and anti-cancer properties. Recent studies have reported to protect the kidney from oxidative damage caused by toxicity induced by anti-microbial agents, through the reinforcement of antioxidant defence of renal tissues.[34]

Saponins

The study carried out by K. Jung et al (2016)[35] investigated the nephroprotective activity of saponins against chemotherapy induced nephrotoxicity in LLC-

PK1 kidney cells. It was found that saponins have protection effect against cisplatin-induced nephrotoxicity by inhibiting MAPKs and exerting Reno protective actions. Studies also suggested that the decreased cell viability due to cisplatin-induced toxicity was recovered significantly after the treatment with ethanolic extract of saponins isolated from the seeds of gac (Momordica cochinchinensis Spreng.).[35]

Kaempferol Glycosides

The other chief constituent present in *Amaranthus roxburghianus* is Kaempferol glycosides which is a flavonolic glycoside. Kaempferol is a natural dietary flavonoid compound with many adaptive biological activities, including antioxidant and estrogenic activity. Investigation was carried out by Z. Wang et al. on effect of kaempferol on mechanisms related to nephrotoxicity in a cisplatin-induced Acute Kidney Injury (AKI) mouse model. It was found that pre-treatment with kaempferol has been observed to reduce kidney damage.[36]

Nephrotoxicity and Biomarkers

Gentamicin is an Aminoglycoside antibiotic used to treat gram-negative bacterial infections. However, its use is restricted due to its nephrotoxic effects on kidney. Various studies have documented that involvement of oxidative stress due to generation of reactive oxygen species (ROS) is one of the reasons for renal injury.[37] The generation of such ROS is also involved in the Gentamicin-induced renal tubular damage (tubular necrosis) and thereby causing acute renal failure.[38] Different mechanisms are involved in GEN-induced nephrotoxicity that causes cell injury and cell death such as DNA damage, lipid peroxidation, inhibition of cellular respiration and creation of adenosine triphosphate, electron transport chain inhibition and destabilization of the tubular cell membrane. The morphological changes that occur in diseased condition in kidney due to deposition of GEN in the renal cortex is very much alike to human beings and experimental animals. Therefore, GEN-induced nephrotoxicity experimental model was selected to study the molecular and pathological mechanism of kidney injury.

Herbal supplementation and natural medicine is gaining more focus in recent times, in a variety of diseased conditions due to their minimal side effects. Hence, the present study was aimed to evaluate the nephroprotective activity of HARE in GEN-induced nephrotoxic rats. The current study reveals that HARE can improve GEN induced kidney tubular inflammation, oxidative stress, histopathological changes and instabilities of kidney function.

Increase in the serum levels of CRE, URE, UAC and TSP are indications of GEN induced renal failure. CRE is a chemical compound that is made by the body to supply energy mainly to muscles. It is produced and left out in energy producing processes in muscles which is later excreted by the kidneys from the body through urine. Serum CRE levels indicate how efficiently the kidney is able to eliminate wastes from the body. Elevated serum CRE levels are observed when there is a significant reduction in the glomerular filtration rate or when urine elimination is obstructed. About 50% of kidney function must be lost before a rise in serum creatinine can be detected. Thus, serum CRE is a late marker of acute kidney injury.[39]

URE is generated in the liver during catabolism of amino acids and other nitrogenous metabolites and is normally excreted into the urine by the kidneys as rapidly as it is produced. Serum URE concentration reflects the balance between urea production in the liver and urea elimination by the kidneys, in urine. Elevated serum URE levels than normal levels may be a sign for improper functioning of kidneys. When renal function is impaired, increasing concentrations of blood URE will steadily accumulate. Often the people who are with early kidney disease may not have any symptoms. The blood URE test reveals about how well the kidneys are working.[40]

Uric acid is a waste product found in blood that is usually formed when the body breaks down chemicals called purines. Hence, UAC is considered as an important tool for screening most of the Purine metabolic disorders. Hyperuricemia accelerates renal progression via a mechanism linked to high systemic BP and COX-2-mediated, thromboxane-induced vascular disease. UAC may be a true mediator of renal disease and progression.[41] Uric acid crystals can form kidney stones in some people.

Serum proteins play an important role in transporting medicines and some other substances through blood and it is important for tissue growth and healing processes. Too much levels of protein or low little protein indicates conditions like liver or kidney disease, infection, inflammation, malnutrition and cancer. TSP test gives measures all the proteins in the blood and also A/G ratio (albumin to globulin ratio). Low TSP indicates that there is a problem in renal function and or it may be that protein isn't being digested or absorbed properly. A high total protein level could indicate dehydration or a certain type of cancer, such as multiple myeloma, that causes protein to accumulate abnormally.[42]

In our study, GEN treated animals showed abnormal serum levels of CRE, URE, UAC and TSP. The animals that are treated with GEN showed significant rise in the serum levels of CRE, URE and UAC, and low levels of TSP. The animals treated with the plant extract (GEN + HARE 200mg/kg and 400mg/kg showed dose dependant decrease in serum CRE, URE, and UAC levels and protein levels being normalized indicating nephroprotective activity.

In this study, the animals treated with GEN for seven days resulted in a significant decline in body weight and elevation in kidney weight when matched with normal

control rats. The decline in the body weight may be associated with renal tubular damage that may in turn result in the failure of renal tubules to reabsorb water. This might result in both dehydration and loss of body weight[43] or increased catabolism and reduced food intake.[44] The increase in kidney weights after the treatment of GEN might be an outcome of inflammation and oedema of tubular cells.[45] The reduction in the body weights and increase in kidney weights of animals were found to be normalized in the groups that were treated with GEN+HARE 200mg/kg & 400mg/kg by the end of the treatment period compared to GEN group.

The normal functioning of kidney was evaluated by measuring the serum biomarker enzymes and antioxidant stress markers as the brush border membrane and other cellular components (lysosomes and mitochondria) are the identified targets of GEN in nephrotoxicity.[46] In our present study, we have accessed the levels of MDA, GSH, and GR as a result of GEN induced oxidative stress. GEN prompted nephrotoxicity is linked with a diminished potential of various antioxidant enzymes (CAT) in the kidney cortex region. Moreover, these reduced levels of antioxidant enzymatic system can indicate the oxidative damage in rats.[47]

The main intracellular reducing agent is glutathione which is known to cause detoxification of lipid peroxides and it is associated with other antioxidants like NADPH, vitamin-C and vitamin-E, protecting against oxidative stress. GSH is a scavenger of hydroxyl radicals and singlet oxygen.[48] Elevated MDA levels indicate increased oxidative stress in GEN induced nephrotoxicity in the rat model.[49] In our present study, the levels of MDA and GR were found to be significantly increased whereas the levels of GSH and CAT were decreased in GEN induced nephrotoxic groups as compared to normal control rats. In animal groups treated with GEN + HARE 200mg/kg & 400mg/kg, the plant extract HARE showed nephroprotective activity by increasing the activity of GSH and CAT enzymes and decreasing the levels of MDA (lipid peroxidation marker) and GR, which ultimately resulted in decreasing the oxidative stress.

In GEN induced nephrotoxicity, histopathological analysis of animal kidneys would show various structural variations in renal tissue. In our current study, the histopathological analysis of kidney tissue showed severe renal tissue damages the in rats receiving GEN treatment along with renal cell necrosis, tubular dilatation and cystic tubular degeneration, tubular inflammation with infiltration of inflammatory cells, which are in accordance to the previously reported studies. The generation of extremely reactive free radicals due to oxidative stress might be the reason behind such a kind of damage caused with GEN treatment to rats. The normal histopathological structures of kidney were found to be restored in the animals treated with HARE 200mg/kg & 400mg/kg along with GEN as compared to GEN treated group. Whereas, mild cystic degeneration, mild tubular dilatation, foci with regeneration of tubules with infiltration of inflammatory cells were observed with normalization of kidney histological structure in rats treated with GM+HARE. These kinds of results support the nephroprotective activity of HARE and reported by many other studies performed previously in GEN induced nephrotoxic rat models using other kinds of drugs.[50]

In summary, our current study demonstrated the nephroprotective activity of HARE in GEN induced nephrotoxicity in wistar rats. The mechanism behind nephroprotective activity of HARE may be significant downregulation of serum biochemical parameters and kidney tissue homogenate oxidative stress markers. Our findings support that HARE showed nephroprotection in GEN-induced nephrotoxicity in rats. However, further studies needed to be carried out to determine the precise mechanism of action of HARE.

SUMMARY

The goal of the current investigation was to determine whether *Amaranthus roxburghianus* Nevski had any nephroprotective effects on Wistar rats whose kidneys had been damaged with Gentamicin. There are more than 800 million people worldwide who have chronic renal disease, or around 10% of the population. 37 million US adults, or 15% of the population, or more than 1 in 7 are thought to have CKD. Every year, millions of individuals pass away because they cannot afford care. The most popular kind of treatment for renal failure is dialysis, which artificially eliminates waste and extra fluid from the body when the kidneys are unable to do so. Kidney transplantation is an alternative to hemodialysis for those with end-stage renal failure. Advanced kidney disease can be treated most successfully with this approach, but it necessitates a large operation and lifelong immunosuppressant medication use to prevent the body from fighting the donor organ. People are choosing ayurvedic treatment for renal failure due to growing public awareness about alternative medicine. By healing damaged kidney tissues, it lessens the need for dialysis. All phases of renal failure can potentially be reversed with ayurvedic drugs. The old natural treatment has little, if any, negative side effects. Seventy-five to eighty percent of the world's population, particularly in underdeveloped nations, depend on herbal treatments. Due to its high acceptability, superior compatibility with the human body, and fewer side effects, it has greater access to primary healthcare. Focusing on the biological activities of plants over the past 10 years, it reveals the presence of many substances with potential nephroprotective effects. The study's goal was to determine whether *Amaranthus roxburghianus'* aerial portions had nephroprotective properties and to investigate potential mechanisms of action. The plant was purchased from a neighbourhood shop in Hyderabad, and hydroalcoholic extract was made using the hot Soxhlet extraction method. A preliminary

phytochemical examination was performed on them. The doses were chosen in accordance with earlier research in which the ideal doses were defined and the acute oral toxicity was assessed using OECD Guideline No. 423. The nephroprotective activity of *A.roxburghianus* was evaluated in GEN-induced nephrotoxic rats. Thirty male wistar rats were divided into 5 groups of 6 animals in each. Group 1 was treated with normal saline 10ml/kg p.o. Group 2 was treated with Gentamicin 100mg/kg i.p. Group 3 was treated with Vitamin-E 250mg/kg p.o along with Gentamicin 100mg/kg i.p. Group 4 was treated with HARE 200mg/kg p.o and Group 5 was treated with 400mg/kg p.o along with Gentamicin 100mg/kg i.p. This treatment schedule was carried out for a period of 7 days. At the end of the treatment i.e. on 8th day the animals were sacrificed and biochemical changes in creatinine, urea, uric acid and total protein were estimated in blood, apart from them physical parameters like change in the animal body weights, wet weight of kidneys were evaluated. Histopathological evaluation of kidney was done. The statistical analysis was done by means of ANOVA followed by Dunnett ‘s multiple comparison test using GraphPad prism 5.0 software. The plant extracts were quantified by estimation of Alkaloids, Flavonoids, Phenolic compounds, Tannins, Glycosides, Amino acids, Terpenoids, Steroids, Lipids, Saponins. In the treatment groups, the hydroalcoholic extract of plant had significantly reduced the increased levels of serum creatinine, urea, and uric acid levels and improved serum total protein levels. The change in body weights and kidney wet weights was normalized in the group that treated with plant extract. The plant extract also normalized the changes in the anti-oxidant parameters. Histological examination of the kidneys revealed that treatment with the extracts restored normal kidney structures in the animals treated with HARE 200mg/kg & 400mg/kg along with GEN as compared to GEN treated group. In contrast, rats treated with GM+HARE showed modest cystic degeneration, mild tubular dilatation, foci with tubule regeneration, and infiltration of inflammatory cells along with stabilisation of the kidney's histological structure. This study has showed that the aerial plant extract of *A.roxburghianus* possess nephroprotective activity.

CONCLUSION

The Hydroalcoholic Aerial Plant Extract of *A. Roxburghianus* Nevski (HARE) possess significant Nephroprotective activity. The present study indicated alkaloids, saponins, flavonoids, phenolic compounds and flavonol glycosides were responsible for Nephroprotective activity of the plant extract. Significant renal impairment caused by GEN100 mg/kg, i.p. was indicated by oxidative stress and histological damage in the renal tissue. HARE was found to produce protection against GEN induced renal toxicity by potent free radical scavenging activity. Thus, HARE is identified in the current investigation as a nephroprotective drug against kidney injury. This study demonstrated a dose-dependent effect of HARE on kidney GEN-induced renal damage, with 400 mg/kg p.o. of HARE being more effective than 200 mg/kg p.o.

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