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Evaluation of Anti-nephrotoxic potential of leaf extracts of *Tamilnadia ulginosa* against Gentamicin-induced Nephrotoxicity

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ABSTRACT

Abstract. Kidney being an essential organ performs various important functions in the body, one of which is the removal of waste products and controlling body's fluid balance. Nephrotoxicity is one of the most common complications of kidney resulting mainly due to the exposure to the toxins which may be drug. Several efforts have been made to reduce nephrotoxicity and natural remedies are gaining great attention as they have lesser side effects as compared to the synthetic drugs.

Purpose. The current study is aimed to investigate anti-nephrotoxic activity of leaf extract of *Tamilnadia ulginosa* in rats.

Research material. The study comprises the results of ethanolic and aqueous extracts of *T. Ulginosa* at two different doses i.e. 200 mg/kg and 400 mg/kg against gentamicin-induced nephro-toxicity in rats.

Results. The ethanolic and aqueous leave extracts showed the presence of alkaloids, flavanoids, tannins etc. Two different doses (200 mg/kg and 400 mg/kg) of each extract were evaluated for the activity. Both the extracts showed significant activity at selected doses while aqueous extract exhibited better activity as compared to other treatment. Thus the ethanolic and aqueous extracts of *T. ulginosa* can be considered as promising drugs in the management of drug induced nephrotoxicity.

Conclusion. The treatment with plant's leaf extracts showed significant efficacy and proves the traditional use of the plant which may be due to the active phytoconstituents present probably by reducing oxidative stress induced by gentamycin.

Keywords

Necrosis, nephrotoxicity, oxidative stress, inflammation, plant extracts, gentamicin, *Tamilnadia ulginosa* leaves

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Introduction

Acute renal failure is a major complication of kidney affecting whole world and people of every age group. The kidney is an essential organ of the body as it has to perform various important functions such as homeostasis maintenance, detoxification and excretion of toxic metabolites and drugs. Constant exposure to drugs or their active metabolites endanger the kidneys and leads to nephrotoxicity, which is most common renal problem. Drug-induced nephrotoxicity (DIN) is a major reason of acute renal injury (ARI). About 20% of hospital admissions and 8-60% of admitted patients due to ARI involve DIN and main cause of mortality and morbidity [1]. Some usual pathways have been suggested for DIN such as change in intra-glomerular hemodynamics, oxidative stress, inflammation, tubular cell toxicity and microangiopathy etc. Additionally, patient specific or drug related factors may also cause DIN in certain patients [2].

Gentamicin is an aminoglycoside antibiotic and was introduced in 1963. Despite its serious side effects such as nephrotoxicity and ototoxicity, it has been successfully used for a long period especially against gram-negative infections because of low cost and bactericidal activity. The exact mechanism of nephrotoxicity by gentamycin is still not understood completely. Etiological basis of getamycin nephrotoxicity relates to the fact that it is a strong cationic drug which accumulates on the biological membranes resulting in the increase in oxidative stress and lipid peroxidation leading to necrosis in renal tubules and subsequently precipitating acute nephrotoxicity [3].

Tamilnadia ulginosa (Family: Rubiaceae and commonly known as Divine jasmine) is found in dry deciduous forests, native to Bangladesh, India, Sri Lanka and Thailand. It is used extensively in various forms in Indian traditional medicines. The different parts of the plant are used for various ailments such as fruits in cholera, diarrhea, dysentery, eye complaints, headache, pimples, sores, as astringent; roots as diuretic, tonic, biliousness, diarrhea, aphrodisiac and in dysentery; roasted pulp as a remedy in diarrhea and dysentery, especially during pregnancy and pulp is also applied on boils; unripe fruit is employed as fish-poison and leaves are boiled and eaten. In recent past, a lot of researches done on *T. ulginosa* plant with respect to its phytochemistry and pharmacological activities, suggested that it possesses anti-anthelmintic activity [4], anti-inflammatory activity [5], anticancer activity [6], anti-diabetic activity [7] etc.

The aim of the present study was to detect the nephroprotective activity from natural resources and to support the traditional uses of *Tamilnadia uliginosa*.

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Materials and Methods

Collection and authentication of Plant Material

The leaves of *Tamilnadia ulginosa* were collected from surrounding areas of Gajwel, Medak district, Andhra Pradesh, India in the month of February. The leaves were authenticated by botanist Dr. Rasingam, Botanical Survey of India (BSI).

Extraction

Fresh leaves of *Tamilnadia ulginosa* were collected, cut into small pieces and dried under shade at room temperature for few days. The dried leaves were powdered and passed through sieve (coarse 60). This powder was used for the preparation of different extracts and 200g of the powder drug was extracted using petroleum ether, chloroform, ethanol and water.

Approximately 200 g of shade-dried powder of *T. ulginosa* leaves was extracted successively with 1200 ml (1:6) of the solvents in an increasing order of polarity viz. petroleum ether, chloroform and ethanol in a soxhlet apparatus for about 14 hours and aqueous extract was prepared by maceration process. Each extract was concentrated to a small volume and allowed to dry. After drying, the respective extracts were weighed and percentage yield of extracts were determined.

Preliminary Phytochemical Screening

The dried powder of leaves of *Tamilnadia ulginosa* was subjected to successive extraction with various organic solvents of increasing polarity and the extracts were then subjected to phytochemical investigation by qualitative chemical identification tests for alkaloids, carbohydrates, glycosides, saponins, flavanoids and steroids/triterpenoids [8].

Animal Selection

Albino rats weighing 150-250 g were procured from Shri Venkateswara Enterprises, Bengaluru for experimental purpose and the animals were acclimatized for 8 days under standard husbandry condition (12:12 h day-night cycle; temperature 25 ± 2^0 C; 45-55% relative humidity) with open access to food and water *ad libitum*. The Institutional Animal Ethical Committe (IAE/SKIPS/2013/JUL20/02/RATS72/MICE24), Sri Krupa Institutre of Pharmaceutical Sciences, approved the experimental protocol as per the guidelines of CPCSEA.

Acute Oral Toxicity

The acute toxicity of leaf extracts of *Tamilnadia ulginosa* was determined in albino rats of either sex weighing between 150-250 g maintained under standard husbandry conditions. The

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animals were fasted 3 h prior to the experiment and "up and down" (OECD Guideline No. 420) were adopted for toxicity studies. The single dose (2000 mg/kg, p.o.) of extracts was administered and observed for its mortality during 48 h study period (short term) toxicity. All the animals were observed for long term toxicity (14 days) [9].

Nephroprotective activity in gentamycin-induced nephrotoxicity in rats

The evaluation of the ethanolic and aqueous extracts for nephroprotective activity was done by gentamycin-induced nephrotoxicity model in rats according to the procedure given in the literature with minor modifications.

Total 36 ratss were taken and randomly divided into 6 groups each containing 6 rats as follows -

Group 1 (Normal control): received distilled water, 1 ml/kg, p.o.

Group 2 (Toxic control): received pretreatment with distilled water for 14 days prior to a single dose of gentamicin (80 mg/kg body weight; i.p. once daily) served as Gentamicin control group

Group 3 (EETU200): were administered with Gentamicin + Ethanolic leaf extract of *T. ulginosa* (200 mg/kg body weight: p.o.) daily for 14 days

Group 4 (EETU400): were administered with Gentamicin + Ethanolic leaf extract of *T. ulginosa* (400 mg/kg body weight: p.o.) daily for 14 days

Group 5 (AETU200): were administered with Gentamicin + Aqueous leaf extract of *T. ulginosa* (200 mg/kg body weight: p.o.) daily for 14 days

Group 6 (AETU400): were administered with Gentamicin + Aqueous leaf extract of *T. ulginosa* (400 mg/kg body weight: p.o.) daily for 14 days

After 48 h, the animals were sacrificed by chloroform anaesthesia. Blood samples were collected by cardiac puncher under diethyl ether anesthesia, using 21 gauge needles mounted on a 5ml syringe into ethylene diamine tetra-acetic acid (EDTA) and analyzed for various parameters [10].

Assessment of physical and biological parameters

Renal parameters such as serum ureic acid and serum creatinine and change in body weight were considered in the study [9]. Serum uric acid is the end product of purine catabolism. So, any defect in the glomerular filtration rate causes the rise in the level of uric acid in the blood. The raise after gentamicin can be attributed to the GFR impairment. The decrease in the elevated uric acid

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by any substance may be due to the antagonism of gentamicin induced disturbance in the glomerulus.

Creatinine clearance gives the ideal about glomerular filtration rate. Administration of gentamicin leads to significant elevation of serum creatinine level indicating injury to the glomerular apparatus. The reversal of the elevation by any substance may be indicative of the reversal of the GFR impairment.

Histological Study

For histological studies, a small transverse piece (3 mm^3 approximately) of tissue from each kidney was dissected out surgically with precision so as to ensure minimal damage to the other part. Kidney tissue samples were fixed by using a formosal solution (10% v/v formaldehyde in normal saline), embedded with paraffin wax followed by preparation of tissue sections using a microtome and stained with Haemotoxylline‑Eosin [11]. Tissue sections were examined (100X) under a polarized‑light‑microscope and photomicrographs were taken using a digital camera.

Data Analysis

The data obtained from the study are presented as mean ± SEM and the level of significance is considered at $p<0.05$. Statistical significance was determined by one-way ANOVA followed by Dunnet's t-test. Gentamycin toxic group was compared with the normal group and the extract treatment groups (EETU200, EETU400, AETU200 and AETU400) were compared with the toxic group.

Results

Percentage Yield of various extracts

The percentage yield and color of various extracts are shown below in Table 1.

Table 1: % Yield of different extracts

S. No.	Solvent	Color of the extract	Percentage Yield
1	Petroleum ether	Deep green	1.44%
2	Chloroform	Deep green	2.5%
3	Ethanol	Dark brown	18%
4	Aqueous	Light orange	25%

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Preliminary phytochemical screening

The preliminary phytochemical analysis of petroleum ether, chloroform, ethanolic and aqueous leaf extracts of *Tamilnadia ulginosa* revealed the presence of various phytoconstituents which are presented in Table 2.

The petroleum ether and chloroform extract showed the presence of fixed oils, gums and mucilages.

The ethanolic extract gave positive results for alkaloids, glycosides, carbohydrates, flavonoids, saponins, steroids and tannins.

The aqueous extract showed the presence of flavonoids, saponins, steroids and tannins.

Table 2: Phytochemical evaluation of different extracts of *Tamilnadia ulginosa* leaves

S. No.	Phytoconstituents	Petroleum ether	Chloroform	Ethanol	Water
1	Alkaloids	- ve	- ve	**+ ve**	+ve
2	Carbohydrates	- ve	**+ ve**	**+ ve**	**+ ve**
3	Flavonoids	- ve	- ve	**+ ve**	**+ ve**
4	Saponins	- ve	- ve	**+ ve**	**+ ve**
5	Steriods	- ve	- ve	**+ ve**	- ve
6	Tannins	**+ ve**	**+ ve**	**+ ve**	**+ ve**
7	Glycosides	- ve	- ve	**+ ve**	- ve

-ve indicates the absence of phytoconstituent and +ve indicates the presence of phytoconstituent

Pharmacological activities

Acute oral toxicity study

The mice treated with ethanolic and aqueous extracts at a dose of 2000 mg/kg, p.o. exhibited normal behaviour, without any signs of passivity, stereotypy and vocalization. Their motor activity and secretory signs were also normal and no sign of depression. EETU and AETU even up to the dose level of 2000 mg/kg body weight did not produce any behavioral symptoms or mortality. So $1/10^{th}$ and $1/5^{th}$ doses of (maximum dose tested for each extract) were selected as medium and high doses and were used in the present study to explore the nephroprotective activity.

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Nephroprotective activity by using gentamicin-induced nephrotoxicity in albino rat model

The results obtained after administration of ethanolic and aqueous leaves extracts of *Tamilnadia ulginosa* in two different doses i.e. 200 mg/kg and 400 mg/kg has shown in Table 3 and Table 4.

Body weight

Gentamicin administered rats (toxic control group) showed significant weight loss in comparison to that of the normal control group. All extract treatment groups showed significant recovery in the loss of body weight when compared to that of the toxic control group. However, aqueous leaf extract (AETU400) at 400 mg/kg dose have most significant effect and ethanolic leaf extract (EETU200) have least significant effect on the recovery of body weight shown in Table 3 and Figure 1.

Table 3: Percentage change in body weight of rats in gentamicin-induced nephrotoxicity model

Groups	Dose (mg)	Percentage change in body weight
Group 1: Normal control	Vehicle	3.26 ± 0.24
Group 2: Toxic control	Gentamicin (60 mg/kg, p.o.)	9.39 ± 0.36***
Group 3: EETU200	200 mg/kg	7.52 ± 0.61[a]
Group 4: EETU400	400 mg/kg	5.92 ± 0.36[b]
Group 5: AETU200	200 mg/kg	5.54 ± 0.57[b]
Group 6: AETU400	400 mg/kg	3.95 ± 0.49[c]

Values expressed in mean ± SEM, where n = 6; Significant at *=P<0.05, **=P<0.01 and ***=P<0.001 when toxic group compared to control group. Level of significance [a]=P<0.05, [b]=P<0.01 and [c]=P<0.001 when treatment groups (EETU200, EETU400, AETU200 and AETU400) were compared with toxic group.

Figure 1: Percentage change in body weight of animals of different groups in Gentamycin-induced Nephrotoxicity model

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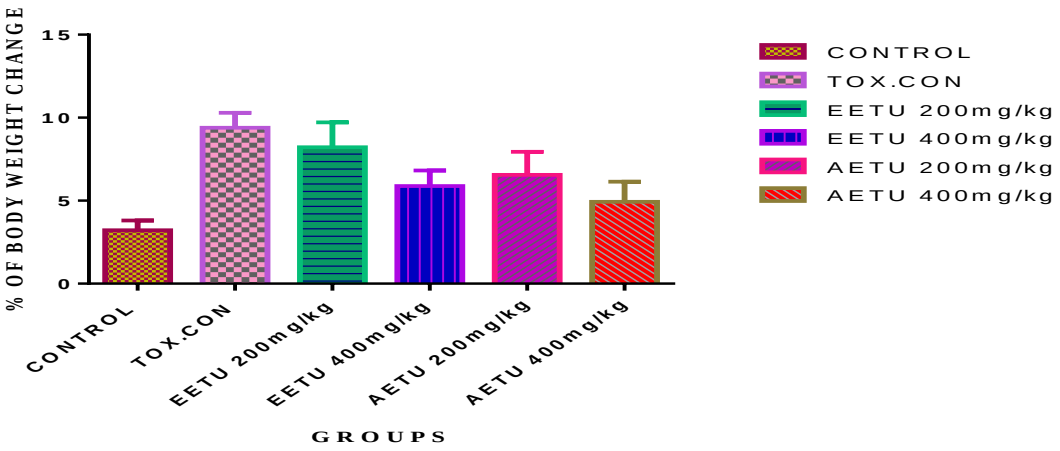

Biochemical Parameters

Toxic control group i.e. gentamycin treatment group results showed significant increase ($P<0.001$) in both blood urea and serum creatinine level in comparison to that of the normal control group. All the extracts treatment groups i.e. EETU200, EETU400, AETU200 and AETU400 results showed significant decrease in the blood urea level when compared to that of the toxic control group. However, aqueous extract (AETU400) at 400 mg/kg dose showed most significant effect and ethanolic extract (EETU200) at 200 mg/kg dose showed less significant results shown in Table 4 and Figure 2.

Table 4: Change in biochemical parameters of albino rats in gentamycin-induced nephrotoxicity model

Groups	Blood urea	Serum creatinine
Normal control	32.88 ± 2.803	1.068 ± 0.060
Toxic control	72.67 ± 1.942***	2.118 ± 0.083***
EETU (200mg/kg)	57.4695 ± 4.674[a]	1.965 ± 0.071
EETU (400mg/kg)	47.175 ± 4.668[b]	1.49 ± 0.115[b]
AETU (200mg/kg)	51.776 ± 1.816[b]	1.735 ± 0.066[a]
AETU (400mg/kg)	39.866 ± 2.656[C]	1.238± 0.056[c]

Values expressed in mean ± SEM, where n = 6; Significant at *=P<0.05, **=P<0.01 and ***=P<0.001 when toxic group compared to control group. Level of significance [a]=P<0.05, [b]=P<0.01 and [c]=P<0.001 when treatment groups (EETU200, EETU400, AETU200 and AETU400) were compared with toxic group.

Fig 2: Blood urea level in different groups in gentamycin-induced hepatotoxicity model

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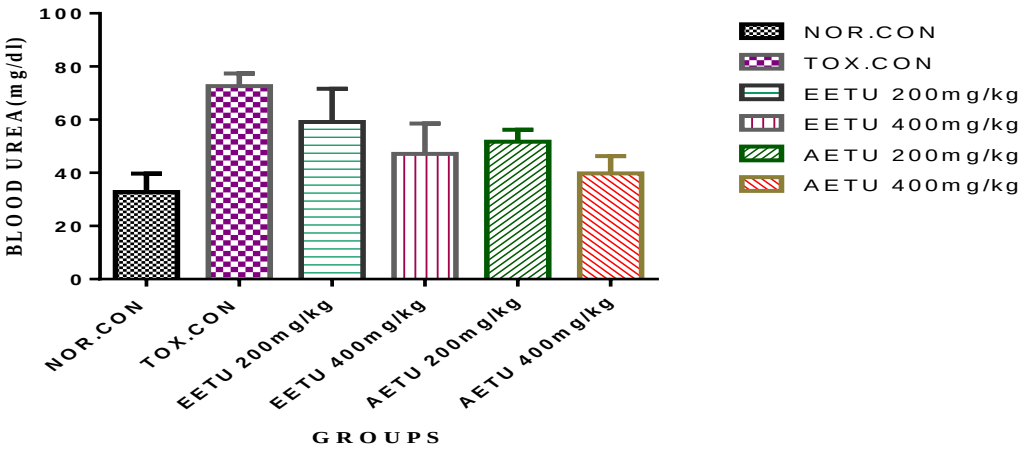
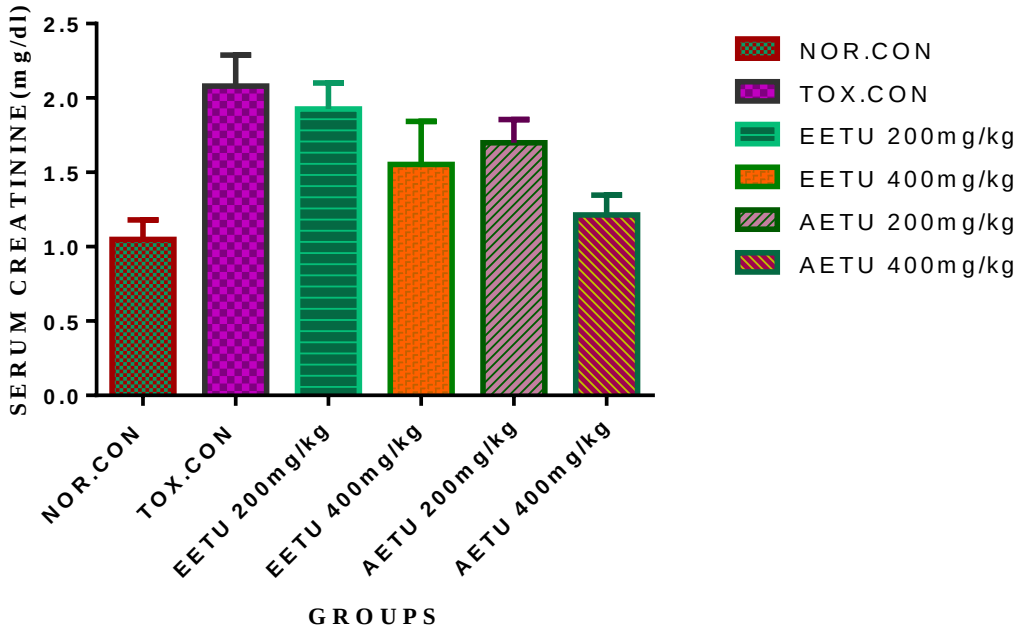


Toxic control group results showed significant (P<0.001) increase in the serum creatinine level in comparison to that of the normal control group. All extract treatment groups except ethanolic extract at 200 mg/kg dose, showed significant effects when compared to that of the toxic control group. However, aqueous extract (AETU400) at 400 mg/kg dose showed the most significant (P<0.001) effect on serum creatinine level shown in Table 4 and Figure 3.

Fig 3: Serum creatinine level in different groups in gentamycin-induced nephrotoxicity model



Histological Study

Photomicrographs of the tissue sections are illustrated in Figure 4. Histological features observed from the toxic control group suggests that gentamicin administered rats had experienced

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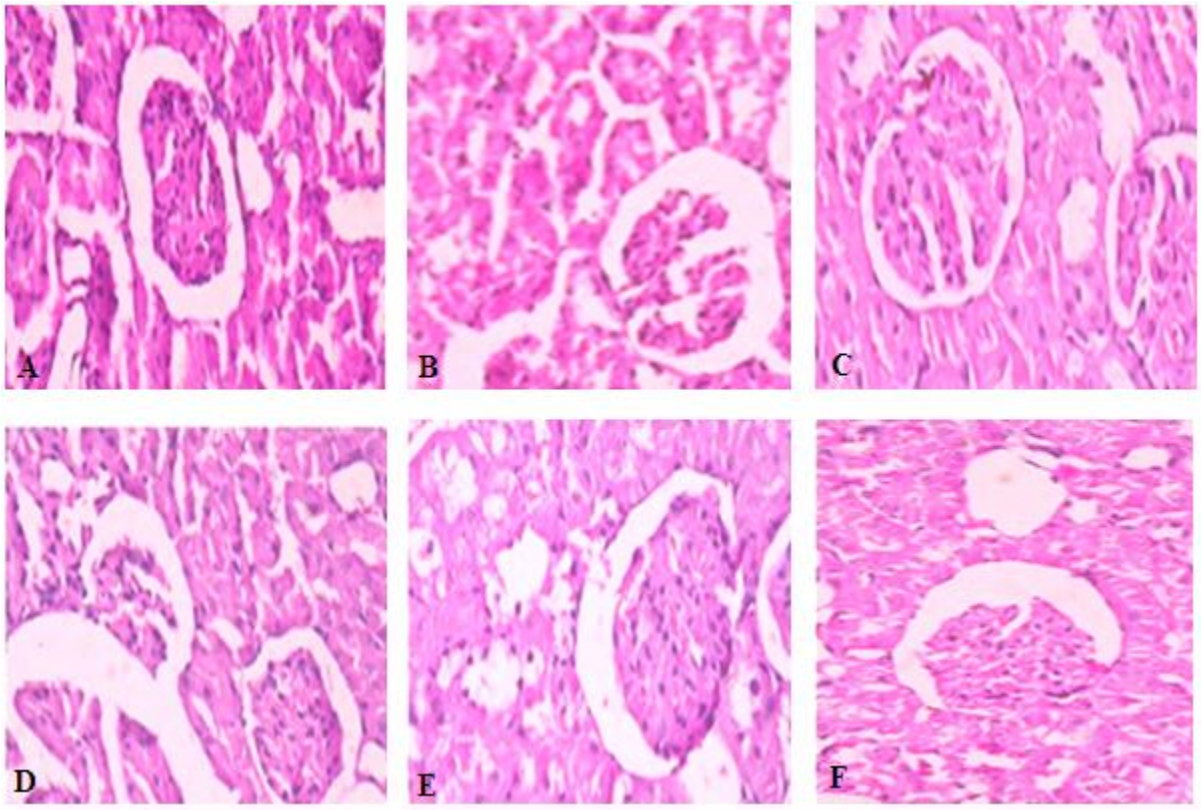
massive histological damage. In contrast to the T.S. of kidney of toxic control rats, all treatment groups except EETU200 showed minimal cellular damages. However, aqueous extract treatment group AETU400 showed most significant reduction in cellular damage.

Figure 4: Photomicrographs of renal tissue sections



(A) Normal control group received distilled water (1 ml/Kg); **(B)** Toxic control group received getamicin (80 mg/Kg); **(C)** EETU200 group received ethanolic leave extract (200 mg/Kg); **(D)** EETU400 group received ethanolic leave extract (400 mg/Kg); **(E)** AETU200 group received aqueous leave extract (200 mg/Kg); **(F)** AETU400 group received aqueous leave extract (400 mg/Kg)

Discussion

Gentamicin is being the most nephro-toxic aminoglycoside antibiotic, its over dosing eventually leads to acute nephrotoxicity and consequently precipitating acute renal failure in human as well as in animals. Biotransformation of the drug leads to the formation of reactive metabolites which act as free radicals and meads to cellular toxicity. The superoxide ion normally formed during oxidation forms hydroxyl radicals which lead to lipid peroxidation. In turn, it causes oxidative deterioration of polyunsaturated lipids of membranes and causes significant modification

in structure and functions. The toxic agent decreases the level of antioxidants, superoxide dismutase, glutathione, catalase etc which protects the tissues by reacting and removing the reactive oxygen species. In this present study, results showed acute renal damage in the toxic group rats after gentamycin administration resulting in reduction in body weight, increase in blood urea and serum creatinine levels in comparison to the normal group animals. Increased levels of serum urea and creatinine in gentamycin intoxicated rats signifies depressed glomerular filtration as well as reduced catalase, glutathione and superoxide dismutase activity resulting in decreased renal antioxidant defence against gentaminin induced oxidative stress causing kidney damage of test animals [12].

Results of renal function tests (body weight, blood urea and serum creatinine) and histological study suggested that the ethanolic and aqueous leave extracts treated rats have shown significant protection against gentamicin induced acute nephropathy.

Phytochemical investigation revealed the presence of alkaloids, flavanoids, saponins, tannins and glycosides in both ethanolic and aqueous extracts of *Tamilnadia ulginosa*. These phyto constituents are already well known for their antioxidant efficacy through various mechanisms. *T. ulginosa* leave extracts also has already been reported for its anti-oxidant property [13].

Thus, we presume that the active phytoconstituents present in leave extracts of *T. ulginosa* might have contributed towards the nephroprotective activities which may be through their antioxidant potential.

Conclusion

The present research work was aimed to investigate the anti-nephrotoxic activity of ethanolic and aqueous leave extracts of *Tamilnadia ulginosa*. The results of the study suggested the nephroprotective activity of *T. ulginosa* leave extracts against gentamycin-induced acute nephrotoxicity in Wistar rats. This may be due to its phytoconstituents, which have antioxidant and anti-inflammatory activity already reported and gave *T. ulginosa* leave extracts a great therapeutic importance.

Limitations and Future Studies

Nature is a rich source of new molecular target for various diseases. Although the use of natural compounds decreased in past few decades which may be due to technical barriers in tracking phytoconstituents against specific target; their complex structure make them difficult to

synthesize or may be costly or compositional variation of natural products. As well as, many potential herbal extracts found less or no efficacy either due to poor solubility profile or improper molecular size or both, causing less bioavailable. Also till the date, plant extracts have low clinical data available for their efficacy.

Phytoconstituents like tannins, flavonoids and alkaloidal compounds are well known for their antioxidant and nephroprotective property, and both the extracts contained the above mentioned constituents. Hence, these can be accounted for the observed activities. However, the exact mechanism responsible for activities is currently unclear. Therefore, further investigations need to be carried out to isolate and identify specific compounds present in the plant extract responsible for these activities and the exact mechanism of their action.

Acknowledgement

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