Int. J. Pharm. Investigation, 2022; 12(3) : 346-350

Original Article

Nephroprotective Activity of Ethanolic Extract of *Alternanthera sessilis* Leaves in Gentamicin-induced Nephrotoxicity in Wistar Albino Rats

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ABSTRACT

Background: Renal disease is a significant issue of global proportions and renal damage is very prevalent as the kidney has the ability to excrete toxic substances to assess the protective impact of the Ethanolic extract of *Alternanthera sessilis* Linn. (EEAS) plant leaves against gentamicin induced nephrotoxicity in rats. **Materials and Methods:** Nephroprotective activity was assessed by inflicting gentamicin (80 mg/kg) in all groups; acute kidney dysfunction is proven by the vital increment of blood serum creatinine, uric acid, BUN and decline in the total protein, albumin, and globulin with various histological damages. **Results:** Treatment with the *Alternanthera sessilis* has appeared critical dosage subordinate change at the measurements of 100 and 200mg/kg by securing the kidney from oxidative stretch. It is additionally recognized that treatment with *Alternanthera sessilis* essentially brought down the level of serum creatinine, uric acid, BUN and increment within the add up to protein, egg whites, globulin when compared to illness gather. Nephroprotective action of EEAS was found when compared with the standard bunch (Vitamin E 250 mg/kg) and control bunch against the infection gather creatures in parameters counting creatinine, urea, uric corrosive, Blood Urea Nitrogen (BUN), add up to protein, egg whites and globulin. The histopathological considers were moreover proving the defensive impact of EEAS. **Conclusion:** From the above results it was concluded that the ethanolic extract of *Alternanthera sessilis* offers nephroprotection.

Keywords: Nephroprotective activity, Gentamicin, *Alternanthera sessilis*, Male wistar albino rats, Serum Creatinine.

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DOI: 10.5530/ijpi.2022.3.58

INTRODUCTION

Nephrotoxicity is the foremost predominant kidney issue that happens when the body is subjected to a sedate of harmful substances.[1] Nephrotoxicity is practically showed by diminished pee concentration, tubular proteinuria, lysosomal protein urea and mid-glomerulus filtration rate, Creatinine clearance and expanded serum blood urea nitrogen (BUN) and serum creatinine levels with morphological changes within the kidney tissue. Numerous restorative specialists, chemicals and overwhelming metals can have an antagonistic impact on the renal working, driving to intense renal disappointment, constant interstitial nephritis and nephritis, which can forever hurt the renal system.[2] The renal brokenness, on the other side, also straightforwardly influences cardiovascular work. Gentamicin may be a classically utilized amino-glycoside anti-microbial for severe infections. Gentamicin nephrotoxicity was considered a tubulopathy where tubular harm and tubular brokenness are the most cause of renal inadequate. This could clarify a few clinical discoveries such as proteinuri, enzymuria and electrolytic changes.[3]

Alternanthera sessilis Linn has a place to the family Amaranthaceae and has been detailed to contain Lupeol, α and β-spinosterol, β-sitosterol, stigmasterol and campesterol.[4] Hexan, ethyl acetic acid derivation, ethanol and watery extricates of clears out of *Alternanthera sessilis* detailed the nearness of alkaloids, glycosides, steroids and terpenoids, tannins, flavonoids, saponins, polyphenols, coumarins, carbohydrates.[5] Customarily *Alternanthera sessilis* could be a verdant vegetable which is utilized as a conventional pharmaceutical in India, China, Taiwan and Sri Lanka. The herb was extricated within the oil and utilized to treat the irresistible wounds. The nutritive esteem of the herb makes it a strong tonic which is utilized as a wide run of applications. The new, damp mass of the *Alternanthera sessilis* is utilized to remedy sprains, skin inflammation, burns and conjunctivitis by applying it on to the influenced range of the body. In India it is utilized for obscured or vague vision and night blindness.[6] Detailed with respect to Nephroprotective impact of *Alternanthera sessilis*. Subsequently, this considers was planned to assess the Nephroprotective impact of ethanolic extricate of *Alternanthera sessilis* Gentamicin actuated nephrotoxicity in rats.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The plant leaves of *Alternanthera sessilis* have been used for the present study. It was collected from the nearby market of Hyderabad, Telangana. The plant was identified, confirmed and authenticated by the Scientist 'F' and HOD Mr. P.V. Prasanna**,** Ministry Of Environment, forest and Climate Change/Botanical survey of India (BSI) Ranga reddy district, Hyderabad, Telangana.

Preparation of Ethanolic extract of *Alternanthera sessilis* (EEAS)

The Leaves of *Alternanthera sessilis* were collected thoroughly washed and shade dried. The dried leaves were made into coarse powder with

the help of mortar and pestle. About 300g of coarse powder of plant material was weighed and subjected to extraction using Ethanol (99%) as the solvent by cold maceration process for a week with an occasional stirring. The plant material soaked in the solvent was separated with the help of muslin cloth, filtered with the help of funnel and filter paper and the solvent was kept undisturbed in a beaker for evaporation at room temperature to obtain a mass of extract. This was transferred into a petri plate and was stored into a desiccator until its use. The extract obtained was dark black in color and was suspended in distilled water using carboxy methyl cellulose as a suspending agent for oral administration to experimental animals.[2]

Experimental Animals

Institutional Animal Ethical Committee (IAEC) has given its approval to the experimental protocol with ethical clearance No: CPCSEA/1657/IAEC/CMRCP/COL-18/66. Adult, healthy, male Albino-Wistar rats with average weight of 150-200gms were used for this study. Animals have been provided with 24-hr access with water and standard nutritional pellets, prior to and during the treatment. They were acclimatized under a time period of one week under approved laboratory environment, i.e., 25°C±1°C temperature, 45-55% RH and also free access to food and water, after which they have been employed in the experiment.

Treatment Protocol

After 1 week of adaptation period. The experimental animals were divided into five groups, of six animals each as described below:

Group 1: Control group was treated with normal saline (2ml/kg, *p.o*).

Group 2: Diseased group was treated with Gentamicin (80 mg/kg/bodyweight, *i.p.*), daily for 8days.

Group 3: Treatment group 1-(low dose) Ethanolic extract of *Alternanthera sessilis* (100mg/kg body weight, *p.o*) for 28 days and simultaneously Gentamicin (80mg/kg body weight, *i.p)* for last 8 days.

Group 4: Treatment group 2-(high dose) Ethanolic extract of *Alternanthera sessilis*(200mg/kg body weight, *p.o*) for 28 days and simultaneously Gentamicin (80mg/kg body weight, *i.p*) for last 8 days.

Group 5: Standard group was treated with Vitamin E-(200mg/kg body weight, *p.o*) for 28 day, simultaneously Gentamicin (80mg/kg body weight, *i.p*) for last 8 days.

A day before the end of the experimental period blood samples will be collected by retro orbital route and all the animals will be sacrificed. The blood samples will be subjected to the centrifuge at a high medium to separate the serum from blood for about 10mins. The serum assigned will be collected carefully with the help of micropipette and collected into the ependroff tubes and analyzed for different biochemical parameters.

Assessment of Kidney Functions

Estimation of biochemical parameters i.e., blood serum parameters (Creatinine, uric acid, urea nitrogen, protein, albumin, globulin) were evaluated according to the methods. The kidney were isolated, cleaned, dried with the help of tissue, weighed and morphological changes were observed and than a portion of kidney was stored into the 10% formalin for histopathological studies.[7-10]

In vivo Antioxidant Studies

The isolated kidneys were homogenated using Ice cold Kcl in tissue homogenizer at 2,000 rpm for 10 min and used for analyzing antioxidant activities like Lipid peroxides (LPO).[11] Catalase (CAT)[12] and Glutathione reductase activity (GSH).[13]

Statistical Analysis of Data

The values were expressed as Mean ± S.E.M, *n*=6 in each group. The statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's test.[14]

RESULTS

Phytochemical Investigation

The preliminary phytochemical screening showed the presence of various phyto-constituents such as flavonoids, phenolic compounds, triterpenoids, tannins, and saponins.

Kidney Weights

The significant values of kidney weights of normal, disease, standard, EEAS100mg/Kg and EEAS 200mg/Kg were found to 1.477±0.1379, 2.6241±0.1369, 1.730±0.05826, 2.246±0.0558 and 2.239±0.0762 respectively on Day 29. There was a significant decrease in kidney weights of animals treated with EEAS 100mg/Kg and 200mg/Kg when compared to disease and normal control. Results were represented in Figure 1.

Serum Creatinine Levels

The significant values of serum creatinine levels of normal, disease, standard, EEAS 100mg/Kg and EEAS 200mg/Kg were found to be 0.668±0.043, 1.515±0.055, 0.685±0.044, 0.655±0.047 and 0.697±0.046 respectively on Day 29. There was a significant decrease in serum creatinine of animals treated with EEAS 100mg/Kg and 200mg/Kg when compared to disease control. Results were represented in Figure 2.

Serum Uric Acid Level

The significant values of serum uric acid levels of normal, disease, standard, EEAS 100mg/Kg and EEAS 200mg/Kg were found to be 3.038±0.155, 5.610±0.147, 3.473±0.171, 3.507±0.131 and 3.672±0.166 respectively on Day 29. There was a significant decrease in Serum uric acid of animals treated with EEAS 100mg/Kg and 200mg/Kg when compared to disease control. Results were showed in Figure 3.

Serum BUN Levels

The significant values of serum BUN levels of normal, disease, standard, EEAS 100mg/Kg and EEAS 200mg/Kg were found to be 16.13±0.706, 61.65±1.801, 24.48±1.243, 28.04±1.764 and 32.96±1.225 respectively on Day 29. There was a significant decrease in serum BUN of animals

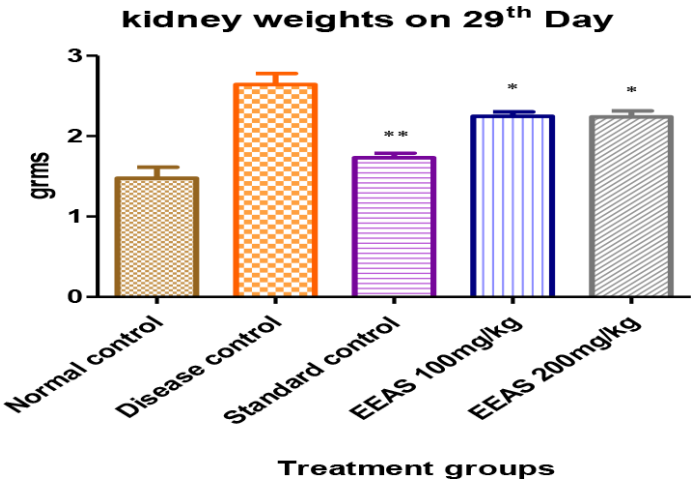

Figure 1: Kidney weight's on 29th day.

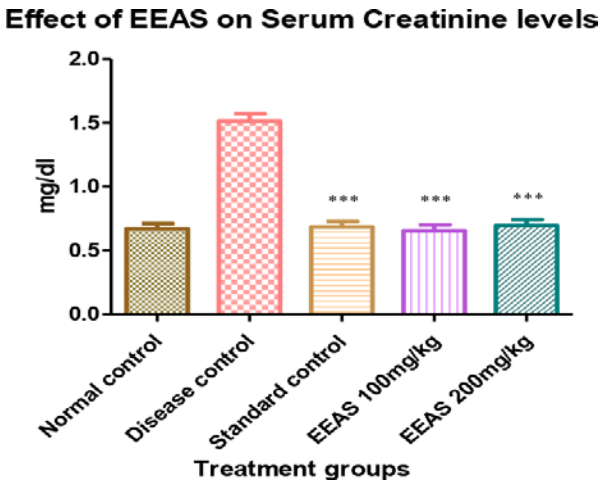

Figure 2: Effects of EEAS on Serum Creatinine.

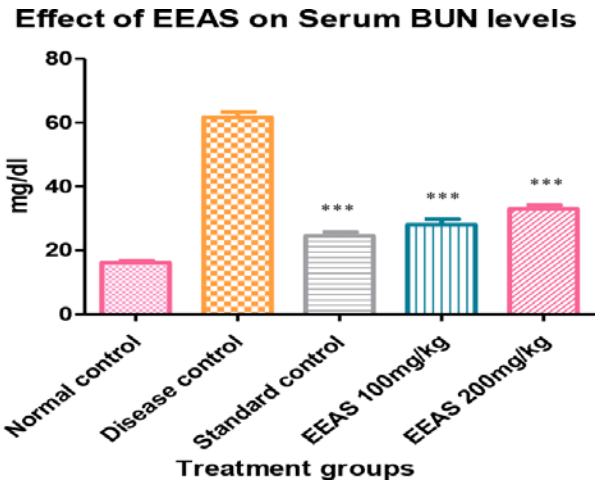

Figure 4: Effect of EEAS on BUN levels.

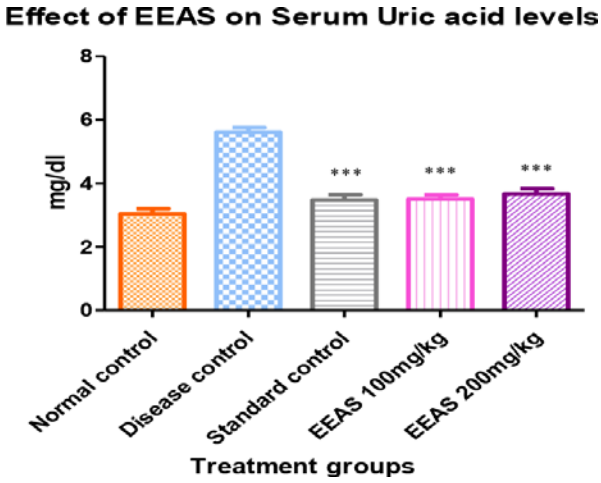

Figure 3: Effect of EEAS on Serum uric acid levels.

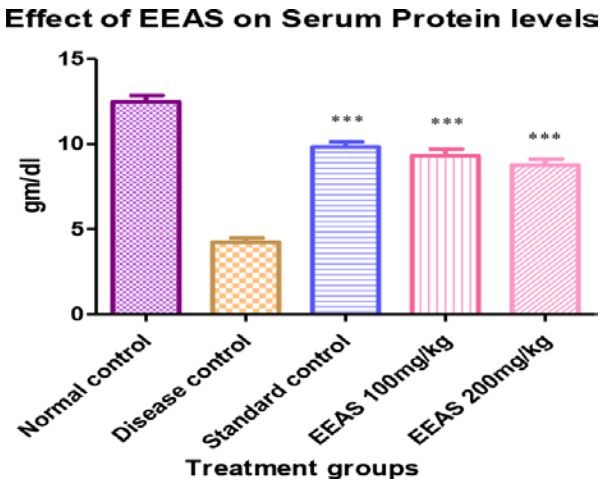

Figure 5: Effect of EEAS on Serum Protein levels.

treated with EEAS 100mg/Kg and 200mg/Kg when compared to disease control. Results were showed in Figure 4.

Serum Protein Levels

The significant values of serum protein levels of normal, disease, standard, EEAS 100mg/Kg and EEAS 200mg/Kg were found to be 12.462±0.387, 4.235±0.254, 9.833±0.289, 9.310±0.383 and 8.765±0.350 respectively on Day 29. There was a significant decrease in serum protein of animals treated with EEAS 100mg/Kg and 200mg/Kg when compared to disease control. Results were showed in Figure 5.

Serum Albumin Levels

The significant values of serum albumin levels of normal, disease, standard, EEAS 100mg/Kg and EEAS 200mg/Kg were found to be 8.255±0.236, 2.868±0.182, 5.978± 0.275, 5.558±0.255 and 5.065±0.257 respectively on Day 29. There was a significant decrease in serum albumin of animals treated with EEAS 100mg/Kg and 200mg/Kg when compared to disease control. Results were showed in Figure 6.

Serum Globulin Levels

The significant values of serum Globulin levels of normal, disease, standard, EEAS 100mg/Kg and EEAS 200mg/Kg were found to be 4.210±0.097, 1.372±0.153, 3.863±0.085, 3.843±0.134 and 3.710±0.097 respectively on Day 29. There was a significant decrease in serum globulin of animals treated with EEAS 100mg/Kg and 200mg/Kg when compared to disease control. Results were showed in Figure 7.

In vivo Antioxidant Studies

The significant values of lipid per oxidation of normal, disease, standard, EEAS 100mg/Kg and EEAS 200mg/Kg were found to be 2.848±0.130, 5.036±0.116, 2.945±0.13, 1.784±0.131, 2.639±0.131 respectively on Day 29. There is a significant decrease in LPO of animals treated with EEAS 100mg/Kg and 200mg/Kg when compared to disease control (Figure 8).

The significant values of glutathione of normal, disease, standard, EEAS 100mg/Kg and EEAS 200mg/Kg were found to be 4.178±0.135, 3.082±0.141, 1.999±0.138, 1.820±0.103, 1.899±0.147 respectively on Day 29. There is a significant increase in gluthathione of animals treated with EEAS 100mg/Kg and 200mg/Kg when compared to disease control (Figure 9).

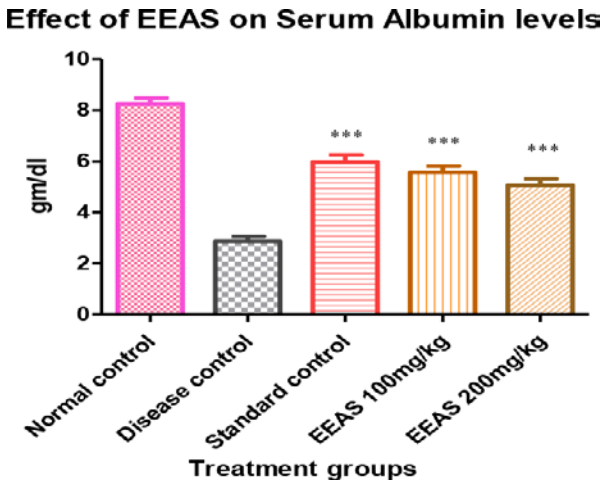

Figure 6: Effect of EEAS on Serum Albumin levels.

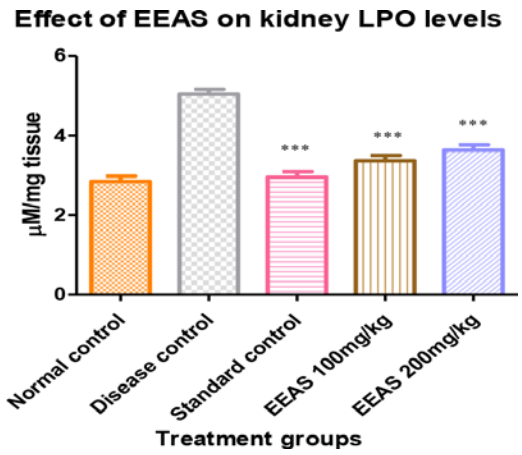

Figure 8: Effects of EEAS on LPO.

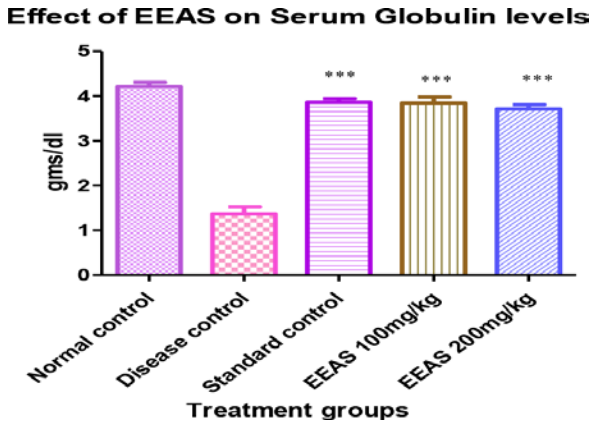

Figure 7: Effect of EEAS on Serum Globulin levels.

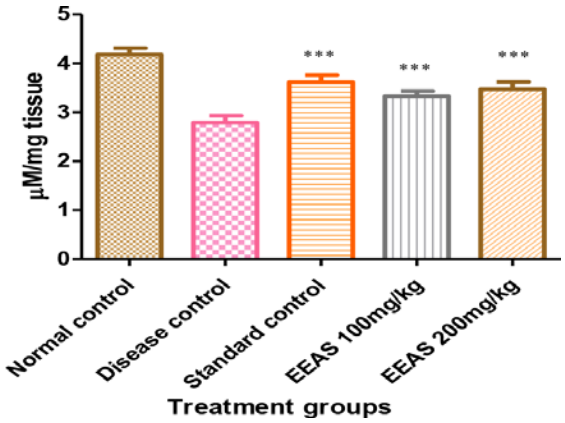

Figure 9: Effects of EEAS on GSH.

The significant values of catalase of normal, disease, standard, EEAS 100mg/Kg and EEAS 200mg/Kg were found to be 0.720±0.026, 0.501±0.029, 0.690±0.022, 0.693±0.026, 0.683±0.029 respectively on Day 29. There is a significant increase in catalase of animals treated with EEAS 100mg/Kg and 200mg/Kg when compared to disease control (Figure 10).

Histopathological Studies

The normal group(a) showed a normal glomerulus and tubules while the kidney sections of disease control group(b) showed acute tubular necrosis, interstitial oedema, peri-tubular inflammation and interstitial haemorrhage. The kidney sections of standard control group(c), EEAS 100mg/kg (d) and 200 mg/kg (e) showed normal glomerulus, mild interstitial congestion, peri-tubular inflammation and interstitial haemorrhage. The histopathological changes in groups pre-treated with EEAS showed protective effects when compared to group treated with that of the gentamicin alone (Figure 11).

DISCUSSION

There are two fundamental variables for kidney harm actuated by Gentamicin (GM), its amassing in proximal tubular cells and interaction with cell films and organelles. The cells of brush border are uncovered to concentration of Gentamicin higher than the one in serum since kidney is primary excretory organ for GM. There's prove that the concentration of Gentamicin in cells of proximal tubules isn't related to nephrotoxicity, in spite of the fact that tubular rot is measurements dependent.[15] Later *in vitro* thinks about appeared that the key angle of GM cytotoxicity is its concentration in cytoplasm, not aggregation in lysosomes as thought earlier.[16] Moreover it was demonstrated that little sum of GM straightforwardly enters the cytoplasm, autonomously of megalin-cubilin interceded endocytosis.[17] Other than, it was illustrated that GM can enter the cells of tubules *in vitro* through nonspecific cationic channel TRPV4.[18] In any case, this channel is show as it were in distal tubules and its commitment to GM cell section is small.

In the present study drug induced Nephrotoxicity was established by single daily dose of Gentamicin, for 8 days. This poisonous quality is characterized by checked height within the serum levels of creatinine, uric acid, BUN and diminish within the add up to protein, egg whites and globulin. Be that as it may these changes were credited by concomitant treatment with single dosage evaluated measurements of ethanolic extricate of *Alternanthera sessilis* for 28 days. the plant extract altogether diminishes the creatinine, uric acid and BUN levels in both the treatment gather when compared to that of toxicant bunch.

The prevailing experiment evidence indicates that Gentamicinconsists of oxidative stress is due to the era of hydroxyl radicals within the kidney tissues. The generated reactive oxygen species inclusive of super oxide and hydroxyl radicals are capable to cause damage to intercellular components. Kidney tissue is susceptible to free radical damage, as it

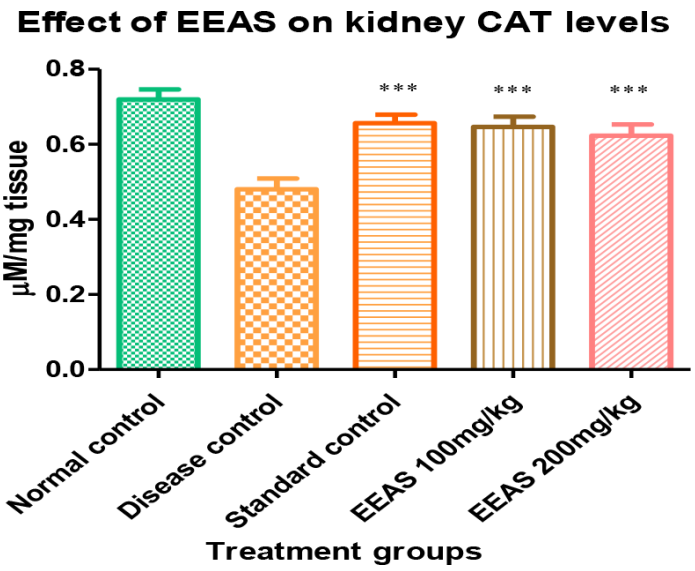

Figure 10: Effects of EEAS on CAT.

consists of low levels of free radicals detoxifying enzymes like GSH, CAT and excessive levels of LPO.

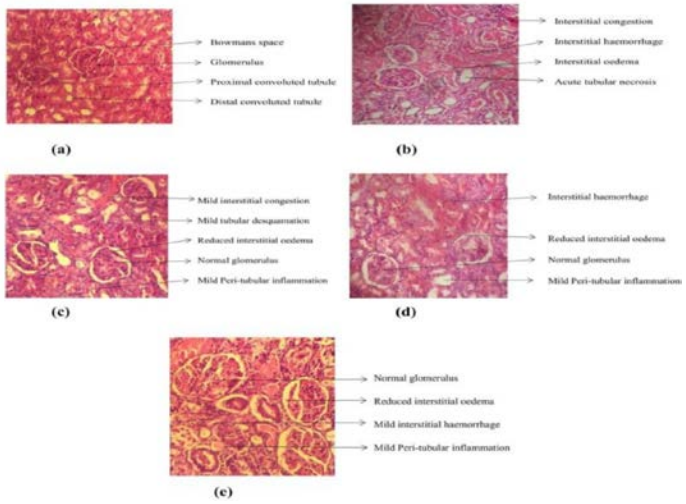

Figure 11: Histopathological studies (a) Normal group, (b) Disease group, (c) Standard group, (d) Ethanolic extract of *A. sessilis* (EEAS) 100mg/kg, (e) EEAS 200mg/kg.

The histopathological results obtained well with the biochemical results the standard and the EEAS treated groups showed significant improvement when compared to that of the disease control.

Hence, it is proved that *Alternanthera sessilis* Linn offers nehroprotective activity against Gentamicin- induced nephrotoxicity.

CONCLUSION

In the present study, the ethanolic extract of *Alternanthera sessilis* significantly reduced the Gentamicin induced elevated levels of serum markers and increases the levels of protein, albumin, globulin and restored altered antioxidant parameters. Hence, it was concluded that the ethanolic extract of *Alternanthera sessilis* offers nephroprotection.

ACKNOWLEDGEMENT

The authors acknowledge the support of Dr. Ch. Gopal Reddy, Correspondent and secretary of CMR College of Pharmacy for providing all the required facilities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ANOVA: Analysis of variance; **EEAS:** Ethanolic extract of *Alternanthera sessilis* Linn; **BUN:** Blood Urea Nitrogen; **LPO:** Lipid peroxides; **CAT:** Catalase; **GSH:** Glutathione reductase activity.

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Article History: Submission Date : 19-01-2022; Revised Date : 08-03-2022; Acceptance Date : 18-04-2022.
Cite this article: Rajesham VV, Jabeen R, Raghavendra M, Ali PR, Rao TR. Nephroprotective Activity of Ethanolic Extract of *Alternanthera sessilis* Leaves in Gentamicin-induced Nephrotoxicity in Wistar Albino Rats. Int. J. Pharm. Investigation. 2022;12(3):346-50.