EVALUATION OF NEPHROPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF CYPREUS SQUARROSUS AGAINST GENTAMICIN INDUCED NEPHROTOXICITY IN RATS

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Abstract

The present study was undertaken to evaluate the ethanolic extract of *Cyperus squarrosus* for its protective effects on gentamicin induced nephrotoxicity in rats. Rats were divided into 5 groups; normal saline, gentamicin 80 mg/kg, i.p. for 8 days, ethanolic extract of *Cyperus squarrosus* 200mg/kg and 400 mg/kg p.o 15 days and concurrently with gentamicin for 8 days. Body weight, urine volume and kidney weights were measured. Serum protein, serum creatinine, serum urea, blood urea nitrogen levels were estimated. Kidneys were collected to perform histopathological examination after the treatment. Gentamicin treatment caused nephrotoxicity as evidenced by marked changes in physical parameters, urinary and blood parameters. Co-administration of ethanolic extract of *Cyperus squarrosus* with gentamicin have markedly improved all the parameters. Histopathological reports showed reduction in the damage of kidneys when treated with the extract. These results suggest that the ethanolic extract of *Cyperus squarrosus* may be useful in reducing the gentamicin induced nephrotoxicity.

Introduction

Nephrotoxicity can be characterized as renal dysfunction. Some substances, both toxic chemicals and medications, have a negative effect on the kidneys (Nephron-toxins are nephrotoxic chemicals). Nephrotoxic effects are also present in patients who are already suffering from renal dysfunction. It leads to apoptosis, inflammatory process, and reactive species generation and increased in plasma creatinine and urea with severe proximal renal tubular necrosis[1]. If nephrotoxicity occurs, the kidneys do not absorb excess urine and waste. Kidneys are the most essential organs of the body involved in the excretion of waste materials. Nephrotoxicity can be characterized as an adverse effect of certain substances on renal function. The substances mentioned are molds and fungi, medicinal substances such as cisplatin and amino glycosides such as gentamicin. Nephrotoxicity is a reduction of renal function as measured by changes in the rate of glomerular filtration rate (GFR), blood urea nitrogen (BUN), serum creatinine (SC), and urine output[2]

Gentamicin is widely used as an antibiotic amino glycoside to treat various bacterial infections, especially Gram-negative bacteria. Gentamicin can cause tissue damage such as ototoxicity, nephrotoxicity, and liver toxicity by producing free oxygen radicals. Gentamicin nephrotoxicity due to its accumulation in renal cortical tubular epithelial cells[7]. Gentamicin nephrotoxicity is characterized by increased blood urea nitrogen, serum creatinine, and reduced glomerular filtration rate (GFR) [3]. Gentamicin nephrotoxicity is based on the use of various antioxidants.

Certain Indian Medicinal plants have been reported to exhibit protective effect of renal tissues against injuries3. Since there are only few researches made on this field of nephroprotection, Medicinal plants have curative properties due to the presence of various complex chemical substances. In the Indian traditional system of medicine, several medicinal plants are prescribed for alleviating renal damage and treating kidney complications and early literature has prescribed various herbs for the cure of renal disorders[4,5].In the present study, the ethanolic extract of Indian medicinal plant was selected to investigate the nephroprotective potential.

Material and methods

Collection of plant material:

Leaves of *Cyperus squarrosus* was obtained from Tirumala Hills, Andhra Pradesh, India and certified by Prof. Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupathi. The entire plant was dried in shade; leaves were removed and pulverized to create a dense powder.The resulting powder was then used for extraction.

The dried powder of fplant were extracted with 70% ethanolic using soxhlet apparatus. The extract was concentrated under reduced pressure using rate flash evaporator which resulted with a yield of 15.54%w/w. The extract was stored in airtight container in refrigerator. Ethanolic extract of *Cyperus squarrosus* (EECS) was further used for Preliminary Phytochemical & pharmacological evaluation.

Preliminary Phytochemical Screening

the *Cyperus squarrosus* extracts were then subjected to phytochemical investigation by qualitative chemical identification tests for alkaloids, carbohydrates, glycosides, saponins, flavanoids and steroids/triterpenoids[6].

Selection and acclimatization of animals

Adult male wistar rats weighing 180–220g were used in the experiments after allowing 15 days acclimatization. The animals were allocated four per polycarbonate cage in a temperature (20–25◦C) and humidity (40–45%) controlled room. The light: dark cycle was 12 hr: 12 hr and normal rodent pellet diet and water were supplied during acclimatization, free to access The animal house approved by the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA)-Registration number – (1330/AC/10/CPCSEA). The study was carried out after the approval by the institutional animal ethical committee (IACE)

Acute toxicity studies

Rats were kept overnight fasting prior to drug administration. Animals received a single oral dose (2000 and 5000 mg/kg, bw) of ethanoloic extract of *Cyperus squarrosus* .After the administration of *Cyperus squarrosus* extract, food was withheld for further 3-4 h. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence, and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes. Mortality, if any, was determined over a period of two weeks [7].

Experimental protocol for Gentamicin mediated nephrotoxicity in wistar albino rats[8]

Male albino Wistar rats weighing 180-220 g were used for the study. The *EECS* lower dose was 100mg/kg, median dose was 200mg/kg, and the high dose was 400mg/kg. The species have been classified into five groups. There were six animals in each group. The research was begun prior to gentamicin injection, and the dosage was continued for eight days with gentamicin therapy. The protocol has been accepted by the IAEC,

Group- 1: Normal

Group- 2: Disease control treated with gentamicin (80mg/kg, body wt., i.p) for 15 days.

Group-3: Rats were treated with the ethanolic extract of leaves of *Cyperus squarrosus* (100mg/kg, body wt.) for consecutive 8 days along with gentamicin (80mg/kg body wt., i.p) and the *EECS* dose continued up to 15 days.

Group- 4: Rats were treated with ethanolic extract *Cyperus squarrosus* (200mg/kg, body wt.) for 8 days along with gentamicin (80mg/kg body wt., i.p) and the *EECS* dose continued up to 15 days.

Group- 5: Rats were treated with ethanolic extract of leaves of *Cyperus squarrosus* (400mg/kg, body wt.) for 8 days along with gentamicin (80mg/kg body wt., i.p) and the *EECS* dose continued up to 15 days.

Biochemical analysis

Serum analysis

Blood samples of wistar rats were collected on the 0th and 15th day by retro-orbital under anesthetic conditions and subjected to centrifugation at 3000 rpm for 15 min and segregated by serum. Serum samples are subjected to estimation of creatinine, urea, uric acid and sodium, potassium, chlorides, membrane bound enzymes (Na^{+}- k^{+}ATPase, Mg^{+2}ATPase and Ca^{+2}ATPase) and antioxidant parameters (SOD, MDA, GSH, Catalase) were tested in serum samples.

Urine analysis

At the end of the experiment, i.e., on the 15th day after collection of blood samples animals were placed in metabolic cages for collection of urine samples. Urine samples were subjected to estimation of creatinine, urea, uric acid and sodium, potassium, chlorides, membrane bound enzymes (Na^{+}-k^{+}ATPase, Mg^{+2}ATPase and Ca^{+2}ATPase) and antioxidant parameters (SOD, MDA, GSH, Catalase) were tested in urine samples.

Kidney homogenate analysis:

The rats were subjected to carbon dioxide gas euthanasia on the 15th day of the study. The stomach was incised, opened to take out the two kidneys to every animal. The isolated kidneys have been washed to remove abnormal tissue. One kidney has been checked for homogeneous renal function, and another kidney has been checked for histopathology.

Histopathology:

Animals were sacrificed on the day of withdrawal of the blood; kidneys were isolated and were washed with ice cold water, then fixed in 10% neutral formalin buffer. Partitioned at 5μm breadth and discolored with hematoxylin-eosin dye and straddling with Canada balsam. The histopathological inspection of photographs was made underneath an unadorned and undifferentiated light microscope (40X) and shot by an Olympus Digital Camera.

Statistical Analysis:

All values are listed as the mean SEM. Using one-way ANOVA, the data were statistically analyzed following Dunnett's analysis.

Results and discussion

Preliminary Phytochemical Analysis:

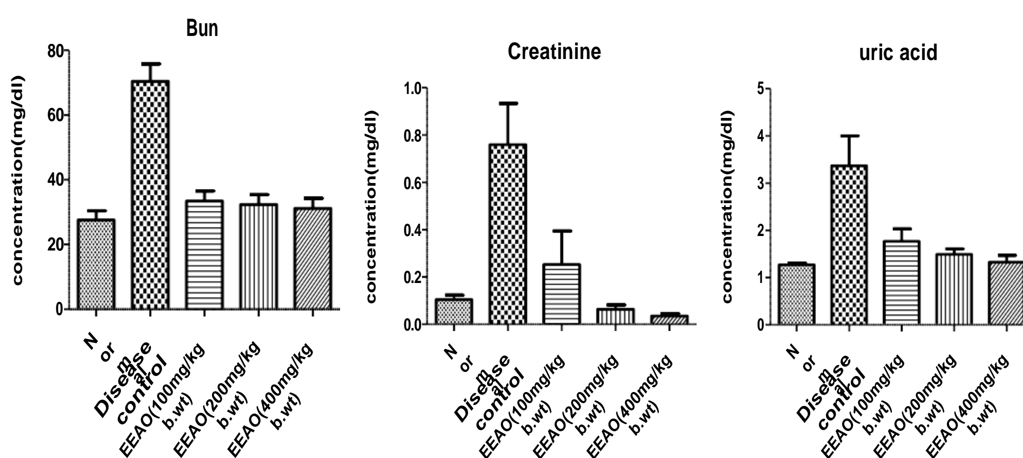
The grades of qualitative phytochemical studies indicate that the maximum number of chemical constituents present in the ethanol extract when compared to other extracts.

Table:1 Phytochemical Analysis of *Cyperus squarrosus* leaf extract:

Phytoconstituents	Tests	Ethanol
Alkaloids	Mayer's test Hager's test Dragendorff's test Wagner's test	- - -
Glycosides	Borntrager's test Liebermann's buchard test Baljet test Keller-killani test	- + +
Flavanoids	Alkaline reagent Lead acetate test $FeCl_3$ test Shinoda test	+ + +
Terpenoids	Salkowski test Liebermann's buchard test	- -
Tannins	Gelatin test Chlorogenic acid test	-

'+' indicates the presence '-'indicates the absence

Graph :1 Effect of *EECS* on serum levels of BUN, Creatinine, Uric acid in gentamicin induced nephrotoxicity in rats:

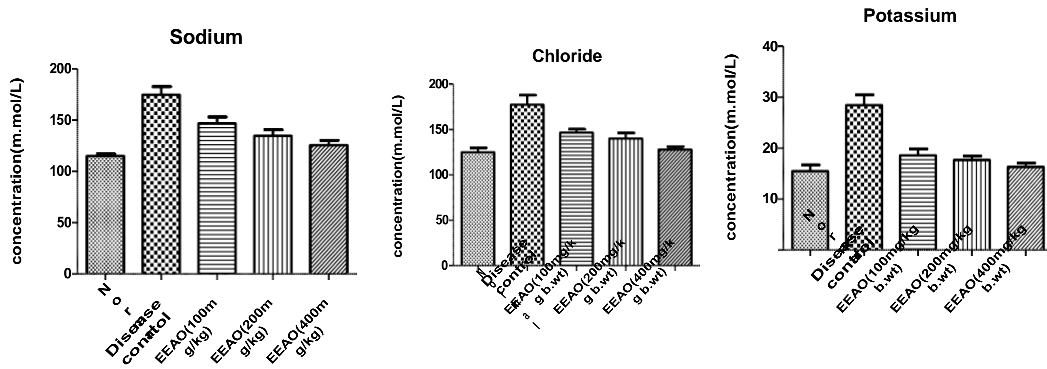


All values are expressed as mean ± S.E.M for six rats in each group. Comparisons made between P<0.001$^{###}$, p<0.01$^{##}$, p<0.05$^{#}$; Disease control Vs Normal, P<0.001***, p<0.01**, p<0.05*; Treatment Vs Disease control, One-way ANOVA followed by Dunnett's-t test.

Gentamicin treated rats exhibited significant increase in serum BUN (70.41±5.422: P<0.001$^{###}$), Creatinine (0.759±0.174: P<0.001$^{###}$), Uric acid (3.370±0.633: P<0.001$^{###}$) when compared with normal rats BUN (27.60±2.834), creatinine (0.104±0.018), uric acid (1.274±0.031).

At the dose of *EECS* 400mg/kg, bd.wt treated rats serum BUN (32.35±3.149: P<0.001***), Creatinine (0.063±0.0182: P<0.001***), Uric acid (1.329±0.143: P<0.001***) levels were significantly decreased compared to gentamicin treated rats.

Graph:2 Effect of *EECS* on serum levels of sodium, chloride, and potassium in gentamicin induced nephrotoxicity in rats:

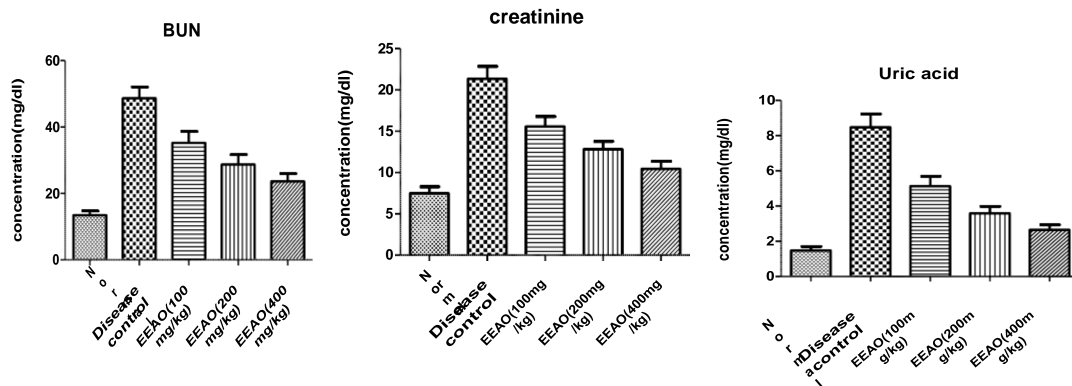


All values are expressed as mean ± S.E.M for six rats in each group. Comparisons made between $P<0.001^{\#\#\#}$, $p<0.01^{\#\#}$, $p<0.05^{\#}$; Disease control Vs Normal, $P<0.001^{***}$, $p<0.01^{**}$, $p<0.05^{*}$; Treatment Vs Disease control, One-way ANOVA followed by Dunnett's-t test.

Gentamicin treated rats exhibited significant increase in serum Sodium (184.1±8.566: $P<0.001^{\#\#\#}$), Chloride (177.4±10.48: $P<0.001^{\#\#\#}$), Potassium (28.45±2.077: $P<0.001^{\#\#\#}$) when compared with normal rats Sodium (131.2±7.367), creatinine (125.0±4.934), uric acid (15.48±1.281).

At the dose of *EECS* 400mg/kg, bd.wt treated rats serum Sodium (136.8±4.895: $P<0.001^{***}$), Chlorides (128.0±3.329: $P<0.001^{***}$), Potassium (16.31±0.7611: $P<0.001^{***}$) levels were significantly decreased compared to gentamicin treated rats.

Graph 3: Effect of *EECS* on urinary levels of BUN, Creatinine, Uric acid in gentamicin induced nephrotoxicity in rats:

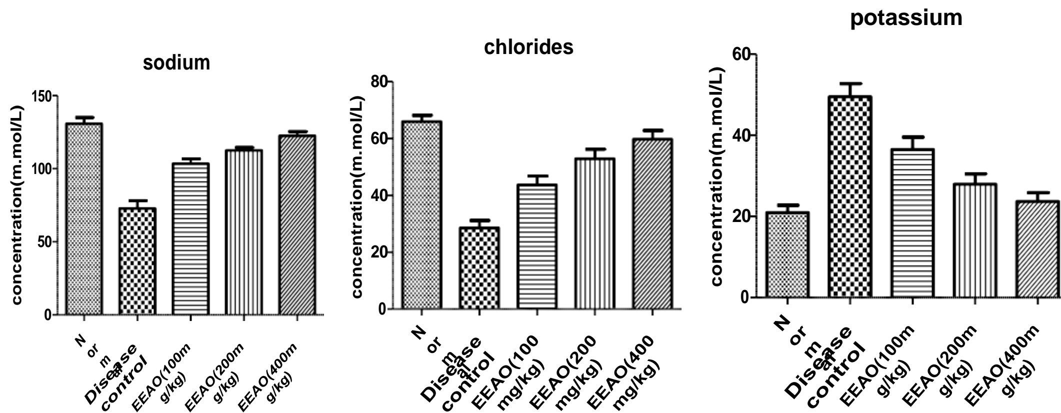


All values are expressed as mean ± S.E.M for six rats in each group. Comparisons made between P<0.001###, p<0.01##, p<0.05#; Disease control Vs Normal, P<0.001***, p<0.01**, p<0.05*; Treatment Vs Disease control, One-way ANOVA followed by Dunnett's-t test.

Gentamicin treated rats exhibited significant increase in Urinary BUN (48.68±3.379: $P<0.001^{\#\#\#}$), Creatinine (21.34±1.152: $P<0.001^{\#\#\#}$), Uric acid (8.479±0.7602: $P<0.001^{\#\#\#}$) when compared with normal rats BUN (13.47±1.272), Creatinine (7.484±0.7989), Uric acid (1.475±0.2292).

At the dose of *EECS* 400mg/kg, bd.wt treated rats Urinary BUN (23.62±2.364: $P<0.001^{***}$), Creatinine (10.44±0.9274: $P<0.001^{***}$), Uric acid (2.651±0.2752: $P<0.001^{***}$) levels were significantly decreased compared to gentamicin treated rats.

Graph:4 Effect of *EECS* on urine levels of sodium, chloride, and potassium in gentamicin induced nephrotoxicity in rats



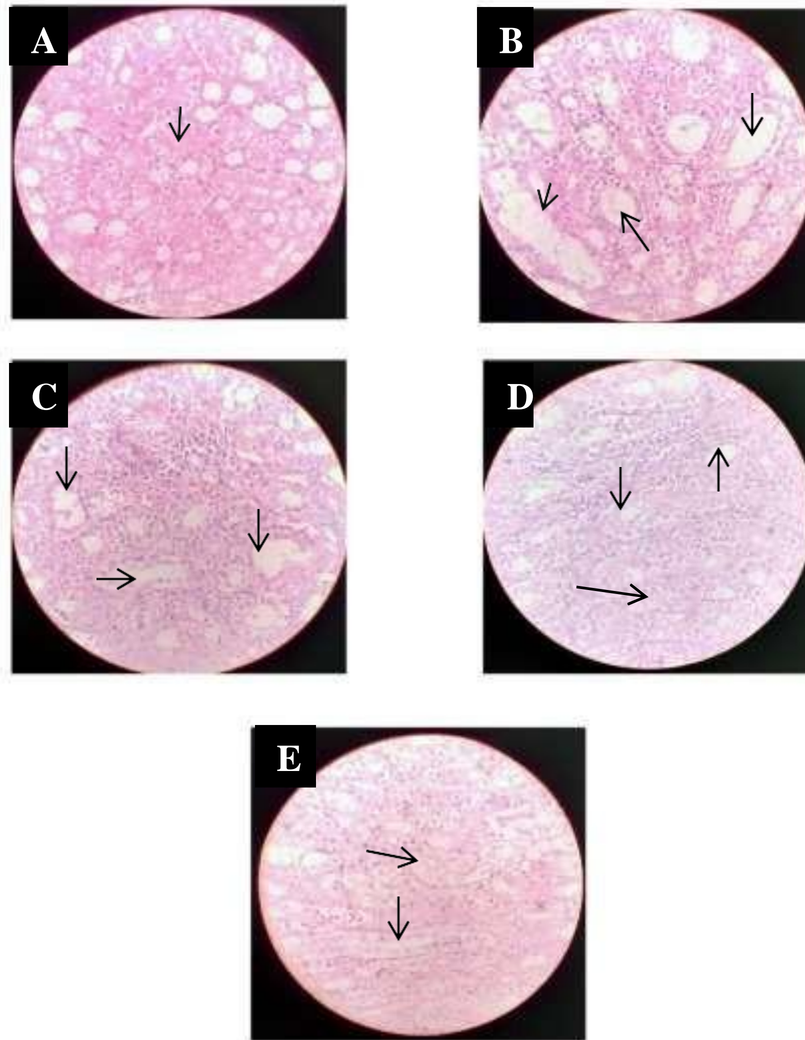
All values are expressed as mean ± S.E.M for six rats in each group. Comparisons made between $P<0.001^{###}$, $p<0.01^{##}$, $p<0.05^{#}$; Disease control Vs Normal, $P<0.001^{***}$, $p<0.01^{**}$, $p<0.05^{*}$; Treatment Vs Disease control, One-way ANOVA followed by Dunnett's-t test.

Gentamicin treated rats exhibited significant decrease in Urinary Sodium (72.81±5.383: $P<0.001^{###}$), Chlorides (28.58±2.560: $P<0.001^{###}$), and increase in Potassium (49.53±3.221: $P<0.001^{###}$) when compared with normal rats Sodium (130.8±4.262), Chlorides (65.95±2.204), Potassium (20.97±1.799).

At the dose of *EEAO* 400mg/kg, bd.wt treated rats increased in Urinary Sodium (122.6±2.910: $P<0.001^{***}$), Chlorides (59.79±3.065: $P<0.001^{***}$), and decrease in Potassium (23.73±2.155: $P<0.001^{***}$) levels were significantly increased when compared to gentamicin treated rats.

Histological studies(40X):

Figure 1: Histopathology of nephroprotective activity of *EECS* in gentamicin induced nephrotoxicity in rats:



A – Normal group, **B** – Disease control(Gentamicin 80mg/kg bd.wt),

C – *EECS (*100mg/kg bd.wt), **D** – *EECS (*200mg/kg bd.wt), **E** – *EECS (*400mg/kg bd.wt)

Histological studies

Histopathology of nephroprotective activity of *EECS* in gentamicin induced nephrotoxicity in rats:

In figure 1 Section of kidneys were taken and studied for histopathological changes. The kidney of the control rats were showed normal glomerular apparatus, tubules and renal parenchyma. Disease control group (gentamicin) rats showed tubular dilatation and glomerular necrosis and the rats treated with extract (*EECS* with doses 100, 200, 400 mg/kg bd.wt) reduction in features of tubular dilatation and glomerular necrosis compared to disease control group.

Discussion

One of the most common causes of acute renal failure is the nephrotoxicity of antibiotics. The role of oxidative stress in renal damage[9] has been recorded in several studies. The mediation of reactive oxygen species (ROS) in the renal effects of antibiotic is strongly indicated by previous research. In addition, ROS is also implicated in proximal tubular necrosis and acute renal failure caused by antibiotic, causing cellular damage and death by various mechanisms due to superoxide anions and hydroxyl radicals, including electron transport chain inhibition, cellular respiration suppression and production of ATP; damage to DNA; lipid peroxidation; and cell membrane destabilization. Gentamicin accumulation in the renal cortex causes renal morphological changes in humans and laboratory animals where the ultimate syndrome is somewhat similar. In order to research the pathophysiological and molecular mechanisms underlying gentamicin nephrotoxicity, the gentamicin- induced nephropathy model was therefore chosen[10].

Depending on the drug dosage and length of exposure, cisplatin induces tubular cell death by either necrosis or apoptosis. Mitochondrial dysfunction and oxidative stress are implicated in many of the mechanisms involved in tumor cell death.

Several studies have centered on using natural and synthetic Reno-protection antioxidants or ROS scavengers[11]. Protective effects of several natural and synthetic antioxidants have been documented in in vivo studies in cisplatin-

induced nephrotoxicity[12].

The renal function degradation caused by gentamicin is increased beyond normal limits in serum levels of creatinine and urea. In our study, abnormal serum and urine BUN, creatinine, uric acid Na^{+}, K^{+} and Cl^{-}, membrane bound enzymes and antioxidant parameter values were shown by the negative control rats as well as the gentamicin and cisplatin community. Variable levels of nephroprotection were demonstrated by *EECS* treatment groups. Doses of 100, 200 and 400 mg/kg at the *EECS* dose stage as indicated by a comparative decrease in serum and urine values; attenuation of the nephrotoxic insult was demonstrated. In addition, the *EECS* 's protective effect on creatinine clearance may be due to its antioxidant capacity because it was found that ROS was involved in impairment of the rate of glomerular filtration[72]. Na^{+}- K^{+}, $Ca^{2+,}$ Mg^{2+} ATPase activity was inhibited by gentamicin and.

The histopathological results in connection with protective effects of plant extracts against gentamicin induced nephrotoxicity were in agreement with published reports[13]. In the present study, there is a good correlation between *in vivo*, membrane bound enzymes, and anti-oxidant parameters, thus it is plausible to suggest that the attenuation of gentamicin induced nephrotoxicity by *EECS.*

Conclusion

The present study demonstrated the nephroprotective activity of ethanolic extract of *Cyperus squarrosus* leaf extract. The promosing results obtained in the present research have led to a scientific proof for the ethnopharmacological data on *Cyperus squarrosus* leaves which may be due to the exsistence of various phytoconstituents like, flavonoids, terpenoids, alkaloids, glycosides and tannins. Hence further studies are required for find promising lead molecule and targeted protein.

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