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Protective Effect of *Punica granatum* L. on Gentamicin Induced Acute Renal Failure in Adult Rats

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Abstract

Aim: Study was undertaken to evaluate the effect of *Punica granatum* L. on Gentamicin-induced renal failure in rats.

Materials and methods: Gr. I rats served as normal, received 0.5 ml of 5% Tween-80 in distilled water, Gr. II was injected with Gentamicin (100 mg/kg, IP), Gr. III was injected with Gentamicin and selenium (2 mg/kg, IP), and Gr. IV-IX were given orally *Punica granatum* fruit chloroform extract (PGCE) and *Punica granatum* methanol extract (PGME) at the doses of 100, 200 and 400 mg/kg, respectively, were administered for eight days in Gentamicin-induced renal failure rats. On last day, Blood and 24 h urine was collected and used for estimation of serum and urine creatinine, urea, uric acid levels. The kidney homogenate was used for the estimation of LPO, SOD, CAT and GSH levels and Kidney sections were analyzed for histopathology.

Results: Gentamicin-induced (Gr. II) had significant increase in levels of serum and urine creatinine, urea, uric acid, lipid peroxidation and significantly decrease in SOD, CAT and GSH levels as compared to normal (Gr. I). The treatment of PGCE and PGME 100, 200 and 400 mg/kg doses (Gr. IV-IX), significantly decreases serum and urine creatinine, urea, uric acid and significantly increases SOD, CAT and GSH levels in kidney homogenate with significant decrease in lipid peroxidation as compared to Gr. II.

Conclusions: The PGCE and PGME at the doses of 400 mg/kg, found to be more effective in protecting the Gentamicin-induced renal failure in rats.

Keywords: Gentamicin; *Punica granatum*; Renal failure; Lipid peroxidation; Antioxidant

Introduction

A number of environmental contaminants, chemicals and drugs including antibiotics dramatically alter the structure and function of various tissues and produce multiple adverse effects in the liver and kidney, heart and intestine. Aminoglycoside antibiotics are commonly used for the treatment of life threatening several Gram-negative bacterial infections. Perhaps the most widely used drug in this category is Gentamicin (GEN) in the treatment of the acute abdomen and urinary tract infections. A major complication of Gentamicin treatment is nephrotoxicity, which account for 10-20% of all cases of acute renal failure.

Nephrotoxicity induced by GEN is a complex phenomenon characterized by an increase in serum creatinine (Cr) and Blood urea nitrogen (BUN) concentration and severe proximal renal tubular necrosis, followed by deterioration and renal failure [1]. The toxicity of GEN seems to relate to the generation of destructive reactive oxygen species (ROS) in these cells [2]. ROS have been proposed as a causative agent of cell death in many different pathological states. Traditional use of *Punica granatum* has been reported to regulate urine discharge and controls the burning sensation of urine [3]. On time to time various researchers have work on antioxidant, anti-inflammatory, and anti-microbial, urolithiasis effects [4-7].

To date there is no pharmacological validation of the traditional use of *Punica granatum* (PG) fruit, a potent antioxidant [8,9] against gentamicin induced nephrotoxicity. This study was planned to investigate the possible protective effect of PG on GEN induced nephrotoxicity in rat model.

Materials and Methods

Plant material

The *Punica granatum* fruit were collected from Bagalkot districts, region of North Karnataka. The Plant was authenticated at Department of Botany, B.V.V. Sangha's Science College, Bagalkot. A voucher specimen 23/2010 was deposited in the same institute. The fruits of *Punica granatum* was shade-dried and uniformly powdered and subjected to hot continuous solvent extraction with petroleum ether (40-60°C) to defat, followed by chloroform and methanol extraction. The solvent was completely removed by using rotary flash evaporator and dried in lyophilizer (Mini Lyotrap, Serial No. J8199/5, LET Scientific Ltd, UK) [10]. The percentage of extract yield of PG chloroform and methanol extracts was 3.23% and 5.31%, respectively. It was calculated in terms of dried weight. These dried powdered

extract were formulated as suspension in distilled water using 5% Tween-80 as suspending agent.

All the chemicals were purchased from Hi-media, Mumbai and Sigma Chemicals Co St Louis, USA and were of analytical reagent grade.

Animals

Experiments were performed in accordance with the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA). The experimental protocol in the study was approved by the Institutional Animals Ethical Committee (HSKCP/IAEC, Clear/ 2010-2011).

The male albino rats of Wister strain, weighing 200-250 g, were obtained from central animal house of HSK College of Pharmacy, Bagalkot. The rats were housed at temperature (25 ± 1°C) with 50 ± 55% of relative humidity. Rats were fed on standard chow diet and water ad libitum. Animals were acclimatized in institutional animal house and were exposed to 12 h day and night cycles.

***In vivo* gentamicin induced nephrotoxicity in rats:** The animals were divided into 9 groups, each group containing six rats.

Experimental design: Group 1: Served as normal, received 0.5 ml of 5% Tween-80 in distilled water treated orally for 8 days.

Group 2: Served as control, received 0.5 ml of 5% Tween-80 in distilled water treated orally for 8 days and simultaneously injected IP with GEN 100 mg/kg in normal saline.

Group 3: Treated orally for 8 days with Selenium 2 mg/kg in distilled water and simultaneously injected IP with GEN 100 mg/kg in normal saline for 8 days.

Groups 4, 5 and 6: Treated orally for 8 days with PG Chloroform extract (PGCE) at a dose of 100, 200 and 400 mg/kg suspended in 0.5 ml of 5% Tween-80 in distilled water and simultaneously injected IP with GEN 100 mg/kg in normal saline for 8 days, respectively.

Groups 7, 8 and 9: treated orally for 8 days with PG Methanol extract (PGME) at a dose of 100, 200 and 400 mg/kg suspended in 0.5 ml of 5% Tween-80 in distilled water and simultaneously injected IP with GEN 100 mg/kg in normal saline 8 days, respectively.

Biochemical parameters

Analysis of urine samples: All the animals were kept in individual all glass metabolic cages and the urine sample of 24 h was collected on 8th days and acidified by the addition of 3N HCl, than centrifuged at 1500 rpm for 10 min. using refrigerated research centrifuge (Remi centrifuge instrument, Mumbai) to remove debris and supernatant were stored at-20°C until analyzed using reagent kits (ERBA diagnostics, Mannheim, GmbH, Germany).

Analysis of blood samples: The 2 ml blood samples were collected by puncturing the retro orbital venous plexus from each animal in centrifuge tube without anticoagulant and allowed to clot at room temperature. The serum was separated by centrifugation at 1500 rpm for 15 min. in Remi's refrigerated research centrifuge and used for estimation of serum creatinine, urea and uric acid using commercially available kits (ERBA, Diagnostic, Mannheim), using Star-21 plus semi-auto analyzer.

Analysis of kidney sample: After 24 h from the last injection, rats was sacrificed under anesthesia and after dissection; both kidneys were removed and washed with cold 0.15M KCl. The right kidney was minced with scissors homogenized in cold phosphate buffer (0.1M, pH 7.4). The homogenate was centrifuged at 1500 rpm for 10 min at 4°C using Remi's refrigerated research centrifuge, the kidney homogenate was used to estimate the biochemical parameters viz. Lipid peroxidation (LPO) was measured by the method of Assay [11]. The antioxidant enzymes viz. Superoxide dismutase (SOD), Catalase and reduced Glutathione were estimated by the methods of Beauchamp, Sinha, Ellman, respectively [12-14].

Hispathological analysis

The left kidney was fixed in a 10% solution of buffered formalin (pH 7.4). The tissue was embedded in paraffin and the sections of 5 µm were taken using MAC microtome (Macro scientific works, Delhi) and stained with hematoxylin-eosin. The slides were examined for histological variations under microscope, Morphometric measurements were completed on Olympus PM-10ADS automatic light microscope (Olympus optical Co., Tokyo, Japan) with a 40X and 10X objectives, calibrated ocular micrometer.

Statistical Analysis

All the statistical comparison between the groups are made by means of One Way Analysis of Variance (ANOVA) and followed by Dunnett's Multiple Comparison test. The P<0.05 regarded as significant using, GraphPad Prism 5.01 Software (GraphPad software, San diego, CA, USA). The data expressed are Mean ± standard error of mean (S.E.M.).

Results

Renal function of gentamicin induced nephrotoxicity in rats was assessed by measuring serum creatinine, urea and uric acid; in normal, control and treated rats of Gr. I-IX, were shown in (Table 1) and urine creatinine, urea and uric acid; in normal, control and treated rats of Gr. I-IX, were shown in (Table 2). Values given in parenthesis are percent change. In the present study, we find that the administration of Gentamicin in Gr. II (control) rats resulted in significantly (P<0.001 vs. Gr. I) increased levels of serum creatinine, urea and uric acid by (↑316, ↑220 and ↑672 %) respectively and urine creatinine, urea and uric acid by (↑198, ↑45 and ↑224%) respectively, indicating renal damage.

Selenium was used as antioxidant in Gr. III. We find that the serum creatinine and uric acid levels were significantly (P<0.001 vs. Gr. II) decreased by (↓73 and ↓84%), respectively. However, urea levels were decreased significantly (P<0.01 vs. Gr. II) by ↓20%.

Treatment with PGCE at the doses of 100, 200 and 400 mg/kg causes, significant decrease (P<0.001 vs. control) in serum creatinine (↓62%, ↓63% and ↓66%), and uric acid (↓59%, ↓56% and ↓63%) levels whereas urea levels were decreased significantly (P<0.05 vs. control) at 100 and 200 mg/kg (↓14% and ↓14%) and ↓17% at 400 mg/kg (P<0.01 vs. control). On the other hand PGME treatment at the doses of 100, 200 and 400 mg/kg also caused significant decrease (P<0.001 vs. control) in serum creatinine (↓58%, ↓67% and ↓69%) and uric acid (↓79%, ↓83%, ↓84%) levels, whereas urea levels were decreased (↓16%) significantly (P<0.05 vs. control) at 100 mg/kg. At the dose of 200 and 400 mg/kg urea was decreased significantly (P<0.01 vs. control) by

(↓19% and ↓19%). These results were at par with selenium treated rats (Gr. III).

S. No	Groups	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
1	Normal	1.530 ± 0.072	36.25 ± 1.341	2.107 ± 0.357
2	Control	6.364 ± 0.605$^{\neq}$ (↑316%)	116.10 ± 2.561$^{\neq}$ (↑220%)	16.270 ± 1.982$^{\neq}$ (↑672%)
3	STD	1.731± 0.3484$^{\neq}$ (↓73%)	92.80 ± 4.979$^{¥}$ (↓20%)	2.610 ± 0.6618$^{\neq}$ (↓84%)
4	PGCE 100 mg/kg	2.403 ± 0.379$^{\neq}$ (↓62%)	100.10 ± 4.778$^{€}$ (↓14%)	6.741 ± 0.811$^{\neq}$ (↓59%)
5	PGCE 200 mg/kg	2.350 ± 0.393$^{\neq}$ (↓63%)	99.69 ± 4.310$^{€}$ (↓14%)	7.104 ± 0.656$^{\neq}$ (↓56%)
6	PGCE 400 mg/kg	1.988 ± 0.263$^{\neq}$ (↓69%)	95.87 ± 3.287$^{¥}$ (↓17%)	6.027 ± 0.865$^{\neq}$ (↓63%)
7	PGME 100 mg/kg	2.656 ± 0.340$^{\neq}$ (↓58%)	97.52 ± 2.874$^{€}$ (↓16%)	3.353 ± 0.553$^{\neq}$ (↓79%)
8	PGME 200 mg/kg	2.121 ± 0.180$^{\neq}$ (↓67%)	93.80 ± 3.880$^{¥}$ (↓19%)	2.802 ± 0.205$^{\neq}$ (↓83%)
9	PGME 400 mg/kg	2.138 ± 0.403$^{\neq}$ (↓66%)	94.36 ± 6.399$^{¥}$ (↓19%)	2.596 ± 0.410$^{\neq}$ (↓84%)

All value are mean ± SEM (n=6) One way analysis of variance test (ANOVA) followed by Dunnette's multiple comparison test. P<0.05$^{€}$, P<0.01$^{¥}$, P<0.001$^{\neq}$, when compared with control. Values given in parenthesis are percent change. PGCE; *Punica granatum* chloroform extract; PGME; *Punica granatum* Methanol extract.

Table 1: Effect of PGCE and PGME on serum creatinine, urea and uric acid gentamicin induced renal failure in rats.

Sl. No	Groups	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
1	Normal	5.097 ± 0.687	114.4 ± 2.312	6.812 ± 0.491
2	Control	15.200 ± 1.056$^{\neq}$ (↑198%)	166.2 ± 1.250$^{\neq}$ (↑45%)	22.08 ± 0.479$^{\neq}$ (↑224%)
3	STD	7.306 ± 0.5870$^{\neq}$ (↓52%)	126.8 ± 2.120$^{\neq}$ (↓24%)	8.01 ± 0.4186$^{\neq}$ (↓64%)
4	PGCE 100 mg/kg	11.810 ± 0.598$^{¥}$ (↓22%)	152.8 ± 2.134$^{¥}$ (↓8%)	17.14 ± 0.467$^{\neq}$ (↓22%)
5	PGCE 200 mg/kg	8.438 ± 0.393$^{\neq}$ (↓44%)	135.2 ± 1.290$^{\neq}$ ↓19%)	10.86 ± 0.512$^{\neq}$ (↓51%)
6	PGCE 400 mg/kg	5.274 ± 0.344$^{\neq}$ ↓65%)	128.5 ± 3.237$^{\neq}$ ↓23%)	8.717 ± 0.427$^{\neq}$ (↓61%)
7	PGME 100 mg/kg	9.550 ± 0.973$^{\neq}$ (↓37%)	146.2 ± 2.939 $^{\neq}$ (↓12%)	15.48 ± 0.418$^{\neq}$ (↓30%)
8	PGME 200 mg/kg	6.066 ± 0.438$^{\neq}$ (↓60%)	140.7 ± 0.969$^{\neq}$ ↓15%)	12.17 ± 0.749$^{\neq}$ (↓45%)
9	PGME 400 mg/kg	4.725 ± 0.674$^{\neq}$ (↓69%)	126.3 ± 3.816$^{\neq}$ (↓24%)	8.279 ± 0.484$^{\neq}$ (↓62%)

All value are mean ± SEM (n=6) One way analysis of variance test (ANOVA) followed by Dunnette's multiple comparison test. P<0.01$^{¥}$, P<0.001$^{\neq}$, when compared with control. Values given in parenthesis are percent change. PGCE; *Punica granatum* chloroform extract; PGME; *Punica granatum* Methanol extract.

Table 2: Effect of PGCE and PGME on urine creatinine, urea and uric acid gentamicin induced renal failure in rats.

Gr. III treated rats with selenium, showed a significant (P<0.001 vs. Gr. II) decrease in urine creatinine, urea and uric acid by (↓52, ↓24 and ↓64%) respectively.

The treatment with PGCE at the doses of 100 mg/kg showed significant (P<0.01 vs. control) decrease in urine creatinine (↓22%) and urea (8%) whereas in uric acid a significant (P<0.001 vs. control) decrease (↓22%) was observed. At 200 and 400 mg/kg, PGCE causes significant (P<0.001 vs. control) decrease in creatinine (↓44% and ↓65%), urea (↓19% and ↓23%) and uric acid (↓51% and ↓61%) levels. On the other hand PGME treatment at the doses of 100, 200 and 400 mg/kg caused significant (P<0.001 vs. control) decrease in urine creatinine (↓37%,↓60%,↓69%), urea (↓12%,↓15%,↓24%) and uric acid (↓30%,↓45%,↓62%) levels. These data showed PGME extract of PG has better protection in regulating the renal damage in a dose dependent manner and at 400mg/kg has better protection than selenium treated rats (Gr. III).

In kidney homogenate, GEN-treatment showed a significantly (P<0.001 vs. Gr. I) increased levels in renal lipid per-oxidation as evidenced by enhanced MDA levels (↑149%) which is associated with the significantly (P<0.001 vs. Gr. I) decreased levels of antioxidant enzymes viz. SOD and GSH (↓59% and↓53%) and in CAT, a significant (P<0.01 vs. control) decrease (↓42%) was observed (Table 3).

Sl. No	Groups	MDA	SOD	CAT	GSH
1	Normal	178.7 ± 5.12	837.7 ± 13.76	8.03 ± 0.71	23.24 ± 1.48
2	Control	445.0 ± 7.45≠ (↑149%)	347.4 ± 12.11≠ (↓59%)	4.68 ± 0.42¥ (↓42%)	10.82 ± 0.75≠ (↓53%)
3	STD	201.3 ± 6.32≠ (↓55%)	623.2 ± 11.23≠ (↑79%)	7.631 ± 0.59€ (↑63%)	18.26 ± 1.36€ (↑69%)
4	PGCE 100 mg/kg	274.7 ± 10.05≠ (↓38%)	617.1 ± 18.33≠ (↑78%)	617.1 ± 18.33≠ (↑78%)	19.79 ± 1.82¥ (↑83%)
5	PGCE 200 mg/kg	265.6 ± 6.50≠ (↓40%)	652.7 ± 12.40≠ (↑88%)	8.30 ± 0.51¥ (↑77%)	19.94 ± 2.41¥ (↑84%)
6	PGCE 400 mg/kg	222.1 ± 7.85≠ (↓50%)	790.0 ± 20.38≠ (↑127%)	8.33 ± 0.88¥ (↑78%)	21.14 ± 1.79¥ (↑95%)
7	PGME 100 mg/kg	334.5 ± 7.66≠ (↓25%)	634.9 ± 14.29≠ (↑83%)	7.392 ± 0.52€ (↑58%)	18.73 ± 2.66€ (↑73%)
8	PGME 200 mg/kg	264.2 ± 5.75≠ (↓41%)	749.5 ± 12.40 ¥ (↑116%)	7.51 ± 0.41€ (↑60%)	19.54 ± 1.25¥ (↑81%)
9	PGME 400 mg/kg	246.1 ± 6.92≠ (↓45%)	795.1 ± 19.97≠ (↑129%)	7.55 ± 0.51€ (↑61%)	20.37 ± 1.96¥ (↑88%)

All value are mean ± SEM (n=6) One way analysis of variance test (ANOVA) followed by Dunnette's multiple comparison test. P<0.05€, P<0.01¥, P<0.001≠, when compared with control. Values given in parenthesis are percent change.

SOD: (Units/mg of protein), CAT: (Units/mg of protein), GSH: µMol/mg protein, MDA: (Units/mg of protein).

Table 3: Effect of PGCE and PGME on lipid per-oxidation (mda) and anti-oxidant enzymes (sod, cat and GSH) in kidney homogenate of gentamicin induced renal failure in rats.

Treatment with PGCE and PGME at 100, 200, 400 mg/kg causes a significant (P<0.001 vs. Gr. II) decrease in lipid per-oxidation levels by (↓25, ↓41, ↓45%) and (↓38, ↓40, ↓50%) respectively. The SOD levels were significantly (P<0.001 vs. Gr. II) increased by (↑78, ↑88 and ↑127%) and (↑83, ↑116 and ↑129%) respectively. The CAT levels at 100 and 200 mg/kg were increased significantly (P<0.05 vs. Gr. II) by PGCE (↑58 and ↑60%) and PGME (↑61 and ↑77%) while at 400 mg/kg, it is increased significantly (P<0.01 vs. Gr. II) by (127%, 129%) respectively. The GSH levels were significantly (P<0.01 vs. Gr. II) increased by (↑73, ↑81 and ↑88%) and (↑83, ↑84 and ↑95%) respectively. These results suggest that PGME at 400mg/kg has better protection than PGCE and standard antioxidant selenium, as it decreases the lipid per-oxidation at par with selenium and increases the antioxidant enzymes better than selenium, indicating improved marked level of protection against oxidative damage in renal tissues H_2O_2. *in vivo*

Discussion

The traditional use of *Punica granatum* has been reported to regulate urine discharge and controls the burning sensation of urine [3]. In our study, we evaluated the following renal biomarkers for evaluation of renal damage: I. Renal hemodynamics by estimating serum and urine creatinine, urea and uric acid levels, II. Determination of antioxidant enzymes activities and lipid peroxidation in kidney homogenate, and III. kidney histopathology. However, it was suggested by researcher that Gentamicin being the most nephro-toxic antibiotic amongst all aminoglycosides, its consider as an acute nephrotoxicity and consequently precipitating ARF in human as well as animal subjects. The intracellular metabolism of drugs leads to the formation of reactive metabolites, which are toxic for cell, as are free radicals. 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 the dramatic modification of structure and function. The toxic agent reduces the concentration of antioxidants, superoxide dismutase, glutathione, catalase, and vitamin-E, ascorbic acid which is the protective tissues that reacts and removes reactive oxygen species [15]. Many studies have suggested that free radicals are important mediators in GM-induced renal damage [16,17]. In addition, several other researchers have attributed the nephroprotective effect of medicinal plants to the antioxidant compounds present in these *Punica granatum* L. fruits.

In our study administration of GEN at the dose of 100 mg/kg for 8 days IP, caused marked nephrotoxicity due to significant increased levels of creatinine, urea and uric acid in serum and urine (Gr. II). The toxicity of GEN seems to relate to the generation of destructive reactive oxygen species (ROS) in renal cells [18,19]. It has been found that GEN induces formation of superoxide O_2^- anion, Hydrogen-peroxide (H2O2) and hydroxyl radical (OH^-) in renal mitochondria, and lipid peroxidation are increased. Due to this the production of O_2^- could lead to a decrease in glomerular filtration rates (GFR) by another mechanism [20,21]. The elevated level of MDA, a marker of lipid peroxidation, indicates increased free radical generation in the GEN-induced nephrotoxicity [22]. As treatment with some antioxidants protects against GEN-induced renal injury, we studied here the effect of Punica granatum, a potent antioxidant and free radical scavenger. Many researchers have reported that Punica granatum fruit is rich in diverse classes of potentially antioxidant compounds such as β-carotenes, vitamin C, vitamin E and polyphenolic compound such as anthocyanins, punicalagin, ellagic and gallic acid [23-25]. Our results shows that the extracts of Punica granatum (PGCE and PGME) acts as antioxidants in vivo, preventing ROS formation and lipid peroxidation in cells and tissues, thus preventing the oxidant induced apoptosis. Considering the new fact the decreased concentration of serum and urine creatinine, urea and uric acid in PGCE and PGME treated rats were attributed to rich antioxidants present in these extracts. The serum creatinine concentration is more significant than urea levels in the earlier phases of kidney disease. The oxidant-antioxidant system is in equilibrium in normal conditions. Intracellular antioxidant

enzymes, SOD, rapidly and specially reduce O_2^- to hydrogen peroxide (H2O2). In addition the PGCE and PGME extracts may also inhibit the lipid peroxidation by inducing the SOD or scavenging and inactivating H2O2 and OH^- by CAT and GSH.

Peroxisome proliferator-activated receptor (PPAR-γ) have been localized in urinary system including glomerulus, collecting ducts, proximal tubules, and renal. There have been reports suggesting that activation of PPAR-γ receptors triggers protection in the different models of renal failure like chronic renal allograft damage and renal ischemia perfusion injury. The studies have shown that activation of PPAR-γ receptors directly attenuates glomerular disease possibly by inhibiting mesangial growth which occurs early in the process of nephropathy. The PGCE and PGME extracts of *Punica granatum* may activate the PPAR-γ receptors in restoring the renal tissue damage as observed in histopathology (Figure 1).

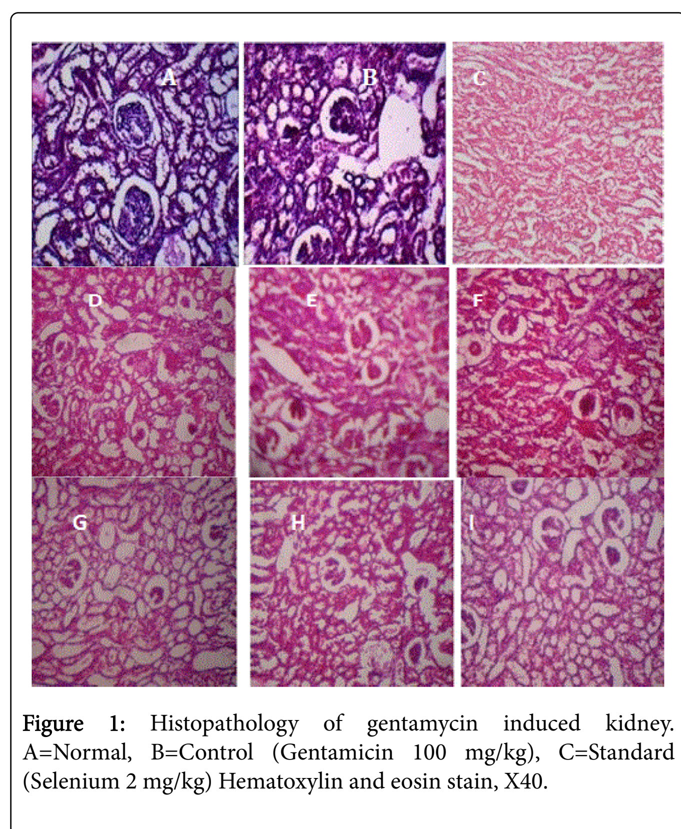

Figure 1: Histopathology of gentamycin induced kidney. A=Normal, B=Control (Gentamicin 100 mg/kg), C=Standard (Selenium 2 mg/kg) Hematoxylin and eosin stain, X40.

Normal: Normal rats (A) show prominent critical tubules and bowman's capsules. The normal kidney showing normal glomerular structure when compare to control groups.

Control: In control rats (B) administration of Gentamicin (100 mg/kg) for the first 8th days produced Renal tubular damage, hemorrhages, tubular dilatation, and glomerular atrophy. Interruption in the basement membrane around the necrotic tubules and narrowing of the Bowman's space.

Treatment: Treatment with Standard (Selenium 2 mg/kg) Figure C showing marked reduced the renal tubular and some of them show intracellular edema membrane, hemorrhages, tubular dilatation, and glomerular atrophy when compared to control kidney sections.

D=PGCE 100, E=PGCE 200, F=PGCE 400, G=PGME100, H=PGME 200, I=PGME 400, Hematoxylin and eosin stain, X10.

Treatment: Treatment with PGCE, PEME 100, 200, 400 mg/kg respectively, Figures D-I showing marked reduced the renal tubular and some of them show intracellular edema membrane, hemorrhages, tubular dilatation, and glomerular atrophy when compared to control kidney sections.

Conclusion

The results of our study confirmed that the PGCE and PGME extracts at the dose of 400 mg/kg shows good nephroprotective activity, as evidence by decrease in serum creatinine, urea and uric acid levels. Further control studies are necessary to know the mechanism of action of PGME extracts in the activation of PPAR-γ receptors in restoring the renal tissue damage.

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