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Nephroprotective Potential of Ethanolic Extract of Barks of Tricholepis Glaberrima Against Gentamicin Induced Nephrotoxicity

Mihir Y Parmar*[1], Sindhuja S[2], Mounika B[3] and Dinesh Pore[4]

Bharat Institute of Technology, Jawaharlal Nehru Technological University, India

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***Corresponding author:** Mihir Y Parmar, Bharat Institute of Technology, Jawaharlal Nehru Technological University, Hyderabad, Telangana, India

Abstract

To scrutinize the nephroprotective potential of Ethanolic Extract of Tricholepis Glaberrima (EETG) against Gentamicin (100mg/kg, i.p. 7 days) induced kidney damage in rats. All rats were pre-treated with EETG (200 & 400mg/kg, p.o) 30min prior to Gentamicin administration for consecutive 7 days. After the last dosing of seventh day after 24 h Serum was analyzed for biochemical parameters. There was no morbidity and mortality observed throughout the study. EETG was found not toxic to the experimental animals up to the dose of 4g/kg. The degree of protection was deliberate using levels of serum enzymes like creatinine, uric acid and Blood Urea Nitrogen (BUN). Protection on Liver can be measured by Liver Function Test Parameters such (LFTs); as Serum Glutamate Oxaloacetate Transaminase (SGOT); Serum Glutamate Pyruvate Transaminase (SGPT) and Alkaline Phosphatase (ALP); Oxidative stress parameters such as levels of Malondialdehyde (MDA); reduced glutathione (GSH); Superoxide Dismutase (SOD); Catalase (CAT) along with histological evaluation of kidney sections as a supplementary data for induction of kidney scratch and nephroprotective potential. The substantially elevated physical parameters such as kidney weight and biochemical parameters; serum enzyme levels of creatinine, uric acid and BUN as well as LFTs parameters in Gentamicin treated animals were observed as compared to control. Oxidative stress parameters MDA, GSH levels and SOD; CAT activities and all above biochemical parameters were found to be restored towards normalization by EETG comparable with silymarin standard. Pathological changes were in same line to supports finding of biochemical evidences of nephroprotection. EETG possess a remarkable nephroprotective potential against Gentamicin induced kidney damage as evidenced by physical, biochemical and histological observation.

Keywords: Antioxidant; Gentamicin; Kidney; Liver; Nephroprotective; Oxidative stress; Silymarin

Abbreviations: BUN: Blood Urea Nitrogen; SGOT: Serum Glutamate Oxaloacetate Transaminase; SGPT; Serum Glutamate Pyruvate Transaminase; ALP: Alkaline Phosphatase; MDA: Malondialdehyde; GSH : Reduced Glutathione; SOD: Superoxide Dismutase; RO: Reactive Oxygen species; IAEC: Institutional Animal Ethics Committee; EETG: Ethanolic Extract Of *Tricholepis Glaberrima*; GFR :Glomerular Filtration Rate; LFTs : Liver Function Test; DMCT : Dunnett's Multiple Comparison Test; ROS: Reactive Oxygen Species ; GFR: Glomerular Filtration Rate ; SGOT: Serum Glutamate Pyruvate Transaminase

Introduction

Kidney crash is a medical event in which the kidneys fall short to adequately filter toxins and waste products from the blood. There are two forms one is acute and other is chronic; a number of other diseases or health problems may cause either form of renal failure to occur. Chronic kidney disease attacks the kidneys slowly and progressively over a period of time. It can take years for the harm to these organs to be noticeable because there are no symptoms, which is why the disease is often called the "silent killer" [1].Nephrotoxicity is caused by class of drugs or xenobiotics like anticancer drug cisplatin and amino glycoside antibiotics are the chief culprit for approximately 20-40% of all acute renal failure cases in intensive care units [2]. Gentamicin is widely used aminoglycoside antibiotics against gram-negative bacteria infections [3]. About 30-35% of the patients, undergone gentamicin treatment for more than seven days, shows signs and symptoms of kidney toxicity [4]. The cellular and molecular mechanism/s of Gentamicin-induced nephrotoxicity is not clearly understood. However Reactive oxygen species (ROS) have important role in pathological mechanisms of Gentamicin-induced acute renal failure. Production as well as amassing of ROS resulted in induction of apoptosis, tubular necrosis and increased infiltration of leukocyte [5]. This Gentamicin-induced acute renal failure is clinically characterized by an increase in serum

creatinine and uric acid levels and urea nitrogen, a reduction in the glomerular filtration rate (GFR) and urine osmolality [6]. There are many natural products such as plant and traditional herbal formulation available for the protective effect on kidney against damage induced by toxin and drugs. More than 600 commercial herbal products with claimed nephroprotective role are being sold in all over the world. Around 170 phytoconstituents isolated from 110 plants belonging to 55 families have been reported to show nephroprotective role. However, only a small proportion of nephroprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their safety and efficacy [7-8]. Renal involvement has also been involved in many cardiovascular diseases, such as diabetes mellitus and regarding the impact of kidney lesions in diabetic nephropathy [9]. In addition, it is becoming highly risk factor to use synthetic drug because of their adverse drug reaction, toxicity and drug-drug interact. Therefore, scientists are fascinated for new herbal molecule with good safety and effective profile. Researches in their previous reports reported that plants possessing polyphenolic compounds, flavonoids and tannins are useful as antioxidants and further it acts as organ protectant [10]. Keeping this consideration in view, we found a medium sized tree, namely *Tricholepis Glaberrima* of family Asteraceae commonly known as "Brahmadanda", which is planted in gardens and avenues, and reported to be useful as diuretic, anti-inflammatory, antedate, enemas, antisecretolytic, antiparasitic, antimalarial, anti-HIV and diabetic. It is further reported that the plant is useful in the treatment of kidney diseases, herpes, stomachache, urethral inflammations and fungal skin diseases. The literature survey of this plant revealed that this plant possesses quercetin caffeic acids, oleanolic aced, steroids, polyphones, flavonoids, tannins and cardiac glycosides. Herbs are reported to contain phenolic compounds these phenolic components are known as antioxidants, are reported to have organ protective properties [11-12]. Hence in our scientific study an effort was made find out nephroprotective potential of Ethanolic extract of bark of *Tricholepis Glaberrima* against Gentamicin induced nephrotoxicity in rat.

Experiential Protocols

Plant material

Bark of *Tricholepis Glaberrima* used was collected from Chitoor district of Andhra Pradesh. The plant was taxonomically identified and authenticated by Dr. Madhav Shetty, Department of Botany, Sri Venkateshwara University, Tirupathi where the voucher specimen for the same is restored under the reference number SS-01.

Preparation of the extracts

The barks were cut into small pieces were cleaned and dried under shade at room temperature for several days and powdered. The resulting powder was then used for extraction. The dried powder of barks were defatted with petroleum ether and then extracted with 70% ethanolic using Soxhlet apparatus. The extract was concentrated under reduced pressure using rota flash evaporator which resulted with a yield of 7.84% w/w. The extract was stored in airtight container in refrigerator. Ethanolic extract of *Tricholepis Glaberrima* (EETG) was further used for pharmacological evaluation. The preliminary phytochemical screening was carried out on 70% ethanolic extract of *Tricholepis Glaberrima* for qualitative identification of type of phytoconstituents present.

Drugs and Chemicals

Silymarin were obtained from Micro Labs, Bangalore, India and Gentamicin was procured from Piramal Health Care, Ahmedabad, India. Urea, Uric acid and Creatinine kits were obtained from Span Diagnostics, Surat, India. All other chemicals used in this study were obtained commercially and were of analytical grade.

Experimental animals

Wistar albino rats (200-250g) of either sex was maintained under controlled conditions of temperature (27± 2 °C) and humidity (50±5%) and a 12-hour light-dark cycle, were used for the experiment. The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard rat pellet diet and water *ad libitum*. The animals were given a week's time to get acclimatized with the laboratory conditions. All the experimental procedures were performed according to the committee for the purpose of control and supervision of experiments on animals (CPCSEA-1015/PO/Re/S/06), ministry of social justice and empowerment Government of India, norms and approved by the Institutional Animal Ethics Committee (IAEC).

Acute toxicity studies

Rats were kept overnight fasting prior to drug administration. Animals received a single oral dose (2000 and 4000mg/kg, b.w.) of ethanoloic extract of barks of *Tricholepis glaberrima*. After the administration of *Tricholepis Glaberrima* extract, food was withheld for further 3-4h. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24h (with special attention during the first 4h) 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 [13].

Selection of dose of the extract

LD50 was done as per OECD guidelines for fixing the dose for biological evaluation. The LD50 of *Tricholepis Glaberrima* extract as per OECD guidelines falls under class four values with no

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signs of acute toxicity at 4,000mg/kg. The biological evaluation was carried out at doses of 200 and 400mg/ kg body weight [13].

Gentamicin-induced Nephrotoxicity in Rats [14]

Thirty Wistar albino rats of either sex was assigned to five groups (n= 6):

a. Group I: Rats in this group were injected with normal saline, intraperitoneally and served as control;

b. Group II: Rats in this group were injected with gentamicin (100mg/ kg, i.p) for seven consecutive days; and served as model control

c. Group III: Rats in this group were injected with gentamicin (100mg/kg, i.p) and administered extract of EETG (200mg/ kg, p.o) for seven consecutive days;

d. Group IV: Rats in this group were injected with gentamicin (100mg/kg, i.p) and administered extract of EETG (400mg/ kg, p.o) for seven consecutive days.

e. Group V: Rats in this group were injected with gentamicin (100mg/kg, i.p) and administered silymarin (25mg/ kg, p.o) for seven consecutive days.

After the last dosing of seventh day after 24h the blood sample were collected by puncturing retro-orbital plexus and serum was separated by centrifugation. Serum was analysed for Blood urea nitrogen, uric acid and creatinine and Liver function Test (LFTs) Parameters such as Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) using standard kits [15]. After collection of blood samples, the rats were sacrificed by light ether anesthesia and their kidneys were excised, rinsed in ice cold normal saline, followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. A small 10% (w/v) portion of the kidney was homogenized in chilled Phosphate buffered saline (50mM, pH 7.4) using a Potter Elvehjehm Teflon homogenizer. The homogenate obtained was centrifuged in a cooling centrifuge at 1,000×g for 10min at 4°C to remove nuclei and unbroken cells. The pellet was discarded, and portion of supernatant was again centrifuged at 12,000×g for 20 min at 4°C obtain a post- mitochondrial supernatant which was used for enzyme analysis. The contents of malondialdehyde (MDA), reduced glutathione (GSH), Superoxide dismutase (SOD) and Catalase (CAT) activity were estimated spectrophotometrically using above post-mitochondrial supernatant. Rats some parts of kidneys preserved in 10% formalin for histopathological study [16].

Histopathological Studies

Portions of the kidney from all the experimental groups were fixed in 10% formalin, dehydrated in alcohol and then embedded in paraffin. Microtome sections (5µm thick) were prepared from each liver sample and stained with hematoxylin-eosin (H&E) dye. The sections were examined for the pathological findings.

Statistical Analysis

The experimental outcomes were expressed as Mean±SEM for six animals in all groups. All parameters were analyzed statistically using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test (DMCT) using Graph Pad prism 5.0 software [17]. Data were considered statistically significant at $P<0.05$.

Results

Acute Toxicity Study

In present study, the ethanolic extract of bark of *Tricholepis Glaberrima* was tested for its acute toxicity study to find out the LD_{50}. It was found that acute administration of the extract using 2000 and 4000mg/kg dose did not cause any mortality in rats, hence the LD_{50} of the extract is considered as 4000mg/kg. Based on the recorded LD_{50} dose, $1/5^{th}$ and $1/10^{th}$ of the dose is considered as therapeutic dose, and therefore a dose of 200mg/kg and 400mg/kg is selected for the evaluation of its nephroprotective activity.

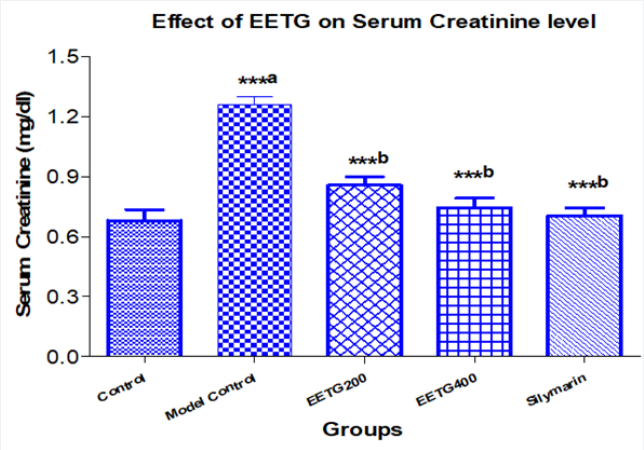

Figure 1: Effect of EETG on level of serum creatinine, Blood Urea Nitrogen & Uric Acid.

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Effect of EETG on the Level of Serum Creatinine, Blood Urea Nitrogen and Uric Acid of Treated Rats

The effect of EETG on the level of creatinine, blood urea and uric acid were done. The effect found in the level of creatinine, blood urea and uric acid are mentioned. Oral administration of ethanolic extract of bark of *Tricholepis Glaberrima* at dose of 200 and 400mg/kg found equally significant in reducing the increased level of creatinine and blood urea (*** P<0.001), however, the reduction in the level of uric acid was more effectively reduced by 400mg/kg as compared to 200mg/kg (*P<0.05 and **P<0.01 respectively). The standard drug silymarin (25mg/kg, i.p.) produced more effective reduction (***P<0.001) in the uric acid level as compared to both doses of the ethanolic extract of *Tricholepis Glaberrima* bark, however silymarin showed similar reduction in the level of serum creatinine and blood urea of gentamicin induced nephrotoxic rats (Figures 1-3).

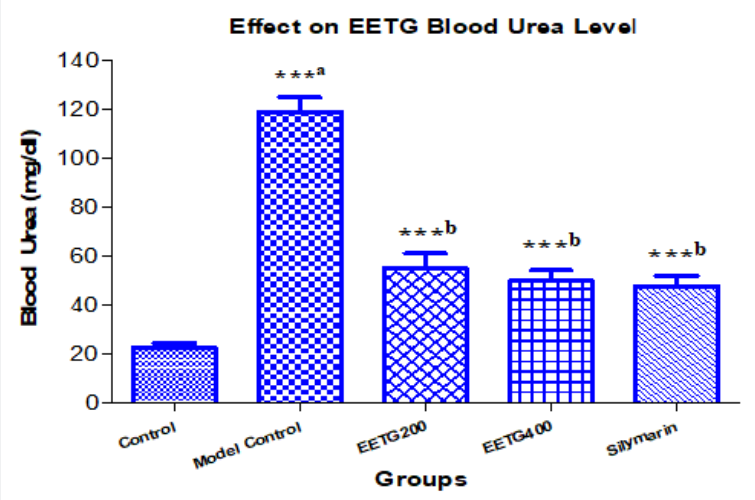

Figure 2: Effect of EETG on level of serum creatinine, Blood Urea Nitrogen & Uric Acid.

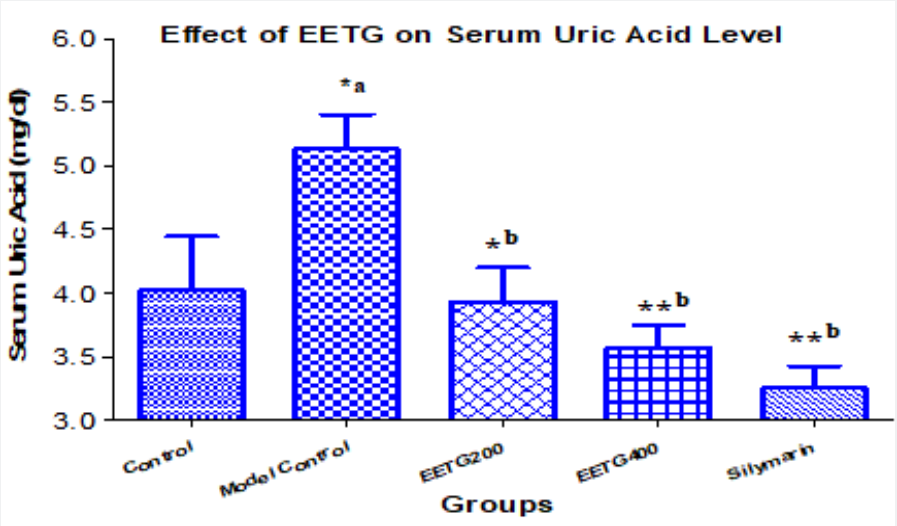

Figure 3: Effect of EETG on level of serum creatinine, blood urea nitrogen & uric acid.

Effect of EETG on Kidney Weight of Treated Rats

After 7 days of treatment of gentamicin induced nephrotoxicity in rats, we found that the kidney weight is increased significantly (###P<0.001) in control group by gentamicin administration as compared to normal group of rats (0.71±0.014 and 0.57±0.014 respectively). Treatment with the EETG using dose of 200 mg/kg and 400 mg/kg produced similar significant reduction (***P<0.001; 0.60±0.015 and 0.57±0.01 respectively) in the increased weight of kidney of gentamicin induced nephrotoxic rats. Silymarin also produced similar and significant reduction (***P<0.001; 0.55±0.01) in the kidney weight as produced by EETG at both doses (Figure 4).

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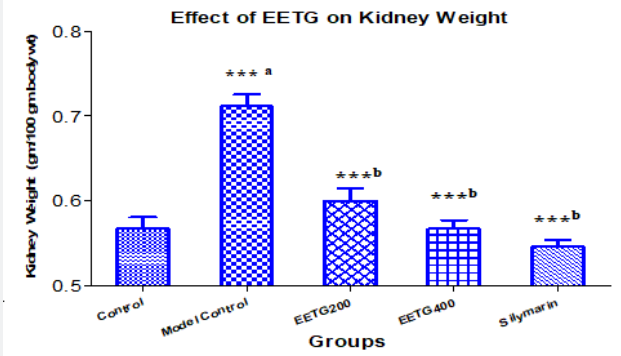

Figure 4: Effect of EETG on kidney weight of gentamicin induced nephrotoxicity in rats.

Effect of EETG on Serum Level Of SGPT, SGOT and ALP

After 7 days, the level of SGPT, SGOT and ALP in the serum were also found, and it was found that, administration of gentamicin (100mg/kg, i.p.) only for 7 days produced significant increase (### P<0.001) in the level of SGPT, SGOT and ALP (93.45, 86.19 and 78.52 U/L, respectively) as compared to the these levels in untreated rats (42.68, 45.25 and 36.56U/L, respectively) (Figures 5-7). It was observed that SGPT level was effectively reduced by both selected doses of ethanolic extract of *Tricholepis Glaberrima* bark, however, EETG400 mg/kg dose produced highly significant reduction(***P<0.001) in SGPT level as compared to EETG 200mg/kg (**P<0.01). EETG 400mg/kg showed similar significant reduction in SGPT level as reduction (***P<0.001) produced by standard drug silymarin (25mg/kg) (Figure 5). Similarly, for SGOT level, it was found that EETG 400mg/kg dose produced higher significant reduction (***P<0.001) in SGOT level as compared to EETG 400mg/kg dose (**P<0.01), and equivalent reduction as silymarin (Figure 6).

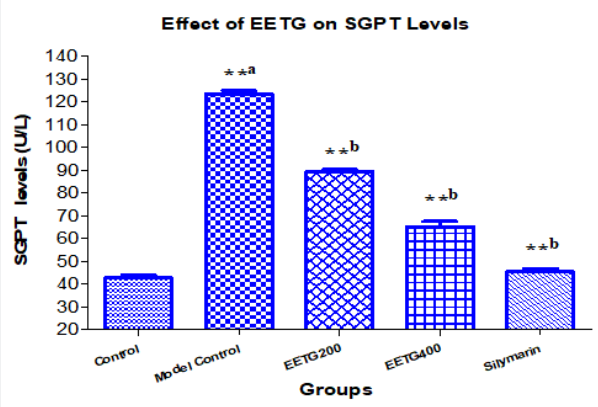

Figure 5: It was observed that SGPT level was effectively reduced by both selected doses of ethanolic extract of *Tricholepis Glaberrima* bark, however, EETG 400mg/kg dose produced highly significant reduction(***P<0.001) in SGPT level as compared to EETG 200mg/kg (**P<0.01). EETG 400mg/kg showed similar significant reduction in SGPT level as reduction (***P<0.001) produced by standard drug silymarin (25 mg/kg).

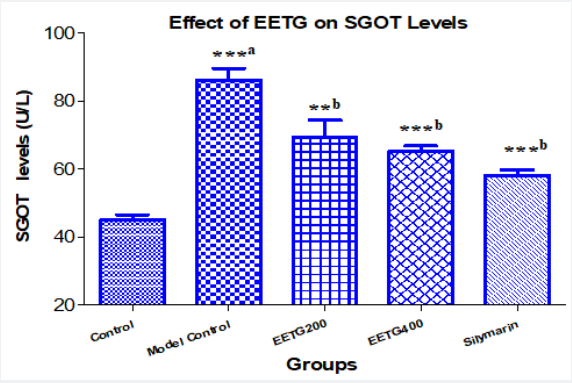

Figure 6: Similarly, for SGOT level, it was found that EETG 400mg/kg dose produced higher significant reduction (***P<0.001) in SGOT level as compared to EETG 400 mg/kg dose (**P<0.01), and equivalent reduction as silymarin.

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Effect of EETG on ALP of Gentamicin Induced Nephrotoxicity in Rats

The effect of EETG on the levels of ALP of the treated rats were also recoded, and it was found that administration of gentamicin to the rats for 10 days produced significant increase (###P<0.001) in the level of ALP. Simultaneous treatment with EETG (200mg/kg and 400mg/kg) and silymarin caused significant reduction in the ALP level, but EETG 400mg/kg was more effective as compared to EETG 200mg/kg, however, lesser effective than the standard drug silymarin (25mg/kg) in the reduction of ALP (Figure 7).

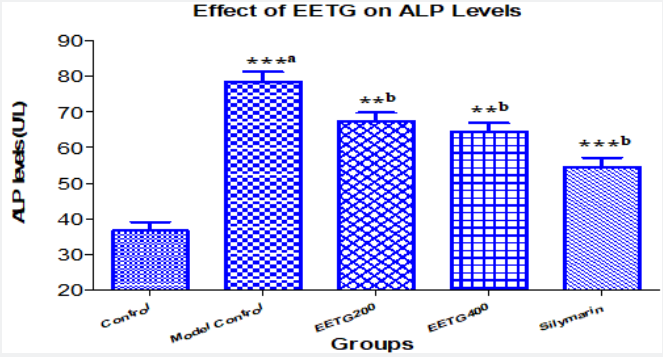

Figure 7: Effect of EETG on ALP of gentamicin induced nephrotoxicity in rats.

Effects of EETG on Oxidative Stress Parameters in Different Groups

Oxidative stress parameters of kidneys were measured. A significant (P<0.001) increase in MDA while declines in GSH levels (P<0.01), SOD (P<0.001) and CAT (P<0.001) activities were found in control as compared to normal. Treatments with EETG (200 & 400mg/kg) and Silymarin (25mg/kg) exhibited significant decrease in MDA levels while significant elevation in GSH level, SOD and CAT activity as compared to control (Figure 8-11).

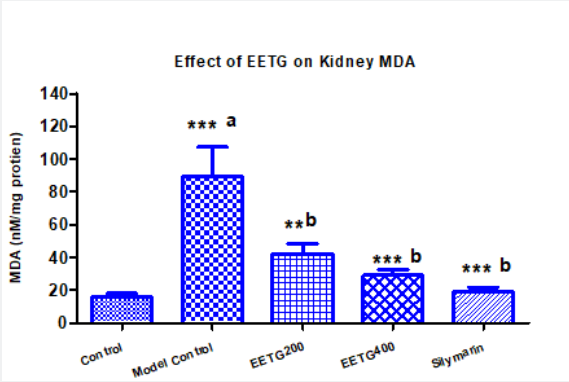

Figure 8: Effect of EETG on MDA, GSH, SOD assnd CAT.

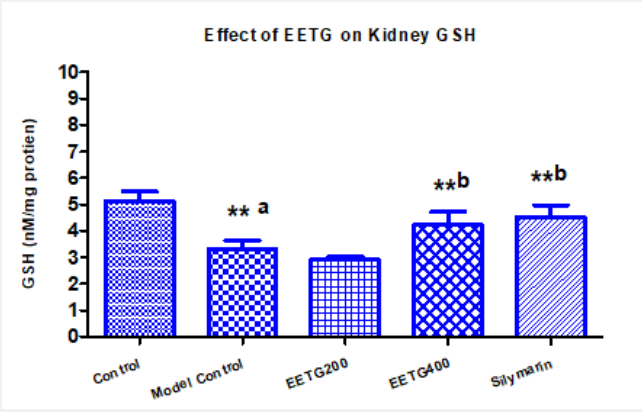

Figure 9: Effect of EETG on MDA, GSH, SOD assnd CAT.

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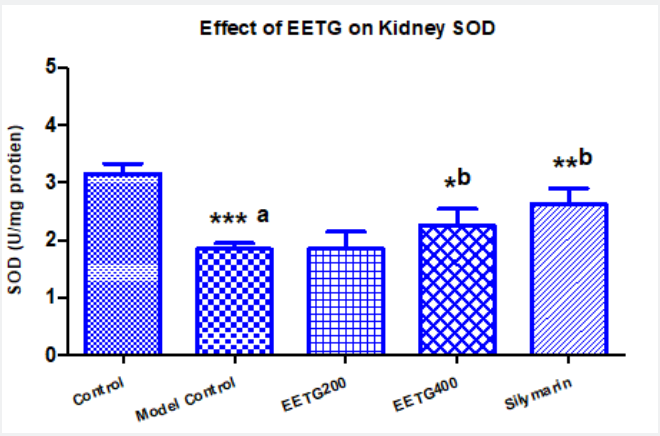

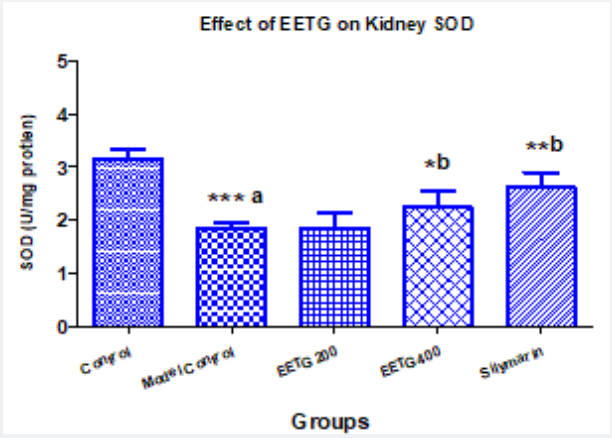

Figure 10: Effect of EETG on MDA, GSH, SOD assnd CAT.

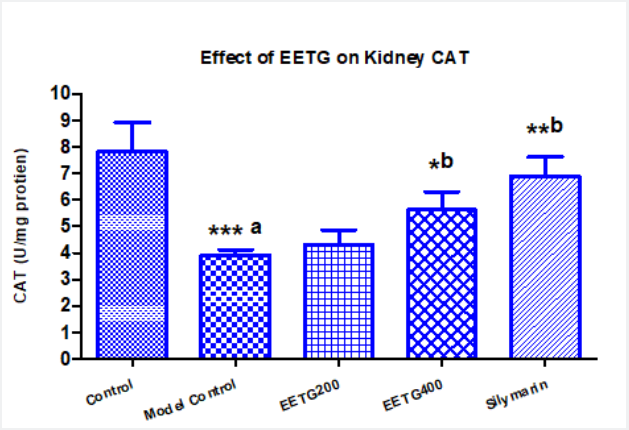

Figure 11: Effect of EETG on MDA, GSH, SOD assnd CAT.

Effects of EETG on Histopathological Changes of Different Groups

In Gentamicin induce nephrotoxicity model normal control rats (Figure 12A) showed normal glomerular and tubular histology whereas gentamicin treated group was found to cause distorted tubular shape, cellular infiltration of the tubules (tubulitis), glomerular and blood vessel congestion, and also result in the presence of inflammatory cells in kidney sections (Figure 12B). The concurrent treatment with the EETG (250 & 400mg/kg, p.o) reduced such changes in kidney histology (Figure 12C & 12D, 12E). Discussions Nephrotoxicity induced by gentamicin is characterized by a decrease in the glomerular filtration rate and tubular injury due to the formation of reactive oxygen species (ROS), which may be directly involved in membrane lipid peroxidation, mesangial cells contraction, which alters the filtration surface area and modify the ultrafiltration coefficient and decrease the glomerular filtration rate [18]. Management of such renal hemodynamic abnormality and reduction of the same are important to prevent the deterioration of normal functions of kidney. Formation of non-protein nitrogenous compound such as urea, uric acid and creatinine takes place due to degradation of these protein and nucleic acids [19]. Changes in the levels of serum urea, creatinine and uric acid concentrations and LFTs strongly suggested impairment of kidney and liver function in nephrotoxicity. Administration of EETG dose dependent manner decreased the levels of serum urea, creatinine and uric acid and LFTs parameters in treated groups significantly.

Several experimental evidences have suggested that gentamicin causes cell damage in the kidney by stimulating ROS production [20-21]. Moreover, when exposed to ROS, the kidneys of the rats that received gentamicin, suffered more because of reduced antioxidant defense system enzymes [22]. In the present study Oxidative stress parameters of kidney homogenate were measured. A significant increase in MDA while declines in GSH content, SOD and CAT activities were found in Gentamicin treated group. Administration of with EETG (200 & 400 mg/kg, p.o) significantly decrease MDA levels while significant elevation in GSH level, SOD and CAT activity dose dependent manner as compared to Gentamicin control. Therefore, it seemed that EECS, due to its antioxidant properties, reduced cellular damages in kidneys. This nephroprotective activity of the EECS may be due to antioxidant activity which may be due to the presence of fla-

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vonoids and phenolic compounds as reported in our study. The results of our study reveal the nephroprotective activity of EETG. The probable mechanism for its protection against cellular damage may be due to its antioxidant activity as evident by its in vivo stress parameters improvements.

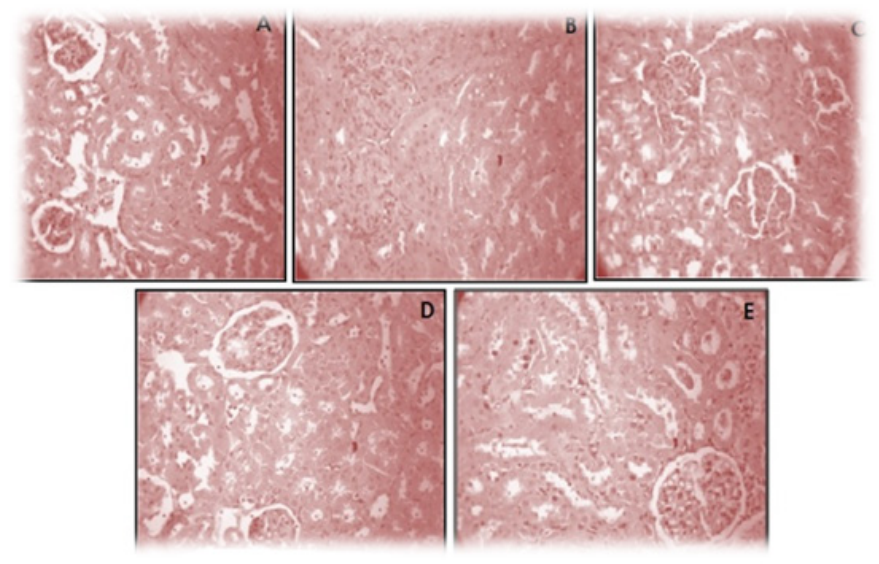

Figure 12: Nephrotoxicity Model Normal Control Rats.

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Conflict of Interest

The authors declare no competing financial interest.

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