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NEPHROPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF CUCUMIS MELO LINN. IN GENTAMICIN INDUCED NEPHROTOXICITY

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ABSTRACT

The seed of Cucumis melo Linn. is having diuretic, lithotriptic, laxative, demulcent and cooling properties. The present investigation was carried out to evaluate the nephroprotective activity of methanolic extract of Cucumis melo (ME-CM) seed kernel in gentamicin-induced nephrotoxicity. The ME-CM was administrated orally (190 mg/kg/d) for 8 days. Gentamicin was administrated at the dose of 100 mg/kg daily in neck region subcutaneously from 4th to 8th day. Gentamicin (alone) treated group showed increased levels of blood urea nitrogen and serum creatinine, which were significantly retrieved in group pretreated with ME-CM. The antioxidant study revealed that the level of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) were increased with decrease in malondialdehyde (MDA) content in ME-CM pretreated group when compared with gentamicin alone treated group. The histopathological analysis also showed the protective nature of ME-CM in gentamicin-induced renal damage. In conclusion, the histopathological and biochemical parameters confirmed that the ME-CM protect against gentamicin induced renal damage probably through its antioxidant activity.

Key words: Antioxidant, Cucumis melo, gentamicin, nephrotoxicity

INTRODUCTION

Cucumis melo Linn. belongs to the family cucurbitaceae. The seeds are having diuretic, lithotriptic, laxative, demulcent and cooling properties. In traditional system of medicine (Unani medicine) seed kernel is commonly used in renal disorders such as kidney and bladder stones, painful and burning micturiation, ulcers in the urinary tract, suppression of urine and it has also been used for other ailments like jaundice, vitiligo, ascites, chronic fevers, inflammation of the liver and kidney, and in general debility [1-3]. Phytochemical studies revealed that the seeds of Cucumis melo contain chromone derivatives, phenolic glycoside, arginine, aspartic and glutamic acids, alpha-galactosidases, dihydroxy triterpenes, sitosterol 2, and beta-sitosterol, etc.[4-8].

Number of chemicals and therapeutic agents are associated with the development of renal failure. One of them is Gentamicin. It is an aminoglycoside antibiotic, widely used in the treatment of Gram-negative infections.

Study carried out by Erbay et al. [9] has mentioned that the aminoglycosides antibiotics were prescribed in 12.1% of the cases in the intensive care unit where an antibiotic was necessary. Even though gentamicin causes the major adverse effects of nephrotoxicity and ototoxicity, this is being continuously used in clinical practice due to its high bactericidal efficacy, low-cost and limited bacterial resistance. Aminoglycoside-induced nephrotoxicity is typically characterized by tubular necrosis, without gross morphological changes in glomerular structures [10] and increase in plasma creatinine and urea levels leading to severe renal failure [11].

There are several mechanisms have been suggested by various researches regarding gentamicin induced nephrotoxicity. One of the mechanisms is supposed to be related with generation of reactive oxygen species (ROS) in kidney [10, 12-14]. Since the gentamicin induced renal damage cause by generation of ROS, a number of antioxidant agents have been showed to prevent gentamicin induced nephrotoxicity in various studies [15-17].

The body has developed several endogenous antioxidant defense systems classified into two groups such as enzymatic and non enzymatic. The enzymatic defense system includes different endogenous enzymes like SOD, CAT, GPx, glutathione reductase (GR) and non enzymatic defense system are vitamin E, vitamin C and GSH [18-19]. Large numbers of research studies have been proved that gentamicin altering the concentrations of antioxidant systems which is responsible for renal injury and co-administration of plant extract and phytoconstituents ameliorated the gentamicin-induced change in the antioxidant system [20-23].

Previous studies have proved the antioxidant properties [24-28] and diuretic activity [29] of Cucumis melo seed. But nephroprotective activity of this seed has not been studied till date. Considering the beneficial role of the seed in kidney disorders and its phytoconstituents, the present study aimed to evaluate nephroprotective activity of methanolic extract of Cucumis melo seed kernel against gentamicin induced nephrotoxicity.

Materials & Methods

Chemicals and equipments

All the equipments obtained from Scientific Systems, New Delhi and Remi Motors Ltd, Mumbai. The gentamicin purchased from Fulford (India) Ltd., Hyderabad and all the other chemicals from Sigma Chemicals Co., and E. Merck, India.

Plant material

Cucumis melo Linn. seed kernels were purchased from Khari Baoli, local market of Delhi and authenticated by Dr. H. B. Singh, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. A voucher specimen No. NISCAIR/RHMD/Consult/-2010-11/1657/255. Sample specimen is deposited in the herbarium of NISCAIR, New Delhi.

Extraction of test drug

100 g crushed seed kernels of Cucumis melo Linn. were extracted by refluxing with 100% methanol in distillation flask over boiling water bath for 6 – 8 h. It was removed from the water bath and allowed to cool at room temperature and filtered. The plant material obtained after filtration was re-extracted twice by the same procedure. All three extracts were collected together (methanolic extracts) and methanol was recovered by distillation method under reduced pressure. The methanolic extract left after removal of the solvent, was coded as ME-CM (Methanolic extract of Cucumis melo) and this was 10.84 % w/w.

Experimental animals

All the experiments were carried out in albino rats of Wistar strain, weighing between 150-180 g, supplied by the Central Animal House Facility of Jamia Hamdard, New Delhi (Registration no. 173/CPCSEA). All animals were housed in groups in polypropylene cage and maintained on a standard pellet diet (Amrut Laboratory rat and mice feed, New Maharashtra Chakan Oil Mills Ltd., Mumbai) and water ad libitum. The animals were kept under standard laboratory conditions at $25 \pm 1^{\circ}C$ temperature.

Experimental design

Experiments were performed in accordance with the guidelines for the care and use of laboratory animals, laid down by the Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA), Ministry of Social Justice and Empowerment, Govt. of India, Jan. 2000. The rats were divided into four groups of six animals each. Group 1 (Control) received 1% CMC in distilled water (10 mg/kg/d, p.o), for 8 days. Group II (Toxicant) received 1% CMC in distilled water (10 mg/kg/d) for 8 days. Group III received 190 mg/kg/d of ME-CM (p.o), suspended in vehicle, 10 ml/kg for 8 days. Group IV received 190 mg/kg/d of ME-CM (p.o), suspended in vehicle, 10 ml/kg for 8 days. In addition to this, the animals in groups II and III were co-administrated subcutaneously daily with gentamicin in a dose of 100 mg/kg in neck region in a volume of 1 ml/kg for last 5 days [30-31].

Estimation of different parameters

At the end of the experiments on 9th day the animals were anaesthetized with anesthetic ether and sacrificed. The blood samples were collected from retro-orbital plexus and left at room temperature for 2 h. The blood samples were centrifuged for 30 minuets at 5000 rpm to separate the serum. The sera were estimated for concentration of BUN and serum creatinine. Kidneys were removed for estimation of malondialdehyde (MDA) formation, Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Reduced glutathione (GSH), and for histopathological examination.

Estimation of Blood Urea Nitrogen (BUN)

BUN was estimated by diacetyl monoxime method [32].

Estimation of serum creatinine (Scr)

Scr was estimated by the alkaline picrate method [33].

Post-mitochondrial supernatant (PMS) preparation

Kidneys were removed quickly, cleaned free of extraneous material and immediately perfused with ice-cold saline (0.85% sodium chloride). The kidneys were homogenized in chilled phosphate buffer (0.1 M, pH 7.4) containing KCI (1.17%) using a Potter Elvehjen homogenizer. The homogenate was filtered through muslin cloth, and was centrifuged at 3000 rpm for 10 min at 4 ºC by Eltek Refrigerated Centrifuge (model RC 4100 D) to separate the nuclear debris. The aliquot so obtained was centrifuged at 12000 rpm for 20 min at 4 ºC to obtain post-mitochondrial supernatant (PMS), which was used as a source of enzymes.

Estimation of MDA formation (TBARS)

The assay for microsomal lipid peroxidation was done following the method of Wright et al. [34] as modified by Khan et al. [35]. The reaction mixture in a total volume of 1.0 ml contained 0.58 ml phosphate buffer (0.1 M, pH 7.4), 0.2 ml microsomes, 0.2 ml ascorbic acid (100 mM) and 0.02 ml ferric chloride (100 mM). The reaction mixture was incubated at 37 ºC in a shaking water bath for 1 h. The reaction was stopped by addition of 1.0 ml 10% trichloroacetic acid (TCA). Following addition of 1.0 ml 0.67% thiobarbituric acid (TBA), all the tubes were placed in boiling water-bath for 20 min and then shifted to crushed ice-bath before centrifuging at 2500 X g for 10 min. The amount of malondialdehyde formed in each of the samples was assessed by measuring optical density of the supernatant at 535 nm using spectrophotometer (Milton Roy 21 D) against a reagent blank. The results were expressed as nmol MDA formed/h/g tissue at 37 ºC using molar extinction coefficient of 1.56 X 10^5/M/cm.

Superoxide dismutase (SOD) activity

SOD activity was measured by Stevens et al. [36].

Assay for catalase activity (CAT)

Catalase activity was assayed by the method of Claiborne [37].

Assay for glutathione peroxidase activity (GP_X)

Glutathione peroxidase activity was assayed by the method of Mohandas et al. [38].

Estimation of reduced glutathione (GSH)

Reduced glutathione was determined by the method of Jollow et al. [39].

Histopathological analysis

The kidney tissue in all the study groups was fixed by immersion in buffered formalin and processed through the sequential steps of dehydration in graded alcohol, clearing in xylol and finally embedded in paraffin for histopathological evaluation. 3µm thick sections were cut, mounted on glass slides stained with Hematoxylin and Eosin. Examination was done under the light microscope (Olympus Cx21i).

Statistical analysis

Statistical evaluation was done by one way analysis of variance (ANOVA) followed by Dunnett's post hoc t-test. The P-values of less than 0.05 have been considered significant.

Results

Protective effect of BUN and Serum creatinine was observed. Significant changes observed in BUN (28.35 ± 2.79, $p<0.01$) and Scr (1.562 ± 0.169, $p<0.01$) in gentamicin (toxicant) group when compared with control (BUN: 10.14 ± 0.904; Scr: 0.739± 0.033). Pretreatment with ME-CM showed significant inhibition in gentamicin induced BUN (11.38 ± 1.05, $p<0.01$) and Scr (0.810 ± 0.058, $p<0.01$) when compared with the toxicant group. ME-CM alone treatment didn't show any significant changes as compared with control.

MDA formation was measured to detect renal injury by lipid peroxidation due to oxidative stress damage by gentamicin. The effect of methanolic extract of Cucumis melo (ME-CM) (p.o.) on the levels of LPO in normal rats' kidney PMS is showed in table 1. Gentamicin treatment alone raised renal MDA levels from 3.29 ± 0.27 in control group to 9.20 ± 0.32 ($p<0.01$) in gentamicin 100 mg/kg/d treated rats (Table 1). Increased levels of MDA in gentamicin treated group were reduced by co-treatment with ME-CM, 190 mg/kg (4.72 ± 0.49; $p<0.01$). There was no significant different in MDA level control and ME-CM alone treated group.

Renal GSH content and antioxidant enzymes (CAT, SOD, GPx) level were estimated to check restoration of these parameters by pretreatment of ME-CM, 190 mg/kg in gentamicin induced nephrotoxicity. GSH content (0.65 ± 0.04) and antioxidant enzymes level [CAT (141.9 ± 6.98); SOD (146.3 ± 3.9), GPx (268.2 ± 16.2)] of kidney PMS in control group is shown in Table 3. The gentamicin (alone) treatment significantly diminished the level of GSH content (0.32 ± 0.04; $p<0.001$) and antioxidant enzymes level [CAT (54.51 ± 4.2, $p<0.001$); SOD (101.9 ± 2.4, $p<0.001$), GPx (128.1 ± 9.0, $p<0.001$)] as compared with control. However, co-administration ME-CM, 190 mg/kg, showed significant increased level of GSH (0.56 ± 0.06, $p<0.01$) and antioxidant enzymes [CAT (120.1 ± 5.2, $p<0.01$); SOD (138.2 ± 3.0, $p<0.01$), GPx (229 ± 18.0, $p<0.01$) when compared with toxicant group. There were no significant different found in control and ME-CM alone treated group in GSH content and antioxidant enzymes level.

Histopathological findings of gentamicin treated group showed destruction of tubular epithelial cells and formation of casts in tubular lumen with normal glomerular limits (Fig. 1). In ME-CM pretreated group showed mild damage to tubular epithelial cells with no changes in glomeruli (Fig. 2). Control (Fig. 3) and ME-CM (Fig. 4) alone treated groups showed a normal tubules and glomeruli.

Discussion

Over the last several years it has become more apparent that the kidney is seriously affected by variety of chemicals and therapeutic agents. There are large numbers of medicinal plants extracts, phytoconstituents and dietary antioxidants have been reported to show protective effects against various nephrotoxins.

Aminoglycosides are natural or semi-synthetic antibiotics with a heterocyclic structure formed by two or more amino-sugars linked by glycoside bonds to an aminocyclitol ring [10]. They show a poor degree of oral absorption and hence intravenous administration is the route usually used in patients with severe infections. A relatively large amount (about a 10%) of the intravenously administered dose is accumulated in the kidney, whereas little distribution of aminoglycosides into other tissues is observed [10]. Despite nephrotoxicity and ototoxicity, the aminoglycosides are continuously being used in clinical practice because of their bactericidal efficacy, synergism with β-lactam agents, low-cost, limited bacterial resistance and post-antibiotic effects [40].

Gentamicin induced nephrotoxicity is a model of acute renal failure caused by oxidative stress [41] generated through the induction of superoxide. The toxicity of gentamicin is believed to relate with generation of reactive oxygen species (ROS) in kidney [10, 12]. It has been demonstrated that gentamicin-induced nephrotoxicity is characterized by direct tubular necrosis, which is localized mainly in the proximal tubules.

Several pharmacological uses of Cucumis melo have been documented in past. However, the nephroprotective activity of seeds of Cucumis melo have not been proved scientifically till date but this has been used in traditional system of medicine to cure various kidney disorders since long time. Therefore, in the present study the effect of methanolic extract of Cucumis melo seed kernel was investigated on gentamicin-induced nephrotoxicity in rats. The protective effect of renal was evaluated by biochemical parameters such as blood urea nitrogen and serum creatinine, MDA, GSH content, SOD, GPx and CAT activity and by histopathological examination of kidney.

Gentamicin in a dose of 100 mg/kg induced nephrotoxicity has been documented in several studies [30-31]. Urea is the principal final product of protein metabolism in the body and elevation of the urea concentration in blood signifies inadequate kidney function. In kidney dysfunction, creatinine is retained in the blood and plasma creatinine level may be grossly elevated. Knowledge of slight elevation is valuable to diagnosis of renal disease. Previous studies demonstrate that the gentamicin reduces the glomerular filtration rate which leads impairment in glomerular function which is accompanied with increase level of blood urea and serum creatinine [20-23]. In present study, subcutaneous administration of gentamicin at 100 mg/kg daily for 8 days caused renal dysfunction, which was confirmed by increase level of blood urea (279.58%) and serum creatinine (211.36%). Co-administration of higher dose of ME-CM (190 mg/kg), (p.o.) with gentamicin subcutaneously prevented the rise of blood urea nitrogen and serum creatinine level 93.19 and 91.37 % respectively (Table 1). This shows protective effect of ME-CM (190 mg/kg) against gentamicin induced renal injury.

Lipid peroxidation refers to the oxidative degradation of lipids [42]. The products of lipid peroxidation are highly destructive. The malondialdehyde is often used to assess oxidative stress. Gentamicin induced oxidative damage was evident by increase in the MDA level in gentamicin (alone) treated group which was inhibited (75.8%) by co-administration of ME-CM (190 mg/kg) with gentamicin treated group.

Antioxidants are any substance that delay or inhibits oxidative damage to a target molecule. At a time one antioxidant molecule can react with single free radicals and are capable to neutralize free radicals by donating one of their own electrons, ending the carbon-stealing reaction [18]. The body has developed several endogenous antioxidant defense includes different endogenous enzymes like SOD, CAT, GPx, glutathione reductase (GR) and non enzymatic defense system which include vitamin E, vitamin C and GSH [18-19].

Catalase is a common enzyme found in nearly all living organisms that are exposed to oxygen, where it catalyzes the decomposition of hydrogen peroxide to water and oxygen [43]. Superoxide dismutase (SOD) is an important endogenous antioxidant enzyme act as the first line defense system against ROS which catalyse the breakdown of the superoxide anion into oxygen and hydrogen peroxide [18, 43]. As such, they are an important antioxidant defense nearly in all cells exposed to oxygen. Glutathione peroxidase (GPx) is an enzyme that catalyzes the breakdown of hydrogen peroxide and organic hydro peroxides [18, 43]. GSH is a tripeptide and a powerful antioxidant present within the cytosol of cells and is the major intracellular nonprotein thiol compound [18]. Thus, the activities of these enzymatic and non enzymatic anti-oxidants are used as indicator for oxidative stress. In present study gentamicin induced decrease in CAT, SOD, GPx and GSH were significantly inhibited by co-administration of methanolic extract of Cucumis melo (ME-CM). This shows the free radical scavenging potential of ME-CM.

Effect of 8 days treatment of methanolic extract of Cucumis melo (ME-CM) (190 mg/kg) on renal function was investigated in normal rats. No significant decrease or increase was found in biochemical parameters in the test drug treated animals compared with control animals (Table 1and 2). Therefore, it is clear that there is no any adverse effect in kidney due to use of methanolic extract of Cucumis melo.

Further, the histopathological changes induced by gentamicin administration significantly protected by pretreatment with ME-CM which correlates with biochemical parameters. Hence it is suggested that methanolic extract of Cucumis melo is potentially useful for the prevention of renal toxicity during gentamicin chemotherapy. However, further studies are necessary with fractionations of this seed extract to isolate and characterize the phytoconstituents responsible for this activity of Cucumis melo seed kernel.

Conclusion

The toxicity of gentamicin is believed to relate with generation of reactive oxygen species (ROS) in kidney and administration of antioxidants have proved marked protection against gentamicin-induced impairment of renal function. Hence, it may be concluded that methanolic extract of Cucumis melo seeds kernel showed nephroprotective action against gentamicin nephrotoxicity possibly through its antioxidant property. This is further supported by the results of histopathological and biochemical parameters of this study.

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Tables and figures

Table 1: Results methanolic extract of seed kernel of Cucumis melo on BUN, Scr and MDA in gentamicin induced nephrotoxicity.

Groups	Treatment	BUN (mg/dl) Mean ± SE	Scr (mg/dl) Mean ± SE	MDA (nmol of MDA formed/g tissue) Mean ± SEM
Group I (Control)	Vehicle	10.14 ± 0.904	0.739± 0.033	3.270.07
Group II (Toxicant)	Gentamicin	28.35 ± 2.79##	1.562 ± 0.169##	9.20 ± 0.32##
Group III (Test drug + Toxicant)	ME-CM + Gentamicin	11.38 ± 1.05** (93.19 %)	0.810 ± 0.058** (91.37 %)	4.72 ± 0.49** (75.80 %)
Group IV (Only test drug)	ME-CM only	10.01 ± 0.697	0.742 ± 0.052	3.13 ± 0.79

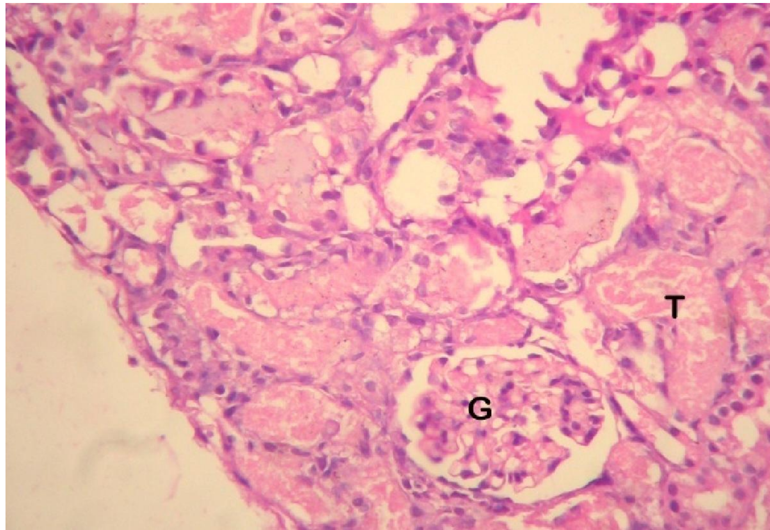
Each value represents the mean ± S.E.M from 6 animals in each group. ##p<0.01, when compared with control group; **p<0.01, when compared with toxicant group. Values given in the parenthesis indicate inhibition.

Table 2: Effect of higher dose of methanolic extract of Cucumis melo (ME-CM) on CAT, SOD, GP_X and GSH in gentamicin induced nephrotoxicity

Groups	Treatment	Catalase [nmol H_2O_2 consumed/ min/mg protein]	SOD (μM epinephrine oxidized/min/ mg protein)	Glutathione peroxidase [nmol NADPH oxidized/min/ mg protein]	Reduced glutathione [nmol GSH/g tissue]
Group I (Control)	Vehicle 10 ml/kg	141.9 ± 6.98	146.3 ± 3.9	268.2 ± 16.2	0.65 ± 0.04
Group II (Toxicant)	Gentamicin 100 mg/kg	54.51 ± 4.2$^{###}$	101.9 ± 2.4$^{###}$	128.1 ± 9.0$^{###}$	0.32 ± 0.04$^{###}$
Group III (Test drug + Toxicant)	ME-CM (190 mg/kg) + Gentamicin	120.1 ± 5.2**	138.2 ± 3.0**	229 ± 18.0**	0.56 ± 0.06**
Group IV (Only test drug)	ME-CM 190 mg/kg	141.2± 4.65	145.6± 4.70	266.4±12.4	0.65± 0.06

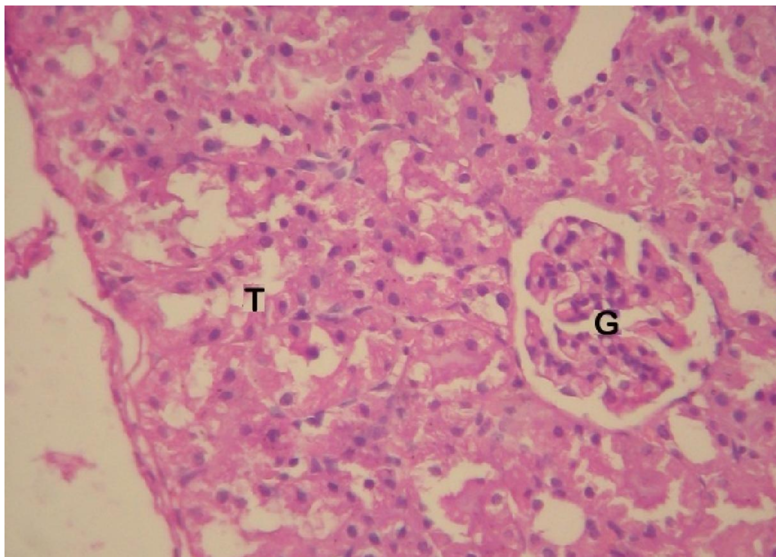
Each value represents the mean ± S.E.M from 6 animals in each group. $^{###}p<0.001$, when compared with control group; $^{**}p<0.01$, when compared with toxicant group.

Fig. 1 Histopathological findings of gentamicin alone group



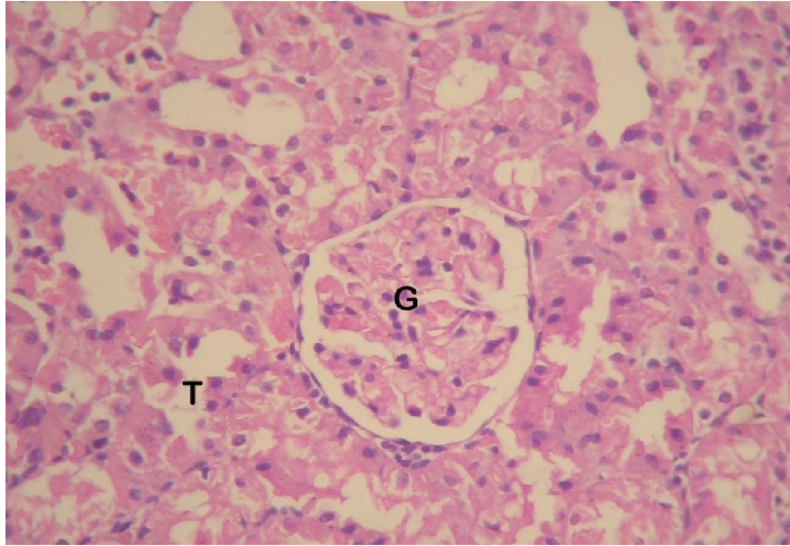
High power photomicrograph of kidney showing destruction of tubular (T) epithelial cells and formation of casts in tubular lumen, glomerulus (G) is within normal limits.

Fig. 2 Histopathological findings of pretreatment with ME-CM prior to gentamicin administration



High power photomicrograph of kidney showing mild damage to tubular (T) epithelial cells. No casts are seen. The glomeruli (G) do not show any damage.

Fig. 3 histopathological findings of control group



High power photomicrograph of kidney showing a normal tubules (T) and glomeruli (G).

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