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# Nephrotoxicity and Urolithiasis Activity of *Solanum nigrum*

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## ABSTRACT

Nephrotoxicity and Urolithiasis was caused due to risk factors like age, sex, diet, geographic location, systemic local medical condition, genetic predisposition and urinary composition. The objective of the study was to assess the Nephroprotective and Antirolithiasis activity of *Solanum nigrum* Linn. Leaves against Gentamycin induced nephrotoxicity in wistar rats. Albino Wistar rats (30) of male sex were divided into 5 groups of six animals each. Group II administered with Gentamycin (40mg/kg, I.P) for 30 days along with Calculi Producing diet (CPD) which serves as negative control group. Groups III and IV were given consecutive doses of plant extract (200mg/kg; 400mg/kg, p. o.,) respectively until 30 days. The study evaluates Serum Urea and Creatinine, total protein in urine, urinary pH and wet kidney weight and Antioxidant enzyme levels such as SOD, CAT and LPO in renal tissue homogenate. The 400mg/kg EESN showed significant Nephroprotective and Antiurolithiatic activity which may be owed to the presence of important constituents like glycoproteins, polyphenolic compounds and flavonoids.

**KEY WARDS:** Solanum nigrum Linn, Nephrotoxicity, Urolithiasis, Gentamycin, Flavonoids.

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## INTRODUCTION

Nephrotoxicity denotes the disruption of normal cellular functions of mitochondria and membrane integrity in the epithelial cells lining glomerulus and renal tubules. It induces renal injury through intratubular obstruction such as crystal disposition and promotes cellular swelling and tubular luminal occlusion (osmotic effects). Medications can also cause chronic renal failure leading to chronic interstitial injury and papillary necrosis [1, 2] A Kidney stone is also known as renal calculus is a solid concretion or crystal aggregation formed in the kidneys from dietary minerals in the urine. Kidney stones typically leave the body by passage in the urine stream. Ureteral obstruction causes post renal azotemia and hydronephrosis which leads to pain in the flank, lower abdomen and groin (a condition called renal colic). Surgical therapy includes Ureteral Stent Placement, Percutaneous nephrostomy [3]. Though these drugs are effective in the treatment of nephrolithiasis, they will contribute sufficiently for enough side effects, hence the usage of herbal medicines has increased because of lesser side effects. Herbalism, a traditional medicinal or folk medicinal practice based on the use of plants and plant extracts. Hrebalism is also known as Phytotherapy. The scope of herbal medicine is extended as a way to learn about potential future medicines [4]. Herbs showing nephroprotective and antiurolithiasis activity have been studied and some significant observations have been reported (Rubia cordifolia, Moringa oleifera, Didymocarpus pedicellata, Aerva lanata, Helianthus annus Linn, Arachis hypogeal Linn, Hemidesmus indicus). The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability. Solanum nigrum Linn. is well known plant tradinally it has been used as Anti-Inflammatory, Antioxidant, Antitumorigenic, Antiulcerogenic, Hepatoprotective, Antipyretic, Diuretic and Nephroprotective. But Nephroprotective activity is not explored scientifically so the present study is to evaluate the Nephroprotective and Antiurolithiasis activity of *Solanum nigrum* Linn. leaves in Wistar rats.

## MATERIALS AND METHODS

The leaves of the plant *Solanum nigrum* Linn, were collected and authenticated by Prof. P. Jayaraman. Ph.D. Director, Plant Anatomy Research Center (PARC). The voucher specimen no. is PARC/2011/1020 and it was submitted to SRM College of Pharmacy. The leaves of *Solanum nigrum* Linn. were dried in shade and powdered by mechanical grinder. The powder of the leaves were initially defated with petroleum ether ( 60 - 80˚C) followed by 1000 ml of ethanol (95%) by using a Soxhlet

extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extract was filtered using whattman filter paper (No 1) and then concentrated in vaccum and dried at 45˚C for ethanol elimination and the extract was kept in a sterile bottle under refrigeration condition of about 2-8˚C[5]. The animals were maintained as per the norms of CPCSEA/ORG/CH/2008/Regd.No.1219 and cleared by CPCSEA each experimental group consisted of five animals housed in separate cages.

## *Test for phytochemical analysis*

The extracts were analyzed for the presence of alkaloids, terpenoids, reducing sugars, Saponins, tannins, Carbonyls, Flavonoids, Phlobatannis and steroids [5] .

## *Experimental animals*

Albino Wistar rats of male sex (150-200g) were obtained from King Institute, Guindy.

## *Chemicals*

Gentamicin, Ammonium oxalate, ethanol, thiobarbituric acid, Nitro blue tetrazolium chloride (NBT), Trichloro acetic acid (TCA), 5, 5-dithio bis-2-nitrobenzoic acid (DTNB), picric acid.

## *Estimation kits*

Urea, Creatinine and total protein were estimated by commercially available kits.

## *Induction of nephrolithiasis*

Male Wistar rats weighing 150-200g were administered with Gentamicin subcutaneously with a single dose of 40 mg/kg body weight and Calculi Producing Diet (CPD) in rat feed for 30 days to induce Nephrolithiasis.

## *Methodology*

Group I After acclimatization of animals, they were administered with vehicle for 30 days of experimental period.

Group II Animals were administered with 40mg/kg/day of Gentamicin subcutaneously along with CPD and vehicle for 30 days.

Group III Animals were administered with inducing agents along with EESN (200mg/kg) for 30 days.

Group IV Animals were administered with inducing agents along with EESN (400mg/kg) for 30 days.

Group V Animals were administered with inducing agents along with standard drug (cystone) for 30 days. After experimental period all biochemical parameters and histopathological studies were evaluated by respective methods.

## *Collection of Blood*

Blood will be collected from the animals at the end of experimental period by Retro-Orbital puncture in Eppendorf tubes containing anticoagulant to obtain serum.

## *Estimation of Serum Creatinine and Urea*

At the end of the experimental period, animals were given mild anaesthesia and blood was collected by retro-orbital sinus puncture into the vials. Serum was obtained by cold centrifugation ($4^0$ C) of the vials for 10min at 3000 rpm. Serum Creatinine and Urea levels were evaluated using commercially available kit procedure in Semi Auto Analyzer [6]

## *Collection of Urine Samples*

Urine samples were collected from the animals after experimental period using Metabolic Cages.

### 1. Estimation of Total Protein

Urine samples were collected and evaluated using commercially available kit procedure in Semi Auto Analyzer [7]

### 2. Estimation of Urinary pH

Urinary pH values were measured using a microelectrode and an Orion pH meter. [8]

### 3. Estimation of Wet Kidney Weight

After experimental period animal were sacrificed and kidneys were excised. The wet weight of the kidneys was evaluated [9]

## *Preparation of kidney homogenate*

Kidneys were excised, weighed and homogenized in chilled Tris buffer (10mM, pH 7.4) at a Concentration of 10% (w/v) (using Remi tissue homogenizer in a serrated pestle). Homogenates were centrifuged at 10,000rpm for 20 min ($4^oC$) in a high speed cooling centrifuge and supernatant was used for further estimation of Antioxidant Parameters.

## *Antioxidant parameters*

### Estimation of lipid peroxidation

**Procedure:** Lipid peroxidation products of tissue homogenate were determined as thiobarbituric acid reactive substances (TBARS). Kidneys of control and treated groups were homogenised in ice-cold 0.9% saline to get 10% homogenate. 0.2ml of homogenate was added with tricarboxilic acid (TCA) ,mixed well and centrifuged at 4000rpm for 20 min. To the supernatant thiobarbituric acid was added and heated for 20 min in boiling water bath. The test tubes were cooled and used for determination of TBARS at 532nm against a reagent blank. TBARS was expressed as n.mol/g protein [10]

### Estimation of SOD

**Procedure:** 0.1ml of tissue homogenate was taken in a test tube containing 1.2ml of sodium pyrophosphate buffer ($p^H$ 8.3, 0.052M). To this mixture 0.1ml of Phenazine methosulphate (186 µM) and 0.3ml of 300µM Nitroblue tetrazolium were added. The reaction was started by addition of 0.2ml of NADH (750µM) the reaction mixture was incubated for 90seconds at $30^0$C. The reaction was stopped by addition of 0.1ml glacial acetic acid. The inhibition rate of NBT reduction was spectrophotometrically determined at 560nm. One unit of SOD is defined as the amount of enzyme required to reduce the NBT by 50%. The activity of SOD was expressed as units/mg protein [11].

### Estimation of catalase

**Procedure:** The tissue homogenate was added with buffer and $H_2O_2$ solution. The absorbance at 240nm was monitored. The activity of Catalase was expressed as Units/mg protein [12]

### Histopathological findings

After the experimental period the Kidney sections of 4 µm of thickness were obtained and fixed in formalin solution. They were stained with hematoxilin-eosin (H&E) [13] and observed under microscope. The tubular area with histopathological alterations like cytoplasmic vacuolization, necrosis and stones was examined.

### Statistical analysis

All the values were expressed as mean ± S.E.M. All the data was analyzed using one way analysis of variance (ANOVA) followed by Bonferroni Multiple Comparison Test.

# RESULTS

## *Percentage yield of EESN*

The shade dried leaves of Solanum nigrum Linn. Weighing about 300g was extracted by Soxlet extraction method using 95% ethanol and the extract was evaporated to dryness using Rotatory Vaccum Evaporator. The weight of ethanolic extract obtained was 24.77g. Its percentage yield is calculated by the following formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract obtained}}{\text{Weight of Crude powder}} \times 100$$

The percentage yield of ethanolic extract of Solanum nigrum (EESN) is 8.3% w/w.

## *Phytochemical constituents*

The Phytochemical studies revealed the presence of alkaloids, steroids, glycosides, reducing sugar, resins, flavonoids, and phlbotannins the absence of, Carbonyl Compounds, saponins terpenoids and cardiac glycosides.

## *Effect of EESN on urinary parameters in rats*

The effect of EESN on urinary parameters showed a significant decrease in the levels of total protein, increase in pH of urine and decrease in the kidney weight when compared to the negative control group, which is tabulated in Table No.6.1 and the graphical representation of these parameters was depicted in Figure 6.1, and 6.2 respectively.

## *Effect of EESN on serum biochemical parameters*

A significant reversion of elevated levels of serum urea and creatinine in groups treated with EESN as compared to negative control group was tabulated in Table No. 6.2 and their graphical presentation was showed in Figure 6.4 and 6.5 respectively.

## *Effect of EESN on antioxidant enzyme levels in renal tissue*

Groups treated with extract showed a significant increase in the levels of antioxidant enzymes such as SOD and Catalase when compared to negative control group and decreasing LPO indicated by decreased levels of Malondialdehyde. The results are tabulated in Table No.6.3 and graphically represented in Figure 6.6, 6.7 and 6.8 respectively.

**Table no. 6.1: effect of EESN on urinary parameters in rats**

| S. No. | Treatment | Total protein mg/dl | Urinary pH | Wet Weight of Kidney (g) |
|---|---|---|---|---|
| 1 | Control | 17.26 ± 0.03 | 7.34 ± 0.03 | 0.59 ± 0.008 |
| 2 | Negative Control | 40.79 ± 0.05[a]*** | 6.11 ± 0.006[a]*** | 0.76 ± 0.006[a]*** |
| 3 | EESN 200mg/kg | 34.21 ± 0.04[b]*** | 6.73 ± 0.01[b]*** | 0.70 ± 0.009[b]*** |
| 4 | EESN 400mg/kg | 20.09 ± 0.02[b]*** | 7.11 ± 0.008[b]*** | 0.64 ± 0.007[b]*** |
| 5 | Standard Drug | 18.40 ± 0.15[b]*** | 7.23 ± 0.008[b]*** | 0.61 ± 0.006[b]*** |

All values are expressed as mean ± SEM (n=6); Data were analysed by ANOVA followed by Bonferroni Multiple Comparison Test. Compared Control Vs Negative control: [a]****p< 0.05*; Compared Negative control Vs Group III, IV and V: [b]****p<0.05*; ns- Statistically not significant.

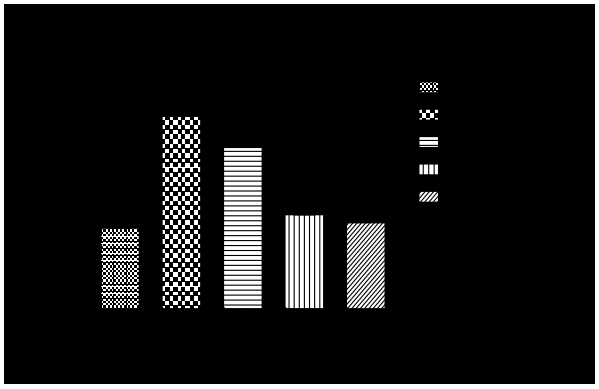


**Figure 6.1: Effect of EESN on protein content**

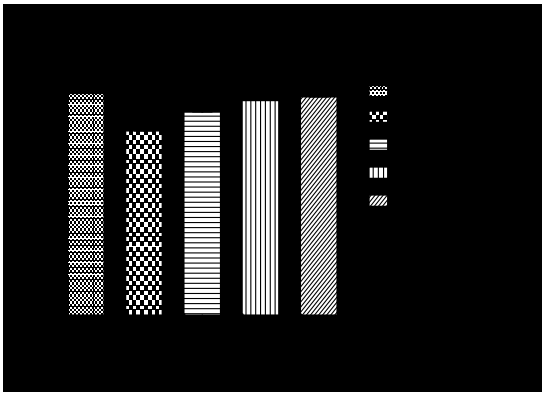


**Figure 6.2: Effect of EESN on urinary PH**

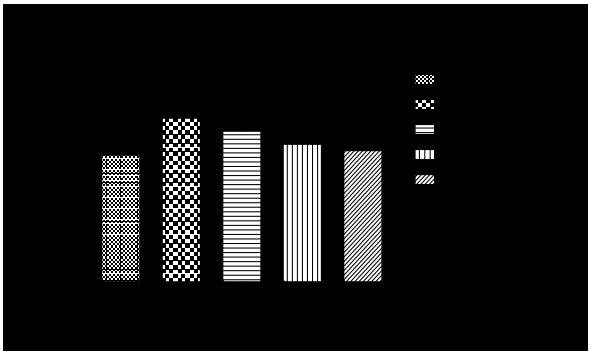


**Figure 6.3 Effect of EESN on wet weight of kidney**

**Table no. 6.2: effect of EESN on serum biochemical parameters**

| S. No | Treatment | Urea (mg/dl) | Creatinine (mg/dl) |
|---|---|---|---|
| 1 | Control | 27.42 ± 0.04 | 0.94 ± 0.01 |
| 2 | Negative Control | 46.08 ± 0.05[a]*** | 4.05 ± 0.06[a]*** |
| 3 | EESN 200 mg/kg | 40.10 ± 0.30[b]*** | 3.12 ± 0.01[b]*** |
| 4 | EESN 400 mg/kg | 33.58 ± 0.13[b]*** | 2.36 ± 0.03[b]*** |
| 5 | Standard | 29.72 ± 0.11[b]*** | 1.07 ± 0.01[b]*** |

All values are expressed as mean ± SEM (n=6); Data were analysed by ANOVA followed by Bonferroni Multiple Comparison Test. Compared Control Vs Negative control: [a]****p< 0.05*; Compared Negative control Vs Group III, IV and V: [b]****p<0.05*; ns- Statistically not significant.

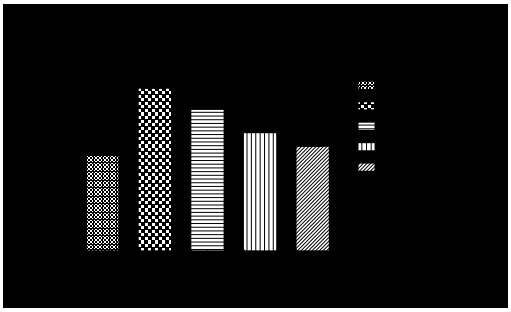


**Figure 6.4 effect of EESN on serum urea levels**

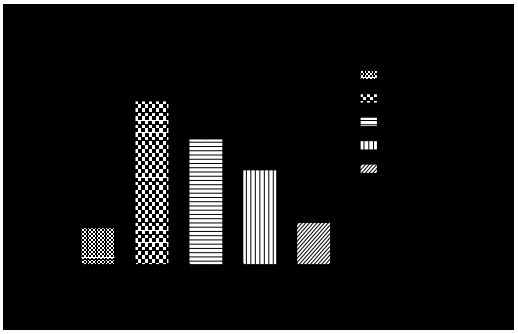


**Figure 6.5 Effect of EESN on serum Creatinine levels**

**Table no. 6.3: effect of EESN on antioxidant enzyme levels in renal tissue**

| S. No. | Treatment | SOD (U/mg protein) | Catalase (U/mg protein) | MDA (no. moles/mg protein) |
|---|---|---|---|---|
| 1 | Control | 9.75 ± 0.16 | 4.49 ± 0.08 | 4.49 ± 0.17 |
| 2 | Negative Control | 4.33 ±0.07[a]*** | 1.02 ± 0.03[a]*** | 10.86 ± 0.28[a]*** |
| 3 | EESN 200 mg/kg | 4.84 ± 0.07[b ns] | 2.23 ± 0.07[b]*** | 9.08 ± 0.17[b]*** |
| 4 | EESN 400 mg/kg | 7.11 ± 0.10[b]*** | 3.11 ± 0.07[b]*** | 6.12 ± 0.12[b]*** |
| 5 | Standard | 8.24 ± 0.21[b]*** | 3.49 ± 0.09[b]*** | 5.30 ± 0.21[b]*** |

All values are expressed as mean ± SEM (n=6); Data were analyzed by ANOVA followed by Bonferroni Multiple Comparison Test. Compared Control Vs Negative control: [a]****p< 0.05*; Compared Negative control Vs Group III, IV and V: [b]****p<0.05*; ns- Statistically not significant.

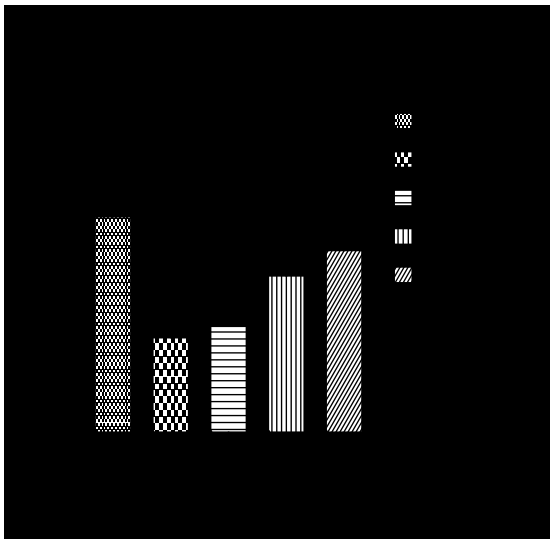
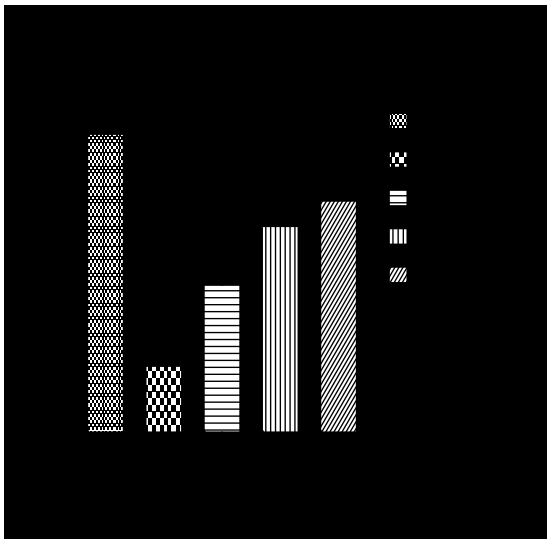


**Figure 6.6 effect of EESN on sod enzyme levels**



**6.7 Effect of EESN on catalase enzyme levels**

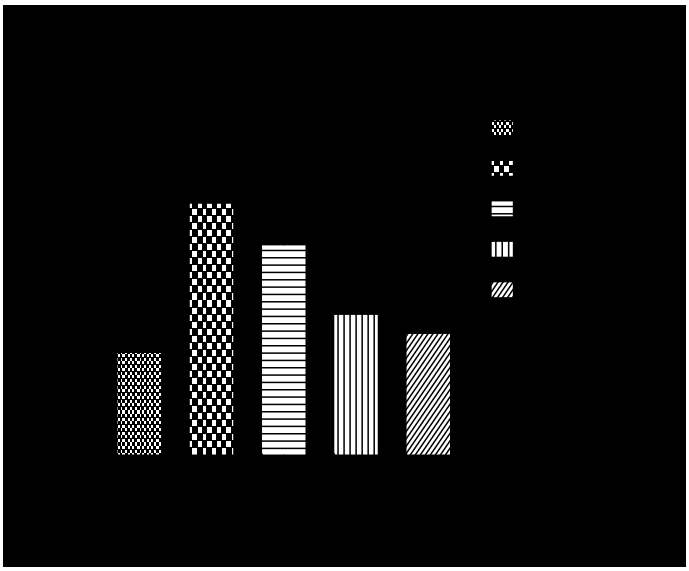


**Figure 6.8 Effect of EESN on MDA levels**

### *Histopathology*

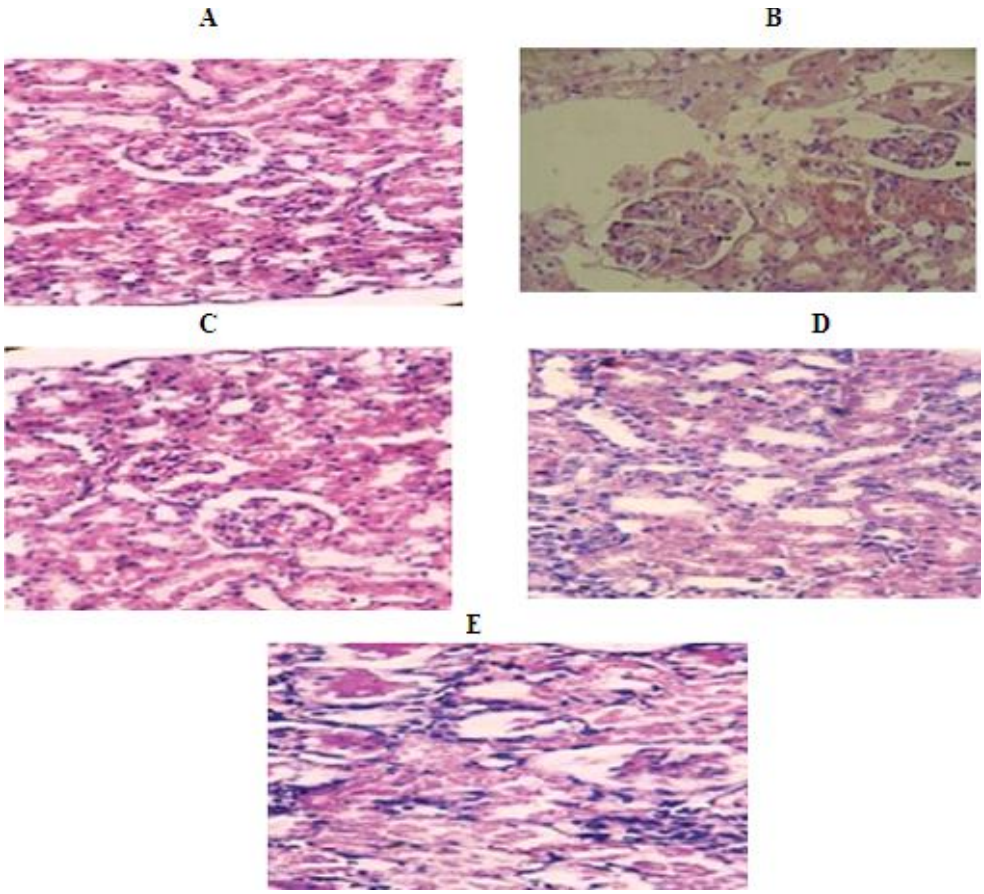



**Figure 6.9:** Histological Sections of Kidney. **A.** Control group-Normal glomerular structure. **B.** Negative control-Showing necrosis of renal tubular membrane, vacuoles. **C.** EESN 200mg/kg- Showing regeneration of renal tissue and reduction in swelling. **D.** EESN 400mg/kg-Showing recovery of renal tubular membrane from necrosis and dialated tubules **E.** Standard-showing regeneration of the renal tissue and normal glomerulus.

## DISCUSSION

Gentamycin (40mg/kg) a widely used aminoglycoside antibiotic for the treatment of gram-negative bacterial infections. Treatment with EESN caused a significant reduction in urinary excretion of calcium and oxalate therefore reducing the supersaturation of urine. This might be responsible for dissolving and also in preventing the formation of stones. This is supported by the histological studies and wet kidney weight. On histological examination, the negative control group (II) showed calcium oxalate crystals in majority of tubules and weight of kidneys when compared to the control group. Group III and IV showed a very few crystals, indication the ability of EESN in dissolving the calculi and comparatively

decrease in kidney weight. Group V administered with standard showed less stone formation when compared to negative control group.

In the present work the pH of the negative control group II is decreased to 5.0-6.0 when compared to control group I (pH 6.0-7.0). EESN treated groups III and IV reversed the acidic pH to normal. This increase in urinary pH might be responsible for dissolution of complexes which contributes to their significant antiurolithiatic activity. In the current work antiurolithiatic activity of *Solanum nigrum* may be due to its diuretic activity which is attributed to the presence of glycoprotein's and flavanoids.

Parameters such total protein content in urine are used to study the extent of renal damage induced by GM and CPD. In group II there is significant increase in urinary excretion of total protein when compared to group I, which could be associated with necrosis of proximal tubules, the primary site of drug accumulation. In groups treated with EESN (III, IV) and group V there is reduction in levels of total protein in urine as compared to group II, which could be due to the ability of EESN to partially ameliorate the tubular necrosis.

In group II negative control there is significant increase in the levels of N-compounds such as urea and creatinine in blood, condition called Azotemia which is due to in sufficient filtration of blood by the kidneys when compared to group I. In group III, IV and V there is significant reduction in blood levels of urea and creatinine when compared to group II.

In the present study, we evaluated the protective effects of EESN against GM and CPD induced NepHrotoxicity in rats. In our study administration of GM resulted in oxidative damage to the lipids and proteins of the kidney in rats. There was a significant decrease in SOD and Catalase activities in rats treated with GM and CPD (Group II). However rats treated with extract (Group III, IV) and group V could restore the antioxidant capacity when compared to group II.

The levels of TBARS were significantly increased negative control group II when compared to group I. In group III and IV there is a significant decrease in TBARS levels as compared to group II. In the current work the antioxidant property of *Solanum nigrum* may be owed to the presence of polypHenolic compounds, glycoproteins and flavanoids.

The plant extract of doses 200mg/kg and 400mg/kg along with standard group V showed significant decrease in total protein, wet weight of kidney, urea, creatinine and lipid peroxidation and significant increase in Urinary pH, SOD and catalase in a dose dependent manner at $p<0.05$ when compared to negative control group. The extract dose at 400mg/kg showed more significant results when compared to 200mg/kg and showed similar results as compared to standard group.

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