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Evaluation of Nephroprotective and Antioxidant Activities of Leaves of Tribulus Terrestris

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ABSTRACT

The present investigation was done in order to evaluate the nephroprotective activity of the alcoholic extract of *Tribulus terrestris* leaves on gentamicin-induced nephrotoxic rat model. Thirty Wistar rats were evenly divided into 5 different groups. Group I served as normal control, group II as diseased control, while group III were the treated group with standard drug vitamin-E. Group IV was pretreated with 250 mg/kg and group V with 500 mg/kg BW per day of *Tribulus terrestris*, one hour before each dose of the nephrotoxicants. On the 11th day, samples of blood and urine were taken for renal function test and urine analysis. Along with it, antioxidant studies were performed using the homogenate of the renal tissue of each group. Plant extract treated group showed a decrease in elevated serum creatinine, blood urea, total protein, urinary urea, urinary creatinine, urinary uric acid, which was further confirmed by histopathological study. It was observed that the high dose of 500mg/kg of *Tribulus terrestris* was more effective than the low dose of 250mg/kg. This indicates that the high dose of the plant extract possesses significant nephroprotective activity.

KEYWORDS: Nephrotoxicants, gentamicin, renal function test, antioxidant studies, *Tribulus terrestris.*

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INTRODUCTION

Healthy kidneys remove excess fluid, minerals, and wastes thereby cleaning the blood. They also produce hormones which keep the bones strong and blood healthy. But if the kidneys are damaged, they do not work properly. This is called renal failure[1].Nephrotoxicity occurs whenever the body is exposed to a drug or toxin that causes kidney damage. The kidney becomes unable to get rid of excess urine, and wastes from the body. Drugs cause approximately 20% of community and hospital-acquired episodes of acute renal failure[2].

Aminoglycosides like gentamicin cause nephrotoxicity by affecting the proximal tubular cells. Upon reaching the proximal tubule of the nephron, these agents undergo endocytosis and concentrate in lysosomes, Golgi body, and endoplasmic reticulum. Once a threshold is reached, the aminoglycosides empty into the cytosol and act on the mitochondria to induce apoptosis and necrosis. Furthermore, a number of transporters are inhibited in the proximal tubule, which affects tubular reabsorption and compromises cell viability. With continued damage, increased secretion of potassium and sodium can be observed with increased serum creatinine[3]. The injured kidney fails to excrete normal concentrations of urea from the blood. Hence serum urea level increases, suggesting the renal injury by the aminoglycoside therapy[4].

Several studies demonstrated that ROS (reactive oxygen species) may be important mediators in GM-induced nephrotoxicity. Abnormal production of ROS directly damages some macromolecules and induces cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation, and DNA damage [5].

Since administration of antioxidants attenuated the reduction in glomerular filtration rate. The administration of superoxide dismutase (SOD) for gentamicin-treated rats has been associated with a marked increase in renal blood flow, suggesting that oxygen is believed to be responsible for gentamicin-induced vasoconstriction. Laboratory experiments have shown that gentamicin increases ROS production and that renal cortical mitochondria are the source of ROS. Administration of antioxidants is beneficial in arresting renal damage produced by gentamicin[6].

Therefore, exogenous administration of antioxidant substances would have a beneficial effect on the cell's antioxidant system. In accordance, there are growing interests in using natural compounds to treat nephrotoxicity[7-8]. The medicinal plant *Tribulus terrestris* belongs to the family Zygophyllaceae. The plant is known by common names like puncture vine, yellow vine, and goathead. It can be found in Southern Europe, Southern Asia, throughout Africa and Australia[9]. Several studies highlight the protective effects of *Tribulus terrestris* and some of its biologically active molecules, mainly because it contains various chemical components such as steroidal saponin, flavonoids, flavonol glycosides, alkaloids, and tannins[10]. In Ayurveda, the plant has been used for

centuries for the treatment of impotence, sexual debility and venereal diseases[11]. To our knowledge, this is the first study to evaluate *Tribulus terrestris* effects against gentamicin-induced nephrotoxicity in rats. Therefore, this study aims to investigate the potential protective effects of *Tribulus terrestris* against kidney damage induced by administration of gentamicin.

MATERIALS AND METHODS

All reagents and chemical products that have been used in this study were of analytical grade. Lead acetate, Tween 80, Hydroxylamine Hydrochloric acid, 10% Formalin were purchased from Yarrow Chem Product Mumbai-400037 (India). Phosphoric acid, Ferrous sulfate, Thiobarbituric acid, Ascorbic acid were purchased from Loba Chemie Pvt. Ltd.

Preparation of the plant extract:

The leaves of the plant *Tribulus terrestris* was gathered from the Tirupathi, Andhra Pradesh, India in the month of June 2018. The plant species were validated by Dr. Mudha Chishti, Associate Professor, Department of Botany, Sri Padmavati Women's University, Tirupati, Andhra Pradesh. A voucher sample was submitted in the Rajiv Gandhi University of Health Science. The collected leaves were washedwith running water to remove the adhering soil, mud, and debris. The leaves were dried in the shade at room temperature to a fixed mass. Then the dried leaves were coarsely powdered using a blender. The final product was kept in an airtight container and was protected from light.

The coarse ground powder of *Tribulus terrestris* was placed into the extraction glass and the plant material was loaded into the main chamber of the Soxhlet extractor. The ground coarse powder was packed tightly in the Soxhlet extractor. For the extraction of the *Tribulus terrestris* leaves powder, methanol was used as a solvent. In this extraction process, 250 ml solvent was used and was carried for about 6 hours. The extract was again re-extracted under the same conditions to ensure complete extraction. The methanol was filled into the solvent vessel and extracted at a temperature of 75^{o}C for 6 hours. The solvent was drained into a beaker and then withdrawn from the extractor and dried. The final product was stored in dry airtight bottles for the pharmacological studies [12].

Wistar rats weighing between 150-200 gm and albino mice weighing between 20-25 gm were retained under standard laboratory conditions at room temperature (25±2 °C) with 12 hr light/dark cycle. The animals were given pellet chow and water ad libitum except during experimentation. The Institutional Animal Ethics Committee (IAEC) at Karnataka College of Pharmacy, Bangalore has given the acquiescence for study protocol. Studies were conducted as per the CPCSEA guidelines Reg No: 1564/PO/RE/S/11CPCSEA[13].

Acute toxicity study:

An acute toxicity study was conducted for the metabolic extract leaves of *Tibullus terrestris* as per OECD guidelines 425 using Swiss albino mice. Each animal was administered metabolic extracts by the oral route. The mice were observed for any changes continuously for the first 2 hours and up to 24 hours for mortality. No signs of mortality and noticeable behavioral changes were seen in any of the groups tested. The extract was found to be safe up to 5000 mg/kg body weight. A dose of 1/10th and 1/20th of 5000mg/kg were treated to be a high dose and low dose prepared by suspending in 2% twin 80. The doses were prepared according to the OECD guideline no. 425 [14].

Experimental procedure:

Animals were divided into 5 groups with six animals in each group. Group I- Control (untreated) treated with normal saline solution for 10 days, group II- diseased control, rats were injected (I.P.) with gentamicin only (80mg/kg body weight) for 10 days, group III– rats were treated with vitamin E, 250 mg/kg as standard nephroprotective agent, one hour before the I.P. injection of gentamicin (80mg/kg) for 10 days, group IV – rats were treated with *Tribulus terrestris* low dose (250mg/kg body weight) one hour before the I.P. injection of gentamicin (80mg/kg body weight) for 10 days, group V- rats were treated with *Tribulus terrestris* high dose (500mg/kg body weight) one hour before the I.P. injection of Gentamicin (80mg/kg body weight) for 10 days [15].

Urine sample collection:

The experimental animals were transferred to separate metabolic cages after the last day administration. For twenty-four hours, urine was collected. A drop of concentrated HCl was mixed with the collected urine, for prevention of growth of microbes and metal hydrolysis. The collected urine was measured and placed in a cleaned airtight container and used for the urine analysis [16].

Collection of blood samples:

After urine was collected from the animals, blood was withdrawn by cardiac puncture under mild ether anesthesia. The collected blood samples were allowed to coagulate for 10 minutes at room temperature and then centrifuged at 3000 rpm for 10 minutes. The supernatant was used for the study of biochemical parameters such as total protein, blood urea nitrogen, uric acid, creatinine, electrolytes [17].

Collection of tissue:

Light ether anesthesia was used to sacrifice the rats. The kidneys were removed, the left kidney was used for homogenate preparation and the right kidney was preserved in 10% formalin solution for histopathological examinations [18].

Renal function test:

The blood serum was collected and sent to the diagnostic center for the total renal function test. The Blood urea nitrogen, serum uric acid, serum creatinine[19],serum total protein, serum Albumin, serum electrolytes[20] and urine samples were collected from each group for estimation of urea[19], and urinary total protein[20].

Estimation of Superoxide dismutase (SOD):

Superoxide dismutase is an enzyme which increases the antioxidant defense against ROS by decreasing the steady state level oxygen. It scavenges the superoxide ions produced as cellular by-products. The ability of the enzyme to inhibit the auto-oxidation of pyrogallol is used in the determination of SOD activity[21].

Estimation of Catalase:

Through dismutation reaction, hydrogen peroxide is produced. CAT reduces it and also prevents the generation of hydroxyl radicals thereby it protects the cellular constituents from oxidative damage in peroxisome. The decomposition of H_2O_2 to H_2O and oxygen is catalyzed by the enzyme [22].

Estimation of Lipid Peroxidation:

Lipid peroxidation occurs because of oxidative stress in cellular lipids. By measuring thiobarbituric acid reacting substance (TBARS), oxidative stress can be measured. Higher the concentration of lipid peroxidation products, higher is the degree of oxidative stress. The increased level of TBARS attacks the polyunsaturated fatty acids in cell membranes and cause lipid peroxidation[23].

Estimation of glutathione peroxidase:

A known amount of enzyme preparation and hydrogen peroxide were mixed in the presence of glutathione for a specified time period. The remaining glutathione was then measured by Ellman's method [24].

Histopathological Analysis:

The kidneys were excised from the animals and washed with the normal saline. The whole of the kidney was placed in 10% neutral formalin for 12-24 hours. Then it was dehydrated and cleared with xylene and ethanol, respectively. This was followed by embedding in paraffin wax for preparation of blocks.A microtome was used to prepare sections of 5 µm thickness from blocks. These were processed in alcohol-xylene series and were stained with Harris hematoxylin and eosin stain and subjected to histopathological examination[25].

Statistical analysis:

The results are expressed as mean ± S.E.M from n=6 rats in each group. One-way analysis of variance (ANOVA) was used to assess the significance of difference among the groups, followed by Tukey's test compared between Normal control (Untreated) vs. all groups $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Animals treated with gentamicin showed a decrease in body weight when compared with the control group. However, animals treated with methanol extract of *Tibullus terrestris* (METT) and gentamicin showed an increase in body weight proportional to the dose compared to the gentamicin group (Table 1). There was a significant ($P <0.001$) increase in kidney weight in the gentamicin-treated group compared to the control group and dose-related decreases ($P <0.01$) and $P <0.001$ in kidney weight among animals treated with METT along with gentamicin. (Table 1)

A significant increase ($P<0.005$) in blood urea nitrogen, serum uric acid, serum creatinine, serum total protein, serum albumin, serum chloride, urea, urinary total protein were observed in gentamicin treated group compared to the control group and dose-dependent decrease ($P<0.005$) were seen on animals pretreated with METT along with gentamicin (Table 2).

A significant decrease ($P<0.005$) in superoxide dismutase, catalase, MDA levels, glutathione peroxidase were observed in gentamicin treated group compared to the control group and dose-dependent increase ($P<0.05$) and ($P<0.005$) were seen on animals pretreated with METT along with gentamicin (Table 3).

Table 1: "Body Weight and Kidney Weight of Rats."

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Initial body weight (g)	160.43±0.03	210.41 ± 0.02***	161.41 ±0.05##	147.01 ±0.01##	140.41 ±0.04##
Final body weight (g)	177.25± 0.04	180.52 ± 0.01***	170.02 ±0.06##	125.23±0.03##	130.23±0.09##
Kidneys weight (g)	1.39±0.05	2.11±0.07***	1.21±0.09 ###	1.59±0.05 ##	1.30±0.02 ###

The data are expressed as Mean ±S.E.M (n=6) rats in each group. *Values differ significantly ($P<0.001$) from the normal control group. # Values differ significantly ($P<0.001$) from the toxic control group.

Table 2: "Plasma Levels Of Blood Urea Nitrogen, Serum Uric Acid, Serum Creatinine, Total Protein, Albumin, Electrolytes, Urea And Urinary Total Protein of Rats."

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Blood urea nitrogen (mg/dL)	50.70± 0.94	129.55±0.92***	48.38±0.96###	65.40±0.91###	47.39±0.93###
Serum uric acid(mg/dl)	2.44±0.16	3.82±0.16***	2.54±0.17###	3.00±0.18##	2.72±0.19###

Serum creatinine (mg/dL)	1.31±0.06	2.94±0.06***	1.41±0.06###	2.01±0.09##	1.59±0.08###
Total protein(g/dl)	5.77±0.10	7.79±0.18**	5.98±0.11##	6.85±0.08#	6.41±0.11#
Albumin(g/dL)	1.86±0.09	3.08±0.09***	2.05±0.07###	2.46±0.09##	2.23±0.07##
Serum chloride(mmol/l)	198.7±0.03	246.7±0.03**	218.5±0.02##	238.7±0.03#	224.0±0.05##
Urea(mg/dL)	37.60±0.03	93.75±0.02***	40.36±0.04###	53.32±0.01###	41.63±0.03###
Urinary total protein(g/L)	2.19±0.06	3.60±0.05***	2.60±0.05##	3.09±0.05#	2.82±0.08##

The data are expressed as Mean ±S.E.M (n=6) rats in each group. *Values differ significantly (P<0.005) from the normal control group. # Values differ significantly (P<0.005) from the toxic control group.

Table 3: "SOD, CAT, MDA, and GSH contents in Kidney of Rats."

Parameters	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**
SOD (units/mg protein)	9.61±0.01	6.20±0.01***	9.00±0.01###	6.90±0.01##	8.52±0.01###
CAT (µmol H_2O_2/min/mg protein)	0.90±0.06	0.59±0.06***	1.10±0.06###	0.69±0.06##	1.00±0.06###
MDA level (mM)	5.09±0.08	11.32±0.09***	5.71±0.10###	8.31±0.09##	6.11±0.09###
GSH (µmol/g wet tissue)	5.18±0.06	2.87±0.06***	4.26±0.07###	3.48±0.06##	3.98±0.06###

The data are expressed as Mean ±S.E.M (n=6) rats in each group. *Values differ significantly (P<0.005) from the normal control group. #Values differ significantly (P<0.005) from the toxic control group.

The histopathological examination of the kidney of control group shows the normal structure of glomerulus with a tuft of capillaries, surrounded by Bowman's capsule [Fig. 1]. The group treated only with gentamicin shows loss of normal structure of glomerulus. Congestion of PCT and DCT shows deteriorative changes with loss of columnar epithelial cells and infiltration of mononuclear cells [Fig. 2]. Group treated with gentamicin and standard drug shows the normal structure of glomerulus with a tuft of capillaries surrounded by Bowman's capsule, proximal and distal convoluted tubules shows recovery from deteriorative changes and lined by columnar epithelial cells [Fig.3]. Group treated with gentamicin and a lower dose of METT shows the normal structure of glomerulus with a tuft of capillaries surrounded by Bowman's capsule with mild toxic changes,

proximal and distal convoluted tubules shows recovery from deteriorative changes and lined by columnar epithelial cells [Fig. 4]. Group treated with gentamicin and a higher dose of METT shows the normal structure of glomerulus with a tuft of capillaries surrounded by Bowman's capsule, proximal and distal convoluted tubules shows recovery from deteriorative changes with normal structure and lined by columnar epithelial cells [Fig. 5].

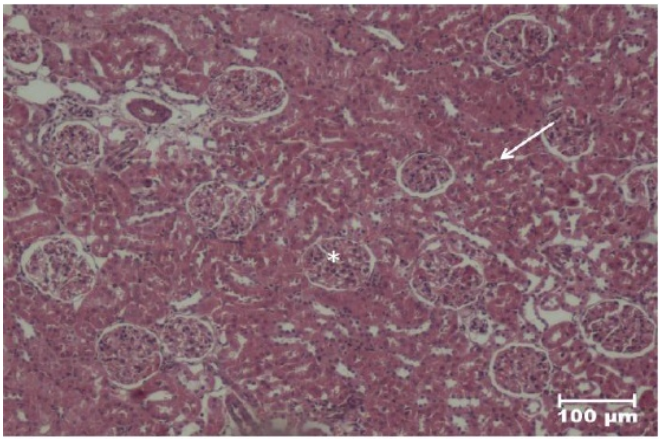

Figure 1: Histograms (×100) of kidney sections of rats in the normal control group.

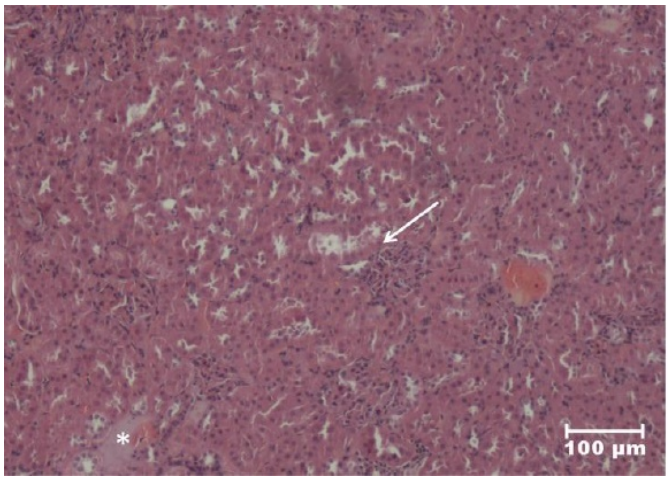

Figure 2: Histograms (×100) of kidney sections of rats in the gentamicin treated group.

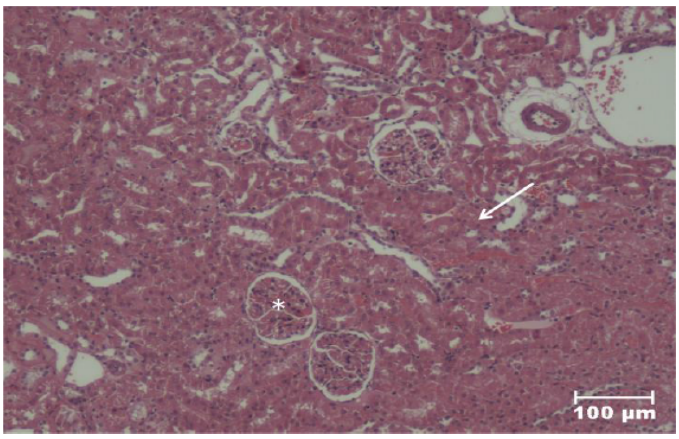

Figure 3:Histograms (×100) of kidney sections of rats in the Vitamin E treated group.

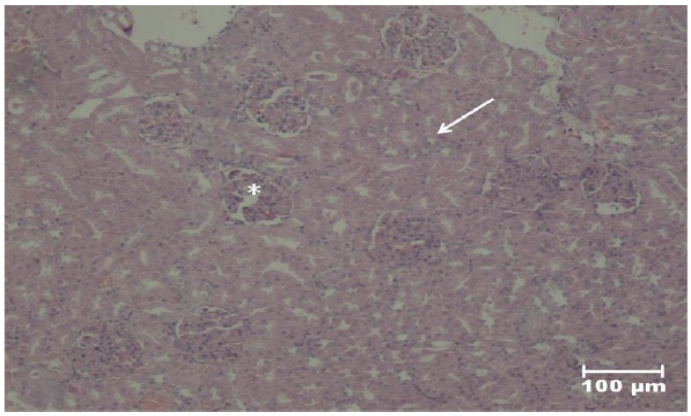

Figure 4: Histograms (×100) of kidney sections of rats in the METT treated group (250 mg/kg).

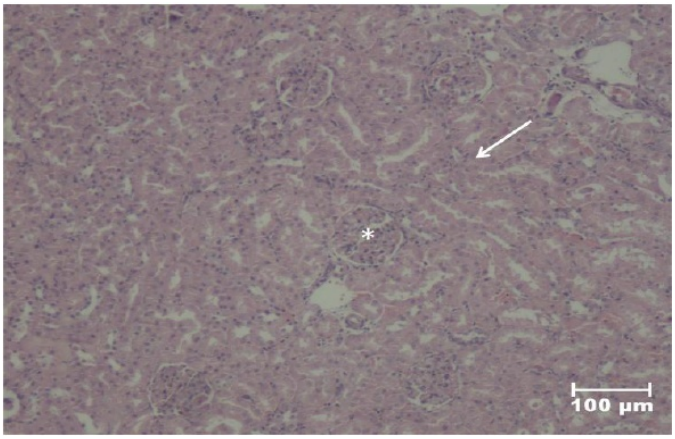

Figure 5: Histograms (×100) of kidney sections of rats in the METT treated group (500 mg/kg).

About 8-10% of the adult population suffers from kidney damage and millions of people die prematurely each year from complications associated with kidney failure[26].Since kidney failure has become one of the leading causes of death in the world, the development and treatment of kidney diseases have become a priority.

Thus the current investigation was carried out to evaluate the nephroprotective and anti-oxidant activity of methanolic extract of leaves of Tribulus terrestris in gentamicin-induced nephrotoxic rat. Upon initial phytochemical examination of the plant, it was found to be rich in antioxidant properties due to the presence of chemical components like steroidal saponins, flavonoids, flavonol glycosides, alkaloids, and tannins. As a result of which, the leaves of the plant *Tribulus terrestris* may possess nephroprotective activity.

Gentamicin belongs to aminoglycoside broad-spectrum antibiotics, useful in the treatment of infections caused by the gram-negative organism. When a dose 80mg/kg is given to rat for a period of 10 days, it causes nephrotoxicity. Gentamicin leads to a broad range of biochemical changes in the body like the elevation of serum creatinine, serum urea, serum uric acid, serum total protein, urinary urea, urinary uric acid, urinary total protein, and a decline in the levels of antioxidant enzymes like

superoxide dismutase, catalase, and glutathione peroxidase. This may be possible because of the marked necrosis of tubules, decreased glomerular filtration and loss of interstitium architecture[27].

Another mechanism involved in the initiation of nephrotoxicity is the accumulation of gentamicin in the nephron tubes, which subsequently results in ROS production, resulting in increased oxidative stress, reduced renal function, and total antioxidant activity in serum in gentamicin-treated animals compared to control animals[28].

The leaves of the plant *Tribulus terrestris* has antioxidant properties, which allow it to lower the lipid peroxidation by introducing an extra electron, as a result of which it stabilizes the unstable reactive oxygen species and ultimately prevents its generation. Thus, pre-treatment of rats with METT showed a significant reduction in elevated serum urea, serum uric acid, urinary total protein, serum total protein, serum creatinine, urinary uric acid and there was a marked reduction in necrosis of tubules.

The study result indicated that the administration of methanolic extracts of *Tribulus terrestris,*when givenat the dose of 250 and 500mg/kg body weight, possess nephroprotective activity in gentamicin-induced nephrotoxicity in rats. The plant extract contains various phytoconstituents which are rich in flavonoids, anthraquinones, and phenolic compounds. It is because of these constituents that it is able to show its protective activity. They are able to donate electrons to reactive radicals, converting them to more stable species, thereby preventing them from reaching the biomolecules like DNA amino acids, polyunsaturated fatty acids, lipoproteins, sugar, and proteins in biological systems. The nephroprotective effect of METT was confirmed by its prevention over the GM-induced toxicity. The METT reduced elevated kidney weight, serum potassium, chloride, serum urea and creatinine in gentamicin-treated rats. Histopathological studies proved that animal pretreated with METT decreased the gentamicin-induced induced renal damage. METT also possess the protective effect against the oxidative induced stress which may be due anti-oxidant property of the drug

In conclusion, all the observations in the present study may indicate that METT act as a protective agent against gentamicin-induced nephrotoxicity. But the experimental study should be followed by further experimental and clinical research to establish and exploit its protective role in drug-induced kidney injury.

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