

Antiinflammatory and Antioxidant Mediated Nephroprotection of Melatonin and Rosuvastatin in Carboplatin Induced Nephrotoxicity: An Experimental Study

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Platinum compounds like cisplatin, carboplatin used as anticancer drugs are known to cause nephrotoxicity. There is a need of drugs to prevent the drug induced nephrotoxicity. This experimental study was done to assess the nephroprotective role of rosuvastatin in carboplatin induced nephrotoxicity models and to compare it with melatonin. A total of 36 albino rats were randomly divided into 6 groups (n=6). Group I was control group. In Groups II to VI nephrotoxicity was induced by intraperitoneal administration of a single dose of Carboplatin 128mg/kg on day 5. In Group II, no drug was administered. In group III and IV melatonin 5mg/kg and 10mg/kg was administered orally once a day for 10 days. In group V and VI, rosuvastatin 10mg/kg and 20mg/kg was administered orally once a day for 10 days. Blood was collected on day 0 for the baseline values and at an interval of 7 and 11 days for biochemical and antioxidant estimation. Kidneys were dissected at the end of study, weighed and renal tissue was subjected to histopathological analysis. There was a significant decrease in urea, creatinine and uric acid in all the treatment groups. Also a significant decrease (p<0.05) was seen in MDA levels in treatment groups as compared to the negative control. A non-significant decrease was observed in IL-18 levels in the treatment groups. Also, histopathology of kidney tissues showed that in treatment groups, there were less changes in interstitium and vessels. Melatonin and rosuvastatin has shown a nephroprotective effect in carboplatin induced nephrotoxicity in terms of improved renal function tests, reduced IL-18 showing anti-inflammatory action, antioxidant action by decreasing MDA and increasing GSH and by histopathological studies of kidney tissue.

Keywords: Antioxidant; Carboplatin induced nephropathy; Melatonin; Rosuvastatin.

Carboplatin (cis-diammine-1,1-cyclobutane dicarboxylate platinum II), a platinum coordination compound and alkylating agent is used in ovarian, head, neck and lung carcinomas. Carboplatin has been reported to cause

hematological side effects like anemia, neutropenia leucopenia, and minimal nephrotoxicity. ¹The renal effects of carboplatin have been reported in high dose regimens and in the presence of concomitant risk factors. ²Though nephrotoxicity

is dose related in both cisplatin- and carboplatin, carboplatin is less nephrotoxic but still leads to elevated serum creatinine in 10% of the patients.³ Cumulative doses of carboplatin have shown evidence of renal damage qualitatively like but less severe than that caused by cisplatin. Authors opine that proper monitoring of patients is required if high doses of carboplatin are used or carboplatin is combined with other nephrotoxic chemotherapy.⁴ However, there is a need for drugs that can prevent carboplatin-induced nephrotoxicity.

Melatonin is currently used in treatment of jet lag and readjustment of sleep wake cycle of shift workers. It gives protection against oxidative damage secondary to various factors like strenuous exercise, toxins primarily by scavenging the free radicals.⁵ Its role in attenuating the nephrotoxicity induced by drugs like vancomycin, amikacin, and cisplatin which cause oxidative stress in kidneys has been reported.⁶ Rosuvastatin, a hypolipidemic drug, decreases cholesterol levels by inhibiting the enzyme 3HMGCoA (3-hydroxy-3-methylglutaryl coenzyme) reductase. In addition, rosuvastatin has anti-inflammatory, immunomodulatory and antioxidant activities. Rosuvastatin like other statins has been reported to attenuate inflammation through inhibition of inflammatory cytokines as well as recruitment of inflammatory cells. Studies have shown the protective role of rosuvastatin in cisplatin-induced nephrotoxicity.⁷

In consideration of the above reports the present experimental study was done to assess and compare the nephroprotective effect of melatonin and rosuvastatin at low and high doses in carboplatin induced nephrotoxicity in rats.

MATERIAL AND METHODS

Animals

The study was conducted after approval by the Institutional Animal Ethics Committee (IAEC/KMC/109/2020). Animals were obtained from the Central Animal House of the institute in accordance with the committee for Control and supervision of experimentation on animal (CPCSEA) guidelines. Thirty-six female albino Wistar rats of weight ranging 150-250g, aged around 8-10 weeks were used for the study. The animals were housed under standard conditions, 12-hour light-dark cycle, 50% humidity, and 28°C

temperature and provided with food and water *ad libitum*.

Drugs/Chemicals

Carboplatin (manufactured by Fresenius Kabi Oncology Ltd), Melatonin 3mg tab (manufactured in India by Sun Pharma Laboratories Ltd.), and Rosuvastatin 20mg tab (manufactured in India by Sun Pharma Laboratories Ltd.) was purchased from Manipal Hospital Pharmacy. All the other reagents used were purchased from Coral Clinical Systems and were of analytical grade. Dimethylsulphoxide (DMSO) was used as vehicle. The doses of drugs were selected as per previous studies.^{5,8,9}

Experimental procedure

In the experiment, a total of 36 rats were randomly divided into 6 groups (n=6). The different groups received treatments as mentioned in Table 1. All groups other than control group were administered Carboplatin 128mg/kg intraperitoneal single dose on day 5 to induce nephrotoxicity.

The animals were weighed on day 0 and 11. Retro orbital blood collection from inner canthus of the eye using capillary tubes was done on day 0, 7 and 11 which was used for biochemical and antioxidant estimations. After 11th day, kidneys were dissected and weighed. The renal tissue was used for histopathological analysis.

Biochemical estimations

Blood Urea was estimated using GLDH kinetic method¹⁰ and serum creatinine was estimated by modified Jaffe's Kinetic method.¹¹ Uric acid estimation was done by uricase/PAP method.¹² Serum IL-18 was measured using ELISA.¹³ The antioxidants glutathione (GSH) and malondialdehyde (MDA) were assayed using methods described earlier.⁹

Histopathological Examination

The histological examination of kidney tissues was done using paraffin-embedded specimens. The specimens were cut into 6 mm thickness and stained with Hematoxylin-Eosin for light microscope examination (Olympus, BH-2, Tokyo, Japan). The characteristic histological changes in sections was observed and were analyzed semi-quantitatively using the technique of Houghton et al.¹⁴ The lesions were graded as 0, 1 or 2 if the histology showed normal structure, areas of focal granular-vacuolar epithelial cell degeneration or tubular epithelial necrosis respectively

RESULTS

The effect of melatonin and Rosuvastatin on body weight and weight of kidneys in carboplatin induced nephrotoxicity is shown in table 2. No significant difference was observed in the weight of animals on day 0 and day 11. There was no significant change in the weight of kidneys in treatment groups as compared to control.

Biochemical estimations

Blood urea

The blood urea in six groups is shown in Table 3. On day 11 Carboplatin showed a significant increase in blood urea levels as compared to control ($p < 0.05$). Also on day 11, all treatment groups showed a significant decrease in blood urea levels as compared to the carboplatin group (Table 3)

Serum creatinine

The serum creatinine levels of different groups is shown in table 4. On day 11 carboplatin group showed a significant increase in serum creatinine levels as compared to control ($p < 0.05$).

There is a significant decrease in creatinine levels as compared to carboplatin in all treatment groups. On comparing day 11 and day 7 levels with day 0 in carboplatin group, a significant increase is observed on day 11 and day 7 ($p < 0.05$).

Uric acid

On day 11 carboplatin group showed a significant increase in uric acid levels as compared to control ($p < 0.05$). There was a significant decrease in uric acid levels as compared to carboplatin in rosuvastatin and high dose of melatonin treatment groups (table 5). On comparing day 11 and day 7 levels with day 0 in carboplatin group, a significant increase is observed on day 11 and day 7 ($p < 0.05$).

IL-18 levels

The IL-18 levels were significantly more in carboplatin group on day 11 as compared to control group ($p < 0.05$). On day 11, all treatment groups showed a decrease in IL-18 levels as compared to carboplatin but the decrease was not statistically significant ($p > 0.05$) as seen in table 6.

Table 1. Treatment groups

Group I (normal control)	Dimethylsulfoxide (DMSO) as vehicle from day 0 to day 10 once a day
Group II (Carboplatin control)	DMSO: 1ml/kg/day p.o for 10 days from day 0 to day 10 once a day
Group III (Carboplatin + Melatonin low dose) (Car +L.M)	Melatonin: 5mg/kg p.o for 10 days from day 0 to day 10 once a day
Group IV (Carboplatin + Melatonin high dose) (Car +H.M)	Melatonin: 10mg/kg p.o for 10 days from day 0 to day 10 once a day
Group V (Carboplatin + Rosuvastatin low dose) (Car +L.R)	Rosuvastatin: 10mg/kg p.o for 10 days from day 0 to day 10 once a day
Group VI (Carboplatin + Rosuvastatin high dose) (Car +H.R)	Rosuvastatin: 20mg/kg p.o for 10 days from day 0 to day 10 once a day

Table 2. Body weight of rats on day 0 and day 11 and weight of kidney on day 11

Groups	Body weight in (g) DAY0	Body weight in (g) DAY11	Kidney weight (g)Day 11	Organ weight/ Bodyweight
1.Control	153.33±8.16	182.00±16.92	0.62 ± 0.074	0.003
2.Carboplatin	175.00±13.52	172.67±10.07	0.59 ± 0.028	0.003
3.Car+ L.M	174.00±25.27	164.00±26.58	0.67 ± 0.079	0.003
4.Car +H.M	177.33±20.20	182.67±32.40	0.65 ± 0.096	0.003
5.Car +L.R	184.17±19.33	182.67±32.40	0.70 ± 0.083	0.003
6.Car +H.R	187.33±24.54	173.33±28.92	0.64 ± 0.062	0.003

All values are expressed as Mean±S.D

Antioxidant estimation**GSH assay**

There is a non- significant decrease in GSH levels in carboplatin treated group as compared to control on day11 (table 7). GSH levels in the other treatment groups were comparable to control ($p>0.05$)

MDA assay

There was significant increase in MDA levels in carboplatin treated group in comparison to the control group. The MDA levels in the treatment groups were significantly reduced in comparison to the carboplatin treated group as shown in table 8.

Table 3. Blood urea levels in different groups on day 0, 7 and 11

Groups	Urea at DAY 0 mg/dl	Urea at DAY 7 mg/dl	Urea at DAY 11 mg/dl
1.Control	1.06 ± 0.07	1.35 ± 0.33	1.21± 0.13 ^a
2.Carboplatin	1.23 ± 0.13	3.07 ±0.28 ^a	3.38± 0.13 ^{*a}
3.Car+ L.M	1.17 ± 0.15	1.56 ±0.36 ^a	1.22± 0.11 ⁺
4.Car +H.M	1.01 ± 0.27	1.46 ± 0.21 ^a	1.15 ± 0.19 ⁺
5.Car +L.R	1.23 ± 0.03	1.73 ± 0.54	1.15 ± 0.20 ⁺
6.Car +H.R	1.24 ± 0.03	1.25 ±0.35	1.06 ± 0.32 ⁺

Repeated measures of ANOVA $p<0.05$, post hoc Bonferroni's test $^*p<0.05$ vs control, ^a vs day 0, One way ANOVA on day 11, post hoc Tukey test $^*p<0.05$ vs carboplatin. All values are expressed as Mean±S.D

Table 4. Serum creatinine levels in different groups on day0, 7 and 11

Groups	Creatinine at DAY 0 mg/dl	Creatinine at DAY 7 mg/dl	Creatinine at DAY 11 mg/dl
1.Control	0.24 ± 0.03	0.23 ± 0.03	0.24 ± 0.03
2.Carboplatin	0.24 ± 0.03	1.60 ±0.66 ^a	1.86 ± 0.24 ^{*a}
3.Car+ L.M	0.23 ± 0.03	0.64 ±0.40	0.28 ± 0.09 ⁺
4.Car +H.M	0.24 ±0.03	0.37 ± 0.21	0.22 ± 0.11 ⁺
5.Car +L.R	0.23 ± 0.03	0.38 ± 0.20	0.26 ± 0.12 ⁺
6.Car +H.R	0.24 ± 0.03	0.32 ± 0.27	0.22 ± 0.14 ⁺

Repeated measures of ANOVA, post hoc Bonferroni's test $p<0.05$, ^{*} vs control, ⁺ vs carboplatin, One way ANOVA on day 11, post hoc Tukey test, ^a vs day 0. All values are expressed as Mean±S.D

Table 5. Uric acid levels in different groups on day 0, 7 and 11

Groups	Uric acid at DAY 0 mg/dl	Uric acid at DAY 7 mg/dl	Uric acid at DAY 11mg/dl
1.Control	0.70 ± 0.21	0.82 ±0.34	0.96 ±0.18
2.Carboplatin	0.48 ±0.17	1.40 ±0.21 ^a	2.57 ± 1.07 ^{*a}
3.Car+ L.M	0.82 ± 0.43	1.37 ±0.64	1.93 ± 0.54
4.Car +H.M	0.89 ± 0.29	1.01 ±0.40	0.88 ±0.38 ⁺
5.Car +L.R	0.63 ± 0.23	0.82 ±0.43	1.01 ± 0.40 ⁺
6.Car +H.R	0.75 ± 0.38	0.76 ±0.29	0.88 ± 0.45 ⁺

Repeated measure of ANOVA, post hoc Bonferroni's test $^*p<0.05$ vs day 0, ⁺ vs carboplatin. One way ANOVA on day 11, post hoc Tukey test $^*p<0.05$ vs day 0. All values are expressed as Mean±S.D

Histopathological examinations

Microphotographs of rat kidneys stained by Hematoxylin and Eosin (200X) is shown in the figure 1.

In control group (A) there are no changes in glomerulus, tubules and vessels, but there was mild small lymphocytic infiltration in interstitium. In carboplatin group (B) glomerular atrophy, vacuolar degeneration, hyaline casts were observed in the tubules. In car+ L.M group (C) glomerular atrophy, vacuolar degeneration, hyaline casts in tubules and moderate to severe small lymphocytic infiltration was seen in interstitium, but no changes were observed in the vessels. In car+ H.M (D) infiltration by small lymphoid cells are present. In car+ L.R (E) RBC casts seen inside the tubules. In Car + H.R (F) RBC casts seen inside the tubules

along with glomerular atrophy and vacuolar degeneration but had no changes in interstitium and vessels.

DISCUSSION

In the present study, nephroprotective effects of melatonin and rosuvastatin in carboplatin induced nephrotoxic model were evaluated. Carboplatin 128mg/kg single dose i.p induced nephrotoxicity which was manifested as increase in blood urea, serum creatinine and serum uric acid levels. This was further confirmed by the glomerular atrophy, vacuolar degeneration and hyaline casts in kidney tubules seen in histopathology which was similar to a previous study.¹⁵ Carboplatin caused dose-dependent renal injury in rats by depleting

Table 6. IL-18 levels on day0, 7 and 11

Groups	DAY 0 ng/l	DAY 7 ng/l	DAY 11 ng/l
1.Control	11.83 ± 0.72	11.91 ± 0.79	11.89 ± 0.76
2.Carboplatin	11.11 ± 0.84	14.06 ± 0.82	14.63 ± 0.82*
3.Car+ L.M	11.24 ± 0.76	12.95 ± 0.52	13.49 ± 0.94
4.Car +H.M	10.91 ± 0.64	13.38 ± 0.75	12.78 ± 1.30
5.Car +L.R	11.77 ± 0.53	12.84 ± 0.48	13.38 ± 1.11
6.Car +H.R	11.76 ± 0.68	12.19 ± 0.94	12.86 ± 1.55

Repeated measures of ANOVA, post hoc Bonferroni's test * $p < 0.05$ vs carboplatin. Remaining groups were tested but not significantly comparable to control group. All values are expressed as Mean±S.D

Table 7. GSH levels in kidney tissue of rats

Groups	GSH levels in μM/mg tissue
1.Control	1.312 ± 0.13
2.Carboplatin	1.235 ± 0.13
3.Car+ L.M	1.463 ± 0.26
4.Car +H.M	1.538 ± 0.46
5.Car +L.R	1.354 ± 0.35
6.Car +H.R	1.248 ± 0.19

All values are expressed as Mean±S.D

Table 8. MDA levels in kidney tissue of rats

Groups	MDA levels in nM/mg tissue
1.Control	51.15 ± 0.599
2.Carboplatin	63.83 ± 1.23*
3.Car+ L.M	54.73±3.31+
4.Car +H.M	48.61±5.89+
5.Car +L.R	47.32±4.61+
6.Car +H.R	47.33±5.19+

All values are expressed as Mean±S.D
One way ANOVA, $p < 0.05$; post hoc Tukey test, * $p < 0.05$ vs control, + $p < 0.05$ vs carboplatin

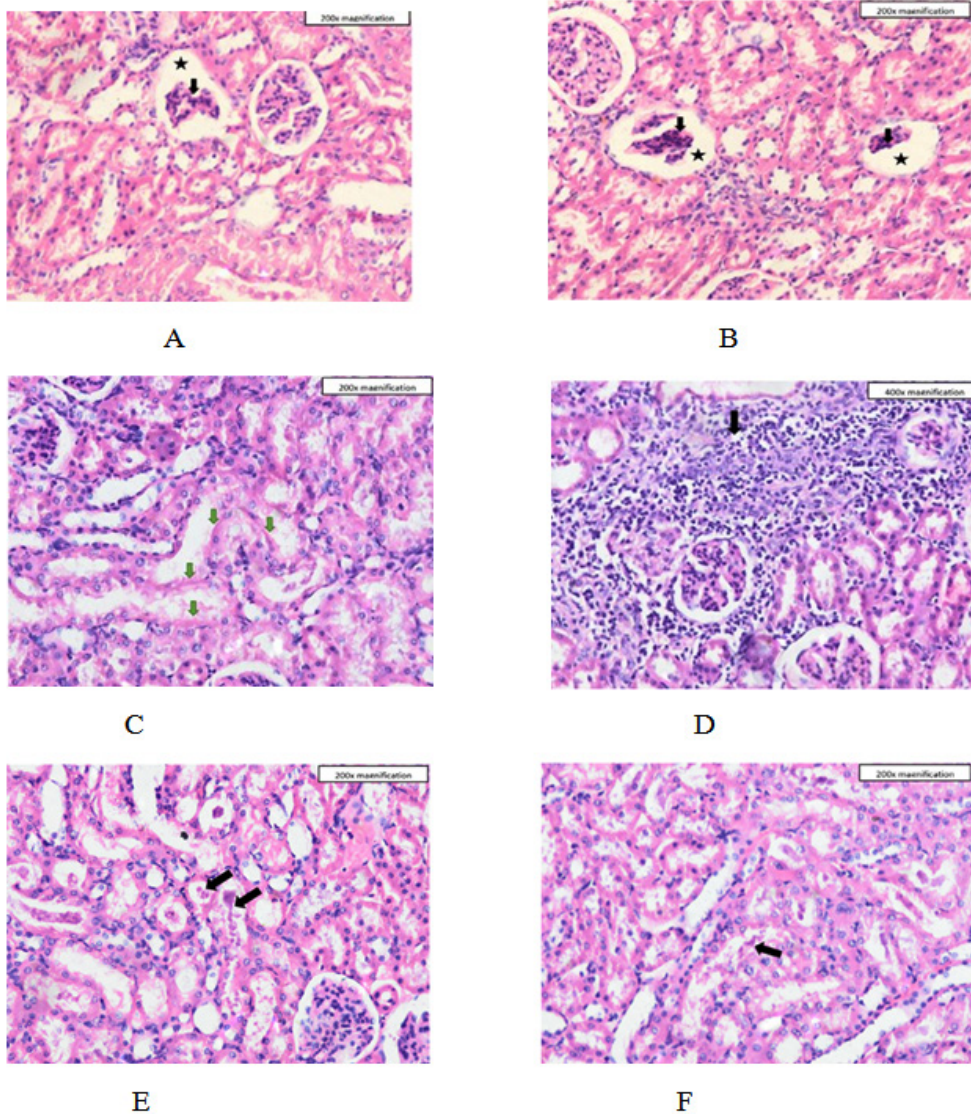


Fig. 1. Histopathology of renal tissue in different treatment groups

renal antioxidants, enhancing lipid peroxidation in addition to platinum content.¹⁶

Treatment with low and high doses of melatonin and carboplatin prevented the rise in blood urea, serum creatinine and uric acid levels on day 7 and day 11, which was suggestive of their nephroprotective property. This was confirmed by histopathological studies where there was only mild infiltration in interstitium and no changes in blood vessels.

Melatonin attenuates the nephrotoxicity induced by drugs by its antioxidant action as it strengthens the antioxidant enzymes and causes free radical scavenging directly. In addition, it modulates inflammatory cytokines which in turn reestablishes the balance of apoptosis and cell survival.¹⁷

Treatment with low and high doses of rosuvastatin and carboplatin prevented the rise in blood urea, serum creatinine and uric acid on

day 7 and day 11 which is suggestive of their nephroprotective effect as well. Histopathology of rosuvastatin groups did not show any changes in interstitium and vessels. This effect was predominantly seen at the high dose of rosuvastatin. Statins in general produce nephroprotective effect by inhibiting receptor-mediated endocytosis that is responsible for the protein uptake in proximal tubular cells in experimental studies.¹⁸

Clinical studies have shown that statins produce nephroprotective effect secondary to pleiotropic effects like antithrombotic, anti-inflammatory and decreasing endothelial nitric oxide synthase.¹⁹

Though some studies report the superiority of lipophilic statins like atorvastatin over rosuvastatin in terms of nephroprotective effect²⁰, other studies refute it and claim rosuvastatin to be better.

Comparison of melatonin and rosuvastatin did not show any significant difference in terms of nephroprotective activity and histopathological changes. This implicates that the nephroprotective action of melatonin is comparable to that of rosuvastatin. There was no significant difference in the nephroprotective activity of different doses of rosuvastatin and melatonin except that the low dose of melatonin did not show a significant decrease.

Studies have shown that different statins like atorvastatin and rosuvastatin have same efficacy in preventing contrast induced nephropathy.²¹

IL-18 is an inflammatory marker produced during acute kidney injury. IL-18, macrophage-derived cytokine, is expressed in cells like macrophages, dendritic cells, T cells, and B cells in addition to parenchymal kidney cells i.e. tubular epithelial cells, podocytes, and mesangial cells.²² IL-18 plays an important role in processes that aggravate renal injury in extension phase of acute kidney injury.²³ Animal studies have shown that drugs that target IL-18–signaling axis can attenuate renal injury.²⁴ In the present study, melatonin and rosuvastatin reduced the IL-18 levels suggesting their anti-inflammatory action. The exact mechanism of IL-18 in renal injury is not clear but studies have shown that it has an important role in tubular injury and renal dysfunction and thus IL-18 as a target has a wide therapeutic potential.²⁴

Melatonin has shown to ameliorate

antibiotic induced nephrotoxicity by reducing oxidative stress in renal tissues.²⁵ Melatonin and its metabolites scavenge free radicals. In addition to stimulating the synthesis of antioxidant enzymes it protects oxidative damage of antioxidant enzymes.²⁶ Furthermore, melatonin also exerts an anti-apoptotic effect on various types of cells.²⁷ The mechanism postulated by Dai W *et al* is that melatonin mediated cell apoptosis by inhibiting the Parkin1 signaling pathway induced by NLRP3 inflammasomes reduces the secretion of inflammatory cytokines like IL-18.²⁸

Rosuvastatin produced significant renoprotective effect in gentamicin induced nephrotoxicity in Sprague Dolly rats by reducing kidney injury molecule-1 and interleukin-18.²⁹ In addition to antioxidant action, the renoprotective effect of rosuvastatin can be mediated by lipid lowering action. prevention of lipid accumulation in renal tubules can also be contributing to renoprotective action of statins.⁷ Studies have shown that rosuvastatin produces nephroprotection by hemin-like effect on hemeoxygenase 1 which has a vital role to play in oxidative stress against antioxidants.³⁰ Also, when comparing between different statins, rosuvastatin showed a significant renoprotective effect as compared to simvastatin.⁷

As oxidative stress is a major mechanism implicated in platinum compounds induced nephrotoxicity, malondialdehyde and reduced glutathione assay was done in kidney tissue to measure lipid peroxidation and glutathione content. Carboplatin induced a significant increase in MDA and a decrease in GSH (though not significant). A significant decrease in MDA by both melatonin and rosuvastatin suggests their antioxidant mechanism.³¹ GSH is also a biomarker for oxidative stress and its increase in GSH in treatment groups was comparable to control group.

Both melatonin and rosuvastatin increased GSH levels most probably by inhibiting lipid peroxidation

This study has certain limitations. Urinary markers of kidney injury and other serum markers like beta 2 microglobulin which is an early marker of renal injury were not estimated.

CONCLUSION

To conclude, rosuvastatin and melatonin

demonstrated an equi-efficacious nephroprotective effects mediated by anti-inflammatory and antioxidant properties. The anti-inflammatory action was primarily mediated by reducing IL-18 and antioxidant action by decreasing MDA and increasing GSH levels. Further long-term studies are warranted to substantiate these findings.

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Conflict of Interest

The authors declare that there are no competing interests.

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Ethics committee

The study was conducted after approval by the Institutional Animal Ethics Committee (IAEC/KMC/109/2020).

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