Research article

A combination of generated hydrogen sulfide and nitric oxide activity has a potentiated protectant effect against cisplatin induced nephrotoxicity

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ABSTRACT

Aim: Hydrogen sulfide and nitric oxide possess cytoprotective activity and in vivo, they are generated from exogenous sodium hydrosulfide and L-arginine respectively. Cisplatin is a major chemotherapeutic agent used to treat cancer and has a high incidence of nephrotoxicity as a side effect. The study aim was to explore the effects of NaHS and L-arginine or their combination on cisplatin induced nephrotoxicity in rats.

Methods: Wistar Kyoto rats were given a single intraperitoneal dose of cisplatin (5 mg/kg) followed either by NaHS (56 μmol/kg, i. p.), L-arginine (1.25 g/L in drinking water) or their combination daily for 28-days. Post-mortem plasma, urine and kidney samples were collected for biochemical assays and histopathological analysis.

Results: Cisplatin decreased body weights and increased urinary output, while plasma creatinine and urea levels were elevated, but sodium and potassium concentrations were diminished. The renal function parameters, blood urea nitrogen and creatinine clearance, were raised and decreased respectively. Regarding markers of reactive oxygen species, plasma total superoxide dismutase was reduced, whereas malondiadehyde was augmented.

Cisplatin also diminished plasma and urinary H_2S as well as plasma NO, while NaHS and L-arginine counteracted this activity on both redox-active molecules. Cisplatin cotreatment with NaHS, and/or L-arginine exhibited a reversal of all other measured parameters.

Conclusion: In current study, NaHS and L-arginine as monotherapy protected the rats from cisplatin-induced nephrotoxicity but the combination of both worked more effectively suggesting

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the augmented anti-inflammatory and antioxidative potential of test treatments when administered together.

1. Introduction

Nephrotoxicity leading. to renal damage is a condition in which the kidneys are unable to clear metabolic waste products from plasma. A failure to maintain pH, electrolytes and fluid balance ensues and subsequently, there is a reduction in glomerular filtration rate. Such a renal failure can either be acute or chronic in nature and it may well arise from systemic and/or renal defects. Acute renal failure often occurs suddenly and is largely reversible, if diagnosed at an early stage. In contrast, chronic renal failure invariably causes permanent kidney damage over a period of time [1].

Hydrogen sulphide (H_2S) and nitric oxide (NO) have been historically regarded as environmental gases possessing potentially toxic effects. However, it has been shown that they are also important biologically active endogenous molecules, capable of modulating various signalling pathways, and in this respect, they resemble carbon monoxide [2]. In mammalian tissues, endogenous H_2S is generated from biosynthetic pathways by the pyridoxal-5′-phosphate- (PLP-) dependent enzymes: cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS), 3-mercaptopyruvate sulfurtransferase (3-MST) and cysteine aminotransferase (CAT), which utilise L-cysteine or homocysteine as substrate. They occur abundantly in peripheral tissues such as smooth muscles, liver, kidneys and blood vessels where they participate in H_2S production [3]. A cytoprotective effect of H_2S has been directly linked to its ability to scavenge reactive oxygen species (ROS), decreasing apoptotic signalling, promoting vascular smooth muscle relaxation and a reversible modulatory effect on mitochondrial respiration. It has previously been shown that both endogenous and exogenous H_2S play a protective role against renal ischemia-reperfusion injury [4]. In addition, H_2S has been reported to regulate autophagy in a variety of cells including hepatocytes, cardiomyocytes and epithelial cells along with a neuroprotective effect accredited to its antioxidant capability. Hence H_2S donation by sodium hydrosulfide (NaHS), has been reported to modulate autophagy and enhance cell survival [5,6]. Moreover, H_2S induces vasodilation during augmented glomerular filtration by inhibiting tubular basment membrane Na^+/K^+ ATPase and the Na^+/K^+2Cl^{2-} apical membrane cosporter producing natriuresis and kaliuresis. Baseline renal H_2S production can also regulate the renin-angiotensin system (RAS) either directly by altering cAMP synthesis or indirectly through ROS maintenance [7]. It has been observed that H_2S in glomerular epithelial cells is necessary for the regulation of globular protein production by means of AMP-activated protein kinase. Due to the paracrine signalling nature and permeability properties of H_2S, its production in vascular endothelial cells and proximal tubular epithelial cells is sufficient to fulfill the H_2S requirement in glomerular epithelial cells [8,9]. H_2S can ameliorate hypertension and reduced kidney function, and its concentration has been found to be substantially decreased during acute or chronic renal damage. Similarly, the vascular endothelium generates nitric oxide (NO) which penetrates adjacent vascular smooth muscle cells stimulating a cyclic GMP-dependent relaxation. Nitric oxide is produced in the body by three isoforms of nitric oxide synthase (NOS) which uses L-arginine and oxygen as substrates [10]. Neuronal NOS (n-NOS or NOS–I) and endothelial NOS (e-NOS or NOS-III) are generated in neuronal and endothelial cells respectively. Inducible NOS (i-NOS or NOS-II) in contrast, is stimulated by proinflammatory cytokines, and excessive synthesis of NO in this instance, is responsible for pathophysiological inflammation [11].

H_2S causes relaxation of vascular smooth muscle cells by decreasing angiotensin II type1 (AT_1) receptor binding together with binding affinity opening of K-ATPase channels [12] and also by enhancement of NO effects [13]. eNOS conversely, depends upon calcium/calmodulin (CaM), and is activated by a stimulatory response of different Ca^{2+} rallying cell surface receptors in the vascular endothelial layer [14].

Cisplatin (*cis*-diamminedichloroplatinum-II) is a chemotherpeutic drug used in the treatment of a range of cancers [15]. Upon administration, cisplatin is converted into a nephrotoxin inducing oxidative stress, inflammation and mitochondrial DNA damage. The process gives rise to activation of the apoptotic pathway [16] and there appears to be a fine balance between renoprotection and tumor toxicity [17]. Moreover, since cisplatin causes such a high incidence of nephrotoxicity [17], the present aim was to establish any possible nephroprotective activity of H_2S and NO generated from NaHS and/or L-arginine concurrently administered with cisplatin.

2. Material and methods

2.1. Chemicals

Cisplatin (Pharmax Life Sciences), ketamine (Global Pharmaceuticals), xylazine (Prix Pharmaceuticals), Sodium hydrosulfide (NaHS) (Daejing Chemicals, Korea), L-Arginine, Trichloroacetic acid and Ferric chloride (BDH, Prolab), 4-Amino-benzene-sulfonic acid, diethylenetriamine penta-acetic acid (DTPA), N-(1-naphthyl) ethyl-ene-diamine (NED), sodium nitrate, sodium nitrite, NADPH, tris(hydroxymethyl)-amino-methane, Vanadium chloride, N-2 dimethyl-p-phenylenediamine sulfate, zinc acetate dihydrate and ethanol of research grade were purchased from Sigma Aldrich, Germany.

2.2. Assay kits

The reactive oxygen species markers, malondialdehyde (MDA) and total superoxide dismutase (T-SOD) assay kits were obtained from Elabscience Biotechnology, USA.

2.3. Experimental animals

Thirty Wistar-Kyoto (WKY) rats (200–250 g) were supplied and maintained in the animal house of the Department of Pharmacology, Faculty of Pharmacy, The Islamia University of Bahawalpur. The animals (6/cage) were housed in polycarbonate home cages at a temperature of $24 \pm 4\ ^{\circ}C$ and humidity >66%, on a 12 h–12 h light-dark cycle with access to standard diet and water ad libitum. Fresh cage sawdust was changed every 48-h, and the animals were treated as per standard criteria, according to the National Institute of Health guidlines for the Care and Use of Laboratory Animals. The study design was reviewed and approved by the Pharmacy Animal Ethics Committee (approval number PAEC/2020/31).

2.4. Experimental protocol

Animals were randomly assigned to 5 groups for the 28-day experimental treatment protocol as follows: Group-I: vehicle control, Group-II: cisplatin, Group-III: cisplatin + NaHS, Group-IV: cisplatin + L-arginine and Group-V: cisplatin + NaHS + L-arginine.

Group-I was administered Saline (5 mg/kg) intraperitonially (i.p) once on day 1 of the protocol. Groups II, III, IV and V were administered a single dose of cisplatin (5 mg/kg) intraperitoneally (i.p.) [18] at the start of the protocol. In addition, the H_2S donor, NaHS, was given daily (56 μmol/kg, i. p) [12] and the NO donor, L-arginine (1.25 g/L daily in the drinking water) [19] during the 28-day protocol. At the end of the protocol, animals were subjected to a 12-h overnight fast prior to dissection. All the animals were euthanised using a mixture of ketamine and xylazine (10:1) at a dose of (0.2 ml/100 g) [20]and the kidneys were removed immediately, flushed with 0.9% NaCl and then stored in 10% formalin solution for analysis.

2.5. Sample collection

Blood and urine samples were obtained on days 0, 14 and 28 of the protocol. For the collection of urine samples, animals were housed in metabolic cages for 24-h [19]. Blood samples were obtained by retro-orbital puncture and collected in heparinized capillary tubes [20]. The samples were then centrifuged at 3000 rpm for 15 min and the plasma supernatant was separated and stored at $-20\ ^{\circ}C$ for further investigation.

2.6. Determination of H_2S levels in plasma and urine

H_2S levels in both plasma and urine were determined by spectophotometry. Samples of 100 ml (urine and plasma) were mixed with 50 μl of distilled water in microcentrifuge tubes along with 30 μl of zinc-acetate" (1% w/v). After 5 min, 200 μl of N,N-2-dimethyl-p-phenylene diamine sulfate (20 mM in 7.2 M HCl) was rapidly added, followed by the addition of 200 μl of $FeCl_3$ (30 mM in 1.2 M HCl). The mixture was placed in the dark for the next 20 min. Then, 150 μl of trichloroacetic acid (10% w/v) was added for protein precipitation, followed by centrifugation at 10,000 rpm for 10 min. The supernatant was collected in 96-well plates for measuring at an absorbance of 670 nm in a plate reader. All the samples under consideration were assayed in duplicate. A calibration curve was plotted, and the concentration of H_2S in plasma and urinary samples from H_2S standard solutions were calculated (NaHS: 3.125–100 μM) [21].

2.7. Nitric oxide determination

2.7.1. Reduction of nitrate

A 100 ml plasma sample was mixed with 20 mmol/l Tris buffer followed by incubation with nitrate reductase, vanadium chloride (8 mg/ml) and NADPH diluted in Tris buffer for half an hour at 37 $^{\circ}C$. The control sample was examined with an external standard (sodium nitrate at a concentration of 50 mmol/l).

2.7.2. Deproteinization

After nitrate reduction, 100 ml of serum was placed in an incubator for 12 h with 900 ml of methanol:diethylether (3:1 mixture v/v). Following incubation, the samples underwent centrifugation at 10,000 rpm for 10 min at 48 $^{\circ}C$, and the supernatant was extracted to determine the nitrite concentration.

2.7.3. Determination of nitric oxide

Fifty ml of HCl (6.5 mol/l) plus 50 ml of sulphanilic acid (37.5 mmol/l) was added to 200 ml of supernatant of de-proteinizated sample and incubated for 10 min at 48 $^{\circ}C$, followed by addition of 50 ml of N-1-naphthylethylenediamine (NED; 12.5 mmol/l)). After 30 min of incubation at 48 $^{\circ}C$, the mixture was subjected to centrifugation at 10,000 rpm for 10 min. Absorbance was measured at 540 nm by micro-plate reader [22].

2.8. Biochemical parameters

2.8.1. Estimation of creatinine

To a 100 μl of sample was added 0.2 ml of reagent (0.75 N NaOH and 1% picric acid) and mixed at 30 $^{\circ}C$, and the absorbance difference at 20 s and 80 s at 515 nm was checked by spectrophotometry. Concentrations were calculated against a standard creatinine curve [23].

2.8.2. Estimation of urea

The concentration of urea was calculated by taking 0.2 ml of sample and mixing it with 1.0 ml of water plus 1.0 ml of trichloroacetic acid and centrifuging for 10 min. Then 0.2 ml of supernatant was added to 3 ml of reagent and kept on a boiling water bath for 20 min. Afterwards, it was allowed to cool and followed by measurement of the absorbance at 520 nm within 15 min [24].

2.9. Oxidative stress markers

The oxidative stress markers, malondialdehyde (MDA) and total superoxide dismutase (T-SOD) were determined by the Elab Sciences kit method.

2.10. Estimation of plasma and urinary sodium and potassium

The samples of plasma and urine were subjected to the flame photometric method for the calculation of sodium and potassium levels. For estimation of plasma and urinary sodium, the samples were further diluted 1:200 and a similar dilution was used for the calculation of the potassium concentration in plasma. However, for evaluation of the potassium level in urine, the sample was subjected to a dilution of 1:1000. All samples under observation were assessed in duplicate for the measurement of sodium potassium levels. The flame photometer was calibrated against standard sodium chloride (NaCl) and potassium chloride (KCl) solutions prior to evaluation of plasma and urinary samples.

2.11. Histopathological analysis

Kidney sections were dipped in (10%) formalin solution for 72-h. The kidney tissues were processed by standard histopathological methods by applying ethanol, fixing with xylene and embedding them in paraffin wax, then stained with hematoxylin and eosin for future studies [25].

2.12. Statistical analysis

All data was expressed as mean ± SEM, and statistical significance between different groups was assessed by applying One-way analysis of variance (ANOVA) with post hoc Bonferroni's test using Graphpad Prism version 8.0. statistical package Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Effect of cisplatin coadministration with either sodium hydrosulfide, L-arginine or their combination on animal body weight

On day 0, at the start of the protocol, animal body weights were recorded before cisplatin treatment, there being no significant difference between groups, and there was a lack of weight change shown in the control group up to day 28. However, administration of cisplatin initiated a marked and progressive reduction in body weights throughout the protocol as shown in Table 1. In the remaining treatment groups, (cisplatin + NaHS, cisplatin + L-Arginine and cisplatin + NaHS + L-Arginine) there was a reversal of the cisplatin induced weight loss on protocol days 14 and 28 (Table 1).

3.1.1. Effect of cisplatin coadministration with either sodium hydrosulfide, L-arginine or their combination on animal kidney weight and body weight ratio

At the 28th day, the animal group with administration of cisplatin shown a marked and progressive elevation in ratio of kidney

Table 1

Effect of cisplatin treatment combined with either sodium hydrosulfide (NaHS), L-arginine or NaHS + L-arginine on body weight and 24-h urinary output in rats.

Treatment Groups	Body Weight (g)			Urinary Output (ml/24 h)		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
Control	230.8 ± 4.39	230.8 ± 4.43	231.8 ± 4.75	6.83 ± 0.30	8.00 ± 0.68	8.33 ± 0.71
Cisplatin	239.0 ± 3.22	196.2 ± 1.42***	174.3 ± 3.16***	8.16 ± 0.40	36.00 ± 0.36[a]	38.33 ± 1.33[a]
Cisplatin + NaHS	242.2 ± 5.12	218.3 ± 0.88[b]	208.8 ± 0.60**,[b]	8.50 ± 0.71	17.67 ± 0.55[a,b]	21.50 ± 0.42[a,b]
Cisplatin + L-arginine	241.3 ± 6.00	208.8 ± 1.04**	200.5 ± 1.30***,[b]	8.00 ± 1.09	21.17 ± 0.60[a,b]	25.17 ± 0.40[a,b]
Cisplatin + NaHS + L-arginine	238.2 ± 3.62	229.5 ± 3.63[b,d]	220.3 ± 0.71[b,d]	7.33 ± 0.84	13.00 ± 0.57[a,b,c,d]	16.83 ± 0.60[a,b,c,d]

All values are expressed as mean ± SEM. One-way analysis of variance (ANOVA) was applied for statistical analysis with post hoc Bonferroni test.

[a] $p < 0.05$ versus control.

[b] $p < 0.05$ versus cisplatin.

[c] $p < 0.05$ versus cisplatin + NaHS.

[d] $p < 0.05$ versus cisplatin + L-arginine

weight and body weight (g/g) versus control (p < 0.05) as shown in Fig. 1. While in the remaining treatment groups, (cisplatin + NaHS, cisplatin + L-Arginine and cisplatin + NaHS + L-Arginine) there was a reversal of the cisplatin induced kidney weight and body weight ratio on protocol day 28 (Fig. 1).

3.2. Effect of cisplatin coadministration with either sodium hydrosulfide, L-arginine or their combination on animal urinary output

Animals in the control group did not display any significant modification of urinary output during the course of the protocol. However, cisplatin (treatment elevated (p < 0.05) the urinary output in comparison with controls on days 14 and 28 of the protocol as shown in Table 1. Conversely, in those rats coadministered cisplatin + NaHS, cisplatin + L-arginine and cisplatin + NaHS + L-arginine there was a urinary output decrease in comparison with the cisplatin alone augmented output. In regard to this, combined treatment with cisplatin + NaHS + L-arginine expressed a greater reduction in urinary output than that diplayed by separate combination of either NaHS or L-arginine with cisplatin (Table 1).

3.3. Effect of cisplatin coadministration with either sodium hydrosulfide, L-arginine or their combination on animal plasma and urinary H_2S levels

In the control group of rats, both plasma and urinary H_2S levels remained unchanged from day 0 up to the end of the protocol. Cisplatin administration, however, reduced H_2S levels in both plasma (by 56.7% [day 14] and 59.8% [day 28]) and urine (by 52.7% [day 14] and 59.7% [day 28]), and urinary H_2S levels most probably as a result of renal injury. All three cisplatin groups cotreated with NaHS, L-arginine or the NaHS + L-arginine combination, reversed the cisplatin impaired H_2S plasma and urinary concentrations on protocol days 14 and 28. In the case of the cisplatin + NaHS + L-arginine treatment group on day 14, the urinary H_2S outcome concentration was significantly larger (P < 0.05) than that induced by cisplatin + L-arginine (Figs. 2 and 3).

3.4. Effect of cisplatin coadministration with either sodium hydrosulfide, L-arginine or their combination on animal plasma NO levels

Plasma NO levels in the control group were not modified during the course of the 28-day protocol. In contrast, treatment with cisplatin decreased NO concentrations in plasma by 55.1% by day 28 of the protocol and this effect was reversed in the cisplatin animal groups coadministered cisplatin with NaHS, L-arginine or NaHS + L-arginine (see Fig. 4). It was noticeable that on day 14 and 28, the reversal was greater following cisplatin + NaHS + L-arginine treatment than that recoded in the cisplatin group treated with NaHS alone (Fig. 4).

3.5. Biochemical assays

3.5.1. Effect of cisplatin coadministration with either sodium hydrosulfide, L-arginine or their combination on animal plasma and urinary creatinine concentrations and creatinine clearance

Following cisplatin dosing, there was a marked increase in plasma levels of creatinine as compared to those in the control group which continued to be unmodified throughout the 28-day experimental protocol. Additionally, There was a preventative effect of NaHS, L-arginine or their combination on cisplatin elevated plasma creatinine, which was more pronounced when the effect of the cisplatin + NaHS + L-arginine combination was compared with cisplatin + NaHS by itself (day 28) (Table 2) Conversely, urinary creatinine concentrations were significantly decreased with time after administration of cisplatin in comparision with the unchanged creatinine levels observed in the control group. In animals given cisplatin in combination with NaHS, L-arginine or NaHS with L-
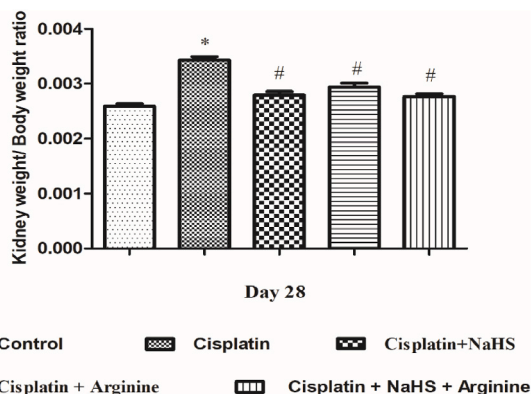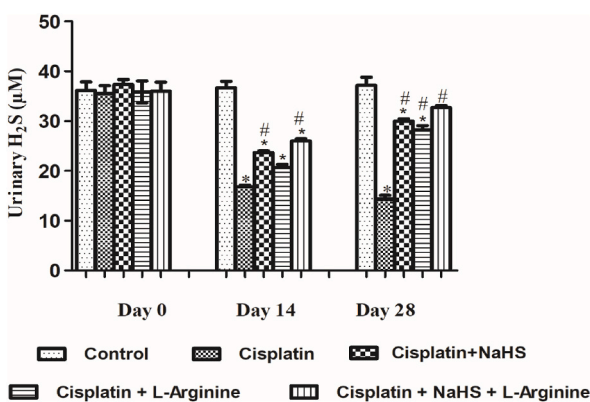

Fig. 1. Effect of cisplatin treatment combined with either sodium hydrosulfide (NaHS), L-arginine or NaHS + L-arginine during a 28-day protocol, on urinary H_2S concentrations in rats.

All values are mean ± SEM. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by post hoc Bonferroni test. *p < 0.05 versus control; #p < 0.05 versus cisplatin.
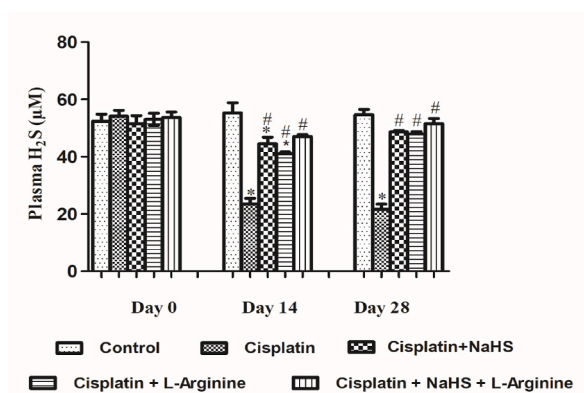
Fig. 2. Effect of cisplatin treatment combined with either sodium hydrosulfide (NaHS), L-arginine or NaHS + L-arginine during a 28-day protocol, on plasma H_2S concentrations in rats.
All values are expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) with post hoc Bonferroni test. *p < 0.05 versus control; #p < 0.05 versus cisplatin.
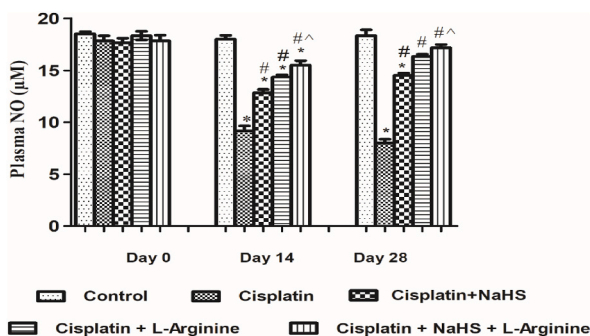

Fig. 3. Effect of cisplatin treatment combined with either sodium hydrosulfide (NaHS), L-arginine or NaHS + L-arginine during a 28-day protocol, on urinary H_2S concentrations in rats.
All values are mean ± SEM. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by post hoc Bonferroni test. *p < 0.05 versus control; #p < 0.05 versus cisplatin.

Fig. 4. Effect of cisplatin treatment combined with either sodium hydrosulfide (NaHS), L-arginine or NaHS + L-arginine during a 28-day protocol, on rat plasma NO concentrations in rats. All values are expressed as mean ± SEM and statistical analysis was performed by one way analysis of variance (ANOVA) with post hoc Bonferroni test. *p < 0.05 versus control; #p < 0.05 versus cisplatin; ^p < 0.05 versus cisplatin + NaHS.

arginine, there was a consistent elevation of cisplatin-impaired levels of urinary creatinine (Table 2) Similarly, the creatinine clearance after cisplatin treatment was considerably decreased versus the control group on days 14 and 28. This cisplatin degradation of creatinine clearance was subsequently reversed by the cisplatin cotreatments with NaHS, L-arginine or the NaHS + L-arginine

Table 2

Effect of cisplatin treatment combined with either sodium hydrosulfide (NaHS), L-arginine or NaHS + L-arginine on plasma or urinary creatinine concentrations in rats.

Treatment Groups	Plasma Creatinine (mg/dl)			Urinary Creatinine (mg/dl)		
	Day 0	Day 14	Day 28	Day 0	14th Day	Day 28
Control	0.84 ± 0.11	1.04 ± 0.26	0.88 ± 0.15	4.40 ± 0.14	4.23 ± 0.16	4.36 ± 0.15
Cisplatin	0.74 ± 0.10	3.77 ± 0.26***	3.84 ± 0.33***	4.31 ± 0.16	2.09 ± 0.08[a]	1.68 ± 0.04[a]
Cisplatin + NaHS	0.86 ± 0.11	2.23 ± 0.20[a,b]	1.71 ± 0.22[###]	4.51 ± 0.08	2.54 ± 0.04[a]	2.81 ± 0.02[a, b]
Cisplatin + L-Arginine	0.85 ± 0.12	2.90 ± 0.26***	2.36 ± 0.42[a, ##]	4.45 ± 0.14	2.23 ± 0.02[a]	2.67 ± 0.02[a, b]
Cisplatin + NaHS + L-Arginine	0.90 ± 0.08	2.62 ± 0.36**	1.80 ± 0.38[###]	4.51 ± 0.10	2.72 ± 0.07[a,b]	3.06 ± 0.08[a,b]

All values are expressed as mean ± SEM. One-way analysis of variance (ANOVA) was performed for statistical analysis with post hoc Bonferroni test. ˆ $p < 0.05$ versus cisplatin + NaHS.

[a] $p < 0.05$ versus control.

[b] $p < 0.05$ versus cisplatin.

combination (Table 3).

3.5.2. Effect of cisplatin coadministration with either sodium hydrosulfide, L-arginine or their combination on animal plasma and urinary urea as well as blood urea nitrogen (BUN)

Following administration of cisplatin, the plasma levels of urea were considerably elevated ($p < 0.05$) in comparison with the control animal group whose levels were unaltered all through the protocol. All the combination treatment groups displayed a preventative outcome on cisplatin raised plasma urea, the cisplatin + NaHS + L-arginine combined treatment exhibiting a bigger effect in this regard on day 28, than the other two cisplatin single cotreatments. (Table 4). The levels of urinary urea were significantly reduced in response to cisplatin while this effect was reversed in all the cotreatment groups. However, the cisplatin + NaHS + L-arginine concomitant treatment group exhibited a greater reversal than the other two combined treatments (Table 4). In addition, blood urea nitrogen (BUN) levels were increased by cisplatin and reduced by the cotreatments, and once again, the cisplatin + NaHS + L-arginine reversal was bigger than the other combination treatments (Table 5).

3.6. Effect of cisplatin coadministration with either sodium hydrosulfide, L-arginine or their combination on oxidative stress markers in rats

3.6.1. Effect of cisplatin coadministration with either sodium hydrosulfide, L-arginine or their combination on plasma biochemical markers

Although plasma T-SOD levels in the control animals were not modified during the protocol, there was a decrease (by 40% [day 14] and 60% [day28]) in the concentration of this oxidative stress marker in the group treated with cisplatin alone. All three cisplatin cotreatment groups (i.e., cisplatin plus either NaHS, L-arginine or their combination) induced significant reversals of the cisplatin evoked plasma T-SOD decline (Fig. 5A).

Plasma MDA levels were found to be substantially increased on protocol days 14 and 28 in cisplatin alone treated rats compared with controls All the cotreatment groups disclosed a reversal of cisplatin boosted MDA plasma levels, but the group that received the cisplatin + NaHS + L-arginine combination divulged the most substantial reversal effect on protocol day 14 (Fig. 5B).

3.7. Effect of cisplatin coadministration with either sodium hydrosulfide, L-arginine or their combination on plasma and urinary sodium and potassium

Cisplatin given alone, significantly decreased the levels of plasma sodium versus the controls, which were unaffected throughout the protocol. On the other hand, concomitant treatment with either NaHS, L-arginine or their combination revoked cisplatin suppressed plasma sodium levels, the largest reversal occurring in the cisplatin + NaHS + L-arginine combined treatment group (Table 6).

Analogously, treatment with cisplatin by itself, also diminished potassium plasma levels compared to the control in which plasma potassium was not altered during the protocol (Table 6). By protocol day 28, NaHS, L-arginine and their combination had a reversal

Table 3

Effect of cisplatin treatment combined with either sodium hydrosulfide (NaHS), L-arginine or NaHS + L-arginine on creatinine clearance in rats.

Treatment Groups	Creatinine Clearance (ml/min)		
	Day 0	Day 14	Day 28
Control	0.24 ± 0.02	0.28 ± 0.02	0.34 ± 0.01
Cisplatin	0.32 ± 0.01	0.13 ± 0.08[a]	0.11 ± 0.04[a]
Cisplatin + NaHS	0.30 ± 0.03	0.14 ± 0.01[a]	0.24 ± 0.05[a, b]
Cisplatin + L-arginine	0.29 ± 0.01	0.15 ± 0.03[a]	0.27 ± 0.01[a, b]
Cisplatin + NaHS + L-arginine	0.25 ± 0.08	0.16 ± 0.07[a]	0.28 ± 0.03[a, b]

All values are expressed as mean ± SEM. One-way analysis of variance (ANOVA) was performed for statistical analysis with post hoc Bonferroni test.

[a] $p < 0.05$ versus control.

[b] $p < 0.05$ versus cisplatin.

Table 4

Effect of cisplatin treatment combined with either sodium hydrosulfide (NaHS), L-arginine or NaHS + L-arginine on plasma and urinary urea in rats.

Groups	Plasma Urea (mg/dl)			Urinary Urea (mg/dl)		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
Control	32.83 ± 0.40	33.17 ± 1.40	33.83 ± 1.90	72.67 ± 2.01	73.00 ± 1.00	70.67 ± 1.94
Cisplatin	30.17 ± 1.47	93.17 ± 2.71[a]	101.5 ± 0.88[a],[b]	71.00 ± 3.26	41.00 ± 0.51[a]	24.00 ± 1.00[a]
Cisplatin + NaHS	25.17 ± 1.24	71.50 ± 0.99[a],[b]	53.67 ± 0.81[a],[b,d]	71.50 ± 2.39	46.33 ± 0.33[a],[b]	53.67 ± 0.88[a],[b]
Cisplatin + L-Arginine	27.50 ± 4.61	78.17 ± 1.68[a],[b]	63.83 ± 0.65[a],[b,d]	73.50 ± 1.70	45.67 ± 1.11[a],[b]	51.17 ± 1.42[a],[b]
Cisplatin + NaHS + L-Arginine	31.83 ± 3.20	63.00 ± 2.69[a],[b,d]	43.17 ± 1.16[a],[b,c,d]	69.33 ± 3.54	53.83 ± 0.83[a],[b,c,d]	62.67 ± 0.80[a],[b,c,d]

All the values are expressed as mean ± SEM. One-way analysis of variance (ANOVA) was performed for statistical analysis with post hoc Bonferroni test.

[a] p < 0.05 versus control.
[b] p < 0.05 versus cisplatin.
[c] p < 0.05 versus cisplatin + NaHS.
[d] p < 0.05 versus cisplatin + L-arginine.

Table 5

Effect of cisplatin treatment combined with either sodium hydrosulfide (NaHS), L-arginine or NaHS + L-arginine on BUN in rats.

Groups	BUN (mg/dl)		
	Day 0	Day 14	Day 28
Control	15.34 ± 0.42	15.50 ± 0.30	15.80 ± 0.33
Cisplatin	14.09 ± 0.36	43.53 ± 0.44[a]	47.42 ± 0.30[a]
Cisplatin + NaHS	11.76 ± 0.30	33.41 ± 0.71[a],[b]	25.07 ± 0.25[a],[b,d]
Cisplatin + L-Arginine	12.85 ± 0.27	36.52 ± 0.30[a],[b]	29.82 ± 0.30[a],[b,c]
Cisplatin + NaHS + L-Arginine	14.87 ± 0.21	29.43 ± 0.42[a],[b,c,d]	20.17 ± 0.42[a],[b,c,d]

All the values expressed as mean ± SEM. One-way analysis of variance (ANOVA) was performed for statistical analysis with post hoc Bonferroni test.

[a] p < "0.05 versus control.
[b] p < 0.05 versus cisplatin.
[c] p < 0.05 versus cisplatin + NaHS.
[d] p < 0.05 versus cisplatin + L-arginine.

effect on the cisplatin incited declination in plasma potassium (Table 6).

In contrast, cisplatin administration on its own, caused an upsurge in both urinary sodium and potassium levels which were subsequently reversed by coadministration of either NaHS, L-arginine or their combination with cisplatin (Table 7). In the case of the animals given cisplatin + NaHS + L-arginine, there was a significantly greater reversal of the urinary sodium decrement compared with cisplatin + L-arginine (Table 7).

3.8. Histopathology

The histopathological studies revealed that control rats retained intact parenchymal cells with regular tubular structure and glomeruli (Fig. 6-A). The cisplatin treatment resulted in chronic inflammation as focal parenchymal primary lesions and focci of both tubular necrosis and atrophy were evident with marked interestitial nephritis (Fig. 6-B). However, there was noticeably less observable parenchymal damage in rats treated with cisplatin + NaHS (Fig. 6-C) and cisplatin + L-arginine (Fig. 6-D). However, when rats were co-administered with NaHS + L-arginine, a prominent protection from cisplatin-induced necrosis, focal granulation formation and fibros is was noted (Fig. 6-E).

All parts (A - E) of Fig. 6 are also provided individually in a supplementary data file labelled as Fig. S.

4. Discussion

Nephrotoxicity causing kidney damage provides, a challenge to the quest for new therapies. A natural target for toxic xenobiotics is the renal sytem, due to its ability to excrete and concentrate toxins. This study was designed to determine any preventative effects of H_2S and NO generated from NaHS and/or L-arginine on cisplatin induced nephrotoxicity as evidenced by kidney dysfunction.

Cisplatin, a chemotherapeutic agent is used for cancer treatment with a high probability of inducing nephrotoxicity and a number of other toxicities including ototoxicity, myelosupression, allergic reactions and gastrotoxicity. In this context, the degree of cisplatin accumulation in renal tissues, especially the terminal portion of the proximal tubule, is much higher compared to other organs [26,27].

The drug is activated to release a nephrotoxicant which alters cellular transport, triggers inflammation, mitochondrial damage, oxidative stress, and apoptosis [28]. The kidney, in response, generates reactive oxygen species (ROS), while endogenous antioxidants such as glutathione (GSH), catalase, peroxidase and superoxide dismutase (SOD) neutralize excess ROS to prevent or limit damage. Cisplatin triggers acute kidney failure due to increased production of ROS in mitochondria, consequently cellular xanthine oxidase and the NADPH oxidase system become compromised [29]. Studies shown that Cisplatin introduction to animals causes decrease in overall
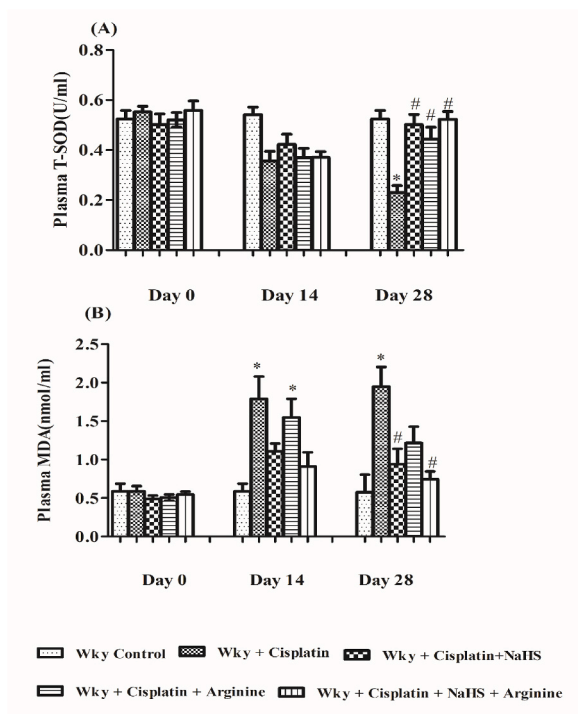
Fig. 5. Effect of cisplatin treatment combined with either sodium hydrosulfide (NaHS), L-arginine or NaHS + L-arginine during a 28-day protocol, on rat plasma (**A**) T-SOD and (**B**) MDA in rats. All values are mean ± SEM and statistical analysis was performed by one way analysis of variance (ANOVA) with post hoc Bonferroni test. ∗ p < 0.05 versus control; #p < 0.05 versus cisplatin.

Table 6
Effect of cisplatin treatment combined with either sodium hydrosulfide (NaHS), L-arginine or NaHS + L-arginine on plasma sodium and potassium levels in rats.

Groups	Plasma Sodium (mEq/L)			Plasma Potassium (mEq/L)		
	Day 0	Day 14	Day 28	Day0	Day 14	Day 28
Control	144.5 ± 1.70	148.7 ± 2.15	146.7 ± 2.27	5.83 ± 0.30	6.66 ± 0.33	6.50 ± 0.22
Cisplatin	147.7 ± 2.02	100.7 ± 2.23[a]	88.67 ± 2.71[a]	6.16 ± 0.40	2.10 ± 0.20[a]	1.80 ± 0.05[a]
Cisplatin + NaHS	144.8 ± 2.08	119.0 ± 1.48[a, b]	125.3 ± 0.88[a, b]	5.16 ± 0.60	2.65 ± 0.06[a]	3.73 ± 0.03[a, b]
Cisplatin + L-Arginine	150.0 ± 2.30	115.0 ± 1.52[a,b]	124.7 ± 1.76[a, b]	5.83 ± 0.70	2.60 ± 0.07[a]	3.63 ± 0.04[a, b]
Cisplatin + NaHS + L-Arginine	142.7 ± 4.15	127.3 ± 1.05[a, b,d]	134.7 ± 0.84[a, b,c,d]	6.50 ± 0.34	3.10 ± 0.57[a, b]	4.16 ± 0.04[a, b]

All the values are expressed as mean ± SEM. One-way analysis of variance (ANOVA) was performed for statistical analysis with post hoc Bonferroni test.

[a] p < 0.05 versus control.
[b] p < 0.05 versus cisplatin.
[c] p < 0.05 versus cisplatin + NaHS.
[d] p < 0.05 versus cisplatin + L-arginine.

renal NO_x content and this leads to the fact that cisplatin is known to distress DNA, RNA and Protein synthesis which in-turn reduces NOS isoenzyme level [30].

After cisplatin administration, inflammatory mediators are increased several fold in the kidney, and this is invariably followed by ischemic insult [31]. Cisplatin nephrotoxicity has been confirmed using a variety of parameters such as body weight, ratio of kidney weight and body weight, urinary output, levels of creatinine, urea, sodium, potassium and reactive oxygen species markers, all of which may contribute to morbidity or mortality. NaHS is a natural donor of exogenous H_2S as an antioxidant modulating different physiological functions such as vascular relaxation, neurotrasmission and insulin sensitivity [32]. Whereas NO is produced from L-arginine by the action of nitric oxide synthase and it is a vasoctive factor important in regulating vascular tone. It is also considerd to be involved in the regulation of renal hemodynamics, renin production, water and electrolyte balance, ischemia prevention, glomerular thrombosis, mesangial cell proliferation and extracellular matrix deposition. In addition to these actions, it induces relaxation of pre-glomerular arteries to improve renal blood flow. Regarding nephrotoxicity, it can be aggravated by NO blockade and ameliorated by NO enhancement, suggesting that NO maintains a nephroprotective function [33].

Table 7

Effect of cisplatin treatment combined with either sodium hydrosulfide (NaHS), L-arginine or NaHS + L-arginine on urinary sodium and potassium levels in rats.

| Groups	Urinary Sodium (mEq/24 h)			Urinary Potassium (mEq/24 h)		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
Control	168.5 ± 1.25	171.7 ± 2.80	173.5 ± 2.48	3.50 ± 0.22	4.50 ± 0.34	4.16 ± 0.30
Cisplatin	167.2 ± 3.19	310.8 ± 3.11[a]	352.0 ± 4.38[a]	4.33 ± 0.61	6.83 ± 0.47[a]	6.91 ± 0.49[a]
Cisplatin + NaHS	166.2 ± 4.59	257.7 ± 2.84[b,a]	264.5 ± 4.10[b,a]	4.35 ± 0.42	5.10 ± 0.05	5.52 ± 0.02
Cisplatin + L-Arginine	174.7 ± 1.05	246.8 ± 2.52[b,a]	270.8 ± 1.86[b,a]	4.00 ± 0.51	5.18 ± 0.16	5.50 ± 0.05
Cisplatin + NaHS + L-Arginine	164.8 ± 5.55	209.2 ± 3.27[b,a,c,d]	238.2 ± 2.52[b,a,d,c]	4.16 ± 0.60	5.01 ± 0.01[b]	5.11 ± 0.09[b]

All the values are expressed as mean ± SEM. One-way analysis of variance (ANOVA) was applied for statistical analysis with post hoc Bonferroni test.

[a] $p < 0.05$ versus control.

[b] $p < 0.05$ versus cisplatin.

[c] $p < 0.05$ versus cisplatin + NaHS.

[d] $p < 0.05$ versus cisplatin + L-arginine.
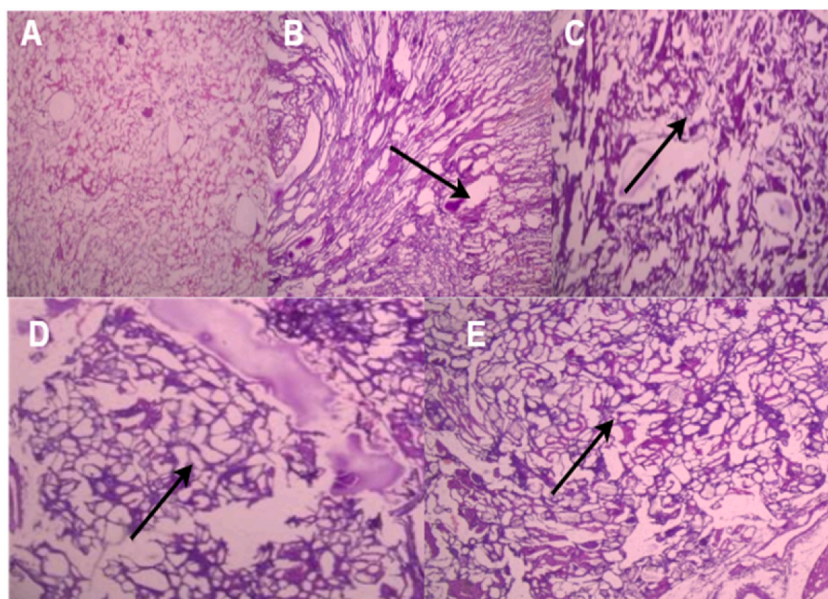
Fig. 6. Typical histopathological sections of the rat kidney from: (A) control group showing healthy parenchymal structure, (B) cisplatin group revealing necrosis and atrophy, (C) cisplatin + NaHS group, (D) cisplatin + L-arginine group and (E) cisplatin + NaHS + L-arginine group showed comparatively marked protection from tubular damage.

Initially in the study, basic parameters were monitored, and cisplatin induced a reduction in body weight with an increased urinary output along with the increase in kidney weight and body weight ratio (KW/BW) that might be because of extra-cellular matrix accretion and renal cell abnormalities. The loss of body weight may have possibly been attributable to damaged renal tubules with a subsequent loss of kidney cells necessary to reabsorb water causing dehydration and decreased body weight. Similarly, urinary volume was enhanced in cisplatin treated animals due to induction of nonoliguric acute kidney failure. Cisplatin is considered to enhance the urinary output linked to decreased gene expression of aquaporins and their density in proximal tubules [4]. During treatment, generation of both H_2S and NO reversed cisplatin induced weight loss and increased urinary output which may well have been derived from their cytoprotective and antioxidant activities and also decreased in KW/BW in treatment groups, which is showing confirmation of the reversal of renal structural changes. In combination, synergistic effects were observed between the two gasotransmitters, which most likely stemmed from an additive prevention of cellular damage.

Plasma creatinine and urea levels were increased along with decreased sodium and potassium concentrations in the cisplatin treated animal groups [34]. This outcome clearly evidenced the impaired capability of the kidney to excrete surplus materials and to conserve valuable cations. An electrolyte imbalance followed by decreased activity of Na^+/K^+ ATPase was directly associated with cisplatin induced renal damage which then instigated hypokalemia and hyponatremia [35]. The renal functional parameter BUN, was also raised with a concurrent fall in creatinine clearance in response to cisplatin due to a general inability of the kidney to excrete waste materials.

Nitric oxide and hydrogen sulphide effect the liberation of each other through the enzymetic expression and after that through downstream signalling pathway [3]. Research on H_2S– NO interaction in inflammation has been less extensive and could be different

from their effect individually. Studies has attenuated that H_2S (or its donor) causes the inhibition of production iNOS isoenzyme expre-ssion thru expre-ssion of heme oxygenase 1 (HO-1) in RAW264. HO-1 expression is stimulated via stimulation of extra cellular signal-regulated kinase (ERK) which in turn is responsible for iNOS expre-ssion. Previous inflammatory models attributes that NaHS attenuates neutrophil infiltration and inflammation and also iNOS [36]. As nitric oxide is considered as dual nature in case of kidney physiology, NO produced by eNOS is considered as "good face" as it appears as a relaxant while iNOS is considered as "bad face" as it produces NO is involved in renal inflammatory conditions by synthesis of peroxynitrite (ONOO-) a pro-inflammatory cytokine in-teracts with the superoxide anion (O_2) [37]. Previous studies shows that NO impart role in regulation of cell-death resis-tance to Cis -diamminedichloroplatinum (II) CDDP. CDDP persuades down-regulation of BCl-2 (B cell lymphoma 2) through proteasome-mediated degradation. NO regulates it negatively through its protein nitrosylation process and inhibition of ubiquitination [38]. NO released from nitric oxide donor, control the other agent toxicity so the released nitric oxide is helpful in numerous modalities of chemotherapy [39].

Hydogen sulfide shown the responsibility of expression of eNOS protein expression both invivo as well as invitro experimental models. Hence the both have synergism effect in Cisplatin induced kidney failure by causing decrease in kidney inflammatory markers levels (serum creatinine and serum BUN and urea).

During cotreatment, donated H_2S and NO activity reversed cisplatin boosted plasma creatinine, and urea, in addition to BUN, while subsequently increasing creatinine clearance. This outcome can be linked to the ability of both molecules to produce pre-glomerular vasodilation thereby enhancing the clearance of waste materials. What is more, the cisplatin evoked sodium and potassium imbalance was most likely initiated through impairment of the proximal tubule sodium-potassium membrane pump brought about by cellular necrosis. The antioxidant and anti-inflammatory activity present in the cotreatments, would have contributed towards the prevention of cellular damage and any ensuing inflammation and necrosis in this tubular region. Similarly, cisplatin instigated a reductionn in plasma T-SOD levels, with an attendent increase in MDA concentration which has been associated with enhanced lipid peroxidation [40] and the production of reactive oxygen species (ROS). The cotreatments reversed the perturbed levels of these oxidative stress markers and this was ascribed to their ability to scavenge ROS and diminish oxidative stress [41].

In the histopathological examination, cisplatin treated renal tissues clearly divulged chronic inflammatory cells with mild infil-tration cisplatin itself or its metabolites and expansion of mesangial cells with diffused glomerulo-sclerosis. In the cotreated tissues however, the evidence of renal damage was minimal and almost certainly linked to the free radical neutralizing capability of H_2S and NO.

Taken as a whole, NaHS and L-arginine cotreatment extensively reversed the kidney dysfunctions developed from cisplatin induced nephrotoxicity. It was also noted, that during cisplatin nephrotoxicity, alterations in urinary output, plasma/urinary sodium and urea, MDA and BUN, were all reversed more effectively by the NaHS/L-arginine combination than either of these agents coadministered alone. Accordingly, combined H_2S and NO generated activity has had a potentiated nephroprotectant effect against cisplatin, and this correlates with a synergistic or cooperative effect of cross-talk [3] between both signalling molecules [42,43].

5. Conclusion

The study showed that cisplatin treatment negatively affected several renal parameters depicting nephrotoxicity. This cisplatin-induced nephrotoxicity was reduced by NaHS and L-arginine as single treatrment. However, when rats were coadministered with both treatments, the outcomes were notably improved revealing the simultaneous anti-inflammatory and antioxidant potential of both treatments proved more effective in ameliorating the cisplani-induced renal damage. However, it is noteworthy that current study is in preclinical stage and further studies are necessary to better understand the mechanism through which treatments exerted the outcomes.

Histopathological outcomes have been enlisted as a limitation of the study. In the future, the effect of test treatments on cisplatin-induced nephrotoxicity might be better elaborated by using adequate animals for quantitative and qualitative presentations of his-topathological outcomes.

Ethics statement

The study design was reviewed and approved by the Pharmacy Animal Ethics Committee (approval number PAEC/2020/31).

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Data availability statement

There are no publicly accessible archives, but we are happy to provide any primary data upon request.

Informed consent statement

Not applicable.

CRediT authorship contribution statement

Faria Khurshid: Writing – original draft, Visualization, Software, Methodology, Investigation, Conceptualization. **Javeid Iqbal:** Validation, Supervision, Conceptualization. **Fiaz-ud-Din Ahmad:** Supervision, Project administration, Conceptualization. **Arslan Hussain Lodhi:** Writing – review & editing. **Abdul Malik:** Validation. **Suhail Akhtar:** Visualization. **Azmat Ali Khan:** Validation. **Marvi Imam Bux:** Writing – review & editing. **Mohammed Younis:** Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Abdul Malik reports financial support was provided by King Saud University, Saudi Arabia. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29513.

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