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PROTECTIVE EFFECT OF THE EXOGENOUS HYDROGEN SULFIDE DONOR, PARKIA SPECIOSA HASSK. AGAINST HYPERTENSIVE NEPHROPATHY

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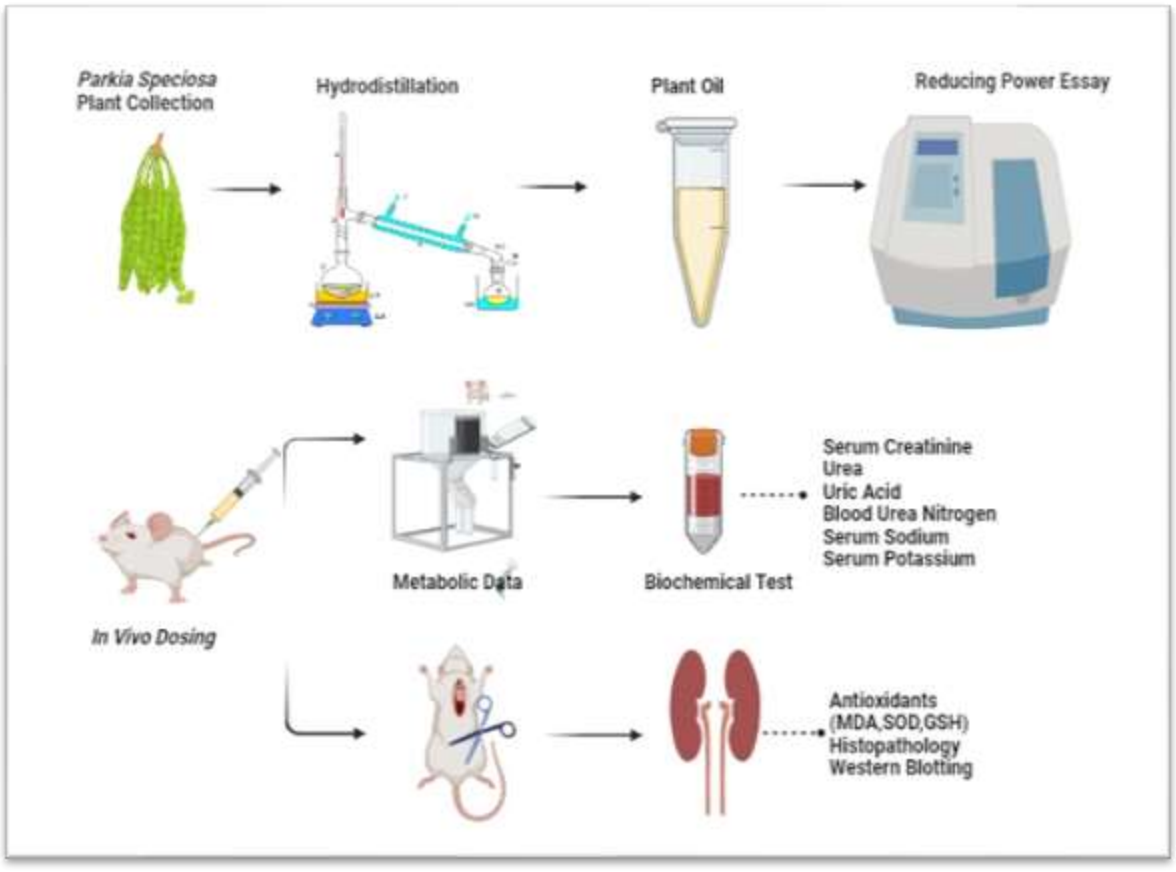
Graphical Abstract



Abstract
The plant *Parkia speciosa* Hassk. (*Ps*H) belongs to the family Fabaceae, and it is endemic to Southeast Asia. Its seeds have been used traditionally to treat various ailments, and several investigations have been undertaken to assess its the pharmacological activity. The aim was to ascertain whether *Ps*H, as a hydrogen sulfide donor, could prevent hypertensive nephropathy. We employed a combined in-vitro and in-vivo approach to evaluate the effect of the plant oil on isoproterenol (ISO)-induced cardiac failure in rats. The oil was prepared by hydrodistillation and the antioxidant potential was evaluated by a reducing power assay. Serum kidney biochemical parameters (serum creatinine, urea, uric acid, blood urea nitrogen), serum electrolytes (serum sodium and potassium), and antioxidant biomarkers (malondialdehyde, superoxide dismutase and, glutathione) were measured while (cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) were examined in kidney tissue by western blot. Dissected kidney sections were also examined histopathologically with eosin-hematoxylin staining and microscopy. The plant oil possessed a maximum reducing power at a concentration of 100µM. Subsequently, in-vivo findings revealed that the oil reversed ISO modified metabolic data, serum kidney biochemical parameters, serum electrolytes, and antioxidant biomarkers in renal tissues. The oil also reversed the ISO-inhibited endogenous H_2S generating enzymes, CBS and CSE as disclosed by western blot. Additionally, all findings were supported by tissue histopathology. Arising from the study outcomes, *Ps*H oil may have value as an H_2S donating nutrapharmaceutical in reducing biochemical aspects of hypertensive nephropathy.

Keywords: *Parkia speciosa* Hassk., hydrogen sulfide, isoproterenol, hypertensive nephropathy, renal protection, western blotting

Introduction

Hypertensive renal disease is a leading cause of kidney failure after diabetes mellitus. Persistent and uncontrolled high blood pressure usually causes moderate hypertensive nephrosclerosis and eventually, kidney failure. An increasing number of patients with hypertensive nephropathy is attributable to aging and cardiovascular complications (1). The prevalence of hypertensive nephropathy varies from country to country, the highest reported occurrence being in France (27%) and the lowest incidence in Japan (6%) (2). Pathologically, hypertension induces vascular remodeling and an alteration in renal hemodynamics that produces an elevation in total vascular resistance and a reduced plasma flow to kidney tissues. A prolonged history of uncontrolled hypertension induces anatomical changes in arcuate and interlobular arteries in addition to hypertrophy, and proliferation of smooth muscle cells in renal arterioles. Therefore, the key objective in combating hypertensive nephropathy is to shield nephrons and provide defense against renal ischemia, which can be accomplished by maintaining the stability of arterial pressure (3).

Hydrogen sulfide (H_2S) was the third gasotransmitter identified after nitric oxide (NO) and carbon monoxide (CO), and it plays an important function in controlling blood pressure and renal physiology (4). In mammals, H_2S is formed in the cytosol from L-cysteine via the enzymes cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulphurtransferase (3-MST) working in conjunction with cysteine aminotransferase (CAT). L-cysteine is a substrate for CBS and CSE in generating H_2S although, other substrates can also serve in this regard (5). H_2S itself has diversity in terms of its biological functions which include vasodilatation, mitochondrial bioenergetics, metabolic regulation, kidney excretion and angiogenesis, in addition to anti-inflammatory, free radical scavenger, and antiapoptotic-activity. Moreover, a deficiency of H_2S can produce multiple kidney pathologies, for example, renal vascular lesions, renal reperfusion injury, uropathy, diabetic kidney disease and hypertensive nephropathy (4). In experimental models of ischemia/reperfusion injury, the expression of CBS and CSE in nephrons is reduced, which lowers the endogenous production of H_2S within the body. Consequently, it exacerbates inflammation, redox imbalance, apoptosis of tubular epithelial cells, and vasculopathy **(Fig. 1).** Therefore, administration

of an H_2S donor after ischemic injury restores the level of hydrogen sulfide producing enzymes and its subsequent production (5).

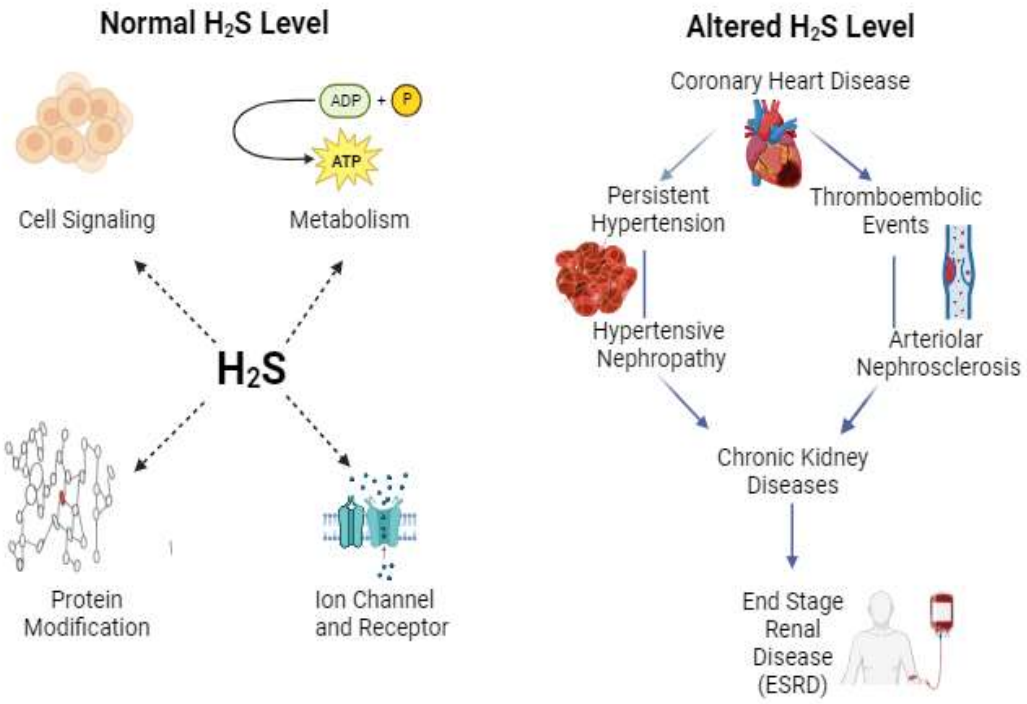


Figure 1. Molecular targets of H_2S include cell signaling, protein modification, metabolism, ion channels and receptors. In relation to these phenomena, altered levels of H_2S impair the cardiovascular system leading to chronic kidney diseases

Natural plant derived compounds possessing polysulfides can be precursors of H_2S. These polysulfides are capable of stimulating the production of H_2S and promoting the regulation of endothelial nitric oxide (NO) which in turn induces relaxation of smooth muscles, vasodilation, and reduction of hypertension (6). Previous studies have shown that among many polysulfide-enriched fruits and vegetables, *Parkia speciosa* Hassk. (*Ps*H) seeds (commonly known as stinky beans) have the most potent H_2S-releasing potential (7). *Ps*H produces pods with green seeds which are consumed as food in the Southeast Asian continent including Malaysia and Northeast India (8). Traditionally, the seeds of this plant are consumed by local people to treat multiple ailments including diabetes, kidney disorders, hypertension, and headache (9).
Investigative studies have reported *Ps*H to exhibit antioxidant, antimicrobial (10), hypoglycemic (11), hypolipidemic (12), anti-inflammatory (13) and anti-hypertensive (14) properties. However, there is no available evidence concerning any positive effects of this potential phytomedicine on hypertensive nephropathy. Therefore, the present study was designed to evaluate any conceivable protective activity of *Ps*H against hypertensive nephropathy in an isoproterenol (ISO)-induced myocardial failure model in rodents. To accomplish this, metabolic data, renal parameters, antioxidant enzyme levels, and western blotting, along with histopathology, were employed to probe the issue.

Material and Method

Preparation of plant oil by hydrodistillation

One kilograms of the plant seeds were imported from Malaysia. After identification, its specimen was preserved in the herbarium of the Faculty of Pharmacy and Alternative Medicine, the Islamia University of Bahawalpur, Pakistan, with a voucher number (PS-SD-08-21-199) for future reference.

After chopping the seeds, hydrodistillation of the product was carried out (15) and the oil obtained was stored for further use.

Chemicals

Chemicals including isoproterenol, methanol, butylated hydroxytoluene (BHT), hydrochloric acid, and vitamin c were obtained from Sigma Aldrich, Germany. Sodium hydrosulfide (Daejing Chemicals, Korea), xylazine (Prix Pharmaceuticals, Pakistan), and ketamine (Global Pharmaceuticals) were used. Serum creatinine (BioSystems), urea (BioSystems), blood urea nitrogen (BioSystems), serum sodium (spectrum), and serum potassium (spectrum) kits were used for biochemical analysis. Antioxidants biomarker kits including malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione (GSH) kits were obtained from Solarbio Life Sciences (China). Sigma Aldrich, Germany, provided the beta-actin antibodies (A5441), anti-CSE antibodies (HPA038053), and anti-CBS antibodies (SAB1405568).

Ferric reducing/antioxidant power (FRAP) Assay

The reducing power of *Ps*H oil was detected by the reduction of ferric (Fe^{+3}) to ferrous (Fe^{+2}) ions producing a chemical color change owing to the generation of Perls' Prussian Blue complex. The colorimetric absorption of the chemical mixture is directly proportional to the reduction potential of the compounds. Different concentrations of plant oil (20-100µM)/10ml were prepared in methanol (16). Then, to 1.0 ml of solution was added 1.25ml of 0.2M phosphate buffer solution (pH 6.6) and 1.25ml of 1% potassium ferricyanide [$K_3[Fe(CN)_6]$. The mixture was kept at 50°C for 20 minutes acidified with 1.25ml of 10% TCA. Finally, 0.5ml of $FeCl_3$ (0.1%) was added to this solution and sample absorbance was measured spectrophotometrically at 700nm (17).

Experimental Animals

Normal healthy adult *Wistar* albino rats (n = 54) weighing 200 ± 20 gm were supplied by the vivarium of the Faculty of Pharmacy, the Islamia University of Bahawalpur, Pakistan. After acclimatization, the animals were randomly assigned to nine groups (n = 6). The rats were fed on standard rodent diet and given free access to water. In the laboratory, conditions were maintained at a temperature of 25 ± 3.0◦C (humidity > 65%), on an alternating 12h/12h light/dark cycle. The study and protocol were approved by the Pharmacy Animal Ethics Committee, Faculty of Pharmacy, Islamia University of Bahawalpur, (certificate #PAEC/21/68).

Experimental protocol

Experimental heart failure was induced by the administration of ISO (5.0 mg/kg, i.p) daily for seven days (18). The H_2S releasing capacity of *Ps*H oil was calculated according to (7). The oil was mixed with Tween 80 (1%) and normal saline before i.p administration (19). The animals were grouped as follows:

Group A: vehicle intraperitoneally (i.p) with no further treatment, Group B: ISO alone to induce experimental heart failure, Group C: ISO plus orally administered (p.o) vitamin C (50mg/kg), Group D: ISO plus digitalis (0.0225 mg/kg) orally, Group E: ISO plus sodium hydrogen sulfide (NaHS; 56 µmole/kg, i.p.), Group F: ISO plus *Ps*H oil (40µmole/kg), Group G: ISO plus *Ps*H (60 µmole/kg i.p), Group H: ISO plus *Ps*H oil (80 µmole/kg i.p), Group I: ISO plus digitalis (0.01125 mg/kg p.o) along with *Ps*H oil (40µmole/kg i.p). Subsequently, all of the animals were fasted overnight, then weighed, and killed using a xylazine-ketamine anesthetic mixture (1:10). Postmortem animal kidneys were immediately dissected, washed with normal saline, and stored for further investigation.

Collecting metabolic data and serum

Samples for metabolic studies and serum were obtained on protocol study days 7, 14, and 28. In the metabolic study, experimental animals were kept in metabolic cages for 24 hours, food and water intake, along with urinary output volume being observed.

Serum was collected in heparinized centrifuge tubes using the retro-orbital puncture method. Sample tubes were then centrifuged for 15 minutes at 3000 rpm to obtain serum. All the collected serum and urine samples were refrigerated till further analysis.

Western blotting

The expression of CBS and CSE was investigated in kidney tissues by western blotting. After chopping the tissue samples, they were lysed in ice-cold NP-40 lysis buffer, followed by homogenization on ice and then centrifuged (4ºC for 10 minutes at 12000 rpm) yielding the lysate in the supernatant. Next, MagicMark™ XP (3uL) and 13µL of sample (mixed with NuPAGE™ sample reducing agent [10X] and NuPAGE™ LDS sample buffer [4X]) were loaded on a 10 well NuPAGE 10% Bis-Tris gel. The samples were separated on the NuPAGE gel which was run for 35 minutes at 150V. The gel was then transferred to a PVDF membrane after an hour. Following blotting, the membrane was kept in a blocking buffer overnight at room temperature. Initial incubation of the membrane was done with primary antibody to CSE (dilution 1:1000) for 60 minutes and then followed by antibody to CBS (dilution 1:450). After the removal of primary antibodies with wash buffer, the membrane was incubated with secondary antibody (dilution 1:1000) for an hour. After wash buffer, an ECL plus western blot detection kit was used to reveal the presence of proteins by using Gel capture software. Afterwards, the PVDF membrane was reprobed using a loading control beta-actin antibody (1:1000) for an hour, and then the same method was used to incubate a secondary antibody. The presence of beta-actin was then ascertained using the ECL plus western blot detection kit.

Histopathological analysis

After dissection, sections of kidney tissues were preserved in a 10% formalin solution. The tissues were then subjected to histopathological analysis including graded methanol, fixation with xylene followed by embedding in paraffin wax. Staining of slides was performed with eosin and hematoxylin, and images were viewed on an optical microscope at 10X resolution.

Statistical Analysis

Data were presented as mean ± SEM (n=6). Statistical analysis among the experimental groups was evaluated using one-way analysis of variance (ANOVA) with Bonferroni post hoc test. Values of $p < 0.05$ were considered statistically significant.

Results

Reducing power Assay

The reducing power assay was performed with the plant seed oil and BHT (standard positive comparator) over the concentration range 20-100 µM. As can be seen in **Figs. 2(A) and 2(B)**, the absorbance of BHT and *Ps*H oil was measured, and a linear concentration-related graph was created by plotting the absorbance on the y-axis and concentration on the x-axis.

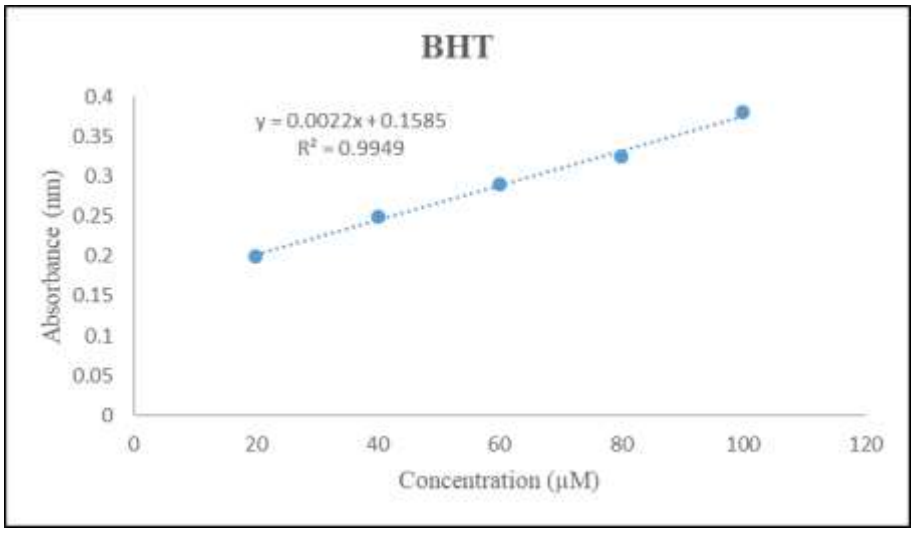


Figure 2 (A): Reducing Potential of BHT at different concentrations

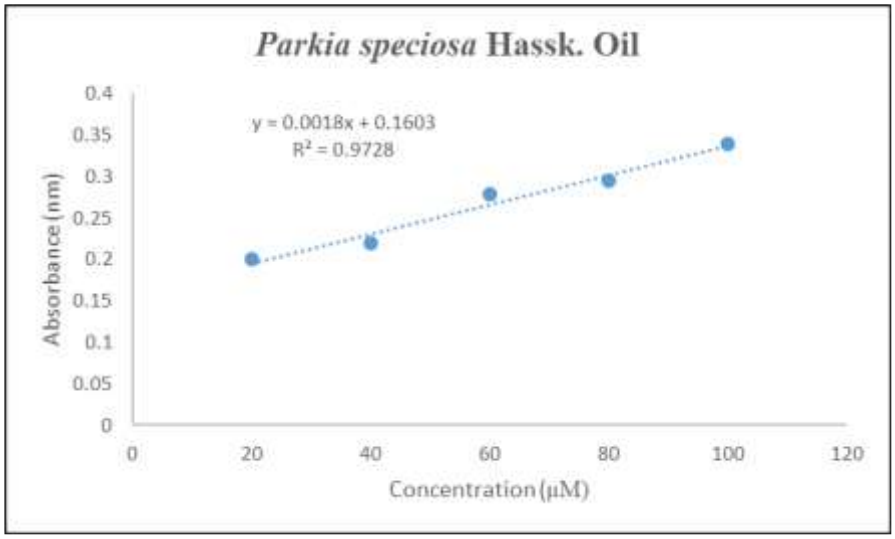


Figure 2 (B): Reducing Potential of *Parkia* speciosa Hassk. oil at different concentrations

Collection of metabolic data

Metabolic data, including water intake, urinary volume, and food intake were collected, and the mean results from of each group are presented in Tables 1, 2 and 3.

Effect of treatments on water intake

Animal water intake was found to be increased on protocol days 7, 14, and 28 in the group receiving ISO alone. Cotreatment with the standard drugs and varied doses of plant seed oil decreased the water intake on the 14th and 28th days of the protocol (see Table 1).

Table 1. Water intake of control, ISO, and ISO + various treatment groups of rats. The values (n = 6) are shown as mean ± SEM. The Bonferroni post hoc test was used in conjunction with one-way ANOVA for statistical analysis. *p < 0.05 versus control

Water Intake (ml)	**7th Day**	**14th Day**	**28th Day**
Control (4 ml/kg saline, p.o.)	18.43 ± 9.41	20.15 ± 6.35	19.56 ± 6.16
Isoproterenol (ISO; 5 mg/kg i.p.)	35.12 ± 2.91*	40.35 ± 5.07*	46.68 ± 3.71*
Vitamin C (50 mg/kg p.o.)	27.31 ± 2.69	30.12 ± 3.30	20.65 ± 5.61
Digitalis (0.01125 mg/kg p.o.)	25.84 ± 4.44	27.10 ± 2.64	21.45 ± 1.72
NaHS (56 µmol/kg i.p.)	28.62 ± 4.13	31.70 ± 5.52	23.30 ± 3.71

*Ps*H oil (40 µmol/kg i.p.)	30.83 ± 1.86	37.5 ± 9.89*	30.83 ± 5.33*
*Ps*H oil (60 µmol/kg i.p.)	28.16 ± 4.14	35.16 ± 2.73*	25.33 ± 4.53*
*Ps*H oil (80 µmol/kg i.p.)	27.50 ± 2.5	30.83 ± 10.57	20.83 ± 13.66
*Ps*H oil (40 µmol/kg i.p.) + Digitalis (0.01125 mg/kg p.o.)	29.83 ± 4.09	36.66 ± 4.71*	28.33 ± 4.71*

Effect of treatments on urine output volume

For every animal in the study group, the urine output volume was recorded on days 7, 14, and 28 during the study. No significant difference was noticed by any treatments during the course of protocol (see Table 2).

Table 2. The urine output volume of control, ISO, and ISO + various treatment groups. The values (n = 6) are shown as mean ± SEM. The Bonferroni post hoc test was used in conjunction with one-way ANOVA for statistical analysis.

Urine Volume (ml)	**7TH Day**	**14th Day**	**28th Day**
Control (4 ml/kg saline, p.o.)	4.01 ± 0.56	4.18 ± 0.90	5.02 ± 1.90
Isoproterenol (ISO; 5 mg/kg i.p.)	8.00 ± 3.84	10.35 ± 1.89	12.34 ± 0.75
Vitamin C (50 mg/kg p.o.)	7.01 ± 1.55	8.00 ± 1.16	5.09 ± 1.54
Digitalis (0.01125 mg/kg p.o.)	6.35 ± 2.78	9.34 ± 3.60	5.67 ± 2.70
NaHS (56 µmol/kg i.p.)	6.01 ± 2.32	7.65 ± 5.72	5.52 ± 2.67
*Ps*H oil (40 µmol/kg i.p.)	7.83 ± 3.28	9.33 ± 4.71	6.33 ± 1.79
*Ps*H oil (60 µmol/kg i.p.)	6.66 ± 2.74	8.33 ± 2.42	5.83 ± 1.67
*Ps*H oil (80 µmol/kg i.p.)	6.33 ± 2.13	7.66 ± 1.79	5.33 ± 1.49
*Ps*H oil (40 µmol/kg i.p.) + Digitalis (0.01125 mg/kg p.o.)	7.33 ± 9.35	9.16 ± 2.33	6.00 ± 2.58

Effect of treatments on food intake

The food intake of rats was monitored on protocol days 7, 14 and 28. There was a reduction of food intake in the ISO alone treated group compared with the saline controls throughout the protocol up to 28 days. However, there was a reversal of the ISO suppression of food intake by combined treatment of ISO with vitamin C, digitalis, NaHS and all three doses of *Ps*H oil (see Table 3).

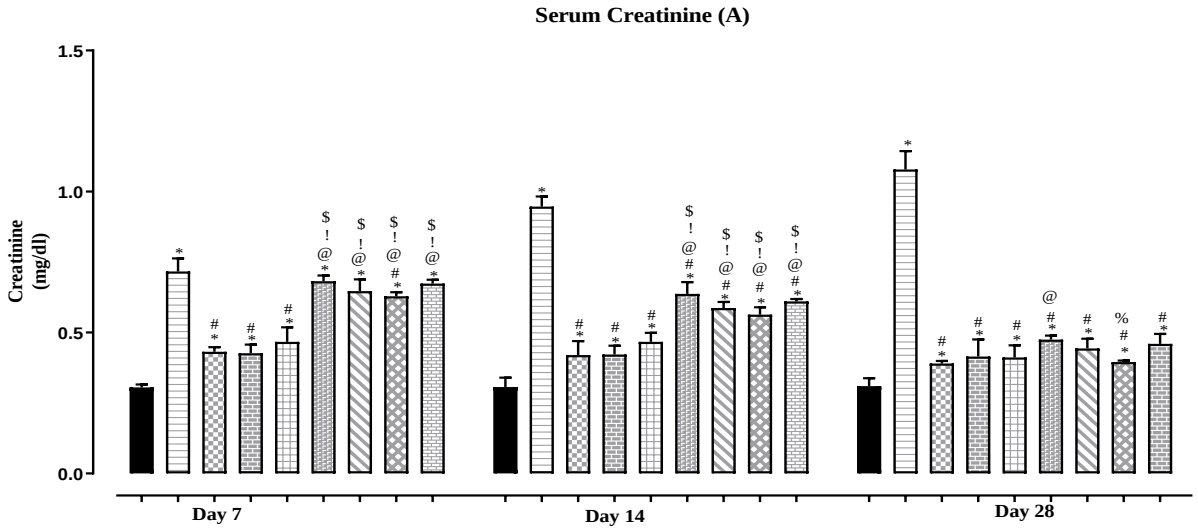
Table 3. The food intake of control, ISO, and ISO + various treatment groups of rats. The values (n = 6) are shown as mean ± SEM. The Bonferroni post hoc test was used in conjunction with one-way ANOVA for statistical analysis. *$p < 0.05$ versus control; #$p < 0.05$ versus ISO

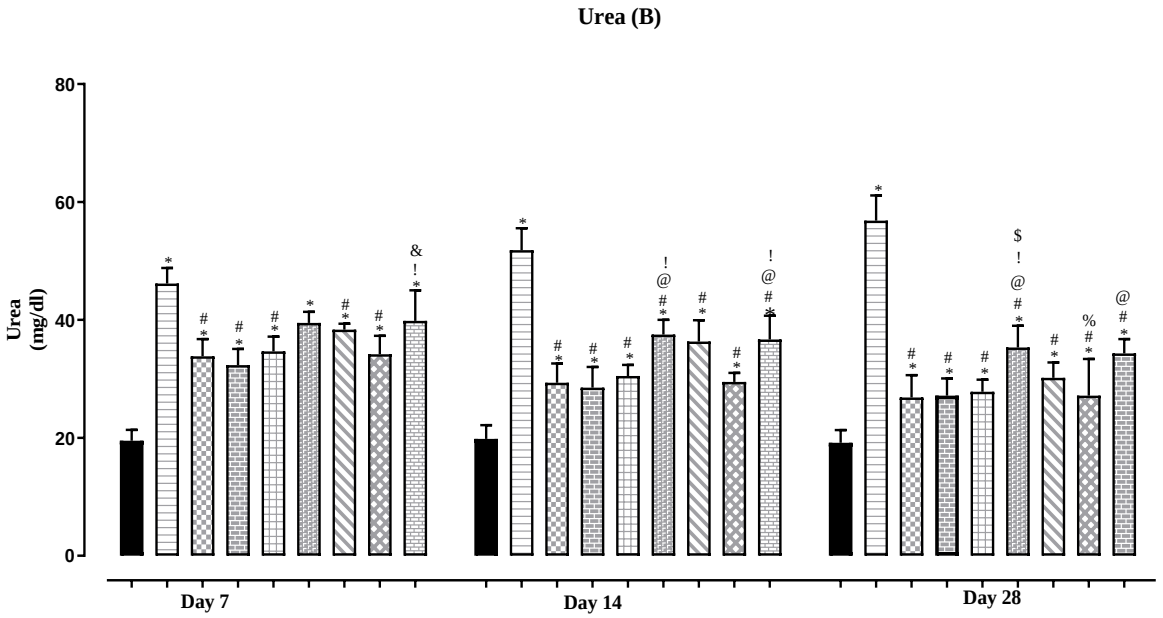
Food Intake (gm)	**7th Day**	**14th Day**	**28th Day**
Control (4 ml/kg saline, p.o.)	20.65 ± 1.11	21.35 ± 1.50	22.34 ± 1.70
Isoproterenol (ISO; 5 mg/kg i.p.)	14.01 ± 2.09*	12.01 ± 1.62*	10.17 ± 1.08*
Vitamin C (50 mg/kg p.o.)	15.50 ± 1.39	17.35 ± 2.07#	19.64 ± 1.60#
Digitalis (0.01125 mg/kg p.o.)	17.67 ± 2.45	20.34 ± 4.19#	21.34 ± 1.90#
NaHS (56 µmol/kg i.p.)	16.85 ± 1.65	18.64 ± 1.67#	20.18 ± 2.20#
*Ps*H oil (40 µmol/kg i.p.)	14.33 ± 2.28*	15.83 ± 2.11#	17.00 ± 2.23*#
*Ps*H oil (60 µmol/kg i.p.)	15.66 ± 1.10	17.16 ± 1.57	19.66 ± 2.56#
*Ps*H oil (80 µmol/kg i.p.)	17.16 ± 1.46	19.33 ± 1.69#	21.16 ± 3.02#
*Ps*H oil (40 µmol/kg i.p.) + Digitalis (0.01125 mg/kg p.o.)	14.5 ± 1.70*	16.16 ± 2.11	18.16 ± 3.33#

Effect of treatments on renal parameters

The main markers of renal impairment were blood urea nitrogen (BUN), urea, uric acid, and serum creatinine. Hence, our results showed that in rats treated with ISO alone, renal biochemical impairment indicators were subsequently raised and the aforementioned parameters were reversed

when these animals were variously cotreated or administered different doses of *Ps*H oil **(Fig. 3A, B, C, D).**





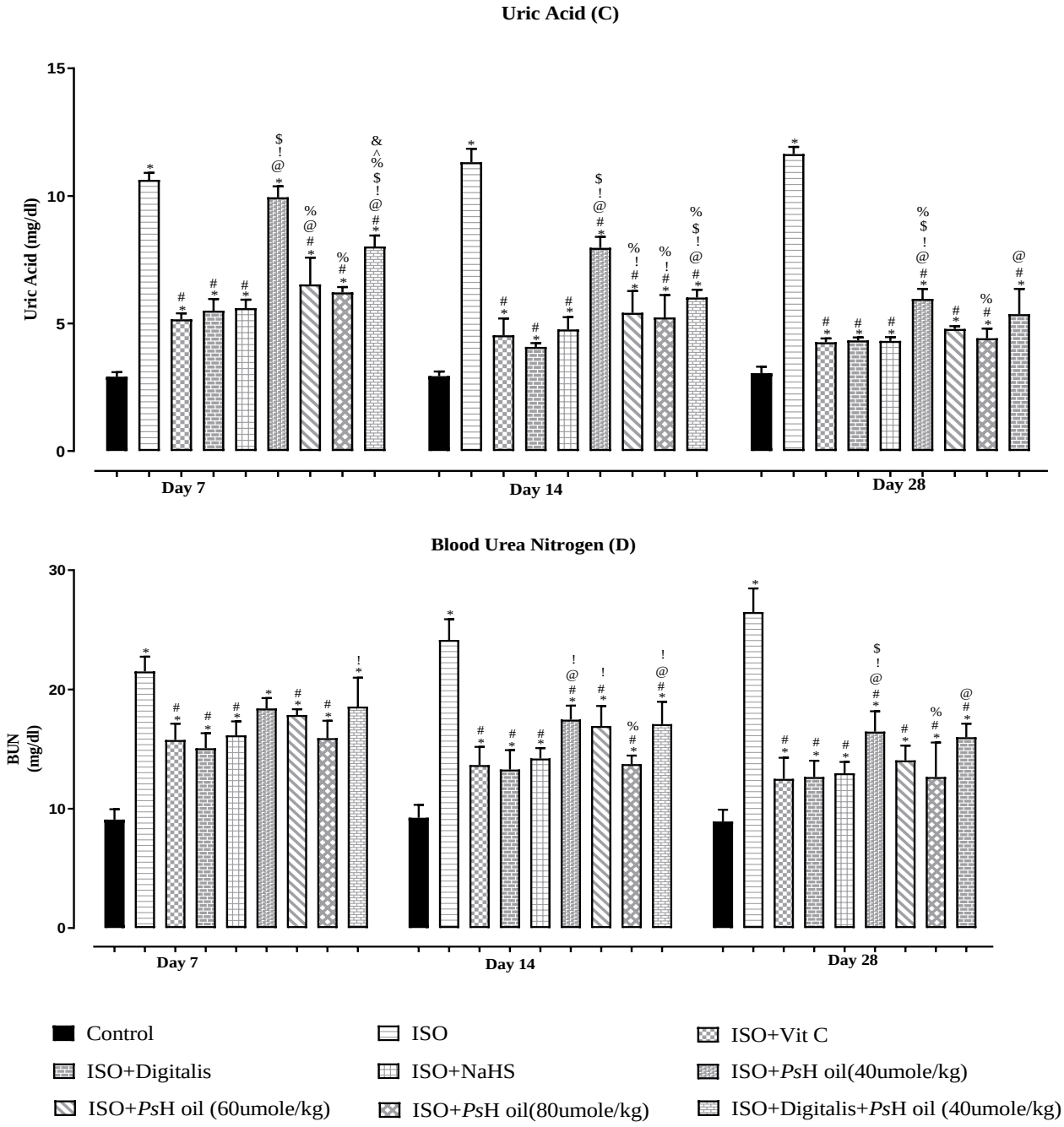


Figure 3. (A)The creatinine, (B) urea, (C) uric acid and (D) BUN levels of control, ISO, and ISO + treatment groups of rats [ISO + vitamin C, ISO + digitalis, ISO + NaHS, ISO + *Ps*H oil (40 µmole/kg), ISO + *Ps*H (60 µmol/kg), ISO + *Ps*H oil (80 µmol/kg) and ISO + Digitalis + *Ps*H oil (40 µmol/kg)]. The values (n = 6) are shown as mean ± SEM. The Bonferroni post hoc test was used in conjunction with one-way ANOVA for statistical analysis. *$p < 0.05$ versus control; $^{\#}p < 0.05$ versus ISO; $^{@}p < 0.05$ versus ISO + vitamin C; $^{!}p < 0.05$ versus ISO + Digitalis; $^{\$}p < 0.05$ versus ISO + NaHS; $^{\%}p < 0.05$ versus ISO + *Ps*H oil (40umole/kg); $^{\wedge}p < 0.05$ versus ISO + *Ps*H oil (60umole/kg); $^{\&}p < 0.05$ versus ISO + *Ps*H oil (80umole/kg).

Effect of treatments on serum electrolytes

Serum potassium and sodium levels in rats were measured on protocol days 7, 14, and 28 after ISO administration and they exhibited lower potassium and elevated sodium serum levels. Cotreatment

with either standard comparator drugs or different doses of *Ps*H oil facilitated the normalization of the ISO-modified levels of serum electrolytes (**Fig. 4A, B**).

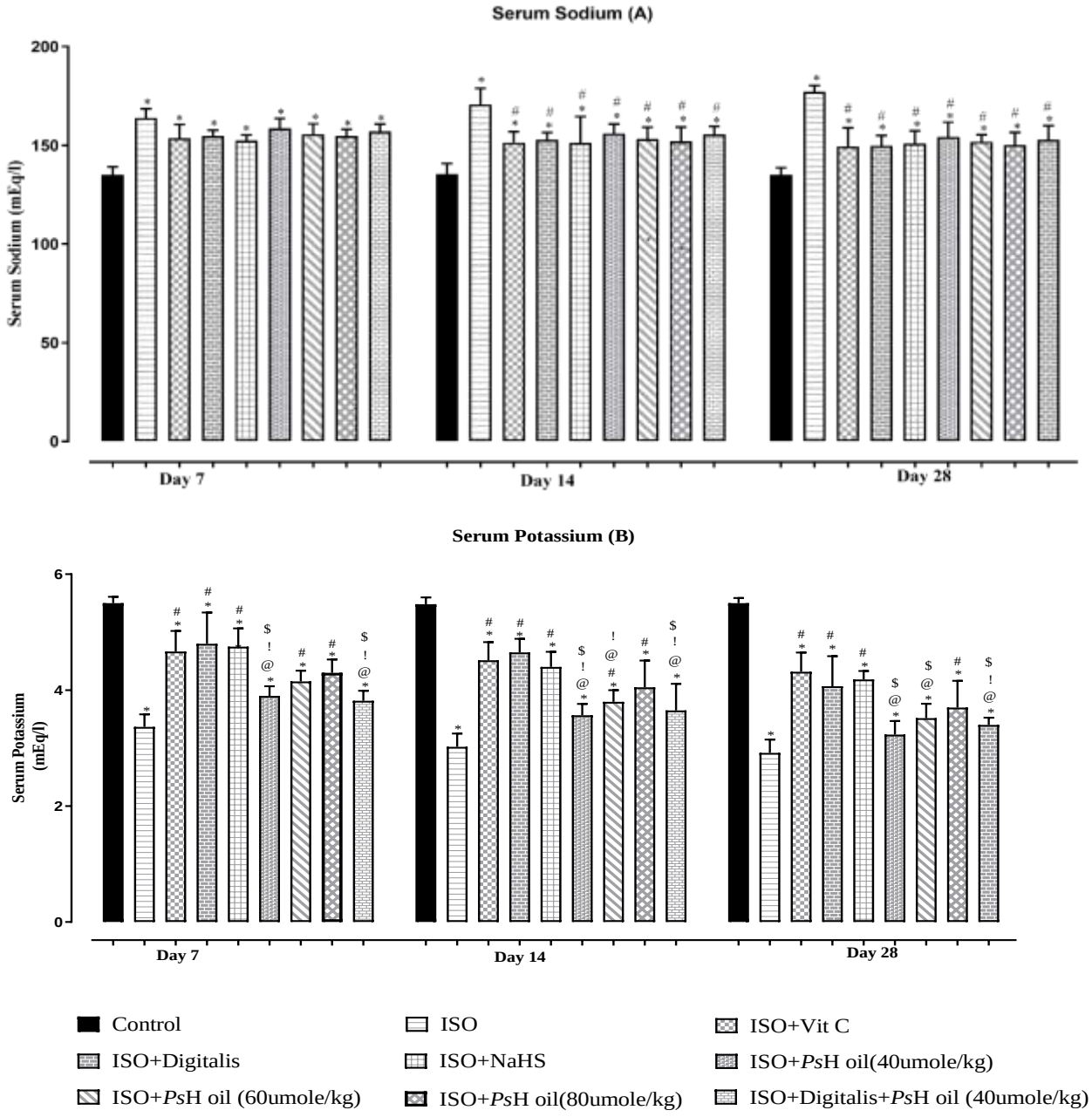
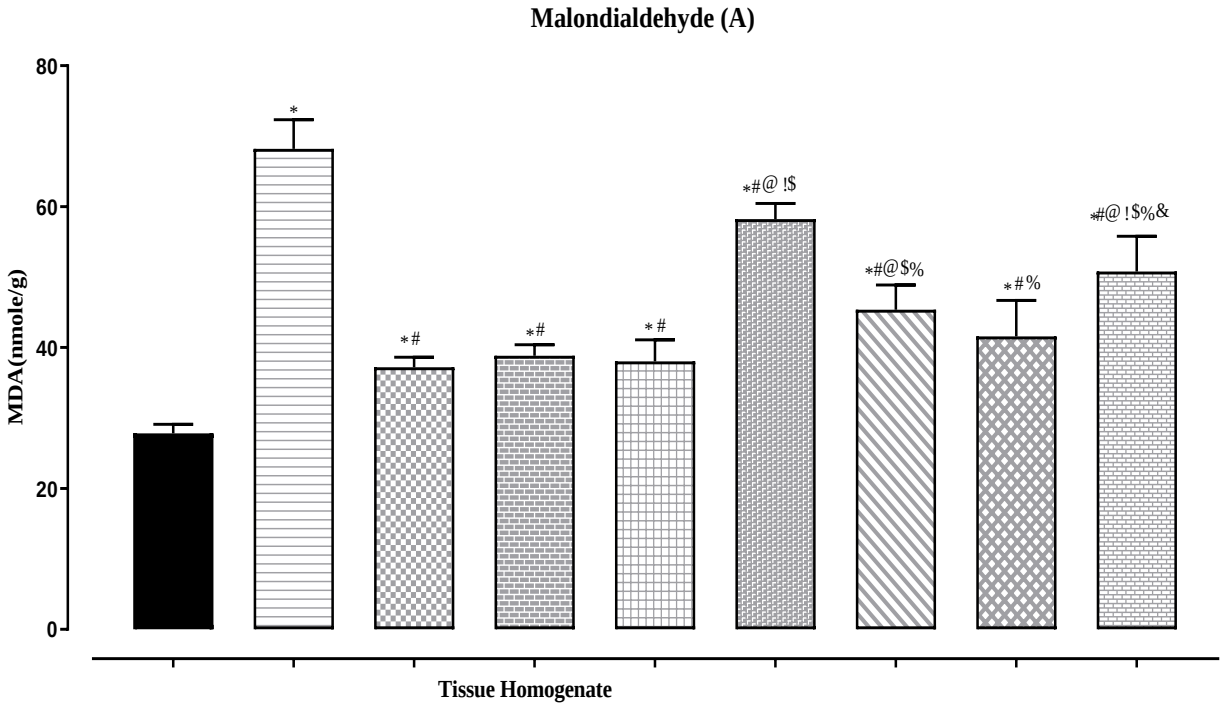


Figure 4. (A) Sodium, and (B) potassium serum levels of control, ISO, and ISO + cotreatment groups of rats [ISO + vitamin C, ISO + digitalis, ISO + NaHS, ISO + *Ps*H oil (40 µmole/kg), ISO + *Ps*H oil (60 µmol/kg), ISO + *Ps*H oil (80 µmol/kg) and ISO + Digitalis + *Ps*H oil (40 µmol/kg)]. The values (n = 6) are shown as mean ± SEM. The Bonferroni post hoc test was used in conjunction with one-way ANOVA for statistical analysis. *$p < 0.05$ versus control; $^{\#}p < 0.05$ versus ISO; $^{@}p < 0.05$ versus ISO + Vitamin C; $^{!}p < 0.05$ versus ISO + Digitalis; $^{\$}p < 0.05$ versus ISO + NaHS.

Effects of treatments on antioxidant enzymes

In comparison with the control group, the ISO-treated animals displayed higher renal tissue MDA concentrations but lower SOD and GSH levels (**Fig. 5A, B** and **C**). Coadministration of ISO with standard drugs (vitamin C, digitalis, NaHS) or different doses of *Ps*H oil, reduced the level of MDA and increased ISO-suppressed tissue levels of SOD and GSH in a concentration dependent manner.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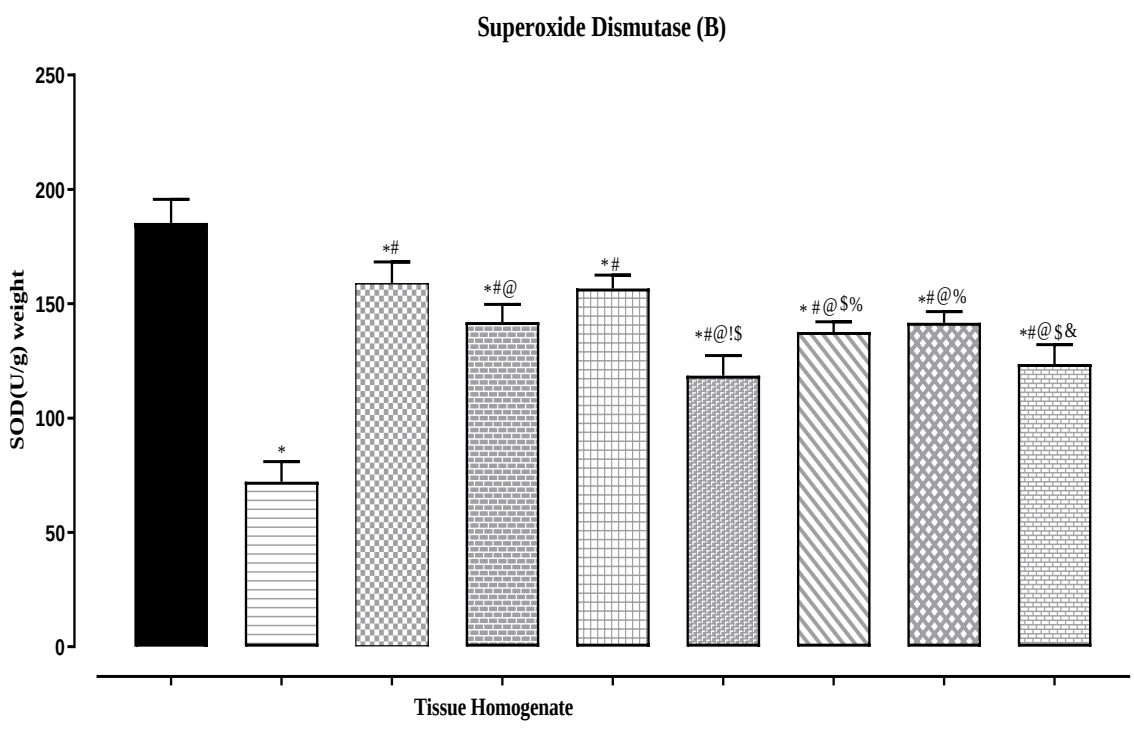
Tissue Homogenate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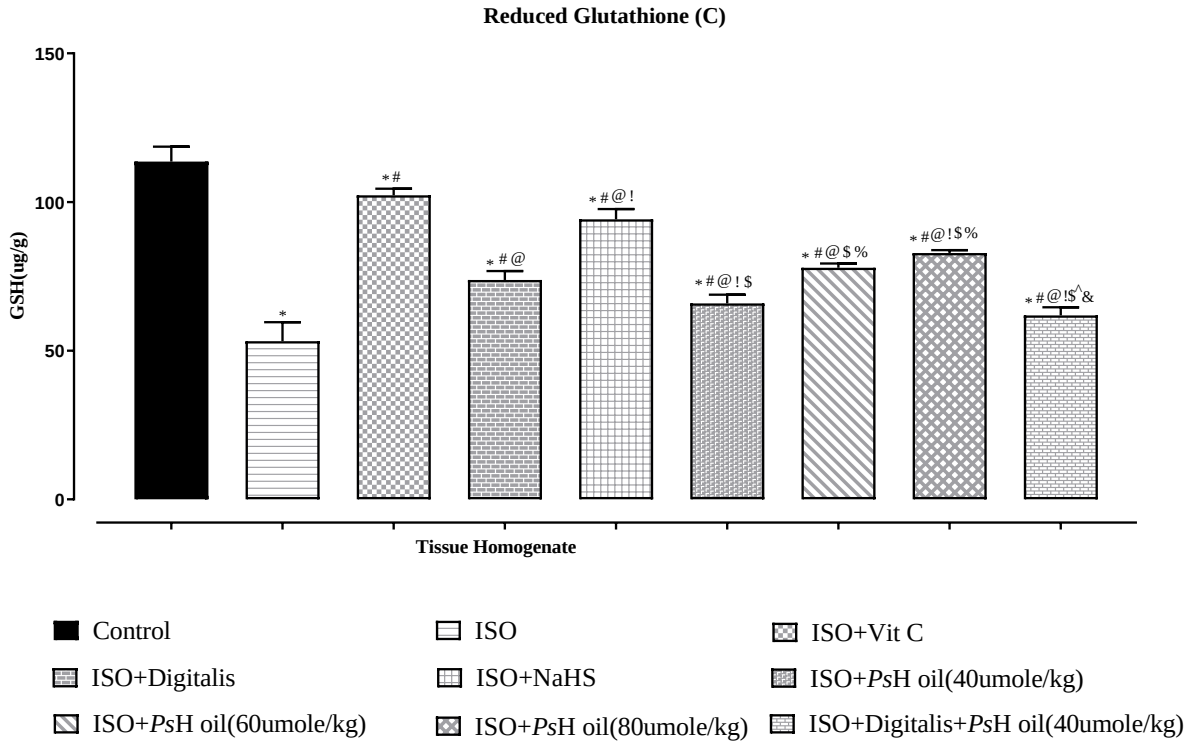

Figure 5. (A) The raised MDA levels, (B) reduced SOD concentrations, and (C) diminished GSH levels in renal tissues of control, ISO and ISO + cotreatment rat groups. [ISO + vitamin C, ISO + digitalis, ISO + NaHS, ISO + *Ps*H oil (40 µmole/kg), ISO + *Ps*H oil (60 µmole/kg), ISO + *Ps*H oil (80 µmole/kg) and ISO + Digitalis + *Ps*H oil (40 µmole/kg)]. The values (n = 6) are shown as mean ± SEM. The Bonferroni post hoc test was used in conjunction with a one-way ANOVA for statistical analysis. *$p < 0.05$ versus control; $^{\#}p < 0.05$ versus ISO; $^{@}p < 0.05$ versus ISO + Vitamin C; $^{!}p < 0.05$ versus ISO + Digitalis; $^{\$}p < 0.05$ versus ISO + NaHS; $^{\%}p < 0.05$ versus ISO + *Ps*H oil (40umol/kg); $^{\wedge}p < 0.05$ versus ISO + *Ps*H oil (60umol/kg); $^{\&}p < 0.05$ versus ISO + *Ps*H oil (80umol/kg).

Effects of treatments on CBS and CSE enzyme expression in kidney tissue detected by western blot

In **Fig. 6**, the expression of CBS and CSE in rat kidney tissues is presented. In both experimental groups, CBS expression was measured at 61 kDa and CSE expression at 45 kDa. Each western blot utilized beta actin as the loading control, which was found at 43 kDa. Following ISO treatment, there was a substantial decrease in the expression of both CBS and CSE enzymes. Subsequently, there was a tendency to normalize the expression of the two enzymes by ISO cotreatment with vitamin C, NaHS and *Ps*H oil at the highest dose.

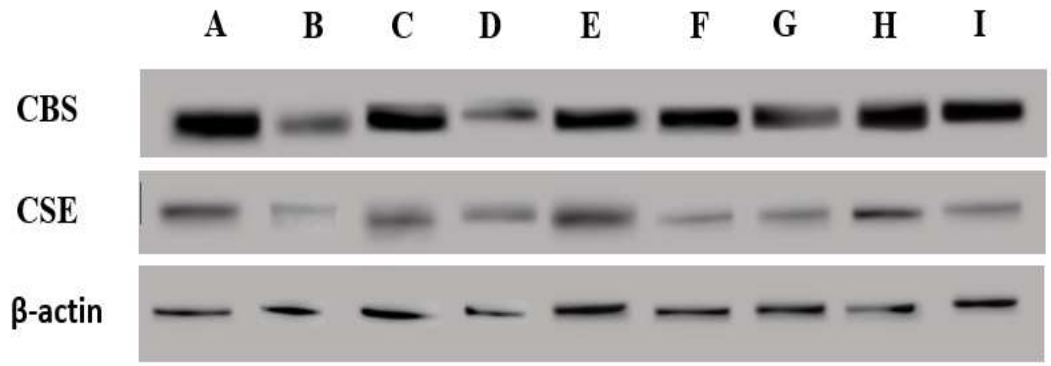

Figure 6. Expression of CBS and CSE enzyme in rat kidney tissues evaluated by western blot. A = Control, B = Isoproterenol, C = ISO + Vitamin C, D = ISO + Digitalis, E = ISO + NaHS, F = ISO + 40µmol/kg *Parkia speciosa* oil, G = ISO + 60µmol/kg *Parkia speciosa* oil, H = ISO + 80µmol/kg *Parkia speciosa* oil, I = ISO + Digitalis + 40µmol/kg *Parkia speciosa* oil. Each western blot utilized beta actin as the loading control, which was found at 43 kDa.

Effect of treatment on rat kidney histopathology

In kidney tissues, the histology of controls is shown in (**Fig. 7A)**. Following ISO treatment, aggregates of inflammatory cells (arrow) hemorrhages, local accumulation of edematous fluid (star) were observed in renal tubules (**Fig. 7B**). Coadministration of ISO and vitamin C, evoked vascular congestion (arrow), swelling of tubules with decreased lumen (arrow head) (**Fig. 7C**). Kidney histology also revealed some vascular degeneration in the renal tubules and mild glomerular atrophy after combined treatment with ISO and digitalis (**Fig. 7D**), while there was a normal histological structure of renal tissues with a slight degeneration of tubules following ISO plus NaHS combined treatment (**Fig. 7E**). In addition, coadministration of ISO with *Ps*H oil at the two lowest doses (40 and 60 µmole/kg), did induce some tubular and glomerular injury (star) in the renal cortex (**Fig.7F** and **G**). In contrast, animals treated with ISO plus the highest plant oil dose (80 µmol/kg) divulged a relatively normal kidney tissue architecture, with slight degeneration of renal tubules and hemorrhages in the renal medullary area (**Fig. 7H**). Tissues from the animals cotreated with ISO, digitalis and the plant oil (40 µmol/kg) displayed glomerular atrophy with dilated Bowman's capsule, renal congestion and desquamation of renal tubules (**Fig. 7I**).

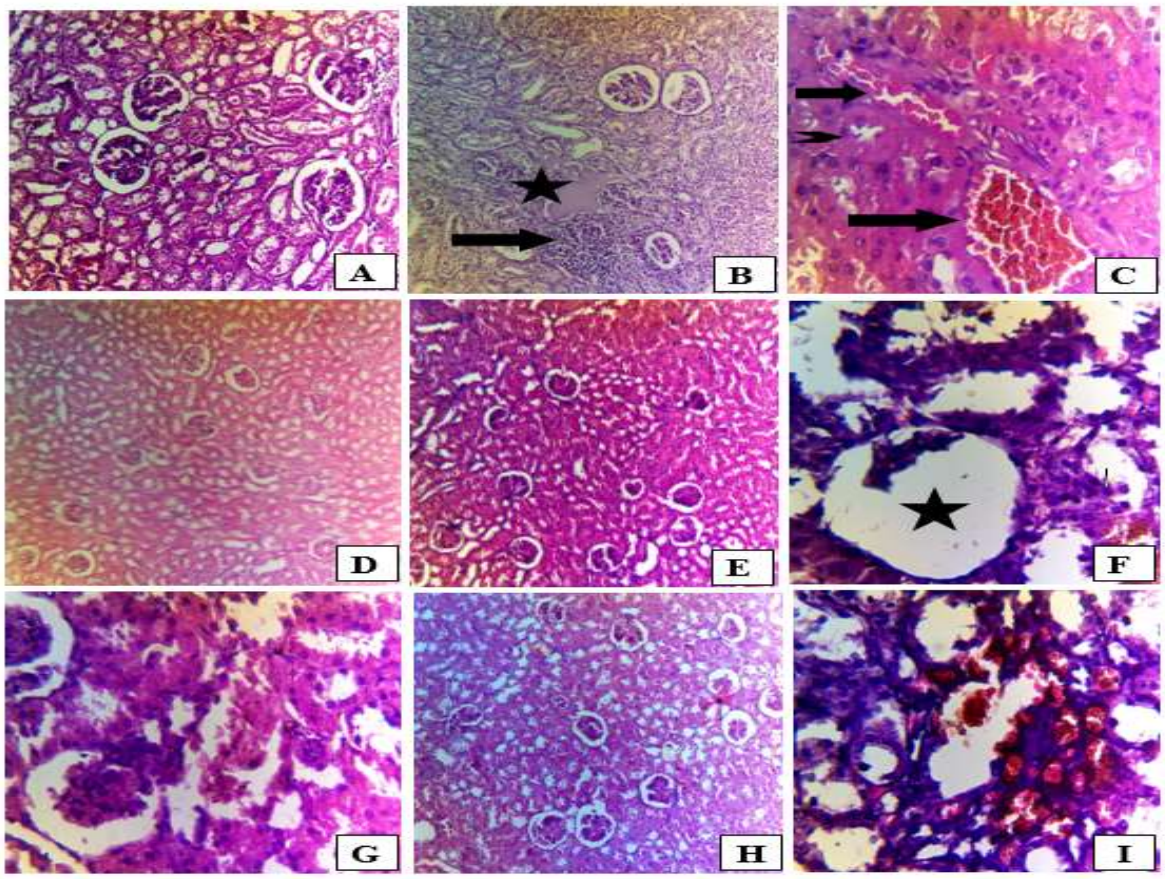

Figure 7. Renal histopathology sections taken from the following treatment groups. (A) control, (B) ISO, (C) ISO + vitamin C, (D) ISO + digitalis, (E) ISO + NaHS, (F) ISO + *P. speciosa* oil (*Ps*H) (40

µmole/kg), (G) ISO + *P. speciosa* oil (*Ps*H) (60 µmole/kg), (H) ISO + *P. speciosa* oil (*Ps*H) (80 µmole/kg) and (I) ISO + Digitalis + *P. speciosa* oil (*Ps*H) (40 µmole/kg).

Discussion

The primary objective of the study was to scrutinize the renoprotective potential of the H_2S donor Parkia speciosa Hassk. against hypertensive nephropathy in experimental animals. It has previously been reported that variation in the levels of H_2S leads to several kidney disorders including renal perfusion, obstructive nephropathy, diabetic nephropathy and hypertensive nephropathy. Consequently, administration of H_2S-releasing donors may represent a valuable treatment option for some of these kidney diseases (20). Arising from the fact that the medicinal plant, *Ps*H contains enriched cyclic sulfur compounds capable of releasing H_2S in biological conditions (7), this may facilitate the restoration of kidney function possibly advocating a natural therapeutic strategy.

Phytochemical quantification of stinky bean constituents by high performance liquid chromatography (HPLC) has demonstrated the presence of caffeic acid, gallic acid, rutin and quercetin. These components are reported to have anti-inflammatory, antioxidant, antiproliferative, antimicrobial, anti-hypertensive and anti-cancer properties (21-23). Similarly, gas chromatography-mass spectrometry (GC-MS) analysis has identified one acyclic and nine cyclic organosulfide constituents including 1,2,4-trithiolane polysulfide, that are capable of acting as hydrogen sulfide donors (15). Hence the presence of such phytochemicals warrants a need to further evaluate the therapeutic potential of this medicinal herb.

For *in-vitro studies*, a reducing power assay of stinky beans was performed. The reducing power of a compound is a strong predictor of its antioxidant potential (17), and it typically rises as the concentration of the sample increases. At a given concentration, a high absorbance at 700 nm indicates that the sample has a strong reducing power (24). The so-called reductones can generate antioxidant activity by donating a hydrogen atom thereby breaking free radical chains (25). In this regard, the oil in *Ps*H produced a maximum reducing power at a concentration of 100 µM and this activity was concentration dependent.

ISO-induced heart failure has been used as an in-vivo model for myocardial infarction ensued by a secondary impairment of kidney function in experimental animals. Our investigation demonstrated that biochemical parameters in the kidney, such as the concentrations of serum creatinine, urea, uric acid, BUN, and serum sodium were elevated following ISO treatment, while potassium levels were decreased. Interestingly, treatment with the plant oil sulfide donor, significantly reversed the above-mentioned parameters. Exogenous sulfide donors are thought to stimulate the Nrf_2 signaling pathway, and in line with previous findings, diallyl sulfide, a natural H_2S donor in garlic, boosts antioxidant defenses and reduces inflammatory cytokines in gentamicin-induced nephrotoxicity by activating Nrf_2 (26). In relation to this, Nrf_2 increases the transcription of protective genes by interacting with antioxidant response elements, which is essential for the maintenance of antioxidants and the prevention of disorders connected with them. It can also modify reactive oxygen species production through NADPH oxidase and mitochondrial regulation (27).

Administration of ISO by itself, reduced the levels of the antioxidant enzymes GSH and SOD, whereas it elevated the level of MDA in renal tissues. Previous studies have revealed that a progressive rise in the levels of reactive oxygen species diminished antioxidant enzymes and it is a key element of the pathophysiology of kidney damage. As a consequence, an increase in oxidative stress may promote cell hypertrophy and proliferation, as well as inflammatory cell infiltration (28). In respect of this, our studies have shown that in all the ISO cotreatment groups, there was an elevation of these protective enzymes in renal tissues. The oil from *Ps*H is a slow H_2S-releasing donor (29). Therefore, it may be concluded that liberated H_2S may well promote the expression of GSH, and SOD by upgrading the transcription of antioxidants via activation of an antioxidant pathway. In this context, this would reduce oxidative stress, increase expression of antioxidant enzyme, and restore the balance between anti- and pro-inflammatory cytokines (30).

Our, western blotting experiments with kidney tissues, disclosed a downregulation of CBS and CSE protein expression by ISO treatment. Such an outcome corroborates the finding that renal ischemia/reperfusion reduces the renal H_2S level along with decreased CBS and CSE expression in rodents (31). Subsequent coadministration of our H_2S donor (*Ps*H) restored the expression of these enzyme proteins towards control levels likely to be conducive to normal kidney function. These results support the concept that exogenous sources of H_2S such as *Ps*H, are renoprotective in the ISO-induced heart failure model. The findings are also reinforced by the histopathological study in renal tissue. Hence, following ISO treatment, kidney tissue divulged evidence of inflammation, nephrotic gangrene, decentralization of cells, and loss of sarcoplasm. In contrast, all the other treatment groups receiving standard comparator drugs or *Ps*H were protected against some of the renal tissue damage by neutralization of the pathological cellular events throughout the latter part of the 28-day protocol.

Conclusion

In conclusion, the oil from stinky beans can confer renoprotection in the ISO-induced heart failure model in albino rats. The renoprotective propensity of the plant oil may stem from an ability to decrease the redox imbalance and increase serum H_2S levels released by its constituent organosulphur compounds. In respect of these findings, clinical outcomes may be improved by combination therapy comprising an H_2S donor along with hypertension-lowering medicines. However, further study is warranted to establish more information regarding H_2S mediated novel therapies that could possibly be employed alongside antihypertensive drugs to inhibit the onset of hypertensive nephropathy.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Credit authorship contribution statement

Ammara Asif: Data curation; formal analysis; investigation; methodology, writing original draft, **Fiaz-ud-Din Ahmad:** project administration; supervision, **Osman Asghar Mirza:** formal analysis; supervision, **Ruyu Yan:** validation; visualization, **Robert D. E. Sewell:** writing, review and editing.

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