Al-Awar et al., UST J Med Sci 2026;4:10.
https://doi.org/10.59222/ustjms.4.10

UST JOURNAL FOR MEDICAL SCIENCES
Published by
University of Science and Technology, Sana'a, Yemen

ORIGINAL ARTICLE

Supporting OPEN-ACCESS Publishing

Nephroprotective Effect of Ethanolic *Psiadia punctulata* Leaf Extract on Iohexol-Induced Nephropathy in a Rat Model

Mostafa M. S. Al-Awar[1*], Nabil A. Al-Baser[2], Mogahed A. Al-Shawia[3]

[1]Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Science and Technology (USTY), Sana'a, Yemen
[2]Department of Pharmacology, Faculty of Medicine and Health Sciences, Mahweet University, Al-Mahweet, Yemen
[3]Department of Biological Sciences, Faculty of Science, Sana'a University, Sana'a, Yemen

*** Corresponding author:** Email: mostafaalawaar@gmail.com

ABSTRACT

Background: *Psiadia* species is a medicinal plant found in Yemen and several African and Asian countries. It belongs to the family Asteraceae, and *Psiadia incana*, *Psiadia punctulata*, and *Psiadia schweinfurthii* are among the most common species in Yemen. Traditionally, *P. punctulata* has been used to treat fever, malaria, skin infections, pain, renal calculi, and bone injuries. This study aimed to assess the nephroprotective effect of the ethanolic extract of *P. punctulata* leaf on iohexol-induced nephropathy in a rat model.

Methods: Thirty-five adult male rats (180 ± 20 g) were used in this study. After one-week acclimatization, the rats were randomly divided into five groups (n = 7 per group). Group 1 received normal saline (1 mL/rat, per os [PO]) for 14 days as the normal control. Group 2 received a single dose of iohexol (15 mg/kg, intraperitoneally [IP]) on day 17. Group 3 received N-acetylcysteine (NAC; 150 mg/kg, IP) for 14 days as a reference drug. Groups 4 and 5 received *P. punctulata* leaf extract (200 and 400 mg/kg, PO, respectively) for 14 days. All groups were exposed to 72 h of dehydration, and all groups except the normal control received furosemide before iohexol administration. Serum and urine samples were analyzed for renal function biomarkers, including serum creatinine, blood urea nitrogen (BUN), potassium, serum total protein (TP), urine volume, urine creatinine, inflammatory markers, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), oxidative stress biomarkers, including malondialdehyde (MDA), glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), and histopathological changes. Data were analyzed using analysis of variance followed by Tukey's multiple-comparison test, with P <0.05 considered statistically significant.

Results: Iohexol administration caused no significant differences in body weight or kidney weight index compared with the other groups. The iohexol group showed significantly elevated renal biomarkers (serum creatinine, BUN, and serum urea), together with significantly decreased urine creatinine and creatinine clearance, compared with the control group. Iohexol also significantly increased pro-inflammatory markers (IL-6 and TNF- α) and the oxidative stress marker MDA, whereas antioxidant biomarkers (GPx, SOD, and CAT) significantly decreased. Treatment with NAC and both doses of *P. punctulata* extract significantly decreased serum creatinine, BUN, and serum urea compared with the iohexol group and significantly increased urine creatinine, creatinine clearance, and antioxidant biomarkers (GPx, SOD, and CAT). The high dose of *P. punctulata* (400 mg/kg) showed the strongest protective effect and significantly increased serum TP levels compared with the iohexol group. This dose also significantly increased serum potassium levels compared with NAC and the lower dose of *P. punctulata* (200 mg/kg). Histopathological findings supported the biochemical results by showing reduced renal tissue damage in the treated groups.

Online ISSN: 2959-4146
Print ISSN: 2959-4138

Conclusion: These findings indicate that *P. punctulata* extract may exert nephroprotective effects against contrast-induced nephropathy (CIN), primarily through antioxidant and anti-inflammatory mechanisms. The rich phenolic and flavonoid content of the extract warrants further preclinical investigation for preventing CIN.

Keywords: *Psiadia punctulata* ▪ Iohexol ▪ Contrast-induced nephropathy ▪ Nephroprotection ▪ Rat model

1. Introduction

Contrast-induced acute kidney injury, or contrast-induced nephropathy (CIN), is a serious complication linked to iodinated contrast media such as iohexol, which is commonly used in diagnostic imaging.[1] It is characterized by acute deterioration in renal function, manifested by elevated serum creatinine and blood urea nitrogen (BUN) levels within 48–72 h after contrast exposure.[2] The incidence of CIN has increased because of the widespread use of contrast media in modern diagnostic and interventional procedures.[3] CIN is considered one of the leading causes of hospital-acquired acute kidney injury. Major risk factors for CIN include older age, pre-existing kidney dysfunction, especially in diabetic patients, high contrast doses, and the use of nephrotoxic drugs. Current preventive strategies rely mainly on hydration with isotonic saline or sodium bicarbonate; however, these measures provide limited protection, highlighting the need for safer and more effective alternatives.[4]

Medicinal plants have long been used in traditional medicine, particularly in Yemen, which is rich in medicinal flora. *P. punctulata*, a member of the Asteraceae family, is widely distributed in Yemen and other African and Asian countries.[5] Traditionally, it has been used to treat fever, malaria, pain, infections, and renal disorders.[6] Phytochemical studies have identified flavonoids, diterpenes, and phenylpropanoids with antioxidant and anti-inflammatory properties.[7] Despite its traditional use, scientific evidence supporting its nephroprotective effects remains limited. Therefore, this study aimed to evaluate the protective potential of *P. punctulata* ethanolic extract against iohexol-induced kidney injury in a rat model and to explore its possible antioxidant and anti-inflammatory mechanisms against CIN.

2. Methods

2.1. Experimental animals

Thirty-five male albino rats, aged 10–12 weeks and weighing 180 ± 20 g, were purchased from the Animal House, Department of Biology, Faculty of Science, Sana'a University. One week before treatment, the rats were acclimatized to the laboratory environment. Seven rats were housed per cage under a 12-hour light/dark cycle. The rats had free access to water and a standard diet and were maintained at 20–25°C and 50–60% humidity.

2.2. Experimental design

The rats were randomly allocated to five groups, with seven rats in each group. Group 1 (normal control) received normal saline (1 mL orally [PO]) for 14 days. Group 2 (positive control) received iohexol (15 mg/kg, intraperitoneally [IP]) as a single dose on day 17 after 72 h of dehydration and furosemide administration.[8] Group 3 received N-acetylcysteine (NAC; 150 mg/kg, IP) for 14 days,[9] whereas Groups 4 and 5 received *P. punctulata* leaf extract at doses of 200 mg/kg and 400 mg/kg, respectively, PO for 14 days.[10] All groups were subjected to 72 h of dehydration, and all groups except the normal control received furosemide (10 mg/kg, IP) before iohexol administration.[8]

2.3. Preparation of *P. punctulata* leaf extract

Leaves of *P. punctulata* were collected from Mubeen village, Hajjah Governorate, Yemen, in October 2024. The plant was taxonomically identified at the Faculty

Online ISSN: 2959-4146
Print ISSN: 2959-4138

of Science, Hajjah University, Hajjah, Yemen. A voucher specimen was deposited in the Faculty of Science, Hajjah University. The leaves were collected using sterilized tools and transported to the laboratory in clean, dry containers. After washing with distilled water, the leaves were air-dried, cut into small pieces, and accurately weighed. The leaves were then macerated in 96% ethanol for three days and then filtered, and the ethanol was evaporated at below 45°C using a rotary evaporator. The resulting extract was freeze-dried and stored in airtight containers at -4°C. A total of 850 g of leaves yielded a 20.6% extract, which was suitable for further experiments.[11–13]

2.4. Phytochemical screening of *P. punctulata* ethanolic extract

The ethanolic extract of P. punctulata leaves was subjected to qualitative phytochemical screening using Fourier Transform Infrared Spectroscopy (FTIR) to detect alkaloids, glycosides, tannins, saponins, flavonoids, proteins, phenols, and carbohydrates.[14]

2.5. Determination of body weight and relative organ weight

Body weight was measured before, during, and after the experimental period to assess changes associated with iohexol administration and treatment with *P. punctulata* extract or NAC. At the end of the experiment, all animals were weighed, and the kidneys were excised and weighed. Relative kidney weight was calculated as an index of renal toxicity using the following formula:[15]

$$\text{Relative kidney weight (\%)} = \text{kidney weight (g)} / \text{body weight (g)} \times 100$$

2.6. Collection of blood and organ samples

At the end of the study period, the rats were anesthetized with diethyl ether and then euthanized. Blood samples were collected by cardiac puncture, and serum was separated by centrifuging the samples at 3000 rpm for 20 min. Kidneys were collected, washed with normal saline, and weighed to determine their relative weight. Each kidney was then divided into two parts: one was fixed in 10% formal saline for histopathological examination, while the other was stored at -80°C for the assessment of oxidative stress and antioxidant parameters.

2.7. Biochemical analysis

Serum samples were used to measure clinical biochemical parameters, including serum creatinine (mg/dL), BUN (mg/dL), potassium (mg/dL), and serum total protein TP in g/dL. Urine samples were collected for 24 h to assess urine volume (mL) and urine creatinine (mg/dL). All biochemical analyses were performed using a spectrophotometer (RYOTEC-9200, Germany). Creatinine clearance was determined using the following formula:[16]

$$\text{Creatinine clearance (mL/min)} = [\text{urine creatinine (mg/dL)} \times \text{urine volume(mL)}] / \text{plasma creatinine (mg/dL)} \times \text{time (min)}$$

2.8. Measurement of pro-inflammatory markers

Kidney tissue homogenates were used to measure TNF-α (ng/ml) and IL-6 (ng/ml). Measurements were performed using enzyme-linked immunosorbent assay (ELISA) kits (Bioassay Technology Laboratory, Shanghai Korain Biotech Co., Ltd., Shanghai, China), in accordance to the manufacturer's instructions and read using a Readwell Touch ELISA plate analyzer (RT-0250816RBK, Robonik, India).

2.9. Measurement of oxidative stress and antioxidant parameters

Kidney tissue samples were homogenized in phosphate-buffered saline (PBS; pH 7) using a handheld homogenizer (Omni International, Kennesaw, GA, USA), with 0.1 g tissue added to 1 mL PBS. The

Online ISSN: 2959-4146
Print ISSN: 2959-4138

homogenate was then centrifuged at 4000 g for 5 min at 4°C. The supernatant was then used to measure glutathione peroxidase (GPx), MDA, catalase (CAT), and superoxide dismutase (SOD) levels in U/g using commercial colorimetric assay kits (Bio-Diagnostics, Giza, Egypt) with RYOTEC-9200 spectrophotometer (RYOTEC, Germany).

2.10. Histopathological examination

Kidney tissues were fixed in 10% formalin, dehydrated using graded ethanol concentrations, cleared with xylene, embedded in paraffin, and cut into 4-µm-thick sections. The tissue sections were then stained with hematoxylin and eosin (H&E) and periodic acid–Schiff (PAS) stains. The prepared slides were examined using an Olympus BX53 digital microscope (Olympus Corporation, Tokyo, Japan).

2.11. Data analysis

Statistical analysis was conducted using GraphPad Prism software, version 8.1.4 (GraphPad Software, Boston, MA, USA). The results were presented as mean ± standard deviation (SD). Data were analyzed using one-way analysis pf variance (ANOVA), followed by Tukey's multiple-comparison test. Body weight changes over time were analyzed using two-way repeated-measures ANOVA, if applicable. A *P*-value <0.05 was considered statistically significant.

3. Results

3.1. Qualitative phytochemical constituents of P. punctulata plant extract

Phytochemical screening of *P. punctulata* extract revealed alkaloids, glycosides, tannins, saponins, flavonoids, phenols, proteins, and carbohydrates. FTIR analysis showed characteristic absorption bands for major functional groups. The broad band at 3398.92 cm^{-1} indicated O–H/N–H stretching, while peaks at 2925.87 and 2852.59 cm^{-1} corresponded to aliphatic C–H stretching. The band at 1631.05 cm^{-1} was assigned to C=C/C=O stretching, and peaks at 1440.04 and 1385.18 cm^{-1} indicated C–H bending. Bands at 1253.30 and 1072.40 cm^{-1} corresponded to C–O/C–N stretching, suggesting the presence of phenolic compounds, flavonoids, alkaloids, ethers, and carbohydrate-related compounds.

Qualitative screening and FTIR confirmed these constituents through characteristic reactions, including precipitate formation for alkaloids, blue-black coloration for tannins, persistent foam for saponins, yellow coloration for flavonoids and proteins, reddish-brown coloration for glycosides, red coloration for phenols, and ring formation for carbohydrates.

3.2. Effect of iohexol, NAC and *P. punctulata* on body weight and relative kidney index

The effects of iohexol, NAC, and *P. punctulata* extract on body weight are shown in Figure 1A. The normal control group showed a steady increase in body weight throughout the study. In contrast, the iohexol-treated group showed a reduction in body weight, particularly during the third week; however, this change was not statistically significant. The iohexol *plus* NAC, iohexol *plus P. punctulata* 200 mg/kg, and iohexol *plus P. punctulata* 400 mg/kg groups showed only minor, non-significant changes in body weight. The relative kidney index is shown in Figure 1B. Iohexol administration did not result in a significant change in kidney index compared with the normal control group. Similarly, treatment with NAC or *P. punctulata* extract at doses of 200 and 400 mg/kg did not significantly alter the kidney index compared with either the control or iohexol group. These findings indicate that iohexol-induced nephropathy was not associated with significant changes in gross kidney weight relative to body weight.

Online ISSN: 2959-4146
Print ISSN: 2959-4138

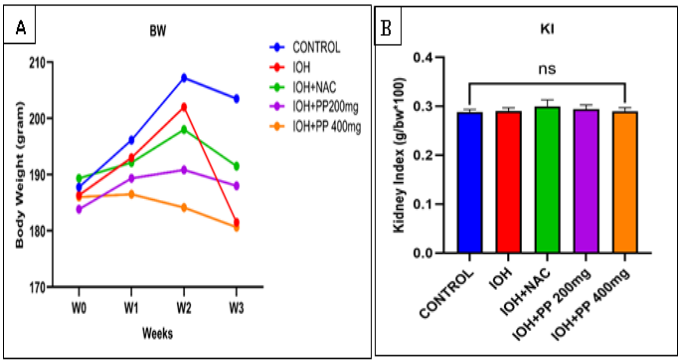

Figure 1: Effect of *P. punctulata* and NAC on body weight (A) and relative kidney index (B). Data are presented as mean ± SD, *n* = 7 rats/group. IOH, iohexol; NAC, N-acetylcysteine; PP, *P. punctulata*.

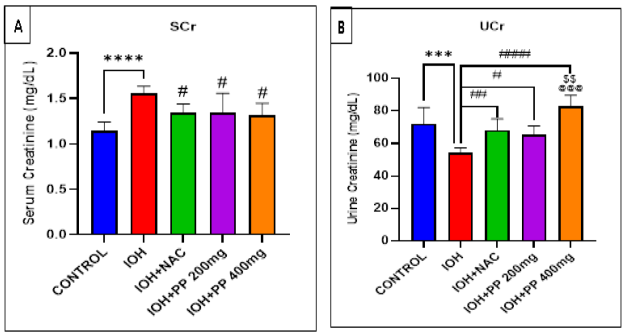

Figure 2: Effect of *P. punctulata* and NAC on serum creatinine (A) and urine creatinine (B) in rats with iohexol-induced nephropathy. Data are presented as mean ± SD, *n* = 7 rats/group. IOH, iohexol; NAC, N-acetylcysteine; PP, *P. punctulata*.

3.3. Effect of iohexol, NAC and *P. punctulata* on renal function and serum biochemistry

Iohexol caused significant alterations in renal function biomarkers compared with the normal control group. Serum creatinine was significantly higher in the iohexol group compared with the control group (1.6 ± 0.1 *vs.* 1.1 ± 0.01 mg/dL; *P* <0.001). Treatment with NAC, *P. punctulata* (200 mg/kg), and *P. punctulata* (400 mg/kg) significantly reduced serum creatinine compared with the iohexol group, with values of 1.3 ± 0.1 mg/dL (*P* = 0.040), 1.3 ± 0.2 mg/dL (*P* = 0.040), and 1.3 ± 0.2 mg/dL (*P* = 0.016), respectively. However, no statistically significant differences were detected between NAC and either *P. punctulata* dose (200 mg/kg) or between the two *P. punctulata* doses (Figure 2A).

Urine creatinine significantly decreased in the iohexol group compared with the control group (54.1 ± 3.3 vs. 72.1 ± 10.1 mg/dL; *P* <0.001). Treatment with NAC, *P. punctulata* (200 mg/kg), and *P. punctulata* (400 mg/kg) significantly increased urine creatinine compared with the iohexol group, with values of 67.8 ± 7.3 mg/dL (*P* = 0.008), 65.3 ± 5.5 mg/dL (*P* = 0.039), and 82.8 ± 6.9 mg/dL (*P* <0.001), respectively. No significant difference was observed between NAC and *P. punctulata* 200 mg/kg, whereas *P. punctulata* 400 mg/kg produced significantly higher urine creatinine than NAC and *P. punctulata* 200 mg/kg, as shown in Figure 2B.

Serum urea was significantly elevated in the iohexol group compared with the control group (61.0 ± 15.8 *vs.* 40.6 ± 3.6 mg/dL; *P* = 0.002). NAC, *P. punctulata* (200 mg/kg), and *P. punctulata* (400 mg/kg) significantly reduced serum urea compared with the iohexol group, with values of 43.8 ± 6.7 mg/dL (*P* = 0.012), 43.8 ± 7.2 mg/dL (*P* = 0.012), and 42.0 ± 8.1 mg/dL (*P* = 0.005) respectively. Conversely, no statistically significant differences were detected among the treatment groups (Figure 3).

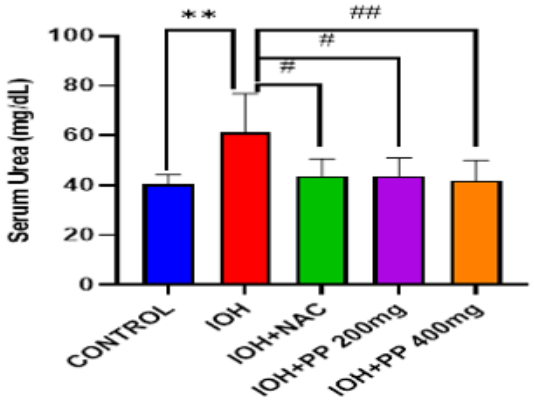

Figure 3: Effect of *P. punctulata* and NAC on serum urea level. Data are presented as mean ± SD, *n* = 7 rats/group. IOH, iohexol; NAC, N-acetylcysteine; PP, *P. punctulata*.

Creatinine clearance significantly decreased in the iohexol group compared with the control group (0.3 ± 0.1 vs. 1.0 ± 0.1 mL/min; *P* <0.001). Treatment with NAC, *P. punctulata* (200 mg/kg), and *P. punctulata* (400 mg/kg) significantly increased creatinine

Online ISSN: 2959-4146
Print ISSN: 2959-4138

clearance compared with the iohexol group, with values of 0.5 ± 0.1 mL/min (P = 0.004), 0.5 ± 0.1 mL/min (P = 0.007), and 0.7 ± 0.1 mL/min (P <0.001), respectively. The improvement observed with *P. punctulata* (400 mg/kg) was significantly greater than that observed with NAC (P = 0.007) and *P. punctulata* (200 mg/kg; P = 0.004), as shown in Figure 4.

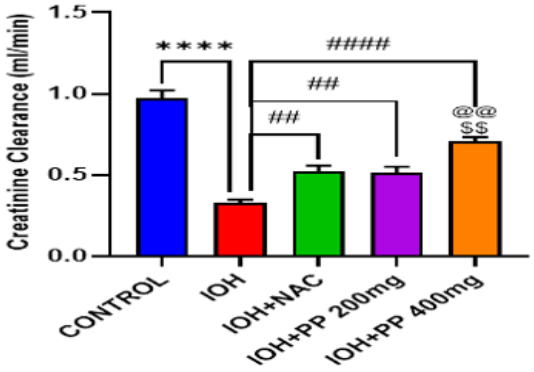

Figure 4: Effect of *P. punctulata* and NAC on creatinine clearance. Data are presented as mean ± SD, n = 7 rats/group. IOH, iohexol; NAC, N-acetylcysteine; PP, *P. punctulata*.

Serum TP showed a non-significant decrease in the iohexol group compared with the control group (4.4 ± 0.7 *vs.* 5.4 ± 0.5 g/dL; P = 0.117). NAC and *P. punctulata* (200 mg/kg) did not significantly alter serum TP levels compared with the iohexol group. However, *P. punctulata* 400 mg/kg significantly increased serum TP compared with the iohexol group (5.9 ± 0.7 g/dL; P = 0.009) and produced significantly higher levels than *P. punctulata* 200 mg/kg (P = 0.014).

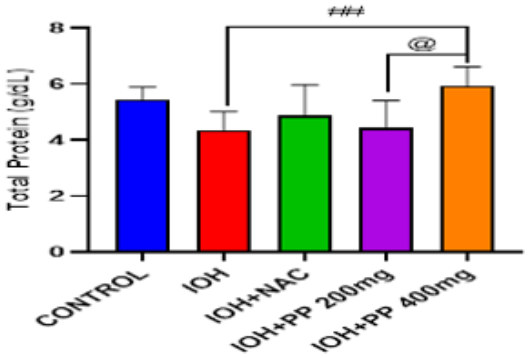

Figure 5: Effect of *P. punctulata* and NAC on TP. Data are presented as mean ± SD, n = 7 rats/group. IOH, iohexol; NAC, N-acetylcysteine; PP, *P. punctulata*.

Potassium levels did not differ significantly between the iohexol and control groups (5.6 ± 0.9 *vs.* 5.1 ± 0.2 mg/dL; P = 0.662). Similarly, NAC, *P. punctulata* (200 mg/kg), and *P. punctulata* (400 mg/kg) did not significantly alter potassium levels compared with the iohexol group. However, *P. punctulata* 400 mg/kg produced significantly higher potassium levels than NAC (P = 0.003) and *P. punctulata* 200 mg/kg (P = 0.018), as shown in Figure 6.

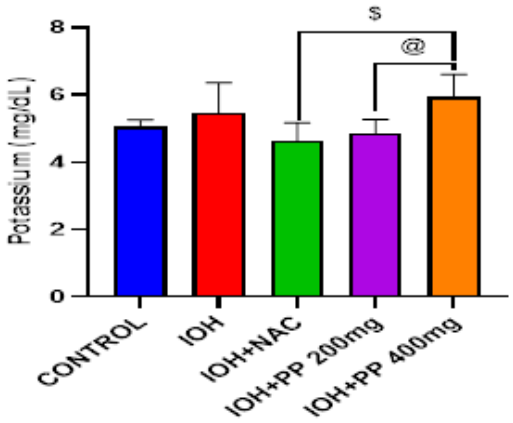

Figure 6: Effect of *P. punctulata* and NAC on serum potassium level. Data are presented as mean ± SD, n = 7 rats/group. IOH, iohexol; NAC, N-acetylcysteine; PP, *P. punctulata*.

BUN was significantly increased in the iohexol group compared with the control group (28.5 ± 7.4 *vs.* 19.0 ± 1.7 mg/dL; P = 0.002). Treatment with NAC, *P. punctulata* (200 mg/kg), and *P. punctulata* (400 mg/kg) significantly decreased BUN compared with the iohexol group, with values of 20.4 ± 3.2 mg/dL (P = 0.012), 20.4 ± 3.3 mg/dL (P = 0.011), and 19.6 ± 3.7 mg/dL (P = 0.005), respectively. In contrast, no statistically significant differences were detected among the treatment groups (Figure 7).

Online ISSN: 2959-4146
Print ISSN: 2959-4138

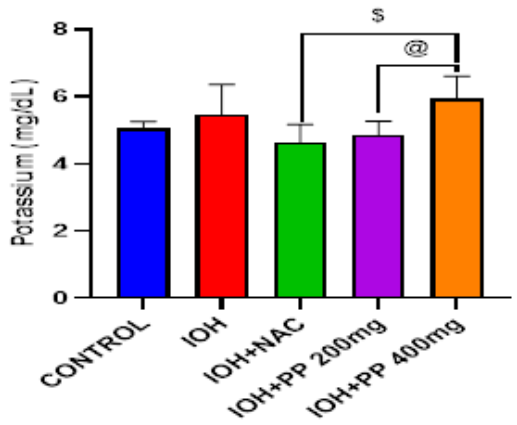

Figure 7: Effect of *P. punctulata* and NAC on BUN. Data are presented as mean ± SD, *n* = 7 rats/group. IOH, iohexol; NAC, N-acetylcysteine; PP, *P. punctulata*.

3.4. Effects of *P. punctulata* and NAC on pro-inflammatory cytokines

The effects of NAC and *P. punctulata* extract on TNF-α and IL-6 are shown in Figure 8A and 8B. TNF-α significantly increased in the iohexol group compared with the control group (59.3 ± 1.3 compared with 50.0 ± 5.3 ng/ml; *P* = 0.047). NAC and *P. punctulata* (200 mg/kg) reduced TNF-α levels compared with the iohexol group, but these reductions were not statistically significant. In contrast, *P. punctulata* (400 mg/kg) significantly reduced TNF-α levels compared with the iohexol group (48.7 ± 8.7 ng/ml; P = 0.018), as shown in Figure 8A.

IL-6 also significantly increased in the iohexol group compared with the control group (50.2 ± 4.1 compared with 40.1 ± 3.8 ng/ml; *P* = 0.006). Treatment with NAC, *P. punctulata* (200 mg/kg), and *P. punctulata* (400 mg/kg) resulted in non-significant reductions in IL-6 compared with the iohexol group, with values of 43.5 ± 4.9 ng/ml, 46.7 ± 6.8 ng/ml, and 45.4 ± 5.1 ng/ml, respectively (Figure 8B).

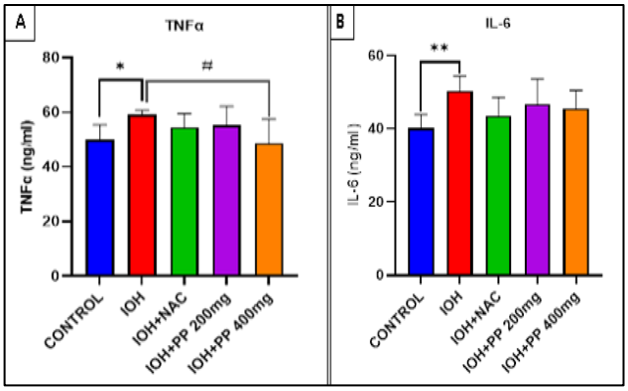

Figure 8: Effects of *P. punctulata* and NAC on TNF-α level (A) and IL-6 level (B). Data are presented as mean ± SD, *n* = 7 rats/group. IOH, iohexol; NAC, N-acetylcysteine; PP, *P. punctulata*.

3.5. Effects of *P. punctulata* and NAC on oxidative stress and antioxidant parameters

Iohexol significantly altered oxidative stress and antioxidant parameters in kidney tissue. GPx activity significantly decreased in the iohexol group compared with the control group (44.4 ± 4.9 *vs.* 50.6 ± 1.3 U/g; *P* = 0.003). Treatment with NAC, *P. punctulata* (200 mg/kg), and *P. punctulata* (400 mg/kg) significantly increased GPx activity compared with the iohexol group, with values of 50.7 ± 1.1 U/g (*P* = 0.002), 48.9 ± 3.5 U/g (*P* = 0.048), and 52.4 ± 1.1 U/g (*P* = 0.001), respectively, as shown in Figure 9A. SOD activity significantly decreased in the iohexol group compared with the control group (263.8 ± 26.4 compared with 324.3 ± 13.4 U/g; *P* <0.001). NAC, *P. punctulata* (200 mg/kg), and *P. punctulata* (400 mg/kg) significantly increased SOD activity compared with the iohexol group, with values of 307.1 ± 24.7 U/g (*P* = 0.002), 293.2 ± 4.4 U/g (*P* = 0.049), and 304.5 ± 16.5 U/g (*P* = 0.003), respectively, as shown in Figure 9B. MDA significantly increased in the iohexol group compared with the control group (13.4 ± 3.4 compared with 9.4 ± 0.9 nmol/g; *P* = 0.014). Treatment with NAC, *P. punctulata* (200 mg/kg), and *P. punctulata* (400 mg/kg) reduced MDA levels compared with the iohexol group, although these reductions were not statistically significant, as shown in Figure 9C. CAT activity

Online ISSN: 2959-4146
Print ISSN: 2959-4138

significantly decreased in the iohexol group compared with the control group (3.6 ± 0.3 compared with 5.1 ± 0.4 U/g; *P* <0.001). Treatment with NAC, *P. punctulata* (200 mg/kg), and *P. punctulata* (400 mg/kg) significantly increased CAT activity compared with the iohexol group, with values of 5.1 ± 0.3 U/g (*P* <0.001), 4.5 ± 0.6 U/g (*P* = 0.008), and 5.1 ± 0.5 U/g (*P* <0.001), respectively, as shown in Figure 9D.

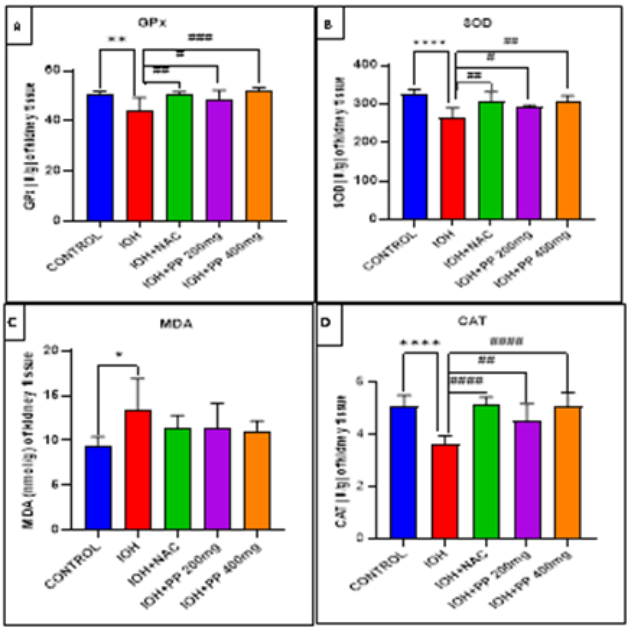

Figure 9: Effects of NAC and *P. punctulata* leaf extract on the levels of GPx (A), SOD (B), MDA (C), and CAT (D) in kidney tissue.

3.6. Histopathological findings in kidney tissue sections stained with H&E

Histopathological examination of kidney tissue using H&E from the normal control group showed normal medullary tubules (T). The renal corpuscles exhibited normal morphology, with an intact Bowman's capsule (black arrow), and Bowman's space (*) was of normal width with no cellular infiltration (Figure 10A and 10B; 200× and 400×, respectively).

Kidney sections from the iohexol-treated group showed marked histopathological alterations, including cytoplasmic vacuolization of proximal tubular cells (black arrows), tubular necrosis (TN), intratubular red casts (#), cellular infiltration (yellow arrows), tubular dilatation (TD), and widening of Bowman's space (*) due to damage to the glomerular filtration barrier (Figure 10C and 10D; 200× and 400×, respectively).

The group treated with iohexol plus NAC showed mild tubular dilatation (TD), mild cytoplasmic vacuolation (blue arrows), mild tubular degeneration (black arrows), and a notable reduction in Bowman's space (*) compared with the iohexol group (Figure 10E and 10F; 200× and 400×, respectively).

The group treated with iohexol *plus P. punctulata* extract (200 mg/kg) showed mild inflammatory cell infiltration (yellow arrows). The glomeruli exhibited a moderately widened Bowman's space (BS), with limited epithelial cell lysis (Figure 10G and 10H; 200× and 400×, respectively).

The group treated with iohexol *plus P. punctulata* extract (400 mg/kg) showed notable histological amelioration. Most renal tubules retained a near-normal structure, with minimal degenerative changes (D). The glomeruli appeared nearly normal (G), and inflammatory cell infiltration was reduced compared with the iohexol and low-dose extract groups (Figure 10I and 10J; 200× and 400×, respectively).

3.7. Histopathological findings in kidney tissue sections stained with PAS

Histopathological examination of kidney tissue stained with PAS from the normal control group showed normal proximal tubules with abundant cytoplasm and a clearly identifiable brush border. The glomeruli and basement membrane also appeared normal (Figure 11A and 11B). The group treated with iohexol showed marked renal injury, including loss of the brush border, acute tubular necrosis, tubular dilatation, hyaline casts, vacuolated tubular cells, increased Bowman's space, and glomerular shrinkage (Figure 11C and 11D).

Online ISSN: 2959-4146
Print ISSN: 2959-4138

The group treated with iohexol plus NAC showed partial improvement, with mostly preserved tubules and no evident tubular necrosis or dilatation. However, mild glomerular shrinkage/degeneration and proximal tubular enlargement were observed (Figure 11E and 11F). The group treated with iohexol plus *P. punctulata* extract (200 mg/kg) showed mild tubular injury, including slight loss of the brush border, a few necrotic tubules, vacuolated cytoplasm, and a few casts, with nearly normal glomeruli (Figure 11G and 11H). The group treated with iohexol plus *P. punctulata* extract (400 mg/kg) showed similar but milder changes, including slight loss of the brush border and no casts, with nearly normal glomeruli (Figure 11I and 11J).

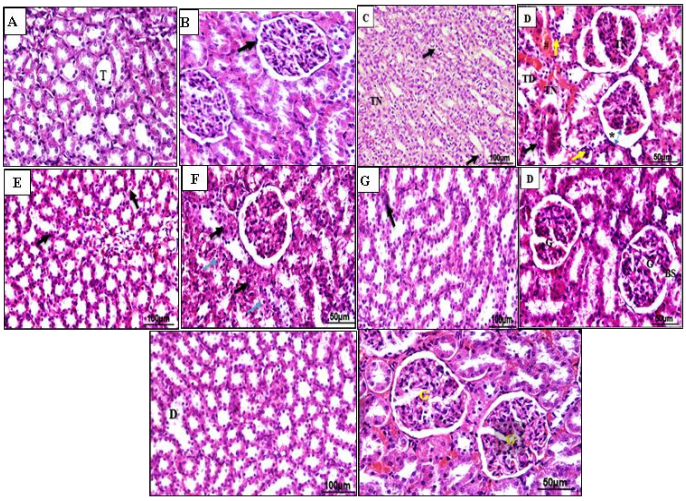

Figure 10: Representative H&E-stained photomicrographs of kidney tissue sections of normal control, NAC-treated and *P. punctulata*-treated groups.

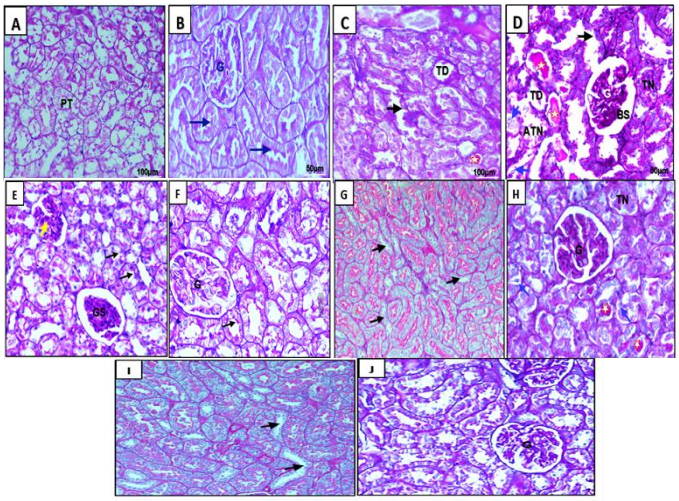

Figure 11: Representative PAS-stained photomicrographs of kidney tissue sections of normal control, NAC-treated and *P. punctulata*-treated groups.

4. Discussion

In the present study, iohexol administration resulted in a progressive reduction in body weight, particularly by the third week, although this change was not statistically significant. This reduction was accompanied by clear evidence of renal injury, including tubular necrosis, cytoplasmic vacuolization, tubular dilatation, and inflammatory cell infiltration. Treatment with *P. punctulata* extract at both 200 and 400 mg/kg, as well NAC, attenuated these changes and helped maintain relatively stable body weight. Histopathological findings showed reduced tubular damage and vacuolization in the treated groups. The kidney index showed a non-significant increase in the iohexol group and remained statistically unchanged after treatment with NAC or *P. punctulata*. This finding indicates the absence of marked nephromegaly or severe tissue edema during the experimental period, consistent with prior studies on CIN.[17, 18]

Iohexol markedly increased serum creatinine and decreased urine creatinine, indicating renal functional impairment. These biochemical changes were consistent with the histopathological evidence of pronounced tubular necrosis, cytoplasmic vacuolization, and tubular structural damage, confirming the successful induction of contrast-induced renal injury. Treatment with both doses of *P. punctulata* extract and NAC significantly lowered serum creatinine and improved urine creatinine, with *P. punctulata* (400 mg/kg) showing the strongest improvement, suggesting a possible dose-dependent effect. These findings are consistent with previous studies showing that plant extracts rich in antioxidant and anti-inflammatory compounds can help restore creatinine

Online ISSN: 2959-4146
Print ISSN: 2959-4138

balance following nephrotoxic injury.[10,19] NAC efficacy is also well documented,[20–24] and the comparable effect of *P. punctulata* suggests strong nephroprotective phytochemicals.

The nephroprotective effect of *P. punctulata* may be attributed to its phenolic and flavonoid constituents, which reduce oxidative stress, suppress inflammation, and preserve glomerular filtration. NAC protects mainly through glutathione replenishment and reactive oxygen species scavenging. Both NAC and *P. punctulata*, particularly the 400 mg/kg dose, significantly mitigated iohexol-induced nephrotoxicity by improving nitrogenous waste homeostasis, creatinine clearance, renal antioxidant defense, and kidney structure. Iohexol markedly reduced creatinine clearance and caused tubular necrosis, tubular dilatation, and impairment of the glomerular filtration barrier, whereas NAC and *P. punctulata* improved renal clearance and histological architecture. The 400 mg/kg *P. punctulata* group showed near-normal recovery with minimal degenerative changes, suggesting a dose-dependent nephroprotective effect consistent with previous reports on NAC in CIN[25–27] and plant-derived flavonoids in nephrotoxic models.[28,29]

Iohexol caused a non-significant decrease in serum TP, whereas *P. punctulata* 400 mg/kg significantly increased TP compared with the iohexol group, possibly reflecting improved protein metabolism and reduced renal protein loss, consistent with previous findings on bioactive plant extracts.[13] Serum potassium did not significantly change after iohexol, in agreement with a previous reports that potassium disturbances in CIN may remain minimal unless tubular dysfunction is severe.[30] However, potassium was significantly higher in the *P. punctulata* 400 mg/kg group than in the NAC and *P. punctulata* 200 mg/kg groups, suggesting a mild dose-related effect on electrolyte homeostasis.

Iohexol significantly increased IL-6 and TNF-α levels, indicating renal inflammation, consistent with histopathological findings of tubular necrosis, cytoplasmic vacuolization, inflammatory cell infiltration, and disrupted renal architecture. NAC and both doses of *P. punctulata* reduced IL-6 and TNF-α, but only *P. punctulata* 400 mg/kg significantly reduced TNF-α. This improvement was supported by reduced inflammatory infiltration, preserved tubular structure, and near-normal glomerular morphology, especially in the *P. punctulata* 400 mg/kg group. These findings agree with reports that natural products and antioxidants can suppress pro-inflammatory cytokines in contrast- or drug-induced nephrotoxicity,[31] while the anti-inflammatory effect of *P. punctulata* may relate to its flavonoid and alkaloid content.[29]

Iohexol markedly reduced renal GPx, SOD, and CAT activities and increased MDA levels, confirming oxidative stress, antioxidant depletion, and lipid peroxidation. These changes were consistent with severe histopathological damage, including tubular degeneration, necrosis, cytoplasmic vacuolization, and loss of normal renal architecture. Treatment with NAC or *P. punctulata* improved antioxidant status and renal histology, with *P. punctulata* 400 mg/kg showing the strongest dose-dependent protection. GPx was markedly restored, SOD recovered to near-normal levels, CAT returned to control values, and MDA decreased, although not significantly. These improvements were accompanied by reduced tubular injury, less vacuolization, and preserved renal architecture. These findings support previous reports that NAC restores glutathione and antioxidant enzymes, including GPx,[22] and that medicinal plant extracts enhance antioxidant defenses and reduce oxidative/inflammatory renal injury.[32]

The SOD findings are consistent with plant-derived antioxidant protection in nephrotoxicity models.[33] The MDA trend also agrees with studies

Online ISSN: 2959-4146
Print ISSN: 2959-4138

showing reduced lipid peroxidation by plant antioxidants[34] and the antioxidant potential of *P. punctulata*.[35] Similarly, CAT recovery supports reports linking phenolic- and flavonoid-rich extracts to CAT upregulation, partly through nuclear factor erythroid 2-related factor 2 (NRF2)-related mechanisms, in kidney injury.[36–38]

Overall, *P. punctulata* showed strong nephroprotective effects against iohexol-induced renal injury, comparable to or greater than NAC in several parameters. These effects appear to involve improved renal function, reduced inflammation and oxidative stress, enhanced antioxidant defenses, and preservation of renal tissue integrity, consistent with evidence on plant-based nephroprotection.[6,10]

This study has several limitations, including the relatively small sample size, the short duration of experiment, lack of molecular analyses to confirm the signaling pathways involved, and absence of isolation or quantification of the specific bioactive bioactive constituents underlying the protective effects. Nevertheless, the study has important strengths. It used an established iohexol-induced nephropathy model to assess renal protection using complementary outcomes, including renal function biomarkers, inflammatory cytokines, oxidative stress markers, antioxidant enzymes, and histopathology. The inclusion of NAC as a reference drug also enabled comparison with a commonly used nephroprotective agent.

5. Conclusion

These findings suggest that *P. punctulata* extract may have nephroprotective effects against CIN, primarily through antioxidant and anti-inflammatory mechanisms. The extract improves renal function, enhances antioxidant defenses, reduces inflammatory responses, and preserves renal tissue architecture. Therefore, *P. punctulata* may represent a promising natural candidate for the prevention of CIN. However, further studies are required to confirm its efficacy, safety, active constituents, and underlying molecular mechanisms.

Acknowledgments

The authors thank the Deanship of the Faculty of Medicine and Health Sciences, Mahweet University, Yemen, for support and facilities during this study. The authors also thank the staff of the Animal House, Department of Biology, Faculty of Science, Sana'a University, for providing the experimental animals. Special thanks are due to Dr. Hussin Sogil for identifying the plant material.

Ethical approval

The study protocol was approved by the Research Ethics Committee of the University of Science and Technology in Sana'a, Yemen (Ethical clearance No.: 1447/0076/UREC/UST). All experimental procedures involving animals followed the Guide for the Care and Use of Laboratory Animals.

Conflict of interest

The authors declare no conflict of interest.

Funding

None.

References

1. Chen HY, Wu YH, Wei CY, Liao ZY, Wu HT, Chen YC, et al. Incomplete recovery from the radiocontrast-induced dysregulated cell cycle, adhesion, and fibrogenesis in renal tubular cells after radiocontrast (iohexol) removal. Int J Mol Sci. 2023;24(13):10945. DOI • PubMed • Google Scholar
2. Wang L, Huang S, Zhu T, Ge X, Pei C, Hong G, et al. Metabolomic study on iohexol-induced nephrotoxicity in rats based on NMR and LC-MS analyses. Chem Res Toxicol. 2022;35(2):244–53. DOI • PubMed • Google Scholar
3. Solomon R, DuMouchel W. Contrast media and nephropathy: findings from systematic analysis and Food and Drug Administration reports of adverse effects. Invest Radiol. 2006;41(8):651–60. DOI • PubMed • Google Scholar
4. Wang Z, Wang Q, Gong X. Unveiling the mysteries of contrast-induced acute kidney injury: new horizons in pathogenesis and prevention. Toxics. 2024;12(8):620. DOI • PubMed • Google Scholar
5. Ogweno Midiwo J, Owuor FAO, Juma BF, Waterman PG. Diterpenes from the leaf exudate of *Psiadia punctulata*. Phytochemistry. 1997;45(1):117–20. DOI • Google Scholar
6. Mahadeo K, Grondin I, Kodja H, Soulange Govinden J, Jhaumeer Laulloo S, Frederich M, et al. The genus *Psiadia*: review of traditional uses, phytochemistry and pharmacology. J Ethnopharmacol. 2018;210:48–68. DOI • PubMed • Google Scholar

Online ISSN: 2959-4146
Print ISSN: 2959-4138

7. Bushra S. Samer, Hadi Al-Mausmi, Amar Gailan, Sultan Ziad, Mohammed Saleh, Abdullah Shalan, et al. Phytochemical screening and antibacterial activity of leaf extracts from *Psiadia punctulata*. Thamar Univ J Nat Appl Sci. 2024;9(1):36–8. DOI ● Google Scholar
8. Liu K, Zhou LY, Li DY, Cheng WJ, Yin WJ, Hu C, et al. A novel rat model of contrast-induced nephropathy based on dehydration. J Pharmacol Sci. 2019;141(1):49–55. DOI ● PubMed ● Google Scholar
9. Kaya S, Yalçın T, Boydak M, Dönmez HH. Protective effect of N-acetylcysteine against aluminum-induced kidney tissue damage in rats. Biol Trace Elem Res. 2023;201(4):1806–15. DOI ● PubMed ● Google Scholar
10. Al-Awar M. Anti-urolithiatic effect of *Psiadia punctulata* leaves ethanolic extracts against sodium oxalate-induced urolithiasis in rats. J Amran Univ. 2024;4:12. DOI ● Google Scholar
11. Handa SS, Khanuja SPS, Longo G, Rakesh DD, editors. Extraction technologies for medicinal and aromatic plants. Trieste: International Centre for Science and High Technology; 2008. Google Scholar
12. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med. 2011;8(1):1–10. PubMed ● Google Scholar
13. Al-Mahbashi H, Moharram BA, Al-Maqtari T. Phytochemical, anti-inflammatory, analgesic, antipyretic and acute toxicity of *Psiadia punctulata* growing in Yemen. Univers J Pharma Res. 2020;5:61–6. DOI ●Google Scholar
14. Shaheen G, Ashfaq A, Khawar A, Jamil QA, Parveen R, Hadi F, et al. Fourier transform infrared spectrometer analysis and antimicrobial screening of ethanolic extract of *Operculina terpathum* from cholistan desert. Pharm Pract (Granada). 2022;20(2):2647. DOI ● PubMed ● Google Scholar
15. Cunha DF, Cunha SF, Reis MA, Teixeira Vde P. Heart weight and heart weight/body weight coefficient in malnourished adults. Arq Bras Cardiol. 2002;78(4):382–7. DOI ● PubMed ● Google Scholar
16. Garud MS, Kulkarni YA. Attenuation of renal damage in type I diabetic rats by umbelliferone – a coumarin derivative. Pharmacol Rep. 2017;69(6):1263–9. DOI ● PubMed ● Google Scholar
17. Liu B, Chai Y, Guo W, Lin K, Chen S, Liu J, et al. MicroRNA-188 aggravates contrast-induced apoptosis by targeting SRSF7 in novel isotonic contrast-induced acute kidney injury rat models and renal tubular epithelial cells. Ann Transl Med. 2019;7(16):378. DOI ● PubMed ● Google Scholar
18. Rakowski T, Dziewierz A, Węgiel M, Siudak Z, Zasada W, Jąkała J, et al. Risk factors of contrast-induced nephropathy in patients with acute coronary syndrome. Kardiol Pol. 2022;80(7–8):760–4. DOI ● PubMed ● Google Scholar
19. Pandhare RB, Shende RR, Avhad MS, Deshmukh VK, Mohite PB, Sangameswaran B, et al. Anti-urolithiatic activity of *Bryophyllum pinnatum* Lam. hydroalcoholic extract in sodium oxalate-induced urolithiasis in rats. J Tradit Complement Med. 2021;11(6):545–51. DOI ● PubMed ● Google Scholar
20. Kelly AM, Dwamena B, Cronin P, Bernstein SJ, Carlos RC. Meta-analysis: effectiveness of drugs for preventing contrast-induced nephropathy. Ann Intern Med. 2008;148(4):284–94. DOI ● PubMed ● Google Scholar
21. Tenório M, Graciliano NG, Moura FA, Oliveira ACM, Goulart MOF. N-Acetylcysteine (NAC): Impacts on human health. Antioxidants (Basel). 2021;10(6):967. DOI ● PubMed ● Google Scholar
22. Hernández-Cruz EY, Aparicio-Trejo OE, Hammami FA, Bar-Shalom D, Tepel M, Pedraza-Chaverri J, et al. N-acetylcysteine in kidney disease: molecular mechanisms, pharmacokinetics, and clinical effectiveness. Kidney Int Rep. 2024;9(10):2883–903. DOI ● PubMed ● Google Scholar
23. Josa E, Barril G, Ruperto M. Potential effects of bioactive compounds of plant-based foods and medicinal plants in chronic kidney disease and dialysis: a systematic review. Nutrients. 2024;16(24). DOI ● PubMed ● Google Scholar
24. Milutinović M, Ćujić Nikolić N, Cvetković T, Šavikin K, Djordjević I, Janković Veličković L, et al. Chokeberry products and by-products as the potential pharmaceuticals for kidney protection—An experimental study in rats. Plants (Basel). 2024;13(22):3136. DOI ● PubMed ● Google Scholar
25. Shekoufeh A, Hassanali A, Nazanin SJ, Mohammad Aref B, Jamileh S, Amirashkan M, et al. Effect of high doses of salep aqueous extract on serum levels of urea nitrogen, creatinine, uric acid, and kidney histopathological changes in adult male Wistar rats. Arch Razi Inst. 2023;78(5):1451–61. DOI ● PubMed ● Google Scholar
26. Marenzi G, Assanelli E, Marana I, Lauri G, Campodonico J, Grazi M, et al. N-acetylcysteine and contrast-induced nephropathy in primary angioplasty. N Engl J Med. 2006;354(26):2773–82. DOI ● PubMed ● Google Scholar
27. Zhu R, Zheng R, Deng B, Liu P, Wang Y. Association of N-acetylcysteine use with contrast-induced nephropathy: an umbrella review of meta-analyses of randomized clinical trials. Front Med (Lausanne). 2023;10:1235023. DOI ● PubMed ● Google Scholar
28. Alsawaf S, Alnuaimi F, Afzal S, Thomas RM, Chelakkot AL, Ramadan WS, et al. Plant flavonoids on oxidative stress-mediated kidney inflammation. Biology (Basel). 2022;11(12):1717. DOI ● PubMed ● Google Scholar
29. Vargas F, Romecín P, García-Guillén AI, Wangesteen R, Vargas-Tendero P, Paredes MD, et al. Flavonoids in kidney health and disease. Front Physiol. 2018;9:394. DOI ● PubMed ● Google Scholar
30. Pattharanitima P, Tasanarong A. Pharmacological strategies to prevent contrast-induced acute kidney injury. Biomed Res Int. 2014;2014:236930. DOI ● PubMed ● Google Scholar
31. Ngum JA, Tatang FJ, Toumeni MH, Nguengo SN, Simo USF, Mezajou CF, et al. An overview of natural products that modulate the expression of non-coding RNAs involved in oxidative stress and inflammation-associated disorders. Front Pharmacol. 2023;14:1144836. DOI ● PubMed ● Google Scholar
32. Tienda-Vázquez MA, Morreeuw ZP, Sosa-Hernández JE, Cardador-Martínez A, Sabath E, Melchor-Martínez EM, et al. Nephroprotective plants: a review on the use in pre-renal and post-renal diseases. Plants (Basel). 2022;11(6):818. DOI ● PubMed ● Google Scholar
33. Paes AS, Koga RdCR, Sales PF, Santos Almeida HK, Teixeira TACC, Carvalho JCT. Phytocompounds from Amazonian plant

Online ISSN: 2959-4146
Print ISSN: 2959-4138

species against acute kidney injury: potential nephroprotective effects. Molecules. 2023;28(17):6411. DOI ● PubMed ● Google Scholar

34. Rostamkhani H, Faghfouri AH, Veisi P, Rahmani A, Noshadi N, Ghoreishi Z. The protective antioxidant activity of ginger extracts (*Zingiber officinale*) in acute kidney injury: a systematic review and meta-analysis of animal studies. Journal of Functional Foods. 2022;94:105111. DOI ● Google Scholar
35. Jonville MC, Kodja H, Strasberg D, Pichette A, Ollivier E, Frédérich M, et al. Antiplasmodial, anti-inflammatory and cytotoxic activities of various plant extracts from the Mascarene Archipelago. J Ethnopharmacol. 2011;136(3):525–31. DOI ● PubMed ● Google Scholar
36. Avila-Carrasco L, García-Mayorga EA, Díaz-Avila DL, Garza-Veloz I, Martinez-Fierro ML, González-Mateo GT. Potential therapeutic effects of natural plant compounds in kidney disease. Molecules. 2021;26(20):6096. DOI ● PubMed ● Google Scholar
37. Anwar S, Alrumaihi F, Sarwar T, Babiker AY, Khan AA, Prabhu SV, et al. Exploring therapeutic potential of catalase: strategies in disease prevention and management. Biomolecules. 2024;14(6):697. DOI ● PubMed ● Google Scholar
38. Ajiboye BO, Famusiwa CD, Nifemi DM, Ayodele BM, Akinlolu OS, Fatoki TH, et al. Nephroprotective effect of *Hibiscus sabdariffa* leaf flavonoid extracts via KIM-1 and TGF-1β signaling pathways in streptozotocin-induced rats. ACS Omega. 2024;9(17):19334–44. DOI ● PubMed ● Google Scholar

To cite this article...

Al-Awar MMS, Al-Baser NA. Nephroprotective effect of ethanolic *Psiadia punctulata* leaf extract on iohexol-induced nephropathy in a rat model. UST J Med Sci. 2026;4:10. https://doi.org/10.59222/ustjms.4.10.

To publish in this journal...

Please submit your manuscript via the online submission system available at: https://journals.ust.edu.ye/USTJMS/about/submissions.

Online ISSN: 2959-4146
Print ISSN: 2959-4138