Article Info

Original article

Chemical characterization and evaluation of the nephroprotective potential of Parrotiopsis jacquemontiana (Decne) Rehder and Periploca hydaspidis Falc crude extract in CCl4-induced Male Sprague-Dawley Rats

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ABSTRACT

Biochemical, antioxidant, serum, and urine profiles together with physical examination can deliver important information regarding animal health status, and are vital in the diagnosis and treatment of patients. CCl₄, a potent nephrotoxin, was used for causing toxicity in rat kidneys. The present study aimed at exploring the nephroprotective potential of P. jacquemontiana leaves methanol extract (PJM) and P. hydaspidis whole-plant methanol extract (PHM) on kidney cells of male rats after oxidative stress and DNA damage was instigated by CCl₄. Various parameters including enzymatic levels, serum profiles, urine profiles, genotoxicity, and histological studies were conducted. In renal samples of rats treated with CCl₄, the antioxidant enzymes (POD, SOD, CAT), PH level, protein level, and glutathione contents were significantly (p < 0.05) declined whereas renal biochemicals (H₂O₂, TBARS, and nitrite), specific gravity, level of urea, urobilinogen, serum BUN and creatinine were markedly (p < 0.05) increased relative to control group. Co-administration of PJM and PHM with CCl₄ displayed protective ability against CCl₄ intoxication by restoring activities of antioxidant enzymes, urine profile, biochemical parameters, and serum profile in rats. CCl₄ also induced prominent DNA damages and glomerular atrophy with abnormal appearance of glomerulus and Bowman’s capsule. These damages results in impaired cortical sections, edema in Bowman’s capsule, accumulation of necrotic cells, dilation of convoluted tubules, and narrowing of space between Bowman’s capsule, which were successfully ameliorated after co-administration of PJM and PHM fractions in a dose-dependent manner (200 and 400 mg/kg b.w.). The results obtained suggest the therapeutic role of PJM and PHM in oxidative-stress related disorders of kidney and may be helpful in kidney trauma.

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1. Introduction

CCl₄-induced tissue injury is documented as the most commonly studied and validated model deliberated in rats so far. The toxicant; CCl₄ causes tissue damage via generating and elevating ROS (reactive oxygen species) levels under oxidative stress (Alsheblak et al., 2016). This oxidative stress underlies severe pathological conditions e.g. hypertension, diabetes mellitus, cancer, hepatic ailments, renal disorders, and death (Al-Olayan et al., 2014; Usman et al., 2020). CCl₄ is not only declared hepatotoxic
to humans but also nephrotoxic. It mainly damages lungs, heart, brain, liver, testes, and kidney with maximum effect on liver cells followed by kidney cells. CCL₄-induced oxidative stress contributes to nephrotoxicity after inducing acute and chronic renal deteriorations leading to a variety of pathological conditions (Sajid et al., 2016; Rahmouni et al., 2017). The mechanism in either case includes the initiation of free-radicals leading to an increase in LPO (lipid peroxidation) products i.e. TBARS (thio-barbituric acid substances), H₂O₂ (hydrogen peroxide), and nitrite (Ali et al., 2021a). Biochemical parameters including AST (aspartate transaminase), ALT (alanine transaminase), and ALP (alkaline phosphatase) are altered whereas endogenous levels of antioxidant enzymes i.e. CAT (catalase), SOD (superoxide dismutase), GSH (glutathione), and POD (peroxidase) are decreased. These changes lead to degradation of cellular molecules (DNA, lipids, proteins) resulting in failure of that respective organ.

These CCL₄-induced cell damages are evaluated by its possible effects on genotoxicity, histology, and activity/expression of antioxidant enzymes machinery (Aayadi et al., 2017). It not only prompts the lipid peroxidation (LPO) and altered redox status of tissues but also cause serious DNA damage and histopathological appearance (Ali et al., 2021a, Ali et al., 2021b). A single injection of CCL₄ instigates DNA oxidative damage via generating DNA-strand breaks in blood lymphocytes detected by comet assay (Chu et al., 2016) whereas interstitial mononuclear cell-infiltration, renal-tubule dilation, glomerular atrophy, and fibrosis in tissue samples (Hozzein et al., 2019). Wide-spread DNA-strand breaks caused via CCL₄ toxicity may cause cell transformation and ultimate death of that cell (Sajid et al., 2016). Comet assay has emerged as a broadly-used tool for in vitro genotoxic testing including the assessment of mutagenicity and genotoxic potential of several chemicals and herbal products in vivo (Abou Gabal et al., 2015).

All living organisms have naturally-embedded defense system which fights against oxidative stress generated via CCL₄ (Karakuş et al., 2017). It acts by increasing the level of endogenous antioxidant enzymes and decreasing LPO (Bellassoued et al., 2018), Allah Almighty has blessed Pakistan with great diversity of medicinal plants which are rich in natural bioactive compounds such as alkaloids, flavonoids, terpenoids, tannins etc (Abbasi et al., 2019, Abbasi et al., 2020, Abbasi et al., 2021). Medicinal plants are highly rich in different antioxidants to combat various types of reactive oxygen species (Iqbal et al., 2019, Iqbal et al., 2020). Antioxidants such as vitamin-E, n-acetyl cysteine, naringenin, silymarin, rhein, and quercetin display health-promoting effects against oxidative damage via declining LPO and partially improving tissue injury (Saheer et al., 2015, Iqbal et al., 2017). Black-tea extract, Gingko-biloba, vitamin-C & E, and melatonin are well-known antioxidants reported for reducing CCL₄-induced renal toxicity (Ogeturk et al., 2005). Plant materials and products have abundant amounts of these antioxidant compounds (Iqbal et al., 2018a, Iqbal et al., 2018b, Iqbal et al., 2018c). Many plant-containing phytochemicals have been used in the treatment of hepatic and renal diseases, either given independently or collectively (Karakuş et al., 2017; Usman et al., 2020, Hameed et al., 2021, Uddin et al., 2021). Two such phytochemical-rich and antioxidant-rich medicinal plants reported for their significant antioxidant, anti-inflammatory, hepatoprotective (Ali et al., 2017; Ali et al., 2018b), antimicrobial (Ali et al., 2018a; Ullah et al., 2015), anti-cancer and wound healing (Ali et al., 2021c, Ali et al., 2021d) potential is Parrottopsis jacquemontiana (Decne) Rehder and Periploca hydaspidis Falc. The current study evaluates the plants of interest for its nephroprotective activities after CCL₄-induced oxidative stress was generated in male experimental rats.

2. Materials and methods

2.1. Collection and identification of plant sample

The leaves of P. jacquemontiana were sampled from Upper Dir district, Pakistan from May to June 2019. The global positing system (GPS) coordinates calculated for the respective plant was 35.34°N and 71.96°E, whereas whole plant of P. hydaspidis was collected from Charbagh (coordinates: 34°49′33″N 72°27′02″E) district Swat from April to June 2019. The identification and collection of plants were done by Dr. Irfan Ullah Farhad and Dr. Syed Afzal Shah from biological sciences department of Quaid-i-Azam University (QAU), Islamabad and voucher # 063214 and 23651 were allotted at Herbarium of Pakistan, Quaid-i-Azam University, Islamabad, Pakistan.

2.2. Processing of plant samples

The leaves of P. jacquemontiana and whole plant of P. hydaspidis were properly washed using distilled water to remove dust and other debris. The plants were air dried at room temperature and stored under shade for two to four weeks. Electric grinder was used to get fine powder of plant material. Powdered plant material was soaked in pure methanol for one week. Filtration was done through Whatman number.1 filter paper to get refined methanolic plant extract. Rotary evaporator was used to evaporate the methanol solvent from respective fractions to obtain P. jacquemontiana methanol extract (PJM) and P. hydaspidis methanol extract (PHM). For in vivo evaluation assays, the extract was further dried and stored at 4°C.

2.3. CCL₄-induced toxicity studies

Crude methanol extracts of P. jacquemontiana and P. hydaspidis were used for its protective potential on CCL₄-induced renal damages in rats. Toxicity was induced by CCL₄ which was monitored by quantifying the levels of enzymes (antioxidant and serum) and other parameters (histological and comet).

2.3.1. Experimental rats used

Male Sprague-dawley rats (180–250 g) obtained from primate facility of QAU, Islamabad were used in the current study and the protocols regarding different assays were approved (BCH-275) by the ethical committee of QAU. The guidelines of NIH (national institute of health) were strictly followed and the experimental rats were gently treated, grown in ventilated cages, and fed with clean water and standard laboratory feed for rats daily.

2.3.2. Acute-toxicity testing

For acute-toxicity screening, the methanol fraction of P. jacquemontiana (PJM) and P. hydaspidis (PHM) was administered intra-gastrically (50 mg/kg b.wt) to each of three male experimental rats and observed for two weeks to check the behavioral changes and mortality risks. Although no toxicity signs and mortality were observed yet, the augmented order of fraction doses ranging from 100 to 4000 mg/kg b.wt was administered intra-gastrically to experimental rats. Toxicity signs, altered behavior and mortality was not even recorded against the highest dose administered of the fraction. Therefore 1/10th of 4000 mg/kg served as high dose (400 mg/kg b.wt) and 1/20th of 4000 mg/kg served as low dose (200 mg/kg b.wt) for the evaluation of nephroprotective potential of PJM and PHM in rats.
2.3.3. Experimental design of rat groups

A total of eight groups (having 7 rats each = 8) comprising Sprague-dawley rats (180–250 g) was designed for each nephrotoxicity study. The first group (Group I) was not administered with any treatment and characterized the control group. Group II was treated with olive oil and DMSO in equal ratio (1 mL/kg body wt.; 1:1 v/v) whereas Group III was intra-peritoneally injected with CCl₄ in olive oil (1 mL/kg body wt.; 2:8 v/v). Group IV was treated with reference control; silymarin (50 mg/kg body wt.) after 24 h following CCl₄ treatment. In Group V and VI, PJM/PHM (200 mg/kg and 400 mg/kg body wt.) doses were administered orally following CCl₄ treatment after 24 h whereas in Group VII and VIII, the rats were only administered with PJM/PHM (200 mg/kg and 400 mg/kg body wt.) doses. The respective treatments were continued for 4 weeks given on alternative days (three times a week) in morning time. After experiment completion, the rats were treated with ether anesthesia, euthanized, and blood was withdrawn from their heart. The kidney tissue homogenates were analyzed for the determination of following parameters.

2.3.4. Anti-oxidant enzymes study of kidney

Tissue homogenate (10%) was prepared in KH₂PO₄ buffer (100 mM) containing EDTA (1 mM; PH 7.4) followed by centrifugation (12,000g) at 4 °C for half an hour. The collected supernatant was used for the determination of following parameters.

2.3.4.1. Catalase assay. Catalase (CAT) is commonly located in cellular organelles and peroxisomes. CAT undergoes the conversion of H₂O₂ into O₂ and H₂O. Different tumors have the reduced potential within tissues was examined by following the protocol of Lowry et al. (1951). 80 mg of kidney portion was weighed and homogenized in potassium phosphate buffer. The reaction mixture was centrifuged at 10,000g for 15–20 min at 4 °C to obtain supernatant. The supernatant (0.1 mL) was incubated for 10 min and dissolved in 1 mL of alkaline copper solution. Similar proportion (1:1) Folin Ciocalteu Phenol reagent was added to the dissolved supernatant and mixed carefully by vortex machine. Incubation was done at room temperature for half an hour and absorbance was taken at 595 nm via spectrophotometer. The total solubilized protein was deliberated by standard curve of bovine serum albumin.

2.3.4.2. Peroxidase assay. Protocol of Chance and Maehly, (1955) was utilized to investigate the activity of POD with slight modifications. The reaction mixture consisted of 40 mL of 300 μL H₂O₂, 20 mM of 100 μL of guaiacol, 25 mM of 2500 μL of phosphate buffer (pH 5) and 1000 μL of supernatant. Inhibitory results were recorded at 520 nm after 1 min, where one unit CAT activity meant change in absorbance of 0.01 units/min.

2.3.4.3. Superoxide dismutase assay. Activity of SOD was determined by following the protocol of Kakkar et al., (1984). Reaction mixture of the assay consisted of 186 mM of 0.1 mL of phenazine methosulphate, 0.052 mM of 1.2 mL of sodium pyrophosphate buffer and 0.3 mL of supernatant. Centrifugation of the mixture was done at 1500g for 10 min and then once more centrifuged at 10,000g for 15 min. Enzymatic reaction in the mixture was started the addition of of 0.2 mL of 780 μM NADH. The optical density was determined at 560 nm and results were assessed in units/mg protein.

2.3.5. Biochemical parameter studies of kidney

The kidney tissue homogenates were analyzed for the assessment of total protein, GSH content, TBARS, H₂O₂, and nitrite quantification assays.

2.3.5.1. Total protein assessment. Estimation of total soluble protein within tissues was examined by following the protocol of Lowry et al. (1951). The oxidation of phenol red is stimulated by H₂O₂ mediated horse-radish peroxidase enzyme. The reaction mixture contained 2 mL of tissue homogenate dissolved in 0.28 nM of 1 mL of phenol red, 5.5 nM dextrose, 8.5 units of horse radish peroxidase, and 0.05 mM phosphate buffer. The whole mixture was incubated for 60 min in shaking water bath at 37 °C. Finally 1% of 1000 μL trichloroacetic acid (TCA) and 0.67% of 500 μL of thiobarbituric acid (TBA) were added. The reaction tubes were put in boiling water bath for 20 min and then stored in crushed ice bath for 5 min. Centrifugation was done at 1200g for 10 min to obtain supernatant and the amount of TBARS was quantified by taking absorbance of supernatant at 535 nm in comparison with blank reagent. Lipid peroxidation activity was determined as nM TBARS/min/mg at 37 °C by using molar extinction coefficient (1.56 × 105 M−1 cm−1).

2.3.5.4. Hydrogen peroxide (H₂O₂) assay. The activity of H₂O₂ was determined by following the protocol of Pick and Keisari, (1981). The oxidation of phenol red is stimulated by H₂O₂ mediated horse-radish peroxidase enzyme. The reaction mixture contained 2 mL of tissue homogenate dissolved in 0.28 nM of 1 mL of phenol red, 5.5 nM dextrose, 8.5 units of horse radish peroxidase, and 0.05 mM phosphate buffer. The whole mixture was incubated for 60 min at optimum temperature (37 °C). After incubation 10 N of 10 μL NaOH was added to impede the reaction and centrifugation was done for 5 min at 800g. The supernatant was collected and optical density was measured at 610 nm against blank reagent. The production of H₂O₂ was measured in nM H₂O₂/min/mg tissue using oxidized phenol red as standard curve.

2.3.5.5. Nitrite quantification assay. Griess reagent was utilized for evaluation of nitrite assay by following the protocol of Grisham et al., (1996). The master mix comprised of 1 mL of tissue homogenate which was dissolved in 3 mL of 100 μL H₂O₂ and 5% solution. The samples were centrifuged at 10,000g for 15–20 min. The obtained supernatant (20 μL) was dissolved in 1 mL Griess reagent. Change in color was observed and absorbance was measured at 540 nm. For estimation of nitrite concentration in tissue samples, standard curve of sodium nitrite was used.

2.3.6. Serum and urine analyses studies of kidney

The level of serum markers (total protein, albumin, BUN, and creatinine) and urine analysis profile (PH, specific gravity, urea, creatinine, albumin, and urobiligenon) was determined following evaluations.

2.3.5.2. Reduced glutathione assay. Reduced glutathione (GSH) activity was evaluated using the methodology of Jollow et al., (1974) with some modifications. First 1000 μL of homogenate mixture was taken and dissolved in 1000 μL of 4% sulfoalicylic acid, then this solution was incubated at 4 °C for one hour followed by centrifugation at 4 °C for 20 min at 1200g. 100 μL of supernatant was poured in 2.7 mL of phosphate buffer (pH 7.4) and 200 μL of 100 mM DTNB was added into it. The reaction between GSH and DTNB produced yellow color indicating reduced glutathione. The optical density was measured via spectrophotometer at 412 nm.
2.3.6.1. Quantification of urea. Reagent-1 consisted of urease (140U/mL), biocides (120), 2-oxoglutarate (10 mM/L), Tris buffer (125 mM/L) and GIDH (U/mL) whereas Reagent-2 consisted of NADH (1.5 mM/L). Briefly, 800 µL of Reagent-1 and 200 µL of Reagent-2 were dissolved in 10 µL of serum sample. After 1 to 2 min, the optical density was recorded at 340 nm. Distilled water was used as blank whereas 50 mg/dL urea served as standard. Urea was estimated by the following formula:

\[
\text{Urea (mg/dL)} = \left( \frac{\text{Abs (sample)}}{\text{Abs (standard)}} \right) \times n
\]

2.3.6.2. Quantification of creatinine. According to the AMP diagnostic kit procedure, 1 mL of working reagent solution was mixed with 0.1 mL of sample/standard and the absorbance was recorded immediately after 25 s at 500 nm as an initial reading. The second absorbance reading was noted after 2 min and creatinine was estimated using the following equation:

\[
\text{Creatinine (mg/dL)} = \left( \frac{A_2 - A_1}{A_2 - A_1(\text{standard})} \right) \times n
\]

2.3.6.3. Quantification of albumin. Colorimetric method was performed to estimate serum albumin by utilizing bromocresol green (BCG) at pH (4.20). According to AMP company kit procedure, the formed to estimate serum albumin by utilizing bromocresol green staining. Light microscope was used for slide examination at 40X and HDCE-50B was used for photography.

2.6. Statistical analysis

The results of all assays were communicated as average ± standard deviation. Graphpad-prism-5 software calculated inhibition percentages. Statistix-8 sofware derived sample differences between different treatment groups at p ≤ 0.05 by applying one-way ANOVA and Tukey’s-HSD multi-comparison tests. CASP-1.2.3.b software was used to determine the amount of DNA damage in comet assay.

3. Results

3.1. Protective effects of PJM and PHM on nephro-toxicity

3.1.1. Effect on anti-oxidant enzymes of kidney

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\text{CCl}_4 \text{ treatment generated ROS which considerably declined the activity of antioxidant enzymes of kidney. Tables 1 and 6 displays a significant decrease (p < 0.05) in enzyme levels of SOD, CAT, and POD after CCl}_4 \text{ treatment relative to control group, while the treatment with PJM, PHM and silymarin markedly reduced (p < 0.05) the toxicity caused by CCl}_4 \text{ by restoring the renal level of SOD (4.49 ± 0.30, 4.43 ± 0.33 and 4.68 ± 0.41 U/milligram), CAT (4.53 ± 0.29, 4.61 ± 0.61 and 4.64 ± 0.53 U/minute), and POD (8.11 ± 0.57, 8.05 ± 0.58 and 8.56 ± 0.30 U/minute) towards normal, similar to control rat group (5.23 ± 0.31 U/milligram, 6.06 ± 0.48 U/minute, and 10.61 ± 0.60 U/minute). After conducting multiple comparison of various treatment groups under study, it was observed that PJM and PHM (200 mg/kg) was least effective compared to control group whereas it’s higher dose: PJM/PHM (400 mg/kg) and reference drug; silymarin successfully restored all enzymatic levels and hence the antioxidant enzyme activity. However, when PJM and PHM was treated (200 and 400 mg/kg) alone, no alteration was caused in the above parameters, similar to control group (Tables 1 and 6).}

| Treatment                                      | CAT (U/min) | POD (U/min) | SOD (U/mg protein) |
|------------------------------------------------|-------------|-------------|--------------------|
| Control                                        | 6.06 ± 0.48a  | 10.6 ± 0.60a | 5.23 ± 0.31a       |
| Control (vehicle)                              | 5.79 ± 0.28b  | 10.2 ± 0.54a | 5.05 ± 0.45bc      |
| CCl4 (1 mL/kg)                                 | 1.53 ± 0.42bc | 2.37 ± 0.49a | 1.36 ± 0.24c       |
| CCl4 + Silymarin                               | 4.64 ± 0.53c  | 8.56 ± 0.30b | 4.68 ± 0.41bc      |
| CCl4 + PJM (200 mg/kg)                         | 3.43 ± 0.40b  | 6.39 ± 0.25b | 3.60 ± 0.32bc      |
| CCl4 + PJM (400 mg/kg)                         | 4.53 ± 0.29c  | 8.11 ± 0.57b | 4.49 ± 0.30c       |
| PJM (200 mg/kg)                                | 5.64 ± 0.65b  | 9.97 ± 0.74a | 5.01 ± 0.31bc      |
| PJM (400 mg/kg)                                | 6.62 ± 0.26c  | 10.4 ± 0.38a | 5.45 ± 0.35c       |

Mean ± SD (n = 7) having different superscript letters within a column denote significance at p < 0.05. PJM: Parrotiopsis jacquemontiana methanol fraction, CCl4: Carbon tetra-chloride.
group. Co-treatment of PJM (200 and 400 mg/kg) markedly reduced the levels of TBARS (41.1 ± 2.11 and 33.8 ± 1.84 nM/minute/mg proteins), H2O2 (12.7 ± 0.50 and 10.8 ± 0.48 μM/mL), and nitrite (91.9 ± 2.17 and 75.3 ± 2.16 μM/mL) compared to CCl4-treated group (49.8 ± 1.73 nM/min/mg proteins, 16.6 ± 0.99 nM/minute/mg tissue, and 110.5 ± 2.57 μM/mL). Similar results were shown by the coadministration of PHM (200 and 400 mg/kg) which evidently decreased the quantity of TBARS (38.1 ± 0.93 and 32.6 ± 1.22 nM/minute/mg tissue), H2O2 (11.7 ± 0.62 and 10.3 ± 0.59 μM/minute/mg tissue), and nitrite (89.9 ± 2.16 and 72.1 ± 1.44 μM/μL) relative to CCl4 group, respectively. The total content of total protein (6.61 ± 0.41, 6.91 ± 0.53 and 7.28 ± 0.52 mg/g tissue) and GSH (22.7 ± 1.12/23.4 ± 0.79 and 27.3 ± 1.46 nM/minute/mg tissue) in kidney tissue was also elevated substantially (p < 0.05) upon co-administration of PJM/PHM and silymarin with CCl4 relative to CCl4 only treated group. After conducting multiple comparisons of various treatment groups under study, it was observed that PJM and PHM (400 mg/kg) had a restorative potential on the content of GSH, TBARS, H2O2, nitrite, and total protein which was found to be statistically similar to silymarin (standard drug). However, when PJM/PHM was treated (200 and 400 mg/kg) alone, then no alteration (p > 0.05) was caused in the aforementioned kidney parameters, similar to control group (Tables 2 and 7).

3.1.4. Effect on urine profile of kidney

PJM and PHM presented a protective effect on urine profile of kidney as presented in Tables 4 and 9. CCl4 injected group showed reduced level of urinary albumin (4.01 ± 0.76 mg/dl) and PH (6.41 ± 0.38) whereas an increased level of urea (39.2 ± 1.54 mg/dl), specific gravity (1.18 ± 0.09), urobilinogen (8.18 ± 0.74 mg/dl) and urinary creatinine (5.76 ± 0.25 mg/dl) relative to control rat group, respectively. Co-treatment with PJM and PHM (400 mg/kg) reversed CCl4-induced nephro-toxic effects by significantly (p < 0.05) elevating urinary albumin (6.95 ± 0.52, and 7.28 ± 0.65 mg/dl) and PH (7.05 ± 0.02 and 6.91 ± 0.31) and decreasing urea (74.7 ± 1.99, and 72.3 ± 2.10 mg/dl), specific gravity (1.16 ± 0.04 and 1.17 ± 0.07), urobilinogen (5.17 ± 0.24 and 5.28 ± 0.40 mg/dl), and urinary creatinine (3.29 ± 0.16 and 3.15 ± 0.36 mg/dl), similar to control rat group. Treatment with PJM/PHM (200 and 400 mg/kg) alone caused no significant change (p > 0.05) in aforementioned urine parameters, unlike CCl4 treated group (Tables 4 and 9).

3.1.5. Effect on comet parameters

Tables (5 and 10) and Figs. (1 and 3) display the ameliorative effect of PJM and PHM on comet parameters post CCl4-induced nephrotoxicity compared to control group. The extent of DNA damage was evident in renal cells of CCl4-administered group rats (Figs. 1c and 3c). The total amount of DNA migrated from head of comet towards the tail in CCl4-treated group was 30.6% whereas PJM and PHM administration (400 mg/kg) reduced the DNA migration to 2.35 and

Table 2
Effect of PJM on kidney biochemical parameters:

| Treatment               | Protein (μg/mg tissue) | GSH (nM/min/mg protein) | TBARS (nM/min/mg protein) | H2O2 (nM/mL/mg tissue) | Nitrite (μM/mL) |
|-------------------------|------------------------|-------------------------|---------------------------|------------------------|----------------|
| Control                 | 8.52 ± 0.36a           | 28.4 ± 0.93c            | 25.5 ± 1.04e              | 6.11 ± 0.33f           | 58.8 ± 1.13g   |
| Control (vehicle)       | 8.21 ± 0.42a           | 30.7 ± 0.98b            | 26.0 ± 0.94d              | 6.04 ± 0.17a           | 59.0 ± 2.18b   |
| CCl4 (1 ml/kg)          | 2.38 ± 0.38a           | 11.6 ± 1.18d            | 49.8 ± 1.73a              | 16.6 ± 0.99a           | 110.5 ± 2.57a  |
| CCl4 + Silymarin        | 7.28 ± 0.52b           | 27.3 ± 1.46e            | 34.5 ± 1.16e              | 8.63 ± 0.32d           | 71.3 ± 1.71d   |
| CCl4 + PJM (200 mg/kg)  | 5.34 ± 0.33            | 16.5 ± 1.11d            | 41.1 ± 2.11f              | 12.7 ± 0.50            | 91.9 ± 2.17d   |
| CCl4 + PJM (400 mg/kg)  | 6.61 ± 0.41b           | 22.7 ± 1.12d            | 33.8 ± 1.84h              | 10.8 ± 0.49c           | 75.3 ± 2.16e   |
| PJM (200 mg/kg)         | 8.14 ± 0.17a           | 31.6 ± 1.04h            | 25.0 ± 2.05d              | 7.17 ± 0.34d           | 59.7 ± 1.30g   |
| PJM (400 mg/kg)         | 8.49 ± 0.40a           | 32.6 ± 0.72i            | 24.5 ± 2.01d              | 6.87 ± 0.29f           | 58.3 ± 1.06i   |

Mean ± SD (n = 7) having different superscript letters within a column denote significance at p < 0.05. PJM: Parrotiopsis jacquemontiana methanol fraction, CCl4: Carbon tetra-chloride.

Table 3
Effect of PJM on serum parameters of kidney:

| Treatment               | Protein mg/dl | Albumin mg/dl | BUN mg/dl | Creatinine mg/dl |
|-------------------------|---------------|--------------|-----------|------------------|
| Control                 | 98.3 ± 2.18a  | 22.0 ± 0.67a | 43.5 ± 1.77e | 3.70 ± 0.64f     |
| Control (vehicle)       | 96.4 ± 3.00b  | 21.8 ± 0.93b | 44.1 ± 2.37c | 4.35 ± 0.27d     |
| CCl4 (1 ml/kg)          | 47.2 ± 2.01b  | 9.93 ± 0.60d | 68.4 ± 2.04a | 8.15 ± 0.70b     |
| CCl4 + Silymarin        | 85.0 ± 1.82c  | 18.7 ± 0.72c | 49.9 ± 2.79c | 4.96 ± 0.67c     |
| CCl4 + PJM (200 mg/kg)  | 71.7 ± 2.17a  | 13.5 ± 0.65a | 57.5 ± 3.17c | 5.55 ± 0.35c     |
| CCl4 + PJM (400 mg/kg)  | 88.3 ± 1.87c  | 16.3 ± 0.78d | 52.0 ± 2.14c | 5.24 ± 0.19c     |
| PJM (200 mg/kg)         | 98.4 ± 2.42a  | 22.6 ± 0.80bc | 43.9 ± 2.10d | 4.34 ± 0.22c     |
| PJM (400 mg/kg)         | 99.9 ± 2.20a  | 23.2 ± 0.76a | 42.8 ± 1.60d | 4.21 ± 0.30c     |

Mean ± SD (n = 7) having different superscript letters within a column denote significance at p < 0.05. PJM: Parrotiopsis jacquemontiana methanol fraction, CCl4: Carbon tetra-chloride.
5.45%, respectively. Although the least quantity of migrated DNA was recorded for silymarin treated rat group (0.98%) compared to control group yet, PJM and PHM administration (200 and 400 mg/kg) lessened the toxic effects induced by CCl₄ and retreated all altered parameters back to normal, resembling control group. After conducting multiple comparisons between various treatment groups, the higher dose of PJM/PHM (400 mg/kg) was directed to significantly (p < 0.05) restore all the comet parameters (Tables 5 and 10) and display protective effect (Figs. 1f and 3f), similar to silymarin administered group (Figs. 1d and 3d). Treatment with PJM/PHM (200 and 400 mg/kg) alone caused no significant alteration (p > 0.05) in comet parameters, similar to control group.

Table 4
Effect of PJM on urine profile of kidney:

| Treatment                  | PH     | Specific gravity | Urea mg/mL | Creatinine mg/mL | Albumin mg/mL | Urobilinogen mg/mL |
|----------------------------|--------|-----------------|------------|------------------|--------------|-------------------|
| Control                    | 7.25 ± 0.15<sup>b</sup> | 1.04 ± 0.05<sup>d</sup> | 65.6 ± 1.34<sup>f</sup> | 2.81 ± 0.11<sup>d</sup> | 8.48 ± 0.33<sup>f</sup> | 3.85 ± 0.58<sup>d</sup> |
| Control (vehicle)          | 7.34 ± 0.27<sup>b</sup> | 1.01 ± 0.06<sup>d</sup> | 66.2 ± 1.71<sup>f</sup> | 2.83 ± 0.10<sup>d</sup> | 8.22 ± 0.59<sup>f</sup> | 4.17 ± 0.19<sup>d</sup> |
| CCl₄ (1 mL/kg)             | 6.41 ± 0.38<sup>d</sup> | 1.38 ± 0.09<sup>d</sup> | 92.9 ± 1.54<sup>f</sup> | 5.76 ± 0.25<sup>d</sup> | 4.01 ± 0.76<sup>d</sup> | 8.18 ± 0.74<sup>d</sup> |
| CCl₄ + Silymarin           | 6.98 ± 0.04<sup>d</sup> | 1.10 ± 0.05<sup>d</sup> | 69.6 ± 2.24<sup>d</sup> | 3.01 ± 0.40<sup>d</sup> | 7.55 ± 0.55<sup>d</sup> | 4.87 ± 0.72<sup>e</sup> |
| CCl₄ + PJM (200 mg/kg)     | 6.70 ± 0.06<sup>d</sup> | 1.27 ± 0.06<sup>d</sup> | 79.0 ± 1.29<sup>d</sup> | 3.76 ± 0.28<sup>d</sup> | 6.43 ± 0.56<sup>d</sup> | 5.60 ± 0.43<sup>d</sup> |
| CCl₄ + PJM (400 mg/kg)     | 7.02 ± 0.02<sup>d</sup> | 1.16 ± 0.04<sup>d</sup> | 74.7 ± 1.99<sup>d</sup> | 3.39 ± 0.16<sup>d</sup> | 6.95 ± 0.52<sup>d</sup> | 5.17 ± 0.24<sup>d</sup> |
| PJM (200 mg/kg)            | 7.11 ± 0.05<sup>d</sup> | 1.07 ± 0.03<sup>d</sup> | 65.2 ± 1.64<sup>d</sup> | 2.82 ± 0.11<sup>d</sup> | 8.31 ± 0.70<sup>d</sup> | 4.02 ± 0.29<sup>d</sup> |
| PJM (400 mg/kg)            | 7.29 ± 0.18<sup>d</sup> | 1.06 ± 0.01<sup>d</sup> | 64.4 ± 1.84<sup>d</sup> | 2.81 ± 0.18<sup>d</sup> | 8.36 ± 0.11<sup>d</sup> | 3.93 ± 0.19<sup>d</sup> |

Mean ± SD (n = 7) having different superscript letters within a column denote significance at p < 0.05. PJM: Parrotiopsis jacquemontiana methanol fraction, CCl₄: Carbon tetra-chloride.

Table 5
Effect of PJM on DNA damages of kidney cells employing comet assay:

| Treatment                  | Comet length (μm) | Head length (μm) | Tail length (μm) | % DNA in head | % DNA in tail | Tail moment |
|----------------------------|-------------------|-----------------|-----------------|--------------|--------------|-------------|
| Control                    | 45.2 ± 2.21<sup>d</sup> | 36.4 ± 1.36<sup>f</sup> | 8.72 ± 1.47<sup>d</sup> | 91.1 ± 1.96<sup>f</sup> | 9.95 ± 1.25<sup>d</sup> | 0.86 ± 0.09<sup>d</sup> |
| Control (vehicle)          | 44.5 ± 2.56<sup>d</sup> | 35.5 ± 2.20<sup>d</sup> | 8.97 ± 2.28<sup>d</sup> | 90.3 ± 2.42<sup>d</sup> | 10.6 ± 1.30<sup>d</sup> | 0.95 ± 0.11<sup>d</sup> |
| CCl₄ (1 mL/kg)             | 64.2 ± 2.25<sup>f</sup> | 22.4 ± 1.98<sup>f</sup> | 41.8 ± 2.94<sup>f</sup> | 75.3 ± 1.82<sup>f</sup> | 24.9 ± 2.17<sup>f</sup> | 10.4 ± 0.47<sup>f</sup> |
| CCl₄ + Silymarin           | 46.2 ± 2.44<sup>d</sup> | 36.3 ± 1.93<sup>f</sup> | 9.85 ± 1.95<sup>d</sup> | 90.13 ± 2.10<sup>d</sup> | 9.70 ± 1.69<sup>d</sup> | 0.95 ± 0.15<sup>d</sup> |
| CCl₄ + PJM (200)           | 59.1 ± 2.57<sup>b</sup> | 27.6 ± 1.69<sup>ab</sup> | 31.5 ± 1.72<sup>d</sup> | 85.8 ± 1.56<sup>b</sup> | 14.6 ± 1.44<sup>b</sup> | 4.61 ± 0.43<sup>b</sup> |
| CCl₄ + PJM (400)           | 49.0 ± 3.04<sup>b</sup> | 32.9 ± 1.62<sup>b</sup> | 16.1 ± 1.77<sup>b</sup> | 88.2 ± 1.49<sup>b</sup> | 12.3 ± 1.63<sup>b</sup> | 1.98 ± 0.16<sup>b</sup> |
| PJM (200)                  | 45.6 ± 2.36<sup>b</sup> | 36.3 ± 2.37<sup>b</sup> | 9.26 ± 1.26<sup>b</sup> | 90.6 ± 1.59<sup>b</sup> | 10.7 ± 1.81<sup>b</sup> | 0.99 ± 0.14<sup>b</sup> |
| PJM (400)                  | 45.2 ± 2.43<sup>b</sup> | 36.8 ± 1.34<sup>b</sup> | 8.43 ± 1.20<sup>b</sup> | 91.2 ± 1.68<sup>b</sup> | 8.97 ± 1.79<sup>b</sup> | 0.81 ± 0.25<sup>b</sup> |

Mean ± SD (n = 7) having different superscript letters within a column denote significance at p < 0.05. PJM: Parrotiopsis jacquemontiana methanol extract, D.DNA: Damaged DNA.

3.1.6. Effect on histo-architecture of kidney
Histopathological alterations in the architecture of renal tissues are presented in Figs. 2 and 4. The control and vehicle group of rats displayed normal morphology and histo-architecture of renal tissues (Figs. 2a,1b and 4a,b) with prominent glomeruli, Bowman’s capsule, distal and proximal convoluted tubules etc. whereas severe pathological abnormalities were observed in CCl₄ administered group i.e. glomeruli disruption with impaired corticular sections, accumulation of necrotic cells, distortion of Bowman’s capsule and the narrowing of space between it (Figs. 2c and 4c). However, these renal damages were successfully ameliorated by the co-administration of PJM (400 mg/kg) (Fig. 2f), PHM (400 mg/kg) (Fig. 4f), and silymarin (Figs. 2d and 4d) with CCl₄ in such a way...
that its histo-architecture resembled the control group. Though all co-treated groups showed slight edema but glomerular hypertrophy was obvious in PJM and PHM high-dose administered groups only (Figs. 2f and 4f). On the other hand, both PJM and PHM treated low doses (200 mg/kg) groups exhibited edema and glomerular atrophy as presented in Figs. 2e and 4e. PJM/PHM treatment alone (200 and 400 mg/kg) did not instigate any histopathological changes in renal tissues (Figs. 2g,h and 4g,h) and resembled the control group.

4. Discussion

As herbal plants have been used since ancient times for the management of human ailments without having adverse effects on the body/living organs and keeping in mind the current treatments available in developed and underdeveloped countries which are highly costly, time-consuming, and with serious side-effects (Batool et al., 2019, Batool et al., 2020, Zahra et al., 2021a, Zahra et al., 2016). The present study demonstrates the protective potential of P. jacquemontiana and P. hydaspidis on CCl4-intoxicated kidney cells of male experimental rats for the first time.

The toxicity of CCl4 to kidneys depends upon inflammatory processes, free-radical generation, and oxidative stress. Single-dose administration of CCl4 can cause oxidative stress and LPO resulting in cellular damage and leakage of enzymes to blood-stream (Haskoury et al., 2018). Keeping in view the high sensitivity of kidney to CCl4, the formation of less urine by kidneys may cause H2O2 accumulation in cellular damage and leakage of enzymes to blood-stream (El-Haskoury et al., 2018). Our study showed declined levels of POD, SOD, GSH, and CAT in CCl4-injected group while decreased concentrations of albumin and proteins in serum of CCl4-intoxicated rats. Normally, creatinine and urea are measured for assessing kidney function and protein metabolism whereby its increased level in blood is deliberated as a marker for kidney impairment (Usman et al., 2020). CCl4-administered group revealed high creatinine levels, which is considered an investigative marker for cell-membrane injury and cellular leakage in kidney tissues (Mika and Guruvayoorappan, 2013). A recent study reported similar results demonstrating elevated level of uric acid and creatinine as indices of nephro-toxicity from the fact that they are final products of nitrological base (purine) and can modify glomerular filtration-rate. Any alteration occurring in glomerular filtration-rate elevates uric acid and serum creatinine levels which are associated with kidney damage (Ullah et al., 2013).

The physiological integrity and functional condition of kidney is also shown by urine analysis as reported in earlier studies, where malfunctioned kidneys demonstrated distressed urine profiles. The detection of urobilinogen, RBC’s, and acidic pH in urine profile with increased level of urinary creatinine, WBC’s, and urea whereas declined levels of albumin and urinary protein indicated serious renal damage (Sahreen et al., 2015). The obtained data exhibited the manifestation of aforementioned parameters in urine analysis (p > 0.001) increase in antioxidant (POD, SOD, GSH, GPX, GR, and CAT) enzymes after co-treatment of Zingerone (100 mg/kg) with CCl4 relative to CCl4-treated group only. Under reduced levels of CAT, SOD, and GSH oxidative stress may take place. Diminished GSH content causes impairment in H2O2 clearance and promotes formation of hydroxyl radicals. Reduced SOD levels are unable to catalyze dismutation of superoxide radicals while reduced levels of CAT are unable to reduce H2O2 → H2O and O2, hence being incapable of preventing cell damage induced via oxidative stress (Safhi, 2018).

Xenobiotic consumption elevates lipid peroxidation (TBARS) and leads to overproduction of superoxide that causes dismutation of H2O2 and singlet O2 which can be readily converted into highly reactive hydroxyl (OH) radicals. This singlet O2 and OH radicals have a high potential in initiating long-chain reactions of free-radicals linked to LPO (Suzek et al., 2017).

Mean ± SD (n = 7) having different superscript letters within a column denote significance at p < 0.05. PHM: Periploca hydaspidis methanol fraction, CCl4: Carbon tetra-chloride.
of CCl4-treated rat group, henceforth verifying that damage had taken place. Also, the increased specific gravity of urine found in CCl4-treated rat group, henceforth verifying that damage had occurred. Mean ± SD (n = 7) having different superscript letters within a column denote significance at p < 0.05. PHM: Periploca hydaspidis methanol fraction, CCl4: Carbon tetra-chloride.

### Table 8
Effect of PHM on serum parameters of kidney:

| Treatment                | Protein mg/dl | Albumin mg/dl | BUN mg/dl | Creatinine mg/dl |
|-------------------------|---------------|---------------|-----------|------------------|
| Control                 | 98.3 ± 2.18a  | 22.0 ± 0.67a  | 43.5 ± 1.77a | 3.70 ± 0.64d    |
| Control (vehicle)       | 96.4 ± 3.00e  | 21.8 ± 0.93a  | 44.1 ± 2.37a | 4.35 ± 0.27ed   |
| CCl4 (1 mL/kg)          | 47.2 ± 2.09f  | 9.93 ± 0.60f  | 68.4 ± 2.04a | 8.19 ± 0.70a    |
| CCl4 + Silymarin        | 85.0 ± 1.82e  | 18.7 ± 0.72f  | 49.9 ± 2.79f | 4.96 ± 0.66ef   |
| CCl4 + PHM (200 mg/kg)  | 69.7 ± 2.33f  | 15.5 ± 0.69f  | 60.1 ± 1.89f | 5.36 ± 0.19f    |
| CCl4 + PHM (400 mg/kg)  | 86.8 ± 3.19f  | 17.7 ± 0.65f  | 54.6 ± 2.06f | 5.10 ± 0.14f    |
| PHM (200 mg/kg)         | 99.7 ± 2.76f  | 21.6 ± 0.79f  | 45.9 ± 1.36f | 4.41 ± 0.15fd   |
| PHM (400 mg/kg)         | 100.5 ± 2.15f | 22.7 ± 0.82f  | 44.6 ± 2.15f | 4.09 ± 0.18f    |

Mean ± SD (n = 7) having different superscript letters within a column denote significance at p < 0.05. PHM: Periploca hydaspidis methanol fraction, CCl4: Carbon tetra-chloride.

### Table 9
Effect of PHM on urine profile of kidney:

| Treatment                | pH       | Specific gravity | Urea mg/dl | Creatinine mg/dl | Albumin mg/dl | Urobilinogen mg/dl |
|-------------------------|----------|-----------------|------------|------------------|---------------|---------------------|
| Control                 | 7.25 ± 0.15ab | 1.04 ± 0.05d   | 65.6 ± 1.34f | 2.81 ± 0.111   | 8.48 ± 0.33abc | 3.85 ± 0.58a        |
| Control (vehicle)       | 7.34 ± 0.27a | 1.01 ± 0.06e   | 66.2 ± 1.71a | 2.83 ± 0.10e   | 8.22 ± 0.59abcd | 4.17 ± 0.19ab       |
| CCl4 (1 mL/kg)          | 6.41 ± 0.38d | 1.38 ± 0.09f   | 92.9 ± 1.54f | 5.76 ± 0.25f   | 4.01 ± 0.76f   | 8.18 ± 0.74f        |
| CCl4 + Silymarin        | 6.98 ± 0.04bc | 1.10 ± 0.05cd  | 69.6 ± 2.24d | 3.01 ± 0.40bc  | 7.55 ± 0.55cd   | 4.87 ± 0.72cd       |
| CCl4 + PHM (200 mg/kg)  | 6.68 ± 0.08cd | 1.24 ± 0.05b   | 75.8 ± 2.11b | 3.46 ± 0.37b   | 6.76 ± 0.77b   | 5.76 ± 0.45b        |
| CCl4 + PHM (400 mg/kg)  | 6.91 ± 0.31bc | 1.17 ± 0.07bc  | 72.3 ± 2.10b | 3.15 ± 0.36bc  | 7.28 ± 0.65bc   | 5.28 ± 0.40bc       |
| PHM (200 mg/kg)         | 7.14 ± 0.32ab | 1.09 ± 0.02cd  | 66.4 ± 1.77f | 2.90 ± 0.40f   | 8.73 ± 0.27ab   | 4.35 ± 0.31ab       |
| PHM (400 mg/kg)         | 7.19 ± 0.11ab | 1.08 ± 0.02cd  | 67.1 ± 1.32f | 2.88 ± 0.31f   | 9.19 ± 0.15ab   | 4.11 ± 0.15ab       |

Mean ± SD (n = 7) having different superscript letters within a column denote significance at p < 0.05. PHM: Periploca hydaspidis methanol fraction, CCl4: Carbon tetra-chloride.

### Table 10
Effect of PHM on DNA damages of kidney cells employing comet assay:

| Treatment                | Comet length (μm) | Head length (μm) | Tail length (μm) | % DNA in head | % DNA in tail | Tail moment |
|-------------------------|--------------------|------------------|------------------|--------------|--------------|-------------|
| Control                 | 46.4 ± 1.39d       | 42.7 ± 1.69f     | 3.74 ± 0.58f     | 95.7 ± 1.33a | 4.67 ± 0.49f | 0.17 ± 0.09f |
| Control (vehicle)       | 45.9 ± 2.17f       | 42.0 ± 2.41i     | 3.95 ± 0.79f     | 95.5 ± 1.73a | 5.61 ± 0.71f | 0.22 ± 0.11f |
| CCl4 (1 mL/kg)          | 73.4 ± 2.37f       | 23.6 ± 1.67f     | 49.8 ± 1.50f     | 64.6 ± 1.02d | 35.3 ± 2.25s | 17.5 ± 0.50s |
| CCl4 + Silymarin        | 45.4 ± 1.65f       | 40.5 ± 1.62f     | 4.91 ± 0.63f     | 94.7 ± 2.05f | 5.65 ± 1.00f | 0.27 ± 0.12f |
| CCl4 + PHM (200)        | 61.2 ± 2.69e       | 33.9 ± 2.14d     | 27.2 ± 1.53c     | 81.2 ± 2.10c | 19.7 ± 1.70c | 5.38 ± 0.53c   |
| CCl4 + PHM (400)        | 51.4 ± 2.11c       | 39.6 ± 1.93c     | 11.8 ± 0.82c     | 89.8 ± 2.24c | 10.2 ± 0.84c | 1.20 ± 0.30c   |
| PHM (200)               | 47.0 ± 1.27d       | 42.3 ± 1.33d     | 4.68 ± 0.67f     | 94.5 ± 1.55s | 5.64 ± 0.47f | 0.28 ± 0.10d   |
| PHM (400)               | 46.2 ± 1.47d       | 42.3 ± 1.84d     | 3.87 ± 0.38f     | 95.6 ± 2.01a | 4.83 ± 0.43d | 0.18 ± 0.07d   |

Mean ± SD (n = 7) having different superscript letters within a column denote significance at p < 0.05. PHM: Periploca hydaspidis methanol fraction, CCl4: Carbon tetra-chloride.
displayed abnormal appearance of glomeruli and Bowman’s capsule with impaired corticull segments, aggregation of necrotic cells, dilation of convoluted tubules, edema in Bowman’s capsule, and space narrowing between Bowman’s capsule. Earlier study accomplished by Kalantari et al., 2018 revealed the histopathology of CCl4 group to be characterized by epithelial cell detachment, widening of tubular lumen, vacuolization, atrophy, and tubular necrosis which modifies the nephron functioning by altering the capability of tubular absorption leading to kidney dysfunction and injury. This kidney injury was successfully reversed by co-treatment of PJM/PHM (particularly 400 mg/kg) fraction, thereby exhibiting normal architecture of renal cells, resembling control group. Hypertrophy of glomerulus was observed in PJM and PHM high doses which may prove favorable, as reported elsewhere that hyperplasia and hypertrophy when present after tissue injury considerably contributes to the process of regeneration (Marongiu et al., 2017). Our study is in match with the findings of AL-Ghamdi et al., 2019, who reported the co-administration (200 mg/kg) of methanol (ZS-1) and aqueous (ZS-4) fractions of Zizyphus spina-christi (L) to display characteristic glomerular and tubular structures of kidney resembling control group.

5. Conclusion

The present findings provide additional scientific evidence unravelling P. jacquemontiana and P. hydaspidis as strong antioxidants capable of defending the kidney from CCl4-induced damages. These medicinal plants are declared safe after no toxicity was observed in experimental rats, even when provided at higher doses. The in vitro high free-radical scavenging capability assessed earlier was hereby implicated endogenously on rat model in vivo. P. jacquemontiana and P. hydaspidis manifest the compounds which

![Fig. 2. Protective potential of PJM on renal histopathology of rats (40X magnification using hematoxylin-eosin stain). (a) Control group (b) Vehicle control (c) CCl4 (1 mL/kg bw) (d) CCl4 + Silymarin (e) CCl4 + PJM (200 mg/kg) (f) CCl4 + PJM (400 mg/kg) (g) PJM (200 mg/kg) (h) PJM (400 mg/kg). BC: Bowman’s capsule, G: Glomerulus, PCT: Proximal convoluted tubule, DCT: Distal convoluted tubule, N: Necrosis, GD: Glomerular disruption, E: Edema, DPCT: Dilated proximal convoluted tubule, GH: Glomerular hypertrophy, GA: Glomerular atrophy, PJM: P.jacquemontiana methanol extract.](image)

![Fig. 3. Fluorescence micrograph of kidney cells and protective effect of PHM on genotoxicity (a) Control group (b) Vehicle control (c) CCl4 (1 mL/kg bw) (d) CCl4 + Silymarin (e) CCl4 + PHM (200 mg/kg) (f) CCl4 + PHM (400 mg/kg) (g) PHM (200 mg/kg) (h) PHM (400 mg/kg). PHM: P. hydaspidis methanol extract, D.DNA: Damaged DNA.](image)
Fig. 4. Protective potential of PHM on renal histopathology of rats (40X magnification using hematoxylin-eosin stain). (a) Control group (b) Vehicle control (c) CCl4 (1 mL/kg bw) (d) CCl4 + Silymarin (e) CCl4 + PHM (200 mg/kg) (f) CCl4 + PHM (400 mg/kg) (g) PHM (200 mg/kg) (h) PHM (400 mg/kg). BC: Bowman’s capsule, G: Glomerulus, PCT: Proximal convoluted tubule, N: Necrosis, GD: Glomerular disruption, BCD: Bowman’s capsule disruption, NBC: Narrowing of Bowman’s capsule, E: Edema, DPCT: Dilated proximal convoluted tubule, GH: Glomerular hypertrophy, GA: Glomerular atrophy, FJM: P-hydaspis methanol extract.

efficiently work on kidney to keep it functioning normally. It appears to effectively reserve the structural integrity of glomerulus, Bowman’s capsule, proximal and distal convoluted tubules when injury takes place via inhibiting CCl4-instigated oxidative stress chain reactions, preventing DNA damage, and restoring cellular antioxidant status. The current studies confirmed that it may be promising candidates for phytomedicine development against various renal ailments. However, additional in vivo and clinical trials are needed to further justify and evaluate the effectiveness and biocompatibility of these plants as nephroprotective agents in humans.

CRediT authorship contribution statement

Saima Ali: Conceptualization, Formal analysis, Investigation, Methodology, Writing -original draft. Muhammad Rashid Khan: Project administration, Resources. Javed Iqbal: Conceptualization, Investigation, Methodology, Validation. Banzeer Ahsan Abbasi: Validation. Tabassum Yaseen: Data curation. Riffat Batool: Formal analysis. Muhammad Delwar Hussain: Funding acquisition. Mohsin Kazi: Funding acquisition. All authors have read and agreed to the published version of the manuscript.

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.

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