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Research article

Fagonia indica attenuates chromium-induced nephrotoxicity via antioxidant and anti-inflammatory activities in mice

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ABSTRACT

Chromium (Cr) is a common environmental pollutant that has wide-ranging toxic manifestations. *Fagonia indica* (*F. indica*) is an herbal medicine with anti-inflammatory properties and antioxidant activity. This study aims to evaluate the protective role of *F. indica* (whole plant) in attenuating Cr-induced nephrotoxicity in Swiss mice. Swiss albino mice were divided into five groups (10 mice in each): group I (control); group II (*F. indica*-treated); group III (Cr-intoxicated); group IV (Cr- and saline-intoxicated); and group V (Cr-intoxicated and *F. indica*-treated). Blood samples were drawn after sacrifice for biochemical examinations. Kidney specimens were collected to examine antioxidant activities and conduct histological and immunohistochemical studies for all groups. Mice intoxicated with Cr at 15 mg/kg/b.wt showed a decrease in superoxide dismutase (SOD), glutathione S-transferase (GST), and glutathione peroxidase (GSH-Px) levels compared to the control group, followed by an elevation in the serum IL-6 level. The data revealed severe damage to the renal tubular epithelial cells as well as marked congestion and positive, diffuse, and strong expression of caspase-3 in the dilated tubules. Additionally, the data disclosed an increase in the serum level of blood urea nitrogen (BUN) and creatinine in group III compared with group I. Group V, treated with *F. indica* at a selected dose of 120 mg/kg/b.wt, showed an improvement in antioxidant activity, attenuation of the IL-6 level, fewer histopathological disturbances, and a statistically significant decrease in the serum level of BUN and creatinine compared with group III. Such changes may be attributed to the antioxidant and anti-inflammatory effects of *F. indica*. Therefore, our investigation revealed that *F. indica* effectively protects against Cr-induced nephrotoxicity.

1. Introduction

Herbal medicines have been used for centuries within different cultures to treat various diseases. Recently, there has been a great deal of interest in herbal medicine, the use of which has increased tremendously over the past three decades. The reason for the use of alternative medicine rather than conventional medicine is that patients are seeking ways to improve their well-being, alleviate their symptoms, or avoid the side effects of conventional therapies. More recently, however, herbs and spices have been identified as sources of various phytochemicals, many of which possess powerful antioxidants. Thus, herbs and spices may have a role in antioxidant defense and redox signaling [1]. *Fagonia indica* (*F. indica*) is an important medicinal plant with a multitude of potential therapeutic applications. It is a small spiny under-shrub. Perfused branched, pale-green, glandular, annual or perennial shurblets up to 60 cm high; internodes 2.5–5.0 cm long. Leaves all uni or lower ones trifolicates; leaflets 8–16 × 3–4 mm, narrowly ovate-lanceolate, mucronate, distinctly articulate at the base; stipular spines suberect, equal to or shorter than leaves. *F. indica* is widely found in the deserts of Asia and Africa, the plant contains a variety of bioactive flavonoids, sterols, and triterpenoids. Flavonoids are the most abundant polyphenols in plants, with anti-diabetic, anti-inflammatory, anti-allergenic, hepatoprotective, antioxidant, anti-atherogenic, anti-cancer, antimicrobial, vasodilatory, cardio-protective, and antithrombotic properties [2].

Due to their essential function in numerous physiological situations, as well as their involvement in a wide range of disorders, free radicals and other oxidants have acquired prominence in biology. Endogenous stress is caused by a buildup of reactive oxygen species (ROS), which can harm cell growth and development. Moreover, ROS molecules decrease the expression of antioxidants (superoxide dismutase (SOD), glutathione

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peroxidase (GSH-Px), and catalase) thus propagating pathological tissue destruction [3]. ROS potently affect adaptive immune maturation by promoting the synthesis of pro-inflammatory cytokines, such as TNF-α and IL-6. These cytokines could induce cell death, which occurs by necrosis or apoptosis [4]. Besides, heavy metals are reported to enhance ROS production and accumulation. Chromium (Cr), a common heavy metal, is found in many forms, including a range of oxidation numbers [Cr (II) to Cr (VI)]. Cr is also extensively used in industries and released into the environment, where, since it is non-degradable, it remains for a long period. The industries of leather tanning, metallurgy, electroplating, alloying, ceramic glazes, wood preservation, water corrosion inhibition, refractory bricks, pressure-treated lumber, textile dyes, pigments, paints, and paper and pulp production all contribute to Cr hyperaccumulation in the environment. Furthermore, the most important Cr sources include the dumping of Cr-contaminated liquids and solid wastes, the emission of Cr from industry cooling towers, and the dust rising from roadways and roadsides [5].

Nephrotoxicity, which could be a major adverse effect of Cr poisoning, is attributed primarily to oxidative stress. Renal failure has been described in both acute and chronic exposure [6]. The role of inflammation in acute kidney organ injury has been observed to progressively involve leukocytes, adhesion molecules, chemokines, and cytokines. IL-6 is a pleiotropic cytokine that impacts hematopoiesis, metabolism, and organ development in addition to immunological and inflammatory responses. Moreover, it induces separate or even opposing physiopathological processes at the same time, which are likely differentiated by signaling pathway cascades referred to as classic and trans-signaling. Aside from its numerous physiological functions, dysregulated IL-6 has been linked to a variety of autoimmune and inflammatory disorders, metabolic abnormalities, and cancers [7].

The main aim of this study is to assess the antioxidant and anti-inflammatory properties upon administration of *F. indica* traditional medicinal herbal extracts in Cr-induced nephrotoxicity in Swiss mice. Furthermore, the level of pro-inflammatory cytokine IL-6 and its cellular roles will be correlated with serum levels of blood urea nitrogen (BUN), serum creatinine, and markers related to oxidative stress SOD, glutathione S-transferase (GST), and GSH-Px to evaluate nephrotoxicity regression outcomes. Moreover, the histopathology and immunohistochemistry of the kidneys of all groups of mice will be assessed and analyzed in detail.

2. Materials and methods

2.1. Animals

A total of 140 adult male Swiss mice weighing 26–28 g was used during the experiments, obtained from the animal household of the King Abdulaziz University, Jeddah, Saudi Arabia. The animals were first acclimatized for five days at the experiment site (Biochemistry Department, Faculty Medicine, Albaha University) under optimal environmental conditions (12-hour light–dark cycles, temperature [20 ± 2 °C] with moderate humidity [60 ± 5%]), and food and water available *ad libitum*. The recommended ethical standards were strictly observed throughout the animal experimentation. Albaha university ethical committee, project No: 32/1442.

2.2. Chemicals and herbal plant

2.2.1. Potassium dichromate

Potassium dichromate ($K_2Cr_2O_7$), (POD) was purchased from (Sigma, St. Louis, MO, USA; Cat. No. SKU-207802). It was dissolved in water with different concentrations. Thirty mice were intoxicated daily by intraperitoneal (i.p.) injected doses (15, 25, and 35) mg/kg/b.wt (N = 10/group) respectively. A significant change of renal tubules in mice and toxicity was achieved at 15 mg/kg/b.wt after 10 consecutive days of i.p. injections. The dose of 15 mg/kg/b.wt was approved in the following experiments, conforming to the dose and time-dependent curve [8]. Doses of 25 and 35 mg/kg/b.wt. caused a high mortality rate, of 50% and 80%, respectively.

2.2.2. F. indica

The *F. indica* was freshly gathered in Albaha province in Saudi Arabia. The plant was identified by the Department of Botany, Faculty of Science, Albaha University. The whole plant was ground, using a dry mill. Particles should be small in size in order to extract simply in solvent. The *F. indica* plant was collected and dried in a dim environment before grinding. Using the protocol of soaking and extracting, the plant was soaked in 70% ethanol concentration, the solution was filtered, and the solvents were evaporated at a standard temperature. The ratio between the solvent and plant should be 1:10, and the plant should be regularly stirred with the solvent for 72 h. The dried extract was stored at −20 °C until use [9].

2.2.3. DPPH radical scavenging assay

Blois [10] and Desmarchelier et al. [11] used the 2,2-diphenyl-1 picrylhydrazyl (DPPH) radical scavenging assay to determine the extracts' potential to scavenge free radicals. The ability of plant extract to donate hydrogen atoms was tested by decolorizing a methanol solution of DPPH. In methanol solution, DPPH creates a violet/purple color, which fades to shades of yellow in the presence of antioxidants. A 0.1 mM DPPH in methanol solution was formed, 2.4 mL of which was combined with 1.6 mL of extract in methanol at various doses (15–150 g/mL). The reaction mixture was completely vortexed and kept at room temperature for 30 min. At 517 nm, the mixture's absorbance was determined spectrophotometrically. BHT (butylated hydroxytoluene) was used as reference.

The following equation was used to compute percent DPPH radical scavenging activity: percent DPPH radical scavenging activity = $\{(A_0-A_1)/A_0\} \times 100$. The absorbance of the control is A_0, whereas the absorbance of the extract/standard is A_1. The percent of inhibition was then plotted against concentration, and the IC_{50} was derived from the graph. At each concentration, the experiment was performed three times.

2.3. Lethality study in Swiss albino mice

Sixty male Swiss albino mice were treated with different concentrations of *F. indica* extract. LD_{50} of the plant extract was calculated using 50% death through three days following i.p. injection with altered dosages. The percentage of mice that died was calculated graphically through the change in the number of dead mice against time. The test was applied to establish LD_{50} by using percentage mice loss per group versus dosage log [12].

2.4. Experimental design

Swiss mice were divided randomly into five groups, each comprising 10 mice. Group I was kept as normal control. Group II administered an extract of *F. indica* only. Groups III, IV, and V administered an i.p. injection of POD at a dose of 15 mg/kg/b.wt for 10 succeeding days. Group IV administered saline and group V was treated with *F. indica* intraperitoneally for 12 successive days with 120 mg/kg/b.wt. (treated group) [13,14]. The nephrotoxicity induction achievement for mice groups was confirmed by kidney function tests and histological inspection. On the last day of the experiments, the mice were weighed and scarified (a process of euthanasia used on mice under anesthesia) 24 h after the last treatment, blood samples were withdrawn from all groups, and serum was reserved for analyzing kidney function parameters and IL-6 determination. Both kidneys were removed, washed, and prepared for histologic inspection and oxidative stress sign activities (SOD, GST, and GSH-Px in tissue). Furthermore, the remaining mice kidneys were kept in 10% formalin for immunohistochemical examination.

2.5. Determination of SOD, GST, and GSH-Px activity in kidney homogenate

The kidney tissue was cut into small pieces and washed by phosphate-buffered saline. Furthermore, it was ground in a homogenization buffer {0.05 M Tris-HCl pH 7.9, 25% glycerol, 0.1 mM EDTA, and 0.32 M $(NH_4)_2SO_4$} containing a protease inhibitor tablet (Roche, Germany; SKU 11697498001). The lysates were homogenized on ice using a homogenizer (HG-15D, Witeg Labortechnik Gmbh; Germany). The solution was sonicated in an ice bath to prevent overheating for 15 s, followed by centrifugation at 12000 rpm, 4 °C for 5 min. The supernatant was aliquoted and stored at −80 °C, and homogenate was assessed for SOD, GST and GSH-Px activity.

The protein concentrations in the samples were measured using Lowry et al.'s [15] technique. SOD activity was measured by autoxidation adrenaline to adrenochrome, as described by Misra and Fridovich [16]. GST activity was measured by reacting the –SH group of GSH with 1 chloro-2,4-dinitrobenzene (CDNB) [17]. Tamura et al. [18] used a method to track the reduction of t-butyl hydroperoxide with nicotinamide adenine dinucleotide phosphate (NADPH) to determine GSH-Px activity. All enzyme activity was measured in units per gram. All measurements were taken with a Spectro 24RS VIS-spectrophotometer (S.N. 24-13061, Labomed Inc, USA) equipped with a temperature-controlled cuvette holder at 19 °C. The following were the wavelengths used in the analyses: 480 nm for SOD, 340 nm for GST, and 412 nm for GSH-Px.

2.6. Determination of BUN and creatinine in serum

Blood samples were withdrawn from mice after scarification through a heart puncher. Blood samples were centrifuged at 3000 rpm for 10 min at 4 °C and aliquoted for the respective analytical determinations. Serum was used for estimation of BUN and creatinine levels, determined using a Colorimetric Assay Kit (Urease Method) (Cat. No. E-BC-K183-S, Elabscience, France). Serum creatinine concentration was determined by a Creatinine Serum Detection Kit (Arbor Assays, MI, USA, Cat. No. KB02-H1) according to the manufacturer's protocol.

2.7. Quantitative determination of IL-6

The IL-6 level was determined based on the method of El Naaa et al. [19]. Briefly, diluted samples or IL-6 standards (10 μl) were mixed with 100 μl of 50 μg/l anti-IL-6 immunoglobulin G labeled with horseradish peroxidase in 10 mmol/l ethylenediaminetetraacetic acid. A 100-μl aliquot of the mixture was transferred to each of the wells. The plate was then incubated for 60 min at 37 °C without shaking and then rinsed three times with 10 mmol/l phosphate-buffered saline (PBS) (pH 7.0) containing 0.1 mol/l NaCl. The micro-plate-bound horseradish peroxidase activity was determined by the addition of 100 μl of 0.15 mol/l citric acid sodium phosphate buffer (pH 4.9), containing 2.0 g/l of o-phenylenediamine and 0.02% (v/v) hydrogen peroxide, incubated for 20 min at 37 °C. To discontinue the reaction, 100 μl of 1 mol/l sulfuric acid was added. The absorbance was determined with a microplate reader at a wavelength of 450 nm. Throughout the standard curve, IL-6 level in mice serum was measured. The results were expressed as picogram/milliliters (pg/ml).

2.8. Immunohistochemical examination of caspase-3

The immunohistochemical staining procedure for caspase-3 was performed by the streptavidin–biotin immunoperoxidase method (Dako-Cytomation, Glostrup, Denmark; Cat. No. SA10-10). Tissue sections were cut at 3–5 μm thickness from the blocks on positively charged slides, then xylene was used to remove the paraffin and rehydrated by graded alcohol. After this, sections were heated in buffered citrate (pH 6.0) for 20 min and then washed in PBS (pH 7.3). Endogenous peroxidase activity was blocked using 6% H_2O_2 in methanol. The immunohistochemical staining for caspase-3 was performed with a ready-to-use rabbit polyclonal antibody (Lab Vision, New York, USA; Cat. No. RB - 1197). The slides were incubated for 2 h with the primary antibody at room temperature and washed using PBS. After rinsing in PBS, slides were immersed with a biotin-conjugated secondary antibody (Lab Vision Corporation, Fermont, USA; Cat. No. BA1001). DAB and Mayer's hematoxylin were used as a chromogen and counterstain, respectively. The slides were washed with distilled water and PBS. Positive and negative controls were stained with the same setting as the studied cases. The negative controls were carried out using the same tissue, with the omission of the primary antibody. Staining was noted as absent, weak, moderate, or strong, whereas positivity was expressed as the percentage of immunoreactive cells over the total number of cells [20].

2.9. Histopathological examination of the kidneys

The kidney was set in 10% paraformaldehyde, dehydrated, fixed in paraffin, and sectioned at 5 μm. Hematoxylin and eosin were used to stain the sections. The morphological changes were examined under a microscope (Eclipse 80i, Nikon, Japan), and the pictures were captured with a video camera (DS-Fi1 digital microscope camera, Nikon, Japan).

2.10. Morphometric analysis

For semi-quantitative assessment of immunohistochemistry reaction, images were digitally imported to an image analyzer software (Image J 1.47 v Software, National Institutes of Health, Bethesda, MD, United States). All sections were randomly evaluated under blindfold manner. Images were captured by a digital camera connected to a CX31 light microscope (Olympus, Japan) and attached to a computer. Ten separate fields of DAB-chromogen stain sections examined (at different time periods the experiment). The mean percentage area per examined field was calculated as the average of the pooled readings from these fields in each specimen. Also, for semi-quantitative assessment of histopathology of renal tubules, the mean of renal tubules percentage area per examined field was calculated as the average of the pooled readings from these fields in each specimen. For histopathology of renal glomeruli, perimeter (in pixels) & area (in pixels) per examined field were calculated as the average of the pooled readings from these fields in each specimen.

3. Statistical analysis

Data were analyzed using the Statistical Package for Social Science software computer program version 26 (SPSS, Inc., Chicago, IL, USA). Data were parametric and presented in mean and standard deviation. A one-way analysis of variance (ANOVA) and Tukey test were used to compare parametric data. The Monte Carlo test was used to compare frequency of cross tabulation ($>2 \times 2$ cells). A Kruskal–Wallis test followed by a post-hoc Dunn's was used to compare quantitative non-parametric data. A p-value of less than 0.05% reflected statistical significance.

4. Results

4.1. Toxicity of F. Indica

The mice treated with *F. indica* (50, 100, 150, 200, 250, and 300 mg/kg) (i.p) did not show any indication of harmfulness on their body weight and overall appearance. The LD_{50} dose of *F. indica* was 150 mg/kg. The mice improved after treatment and have been surviving without any signs of adverse effects. On the other hand, on day 10 of POD injection, group V was treated with *F. indica* 120 mg/kg/b.wt. for 12 succeeding days and saw a median survival time of 28 days with improved health. No mouse was completely cured. However, those mice treated with *F. indica* 100 mg/kg/b.wt. for 12 consecutive days did not show any notable changes in activity. It was also observed that the given treatment with

200 mg/kg caused sores and increased irritability in the mice, and three mice out of 10 died due to toxicity.

Previous Gas chromatography–mass spectrometry (GC-MS) study shown that *F. indica* possesses intriguing pharmacological and physiological effects. It was verified that the presence of six compounds, Octadecanoic acid, 4-hydroxybutyl ester, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethylester and Hexadecanoic acid1-[(2-aminoethoxy) hydroxyphosphinyl]oxy] methyl]-1,2-ethanediyl ester [21].

4.2. DPPH radical scavenging activity

The free radical scavenging activity of the *F. indica* extract was highest at a concentration of 100 μg/ml (90.71 ± 0.87), whereas at the same concentration, the standard BHT was 93.31 ± 0.36%. The IC_{50} of *F. indica* of was 8.3 ± 0.12, and that of BHT was 6.32 ± 0.24 μg/ml.

4.3. Effect of F. indica on the kidney homogenate SOD, GST, and GSH-Px activities

The SOD, GST, and GSH-Px levels in kidney homogenates presented in Table 1 revealed a significant decrease of these parameter levels in group III compared with group I. The outcome data show that the 120 mg/kg/b.wt dose of *F. indica* given to group V. i.p. provoked a significant increase in the level of SOD, GST, and GSH-Px enzymes activities respectively; compared with the intoxicated group III ($P < 0.001$). These results suggest that the treatment with *F. indica* at a selected dose resulted in an increase of ROS removal in mouse kidneys. No significant changes were observed in the antioxidant activities in the saline-treated group IV. Moreover, group II, injected with *F. indica,* did not show any signs of irritability, weight loss, or sores, and no death was recorded throughout the experiments. Additionally, treated group V revealed a significant improvement in parameters assessed through mice serum and kidney tissue homogenate compared with the untreated group III.

4.4. Effect of F. indica on BUN and creatinine serum levels in different groups of mice

Serum biochemical findings of all groups are shown in Table 2. The mice BUN and creatinine measurements were performed on separately serum samples withdrawn from each mice/group used in the experiments. The levels of BUN and creatinine for group III and group IV were higher than those for control group I ($P < 0.001$). The serum BUN was 50.01 ± 0.62 mg/dl in group III, while that of control group I was 24.71 ± 0.43 mg/dl. Group V, treated with *F. indica*, showed improvement and reduction of serum BUN, to 26.80 ± 0.41 mg/dl ($P < 0.001$). The creatinine level showed an overestimation of the serum levels up to 2.34 ± 0.08 mg/dl in group III compared with group I (0.82 ± 0.09 mg/dl). Group V showed a significant reduction in creatinine levels, to 0.95 ± 0.05 mg/dl ($P < 0.001$).

4.5. Effect of F. indica on serum interleukin-6

The administration of *F. indica* decreased the level of IL-6 in serum to 22.8 ± .6 pg/ml in group V, while the level of group III was 41.4 ± .9 pg/ml. Furthermore, group III showed a significant increase of IL-6 levels compared with group I, 19.3 ± 1.2 pg/ml ($P < 0.001$) (Table 3). A positive correlation was found between the pro-inflammatory cytokine IL-6 and the parameters, including SOD, GST, GSH-Px, BUN, and creatinine levels (Figure 1).

4.6. Effect of F. indica on immunohistochemical results (light microscopic detection of caspase-3)

In regard to caspase-3 immunohistochemical expression, the stained sections of the kidneys of control group I and *F. indica*-treated mice group II showed a normal appearance, with a negative expression of caspase-3 in the renal tubules and interstitial cells (Figure 2A and B). In the POD-treated group (group III), the kidney showed a positive diffuse strong expression of caspase-3 in dilated tubules; glomerulus loop of Henle and interstitial cells (Figure 2C). In the POD-treated group with saline (group IV), the kidney showed a positive diffuse strong expression of caspase-3 in dilated tubules, glomerulus loop of Henle and interstitial cells (Figure 2D). In group V, treated with POD and *F. indica*, the kidney showed a weakly positive patchy (focal) caspase-3 immunostaining in kidney tubules and interstitial cells (Figure 2E). There was a statistically significant difference between groups and quantified data of immunohistochemical results in different mice groups were shown in (Table 4).

Table 3. Effect of *F. indica* on serum Il-6 level (pg/ml), in mice different groups.

	Group I	Group II	Group III	Group IV	Group V
Il-6 (pg/ml)	19.3 ± 1.2	21.1 ± .6*	41.4 ± .9*#	41.1 ± 1.2*#	22.8 ± .6*#¶€

Data expressed as mean ± SD, significance <0.05.
*: significance vs Group I., #: significance vs Group II., ¶: significance vs Group III., €: significance vs Group IV.

Table 1. Effect of *F. indica* on SOD, GST and GSH-Px levels (U/g) wet tissue in mice different groups.

	Group I	Group II	Group III	Group IV	Group V
SOD (U/g)	56.42 ± .64	54.36 ± .96*	27.52 ± .78*#	28.22 ± .71*#	48.36 ± .76*#¶€
GST (U/g)	63.16 ± 1.26	60.89 ± 1.74*	30.42 ± 1.04*#	30.74 ± 1.01*#	58.92 ± .78*#¶€
GSH-Px (U/g)	80.04 ± .79	79.35 ± 1.31	43.42 ± .78*#	43.63 ± 1.02*#	72.23 ± 1.22*#¶€

Data expressed as mean ± SD, significance <0.05.
*: significance vs Group I., #: significance vs Group II., ¶: significance vs Group III., €: significance vs Group IV.

Table 2. Effect of *F. indica* on serum BUN and creatinine levels (mg/dl) in mice different groups.

	Group I	Group II	Group III	Group IV	Group V
BUN (mg/dl)	24.71 ± .43	24.89 ± .38	50.01 ± .62*#	50.56 ± .89*#	26.80 ± .41*#¶€
Creatinine (mg/dl)	0.82 ± .09	.84 ± .05	2.34 ± .08*#	2.34 ± .07*#	.95 ± .05*#¶€

Data expressed as mean ± SD, significance <0.05.
*: significance vs Group I., #: significance vs Group II., ¶: significance vs Group III., €: significance vs Group IV.

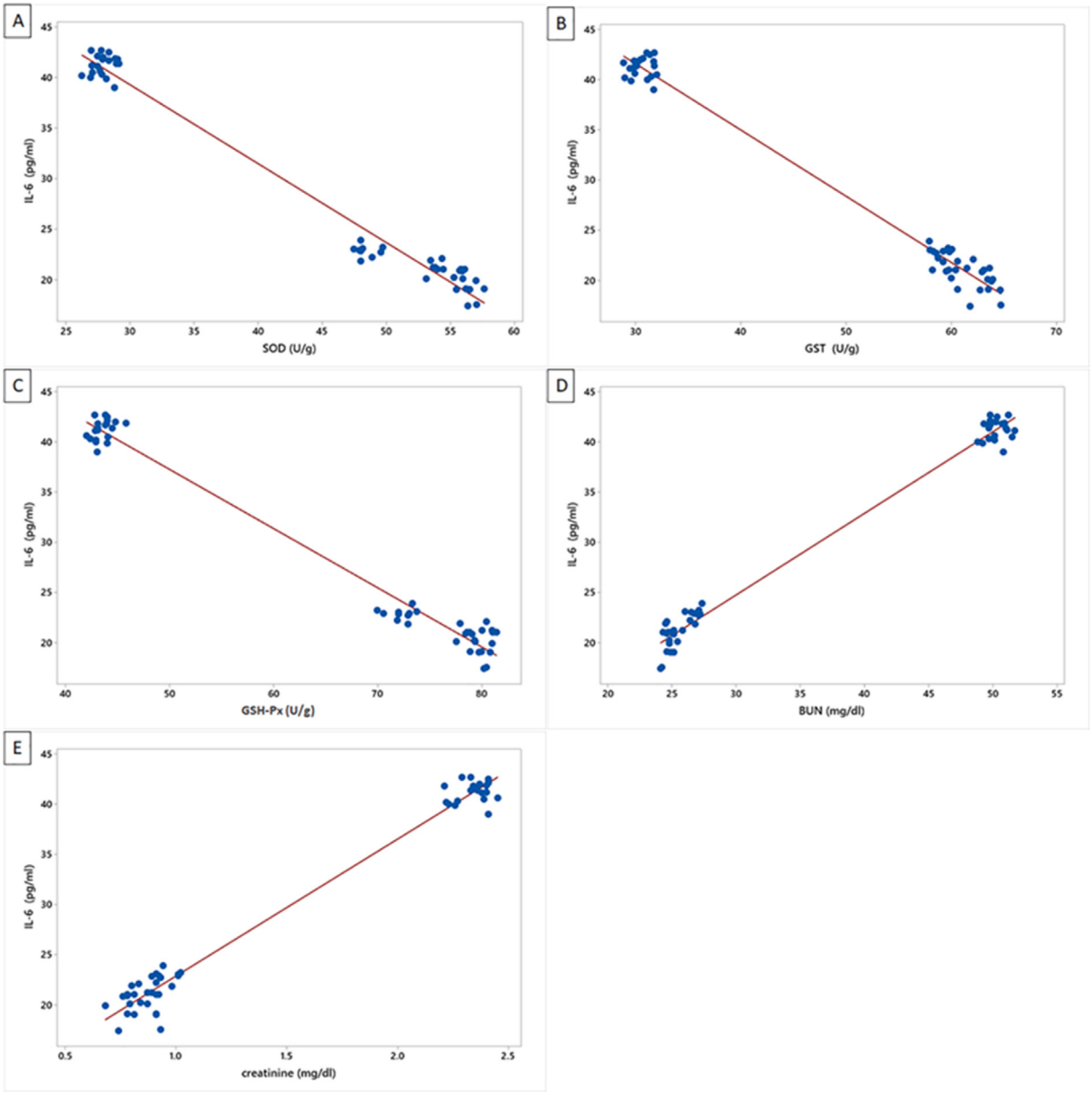

Figure 1. Correlations of IL-6 with (A) Il-6 (pg/ml) vs SOD (U/g), (B) Il-6 (pg/ml) vs GST (U/g), (C) Il-6 (pg/ml) vs GSH-Px (U/g), (D) Il-6 (pg/ml) vs BUN (mg/dl), (E) Il-6 (pg/ml) vs creatinine (mg/dl).

4.7. Effect of F. indica on histopathological results (light microscopic examination of H&E-stained sections)

The microscopic examination of H&E-stained sections of the kidneys for control group I and group II showed normal glomeruli and tubules (Figure 3A and B). In groups III and IV, the kidney showed severely damaged renal tubular epithelial cells and marked congestion. Severe renal interstitial congestion and hemorrhage and coagulative necrosis of renal tubules is shown in (Figure 3C and D). In group V, the kidneys presented a mild hydropic generation of renal tubular epithelial, rarified renal tubular epithelial cells with small pyknotic nuclei, and renal tubular epithelial hyperplasia with regenerative changes (Figure 3E and F). The quantified data of histopathological results in different mice groups were shown in (Table 5). The schematic diagram of the experimental design and different groups classification/mice applied throughout the experiments are shown in (Figure 4).

5. Discussion

The term "nephrotoxicity" refers to a rapid decline in kidney function caused by the toxic effects of drugs and substances. There are several types of nephrotoxicity, and some medicines may have multiple effects on renal function. Renal tubular toxicity, inflammation, glomerular injury, crystal nephropathy, and thrombotic microangiopathy are some of the mechanisms that cause nephrotoxicity [22]. The kidney is a target organ for systemically absorbed chromate, and nephrotoxicity, or total renal shutdown, may be the main cause of death in acute Cr exposure [23].

Since ancient times, a great variety of plants has been used for therapeutic purposes. Most parts of plants have been used as extracts and may possess anti-inflammatory and antioxidant properties related to diseases such as diabetes, atherosclerosis, neurodegenerative conditions, and cancer [24]. *F. indica* is one of a genus of wild flowering plants in the

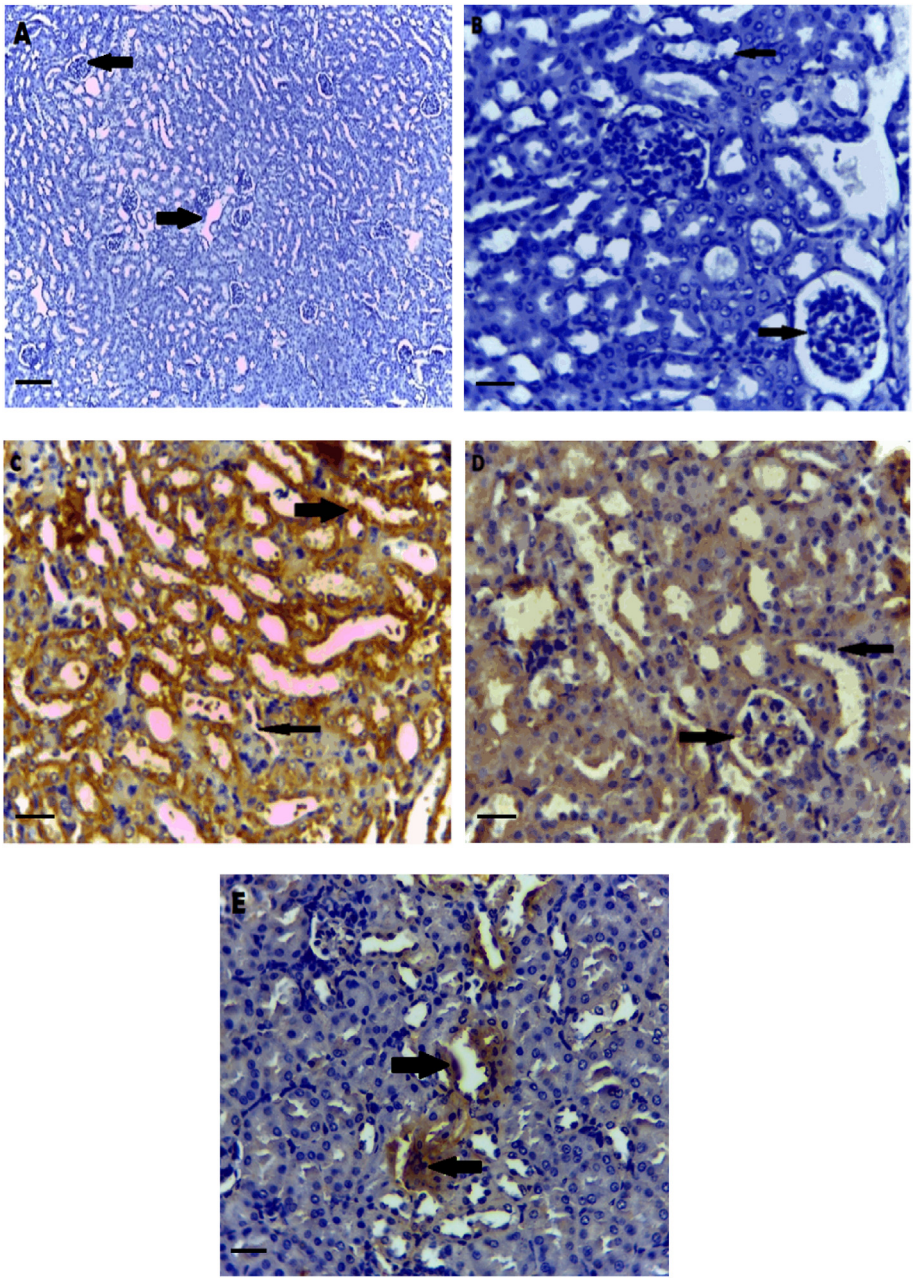

Figure 2. Immunohistochemical staining caspase-3 in kidney. (A, B) section from control kidney and *F. indica* treated kidney (groups I, II) showing negative expression (no immune-reactive cells) (×200). (C, D) section from group III (Chromium-intoxicated) & IV (Chromium-intoxicated and saline) showing diffuse strong expression in dilated tubules, glomerulus and interstitial cells. (×200). (E) section from group V (Chromium-intoxicated treated *F. indica*) showing weak patchy (focal) expression in kidney tubules and interstitial cells (×200).

Table 4. The quantified comparison of immunohistochemical staining in mice different groups.

	Group I	Group II	Group III	Group IV	Group V
Percent area	0 + 0	0 + 0	24.14 ± 4.78*#	22.14 ± 5.65*#	7.23 ± 1.31*#¶

Data expressed as mean ± SD significance <0.05.
*: significance vs Group I., #: significance vs Group II., ¶: significance vs Group III.

caltrop family, *Zygophyllaceae*, which is a rich source of pharmacologically active compounds [25, 26, 27]. However, our outcomes revealed the potent antioxidant potential and scavenging activities of *F. indica*. This data achieved in DPPH evaluation to measure free radical scavenging activities.

In the current study, at 15 mg/kg/b.wt of POD-intoxicated group III, SOD, GST, and GSH-Px showed a significant decrease compared to control group I (Table 1). These results agree with those of Bashandy et al. [28], who showed that daily oral POD treatment for eight weeks in rats caused oxidative stress and a significant reduction in SOD, CAT, and GSH-Px. Additionally, our findings align with those of Kotyk and Iskra [29], who declared that the i.p. action of POD at a dose of 20 mg/kg body weight causes a decrease of GSH-Px content and an increase of the lipid peroxidation process in rat kidneys. Moreover, this study harmonizes with the research of Aboulhoda et al. [26], who stated that the oxidative stress state is induced by POD. This was evidenced by the significant increase in the levels of ROS-generating enzyme NADPH oxidase, increase in lipid peroxidation product MDA, and decrease in antioxidant enzymes GSH-Px, SOD, and Trx reductase in the POD-treated group.

Controversially, our findings disagree with those of Momo et al. [30], whose results showed that treatment with POD induced oxidative stress, evidenced by a significant decrease in CAT and peroxidase activities and an increase in MDA and SOD as compared to control values. These results might be in response to increased oxidative stress and lipid peroxidation brought about by the presence of a heavy metal.

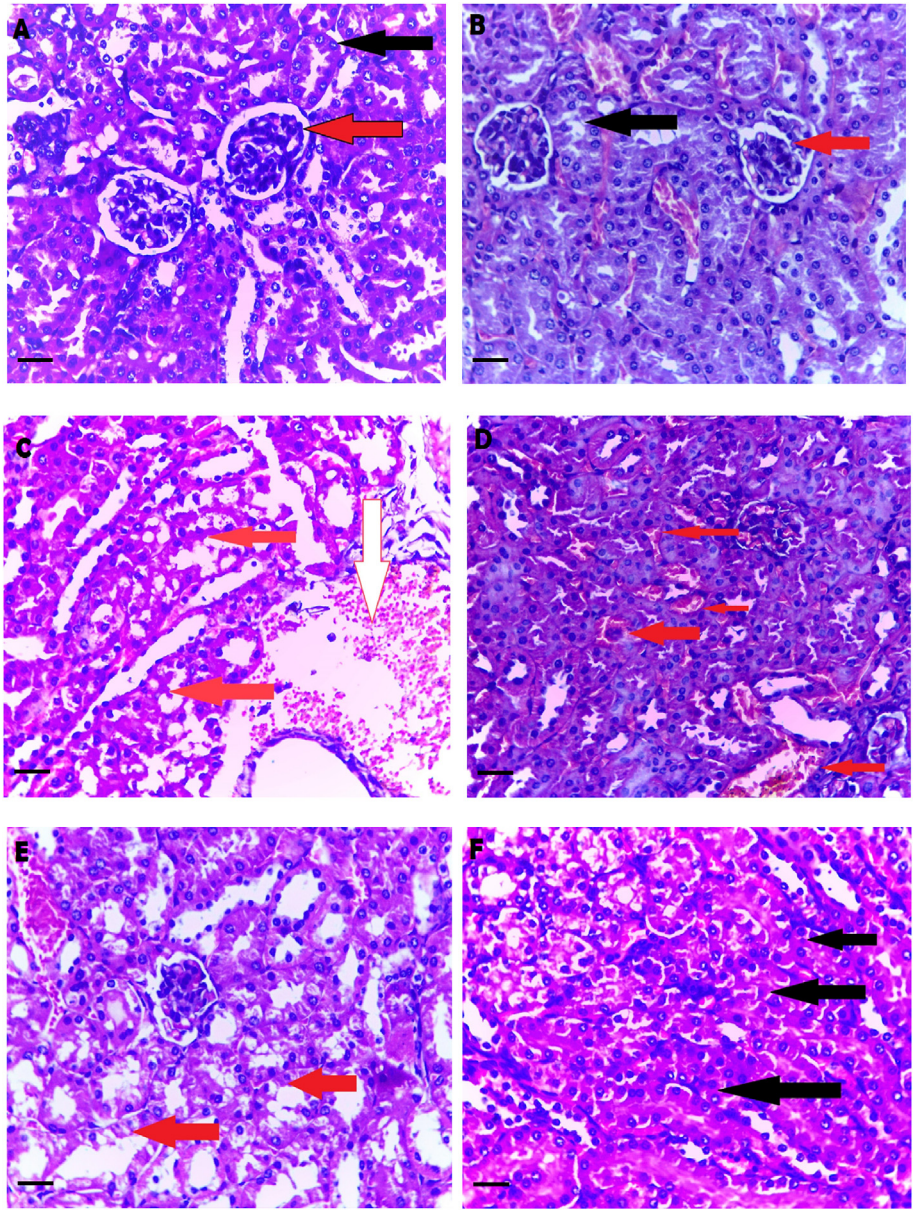

Figure 3. Histopathological examination of the kidney showing (A, B) section from control kidney and *F. indica* treated kidney (group I & II) showing normal glomeruli (red arrow) and tubules (black arrow) (H&E ×200). (c) section from group III (Chromium-intoxicated) showing severely damaged renal tubular epithelial cells (red arrow) and marked congestion (white arrow) (H&E ×200). (D) section from group IV (Chromium-intoxicated and saline) showing severely renal severe interstitial congestion and hemorrhage (red arrows) (H&E ×200). (E) section from group V showing mild hydropic generation of renal tubular epithelial (red arrow) (H&E ×200). (F) section from group V (Chromium-intoxicated treated *F. indica*) showing renal tubular epithelial hyperplasia with regenerative changes (black arrows) (H&E ×200).

Table 5. The quantified comparison of histopathological staining in mice different groups.

	Group I	Group II	Group III	Group IV	Group V
Percent area of renal tubules (%)	13.60 ± 3.22	12.56 ± 2.87	52.11 ± 11.46*#	49.32 ± 10.21*#	16.79 ± 4.38¶€
Perimeter of Renal Glomerulus (pixels)	143818.3 ± 28764.85	137349.6 ± 23470.42	52078.1 ± 10416.37*#	48128.1 ± 9627.41*#	94344.9 ± 18869.44*#¶€
Area of Renal Glomerulus (pixels)	1514.7 ± 302.93	1333.4 ± 266.76	922.1 ± 104.4*#	910.7 ± 97.6*#	1230.8 ± 96.90¶€

Data expressed as mean ± SD, significance <0.05.
*: significance vs Group I., #: significance vs Group II., ¶: significance vs Group III.

The results of this study showed that *F. indica* at a dose of 120 mg/kg/b.wt in group V provoked a significant increase in the level of SOD, GST, and GSH-Px enzyme activities, respectively; when compared with the intoxicated group III (Table 1). These results suggest that treatment with *F. indica* results in increased ROS removal in mouse kidneys. These results harmonize with those of Azam et al., who declared that the assessment of DNA damage also suggests that *F. indica* plays a key role in the restoration of normal tissue structure and function by reducing DNA damage. This could be due to the plant's antioxidant activity against oxidative DNA damage. Several prior studies have stated that antioxidant activities of several phytomedicinal plant extracts and their constituents inhibit oxidative DNA degradation [27]. The extract of *F. indica* effectively reduced free radical levels by mechanisms involving increased expression of Cu-Zn SOD, decreased expression of iNOS, and simultaneous scavenging of the free radicals, such as O_2, OH, NO, and ONOO [31].

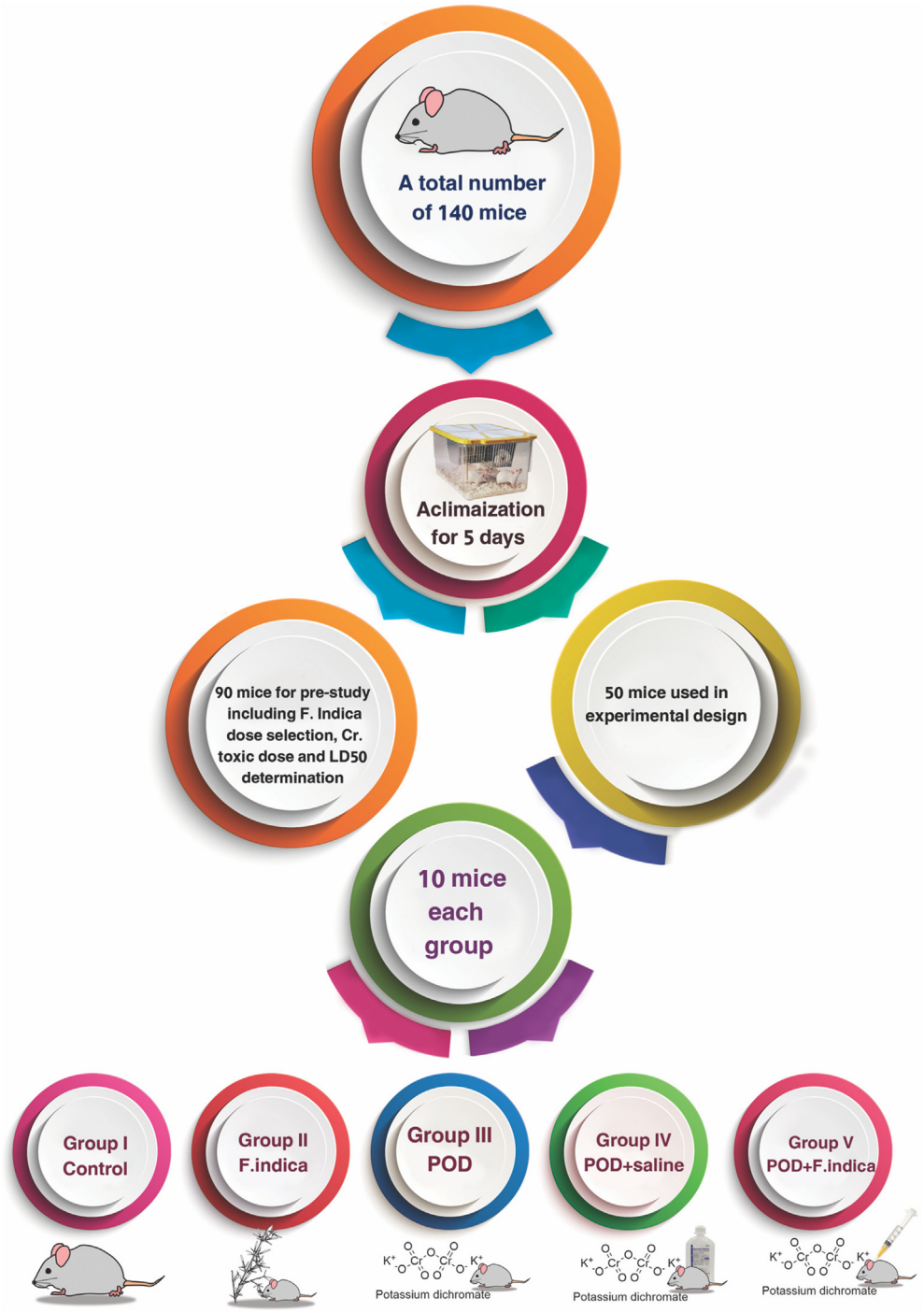

Figure 4. Schematic diagram of the experimental design.

Moreover, the flavonoid content of *F. indica* exceeds that of the other plants; Calotropis procera, Zygophylum hamiense Schweinf and Salsola imbricata Forssk, 10- to 30-fold. Thus, it was found that *F. indica* possesses protective effects by preventing the induction of oxidative stress and enhancing the antioxidant defenses involving CAT, GSH-Px, and SOD enzymes [32].

Our results also revealed a significant and abnormal increase in total BUN and creatinine levels in mice intoxicated with POD (group III) over control groups I and group II. These results align with those of Hassan et al. [23], who stated that a single-dose POD injection for 14 days induced acute kidney injury in rats, as evidenced by significant renal function test alterations. When compared with other groups, there was a statistically significant increase in levels of urea and creatinine in the POD-treated group, which may be attributed to a loss of functional integrity in the kidney and renal tubule distortion as a consequence of Cr administration [33]. Our study also agrees with that of Salama and Elmalt, who confirmed that, when compared to the normal control group, serum creatinine and BUN levels were elevated by 76% and 87%, respectively, after induction of acute renal damage by POD [34].

According to our findings, group V (treated with *F. indica*) revealed a highly significant level of improvement and reduction of BUN and creatinine compared to the high level of these two substances seen in the intoxicated group III (Table 2). This was in accordance with Kamran et al. [35], who showed improvement in kidney function with *Fagonia cretica*

(*Zygophyllaceae*). Serum creatinine and urea were reduced significantly through selected remedies. It was found that the extract of *Tribulus terrestris* (TT) (*Zygophyllaceae*) showed a dose-dependent effect and a highly significant reduction in the urea and creatinine levels [36]. Furthermore, plasma creatinine and urea nitrogen concentrations in the TT group significantly decreased in comparison with the acute kidney injury group [37]. Additionally, Kilany et al. [38] discussed TT pre-administration at a dose of 200 mg/kg that significantly decreases serum renal products, BUN, creatinine, and uric acid. The plant extracts mentioned are attributed to the same *F. indica* genus family *Zygophyllaceae*.

In the current study, IL-6 level increased significantly with the doses of POD administered (Table 3). These data are in harmony with the results of Balabekova et al. [39], who showed that prolonged exposure to Cr is associated with chronic inflammation. Our results showed that the inflammatory process was characterized by up-regulated production of IL-6 in the initial phase. Furthermore, our findings revealed that *F. indica* treatment regulates the level of IL-6. The administration of *F. indica* significantly decreased the concentration of IL-6 in treated group V compared to group III. These results are in accordance with Ajuwon et al. [40], who showed that acute exposure to toxicants leads to an upregulation of pro-inflammatory markers within a few hours. IL-6 production and secretion are induced under pathological situations, for instance when cells are stimulated by interleukin-1 [41].

Moreover, the present data revealed a positive diffuse strong expression of caspase-3 in dilated tubules, glomerulus loop of Henle, and interstitial cells. This is in agreement with Ahmed et al. [42], who described the mean values of the area percentage of immune reaction for caspase-3 in the renal interstitium and discussed the statistically significant increase in the mean area percentage of caspase-3 reaction. According to our findings, *F. indica*-treated group V showed a weakly positive patchy (focal) caspase-3 immunostaining in kidney tubules and interstitial cells. This finding is in accordance with Kilany et al. [38], who showed that *Tribulus terrestris* -treated gentamicin nephrotoxicated tubular epithelial cells had mild focal positive expression of caspase-3, which was accentuated by cytoplasmic brown DAB staining.

Furthermore, group III exhibited severe damage of renal tubular epithelial cells and marked congestion, severe renal interstitial congestion and hemorrhage, and coagulative necrosis of renal tubules. Stained sections from group III revealed massive destructive damage to the renal cortex. Fatty cell infiltration and areas of hemorrhage were seen within a thick renal capsule. These results were in accordance with De-Vries et al. [43], who claimed that there was a link between intrarenal lipid component accumulation and renal damage in both *in vivo* and *in vitro* investigations. Furthermore, Shanmugam et al. [44] revealed fatty infiltration in diabetic rat renal tissue, which they attributed to oxidative stress.

Several studies in line with our findings have clarified that Cr-induced renal dysfunction could be due to Cr renal tubular damage and cell debris. Salama and Elmalt [34], through an examination of the renal histopathology of the POD group, showed that Bowman's space in the renal corpuscle showed hypercellularity, pyknosis, and build-up of hyaline materials, while the proximal convoluted tubules showed dilatation, degeneration, and necrosis of the epithelial lining cells as well as hyaline casts in the lumen of other tubules.

Our outcomes revealed that *F. indica*-treated group V showed, on histopathology examination of the kidneys, expression of mild hydropic generation of renal tubular epithelial, rarified renal tubular epithelial cells with small pyknotic nuclei, and renal tubular epithelial hyperplasia with regenerative changes. These histopathological changes were achieved and, in accordance with Karman et al., the kidney was treated with *Fagonia cretica* extract; one of the *Fagonia* family showed less damage to the Bowman's capsules and preservation of cellular structures of nephron clear [35]. Additionally, kidney cells of rats treated with CCl4 and TT showed fewer signs of toxicity. Most of the cells were protected and appeared normal, and few cells were damaged [36]. Similarly, according to Najafi et al., the kidney damage partially improved in the TT-treated group when compared with the toxic group [37].

Distinctive studies regarding occurrence, phytochemistry, traditional uses, biochemical constituents and therapeutic uses of *F. indica* have been done and clearly documented about the presence of flavonoids, saponins, tannins, glycosides, pectin and alkaloids. These confirmed by different researchers. The *F. indica* is a very imperative medicinal plant because it has different therapeutic and traditional uses such as antidiabetic, anticancer, anti-leishmanial, antipyretic, anti-inflammatory, laxative, gastroprotective, hepatoprotective and antioxidant effects. Moreover, *F. indica* extract also found to demonstrate antiadhesion and antibiofilm agents against MDR bacteria. Biofilm formation is considered to be a major public health worries, since more than 80% of microbial infections of human beings comprise biofilm formers. Further *in vitro* and *in vivo* studies should be carried out to find the exact mechanisms of actions for better scientific evidence for future applied on human clinical trials [45,46].

Consequently, the results gained from this research work advice to reduce exposure to chromium compounds in groundwater and it should have water analysis by an accredited laboratory before used for drinking, cooking, bathing, washing, and gardening. Also, avoid smoking in enclosed spaces to limit exposure to chromium. Moreover, chromium compounds marketed in dietary supplements as beneficial to health as weight loss supplements. These claims are not supported yet by scientist.

Furthermore, this research work conducted for the first time, in quantitative study of *F. indica* effects on nephrotoxicity throughout different biochemical parameters as well the prominence of these parameters when correlate with pro-inflammatory cytokines and antioxidant activity of *F. Indica* on animal model to clear the importance and medical application of such medicinal plant for future pharmacological purpose uses. Moreover, to preserve traditional knowledge, and also to motivate the researchers against the vanishing wealth of traditional knowledge of the herbal medicine like *F. Indica*. The vast use of medicinal plants reports shows the significance of traditional herbal preparations in health care, interest in medicinal plant use. The indigenous use of plants needs conservational strategies and further investigation for better utilization of natural resources after official agreement, medical and research approvals.

6. Conclusion

Our findings reveal that *F. indica* has an effective role in protecting against Cr-induced nephrotoxicity. The results show promise and signify that *F. indica* extract can play an important role in monitoring oxidative stress, improving the nature of renal biochemical parameters and regulating pro-inflammatory cytokine and tissue intelligibility. Such changes may be attributed to the antioxidant and anti-inflammatory activity of *F. indica*, which is a useful herbal medicine and may be applied in future in the treatment of different diseases. These conclusions should be taken into consideration when debating the exploitation and effectiveness of *F. indica*. It would be interesting for further studies to explore *F. indica's* biological effects and its mode of action at the cellular and molecular level in greater detail.

Declarations

Author contribution statement

Abdullah Almilaibary: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Eman A. A. Abdallah: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

El-Refaei MF: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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References

[1] N. Shehab, A. Shahiwala, I. Benouared, R. Khan, Preparation and antihepatotoxicity activity of Fagonia indica extract and its solid dispersion formulation, Pak. J. Pharm. Sci. 33 (3) (2020) 1025–1032.
[2] B. Abbasi, T. Khan, R. Khurshid, M. Nadeem, S. Drouet, C. Hano, UV-C mediated accumulation of pharmacologically significant phytochemicals under light regimes in in vitro culture of Fagonia indica (L.), Sci. Rep. 11 (2021) 679.
[3] E. Abdallah, M. El-Refaei, Caffeic acid phenethyl ester mitigates infertility: a crucial role of metalloproteinase and angiogenic factor in cadmium-induced testicular damage, J. Biochem. Mol. Toxicol. (2021), e22960.
[4] L.E. Padgett, K.A. Broniowska, P.A. Hansen, J.A. Corbett, H.M. Tse, The role of reactive oxygen species and proinflammatory cytokines in type 1 diabetes pathogenesis Ann, N.Y. Acad. Sci. 1281 (2013) 16–35.
[5] A. Wakeel, M. Xu, Y. Gan, Chromium-induced reactive oxygen species accumulation by altering the enzymatic antioxidant system and associated cytotoxic, genotoxic, ultrastructural, and photosynthetic changes in plants, Int. J. Mol. Sci. 21 (2020) 728.
[6] M. Manjeera, I. Ramya, S. Sathyendra, Occupation-related chromium toxicity a rare cause of renal failure and rhabdomyolysis, Indian J. Occup. Environ. Med. 20 (2020) 150–152.
[7] H. Su, C. Lei, C. Zhang, Interleukin-6 signaling pathway and its role in kidney disease: an update, Front. Immunol. 8 (2017) 405.
[8] R. Hegazy, A. Salama, D. Mansour, A. Hassan, Renoprotective effect of lactoferrin against chromium-induced acute kidney injury in rats: involvement of IL-18 and IGF-1 inhibition, PLoS One 11 (3) (2016), e0151486.
[9] R. Nagappan, Evaluation of aqueous and ethanol extract of bioactive medicinal plant, Cassia didymobotrya (Fresenius) Irwin & Barneby against immature stages of filarial vector, Culex quinquefasciatus Say (Diptera: Culicidae), Asian Pac. J. Trop. Biomed. 9 (2) (2012) 707–711.
[10] M.S. Blois, Antioxidant determinations by the use of a stable free radical, Nature 181 (1958) 1199–1200.
[11] C. Desmarchelier, M.J.N. Bermudez, J. Coussio, G. Ciccia, A. Boveris, Antioxidant and prooxidant activities in aqueous extract of Argentine plants, Int. J. Pharmacogn. 35 (1997) 116–120.
[12] D. Lorke, A new approach to practical acute toxicity testing, Arch. Toxicol. 4 (54) (1983) 275–287.
[13] M. Al Yahya, A. Eltaher, H. Kamal ElDin, Fagonia Bruguieri Freez Dried Extract as Anti-allergic Treatment, World Intellectual Property Organization, 2007.
[14] N. Shehab, A. Mahdy, S. Khan, S. Noureddin, Chemical constituents and biological activities of Fagonia indica burm F, Res. J. Med. Plant 5 (5) (2011) 531–546.
[15] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[16] H.P. Misra, I. Fridovich, The role of superoxide anion in the autoxidation of epinephrine and simple assay for superoxide dismutase, J. Biol. Chem. 247 (1972) 3170–3175.
[17] W.H. Habig, M.J. Pabst, W.B. Jakoby, Glutathione S-transferases. The first enzymatic step in mercapturic acid formation, J. Biol. Chem. 249 (1974) 7130–7139.
[18] M. Tamura, N. Oshino, B. Chance, Some characteristics of hydrogen- and alkylhydroperoxides metabolizing systems in cardiac tissue, J. Biochem. 92 (1982) 1019–1031.
[19] M. El-Naaa, M. El-Refaei, W. Nasif, S. Abduljewad, A. El-Brairya, M. El-Readic, In-vivo antioxidant and anti-inflammatory activity of,rosiglitazone, a peroxisome proliferator-activated receptor-gamma (PPAR-γ) agonists in animal model of bronchial asthma, J. Pharm. Pharmacol. 67 (2015) 1421–1430.
[20] B. Omran, E. Abdallah, M. Abdelwahab, Study of probable toxic effects of bisphenol A & the protective role of vitamin E on testes and prostate of adult male albino rats, Ain Shams J. Forens. Med. Clin. Toxicol 29 (2017) 7–19.
[21] A. Rehman, F. Talib, T. Aftab, FTIR, HPLC, GC-MS analysis and investigation of hypoglycemic effects of leaves extracts of Fagonia indica, Pharmacogn. Commn. 11 (2) (2021) 109–118.
[22] M. Al-Naimi, H. Rasheed, N. Hussien, H. Al-Kuraishy, A. Al-Gareeb, Role and significance of renal biomarkers in the early detection of acute renal injury, Nephrotoxicity 10 (3) (2019) 95–99.
[23] M. Hassan, D. Zaghloul, M. Mahmoud, Z. Moaty, R. Toghan, Protective effect of lactoferrin against chromium induced adverse renal changes in rats: oxidative stress theory, Am. J. Biochem. Biotechnol. 17 (2) (2021) 181–191.
[24] M. Yoldi, Anti-inflammatory and antioxidant properties of plant extracts, Antioxidants 10 (2021) 921.
[25] K. Ali, H. Khan, Fagonia indica; A review on chemical constituents, traditional uses and pharmacological activities, Curr. Pharm. Des 27 (22) (2021) 2648–2660.
[26] B. Aboulhoda, S. Saad El-Din, M. Khalifa, S. Arsanyos, A. Motawie, M. Sedeek, G. Abdelfattah, W. Abdelgalil, Histological, immunohistochemical, and molecular investigation on the hepatotoxic effect of POD and the ameliorating role of Persea americana mill pulp extract, Microsc. Res. Tech. (2021) 1–17.
[27] F. Azam, N. Sheikh, G. Ali, A. Tayyeb, Fagonia indica repairs hepatic damage through expression regulation of toll-like receptors in a liver injury model, J. Immunol. Res 12 (2018), 7967135.
[28] S. Bashandy, H. Ebaid, J. Al-Tamimi, A. Omar, A. Farid, E. Omara, I. Alhazza, Melatonin alleviated potassium dichromate-induced oxidative stress and reprotoxicity in male rats, BioMed Res. Int. 12 (2021), 3565360.
[29] B. Kotyk, R. Iskra, Effects of ethylthiosulfanyalate and chromium (VI) on the state of glutathione antioxidant system and oxidative stress marker content in the rat kidney, Curr. Appl. Sci. Technol 21 (4) (2021).
[30] C. Momo, M. Pascal, N. Pasima, Effects of crassocephalum bauchiense ethanolic extract on reproductive parameters in rabbit does exposed to potassium dichromate, Am. J. Anim. Vet. Sci. 16 (2) (2021) 151–161.
[31] P. Anil, B. Nikhil, G. Manoj, N. Prakash, Phytochemicals and biological activities of Fagonia indica, Int. Res. J. Pharm. 3 (6) (2012).
[32] N. Shehabn, E. Abu-Gharbieh, F. Bayoumi, Impact of phenolic composition on hepatoprotective and antioxidant effects of four desert medicinal plants, BMC Compl. Alternative Med. 15 (2015) 401.
[33] C. Venter, H.M. Oberholzer, F.R. Cummings, M.J. Bester, Effects of metals cadmium and chromium alone and in combination on the liver and kidney tissue of male Spraque-Dawley rats: an ultrastructural and -energy-loss spectroscopy investigation, Microsc. Res. Tech. 80 (8) (2017) 878–888.
[34] A. Salamaa, H. Elmalt, Aescin ameliorates acute kidney injury induced by POD in rat:involvement of TLR 4/TNF-α pathway, Egypt, J. Chem. 64 (4) (2021) 2067–2074.
[35] S.H. Kamran, R.M. Shoaib, M. Ahmad, S. Ishtiaq, R. Anwar, Antidiabetic and renoprotective effect of Fagonia cretica L. methanolic extract and Citrus paradise Macfad juice in alloxan induced diabetic rabbits, J. Pharm. Pharmacog. Res 5 (2017) 365–380.
[36] M. Abdel-Kader, A. Al-Qutaym, A. Saeedan, A. Hamad, M. Alkharfy, Nephroprotective and hepatoprotective effects of Tribulus terrestris L. growing in Saudi Arabia, J. Pharm. Pharmacog. Res 4 (4) (2016) 144–152.
[37] H. Najafi, R. Firouzifar, O. Shafaat, S. Ashtiyani, N. Hosseini, Protective effects of Tribulus terrestris L extract against acute kidney injury induced by reperfusion injury in rats, Iran. J. Kidney Dis 8 (4) (2014).
[38] O. Kilany, A. El-Beltagy, H. Mohammad, Protective effects of Tribulus terrestris against gentamicin mediated nephrotoxicity, oxidative damage and apoptosis in male rats/Egypt, Acad. J. Biolog. Sci. 12 (1) (2020) 41–58.
[39] M. Balabekova, Y. Ostapchuk, Y. Perfilyeva, Oral administration of ammonium metavanadate and POD distorts the inflammatory reaction induced by turpentine oil injection in male rats, Drug Chem. Toxicol. (2021) 1525–6014.
[40] J.L. Ajuwon, M. Oguntibeju, Amelioration of lipopolysaccharide-induced liver injury by aqueous rooibos (Aspalathus linearis) extract via inhibition of pro-inflammatory cytokines and oxidative stress, BMC Compl. Alternative Med. 14 (1) (2014) 392.
[41] D. Schmidt-Arras, S. Rose-John, IL-6 pathway in the liver: from physiopathology to therapy, J. Hepatol. 64 (6) (2016) 1403–1415.
[42] A.O. Ahmed, H.E. Mokhtar, H.O. Helmy, S.S. Abd el-fatah, Structural changes induced by POD in renal cortex of adult male albino rats and the possible protective role of selenium, Med. J. Cairo Univ. 87 (1) (2019).
[43] A.P. De Vries, P. Ruggenenti, X. Ruan, M. Praga, J. Cruzado, I. Bajema, M. Abbate, Fatty kidney, Emerging role of ectopic lipid in obesityrelated renal disease, Lancet Diabet. Endocrinol. 2 (5) (2014) 417–426.
[44] K.R. Shanmugam, C.H. Ramakrishna, K. Mallikarjuna, K.S. Reddy, Perturbation in kidney lipid metabolic profiles in diabetic rats wit reference to alcoholic oxidative stress, Indian J. Nephrol. 19 (3) (2009) 101–106.
[45] T. Khan, M.A.A. Khan, P. Nisar, S.A. Jan, S. Afridi, Z.K. Shinwari, Anticancer plants: a review of the active phytochemicals, applications in animal models, and regulatory aspects, Biomolecules 10 (1) (2020) 47.
[46] N. Aslam, S. Hayat, T. Ali, M. Waseem, M.H. Siddique, M. Afzal, A. Muzammil, G. Naz, A. Sarwar, S. Muzammil, Antiadhesion and antibiofilm potential of Fagonia indica from Cholistan desert against clinical multidrug resistant bacteria, Braz. J. Biol. 28 (2021), e239991.