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Abatement by *Alhagi maurorum* of lead-induced nephrotoxicity in rats: emphasis on Nrf2/HO-1

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Abstract

Background Lead (Pb) is a heavy metal with extreme toxicity and numerous industrial uses. It produces nephrotoxicity with notable changes in renal architecture and function. The *Alhagi maurorum* (AM) belongs to the family *Fabaceae* and is native to the Middle East area. In the present study, the potential nephroprotective effects of AM in Pb-intoxicated rats were evaluated.

Results AM methanolic extract was standardized in reference to its main phenolic compounds orientin, rutin, and quercetin. Acute oral toxicity studies indicated that the extract is safe for animals. Rats were allocated into five groups and treated for 28 days as follows: control, AM (200 mg/kg, orally), Pb (15 mg/kg, orally), Pb + AM (100 mg/kg), and Pb + AM (200 mg/kg). Pb administration markedly increased serum cystatin C, urea, creatinine levels, and urinary NAG. Pb also caused renal histopathological alterations. However, AM o-treatment ameliorated such pathological changes. In addition, AM treatment prevented Pb-induced accumulation of malondialdehyde (MDA), attenuated glutathione (GSH) depletion, and catalase (CAT) and superoxide dismutase (SOD) exhaustion. AM guarded against Pb-induced enhanced the protein expression of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), cyclooxygenase-2 (COX-2), and nuclear factor-kappa B (NF-κB). Further, AM significantly prevented Pb-induced upregulation of mRNA levels of Bax and downregulation of Bcl-2. These effects were associated with increased levels of heme oxygenase-1 (HO-1) and nuclear factor erythroid 2-related factor 2 (Nrf2) in renal tissue.

Conclusion AM guards against Pb-induced nephrotoxicity in rats that involve, at least partly, the antioxidant, anti-inflammatory, and anti-apoptotic activities as well as the modulation of HO-1 and Nrf2 expression.

Keywords Lead, *Alhagi maurorum*, Nephrotoxicity, HO-1, Nrf2

Background

Lead (Pb) is an extremely toxic metal which is frequently utilized in several industries [1]. Pb is non-decomposable and stays for long durations and is found at a marked level in ecological and biological systems [2]. It is immensely toxic to various body systems including the nervous, hematopoietic, hepatic, reproductive systems, and kidneys [3]. Exposure to Pb induces damage to renal tubules and renal failure, and chronic lead nephropathy is an outcome of its long-term exposure [4]. Toxicodynamics of renal toxicity by Pb has been previously reviewed [5]. Preclinical studies indicated that oxidative stress, inflammation, and apoptosis mediate Pb nephrotoxicity which can be demonstrated histopathologically as karyomegaly, and apoptosis of proximal tubular cells and renal tubular epithelial cells [6, 7].

Over 35,000 medicinal plant species are utilized globally for medical purposes due to their effectiveness together with almost no unwanted effects compared to conventional therapy [8]. In general, herbal products

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have proven effective in heavy metal detoxification [9]. Also, natural products have been reported to exhibit detoxification via upregulation of phase II and antioxidant enzymes [10]. *Alhagi* is a plant genus rich in pharmacologically active constituents [11]. *Alhagi maurorum* (AM) belongs to the *Fabaceae* family and is a traditional medicine for rheumatic aches, bilharziasis, liver conditions, and different gastrointestinal discomforts. It is utilized as an aphrodisiac, antimicrobial and to treat hemorrhoids and uterine complications [12]. Phytochemically, AM has been reported to be rich in flavonoids [13]. Pharmacologically, AM exhibited antioxidant and anti-inflammatory properties [13, 14]. Furthermore, AM ameliorated cisplatin-induced nephrotoxicity [15]. Therefore, the current study was designed to evaluate the capacity of AM to protect against lead acetate-induced nephrotoxicity in rats.

Methods

Chemicals

Lead acetate (PbAc) was obtained from Merck (Darmstadt, Hesse, Germany). The rest of the utilized reagents or chemicals in the current experiment were of an analytical quality.

Plant material

AM plants were procured from the Al-Qassim region of Saudi Arabia and authenticated by the Department of Biological Science. The aerial parts were dried in the shade. The dried parts were then grounded, and methanol solvent was used to extract the fine powder by means of IKA ULTRA-TURRAX® T 25 (IKA Labortechnik, Stauten, Germany) as per the producer guidelines. The whole process was carried out at room temperature. The distillation of the solvent was performed at 40 °C under decreased pressure to yield a greenish semisolid deposit. The obtained extract was stored at − 80 °C.

Standardization of AM extract

AM extract was standardized according to its main phenolic compounds, namely, orientin, rutin, and quercetin by HPLC technique. The HPLC system consisted of an Agilent 1260 system. ChemStation has been employed to control the HPLC system and data processing. Separation was made on Kromasil 100 C18, 5 µ, 250 × 4.6 mm column (Teknokroma, S. Coop. C. Ltd., Barcelona, Spain), retained at 35 ± 2 °C. DAD; 248, 330, and 360 nm. Loratadine, 20 ng/µL, 248 nm, was used as the internal standard. The program of the HPLC system was fixed to deliver mobile systems as; mobile A: formic acid in water (0.2%) and mobile B: acetonitrile. Gradient elution program: 0–10 min, 5% B; 10–60 min, 5–38% B; 60–61 min, 38%–100% B; 61–65 min, 100% B; 65–66 min, to 5% B; run time 72 min, and flow rate, 0.9 mL/min. A weight of 10 mg quercetin was conveyed to a measuring flask of 10 mL to dissolve in methanol (10 mL). The dilutions of the calibration curve were planned in methanol to obtain the concentrations; 5, 15, 30, 50, and 65 ng/µL with fixed concentration, 100 ng/µL, of the InSt (loratadine). Calibration levels were examined in triplets. Loratadine represented the internal standard at 50 ng/µL final concentration (Rt, 56.89 min). Separately, each reference compound was injected using the scan mode DAD from 190 to 520 nm. The UV–Vis scans of all compounds were conserved and paired with the identified compounds in every sample. The characterization of the unknown flavones was performed by two or three maxima at the UV and visible ranges. Besides, the concentration of the unknown flavanols was determined as quercetin. In a clean, dry test tube, a 10 mg sample was put and mixed with 1 mL methanol. The mixture was vortexed for 30 s, sonicated for 10 min, and passed through a Nylon syringe filter membrane (0.2 u). A 100 µL from the extract solution was blended with 100 µL InSt solution, and a volume of 5 µL was instilled for HPLC-ion trap mass spectrometry-DAD analysis. The DAD data were retrieved at 246, 326, 356, and 372 nm. The study of mass spectral and production data permitted the detection of 10 flavonoids. The profile was illustrated by the manifestation of three major flavonols recognized as orientin (1.18 mg/g), rutin (0.57 mg/g), and quercetin (0.18 mg/g).

Animals

Male Wistar rats (8–10 weeks old and 200 ± 25 g) were procured from the local animal facility. Rats were kept in a regular workroom environment: room temperature (22 ± 2 °C), humidity (50 ± 10%), and 12/12 h of light/dark cycles and placed in polypropylene cages. Rats had free access to the regular food pellets and water. Procedures involving animal handling were approved by local Ethics Committee.

Acute oral toxicity

An acute oral toxicity assay was made using three rats following the OECD guideline No. 423 [16]. Overnight fasted rats received the AM extract, orally at a dose of 2 g/kg and 10 ml/kg volume. The mortality, if any, was observed.

Experimental design

Animals were separated into five groups (n = 6). Group 1 (control) orally administered 0.5% sodium carboxymethyl cellulose (CMC); Group 2 (AM) was orally administered 200 mg/kg of AM suspended in CMC. Group 3 lead (Pb) group was given 15 mg/kg PbAc orally. PbAc dose was selected based on a pilot study. Groups 4 and 5 (Pb + AM

groups) were orally administrated with AM (100 and 200 mg/kg, respectively) and PbAc (15 mg/kg. p.o.). The chosen doses were based on a pilot experiment and are also consistent with those in the literature [14, 17, 18].

Rats in all groups received respective treatments one time every day for 28 successive days. On the 28th day, all rats were transferred to metabolic cages and kept for 24 h, and urine was collected after centrifugation at 3000xg and kept at − 80 °C. On the 29th day, rats were anesthetized using IP injection of ketamine (80 mg/kg), and blood samples were taken via the retro-orbital plexus. Sera were separated and preserved for further biochemical tests. Animals were sacrificed by decapitation, and kidneys were dissected out. The right kidneys were suddenly frozen using liquid nitrogen and kept at − 80 °C for biochemical analyses. The left kidneys were preserved in formalin for the histological and immunohistochemical assessments.

Markers of kidney function

Serum levels of creatinine, urea, and urinary concentration of *N*-acetyl-beta-D-glucosaminidase (NAG) were evaluated using commercial kits (cat # MBS749827, MBS8807933, and MBS702746, respectively, MyBioSource, Inc., San Diego, USA). Serum cystatin C (Cys C) was determined using a commercial kit (Cat # CSB E08385r, CUSABIO, Houston, TX, USA) according to the producer instructions.

Markers of oxidative status

Kidney tissues were homogenized and centrifuged to separate the supernatant on which the markers of oxidative status were assessed**.** The levels of malondialdehyde (MDA) and reduced glutathione (GSH) and the activities of the enzymes superoxide dismutase (SOD), and catalase (CAT) in kidney homogenates were determined spectrophotometrically using commercially available kits (Cat # MBS8807536, MBS265966, MBS036924, and MBS726781, respectively, MyBioSource, Inc., San Diego, USA).

Histopathological examination

Kidneys preserved in formalin were embedded in paraffin, and serial sections were sliced (5 µm thickness) for histopathological examination and stained with hematoxylin and eosin or Sirius red. The stained sections were checked under a light microscope.

Immunohistochemical evaluation of IL-6, COX-2, TNF-α, NF-κB, Nrf2, and HO-1

Tissue sects. (5 µm) were deparaffinized and rehydrated then boiled for 10 min in 0.1 M citrate buffer (pH 6.0). Afterward, for 2 hours, tissues were put in a 5% bovine serum albumin (BSA) solution dissolved in tris-buffered saline (TBS) to be then incubated with the primary antibodies of interleukin-6 (IL-6), cyclooxygenase-2 (COX-2), tumor necrosis factor-alpha (TNF-α), nuclear factor erythroid 2-related factor 2 (Nrf2), heme oxygenase-1 (HO-1) with Catalog # ab9324, ab179800, ab307164, ab313825, and ab189491, respectively (Abcam, Cambridge, UK), and NF-κB p65 (Catalog # sc-8008, Santa Cruz Biotechnology, Dallas, TX, US) at 4 °C for 12 h. Then flushed with TBS and Mouse and the Rabbit Specific HRP/DAB Detection IHC kit (ab64264, Abcam, Cambridge, UK), according to the primary antibody type, were used for the detection of the immune reactions in tissue sections. The image analysis software (ImageJ, 1.46a, NIH, Bethesda, MD, USA) was used for image analysis and quantification of the optical density (OD) with a minimum of three sections (two different fields/section) per group.

Real-time PCR assessment of mRNA expression of Bax and Bcl2

The total RNA content was extracted using TRIzol. In triplicates, by reverse transcription, RNA was converted to cDNA using the Multi scribe RT enzyme kit. The Power SYBR Green PCR Master Mix (Applied Biosystems, CA, USA) was employed to undergo the RT-PCT reaction on a 7500 RT-PCR System (Applied Biosystems, Foster City, CA, USA). Using the thermal cycler, the temperature was set at 95 °C for 4 min, 40 cycles at 95 °C for 10 s, 60 °C for 30 s, and 72 °C for 10 s. The relative fold alteration in the Bax and Bcl2 mRNA was measured relative to the control. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to standardize the fold change in mRNA of the target genes. The used primers used are shown in Table 1.

Table 1 Primer sequence

Name	Accession number	Forward primer	Reverse primer
Bcl2	NM_016993.1	GTACCTGAACCGGCATCTG	GGGGCCATATAGTTCCACAA
Bax	NM_017059.2	GGCTGGACACTGGACTTC	CAGATGGTGAGTGAGGCA
GAPDH	NM_017008.4	GACCCCTTCATTGACCTCAAC	CGCTCCTGGAAGATGGTGATGGG

Statistical analysis

Data are stated as the mean S. Normality of data was confirmed by Kolmogorov–Smirnov test. One-way ANOVA followed by Tukey's test was used to analyze the data and test for significance among different groups. Differences are considered statistically significant at $p < 0.05$.

Results

Acute oral toxicity test

One day after the oral administration of 2000 mg/kg of the AM extract to three rats, the extract did not cause any mortality. The test was repeated at the same dose using three more animals, and the same result was observed. According to OECD guideline No. 423, AM is considered safe and belongs to category 5 with LD50 > 2000 mg/kg.

Markers of kidney function

Our data indicated that administration of lead (15 mg/kg/day) resulted in severe nephrotoxicity as supported by significant elevation of serum creatinine, urea, and cystatin C by 228.69%, 578%, and 533.38% as compared to the control group, respectively (Fig. 1A, 1B, 1C). Also, challenging rats with lead resulted in a significant rise in urinary NAG by 223.80%. However, the co-treatment with Alhagi 100 and 200 mg/kg prevented the rise in serum levels of creatinine (41.47% and 56.57%), urea (31.53%, 57%), and cystatin C (36.22% and 68.42%) as compared to lead group, respectively. Also, the urinary NAG concentration was significantly lowered by the administration of the two doses of AM by 30.72% and 53.93% as compared to animals in the lead group, respectively (Fig. 1D).

Histopathological examination

Microscopic assessment of the H and E-stained kidney tissues from the control and AM-alone groups displayed the usual architecture of both renal cortex and medulla (Fig. 2, upper panel). On the contrary, kidneys from animals in the Pb group showed widespread severe necrosis of the renal tubules in both medulla and cortex. The renal tubular epithelium suffered from noticeable injuries with deeply eosinophilic cytoplasm and dark pyknotic nuclei. The interstitial tissue showed abundant edema with a variable number of inflammatory cell infiltration. Examination of renal sections from the Pb + AM (100 mg/kg) group showed multifocal

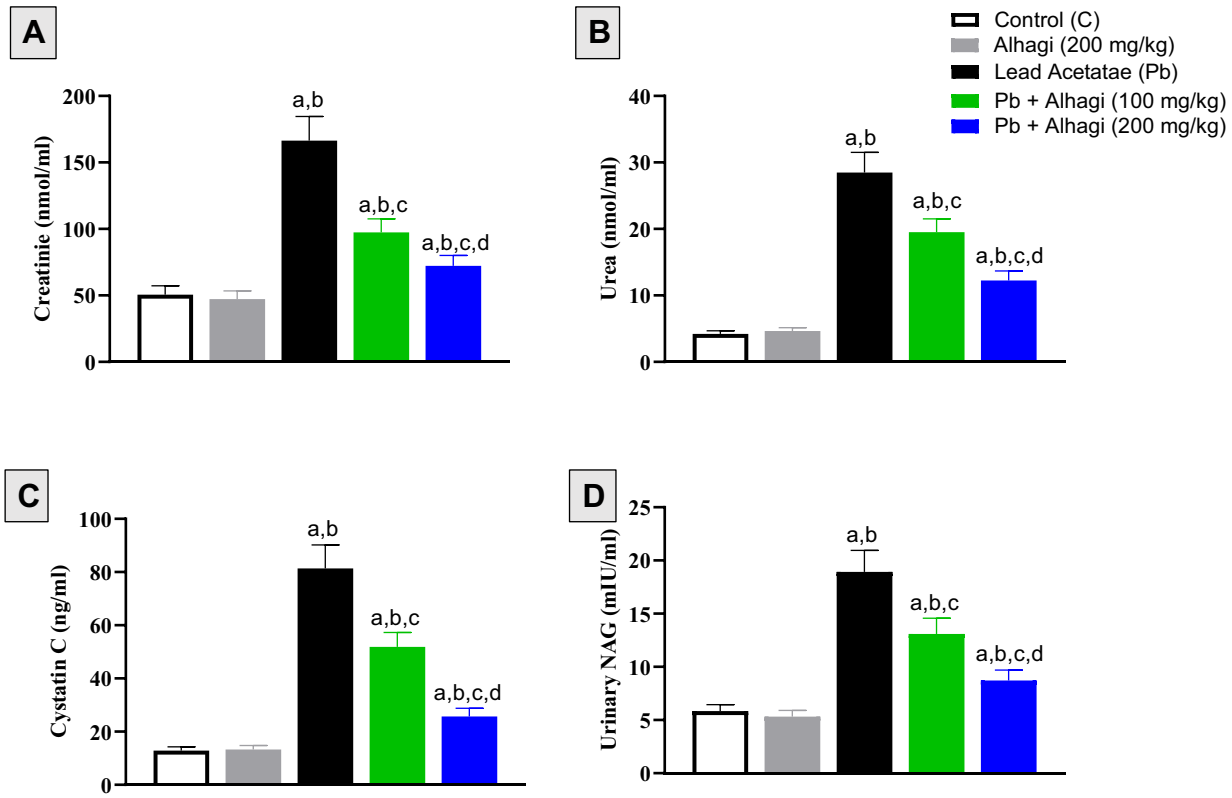


Fig. 1 The effect of *Alhagi maurorum* (AM) on the markers of renal toxicity induced by lead (Pb) in rats: serum levels of creatinine (**A**), urea (**B**), and cystatin (**C**) and the urinary concentration of *N*-acetyl-beta-D-glucosaminidase (NAG). Data are stated as mean ± SD ($n = 6$). a, b, c, and d represent statistical significance compared to the control, AM (200 mg/kg), Pb, and Pb + AM (100 mg/kg), respectively, at $p < 0.05$

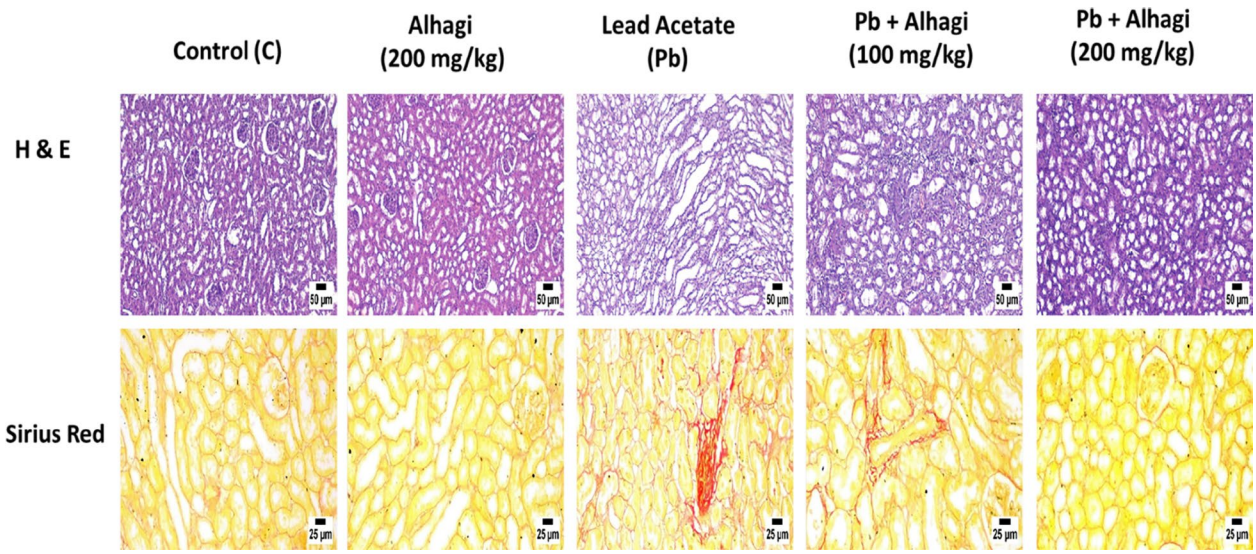


Fig. 2 The effects of *Alhagi maurorum* (AM) on lead (Pb)-induced kidney damage using hematoxylin and eosin (H and E) and Sirius red staining. Red color in the Sirius red panel indicates collagen deposition

interstitial nephritis in the renal cortex and medulla that were characterized by aggregations of a number of mononuclear inflammatory cells accompanied with necrobiotic changes in the renal tubular epithelium. Marked improvement of the renal parenchyma was observed in rat kidneys from the Pb + AM (200 mg/kg) group. The renal cortex and medulla revealed almost normal renal tubules with mild inflammatory cell infiltration. To further assess the effects of AM on collagen deposition, kidney sections were stained with Sirius red. As shown in Fig. 2 lower panel, Pb nephrotoxicity was associated with extensive deposition of collagen fibers, particularly in the renal cortex as compared to control and AM-alone groups. However, co-treatment of rats with AM (100 or 200 mg/kg) obviously inhibited collagen deposition in a dose-related manner.

Effect of AM on oxidative status

Challenging rats with Pb caused a state of oxidative stress as demonstrated by the accumulation of MDA, GSH depletion, and CAT and SOD exhaustion by 758.33%, 65%, 62%, and 65.60% as compared to the control group, respectively. However, administering AM (100 or 200 mg/kg) resulted in significant protection as MDA was reduced by 32% and 49%, respectively. GSH content was enhanced by 23.52% and 57.30% (Fig. 3A, B). The high dose of AM (200 mg/kg) enhanced both CAT and SOD activities by 48.46% and 48% in comparison with the Pb-treated animals, respectively (Fig. 3A, B, C, and D).

Immunohistochemical assessment of inflammatory markers

Immunohistochemical staining of kidney sections of rats subjected to Pb showed a significant elevation in IL-6, TNF-α, COX-2, and NFκB expression by 111%, 127%, 100%, and 107%, respectively, as compared to the control values. Treatment of animals with AM (100 mg/kg) resulted in a respective decrease in their expression by 15.78%, 27.62%, 19.62%, and 9.78% as compared to Pb values, respectively. Also, AM (200 mg/kg) treatment caused a respective decline in their expression by 49.21%, 50%, 45.10%, and 41.03% compared to lead values, respectively (Fig. 4).

Assessment of Bax and Bcl2 mRNA expression

Rats exposed to Pb showed a substantial increase in Bax mRNA by 290% compared to the control values (Fig. 5A). AM (100 or 200 mg/kg) prevented Bax upregulation by 21% and 41.41%, respectively. On the other hand, rats exposed to lead showed a significant reduction in Bcl2 mRNA by 23% compared to control values (Fig. 5B). AM (100 or 200 mg/kg) prevented Bcl2 downregulation by 15.58% and 19.87%, respectively.

Immunohistochemical assessment of HO-1 and Nrf2 expression

The immunohistochemical staining of the kidney sections of rats subjected to Pb showed a significant increase in HO-1 and a decrease in Nrf2 expression by 13% and 36%, respectively, upon comparing to the control group.

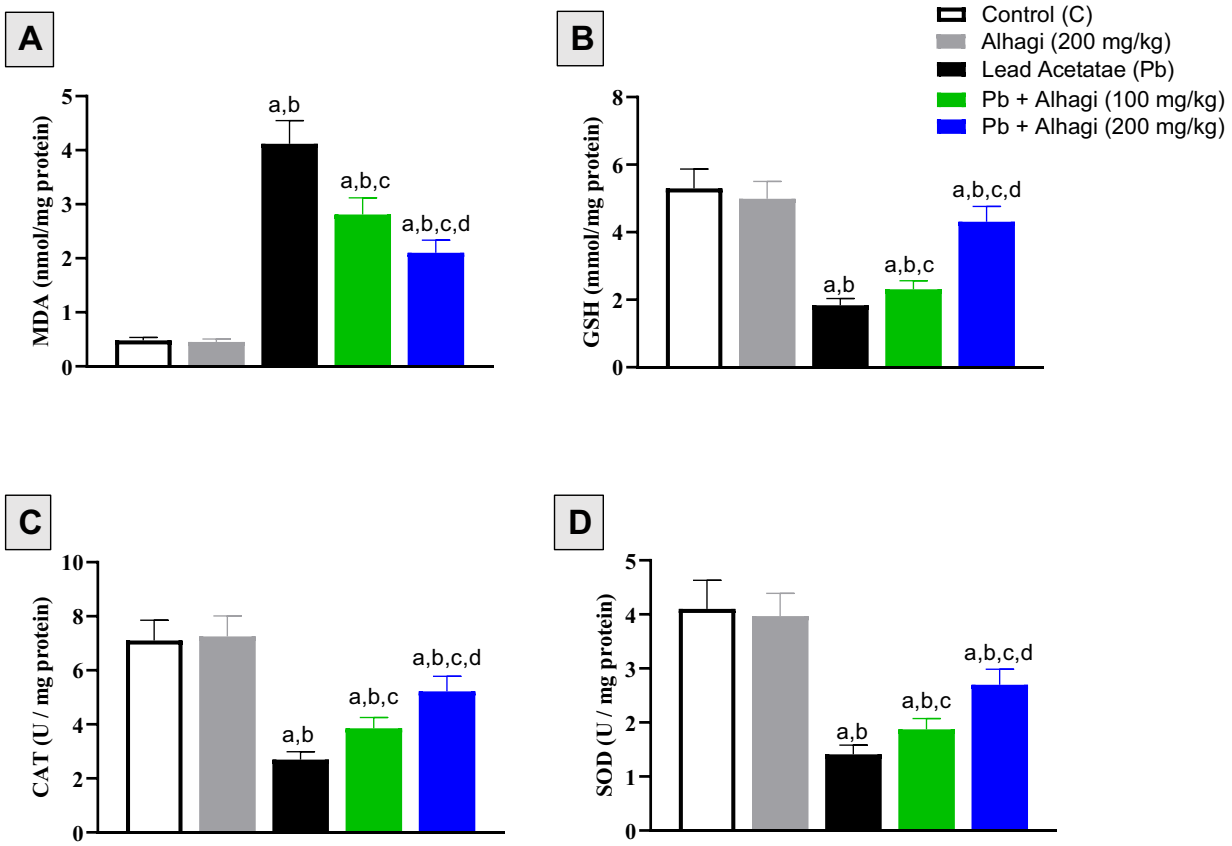


Fig. 3 The effect of *Alhagi maurorum* (AM) on the oxidative status in lead (Pb)-induced nephrotoxicity in rats. **A** Malondialdehyde (MDA), **B** reduced glutathione (GSH), **C** superoxide dismutase (SOD), and **D** catalase (CAT). Data are presented as mean ± SD ($n = 6$). a, b, c, and d represent statistical significance compared to the control, AM (200 mg/kg), Pb, and Pb + AM (100 mg/kg), respectively, at $p < 0.05$

Treatment of rats with AM (100 or 200 mg/kg) resulted in a significant enhancement of HO-1 by 29% and 32% compared to the Pb-treated animals, respectively. Also, AM (100 or 200 mg/kg) increased in the Nrf2 expression by 17.% and 35% compared to lead values, respectively (Fig. 6).

Discussion

Lead (Pb) is one of the important environmental chemicals with multiple deleterious effects on human health [19, 20]. In particular, Pb-induced nephrotoxicity has raised great concern [21]. In this regard, natural products have been proposed as safe and effective tools for metal detoxification. The present study aimed to assess the probable defensive activities of AM extract against Pb-induced nephrotoxicity in rats. Our data indicated that challenging rats with Pb acetate resulted in a decline in kidney function as evidenced by raised serum levels of the nephrotoxicity markers: creatinine, urea, and cystatin C, as well as the urinary NAG. This is consistent with the previous studies on Pb nephrotoxicity in rats [22, 23]. Meanwhile, co-administration of AM significantly ameliorated the rise in such biochemical indicators suggesting a protective activity for the extract against Pb nephrotoxicity. These protective activities were further substantiated by histological examinations of kidney sections. Exposure to Pb caused tubular, glomerular, and interstitial damage. The interstitial tissue showed abundant edema with a variable number of inflammatory cell infiltration. These data are in line with the findings of earlier researches that reported the pathological activities of Pb in kidney architecture [24, 25]. Interestingly, administration of AM significantly ameliorated Pb-induced histopathological action in kidney tissues. This gains support from the ability of Alhagi to prevent hepato- and neurotoxicity of Pb in rats [26] and the traditional use of the Alhagi species to combat metal and chemical toxicities [27].

Exposure to lead is identified to disturb the pro-oxidant/antioxidant balance present within the mammal cells [28, 29]. Pb has a high affinity for the sulfhydryl group which acts as the functional group of the

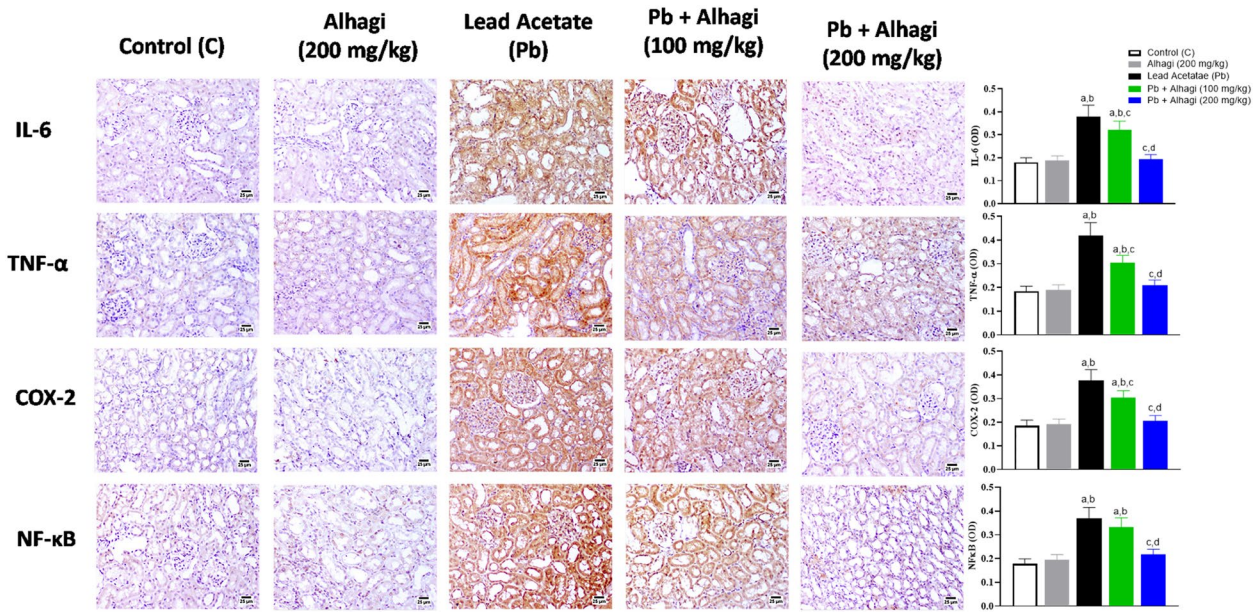


Fig. 4 The effect of *Alhagi maurorum* (AM) on the immunohistochemical reactivity of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), cyclooxygenase-2 (COX-2), and nuclear factor-kappa B (NF-κB) in kidney tissues of lead (Pb)-challenged rats. Data are presented as means of the optical density (OD) ± SD ($n = 6$). a, b, c, and d represent statistical significance compared to the control, AM (200 mg/kg), Pb, and Pb + AM (100 mg/kg), respectively, at $p < 0.05$

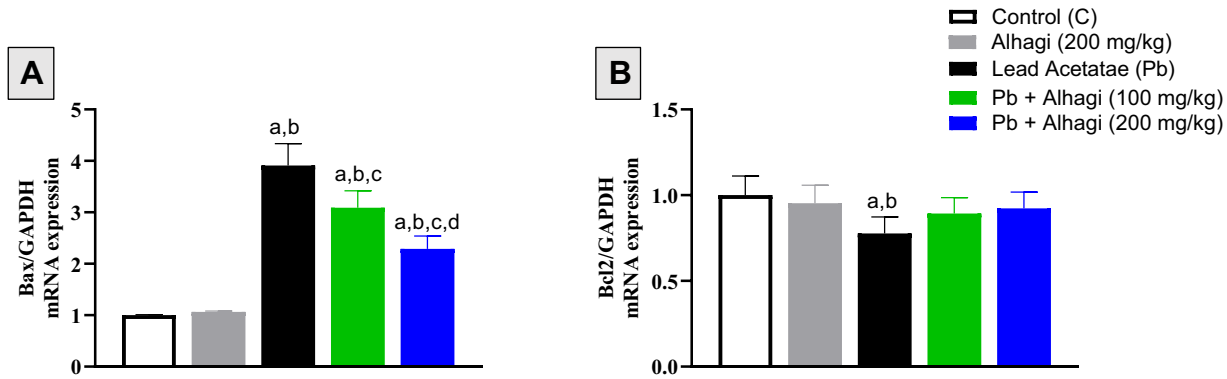


Fig. 5 Effects of *Alhagi maurorum* (AM) treatment on Bax (**A**) and Bcl2 (**B**) mRNA expression in kidney tissues of Pb-exposed rats. Data are presented as mean ± SD ($n = 6$). a, b, c, and d represent statistical significance compared to the control, AM (200 mg/kg), Pb, and Pb + AM (100 mg/kg), respectively, at $p < 0.05$

antioxidant enzyme [30]. Thus, Pb interferes with the enzymes' antioxidant capacity leading to the accumulation of reactive oxygen species (ROS) that contribute to renal cell damage through lipid peroxidation [31]. The phytotherapy researchers' attention is directed toward using medicinal plants with antioxidant capacity to protect against heavy metals toxicity [32, 33].

In the current study, the decreased activities of SOD and CAT and decreased levels of GSH reflect the deleterious effects of Pb on antioxidant enzymes. Besides, the increased production of MDA evidenced lipid peroxidation. However, co-treatment with AM reduced lipid peroxidation and enhanced non-enzymatic and enzymatic antioxidants. These observations are in line with a previous study that proved that AM extract ameliorated oxidative stress by increasing the tissue content of GSH, enhancing SOD and CAT activities, and preventing the accumulation of MDA [18].

Induction of inflammatory response is one of the major perturbations induced by Pb on the kidneys. Our

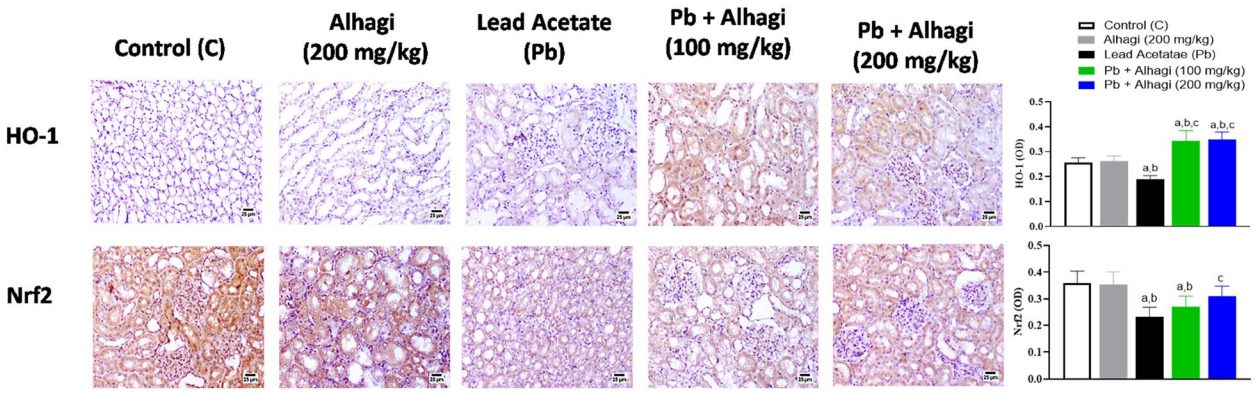


Fig. 6 Effect of *Alhagi maurorum* (AM) on the immunohistochemical reactivity HO-1 and Nrf2 in the kidney tissues of lead (Pb)-challenged rats. Data are presented as means of the optical density (OD) ± SD ($n = 6$). a, b, and c represent statistical significance compared to the control, AM (200 mg/kg), and Pb, respectively, at $p < 0.05$

data indicated a significant increase in the expression of pro-inflammatory cytokines IL-6, TNF-α, COX-2, and NF-κB in the Pb alone-treated group. These results are in accordance with the earlier studies that stated that exposing rats to Pb raised the levels of pro-inflammatory cytokines in the kidneys [34]. In addition, Pb is implicated in the activation of NF-κB, which regulates the expression of a number of genes involved in inflammation [35, 36]. Interestingly, AM treatment protected against the increase in pro-inflammatory mediators in the kidneys of Pb-intoxicated rats. Additionally, Pb is known to increase the levels of the inducible COX-2 that, in turn, contributes to the evolution of the inflammatory process [37]. In the current study, rats treated with AM together with Pb demonstrated a marked waning in inflammatory markers compared to the Pb-only-treated animals.

Initiation of apoptosis caused by Pb is an important underlying mechanism of Pb-induced nephrotoxicity [38]. The apoptotic proteins, the proapoptotic Bax, and the anti-apoptotic Bcl-2 regulate this process [39]. Similar to our findings, the previous studies evidenced that Pb-induced apoptotic alterations in the kidneys through the significant rise in Bax and a marked decrease in Bcl-2 mRNA expression [40]. Interestingly, oral administration of AM extracts significantly changed the expression of apoptotic mediators in kidney tissue in favor of anti-apoptosis. The observed anti-apoptotic effects of the plant are in line with the previous reports [26].

Pb-induced apoptosis has been proposed as an immediate consequence of oxidative stress [41]. Furthermore, Pb administration was proven to downregulate the renal tissue expression of the antioxidant Nrf2 and HO-1 [22]. Nrf2 and HO-1 confer cellular protection against toxic metals by stimulating the antioxidant and detoxifying defense system. Therefore, following Pb intoxication, the decline in Nrf2 and HO-1 expressions can justify the deactivation of antioxidant enzymes in this study. These data are in harmony with a former report by [42]. Remarkably, the administration of AM directed the redox homeostasis in the rat kidneys that can be simplified in the decline in the markers of oxidation and augment in the activities of antioxidant enzymes that can be returned to the increased gene transcription of the Nrf2 and HO-1. Moreover, the increased Nrf2 and HO-1 explain the anti-inflammatory activities offered by AM. Further, the beneficial protective effects may be attributed to its content of polyphenolics as well as other biologically active compounds [13, 43–46].

Conclusion

It can be concluded that AM protects against Pb-induced nephrotoxicity. This is referred, at least partly, to its antioxidant, anti-inflammatory, and anti-apoptotic activities as well as its ability to activate the HO-1/Nrf2 axis.

Limitations

Detailed toxicity studies are needed, and the exact active constituents are yet to be determined.

Abbreviations

Pb	Lead
MDA	Malondialdehyde
GSH	Glutathione reduced form
CAT	Catalase
SOD	Superoxide dismutase
UL-6	Interleukin-6
TNF-α	Tumor necrosis factor-alpha
COX-2	Cyclooxygenase-2
NF-κB	Nuclear factor-kappa B
HO-1	Heme oxygenase-1
Nrf2	Nuclear factor erythroid 2-related factor 2

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Author contributions
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Availability of data and materials
The datasets of the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Procedures involving animal handling were permitted by the Research Ethics Committee, College of Pharmacy, King Abdulaziz University (Reference No. PH-1443-32).

Consent for publication
Non-applicable.

Competing interests
The author declares that no competing interests exist.

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