ORIGINAL ARTICLE

Ameliorating effects of *Rhamnus alaternus* L. aqueous extract on aluminum chloride-induced nephrotoxicity via attenuation of oxidative stress in male Wistar rats

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Abstract

Recently, the use of medicinal plants including effective therapeutic molecules has become of the highest priority in treating various diseases and toxicities. The aim of the present study was to undertake the beneficial effect of *Rhamnus alaternus* L. aqueous extract (RAAE) against aluminum chloride-induced sub-chronic hematotoxicity and renal oxidative damage in rats. DPPH free radical scavenging, β-carotene bleaching, ferric-reducing antioxidant power (FRAP), phenolic, flavonoid, and tannin contents were measured in RAAE. Twenty-four male rats were divided into four groups. The first group was used as controls, and the other three groups received daily orally 50-mg $AlCl_3$/kg b. wt, 250-mg RAAE/kg b. wt, and AlC_3 plus RAAE, respectively, for 4 weeks. The findings indicated the presence of an important amount of total phenolic, flavonoids, and tannins and high-capacity antioxidant activity. The administration of $AlCl_3$ caused induction of hematotoxicity evidenced by a significant decrease in hematocrit (Ht), hemoglobin concentration (Hb), red blood cell count (RBC), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC). In addition, $AlCL_3$ led to nephrotoxicity and oxidative stress occurrence, which were revealed by an increase of urea, creatinine, and uric acid, depletion of reduced glutathione concentration, superoxide dismutase, catalase, and glutathione peroxidase activities along with an increased level of the malondialdehyde level. However, the supplementation of RAAE significantly restored the previous mentioned parameters approximately to their normal values. These results were identical with the histological observations. In conclusion, the results showed that RAAE had efficient antioxidant properties due to its richness of antioxidant compounds, which played an important role against $AlCl_3$-induced sub-chronic hematotoxicity and oxidative nephrotoxicity.

Keywords Aluminum chloride · *Rhamnus alaternus* L · Nephrotoxicity · Oxidative stress · Antioxidants

Introduction

Recently, the use of medicinal plants including effective therapeutic molecules has become of the highest priority in treating various diseases and toxicities (El-Demerdash et al. 2018). In this context, several studies have reported heavy metal pollution as a serious worldwide environmental problem, whose activities can undesirably affect human and animal health, through inducing organ and system toxicities (Akintunde et al. 2020; Mishra et al. 2021). Aluminum (Al) is a reactive metal naturally found in the environment and commonly used in water-treatment processes, food-processing

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sectors, pharmaceuticals, and cosmetics (Tietz et al. 2019). However, occupational exposure to acute high levels of aluminum primarily leads to various diseases and cancers (Exley 2016). This metal cannot be metabolized in the body, and so it potentially accumulates in mammalian soft tissues, such as hematopoietic, liver, brain, bones, and renal tissues (Cheng et al. 2017; Al-Kahtani and Morsy 2019). The human population is continually exposed to Al compounds via ingestion of contaminated water and foodstuffs and excretion principally through the urine, making the kidney an aluminum-exposed organ (Exley 2013; Panhwar et al. 2016).

Kidneys are vital organs whose main roles are to efflux toxic wastes through urine and to maintain blood homeostasis (Pizzorno 2015), since these functions can be strongly affected by various toxicants leading to kidney diseases and pathogenesis (Cao et al. 2018; El-Demerdash et al. 2020). Previous in vivo studies investigating Al toxicities have reported kidney dysfunction evidenced mainly by disruption of the cellular calcium flow and increased risk of aluminum retention and accumulation in the renal tubules (Saber et al. 2015; Hasona and Ahmed 2017). Furthermore, the cytotoxic effects of Al were reported to alter iron homeostasis and consequently lead to increased levels of iron accumulation in tissues (Ward et al. 2001). Also, aluminum compounds were found to induce hematologic disorders (Mir et al. 2015; Sharma et al. 2016) through induction of hypochromic microcytic anemia and alterations of hematological profiles (Al-Qayim and Mashi 2014).

The underlying toxic effects of Al involve the generation of reactive oxygen species (ROS), causing DNA damage, oxidation of cell molecules (lipids, proteins, and nucleic acids), and cell apoptosis (Hasona and Ahmed 2017; Liu et al. 2016). As a result, several antioxidant defense systems, including glutathione, catalase, glutathione peroxidase, and superoxide dismutase found in the cell, can reduce the severity of ROS effects (Saber et al. 2015). In this context, the use of antioxidant supplementation to reduce tissue oxidative injuries and reinforce endogenous antioxidant systems (Hong et al. 2021) and, indeed, an exogenous intake of antioxidants was reported to reduce oxidative damage. Additionally, the abundance of phenolic compounds in plants was found to play an effective protective antioxidant role against oxidative damages (Tahari et al. 2016; Balgoon 2019).

Rhamnus alaternus L., a shrub belonging to the Rhamnaceae family and commonly known as M'lilez, is distributed in the Mediterranean region and mainly in Algeria. The leaves and bark of *Rhamnus alaternus* L. display many therapeutic properties in traditional medicine as a remedy for diseases such as diabetes, dyslipidemia, liver complications, and cardiovascular diseases (Boussahel et al. 2015; Halzoune et al. 2020). Several antioxidant compounds of *Rhamnus alaternus* have been identified as anthraquinones: emodin, chrysophanol, and flavonoids

(e.g., Kaempferol 3-O-bisorhamninoside (Ammar et al. 2009; Bhouri et al. 2011). Additionally, this plant was previously reported to have effective antiradical, antiproliferative, cytotoxic, and antibacterial activities (Ammar et al. 2009); however, the prophylactic properties of the extract against metallic trace elements (MTE) toxicity have not yet been fully elucidated. Based on the typical biological properties of *Rhamnus alaternus* and the wide distribution, use, and, consequently, the increased human exposure risk to Al, the present study conducted on *Rhamnus alaternus* aqueous extract was to determine the contents of total phenolic, flavonoids, and tannin, and to evaluate their in vitro antioxidant activity by using DPPH radical scavenging, inhibition of β-carotene bleaching, and reduction of power assays, as well as to study the possible protection against $AlCl_3$-induced hematologic disorders and kidney damage in male Wistar rats.

Materials and methods

Chemicals and reagents

Aluminum chloride ($AlCl_3$) reagent grade, 98%; quercetin; catechin; free stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH); Folin–Ciocalteu phenol reagent; reduced glutathione (GSH); butylated hydroxytoluene; and thiobarbituric acid (TBA and DTNB [5,5′-dithiobis-2-nitrobenzoic acid]) were obtained from Sigma Chemical Co. (St. Louis, MI, USA).

Plant material

Rhamnus alaternus L. plant specimens were collected during February 2019 from the Tigzirt district of the Tizi-Ouzou city (Northeast Algeria). The plant species was botanically identified based on the work of Quezel and Santa (1962-1963) and validated by Dr. Tarek Hamel from Department of Plant Biology and Environment, Badji Mokhtar University, Annaba, Algeria.

Plant extract preparation

The stems and leaves were washed with distilled water, dried in the shade, and then pulverized in a mill to get a fine powder to be used for plant extract preparation (Berroukche et al. 2015). Briefly, 500 g of *R. alaternus* aerial part powder was added to 2000 ml of distilled water, and the decoction was maintained at continuous reflux for 2 h at 80 °C. Following filtration, the recovered extract (decoct) was centrifuged for 5 min at $2500 \times g$, and then evaporated under a vacuum by means of a Rota-vapor. The residue was collected and weighed to determine the yield, expressed in percentages.

Total phenolic contents

The amount of total polyphenols in the *R. alaternus L* aqueous extract was spectrophotometrically determined according to the Folin–Ciocalteu reagent method (Zhu et al. 2011), with a slight modification. Briefly, 100 μl of the extract (1 mg/ml) was mixed with 0.5 ml of diluted Folin–Ciocalteu reagent (1:10 with water), in which 400 μl (7.5%, m/v) of sodium carbonate solution was added. Thereafter, the mixture was stirred and incubated in the dark for 90 min at 30 °C, with the absorbance read at 765 nm. Results are expressed as micrograms of gallic acid equivalent per milligram of extract (μg of EqAG/mg of extract) according to the following equation: $Y = 0.0138\,x + 0.0352$ ($R^2 = 0.992$).

Total flavonoid contents

The flavonoid levels of *R. alaternus L* were determined using the aluminum trichloride method, as described elsewhere (Pourmand et al. 2006). Briefly, 50 μl of *R. alaternus* extract (1 mg/ml) was added to a mixture containing 1500 μl of methanol, 100 μl of aluminum chloride reagent, 100 μl of sodium acetate (1 M), and 2.8 ml of distilled water. The mixture was then stirred and subsequently incubated in the dark for 30 min at 22 °C. Absorbance was read at 430 nm. Flavonoid concentration was determined in terms of μg of quercetin equivalent (EQ)/mg of extract, using the following equation: $Y = 0.0111\,x + 0.0116$ ($R^2 = 0.994$).

Total condensed tannin contents

The levels of condensed tannins in the aqueous extract were determined as previously reported (Julkunen-Tiitto 1985). For this purpose, 50 μl of aqueous extract was added to 1500 μl of vanillin/methanol solution (4%, m/v). The mixture was then supplemented with 750 μl of concentrated hydrochloric acid (HCl) and incubated for 20 min at room temperature. The absorbance was read at 550 nm, and, hence, the tannin concentration was determined using a catechin standard curve, with the following equation: $Y = 0.0111\,x + 0.0741$ ($R^2 = 0.984$).

DPPH free radical scavenging assay

A DPPH assay was used to evaluate the free-radical DPPH-scavenging capacity of the extract and performed as previously described (Sánchez-Moreno et al. 1998). Briefly, 50 μl of various concentrations (expressed in mg/ml) of either the extract or the standard (ascorbic acid and butylated hydroxytoluene, BHT) was added to 1.950 ml of DPPH methanolic solution (2.5 mg/l00 ml). The mixture of various sample concentrations and DPPH was kept in the dark for 30 min at room temperature. The absorbance was read at 517 nm, and, thereby, the percentage of antiradical activity was determined according to the following formula: antiradical activity (%) $= 100 \times [(Ac - Ae)/Ac]$, where Ac represents the absorbance of the control and Ae the absorbance of the sample test.

β-carotene-linoleic acid assay

The potential of the aqueous extract to prevent β-carotene discoloration was investigated (Ismail et al. 2004). Briefly, 2 mg of β-carotene was dissolved in 10 ml of chloroform, from which 1 ml was mixed with 2 ml of Tween-20 and 0.02 ml of linoleic acid. The resulting mixture was then evaporated using a rotary evaporator for 10 min at 40 °C. Thereafter, a volume of 100 ml of distilled water was slowly added to the flask, containing an emulsified mixture. Five-milliliter aliquots of this emulsion were transferred to different test tubes containing 0.2 ml of either the extract or the synthetic antioxidant (BHT) at various concentrations. The tubes were then gently shaken and incubated for 2 h in a water bath at 45 °C. The absorbance of each solution was measured every 15 min and was used to determine antioxidant activity (AA) of the RAAE as follows: AA (%) $= [1 - (At_0 - At_{120})\text{ test}/(At_0 - At_{120})\text{ control}] \times 100$, where AA (%) represents the antioxidant activity, At_0 the absorbance at time zero, and At_{120} the absorbance at time 120 min.

Reducing power assay

The ferric-reducing power of the RAAE was determined using a previously described protocol (Pan et al. 2008). One milliliter of various extract dilutions was mixed with 0.25 ml of phosphate buffer solution (0.2 M; pH 6.6) and 2.5 ml of potassium ferrocyanide solution (10%; m/v). The tubes were incubated thereafter for 20 min at 50 °C. After cooling, 2.5 ml of 10% trichloroacetic acid was added to the tubes, which were then centrifuged for 10 min at 3000 g. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%) and used to determine the absorbance at 700 nm. BHA and ascorbic acid at various concentrations were used as positive controls under the same experimental conditions.

Animals and treatments

All of the experimental procedures followed the International Guidelines for Care and Use of Laboratory Animals (Council of European Communities 1986) and were approved by the Ethical Committee of Directorate General for Scientific Research and Technological Development of the Algerian Ministry of Higher Education and Scientific Research under the ethical number of PNR ANDRS 8/u23/345.

The entire study was performed on male Wistar rats (Pastor Institute, Algiers, Algeria), weighing 220 ± 2 g and aged 10 weeks old. The animals were housed under constant temperature (22 ± 2 °C) in a 12-h light/12-h dark cycle, with free access to water and an energetically balanced diet (ONAB, Bejaia, Algeria). After 2 weeks of adaptation, the animals were equally divided into four groups (6 animals/group). The control group received distilled water; the AlCl$_3$ group received 50 mg of AlCl$_3$/kg of body weight (bw)/day; the RAAE group received 250 mg of RAAE/kg of body weight (bw)/day; and, finally, the AlCl$_3$ + RAAE group received a combined treatment consisting of 50 mg of AlCl$_3$/kg and 250 mg RAAE/kg of body weight. Treatment was performed by oral gavage once a day for 4 weeks. The selected dose of AlC$_3$ was chosen according to Aita (2014), while that of RAAE was according to Berroukche et al. (2015).

At the end of the treatment period, the animals were sacrificed by decapitation, and blood samples were immediately collected under heparin or EDTA. The blood with heparin was used to collect plasma for the biochemical analyses, and the other one containing EDTA was used to determine blood count parameters. The kidneys were also carefully removed from the rat cadavers under ice and weighed. Each kidney was sectioned into two-halves: one-half was fixed in 10% buffered formalin for histopathological evaluation, and the other half was frozen at −80 °C until being used for oxidative stress analyses.

Complete blood count and biochemical parameters

The complete blood count parameters were determined by means of a hematological automatic analyzer (Erma Coulter, Inc., model PCE-210 N). The analyzed parameters include white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC). The biochemical parameters reflecting the renal tests (urea, creatinine, and uric acid) were determined using commercially available kits (SpinReact, Girona, Spain).

Kidney homogenate preparation

One gram of kidney tissue from each animal was crushed, homogenated in 2 ml of phosphate buffer solution (w/v; 1-g tissue with 2 ml of PBS, pH 7.4), and the resulting tissue suspension was centrifuged for 15 min at $10,000 \times g$ and 4 °C. The obtained supernatant was used to determine GSH, lipid peroxidation, SOD, and GPx activity.

Reduced glutathione (GSH) content

GSH content was determined in the homogenates as previously reported by Jollow et al. (1974). Briefly, 0.8 ml of the renal supernatant was mixed with 0.3 ml of sulfosalicylic acid solution (25%) and centrifuged at $2500 \times g$ for 15 min. The collected supernatant (0.5 ml) was then mixed with 1 ml of a phosphate buffer (0.1 M, pH 7.4) and 0.025 ml of 0.01 M DNTB. This mixture was used to measure the concentration of GSH using a spectrophotometer at 412 nm. Results were expressed in μmol GSH/mg of protein, as previously reported by Bradford (1976).

Lipid peroxidation levels

Lipid peroxidation was assessed by measuring the level of malondialdehyde (MDA) in the renal homogenates (Buege and Aust 1984). For this purpose, 375 μl of the kidney homogenate was mixed with 150 μl of tris buffer solution (Tris, 50 mM; NaCl, 150 mM; pH, 7.4) and 375 μl of TCA-BHT solution (TCA, 20%; BHT, 1%). After stirring and centrifugation for 10 min at $1000 \times g$ and 4 °C, 400 μl of the solution was taken and added to 80 μl of HCl (0.6 M) and 320 μl of the Tris-TBA solution (Tris, 26 mM; TBA, 120 mM). The reaction medium was mixed and incubated thereafter for 10 min at 80 °C with the absorbance of the TBA-MDA complex read at 532 nm.

Determination of superoxide dismutase (SOD) activity

The enzymatic activity of superoxide dismutase was estimated according to the technique of Beyer and Fridovich (1987). Kidney tissue homogenate (50 μl) was supplemented with a reaction medium containing EDTA (0.1 mM), L-methionine (13 mM), a phosphate buffer (50 mM; pH, 7.8), nitro blue tetrazolium (75 mM), and riboflavin (2 mM). The resulting solution was then incubated for 5 min at 37 °C and subsequently used to determine the absorbance at 560 nm. SOD activity was expressed as units/mg of protein.

Determination of glutathione peroxidase (GPx) activity

Glutathione peroxidase activity was measured by the method of Flohe and Gunzle (1984). Here, kidney tissue homogenate (0.3 ml) was mixed with 0.2 ml of GSH (2 mM), 0.3 ml of phosphate buffer solution (0.1 M; pH, 7.4), and 0.1 ml of sodium azide (10 mM), and incubated for 5 min at 37 °C. Thereafter, 0.5 ml of TCA (5%) was added to the mixture, which was placed in an ice bath for 30 min to stop the reaction. The mixture was then centrifuged at $3000 \times g$ for 10 min,

after which 0.1 ml of the collected supernatant was supplemented with 0.2 ml of phosphate buffer solution (0.1 M; pH, 7.4) and 0.7 ml of DTNB (0.4 mg/ml), incubated for 5 min, and subsequently used to measure the absorbance at 412 nm.

Determination of catalase activity

Catalase activity was estimated according to the method of Aebi (1984). Absorbances were measured at 240 nm by the change in optical density, following the hydrolysis of hydrogen peroxide (H_2O) by reacting 200 µl of H_2O and 20 µl of homogenate in a phosphate buffer (100 mM; pH, 7.4) for 1 min at an incubation temperature of 25 °C. The enzyme activity was expressed as µmol of H_2O/min/mg of protein.

Determination of oxidative stress index

The oxidative stress index was expressed in terms of the prooxidant (P)/antioxidant (A) ratio and was calculated by the following formula:

Oxidative stress index = levels of MDA/levels of activity of SOD + CAT + GPx.

Histological analyses

Kidney tissues were fixed in 10% formalin for 24 h, embedded in paraffin, and cut into 5-mm-thick sections. After being deparaffinized and rehydrated, the tissue sections were stained with hematoxylin and eosin (H and E) for observation by optical microscopy (Haoult 1984).

Statistical study

Results were displayed as the means of three independent replicates (triplicate) (mean ± SEM) (in vitro study).

All data were expressed as mean ± SEM for six rats in each group. Multiple comparisons of the different batches were tested by variance (ANOVA) with the Tukey post hoc test, using GraphPad Prism software (Prism7, version 7.00, GraphPad Software). The differences $P < 0.05$ (*) and $P < 0.01$ (**) were considered significant and highly significant, respectively.

Results

Phytochemical analysis and antioxidant activity of RAAE

The collected leaves and stems of the *R. alaternus L* plant showed a polyphenol level of 64.11 ± 3.18 µg/mg, a flavonoid level of 12.62 ± 0.85 µg/mg, and a tannin level of 8.39 ± 0.48 µg/mg of extract.

The RAAE extract displayed high antioxidant activity. As shown in (Fig. 1a), the RAAE at 200 µg/ml inhibited the DPPH radical by 65.42% with an $IC_{50} = 60.08 \pm 2.09$ µg/ml. The standards (used as controls) vitamin C and BHT recorded inhibition percentages of 95.10% and 75.96%, respectively. The IC_{50}s were 7.24 ± 0.45 µg/ml for vitamin C and 37.85 ± 1.12 µg/ml for BHT. Comparing the IC_{50}s and the standard revealed that RAAE showed lower β-carotene bleaching inhibitory activity (higher EC_{50} values; $IC_{50} = 52.34 \pm 1.16$ µg/ml) than that of BHT (20.46 ± 0.84 µg/ml). Furthermore, RAAE showed higher-reducing power activity with an $IC_{50} = 43.06 \pm 1.90$ µg/ml than that of the BHT ($IC_{50} = 56.13 \pm 1.22$ µg/ml) and lower-reducing power when compared to that of vitamin C ($IC_{50} = 8.52 \pm 0.63$ µg/ml) (Fig. 1b).
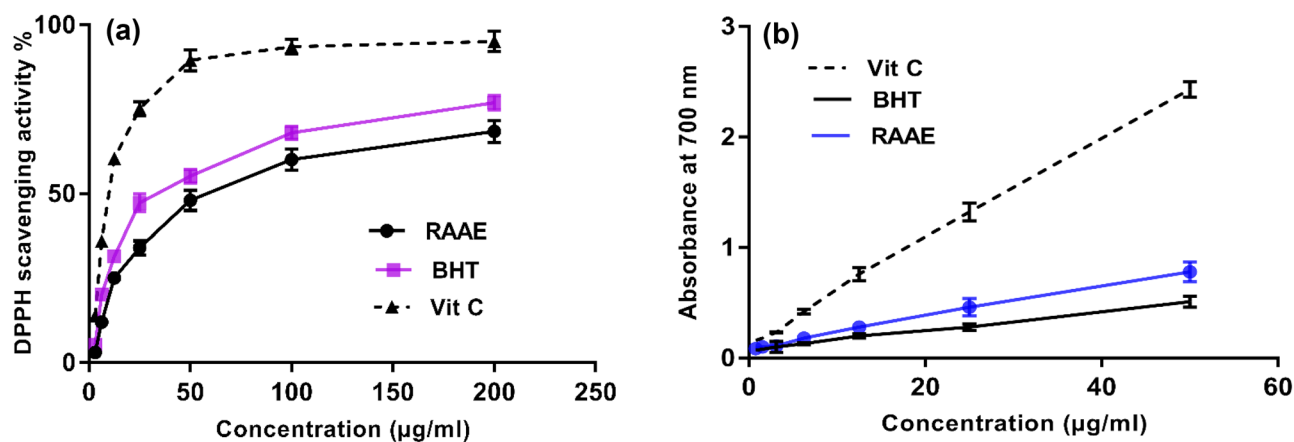
Fig. 1 DPPH radical scavenging activity (%) (**a**) and reducing power activity (**b**) of aqueous extract of *Rhamnus alaternus* (RAAE). Vitamin C and butyl hydroxytoluene (BHT) were used as positive control. Values are expressed as mean ± SEM of triplicate replications

Table 1 Hematological parameter values of rats treated with AlC3, RAAE, or their combination (AlCl3/RAAE)

Parameters	Experimental groups			
	Control	$AlCl_3$	RAAE	$AlCl_3$ /RAAE
WBC (10^3/µl)	9.78 ± 0.95	$12.46 \pm 1.68^*$	$9.84 \pm 0.76^{\#}$	10.22 ± 1.03
RBCs ($\times 10^6$/µl)	8.02 ± 0.78	$6.75 \pm 0.31^*$	$8.14 \pm 0.93^{\#}$	7.47 ± 0.82
Hb (g/dl)	16.28 ± 1.12	$12.83 \pm 0.90^*$	$16.54 \pm 1.34^{\#}$	$16.14 \pm 1.08^{\#}$
HCT (%)	46.33 ± 1.28	$37.60 \pm 1.43^*$	$47.82 \pm 1.90^{\#}$	43.76 ± 2.14
MCV (fl)	55.12 ± 1.07	51.34 ± 2.95	55.63 ± 2.38	53.86 ± 1.20
MCHC (g/dl)	38.17 ± 1.74	37.22 ± 2.09	38.45 ± 1.63	37.84 ± 1.92

Values are mean ± SEM for groups of 6 animals each. *AlCl₃*, aluminum chloride, *RAAE Rhamnus alaternus* L. aqueous extract, *WBC* white blood cell count, *RBCs* red blood cell count, *Hb* hemoglobin concentration, *HCT* hematocrit, *MCV* mean corpuscular volume, *MCHC* mean corpuscular hemoglobin concentration

Significant difference compared to the control group ($^*p < 0.05$)

Significant difference compared to the $AlCl_3$-treated group ($^{\#}p < 0.05$)

Effect of treatment on hematological parameters

As shown in Table 1, the $AlCl_3$ treatment caused a significant decrease ($p < 0.05$) in hematocrit (Ht), hemoglobin concentration (Hb), and red blood cell count (RBC). Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) did not show a significant difference compared to those of the control, while the white blood cells count (WBC) was significantly increased ($p < 0.05$). Treatment with RAAE alone produced results similar to those obtained with the control. Interestingly, the RAAE extract concomitantly with $AlCl_3$ showed a significant recovery of the hematological parameters deregulated by the ingested $AlCl_3$. Indeed, these subjects demonstrated an increased Hb level as well as RBC, MCV, MCHC, and WBC levels, which are comparable to those observed in both the control and the group receiving only the RAAE extract.

Effect of treatment on serum biochemical parameters

Table 2 confirms a highly significant ($p < 0.01$) increase in the plasma levels of urea and creatinine and a significant increase in uric acid, following treatment with $AlCl_3$ as compared to the control ($p < 0.05$). However, the rats exposed to $AlCl_3$ in combination with RAAE extract showed a greater ($p < 0.05$) recovery of the urea, creatinine, and uric acid levels than did the $AlCl_3$-treated rats.

Effect of treatment on lipid peroxidation GSH contents in kidney

The analysis of the renal redox status (Fig. 2) of the $AlCl_3$-treated rats shows a significant increase ($p < 0.01$) in the level of malondialdehyde (MDA) (Fig. 2a) and a significant depletion ($p < 0.01$) of reduced glutathione (GSH) content (Fig. 2b) as compared with the controls. The levels of MDA and GSH in the rats treated with RAAE extract alone were similar to those in the control animals. Hence, the rats treated with the $AlCl_3$ + RAAE combination displayed improved antioxidant parameters with a significant decrease in the MDA level and an increase in GSH content ($p < 0.05$) compared to those seen in the $AlCl_3$-treated rats (Fig. 2a and b).

**Table 2** Biochemical parameter values of rats treated with AlC_3, RAAE, or their combination (AlCl3/RAAE)

Parameters	Experimental groups			
	Control	$Alcl_3$	RAAE	$Alcl_3$ /RAAE
Urea (mg/dl)	34.16 ± 1.83	$53.19 \pm 1.20^{**}$	$37.46 \pm 1.75^{\#\#}$	$43.5 \pm 1.60^*$
Creatinine (mg/dl)	0.61 ± 08	$0.86 \pm 0.10^{**}$	0.64 ± 0.09	$0.72 \pm 0.05^{*\#}$
Uric acid (mg/dl)	2.17 ± 0.21	$3.86 \pm 0.43^*$	$2.28 \pm 0.42^{\#}$	2.95 ± 0.36

Values are mean ± SEM for groups of 6 animals each

AlCl₃, aluminum chloride, *RAAE Rhamnus alaternus* L. aqueous extract

Significant difference compared to the control group ($^*p < 0.05$, $^{**}p < 0.01$)

Significant difference compared to the $AlCl_3$-treated group ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$)
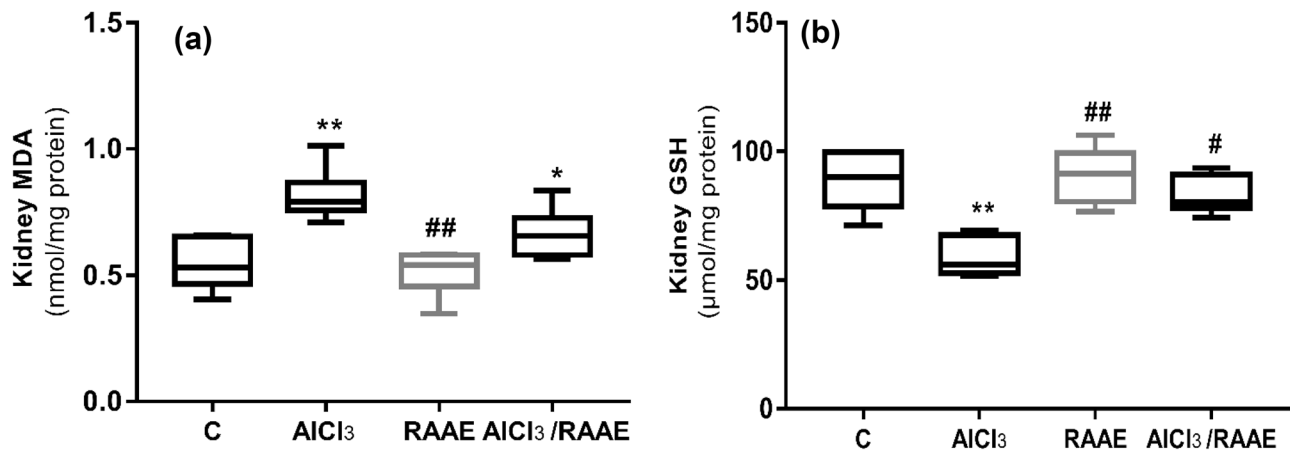
Fig. 2 Kidney malondialdehyde (MDA) (**a**) and reduced glutathione (GSH) (**b**) levels in control and experimental groups. Values are mean ± SEM for groups of 6 animals each. AlCl$_3$, aluminum chloride; RAAE, *Rhamnus alaternus* L. aqueous extract. Significant difference compared to the control group (*$p < 0.05$, **$p < 0.01$); significant difference compared to the AlCl$_3$-treated group ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$)

Effect of treatment on antioxidant enzyme activities in kidney

Treatment with AlCl$_3$ produced significant adverse effects on the redox status of the kidneys, revealed by a significant reduction in the enzymatic activity of SOD ($p < 0.01$), GPx ($p < 0.05$), and CAT ($p < 0.05$) when compared with controls (Fig. 3a, b and c). The rats receiving the RAAE extract alone recorded SOD, GPx, and CAT data similar to those in the non-treated rats. Accordingly, the use of RAAE in the rats treated with AlCl$_3$ reversed the deregulatory effect of the AlCl$_3$ and significantly modulated the SOD, GPx, and CAT activities (Fig. 3a, b and c). These results point to the positive effect of RAAE in repairing metal toxicity damage in the kidney tissue.

Oxidative stress index

Results listed in Table 3 show oxidative stress index (OSI) in terms of the prooxidant (P)/antioxidant (A) ratio in rat kidney tissue homogenates. The value of P/A in the kidney was higher in the animals exposed to AlCl$_3$. In contrast, co-administration of RAAE in association with AlCl$_3$ attenuated the increase of P/A in kidney tissue.

Kidney histology

Microscopic observation of sections of renal tissue from the control rats revealed a normal architecture characterized by normal renal parenchyma and intact renal glomeruli and tubules (Fig. 4a and b). No structural modifications were observed in the kidneys of the RAAE-treated rats compared to the controls (Fig. 4c and d). In contrast, kidney sections from AlCl$_3$-treated rats showed severe tissue damage

evidenced by tissue congestion, the presence of necrotic areas, a high mononuclear cell infiltration of the kidney tissue, dilation of Bowman's space, and an alteration of the tubular structure (Fig. 4e, f and g). Of note, RAAE showed marked prevention against AlCl$_3$-induced kidney tissue damages (Fig. 4h, i and j).

Discussion

The current study was conducted to investigate the possible protective effect of aqueous extract of *Rhamnus alaternus* L. against AlCl$_3$-induced nephrotoxicity, hematotoxicity, and oxidative stress in adult rats. For this reason, phytochemical analyses and in vitro antioxidant tests were performed on the aqueous extract of *Rhamnus* alaternus to estimate the possible beneficial effects. The quantification of the phenolic compounds, indeed, showed RAAE to contain marked levels of total flavonoids, phenols, and tannins, and this concords with previous findings (Boussahel et al. 2013, 2015). These bioactive components are included among the major phytochemical classes responsible for antioxidant properties in plants (Tichati et al. 2021). Accordingly, the antioxidant activity of RAAE was estimated by the scavenging of DPPH radicals, inhibition of bleaching of β-carotene, and reducing power. Findings showed that the extract can act as a radical scavenger and a reductive power, along with inhibitory activity against lipid peroxidation, owing to its ability to donate hydrogen atoms or electrons. Similar results have been reported by Boussahel et al. (2015) and Ammar et al. (2009), who proved that the antioxidant capacity of *Rhamnus alaternus* is due to its richness in phenolic compounds such as phenolic acids and flavonoids (Bakour et al. 2017). Our overall in vitro data suggest the possible use of
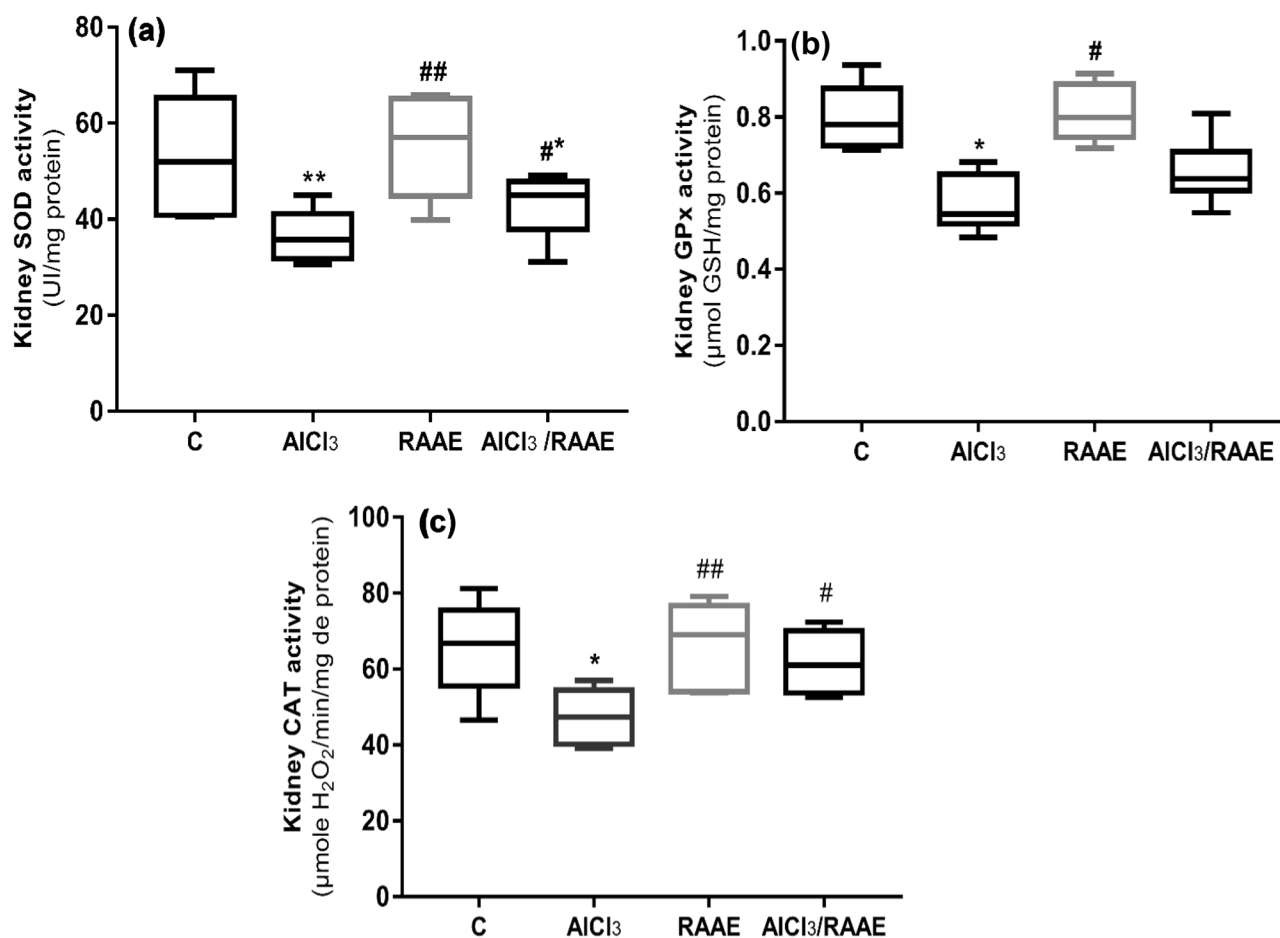
Fig. 3 Kidney antioxidant enzyme activities: superoxide dismutase: SOD (**a**); glutathione peroxidase GPx (**b**) and catalase: CAT (**c**) in control and experimental groups. Values are mean ± SEM for groups of 6 animals each. $AlCl_3$, aluminum chloride; RAAE, *Rhamnus alaternus* L. aqueous extract. Significant difference compared to the control group ($^{**}p < 0.01$, $^{*}p < 0.05$); significant difference compared to the $AlCl_3$ treated group ($^{##}p < 0.01$, $^{#}p < 0.05$)

RAAE to prevent/restore toxic damage caused by ingested aluminum-contaminated diets.

In our study, treatment with $AlCl_3$ caused a disturbance of the hematological profile of rats by decreasing the number of red blood cells, the levels of Hb, and Ht compared to the control group. The deregulation of hematological parameters can be explained by the alteration of the erythropoiesis process via the modulation of renal erythropoietin biosynthesis.

Table 3 The oxidative stress index expressed as the ratio of prooxidant (P)/antioxidants (A) in the kidney of control and experimental rats

Parameters	Experimental groups			
	Control	$AlCl_3$	RAAE	$AlCl_3$/RAAE
P/A	0.0045	0.0096	0.0041	0.0063

P, prooxidant, *A* antioxidants, the oxidative stress index was calculated by determining the prooxidant (P)/antioxidant (A)

$AlCl_3$ may thus have a direct effect on circulating erythrocytes or by interfering with iron cell metabolism, as previously suggested (Bakour et al. 2017). In fact, aluminum can interact in several ways with the hematopoietic system by inhibiting the synthesis of hemoglobin and accelerating the destruction of erythrocytes (Geyikoglu et al. 2013). In addition, this microcytic anemia (modified MCV) may be the result of the effects of $AlCl_3$-generating free radicals that can damage red blood cells through lipid peroxidation. High concentrations of these radicals also make hemoglobin easily oxidized to methemoglobin, thus inhibiting the binding of oxygen to ferric iron (Bakour et al. 2017).

Co-administration of RAAE in the present study restored hematological parameters to comparable values with those obtained in controls. The beneficial effects of RAAE on restoring the toxic effect of $AlCl_3$ could be attributed to the bioactive compounds present in RAAE, including flavonoids such as quercetin and kaempferol (Bhouri et al. 2011; Boussahel et al. 2015). In this regard, recent studies have shown that polyphenols
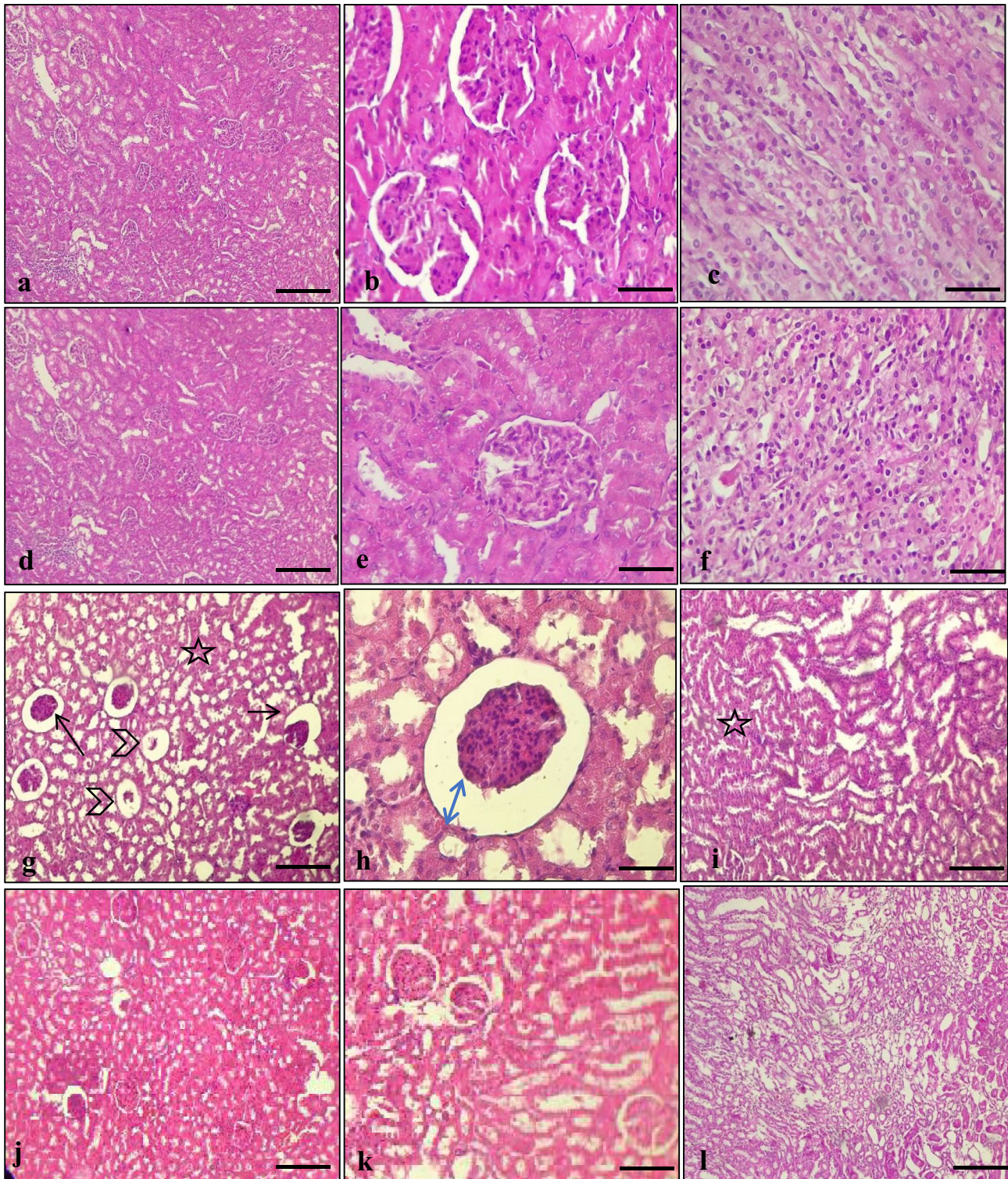
Fig. 4 Photomicrographs of the histological section of kidney tissue in control rats (**a**, **b**, **c**), after administration of RAAE (**d**, **e**, **f**), aluminum (**g**, **h**, **i**) and aluminum RAAE (**j**, **k**, **l**). In control and AE rats, the sections show normal renal parenchyma (**a**, **b** and **d**, **e**) with normal glomerular (**b**, **e**) and normal tubular structure (**c**, **f**). In rats treated with aluminum, the renal tissue shows severe degenerative alterations of glomerule (arrow), congestion (arrowhead) (**g**) with Bowman's space dilatation associated with glomerular atrophy (**g**, **h**, arrow) and tubular hydropic degeneration with presence of tubular necrosis (asterix, **g**, **i**). In aluminum-RAAE-treated rats, slices showed relatively normal renal tissue (**j**, **k**) and tubular structure (l) (H and E staining, magnification×150 and×400, scale bar=50 μm)

and flavonoids repair damaged stem cells and protect against possible hemolysis of erythrocytes by free radicals (Mladenović et al. 2014).

In this study, AlCl$_3$ nephrotoxicity is supported by the kidney function damage evidenced by an incredible increase in the levels of urea, creatinine, and uric acid compared to the controls (AlCl$_3$-free rats), and this was exactly found in a previous study (Al-Kahtani and Morsy 2019). This nephrotoxicity may be due to a significant reduction in the rate of glomerular filtration, resulting in the retention of urea and an accumulation of creatinine in the blood (Al-Qayim and Mashi 2014). The uric acid considered as an indicator of cellular defense against the deleterious effects of oxygenated free radicals (OFRs) can be increased in serum, following the increase of the production of the endogenous antioxidant. These antioxidants may prevent oxidative changes in endothelial enzymes and preserve the ability of the endothelium to mediate vascular dilations to cope with oxidative stress (El Ridi and Tallima 2017).

The increased concentrations of urea and creatinine also show the alteration of the glomeruli and tubules (Al-Kahtani and Morsy 2019), which goes hand in hand with kidney histopathological changes. In accordance with the previous studies (Al-Qayim and Mashi 2014; Al-Qhtani and Farran 2017), AlCl$_3$ ingestion caused tubular and glomerular degenerative aspects accompanied by tubular necrosis and interstitial edema.

Our study, therefore, demonstrates that RAAE supplementation alleviated AlCl$_3$ damage to the kidneys by restoring the glomerular filtration rate, as manifested by notable reductions observed in the levels of urea, creatinine, and uric acid. This nephroprotective effect of RAAE can be attributed to its rich content in phenolic compounds as suggested in other studies (Halzoune et al. 2020). In fact, our in vitro experiments confirm the antioxidant power of *Rhamnus alaternus* in terms of electron donation and its strong potential in preventing oxidation. Furthermore, Boussahel et al. (2015) reported that the predominant flavonoids in RAAE were quercetin and kaempferol. These flavonoids display major healthy features, including diuretic and natriuretic attributes and antiapoptotic and antifibrotic properties (Vargas et al. 2018).

Aluminum exerts its toxic activity through the generation of reactive oxygen species, leading to the disruption of the renal redox status (Al-Qhtani and Farran 2017; El-Demerdash et al. 2020). In our study, treatment with AlCl$_3$ deregulated the pro-oxidant/antioxidant balance, as revealed by the increase in the rate of lipid peroxidation through an over-expression of a high level of MDA, depletion of GSH, and a decrease in the enzymatic expression of SOD, GPx, and CAT involved in tissue damage (Saber et al. 2015; Al-Kahtani and Morsy 2019). The decrease in SOD activity in kidneys can be explained by the overuse of this enzyme, contributing consequently to insufficient elimination of superoxide anion radicals from the cell media and thus leads to an excess of ROS. The overproduction of these radicals has an inhibitory effect on the enzymes responsible for the elimination of ROS such as GPx and CAT (Al-Kahtani and Morsy 2019).

The addition of RAAE thus prevented or restored the side effect of AlCl$_3$. Indeed, the RAAE-treated rats demonstrated a recovery of GSH content, a decreased concentration of MDA, and restored antioxidant enzymatic activity by both SOD, GPx, and CAT. Our findings thus confirm the ability of RAAE to restore the imbalances caused by AlCl$_3$. These effects may be partly due to phenolic compounds that are capable of interacting with biological systems (Trea et al. 2020), which have been shown to decrease oxidative stress and possibly inhibit the oxidation of lipids through direct free radical scavenging, metal chelation, activation of antioxidant enzymes, and inhibition of enzymes associated with a free radical generation (Salehi et al. 2020).

Conclusion

The results of this study showed that AlCl$_3$ induced hematological changes, kidney damage, and oxidative stress in the kidneys of male rats; co-administration of RAAE alleviated the adverse effects of AlCl$_3$, resulting in improved hematological and biochemical/antioxidant parameters of the kidneys. The mechanism of this protective effect is due to its activity of inhibiting lipid peroxidation and activation of antioxidant enzymes. The bioactive compounds of RAAE that are responsible for these effects have not been isolated in the present study. Therefore, additional studies should be carried out to identify these active compounds.

Author contribution Tichati L.: conducting a research and investigation process, specifically performing the experiments. Benzaid C: collected and identified the plant, performed the extraction of *Rhamnusalaternus*aqueous extract and phytochemical analysis. Trea F.: involved in antioxidant marker determination, histopathological analysis, and participated in the discussion of results. Rouabhia M.: application of statistical and review. Ouali K.: supervised the project, wrote and submitted the manuscript, as well as responded to the reviewer's comments and suggestions. All the authors have read and approved this manuscript before submission.

Declarations

Ethical approval and consent to participate All the protocols used in this study were conducted according to the International Guidelines for Laboratory Animal Care and Use (Council of European Communities) (JO86/609/CEE) and approved by the Ethical Committee of Directorate General for Scientific Research and Technological Development at Algerian Ministry of Higher Education and Scientific Research, permit no. PNR/SF 08/2012.

Consent for publication All the authors consent to the publication of the manuscript.

Conflict of interest The authors declare no competing interests.

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