


ORIGINAL CONTRIBUTION

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Rhamnus alaternus aqueous extract enhances the capacity of system redox defence and protects hepatocytes against aluminum chloride toxicity in rats

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Abstract

Background: This study was designed to evaluate the protective effects of a *Rhamnus alaternus* aqueous extract (RAAE) on aluminum chloride-induced hepatotoxicity in rats. A preliminary phytochemical study and antioxidant activity tests of the extract were performed.

Methods: A preliminary phytochemical study and antioxidant activity tests of the extract were performed. For the in vivo study, twenty-four male rats were divided into four groups. The control group (C); the RAAE group treated with 250 mg/kg b.w RAAE; the AlCl₃ group, which received 50 mg/kg b.w AlCl₃; and the AlCl₃/RAAE group that was treated with AlCl₃ plus RAAE.

Results: The RAAE contains several phenolic compounds. This plant extract showed a high radical scavenging effect and high antioxidant activity. Administration of AlCl₃ resulted in a significant increase in the activities of aspartate aminotransferase and alanine aminotransferase (AST, ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) and significant decreases in the plasma concentrations of total proteins and albumin. Moreover, AlCl₃ induced a hepatic pro-oxidant effect leading to an increase in malonaldehyde (MDA) and carbonyl protein contents, the depletion of the content of reduced glutathione (GSH) and a decrease in the antioxidant enzymatic activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). However, RAAE supplementation with AlCl₃ treatment significantly decreased the levels of MDA and carbonyl proteins and markedly restored the activities of the antioxidant enzymes. These results are supported by the improvement in liver tissue restoration.

Conclusions: The *Rhamnus alaternus* aqueous extract was shown to have effective antioxidant activity owing to its phenolic compounds protecting against AlCl₃-induced liver oxidative damage.

Keywords: Aluminum Chloride, *Rhamnus alaternus* Aqueous Extract, Oxidative Stress, Antioxidant, Hepatotoxicity, Rat

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Introduction

Aluminum (Al) is a ubiquitous metal found in our daily life due to its presence in the natural environment and its large use in food, cosmetic products, and water purification and for therapeutic purposes [1, 2]. Human tissues such as the skin, respiratory tract and intestine are barriers to Al entry into the blood. However, Al contamination can occur through fluids given parenterally [3]. Inappropriate levels of Al in the human body could cause serious health safety problems [4, 5], and aluminum may accumulate in the liver [6], kidneys [7] and brain [8]. It has been reported that the presence of Al in these organs causes damage through the generation of reactive oxygen species [9]. ROS can induce the oxidation of cell macromolecules (lipids, proteins, and nucleic acids) and alter the normal redox status of the cell [10, 11]. The liver is a vital metabolic organ that is able to maintain energy levels, metabolize xenobiotics and ensure the structural stability of the body [12]. Liver dysfunction or failure usually leads to health disorders [13]. Al can affect the liver by causing a loss in redox homeostasis, which consequently leads to pro-/antioxidant imbalance resulting in the production of high levels of ROS [9, 14]. Al hepatotoxicity is associated with histopathological alterations, including cell necrosis and increased inflammatory cell infiltration [15, 1]. Al intoxication treatment is performed with the chelating agent deferoxamine or malic acid [16, 17]. Experimental studies have suggested that the use of antioxidants and free radical scavengers such as selenium, melatonin, boric acid, and vitamin C can prevent the deleterious effects of free radicals produced as a result of Al intoxication [18, 19]. Plant extracts may ameliorate the toxicosis caused by Al exposure, as they contain several antioxidant molecules [20, 21]. One plant used against Al toxicosis could be *Rhamnus alaternus* L. This shrub belongs to the Rhamnaceae family, known under the non-vernacular of Nerprun and Tamazight Amliles. It is widely used in popular medicine for its digestive, diuretic, laxative, astringent, and hypotensive activities and for treating hepatic (jaundice) and dermatological complications [22]. Previous studies have shown that the crude extract of *R. alaternus* has multiple biological properties, including antioxidant, anti-mutagenic, antiproliferative, anti-genotoxic [23–25], and antimicrobial properties, and it is rich in polyphenols [26] and flavonoids [27]. Therefore, the present study aimed to investigate the protective antioxidant effects of the *R. alaternus* aqueous extract against sub-chronic exposure to aluminum chloride-induced oxidative hepatotoxicity in rats.

Materials and methods

Chemicals and reagents

Reagent grade 98 % pure aluminum chloride ($AlCl_3$), the Folin–Ciocalteu phenol reagent, catechin, the free stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene, thiobarbituric acid (TBA), reduced

glutathione (GSH), and DTNB [5,5'-dithiobis-2-nitrobenzoic acid] were purchased from Sigma Chemical Co. (St. Louis, France).

Plant materials

The study was conducted on the aerial parts of *R. alaternus* plant leaves and stems that were collected in February 2019 from the town of Tizirt (Tizi-Ouzou city, Northeast Algeria).

The aerial parts (leaves and stems) were cleaned with distilled water, dried in the dark, and then pulverized in a mechanical grinder to obtain a fine powder. The aqueous extract of *R. alaternus* leaves was prepared by the decoction method [28]. Briefly, 500 g of plant powder was used in a flask containing 2000 ml of distilled water, and the decoction continuously refluxed for two hours.

Thereafter, the decoct was filtered through Whatman No. 1 filter paper, centrifuged at 2500 g for 5 min, and evaporated in vacuo using rotary evaporator rotavapor. The residue was collected, weighed to determine the yield, and then maintained under sterile conditions at $-20\text{ }^{\circ}\text{C}$ until use.

Determination of total phenolic compounds

The determination of total polyphenol content was performed by the Folin–Ciocalteu colorimetric method as described elsewhere [29]. The concentration of the total polyphenols was determined from the linear regression equation of the calibration curve with gallic acid: $Y = 0.0138x + 0.0352$, $R^2 = 0.9927$, and expressed as micrograms of gallic acid equivalents (GE) per milligram of extract ($\mu\text{g GE}/\text{mg}$ of extract).

Determination of total flavonoids

Quantification of the flavonoids in the extract was performed by the aluminum trichloride method as described by Pourmorad et al. [30]. The level of flavonoids was deduced from the calibration curve of quercetin ($Y = 0.0111x + 0.0116$, $R^2 = 0.994$) and expressed as micrograms of quercetin equivalents (QE) per milligram of extract ($\mu\text{g QE}/\text{mg}$ of extract).

Determination of condensed tannins

The contents of condensed tannins were determined by the acidic vanillin method [31]. The level of condensed tannins was deduced from the calibration curve of catechin (standard solution) according to the equation $Y = 0.0111x + 0.0741$, $R^2 = 0.9847$, and expressed as micrograms of catechin equivalents (CE) per milligram of extract ($\mu\text{g CE}/\text{mg}$ of extract).

DPPH radical scavenging activity

The anti-free radical activity of *R. alaternus* plant extract was evaluated by a 1,1-diphenyl-2-picrylhydrazyl (DPPH)

trapping test. Of note, DPPH is defined as a relatively stable free radical [32]. The antioxidant activity, indicating the free-radical scavenging ability of the extract, is expressed as the percent DPPH discoloration according to the following equation: $AA (%) = [(Abs\ Control - Abs\ sample) / Abs\ control] \times 100$. $AA (%)$ refers to the antioxidant activity of DPPH and Abs refers to the absorbance at 517 nm.

Reducing power assay

The reducing power of the extract was determined according to the method of Pan et al. [33] based on the chemical reduction of Fe^{3+} in the complex $K_3Fe(CN)_6$ to form Fe^{2+} .

β -carotene bleaching assay

The ability of the *R. alaternus* aqueous extract to prevent β -carotene bleaching was determined according to the method of Ismail et al. [34]. The antioxidant activity ($AA (%)$) is expressed as an inhibition percent in relation to the negative control using the following equation:

$$AA\% = [1 - (At_0 - At_{120})_{test} / (At_0 - At_{120})_{control}] \times 100,$$

where At_0 refers to the absorbance at time zero, and At_{120} refers to the absorbance at 120 min.

Animal studies

Twenty-four male Wistar albino rats weighing 220 ± 2 g were obtained from Pasteur Institute (Algiers, Algeria). They were kept in the animal house of our institution (Department of Biology, Badji-Mokhtar, Annaba University) under an adequate atmosphere: a temperature of 22 ± 2 °C, a 12 h/12 h light/dark cycle, and stable hygrometry. Animals were provided free access to water and an energetically balanced and healthy diet (ONAB; Bejaia, Algeria).

Study design

After two weeks of adaptation, the animals were equally divided into four main groups and then subjected to various treatments for four weeks. Each treatment consisted of the delivery of one millilitre of the appropriate solution through daily gavage.

Group 1 (control group): rats received distilled water; Group 2 (RAAE): rats were orally administered 250 mg of *R. alaternus* aqueous extract (RAAE) per kg body weight (b.w); Group 3 ($AlCl_3$): rats were orally administered 50 mg $AlCl_3$ /kg b.w; Group 4 ($AlCl_3$ + RAAE): rats received combined treatment (50 mg $AlCl_3$ + 250 mg RAAE/kg b.w).

The selected doses of RAAE and $AlCl_3$ were determined from what has been previously used in other

studies [28] and [35]. All experimental procedures were performed according to international guidelines for the care and use of laboratory animals [36]. After the treatment period (4 weeks), the rats were sacrificed by decapitation, and blood samples were collected into heparin tubes followed by 10 min of centrifugation at 4000 g and 4 °C for biochemical analyses. Additionally, the liver of each animal was removed, weighed, and divided into two pieces: one piece was placed in buffered formalin for histological examination, and the other piece was kept in a -80 °C freezer until antioxidant marker analysis.

Biochemical parameters

The plasma biochemical parameters (total proteins, albumin, transaminases (AST, ALT), alkaline phosphatase (ALP), total bilirubin, gamma-glutamyl transferase (γ -GGT), and lactate dehydrogenase (LDH)) were determined by kinetic-colorimetric methods using commercially available kits from SpinReact (Spain).

Homogenate preparation

Liver samples were homogenized in the presence of phosphate-buffered saline. Briefly, 1 g of liver tissue was ground, homogenized in 2 ml of phosphate buffer (1/2 w/v; 1 g tissue with 2 ml PBS, pH = 7.4), and then centrifuged at 10,000 g, and 4 °C for 15 min. The obtained supernatant was used for the determination of the malondialdehyde (MDA), reduced glutathione (GSH), protein carbonyl (PCO), and protein levels. We also measured SOD, GPx, and CAT enzymatic activities using the collected supernatants.

Non-enzymatic antioxidant parameters

The hepatic reduced glutathione (GSH) content was assayed according to the method of Ellman modified by Jollow et al. [37]. The hepatic malondialdehyde (MDA) content was determined following the method of Buege and Aust [38]. The level of carbonyl proteins (CPO) was measured by the method of Levine et al. [39], and the level of hepatic proteins was determined according to the method of Bradford [40] using Coomassie blue reagent.

Enzymatic antioxidant activity parameters

The enzymatic activity of dismutase (SOD) was determined according to the method of Beyer and Fridovich [41]. Glutathione peroxidase (GPx) and catalase (CAT) were determined according to the protocols outlined by Flohe and Gunzler [42] and Aebi [43], respectively.

Histopathological examination

Histological study of the liver was performed according to the routine technique described by Hould [44]. In

brief, liver tissue specimens of each animal were fixed in 10 % buffered formalin solution for 24 h, dehydrated, and embedded in paraffin. Thereafter, the liver sections were sliced into 5 μm thick sections, mounted on slides, stained with haematoxylin-eosin and subjected to light microscopy (LEICA DM, 1000) observation.

Statistical study

Experiments were performed with three repetitions in a fully randomized design and are displayed as the mean \pm SEM. Comparisons of multiple groups were analysed for statistical significance by one-way analysis of variance (ANOVA) with Tukey's post hoc test. Statistical tests were conducted using GraphPad Prism (Ver 7, California, USA), where $p < 0.05$ was considered significant.

Results

Yield and total phenolic, flavonoid and tannin contents of the RAAE

Following phytochemical screening analyses, we determined that the yield of the aqueous extract of *R. alaternus* was 15.73 % (Table 1). The level/mg of dry extract for polyphenols was 64.11 μg GE/mg of extract; for flavonoids, this value was 12.62 μg QE/mg of extract; and for tannins, it was 8.39 μg CE/mg of extract (Table 1).

Antioxidant activity of RAAE

As shown in Table 2, the aqueous extract of *R. alaternus* had a marked free-radical scavenging effect by DPPH assay with an IC_{50} value of 60.08 $\mu\text{g}/\text{ml}$. The standard antioxidants ascorbic acid and BHT showed IC_{50} values of 7.24 $\mu\text{g}/\text{ml}$ and 37.85 $\mu\text{g}/\text{ml}$, respectively. Additionally, the aqueous extract exhibited a reducing iron power ($\text{EC}_{50} = 43.06 \mu\text{g}/\text{ml}$) that was superior to that of BHT (56.13 $\mu\text{g}/\text{ml}$), and the extract's inhibition of β -carotene oxidation showed an IC_{50} of approximately 52.34 $\mu\text{g}/\text{ml}$.

Effect of treatment on hepatic functional biochemical markers

Data related to the effects of the treatments on the variation in biochemical parameters of hepatic function are reported in Table 3. Treatment with AlCl_3 caused a significant decrease ($p < 0.05$) in the plasma concentrations of total proteins and albumin compared to the control. In addition, the level of total bilirubin and the enzymatic activities of AST, ALP, LDH, ALT, and γ -GT increased in the AlCl_3 -treated group compared to the control

group (Table 3). Interestingly, co-administration of RAAE to rats attenuated the hepatic biochemical alterations observed in rats receiving AlCl_3 alone. Moreover, the co-treatment group showed improvements in the enzymatic activity status, the plasma levels of total proteins and albumin levels.

Effect of treatments on hepatic malondialdehyde and carbonyl proteins

As shown in Fig. 1a and b, AlCl_3 treatment caused depletion in hepatic antioxidant capacity, as evidenced by a significant increase (more than 49.67 %; $p < 0.01$) in MDA (lipid peroxidation marker) and CPO levels (+28.64 %; $p < 0.01$) compared to the control. However, the use of *R. alaternus* at the same time as AlCl_3 significantly ($p < 0.05$) decreased lipid peroxidation, as the level of hepatic MDA decreased from 49.67 to 16.43 % and the level of CPO decreased from 28.64 to 13.86 % compared to AlCl_3 treatment alone (Fig. 1).

Effect of treatments on hepatic reduced glutathione content and antioxidant enzymes

As shown in Fig. 2a, b, c and d, treatment with AlCl_3 caused a highly significant ($p < 0.01$) decrease in the GSH content and enzymatic activities of SOD and GPx, and a significant ($p < 0.05$) decrease in catalase activity in liver homogenates compared to control rats. The use of *Rhamnus R. alaternus* extract alone had no adverse effects on hepatic GSH or the enzymatic activities of SOD, GPx, or catalase (CAT). Interestingly, co-administration of RAAE with AlCl_3 restored the antioxidant enzymatic activities and GSH levels compared with AlCl_3 alone.

Histological evaluation

The control group (Fig. 3 A and B) exhibited normal hepatic histological architecture characterized by a visible central vein and normal hepatocytes with granular cytoplasm. Histological analyses of hepatic tissues from rats fed the aqueous extract of *R. alaternus* showed no structural changes or cell damage (Fig. 3 C and D). However, the AlCl_3 -treated group showed severe hepatic tissue alterations consisting of hepatic degeneration, sinusoidal dilatation with infiltration of mononuclear cells, dilated centrilobular veins, and cell necrosis

Table 1 Yield, total phenolic, flavonoid and tannins contents of RAAE

Extract	Yields (%)	Total phenolics (μg GE/mg DE)	Total flavonoids (μg QE/mg DE)	Condensed tannins (μg CE/mg DE)
RAAE	15.73 \pm 0.22	64.11 \pm 3.18	12.62 \pm 0.85	8.39 \pm 0.48

Values are means of three replications \pm SEM

RAAE: *R. alaternus* aqueous extract; GE, gallic acid equivalents; QE, quercetin equivalents; CE, catechin equivalents; DE, dry extract

Table 2 DPPH free radical scavenging activity, reducing power, and β -carotene bleaching inhibition of RAAE

Extract/Standard	EC ₅₀ values (μ g/ml)		
	DPPH scavengingActivity	β - Carotenebleaching inhibition	Reducing power
AA	7.24 \pm 0.45	NT	8.52 \pm 0.63
BHT	37.85 \pm 1.12	20.46 \pm 0.84	56.13 \pm 1.22
RAAE	60.08 \pm 2.09	52.34 \pm 1.16	43.06 \pm 1.90

Values are means of three replications \pm SEM; NT: Not tested

AA Ascorbic acid, BHT Butyl hydroxytoluene, RAAE *R. alaternus* aqueous extract; EC₅₀: Extract concentration corresponding to 50% of the antioxidant activity or 0.5 of absorbance in reducing power assay

(Fig. 3E, F and G). These adverse effects due to AlCl₃ were almost restored after *Rhamnus al R. alaternus* extract treatment. There was also a decrease in the inflammatory response with the use of the *R. alaternus* extract (Fig. 3 H and I).

Discussion

In this in vitro and in vivo (animal model) study, we demonstrated a possible protective effect of the *R. alaternus* aqueous extract (RAAE) against AlCl₃-induced oxidative liver damage as previously reported [27]. The beneficial antioxidant properties of the *R. Alaternus* aqueous extract can be attributed to the phenolic compounds present, as previously reported for various plant extracts [25]. The *R. alaternus* extract we used in this study had strong DPPH free-radical scavenging activity, owing to its ability to provide hydrogen atoms [23] and is considered a primary antioxidant that is able to delay or inhibit β -carotene bleaching [45]. Additionally, its reducing power is a significant indicator of its potential antioxidant activity [46]. Moreover, sub-chronic aluminum chloride-exposed rats (50 mg/kg bw for 30 days) exhibited marked hepatotoxicity that manifested as a significant decrease in the levels of total proteins and albumin.

This can be attributed to the increased intracellular concentration of aluminum in the liver that caused a decrease in protein synthesis [35]. The inhibitory effects of AlCl₃ on the protein profile demonstrated in this study are in accordance with those reported by Albendea et al. [47], who showed that proteins react with free radicals generated by AlCl₃. These proteins can be denatured, fragmented, or lose their chemical structure, and thus, lose their biological activities. We demonstrated that the decrease in the levels of protein transporters is associated with liver tissue damage, as evidenced by a significant increase in AST, ALT and LDH activity. This finding could support the hepatic histopathological modifications found, resulting in leakage of liver enzymes into the bloodstream following alterations in cell membrane permeability [48, 14]. Furthermore, increased enzymatic activity of alkaline phosphatase (ALP) in the blood generally describes primary liver biliary cirrhosis or an alteration of the hepatic architecture [49], since the rise in total bilirubin level might be due to erythrocyte haemolysis or disruption of hepatic function [2]. Remarkable AlCl₃-induced alterations in biochemical parameters (decreased plasma concentrations of total proteins and albumin and increased AST, ALT and PAL activities) were confirmed by the

Table 3 Plasma hepatic function parameters

Parameters	Experimental groups			
	Control	AlCl ₃	RAAE	AlCl ₃ /RAAE
TP (g/dl)	8.95 \pm 0.56	7.08 \pm 0.38*	9.02 \pm 0.64#	8.27 \pm 0.43
ALB (g/dl)	5.23 \pm 0.28	3.44 \pm 0.17*	5.32 \pm 0.26#	4.57 \pm 0.20
TB (mg/L)	01.05 \pm 0.07	2.13 \pm 0.15**	01.12 \pm 0.10##	1.53 \pm 0.14*
AST (U/L)	114.34 \pm 7.03	146.53 \pm 9.64**	111.09 \pm 6.75**	123.43 \pm 6.12#
ALT (U/L)	43.26 \pm 2.64	56.23 \pm 3.17*	41.09 \pm 3.01#	50.32 \pm 2.87
ALP (U/L)	108.39 \pm 7.03	168.05 \pm 11.92**	113.12 \pm 5.36##	135.60 \pm 8.35*#
γ -GT (U/L)	02.13 \pm 0.12	2.94 \pm 0.20*	02.10 \pm 0.09##	02.45 \pm 0.16
LDH (U/L)	538.12 \pm 31.95	935.40 \pm 56.32**	548.44 \pm 35.19##	796 \pm 45.76*

Data are expressed as mean \pm SEM (n = 6 rats/group)

AlCl₃: Aluminum Chloride; RAAE: *R. alaternus* aqueous extract; TP: total protein; ALB: albumin; TB: total bilirubin; AST: aspartate transaminase; ALT: alanine transaminase; ALP: alkaline phosphatase; γ -GT: gamma-glutamyltransferase; LDH: lactate dehydrogenase

Significant difference compared to the control group (* p >0.05, ** p >0.01)

Significant difference compared to the AlCl₃ treated group (# p >0.05, ## p >0.01)

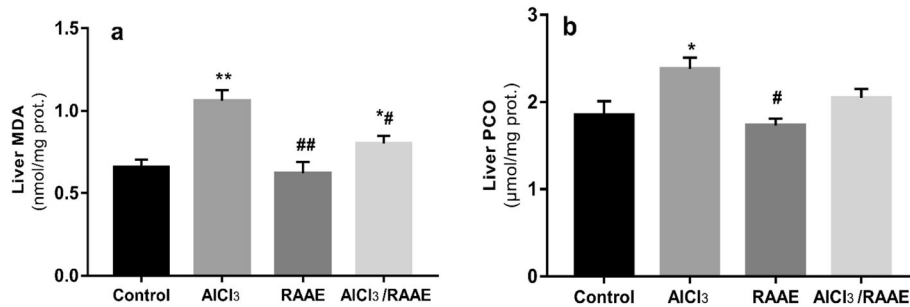


Fig. 1 Hepatic malondialdehyde (MDA) and carbonyl protein (PCO) levels in control and experimental groups. Data are expressed as mean \pm SEM (n = 6 rats/group). AlCl₃: Aluminium Chloride; RAAE: *R. alaternus* aqueous extract; Significant difference compared to the control group (* p <0.05, ** p <0.01); Significant difference compared to the AlCl₃; Significant difference compared to the AlCl₃ treated group (# p <0.05, ## p <0.01)

histopathological observations reported in this study, showing cell necrosis, tissue degeneration, sinusoidal dilatation, and congestion of the centrilobular vein. These results are in line with those previously reported [14, 21].

It should be noted that co-ingestion of RAAE and AlCl₃ restored the levels of total proteins and albumin. RAAE decreased the activity of hepatic enzyme markers, contributing to the protection of the structural integrity of the hepatocellular membrane. The beneficial effects of RAAE could be attributed to the high level of phenolic compounds, including alkaloids and flavonoids [22, 27], confirming its effectiveness in preventing liver damage induced by various xenobiotics [28, 50].

We showed that the over-induction of oxidative stress due to AlCl₃ intoxication is strengthened by our study of lipid peroxidation and protein carbonyls, which are correlated with decreased hepatic antioxidant enzyme activities. In addition, AlCl₃ treatment caused alterations in the antioxidant status, resulting in a significant increase in the levels of MDA and PCO, which reflects the oxidation of lipids and proteins shown previously [10, 51]. We demonstrated that AlCl₃ decreased the levels of hepatic glutathione (GSH), leading to a pro-/antioxidant imbalance, as previously reported [35, 14]. Glutathione, an abundant tripeptide, plays a key role in detoxifying free radical species, maintaining the intracellular redox state, and

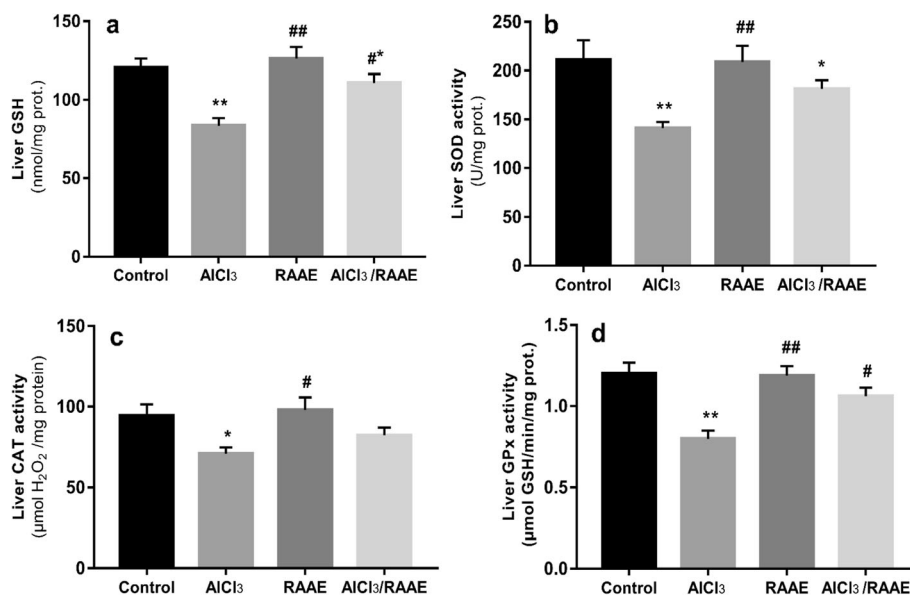


Fig. 2 Reduced glutathione content (a), and SOD (b), CAT(c), and GPx (d) activities in liver of control and experimental groups. Data are expressed as mean \pm SEM (n = 6 rats/group). AlCl₃, Aluminium Chloride; RAAE, *R. alaternus* aqueous extract; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase. Significant difference compared to the control group (** p <0.01, * p <0.05); Significant difference compared to the AlCl₃ treated group (## p <0.01, # p <0.05)

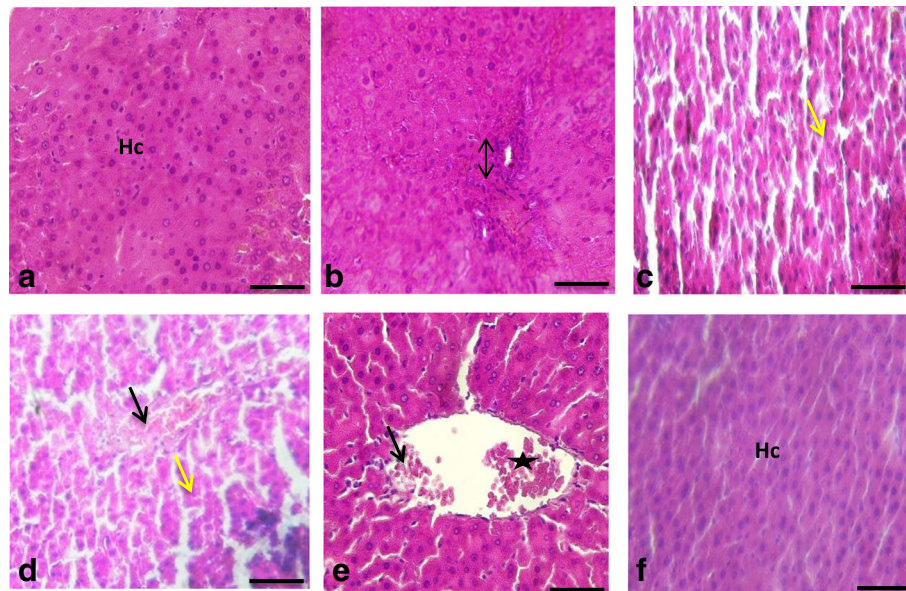


Fig. 3 Photomicrograph of H&E sections of liver of control and AE treated rats showing normal hepatic cells with well-defined nuclei and cytoplasm (Hc), normal hepatic vein(double arrow) (a, b). Section of Aluminum treated rat liver showing severe degenerative alterations with a necrotic anuclear hepatocytes (yellow arrows), infiltration of lymphocyte and Kupffer cells (black arrows), congestion and presence of necrotic area (*) (c, d, e). Section of Aluminum-AE treated rat liver showing a clear improvement compared to the control and AE groups (f) with a reorganization of hepatocytes structure (Hc) (H&E staining, magnification 150X and 300X, scale bar = 50 μ m)

enabling the regeneration of some anti-radical molecules from their oxidized forms (e.g., vitamins C or E); its decrease compromises hepatic function [52]. In our study, the decrease in the hepatic antioxidant defence system was confirmed by the decreased activities of antioxidant enzymes (SOD, CAT, and GPx), which are considered the first line of defence against oxidative damage [53].

Interestingly, co-administration of RAAE and AlCl_3 significantly decreased the levels of MDA and carbonylated proteins while improving the level of GSH and the activities of antioxidant enzymes. The beneficial effects of RAAE could be due to the presence of bioactive molecules, including flavonoids, as previously reported [23, 27]. Flavonoids have been reported to prevent the depletion of GSH content using glutathione disulfide (GSSG) following ROS trapping [54, 55]. Additionally, flavonoids prevent lipid peroxidation through their redox properties, as highlighted by their ability to provide electrons and hydrogen atoms. Additionally, they could act by chelating transition metals, such as copper and iron [56, 57]. These last results confirmed the efficacy of the *R. alaternus* extract in reducing/preventing the adverse effects of AlCl_3 with an in vivo study. Indeed, rats receiving RAAE showed decreased hepatic histological damage induced by AlCl_3 . These results suggest hepatocyte cell repair and strengthens the beneficial use of RAAE to overcome the hepatic toxicity induced by AlCl_3 .

Conclusions

This study showed that the aqueous extract of *R. alaternus* attenuated oxidative damage and lipid peroxidation induced by AlCl_3 . The mechanism of this hepatoprotective effect could be linked to its antioxidant potential and its ability to increase the activity of antioxidant enzymes.

Abbreviations

RAAE: *Rhamnus alaternus* aqueous extract; AlCl_3 : Aluminum chloride; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; LDH: Lactate dehydrogenase; MDA: Malonaldehyde; GSH: Reduced glutathione; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; CAT: Catalase

Authors' contributions

Benzaid C: Conducting a research and investigation process, specifically, performing the experiments. Tichati L: collected and identified the plant, and performed the extraction of *Rhamnus alaternus* aqueous extract and phytochemical analysis. Trea F: Involved in antioxidants markers determination, histopathological analysis and participated in the discussion of results. Rouabhia M: Application of statistical and reviewing. Ouali K: supervised the project, wrote and submitted the manuscript, as well as responded to the reviewer's comments and suggestions. All authors have read and approved this manuscript before submission.

Funding

This research received no specific grant from any funding agency in commercial or not-for-profit sectors. This research was supported by the National Fund for Scientific Research of Algeria (Laboratory of Environmental Bio surveillance) and by the Ministry of Higher Education and Scientific Research of Algeria (PRFU project D01N01UN230120180020).

Availability of data and materials

All data and analyzed outcomes are available with the corresponding author if requested.

Declarations

Ethics approval and consent to participate

The study was 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.

Consent for publication

All authors consent to the publication of the manuscript.

Competing interests

The authors declare that there are no conflicts of interest.

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Received: 6 August 2020 Accepted: 11 July 2021

Published online: 23 July 2021

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