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Nigella sativa Oil Reduces Aluminium Chloride-Induced Oxidative Injury in Liver and Erythrocytes of Rats

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Abstract The present study was planned to investigate the protective effects of *Nigella sativa* oil (NSO) supplementation against aluminium chloride ($AlCl_3$)-induced oxidative damage in liver and erythrocytes of rats. Simultaneously, a preliminary phytochemical study was affected in order to characterize the bioactive components containing in the NSO using chemical assays. The antioxidant capacities of NSO were evaluated by DPPH assay. The results showed that NSO was found to contain large amounts of total phenolics, flavonoids and tannins. Twenty-four rats were equally divided into two groups, in which group A received standard diet, whereas group B treated daily with an oral gavage dose of 2 ml NSO/kg body weight. After 5 weeks pretreatment, both groups were divided again into two subgroups (A and B) of six animals each and treated for other 3 weeks. Therefore, subgroup A1 was served as a control which received standard diet, but subgroup A2 received $AlCl_3$ (34 mg/kg bw mixed with food). Subgroup B1 received both $AlCl_3$ and NSO; however, subgroup B2 received NSO only. Results showed that $AlCl_3$ exhibited an increase in white blood cell counts and a marked decrease in erythrocyte counts and haemoglobin content. Plasma aspartate transaminase, alanine transaminase, alkaline phosphatase and lactate dehydrogenase activities and total bilirubin concentration were higher in $AlCl_3$ group than those of the control, while albumin and total protein concentration were significantly lower. Compared to the control, a significant raise of hepatic and erythrocyte malondialdehyde level associated with a decrease in reduced glutathione content, glutathione peroxidase, superoxide dismutase and catalase, activities of $AlCl_3$ treated rats. However, the administration of NSO alone or combined with $AlCl_3$ has improved the status of all parameters studied. It can be concluded that $AlCl_3$ has induced the oxidative stress, altered the biochemical parameters and the hepatic histological profile, but the supplementation of NSO has alleviated such toxicity.

Keywords Aluminium chloride · *Nigella sativa* oil · Rats · Liver · Erythrocytes · Oxidative stress · Biochemical parameters

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Introduction

Aluminium (Al) is a very abundant metal in the earth's crust which constitutes 8.13 %. It is a constituent of cooking utensils, medicines such as antacids, cosmetics such as deodorants and food additives. Also, it can be found in food especially corn, yellow cheese, salt, herbs, spices and tea. In addition, Aluminium salts are widely used as flocculants in the treatment of drinking water for purification purposes which allowed its easy access into the body via gastrointestinal tract and lung tissue [1, 2]. It accumulates particularly in liver and brain than in bone, muscles, heart or lungs. Liver is the major organ responsible for metabolism, detoxification and secretory functions of the body. Hence, it regulates various important metabolic functions in mammalian systems. Some papers

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indicate that Al accumulates in liver and causes harmful effect [3, 4]. Exposure of rats to Al induced oxidative stress with an increase in hepatic malondialdehyde (MDA) levels, a decrease in glutathione (GSH) concentrations and in glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) activities [5, 6]. In addition, Al induced changes in haemato-biochemical parameters of animal models especially rats and rabbits [8].

Several previous studies were carried out to clarify the exact mechanism of Al cytotoxicity. These data reported that Al potentiates the activity of Fe^{+2} and Fe^{+3} ions and disturb iron metabolism; in fact, it is able to be bound to transferrin because of most closely Al^{+3} ionic radii resemble those of Fe^{+3} induce Fe^{+2} accumulation in cells, which cause an excessive free radical generation, resulting in oxidative deterioration of lipids, proteins and DNA [1, 9]. These disturbances are correlated to oxidative stress which is defined as an impairment of the physiological prooxidant/antioxidant balance. Several authors [2, 10, 11] indicated that an excessive and prolonged Al exposure is directly related to a generation of many health disorders.

Liver diseases are among the most serious health problems in the world today, their prevention and treatment options still remain scarce despite tremendous advances in modern medicine. The pathogenesis of hepatic diseases as well as the role of oxidative stress and inflammation therein is well recognized [12]. Consequently, blocking or retarding the chain reactions of oxidation and inflammation development could be promising therapeutic strategies for prevention and treatment of liver injury. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant and the incidence of oxidative stress damages [13, 14]. Diet supplementation with natural antioxidants can serve as a type of preventive medicine; for this reason, researches that deal with the determination of natural antioxidant sources are important [5, 14, 15]. Plants have been the basis of traditional medicines throughout the world for thousands of years. Therefore, the number of reports focused on identification, isolation and testing of natural antioxidants from plants has increased immensely during the last decade [16, 17].

Nigella sativa commonly known as the black seed is an annual herbaceous plant belonging to the Ranunculaceae family. It was used in the Middle and Far East as a natural remedy since ancient times. It is one of the most famous herbs known for its wide range of the healing capabilities which are well known for more than 2,000 years. Recently, the effect of *Nigella sativa* oil (NSO) has been evaluated in animal studies [16–18]. Many reports are focused to investigate the pharmacological and therapeutic effects of different parts of this plant. Studies have reported the occurrence of fats, fatty acids, essential oils, enzymes, proteins, peptides, alkaloids, saponins, phenols and polyols in seeds of *N. sativa* and its oil [19–21]. Also, the same researchers have been shown that these compounds are thought to be responsible for multisystemic beneficial actions including hypoglycaemic, hypocholesterolemic and antioxidant effects. Bourgou et al. [21] indicate that phenolic compounds are the most important pharmacologically active constituents of *N. sativa* seeds and its oil, that have been shown to be capable of scavenging free radicals and protecting lipids from being oxidized or destroyed during oxidative damage. The role of NSO against aluminium toxicity has not so far been studied. Therefore, the present study was undertaken to investigate the preventive effect of NSO against aluminium chloride ($AlCl_3$)-induced oxidative stress in rats' liver and erythrocytes, and to determine the possible use of this oil in alleviating $AlCl_3$ damages.

Materials and Methods

Plant

Nigella sativa Oil Resource

N. sativa oil was provided from a local commercial market (it was produced by Ets Aboulkacem Company, BPN°118, les sept merveilles, Algiers, Algeria). The company produces NSO by cold pressing of fresh seeds without using chemicals.

Total Phenolic Contents

Total phenolic content was determined using the Folin-Ciocalteu method of Wolfe et al. [22]. Briefly, 10 μl diluted extract solution was shaken for 5 min with 50 μl of the Folin-Ciocalteu reagent. Then 150 μl of 20 % Na_2CO_3 was added, and the mixture was shaken once again for 1 min. Finally, the solution was brought up to 790 μl by adding distilled water. After 90 min, the absorbance at 760 nm was measured. Gallic acid was used as a standard for the calibration curve. The phenolic content was expressed as milligram gallic acid equivalent/100 g of 100 of NSO using the linear equation based on the calibration curve.

Measurement of Free Radical-Scavenging Action

This assay was determined following the method of Blois [23] with some modifications. Various dilutions of NSO were mixed with ethanolic diphenylpicrylhydrazyl (DPPH) solution. After an incubation period of 30 min at 25 °C in the dark, the absorbance at 515 nm was recorded as A_{sample}. A_{blank}. Experiment was also carried out applying the same procedure to a solution without the test material. The free radical-scavenging activity of each solution was then calculated as percent inhibition according to the following equation: % inhibition=100 $(A_{blank}-A_{sample})/A_{blank}$. The antioxidant activities of NSO were expressed as IC_{50}, defined as the

concentration of the test material required to cause a 50 % decrease in initial DPPH concentration.

Scavenging of Superoxide Anion

Scavenging of superoxide anion was determined by the method of Yen and Chen [24] with some modifications. The superoxide anion was assayed by the oxidation of riboflavine and the reduction of nitro blue tetrazolium (NBT), where the absorbance was read at 560 nm. All solutions were prepared in a phosphate buffer (1 M, pH 7.4). The scavenging activity was calculated as follows:

PI %=$[(A_{blank}-A_{sample})/A_{blank}]\times 100$, where A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of NSO. The IC_{50} was calculated from the plot of the inhibition percentage against the NSO concentration.

Total Flavonoid Contents

Flavonoid contents were determined according to the method of Zhishen et al. [25]. Two hundred fifty microlitres of NSO or standard solution was mixed with 1.25 ml of distilled water and 75 μl of 5 % $NaNO_2$ solution. After 6 min, 150 μl of 10 % $AlCl_3$ solution were added. Five minutes later, 0.5 ml of 1 M NaOH solution was added and then the total volume was made up to 2.5 ml with H_2O. Following thorough mixing of the solution, the absorbance against blank was determined at 510 nm. Quercetin was used for standard curve construction (0.05–0.5 mg/ml, $y=4.032x+0.004$, $r^2=0.99$, where y is the absorbance and x is the standard concentration). The results were expressed as milligram quercetin equivalents (QE)/g extract.

Total Tannin Contents

The method of Julkunen-Tiitto [26] was followed in this assay. An aliquot (50 μl) of NSO or standard solution was mixed with 1.5 ml of 4 % vanillin (prepared with methanol), then 750 μl of HCl (12 M) were added. The well-mixed solution was incubated in the dark at ambient temperature for 20 min. The absorbance against blank was read at 500 nm. Catechin was used to make the standard curve. The results were expressed as milligram catechin equivalents (CE/g extract).

Animals and Experimental Procedure

Twenty-four Wistar albino male rats weighing 235±10 g (7–9 weeks), obtained from Pasteur institute (Algiers, Algeria) were used for the experimental procedures. Animals were acclimated for 2 weeks under the same laboratory conditions of photoperiod, and an average relative humidity of 40 % and room temperature of 23±2 °C. Food (standard food, supplied by the "ONAB, El Harrouch", Algeria) and water were available ad libitum.

Study Groups

Animals were randomized into two groups of 12 animals each. Group A comprised of rats which received standard diet, but group B received daily 2 ml/kg bw of NSO by oral gavage (force-feeding) once per day for 5 weeks.

After 5 weeks of pretreatment, rats of group A were divided again into two subgroups of six individuals each as follows:

- Subgroup A1 (control group): served as control rats.
- Subgroup A2 ($AlCl_3$-treated group): received 34 mg $AlCl_3$/kg bw mixed with food.

Similarly, rats of group B were divided into two subgroups of six animals each as follows:

- Subgroup B1 ($AlCl_3$+NSO-treated group): received both $AlCl_3$ (34 mg $AlCl_3$/kg bw) and NSO (2 ml/kg bw).
- Subgroup B2 (NSO-treated group): received NSO alone.

Though, both subgroups A and B were treated for other additional 3 weeks.

The doses of $AlCl_3$ and NSO were selected on the basis of previous works [5, 8] and [16, 27], respectively.

Samples Preparation

Blood Collection

At the end of the experimental period, animals were overnight fasted, then weighed and they sacrificed by cervical decapitation. Blood samples were immediately collected into two groups of ice-cold polypropylene tubes. The first one contained EDTA and used for determination of haematological parameters. The second group contained heparin which has been centrifuged at 2,200×*g* for 15 min. Plasma samples were stored at −20 °C prior to use for assaying aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total bilirubin, albumin and total proteins.

Preparation of Erythrocytes and Liver Homogenates

The sediment containing erythrocytes were twice suspended in phosphate buffer saline (0.9 % NaCl in 0.01 M phosphate buffer pH 7.4) and centrifuged at 3,000×*g* for 15 min. The hemolysats were then aliquoted and stored at −20 °C before use for antioxidant enzyme activities, and the determination of

malondialdehyde (MDA) and reduced glutathione (GSH) levels.

Livers were quickly removed, washed in 0.9 % NaCl solution and weighed after the careful removal of the surrounding connective tissues, and then, 1 g was homogenized in 2 ml of phosphate buffer solution (PBS: 50 mm Tris, 150 mm NaCl, pH 7.4) in ice-cold condition. Homogenates were centrifuged at 10,000×*g* for 15 min at 4 °C; the supernatants were divided into aliquots and then stored at −20 °C.

Haematological Variables

Haematological parameters (erythrocytes, WBC, HT, PLT) were evaluated by electronic haematological counter (Selectra coulter, Germany).

Biochemical Assays and Analysis

Different biochemical parameters were assayed sepectrophotometrically according to appropriate standardized procedures, using commercially available kits from Spinreact (Spain, refs: AST-1001160–1001161, ALT-1001170–1001171, ALP-1001130–1001131, LDH-1001260, Total bilirubin-1001044, Albumin-1001020–1001023 and Total proteins-1001291).

Estimation of Lipid Peroxidation Levels

The lipid peroxidation (LPO) levels of liver and erythrocyte homogenates were measured as MDA, the end product of lipid peroxidation, which react with thiobarbituric acid (TBA) as a TBA reactive substance (TBARS) to produce a red-coloured complex at 532 nm according to Buege and Aust [28]. The absorbance was read at 530 nm.

Reduced Glutathione (GSH) Levels

Reduced glutathione contents of liver and erythrocyte homogenates were estimated using a colorimetric technique, as mentioned by Jollow et al. [29] which based on the development of a yellow colour when DTNB [5,5′-dithiobis-(2-nitrobenzoic acid)] was added to compounds containing sulfhydryl groups. The absorbance was recorded at 412 nm.

Estimation of Antioxidant Enzymes Activities

Glutathione peroxidase (GPx) activity was measured according to the procedure of Flohe and Gunzler [30] at 420 nm.

Catalase activity (CAT) was measured according to the method of Aebi [31]. This assay is based on the ability of the enzyme to induce the disappearance of hydrogen peroxide monitored by following the decrease in the absorbance at 240 nm for 1 min.

The superoxide dismutase (SOD) activity was determined using the method of Asada et al. [32]. SOD activity was evaluated by measuring of its ability to inhibit the photoreduction of nitro blue tetrazolium (NBT). The reaction started by switching on the light, changes in absorbance was recorded at 560 nm after 20 min.

Protein Assays

Protein supernatants concentration of liver and erythrocytes was measured spectrophotometrically at 595 nm according to the method of Bradford [33], using bovine serum albumin as standard.

Histopathological Examination

Liver was dissected and immediately fixed in formol solution for 24 h, processed by using a graded ethanol series, and then embedded in paraffin. The paraffin sections were cut into 5-μm thick slices and stained with hematoxylin and eosin (H&E) for light microscopic examination by Hould [34]. The sections were viewed and photographed.

Statistical Analysis

All data are expressed as mean±SD for six rats of each group. Significant differences between the group's means were determined by ANOVA followed by Student's *t* test. The statistical signification of difference was taken as $p \leq 0.05$.

Results

Antioxidant Activity of *Nigella sativa* Oil

The assessment of antioxidant activity (DPPH) showed an IC_{50} value of 59.90 % (Table 1). However, the IC_{50} value of NSO on superoxide radical-scavenging activity was 86.10 %.

Table 1 Amounts of antiradicalar DPPH, anion superoxide-scavenging activity, total phenols content, total flavonoids and condensed tannins in *Nigella sativa* oil

Total phenolic content (mg GAE/100 g of NSO)	0.38±0.01
50 % scavenging concentration (mg/ml) on DPPH radical	59.90±2.52
50 % scavenging concentration (mg/ml) on superoxide anion	86.10±2.23
Total flavonoid content (mg QE/g NSO)	0.50±0.01
Condensed tannins (mg CE/g NSO)	0.40±0.01

GAE gallique acide equivalents

QE quercetin equivalents

CE catechin equivalents

The total phenolic content of liophylic NSO product was 0.38 mg of CAE/100 g of extract as also shown in Table 1. The total flavonoid content was expressed as 0.50 mg of quercetin equivalents per gram of NSO. Besides, the results showed that NSO contained 0.40 mg of condensed tannins, which expressed as milligrams of catechin equivalents per gram.

Effects of Treatments on Haematological Parameters

Data of haematological parameters in control and treated groups are shown in Table 2. Compared to the control, rats treated with $AlCl_3$ had significantly lower erythrocyte counts and Hb content; however, a significant increase was noted in WBC counts. No statistically significant changes were observed in all haematological parameters when control group was compared with $AlCl_3$+NSO group.

Effects of Treatments on Plasma Biochemical Markers

$AlCl_3$-treated rats showed a significant increase in plasma AST, ALT, ALP, LDH and total bilirubin compared to those of controls (Table 3). Fewer perturbations were noted concerning these parameters in the $AlCl_3$+NSO group compared to $AlCl_3$ group. Albumin and total protein concentrations were decreased in $AlCl_3$-treated group compared to the control and also to the $AlCl_3$+NSO group. In contrast, treatment with NSO alone resulted in a significant amelioration of these parameters.

Effects of Treatments on Lipid Peroxidation

Figure 1 revealed that liver and erythrocyte malonaldehyde (MDA) contents were significantly increased in $AlCl_3$ group compared to those of control groups. NSO administered to rats of ($AlCl_3$+NSO) group alleviated lipid peroxidation induced by $AlCl_3$ treatment and modulated significantly the levels of MDA in both tissues compared to $AlCl_3$ group.

Effects of Treatments on GSH Contents

A significant decrease of hepatic and erythrocyte glutathione (GSH) levels was evident in rats exposed to $AlCl_3$ (Fig. 2). Administration of NSO ameliorated GSH levels in ($AlCl_3$+NSO) group compared to that of $AlCl_3$.

Effects of Treatments on Antioxidant Enzyme Activities

Data concerning liver antioxidant enzyme activities (GPx, SOD and CAT) are presented in Table 4. In $AlCl_3$ group, GPx, SOD and CAT activities were decreased significantly compared to the control. Administration of NSO improved enzyme activities in $AlCl_3$+NSO group compared to the $AlCl_3$ group.

Results of various antioxidant enzyme activities measured in erythrocytes are presented in Table 5. In $AlCl_3$ group, GPx, SOD and CAT activities were declined compared to the control, but the supplementation of NSO has lessened such decline.

Histopathological Results

Histopathological examination of $AlCl_3$-treated rats' liver (Table 6 and Fig. 3) revealed an inflammatory cell infiltration (white arrow) with degenerative changes in hepatocytes (black arrow), loss of typical hepatic cord organization and sinusoidal dilatation (star) (Fig. 3b). In contrast, combined treatment of $AlCl_3$+NSO revealed inflammatory cell infiltration (white arrow), a lesser degree of sinusoidal dilatation (star) and normal cells morphology compared to $AlCl_3$-treated group (Fig. 3c). Furthermore, no histological alterations were observed in the liver of NSO-treated group (Fig. 3d) when compared to the control (Fig. 3a).

Discussion

Preliminary phytochemical analysis showed that phenols, flavonoids and condensed tannin existed in the NSO. It was

Table 2 Changes on haematological parameters of control and treated rats

Parameters and treatments	Control	$AlCl_3$	$AlCl_3$+NSO	NSO
Erythrocytes (10^6/μl)	8.96±0.31	7.26±0.32*	8.81±0.23$^{\#}$	8.94±0.56
WBC (10^3/μl)	10.31±1.81	13.01±0.72*	11.31±1.10$^{\#\#}$	12.31±1.00*
Hb (g/dl)	14.81±0.58	12.18±0.53**	14.63±0.50$^{\#\#}$	15.56±1.17
HT (%)	44.55±1.33	43.60±1.50	43.65±0.90	47.01±1.42*
PLT (10^3/μl)	721.66±7.99	688.00±7.11	704.33±6.44	718.66±3.73

Values are given as mean±SD for groups of six animals each. Significant difference: all treated groups compared to the control one (*$p \leq 0.05$, **$p \leq 0.01$), $AlCl_3$+NSO group compared to the $AlCl_3$ treated one ($^{\#}p \leq 0.05$, $^{\#\#}p \leq 0.01$)

Table 3 Effects of treatments on some biochemical parameters in plasma of control and treated rats

Parameters and treatments	Control	$AlCl_3$	$AlCl_3$+NSO	NSO
AST (U/L)	152.71±4.72	296.57±1.87**	221.55±4.04*, #	160.77±4.22
ALT (U/L)	162.36±3.19	209.50±5.67*	141.33±4.31#	132.61±2.28
ALP (U/L)	113.25±2.10	177.47±8.10***	111.80±8.29###	96.00±6.67
LDH (U/L)	704.83±5.14	964.50±5.97***	879.33±2.69**, #	660.00±4.75
Total bilirubin (mg/l)	1.60±0.17	2.05±0.28*	1.50±0.14#	1.33±0.32
Albumin (g/dl)	43.09±3.20	31.19±7.14**	45.30±1.10##	43.73±1.50
Total protein (g/dl)	92.69±4.20	82.26±5.93**	89.58±2.83#	90.38±2.19

Values are given as mean±SD for groups of 6 animals each. Significant difference: all treated groups compared to the control one (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$), $AlCl_3$+NSO group compared to the $AlCl_3$ treated one (#$p \leq 0.05$, ##$p \leq 0.01$, ###$p \leq 0.001$)

apparent that the total flavonoids were the dominant phenolic compounds of the NSO. The stable DPPH radical is widely used to evaluate the free radical-scavenging activity in many plant extracts. The assessment of antioxidant activity showed that the oil extracted from NS was able to scavenge this radical. Results demonstrated also that NSO exhibited significant superoxide anion-scavenging capacity [20, 21, 35].

Compared to the control, administration of $AlCl_3$ induced significant decrease in erythrocytes and Hb count, indicated occurrence of microcytic anemia. This anemia could be explained by the inhibition of erythropoiesis, haemoglobin synthesis reduction and/or increase in the rate of erythrocyte destruction in haematopoietic organs. Also, it could be associated to $AlCl_3$ toxicity which induces iron deficiency and a reduction in heme synthesis. On the other hand, haemoglobin of erythrocytes is a major source of radical production when it interacts with redox drugs or xenobiotics giving rise to superoxide radicals, hydrogen peroxide and in certain cases peroxy radicals leading to membrane lipid peroxidation and hemolysis. Similar results were found in rat erythrocytes exposed to diazinon for 3 weeks [15].

In addition, $AlCl_3$ treatment induced a significant increase in WBC counts, which might be indicative of immune system activation and that may reflect the incidence of tissue oedema and inflammation [7, 8, 36]. The present results were in line with previous reports which demonstrated that heavy metal

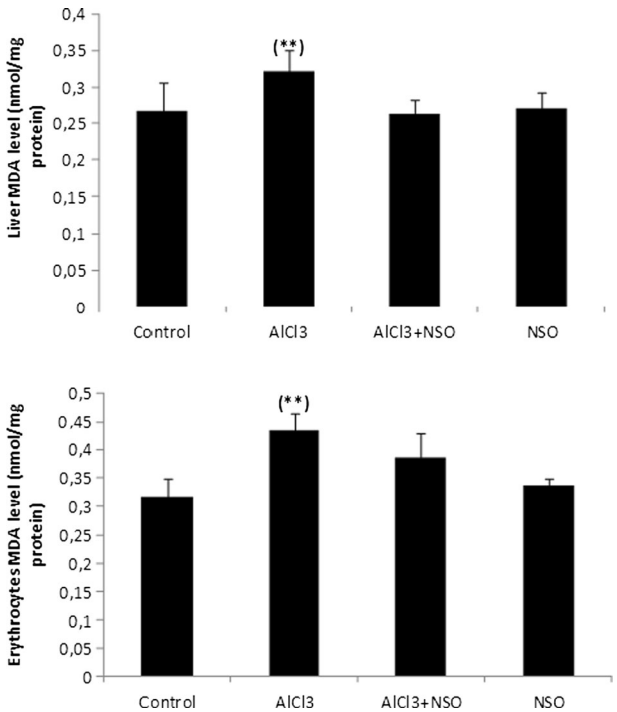

Fig. 1 Malondialdehyde (nmol/mg protein) levels in liver and erythrocytes of control and treated rats. Values are given as mean±SD for groups of six animals each. Significant difference: all treated groups compared to the controls one (**$p \leq 0.01$)

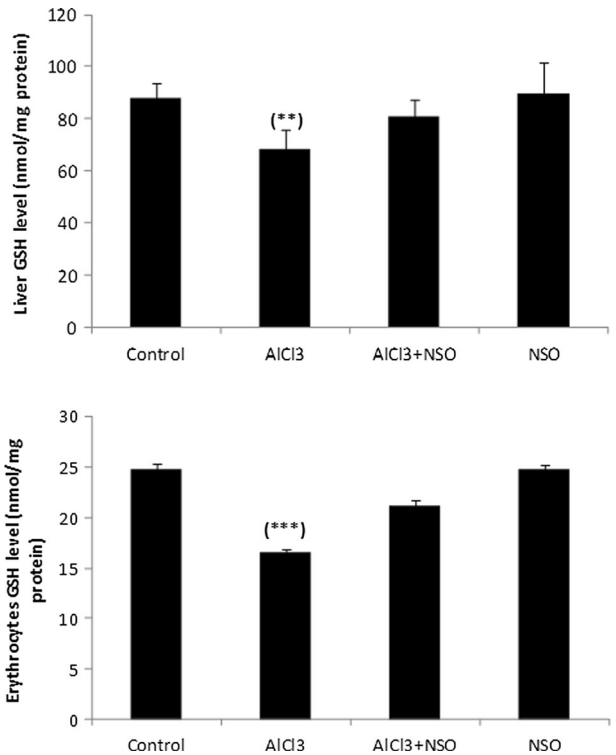

Fig. 2 Reduced glutathione (nmol/mg protein) levels in liver and erythrocyte of control and treated rats. Values are given as mean±SD for groups of six animals each. Significant difference: all treated groups compared to the controls one (**$p \leq 0.01$, ***$p \leq 0.001$)

Table 4 Antioxidant enzyme activities in liver of control and treated rats

Parameters studied	Control	$AlCl_3$	$AlCl_3$+NSO	NSO
Glutathione peroxidase[a]	0.17±0.006	0.14±0.003**	0.16±0.01	0.17±0.01
Superoxide dismutase[b]	0.81±0.09	0.36±0.06***	0.87±0.03$^{\#}$	0.91±0.03
Catalase[c]	7.05±0.09	5.66±0.20*	6.85±0.20	7.03±0.12

[a] Glutathione peroxidase: μmoles of GSH/min/mg protein, [b] superoxide dismutase : units represent the amount of enzyme that inhibits the oxidation of NBT by 50 %/mg of protein. [c] Catalase: μmoles H_2O_2 degraded/min/mg protein

Values are given as mean±SD for groups of six animals each. Significant difference: all treated groups compared to the control one (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$), $AlCl_3$+NSO group compared to the $AlCl_3$ treated one ($^{\#}p \leq 0.05$)

exposure altered erythropoiesis in rats [37, 38]. Thus, the NSO supplementation prevents this alteration, stimulate erythropoietine production and restore the erythropoiesis mechanism. In fact, NSO beneficial effects are probably due to its antioxidant activity causing a decrease of $AlCl_3$ concentration in blood cells, inhibit its entry into erythrocytes and facilitating iron incorporation to the heme group. It is could be also related to the anti-inflammatory effects of NSO reported in several prior studies [18, 39, 40]. The same reports indicate that NSO inhibits eicosanoid generation as well as leukotrienes and histamine release. Though, nigellone is the exact component responsible for this effect [16, 17].

Results obtained from the present work showed a significant increase in plasma AST, ALT, ALP and LDH in $AlCl_3$-treated rats, these findings are in accordance with the previous studies [5, 8]. The increases in enzyme activity may be due to the liver injury or dysfunction which results in the leakage of these enzymes from the liver cytosol into the blood and/or to the disturbance in the balance between biosynthesis and degradation of these enzymes with increased permeability of hepatocyte membrane [5, 41]. Additional effects of $AlCl_3$ treatment revealed a decreased plasma albumin, and increased total bilirubin. Therefore, the significant decrease in albumin concentration could be attributed on the one hand to an under nutrition and on the other hand to a reduction of hepatic protein synthesis, a decreasing plasma total proteins confirm the direct damaging effect of $AlCl_3$. The increase in plasma bilirubin may result from decreased liver uptake, conjugation or increased bilirubin production from haemolysis [5, 8, 11]. In parallel, the present study demonstrates that the supplementation of NSO with $AlCl_3$ produced an effective action against the hepatocyte damages as shown by a decrease of the elevated plasma hepatic key enzymes, total bilirubin levels and by a normalization of both albumin and total proteins. Similar observation was reported by Mahmoud et al., [16], Krishnan and Muthukrishnan [18] and Kokdil et al. [27]. This action could be explained by the ability of NSO and its bioactive constituents to prevent hepatocyte damages, such as cellular necrosis as well as the membrane function failure.

The elevated level of MDA in $AlCl_3$-treated rats could be linked to the peroxidation damages of biological membranes, caused by an increased reactive Fe^{+2} and/or inactivation of enzymes involved in antioxidant defence, a decreased GPx, SOD and CAT activities showed in these results confirmed this theory. Similar findings are reported by Newairy et al. [8], Lukyanenko et al. [11] and Shrivastava [41] who had indicated that aluminium intake produced oxidative stress. Besides, Zhu et al. [37], Nehru and Anand [42] reported that $AlCl_3$ accumulation induced alteration of zinc and copper homeostasis and decreased their binding ability to the antioxidant enzymes which caused antioxidant enzyme dysfunction. Moreover, the decreasing GSH activity might be related to the inhibitor effect of $AlCl_3$ on glutamyl-cysteine-synthetase activity, the enzyme that controls the biosynthesis of glutathione in liver and/or the inhibition of NADPH-generating enzymes such as glucose-6-phosphate dehydrogenase and NADP-isocitrate dehydrogenase [8, 41, 43]. In fact, NSO prevents the formation of reactive oxygen species, causes reduction of lipid peroxidation and stimulates antioxidant

Table 5 Antioxidant enzyme activities in erythrocytes of control and treated rats

Parameters studied	Control	$AlCl_3$	$AlCl_3$+NSO	NSO
Glutathione peroxidase[a]	0.19±0.007	0.15±0.004**	0.18±0.004$^{\#}$	0.20±0.004
Superoxide dismutase[b]	1.36±0.02	0.40±0.06***	0.70±0.01**	1.40±0.01
Catalase[c]	1.86±0.01	1.56±0.05**	1.75±0.04	1.90±0.07

[a] Glutathione peroxidase: μmoles of GSH/min/mg protein, [b] superoxide dismutase : units represent the amount of enzyme that inhibits the oxidation of NBT by 50 %/mg of protein. [c] Catalase: μmoles H_2O_2 degraded/min/mg protein

Values are given as mean±SD for groups of six animals each. Significant difference: all treated groups compared to the control one (**$p \leq 0.01$, ***$p \leq 0.001$), $AlCl_3$+NSO group compared to the $AlCl_3$ treated one ($^{\#}p \leq 0.05$)

Table 6 Semiquantitative scoring of architectural damage on histopathological examination of the rat livers in the different treatment groups

Treatments	Control	$AlCl_3$	$AlCl_3$+NSO	NSO
Degeneration of hepatocytes	−	++	−	−
Sinusoidal dilatation	−	++	+	−
Inflammatory cells infiltration	−	+++	++	−
Loss of typical hepatic cords organization	−	+	−	−

(−) indicates normal, (+) indicates mild, (++) indicates moderate and (+++) indicates severe

defence system [17, 18]. Moreover, several recent studies attributed the antioxidant activity of NSO to their total polyphenolic contents. It has been reported that the antioxidative activity of polyphenols is related to their ability to chelate metal ions and scavenge reactive oxygen species [21, 39].

The biochemical parameters were correlated with the liver histological studies. In fact, the liver histoarchitecture of the $AlCl_3$ treated rats presented by inflammatory cells, infiltration with degenerative changes, loss of typical hepatic cords organization and sinusoidal dilatation. Accordingly, hydroperoxides accumulated in liver could cause cytotoxicity associated with membrane phospholipid peroxidation, the basis for liver cellular damage. These results are in accordance with those obtained by other studies which indicate that metals cause histopathological and enzymatic changes in rats [10, 18]. The co-treatment of NSO improved the histological alterations induced by $AlCl_3$, which could be related to the antioxidant and metal-chelating efficacy of the NSO. This protective effect of black seed oil was reported by Mahdy and Farrag [10] and Krishnan and Muthukrishnan [18] who found that black seed may be successful in the protection of rat liver necrosis.

In the present study, the NSO has been demonstrated to possess excellent antioxidant activities by various in vitro and in vivo assays. The various in vitro antioxidant tests proved that NSO possesses components such as polyphenols and flavonoids with scavengers of free radicals. Also, this study clearly indicates that $AlCl_3$ affects both haematological and biochemical parameters as well as antioxidative system inducing oxidative stress. Co-administration of NSO reduces this disturbance particularly hepatotoxicity due to its large therapeutic proprieties linked to the presence of several phenolic compounds, such as flavonoids. Further biochemical investigations are needed to ascertain the precise mechanisms of its action.

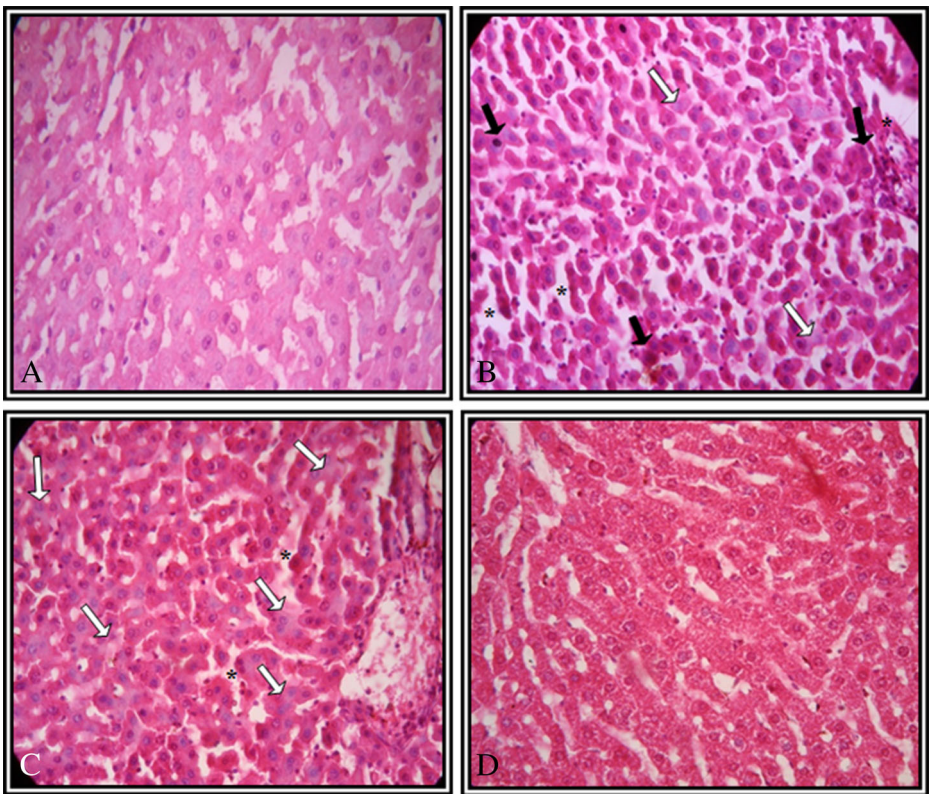

Fig. 3 Photomicrograph of H&E stained sections of liver from control rat showing normal histological structure (Fig. 3a× 400). $AlCl_3$-treated rat liver showing inflammatory cell infiltration (*white arrow*) with degenerative changes in hepatocytes (*black arrow*), loss of typical hepatic cords organization and sinusoidal dilatation (*star*) (Fig. 3b×400). NSO co-administrated with $AlCl_3$-treated rat liver showing inflammatory cell infiltration (*white arrow*), a lesser degree of sinusoidal dilatation (*star*) and normal cells morphology compared to $AlCl_3$-treated group (Fig. 3c×400). NSO-treated rat liver showing normal appearance of hepatocytes (Fig. 3d×400)

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Conflict of Interest The authors declare that there are no conflicts of interest.

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Article 2

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Research Article

Antioxidant Effect of Alpha Lipoic Acid on Hepatotoxicity Induced by Aluminium Chloride in Rats

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ABSTRACT

The present study was planned to investigate the ameliorative effects of α-lipoic acid (α-LA) supplementation against aluminium chloride ($AlCl_3$) induced hepatotoxicity in rats. The results showed that rats consuming diets with $AlCl_3$ added had poor growth performance, and most serum hematological indexes were significantly altered compared to the control. Biochemical results showed that lipid peroxidation increased significantly in Al-treated rats, as evidenced by high liver malondialdehyde (MDA) levels. Alteration of the antioxidant system in treated group was confirmed by the significant decline of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) activities and reduced glutathione (GSH) content in liver. Moreover, $AlCl_3$ exposure induced an increase in the activities of the aspartate transaminase (AST), alanine transaminase (ALT), lactate deshydrogenase (LDH) and bilirubin levels, while albumin and total protein were significantly decreased. These results strongly suggest that aluminium affected antioxidant defence system and both haematological and biochemical parameters, co-administration of α-lipoic acid exerted a protective effect against aluminium induced oxidative stress.

Keywords: Aluminium chloride, Alpha-lipoic acid, Biochemical studies, Liver, Oxidative stress, Rat.

INTRODUCTION

Aluminium is very abundant metal in the earth's crust which constitutes 8.13%. It is a constituent of cooking utensils, medicines such as antacids, cosmetics such as deodorants, and food additives. Also it can be found in food especially corn, yellow cheese, salt, herbs, spices and tea. In addition, aluminium salts are widely used as flocculants in the treatment of drinking water for purification purposes.[1,2] Considering the large utilisation of aluminium in different fields, many available data reported that aluminium exposure increased recently, which have allowed its easy access into the body via gastrointestinal tract and lung tissue.[3] A several authors indicate that an excessive and prolonged aluminium exposure affects directly haematological and biochemical parameters, disturbs lipid peroxidation and attenuate the activities of the antioxidant enzymes in plasma and tissues of animals models especially rats and rabbits.[2,4,5] This impairment of the physiological prooxidant/antioxidant balance causes oxidative stress.

Lipoic acid (1, 2-dithiolane-3-pentanoic acid, LA) has been known for a long time as a cofactor of α-ketoacid dehydrogenases.[6,7] This compound is found naturally in our diets but it is synthesized in human cells. In vivo lipoic acid is rapidly converted into its reduced form, dihydrolipoic acid (DHLA).[8,10] Recent studies demonstrated that LA and dihydrolipoic acid can act as potent antioxidants. They can scavenge a number of free radicals both in hydrophilic and lipophilic phases of cell.[11,12] In addition, they were found to be capable of regenerating endogenous antioxidants in the body including Vitamin C, Vitamin E and intracellular reduced glutathione, therefore it has been proposed that both LA and dihydrolipoic acid are a therapeutic agents in the prevention or treatment of pathological conditions mediated via oxidative stress.[13,14] Because of the health problems induced by many environmental pollutants, much effort has been expended in evaluating the relative antioxidant potency of α-LA. Consequently, this study aimed to evaluate (i) the influence whether $AlCl_3$ induced hematological and biochemical perturbations in rats and (ii) the protective role of α-LA in alleviating the detrimental effect of $AlCl_3$ induced toxicity.

MATERIALS AND METHODS

Chemicals

All chemicals used in this study were purchased from Sigma chemical co. (USA).

Animals and experimental procedure

12 rats weighing around 235 ± 10 g were obtained from Pasteur institute (Algiers, Algeria). Animals were acclimated for 2 weeks under the same laboratory conditions of photoperiod (12-h light: 12-h dark cycle), a minimum relative humidity of 40% and room temperature 23±2°C. Food (standard diet, supplied by the "ONAB, El-Harrouch", Algeria) and water were available *ad libitum*. The animals were randomized into three groups of 6 animals each.

- Group **(A, Control group):** served as control rats which received standard diet.

- Group **(B, $AlCl_3$-treated group):** received only $AlCl_3$ (34 mg/kg bw administered in the diet).

- Group **(C, $AlCl_3$+α-LA group):** received both $AlCl_3$ and α-LA (35mg/kg bw) by oral gavage once per day.

During all period of treatment (three weeks), food consumption was measured daily, while the body weights were recorded weekly. The amount of ingested diet was calculated as the difference between the weight of feed that remained in the food bin (D1) and the amount placed 1 day before (D2). These data were then used to calculate the daily average feed intake, according to the formula:

Average feed intake: D_2-D_1

Quantities of $AlCl_3$ ingested by each rat were calculated from daily food consumption. The doses of $AlCl_3$ and α- LA were selected on the basis of previous works[1,15-17] respectively.

Samples preparation

Blood collection

At the end of the experimental period, animals were weighed, overnight fasted and they sacrificed by cervical decapitation. The blood samples were immediately collected into tow ice –cold polypropylene tubes. The first one containing EDTA as anticoagulant and used for determination of haematological parameters. The second tube containing heparin as anticoagulant, which the plasma samples obtained from, by centrifugation (2200g for 15 min) after that the result supernatants were aliquoted and stored at -20°C prior to use for biochemical assay of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatise (ALP), lactate dehydrogenase (LDH), total bilirubin, albumin and total protein.

Preparation of liver homogenate

The liver were quickly removed washed in 0.9% NaCl solution and weighed after the removal of the surrounding-connective tissues carefully, and then, one gram of liver was homogenised in 2 ml of phosphate buffer solution (PBS: 50mm Tris, 150 mm NaCl, pH 7.4) in ice cold condition. Homogenates were centrifuged at 10.000g for 15 min at 4°C, the supernatants were divided into aliquots to use each one for one time and stored at -20°C before being used.

Haematological variables

Haematological parameters were evaluated by electronic haematological counter (selectra coulter, Germany).

Plasma biochemical markers

Transaminases activities, total bilirubin, albumin and total protein levels were determined spectrophotometrically according to appropriate standardised procedures, using commercially available kits from Spinreact (Spain, refs: AST-1001 160-1001161, ALT-1001170-1001171, ALP-1001130-1001131, LDH-1001260, total bilirubin -1001044, albumin -1001020-1001023, total protein -1001291).

Estimation of lipid peroxidation level

The lipid peroxidation (LPO) level in the liver homogenates was measured as malondialdehyde (MDA), which is the end product of lipid peroxidation, and react with TBA as a TBA reactive substance (TBARS) to produce a red colored complex which has peak absorbance at 530nm according to Buege and Aust.[18] 375µl of supernatant were homogenized by sonication with 150µl of PBS, 375µl of TCA-BHT (trichloroacetic acid-butylhydroxytoluene) in order to precipitate proteins and then centrifuged (1000g, 10min, 4°C). 400µl of obtained supernatant were mixed with 80µl of HCl (0.6M) and 320µl of TBA dissolved in Tris solution and the mixture was incubated at 80°C for 10 min. The absorbance of the resultant supernatant was red at 530nm.The amount of MDA was calculated by using an extinction coefficient of 1.56×10^5 $mM^{-1}cm^{-1}$.

Reduced Glutathione level

Reduced glutathione **(**GSH) contents in liver and erythrocyte homogenates was estimated using a colorimetric technique, as mentioned by Ellman[19], modified by Jollow et al.[20], based on the development of a yellow colour when DTNB [(5, 5 dithiobis-(2-nitrobenzoic acid)] was added to compounds containing sulfhydryl groups. In brief, 0.8 ml of liver supernatant was added to 0.2ml of 0.25% sulphosalycylic acid and tubes were centrifuged at 2500xg for 15min. The resulting supernatant (0.5 ml) was mixed with 0.025 ml of 0.01M DTNB and 1ml phosphate buffer (0.1M, pH 7.4). The absorbance at 412 nm was recorded. The amount of GSH was expressed as nmoles of GSH/mg protein.

Antioxidant enzymes activities

Glutathione peroxidase activity

Gutathione peroxidase (GPx) (E.C.1.11.1.9) activity was measured according to the procedure of Flohe and Gunzler.[21] Supernatant obtained after centrifuging 5% liver homogenate at 1500 x g for 10 min followed by 10000 x g for 30 min at 4°C was used for GP_x assay. One ml of reaction mixture was prepared which contained 0.3ml of phosphate buffer (0.1M, pH7.4), 0.2ml of GSH (2mM), 0.1ml of sodium azide (10mM), 0.1ml of H_2O_2 (1mM) and 0.3ml of liver supernatants. After incubation at 37°c for 15min, reaction was determined by addition of 0.5ml 5% TCA. Tubes were centrifuged at 1500 x g for 5 min and the supernatant was collected. 0.2ml of phosphate buffer (0.1M pH7.4) and 0.7ml of DTNB (0.4mg/ml) were added to 0.1ml of reaction supernatant. After mixing, absorbance was recorded at 420 nm.

Catalase activity

The activity of catalase (CAT) (E.C.1.11.1.6) was measured according to the method of Aebi.[22] The reaction mixture 1ml contained a 100mM phosphate buffer (pH 7), 500mM H_2O_2 and liver supernatants. The reaction started by adding H_2O_2 and its decomposition was monitored by following the decreased in absorbance at 240nm for 1

min. The enzyme activity was calculated by using an extinction coefficient of 0.043mM^{-1}cm^{-1}.

Superoxide dismutase activity

The superoxide dismutase (SOD) (E.C.1.15.1.1) activity was determined using a method of Asada et al.,[23] SOD activity was evaluated by measuring of its ability to inhibit the photo reduction of nitro-blue tetrazolium (NBT). One millilitre of homogenate's supernatant was combined 50mM phosphate buffer (pH 7.8), 39 mM methionine, 2.6 mM NBT and 2.7 mM EDTA-Riboflavin, as to obtain a final concentration of 0.26 mM, was added as the last and switching on the light started the reaction, changes in absorbance at 560nm were recorded after 20min. In this assay, one unit of SOD is defined as the amount that inhibits the NBT reaction by 50%. Specific activity was defined as units/mg of protein.

Protein content

Protein supernatants concentration in liver was measured spectrophotometrically at 595 nm according to the method of Bradford[24], using bovine serum albumin as standard.

Statistical analysis

All data are expressed as mean ± SD for six rats in each group. Significant differences between the group's means were determined by paired student's test. The statistical signification of difference was taken as p≤0.05.

RESULTS

Effects of treatments on body and relative liver weights

Changes in total body weight and liver relative's weights are shown in Table1. The total body weight showed a pronounced reduction by 20.09% and 16.068% in rats of $AlCl_3$ treated group compared to the $AlCl_3$+α-LA treated group and to the controls respectively. Besides, a highly significant increase in liver relative weights of $AlCl_3$ treated rats (a hypertrophy of the liver) was noted compared to the $AlCl_3$+ α-LA treated rats and to the controls ones.

Table 1: Changes in body weight (g) and liver relative weights (g/100g bw) of control and treated rats

Parameters studied	Control	$AlCl_3$	$AlCl_3$+ α-LA
Initial body weight (g)	236.33 ± 51.34	242.67 ± 41.63	243.17±29.74
Final body weight (g)	311.17 ± 54.61	261.17 ± 38.20*	326.5±46.85$^{\#}$
Relative liver weight (g/100g bw)	2.63 ± 0.16	3.423 ± 0.48**	2.68±0.22$^{\#}$

Values are given as mean ± SD for groups of 6 animals each. Significant difference; All treated groups compared to the controls one (*p≤0.05, **p≤0.01); $AlCl_3$ + α-LA group compared to the $AlCl_3$ treated one ($^{\#}$p≤0.05).

Effects of treatments on food intake

The decrease in body weight was associated with a reduction in food intake by 10.867% and 12.010% in $AlCl_3$ treated group compared to $AlCl_3$+ α-LA group and to the control respectively (Table 2).

Table 2: Daily food intake and $AlCl_3$ ingested of control and treated rats

Parameters and treatments	Control	$AlCl_3$	$AlCl_3$+ α-LA
Food intake (g/day/rat)	21.14±2.26	18.601±4***	20.87±2.31$^{\#\#\#}$
Quantities of $AlCl_3$ ingested (mg/day/rat)	-	6.324±1.35	7.1±1.35

Values are given as mean ± SD for groups of 6 animals each. Significant difference; All treated groups compared to the controls one (***p≤0.001); $AlCl_3$ + α-LA group compared to the $AlCl_3$ treated one ($^{\#\#\#}$p≤0.001).

Effects of treatments on haematological parameters

As shown in Table 3, hematological parameters in control and treated groups. Red blood cell (RBC), haemoglobin (Hb) content and mean corpuscular haemoglobin concentration (MCHC) in $AlCl_3$ treated group were significantly decreased compared to those in the controls. While, white blood cell number (WBC) were significantly increased than those of the controls. There was no significant effect of adding 34 mg/kg AlCl3 on all haematological indexes in rats treated with $AlCl_3$+ α-LA compared control rats.

Table 3: Changes on haematological parameters of control and treated rats

Parameters and treatments	Control	$AlCl_3$	$AlCl_3$+ α-LA
RBC (10^6/µl)	8.96±0.31	7.26±0.32*	8.80±0.50$^{\#}$
WBC (10^3/µl)	10.31±1.81	13.01±0.72*	11.58±1.12$^{\#}$
Hb (g/dl)	14.81±0.58	12.18±0.53**	14.28±0.57$^{\#\#}$
HT (%)	44.55±1.33	43.6±1.50	43.85±1.78
MCV (mm^3/RBC)	49.66±1.75	49.33±2.16	49.16±1.47
TCMH (pg/RBC)	16.55±0.69	16.30±0.62	16.58±0.39
MCHC (g/dl)	33.26±0.46	31.56±0.60**	33.21±0.62$^{\#}$
PLT (10^3/µl)	721.66±7.99	688.00±7.11	701.83±2.19
VMP	9.50±0.43	9.40±0.42	9.73±0.46

Values are given as mean ± SD for groups of 6 animals each. Significant difference; All treated groups compared to the controls one (*p≤0.05, **p≤0.01); $AlCl_3$ + α-LA group compared to the $AlCl_3$ treated one ($^{\#}$p≤0.05, $^{\#\#}$p≤0.05).

Effects of treatments on plasma biochemical markers

Table 4 showed some biochemical indexes which indicated liver injury in rats. $AlCl_3$ treated rats data

showed a significant increase in plasma AST, ALT, ALP, LDH and total bilirubin by 94%, 29%, 56%, 37%, and 28% respectively compared with control group and then by 50%, 45%, 50% and 22% compared to those of $AlCl_3$+α-LA treated group, while albumin and total protein were decreased in $AlCl_3$ treated group by 27% and 11% compared to the control one and by 28.734% and 3.28% compared to the $AlCl_3$+α-LA group.

Table 4: Effects of treatments on some biochemical parameters in plasma of control and treated rats

Parameters and treatments	Control	$AlCl_3$	$AlCl_3$+ α-LA
AST (U/L)	152.71±4.72	296.571±1.88**	197.32±7.32$^{###}$
ALT (U/L)	162.368±3.94	209.506±5.67*	144.49±10.24$^{###}$
ALP (U/L)	113.253±2.14	177.478±8.09***	117.68±1.17$^{###}$
LDH (U/L)	704.833±5.44	964.5±5.97***	882.66±4.62**
Total bilirubin (mg/l)	1.6±0.01	2.05±0.28*	1.683±0.01$^{##}$
Albumin (g/dl)	43.09±3.20	31.195±7.14**	43.77±2.02$^{##}$
Total protein (g/dl)	92.69±4.12	82.266±5.93**	85.06±5.55

Values are given as mean ± SD for groups of 6 animals each. Significant difference; All treated groups compared to the controls one (*p≤0.05, **p≤0.01, ***p≤0.001); $AlCl_3$ + α-LA group compared to the $AlCl_3$ treated one ($^{##}$p≤0.05, $^{###}$p≤0.001).

Effects of treatments on lipid peroxidation

MDA levels in liver tissue (Figure 1) were increased in $AlCl_3$-treated group compared to those of $AlCl_3$+α-LA-treated group (12%) and the control (21%) ones. $AlCl_3$+α-LA-treated rats did not show any significant changes in liver MDA level compared to the control.

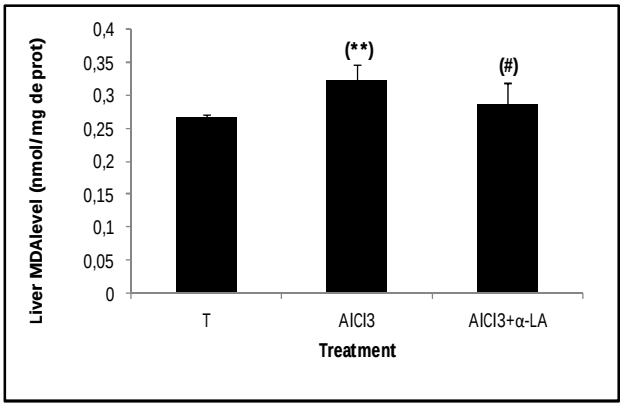


Figure 1: Liver homogenate malondialdehyde (nmol/mg protein) levels of control and treated rats. Values are given as mean ± SD for groups of 6 animals each. Significant difference: All treated groups compared to the controls one (**p≤0 .01). $AlCl_3$+α-LA group compared to the $AlCl_3$ treated one ($^{#}$p≤0.05).

Effects of treatments on GSH content

Results in Figure 2 Showed changes of GSH levels in liver tissue which indicated liver oxidative injury. Exposure rats to $AlCl_3$ produced a significant decline in GSH levels compared to those of $AlCl_3$+α-LA-treated group (14%) and the control (22%) ones.

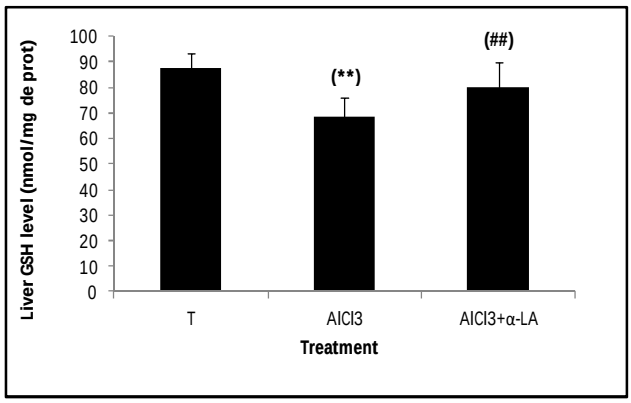
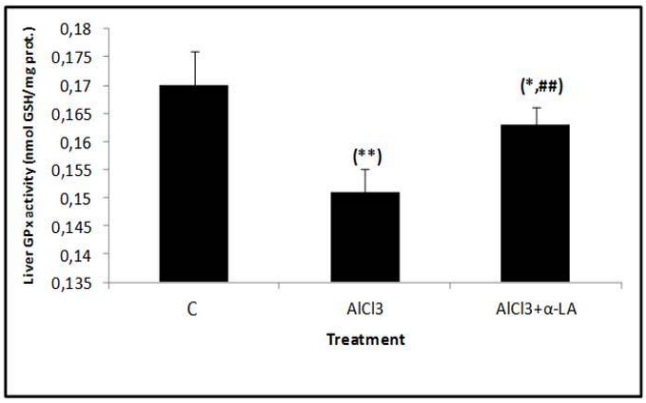
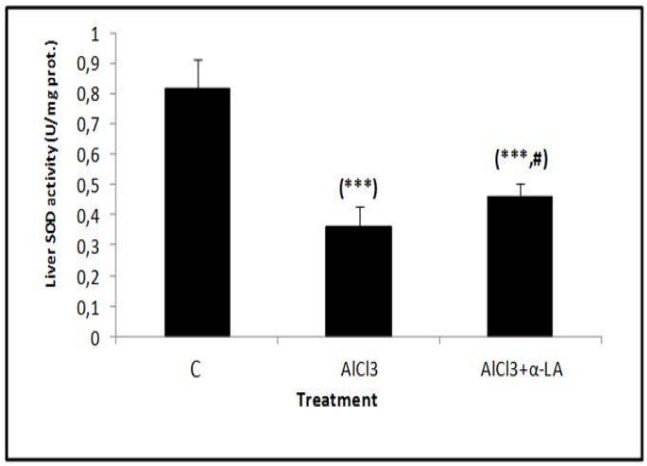


Figure 2: Liver homogenate reduced glutathione (nmol/mg protein) levels of control and treated rats. Values are given as mean±SD for groups of 6 animals each. Significant difference: All treated groups compared to the controls one (**p≤0 .01). $AlCl_3$+ α-LA group compared to the $AlCl_3$ treated one ($^{##}$p≤0.01).

Effects of treatments on antioxidant enzyme activities

Results in Figure 3 showed Changes of GPx, SOD and CAT activities in liver tissue which indicated liver oxidative damage. Exposure rats to $AlCl_3$ produced a significant decline in GPx, SOD and CAT enzyme activities compared to those of control group. In contrast, treatment with 35 mg/kg of α-lipoic acid resulted in a significant amelioration of 4-43% in the enzyme activities (GPx, SOD and CAT).





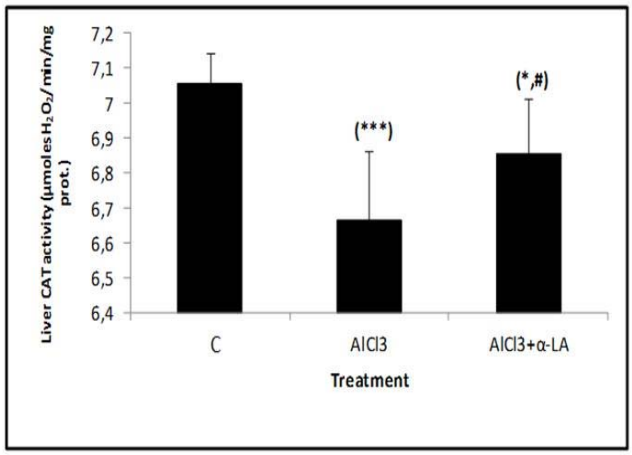


Figure 3: Liver homogenate glutathione peroxidase (nmoles of GSH/min/mg protein), superoxide dismutase (U/mg de protein) and catalase (µmoles H_2O_2/min/mg protein) activities of control and treated rats. Values are given as mean ± SD for groups of 6 animals each. Significant difference: All treated groups compared to the controls one (*p≤0.05), (**p≤0 .01), (***p≤0.001). $AlCl_3$+ α-LA group compared to the $AlCl_3$ treated one ($^{\#}$p≤0.05), ($^{\#\#}$p≤0.01).

DISCUSSION

In our experimental study, reduction in body weight is used as an indicator for the deterioration of rat general health status. It has been reported that $AlCl_3$ could induce toxicological effects and biochemical dysfunctions representing serious health hazards.[2,25,26] The findings from the present work indicate that excessive $AlCl_3$ exposure has changed body weight, absolute and relative liver weights, leading however to significant decrease in animal growth and production performances. This could be probably attributed to the reduction of feed consumption and/or malabsorption of nutrients induced by $AlCl_3$ effects on the gastro-intestinal tract and/or inhibition of protein synthesis.[2,25,26] $AlCl_3$ exposure may account to reduced food intake seen in the $AlCl_3$-treated group. Hence, these findings were similar to the results published by El-Demerdash, and Zhu et al.[1,26] who reported that high Al exposure have significantly induced disturbances of the total body weight, absolute and relative liver weights of rats. Accordingly, among the main approaches used to ameliorate Al-induced hepatotoxicity is the use of agents with powerful antioxidant properties. However, recent studies have reported that lipoic acid showed significant protective effects against tissue damage induced by some xenobiotics like arsenic[8,27], Bisphenol A[28] and adriamycin.[17]

The co-administration of lipoic acid attenuated the in vivo effects of $AlCl_3$ by scavenging or neutralizing ROS. These results indicated that α-LA might have a beneficial role in lowering $AlCl_3$ toxicity probably due to its radical scavenging property. Thus, α-LA treatment corrects the body weight slow-down and the relative liver weight increases. This may be attributed to an increases food intake and /or lipoic acid capability to interfere with the absorption of $AlCl_3$ in the intestine. It can also be suggested that both α-LA and DHLA chelate heavy metals, restricts the molecular damage and reduce hepatic injury.

Exposure rats to $AlCl_3$ decreased hematological parameters (RBCs and Hb) and developed anemia in rats. Our experimental were in line with previous reports which demonstrated that heavy metals exposure altered hematological parameters in rats[29,30] In fact, according to the earlier reports[29,31,32] this anaemia could be explained by the inhibition of erythropoiesis and/or haemoglobin synthesis reduction, the same reports indicate that $AlCl_3$ could be interfering with Fe incorporation to the heme group witch induce Fe deficiency and reduce heme synthesis. Also the observed microcytic anaemia it might be due to an increase in the rate of erythrocytes destruction in haematopoietic organs. So, $AlCl_3$ might be crossing the erythrocyte membrane which is a result of its ability to initiate a lipid peroxidation.[2,5,30] In our study, $AlCl_3$-treated rats also exhibited significantly higher WBC compared with controls rats. This increase might be indicative of the activation of defence and immune system showed that there were oedema and inflammation in the tissues.[5]

The results of the present study showed that α-LA supplementation has potentially beneficial effects on haematological system. Our results are similar to a previous studies reported by Caylak et al.[33] In fact, this beneficial effect are probably due to the direct chelating activity of α-LA which have possibility to decrease the $AlCl_3$ concentration in blood cells and inhibit its entry into erythrocytes, resulting an increase of Fe and facilitate its incorporation to the hem group. It could be also related to the anti-inflammatory effects of α-LA reported in several prior studies.[11,34]

The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from the liver cytosol into the blood due to altered permeability of membrane. In this work, $AlCl_3$-treated rats showed a significant increase in plasma levels AST, ALT, ALP and LDH, which confirmed the liver injury or dysfunction these results are in line with that reported by El-Demerdash[1] and Gaskill et al.[15] Also, increases enzymes plasma levels may be due to the disturbance in the balance between biosynthesis and degradation of these enzymes.[35,36] Additional effects of $AlCl_3$ treatment revealed decreased plasma total protein, albumin and increased total bilirubin as compared to control. This data are in agreement with previous studies.[15,36] So, the significant decrease in the concentration of the albumin could be attributed on the one hand to an under nutrition and on the other hand to a reduction of the protein synthesis in the liver results a decreasing plasma total protein which confirmed the direct damaging effect of $AlCl_3$ on liver cells.[2,36] The increase in plasma total bilirubin may result from decreased liver uptake, conjugation or increased bilirubin production from haemolyse. El-Sharaky et al.[37] found that

the induction rat in bilirubin was associated with free radical production.

On the other hand, lipoic acid prevent the increase in the activities of these enzymes is the primary evidence of their hepatoprotective activity.[7] In the present finding, lipoic acid co-administration produced an effective action against the hepatocytes damaging effects, as shown by a decrease of the elevated plasma hepatic key enzymes (AST, ALT, ALP and LDH) and by a normalisation of albumin, total protein and total bilirubin. The mechanism proposed to explain these results could be attributed to α-LA preventive effects against $AlCl_3$ induced damages in rats hypatocytes.[7,14] So, the α-LA prevents cellular necrosis as well as the membrane failure, this hepatoprotective effects might be related to both its radical scavenging proprieties and indirect effect as a regulator of antioxidative systems.[16, 17, 25]

The increased level of MDA in $AlCl_3$ treated rats could be linked to the peroxidation damages of biological membranes, caused by an increased reactive Fe^{+2}.[38,39] According to Newairy et al.,[15] and Wu et al.[39] study's, aluminium is able to be bound by the Fe^{+3}carying transferrin protein because of most closely Al^{+3} ionic radical resemble those of Fe^{+3}, results an accumulation of Fe^{+2} in cells. Besides[1,38] reported that an increased MDA concentration could be caused by inactivation of enzymes involved in antioxidant system such as GPx, SOD and CAT activities. In fact, $AlCl_3$ accumulation induced alteration of zinc and copper homeostasis and decreasing its binding ability to the antioxidant enzymes which caused antioxidant enzymes dysfunction. The decreasing GSH activity might be related to the inhibitor $AlCl_3$ effect on Glutamyl-cysteine-synthetase activity, the enzyme that controls the biosynthesis of glutathione in liver, thus resulting a reduction in GSH synthesis. This might be coupled to the aluminium inhibiting ability of NADPH-generating enzymes such as glucose 6-phosphate dehydrogenase and NADP-isocitrate dehydrogenase, resulting a slowing down in the GSH regeneration.[15] Oxidative stress occurred as a consequence of imbalance between the production of reactive oxygen species and the antioxidative process in favor of radical production. In the current study, the significant decrease in the antioxidant enzyme activities (GPx, SOD and CAT) in liver proved the failure of antioxidant defense system to overcome the influx of reactive oxygen species generated by Al exposure. α-LA supplementation produced protective effect against antioxidant defense system failure our result is in agreement with data reported from similar studies on rats.[8,28] This study suggested that α-LA antioxidants capacity may be due to its ability to chelate both heavy and transition metals, to scavenge reactive oxygen species by the sulphydryl group to regenerate endogenous antioxidants (such as vitamin C, vitamin E, and GSH) and to repair oxidative damage of cellular macromolecules which prevent the increase in lipid peroxidation level and increasing the antioxidants enzymes activities. Several reports[8,9,14] evidenced that a combination of α-LA could prevent GSH depletion by scavenging reactive oxygen species and /or increasing cysteine uptake, which is a rate limiting step for GSH biosynthesis.

CONCLUSION

This study clearly indicates that $AlCl_3$ affects both haematological and biochemical parameters as well as antioxidative system inducing oxidative stress. Co-administration of α-LA ameliorates this disturbance. In fact, the ameliorative effect of α-LA against oxidative stress in $AlCl_3$ treated rats due to its antioxidant propriety by scavenging free radical and chelating metals as well as regeneration of endogenous antioxidant. Nevertheless, further studies are needed to investigate the precise mechanism(s) of action of α-LA on oxidative stress biomarkers in rat under $AlCl_3$ intoxication.

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