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The effect of *Thymus vulgaris* L. on renal and liver toxicity in wistar rats exposed to aluminum

Nawel Mokrane[1*], Omar Kharoubi[1], Fatima Zohra Tahari[1], Akila Guenzet[2] and Abdelkader Aoues[1]

[1]Laboratory of Experimental Biotoxicology, University of Ahmed Ben Bella "1", Oran, Algeria.
[2]Laboratory of Clinic and Metabolic Nutrition, University of Ahmed Ben Bella "1", Oran, Algeria.

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The main sources of human exposure to aluminum are found in the extensive presence of the metal in the environment and its growing industrial applications. The present study was carried out to determine the effectiveness of the *Thymus vulgaris* L. extract in alleviating aluminum chloride ($AlCl_3$) toxicity on biochemical and antioxidant parameters. The experiment's rats were divided into five groups: a control group; an intoxicated group (300 mg $AlCl_3$/kg bw); and three other groups which were given $AlCl_3$ (300mg/kg/bw) then *T. vulgaris.* L extract *(T.v)*, Malic Acid (MA) and Vitamin E (Vit E) at a concentration of 150mg/kg/bw. Each group was given its respective dosage, daily for 90 days. The results showed a significant decrease in the body/liver/kidney weights, the plasma total protein (T. Protein) and albumin (Alb) levels (p≤0.05) in the intoxicated rats group. However, a significant increase in the plasma uric acid (Uac), alkaline (AlP) and acid phosphatase (AcP) levels was noted (p≤0.05) in the same group. The amount of aluminum, TBARS and nitrate/nitrite (NO) in the liver and kidney tissues of the rats treated with $AlCl_3$ was also found to have increased (p≤0.05), while the levels of glutathione peroxidase (GSH-Px) and glutathione transferase (GSH-St) have decreased significantly (p≤0.05). These altered parameters were restored in the rats treated with the *T. vulgaris* L. extract. Therefore, due to these beneficial effects, *T. vulgaris* L. could potentially be used to antagonize $AlCl_3$ toxicity.

Key words: *Thymus vulgaris* L., aluminum chloride, oxidative stress, malic acid, vitamin E.

INTRODUCTION

Aluminum is a metal used more and more in our daily routine in spite of its toxic impact its biological functions remain unknown. Al has been considered as a toxic agent in many pathological processes such as neurodegenerative diseases (Exley, 2016), mycrocytic anaemia and osteoporosis (Chappard et al., 2016). Once absorbed, Al gathers necessarily in the bones, brain, liver and kidneys (Kinawy, 2019). This metal was considered hurtless since its presence in the trivalent form meant that it would easily attach to ions and forms colloidal polymeric particles. This form of particles is insoluble as a result it was believed that the absorption of the metal would be restricted (Bondy, 2010). In addition, toxic impacts of Al have led to histopathological alterations in

*Corresponding author. E-mail: monawel@hotmail.fr. Tel: +213 (0) 69.73.28.32.

the kidney and liver (Ghorbel et al., 2015). The molecular mechanisms include membrane function disruption, oxidative stress induction and disruption of several metals' metabolism (Hasona and Ahmed, 2017). Therefore, the estimation of antioxidant defense has become an important aspect of investigation of Al toxicity.

There is a growing tendency towards using phytotherapy owing to the general belief that it has no side effects compared to chemotherapy (Geleta et al., 2016). Many natural product extracts have been found to have a variety of pharmacological and antioxidant effects. *Thymus vulgaris* (common thyme), locally known in Algeria as "Zaatar", belonging to the Lamiaceae family, is a perennial herb indigenous in North Africa, Central and Southern Europe. Common Thyme's stems are branching and of a silver-grey color. Its leaves are small, oval and coiled at the edges and very fragrant while its flowers appear from May to September and are of a white or pale pink color. *T. vulgaris* L. has been commonly used since ancient times in the treatment of burns and poisoning caused by snakes and scorpions. It is an aromatic medicinal herb which is widely used in traditional folk medicine for its antimicrobial effects (Hosseinzadeh et al., 2015), anti-inflammatory and antalgic effects, antioxidant benefits (Tural and Turhan, 2017) and its antifungal properties (Benabed et al., 2018). It is also recognized for its antispasmodic, antiseptic, anthelmintic, diuretic and sedative properties (Hosseinzadeh et al., 2015). It is these various benefits of *T. vulgaris* L. that form the motivation behind the current study which aims to evaluate the protective effects of *T. vulgaris* L. against $AlCl_3$ toxicity in rats.

Various acute and chronic metal intoxications treatments are available. These include chelating agents which have been used clinically as antidotes that bind and enhance the excretion of toxic elements. The chelating agent most commonly used in Al disorders is desferrioxamine (DFO), which has a great ability to decrease the Al body toxicity by increasing its excretion (Kruck et al., 2004). However, DFO therapy is associated with toxic side effects, is very expensive and is only efficient intravenously or subcutaneously. Therefore, alternative molecules with the same efficiency as DFO, but ones which can be orally administered were needed. One such alternative is the Malic acid chelator which is a non-toxic and natural compound containing dicarboxylic acid, and magnesium. The chelation abilities of MA-mg against $AlCl_3$ toxicity were assessed when it was administered to mice exposed to Al at about one-fourth of the LD_{50} level. Compared to other chelators, the MA-mg product showed a better therapeutic effectiveness.

Vitamin E (Vit E) (alpha-tocopherol) is a powerful naturel antioxidant. It acts in synergy with other molecules like vitamin C, selenium and zinc. A good intake of vitamin E can neutralize the excess of free radicals and therefore prevent cellular activity disruption which is normally caused by exposure to various stressors. The antioxidant function of this micronutrient could, at least in part, enhance immunity by maintaining the functional and structural integrity of important immune cells (El-Demerdash, 2004; Kutlbay et al., 2007).

The present study was carried out to examine the effects of $AlCl_3$ poisoning on the biochemical and antioxidant parameters of the liver and kidney tissues of rats. Due to the health problems caused by $AlCl_3$ and many other environmental pollutants, various investigations have been undertaken in this study to evaluate the chelator effects offered by the natural Thyme plant and the Malic acid as well as the relative antioxidant potential offered by Vit E. The present study however, focuses on determining the efficiency of these treatments especially that of *T. vulgaris* L. aqueous extract in antagonizing the biochemical alterations and oxidative stress leading to liver and renal dysfunction in male rats.

MATERIALS AND METHODS

Collection of plant material

T. vulgaris L. (Lamiaceae) was collected in June 2014 from the Mostaganem region in the north west of Algeria. The plant's identification was confirmed at the department of Botany of Ahmed Ben Bella University 1 (Oran, Algeria) where a specimen (voucher No. LB 2368) was kept.

Preparation of *T. vulgaris* L. extracts

The aerial parts of the plant (leaves, flowers and stems) were air-dried in the dark away from humidity at room temperature and then ground to fine powder. The powdered aerial parts of *T. vulgaris* L. (5 g) were then extracted with boiled distilled water (500 ml) for 30 min. This water extract was then filtered, lyophilized and stored at -20°C until use. In order to extract the adequate amount of extract for experimental animal, the procedure of preparation was repeated multiple times. The yield of extraction is 20.22%.

Animals and experimental design

Male Wistar rats of four weeks old weighing (70 ± 10 g) were housed in standard cages in groups of six rats each. All groups were kept in a 12 h light/12 h dark cycle, at a room temperature of (22 ± 2°C) and were fed *ad libitum*. All experiments reported in this study were carried out in accordance with current guidelines for the care and use of laboratory animals (8th edition, 2011). All animals within each treatment group were given their respective diet for 90 days as follows:

(i) Group 1 (C): Untreated control group.
(ii) Group 2 ($AlCl_3$): The rats were orally given a solution composed of $AlCl_3 6H_2O$ which was dissolved in distilled water. The daily dose given was 300 mg/kg bw.
(iii) Group 3 ($AlCl_3$ + *T. vulgaris* L.): During the first 45 days, the rats were given to drink by feeding bottle the same $AlCl_3 6H_2O$ solution as Group 2 at a daily dose of 300 mg/kg bw. This was then followed by giving the rats a treatment solution of *T. vulgaris* L. aqueous extract at a daily dose of 150 mg/kg bw for the following 45 days for a total duration of 90 days.
(iv) Group 4 ($AlCl_3$ + MA): The rats in this group were given to drink

the same $AlCl_3 6H_2O$ solution for 45 days which was then followed by a Malate Magnesium chelator solution for 45 days at a concentration of 150 mg/Kg bw.
(v) Group 5 ($AlCl_3$ + Vit E): The rats in this group were given to drink the same $AlCl_3 6H_2O$ solution for 45 days followed by a Vitamin E solution for 45 days at a dose of 150 mg/Kg bw.

T. vulgaris L. (150 mg/Kg) is a tolerated dose and without toxic effect because it represents the (1/30) of LD_{50} (lethal dose of 50% of population) of alcoholic extract which is 5 g/kg of body weight used in the study of El-Newary et al., 2017. In another study (Geleta et al., 2016) researchers tested the dose (10.000 mg/kg) of aqueous extract of *T. shimperi* on acute intoxication also tested the dose of (600 mg/kg) in subchronic toxicity and observed their non-toxicity. Therefore the dose chosen in this study is lower than the LD_{50} previously noted. The animals were observed daily for any signs of toxicity and their body weight was recorded on a weekly basis throughout the experimental period.

Blood collection and tissue sample preparation

At the end of the experimental period and after the administration of the last dose, the rats were left to rest overnight and were sacrificed under pentobarbital anesthesia the following day. The rats' blood samples were then collected, and the liver and kidney organs were harvested, rinsed with a saline solution (0.9% NaCl) and then weighed. The organs' weight ratios were estimated and their relative weight calculated as g/100 g BW. To evaluate the oxidative status, the liver and kidney were homogenized in suitable buffers**:** in 1.15% KCl for thiobarbituric acid reactive substances (TBARS), in a 0.1 M phosphate buffer (pH 7.2) for glutathione transferase (GSH-ST) and glutathione peroxidase (GSH-PX), in a PBS (Phosphate buffered saline, PH 7.4) solution for nitrite estimation In addition, some portions of the liver and kidney were digested with nitric acid to determine the aluminum concentration.

Biochemical parameters

Alkaline phosphatase (AlP), acid phosphatase (AcP), uric acid (Uac) and albumin (Alb), were estimated in serum using Chrono-Lab kits (Spain). Total protein was measured by using bovine serum albumin as a standard (Lowry et al., 1951) Folin and Ciocalteus Phenol reagents were used to develop the blue color that was measured spectrophotometrically at 750 nm.

Lipid peroxidation (TBARS)

Lipid peroxidation in the liver and kidney tissues was estimated by measuring the formed malondialdehyde (MDA) using thiobarbituric acid reactive substances, according to the spectrophotometric method of (Okhawa et al., 1979). Aliquots (0.2 ml) of hepatic or renal homogenates were added to 0.2 ml of 8.1% (w/v) sodium dodecyl sulfate, 1.5 ml of 20% (w/v) acetic acid buffer (pH 3.5), and 1.5 ml of 0.8% (w/v) thiobarbituric acid. After completing the volume with 4 ml of distilled water, the mixture was heated for 1h at 90°C then cooled and centrifuged at 4000 r/min for 10 min. TBARS were measured at 532 nm and expressed as MDA equivalents (nmol/g of Protein) using a molar extinction co-efficient: 1.56×10^5mol/L/cm.

Glutathione transferase (GSH-ST)

Glutathione transferase (GSH-ST) activity in the liver and kidney tissues was assessed using an assay kit provided by Cayman (Chemical, USA).

Glutathione peroxidase (GSH-Px)

Glutathione peroxidase (GSH-Px) activity in the liver and kidney tissues was assessed by the method of Rotruck et al. (1973). Briefly, the reaction mixture contained 0.2 ml of Tris–HCl buffer (0.4 mol/L, pH 7.0), 0.2 ml of reduced GSH (1 mmol/L), 0.1 mL of Sodium Azide (10 mmol/L), 0.1 ml of H_2O_2 (1 mmol/L) and 0.2 ml of tissue homogenates. After incubation of the mixture at 37°C for 10 minutes, the reaction was stopped by the addition of 0.4 ml of 10% trichloroacetic acid, and tubes were subjected to centrifugation at 2400 r/min for 10 min. The supernatant (0.2 ml) was then added with 0.1 ml of Ellman's reagent (0.0198 g of DTNB prepared in 0.1% sodium citrate). The Absorbance was recorded at 340 nm.

Nitrite (NO)

Nitrate Nitrite activity in the liver and kidney tissues was assessed using an assay kit provided by Cayman (Chemical, USA).

Aluminum Measurement

The concentration of $AlCl_3$ was estimated in the liver and kidney organs by using an atomic absorption spectrometer (Shimadzu, AA6200) following a wet acid digestion method as modified for dry-weight samples (Van Ginkel et al., 1990). A fraction of each organ (approximately 100 mg) was heated at 60°C for 24 h to obtain a constant dry weight. This was then placed in weighed flasks and digested with a nitric acid solution (65°) for 8 h. To get a final volume of 4 ml, a nitric acid solution (1% concentration) was then added to determine the aluminum concentration in the sample. The atomic absorption signal was measured by integrating the total absorption profile at 309.3 nm with a spectral bandwidth of 0.5 nm. All the analyses were performed in triplicate, the limit detection to Al is of 0.02 mg/L and the results were expressed in µg/g tissue wet.

Statistical analysis

The results are expressed as a mean ± standard error of the mean (SEM). Data comparison was determined by one-way ANOVA followed by Tukey *post hoc* analysis and the results were considered statistically significant when $p < 0.05$.

RESULTS

Throughout the study, some clinical signs of toxicity were observed in the $AlCl_3$-treated rats at the dose previously indicated. These included; fur loss, urine coloration, as well as a lack of activity (dullness, spirit depression) when compared to the rats in the control group. However, no rat deaths were recorded during the experimental period.

Effect of treatment on body, liver and kidney weights

At the end of the experiment, $AlCl_3$ administration in rats revealed a significant decrease in body weight levels by 19.13% when compared to the control group. A significant decrease ($p \leq 0.05$) is also observed in the absolute liver and kidney tissue weights when compared to the control group as shown in Table 1. However, the group of rats treated with *T. vulgaris* L. showed

Table 1. The Effect of Treatment on Body Weight, Absolute and Relative Liver and Kidney Weights.

Groups	Control	$AlCl_3$	$AlCl_3$ + T.v	$AlCl_3$ + MA	$AlCl_3$ +Vit E
Body weight BW (g)	335.95 ± 15.11	271.65 ± 11.27*	320.53 ± 7.14$^{\$}$	325.95 ± 12.84$^{\$}$	291.18 ±11.48*
Liver Absolute weight (g)	9.690 ± 0.131	7.376 ± 0.244*	8.525 ± 0.399$^{*\$}$	7.668 ± 0.392*	7.616 ± 0.135*
Relative weight (g)	3.007 ± 0.270	2.737 ± 0.155	2.759 ± 0.151	2.573 ± 0.112*	2.659 ± 0.053*
Kidney Absolute weight (g)	2.315 ± 0.054	1.930 ± 0.102*	2.287 ± 0.138$^{\$}$	2.098 ± 0.021*	2.005 ± 0.071*
Relative weight (g)	0.787 ± 0.022	0.716 ± 0.056	0.753 ± 0.041	0.677 ± 0.042*	0.627 ± 0.073$^{*\$}$

Values are expressed as mean ± SEM, n = 6 for each group.
(*) Comparison with the control group. ($) Comparison with AlCl3 group.

Table 2. The Effect of Treatment on Serum Biochemical Parameters.

Parameters	Control	$AlCl_3$	$AlCl_3$ + T.v	$AlCl_3$ + MA	$AlCl_3$ +Vit E
AlP (U/l)	31.81 ± 1.44	47.02 ± 2.3*	31.54 ± 1.73$^{\$}$	36.23 ± 0.83$^{*\$}$	44.56 ± 1.53*
AcP (U/l)	4.94 ± 0.38	13.62 ± 0.44*	5.76 ± 0.87$^{\$}$	8.10 ± 1.06$^{*\$}$	12.97 ± 0.48*
T.Prot (g/l)	54.02 ± 3.64	26.37 ± 3.09*	48.16 ± 1.32$^{*\$}$	32.17 ± 2.36*	29.5 ±2.43*
Alb (g/l)	43.42 ± 4.26	18.81 ± 1.25*	35.83 ± 3.91$^{*\$}$	23.2 ± 2.67*	24.22 ± 3.45*
Uac (g/l)	4.98 ± 0.68	11.55 ± 1.39*	5.89 ± 0.65$^{\$}$	8.98 ± 1.11*	8.3 ± 1.29$^{*\$}$

Values are expressed as mean ± SEM, n = 6 for each group.
(*) Comparison with the control group. ($) Comparison with AlCl3 group.

considerably less weight loss at the end of the experiment ($p \leq 0.05$) when compared to the intoxicated group. A similar improvement, albeit to a lesser extent than the one recorded in the *T. vulgaris* L-treated group, was also observed in the MA and Vit E treated groups.

Effect of treatment on serum biochemical parameters in aluminum-induced hepatic and renal toxicity in rats

Treatment with $AlCl_3$ increased significantly the plasma AlP level by 32.34% when compared to the control group (Table 2). In addition, it was found that the concentration of plasma AcP and Uac has significantly increased by 3-fold in the rats treated with $AlCl_3$. Plasma Alb and T. Protein, however, decreased significantly by (56.68 and 51.18% respectively) in the intoxicated rats group.

Examining the groups treated with either *T. vulgaris* L. or MA, a significant reduction in the plasma AlP (*T. vulgaris* L. 32.92%, MA: 22.95%) and AcP (*T. vulgaris* L. 57.70%, MA: 40.53%) was observed when compared to the intoxicated group. In addition, the Vit E treated group showed a low decrease in the same parameters that soared in the intoxicated group. The concentration of Uac was also significantly reduced by the *T. vulgaris* L. and Vit E treatments (49%, 28.14% respectively). The effect of the MA treatment on this parameter was found to be smaller (22.25%) in comparison. These results were accompanied by an increase in Alb and T. Protein concentrations in the *T. vulgaris* L. treated group (47.50 and 45.24% respectively) when compared to those of the intoxicated group. MA and Vit E treatments only slightly improve these parameters.

Effect of treatment on lipid peroxidation on aluminum-induced hepatic and renal toxicity in rats

As shown in Figure 1, a significant increase in the TBARS level (55.67% in the liver tissue and 62.64% in the kidney tissue) was observed in the intoxicated rats when compared to the control groups. The administration of *T. vulgaris* L. decreased the TBARS production significantly by a rate of 50.64% in the liver and 56.3% in the kidney when compared to the intoxicated rats. Malate and Vitamin E treatments showed less efficient results in term of restoring normal values of TBARS.

Effect of treatment on antioxidant parameters on aluminum-induced hepatic and renal toxicity in rats

The results obtained showed that GST and GPX levels (Figures 2 and 3) were significantly decreased in the liver (62.87 and 57.23% respectively) and kidney (73.23 and 75.18% respectively) of the rats treated with $AlCl_3$. The concentration of NO was also significantly increased ($p \leq 0.05$) as shown in Figure 4. However, liver and kidney GST and GPX levels increased by 2 and 3-fold respectively in the groups treated with *T. vulgaris* L. A significant increase ($p \leq 0.05$) in liver GST and GPX levels

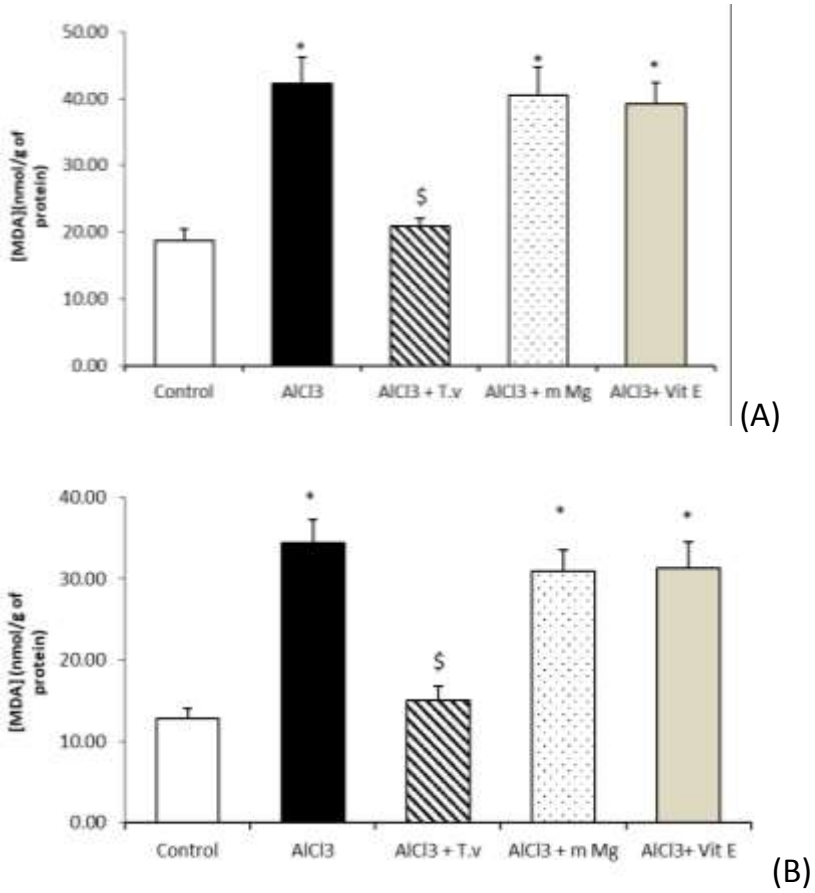

Figure 1. The Effect of Treatment on TBARS level (nmol/g of protein) in the Liver (A) and Kidney (B)
(*) Comparison with the control group. ($) Comparison with AlCl3 group.

was also respectively noted in the groups treated with MA and Vit E when compared to the $AlCl_3$-intoxicated group. In addition, treatment with *T. vulgaris* L. caused a significant decrease in NO concentration (67.46 and 41.96%) in the liver and kidney tissues respectively. Only a slight reduction in liver and kidney ($p \leq 0.05$) NO concentration resulted from the Vit E treatment when compared to the intoxicated group.

Effect of treatment on liver and kidney aluminum content

The $AlCl_3$ concentration in the liver and kidney of rats was measured after 3 months of oral $AlCl_3$ exposure and the results are presented in Table 3. The concentration of $AlCl_3$ in the liver and kidney of the intoxicated group was higher by 3 and 5 fold respectively when compared to the control group. However, this concentration was significantly decreased in liver and kidney of the group treated with *T. vulgaris* L. (32.42 and 59.28%) when compared to the intoxicated group.

DISCUSSION

Aluminum absorbed by the human body via the gastro-intestinal and the respiratory tracts, is known to disrupt the pro-oxidant and antioxidant balance of tissues, leading to various biochemical and physiological dysfunctions (Exley, 2004; Nehru and Bhalla, 2006). In this study, Aluminum Chloride was chosen over other Aluminum compounds because the stomach already contains and utilizes Chloride. Therefore, this form of Aluminum can be introduced with minimal change to gastric fluid composition. The focus of the present study is on the liver and kidney organs where metals are usually accumulated and where toxic effects can be expected.

The results of the study have indicated that body, liver and kidney weights gain in the rats treated with $AlCl_3$ was markedly less ($p \leq 0.05$) when compared to the normal control group. This demonstrates that Al administration has a detrimental effect on the rats' body weight. This finding agrees with the results of other studies where balgoon (2019) noticed a significant reduction of about

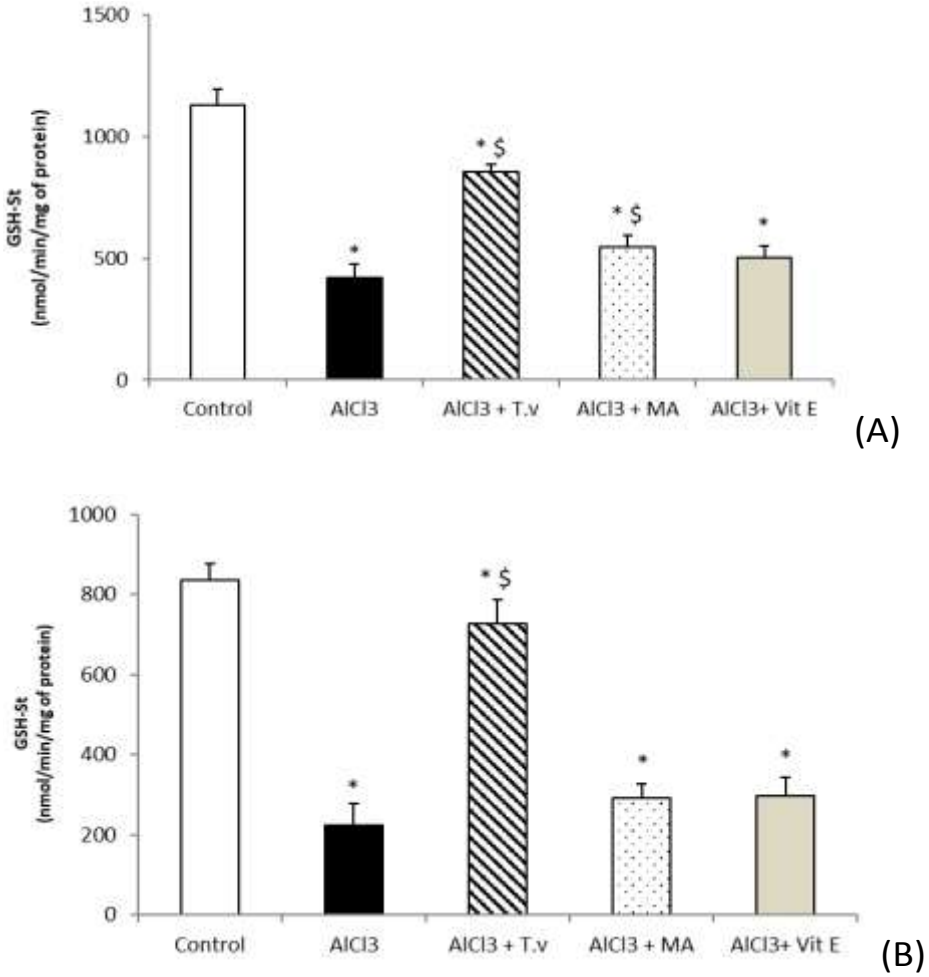

Figure 2. The Effect of Treatment on GSH-St Activity (nmol/min/mg of protein) in the Liver (A) and Kidney (B)
(*) Comparison with the control group. ($) Comparison with AlCl3 group.

3.51% in weight gain in $AlCl_3$-treated rats. Similar results were also obtained by (Singla and Dhawan, 2013). A significant decrease in absolute and relative liver and kidney weights was observed in $AlCl_3$-treated rats which is in agreement with previous reports by (Yeh et al., 2009; Paz et al., 2017). However, the current study has found that rats treated with *T. vulgaris* L. showed a significant improvement in body, liver and kidney weights gain. In the study of Manafi et al. (2014) broilers intoxicated with aflatoxin and treated with Thyme essence showed a significant gain in body weight revealing the same beneficial impact.

Several studies have reported that Al accumulates in mammalian tissues such as brain, bone, liver and kidney (Belaïd-Nouira et al., 2013). As a result, Al causes alterations in the biliary secretory function and an increase in oxidative stress in hepatic tissues (Gonzalez et al., 2004). Such accumulation might be the result of the higher affinity of Al for transferrin, which, in turn, might also explain the interference it causes with iron metabolism (Crichton et al., 2002). The present study results are also consistent with recent findings that showed that chronic Al consumption causes significant plasmatic increases in the activities of AlP and AcP enzymes which could be due to severe damage in the tissue membranes (Esmaeili et al., 2000; Al-Qhtani and Farran, 2017). Moreover, Rahman et al. (2000) suggested that the decrease in the activities of AlP and AcP in different tissues might be due to the increased permeability of the plasma membrane or the cellular necrosis of the liver, kidneys and lungs. In addition, Ochmanski and Barabasz (2000) proposed that the salts of Al could potentially enter inside the cells and might directly bind to the DNA and RNA helices. This prevents their replication and then inhibits the activities of AlP and AcP. In this study, it was shown that an administration of the *Thymus vulgaris* L. aqueous extract has led to a marked reduction in the elevated activities of AlP and AcP in $AlCl_3$ treated rats. In the study of Abd El Kader and Mohamed (2012) rats intoxicated with paracetamol

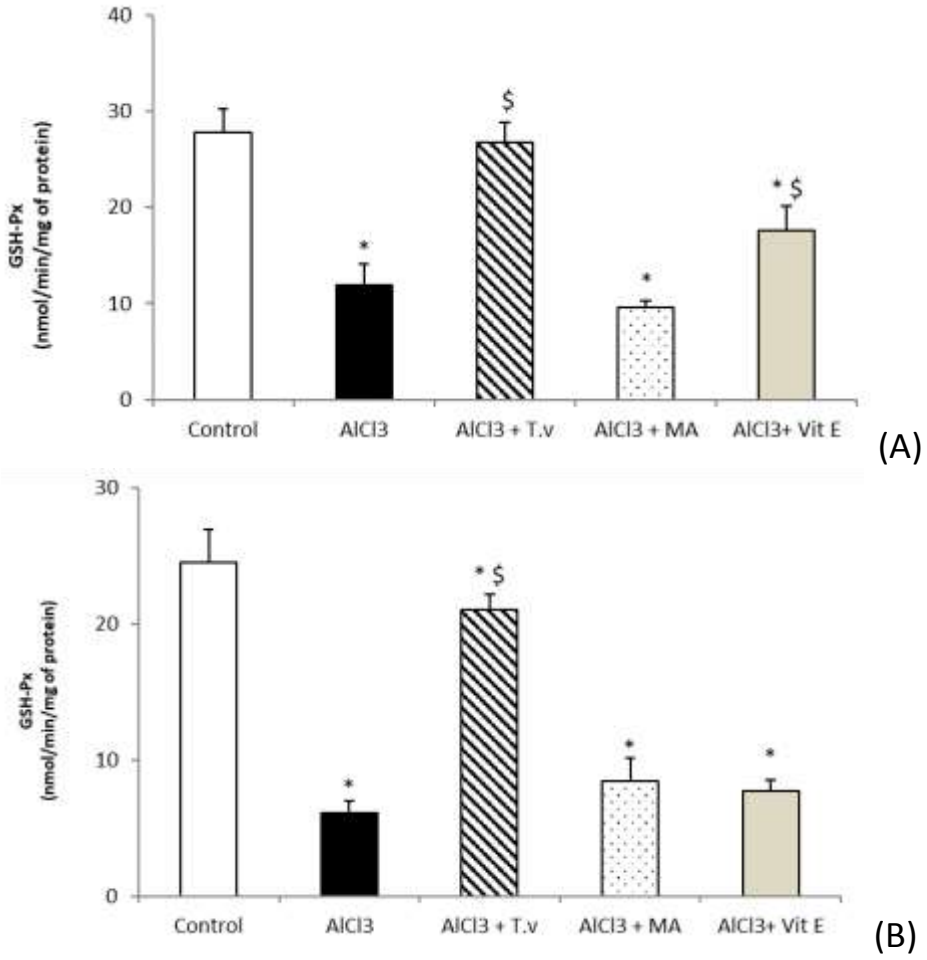

Figure 3. The Effect of Treatment on GSH-Px Activity (nmol/min/mg of protein) in the Liver (A) and Kidney (B)
(*) Comparison with the control group. ($) Comparison with AlCl3 group.

and treated with thyme showed the same effect.

The observed decline in the level of protein in $AlCl_3$ treated rats in this study agrees with the findings of Kinawy (2019) and might be due to changes in the metabolism of proteins, particularly albumin. On one hand, this could be attributed to malnutrition but on the other hand, it could be due to a reduction in the protein synthesis in the liver (Tripathi et al., 2009). The same results also revealed that $AlCl_3$ intoxication caused a significant decrease ($p< 0.05$) in plasma T. Protein and Alb (Massoud et al., 2016), while an increase in AlP parameter was observed. Furthermore, aluminum may promote proteinuria by causing a nephritic syndrome or chronic glomerulonephritis.

The accumulation of Al in the kidney is usually accompanied by renal failure (Ecelbarger et al., 1994). Moreover, Al accumulation in the kidney promotes degeneration in renal tubular cells, inducing nephrotoxicity (Somova et al., 1997; Mohamed et al., 2017). In addition, renal functions can also be altered in the early stages of hepatic damage before the formation of ascites (Monasterolo et al., 1993). Uric acid is the major product of purine bases, adenine and guanine. It is partially considered as a significant marker of renal function. The Plasma Uac (Uric acid) elevation recorded in this study after $AlCl_3$ exposure is in agreement with the majority of researches (Sivakumar et al., 2014; Al-Qhtani and Farran, 2017), and is also supported by the findings of Rudenko et al. (1998) who reported that $AlCl_3$ intensifies the acid–secretion function of the kidney and changes the transport of sodium.

It has been suggested that the toxic effects associated with aluminum are due to the generation of reactive oxygen species, which results in the oxidative deterioration of cellular lipids, proteins and DNA. In the present experiment, we observed a significant increase in liver and kidney lipid peroxidation (TBARS) after $AlCl_3$ exposure. These results are similar to previously published findings demonstrating that aluminum salts intake promoted lipid peroxidation in liver and kidney

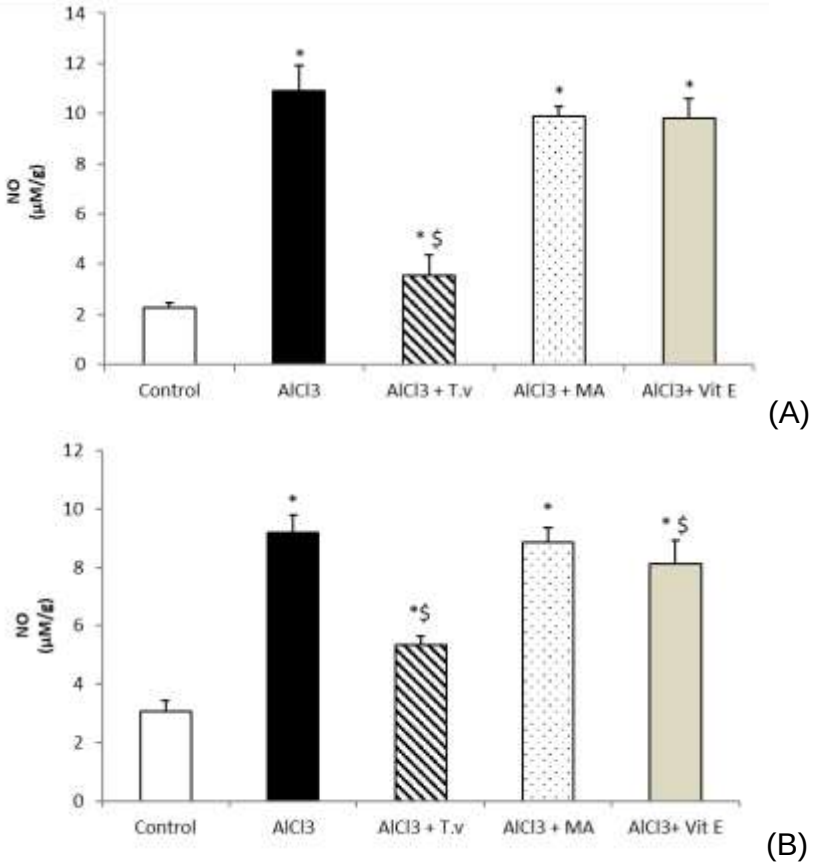

Figure 4. The Effect of Treatment on NO level (nmol/min/mg of protein) in the Liver (A) and Kidney (B)
(*) Comparison with the control group. ($) Comparison with AlCl3 group.

Table 3. The Effect of Treatment on Aluminum Content in the Liver and Kidney Tissues.

Groups	Control	$AlCl_3$	$AlCl_3$ + T.v	$AlCl_3$ + MA	$AlCl_3$ +Vit E
Liver Al (µg/g)	2.63 ± 0.41	8.84 ± 0.57*	3.60 ± 0.45$^{\$}$	8.16 ± 0.57*	8.88 ± 0.38*
Kidney Al(µg/g)	0.91 ± 0.11	4.72 ± 0.42*	3.19 ± 0.43$^{*\$}$	3.90 ± 0.38 $^{*\$}$	4.29 ± 0.31*

Values are expressed as mean ± SEM, n = 6 for each group.
(*) Comparison with the control group. ($^{\$}$) Comparison with AlCl3 group.

tissues (Belaïd-Nouira et al., 2013; Tahari et al., 2016). It was also found out that the consumption of *T. vulgaris* L. induces a significant decrease ($p \leq 0.05$) in TBARS level.

Our study demonstrated that $AlCl_3$ administration caused a reduction in liver and kidney glutathione transferase (GSH-St) and glutathione peroxidase (GSH-PX) activities. The results are in accordance with Newairy et al. (2009). Furthermore, El-Sayed et al. (2011) reported a significant decrease in both enzymes in the liver of about (56 and 80%) respectively. A reduction of the kidney's GSH-Px activity was also noticed by Mahieu et al., 2009.

Nitrite resulted from the oxidation of NO, so Nitrite estimation is indirectly that of nitric oxide (NO) content in the biological samples. It has been suggested that Nitric oxide plays multiple roles in Al intoxication (Guo et al., 2001). The present investigation showed that $AlCl_3$ toxicity excessively increases NO products in the liver and kidney tissues which agree with many studies (Guo et al., 2005).

Recent studies demonstrated that aluminum may alter the activity of tissue antioxidative enzymes as TBARS and NO activity (Taïr et al., 2016; Benyettou et al., 2017). Our data support these results and the levels of these parameters were found to be elevated in $AlCl_3$-treated rats. However, GSH-Px and GSH- St activity were

decreased in the liver and kidney tissues. These observations are similar to previously published findings demonstrating that the aluminum intake promoted oxidative stress while the extract plants administration restored it (Kutlubay et al., 2007; Joshi et al., 2013).

Our results have indicated that a significant accumulation of $AlCl_3$ occurs in the liver and kidney tissues of $AlCl_3$-intoxicated rats. The same results were observed in other studies (Yeh et al., 2009) where $AlCl_3$ accumulation increased by 3 times in the liver and 6 times in the kidney (Missel et al., 2005; Cheng et al., 2014). However, the present study has shown that following *T. vulgaris* L. extract treatment; a significant decrease in $AlCl_3$ accumulation in the liver and kidney tissues was recorded.

It is well documented that the leaves and flowers of plants contain numerous aromatic chemical products which prevent the cellular damage caused by free radical molecules, ions or atoms (Dapkevicius et al., 2002; Al-Asmari et al., 2017). *T. vulgaris* L. is a famous herb cultivated all over the world, used essentially in food as a flavor and in medicine as an anti-microbial, anti-poisonous and deworming agent.

Intoxicated rats treated with thyme extract have shown low AlP and AcP levels in plasma which indicates the thyme's hepatoprotective impact. *T. vulgaris* L. aqueous extract also elevated the total serum protein, Alb and Uac levels. This explains the healing effect of thyme on aluminum toxicity and confirmed its nephroprotective effect as stated by El-Newary et al. (2017). This positive effect could be due to its secondary metabolites composition including alkaloids, polyphenols and flavonoids. The main compounds of *T. vulgaris* L. are the natural terpenoid thymol (12-61%) and its phenol isomer carvacrol (0.4-20.6%) which revealed a high antioxidative, antispasmodic and antibacterial effects (Amiri, 2012). In addition, *T. vulgaris* L. Contains high levels of vitamins B_3, C and E. It is also; very rich in potassium (K), Magnesium (Mg) and others trace elements (Komaki et al., 2016). Due to this composition, thyme is considered as a high regulator of homeostasis. This study also proclaimed that intoxicated rats given aqueous thyme extract showed remarkable lowered levels of MDA and NO and elevated levels of GSH-St and GSH-px in renal and hepatic tissues compared to control group. This implies the efficient and protective impact of thyme which is probably linked to its capacity to reduce oxidative stress as free radical scavenger or as a quencher of singlet oxygen formation preventing peroxidation of lipids.

El-Newary et al. (2017) found out that dietary supplementation of *T. vulgaris* L. reduced all the above measured parameters in the liver and kidney of rats. These results highlighted the benefit of *T. vulgaris* L. as a dietary antioxidant.

Other reports indicate that the essential oil of *T. vulgaris* L. has a potential antioxidant activity and a protective effect against toxicity of aflatoxins (El-Nekeety et al., 2011). The properties of the *T. vulgaris* L. constituents as polyphenols (Thymol and Carvacrol) and flavonoids (caffeic acid, rosmarinic acid, apigenine, thymonine and luteolin) protect the cellular membranes' integrity (Ündeger et al., 2009; Popovic-Milenkovic et al., 2014) from $AlCl_3$-induced oxidative damage and repair the antioxidant system. Moreover, other authors stated that thymol was found to decrease ROS production in dependent concentration and inhibit hepatotoxicity induced by CCl_4 in male Swiss albino mice as assessed by lipid peroxidation and by histopathological examination (Alam et al., 1999).

T. vulgaris L. has noticeably decreased the aluminum content in the liver and kidney. Some authors ascertained the fact that phenolic compounds were not able to chelate metal ions. Melidou et al. (2005) found that intracellular binding of iron is responsible for the protection offered by flavonoids against H_2O_2-induced DNA damage. On the other hand, complexation of plant extracts with metal ions might diminish the absorption of metal ions leading to chelating and blocking metal-mediators generating the ROS (Yang et al., 2013).

In the current study, it was also found that MA and Vit E treatments only partially alleviated the harmful effects of $AlCl_3$ toxicity as observed in previous research by El-Demerdash (2004) and Masoud et al. (2016). These results also concur with previously reported data indicating that administration of Aluminum with some plant preparations such as the *Crocus sativus* L. extract (Shati and Alamri, 2010), the *Zingiber Officinale* extract (Hasona and Ahmed, 2017) or Propolis (Turkez et al., 2010) or Selenium antioxidant products showed a recovery from hepatic damage (Viezeliene et al., 2011). Mahieu et al. (2009) and Yeh et al. (2009) have also revealed the beneficial effects of Melatonin and Taurine antioxidants against $AlCl_3$ nephrotoxicity.

Conclusion

Aluminum has adverse effects on human health and special attention must be paid to the sources of Al in food, water and personal care products. From the results presented in this study, it can be seen that exposure to $AlCl_3$ is capable of inducing significant harmful changes in some biochemical characteristics and redox status in liver and kidney tissues. However, this study has also demonstrated the therapeutic effects of the *T. vulgaris* L. plant in minimizing Aluminum liver and kidney toxicity. This treatment resulted in a significant improvement in body, liver and kidney weights and the plasma biochemical parameters (T. Protein, Alb, AlP, AcP and Uac). In addition, the *T. vulgaris* L. treatment led to a more efficient recovery from the oxidative tissue damage caused by Al intoxication (TBARS, GSH-Px, GSH-St and NO) when compared to the Malate and Vitamin E treatments. Therefore, using *T. vulgaris* L. extract could be a protective and preventative method in overcoming

the detrimental effects of Aluminum toxicity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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