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The protective effect of Vitamin C and Vitamin E on some antioxidants and lipid peroxidation in blood and tissues of male albino rats treated with aluminum

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Abstract: Aluminum is presents in many manufactured foods, medicines and is also added to drinking water for purification purposes. Therefore, the present study included the investigation of the protective effect of vitamin C and vitamin E in hematological parameters, and preventing oxidative stress induced by aluminum chloride ($AlCl_3$), with dose of 40 mg/Kg of body weight, orally by a gavage tube to male albino rats Rattus norvegicus, aged (3-4) months, weighing (200-250) g. Thirty male rats were randomly divided into five groups (6 rats / group), treated orally daily for 30 days as follows: The first group was given distilled water considered as control (untreated group). The second group was treated with 40 mg/Kg B.W. $AlCl_3$ only. Third and fourth groups were treated with 40 mg/Kg $AlCl_3$ plus vitamin C or vitamin E with dose of 400 mg/Kg B.W. respectively. The fifth group was treated with $AlCl_3$ plus combination of vitamin C and vitamin E at the same previously used concentrations. All these groups were given a standard forage and tap water ad libitum.

The results showed that treating with $AlCl_3$ caused a significant decrease at ($P \leq 0.05$) in the hemoglobin (Hb) concentration and packed cell volume (PCV), but a significant increase in total white blood cells count (WBCs) and lymphocytes in the blood of male rats treated as compared with control group. The results also showed a significant decrease in the concentration of glutathione (GSH) and albumin, but a significant increase at ($P \leq 0.05$) in the concentration of each of malondialdehyde (MDA), uric acid, bilirubin, creatinine, superoxide dismutase (SOD), catalase (CAT), [which have a role as non-enzymatic and enzymatic antioxidants], alanine transaminase (ALT) and aspartate transaminase (AST) [liver function enzymes] activities in blood serum of treated rats as compared with control group. A significant decrease in the level of the GSH, increase in the level of MDA in brain tissue were observed which indicates the ability of aluminum to induce oxidative stress in albino rats.

The results also showed that treatment with $AlCl_3$ plus vitamin C or vitamin E and their combination caused a significant increase in GSH level and a significant decrease in MDA level in serum and brain tissue compared with control group. In addition to positive effect on some hematological and biochemical parameters. In conclusion, vitamin C and E had prophylactic capacity that would remove any oxidative stress and toxic effects caused by $AlCl_3$.

Keywords: Aluminum, Vitamin C, Vitamin E, Antioxidants, Lipid peroxidation.

INTRODUCTION

The environmental contamination with heavy metals (Such as lead, cadmium, zinc and aluminum) has increased due to anthropogenic activities [1,2]. Release of these metals are of major concern since they are a serious threat to the health and well-being of humans and animals [3,4]. Evidence for the contribution of aluminum (Al) to environmental pollution with different aluminum containing compounds exposes people to higher than normal levels of aluminum. Al is thus potentially toxic for human [5].

Aluminum, the third most abundant element of Earth's crust. It is one of the trace elements (a non-essential) with moderate toxic effect on living organism [6,7]. The elemental aluminum does not occur in its pure state but is always combined with other elements such as chloride, hydroxide, silicate, sulphate and phosphate [8]. The general population is exposed to aluminum due to its widespread use in water treatment for purification purpose [9], food additives, various aluminum-based pharmaceuticals, from occupational dusts and Al containers, foil, cooking utensils [10,11].

Aluminum is released to the environment by both natural processes and from various anthropogenic sources. The major sources of Al include air, soil, water and food [12,13], and the gastrointestinal tract constitutes the main route of entry into the body. Al is mostly absorbed through the skin, lungs, and intestinal tract and the absorption rate is low in normal human subjects [7,14]. Al is known to cause toxic effects to variety of organ systems including blood, bones (causing brittleness or osteoporosis), kidneys, stomach, liver and brain [15]. Researches suggests that there is a relationship between high levels of Al and risk of a number of neurodegenerative diseases and affects several enzymes and other biomolecules relevant to Alzheimer's disease [16], Parkinson's disease, Dementia and other neurological disorders [17]. It has been shown clearly that Al accumulates in various mammalian tissues such as brain, bone, liver and kidney and is also accompanied by renal failure [18,19]. Aluminum causes oxidative stress within brain tissue [20]. And Al has direct effect on hematopoisis. Excess of Al has been shown to induce microcytic anemia [21]. The toxic effects of Al have been suggested to be due to the generation of Reactive Oxygen Species (ROS) [22], which results in the oxidative deterioration of cellular lipids, proteins and DNA [22,23]. So, these toxic effects of Al appear to be mediated, at least in part, by free radical generation [24]. Several studies have shown that oxidative stress induced by Al modifies the peroxidation of lipids and the activities of anti-oxidative enzymes. Figure (1) proposed a theoretical diagram of relationship among Al, ROS, anti-oxidative enzymes and lipid peroxidation (LPO). It is adapted from the research findings of Exley[25]; Halliwell and Gutteridge [26]. Yuan, et al.,[27] concluded the Al overload increase oxidative stress (H_2O_2) in the different brain tissues of neonatal rats.

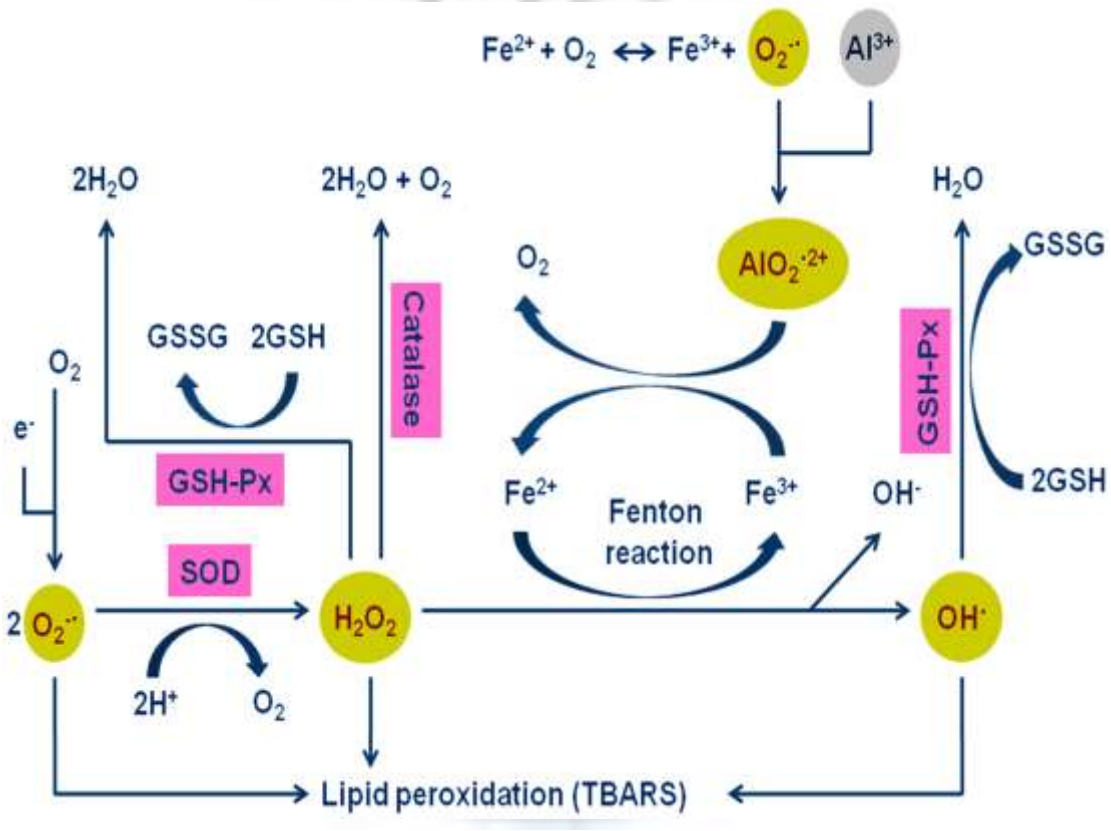

Figure (1): Diagrammatic representation of the relation among the aluminum (gray), reactive oxygen species (yellow), anti-oxidative enzymes (pink) and lipid peroxidation. TBARS = thiobarbituric acid reactive substances; SOD = superoxidase dismutase; GPx = glutathione peroxidase. (It is adapted from the research findings of Exley[25]; Halliwell and Gutteridge, [26]).

Antioxidants play an important protective role against the ROS. Recent studies were carried out to evaluate the potential role of antioxidant vitamins, such as vitamin C and vitamin E [11,28]. Both these vitamins are essential micronutrients for humans and animals. They are involved in the protection of biological membrane against LPO and preventing the free radicals damage to phospholipids membranes and enzyme, and both vitamin C and E helping to prevent degenerative diseases such as cardiovascular diseases, cancers, and cataract, probably through anti-oxidative mechanisms [29,30].

Vitamin C (Ascorbic acid) is an anti-oxidative agent that has been reported to be important for detoxification pathways. Vit. C is an essential micronutrient required for normal metabolic functioning of the body [29]. It acts against the toxic, mutagenic and carcinogenic effects of environmental pollutants by stimulating liver detoxifying enzymes [31]. Vit. C may also be able to regenerate other anti-oxidants such as vitamin E [30].

Vitamin E (α-tocopherol) is the major lipid soluble anti-oxidant that is present in biomembranes. Scavenges free radicals in the early stages of LPO. Vit. C and E are anti-oxidants essential for cell survival in environments containing peroxides [32]. The anti-oxidant function of this of this micronutrient could also, at least in part, enhance the immune reactions by maintenance of the functional and structural integrity of all important immune cells [22]. Indeed, with the health problems that can be induced by many environmental pollutants, efforts have been made towards the evaluation of relative antioxidant potential of vit. C [33], and vit. E [22].

Therefore, the present study investigate the potential for a protective role of vit. C, E and their combination against the toxic effects of $AlCl_3$ on blood components, lipid peroxidation, non-enzymatic, enzymatic antioxidants and liver function enzymes in serum and brain tissue of albino rats.

MATERIALS AND METHODS

Chemicals material: Aluminum Chloride ($AlCl_3$) used in the study, was obtained from British Drug House Company (BDH, chemicals, Itd., Poole) English origin. Vitamin C. (L-ascorbic acid), Vitamin E. (α-tocopherol) powders were obtained from the state company for drug industries and medical appliances Ninavah-Iraq for use as food anti-oxidants (Exogenous).

Experimental animals: Thirty adult male rats Rattus norvegicus used in this study were obtained from the animal house of Medicine college at the University of Mosul. Iraq, at aged (3-4) months,weighing (200-250)g. They were housed in polypropylene cages under controlled conditions of temperature (24 ± 26)º C and lighting (14 hours light/10 hours dark). The rats were supplied a standard pellet diet and tap water ad libitum until the end of the experiment.

Experimental design: After 2 weeks of acclimation prior to the commencement of the experiment. The adult male rats were randomly divided into five groups (6 rats /group with approximately similar weights), placed in separate cages and were treated daily for 30 days as follows:

1. Group (1)- served as **control**: This group includes rats that were given standard forage and tap water, ad libitum until the end of experiment and were given distilled water daily by a gavage tube for 30 days to offset the stress of keeping rats.

2. Group (2)- (**$AlCl_3$**): This group treated with $AlCl_3$ with dose of 40 mg/kg B.W. orally by gavage tube [13,34].

3. Group(3)- **$AlCl_3$ + Vit. C**: This group treated with $AlCl_3$ (40mg/kg B.W.) plus Vit. C with dose of (400mg/kg BW.) orally by a gavage tube [35]..

4. Group(4)- **$AlCl_3$** + **Vit. E**: This group treated with $AlCl_3$ (40mg/kg B.W.) plus Vit. E with doses of (400mg/kg B.W.) orally by a gavage tube [35].. –

5. Group(5)- **$AlCl_3$** + **Vit. C** and **Vit. E**: This group treated with $AlCl_3$ plus vit. C and vit E at the same previously used concentrations.

Collection and preservation of sample and tissues: At the end of the experiments (30 days), blood samples were collected from the eyehole vein of each animal by using a capillary tubes and the blood was divided by a type of examination, 1 ml of blood was put into a plastic tubes with lids containing anticoagulant EDTA in order to do hematological tests. Also blood samples were collected into dry, clean plain tubes and free of any anticoagulant material, allowed to clot. Serum was separated after centrifugation at 3000 g. For 15 min. to obtain blood serum. The blood serum was collected and stored in a deep freeze at (-20º C) for biochemical test purposes. Rats of each group were then killed by dislocated there necks at the end of treatment period. The brains were obtained from the skulls and weighed. minced and homogenized (10 W/V) separately in ice-cold 1.15 % KCl-0.01 mol/l Sodium,, Potassium Phosphate buffer (pH 7.4) in a homogenizer. The homogenate was centrifuged at 10000 g for 30 min. at 4 ºC [22], and the resultant supernatant was used to estimate the level of malondialdehyde (MDA) by using Thiobarbituric acid (TBA) method [36] and glutathione (GSH) by using Ellmanm´ s reagent method [37] at the same day of preparation.

Hematological and Biochemical Tests: The whole blood and serum were used to estimate the levels of a number of hematological and biochemical parameters respectively, using the measurement methods referred to in table (1), as a number of ready tests were used from international companies such as English Randox Company, French Biolabo Company and German Biocon Company, to estimate the concentration of hemoglobin,biochemical parameters that include; albumin, uric acid, bilirubin, creatinine, catalase (CAT) and activities of liver enzymes (ALT, AST). The rest of the hematological and biochemical parameters were estimated by using manual methods.

Table (1): The methods used for estimating the number of hematological and biochemical parameters in the present study.

Measured parameters		Method used	Reference
Blood components	Hemoglobin conc. (Hb)	Drabkin's method	Drabkin and Austin, 1935 [38]
	Packed cell volume (PCV)	Using the haematocrite reader	Talib and Khurana, 1996 [39]
	Total white blood cells (WBCs) count	Using the hemocytometer and Turk's solution	
	Lymphocytes	Using thin blood film and Gimza stain	
Biochemical parameters in serum	Albumin conc.	Bromacresol green method	Doumons et al., 1971 [40]
	Uric acid conc.	Urecase enzymatic method	Newman and Price, 1999 [41]
	Bilirubin conc.	Colorimetric method	Walter and Gerard, 1970 [42]
	Creatinine conc.	Colorimetric method	Tietz, 1999 [43]
	Malondialdehyde (MDA) conc.	Thiobarbituric acid (TBA) modified method	Guidet and Shah, 1989 [44]
	Glutathion (GSH) conc.	Modified Ellman's reagent method	Sedlak and Lindsay, 1968 [45]
	Superoxide dismutase (SOD) activity	Modified photochemical Nitro blue Teterazolum (NBT) method	Brown and Goldstein, 1983 [46]
	Catalase (CAT) activity	Spectrophotometric method	Yoshiji et al., 2001 [47]
	Alanine transaminase (ALT) activity	Colorimetric method	Reithman and Frankel, 1957 [48]
	Aspartate transaminase (AST) activity	Colorimetric method	Reithman and Frankel 1957 [48]

Statistical Analysis: Data were analyzed by using the Complete Radamized Design (C.R.D.). Duncan Multiple Range Test as results was used to test for differences among groups, differences were considered significant if ($P \leq 0.05$), and data were applied by statistical program ready (SPSS) version 18 for windows [49].

RESULTS AND DISCUSSION

The results in table (2) showed the effect of Aluminum, vitamin C, E and their combination on hematological parameters. After 30 days of $AlCl_3$ administration with a dose of 40 mg/Kg B.W., there was a significant decrease ($P \leq 0.05$) in hemoglobin (Hb) concentration and packed cell volume (PCV) compared with control group. In contrast, total white blood cells (WBCs) count and lymphocytes showed a significant increase ($P \leq 0.05$) as compared with control group.

Table 2: The protective effect of vitamin C, E and their combination on hematological parameters in blood of male albino rats treated with aluminum chloride

Treatment groups / Parameters	Mean ± SD*				
	Control	Aluminium (Al)	Al + Vit. C	Al + Vit. E	Al + Vit C + E
Hb (g/dl)	15.44±0.89 a	12.27±0.35 c	13.71±0.38 b	13.01±0.52 bc	12.60±0.33 c
PCV (%)	51.46±2.65 a	40.90±1.15 c	45.70±1.27 b	43.33±1.79 bc	42.00±1.13 c
WBCs count (*10^3 cell/µl)	3.93±0.25 c	6.60±0.10 a	4.33±0.25 bc	4.56±0.05 b	4.50±3.06 b
Lymphocytes (%)	61.16±0.81 b	78.13±0.20 a	62.70±0.95 b	64.90±0.45 b	66.30±0.37 b

* Mean ± SD for 3 reduplications.
Number of animals 6 male rats / group.
Numbers are preceded by different letters horizontally indicate a significant difference at the level of probability (P≤0.05) and correct reverse according Duncan test.

These results are also consistent with the findings of some studies by Neelu et al., [5], Abdel Aziz and Zabut [13] and Al-Mallah et al., [50]. Treatment with 40mg/Kg B.W. $AlCl_3$ led to reduction in blood Hb conc. and PCV while WBCs count and lymphocytes were increased .The reduction in Hb concentration might be due to the increased rate of destruction or reduction in the rate of formation of red blood cells (RBCs).Reduction in Hb and PCV might be attributed hyperactivity of bone marrow. And the decrease in Hb conc. could be not only due to the decrease in RBCs count but also due to the impairment of heme biosynthesis in the bone marrow, leading to the production of RBCs with impaired integrity that are easily destroyed in the circulation. Swartz et al., [51] noted that the Al has a direct effect on haematopoisis. Excess Al has been shown to induce microcytic anemia. From similar results Naylor [52] concluded that anemia resulted from hemodilation, extra vascular hemolysis and toxic dyshemopiosis.

In the same time the increase in WBCs count in rats treated with Al compared with control group is attributed to the fact that the animals exposed to oxidative stress which led to the stimulation of the immune system to form defensive cells [13]. The increase in lymphocytes could be due to the toxic action of the Al ion that stimulates the hemopoietic system to release more of these cells, causing an increase in their number in blood stream [13]. Nevertheless, when male rats were treated with vitamin C or E with a dose of 400 mg/Kg B.W. or thir combination concomitantly with 40 mg/Kg B.W. of $AlCl_3$ during 30 days, it led to a significant increase (P≤ 0.05) in Hb conc. and PCV, while it showed a significant decrease (P≤ 0.05) in WBCs count and lymphocytes compared with a group of rats treated with $AlCl_3$ only. Knowing that all these values are not up to the values of the control group. Vit. C and E counteracted the effect of Al ion in hematological parameters. These results of the present study are also consistent with the finding Abdel Aziz and Zabut [13]. And this is due to the preventive effect of vit. C and vit. E from ROS and the protection of RBCs from oxidative stress. Vit. C and E are the important non-enzymatic antioxidants that work to remove ROS and increase the efficiency of the immune responses [53]. Vit. E and C separately increases the activities of antioxidant enzymes in various tissues of rats, especially liver tissues and also vit. E on bone marrow, where the different blood cells are formed [54]. Vit. C also increase the vital presence of iron, it also increases its absorption in the gastrointestinal tract [55] and stimulates vit. C to secrete the catalyst for the formation of RBCs (Erythropoietin) from the kidney, which stimulates the formation of RBCs from bone marrow[56].

The results in table (3) and (4) showed a significant increase in the conc. of malondialdehyde (MDA), while showed a significant decrease in the conc. of glutathione (GSH) in blood serum and brain tissue respectively of rats treated with $AlCl_3$ as compared with control group. Results of the present study are consistent with the finding of El-Demerdash [22] and Sallam et al., [33], about treatment of rats and rabbits with a conc. of $AlCl_3$ 34mg/Kg B.W. for 30 days. Also Neelu et al.,[5]; Stevanovic et al.[57] and El-Kholy et al., [58] found that the mice and rats treated with different concentrations of $AlCl_3$ for different periods, showed an increase in MDA conc. and a decrease in GSH conc. in brain tissues. The compatibility between the high MDA conc. and low GSH conc. in the blood serum and the brain tissues of rats treated with $AlCl_3$ is one of the oxidative stress indicators compared with control group[58]. Stevanovic et al. [57] suggested the role of Al as a pro-oxidant. Pro-oxidants attack lipids within cell membranes via a process termed LPO. The conc. of MDA has been used as an index of LPO in situations of Al exposure [59]. This study was also supported by Omurtag, et al., [60] found in MDA of animal treated with $AlCl_3$.

Table 3: The protective effect of vitamin C, E and their combination on malondialdehyde (MDA), non-enzymatic antioxidants and enzymatic antioxidants in serum of male albino rats treated with aluminum chloride

Treatment groups / Parameters	Mean ± SD*				
	Control	Aluminium (Al)	Al + Vit. C	Al + Vit. E	Al + Vit C + E
MDA (µmol/L)	2.75±0.21 b	5.73±1.36 a	2.95±0.05 b	2.99±0.03 b	3.11±0.07 b
GSH (µmol/L)	18.46±0.49 b	12.93±1.46 c	20.73±0.15 a	19.96±0.37 a	20.36±0.83 a
Albumin (g/dl)	4.20±0.30 a	3.50±0.36 b	4.10±0.30 ab	3.90±0.26 ab	3.70±0.34 ab
Uric acid (mg/dl)	3.53±0.20 c	5.70±0.20 a	3.61±0.02 C	3.74±0.21 c	4.07±0.06 b
Bilirubin (mg/dl)	0.76±0.02 c	1.08±0.30 a	0.82±0.02 b	0.83±0.05 b	0.86±0.02 b
Creatinine (mg/dl)	0.61±0.01 c	0.77±0.02 a	0.65±0.03 bc	0.66±0.02 b	0.67±0.01 b
Superoxide dismutase [SOD] (ΔO.Δ.)	0.155±0.04 a	0.098±0.00 c	0.142±0.02 b	0.139±0.01 b	0.137±0.05 b
Catalase [CAT] (U/ml)	2.19±0.02 a	3.59±0.56 c	2.18±0.13 a	2.25±0.06 b	2.30±0.03 b

*Mean ± SD for 3 reduplications.
Number of animals 6 male rats / group.
Numbers are preceded by different letters horizontally indicate a significant difference at the level of probability (P≤0.05) and correct reverse according Duncan test.

Table 4: The protective effect of vitamin C, E and their combination on malondialdehyde (MDA), and glutathione (GSH) level in brain tissue of male albino rats treated with aluminum chloride

Treatment groups / Parameters	Mean ± SD*				
	Control	Aluminium (Al)	Al + Vit. C	Al + Vit. E	Al + Vit C + E
MDA (nmol/g Wet tissue)	215.80±3.48 b	289.30±5.71 a	224.53±3.22 ab	229.53±4.53 ab	241.63±1.88 ab
GSH (µmol/ g Wet tissue)	0.54±0.04 d	0.32±0.01 e	0.94±0.03 a	0.74±0.04 b	0.63±0.02 C

*Mean ± SD for 3 reduplications.
Number of animals 6 male rats / group.
Numbers are preceded by different letters horizontally indicate a significant difference at the level of probability (P≤0.05) and correct reverse according Duncan test.

Aluminum has the ability to generate ROS which causes peroxidation of membrane lipids. Usually the deleterious effect of the oxidative stress is counteracted by natural cellular defense mechanism that involves enzymes [glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT)] and water or fat soluble non-enzymatic antioxidants (vit. C and vit. E, GSH and Selenium) or Scavenges of free radicals [5,60]. Glutathione is present in two forms: reduced GSH and oxidized GSH (GSSG). The high reduction-oxidation potential makes the GSH act as a cofactor for enzymatic reactions and antioxidants. The free radical scavenging effect shows the reducing power of GSH [61]. The results of this study showed a decrease in GSH conc. in the blood serum and brain tissue of rats treated with $AlCl_3$. This is due to Al ion that induced the depletion of GSH which has an interesting physiological as well as pharmacological use like detoxification by participation in redox system. Al can cross the semi-permeable membrane of RBCs, though not too much extent but can induce a change in the chemical status of GSH and brought a reduction in the level of reduced GSH [61].

The results in table (3) and (4) showed that when rats were treated with vit. C or vit. E or their combination concomitantly with $AlCl_3$, this causes a significant decrease in MDA conc. in the serum and non significant decrease in MDA conc. in the brain tissue, accompanied by a significant increase in GSH conc. In both serum and brain tissues comparing to rats treated with $AlCl_3$ only. These results are agreed with the findings of [22,33,62,63]. The reason could also be due to the role of each vit. C and E to reduce the toxic effects caused by aluminum..

Vit. C is an important water-soluble vitamin that is found in two biologically active forms: ascorbic acid and its oxidized derivative, dehydroascorbic acid. Vit. C can also act as a hydrogen donor to reverse oxidation and therefore function as an antioxidant that reacts with the free radicals and deactivate them before they cause damages to proteins or lipids [64]. As well as the conversion of oxidized vit. E radical to active reductive form [30], which leads to reduce the oxidative stress occurring due to Al in the body, and then decrease the oxidative stress and tissues damage, and this strengthens the case of antioxidants in the body [31]. Vit. E is a major lipid-soluble antioxidant, which can protect biomembranes and prevented the continuation of LPO of poly unsaturated fatty acids interactions, it prevents and stops the oxidation and converts it to a non-natural compounds. Vitamin E can only prevents the formation of free radicals but it works to excrete aluminum.

The results in table (3) showed changes in albumin, uric acid, bilirubin and creatinine conc. in the experimental groups. While the results showed a significant decrease in albumin con., in contrast, uric acid, bilirubin and creatinine showed a significant increase in the blood serum of rats treated with $AlCl_3$ as compared with control group. And the results showed that the rats treated with vitamin C or E or their combination concomitantly with $AlCl_3$ during 30 days. It led to a non significant increase in albumin conc., and was accompanied by a significant decrease in uric acid, bilirubin and creatinine concentrations as compared with the group of male rats that were treated with $AlCl_3$ alone, knowing that all of these values are not up to the values of the control group. Treatments with vit. C or E or their combination concomitantly with $AlCl_3$ did not restore these compounds to control levels.

These changes in the non-enzymatic antioxidants such as albumin, uric acid, bilirubin and creatinine of the male rats treated with $AlCl_3$ (Table 4) are in agreement with Neelu, et al., [5], Narayanan [7], Abdel Aziz and Zabut [13] and El-Demerdash [22]. Aluminum has been reported to induce LPO, and to alter physiological and biochemical characteristics of biological system, by using the large production of free radicals and increase of the oxidative stress due to the treatment. The decreased conc. of albumin in the serum of rats that were treated with Al (Table 3) might be due to the change in protein biosynthesis and/or metabolism [64]. Albumin is considered as the major and the most sensitive protein for oxidation within blood. It is synthesized exclusively by the liver [65] and as indicated by Neelu, et al., [5] that Al intoxication lead to severe increase in the level of LPO damage in liver cells.

The observed increase in uric acid conc. might be due to extra degradation of purines in the liver, or the inability to excrete uric acid by the kidneys[66]. Kidneys can also be one of the targets to toxic impacts due to Al exposure because kidneys may be exposed to high conc. of Al during the normal process of excretion [67]. Al causes damages to the kidney cells by loss of cell viability, enzyme release and damage to cell brush borders [68]. The increase in serum total bilirubin (Table 4) may result from decreased liver uptake, conjugation or increased bilirubin production from hemolysis [69]. An increase in creatinine con. has been seen, interpreted as caused by a decrease in muscle mass, or an abnormal glomerular function of the kidneys induced by $AlCl_3$ administration [13]. The elevation in serum creatinine conc. is considered as a significant marker of renal dysfunction. Many previous studies have shown that Al has a protective effect on the progress of renal dysfunction [7]. Rudenko, et al., [70] reported that $AlCl_3$ intensifies the acid-secretory function of kidneys and changes the transport of sodium.

The superoxide dismutase [SOD; EC.1.15.1.1] activity is measured by indirect method through emergence of change in the optical density of formazan consisting of reduction of dye nitroblue tetrazolium (NBT), which in turn generated from irradiation of blood serum. The results in table (3) showed a significant decrease in the level of change in absorbance of formazan in the SOD activity in the serum of rats that were treated with $AlCl_3$ only when compared with control group. These results that indicate an increase in enzyme activity, was also indicated by Beyer, et al.,[71]. But these results showed a significant increase in the SOD activity in serum of rats that were treated with vitamin C or E or their combination concomitantly with $AlCl_3$ during 30 days as compared with $AlCl_3$ only. The same results were refemed by Jankeer [72], when the rats treated with drinking water containing 20 or 40 mg lead / L daily for a period of 30 days.

The results in table (3) showed a significant increase in catalase [CAT; EC.1.11.1.6] activity in serum of rats that were treated with $AlCl_3$ only when compared with control group. But these results showed that a significant decrease in the CAT activity in serum of rats that were treated with vit. C or E combination concomitantly with $AlCl_3$ during 30 days compared with $AlCl_3$ only. Knowing that all these values are not up to the values of the control group as shown in table (3). These results are in agreement with Yuan, et al., [27], Stevanovic, et al., [57]. The present study showed that $AlCl_3$ affects some of serum enzyme activities (SOD, CAT) in the rats that were treated with $AlCl_3$ only or vit. C or E their

combination concomitantly with $AlCl_3$ daily for a period of 30 days. SOD is a key enzyme in cellular defense system that disproportionate O^{-2} into oxygen and H_2O_2, with latter being detoxified by GPx or CAT to H_2O molecules and O_2. The present study demonstrated that Al ion resulted in high oxidative stress in the rats treated with $AlCl_3$. A long period of exposure to $AlCl_3$ was reported to produce a significant alteration in enzymatic antioxidants such as SOD and CAT and non-enzymatic antioxidants such as GSH, albumin, bilirubin, creatinine and uric acid that result from oxidative stress [7,13,22,27,35,57,64,72].

Aluminum may directly affect organelles at cellular level in various tissues, which will indirectly influence enzymes activities such as SOD and CAT. Thus, it was shown that some properties of membrane phospholipids were changed [73]. The above mentioned products and other intermediate products reach target tissues and cause much of direct or indirect damage. such damages occurs either by attachment to molecules such as DNA, protein, carbohydrates and their smaller components or different types of enzymatic activity [22,23,33]. In this study, both SOD and CAT enzymes activities were increased in animals exposed to $AlCl_3$ in comparison with control. This is due to that these two enzymes have related functions. SOD catalyzes the dismutation of superoxide anion radical to H_2O_2 and H_2O. The H_2O_2 is detoxified to H_2O and O_2 molecules by CAT. Due to the inhibition effect against oxyradical formation the SOD-CAT system that provide the first defense line against oxygen radical toxicity and usually used as biomarker or as an indicator for ROS production [27].

Transaminase [i.e. alanine transaminase(ALT; EC.2.6.1.2) and aspartate transaminase(AST; EC.2.6.1.1)] are excellent markers of tissue damage especially liver tissue. ALT and AST are important and critical enzymes in biological processes. Treatment with $AlCl_3$, resulted a significant increase in the activities of serum ALT as in table (5) and AST as compared with control group. While, vit. C or Vit. E or their combination concomitantly with **$AlCl_3$** causes a significant decrease in these enzymes as compared with a group of rats treated with **$AlCl_3$** only. Knowing that all these values are not up to the values of the control group

Table 5: The protective effect of vitamin C, E and their combination on some of liver enzymes activities in serum of male albino rats treated with aluminum chloride

Treatment groups / Enzyme activity	Mean ± SD*				
	Control	Aluminium (Al)	Al + Vit. C	Al + Vit. E	Al + Vit C + E
Alanine transaminase [ALT] (IU/L)	45.83±3.02 b	55.90±1.24 a	48.50±0.70 b	49.90±3.06 b	50.20±2.95 b
Aspartate transaminase [AST] (IU/L)	66.10±2.02 c	83.90±2.04 a	72.20±1.38 b	73.40±4.20 b	75.30±2.30 b

*Mean ± SD for 3 reduplications.
Number of animals 6 male rats / group.
Numbers are preceded by different letters horizontally indicate a significant difference at the level of probability ($P \leq 0.05$) and correct reverse according Duncan test.

The increase in serum ALT and AST activities of animals treated with $AlCl_3$ (Table 5) are in agreement with the finding of Neelu, et al.,[5]. Abdel Aziz and Zabut [13] El-Demerdash [22], Sallam,. et al.,[33], Chinoy and Memon [64], They found that the exposure to $AlCl_3$ resulted in liver necrosis, and Al exposure can result in Al accumulation in the liver and the metal can be toxic to the hepatic tissue at high concentration [74]. AST significantly increases in such cases. Also, ALT activity indicates to the existence of liver diseases, as this enzyme is present in large quantities in the liver. The level increases in serum when cellular degeneration or destruction occurs in this organ. Therefore, the increase in the activities of these enzymes in serum are considered to be indicators of liver damage and thus alteration in liver function [5,33].

The decrease in serum ALT and AST activities of rats treated with vit.C or vit.E or their combination concomitantly with $AlCl_3$ could be used as an indication improve of liver function and protection from the toxicity of Al. and vit. C or vit. E separately or together reversed the toxic effects of Al ions on the activities of ALT and AST.

CONCLUSION

Results shows that concurrent use of vit.C and vit.E reduced aluminum concentration considerably, indicating the potential activity in combination against aluminum toxicity in male albino rats. Vit.C and vit.E have positive effects on hematological and biochemical parameters, and this shows that these vitamins have a preventive ability in removing oxidative stress.

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