THE PROTECTIVE ROLE OF CERTAIN ANTIOXIDANTS (VITAMINS C AND E AND OMEGA-3 OIL) AGAINST ALUMINUM CHLORIDE INDUCED BIOCHEMICAL CHANGES IN FEMALE ALBINO RATS (RATTUS RATTUS NORVEGICUS)

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Abstract
The present study was undertaken to evaluate the protective effect of certain antioxidants such as Vitamins C, E and Omega-3 oil on Aluminum induced biochemical changes in the female albino rats. Sixty four female adult rats were divided randomly into two control: (control 1) 0 AlCl₃/Kg body weight (b.w.); (control 2) supplied orally with 0.2 ml/rat sun flower oil and six treated groups: AlCl₃ (60 mg/kg b. w.) ; AlCl₃ (60 mg/kg b. w.) plus 0.2ml/rat of 0.5% Acetic acid; AlCl₃ (60 mg/kg b. w.) plus Vit.C (50 mg/kg); AlCl₃ (60 mg/kg b. w.) plus Vit.E (100 mg/kg); AlCl₃ (60 mg/kg b. w.) plus 0.2 ml/rat of 5% Omega-3 and AlCl₃ (60 mg/kg b. w.) plus Vit.C (50 mg/kg) plus Vit.E (100 mg/kg) plus 0.2ml/rat of 5% Omega-3 ) respectively. Rats were orally administered their respective doses every other day for 35 days. At the end of the experiments, body weights were recorded and blood samples were collected for biochemical tests. Rats treated with aluminum chloride in the presence or absence of acetic acid showed significant decreases in the rate of body weight gain as compared with the control. Antioxidants (Vitamins C, E and omega-3) along with aluminum chloride produced protective effects as the rate of body weight gain approximately was more or less similar to the normal values of the control.

The rats treated with AlCl₃ (in the presence or absence of acetic acid ) showed a significant increase in Alanine aminotransaminase (ALT), Aspartate aminotransaminase (AST), Urea and Creatinine and a significant decrease in serum albumin and total protein as compared with the control. The administration of antioxidants (Vit C, E and Omega-3 oil) along with AlCl₃ showed protective effects on liver and kidney since ALT, AST, Urea, Creatinine, albumin and total protein were tending to return towards their normal levels of control. So, the present study showed that Vit C, E and Omega-3 oil can be effective in the protection of aluminum-induced toxicity.

Key Words: Al toxicity, Vitamin C, Vitamin E, Biochemical, Enzymes.

Introduction
Aluminum (Al) is the third most abundant element comprising approximately 8% of the earth’s crust (Klein, 1991). The main sources of Al include corn, yellow cheese, salt, herbs, spices, tea, cosmetics, and Al cooking utensils (El-Demerdash et al., 2004 and Yousef, 2004). In addition, Al compounds are widely used in medicines such as antacids, phosphate binders, buffered aspirin, vaccines and allergen injections and fluids used in renal dialysis (Kaehny et al., 1997 and Yokel, 2004).Normal adults consume approximately 3-5 mg Al in daily with the diet and variable amounts from drinking water depending on local conditions including alum treatment and acidification (Nordberg et al., 1985). Aluminum sulphate is the most widely used coagulant for clarifying turbid drinking water (Martin, 1986; Ochmanski and Barabasz, 2000).

Aluminum is absorbed through the skin, gastrointestinal tract, lung, and nasal mucosa. After absorption, most Al is transported by the blood to various body organs. Bone, muscle and lung contain the highest Al contents in the normal human being. Al uptake by the brain is linked to the presence of high affinity of transferring receptors (Anane et al., 1997).Aluminum is also accumulates in a number of mammalian tissues, including kidney, liver, brain and bone (Anand et al., 2002). Al accumulation in the kidney promotes the degeneration of the renal tubular cells, and inducing nephrotoxicity (Mansour et al., 2006). Therefore, Al accumulation in the kidney promotes renal failure and the subsequent systemic toxicity (Mahieu et al., 2005). Also, Al accumulation in the liver leads to cholestasis (Osinaka et al., 2004).The toxicological effects of Al on humans include encephalopathy (Alfrey et al., 1976), bone disease (Ward et al., 1978), anemia (Short et al., 1980) and skeletal system disease (Gupta et al., 2005).It may also be a contributing factor for the development of Alzheimer’s disease (AD) (Campbell, 2002). These toxic effects of Al have been suggested to be due to the generation of reactive oxygen species (El-Demerdash, 2007), which results in
the oxidative deterioration of cellular lipids, proteins, and deoxyribonucleic acid (DNA) (El-Demerdash 2004; Mansour et al., 2006). So, these toxic effects of Al appear to be mediated, at least in part, by free-radical generation (Moumen et al., 2001; Anane and Creppy, 2001).

Cronan and Schofield (1979) have shown at neutral pH, Al minerals are insoluble, but solubility increases at lower pH. Thus, acidification of lakes and streams by acid rain mobilized Al from the soil to the aquatic environment. The levels of dissolved Al in water are strongly influenced by pH and the presence of other substances in the water (Browne et al., 1990). Some studies were carried out to evaluate the potential protective role of antioxidant vitamins, such as vitamin C, vitamin E (Yousef et al., 1999; Salem et al., 2001).

Vitamin C (Vit.C) (ascorbic acid) is an essential micronutrient required for normal metabolic functioning of the body. Many biochemicals, clinical and epidemiological studies showed that vitamin C may be of benefit in chronic diseases such as cardiovascular disease, cancer and cataract, probably through antioxidant mechanisms (Carr and Frei, 1999).

Vitamin E (Vit.E) (α-tocopherol) is a naturally occurring antioxidant nutrient that has an important role in animal health through the inactivation of harmful free radicals that are produced during normal cellular activity and under various stress conditions (El-Demerdash, 2007; Yousef, 2004). The antioxidant functions of this micronutrient, also, at least in part, enhance immune reactions by maintenance of the functional and structural integrity of the all-important immune cells (Yousef et al., 2003; El-Demerdash et al., 2004).

Omega-3 poly unsaturated fatty acid from fish and fish oil can protect against chronic heart disease (CHD), both health professional and publics are increasingly interested in its role in the prevention and management of CHD. During multiple pharmacological treatments for cardiovascular disease, many researchers believed that dietary intervention or nutritional supplements may be a more natural and acceptable method of providing benefits (Garrido-Sanchez et al., 2008). The current work aimed to study the effect of AlCl₃ on some biochemical parameters and the protective effects of some antioxidants (Vitamins C and E and Omega-3 oil) on Al induced biochemical changes of liver and kidney tissues.

**Materials and Methods**

**Experimental animals**

Adult female albino rats *Rattus rattus norvegicus* were used during the present study. The rats were 10-12 weeks old with a body weight ranging from 190-210 g. The rats were kept in polypropylene rat’s cages at a rate of 2 animals per cage. The cages were bedded with wood chips and the animals had free access to standard rodent diet and tap water *ad libitum*. The animals were kept in animal house of biology department (Faculty of Science, University of Zakho), maintained under laboratory conditions at a controlled temperature of about 24± 2 °C and exposed to a photoperiod of 12 hrs light followed by 12 hrs of darkness. Animals were acclimated to the laboratory condition for about 7 days before the application of experimental work.

**Experimental design**

Sixty four adult female albino rats were used in this study. The rats were divided randomly into eight groups, each of eight individuals and treated as in (Table 1).
Table (1): The distribution of rats in their experimental groups. b.w., body weight; G, treatment groups.

| Groups                  | Number of Rats | Dose                                      | Duration |
|-------------------------|----------------|-------------------------------------------|----------|
| G1: Control             | 8              | ---                                       | 35 days  |
| G2: Control 2           | 8              | 0.2 ml Oil/rat                            | 35 days  |
| G3: Aluminum chloride   | 8              | 60 mg/kg b.w.                             | 35 days  |
| G4: AlCl₃ + Acetic acid | 8              | 60 mg/kg b.w. + 0.2 ml 0.5% Acetic acid   | 35 days  |
| G5: AlCl₃ + Vitamin C   | 8              | 60 mg/kg b.w. + 50 mg Vit.C/kg b.w.       | 35 days  |
| G6: AlCl₃ + Vitamin E   | 8              | 60 mg/kg b.w. + 100 mg Vit.E/kg b.w.      | 35 days  |
| G7: AlCl₃ + Omega-3     | 8              | 60 mg/kg b.w. + 5% Omega-3                | 35 days  |
| G8: AlCl₃ + Vitamin C + | 8              | 60 mg/kg b.w. + 50 mg Vit.C/kg b.w. + 100 | 35 days  |
| Vitamin E + Omega-3     |                | mg Vit.E/kg b.w. + 5% Omega-3             |          |

The doses of AlCl₃, Vit. C and Vit. E were calculated according to the animal’s body weight before their uptake. The desired doses of AlCl₃, Vit. C, Vit. E, Acetic acid and Omega-3 for each animal were daily intubated into oesopharyngeal region daily, using small syringe connected to thin silicon tube.

**Total body weight**

Total body weight for each animal was measured and recorded twice; first at the beginning of the experiment and second at the end of the experiment using a top loading balance (Adventurer OHAMUS, USA). Finally, the rate of body weight gain was calculated.

**Serum biochemical analysis**

The blood sample was taken from the rat by heart puncture and withdrawn into a dry and clean non-heparinized tube. The sample was allowed to clot at room temperature for 30 minutes. Then the sample was centrifuged at 3000 rpm for 15 minute (Dacie and Lewis, 1984). Serum samples were placed in eppendorf tubes and used for determination of some biochemical parameters such as serum ALT, AST, Albumin, Total protein, Urea and Creatinine by using Auto Analyzer Spectrophotometer (Model Lisa Xs-French).

**Statistical analysis**

For body weight and serum biochemical parameters, all data were expressed as mean ± standard error (M ± S.E.) and statistical analysis was carried out using statistical available software (SPSS version 17.0). One way analysis of variance (ANOVA) was performed to test for significance followed by Duncan’s multiple range comparison tests for comparison between the groups. P values (0.05) and (0.01) were considered significant.
Results

Effects of AlCl₃ alone or along with acetic acid and some antioxidants on the body weight gain.

As shown in Table (2) rats treated with AlCl₃ and AlCl₃ plus acetic acid have shown a significant decrease (P<0.05) in the rate of body weight gain as compared with the control. On the other hand, rats treated with AlCl₃ plus Vit.C, AlCl₃ plus Vit.E, AlCl₃ plus omega-3 and their combinations in comparison with control and control 2 did not produced any significant (P > 0.05) reduction in the rate of body weight gain.

Table (2): Effects of AlCl₃ along with acetic acid, and some antioxidants on the body weight gain of rats.

| Groups                      | Monthly Body Weight Gain(gm) % | Mean ± S.E. |
|-----------------------------|--------------------------------|-------------|
| Control                     | 11.204±1.109b                  |             |
| Control 2                   | 10.276±1.308b                  |             |
| AlCl₃                       | 2.651±1.201a                   |             |
| AlCl₃ + Acetic acid         | 2.462±1.016a                   |             |
| AlCl₃+Vit.C                 | 10.347±1.022b                  |             |
| AlCl₃+Vit.E                 | 8.988±1.772b                   |             |
| AlCl₃+Omega-3               | 8.48±1.76b                     |             |
| AlCl₃+Vit.C+Vit.E+Omega-3   | 8.241±1.557b                   |             |

Note: Different letters represent the presence of a significant difference (P<0.05).

Effects of AlCl₃ and some antioxidants on serum ALT.

As illustrated in Table (3) rats treated with AlCl₃ and AlCl₃ plus acetic acid have shown a significant increase (P<0.01) in serum ALT as compared with control. On the other hand, serum ALT activity in rats treated with AlCl₃ plus Vit.C, AlCl₃ plus Vit.E, AlCl₃ plus Omega-3 and their combinations was not influenced by Al and they showed approximately normal ALT activity which was statistically non-significant when compared with that of the control (P>0.05).

Effects of AlCl₃ and some antioxidants on serum AST.

Rats treated with AlCl₃ in the presence of acetic acid showed a significant increase (P<0.01) in AST activity as compared with the control (Table 3). The presence of individual antioxidants along with Al showed a mild protective effect of body organs, since AST activity was still elevated but to a lesser extent as compared with Al treated rats. However, a combination of Vit.C and E and Omega-3 along with Al showed much better protective effect as indicated by more or less normal AST activity.

Effects of AlCl₃ and some antioxidants on serum Total Protein.

As the results indicate, aluminum in the presence or absence of acid significantly reduced the level of total protein (P < 0.05) when compared with control. On the other hand, in rats supplied with Vit.C and E and Omega-3 and their combination showed protective effects on serum total protein since its level was closely similar to its normal level as shown in table (3).
Effects of AlCl₃ and some antioxidants on serum Albumin.

In rats treated with AlCl₃ and with AlCl₃ plus acetic acid had shown a significant reduction (P<0.01) in the level of albumin as compared with those of the control rats. On the other hand, the level of Albumin in rats treated with Vit. E and Omega-3 and their combinations along with Al returned to more or less to normal values and showed non-significant differences as compared with the control (P > 0.01). However, in rats treated with Vit. C along with Al, the level of albumin was significantly reduced (P < 0.01). As shown in table (3).

Effects of AlCl₃ and some antioxidants on serum Urea.

As the results indicated, rats treated with Al in the presence or absence of acid produced a mild and statistically non-significant elevation (P > 0.05) in the level of serum urea. Furthermore, administration of Vit. C and E and Omega-3 returned the level of serum urea toward the control level as shown in table (3).

Effects of AlCl₃ and some antioxidants on serum Creatinine.

The results of the experiments on the effect of AlCl₃ alone or with acid, Vit.C and E and Omega-3 on serum creatinine level are showed in Table (3). As the results indicate, AlCl₃ alone caused a highly significance (P<0.01) elevation in the level of creatinine as compared with the control. On the other hand, administration of Vitamin C, E, and Omega-3 and their combinations showed a protective effect on the kidney as indicated by the exhibition of approximately the control creatinine values.
**Table (3): Effect of AlCl₃ alone or along with acetic acid and some antioxidants on some biochemical parameter.**

| Groups                                      | SALT ** (IU/L) | SAST ** (IU/L) | T. Protein * (g/dl) | Albumin ** (g/dl) | Urea | Creatinine ** (mg/dl) | (g/dl) | (g/dl) |
|---------------------------------------------|----------------|----------------|---------------------|-------------------|------|-----------------------|--------|--------|
| 1. Control                                  | 64.666±2.027 a | 129.333±1.855 a | 6.333±0.088 b       | 2.533±0.176 b     | 48.666±1.333 a |                  | 0.556±0.0088 a |        |
| 2. Control 2                                | 82.804±3.647 a | 149.800±13.990 ab | 6.620±0.086 b       | 2.568±0.021 b     | 51.400±2.204 a |                  | 0.554±0.0067 a |        |
| 3. AlCl₃                                    | 118.750±8.097 b | 167.200±26.946 ab | 5.797±0.178 a       | 2.230±0.068 a     | 54.400±2.400 a |                  | 0.688±0.0546 b |        |
| 4. AlCl₃ + Acetic acid                      | 122.600±13.786 b | 195.000±8.955 b | 5.820±0.124 a       | 2.152±0.024 a     | 50.400±2.336 a |                  | 0.626±0.0222 ab |        |
| 5. AlCl₃ + Vit.C                            | 97.600±2.227 ab | 157.200±10.165 ab | 6.400±0.192 b       | 2.868±0.069 c     | 49.000±0.707 a |                  | 0.550±0.0204 a |        |
| 6. AlCl₃ + Vit.E                            | 93.166±2.227 ab | 159.000±9.295 ab | 6.475±0.170 b       | 2.653±0.057 bc    | 48.000±0.577 a |                  | 0.540±0.0200 a |        |
| 7. AlCl₃ + Omega-3                          | 92.400±5.045 ab | 161.666±3.343 ab | 6.520±0.106 b       | 2.746±0.036 bc    | 47.400±2.785 a |                  | 0.528±0.0073 a |        |
| 8. AlCl₃ + Vit.C + Vit.E + Omega-3          | 90.000±2.594 ab | 120.666±4.835 a | 6.516±0.203 b       | 2.804±0.048 a     | 49.000±2.260 a |                  | 0.520±0.0089 a |        |

The values represented by mean ± S.E. of Mean, N=8, Duncan's test used to compare between groups, similar letters in the same column refers to non-significant level while different letters represent to significant level: ** (P < 0.01) and * (P < 0.05).
Discussion

In the presented study, oral administration of AlCl$_3$ in the presence or absence of acetic acid for 35 days significantly reduced the rate of body weight gain as compared with the control groups. These results agree with those observed by Sallam et al., (2005) in rats treated with 34mg /kg AlCl$_3$. This reduction in the rate of body weight gain may be due to the elevation of malonaldehyde level by heavy metals and a reduction in the levels of both glutathione and catalase. Variation in the activity of these enzymes may contribute in the maintenance of lipid peroxidation induced by the metals (Corpas et al., 2002). Furthermore, partial disruption of small intestine villi and subsequent malabsorption of nutrients represents another factor that may be responsible for the loss of body weight (Al-Qudah, 2006). This reduction in nutrients transport causes an inhibition in adenosine tri phosphate (ATP) production, active transport in amino acid and subsequent inhibition in protein syntheses (John 1982).

In this study, rats treated with some antioxidants (vitamins C and E and omega-3) along with aluminum, the rate of body weight gain increased as compared with that animals treated with Al in the presence or absence of acid. This may be due to the antagonists effect of above vitamins on the toxic effect of Al and subsequent protection of the body from Al-toxicity (Yousef, 2004 and El-Demerdash, 2007). Furthermore, the antioxidants effect of omega-3 also reduces the aluminum toxicity (Mete et al., 1999).

In the current study, treatment of rats with AlCl$_3$ or AlCl$_3$ with acetic acid significantly increased the activities of both serum ALT and AST. The toxic effect of Al was enhanced in the presence of acid. These results agree with those reported by Al-Sulaivany (2010) in rats treated with Al in the presence or absence of acid. He found significant elevation in serum ALT and AST activities and reduction in their activities in liver and kidney tissues. This indicates that increase in serum enzyme activity is resulted from the leak of the enzyme from body tissues and organ including liver and kidney tissues. Similar results were also observed by Hassoun and Stoths (1995), Chinoy and Memon (2001) and El-Demerdash (2007). They indicated that exposure to Al caused liver necroses and subsequent escape of AST from them to the blood. Furthermore, the increase in ALT level is resulted from the cellular destruction of the body tissues including the liver (Harper et al., 1979).

The presences of vitamins C, E, omega-3 and their combinations along with Al alleviated the toxicity of Al on body tissues since the activities of both ALT and AST tended to return back approximately to the normal levels. Furthermore, combinations of omega-3, vitamins C and E greatly reduced the toxic effect of Al as indicated by the return of the activities of these enzymes to their normal values. Due to the availability of limited information about the protective effects of antioxidant on Al toxicity in rodents, it is difficult to compare the results. However, Al-sulaivany (2010) observed more or less a similar protection effect of antioxidants on ALT and AST in the tissues of rats exposed to Al. Also a similar reduction in the toxic effect of heavy metals in the presence of antioxidants was observed by Tawwab et al. (2004).

In this study, rats treated with Al in the presence or absence of acid significantly elevated the level of serum creatinine. A similar elevation in urea and creatinine level in AlCl$_3$ treated rats was considered as a significant marker for renal dysfunction (El-Demerdash, 2007 and Al-Sulaivany, 2010). Szilagyi et al. (1994) reported that alteration in serum urea may be related to metabolic destruction (e.g. renal function, cation-balance… etc.) produced by heavy metals. In addition, Katyal et al. (1997) reported that Al has been implicated in the pathogenesis of several clinical disorders, including renal dysfunction. Increased urea concentrations in the plasma of animals treated with Al and Al plus acetic acid may be due to its effect on liver function, as urea is the end-product of protein catabolism, and/or referred to kidney dysfunction as indicated by enlargement of the relative weight of kidney. Decreased protein levels in Al-treated rats might be due to changes in protein synthesis and/or metabolism (Chinoy and Memon 2001).

Exposure of rats to AlCl$_3$ in the presence or absence of acid significantly reduced total serum protein and albumin. These results agree with those reported by Al-Sulaivny (2010) and Al-Demerdash (2004). Decreased serum protein in rats exposed to Al might be due to villi disruption and subsequent malabsorption and transport of nutrients (Al-Qudah, 2006). This was followed by depression of protein synthesis and metabolism (Chinoy and Memon, 2001).

The uptake of vitamins C, E and omega-3 produced a protective effective in Al treated rats
since the levels of total protein and albumin returned approximately to their normal values. Similar results were reported by Al-Sulaivany (2010) during administration of antioxidants to Al treated rats. Al intoxicated animals showed a number of indicators of oxidative stress, which includes increases in the level of Thiobarbituric acid reactive substances (TBARS) and decreases in Glutathione (GSH), Glutathione S-transferase (GST) and catalase in the rats testes (Yousef and Salama 2009). Al induced oxidation stress may be resulted from the generation of free radical (Gomez et al., 1997; Yousef, 2004; Yousef et al., 2005). However, Al is considered to be a non-redox active metal, it promotes biological oxidation both in vitro and in vivo because of its pro-oxidant activity (Gomez et al., 2005; Yousef et al., 2007; Turner and Lysiak, 2008). Increased reactive oxygen species (ROS) was reported in previous studies during Al exposure, which was attributed to electron leakage, enhanced mitochondrial activity and increased electron chain activity(Flora et al., 2003). Furthermore, they added that ROS subsequently attack almost all cell components including membrane lipids and producing lipid peroxidation. Therefore, it can be hypothesized that oxidative stress may be one of the contributing factors to Al-induced liver dysfunction (Yousef and Salama, 2009). Finally it was indicated that when rats treated with AlCl3 had undergone a reduction in the body weight gain. These effects were counteracted on administration antioxidants and omega-3 along with AlCl3. and producing lipid peroxidation. Therefore, it can be hypothesized that oxidative stress may be one of the contributing factors to Al-induced liver dysfunction (Yousef and Salama, 2009). Finally it was indicated that when rats treated with AlCl3 had undergone a reduction in the body weight gain. These effects were counteracted on administration antioxidants and omega-3 along with AlCl3. and serum biochemical parameters were returned to more or less normal values when rats were treated with antioxidants and omega-3 along AlCl3.

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