

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*)

Omar A.M. AL. Habbib, Najem S. Gorgees and Reyzan A. Hussein*

Dept. of Biology, Faculty of science, University of Zakho, Kurditan Region – Iraq.

* School of Animal Production, Faculty of Agriculture and Forestry, University of Duhok, Kurditan Region – Iraq.

(Accepted for publication: August 28, 2014)

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 (Osinska *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 biochemical, 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 $AlCl_3$ 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 $24 \pm 2^\circ C$ 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: AlCl ₃ + Acetic acid	8	60 mg/kg b.w. + 0.2 ml 0.5% Acetic acid	35 days
G5: AlCl ₃ + Vitamin C	8	60 mg/kg b.w. + 50 mgVit.C/kg b.w.	35 days
G6: AlCl ₃ + Vitamin E	8	60 mg/kg b.w. + 100 mgVit.E/kg b.w.	35 days
G7: AlCl ₃ + Omega-3	8	60 mg/kg b.w. +5% Omega-3	35 days
G8: AlCl ₃ + Vitamin C + Vitamin E + Omega-3	8	60 mg/kg b.w. + 50 mgVit.C/kg b.w. + 100 mgVit.E/kg b.w. + 5% Omega-3	35 days

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 (Adventure™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±1.109 ^b
Control 2	10.276±1.308 ^b
AlCl ₃	2.651±1.201 ^a
AlCl ₃ + Acetic acid	2.462±1.016 ^a
AlCl ₃ +Vit.C	10.347±1.022 ^b
AlCl ₃ +Vit.E	8.988±1.772 ^b
AlCl ₃ +Omega-3	8.48±1.76 ^b
AlCl ₃ +Vit.C+Vit.E+Omega-3	8.241±1.557 ^b

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 * (mg/dl)	Creatinine ** (mg/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	82.800±12.866 ^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.AlCl ₃	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.AlCl ₃ + 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 ^{a,b}
5.AlCl ₃ + 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.AlCl ₃ + Vit.E	93.166±3.544 ^{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.AlCl ₃ + 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.AlCl ₃ + Vit.C+Vit.E+Omega-3	90.000±2.594 ^{ab}	120.666±4.835 ^a	6.516±0.203 ^b	2.804±0.046 ^{bc}	48.333±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 serum biochemical parameters were returned to more or less normal values when rats were treated with antioxidants and omega-3 along AlCl₃.

References

- Alfrey, A. C.; LeGendre, G. R. and Kaehny, W. D. (1976). The dialysis encephalopathy syndrome: Possible aluminum intoxication. *N. Engl. Jour. Med.* 294:184-188.
- AL-Qudah, M. M. A. (2006). Histological and physiological effect of lead compound on digestive system of swiss mouse. PhD thesis of Physiology. Faculty of Graduate Studies. University of Jordan.
- Al-Sulaivany, B. S. A. (2010). Antioxidant effect of omega-3 and multivitamins on Aluminum-induced changes in some hematological and biochemical parameters in Albino rats. Ms.c thesis of physiology. University of Duhok, Duhok, Iraq.
- Anand, R.; Harry, D.; Holt, S.; Milner, P.; Dashwood, M.; Goodier, D.; Jarmulowicz, M. and Moore, K. (2002). Endothelin is an important determinant of renal function in a rat model of acute liver and renal failure. *Gut.*, 50:111–117.
- Anane, R. and Creppy, E. E. (2001). Lipid peroxidation as a pathway to aluminum cytotoxicity in human skin fibroblast cultures: prevention by superoxide dismutase plus catalase and vitamins E and C. *Hum. Exp. Toxicol.*, 20: 477-481.
- Anane, R.; Bonini, M. and Creppy, E. E. (1997). Transplacental passage of aluminum from pregnant mice to fetus organs after maternal transcutaneous exposure. *Hum. Exp. Toxicol.*, 16:501-504.
- Browne, B. A.; McColl, J. G.; and Driscoll, C.T. (1990). Aluminum speciation using morin: I, II. in humans. *Am. Jour. Clin. Nutr.*, 69: 1086–1107.
- Campbell, A. (2002). The potential role of aluminum in alzheimer's disease. *Nephrol. Dial. Transplant.*, 17: 17-20.
- Carr, A. C. and Frei, B. (1999). Toward a new recommended dietary allowance for Vitamin C based on antioxidant and health effects in humans. *Am. Jour. Clin. Nutr.*, 69:1086–1107.
- Chinoy, N. J. and Memon, M. R. (2001). Beneficial effects of some vitamins and calcium on fluoride and aluminum toxicity on gastrocnemius muscle and liver of male mice. *Fluoride*, 34:21-33.
- Corpas, I.; Benito, M. J.; Marquina, D.; Castillo, M.; Lopez, N. and Antonio, M. T. (2002). Gestational and lactational lead intoxication produces alterations in the hepatic system of rat pups. *Ecotoxicol. Environ. Saf.*, 51: 35-43.
- Cronan, C. S. and Schofield, C. L. (1979). Aluminum leaching response to acid precipitation: Effect on high-elevation watersheds in the Northeast. *Science* 204:304-306.
- Dacie, S. J. V. and Lewis, S. M. (1984). Practical hematology. 6th ed. Churchill Livingstone. Edinburg, London, Melbourne, and New York.
- El-Demerdash, F. M.; Yousef, M. I.; Kedwany, F. S; and Baghdadi, H. H. (2004). Role of α -tocopherol and β -carotene in ameliorating the fenvalerate-induced changes in oxidative stress, hemato-biochemical parameters, and semen quality of male rats. *Jour. Environ. Sci. Health Bull.*, 39:443–459.
- El-Demerdash, F.M. (2007). Antioxidant effect of vitamin E and selenium on lipid peroxidation, enzyme activities and biochemical parameters in rats exposed to aluminum. *Jour. Trace Elem. Med. Biol.*, 18:113–121.
- Flora, S. J. S.; Mehta, A.; Satsangi, K.; Kannan, G. M. and Gupta, M. (2003). Aluminum induced oxidative stress in rat brain: response to combined administration of citric acid and HEDTA. *Comp. Biochem. Physiol.*, 134: 319–328.
- Garrido-Sanchez, L.; García-Fuentes, E.; Rojo-Martínez, G.; Cardona, F.; Soriguer, F.; Tinahones, F.J. (2008). Inverse relation between levels of anti-oxidized-LDL antibodies and eicosapentanoic acid (EPA). *Br. Jour. Nut.* 22: 1–5.

- Gomez, M.; Sanchez, D. J.; Llobet, J. M.; et al. (1997). The effect of age on aluminum retention in rats. *Toxicology*, 116:1-8.
- Gupta, V. B.; Anitha, G.; Hegda, M. L.; Zecca, L.; Garruto, R. M.; Ravid, R.; Shankar, S. K.; Stein, R.; Hanmugavelu, P. and Jagannatha Rao, K.S. (2005). Aluminum in Alzheimer's disease: are we still at a crossroad. *Cell Mol. Life Sc.*, 62: 143-158.
- Harper, H. A. Rodwell, V. W. and Mayers, P. A. (1979). Review of physiological Chemistry. Middle East Edition.
- Hassoun, E. A. and Stohs, S. J. (1995). Comparative studies on oxidative stress as a mechanism for the fetotoxicity of TCDD, endrin and lindane in C57BL/6J and DBA/2 J mice. *Teratology*, 51:186.
- John, N. H. (1982). Nutritional Toxicology: Definition and Scope. In: *Nutritional Toxicology*, academic press, Inc., 1: 1-15.
- Kaehny, W.; Hegg, A. and Alfrey, A. (1997). Gastrointestinal absorption of Aluminum from Aluminum containing antacids. *N. Engl. Jour. Med.*, 296: 1389-1390.
- Katyal, R.; Desigan, B.; Sodhi, C. P. and Ojha, S. (1997). Oral aluminum administration and oxidative injury. *Biol. Trace Elem. Res.*, 57: 125-130.
- Klein, G. L. (1991). The aluminum content of parental solutions: current studies. *Nutrition Reviews*, 49:74-79.
- Mahieu, S. N.; Millen, M.; Gonz'alez, M. C.; Contini and Elias, M. M. (2005). Alterations of the renal function and oxidative stress in renal tissue from rats chronically treated with aluminum during the initial phase of hepatic regeneration. *Jour. Inorg. Biochem.*, 99:1858-1864.
- Mansour, S.; Alan, S.; and Norman, B. R. (2006). Aluminum-induced injury to kidney proximal effects on markers of oxidative damage. *Jour. Trace Elem. Med. Biol.*, 19:267-273.
- Martin, R. B. (1986). The chemistry of aluminum as related to biology and medicine. *Clin. Chem.*, 32: 1797-1806.
- Mete, N.; Isik, B.; Erdinc, L. and Gurkan, F. (1999). The Effect of Fish Oil on Liver and Plasma MDA and Antioxidant Status of Rats. *Tr. Jour. Med. Sci.*, 1-6.
- Moumen, R.; Ait-Oukhatar, N.; Bureau, F.; Fleury, C.; Bougle, D. and Arhan, P. (2001). Aluminum increases xanthine oxidase activity and disturbs antioxidant status in the rat. *Jour. Trace Elem. Med. Biol.*, 15: 89-93.
- Nordberg, G. F.; Goyer, R. A. and Clarkson, T. W. (1985). Impact of effects of acid precipitation on toxicity of metals. *Environ. Health Perspect.*, 63:169-170.
- Ochmanski, W. and Barabasz, W. (2000): Aluminum-occurrence and toxicity for organisms. *Przegl. Lek.*, 57:665-668.
- Osinska, E.; Kanoniuk, D. and Kusiak, A. (2004): Aluminum hemotoxicity mechanisms. *Ann. Univ. Mariae Curie Sklodowska Med.*, 59: 411- 416.
- Salem, M. H.; Kamel, K. I.; Yousef, M. I.; Hassan, G. A. and EL-Nouty, F. D. (2001). Protective role of ascorbic to enhance semen quality of rabbits treated with sublethal doses of aflatoxin B1. *Toxicology*, 162: 209-218.
- Sallam, S. M. A.; Nasser, M. E. A.; Yousef, M. S. H.; El-morsy, A. M.; Mahmoud, S. A. S. and Yousef, M.I. (2005). Influence of aluminum chloride and ascorbic acid on performance, digestibility, caecal microbial activity and biochemical parameters of rabbits. *Agr. and Biol. Sci.*, 1: 10-16.
- Short, A. I. K.; Winney, R. J.; and Robson, J. S. (1980). Reversible microcytic hypochromic anaemia in dialysis patients due to aluminum intoxication. *Proc. Eur. Dial. Transplant. Assoc.* 17: 226-233.
- Szilagyi, M.; Bokori, J.; Fekete, S.; Vetesi, F.; Albert, M. and Kadar, I. (1994). Effects of long-term aluminum exposure on certain serum constituents in broiler chickens. *Eur. Jour. Clin. Chem. Clin. Biochem.*, 32: 485-486.
- Tawwab, M.A.; Mamdouh, A.A. ; Mohammad, H. and Saleh, F.M. (2007). The use of calcium pre-exposure as a protective agent against environmental copper toxicity for juvenile Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture*. 264:236-246.
- Turner, T. T. and Lysiak, J. L. (2008). Oxidative stress: a common factor in testicular dysfunction. *Jour. Androl.*, 29: 488-498.
- Ward, M. K.; Feest, T. G.; Ellis, H. A.; Parkinson, I. S.; Kerr, D. N. S.; Herrington, J. and Goode, G. L. (1978). Osteomalacic dialysis osteodystrophy: Evidence for a water-borne aetiological agent, probably aluminum. *Lancet.*, 1: 841-845.
- Yokel, R. A. (2004). Aluminum. In elements and their compounds in the environment, occurrence, analysis and biological relevance (E. Merian, M. Anke, M. Ihnat, and M. Stoepler, Eds), 2: 635-658. Wiley- VCH, Weinheim, Germany.
- Yousef, M. I. (2004). Aluminum-induced changes in hemato-biochemical parameters, lipid per oxidation and enzyme activities of male rabbits: Protective role of ascorbic acid. *Toxicology*, 199: 47-57.
- Yousef, M. I.; Abdallah, G. A. and Kamel, K. I. (2003). Effect of ascorbic acid and vitamin E supplementation on semen quality and biochemical parameters of male rabbits. *Anim. Reprod. Sci.*, 76:99-111.
- Yousef, M. I.; Afrah, F. and Salama, (2009). Propolis protection from reproductive toxicity caused by aluminum chloride in male rats. *Food and Chem. Toxicol.*, 47: 1168-1175.
- Yousef, M. I.; EL-Demerdash, F. M. and EL-Agamy, E. I. (1999). Effect of ascorbic acid on some biochemical parameters of rabbits affected by aflatoxin B1. *Environ. Nut. Intl.*, 3: 141-153.
- Yousef, M. I.; El-Morsy, A. M. A. and Hassan, M. E. (2005). Aluminum-induced deterioration in

reproductive performance and seminal plasma biochemistry of male rabbits: Protective role of ascorbic acid. *Toxicology*, 215: 97-107.

Yousef, M. I.; Kamel, K. I.; El-Guendi, M. I. and El-Demerdash, F. M. (2007). An in vitro study on

reproductive toxicity of aluminum chloride on rabbit sperm: the protective role of some antioxidants. *Toxicology*, 239: 213-223.

هه لسه نگاندا کارتیکه ریت پاراستنا هنده ك كه رهسیت دژی نه كسه دی وهك فیتامین سی و ی و زهیتامیلاكا نهه نگی ل سهر گهورینا چهند پارامیته ریت خوینی یین جردین سبی نهویت هاتینه توشكرن بو نهلمنیوم كلورایدی

كورتیا فه كولیئی

مهروم ژ فئی فه كولیئی نه وه هه لسه نگاندا کارتیکه ریت پاراستنا هنده ك كه رهسیت دژی نه كسه دی وهك فیتامین سی و ی و زهیتامیلاكا نهه نگی ل سهر گهورینا چهند پارامیته ریت خوینی یین جردین سبی نهویت هاتینه توشكرن بو نهلمنیوم كلورایدی. دفی فه كولیئیدا ۶۴ جردین سبی یین گههستی كوژیی وا دناقیهرا ۱۰-۱۲ حهفتیاندا بوون و كیشاوا دناقیهرا ۱۹۰-۲۱۰ گراما دا بون. نهؤ جرده هاتنه خودان كرن ل ژورا گیانه وهرا ل پشكا بایولوجی، كولیژا زانست، لزانكویا زاخو ل بن كاودانین ستاندارد یین تاقیگههی كویلا گهرماتیی ۲۴ پلهیه ودهمی روناھی ۱۲ دمژمیرن، خارن وئاؤ دهاتنه بهرههفكرن روژانه تا دو ماهیكا فه كولیئی. نهؤ جردین هندی هاتنه دابهشكرن ل سهر ۸ كوما وه کی ل خواری دیار كری: - كوما ئیكی (كونترول).

كومادووی (كونترول 2، دانا ۰،۲، ملیت زهیتا گولبه روژا بو ههر جرده کی بریكا دهفی). كوما سی (دانا ۶۰ ملیگرامیته نهله منیوم كلورایدی بو ههر کیلویه کی ژكیشا لهشی بریكا دهفی). كوما چواری (دانا ۶۰ ملیگرامیته نهله منیوم كلورایدی بو ههر کیلویه کی ژكیشا لهشی دگهل ۰،۲ ملا ۰،۵٪ ژ ترشی خهلیکی بریكا دهفی). كوما پینجی (دانا ۶۰ ملیگرامیته نهله منیوم كلورایدی بو ههر کیلویه کی ژكیشا لهشی دگهل ۰،۵ ملیگرامیته فیتامین سی بو ههر کیلویه کی ژكیشا لهشی بریكا دهفی). كوما شهشی (دانا ۶۰ ملیگرامیته نهله منیوم كلورایدی بو ههر کیلویه کی ژكیشا لهشی دگهل ۱۰۰ ملیگرامیته فیتامین سی بو ههر کیلویه کی ژكیشا لهشی بریكا دهفی). كوما حهفتی (دانا ۶۰ ملیگرامیته نهله منیوم كلورایدی بو ههر کیلویه کی ژكیشا لهشی دگهل ۰،۲ ملا بو ههر جرده کی ژ ۰،۵٪ ژ زهیتامیلاكا نهه نگی بریكا دهفی). كوما ههشتی (۶۰ ملیگرامیته نهله منیوم كلورایدی بو ههر کیلویه کی ژكیشا لهشی دگهل ۰،۵ ملیگرامیته فیتامین سی بو ههر کیلویه کی ژكیشا لهشی دگهل ۱۰۰ ملیگرامیته فیتامین سی بو ههر کیلویه کی ژكیشا لهشی دگهل ۰،۲ ملا بو ههر جرده کی ژ ۰،۵٪ ژ زهیتامیلاكا نهه نگی بریكا دهفی). لدوما هیا فه كولیئی كوبوماوی ۳۵ روژا فه كیشا، كیشین جردا هاتنه تومار كرن، سامپلیت خوینی هاتنه وهرگرتن.

نهو جردین هاتینه توشكرن بو نهلمنیوم كلوراید دگهل ترشی خهلیکی یان ژی بتنی دیار بو كو كیمبونه كا بهرچاؤ هه بو د كیشا لهشین جردا دا. بهلی دانا نهو كه رهسیتین دژی نه كسه دی وهك (فیتامین سی و ی و زهیتامیلاكا نهه نگی) دگهل نهلمنیوم كلوراید دئه نجامدا نهف كیشه زفراندن بو نژیکی كونترول.

نهو جردین هاتینه توشكرن بو نهلمنیوم كلوراید دگهل ترشی خهلیکی یان ژی بتنی دیار بو كویلندوبونه كا بهرچاؤ هه بو دئه نزمین AST,ALT و هروسا بلندبونا یوریا و كریاتینینی دخوینیدا، دیسان كیمبونه كا بهرچاؤ هه بو د پروتینی وئه لیبومینی خوینیدا دهمی نهه بهراوردبكه یین دگهل كونترول.

دانا چهند كهرهسیتین دژی نه كسه دی وهك (فیتامین سی و ی و زهیتامیلاكا نهه نگی) دگهل نهلمنیوم كلوراید دیار بو كوئه نزمین AST,ALT دئاستی سروشتیدا بو وزفرینا ئاستین یوریا و كریاتینین و پروتین وئه لیبومینی بوئاستین سروشتی.