EFFICACY OF CYCLOHEXANE-DIAMINE-TETRAACETIC ACID (CDTA) IN TREATMENT OF ALUMINUM CHLORIDE TOXICITY IN RABBITS

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ABSTRACT

Toxic effects of AlCl3 in rabbits with reference to the efficacy of cyclohexanediaminetetra acetic acid (CDTA) in its treatment were evaluated. Forty male adult New Zealand white rabbits were divided into four groups (n= 10). The first group was received only distilled water and served as control. The second group was dosed 20 mg AlCl3/kg bw orally via drinking water for 3 months. The third group was dosed 20 mg $AlCl_3$ /kg bw orally via drinking water plus 4.2 mmol CDTA /kg bw intraperitoneal (i.p.) daily for 3 months. The fourth group was dosed 20 mg $AlCl_3$/kg bw orally via drinking water for 3 months. then $AlCl_3$ administration was stopped and the animals treated with 4.2 mmol CDTA /kg bw i.p. daily for another 3 months. At the end of the experiment, blood samples were collected for RBCs, WBCs and differential leucocytic counts, Hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). Serum samples were obtained for creatinine, urea, uric acid, glucose, AP, AST, ALT, total protein, albumin, globulin, A/G ratio, triglycerides, lactate dehydrogenase (LDH) cholesterol, Aluminum, Copper, iron, zinc, calcium and phosphorus assay. Results revealed significant decrease in RBCs, WBCs, PCV, Hb and MCHC in group II and slightly decrease in group IV while group III showed no significant difference compared with group I. Increase the number of neutrophils, eosinophils and monocytes in group II followed by groups IV and III while basophils and lymphocytes took an opposite manner. Significant increase in creatinine, urea, uric acid, AP, ALT, AST, triglycerides and LDH concentrations in group II followed by group IV while group III showed no significant difference compared with control. Significant decrease in glucose, cholesterol and total protein and its fractions (albumin and globulin) in group II and III. Significant increase in aluminum and iron in-group II and IV while in-group III there is no different from control (group I). Decreased concentration of Cu, Zn, Ca and P in-groups II and IV while group III show no significant difference. The use of Cyclohexanediamineteraacetic acid (CDTA) in alleviating AlCl3 toxicity was effective firstly in the third group followed by group IV. The use of CDTA in treatment of aluminum toxicity was most effective when used as prophylactic or when administered in the same time with aluminum as appeared clearly in the third group than it administered post aluminum exposure.

INTRODUCTION

Aluminum is found in relatively high concentration in the earth crust, drinking water, and several pharmacological preparations and in many proceeds of food. It is also well known that high dosage of medicaments like antacids containing aluminum hydroxide contribute to the body burden of aluminum (**Spencer *et al.*, 1982**). Other important sources are foodstuffs prepared and/or stored in aluminum utensils. However, the latter has attracted little attention and has been dismissed as trivial compared to the other sources (**Trapp and Cannon, 1981**), despite it had been known for more than half a century that aluminum pots or foils are vulnerable to generation by acidic or alkaline foods (**King *et al.*, 1981**). **Lione, (1983)** had shown that an aluminum pot used to prepare tomato sauce could add up to 4-mg aluminum to each serving. **Lione *et al.*, (1984)** found that coffee brewed in aluminum precolators contained a considerable amount of aluminum. Known sources of aluminum include air (**Epstein, 1987**), antacids and antiperspirants (**Graves *et al.*, 1990**), cosmetics (**Walton, 1992**), dental preparations, Vaccines and allergic extracts (**McLachlan *et al.*, 1991**), food additives (**Greger, 1985**), tea, water and canned drinks (**Michel *et al.*, 1990; Kawachi and Pearce, 1991** and **Aikoh and Nishio, 1996**). The most common foods with substantial amounts of Al-containing additives include some processed cheeses, baking powders, cake mixes, pancake mixes, self-raising flours and pickled vegetables (**Lione, 1983**). With regard to the absorption of aluminum, the gastrointestinal tract constitutes the main route of entry for this metal into the body. However, the absorption rate is low in normal human subjects (**Brown *et al.*, 1986**). Aluminum hydroxide, administered therapeutically in large quantities as an antacid and phosphate binder, has been suggested to contribute to the aluminum accumulation and toxicity (**Nathan and Pedersen, 1980; Cannata *et al.*, 1983** and **Lione, 1985**). A part from the intake of the pharmacological doses of aluminum, the possible impact of low or moderate diet levels on body retention of aluminum has been discussed by **Greger and Baier, (1983)** and **Lione, (1985)**.

Aluminum (Al III) is a well-known toxic agent and represents a severe problem for a variety of medical (**Nicolini *et al.*, 1992**) as well as environmental situations (**Meranger, 1989**). Teratogenesis bioassays in rodents (hamsters, mice, or rats) have yielded positive results for compounds of Al, As, Bo, Cd, Co, Cr, Cu, Ga, Hg, Li, Mn, Ni, Pb, Se, U, V, and Zn, producing fetal and early postnatal deaths, as well as malformations (e.g., exencephaly, eye defects, cleft palate, skeletal anomalies). Exposures to Cd, Co, Cu, CH3Hg, Li, Ni, Pb, and Zn during embryogenesis have been reported to cause developmental malformations of the South African frog, Xenopus laevis e.g., bent tail, craniofacial deformity, ocular abnormalities, intestinal

malrotation, cardiac anomalies (**Sunderman, 1998**). Aluminum is considered to be neurotoxic metal and it often connected with the onset of neurodegenerative diseases (**Toimela &Tahti, 2004**). The subchronic exposure of rats for AlCl3 leads to behavioral and neurophysiological changes (**Baydar *et al.*, 2003**). In Alzheimer's disease (AD), analytical as well as epidemiological studies suggest an implications abnormal focal accumulation of aluminum in the brain. In this disease, aluminum may interfere with various biochemical processes including acetylcholine metabolisand can thus act as a possible etiopathogenic cofactor (**Doll, 1993 and Zatta *et al.*, 2002**). Data point to oligodendrocytes as a CNS cell type particularly sensitive to Al, and myelination as a process disrupted by Al exposure. Al, like Fe, can be taken up by oligodendrocytes via transferrin receptors. The investigator hypothesizes that Al interferes with Fe uptake of oligodendrocytes during the critical period of developmental myelination, and that, in adults, Al in oligodendrocytes becomes localized in myelin where it promotes oxidative damage and myelin loss (**Golub, 1999**). **Berlyne *et al.*, (1972)** attributed liver damage, to the reduction of liver oxygen consumption as a result of aluminum toxicity. They also found that increase in aluminum level was associated with the depression of cell respiration and inhibition of protein synthesis. Oral aluminum administration during pregnancy produces growth retardation and delayed ossification (**Golub and Domingo, 1996**). The evidence implicating Al as a neurotoxin has been continuously mounting. Research with both animals and humans has linked Al with neurocognitive dysfunction and in some cases, death (**Rifat *et al.*, 1990; Kristensen *et al.*, 1990 and Murray *et al.*, 1991**). There is a relationship between Al intake and Alzheimer's (**McLachlan *et al.*, 1991**). The neurotoxic effects of aluminum have been well documented by a number of human and animal studies (**Marquis, 1989** and **Sturman and Wisniewski, 1988**). Elevated aluminum concentration in the central nervous system have been related to impaired motor function and to a number of cognitive deficits in both human (**Bolla *et al.*, 1992** and **Rifat *et al.*, 1990**) and experimental animals (**Comissaris *et al.*, 1982; Connor *et al.*, 1988** and **Gonda *et al.*, 1997**).

The present study was conducted to elucidate the toxic effect of AlCl3 on rabbit and it was taken into consideration to investigate the efficacy of cyclohexane-diamine-tetraacetic acid (CDTA) in its treatment.

MATERIAL AND METHODS

Chemicals:

Aluminum chloride hexahydrate was purchased from Merck Company. Cyclohexanediamineteraacetic acid (CDTA) was obtained from Sigma Chemical Company.

Animals:

Forty male adult white New Zealand rabbits weighing (3-4) kg were examined to ascertain that to be diseased free. They were kept under hygienic conditions, were maintained on 12-hrs light/dark cycle, were fed on balanced ration and were given water ad libitum during the experiment.

Experimental design:

Rabbits were divided into four equal groups (Ten each), animals in the first group were received distilled water alone and were kept as control, and animals in the second group were received 20 mg $AlCl_3$/kg bw orally via drinking water for 3 months, animals in the third group were received 20 mg $AlCl_3$/kg bw orally via drinking water plus 4.2 mmol CDTA /kg bw i.p. daily for 3 months while animals in the fourth group were received 20 mg $AlCl_3$/kg bw orally via drinking water for 3 months, then $AlCl_3$ administration was stopped and the animals treated with 4.2 mmol CDTA /kg bw i.p. daily for another 3 months.

At the end of the experiment whole blood samples were collected for hematological studies as RBCs, WBCs, differential leucocytic counts, hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) determination according to **Coles, (1986)**.

Serum samples were obtained to determine spectrophotometrically using analytical commercial kits the serum activities for creatinine, urea, uric acid, glucose, alkaline phosphatase (AP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) total protein, albumin, Triglycerides, lactate dehydrogenase (LDH) and cholesterol according to **Henry, (1974)**; **Patton and Crouch, (1977)**; **Fossati *et al.*, (1980)**; **Trinder, (1969)**; **Kochmar and Moss, (1976)**; **Schmidt and Schmidt, (1973)**; **IFCC, (1978)**; **Henry, (1964)**; **Grant and Kachmar, (1976)**; **Wahlefeld, (1974)**; **Rec. GSCC (1970)** and **Stein, (1986)**. Serum globulin was determined by subtracting the values of albumin from the values of total protein. Albumin/globulin ratio was determined by dividing the value of serum albumin by the value of globulin concentration. Aluminum, copper, iron, zinc, calcium and phosphorous concentrations in the serum were determined spectrophotometrically according to **Jayman and Sivasubramaniam, (1974)**; **Abe *et al.*, (1989)**; **Dreux (1977)**; **Johnson and Eliasson, (1987)**;

Lehman and Henry, (1984) and **El-Merzabani *et al.*, (1977)**. Student's "t" test was used to determine the statistical significance of the obtained results *(mean ? SE)* according to **Gad and Weil, (1986),** where the probability values were <0.05 and 0.001.

RESULTS

1- Hematological parameters:

Hematological parameters as RBCs, WBCs counts, Hb, packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) of control and different treated groups of rabbits were summarized in (Table 1). Significant decrease in RBCs, WBCs, PCV, Hb and MCHC in group II and slightly decrease in group IV while group III showed no significant difference compared with group I. The differential leucocytic count were summarized in Table (2). Increase the number of neutrophils, eosinophils and monocytes in-group II followed by groups IV and III while basophils and lymphocytes took an opposite manner.

2- Biochemical parameters:

The activities of serum creatinine, urea, uric acid, glucose, AP, ALT and AST in control and different treated groups of rabbits were summarized in Tables (3&4). Significant increase in creatinine, urea, uric acid, AP, ALT, AST, triglycerides and LDH concentrations in group II followed by group IV while group III showed no significant difference compared with control. Significant decrease in glucose, cholesterol and total protein and its fractions (albumin and globulin) in group II and III.

3- Aluminum, copper, iron, zinc, calcium and phosphorous concentrations:

Aluminum, copper, iron, zinc, calcium and phosphorous concentrations in the serum of control and different treated groups of rabbits were summarized in Table (5). Significant increase in aluminum and iron in-group II and IV while in-group III there is no different from control (group I). Decreased concentration of Cu, Zn, Ca and P in groups II and IV while group III show no significant difference.

4- Efficacy of CDTA in Aluminum toxicity treatment:

The use of CDTA in treatment of aluminum toxicity was most effective when used as prophylactic or when administered in the same time with aluminum as appeared clearly in group III followed by IV than it administered post aluminum exposure.

DISCUSSION

The most common foods with substantial amounts of Al-containing additives include some processed cheeses, baking powders, cake mixes, pancake mixes, self-raising flours and pickled vegetables (**Lione, 1983**).

Al is a nonessential metal and is considered an environmental neurotoxin. It can produce toxicity to the central nervous system (dialysis encephalopathy syndrome) **(Sideman and Manor, 1982),** the skeletal system, bones and joints **(Netter *et al.*, 1980; Ott *et al.*, 1982 and Huang, 1993),** the hematopoietic system **(McGonigle and Parsons, 1985),** urinary system and liver **(Wills *et al.*, 1993),** and it have numerous cellular toxic actions **(Crapper McLachlan *et al.*, 1991** and **Exley and Birchall, 1992).**

There were significant decrease in the RBCs & WBCs counts, PCV %, Hb and MCHC concentrations in group II and slight decrease in group IV while group III showed no significant difference compared with control (Table 1). The differential leucocytic count showed an increase in the number of neutrophils, eosinophils and monocytes in group II followed by groups IV and III. Basophils and lymphocytes taked an opposite manner (Table 2). These results are in agreement with that reported by **Touam *et al.*, (1983); Zaman *et al.*, (1993a, b and c); Gonzalez-Revalderia *et al.*, (2000)** and **Zaman *et al.*, (1993a)** found a decrease in RBCs count; Hb level and PCV percentage in rats exposed to $AlCl_3$. They also found a significant decrease of delta aminolevulinic acid dehydratase which plays an important role in heme synthesis. This ihibition might explain the decrease in Hb concentration recorded in the present study , and which is supported by the presence of microcytic hypochromic anemia induced by Al **(Touam *et al.*, 1983)** and **Zaman *et al.*, (1993b,c)** found that Al reduced the deformability of erythrocytes, and such cells are rather frequently retained in the reticuloendothelial system of the spleen and eliminated faster from the blood stream. Al produces peroxidative changes in the RBCs membrane leading to hemolysis. Therefore, the depressed erythrocytic count may be due to the consequence of both the hemolytic action of Al and the shortened time of survival of RBCs. **Zaman *et al.*, (1993c)** reported that Al inhibits heme biosynthesis in vitro. Al affects the cellular metabolism including blood cells that may be affected by different ways, in which Al may combine in vitro with amino acids, peptides, enzymes, cofactors, nucleotides and carbohydrates. The decrease in WBCs count in the present study is in accordance with **Zaman *et al.*, (1993c)** who observed the effect of Al on leukocyte system of animals suggesting the influence of this element on the resistance of the organism. The increase in neutrophils was also similar to that obtained by **Zaman *et al.*, (1993b)**. The investigations in man and animals had supported an association between aluminum exposure and

anemia (**Short *et al.*, 1980 and Kaiser *et al.*, 1984**), due to the effect of aluminum on Hb synthesis (**Altmann *et al.*, 1988 and Chmielnicka *et al.*, 1996**). Al had been shown to cause encephalopathy and microcytic anemia (**Sedman, 1992**). The exact mechanism of Al-induced anemia is unknown, however it appeared to involve inhibition of heme synthesis, either by inhibition of enzyme activity or interference with iron incorporation or utilization (**Kaiser and Schwartz, 1985**). Al intoxication is characterized by periorbital bleeding lethargy anorexia and death in animals (**Berlyne *et al.*, 1972**). Al induce changes in iron metabolism (**Abreo *et al.*, 1991 and Joshi, 1991**), lipid peroxides and cholesterol (**Jain *et al.*, 1995 and Sridhar *et al.*, 1995**).

Liver is a major site of Al accumulation in the experimental animals (**Klein *et al.*, 1993 and Spencer *et al.*, 1995**). Experimental studies in rats had revealed that Al was not only deposited in the subcellular fractions of the **liver (Van der Voet *et al.*, 1992),** but also impaired the microsomal functions of the liver (**Bidlack *et al.*, 1987 and Jeffery *et al.*, 1987**). The effects of Al on hepatic functions had been reported with a number of investigators, where aluminium was reported to produce changes in hepatic functions represented by reduced bile flow (**Galle and Giudicelli, 1982**). **Demircan *et al.*, (1998)** concluded that hepatic accumulation of aluminium could produce hepatobiliary dysfunction characterized by portal inflammation detectable in histological examination of liver tissue. **Klein *et al.*, (1989)** suggested that Al administration was associated with decreased taurine conjugation of bile acids, a phenomenon that might be associated with cholestases. **Xu *et al.*, (1990)** reported that Al could produce an inhibition in hepatic glycolysis. The increase in the serum enzymes was an indicator of hepatocyte injuries during toxicant metabolism (**Panangala *et al.*, 1986**). Increase in serum ALT that considered liver specific enzyme, iused as a marker of hepatocellular necrosis or increased cell membrane permeability (**Hoffmann *et al.*, 1989**). **Benjamin, (1984)** interpreted the increase of AST level in toxicity to hepatocellular disease, skeletal muscle necrosis or cardiac muscle necrosis or degeneration.

Our results summarized in Table (3) revealed an increase in these enzymes, AST, ALT and AP which is different from that recorded by **Marie, (1994)** who found that the effect of aluminum on liver functions showed a progressive decrease in hepatic ALT and hepatic AST as well as total protein in the liver of Nile Catfish. The measurement of ALT and AST activity in the rats treated with $AlCl_3$ showed a toxic effects on these transaminases, the hepatic ALT and AST activity reaching a maximum decrease after 2 weeks of Al administration which was correlated with a decline of serum ALT and with an increase of serum AST (**El-Yamany *et al.*, 1997**). However, **Flora *et***

***al.*, (1991)** observed an inhibtion of hepatic ALT and elevation of serum AST activity indicated hepatoxicity in Al treated rats. **Crowe *et al.*, (1990)** reported that alkaline phosphatse (AP) increased in calves receiving a high alumininim diet and **Krasovski et al. (1979)** who found an increase in AP in AL exposed rats, guinea pigs and rabbits. On the other side, **El-Yamany *et al.*, (1997)** reported that Al lowered both serum and hepatic AP all over the experiment.

Uric acid in this study was increased (Table 3) and this is in accordance with **Schroeder and Michener, (1975)** who found that Al induced changes in serum uric acid in rats.

Glucose level in the present study (Table 3) showed a decrease in its level and this is in agreement with **(Schroeder and Mitchener, 1975)** who reported that Al induced changes in serum glucose in rats. **Gisselbrecht *et al.*, (1957)** reported that Al salts interes with the absorption of glucose from the gastrointestinal tract which ended by decreasing glucose level in serum. **Kortus, (1967)** found that rats treated with $AlCl_3$ showed a decrease in glycogen content of liver and muscles. Glucose is the main source for energy in the body, hexose and glucose 6-phosphate dehydrogenase catalyze the first step in glycolysis 'and hexose monophosphate shunt, both enzymes were inhibited by increased Al concentration **(Lai and Blass, 1984; Cho and Joshi, 1988 and Joshi, 1990 & 1991)**. They reported a reduction of glucose utilization in rats and inhibited liver glycolysis **(Xu *et al.*, 1990)** during Al intoxication.

As shown in (Table, 4), the total protein concentration in serum was decreased 4which inflicted to the all body result in retardation of growth, these results were supported by that obtained by **Cherroret *et al.*, (1996)** who reported that Al inducesa significant decrease in food ingestion, weight gain , and total protein concentration in plasma of male rats. Chronic administration of aluminium induced marked growth retardation in rats **(El-Yamany *et al.*, 1997)** while **Desigan *et al.*, (1995)** reported that $AlCl_3$ administration for rats caused no significant change in the growth rate. Aluminum is capable of producing biphasict in diverse cell systems **(kumar, 1999)**. A high dose of aluminium had been observed, to decrease protein synthesis and a lower dose increased protein synthesis had been reported to be increased at lower dose and decreased at higher dose **(Nicholls *et al.*, 1990 & 1991)**. At early stages of Al intoxication in mice, the effect of Al ions on protein synthesis in muscle tissues differs qualitatively from that one in liver or kidneys. Most noticeable aluminum-induced changes of protein synthesis in organs in vitro occurs within the first 12-20 h after intoxication **(Viezeliene *et al.*, 2002)**. The recorded decrease in cholesterol level in treated

groups compared with control (Table 4) is supported by **Schroeder and Michener, (1975)** who found that Al induced changes in serum cholesterol in rats, **Sridhar *et al.*, (1995)** who recorded that over a period of time, the serum cholesterol levels decreased in chicken exposed to Al in drinking water, and **by El-Yamany *et al.*, (1997) who** reported that the levels of cholesterol in liver and serum decreased in Al treated rats.

Concentrations of Aluminum, copper, iron, zinc, calcium and phosphorous are presented in table 5. Cu, Zn, Ca and P concentrations showed a decrease in groups II and IV while in group III no significant difference was observed when compared with group I (Table 5). The decrease in P concentration was supported by the results recorded by **Clarkson *et al.*, (1972)** who found a decrease in serum phosphorus concentrations in patients treated with $AlOH_3$, **Crowe *et al.*, (1990)** and **Muller *et al.*, (1993)** who reported a decrease in the level of P in Al treated rats and dairy calves. In the same view of results, **Rosa *et al.*, (1982)** who reported that additional dietary phosphorus appeared beneficial in overcoming the adverse effects of high dietary Al in lambs, and **Sooncharemying and Edwards, (1990)** found that supplemental Al to poultry rations decreased body weight while supplmentation with P alleviated some of the toxic effects of Al. The decrease in Ca and Cu concentrations are in agreement with the results of **Yasui *et al.*, (1991b)** who found a decrease in Ca in rats and monkeys exposed to Al, **Crowe *et al.*, (1990)** who found a decrease in Ca in calves, and **Muller *et al.*, (1993)** who showed a decrease in Ca and Cu concentrations in Al exposed rats. The study of radioactive calcium uptake by mice indicates that low molecular mass aluminum complexes induce calcium overload in heart and brain. The efficiency of this process depends on the nature of the ligand. ATP seems to play an important role in this process. The experimental fact that similar iron complexes have shown effects similar to those of aluminum, supports the concept of a general involvement of ATP in metal toxicity, and in its related pathologies **(Anghileri and Thouvenot, 1999)**. The results of this study revealed a decrease in serum zinc level which agreed with the results recorded by **Sugawara et al. (1987) and Sugawara and Sugawara (1992)** who reported that Zn concentration was significantly decreased by Al ingestion. **Yasui *et al.*, (1991a) and Muller *et al.*, (1993)** who found a decrease in Zn level in rats exposed to Al. **Yokel, (2000)** reported that Al increases Fe-induced oxidative injury. Facilitation of Fe-induced oxidative injury and disruption of basic cell processes may mediate primary molecular mechanisms of Al-induced neurotoxicity. **Ward *et al.*, (2001)** recorded in an animal model of Al overload that the increase in tissue Al concent was paralleled by elevation of tissue iron in the kidney, liver, heart and spleen as well as in various regions. The basis of reduced Fe uptake

in Al exposed oligodendrocytes. Downregulation of surface transferrin receptors in oligodendrocytes demonstrated, could be due to altered receptor recycling rates or a decrease in transferrin receptor mRNA expression **(Golub, 1999)**.

In the present study, CDTA appeared as a good Al chelator especially when given in the same time of Al exposure. The good results due to use of CDTA in alleviating Al toxicity are in agreement with **Sutton and Marasas, (1959); Sillen and Martell, (1964); Martell and Smith, (1974) and Yokel, (1994)**. **Sutton and Marasas, (1959)** found that after i.p. injection to Guinea pigs with CDTA, 25-95% of the Al was excreted in the urine, suggesting renal elimination of this complex.

In conclusion, the toxic effects of AlCl3 in rabbits and the use of CDTA in its treatment were recorded. Many further investigations are needed to obtain more detailed picture of Al toxicity. We need to find a reasonable certainty that no harm to sensitive persons will results from dietary exposure to residues of Al. CDTA is more effective in the treatment of aluminum chloride toxicity especially if it is given in the same time of aluminum exposure than after aluminum exposure. CDTA is a highly effective Al Chelator because **(1)** it forms a very stable, water-soluble octahedral complex with Al that does not hydrolyze at physiological pH. **(2)** It has a hard base with a log K with Al that is greater than that of endogenous ligands. **(3)** It has the efficacy to permeate membranes to distribute to intracellular sites of Al storage, and **(4)** it has a high LD_{50}, metal selectivity, resistance to metabolic degradation and lack of toxicity during excretion.

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Table (1): RBCs, WBCs counts, Hb, PCV, MCV and MCHC of control and different treated groups of rabbits.

Groups	RBCs $x10^6/mm^3$	WBCs $x10^3/mm^3$	Hb (g/dl)	PCV (%)	MCV (u3)	MCHC (g/dl)
G I (control)	6.37 ± 0.24	7.78 ± 0.20	12.16 ± 0.41	38.27 ± 2.13	62.94 ± 3.01	35.26 ± 1.55
G II	3.02 ± 0.15[b]	4.93 ± 0.13[b]	6.87 ± 0.22[b]	25.36 ± 1.94[b]	75.08 ± 2.18[a]	26.85 ± 1.80[b]
G III	5.96 ± 0.13[d]	6.81 ± 0.08[b,d]	10.89 ± 0.36[a,d]	34.60 ± 2.08[e]	60.72 ± 1.11[d]	32.99 ± 1.06[d]
G IV	4.41 ± 0.10[b,d,f]	5.02 ± 0.14[b,f]	8.11 ± 0.09[b,d]	30.24 ± 0.99[a,e]	68.45 ± 2.02[c,e]	28.17 ± 1.88[b,e]

a,b: Significantly different from group I at p≤0.05 and 0.001.
c,d: Significantly different from group II at p≤0.05 and 0.001.
e,f: Significantly different from group III at p≤0.05 and 0.001

Table (2): Differential leucocytic counts of control and different treated groups of rabbits.

Groups	Neutrophils (%)	Eosinophils (%)	Basophils (%)	Lymphocytes (%)	Monocytes (%)
G I (control)	41.26 ± 1.27	2.15 ± 0.09	3.07 ± 0.14	44.56 ± 1.68	8.96 ± 0.43
G II	50.18 ± 1.95[a]	6.17 ± 0.15[b]	0.43 ± 0.02[b]	32.08 ± 1.72[b]	11.14 ± 0.63[a]
G III	45.23 ± 1.86	3.01 ± 0.10[b,d]	2.42 ± 0.11[a,d]	39.44 ± 1.21[a,c]	9.90 ± 0.55
G IV	47.20 ± 1.66[a]	4.93 ± 0.17[b,d,e]	2.05 ± 0.19[b,d]	35.73 ± 1.98[a]	10.09 ± 0.47

a,b: Significantly different from group I at p≤0.05 and 0.001.
c,d: Significantly different from group II at p≤0.05 and 0.001.
e: Significantly different from group III at p≤0.001.

Table (3): Creatinine, urea, uric acid, glucose, AP, ALT and AST in the serum of control and different treated groups of rabbits.

Groups	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Glucose (mg/dl)	AP (U/l)	ALT (U/l)	AST (U/l)
G I (control)	1.60 ± 0.42	17.21 ± 0.80	2.53 ± 0.11	126 ± 8.12	10.13 ± 1.04	62.94 ± 1.29	69.86 ± 2.04
G II	3.54 ± 0.14b	28.17 ± 0.63b	5.88 ± 0.27b	71 ± 5.34b	18.77 ± 1.26b	81.22 ± 1.46b	89.54 ± 1.23b
G III	2.01 ± 0.10d	19.23 ± 0.52d	2.81 ± 0.14d	118 ± 6.19d	12.06 ± 1.13d	66.13 ± 1.52d	73.14 ± 1.25d
G IV	2.67±0.19a,c,e	22.44± .97b,d,e	3.26± 0.18a,d	98± 3.27a,d,e	14.98±1.06a,c	74.80 ± 1.90b,e,e	80.76± .08b,d,f

a,b: Significantly different from group I at p≤0.05 and 0.001.
c,d: Significantly different from group II at p≤0.05 and 0.001.
e,f: Significantly different from group III at p≤0.05 and 0.001.

Table (4): Total protein, albumin, globulin, albumin/globulin ratio, triglycerides, LDH and cholesterol levels in the serum of control and different treated groups of rabbits.

Groups	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)	A/G ratio	Triglycerides (mg/dl)	LDH (U/l)	Cholesterol (mg/dl)
G I (control)	67.54 ± 1.12	31.83 ± 0.91	35.71 ± 1.47	0.89	114 ± 3.32	80.52 ± 1.27	102 ± 1.28
G II	60.18 ± 1.05b	27.23 ± 1.01a	32.95 ± 1.00	0.83	138 ± 4.27b	99.63 ± 1.64b	82 ± 3.64b
G III	64.66 ± 1.25c	29.99 ± 0.83c	34.67 ± 1.22	0.87	120 ± 4.83c	82.65 ± 1.90d	96 ± 2.96c
G IV	61.06 ± 1.40a	28.01 ± 0.99a	33.05 ± 1.06	0.85	129 ± 3.51a	94.57 ± 2.13b,f	88 ± 2.12b,e

a,b: Significantly different from group I at p≤0.05 and 0.001.
c,d: Significantly different from group II at p≤0.05 and 0.001.
e,f: Significantly different from group III at p≤0.05 and 0.001.

Table (5): Aluminum, copper, iron, zinc, calcium and phosphorous concentrations in the serum of control and different treated groups of rabbits.

Groups	Al (ppm)	Cu (µg/dl)	Fe (µg/dl)	Zn (µg/dl)	Ca (mg/dl)	P (mg/dl)
G I (control)	0.011 ± 0.001	174 ± 13.51	204 ± 17.21	86 ± 7.05	10.32 ± 1.24	4.37 ± 0.21
G II	0.835 ± 0.021b	121 ± 9.64a	276 ± 13.08a	59 ± 4.22a	6.51 ± 0.75a	2.28 ± 0.18b
G III	0.034 ± 0.008a,d	168 ± 11.72c	189 ± 9.19d	75 ± 6.23c	9.08 ± 0.71c	3.95 ± 0.16d
G IV	0.586 ± 0.030b,d,e	150 ± 12.11	220 ± 18.14c	62 ± 4.16a	7.82 ± 0.52	2.88 ± 0.09b,d,c

a,b: Significantly different from group I at p≤0.05 and 0.001.
c,d: Significantly different from group II at p≤0.05 and 0.001.
e: Significantly different from group III at p≤ 0.001.

الملخص العربى

مقدرة حامض الخليك ، ثنائى ا لأمين ، رباعى الهكسان الحلقى فى علاج التسمم بكلوريد الألومنيوم في الأرانب

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تناولت هذه الدراسة الأثر السام لكلوريد الألمونيوم على الأرانب مع الاهتمام بدور حامض الخليك ثنائى ا لأمين رباعى الهكسان الحلقى فى العلاج. أستخدم عدد أربعون أرنب نيوزيلندى أبيض وزن (٣-٤) كجم قسمت إلى أربعة مجموعات (١٠ بكل مجموعة). تناولت المجموعة الأولى الماء المقطر و استخدمت كمجموعة ضابطة للتجربة وتناولت المجموعة الثانية ٢٠ مجم كلوريد الألمونيوم لكل كجم من وزن الحيوان فى ماء الشرب و لمدة ثلاثة أشهر و تناولت المجموعة الثالثة ٢٠ مجم كلوريد الألمونيوم لكل كجم من وزن الحيوان فى ماء الشرب بالإضافة إلى ٤،٢ مللمول حامض الخليك ثنائى ا لأمين رباعى الهكسان الحلقى لكل كجم من وزن الحيوان حقنا بالغشاء البلورى يوميا لمدة ٣ أشهر و تناولت المجموعة الرابعة ٢٠ مجم كلوريد الألمونيوم /كجم من وزن الحيوان فى ماء الشرب لمدة ثلاثة أشهر ثم توقف إعطاء كلوريد الألمونيوم و أعقبها إعطاء ٤،٢ مللمول حامض الخليك ثنائى ا لأمين رباعى الهكسان الحلقى لكل كجم من وزن الحيوان حقنا بالغشاء البلورى يوميا و لمدة ٣ أشهر.أظهرت نتائج هذه الدراسة انخفاض معنوى فى عدد كريات الدم الحمراء و البيضاء ونسبة الهيموجلوبين و حجم الخلايا المضغوط و تركيز الهيموجلوبين فى الكريات الدموية فى المجموعة الثانية بينما كان انخفاضا أقل فى المجموعة الرابعة و لم تلاحظ أى فروق معنوية فى هذه النتائج بالمجموعة الثالثة مقارنة بنتائج المجموعة الأولى (الضابطة).ارتفاع أعداد النيتروفيل و الايزينوفيل و المونوسيت فى المجموعة الثانية ثم يتبعها المجموعة الرابعة و الثالثة بينما اتخذت أعداد البازوفيل و الليمفوسيت اتجاها مضادا . ارتفاع معدل الكرياتينين و اليوريا وحامض اليوريك و الألكالين فوسفاتيز و الألنين أمينو ترانسفيراز و الأسبرتات أمينو ترانسفيراز و الترايجلسريد و اللاكتات دى هيدروجيناز فى المجموعة الثانية و أعقبها فى المجموعة الرابعة و لم يحدث تغيير يذكر بالمجموعة الثالثة مقارنة بنتائج المجموعة الأولى. انخفاض معنوى فى مستوى الجلوكوز و الكوليسترول و البروتين الكلى (الألبيومين و الجلوبيولين) بالمجموعة الثانية و الثالثة.ارتفاع معنوى بمستوى الألمونيوم و الحديد فى المجموعة الثانية و الرابعة بينما لم يلاحظ أى اختلاف فى المجموعة الثالثة مقارنة بنتائج المجموعة الأولى و انخفاض مستوى النحاس و الزنك و الكالسيوم و الفوسفور فى المجموعتين الثانية و الرابعة بينما لم يكن هناك أى تغيير فى المجموعة الثالثة مقارنة بالمالأولى. كان استخدام حامض الخليك - ثنائى الأمين - رباعى الهكسان الحلقى فى علاج التسمم الألمونيوم مؤثرا فى المجموعة الثالثة يتبعها المجموعة الرابعة.أى أنة كان مؤثرا عندما تم استخدامه كوقاية أو عندما اخذ فى نفس وقت التعرض للتسمم بالألمونيوم اكثر مما هو مفيد فى حالة اعطاءه بعد التعرض للألمونيوم.