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EFFECT OF DIFFERENT SOURCES OF VITAMIN C ON CHOLINESTERASE ACTIVITY AND LIPID PEROXIDATION OF RATS EXPOSED TO SHORT-TERM ALUMINUM STRESS

Emad S. Shaker[1] and Hany M. Helmy[2]

[1]Agricultural Chemistry Dept., Faculty of Agriculture, Minia Univeristy, Minia
[2]Home Economic Dept., Faculty of Special Education, Zagazig University, Zagazig, Egypt

ABSTRACT

The deterioration effects of aluminum chloride $AlCl_3$ medium dose (30 mg/kg body weight/day) *short-term* (40 days) in drinking water were studied on experimental rat brain, serum, bones and kidneys. Vitamin C as reducing agent was given orally to detect the effect of antioxidants to protect rat from suppressing caused by aluminum-stress in drinking water. Vitamin C was used as commercial chemical, pharmacological drug and aqueous balady orange peel extract in the same vitamin concentration (1.4 g/l).

Comparing to negative untreated rat, Al in drinking water (positive control) significantly decreased daily body weight gain, daily feed intake and food efficiency. Causing neurotoxic effect and oxidative damage, Al increased serum cholinesterase ChE insignificantly, brain ChE significantly and lipid peroxidation LP significantly as well. Al showed significant decreased of serum vitamin C content. Serum lipid profile; triglyceride TG, cholesterol CHL, low density lipoprotein LDL and very low density lipoprotein vLDL have been insignificantly increased, and high density lipoprotein HDL was significantly decreased. Kidneys profile changes have been detected in the significant high level of urea and creatinine. Al increased P and decreased Ca contents, insignificantly in serum and significantly in bones. Neurodegenerative changes have been noticed as neurotic plaques and necrosis in the cerebellum. Granulation, vacuolation and necrosis of epithelial lining renal tubules have been noticed in kidneys histology.

Comparison study between the commercial, medical and natural extracted vitamin C effects on deterioration effect of Al has been done. Comparing to positive control, vitamin C sources showed significant increase for food efficiency. Extracted vitamin insignificantly reduced serum ChE activity and improved HDL (8.75% increase), and LDL (13.75% decrease) values, insignificantly. While, medical vitamin showed significant decrease of brain ChE, improved kidney function significantly and increased serum Ca insignificantly. Commercial vitamin increased serum vitamin C content significantly, decreased lipid peroxidation significantly and decreased Al bones, increased Ca in bones, significantly. Apparent and normal histopathological changes have been detected especially with sections of natural vitamin extract.

INTRODUCTION

Aluminum (Al) is the most abundant metallic element, and the third constituent of the earth's crust. The sources of Al vary from food industry, beverage cans, cosmetics drinking water and some common oral pharmaceutical products, such as antacids and antdiarrheic drugs, also contain aluminum. Mechanism by which Al exerts its neurotoxic effects is not understood, however, data suggest that Al interacts with the cholinergic system, acting as a cholinotoxin[(1)]. Alzheimer's disease (AD), the most common neurodegenerative disorder in humans, is characterized by deterioration of mental function. Several factors augment the risk of (AD), including environmental factors, such as metals. Epidemiological studies have indicated a link between increased Al concentration in potable water and AD[(2)]. *In vitro* and *in vivo* effects of Al on acetylcholinesterase (AChE) activity have been described[(3,4)], but both activation and inhibition of AChE activity have been reported. Alterations in the brain and other organ systems caused by AD might increase the penetration of Al into the brain[(5)] and lead to such pathological features as neurofibrillar tangles.

In respect to lipid peroxidation, Al is not a transition metal and does not undergo redox reactions *in vivo*. However, increased reactive oxygen species (ROS) have been reported during Al exposure[(6)]. Besides the fact that Al is a cholinotoxin and a pro-oxidant agent, its neurotoxic effects could be exerted by additional mechanisms such as; the promotion and accumulation of insoluble amyloid ß-protein, the aggregation of hyperphosphorylated tau protein, disruption of calcium regulation and by interacting directly with the genomic structure[(7)].

Meanwhile, direct association has been proved between AD with reactive oxygen species ROS formation[(8)], since the brain is rich in peroxidizable fatty acids[(9)]. Several reports have emphasized the potential therapeutic role that antioxidant agents may play for treatment of Alzheimer's disease AD[(10)]. Vitamin C the most important vitamin for nutrition, has the capacity to eliminate several different reactive oxygen species. It helps to return some biological systems as liver enzymes to normal levels after administration[(11)] with different $Al_2(SO_4)_3$ dosages. Fruits, especially *Citrus* are the best sources of vitamin C. Some other extract compounds evoke antioxidant and protective responses under experimental conditions[(12)]. At a central nervous system CNS level, some constituents reduce edema formation in ischemic rat brain through the inhibition of lipid peroxidation[(13)].

Al found in drinking water accumulated in brain, liver, kidneys and mice spleen by long-term administration of aluminum maltolate[(14)]. Aluminum has been recognized as a cause of bone tissue disorders. At micromolar concentrations, Al affects the intracellular calcium homeostasis. Neurotoxicity of Al may be related to an alteration of the intracellular calcium regulatory system[(15)]. Total bone calcium and breaking-strength decreased with calcium deficiency and aluminum supplementation[(16)]. While, in another

experiment rabbit bones and serum Ca contents were increased along with Al intake increase[17].

Medium level short-term exposure to Al may be a contributing factor in AD and related disorders. Because of the alterations in the cholinergic system and the oxidative injury, we measured both of AChE activity and lipid peroxidation (LP) in rat serum and brain. In comparative study, we investigated the power of preventing agents for balady orange peel extract, medical and commercial vitamin C as common antioxidants and reducing agents. Our research tries to answer the question; whether a purified phytochemical has the same health benefit as does the whole food or mixture of foods in which the phytochemical is present. The effect of Al on Ca and P in rat bones was also determined. Biological analytical determinations and histological studies have been fulfilled in rat serum and brain tissue.

MATERIALS AND METHODS

Commercial vitamin C and hydrated Aluminum chloride were purchased from BDH biochemicals, England; and the medical (Cevilene) contains only vitamin C was produced by Kahira Pharm. Chem. Ind. Co. Balady orange was brought from local market in Cairo. The orange peel was carefully peeled and cut to small pieces. The aqueous peel extracts (200 g/l) was kept in 4°C for experiments.

Biological Evaluation:

Experimental animals: Thirty male albino rats weight 120-140 g were obtained from Experimental Animal House, National Research Center, Giza. Rats were housed in stainless steel wire cages under the normal healthy condition, and were fed on a basal diet for 7 days as adaptation period. The basal diet which fed to all rat groups[18], consisted of 14% casein, 5% cellulose, 4% salt mixture, 1% vitamin mixture, 10% corn oil and 66% starch and salt.

The rats were divided randomly into five groups as follows:

Group (1): were drinking distilled water (dw) (negative control).

Group (2): drinking distilled water with Al 30 mg/kg body weight/day in dw for only four days in each week (positive control).

Group (3) were drinking Al 30 mg/kg bw/d in dw, and treated orally every day with 0.5 ml (1.4 g/l) commercial aqueous vitamin C solution.

Group (4) were drinking Al 30 mg/kg bw/d in dw, and treated orally every day with 0.5 ml aqueous orange peel extract contain (1.4 g/l) vitamin C.

Group (5) rats were drinking Al 30 mg/kg bw/d in dw, and treated orally every day with 0.5 ml medical (1.4 g/l) vitamin C.

Al in drinking water was given to all groups except for negative control, only four days per week. Amount of the given vitamin C was the same in the commercial, orange peel and the medical samples. Vitamin C content in balady orange peel was 7.01 mg/g, so rats of group (4) were given 0.5 ml (200 g/l) of aqueous peel extract. These sources of vitamin C continuously were given orally each day for the experiment time.

After 40 days, the rats were anesthetized with diethyl ether and slaughtered. Serum was separated from blood samples and kept at -18°C until bioassay. Histopathological experiment was done as well for rat brain and kidneys.

Activity of cholinesterase (ChE) was measured every 30 sec in serum and brain tissues at 405 nm to follow the inhibition of the enzyme (Unit/l)[19]. Samples of brain tissue were minced and homogenized in ice cold. The homogenate was centrifuged and the resultant supernatant was used for cholinesterase assay[20]. Antioxidant determinations; Lipid peroxidation measured as malondialdehyde (MDA) (µmol/ml)[21], and vitamin C (mg/l)[22] were examined for rat serum.

Triglycerides (TG), Cholesterol (CHL) and HDL were colorimetrically determined in rat serum using the enzymatic colorimetric methods[23,24]. HDL was calculated[25] (*mg/dl*) as follows;

$$\text{LDL} = \text{Total CHL} - \{\text{HDL} - (\text{TG}/5)\}$$

For kidney function study, serum urea[26] and creatinine[27] were determined (mg/dl) according to colorimetric methods. Ca and Al were determined in bones using UNICAM 929 atomic absorption spectrometer[28]. Phosphorous in bones was determined using colorimetric method[28]. In serum, Ca [29] and P[30] were also determined by colorimetric methods.

Histopathology of kidneys and Brain tissue: Histopathological examination was fulfilled in Pathology Dept., Faculty of Veterinary Medicine, Cairo University. Specimens from the brain and kidneys of rats were fixed in 10% v/v neutral buffer formalin, dehydrated in ethyl alcohol and cleared in xylol. Tissue sections (4-6µ thick) were stained with haematoxylin, eosin stain and examined micro-scopically[31].

Statistical analysis: The obtained data were statistically analyzed using (ANOVA) procedure[32,33]. The levels of significance was accepted with $p<0.05$.

RESULTS AND DISCUSSION

The chosen concentration of $AlCl_3$ in drinking water has been used as medium dose, short-term experiment as mentioned[34]. Chosen vitamin C concentration (1.4 mg/ml) used orally correlats with recommended daily intake and some used practical data [35] (2 mg/ml ascorbic acid).

Weight characteristic measurements:

Table (1) shows that Al treatment significantly decreased body weight gain, daily body weight gain, daily feed intake and food efficiency comparing to negative control. The significant decrease of positive control may be due to anemia or hemolysis, since Al was proven to coagulate human blood. Another study proved that Al treatments had no effects on the gain of body weight [36].

Table (1): Effect of different vitamin C sources on Al stress on body weight gain (g), daily body weight gain (g), daily feed intake (g) and food efficiency (g) in albino rats after 40 days.

Treatment	Body weight gain (g) Mean ± SD	Daily body weight gain (g) Mean ± SD	Daily feed intake (g) Mean ± SD	Food efficiency Mean ± SD
Control	110.33± 7.12[a]	2.74± 0.18[a]	20.44± 0.27[a]	0.13± 0.009[a]
Pos. control	92.5± 13.69[c]	2.32± 0.34[c]	19.9± 0.14[b]	0.12± 0.017[b]
Al+ Commer.	104.67± 7.12[ab]	2.61± 0.18[ab]	18.25± 0.89[d]	0.14± 0.012[a]
Al+ Natural	96.0± 7.48[bc]	2.38± 0.18[bc]	17.42± 0.12[e]	0.14± 0.011[a]
Al+ Medic	103.0± 2.45[ab]	2.58± 0.06[ab]	18.92± 0.29[c]	0.14± 0.004[a]
F. values	4.305*	4.035*	44.974*	4.39*

S.D. Standard Deviation, n=6, * significant at (P<0.05)
Means in a column followed by the same letter are not significantly different at P<0.05

Significantly, negative control was the highest for daily feed intake followed by positive control, medical, commercial and extracted vitamin C. Vitamin supplementations (Table 1) significantly increased food efficiency comparing to positive control. Weight gain was significantly increased for commercial and medical vitamins, and insignificantly increased for natural vitamin.

Biological Determinations: The Al controversies data in research could be explained by the different methods and doses of Al administrations, differences in the biological samples assayed, differing periods of exposure (long-term and short-term) and by the metal speciation.

Cholinsterase (ChE) activity:

It is known that acetylcholine helps carrying messages between nerve cells in the brain. Increase of ChE enzyme activity illustrates the degraded effect on the brain function. Increase of AChE activity was investigated insignificantly in serum of rat treated with commercial vitamin C, followed by $AlCl_3$ treatment in the short term exposure (Table 2). The effect of Al on AChE activity was explained [37] for direct neurotoxic effect of the metal or disarrangement of the plasmatic membrane caused by increased lipid peroxidation. Aluminum lactate produced higher activation on AChE enzyme than did $AlCl_3$ [38].

In serum, medical vitamin surpassed insignificantly the negative control rats, and finally Al with extracted vitamin C (Table 2). Extracted natural vitamin C proved to have potential effect on serum enzyme activity in short term study, but its effect was slow since it did not appear in brain. Extracted vitamin insignificantly increased the enzyme activity in brain than that for positive control. On the other hand, medical vitamin showed specific effect on brain more than on serum. Medical vitamin enzyme activity showed insignificant decrease than negative control, and significant decrease than that for positive control.

Table (2): Effect of different vitamin C sources on Al stress on serum and brain cholinesterase activity (U/l) in albino rats after 40 days.

Treatment	Serum ChE (U/l) Mean ± SD	Brain ChE (U/l) Mean ± SD
Control	463.75±178.7[a]	336.89±41.0[b]
Pos. control	504.72±280.4[a]	398.82±69.5[a]
Al+Commercial	569.38±44.2[a]	397.80±51.7[a]
Al+Natural	429.83±93.2[a]	425.74±41.9[a]
Al+Medical	500.02±151.6[a]	328.44±29.3[b]
F. values	0.565	4.599*

S.D. Standard Deviation, n=6, * significant at (P<0.05)
Means in a column followed by the same letter are not significantly different at P<0.05

Acetylcholinesterase inhibitor AChEI, which enhance cholinergic transmission by reducing the enzymatic degradation of acetylcholine ACh, is the main compound currently approved for the treatment of AD. ChE activity in agreement significantly increased at $4.0x10^{-4}$ followed by $4.4x10^{-4}$ concentrations of $AlCl_3$[39]. Sodium chloride greatly decreased $AlCl_3$-induced ChE activity. Al 0.1 mmol/kg/day for 5 days per week enhanced AChE activity in striatum and elevated lipid peroxidation; decreased AChE in hypothalamus [37].

Lipid peroxidation (LP) determinations:

Al acts as pro-oxidant in neural tissues at 5, 10 mg/kg/day for 8 weeks [40]. In agreement, data showed significant increase of oxidative damage caused by Al treatment comparing to other groups (Table 4). AD might cause low levels of essential nutrients[41]. Antioxidant activities were lower in AD patients and oxidative damage appears to occur as one of the earliest pathophysiological events in AD[42]. Al^{3+} enhances lipid peroxidation of human HDL, and the oxidative damage could be involved in neurological diseases [43]. In agreement, 10 mg/kg bw/d for 4 weeks increased lipid peroxidation and decreased antioxidant enzymes[44]. Significantly, vitamin C supplementations decreased the lipid peroxidation measurement comparing to positive control but didn't reach the negative control. Data showed that commercial, medical and then extracted vit. C decreased serum oxidative damage insignificantly.

Because of the oxidative damaged caused by Al, vitamin C found in the significant lowest level in

positive control (Table 4), followed by negative control. Commercial, medical followed by extracted vitamin C succeeded to increase serum vitamin level (Table 4) comparing to negative and positive controls. Regular consumption of fruits and vegetables is associated with reduced risks of diseases and aging [45].

Table (3): Effect of different vitamin C sources on Al stress on lipid peroxidation determined (MDA) µmol/ml and vitamin C mg/L in albino rats after 40 days.

Treatment	Lipid peroxidation *µmol/ml* Mean ± SD	Vitamin C *mg/L* Mean ± SD
Control	2.89±0.12^{b}	1.24±0.074^{c}
Pos. control	5.78±1.81^{a}	0.95±0.095^{d}
Al+Commercial	3.01±0.89^{b}	1.91±0.23^{a}
Al+Natural vit.	3.99±0.49^{b}	1.7±0.19^{b}
Al+Medical vit.	3.77±0.77^{b}	1.73±0.19^{ab}
F. values	8.187*	34.197*

S.D. Standard Deviation, n=6, * significant at (P<0.05)
Means in a column followed by the same letter are not significantly different at P<0.05

Prevention is a more effective strategy than is treatment of chronic diseases. Vitamin C could improve the toxic effects of thiobarbituric acid-reactive substances of $AlCl_3$ [46]. Tannic acid (50 mg/kg) reduced the oxidative damage in brain tissue of $AlCl_3$ (30 mg/kg bw in drinking water) exposed rats [34].

Lipid profile:

Serum cholesterol CHL, triglyceride TG, low density lipoprotein LDL and very low lipoprotein vLDL showed insignificant increases with Al administration (Table 5), while high density lipoprotein HDL was significantly decreased. In agreement, Al administration caused marked increase in cholesterol levels and a significant decrease in the total lipid, glycolipid and phospholipid content of primate brain [47]. Commercial vitamin C decreased CHL, TG and vLDL levels insignificantly, comparing to negative control. $AlCl_3$ decreases total phospholipid except for phosphatidylcholine and increases CHL content, with correlation to the reported phospholipid profiles of Alzheimer brains [48].

Insignificantly, extracted vitamin C increased 8.75% HDL, decreased 13.75% LDL levels, and decreased LDL/HDL ratio insignificantly (Table 4), comparing to positive control (Table 5). As appeared, this ratio was equal to that for negative control. LDL/HDL is proved to be a remarkable healthy sign for the natural vitamin C. Medical vitamin showed the highest ratio insignificantly, followed by positive control and then vitamin commercial source.

Table (4): Effect of different vitamin C sources on Al stress on TG, CHL, HDL, LDL and vLDL (mg/dl) (Mean ± SD) in albino rats after 40 days.

Treat.	TG *mg/dl*	CHL *mg/dl*	HDL *mg/dl*	LDL *mg/dl*	vLDL *mg/dl*
Control	65.83± 5.27^{a}	64.29± 4.38^{a}	39.55± 3.11^{a}	11.57± 4.85^{a}	13.17± 1.05^{a}
Pos. control	74.08± 5.09^{a}	65.82± 8.79^{a}	34.06± 4.26^{bc}	16.95± 10.61^{a}	14.82± 1.02^{a}
Al+ Commr.	65.22± 6.72^{a}	61.66± 4.41^{a}	33.76± 4.31^{bc}	14.85± 7.15^{a}	13.04± 1.35^{a}
Al+ Nat.	66.02± 11.28^{a}	64.86± 2.41^{a}	37.04± 3.89^{ab}	14.62± 3.81^{a}	13.2± 2.26^{a}
Al+ Med.	71.17± 3.47^{a}	62.72± 2.69^{a}	31.0± 2.32^{c}	17.49± 3.64^{a}	14.23± 0.69^{a}
F. values	1.961	0.648	4.844*	0.762	1.961

S.D. Standard Deviation, n=6, * significant at (P<0.05)
Means in a column followed by the same letter are not significantly different at P<0.05

Table (5): Effect of different vitamin C sources on Al stress on LDL/HDL ratio (*mg/dl*) after 40 days.

Treatment	LDL/HDL ratio Mean ± SD
Control	0.41±0.27^{a}
Pos. control	0.52±0.37^{a}
Al+Commercial	0.46±0.26^{a}
Al+Natural vit.	0.41±0.16^{a}
Al+Medical vit.	0.57±0.14^{a}
F. values	0.477

S.D. Standard Deviation, n=6
Means in a column followed by the same letter are not significantly different at P<0.05

Effects on urea and creatinine levels:

Kidney function is essential for detoxification, and its failure drastically increases Al toxicity.

Table (6): Effect of different vitamin C sources on Al stress on urea (mg/dl) and creatinine (mg/dl) in albino rats after 40 days.

Treatment	Urea *mg/dl* Mean ± SD	Creatinine *mg/dl* Mean ± SD
Control	32.61±2.03^{b}	0.37±0.025^{b}
Pos. Cont.	39.96±7.72^{a}	0.42±0.005^{a}
Al+Commercial	37.22±2.92^{ab}	0.43±0.037^{a}
Al+Nat.	33.49±3.47^{b}	0.41±0.012^{a}
Al+Med.	32.92±3.25^{b}	0.35±0.023^{b}
F. values	3.289*	11.52*

S.D. Standard Deviation, n=6, * significant at (P<0.05)
Means in a column followed by the same letter are not significantly different at P<0.05

Urea and creatinine have been significantly increased with Al administration comparing to negative control, and medical vitamin C significantly improved these kidney function changes (Table 6) more than other vitamin sources. Similar increase in serum urea and creatinine was recorded in rats [49]. Al has been implicated in the pathogenesis of several

clinical disorders, such as dialysis dementia [20], the fulminant neurological disorder that can develop in patients on renal dialysis. The hepatorenal activities would confirmed by the histopathological findings. Vitamin supplementations showed insignificant changes between each other.

Aluminum, calcium and phosphorous determinations in serum and bones:

In serum, Al treatment insignificantly decreased Ca content, while medical vitamin followed by extracted vitamin showed insignificant increase of Ca level comparing to positive control (Table 7). In another study, prolonged oral administration of Al elevates serum Al level, but there were no marked changes in serum Ca [50].

Table (7): Effect of different vitamin C sources on Al stress on Al, Ca and P in serum (mg/dl) and Al (µg/100g), Ca, P (mg/100g) in albino rat bones after 40 days.

Treatment	Al	Ca		P	
	In bones *µg/100g* Mean ± SD	In serum *mg/dl* Mean ± SD	In bones *mg/100g* Mean ± SD	In serum *mg/dl* Mean ± SD	In bones *mg/100g* Mean ± SD
Control	16.55± 2.34^{b}	9.84± 0.25^{a}	3581.4± 480.7^{a}	6.24± 0.15^{a}	46.55± 5.6^{c}
Pos. cont.	21.68± 2.59^{a}	9.53± 0.24^{a}	2596.9± 565.9^{b}	6.42± 0.3^{a}	105.55±1 3.6^{a}
Al+ Commr.	7.39± 1.91^{c}	9.53± 0.35^{a}	3191.76± 271.1^{a}	6.33± 0.21^{a}	100.25±1 0.8^{a}
Al+ Natur.	16.67± 1.42^{b}	9.73± 0.29^{a}	2572.6± 228.1^{b}	6.76± 0.48^{a}	54.1± 7.7^{c}
Al+ Med.	15.99± 3.12^{b}	9.81± 0.13^{a}	2457± 382.7^{b}	6.66± 0.64^{a}	80.6± 9.3^{b}
F. values	29.007*	1.906	8.588*	1.830	44.208*

S.D. Standard Deviation, n=6, * significant at (P<0.05)
Means in a column followed by the same letter are not significantly different at P<0.05

Osteomalacia is due to Al interfering with bone chemistry. It could be initiated from inhibition of Ca uptake into bone or hypophosphatemia from insoluble complex of Al and P which explain the enlargement of the kidneys [52]. Data showed significant increase of Al and significant decrease of Ca contents in bones for positive control comparing to negative control (Table 7). Commercial vitamin has shown successful and significant decrease of Al and increase in Ca contents comparing to positive control. Generally, in brain, liver, spleen, kidney cortex, skeletal muscle and bones, bones had at least 10 times higher values of Al than other organs (0.959 µg/g) [55].

Al treatment insignificantly increased serum P content, but extracted vitamin C (Table 7) showed the highest content insignificantly. Al ingestion may cause hyperphosphatemia in the intact animal, but no significant increase in serum Al concentration [36]. Data showed that Al in drinking water (positive control), followed by commercial vitamin increased P content significantly in bones comparing to negative control (Table 7). An opposite correlation between Ca and P contents was more obvious in bones.

References showed a significant two-fold Al increase in the hippocampus of brain and a significant decrease of Al in the cortex. High levels of intravenous aluminum increased total plasma calcium and decreased ionized calcium[53]. Low dose Al level showed brain Al (0.963 µg/g wet weight) and control (0.717 µg/g wet weight)[54]. While in CNS of patients with AD and in controls, positive correlation has been shown between contents of Ca and Al[51].

Histopathological examination:

Brain: Brain of control untreated rat revealed no histopathological changes (Fig. 1a). While, sections from positive control rat treated only with $AlCl_3$ revealed neurodegenerative changes which represented as neurotic plaques and neuronophagia (Fig. 1b1), Astrogliosis (Fig. 1b2), meningial hemorrhage as well as necrosis of Purkinje cells of the cerebellum (Fig. 1b3), and cerebral hemorrhages were also noticed in all examined cases (Fig. 1b4). Al accumulates in all regions of the brain with maximum accumulation in the hippocampus[56]. Aluminum compartmentalizes in almost all the tissues of the body to varying extents, and the highest accumulation was in the spleen. Aluminum appears to accumulate in most brain cells, decreases spontaneous nervous discharge and suggested to inhibit cholinergic functioning. The elevation of ChE (Table 2) of Al treated rat was confirmed and manifested by brain nerve cell necrosis and neuronophagia.

The total content of Al (µg/g fresh brain tissue) was measured by inductively coupled plasma atomic emission spectrometry (ICP-AES)[57]. Aging CNS is particularly susceptible to Al^{+3} toxic effects which may increase the cell load of oxidative stress and may contribute, as an aggravating factor, to the development of neurodegenerative events as observed in Alzheimer's disease.

However, examined brain of rat treated with commercial vitamin revealed no histopathological changes (Fig. 1c1), except basophilic and pyknotic neurons in some examined sections (Fig. 1c2). Apparent normal neurons were noticed in some examined sections from rat treated with natural vitamin (Fig. 1d1) together with edema in the menninges and necrosis of Purkinje cells of the cerebellum (Fig. 1d2). Histopathological examination of brain of rat treated with medical vitamin revealed neuronophagia of degenerated neurons (Fig. 1e) associated with focal gliosis.

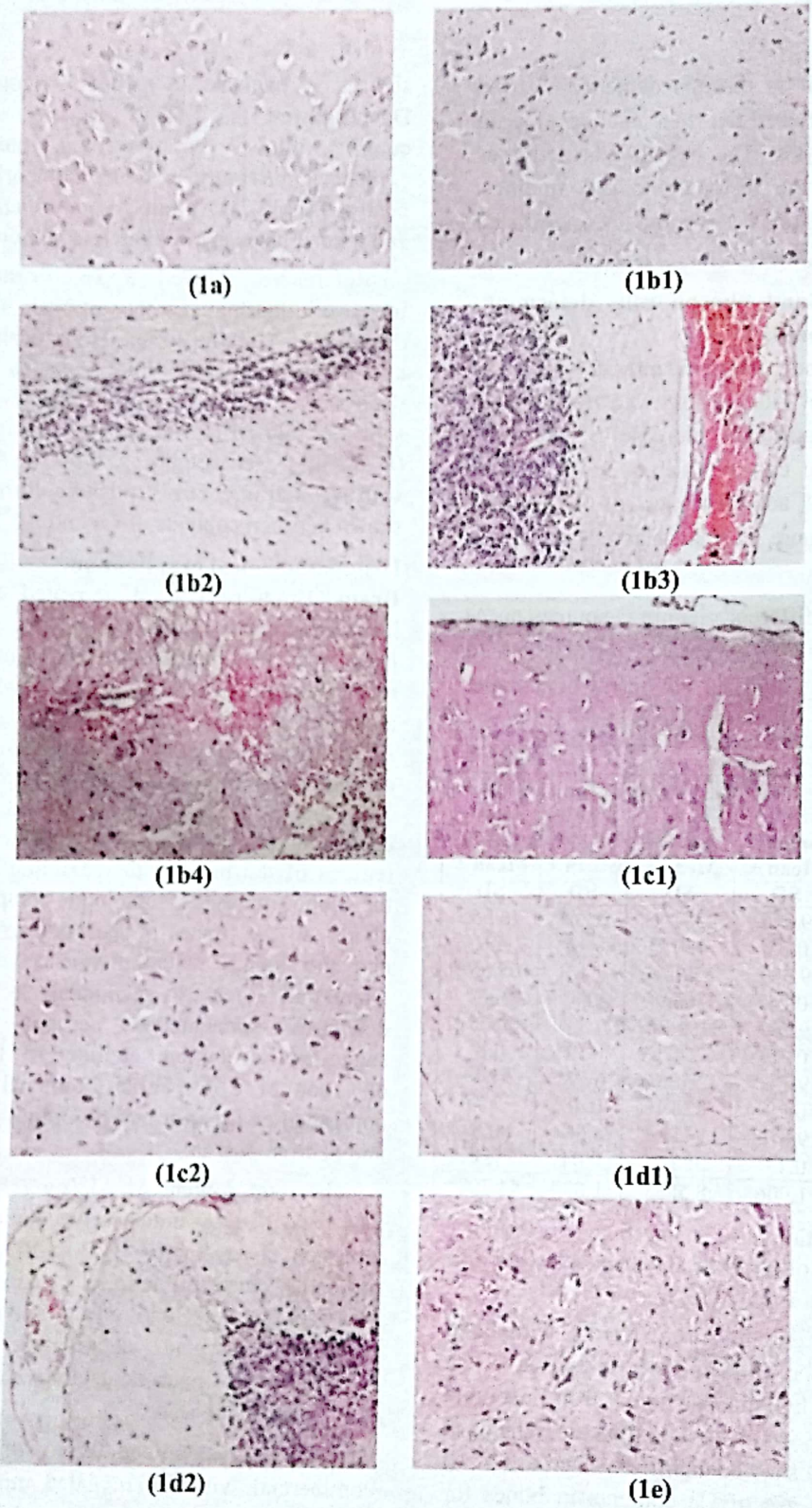
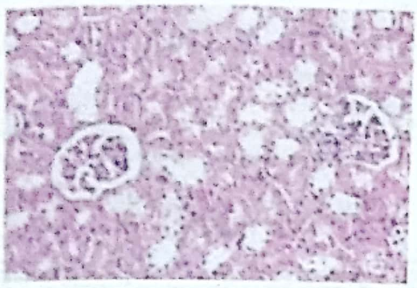


Figure 1 (Brain histograms). Figure 1a: Brain of negative control showing no histopathological changes (H & E X 200). **Figure 1b:** Brain of positive control rat showing (1b1) neurotic plaques and neuronophagia (H & E X 200), (1b2) Astrogliosis (H & E X 200), (1b3) meningial hemorrhage as well as necrosis of Purkinje cells (H & E X 200), (1b4) cerebral hemorrhages (H & E X 200). **Figure 1c:** Brain of rat treated with Al and commercial vitamin showing (1c1) no histopathological changes (H & E X 200), (1c2) basophilic, pyknotic neurons (H & E X 200). **Figure 1d:** Brain of rat treated with Al and natural vitamin showing (1d1) apparent normal neurons (H & E X 200), (1d2) edema in the menninges and necrosis of Purkinje cells of the cerebellum (H & E X 200). **Figure 1e:** Brain of rat treated with Al and medical vitamin showing neuronophagia of degenerated neurons (H & E X 200).



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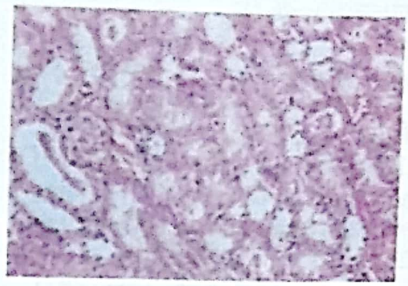

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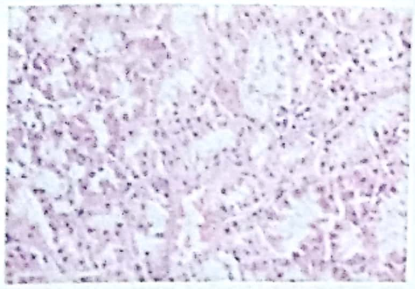

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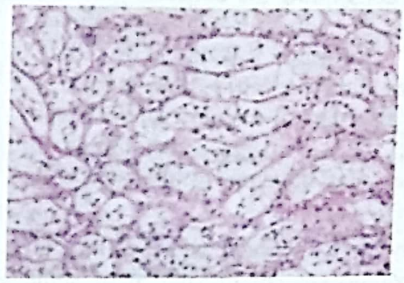

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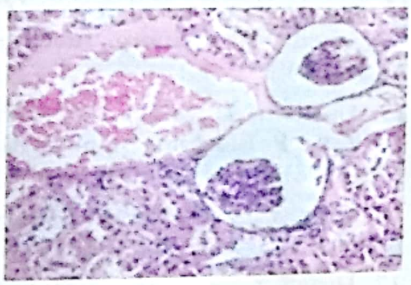

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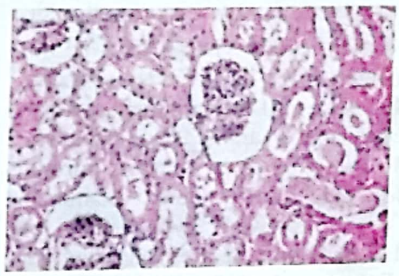
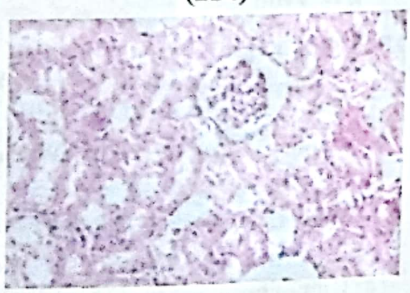
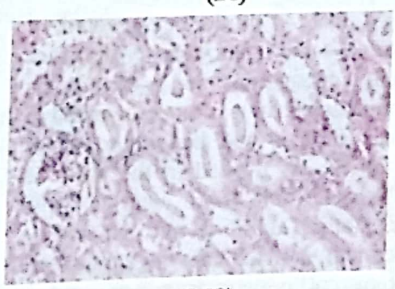


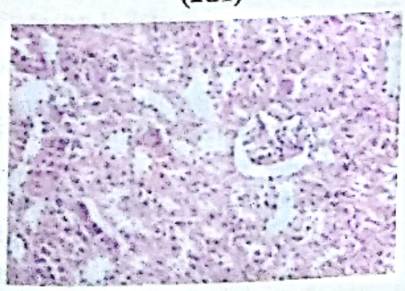
(2c)



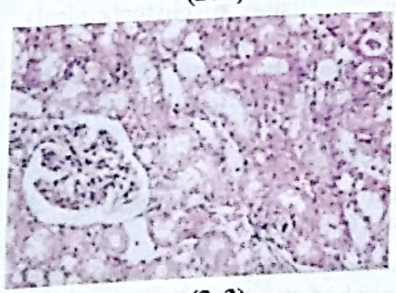
(2d1)



(2d2)



(2e1)



(2e2)

Figure 2 (Kidney histograms). Figure 2a: Kidney of negative control showing the normal histological structure (H & E X 200). **Figure 2b:** Kidney of positive control rat showing (2b1) granulation and vacuolation of epithelial lining renal tubules together with hyaline cast in the lumen of some tubules (H & E X 200), (2b2) pyknotic nuclei of epithelial lining some proximal convoluted tubules (H & E X 200), (2b3) necrosis of epithelial cells lining the collecting tubules (H & E X 200), (2b4) hyalinosis of glomerular tufts associated with distension of Bowman's space (H & E X 200). **Figure 2c:** Kidney of rat treated with Al and commercial vitamin showing hyaline cast in the lumen of some renal tubules as well as distension of Bowman's space of the glomeruli (H & E X 200). **Figure 2d:** Kidney of rat treated with Al and natural vitamin showing (2d1) no histopathological alterations (H & E X 200), (2d2) hyaline cast in the lumen of renal tubules (H & E X 200). **Figure 2e:** Kidney of rat treated with Al and medical vitamin showing (2e1) apparent normal histological structure (H & E X 200), (2e2) hypertrophy of glomerular tuft and vacuolation of their endothelium as well as epithelial lining renal tubules (H & E X 200).

Kidneys: Microscopical examination of kidneys of control untreated rat revealed the normal histological structure of renal parenchyma (Fig. 2a). Meanwhile, apparent enlarged kidneys of rat treated with $AlCl_3$ showed granulation and vacuolation of epithelial lining renal tubules together with hyaline cast in the lumen of some tubules (Fig. 2b1), pyknotic nuclei of epithelial lining some proximal convoluted tubules (Fig. 2b2), necrosis of epithelial cells lining the collecting tubules (Fig. 2b3), hyalinosis of glomerular tufts associated with distension of Bowman's space (Fig. 2b4). Increase of serum urea and creatinine (Table 5) may be attributed to various disorders of the glomerulus which reduce filtration rate [58]. It was stated that fulminant neurological disorder can develop in patients with renal dialysis [20].

Examined kidneys of rat treated with commercial vitamin revealed hyaline cast in the lumen of some renal tubules as well as distension of Bowman's space of the glomeruli (Fig. 2c). However, some examined sections of rat treated with natural vitamin showed no histopathological changes (Fig. 2d1). Other sections revealed presence of hyaline cast in the lumen of renal tubules (Fig. 2d2). Moreover, examined kidneys of rat treated with medical vitamin revealed apparent normal histological structure (Fig. 2e1). Other examined sections of this group showed hypertrophy of glomerular tuft and vacuolation of their endothelium as well as epithelial lining renal tubules (Fig. 2e2).

The study declared that Al could be detected in brain, bones and kidneys in agreement with other results [59]. Aluminum depositions in body tissues could reflect the various deleterious effects recorded in the tested biochemical indices and histopathological studies. Aluminum toxicity as proved in the study is due to both of cholinotoxin and more clear by lipid peroxidation effects. Natural additive and synergistic effects for phytochemical in fruit and vegetables are responsible for potent antioxidant activities, and is attributed to the complex mixture of phytochemicals present in whole foods. More studies are needed for measuring various prospective natural extracts and investigating some of their valuable components.

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تأثير مصادر مختلفة من فيتامين ج على نشاط إنزيم الكولين إستيرازى الأكسدة الليبيدية لفئران معرضة لإجهاد الألمونيوم لفترات قصيرة

عماد صبرى شاكر[1] وهانى حلمى محمد[2]

[1]قسم الكيمياء الزراعية - كلية الزراعة - جامعة المنيا - المنيا

[2]قسم الأقتصاد المنزلى - كلية التربية النوعية - جامعة الزقازيق- الزقازيق - مصر

أجريت هذه التجربة فى محاولة لدراسة تأثير جرعة متوسطة (٣٠ مجم/كجم وزن/اليوم) من الألمونيوم لفترة قصيرة (٤٠ يوم) على مخ ومصل دم وعظام وكلى فئران التجارب. كذلك أجريت فى التجربة بعض الدراسات الحيوية بإستعمال فيتامين ج كمادة مختزلة لدراسة فعل العامل المضاد للأكسدة على الضغط الإجهادى الناتج من الألمونيوم فى ماء الشرب. تنوعت مصادر الفيتامين المستخدمة من الفيتامين التجارى والمصدر الدوائى والمستخلص المائى لقشر البرتقال البلدى بنفس تركيز الفيتامين (١,٠٤ جم/لتر).

أظهرت الفئران المعاملة بالألمونيوم فى ماء الشرب (الكنترول الموجب) مقارنة بالكنترول السالب نقصاً معنوياً حقيقيا لوزن الفئران اليومى والغذاء اليومى المأخوذ وكفاءة الغذاء. كما وضح التأثير السام على المخ وحدوث أكسدة نتيجة زيادة غير معنوية لإنزيم الكولين ستيراز فى مصل الدم و زيادة معنوية للإنزيم فى المخ. وكذلك أدت الأكسدة الفوقية للدهون إلى زيادة معنوية ونقص معنوى لفيتامين ج فى مصل الدم. وأوضحت دراسة المحتوى الدهنى فى مصل الدم زيادة غير معنوية للجلسريدات الثلاثية والكولسترول والليبوبروتين المنخفض والمنخفض جدا للكثافة، وأيضا نقص معنوى لليبوبروتين عالى الكثافة. ظهر تغير وظائف الكلى للفئران من المستوى العالى معنوياً لليوريا والكرياتينين. كذلك ظهر نقصاً للكالسيوم وزيادة للفوسفور (غير معنوية فى مصل الدم ومعنوية فى عظام الفئران). أوضح الفحص الهستولوجى للمخ تدهور صفائح وتجمعات عصبية ودموية بالمخيخ وأظهر هستولوجى الكلى تحبب وتحوصل لبطانة الأنابيب الكلوية للفئران المعاملة بالألمونيوم.

أجريت دراسة للمقارنة بين تأثير فيتامين ج من مصدر تجارى و دوائى و مستخلص طبيعى بتركيز متساوى على التأثير الضار للألمونيوم. مقارنة بالكنترول الموجب, حدثت زيادة معنوية لمصادر الفيتامين المختلفة على كفاءة الغذاء. كذلك فإن المستخلص الطبيعى قلل من نشاط إنزيم الكولين استيراز بمصل الدم نقصاً غير معنوياً وحسن من قيم الليبوبروتين عالى الكثافة بزيادة غير معنوية قدرها ٨,٧٥ % وقلل الليبوبروتين منخفض الكثافة بنقص غير معنوى قدره ١٣,٧٥ %. بينما أظهر الفيتامين فى الصورة الدوائية نقصاً معنوياً لنشاط إنزيم الكولين استيراز بالمخ وتحسن فى وظائف الكلى معنوياً، كما رفع محتوى الكالسيوم فى مصل الدم بزيادة غير معنوية. أما الفيتامين التجارى فزاد من محتوى فيتامين ج فى مصل الدم معنوياً و قلل من الأكسدة الفوقية للدهون معنوياً، كما خفض من مستوى الألمونيوم ورفع مستوى الكالسيوم فى عظم الفئران (معنوياً). وكان الفحص الهستولوجى لمخ وكلى فئران هذه المجموعات طبيعياً فى بعض الشرائح خاصة للفئران المعاملة بمستخلص الفيتامين الطبيعى مع الألمونيوم.