

Potential role of some nutraceuticals in the regression of Alzheimer's disease in an experimental animal model

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Aim: The goal of this study was to evaluate the potential role of some nutraceuticals, coenzyme Q₁₀, vitamin B complex, and lecithin against aluminum-induced neurodegeneration characteristic of Alzheimer's disease.

Materials and methods: Ninety-six male and female Sprague Dawley rats were divided into 2 main groups, namely female and male. Each group was divided into 6 subgroups. Group 1 served as control group. Group 2 was administered AlCl₃ for 4 months. Groups 3, 4, 5, and 6 were administered with AlCl₃ for 4 months then treated with Coenzyme Q₁₀, vitamin B complex, lecithin, or all in combination for 3 months, respectively. Brain acetylcholinesterase (AChE), Na⁺/K⁺-ATPase activities, and vitamin B₁₂, folate, homocysteine (Hcy), lipid peroxidation, glutathione, and plasma nitric oxide (NO) levels were determined. Moreover, histopathological examination of brain tissue was evaluated.

Results: Al intoxication caused a significant increase in brain AChE activity, Hcy, lipid peroxidation, and plasma NO levels, while it produced significant decrease in brain Na⁺/K⁺-ATPase activity, glutathione, vitamin B₁₂, and folate levels. Moreover, histopathological investigation of the brain of Al intoxicated rats showed marked neurodegeneration and deposition of neurofibrillary tangles. Treatment with the selected nutraceuticals revealed an improvement in the neurological damage induced by AlCl₃ as indicated by improvement in most of the biochemical markers and histopathological features.

Conclusion: The selected nutraceuticals (Coenzyme Q₁₀, vitamin B complex, lecithin, and their combination) may play a beneficial role in delaying the progression of neurodegenerative disorders. It is noteworthy that the combined therapy revealed more pronounced effect compared to singular treatments with either one of them.

Key words: Alzheimer's disease, aluminum, rats, CoQ₁₀, vitamin B, lecithin

Introduction

Aluminum (Al) is the third most abundant element and the most common metal in the earth's crust (1). With the global industrialization and consequent pollution, Al is increasingly taken into our bodies through food, air, water, and even drugs (2). Al is present in many manufactured foods and is added as alum for treating drinking water for purification purposes (3,4).

Aluminum (Al) is considered as a potential etiological factor in Alzheimer's disease (AD) (5,6). Excessive Al intake might lead to deposition of A β in central nerve cells and overexpression of β -amyloid precursor protein (APP) (7,8). The neurotoxicity of A β is associated with oxidative stress (9) and with the generation of reactive oxygen species that damage neuronal membrane, lipids, proteins, and nucleic acids. Acetylcholinesterase (AChE) has been found to colocalize with A β deposits and promotes the assembly of A β into amyloid fibrils forming A β -AChE complex that is more toxic than amyloid fibrils (10).

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Nutrition plays an important role in the treatment of many diseases, and the right choice of nutrients can help to prevent disorders and improve the quality of life. The future challenge will be to combine the strategic use of both cosmeceuticals and nutraceuticals in preventing the damaging effects of ultraviolet radiation and environmental pollutants on many biologic processes (11-13).

Coenzyme Q₁₀ is a fat-soluble-vitamin like quinone commonly known as ubiquinone, CoQ, and vitamin Q₁₀. The efficacy of Co Q₁₀ appears most promising for alleviating neurodegenerative disorders (14).

Vitamin B₁₂ plays a role in the pathogenesis of behavioral changes in AD (15). Vitamin B₁₂ deficiency could aggravate or accelerate the course of AD as it possesses neuroprotective and anti-inflammatory properties (16). Vitamin B₁ (thiamine) plays an important role against brain damage. The progress of damage can be stopped by a timely injection of a large dose of thiamine (17).

Lecithin is the major dietary source of choline and, in some circumstances, it can be transformed into ACh (18). Extra consumption of lecithin may reduce the progression of dementia (19). Therefore, this study aimed to investigate the potential role of some nutraceuticals, namely coenzyme Q₁₀, vitamin B complex, and lecithin, against aluminum induced neurodegeneration characteristic of Alzheimer's disease.

Materials and methods

Nutraceuticals

Coenzyme Q₁₀ was obtained from Arab Co. for Pharmaceuticals and Medicinal Plants (MEPACO), Egypt. Vitamin B complex was purchased from Global Napi Pharmaceutical, Egypt. Lecithin was provided by Techno Pharma, Egypt.

Animals

Ninety six (male and female) Sprague Dawley aged rats supplied by the Animal Laboratory House of the National Research Centre, Cairo, Egypt. They were kept in a regulated environment (25 ± 1 °C, 50 ± 2% humidity), with 12 h light/dark cycles. Animal Laboratory Administrative Center and the

Institutional Ethics Committee at the National Research Centre, Cairo, Egypt, approved all the experimental procedures.

Experimental design

The animals were divided into 2 main groups, namely female and male. Each group was subdivided into 6 groups. Group 1 served as control group. Group 2 was administered AlCl₃ (100 mg kg⁻¹ b.w.) (20) for 4 months. Groups 3, 4, 5, and 6 were administered AlCl₃ for 4 months, and then treated with either one of Coenzyme Q₁₀, (200 mg kg⁻¹ b.w.) (21); vitamin B complex (0.2 mg kg⁻¹ b.w.) (22); lecithin (60 mg kg⁻¹ b.w.) (23), or all in combination for 3 months. AlCl₃ and the nutraceuticals were given orally using gastric tube.

At the end of the experiment period, fasting blood samples were collected from retro-orbital venous plexus under diethyl ether anesthesia. Blood samples were collected in heparinized tubes and then centrifuged at 3000 rpm at 4 °C for 15 min to separate plasma, which were used for nitric oxide (NO) determination.

After blood collection, the brains of the rats were dissected, washed in isotonic saline, and dried. Each brain was mid-sagittally divided into 2 portions. The first portion was fixed in formalin buffer for histopathological investigation. The second portion of brain was weighed and homogenized immediately to give 10% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl and 300 mM sucrose. The homogenate was centrifuged at 3000 rpm for 10 min in cooling centrifuge at 4 °C. The supernatant (10%) was used for determination of acetylcholinesterase activity (AChE), Na⁺/K⁺-ATPase activity, and reduced glutathione (GSH), vitamin B₁₂ and folate as well as homocysteine levels. The supernatant (10%) was further diluted to give 5% for determination of lipid peroxidation level.

Biochemical examinations:

A kinetic spectrophotometric method was used to determine AChE activity (24). The Na⁺/K⁺-ATPase activity was determined according to the method described by Tsakiris et al. (25). Lipid peroxidation was estimated according to Satoh (26). GSH level was determined according to the method of Ellman (27).

Determination of plasma nitric oxide (NO) was carried out according to the method of Berkels et al. (28). Determination of brain vitamin B₁₂ and folate were carried out using radioimmunoassay (RIA) technique (29). Homocysteine levels were measured using the method of Frantzen et al. (30).

Histopathological investigation

Brain samples were fixed in buffered formalin solution for 1 week. Then, the brain tissues were washed in running tap water for 24 h, and dehydrated in ascending series of alcohol. The samples were cleared in xylene and immersed in paraffin. The tissues were mounted in blocks and left at 4 °C until the time to be used. The paraffin blocks were sectioned at 5 µm thickness and mounted on clean glass slides. Ordinary hematoxylin and eosin stain was used (31).

Statistical analysis

The obtained data were presented as mean ± standard error. The difference between 2 groups was calculated using independent Student's t-test, while the difference between more than 2 groups was calculated using one way analysis of variance (ANOVA) using MSTAT-C version 4 program according to Snedecor and Cochran (32).

Results

The present study showed that aluminum induced a significant increase in brain AChE while it caused a significant decrease in Na⁺/K⁺-ATPase activities (P < 0.01) compared to the control group in both genders. Treatment with either CoQ₁₀, vitamin B, or lecithin alone or all in combination showed a significant decrease (P < 0.01) in brain AChE activity in both female and male rats compared to Al-intoxicated group. On the other hand, Na⁺/K⁺-ATPase activity showed a significant increase in each of CoQ₁₀, vitamin B, lecithin (P < 0.05), and in the combined therapy groups (P < 0.01) compared to the Al-intoxicated group (Table 1).

Al produced a significant elevation in brain AChE activity in the Al-intoxicated group compared to the other 5 groups [control, CoQ₁₀, vitamin B complex, lecithin, and the combination therapy (CoQ₁₀ + lecithin + vitamin B)], whereas it caused a significant inhibition in brain Na⁺/K⁺-ATPase activity compared to the same 5 groups. Insignificant change in the activity of the 2 enzymes was detected among the treated groups except the presence of a significant elevation in Na⁺/K⁺-ATPase activity in the combined therapy-treated group compared to vitamin B or lecithin-treated groups in both female and male rats (Table 1).

Table 1. Brain acetylcholinesterase and Na⁺/K⁺-ATPase activities among the groups.

Parameters	Acetylcholinesterase (U/mg protein)		Na ⁺ /K ⁺ -ATPase (µmol pi/h/mg protein)	
	Female	Male	Female	Male
Control	518 ± 29.0	415 ± 39.5	7.1 ± 0.37	6.6 ± 0.48
Al intoxication	906 ± 14.0*	600 ± 21.8*	3.6 ± 0.15*	3.2 ± 0.13*
Al→CoQ ₁₀	680 ± 24.7**	469 ± 14.6**	5.2 ± 0.53**	4.4 ± 0.42**
Al→Vitamin B complex	666 ± 14.0**	470 ± 15.4**	5.0 ± 0.18**	4.3 ± 0.32**
Al→Lecithin	700 ± 24.7**	487 ± 26.0**	4.5 ± 0.14**	4.0 ± 0.21**
Al→(CoQ ₁₀ + Vit.B + lecithin)	653 ± 2.80**	446 ± 12.7**	6.0 ± 0.36**	5.1 ± 0.27**

* Significant difference at P < 0.05 as compared with the control group.

** Significant difference at P < 0.05 as compared with the Al intoxicated group.

Concerning brain vitamin B₁₂, folate, and homocysteine levels, the results revealed a significant decrease (P < 0.01) in brain vitamin B₁₂ and folate levels and a significant increase (P < 0.01) in homocysteine level in Al- intoxicated group compared to the control group in both genders. Moreover, there was a significant increase in vitamin B₁₂ level among CoQ₁₀, vitamin B complex, combined therapy (P < 0.01), or lecithin-treated group (P < 0.05) compared to the Al-intoxicated group in both genders. Treatment with CoQ₁₀, vitamin B complex, lecithin, or the combined therapy revealed a significant increase (P < 0.01) in folate levels in both genders compared to the Al-intoxicated group, whereas it showed a significant decrease (P < 0.01) in homocysteine levels in both female and male rats compared to the Al-intoxicated group (Table 2).

The comparison between all groups showed that Al administration revealed significant variations in vitamin B₁₂, folate, and homocysteine levels among the other 5 groups in both genders. Al produced a significant inhibition in brain vitamin B₁₂ and folate levels in the Al-intoxicated group compared to the other 5 groups, whereas it produced a significant increase in brain Hcy levels compared to the same 5 groups. There was a significant change in vitamin B₁₂ and folate levels among the treated groups except in the combination therapy-treated group where there was no significant change in vitamin B₁₂ and folate levels compared to the vitamin B complex-treated

group in both female and male rats. Furthermore, treatment with CoQ₁₀ showed an insignificant change in vitamin B₁₂ levels in both female and male rats and in folate levels in male rats as compared to the lecithin-treated group. The obtained data revealed a significant change in homocysteine levels among the treated groups except in the CoQ₁₀-treated group where there was an insignificant change in Hcy levels compared to the vitamin B complex-treated group in both male and female rats. In addition, there was an insignificant change in Hcy levels in the combined therapy-treated group compared to the CoQ₁₀-treated group in male rats (Table 2).

The results showed a significant increase (P < 0.01) in each of brain lipid peroxidation and plasma nitric oxide levels in the Al-intoxicated group compared to the control group in both female and male rats. Treatment with CoQ₁₀, vitamin B complex, lecithin, or the combined therapy showed a significant decrease (P < 0.01) in brain lipid peroxidation and plasma nitric oxide levels in both female and male rats compared to the Al-treated group. Brain glutathione levels showed a significant decrease in the Al-intoxicated group as compared to the control group in both female and male rats. Treatment with CoQ₁₀, vitamin B complex, lecithin, or the combined therapy revealed a significant increase (P < 0.01) in brain glutathione levels in both female and male rats compared to the Al-intoxicated group (Table 3).

Table 2. Brain vitamin B₁₂, folate, and homocysteine levels among the groups.

Parameters	Vitamin B ₁₂ (pg/mg protein)		Folate (ng/mg protein)		Homocystein (µmol/mg protein)	
	Female	Male	Female	Male	Female	Male
Control	110.3 ± 2.6	109.3 ± 2.3	1.6 ± 0.08	1.6 ± 0.08	5.4 ± 0.14	6.5 ± 0.0 4
Al intoxication	76.7 ± 2.6*	72.8 ± 2.7*	0.8 ± 0.04*	0.8 ± 0.03*	10.1 ± 0.01*	10.6 ± 0.17*
Al→CoQ₁₀	92.2 ± 3.5**	89.2 ± 3.8**	1.2 ± 0.02**	1.1 ± 0.05**	6.5 ± 0.12**	7.5 ± 0.14**
Al→Vitamin B complex	98.9 ± 1.6**	94.0 ± 2.9**	1.4 ± 0.06**	1.3 ± 0.05	6.9 ± 0.23**	8.2 ± 0.01**
Al→Lecithin	84.6 ± 2.2**	86.4 ± 1.2**	1.0 ± 0.02**	1.0 ± 0.04**	7.5 ± 0.27**	7.8 ± 0.08**
Al→(CoQ₁₀ + Vit.B + lecithin)	102.6 ± 2.6**	98.7 ± 2.3**	1.4 ± 0.05**	1.3 ± 0.05	6.1 ± 0.07**	7.4 ± 0.12**

* Significant difference at P < 0.05 as compared with the control group.

** Significant difference at P < 0.05 as compared with the Al intoxicated group.

Table 3. Brain lipid peroxidation, glutathione, and plasma nitric oxide levels among the groups.

Parameters	Nitric oxide ($\mu\text{mol/L}$)		Glutathione ($\mu\text{mol/mg protein}$)		Lipid Peroxidation (nmol/mg protein)	
	Female	Male	Female	Male	Female	Male
Control	30.8 \pm 0.9	30.8 \pm 0.9	0.31 \pm 0.03	0.39 \pm 0.02	4.9 \pm 0.2	4.8 \pm 0.35
Al intoxication	42.7 \pm 1.7*	42.7 \pm 1.7*	0.10 \pm 0.01*	0.26 \pm 0.02*	7.9 \pm 0.2*	7.6 \pm 0.27*
Al \rightarrow CoQ ₁₀	34.6 \pm 0.75**	34.6 \pm 0.75**	0.23 \pm 0.01**	0.39 \pm 0.01**	5.6 \pm 0.2**	5.5 \pm 0.32**
Al \rightarrow Vitamin B complex	36.4 \pm 0.40**	36.4 \pm 0.40**	0.26 \pm 0.01**	0.37 \pm 0.01**	6.1 \pm 0.15**	5.9 \pm 0.26**
Al \rightarrow Lecithin	34.3 \pm 1.6**	34.3 \pm 1.6**	0.17 \pm 0.01**	0.30 \pm 0.02**	5.7 \pm 0.35**	5.5 \pm 0.33**
Al \rightarrow (CoQ ₁₀ + Vit.B + lecithin)	32.7 \pm 0.96**	32.7 \pm 0.96**	0.27 \pm 0.02**	0.39 \pm 0.02**	5.2 \pm 0.36**	5.2 \pm 0.40**

* Significant difference at $P < 0.05$ as compared with the control group.

** Significant difference at $P < 0.05$ as compared with the Al intoxicated group.

The results showed that Al administration caused a significant variation in brain lipid peroxidation, glutathione, and plasma nitric oxide levels among the other 5 groups ($P < 0.01$). Al causes a significant elevation in brain lipid peroxidation and plasma nitric oxide compared to the other 5 groups whereas it caused a significant decrease in brain glutathione level compared to other 5 groups in both female and male rats. There was an insignificant change in brain lipid peroxidation level among the 4 treated groups in both male and female rats. There is an insignificant change in plasma nitric oxide level among the 3 of the treated groups in both female and male rats except in the combined therapy-treated group where there was a significant change in plasma nitric oxide level compared to the vitamin B complex-treated group in both female and male rats. Furthermore, there was a significant change in brain glutathione levels in the lecithin-treated group compared to the other treated groups in both female and male rats (Table 3).

Histopathological investigation

The microscopic examination of the brain sections of control female and male rats showed highly active nerve cells having huge nuclei with relatively pale-stain. The nuclear chromatin and prominent nucleoli are dispersed. The surrounding relatively inactive supporting cells have small nuclei with densely-stain, condensed chromatin, and no visible nucleoli (Figures 1A and 2A).

Supplementation with AlCl₃ for 4 months in both female and male rats showed necrosis of the brain and deposition of neurofibrillary tangles. The normal structure and outlines of the nerve cells and their nucleoli are lost. Some neurons appeared like a ring shape (Figures 1B and 2B). Examination of brain sections of both male and female rats intoxicated with AlCl₃ and treated with CoQ₁₀ for 3 months showed necrosis of few neurons. The normal structure and the outlines of the nerve cells and their nuclei appeared (Figures 1C and 2C).

Following the administration of vitamin B complex for 3 months after AlCl₃ intoxication, the neurons revealed a healthy appearance with small-condensed surrounding cells. Necrosis of some neurons still appeared (Figures 1D and 2D).

Brain sections of female and male rats treated with lecithin after AlCl₃ intoxication showed that the nuclei of the nerve cells were shifted to the periphery. The supporting cells were condensed and surrounded by a shadow-like ring in female rats (Figures 1E and 2E).

The histopathological investigation of the brain sections of the rats supplemented with the combined therapy (CoQ₁₀, vitamin B complex, and lecithin) after AlCl₃ intoxication showed more or less normal neurons in both female and male rats except the appearance of pale supporting cells in both male and female rats (Figures 1F and 2F).

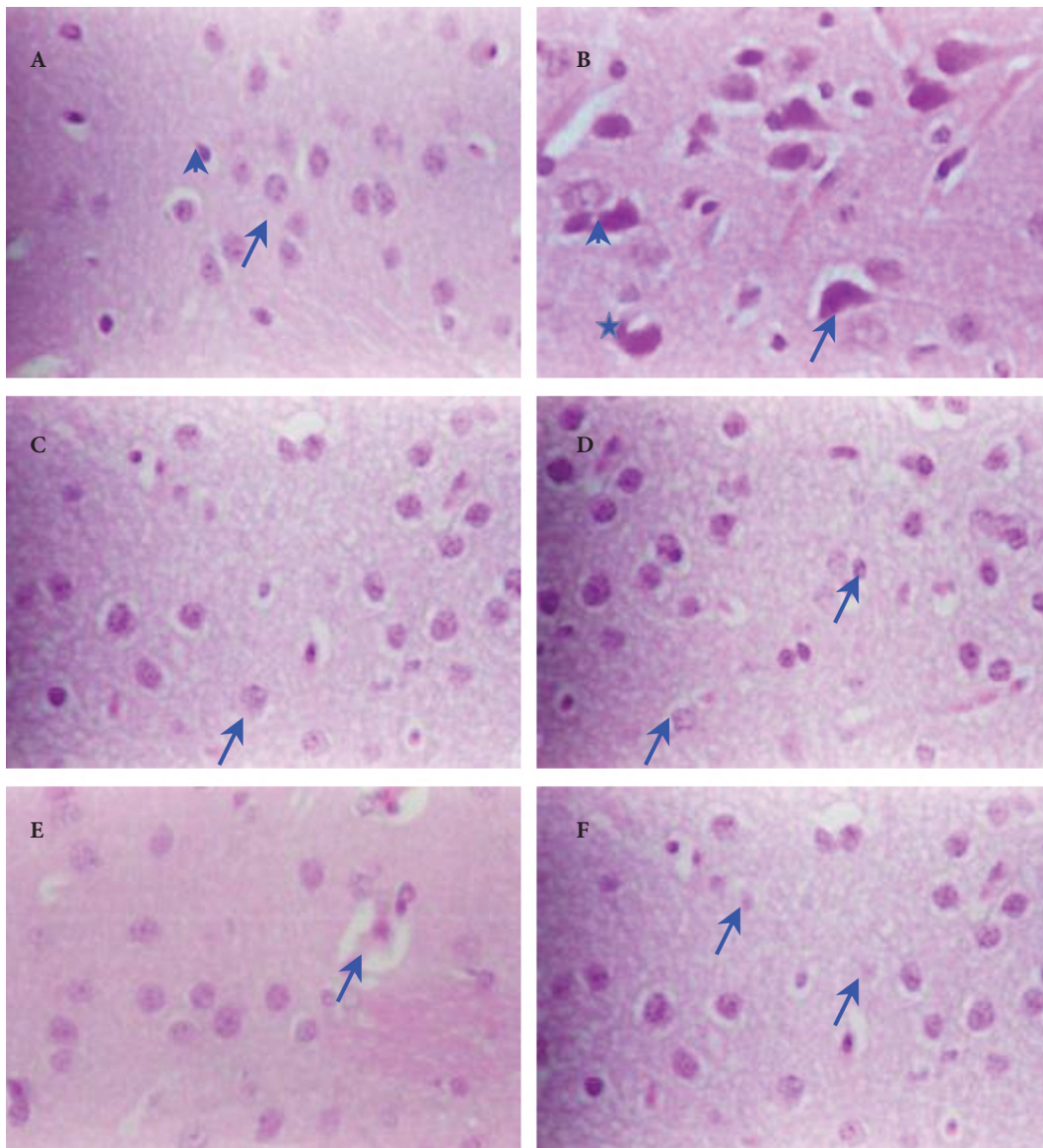


Figure 1. Photomicrographs of brain sections of female rats. (A) Control, shows nerve cells having huge nuclei with relatively pale-stain (arrow) and the supporting cells have small nuclei with densely-stain (arrowhead), (B) Al intoxicated rat shows necrosis of the brain (arrow) and deposition of neurofibrillary tangles (arrowhead). Some neurons appeared like ring shape (*), (C) Al→ CoQ₁₀, shows necrosis of few neurons (arrow), (D) Al→ Vit.B₁₂, the neurons reveal healthy appearance with small-condensed surrounding cells. Necrosis of some neurons still appeared (arrow). (E) Al→ Lecithin and shows that the nuclei of the nerve cells were shifted to the periphery. The supporting cells were condensed and surrounded by shadow like ring in female rats (arrow), (F) Al→ CoQ₁₀, Vit.B₁₂ and Lecithin shows more or less normal neurons except the appearance of pale supporting cells (arrow) (H & E X 400).

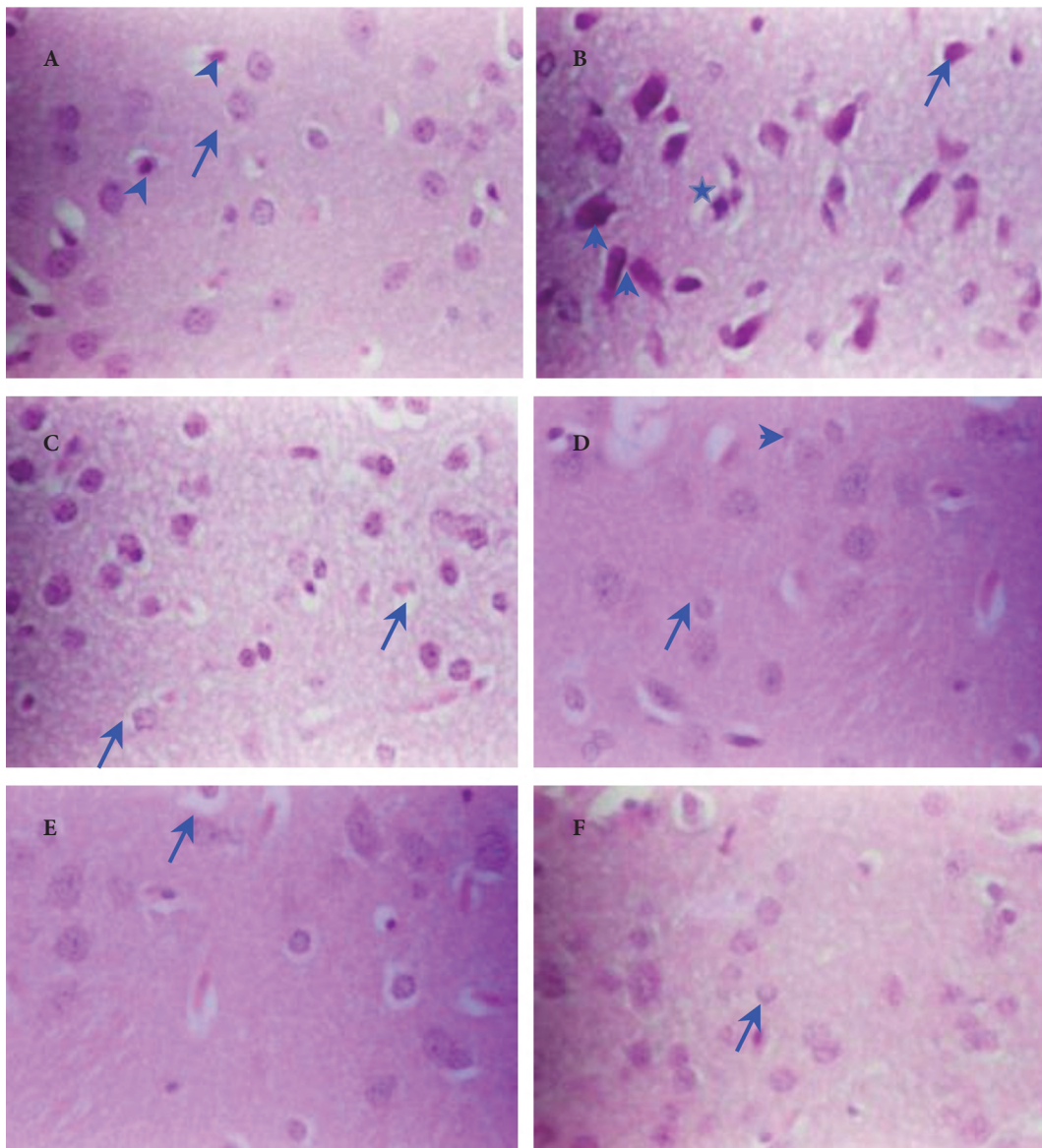


Figure 2. Photomicrographs of brain sections of male rats. (A) Control, shows nerve cells having huge nuclei with relatively pale-stain (arrow) and the supporting cells have small nuclei with densely-stain (arrowhead); (B) Al intoxicated rat shows necrosis of the brain (arrow) and deposition of neurofibrillary tangles (arrowhead). Some neurons appeared like ring shape (*), (C) Al→ CoQ₁₀, shows necrosis of few neurons (arrow) (D) Al→ Vit.B₁₂, the neurons reveal healthy appearance with small-condensed surrounding cells (arrow). Necrosis of some neurons still appeared (arrowhead) (E) Al→ Lecithin and (F) Al→ (CoQ₁₀, Vit.B₁₂ and Lecithin) shows more or less normal neurons except the appearance of pale supporting cells (arrow) (H & E X400).

Discussion

In view of the obtained data, long-term treatment with AlCl₃ caused elevation in the AChE activity. An increase in AChE activity can explain that Al interacts

with the cholinergic system, acting as a cholinotoxin (33). The interference with cholinergic projection functions may represent the way by which Al contributes to pathological processes in AD leading

to learning and memory deficits (34). Besides the fact that Al is a cholinotoxin agent, its neurotoxic effect could be exerted by additional mechanisms, such as induction of oxidative stress (4). The increased production of the AChE may be due to a direct action of A β , which binds to nicotinic receptors or over expression of β -amyloid precursor (APP) and consequently A β induced by Al results in the increased activity of AChE within and around A β plaques (35).

It was found that AChE increment in Al intoxicated female rats was more than that in male. For instance, estrogen efficiencies with advancing age in woman lead to impaired mitochondrial enzymes and were proposed as a key contributor to metabolic imbalance in AD (36). This may help explain gender differences in disease incidence and support a possible use of steroid hormone therapy (37).

Gender may also influence the levels of proinflammatory cytokines as older woman may have low grade inflammation status as compared to older male (38). On the other hand, Li et al. (39) found that AD is more prevalent in female than male.

In the present study, Al administration caused a reduction of brain Na⁺/K⁺ ATPase activity. Lynch et al. (40) stated that self-aggregation of A β due to Al administration leads to generation of hydrogen peroxide and hydroxyl radical via certain chemical reactions. The production of these reactive oxygen species induces membrane lipid peroxidation, which can impair the function of membrane ion-motive ATPase (Na⁺/K⁺- and Ca⁺-ATPases) resulting in membrane depolarization and a decrease in cellular ATP levels.

The present data demonstrate that AlCl₃ reduced brain vitamin B₁₂ and folate levels with concomitant increase in brain homocysteine (Hcy) levels. Impaired vitamin B₁₂ functions and decreased vitamin B₁₂ status have been associated with neurological and cognitive impairment (41). It has been suggested that folate deficiency may precede AD and vascular dementia (42). The suggested mechanism for decreasing vitamin B₁₂ in the present study is that Al potentiates cerebral oxidative stress, which in turn augments the oxidation of an intermediate form of vitamin B₁₂ that is generated in the methionine synthase reaction, thereby impairing the metabolism of Hcy. Oxidative

stress also compromises the intraneuronal reduction of the vitamin to its metabolically active state (43). Deficiencies of folate and vitamin B₁₂ resulted in high concentration of Hcy (44). Superphysiological levels of Hcy are neurotoxic in cell culture and in vivo mouse models (45). Accumulation of Hcy in the brain leads to growth restriction, neural or cognitive dysfunction (46), impaired brain energy metabolism, and the inhibition of Na⁺/K⁺ ATPase activity (47).

The brain is an organ that is especially susceptible to peroxide damage because of several factors, such as its high lipid content, high oxygen turn over, low mitotic rate as well as low antioxidant concentration. However, increased production of reactive oxygen species (ROS) was reported during Al exposure, which is attributed to electron leakage and increased electron chain activity (48). ROS subsequently attack almost all cell components including membrane lipids, producing lipid peroxidation (49). Thus, it can be hypothesized that oxidative stress could be one of the contributing factors for Al-induced central nervous disorders (50). This explains the increased brain lipid peroxidation levels in Al-intoxicated rats in the current study.

The depletion of reduced glutathione (GSH) in the brain of Al intoxicated rats in the current study may be due to the effect of Al on GSH synthesis by decreasing the activity of glutathione synthase (GS), a rate-limiting step of whole reaction, thus leading to reduced GSH content (51). A depletion of cellular GSH can impair cellular defenses against the toxic actions of ROS and other compounds that lead to cellular injury and death (52).

Nitric oxide has been suggested to play multiple roles in Al intoxication (53). Al could stimulate nitric oxide production by activating inducible nitric oxide synthase (iNOS) (54). Furthermore, A β upregulated the cytokine-mediated induction of iNOS and altered the cellular redox in glial cells (55). This explanation suggests the role of peroxynitrite (ONOO⁻; a reaction product of NO[•] and O₂^{-•})-mediated pathology in the AD brain (56).

The present data showed that administration of Coenzyme Q₁₀ in Al-intoxicated rats revealed a significant reduction in brain AChE activity, and homocysteine, lipid peroxidation, and plasma nitric levels where it led to a significant increase in each of

brain Na^+/K^+ ATPase activity, and vitamin B12, folate and glutathione levels.

Coenzyme Q_{10} (CoQ_{10}) is a well known hydrophobic member of the mitochondrial electron transport chain, which is capable of accepting either 1 or 2 electrons. CoQ_{10} acts as a potent natural antioxidant, oxygen-derived free radical scavenger as well as membrane stabilizer (57,58). CoQ_{10} participates as a cofactor of dehydrogenases in the transport of electron and proton as well as in ATP production, so it can stimulate ATPase activity (59). CoQ_{10} is able to inhibit mitochondrial ROS generation and inner mitochondrial depolarization as it has been reported that supplementing the rats with diet containing CoQ_{10} reduced ROS production in the mitochondria (60). Moreover, Gomez-Diaz et al. (61) stated that plasma membrane protection against oxidative stress is increased due to CoQ_{10} supplementation. Based on the link between the rate of $\text{A}\beta$ production and induced oxidative stress, CoQ_{10} exerts its neuroprotective effect via reduction of oxidative stress (62). Taken together, the above-mentioned functions of CoQ_{10} permit this coenzyme to exhibit an improvement in each of the biochemical markers investigated in the present study.

Our results indicated that vitamin B complex administration to Al-intoxicated rats resulted in a significant improvement in the studied biochemical parameters. This could be explained by the efficacy of folate to decrease brain Al accumulation and influence Al influx, efflux, or both from the brain (63). There is a direct relation between folic acid supplementation and Al accumulation so that folate acts on intestinal Al absorption and the presence of folate in the lumen inhibits the transport of Al. This inhibition may occur by a folate-Al complex formation (63). Moreover, the high level of total homocysteine (tHcy) may directly cause the molecular pathology underlying AD, and the use of vitamins to lower tHcy may have a role in the prevention of AD (64). Adequate intake of folate-containing foods and probably relatively good nutritional intake results in a reduction in tHcy-induced production and/or accumulation of $\text{A}\beta$ processes subsequently lead to AD (65). Thus, this study supports the potential role of B vitamins in improvement of the biochemical markers investigated in our study.

AD sufferers have been found to have a lack of the enzyme responsible for converting choline into acetylcholine within the brain (66). Acetylcholine (ACh) is a neurotransmitter derived from choline and coenzyme A. Nervous tissue cannot synthesize choline, which is ultimately derived from the diet and delivered to neurons through the blood stream. ACh released from cholinergic synapses is hydrolyzed by AChE into choline and acetyl coenzyme A. Approximately 50% of choline derived from ACh hydrolysis is recovered through a high affinity transporter. As a consequence of this, neurons require a further supply of choline for ACh synthesis (67). Lecithin is a major dietary source of choline. Replacement of cholinergic function may be of therapeutic benefit to AD patients. Different approaches proposed that the intervention with AChE precursor stimulates ACh release via muscarinic or nicotinic receptor agonists or AChE inhibition (68) have a potential role in management of AD. By this way, treatment of Al-intoxicated rats with lecithin produces moderate effect on the examined neuronal biochemical marker in the current study.

The combined therapy including CoQ_{10} , vitamin B complex, and lecithin exhibits the most pronounced effect as compared to the other treatments in improving the studied biochemical parameters related to the neurological damage, characteristic of AD. This finding could be interpreted as that vitamin B complex is a potent antioxidant agent, which scavenges free radicals, reduces the oxidative stress, and enhances the antioxidant defense system. CoQ_{10} initiates the reaction involves in the production of acetyl Co-A. Lecithin represents the source of choline, the precursor of ACh, in the presence of acetyl Co-A. Therefore, we suggest that the synergistic effect of these nutraceuticals resulted in the observed neuroprotective action of the selected combined therapy.

Histopathological investigation showed that AlCl_3 administration causes neuronal degeneration and brain necrosis in concomitant with the appearance of neurofibrillary tangle-bearing neurons. This finding agrees with the previous report of Lopez and Becker, and Sergeant et al. (69,70). These histopathological features are the hallmarks that characterize AD.

Treatment of Al-intoxicated rats with CoQ₁₀ showed the appearance of more or less normal structure of most neurons. Necrosis of some neurons was also observed. These results are consistent with those of Ostrowski (62), who reported that CoQ₁₀ diminishes neuronal injury in the hippocampal region. Vitamin B administration to Al-intoxicated rats revealed the neurons in healthy appearance with small condensed surrounding cells. Necrosis of some neurons still appeared. These findings are supported by Hoane et al. (71). Al intoxicated rats treated with lecithin showed some degree of degenerative neurons. This result is in agreement with Muma and Rowell (72). The combined therapy improved the structure and

viability of the brain cells with the exception of the appearance of some pale supporting cells in the brain of male rats.

In conclusion, supplementation with the selected nutraceuticals in the current study significantly modulated the biochemical markers of Al-intoxicated rats. These findings were well documented by histopathological investigations. Therefore, we suggest that the synergistic effect of CoQ₁₀, vitamin B complex, and lecithin in the combined therapy results in marked neuroprotective action rather than the individual treatment with either one in reducing the progressive neurological damage characterizing AD.

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