

Comparative Study on the Influence of Epigallocatechin-3-gallate and/or Coenzyme Q10 against Alzheimer's disease Induced by Aluminium in Normally-Fed and Protein Malnourished Rats

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Abstract

Background: Alzheimer's disease (AD) is a neurodegenerative disorder greatly influenced by oxidative stress and mitochondrial dysfunction which may lead to deposition of β -amyloid ($A\beta$) peptides. Protein malnutrition increases oxidative damage in cortex, hippocampus and cerebellum. Epigallocatechin-3-gallate (EGCG) has health-promoting effects in CNS, while Coenzyme Q10 (CoQ10) is intracellular antioxidant and mitochondrial membrane stabilizer.

Objective: To investigate the possible protective effect of EGCG and/or CoQ10 against aluminium-induced neurotoxicity presenting symptoms that mimic AD in both normally-fed (NF) and protein malnourished (PM) rats.

Methods: Ten groups of rats were used; five for NF (20% casein) and the same for PM (10% casein). Both NF and PM groups received daily for four weeks; either saline for control or $AlCl_3$ (70 mg/kg, I.P) for AD induction groups, treated groups received together with $AlCl_3$ either EGCG (10 mg/kg, I.P), CoQ10 (200 mg/kg, P.O) or combination of both. Histopathological changes in the brain and biochemical changes in $A\beta$, Acetyl cholinesterase (ACHE) as well as oxidative parameters (MDA, SOD, TAC) were evaluated for all groups.

Results: The study revealed that, brain neurological damage characterizing induction of AD as indicated by histopathological changes in the brain and the increase in $A\beta$, ACHE and MDA as well as the decrease in SOD and TAC was more pronounced in PM rats. Administration of EGCG and/or CoQ10 during induction of AD showed protective effect in both NF and PM rats as indicated by the decreased $A\beta$, ACHE, MDA together with the increased SOD, TAC and confirmed by histo pathological examinations in different brain regions. However, the effect of combined treatment was more pronounced in both NF and PM rats.

Conclusion: PM is a risk factor in induction of AD, EGCG and CoQ10 combined therapy has marked protective effects during induction of AD in both NF and PM rats rather than each individual treatment.

Keywords: Alzheimer's disease; $AlCl_3$; Protein malnutrition; Oxidative stress; Epigallocatechin-3-gallate; Coenzyme Q10; Rats

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that leads to memory loss and nerve cell death throughout the brain and represents the major cause of dementia in the world [1]. The number of AD patients is expected to reach 106.8 million worldwide by the year 2050; therefore, the disease is considered a growing public health concern with major socioeconomic burden [2]. AD risk factors and disease-modifying factors have gained much attention [3]. A number of factors may increase the chances of developing the disease, some risk factors can be changed or controlled while others cannot [3,4]. Stress, diabetes and depression have been associated with increased risk of AD, while cognitive engagement and physical activities have been linked to decreased risk of AD [4].

Recently, there have been growing evidences supporting the role of nutrition in AD [5]. A number of dietary components such as antioxidants, vitamins and polyphenols have been reported to decrease the risk of AD incidence, while saturated fatty acids, high-calorie intake, heavy smoking and excess alcohol consumption were identified as risk factors [6]. Dietary patterns have emerged to explore the relationship between diet and AD [7]. Patients with AD have a worse nutritional status compared to others and usually suffer from malnutrition and weight loss [8,9]. In addition, weight loss was reported to predict rapid cognitive decline in AD patients [10,11]. Consequently, treatment of weight loss and malnutrition may be important to decrease the risk of

AD. Although the mechanisms of many nutrients such as antioxidants and vitamins to decrease the risk of AD are not clear, but reducing oxidative stress, inflammatory mediators together with both $A\beta$ and tau pathologies can attenuate cognitive deterioration associated AD [12,13].

Aluminum (Al) is considered the third most abundant element and the most common metal in the earth's crust [14]. Al is increasingly taken into our bodies through food, air, water, and even drugs as a result of high rate global industrialization and consequent pollution [15]. It is found in many manufactured foods and is added as alum for treating drinking water for purification purposes [16]. Excessive Al intake can lead to deposition of $A\beta$ in central nerve cells and over expression of β -amyloid precursor protein (APP), so it can be considered as a potential etiological factor in AD [17,18]. It is

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known that the neurotoxicity of A β is associated with oxidative stress; generation of reactive oxygen species and it can lead to the damage of neuronal membrane, lipids, proteins, and nucleic acids [19,20]. Consequently, oxidative stress is thought to play an effective role in pathogenesis of AD [21]. Oxygenate radicals can interrupt the integrity and performance of the cell [22]. The brain tissue also contains a lot of unsaturated fatty acids which are especially vulnerable for free radical attacks [23]. Therefore, antioxidant substances can play an important role in prevention and cure of AD [24]. Acetylcholinesterase (ACHE) has been also found to co localize 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 [20].

Coenzyme Q10 (CoQ10) or ubiquinone, is a fat-soluble, pseudo-vitamin substance that is mainly present in the mitochondria and has an important role in generating ATP as a component of the electron transport chain [25]. It has been documented that CoQ10 acts as a powerful antioxidant and radical scavenger [26,27]. It can also help to regenerate other antioxidants, so it has been used as anti-aging and is effective in the improvement of cognitive disorders [28]. In the body, cells synthesize CoQ10 from the amino acid tyrosine, requiring adequate levels of vitamins such as folic acid [29]. Consequently, folic Acid is considered an essential cofactor for the endogenous synthesis of CoQ10 and any deficiency in folic acid would result in a deficiency in CoQ10 [30]. The efficacy of CoQ10 appears to be promising for alleviating neurodegenerative disorders [31]. It is worthy to note that CoQ10 is reduced in AD patients [32,33].

Epigallocatechin-3-gallate (EGCG) is the most abundant and active compound responsible for most of green tea's role in promoting good health. It acts through different pathways; as antioxidant, anti-inflammatory, anti-atherogenic and also showing gene expression content, functioning through growth factor-mediated pathways [34]. EGCG as antioxidant is highly effective in protecting cells; it also has COX-2 inhibiting property [34,35]. EGCG can attenuate peroxide production in glial cells by either inhibiting the deamination of monoamines or acting as a free radical scavenger [36].

Therefore, the aim of this study was to investigate the effect of protein malnutrition on induction of AD and the possible protective effect of EGCG and/or CoQ10 against aluminium-induced neurotoxicity presenting symptoms that mimic AD in both NF and PM rats.

Materials and Methods

Animals

Eighty male Sprague Dawley rats were used. Rats weighing 180-200 g were obtained from Nile Co. for Pharmaceuticals and Chemical Industries, Cairo, Egypt. They were housed in stainless-steel cages (four per cage); under the same adequate conditions, with alternatively 12 H light and dark cycles, at a temperature of $25 \pm 1^\circ\text{C}$ and water was given ad-libitum. Two weeks before starting the experimental nutrition [standard protein diet for NF rats and low protein diet for PM rats], all rats were daily provided with the dietary requirements of diet pellets (standard); contained protein (20%), fiber (5%), fat (3.5%), ash (6.5%) as well as vitamin mixture (El-Nasr, Abu Zaabal, Cairo, Egypt). On the other hand, each 100 g of the standard protein diet [20% casein diet] contained casein (20 g), sucrose (70 g), salt mixture (4 g), oil and oil-soluble vitamins (5 g) and (0.6 g) vitamin mixture in starch [37]. Low protein diet [10% casein diet, chosen for this study] has the same composition as standard protein diet except that casein was reduced

to 10 g instead of 20 g per each 100 g of the diet. The remained 10 g was replaced by sucrose-starch mixture. In accordance with ethical guidelines of Al-Azhar University (Faculty of Pharmacy) Egypt, the study was conducted.

Drugs and chemicals

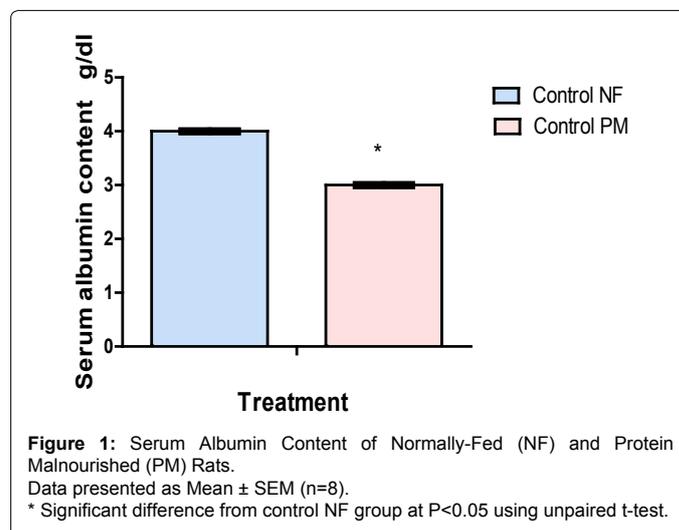
From Sigma Chemical Co. (St. Louis, MO, USA); EGCG, CoQ10 and Aluminum chloride - hydrated ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) were purchased. EGCG and AlCl_3 were freshly dissolved in distilled water while CoQ10 was suspended in 1% aqueous solution of Tween 80; suspensions were freshly prepared every day. All other solvents and chemicals were of the highest grade-commercially available.

Experimental design

Animals were randomly assigned to ten groups (8 rats/group); five groups were maintained feeding on 20% casein diet (NF rats) and five groups on 10% casein diet (PM rats) for four weeks. During these four weeks of experimental nutrition, both groups of NF and PM were treated as follows; group served as control and was given saline daily, the other four groups were injected daily with $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (70 mg/kg I.P) [38]. One of these four groups served as AD model group, while the others three groups were treated with EGCG (10 mg/kg, I.P every other day) [39], CoQ10 (200 mg/kg, P.O daily) [40] and with their combination together with AlCl_3 during the four weeks of AD induction. The dose volume for all administered drugs was not exceeding 0.5 ml/200 g body weight. At the end of the four weeks, blood samples were withdrawn from the retro-orbital venous plexus of control NF and PM groups to ensure the nutrition status by determination of serum albumin content (Figure 1). Serum samples were obtained by centrifugation at 3000 rpm for 10 min. After rats were sacrificed, the brain tissues were dissected. All brain tissues from all groups were subjected immediately for analysis or they were kept frozen until the time of analysis (at -80°C) after washing with ice-cold saline. The homogenates of the brain tissues in saline were used to assess the oxidative stress markers as superoxide dismutase (SOD) and total antioxidant capacity (TAC) as well as lipid peroxides which were expressed as malondialdehyde (MDA). Acetylcholine esterase (ACHE) content and A β content were also determined. For histopathological examinations, specimens from all brain areas were taken from different treated groups.

Biochemical parameters

Protein estimation: The protein content was measured in the brain



homogenates using bovine serum albumin as a standard and according to Bradford method [41].

Determination of serum albumin content: Serum albumin content was assessed by using ready-made kits obtained from Stanbio Laboratory Inc. (San Antonio, USA).

Assessment of oxidative stress biomarkers: In the brain homogenate for each rat, MDA and SOD as well as TAC were measured. By estimating the level of thiobarbituric acid reactive substances (TBARS), lipid peroxidation can be determined as MDA [42]. Relying on the ability of the enzyme to inhibit the phenazine methosulphate mediated reduction of nitroblue tetrazolium dye, SOD content was assessed where the increase in absorbance at 560 nm for 5 min is measured [43]. On the other hand, determination of TAC is performed through the reaction with a defined amount of exogenously provide H_2O_2 . The residual H_2O_2 is colorimetrically determined by the enzymatic reaction that involves the conversion of 3, 5-dichloro-2-hydroxybenzene sulphonate to a colored product [44].

Determination of ACHE content: In the brain tissue homogenate, ACHE content was assessed according to the manufacturer's instructions by using ELISA Kits from Ray Biotech, Inc. (USA).

Determination of A β content: Determination of A β was assessed in brain tissue homogenate according to the manufacturer's instructions by using ELISA Kits (USCN Life Science, Inc., Product Number MBS702915).

Histopathological examination of the brain

In 10% formalin for 24 h, brain specimens were fixed then they were washed with tap water, they were prepared and stained for light microscopy [45]. For dehydration; serial dilutions of alcohol were used (methyl, ethyl and absolute ethyl). In hot air oven at 56°C for 24 h, specimens were cleared in xylene embedded in paraffin. By using microtome at 4 microns thickness, paraffin bees wax tissue blocks were sectioned. Then, sections were collected on glass slides and deparaffinized. They were stained for routine histological examination using Hematoxylin and Eosin stain.

Statistical Analysis

Data are expressed as mean \pm SEM. Multiple comparisons were performed using one-way ANOVA followed by Tukey Kramer as a post hoc test. Unpaired t-test was used to compare two different treatments. As a criterion for significance, 0.05 level of probability was used. By using Instat (version 3) software package, all statistical analyses were performed and by using GraphPad Prism (ISI, USA) software (version 5), graphs were sketched.

Results

Serum albumin content of NF and PM rats

Protein malnutrition induced by feeding rats with low protein diet (10% casein) for 4 weeks produced marked decrease in serum albumin amounted to 75% from the corresponding control NF group.

Brain acetylcholine esterase (ACHE) content

Results are shown in (Figure 2); administration EGCG, CoQ10 and their combination during induction of AD in both NF and PM produced significant decrease in ACHE content amounted to 47.68%, 29.12% and 60.31% respectively in NF groups and to 39.29%, 26.41% and 59.88% respectively in PM groups as compared to corresponding

AD model group. However, PM itself markedly increased ACHE content in all AD model groups either untreated ($AlCl_3$) or treated ($AlCl_3$ +EGCG, $AlCl_3$ +CoQ10 and $AlCl_3$ +EGCG+CoQ10) than their corresponding NF reaching to 127.8%, 148.3%, 140% and 129.22% respectively. It is worthy to note that the base line control level of ACHE content was lower in PM rats than the corresponding control NF group amounted to 77.5%.

Brain oxidative stress biomarkers (MDA, SOD and TAC)

Results are shown in (Figures 3a-3c), administration of EGCG or CoQ10 alone or in combination during induction of AD in NF and PM rats showed marked decrease in MDA content with respect to untreated AD model by 71.96%, 62.71% and 81.68% respectively in NF groups and by 52.59%, 36.83% and 69.96% respectively in PM groups. PM itself induced marked increase in MDA content in all groups (control, un-treated and treated AD model). MDA content reached to 177.23%, 129.56%, 215.16%, 215.54% and 208.62% respectively as compared to their corresponding NF values.

On the other hand, administration of EGCG or CoQ10 alone or in combination during induction of AD in NF and PM rats resulted in marked increase in SOD content with respect to their corresponding AD model group (un-treated) reached to 267.47%, 147.59% and 371.69% respectively in NF rats, while reached to 314.05%, 175.21% and 427.27% respectively in PM rats. In addition, TAC content also increased in response to treatment by 218.89%, 182.41% and 414.44% respectively in NF groups and by 273.77%, 174.09% and 388.85% respectively in PM groups as compared to the un-treated AD model groups. Content of SOD and TAC in all PM groups have been decreased than the corresponding NF groups (control, un-treated and treated AD model) amounted to 81.56%, 61.50%, 82.13%, 81.02% and 81.48% respectively for SOD and to 85.91%, 80.05%, 93.82%, 77.69% and 76.07% respectively for TAC.

Brain β -amyloid (A β) content

Rats treated with EGCG, CoQ10 or their combination during induction of AD in both NF and PM rats showed significant decrease in A β content by 64.94%, 57.05% and 74.12% respectively in NF rats, while in PM rats A β content decreased by 50.15%, 36.07 and 67.22

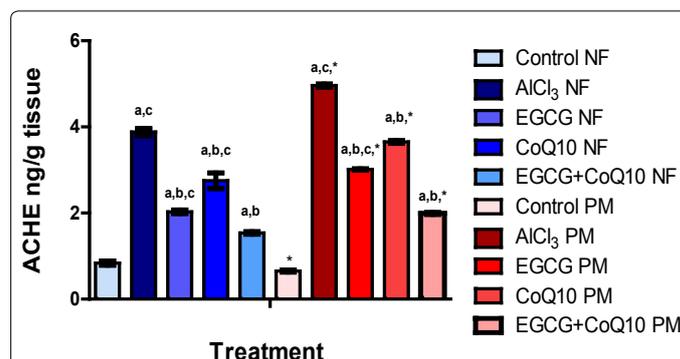


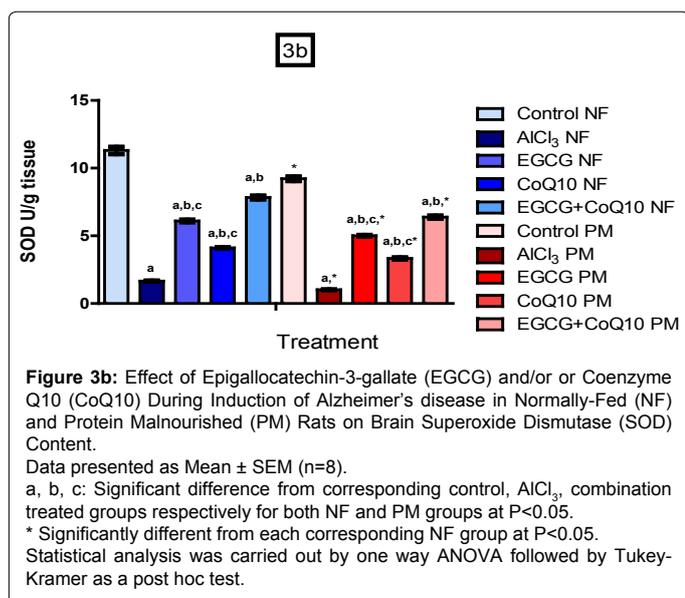
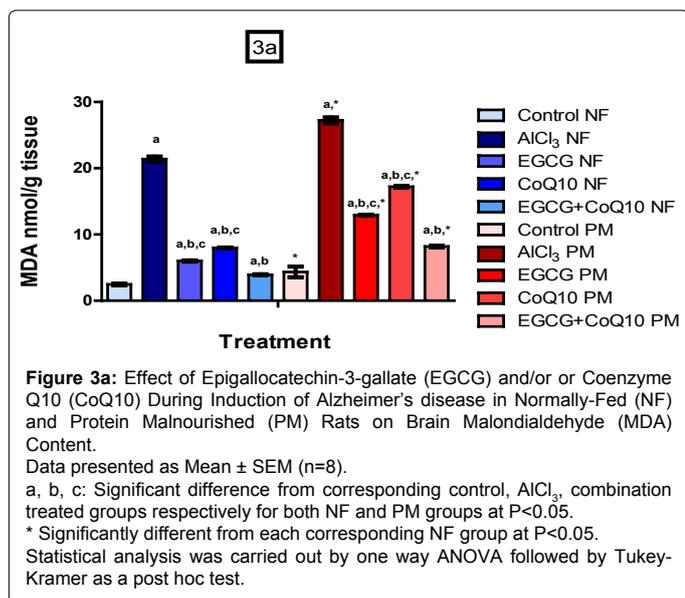
Figure 2: Effect of Epigallocatechin-3-gallate (EGCG) and/or Coenzyme Q10 (CoQ10) During Induction of Alzheimer's disease in Normally-Fed (NF) and Protein Malnourished (PM) Rats on Brain Acetylcholine Esterase (ACHE) Content.

Data presented as Mean \pm SEM (n=8).

a, b, c: Significant difference from corresponding control, $AlCl_3$, combination treated groups respectively for both NF and PM groups at $P < 0.05$.

* Significantly different from each corresponding NF group at $P < 0.05$.

Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer as a post hoc test.



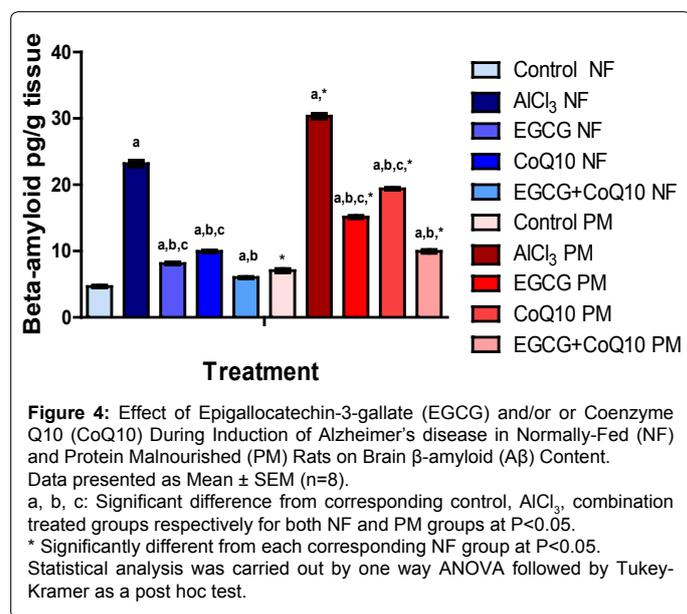
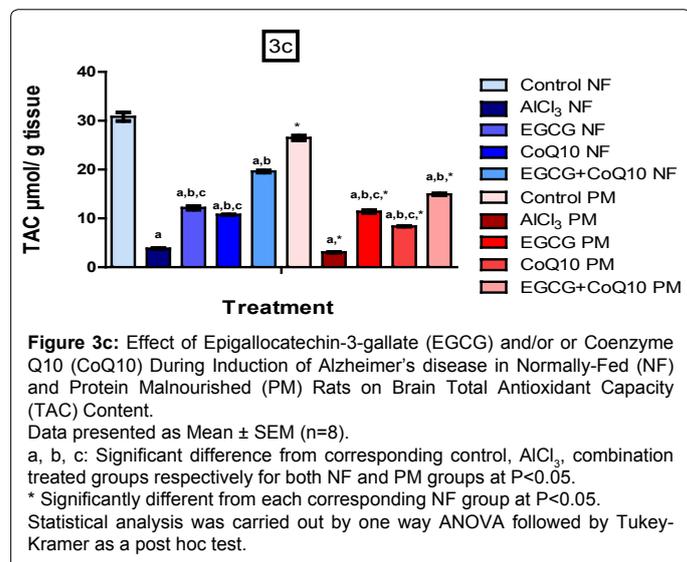
respectively with respect to the corresponding un-treated AD model group. In all groups (control, un-treated and treated AD model), PM produced a significant increase in Aβ content. It reached to 151.82%, 130.09%, 186.1%, 194.78% and 165.83% respectively compared to corresponding NF groups (Figure 4).

Histopathological alterations in the brain

Histopathological alterations in brain specimens from different groups of NF rats are shown in (Figures 5A-5F and 5H) and (Table 1) that illustrates the severity or the intensity of the alterations in the brain of the NF rats. Brain specimens from control NF rats illustrated that the histological structure of the cerebral cortex and the hippocampus was normal while, brain specimens of NF rats injected with AICl₃ for four weeks (model that mimics AD) showed degeneration and pyknosis in hippocampus neurons associated with formation of focal eosinophilic plaques in striatum. Treatment of NF rats with EGCG markedly ameliorated the pathological changes induced by AICl₃, where the

hippocampus was histological intact, while treatment of NF rats with CoQ10 illustrated normal histological structure in both hippocampus and cerebral cortex. Additionally, no histopathological alterations in hippocampus were detected with combined administration of EGCG and CoQ10 to NF rats during the four weeks of AD induction.

Histopathological alterations in brain specimens of PM groups are shown in (Figures 5I, 5K-5O and 5R) and (Table 2) that illustrated the severity or the intensity of these alterations in the brain of the PM rats. Brain specimens of control PM rats showed normal histological structure, while brain specimens of PM rats injected with AICl₃ for four weeks (model that mimics AD) showed very severe pyknosis and degeneration in hippocampus neurons associated with focal eosinophilic plaques formation in striatum. On the other hand, treatment of PM rats with EGCG showed pyknosis in the neurons of the hippocampus associated with plaques formation in the striatum, while treatment with CoQ10 showed normal histological structure in the hippocampus. Moreover, combined treatment of PM rats with EGCG and CoQ10 during the four weeks of AD induction illustrated



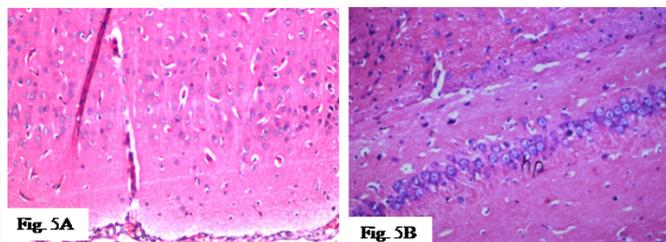


Figure 5A and 5B: Representative photomicrographs (magnification 40 X) of brain sections stained by Hematoxylin-Eosin stain: Sections taken from the brain of control NF group illustrating no histopathological alteration in the cerebral cortex (5A) and also in hippocampus (5B).

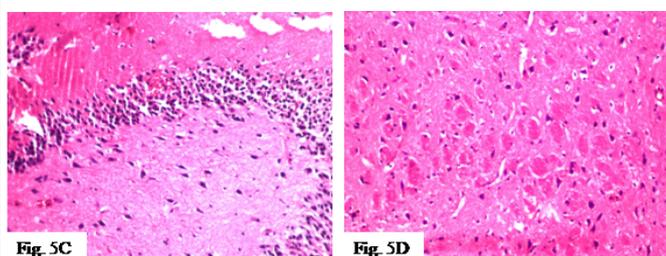


Figure 5C and 5D: Representative photomicrographs (magnification 40 X) of brain sections stained by Hematoxylin-Eosin stain: Sections taken from the brain of un-treated AD model in NF rats illustrating the presence of pyknosis as well as degeneration that were detected in neurons of the hippocampus (5C) associated with formation of focal eosinophilic plaques in striatum (5D).

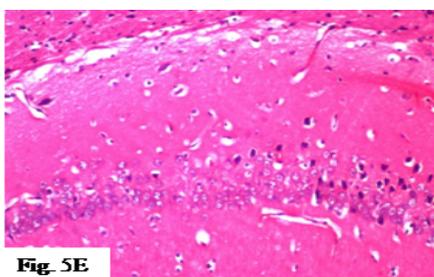


Figure 5E: Representative photomicrograph (magnification 40 X) of brain section stained by Hematoxylin-Eosin stain: Section taken from the brain of NF rats treated with EGCG during the induction of AD that illustrating no histopathological alteration in hippocampus.

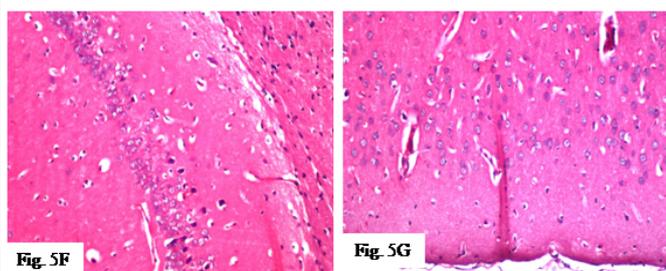


Figure 5F and 5G: Representative photomicrographs (magnification 40 X) of brain sections stained by Hematoxylin-Eosin stain: Sections taken from the brain of NF rats treated with CoQ10 during the induction of AD that illustrating normal histological structure in the hippocampus (5F) and the cerebral cortex (5G).

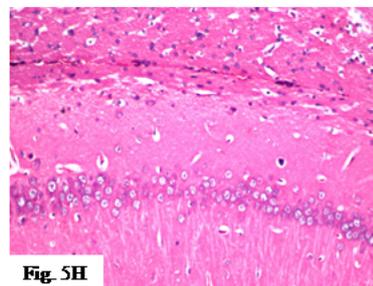


Figure 5H: Representative photomicrograph (magnification 40 X) of brain section stained by Hematoxylin-Eosin stain: Section taken from the brain of NF rats co-administered EGCG and CoQ10 during the induction of AD that illustrating no histopathological alteration in hippocampus.

Histopathological alterations	Control NF	AlCl ₃ 70 mg/kg NF	AlCl ₃ +EGCG NF	AlCl ₃ +CoQ10 NF	AlCl ₃ +CoQ10+EGCG NF
Degeneration and pyknosis in the neurons of hippocampus	-	+++	-	-	-
Formation of eosinophilic plaques in striatum	-	+++	-	-	-

+++ Severe - Nil

Table 1: Effect of epigallocatechin- γ -gallate (EGCG) and/or coenzyme Q₁₀ (CoQ₁₀) during AD induction against the severity of histopathological alterations in the brain of NF rats.

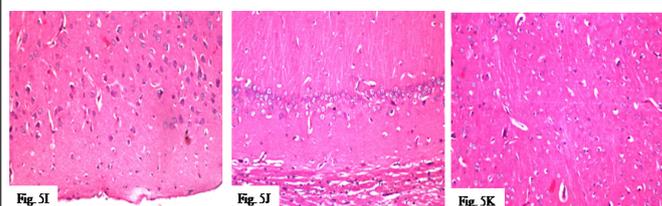


Figure 5 (I-K): Representative photomicrographs (magnification 40 X) of brain sections stained by Hematoxylin-Eosin stain: Sections taken from the brain of control PM rats illustrating normal histological structure of meninges and cerebral cortex (5I), hippocampus (5J) and striatum and cerebellum (5K).

that there was no histopathological alteration in hippocampus and cerebral cortex.

According to the mentioned results, it is clear that the severity of the brain neurological damage induced by AlCl₃ was more pronounced in PM rats than that in NF rats as indicated by the histopathological alterations in different brain regions as well as the biochemical changes in A β , Acetyl cholinesterase (ACHE) and oxidative stress markers. On the other hand, the improvement induced by EGCG was more pronounced in NF rats than in PM rats, while there were marked improvements induced by CoQ10 either alone or in combination with EGCG in both NF and PM rats as indicated by the histopathological alterations in different brain regions.

Discussion

In the present investigation, induction of AD-like behavior in rats was done by injection of AlCl₃ (70 mg/kg, I.P) daily for consecutive four weeks to rats feeding with either standard protein diet containing

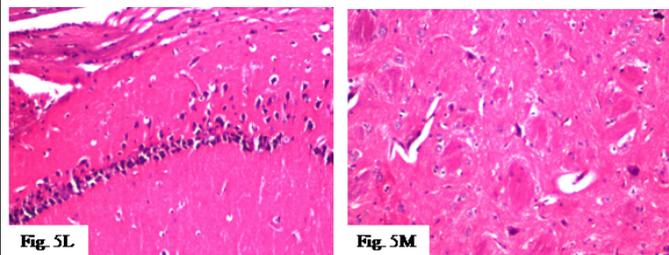


Figure 5L and 5M: Representative photomicrographs (magnification 40 X) of brain sections stained by Hematoxylin-Eosin stain: Sections taken from the brain of un-treated AD model in PM rats illustrating the presence of pyknosis and degeneration that were observed in neuron of hippocampus (5L) associated with focal eosinophilic plaques in striatum (5M).

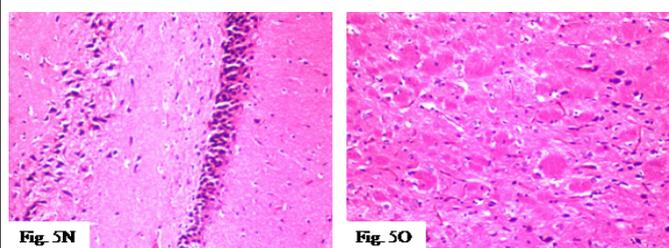


Figure 5N and 5O: Representative photomicrographs (magnification 40 X) of brain sections stained by Hematoxylin-Eosin stain: Sections taken from brain of PM rats treated with EGCG during induction of AD illustrating the presence of pyknosis in the neurons of the hippocampus (5N) associated with plagues formation in the striatum (5O).

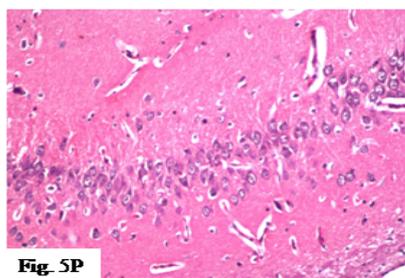


Figure 5P: Representative photomicrographs (magnification 40 X) of brain sections stained by Hematoxylin-Eosin stain: Sections taken from the brain of PM rats treated with CoQ10 during the induction of AD illustrating no histopathological alteration in hippocampus.

20% casein (NF rats) or low protein diet containing 10% casein (PM rats). Al has been suggested as a causal factor in AD, in part because of reports showing the toxicity of Al, the elevation of Al concentrations in the brains of patients with AD, and an association between Al concentrations in water and the prevalence of AD [46]. Also Al is a notable neurotoxicant that causes acceleration of oxidative damage to biomolecules. Furthermore, Al salts have been reported to cause cell depletion in the hippocampus [47] and degeneration of cholinergic terminals in the cortical areas. It accumulates in the cingulate bundle and thereby induces learning deficits [48].

Results of the present study showed that administration of AlCl₃ to both NF and PM rats significantly increased AChE content; a marker of loss of cholinergic neurons in the brain, but PM rats showed significant increase in the AChE content more than NF rats. The present result might be attributed to the ability of Al to alter the blood brain barrier

and produce marked changes in the cholinergic neurotransmission [49]. Al is well known as a potent brain cholinotoxin with slow accumulation rate [50,51]. The present results are also in parallel with those previously recorded a significant increase in AChE content in rats treated with AlCl₃ (50 mg/kg) daily for three months [52]. It is also reported that AChE content in different brain regions increased after administration of AlCl₃ [53]. The elevation in AChE content may be attributed to the direct neurotoxic effect of Al or to the disarrangement of the cell membrane phospholipids caused by the associated increase in lipid peroxidation [54].

The data obtained from this investigation showed that administration of AlCl₃ significantly increased MDA and decreased SOD and TAC activities in both NF and PM rats. However, PM rats showed a significant increase in MDA and decrease in SOD and TAC activities as compared to NF rats. As previously mentioned, Al is a potent pro-oxidant known to enhance lipid peroxides in the cortex and hippocampus [55]. It has been also reported that, Al induces lipid peroxidation and alter physiological and biochemical characteristics of biological systems [55]. Moreover, as oxidative damage is mediated by free radicals, it was requisite to examine the status of endogenous antioxidant enzymes which are the first line of defence against free radical damage under oxidative stress conditions as in cases of malnutrition [6,8]. In this study, administration of AlCl₃ resulted in marked elevation in oxidative stress as indicated by increases in lipid peroxidation (measured as MDA level) and decreases in, SOD and TAC. Al can also reduce axonal mitochondria turnover, disrupt of the Golgi or reduce the synaptic vesicles as a result of the oxidative stress status [56].

Lipid peroxidation is considered one of the main manifestations of oxidative damage and plays an important role in toxicity [57]. Concerning the data obtained in the present work, the elevation of lipid peroxidation in brain of Al-treated rats was evidenced by increased

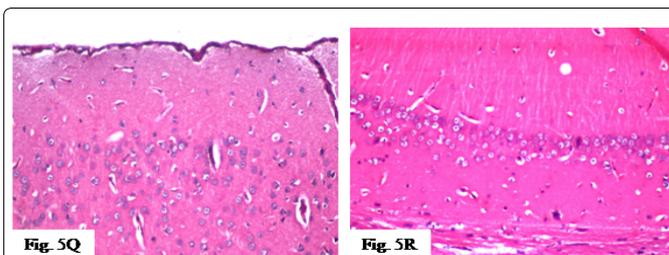


Figure 5Q and 5R: Representative photomicrographs (magnification 40 X) of brain sections stained by Hematoxylin-Eosin stain: Sections taken from the brain of PM rats co-administered EGCG and CoQ10 during the induction of AD illustrating no histopathological alteration in hippocampus (5Q) and cerebral cortex (5R).

Histopathological alterations	Control PM	AlCl ₃ 70 mg/kg PM	AlCl ₃ +EGCG PM	AlCl ₃ +CoQ10 PM	AlCl ₃ +CoQ10+EGCG PM
Degeneration and pyknosis in the neurons of hippocampus	-	++++	+	-	-
Formation of eosinophilic plaques in striatum	-	++++	+	-	-

++++ Very Severe + Mild - Ni

Table 2: Effect of epigallocatechin-γ-gallate (EGCG) and/or coenzyme Q10 (CoQ10) during AD induction against the severity of histopathological alterations in the brain of PM rats.

production of MDA. Similar results stated that daily $AlCl_3$ via drinking water for six weeks induces significant increase in MDA concentration in hippocampus and frontal cortex of rats [58]. Nearly similar findings regarding administration of $AlCl_3$ (50 mg/kg/day) in drinking water for a month induced oxidative damage with accumulation of lipid peroxidation [59]. It is also reported that injection of $AlCl_3$ (I.P) for 60 days at different doses can accelerate lipid peroxidation in rat's brain which may represent one of the most important intoxication mechanisms of Al [60]. The elevation of MDA could be also attributed to the ability of Al itself to accelerate oxidative damage to biomolecules like lipids, protein and nucleic acids [61]. The obtained data revealed also a significant inhibition in the activities of SOD and TAC in brain tissue of $AlCl_3$ treated NF and PM rats. The present findings were consistent with the results of several investigators who revealed marked decrease in endogenous antioxidant after administration of different salts of Al [62]. It is also reported that administration of $AlCl_3$ decreases the content of glutamate-s-transferase [63] and GST as well as the level of sulfhydryl group (SH) in the brain [64] which may be also included in the declining effect of Al on the expression of mRNA of the endogenous antioxidants [65]. Moreover and as a direct effect, intra-hippocampal injections of $AlCl_3$ in rats induce significant increase in MDA concentration [66].

Results of the current study also showed that injection $AlCl_3$ significantly increased $A\beta$ content in the brain of NF and PM rats; PM rats showed significant increase in $A\beta$ content than NF rats. Al is known as a cholinotoxin agent and its neurotoxic effect could be exerted by additional mechanisms such as: induction of oxidative stress, increased production of the ACHE that may be due to a direct action of $A\beta$ which binds to nicotinic receptors or due to over expression of APP. $A\beta$ induced by Al results in increased content of ACHE within and around $A\beta$ plaques [67]. Al is also bound by the $A\beta$ and was found co-localized with it in the AD brain [68]. Amyloid fibrils formed in the presence of Al were slightly thicker, significantly longer and spirally wound around each other. Subsequent studies have been conducted to confirm that amyloid beta sheets will bind up to 4 Al atoms and that binding increased the β - sheet content of the peptide [69]. The neurotoxicity of $A\beta$ in whatever form may involve the formation of reactive oxygen species. Al is a pro-oxidant and is known to promote the oxidation content of $A\beta$ in the presence of iron. It has also been linked to $A\beta$ production through the immune response. It is also linked to activate complement which in turn has been linked to the enhanced aggregation of $A\beta$ [68]. Moreover, self-aggregation of $A\beta$ due to Al administration may lead to generation of hydrogen peroxide and hydroxyl radical via certain chemical reactions and may cause induction of membrane lipid peroxidation [70].

The next step was to confirm the previously mentioned results by the histological examination of different brain regions which showed that administration of $AlCl_3$ (70 mg/kg) for 4 weeks to both NF and PM caused pyknosis and degenerations which were observed in the neuron of the hippocampus and also associated with focal eosinophilic plaques formation in the striatum. Severity of the brain neurological damage induced by Al was more pronounced in PM rats than in NF rats. As in addition to Al neurological damage; protein malnutrition induces deterioration in brain which may be due to increased oxidative stress. Dietary protein is a very important source of essential amino acids that can be used as intracellular antioxidants; therefore its restriction may lead to an increase in oxidative damage by diminishing antioxidant defences of the tissue [71].

On the other hand, cognitive dysfunction and oxidative stress

are strongly correlated; agents that modulate reactive oxygen species may be potentially useful as anti-dementia. Further documents demonstrated that, the nutrients related to CNS development and functions are also those that modify individual differences in cognitive development and cognitive performance [72]. Cognition and behaviors such as learning, memory, anxiety and risk assessment depend on the proper development of the limbic system and associated neural areas [73]. Impaired development of limbic system neurons, which play an important role in normal affect and cognition, occurs when maternal protein intake is reduced with resulting brain abnormalities [74]. In recent years, there has been increasing evidence supporting the role of nutrition in AD [5]. A number of dietary factors such as antioxidants, vitamins and polyphenols have been reported to decrease the risk of AD [6].

Results of the present study also showed that administration of EGCG to NF and PM rats during induction of AD using $AlCl_3$ (70 mg/kg) model significantly decreased $A\beta$ content, ACHE content and MDA level, while significantly increased SOD and TAC content. The present results are in accordance with the results in which EGCG increased SOD content and protected against glycation end products induced neurotoxicity by decreasing ROS and MDA [75]. Results are also in agreement with the results showed that EGCG treatment led to increase in SOD content and decrease in MDA in the hippocampus [76]. In addition, EGCG is able to bind $A\beta$; it may act as an antioxidant and anti-inflammatory against $A\beta$ aggregation in hippocampus and thus have a neuroprotective effect. Actually, $A\beta$ neurotoxicity has been reported to be mediated by free radicals and attenuated by antioxidants and free radical scavengers [77]. Moreover, EGCG has been shown to prevent $A\beta$ induced hippocampal neuronal cell death in cultured hippocampal neurons through its antioxidant properties [78]. In the present work, histological examinations in different brain regions confirmed other findings, where administration of EGCG during induction of AD in rats resulted in: no histopathological alteration in the hippocampus in NF group while, there was mild degeneration and pyknosis in the neurons of the hippocampus associated with plaques formation in the striatum observed in PM group. This result may refer to the marked deterioration caused by protein malnutrition. So, it could be concluded that EGCG has marked protection in NF rats, while in PM rats the protection was limited.

The present results also showed that administration of CoQ10 to NF and PM rats during induction of AD using $AlCl_3$ (70 mg/kg) model significantly decreased $A\beta$ content, ACHE content and MDA level, while significantly increased SOD and TAC content. CoQ10 is a member of the mitochondrial electron transport chain, which is capable of accepting either 1 or 2 electrons. It acts as a potent natural antioxidant, oxygen-derived free radical scavenger and as membrane stabilizer [79]. CoQ10 can stimulate ATPase content and participates in ATP production [80]. In addition, it is able to inhibit mitochondrial ROS generation and inner mitochondrial depolarization [81]. Moreover, plasma membrane protection against oxidative stress is increased due to CoQ10 supplementation [82]. Based on the link between the rate of $A\beta$ production and oxidative stress, CoQ10 exerts its neuroprotective effect via reduction of oxidative stress [83]. The above mentioned functions of CoQ10 permit this coenzyme to exhibit an improvement in each of the biochemical markers as investigated in the present study. It can also protect from severe cholinergic neurons damage in the brain of rats [84]. In the light of what was mentioned; oxidative stress plays a pivotal role in AD [85], damages the neuronal membranes integrity through generation of free radicals [86], reduces the number of nerve cells in aging brain [87] while CoQ10 is a powerful antioxidant and has

free radical scavenging property, consequently CoQ10 can reverse the neurodegenerative damages which characterized AD [88]. It is worthy to note that CoQ10 is reduced in AD patients [32,33].

On the other hand, it is well noticed that CoQ10 which is an essential cofactor in the mitochondrial electron transport pathway and has a powerful antioxidant capacity, thus can reduce the oxidative stress, counteract the oxidative damage and protect from brain neuro degeneration caused by PM together with $AlCl_3$ as investigated in the present results. Moreover, the present results may be also explained based on the potential role of CoQ10 in the treatment of neurodegenerative damages associated AD [89]. It is also reported that pre-treatment with CoQ10 prevents $A\beta$ accumulation; it can reduce the influx of extracellular Ca^{2+} , and Ca^{2+} release from mitochondria due to opening the mitochondrial transition pore after β -amyloid uptake [90]. CoQ10 treatment can also decrease plaque area and number in the hippocampus as well as in the cortex [91] which is in agreement with the results of the histopathological examinations obtained in the current study. It is of great importance to note that, histological examinations of brains of NF and PM groups showed no histo pathological alteration in the hippocampus as well as in the cortex. These findings provide a great evidence of the marked improvement achieved with CoQ10 in NF and PM rats exposed to $AlCl_3$.

Results of the present investigation also showed that combination treatment with EGCG and CoQ10 during induction of AD in both NF and PM rats using $AlCl_3$ (70 mg/kg) model showed marked decrease in ACHE and MDA content as well as in $A\beta$ content together with marked increase in SOD and TAC content. The improvement effect of the combination treatment was more pronounced than that shown with either EGCG or CoQ10 alone. Histological examinations of the different brain regions in both NF and PM groups confirmed these improvement effects.

Therefore, the present study highlights that combination of EGCG and CoQ10 improved all biochemical and histological changes induced by $AlCl_3$ and provided neuro protective in both NF and PM rats, an effect that could be partially correlated with their antioxidant and/or anti-inflammatory properties. However, there is no published data regarding the effect of the combined treatments of EGCG with CoQ10 on AD either in NF or PM animals' models. There is no also published data concerning the effect of either EGCG or CoQ10 alone on AD in PM animals' models.

Consequently, it is clear that the severity of the brain neurological damage induced by $AlCl_3$ was more pronounced in PM rats. It is worthy to note also that the base line level of ACHE was lower in PM rats than NF rats indicating their presence at high-risk with increased chances of developing AD. However, the improvement induced by EGCG (as indicated by the histopathological alterations in different brain regions) was more marked in NF rats than in PM rats, while similar improvements were obtained by CoQ10 or its combination with EGCG in both NF and PM rats.

Conclusion

Protein malnutrition is a risk factor in developing AD. It increases the susceptibility and the severity of the brain neurological degeneration associated the pathogenesis of AD. EGCG and/or CoQ10 are effective in minimizing the hazards of aluminum- induced AD. The combination treatment has more pronounced effect in minimizing the hazards of aluminum- induced AD than either EGCG or CoQ10 alone in both NF and PM rats. It is worthy to mention that, marked improvement is obtained by CoQ10 more than EGCG concerning the

brain histopathological degenerations induced by aluminum in PM rats. However, further researches are needed to improve the quality of evidence relating to the association of AD with PM.

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