

Epigallocatechin-3-Gallate Protects against Aluminum-Induced Neurotoxicity in Rats under Social Isolation, Electric Shock and Inadequate Protein Malnutrition

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Abstract—Epigallocatechin-3-gallate (EGCG) is the most abundant catechin in green tea. This study aims to investigate the influence of EGCG on different stressful conditions as social isolation, Electric Shock (EC) and inadequate nutritional conditions as Protein Malnutrition (PM) on neurotoxicity induced by Al in rats. Rats were randomly categorized into 10 groups. One normal control received saline, and four Al toxicity groups injected daily for three weeks by $AlCl_3$ (70 mg/kg, IP). One of them served as Al toxicity model, two groups subjected to different stresses either by isolation as mild stressful condition or by Electric Shock (EC) as high stressful condition. The last group was maintained on 10% casein diet, a group received EGCG (10 mg/kg, IP) and other 4 groups treated with EGCG and different stress conditions employed in the study. Rats exposed to Al showed brain neurotoxicity and neuronal degenerations. Both mild (SI) and high (EC) stressful conditions as well as inadequate nutrition (PM) enhanced Al-induced neurotoxicity and brain neuronal degenerations. The enhancement induced by stresses especially in its higher conditions (ES) was more pronounced than that of inadequate nutritional conditions (PM) as indicated by the significant increase in $A\beta$, AChE, MDA, $TNF-\alpha$, $IL-1\beta$ together with the significant decrease in SOD, TAC, BDNF. EGCG showed more pronounced protection against hazards of Al in both stressful conditions (SI and EC) rather than in PM. The protective effects of EGCG were indicated by the significant decrease in $A\beta$, AChE, MDA, $TNF-\alpha$, $IL-1\beta$ together with the increase in SOD, TAC, BDNF and confirmed by brain histopathological examinations. EGCG can protect against Al-induced brain neuronal degenerations in all conditions. Consequently, administration of EGCG together with socialization as well as adequate protein nutrition is advised especially on excessive Al-exposure to avoid the severity of its neuronal toxicity.

Index Terms—aluminium, protein malnutrition, neuronal degeneration, epigallocatechin-3-gallate

I. INTRODUCTION

Aluminum (Al) is widely distributed in the environment. Hence, exposure to aluminium toxicity can be very common during daily life [1]. Besides, Al is considered a major constituent of many drugs including antacids, aspirins, some vaccines and injectable allergens [2]. Large amounts of Al inhaled or orally ingested leads to Al toxicity [3], as it can cross the blood brain barrier and consequently accumulates in mammalian tissues especially brain, bone, liver and kidney [4], [5]. The progressive neurodegeneration caused by Al intoxication results in severe memory loss and other cognitive deficits such as orientation, judgment, and reasoning [6]. Additionally, Neuropsychiatric symptoms such as apathy, depression, and agitation/aggression occur in the majority of persons with dementia over the course of the illness [7]. Earlier, it has been reported that Al can generate Reactive Oxygen Species (ROS) leading to neuronal degeneration. The mechanism of action of such toxicity involves oxidative damage to cellular lipids, proteins, and DNAs [8]. Furthermore, Al intoxication lowers the intracellular levels of reduced glutathione [9], thus alters the antioxidant defense status and membrane integrity [10], [11]. It also inhibits alkaline phosphatases and phosphodiesterase enzymes [12]. Moreover, Al induced oxidative stress might act to increase Tumor Necrosis Factor (TNF) production, a group of cytotoxic pro-inflammatory cytokines, which in turn stimulates $TNF-\alpha$ receptors of cell surface, leading to activation of the stress-related protein kinases and consequently, resulting in increased production of additional inflammatory cytokines [13]. Thus, the maintenance of redox homeostasis is supposed to be an effective way in order to prevent tissue damage as proposed in the present study.

Stress is a major risk factor in the onset of several neuropsychiatric disorders including anxiety and

depression. Several studies have shown that social isolation stress induces behavioural and brain molecular changes [14]-[17]. Social Isolation (SI) refers to the absence or insufficient contact with others [16] which represents the major source of mental or psychosocial stress and is associated with the increased prevalence of neurological diseases. It also exacerbates the risk of many neuropsychological disorders especially for elderly [15], [17]. Moreover SI causes depletion of brain glutathione content, an endogenous antioxidant, in association with the rise in lipid peroxidation and marked increase of nitric oxide production in the brains of socially isolated animals [18].

Electric Shock (EC) is another stressful condition that induces a pain-like state. Importantly, it impairs neurotransmitter levels leading to behavioural and physiological alterations in brain function [19].

Protein malnutrition is one of the most common health conditions worldwide. An insufficient protein diet may eventually result in behavioural deficits. Protein malnutrition has a critically long-lasting impact on the hippocampal formation and its role in learning and memory [20]. Several studies indicated that protein malnutrition can interfere with protein synthesis and structure and, hence, alter enzyme activity especially those related to neurotransmitter systems [21], [22]. Recently, severe protein malnutrition (PM) was found to increase the oxidative damage of lipids and proteins from the studied brain areas [23], [24].

Reports have already identified various polyphenols that are potentially antioxidant, anti-inflammatory and anti-amyloidogenic effects [25]. Of these compounds, the green tea flavonoid (-)-Epigallocatechin-3-Gallate (EGCG) which has been found to protect neuron-like cells against A β -mediated toxicity, oxidative stress and pro-inflammatory cytokines [26]. The role of EGCG could be explained through different pathways including gene expression activity and growth factor mediated pathways [27]. Furthermore, the antioxidant properties owing to its effective free radical scavenging activity [28].

Therefore, the present study investigates the neuroprotective action of EGCG in Aluminium Chloride (AlCl₃) induced neurotoxicity in rats by exploring the neurobehavioural, cognition, neuropathological changes in the hippocampus and cortex of rats in different stressful conditions including Social Isolation (SI), Electric Shock (ES) or Protein Malnutrition (PM). We believe that, so far, there are no reports on the use of EGCG to treat neurotoxicity in these stressful conditions.

II. MATERIALS AND METHODS

A. Drugs and Chemicals

Aluminum chloride (AlCl₃·6H₂O) was purchased as faint yellow powder from Sigma-Aldrich Chemical Co., St. Louis, MO, USA.

B. Animals

Adult male albino rats, weighing approximately 300-340 g at the beginning of the experiment, were obtained

from the Nile Co. for Pharmaceuticals and Chemical Industries, Cairo, Egypt. Animals arrived at least two months before use to reach the desired weight and were kept at controlled environmental conditions in terms of constant temperature (24 $\pm$ 1°C) and a 12/12 h light/dark and provided with their daily dietary requirements consisting of standard diet pellets (El-Nasr Chemical Co., Abu Zaabal, Cairo, Egypt) and water was given ad libitum. Rats were housed in stainless-steel cages, three to four per cage and kept at the animal house (at facility of Faculty of Pharmacy, Al-Azhar University "girls"). They were taken to test situation one hr before each experiment for adaptation after removing food and water from the home cages. Experiments were usually carried out at a fixed time around 9 am – 2 pm. Animal experiments followed the national institute of health guidelines for the care and use of laboratory animals (NIH publications No. 8023, revised 1978). The experimental protocol used in this study was approved by the Animal Ethics Committee of the Faculty of Pharmacy, Al-Azhar University, Egypt.

III. EXPERIMENTAL DESIGN

The rats were randomly categorized into 10 groups. One normal control received saline, and four Al toxicity groups injected daily for three weeks by AlCl₃ (50 mg/kg, IP [29]). One of them served as Al toxicity model, two groups subjected to different stresses either by isolation as mild stressful condition (SI-associated Al toxicity model [14]-[16], [30], [31] or by electric shock as high stressful condition (EC-associated Al toxicity model [32]). The last was maintained on 10% casein diet (PM-associated Al toxicity model reference [20]), a group received EGCG (10 mg/kg, IP [33]) and other 4 groups treated with EGCG and different stress conditions employed in the study. Biochemical changes in the brain in addition to histopathological changes in different brain regions were detected for all groups.

IV. BIOCHEMICAL ASSESSMENT

A. Determination of Beta-Amyloid (A β) Content

Determination of rat Beta-amyloid (A β) content was conducted using ELISA kit from Uscn Life Science, Inc., Product Number MBS702915. The assay was conducted following the manufacturer's instructions.

B. Assessment of Brain AChE Activity

Acetylcholinesterase activity was assessed using a kit obtained from Sigma-Aldrich Co., St Louis, MO, USA. Product Number MAK119. The assay was conducted following the manufacturer's instructions.

C. Brain Derived Neurotrophic Factor (BDNF)

It was assessed in brain tissue homogenate using ELISA Kit supplied by (MyBioSource, Inc., San Diego, USA, Product Number MBS494147). The assay was conducted following the manufacturer's instructions.

D. Determination of Oxidative Stress Markers (MDA, SOD and TAC)

1) Lipid peroxides expressed as malondialdehyde (MDA)

The content of MDA in brain tissue homogenate was carried out using commercially available test kit (cat No MD 25 28) supplied by Biodiagnostic, Giza, Egypt. The assay was conducted following the manufacturer's instructions.

2) Assessment of brain SOD enzyme activity

The activity of SOD in brain tissue homogenate was carried out using commercially available test kit (cat No SD 25 20) supplied by Biodiagnostic, Giza, Egypt. The assay was conducted following the manufacturer's instructions.

3) Assessment of brain total antioxidant capacity (TAC)

Total antioxidant capacity (TAC) kit is obtained from Biodiagnostic, Giza, Egypt. The assay was conducted following the manufacturer's instructions.

E. Brain Inflammatory Mediators (IL-1 β , TNF- α)

1) Determination of IL-1 β

IL-1 β assay employs the quantitative sandwich ELISA utilizing a polyclonal antibody specific for rat IL-1 β . Determination of IL-1 β was performed in brain tissue homogenate by using ELISA Kit supplied by RayBiotech, Inc., USA, Product Number (ELR-IL1b). The assay was conducted following the manufacturer's instructions.

2) Tumor necrosis factor alpha

Determination of TNF- α was done in brain tissue homogenate using ELISA Kit. The assay was conducted following the manufacturer's instructions.

F. Determination of 5-Hydroxytryptamine Content in Brain Tissue

To sample tubes 0.2 ml of supernatant was added to 0.2 ml of 0.2 N acetic acid, then 1.2 ml of OPT was added and shaken well. Blank was prepared by adding 0.2 ml of 0.2 N acetic acid instead of supernatant. The tubes were then placed in a boiling water bath for 10 min, and then cooled under tap water. The fluorescence was read at excitation 355 nm and emission at 470 nm using Hitachi (F3010 model) spectrophotofluorometer [34].

V. HISTOPATHOLOGICAL EXAMINATION OF DIFFERENT BRAIN REGIONS

Brain specimens were fixed in 10% formalin for 24 h then washed with tap water. For light microscopy, the specimens were prepared and stained according to the method described by [35]. Sections then were stained with Hematoxylin & Eosin stain for routine histological examination.

VI. STATISTICAL ANALYSIS

Data are expressed as the mean $\pm$ S.E.M. and statistical analysis was carried out by one way ANOVA followed by Tukey multiple comparisons test to calculate

significance of the difference between treatments. Values of $p < 0.05$ were considered significant. All statistical analyses were performed and graphs were sketched using GraphPad Prism (ISI, USA) software (version 5) computer program.

VII. RESULTS

EGCG restored the antioxidant parameters levels of MDA, SOD, TAC. MDA level in AlCl₃ treated group was significantly increased by six-folds in brain tissue homogenate as compared to normal healthy control group (Fig. 1). The elevation level was enhanced significantly by (23 %, 37% and 57%) with the influence of protein malnutrition, social isolation, and electric shock respectively as compared to AlCl₃ treated group. Protective effect markedly decreased MDA level following AlCl₃ treatment in addition to the different stressors employed in the study by (54%, 22%, and 28%) as compared to AlCl₃ treated group.

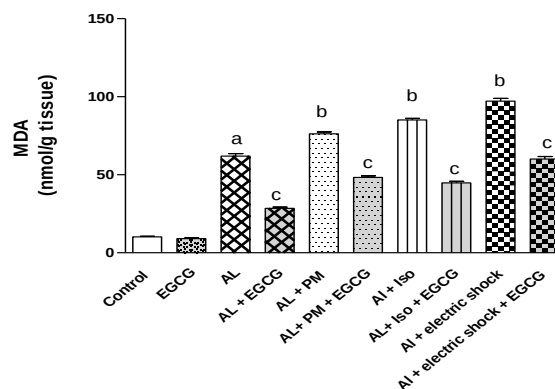


Figure 1. Effect of Al alone and in combination with protein malnutrition, different stressful conditions, and EGCG on brain MDA level in rats. Values were expressed as mean $\pm$ SEM a: significant difference from control at $p < 0.05$, b: significant difference from Al-treated group at $p < 0.05$, c: significant difference from EGCG at $p < 0.05$.

AlCl₃ showed (80%) significant decrease in the level of SOD level in the brain tissue homogenate of the treated rats group as compared with normal healthy control group (Fig. 2). The same significant decrease occurred with the groups under the effect of protein malnutrition, social isolation, and electric shock groups treated with AlCl₃ as compared with normal healthy control. Administration of the protective EGCG increased significantly the SOD expression level in comparison with AlCl₃ treated group.

TAC level was significantly decreased by (74%) in AlCl₃ treated rats group. The same pattern of decrease was also significant in the groups treated with AlCl₃ and accompanied with protein malnutrition, social isolation, and electric shock by (80-85%) as compared with control (Fig. 3). The elevation of TAC level was (100%) following the administration of EGCG as protective in the AlCl₃ treated rats group. The increase was shown as (42%, and 53%, and only 31%) in the groups protected by the administration of EGCG with protein malnutrition, social isolation, and electric shock respectively after

AlCl_3 administration, as compared with AlCl_3 treated group.

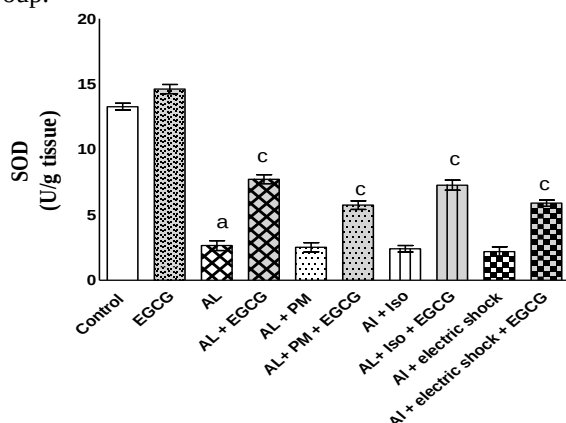


Figure 2. Effect of Al alone and in combination with protein malnutrition, different stressful conditions, and EGCG on brain SOD level in rats. Values were expressed as mean $\pm$ SEM a: significant difference from control at $p < 0.05$, b: significant difference from AL-treated group at $p < 0.05$, c: significant difference from EGCG at $p < 0.05$.

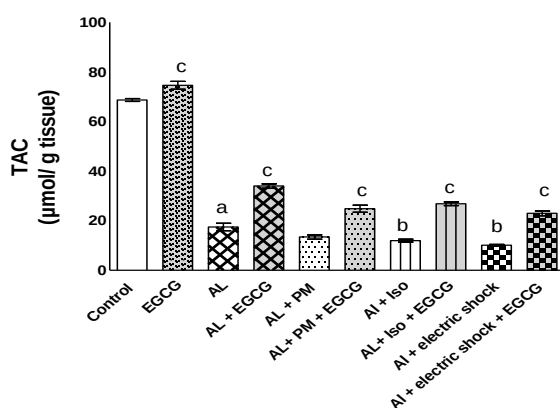


Figure 3. Effect of Al alone and in combination with protein malnutrition, different stressful conditions, and EGCG on brain TAC level in rats. Values were expressed as mean $\pm$ SEM a: significant difference from control at $p < 0.05$, b: significant difference from AL-treated group at $p < 0.05$, c: significant difference from EGCG at $p < 0.05$.

EGCG reduced the elevated levels of the inflammatory biomarkers $\text{TNF-}\alpha$ & $\text{IL-1}\beta$. As shown in Fig. 4, AlCl_3 induced oxidative stress aggravated neuro-inflammatory effects that was indicated as significant elevation in $\text{TNF-}\alpha$ level by about three folds as compared to normal healthy control group. On one hand, the elevation in $\text{TNF-}\alpha$ was enhanced by AlCl_3 in combination with protein malnutrition, social isolation, and electric shock stressors. On the other hand, rats showed a significant decrease (28%) following the administration of EGCG in the groups suffering from intoxication with AlCl_3 together with protein malnutrition, social isolation, and electric shock (15%, 18%, and 10%) respectively as compared to AlCl_3 model.

The inflammatory mediator $\text{IL-1}\beta$ is considered as a marker for AlCl_3 induced oxidative stress in rat's brain neurons. In the current study, the authors were able to show that AlCl_3 administration significantly increased the $\text{IL-1}\beta$ expression level by three folds as compared to

normal rats (Fig. 5). The increase in $\text{IL-1}\beta$ level was slightly enhanced by the influence of protein malnutrition, social isolation, and electric shock. EGCG administration was able to restore these values back compared to the control group.

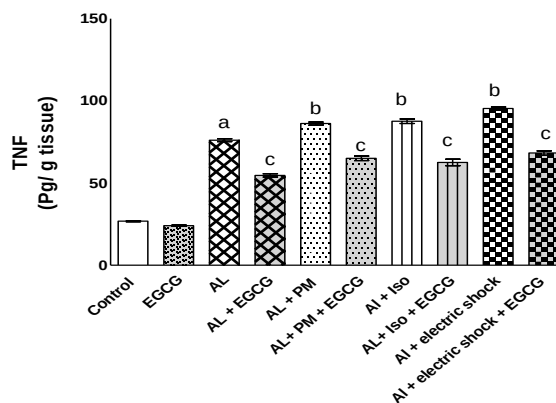


Figure 4. Effect of Al alone and in combination with protein malnutrition, different stressful conditions, and EGCG on brain $\text{TNF-}\alpha$ level in rats. Values were expressed as mean $\pm$ SEM a: significant difference from control at $p < 0.05$, b: significant difference from AL-treated group at $p < 0.05$, c: significant difference from EGCG at $p < 0.05$.

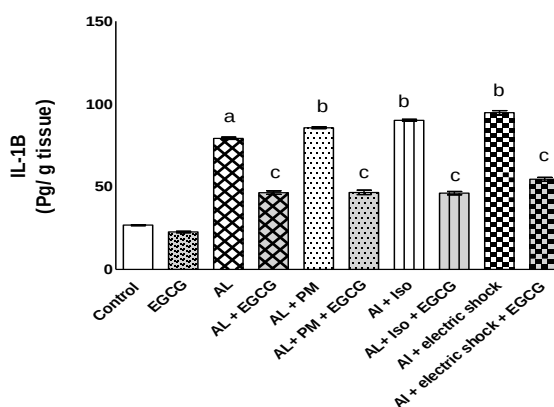


Figure 5. Effect of Al alone and in combination with protein malnutrition, different stressful conditions, and EGCG on brain $\text{IL-1}\beta$ level in rats. Values were expressed as mean $\pm$ SEM a: significant difference from control at $p < 0.05$, b: significant difference from AL-treated group at $p < 0.05$, c: significant difference from EGCG at $p < 0.05$.

EGCG restored the levels of biochemical markers in the brain (AChE, $\text{A}\beta$, and BDNF). AlCl_3 administration showed a marked elevation in AChE level (three folds) as compared to healthy normal control group (Fig. 6). The neurodegeneration was even more elevated in the groups suffering protein malnutrition, social isolation, and electric shock (11%, 31%, and 38%) respectively. By contrast, EGCG administration succeeded to reduce significantly AChE elevated level (32 %). Following EGCG administration in the groups suffering from protein malnutrition, social isolation, and electric shock, the AChE level was significantly reduced (18%, 19%, and only 9%) respectively as compared to AlCl_3 group.

As shown in Fig. 7, the induction of neurotoxicity by AlCl_3 showed a marked elevation in $\text{A}\beta$ level in rats brain tissue (5 folds) as compared to normal healthy control

group. A β level has been increased significantly by combining AlCl₃ with protein malnutrition, social isolation stressors by (6 folds). The electric shock combination exaggerated the increase by (8 folds) as compared to normal healthy control group. Administration of EGCG alone produced a marked reduction (44%) as compared to AlCl₃ group. EGCG reduced the A β level in the groups suffering from Protein malnutrition, and social isolation. EGCG also reduced the A β level in the group suffering from the Electric shock (24%) as compared to AlCl₃ group.

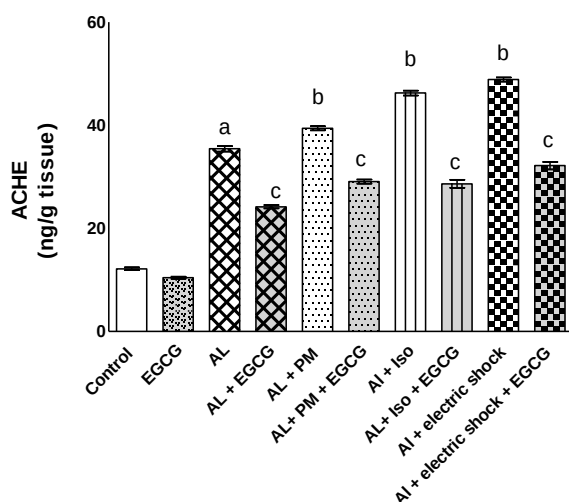


Figure 6. Effect of Al alone and in combination with protein malnutrition, different stressful conditions, and EGCG on brain AChE level in rats. Values were expressed as mean $\pm$ SEM a: significant difference from control at $p < 0.05$, b: significant difference from Al-treated group at $p < 0.05$, c: significant difference from EGCG at $p < 0.05$.

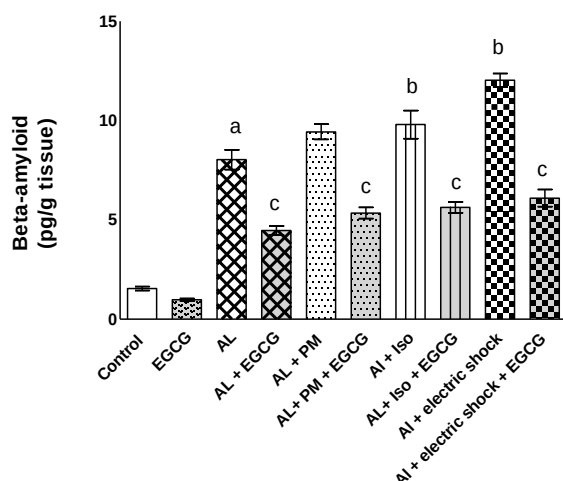


Figure 7. Effect of Al alone and in combination with protein malnutrition, different stressful conditions, and EGCG on brain A β level in rats. Values were expressed as mean $\pm$ SEM a: significant difference from control at $p < 0.05$, b: significant difference from Al-treated group at $p < 0.05$, c: significant difference from EGCG at $p < 0.05$.

BDNF quantification was illustrated in Fig. 8. AlCl₃ resulted in a significant decrease (37%) as compared to normal healthy control group. Co-existence of protein malnutrition, social isolation, and electric shock during AlCl₃ administration augmented the level decrease (45%,

48%, and 50%) respectively. EGCG elevated BDNF expression level significantly (25%), in the groups under different stressors.

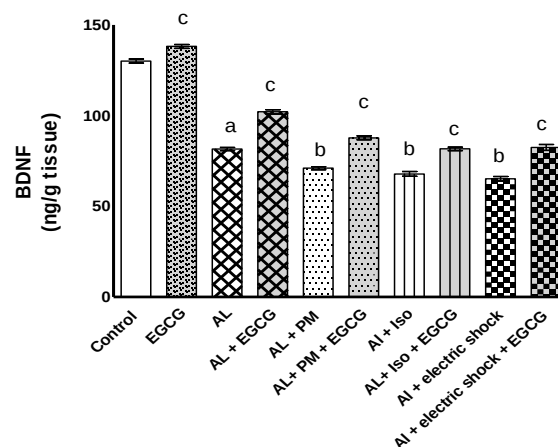


Figure 8. Effect of Al alone and in combination with protein malnutrition, different stressful conditions, and EGCG on brain BDNF level in rats. Values were expressed as mean $\pm$ SEM a: significant difference from control at $p < 0.05$, b: significant difference from AL-treated group at $p < 0.05$, c: significant difference from EGCG at $p < 0.05$.

There was no histopathological alteration in the cerebral cortex, hippocampus, striatum and substantia nigra (Fig. 9a, b, c & d). EGCG: There was no histopathological alteration in the cerebral cortex, hippocampus, striatum and substantia nigra (Fig. 9a, b, c & d). AL: There was no histopathological alteration in the cerebral cortex as well as the hippocampus (Fig. 9e & f). Focal plaques formation as well as intracellular neuronal oedema was detected in striatum (Fig. 9g). Intracellular oedema was observed also in the neurons of substantia nigra (Fig. 9h). AL+EGCG: There were no histopathological findings in the meninges, cerebral cortex and hippocampus (Fig. 9i & j). AL+PM: Nuclear necrosis and degeneration were observed in the neurons of cerebral cortex (Fig. 9k), associated with focal gliosis (Fig. 9l). The pyramidal cells of the hippocampus as well as the neurons of the fascia dentate, striatum and substantia nigra showed nuclear pyknosis and degeneration with congestion in the blood vessels (Fig. 9m, n, o & p). AL+PM+EGCG: There was congestion in the cortical blood vessels (Fig. 9q), but the hippocampus showed nuclear pyknosis in the neurons (Fig. 9r). AL+SI: The cerebral cortex and the subiculum of the hippocampus showed normal histological structure (Fig. 9s & t). There were nuclear pyknosis and degeneration in the neurons of the fascia dentate in the hippocampus (Fig. 9u) and focal plaques formation in the striatum (Fig. 9v). The substantia nigra showed atrophy in the neuronal cells (Fig. 9w). AL+SI+EGCG: There was no histopathological finding in the meninges and cerebral cortex (Fig. 9x), but the hippocampus showed nuclear pyknosis in some neurons (Fig. 9y). AL+ES: There were nuclear pyknosis and degeneration in the neurons of the cerebral cortex (Fig. 9z). The hippocampus and striatum

showed normal histological structure (Fig. 9aa, bb & cc). Atrophy was observed in some neurons of the substantia nigra (Fig. 9dd). Al+ES+EGCG: There was no histopathological alteration in both cerebral cortex as well as hippocampus (Fig. 9ee & ff).

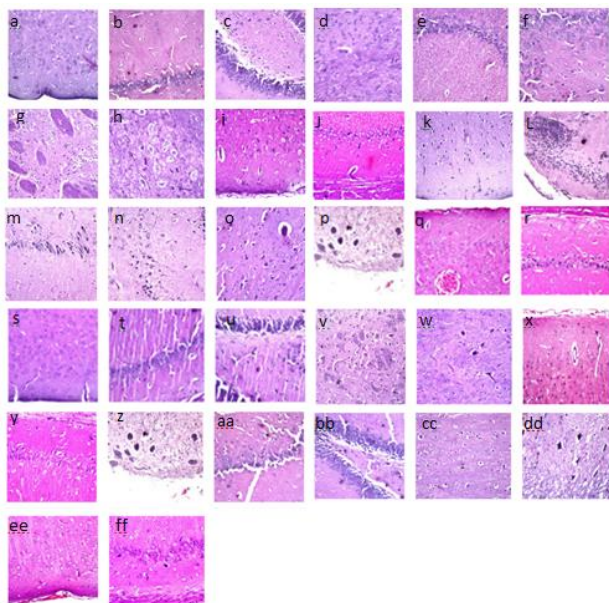


Figure 9. Control group (a, b, c & d). Al: (e, f, g). Al+EGCG group (i & j). Al+PMgroup (k, l, m, n, o & p). Al+PM+EGCG: There was congestion in the cortical blood vessels (q), but the hippocampus showed nuclear pyknosis in the neurons (r). Al+SI group (s & t, w). Al+SI+EGCG group (x, y). Al+ES: (z, aa, bb & cc, dd). Al+ES+EGCG group (ee&ff).

VIII. DISCUSSION

Stressors including protein malnutrition, social isolation and electric shock have been documented to lead to different neuropsychiatric disorders. Electrical injury may cause Central Nervous System (CNS) complications and peripheral nerve disorders. It is reported previously that environmental insults including electrical injury can lead to a pathological process activating a molecular cascade leading to progressive loss of motor neurons [36]. Protein malnutrition is considered a major form of malnutrition worldwide. It was shown previously that protein restriction affects the biochemical, behavioural, and electrophysiological consequences of individuals. Hence, decreases cognitive abilities. Furthermore, social stressors such as social isolation can enhance inflammatory response genes to protect against physical vulnerability [37], [38].

Increased oxidative stress is implicated in the pathogenesis of Alzheimer's Disease (AD). It has been suggested that increased reactive oxygen species occur prior to amyloid- β (A β) deposition which is a peptide driven from the proteolytic processing of the amyloid precursor protein. Hence, it has been previously proposed that antioxidant strategies can be employed in the treatment of AD [39].

Aluminum is a proinflammatory, trivalent metal neurotoxin that has been implicated in the onset of neurodegeneration in AD. Aluminum accumulation

within the Central Nervous System (CNS) induces irreversible brain cell damage and functional decline resulting in deficits in cognition and behavior [1], [2]. Aluminium seems to be responsible for Reactive Oxygen Species (ROS) mediated toxicity. At physiological concentrations, aluminium is capable of contributing to an aluminium-driven neuropathology that is relevant to AD [40]. Aluminium has an easy access to human body through various routes, among which are the use of aluminium utensils, food additives, drugs and deodorants. So far, there is no effective cure for AD, but only symptomatic relief for the disease with no effect on the disease progression. No safe and effective synthetic drug has been reported for cure of the AD. Hence, alternative strategies have been employed in the treatment of AD symptoms. Among these are natural compounds. EGCG, a polyphenol catechin which is well known for its antioxidant/ROS scavenging effects, is a good candidate for the treatment of AD as a radical scavenger. EGCG has also previously been reported to have modulatory effects on synaptic transmission [26].

EGCG decreased significantly the expression levels of the inflammation biomarkers TNF- α and IL-1 β . Inflammation is a hallmark of AD pathogenesis. It was reported in a previous study that inhibition of tumor necrosis factor TNF- α reduces the overall risk of Alzheimer's Disease (AD) [41], [42]. In line with this study, AlCl₃ together with different other stresses employed in the current study induced oxidative stress aggravated neuroinflammatory effects that was shown as significant elevation in TNF- α expression level by about three folds as compared to healthy normal control group. These elevations were shown to decrease significantly following the administration of EGCG.

IL-1 β is a pro-inflammatory cytokine. It has previously been shown that IL-1 β overexpression is associated with the formation of plaques [43], [44]. The inflammatory mediator IL-1 β as marker of AlCl₃ induced oxidative stress in rat's brain neurons. Its expression level was significantly increased as compared to normal rats. The increase in IL-1 β level was slightly enhanced by the influence of protein malnutrition, social isolation, and electric shock. EGCG administration decreased significantly IL-1 β expression level.

Effect of EGCG on different antioxidant enzyme activities. The superoxide radical poses danger to living cells very similar to the negative effects of hydrogen peroxide. SOD converts superoxide radicals to hydrogen peroxide and oxygen. AlCl₃ treatment significantly decreased the SOD activity leading to the increase of the superoxide ions and consequently inactivated glutathione peroxidase and increases the hydrogen peroxide levels in the body. The present study has shown a significant decrease of SOD in the group of rats treated with AlCl₃. Decreased SOD levels were markedly reversed by EGCG. As ROS possess a short half-life, it is really difficult to assess their levels in a direct manner. Therefore, instead, we estimated the byproduct of the damage they cause. One of such byproducts is Thiobarbituric Acid Reactive

Substances (TBARS) which is a by-product of lipid peroxidation. The polyunsaturated fatty acids in cell membranes are liable for oxidation in the presence of ROS. Assay of TBARS measures malondialdehyde (MDA) present in the sample. Increased TBARS levels were markedly reversed by EGCG in treated rats.

Free radical disturbing effects of $AlCl_3$ were also observed in the current study as decrease in Total Antioxidant Capacity (TAC) which was also reversed following EGCG administration.

EGCG reversed the decrease in the level of BDNF in treated rats. Brain Derived Neurotrophic Factor (BDNF) has been identified as a main molecule involved in plastic changes related to learning and memory. Hence, disruption of BDNF biosynthesis has been implicated in the pathogenesis of AD [45]. It has been shown previously that pathological aging and also psychiatric diseases are associated with different variations in BDNF expression [46]. In line with this finding, $AlCl_3$ resulted in a significant decrease in the level of BDNF as compared to normal healthy control group. Co-existence of several types of stresses such as protein malnutrition, social isolation, and electric shock during $AlCl_3$ administration augmented the decrease in the expression level. EGCG restored BDNF restoration significantly.

EGCG reversed the decrease in the level of acetylcholine esterase in treated rats. Acetylcholinesterase is the main enzyme responsible for the hydrolysis of the acetylcholine which results in termination of the transmission of the nerve impulse. Termination of the nerve impulse is usually associated with AD [47]. It was shown previously that high levels of AChE do exist in the brain of AD patients which results in a decrease in acetylcholine level in the brain of AD patients [48], [49]. The increase in activity of AChE by aluminium can be attributed to the allosteric interaction between aluminium chloride as a cation and anionic site of acetylcholinesterase enzyme leading to the alteration in the secondary structure of AChE in the brain. $AlCl_3$ together with protein malnutrition, social isolation, and electric shock administration showed a marked elevation in AChE level three folds as compared to normal healthy control group. EGCG administration was able to reduce significantly AChE elevated level.

All these findings may be associated to the anti-oxidant, anti-inflammatory capacity of this drug type.

EGCG reversed the decrease in the level of $A\beta$ in treated rats. One of the major neuropathological signs of AD pathogenesis is a senile plaque which is formed by the conversion of the $A\beta$ peptide to amyloid in the brain [50], [51]. As shown in Fig. 7, induction of neurotoxicity by $AlCl_3$ showed a marked elevation in $A\beta$ level in rats brain tissue by (5 folds) as compared to normal control group. $A\beta$ level has been increased significantly by the combination of $AlCl_3$ with protein malnutrition, social isolation by (6 folds). While the electric shock combination exaggerated the increase by (8 folds) as compared to normal control group. Administration of

EGCG alone produced a marked reduction (44%) as compared to $AlCl_3$ group. Protein malnutrition, and social isolation combination with EGCG also reduced the $A\beta$ level (33%, and 30%) respectively. Electric shock combination reduced the level only (24%) as compared to $AlCl_3$ group.

IX. CONCLUSION

This study aims to investigate the influence of different stressful conditions as social isolation, Electric Shock (EC) and inadequate nutritional conditions as Protein Malnutrition (PM) on neurotoxicity induced by Al in rats. The study aims also to investigate the possible protective effect of EGCG in these stressful conditions. three weeks by $AlCl_3$ (70 mg/kg, IP).

Rats exposed to Al for three weeks showed brain neurotoxicity and neuronal degenerations. Both mild (SI) and high (EC) stressful conditions as well as inadequate nutrition (PM) enhanced Al-induced neurotoxicity and brain neuronal degenerations. The enhancement induced by stresses especially in its higher conditions (ES) was more pronounced than that of inadequate nutritional conditions (PM) as indicated by the significant increase in $A\beta$, AChE, MDA, $TNF-\alpha$, $IL-1\beta$ together with the significant decrease in SOD, TAC, BDNF. On the other hand, EGCG showed more pronounced protection against hazards of Al in both stressful conditions (SI and EC) rather than in PM.

CONFLICT OF INTEREST

The authors declare have no conflict of interest.

AUTHOR CONTRIBUTIONS

Azza A. Ali was responsible for the study design; Ahmed Wahid was responsible for study design and manuscript writing; The Mona G Khalil and Asmaa A. Mohamed conducted the practical experimental work; Mona M. Kamal made a statistical analysis of the experiment and Karema Abu-Elfotuh Contributed to the practical experimental work and study design.

ETHICS APPROVAL

Animal experiments followed the national institute of health guidelines for the care and use of laboratory animals (NIH publications No. 8023, revised 1978). The experimental protocol used in this study was approved by the Animal Ethics Committee of the Faculty of Pharmacy, Al-Azhar University, Egypt.

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