

Effect of green tea and omega-3 on aluminum chloride neurotoxicity in rabbits: histological and immunohistochemical study

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Background/aim

Aluminum chloride is one of the most common causative factors in many neurodegenerative disorders. The present study aimed to evaluate the effect of green tea and omega-3 on aluminum chloride-induced neurotoxicity in rabbits.

Materials and methods

Twenty-four male rabbits were divided into four groups: control group, aluminum chloride group, in which rabbits were administered aluminum chloride at a dose of 300 mg/kg/day orally for one month, green tea-treated group, in which rabbits were given aluminum chloride plus green tea extract at a dose of 50 mg/kg/day orally for one month, and the omega-3-treated group, in which rabbits were administered aluminum chloride plus omega-3 at a dose of 20 mg/kg/day orally for one month. Then, the brain tissues were examined for histopathological and immunohistochemical changes.

Results

Aluminum chloride-induced distortion in histological structures of the dentate gyrus with a significant increase in caspase-3 antibody reaction compared with the control group, indicating neuronal cell apoptosis. Green tea and omega-3 reduced the neurotoxin effect of aluminum chloride. However, administration of omega-3 led to more improvement in aluminum chloride neurotoxicity than green tea.

Conclusion

Omega-3 administration diminishes the neurotoxin effect of aluminum chloride on the dentate gyrus more than green tea.

Keywords:

aluminum chloride, dentate gyrus, green tea, omega-3

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Introduction

Aluminum compound is usually used for industrial purposes such as in water treatment, pharmaceuticals, food additive colors and papermaking, and used with other metals in food packaging and cookware [1]. Aluminum is considered one of the environmental pathogens that leads to neurodegenerative disorders [2]. The brain is a prospective target for aluminum toxicity [3]. Aluminum can simply enter the blood–brain barrier because of its high affinity to the receptors of transferrin [4].

Aluminum causes histopathological changes in the hippocampus structures and is considered a strong neurotoxin that can induce Alzheimer's disease [5]. Exposure to aluminum could cause cognitive dysfunction, learning and memory deficits and also neurodegenerative diseases [6]; it may also cause neurobehavioral, neuropathological, and neurochemical changes in the brain [7].

Green tea contains several chemical compounds, including polyphenols as catechins, caffeine, and

theanine which have neuroprotective effects [8] the green tea have anti-inflammatory, anti-apoptotic properties and prevents the amyloid-beta accumulation [9]. The polyphenols in green tea are able to deactivate free radicals and can eliminate the damage caused by reactive oxygen species [10]. A large number of studies show that green tea consumption improves cognitive functions and inhibits memory deficits in humans and in various animal models of neurodegenerative disorders [11].

Omega-3 is a polyunsaturated fatty acid that is essential for brain development and performance, especially in the hippocampus [12]. Omega-3 is an essential type of fatty acids and plays an important role in neurological function because it is an essential membrane component and energy substrate [13].

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Omega-3 plays a role in regulating the immune and oxidative stress responses in the brain tissue due to its ability to neutralize the proinflammatory cytokines and production of antioxidant molecules [14]. In turn, this can contribute to continuous cell proliferation and neurogenesis [15]; dietary consumption of omega-3 fatty acids can prevent cognitive deterioration and decrease the physiological disturbances of the brain that are related to aging or neurological disorders, such as Alzheimer's disease and Parkinson's disease [16].

The hippocampus performs many higher brain functions, such as memory and learning. The dentate gyrus is the input area of the hippocampus that plays a major role in these procedures. Granule cells of the dentate gyrus receive excitatory input neurons that are from the entorhinal cortex and send output excitatory neurons to the Cornu Ammonis 3 CA3 region of the hippocampus [17]. The dentate gyrus is responsible for the formation of new memories and one of the brain areas where neurogenesis occurs [18].

This experimental study was designed to evaluate the effect of green tea and omega-3 on aluminum chloride-induced neurotoxicity on the dentate gyrus in rabbits.

Materials and methods

Chemicals

Aluminum chloride

Aluminum chloride was purchased as a powder from Sigma Aldrich Company, Cairo, Egypt (CAS No. 7446-70-0), and dissolved in distilled water.

Green tea

Green tea was obtained as a tablet containing 300 mg dry extract from the Arab Company for Pharmaceuticals and Medicinal plants (MEPACO-MEDIFOOD, Egypt). Tablets were crushed and dissolved in distilled water.

Omega-3 oil

Omega-3 oil was obtained as soft gelatin capsules containing 1000 mg of omega-3 fish oil from SEDICO Pharmaceutical Company, Egypt; the contents of the capsule were aspirated by a syringe.

Animals and ethical consideration

This experimental study was carried out on 24 male New Zealand white rabbits weighing 1–1.5 kg. These rabbits were kept at controlled temperatures of 25°C and had free access to water and diet for 10 days before the initiation of the experiment. The procedures were reviewed and approved by the Research Ethical

Committee of Animal Care of Faculty of Medicine, Benha University, with approval number Rc/6-2-2021.

Experimental design

Twenty-four rabbits were divided into four groups, each with six rabbits, as follows:

- (1) Group I (control group): rabbits were given a standard diet.
- (2) Group II (aluminum chloride group): rabbits were given aluminum chloride powder dissolved in distilled water orally at a dose of 300 mg/kg/day for one month [19].
- (3) Group III (green tea-treated group): rabbits were given aluminum chloride powder with the same dose as group II plus green tea extract at an oral dose of 50 mg/kg/day for one month [20].
- (4) Group IV (omega-3-treated group): rabbits were given aluminum chloride powder with the same dose as group II plus omega-3 at an oral dose of 20 mg mg/kg/day for one month [21].

The animals were sacrificed by ether inhalation; then, craniotomy and laminectomy were performed within 30 min after death. After that, the brains were collected carefully and washed with normal saline. Then, brain tissues prepared for histopathological study.

Histopathology study

Hematoxylin and eosin staining

The brain hemispheres were fixed in 10% formalin, and then dehydrated, cleaned with xylene and embedded in paraffin. Coronal serial sections of the temporal lobe blocks were cut into 5 µm thickness and stained with hematoxylin and eosin [22].

Cresyl violet staining

Sections were stained in a 0.1% cresyl violet solution (dissolved in 0.01% glacial acetic acid) at 37°C for 10 min, and quickly rinsed in tap water to remove excess stain and then in 95% ethyl alcohol for 30 s. Cresyl violet used to stain the ribosomal RNA (rough endoplasmic reticulum) in Nissl substance of neurons that appeared dark blue [23].

Immunohistochemical staining for caspase-3

Caspase-3 antibodies were used to detect apoptosis, which immune-histochemically appeared as yellow to brown cytoplasmic discoloration. Using the avidin-biotin peroxidase method, paraffin sections at a thickness of 4 µm were incubated with a rabbit monoclonal antibody for immunoglobulin G against caspase-3 [24]. Morphometric study was carried out to determine the area% of caspase-3 immunostaining

using the image analysis Leica Q 500 MC (Chicago, USA) image processing program.

Statistical analysis

The collected data were tabulated and analyzed statistically using SPSS software Version 19 (Chicago, USA). The data were presented as the mean $\pm$ SD using the one-way ANOVA test.

Results

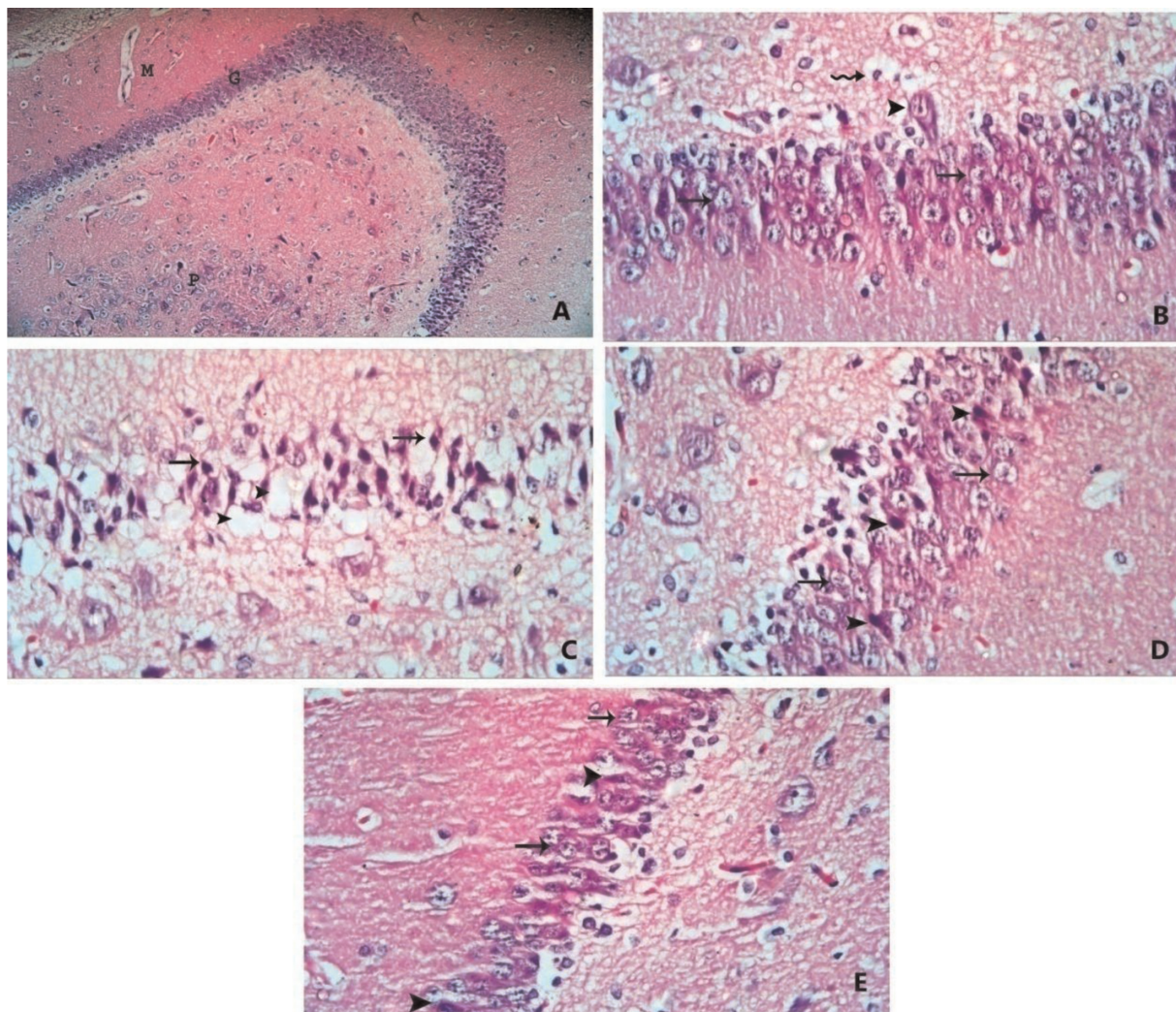
Histopathological examination

Hematoxylin and eosin staining

Hematoxylin and eosin-stained coronal section in the temporal lobe from the control group showed the

dentate gyrus with three layers: the molecular layer, the granular layer, and the polymorphic layer (Fig. 1a). The granular layer showed granular cells with vesicular nuclei and the polymorphic layer contained pyramidal cells and interneuron (Fig. 1b). In group II (aluminum chloride group), distortion of the granular cell layer was observed, numerous cells showed apoptotic shrinkage with chromatin condensation and there was considerable degenerative vacuolization (Fig. 1c). In group III (green tea-treated group), there were normal granular cells with a vesicular nucleus and some other apoptotic shrinkage cells with chromatin condensation (Fig. 1d). Moreover, in group IV (omega-3-treated group), there were normal granular cells with a vesicular nucleus and

Figure 1



Photomicrograph of sections in the temporal lobe from (a) the control group shows that the dentate gyrus consisted of the molecular layer (M), the granular layer (G) and the polymorphic layer (P) (H&E, $\times 100$). (b) The granular layer of the dentate gyrus shows granular cells with vesicular nuclei (arrows); the polymorphic layer contained pyramidal cells (arrow head) and interneuron (wavy arrow) (H&E, $\times 400$). (c) The aluminum chloride group shows distortion of the granular layer with numerous apoptotic shrinkage cells with chromatin condensation (arrows) and considerable degenerative vacuolization (arrow heads) (H&E, $\times 400$). (d) The green tea-treated group shows normal granular cells with a vesicular nucleus (arrows) and some apoptotic shrinkage cells with chromatin condensation (arrows head) (H&E, $\times 400$). (e) The omega-3-treated group shows normal granular cells with a vesicular nucleus (arrows) and a few apoptotic shrinkage cells with chromatin condensation (arrows heads) (H&E, $\times 400$). H&E, hematoxylin and eosin.

few apoptotic shrinkage cells with chromatin condensation (Fig. 1e).

Cresyl violet staining

Cresyl violet stain section from the control group showed granular cells with Nissl granules around the nucleus (Fig. 2a). However, in group II, the granular layer showed numerous apoptotic cells (Fig. 2b), while in group III, the granular layer showed normal granular cells with Nissl granules around the nucleus and some apoptotic cells (Fig. 2c). In addition, the granular layer of group IV showed normal granular cells associated with Nissl granules around the nuclei (Fig. 2d).

Immunohistochemical staining for caspase-3

Examinations of the caspase-3 antibody immunostained section from the control group showed a slight positive reaction (Fig. 3a), while a marked positive caspase-3 reaction was observed in group II (Fig. 3b). However, there was a moderate positive caspase-3 reaction in group III (Fig. 3c) and a mild positive caspase-3 reaction in group IV (Fig. 3d).

Morphometric results

The mean area % of caspase-3 expression for all groups is shown in Table 1. There was a significant decrease ($P \leq 0.05$) in caspase-3 expression in groups III and IV

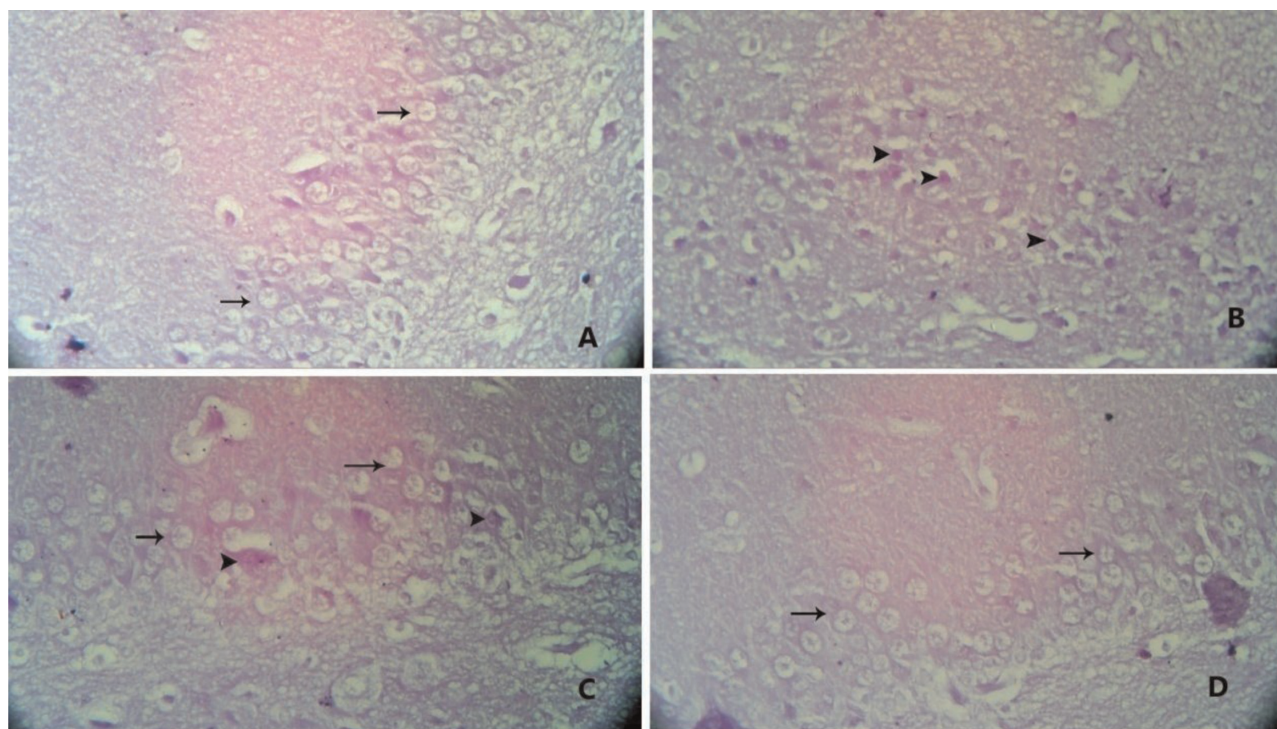
compared with group II and a decrease in group IV than group III.

Discussion

Aluminum chloride can pass through the blood–brain barrier and accumulate in the brain, especially in certain cortical regions such as the hippocampus gyrus. Chronic exposure to the trace element of aluminum chloride exerts a toxic effect on the lung, skeletal muscles, and nervous tissue [25]. Aluminum chloride has been shown to exert numerous undesirable behavioral effects, anxiety, and neurodegenerative histological effects on brain tissue of rats [26].

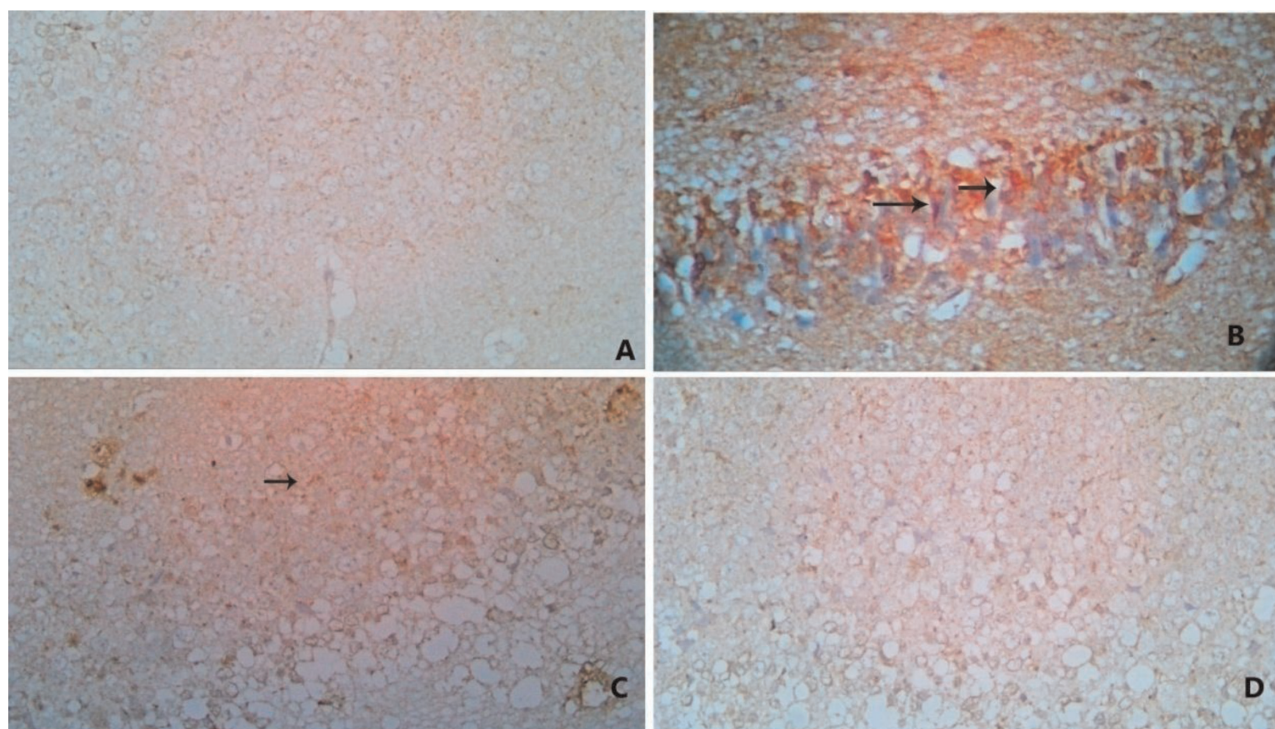
Many previous studies have reported on the effective role of green tea use in neurodegenerative disorders in humans and animals. Green tea improved beta amyloid-impaired memory and decreased oxidative damage of the brain in animals exposed to aluminum chloride [27]. Omega-3 is considered one of the phospholipid components of glial and neuronal membranes and plays an important role in its remodeling and synthesis [28]. This study was designed to evaluate the role of green tea and omega-3 in eliminating aluminum chloride neurotoxicity.

Figure 2



Photomicrograph of sections in the dentate gyrus (a) control group shows normal granular cells with Nissl granules around the nucleus (arrows), (b) the aluminum chloride group shows numerous apoptotic cells (arrow heads), (c) the green tea-treated group shows normal granular cells with Nissl granules around the nucleus (arrow) and apoptotic cells (arrows head) and (d) the omega-3-treated group shows normal granular cells with Nissl granules around the nucleus (arrow) (cresyl violet, $\times 400$).

Figure 3



Photomicrograph of sections in the dentate gyrus from (a) the control group shows a slight positive caspase-3 antibody reaction, (b) the aluminum chloride group shows marked positive caspase-3 antibody reaction (arrow), (c) the green tea-treated group shows moderate positive caspase-3 antibody reaction (arrow) and (d) the omega-3-treated group shows a mild positive caspase-3 antibody reaction (caspase immunostaining, $\times 400$).

Table 1 Mean values of area percent caspase-3 immunoreactivity $\pm$ SD in all groups

Groups	Group I	Group II	Group III	Group IV
Mean $\pm$ SD	3.42 $\pm$ 3.15	40.25 $\pm$ 6.8	18.45 $\pm$ 6.75	6.17 $\pm$ 2.47
Significant $P \leq 0.05$	With group II and III	With group I, III, and IV	With group I and II	With group II

In the present study, the administration of aluminum chloride induced distortion of the granular cell layer of the dentate gyrus with numerous apoptotic cells in cresyl violet-stained sections. Confirmed by a significant increase in caspase-3 antibody reactions compared with that in the control group, these findings indicated the neurodegenerative effect of aluminum chloride in the dentate gyrus. Similarly, Kamel and Mostafa [29] studied the effect of aluminum chloride on the hippocampus in adult rats and found a significant decrease in the number of normal pyramidal cells in the pyramidal layer of the hippocampus, with a significant increase in the apoptotic pyramidal cells. Also, Gazia [19] revealed that aluminum chloride-induced apoptotic features in the ultrastructure of the dentate gyrus neurons in the form of heterochromatic nuclei and dilated cisterna of rough endoplasmic reticulum in their cytoplasm.

A recent study concluded that aluminum chloride reduced memory, learning and locomotion activity as

there was elevation in Malondialdehyde level and decreased in Glutathione (GSH), superoxide dismutase, catalase, and Glutathione peroxidase in the cortex and hippocampus of aluminum chloride animals than the control animals [30].

Other study evaluated the effects of aluminum on long-term memory in rats and the study revealed that, with increasing dose of aluminum chloride, there were marked changes in the neuronal ultrastructure of the hippocampus [31]

Similarly, Auti and Kulkarni [32] found that aluminum chloride-induced multifocal neuronal degeneration with pyknotic nuclei in the hippocampus and cortex compared with the control group. Aluminum chloride-induced toxicity in the brain tissue and the Morris water maze test revealed impairment in spatial memory in rats. Other authors attributed the neurotoxic effects of aluminum chloride to its contribution toward free radical-mediated cellular injury, through inducing

oxidative stress and impairment in the defense system with alteration of the neurochemistry of brain tissue [4]. Also, Liaquat *et al.* [33] detected a significant increase in brain lipid peroxidation and acetylcholine-esterase activity in the aluminum chloride group than the control group.

Caffeine in green tea could inhibit the adenosine that increases dopamine and norepinephrine. Therefore, green tea could improve brain function as well as mood and memory [34]. Also, green tea contains l-theanine, which is an amino acid that can cross the blood-brain barrier [35]

In this study rabbits, treated with green tea showed a mild improvement in the histopathological changes induced by aluminum chloride as there were normal granular cells with a vesicular nucleus and some other apoptotic shrinkage cells with chromatin condensation, with a moderate positive caspase-3 antibody reaction confirmed by a significant decrease in caspase-3 antibody reaction compared with that in the aluminum chloride group. This result is in agreement with the results of Jelenković *et al.* [27], who studied the influence of green tea leaf extract on neurotoxicity of aluminum chloride in rats and concluded that green tea leaf extract decreased aluminum chloride neurotoxicity through its antioxidant activity and improved the function of mitochondria in brain tissue.

A previous study on the neuroregenerative role of green tea found that green tea is a major antioxidant and acts as a modulator in intracellular metabolism and neuronal signaling [11].

Other authors revealed that intrahippocampal application of green tea before administration of aluminum chloride induced a significant increase in the GSH content and a significant reduction of superoxide anions compared to that in the aluminum chloride group [36].

The protective effect of green tea extract was mostly recognized by its antioxidant and antiapoptotic activity, its beneficial role in modulating inflammatory responses and its ability to increase toxin metabolism and neutralize phospholipase A2, hyaluronidase and proteases enzymes [37]. However, omega-3 contained docosa-hexaenoic acid (DHA), which is important for the brain tissue and eicosapentaenoic acid, which augments the effects of DHA. Reduction of DHA levels may induce Alzheimer's disease and memory problems [16].

In this study, rabbits treated with omega-3 showed a moderate improvement in histopathological changes induced by aluminum chloride as there were normal granular cells with a vesicular nucleus and few apoptotic shrinkage cells with chromatin condensation, with a mild positive caspase-3 antibody reaction confirmed by a significant decrease in the caspase-3 antibody reaction compared with that in the aluminum chloride group. Also, there was a decrease in the main area % of the caspase-3 antibody reaction than in the green tea-treated group; these results revealed that the therapeutic effect of omega-3 was better than green tea in eliminating aluminum chloride neurotoxicity. Similarly, Israa and Khabat [38] found that in the omega-3-treated group, there were a large number of normal neurons with a highly antiapoptotic effect of omega-3 against aluminum chloride neurotoxicity in albino rats. This is in agreement with Ali *et al.* [21]; who concluded that omega-3 ameliorated the oxidative stress induced by aluminum chloride in the brain tissue, as there was a significant reduction in free radical levels, and plays a major role in the initiation of brain antioxidant enzymes.

Other authors studied the influence of omega-3 fatty acid supplementation on aluminum-induced toxicity in male albino rats and revealed that aluminum-induced harmful effects on oxidative status and serum biochemical parameters of many organs such as the kidneys, liver, brain, and testis. In addition, omega-3 exerted ameliorative effects on the toxicity induced by aluminum chloride [39].

Also, Albakoush *et al.* [40] found that exposure to aluminum chloride in pregnant rats induced marked alterations in structures of the cerebellar cortex in rat pups and administration of omega-3 during gestation and lactation periods has an ameliorative effect on aluminum chloride toxicity.

Conclusion

Green tea and omega-3 exert neuroprotective effects on aluminum chloride-induced neurotoxicity in rabbits. However, omega-3 administration decreases neurotoxicity on the dentate gyrus more than green tea.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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