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Anti-amnestic properties of Ginkgo biloba extract on impaired memory function induced by aluminum in rats

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ABSTRACT

Aluminum is the most widely used non-ferrous metal. However, recently it is reported to be a neurotoxic agent that could induce biochemical defects in brain by affecting levels of neurotransmitters and generating reactive oxygen species resulting in oxidative stress. This study aimed at evaluating neuroprotective effect of Ginkgo biloba extract[2] (GBE) (200 mg/kg for 28 days) in antagonizing aluminum-induced neurotoxicity through investigating certain parameters such as serum aluminum level, brain aluminum content, brain regional distribution of aluminum, brain oxidative stress biomarkers' content, and brain acetylcholinesterase[3] (AChE) activity. Passive avoidance paradigm was used to assess memory retrieval of rats. Rats' activities were studied using open field test. Results showed that administration of aluminum (10 mg/kg for 28 days) impaired rats' memory retrieval associated with marked elevation of aluminum brain content, serum aluminum level and AChE activity. In addition, aluminum treatment induced significant elevation in its brain content in all tested regions. GBE treatment attenuated neurotoxic effects of aluminum as evidenced by improving rats' performance in passive avoidance and lowering brain AChE activity. Moreover, marked elevation in brain content of oxidized glutathione[4] (GSSG) and malonedialdehyde[5] (MDA) as well as depletion of reduced glutathione[6] (GSH) demonstrated following aluminum administration were reversed reaching normal levels after GBE treatment. Open field test, demonstrated no changes in latency period, number of ambulation, rearing, and grooming following aluminum or other treatments. Therefore, GBE may be a promising therapy ameliorating neurotoxicity of aluminum as an environmental toxic agent.

1. Introduction

Neurotoxicity syndrome is defined as altered nervous system functioning caused by exposure to certain chemicals that affect the nervous system. It is a result of degeneration of the neuronal cells (Troshin, 2009). Symptoms may include brain damage, dementia or amnesia, anxiety, depression, limb weakness, blurred vision (Asada et al., 2010). Neurotoxicity occurs upon the exposure to natural or synthetic toxic substances, called neurotoxins, characterized by their abilities to alter the normal activity of the nervous system causing damage to neurons such as: aluminum, mercury, copper, arsenic, lead and manganese (Pohl et al., 2011; Belyaeva et al., 2012). Most of metals induce neurotoxicity via oxidative stress mechanisms by impairment of cellular metabolic pathways leading to generation of elevated levels of reactive oxygen/nitrogen species (ROS/RNS) (Yin et al., 2011).

Aluminum is the most widely used non-ferrous metal on earth. Its common uses have encountered to high percentage of aluminum intake via oral and inhalational routes. Aluminum compounds are widely used in the manufacturing of several medicinal products such as antacids, antiperspirants, abrasive agents as well as food additives. There is an epidemiological relation between chronic aluminum exposure and the incidence of Alzheimer's disease (Bondy, 2010). The presence of excess aluminum in the brains of patients with Alzheimer's disease has been reported by recent researches (Andrasi et al., 2005). Moreover, recent studies of workers exposed to aluminum dust in industrial exposure have shown evidence of impaired cognitive function of those workers (Meyer-Baron et al., 2007). In addition, a decline in visual memory was observed in haemodialysis patients who exhibited higher serum aluminum levels (Bolla et al., 1992).

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[1] Seconded to The Holding Company for Biological Products and Vaccines as The Advisor to Chairman.
[2] GBE: Ginkgo biloba extract.
[3] AchE: acetylcholinesterase.
[4] GSSG: oxidized glutathione.
[5] MDA: malonedialdehyde.
[6] GSH: reduced glutathione.

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Aluminum reported neurotoxicity was mainly mediated via its pro-oxidant properties as well as its capability to induce/exacerbate oxidative stress and enhanced lipid peroxidation (Bela et al., 2012). A possible suggested mechanism of aluminum-induced neurotoxicity suggested it could be due to its pro-oxidant activity, it promotes biological oxidation in vitro and in vivo, facilitating: (1) iron-induced lipid peroxidation, (2) non-iron-induced lipid peroxidation, (3) non-iron-mediated oxidation of NADH, and (4) non iron-mediated oxidation of the hydroxyl radical (OH^-) (Exley, 2004). Several researches highlighted that aluminum accumulation could be associated with prominent exacerbation in oxidative stress and apoptosis in the tissue where it accumulated. The presence of aluminum implies an increase in OH^- and its precursors (peroxides and $O_2{}^-$) that can only be neutralized by direct free radical scavengers, which change the redox state of the cell (Sharma and Sharma, 2012).

In addition, aluminum was demonstrated to cause remarkable dysfunction in several neurotransmitters (Abu-Taweel et al., 2012), increased amyloid deposition, altered energy metabolism, impaired calcium homeostasis, aggravated inflammatory response as well. Therefore, aluminum induced brain dysfunction could be due to any of these mechanisms or the interaction between them. This reported neurotoxicity and brain dysfunction of aluminum were manifested as significant impairment of memory as well as remarkable deterioration in learning ability of aluminum-treated patients.

Aluminum-induced neurotoxicity is the most important aspect of aluminum toxicities because neurons appear to be particularly vulnerable to be attacked by free radicals for the following reasons: (a) their glutathione (an important natural antioxidant) content is low, (b) their membranes contain a high proportion of polyunsaturated fatty acids, and (c) brain metabolism requires substantial quantities of oxygen (Christen, 2000).

GBE has been used for centuries in traditional chinese medicine. The main active constituents of GBE are: terpenoids, flavonoids, biflavonoids, organic acids and polyprenols. The standardized GBE termed "EGb 761" consists of 22–27% flavone glycosides, 5–7% terpene lactones (of which 2.8–3.4% consists of ginkgolides A, B, and C, 2.6–3.2% bilobalide), and less than 5 mg/kg (5 ppm) ginkgolic acid (Van Beek, 2005). The reported therapeutic efficacy of GBE is likely contributed to the terpene trilactones (ginkolides and bilobalide) and the flavonoid glycosides (DeFeudis, 2003).

GBE may act through several mechanisms including antioxidant effect, inhibition of platelet activating factor, enhancement of hippocampal neurogenesi, protection of the neurons from excitotoxicity, inhibition of glucocorticoid synthesis, scavenging of free radicals, anti-inflammatory activity, and anti-apoptotic activity (Saleem et al., 2008).

Therefore, this study aimed at investigating the neuroprotective effect of GBE on aluminum-induced neurotoxicity via studying rat brain AChE activity and rats' step through latency in the passive avoidance task both of which are important to indicate memory function. This study also investigated brain oxidative stress biomarkers as well as brain regional distribution of aluminum as indicators for neurotoxicity. The open field test was used to detect any confounding effect of the used drugs on the memory task results.

2. Materials and methods

2.1. Chemicals

Aluminum lactate was purchased from Loba plus chemical company, U.K. The GBE, supplied as a dry powder, was obtained from Huisong Corp. – China, (product name: Ginkgo biloba Extract 24/6), (Lot No.: GB 101-071115). Ginkgo biloba was grown in China and the extract was prepared from the leaves. The leaves were dried before extraction with 50% ethanol/H_2O (v/v). The herb-to-extract ratio is 50:1. The extract solutions were prepared according to Good Manufacturing Practice rules and the quality and identity of the constituents were checked by thin-layer chromatography.

The Flavonoe glycoside content of the used GBE was reported to be 24.27% (w/w) [13.68% Quercetin glycosides, 9.34% Kaempferol glycosides and 2.09% Isohamnetin glycosides]. Whilst, the Terpene Lactone content of the used GBE was reported to be 6.81% (w/w) [2.10% Bilobalides, 2.72% Ginkgolide A, 1.37% Ginkgolide B and 0.62% Ginkgolide C]. All other used chemicals were of the highest quality available.

Aluminum toxicity was induced by aluminum lactate dissolved in normal saline (NaCl 0.9%); it was given in a dose of 10 mg/kg/day intraperitoneally for 28 days according to Julka et al. (1995). GBE treatments were started on the first day of induction of aluminum toxicity. It was prepared daily by suspending in normal saline. It was given in a dose of 200 mg/kg/day orally for 28 days (Gong et al., 2006). Each animal received 1 ml/100 g B.W. of the suspension.

2.2. Animals

Male adult Sprague-Dawley rats of (80–100 g body weight) were obtained from the breeding colony maintained at the animal facility of Pharmacology and Toxicology Department, National Organization for Drug Control and Research, NODCAR, Egypt. Animals were caged in groups of seven and were fed on standard diet pellets (El-Nasr Co. Abou-Zaabal, Egypt) with water ad libitum. The animal room was maintained at 21–24 °C and 40–60% relative humidity with 12 h light/dark cycles. Animals were subjected to an adaptation period of 15 days in the animal facility before experiments.

The NODCAR committee for animal care and use approved the protocol, which conforms to the international ethical guidelines for animal care and use.

2.3. Experimental design

In this study animals were classified randomly into four groups (20 rats each). The first group served as control group and received normal saline (NaCl 0.9%) i.p. The second group is the aluminum-treated group which received aluminum lactate (10 mg/kg/day i.p. for 28 days). Whilst, the third group was GBE-treated group receiving (200 mg/kg/day p.o for 28 days). Finally, the fourth group of animals received combined treatment of aluminum lactate (10 mg/kg/day i.p. for 28 days) and GBE (200 mg/kg/day p.o for 28 days).

In this study we had 4 sets of animals each consisted of the four groups mentioned above. The first set of animals was used to test memory function in passive avoidance test. Whilst, second set of animals was used for testing activity in open field test and then rats of the same set were sacrificed and used for the determination of the brain AChE activity. The third set was used for determination of aluminum content in the selected brain regions, rats' brains and for measuring serum aluminum level. The members of the fourth set were used for determination of brain contents of oxidative stress biomarkers namely GSH, GSSG and MDA.

2.4. Behavioral tests

Behavioral tests were in an isolated room between 9:00 and 10:00 am, 24 h after administration of the last dose of aluminum and/or GBE. Rats were weighted every day and tested neurologically with special emphasis on: general condition, deambulation, posture, righting from the side, placing reaction of hindlimbs, geotaxic reaction, avoiding of borders and equilibrium.

2.4.1. Passive avoidance test

2.4.1.1. Apparatus. A step-through passive avoidance apparatus was used. It consists of a Plexiglas box divided into two compartments, light and dark. The light compartment is a rectangular light box with a 25 cm in length and 25 cm in width grid floor and walls of 27 cm height. One of the walls has an 8 cm in length and 8 cm in width opening connecting the light compartment to a dark compartment. The dark compartment has same dimensions of the light one as well as electrifiable grid floor connected to a constant current stimulator. This grid floor consists of 40 parallel bars (0.3 cm in diameter, set 1.2 cm apart). The dark is constructed of black Perspex panels. The connection between the two compartments can be closed with a manually operated door made of Plexiglas. The larger compartment was illuminated with a 220-V 40-W bulb placed 15 cm above the center. Each compartment has a wall, which can be opened or closed for placement and removal of the rats.

2.4.1.2. Procedure. Passive avoidance test was conducted by the method of Narayanan et al. (2010), with modifications. The experiment had three parts: (1) an exploration test, (2) an aversive stimulation and learning test, and (3) a retention test. The exploration test was conducted in three trials. During this session, each rat was kept in the center of the larger compartment facing away from the entrance to the dark compartment. The door between the two compartments was kept open. The rat was allowed to explore the apparatus (both larger and smaller compartments) for 3 min. In each trial, the total time taken by the animal to enter the dark compartment was noted using a stop-watch. At the end of the trial, the rat was replaced in the home cage, where it remained during an inter-trial interval of 5 min. After the last exploration trial, the rat was again kept in the larger compartment as in the trial sessions. When the animal entered the smaller compartment,