Original article   http://dx.doi.org/10.1016/j.joad.2016.08.028

Aluminium-induced acute neurotoxicity in rats: Treatment with aqueous extract of *Arthrophytum* (*Hammada scoparia*)

Kaddour Taïr[1*], Omar Kharoubi[1], Oussama Anouar Taïr[2], Nouria Hellal[1], Iméne Benyettou[1], Abdelkader Aoues[1]

[1]*Laboratory of Experimental Biotoxicology, University of Ahmed Ben Bella "1", Oran, Algeria*

[2]*Faculty of Medicine, University of Ahmed Ben Bella "1", Oran, Algeria*

ARTICLE INFO

ABSTRACT

Objective: To study the antioxidative and protective properties of aqueous extract of *Hammada scoparia* (*H. scoparia*) against the effects of sub-chronic aluminium (Al) intoxication on mnemonic process and some neurochemical markers.
Methods: Al was administered intraperitoneally (50 mg/kg body weight, three times a week), and *H. scoparia* and malic acid were given orally by gavage at a daily dose (100 mg/kg body weight) to rats for 90 days.
Results: Al caused significant short-term and long-term memory disturbances, a decrease in locomotor activity, a significant inhibition of acetylcholinesterase activity in brain and a significant depletion of antioxidant enzymes (catalase, glutathione reductase and glutathione peroxidase) and glutathione. It significantly increased lipid peroxidation levels in cerebrum and cerebellum. However, treatment with *H. scoparia* extract protected efficiently the neurological functions of intoxicated rats by considerably increasing antioxidants levels and decreasing production of thiobarbituric acid reactive substances by 4.26% compared to untreated group. We noted some controversial results with malic acid. It showed some positive results but it was not as efficient as *H. scoparia* extract. Current results were consistent with histopathological observations including neurodegeneration and vacuolated cytoplasm (spongiosis) in Al treated sections when *H. scoparia* and malic acid treated sections showed marked neuroprotection signs.
Conclusions: This study strongly suggested that *H. scoparia* extract could possibly restore the altered neurological capacities and antioxidant power in rats, and it could even be a good alternative to chelating agents or other chemical medicines against Al-induced neurotoxicity.

1. Introduction

Aluminium (Al) is the most abundant metal present on the earth's crust. It is extensively used in daily life and was found in drinking water probably due to water purification procedures[1]. The new 20th century industrial products containing Al salts like antiperspirants are another source of exposure; vaccines adjuvants, phosphate binders, dialysis, total parenteral nutrition solutions and foods provide easy exposure of Al to human being[2–7].

The toxicity of Al is directly linked to its bioavailability. In biological systems, this element has been shown to accumulate in many mammalian tissues such as brain, bone, liver and kidney[8–10], and its elimination half-life from human brain is calculated to be seven years[2].

Al is the most common neurotoxicant[11–16], and the evidences about its implication in developing Alzheimer's disease are getting increased[17–20]. It was also found that this trivalent cation can participate as a factor in the development of neural tube defects in human[21]. Many studies showed that there were neuropathological, neurobehavioral, neurophysical

*Corresponding author: Kaddour Taïr, Laboratory of Experimental Biotoxicology, University of Ahmed Ben Bella "1", Oran 31000, Algeria.
    Tel: +213 0696780294
    E-mail: kadourtair@yahoo.fr

and neurochemical changes after Al exposure[22–27]. The brain is considered to be the most vulnerable to the toxic manifestation of Al, and it is particularly sensitive to oxidative stress due to increased levels of free radicals and decreased levels of antioxidants following toxicity[28–32].

Oxidative events have frequently been linked to neurodegenerative disorders such as Alzheimer's disease[33–36]. Public, academic, and government interest in traditional medicines or their isolated bioactive constituents seems to become amplified because of the adverse drug reactions and economic burden of the modern system of medicine[37].

Hammada scoparia (Pomel) Iljin (*H. scoparia*) belongs to Chenopodiaceae family, and it is a glabrous, grey brown, woody, dwarf shrub usually turning darker or blackish when dried. It grows wild in dry habitats of the Mediterranean region and the Near East[38]. In Algeria, it is commonly known as "rimth". Tunisian colleagues reported that *H. scoparia* possessed a large spectrum of pharmacological and therapeutic activities and it has been shown recently that flavonoid-enriched fraction of the plant has a protective effect on hepatic ischemia/reperfusion injury. A hepatic damage usually happens during liver surgery and transplantation[39]. Another study showed molluscicidal activity of the plant leaves extract against *Galba truncatula*[40]. It also possesses a potent antitumoral activity[41]. On the other hand, malic acid (MA), a naturally occurring, nontoxic and organic dicarboxylic acid, and magnesium are both known to be involved in the processes of generating adenosine triphosphate through Krebs cycle, and they play a pivotal role in mitochondrial adenosine triphosphate synthesis[42]. The MA-magnesium combination presented a big efficacy in treatment of patients having fibromyalgia when served as a dietary supplement[43]. Additionally, the chelation abilities of MA against Al toxicity were assessed at the University of Barcelona when toxicologists administered MA to mice exposed to Al at about one-fourth of the LD_{50} level. LD_{50} is the concentration of compound that will kill 50% of the experimental animals. Compared to other chelators (oxalic acid, malonic acid and succinic acid), MA showed the best therapeutic effectiveness at the same level compared with synthetic deferoxamine mesylate[44].

The lack of data information about the protective properties of *H. scoparia* against Al-induced neurotoxicity pushed us to conduct the present study as an answer to the wonders that if a nutritional strategy like chronic administration of aqueous extract of *H. scoparia* could efficiently prevent Al-induced neurotoxicity in terms of oxidative stress in rat brain as it could be with chelation therapy.

2. Materials and methods

2.1. Preparation of Arthrophytum plant extracts

Whole plants of *H. scoparia* [*Arthrophytum scoparium* (Pomel) Iljin, *Haloxylon articulatum* (Cav.) Bunge, *Haloxylon scoparium* (Pomel)][45,46] were collected from the region of Aïn Sefra, Algeria in June, 2013. The plant was subjected to the identification and authentication at the Herbarium of Botany Directorate in Ahmed Ben-Bella (Oran) University (voucher specimen No. LB0748). Twenty five grams of aerial parts of the plant were extracted with 250 mL of distilled water by the method of continuous hot extraction at 60 °C. Once the filtrate recovered, it was lyophilized and the residue collected (yield 11%) was stored at −20 °C.

2.2. Animals and experimental design

A total of 24 male Wistar rats with weight of (150 ± 10) g were used for the study. The processes of protocols using the experimental animals were in accordance to the Guide for the Care and Use of Laboratory Animals (8th edition, 2011) and approved by the scientific committee of the university. The animals were housed in the cages with six per cage and fed *ad libitum*, and they were exposed to a 10 h light: 14 h dark cycle and the room temperature was maintained at (23 ± 2) °C. Animals were divided into four groups of six animals each. Group 1 (control) served as untreated control and received a intraperitoneal injection of 0.9% saline solution (NaCl); Group 2 consisted of Al intoxicated rats which were given a dose of 50 mg/kg body weight (BW) of Al chloride (AlCl$_3$·6H$_2$O) three times a week; Group 3 was *Arthrophytum* (*H. scoparia*) treated group which received intragastrically aqueous extract at a dose of 100 mg/kg BW of *H. scoparia* simultaneously with an intraperitoneal injection of 50 mg/kg BW of Al chloride (AlCl$_3$·6H$_2$O); Group 4 was MA treated group which was given 100 mg/kg BW of MA by gavage in parallel with intraperitoneal injection of 50 mg/kg BW of Al chloride (AlCl$_3$·6H$_2$O).

All the groups were treated under the same housing conditions for a period of 90 days. The injection solution was prepared in sterilized saline solution. The animals were weighed and behavioural observations were recorded at the end of the experiment, then the animals were sacrificed under pentobarbital anaesthesia. The organs were removed, cleaned, washed with saline (0.9% of sodium chloride) then weighed and the organ weight ratio was estimated, and the relative weight of organs was calculated as g/100 g BW.

2.3. Tissue simple preparation

After anesthetization by intraperitoneal injection of pentobarbital, animals were sacrificed and the brains were immediately removed, placed in ice-cold isotonic saline and dissected into cerebrum and cerebellum which were stored at −80 °C. Later the brain regions were taken and minced into small pieces then homogenized with ten volumes of phosphate buffer (0.1 mol/L, pH 7.4) containing 0.3 mol/L sucrose and 0.08 mol/L potassium chloride using WiseTis® (HG-15A) homogeniser, and the homogenates were then centrifuged at 7 600 r/min for 10 min at 4 °C and the resultant supernatant was further centrifuged at 12 000 r/min for 10 min at 4 °C to yield the supernatant which was later used for the estimation of antioxidants parameters [malondialdehyde (MDA), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR)] and acetylcholinesterase (AChE) activity.

2.4. Behavioural parameters

Behavioural tests were conducted in order to evaluate how Al intoxication can affect locomotor, learning and memory

capacities in the different experimental groups and to assess the ability of either plant antioxidant molecules or chelators to restore a physiological homoeostasis. The following behavioural tests were used.

2.4.1. Open field

The open field test provides simultaneous measures of locomotion and anxiety[47]. The open field used was a square wooden arena (90 cm × 90 cm × 25 cm). The floor was divided by white lines into 36 smaller squares (15 cm × 15 cm). The open field maze was cleaned between each rat to avoid odour cues. The rats were carried to the test room in their home cages and tested once at a time period of 30 min each. Other parameters of exploratory activity such as rearing, grooming and sniffing were carefully observed and time spent in performing each behaviour was recorded. These parameters were defined as follows: rearing was defined as standing on hind legs with paws pressing against the wall of the arena; sniffing was defined as continuously placing nose against the floor for at least 2 s; grooming was defined as using paws or tongue to clean/scratch body[48].

2.4.2. Elevated T-maze

The test was realized according to the procedure described by Viana *et al.*[49]. This behavioural task assessed an effective memory of the experimental model related to the environment due to the open arms of the elevated T-maze apparatus[49–53] which was elevated by 50 cm from the ground and composed of two open arms of equal dimensions (50 cm × 10 cm) and two enclosed arms surrounded by 15 cm high walls. Rodents were found aversive to the characteristics and keeping a vivid memory of the aversive situation through measuring the time spent in the open arms during the test[50,53].

Right after the open field test, rats were placed at the end of the enclosed arms and the time (latency) taken to withdraw from the arm was recorded over 300 s (a cut off time if no changes have been noted). This time was called baseline. Afterwards this step was repeated two successive times at about 30 s of intervals between all attempts and the times to get out from the closed arms were recorded, and these were called inhibitory avoidances (inhibitory avoidance 1, inhibitory avoidance 2). The escape test was performed following the inhibitory avoidance 2, and it was represented by the time used for animal to withdraw from the open arms. To assess long-term memory, inhibitory avoidance and escape were measured again 72 h later[50,53].

2.5. Lipid peroxidation (LPO) levels, reduced GSH and antioxidant enzyme activities

2.5.1. LPO levels [thiobarbituric acid reactive substances (TBARS)]

The LPO levels in cerebrum and cerebellum homogenates were measured colourimetrically as described by Okhawa *et al.*[54] by measuring MDA formation. This is a method based on the reaction of thiobarbituric acid with some products of lipid peroxidation in acidic environment at increased temperature. The formed product was coloured in pink which enabled its spectrophotometric determination.

In brief, 0.2 mL of supernatant prepared from homogenized tissues using 9 mL potassium chloride (1.15%) was added with 0.2 mL of sodium dodecyl sulphate, 1.5 mL of acetic acid and 1.5 mL of thiobarbituric acid. After completing volume with 4 mL of distilled water, the samples were heated in boiling water bath for 60 min, and the samples were then cooled and centrifuged at 4 000 r/min for 10 min. Absorbance was measured at 535 nm. The amount of MDA was calculated using a molar extinction coefficient of 1.56×10^5 mol/L/cm.

2.5.2. Determination of CAT (EC 1.11.16) levels

CAT was assayed by the method of Aebi[55] with slight modification. The rate of H_2O_2 decomposition was followed by monitoring absorption at 420 nm. In brief, 250 μL of phosphate buffer (0.066 mol/L), 250 μL of cerebrum and cerebellum homogenates and 250 μL of 0.03 mol/L H_2O_2 (prepared in phosphate buffer, 0.066 mol/L, pH 7.0) were added in a cuvette. After incubation for 5 min, $TiOSO_4$ was added to the mixture and absorbance was directly measured against phosphate buffer as a blank, and one unit of CAT is equal to 1 mmol H_2O_2 degraded/ mg of protein.

2.5.3. Reduced GSH levels

Reduced GSH was determined using a colourimetric technique as described by Sedlak and Lindsay[56]. The principle was based on reaction of compounds containing sulfydryl groups with 5,5′ dithiobis(2-nitrobenzoic acid) (DTNB) which produced yellow coloured product that absorbed at 412 nm. In brief, 1 mL of cerebrum and cerebellum supernatant (homogenates) was prepared after treatment with 1 mL of 50% trichloroacetic acid-distilled water (1:4), and the supernatant obtained after centrifugation at 2 400 r/min for 15 min was mixed with 0.02 mL of 0.01 mmol/L DTNB and an amount of Tris buffer (0.4 mol/L, pH 8.5). Total GSH content was expressed as nanomoles of GSH per milligram of protein.

2.5.4. GR

GR catalyses the conversion of oxidized glutathione employing nicotinamide adenine dinucleotide phosphate (NADPH) as a substrate, and it was assayed by the procedure adopted by David and Richard[57].

The amount of NADPH utilized was a direct measure of enzyme activity in our tissue homogenates. In brief, the assay system contained 1 mL of phosphate buffer (0.12 mmol/L, pH 7.2), 0.1 mL of 15 mmol/L ethylene diamine-tetra-acetic acid, 0.1 mL of sodium azide (10 mmol/L), 0.1 mL of oxidized glutathione and 0.1 mL of supernatant (cerebrum and cerebellum homogenates) and the volume was made up to 2 mL with distilled water. The reaction was started by the addition of NADPH solution, and the absorbance was read at 340 nm and the enzyme activity was expressed as μmol NADPH oxidized/ mg of protein.

2.5.5. Activity of GPx

GPx (EC 1.11.1.9) activity in brain tissues was assessed by the method of Rotruck *et al.*[58]. Briefly the reaction mixture contained 0.2 mL of Tris–HCl buffer (0.4 mol/L, pH 7.0), 0.2 mL of reduced GSH (1 mmol/L), 0.1 mL of sodium azide

(10 mmol/L), 0.1 mL of H_2O_2 (1 mmol/L) and 0.2 mL of tissue sample.

After incubation at 37 °C for 10 min, reaction was stopped by the addition of 0.4 mL of 10% trichloroacetic acid, and tubes were subjected to centrifugation at 2 400 r/min for 10 min. The supernatant (0.2 mL) was then added with 0.1 mL Ellman's reagent (0.019 8 g of DTNB prepared in 0.1% sodium citrate). Absorbance was recorded at 340 nm.

2.5.6. Estimation of tissue AChE

AChE belongs to cholinesterase family. The enzyme activity was assessed according to the procedure of Ellman et al.[59]. Acetylthiocholine was hydrolysed by AChE to acetic acid and thiocholine. The catalytic activity was measured by following the increase of yellow anion, 5-thio-2-nitrobenzoate, produced from thiocholine when it reacted with DTNB[59] at 410 nm.

In brief, an aliquot of cerebrum and cerebellum homogenate (0.02 mL) was added to tubes containing 3 mL of phosphate buffer (100 mmol/L, pH 8.0), 0.02 mL of acetylthiocholine solution (75 nmol/L) and 0.1 mL of DTNB.

2.5.7. Protein estimation

Protein was measured by the method of Lowry et al.[60] using bovine serum albumin as a standard, and necessary dilutions were realized to get the correct concentrations of the proteins present in tissues.

2.6. Histopathological studies [haematoxylin and eosin (H&E) staining]

Samples (entire brains: cerebrum and cerebellum) from each group were selected, transversely cut and fixed in 10% buffered formaldehyde solution, then conserved in paraffin. Four-micrometre tissue sections were realized and dried at adequate temperature to get paraffin removed from the glass slides. The next step was to rehydrate sections then stain them with haematoxylin and eosin as nuclear and cytoplasmic stains. The sections were analysed using Leica®DM5000B microscope and photographed with Leica EC3 digital camera.

2.7. Statistical analysis

Data were expressed as mean ± SEM with six rats in each group. Data comparisons were carried out by using One-way ANOVA followed by least significant difference (LSD) test to compare means between the different treatment groups, and results were considered statistically significant when $P \leq 0.05$.

3. Results

3.1. Effect of treatment on body, cerebrum and cerebellum weights

As shown in Table 1, there was a significant difference in BWs of all experimental animals compared to controls. However, there was no significant change between Groups 2 and 3. The relative whole brain and cerebrum weights were also significantly lower in Group 2 than in the control group, and administration of H. scoparia in parallel with Al produced a recovery in relative whole brain compared to Group 2. MA had the same effect on relative cerebrum weight.

3.2. Effect of treatment on behavioural parameters

The Al chloride treatment induced significantly decreased ($P \leq 0.05$) locomotor activity as shown in Figure 1. This was concluded through the significant decrease in numbers of crossed squares and the highly significant decrease in rearing and sniffing performed by the animals in intoxicated group compared to the control one (Figure 1C,D).

Treatment with H. scoparia aqueous extract during Al exposure showed a very protective effect by significantly improving some of the previously altered scores in intoxicated rats, and this result was available for MA treatment group.

The mean inhibitory avoidance latency (IAL) and escape latency (ESL) of the elevated plus maze task presented some adverse variations. The IAL was significantly higher in intoxicated animals compared to control (Figures 2–5) ($P \leq 0.05$), and the rats permanence time (IAL1) was three times much longer in enclosed arms compared to baseline. In the next attempt (avoidance 2 test), the results showed a highly significant decrease in term of latencies in the protected arms (169 s was spent by intoxicated rats when controls stayed there over 300 s).

Treatment with Al and H. scoparia simultaneously presented a recovery in term of learning-short term memory capacities, and the animals had been stationary for about 30.8 s more than those in Al treated group.

Table 1

The effects of H. scoparia and MA on BW, absolute whole brain, cerebrum and cerebellum weights of control and rats treated with $AlCl_3$ after 90 days of treatment.

Groups	Initial BW (g)	Final BW (g)	Absolute whole brain weight (g)	Relative whole brain weight (g/100 g BW)	Absolute cerebrum weight (g)	Relative cerebrum weight (g/100 g BW)	Absolute cerebellum weight (g)	Relative cerebellum weight (g/100 g BW)
Control	150.12 ± 4.92	270.52 ± 4.92	2.015 ± 0.086	0.842 ± 0.166	1.498 ± 0.061	0.640 ± 0.021	0.517 ± 0.031	0.198 ± 0.014
AlCl$_3$	150.85 ± 7.45	230.85 ± 4.92[*]	1.879 ± 0.096	0.757 ± 0.166[*]	1.415 ± 0.093	0.553 ± 0.002[*]	0.456 ± 0.022	0.198 ± 0.016
AlCl$_3$+ H. scoparia	151.10 ± 12.61	237.60 ± 15.61[*]	1.936 ± 0.057	0.841 ± 0.160[*]	1.453 ± 0.020	0.588 ± 0.019	0.482 ± 0.034	0.196 ± 0.018
AlCl$_3$ + MA	150.00 ± 8.06	237.50 ± 17.14[*]	1.999 ± 0.060	0.798 ± 0.166	1.547 ± 0.043	0.632 ± 0.016[#]	0.462 ± 0.039	0.208 ± 0.008

Values are given as mean ± SEM each group. [*]: $P \leq 0.05$ compared with control group; [#]: $P \leq 0.05$ compared with AlCl$_3$ group.
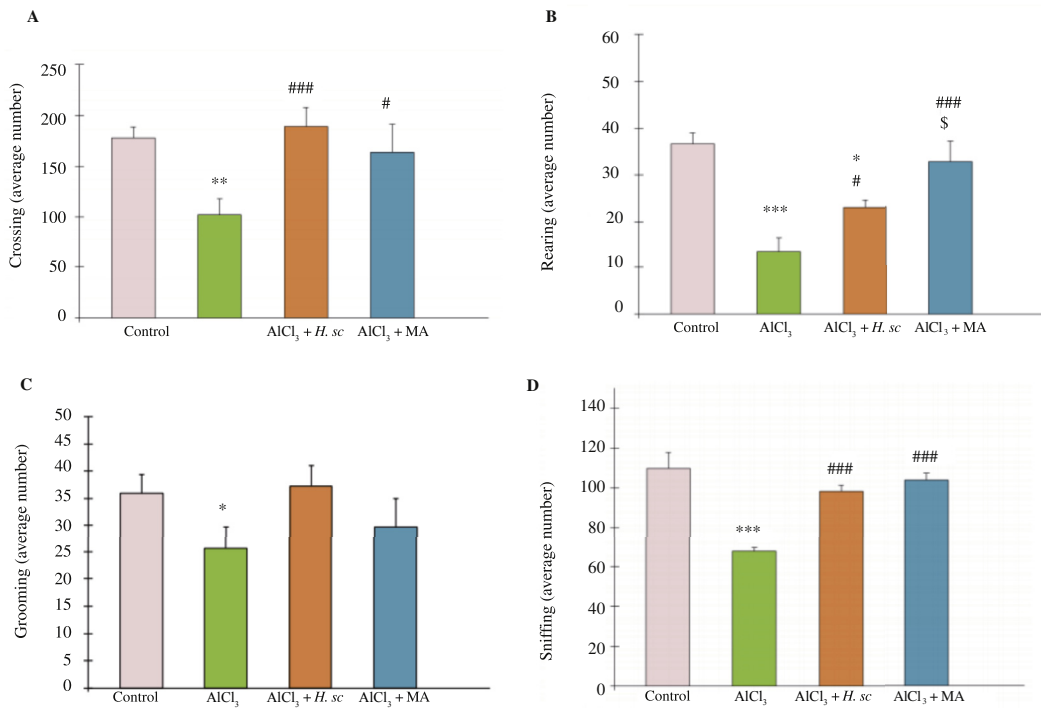
Figure 1. Open field results following $AlCl_3$ treatment during 90 days.

The open field parameters crossing (A), rearing (B), grooming (C) and sniffing (D) were evaluated in the test. Values are given as mean ± SEM. *: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$ compared with control group. #: $P \leq 0.05$, ###: $P \leq 0.001$ compared with $AlCl_3$ group. $: $P \leq 0.05$ compared with $AlCl_3$ + *H. scoparia* group. *H. sc*: *H. scoparia*.

For the first escape trial, rats joined the closed arms of the apparatus within 17 s, 36.61 s, 16.65 s, and 87.88 s for Groups 1–4 respectively.

The long-term memory tests (avoidance 3: IAL3 and escape 2: ESL2) was performed at 3 days after avoidance 2 and escape 1 tests. Control and *H. scoparia* treated rats performed good scores, which were very close to each other even there was no statistical differences between values of both IAL3 and ESL2 for the two groups, when those of Al intoxicated group clearly indicated an impairment in long-term memory process through a highly significant decrease in IAL3 compared to controls (Figure 2).

Finally, MA treatment procedure presented some contradiction since there was no statistical difference between avoidances of Al + *H. scoparia* treated group and Al intoxicated group, but their ESL2 values were favourable for amelioration of the memory process.

3.3. Effect of treatment on AChE activity

Acute/sub-chronic Al chloride administration in rats produced a significant ($P \leq 0.05$) decrease in cerebrum AChE activity by 19.13% as compared to control rats. However, the
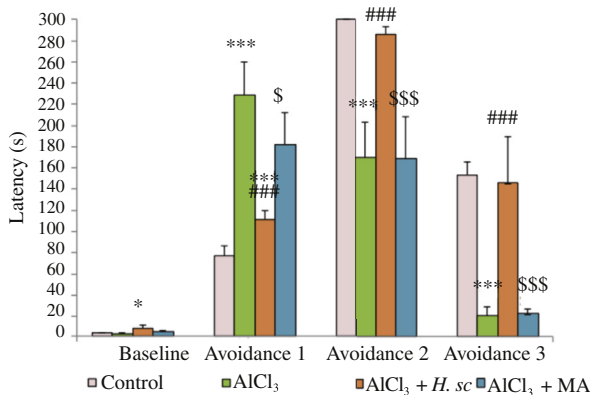

Figure 2. Elevated T-maze results following $AlCl_3$ treatment during 90 days.

Values are given as mean ± SEM. *: $P \leq 0.05$, ***: $P \leq 0.001$ compared with control group. ###: $P \leq 0.001$ compared with $AlCl_3$ group. $: $P \leq 0.05$, $$$: $P \leq 0.001$ compared with $AlCl_3$ + *H. scoparia* group. *H. sc*: *H. scoparia*.
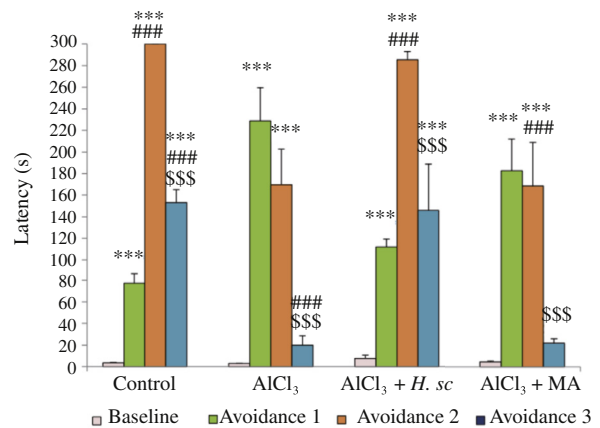

Figure 3. Comparative analysis of avoidance tasks inside each experimental group (elevated T-maze).

Values are given as mean ± SEM. ***: $P \leq 0.001$ compared with baseline. ###: $P \leq 0.001$ compared with Avoidance 1. $$$: $P \leq 0.001$ compared with Avoidance 2. *H. sc*: *H. scoparia*.
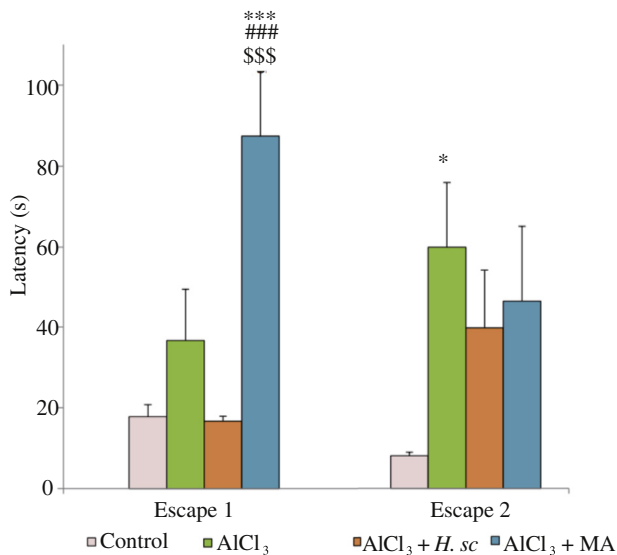
Figure 4. Effects of AlCl$_3$ treatment during 90 days on escape from the open arm of the elevated T-maze.

Escape 1 was measured in all experimental groups immediately after inhibitory avoidance training. Escape 2 was measured 72 h later. Values are given as mean ± SEM. *: $P \leq 0.05$, ***: $P \leq 0.001$ compared with control group. $^{###}$: $P \leq 0.001$ compared with AlCl$_3$ group. $^{\$\$\$}$: $P \leq 0.001$ compared with AlCl$_3$ + H. scoparia group. H. sc: H. scoparia.
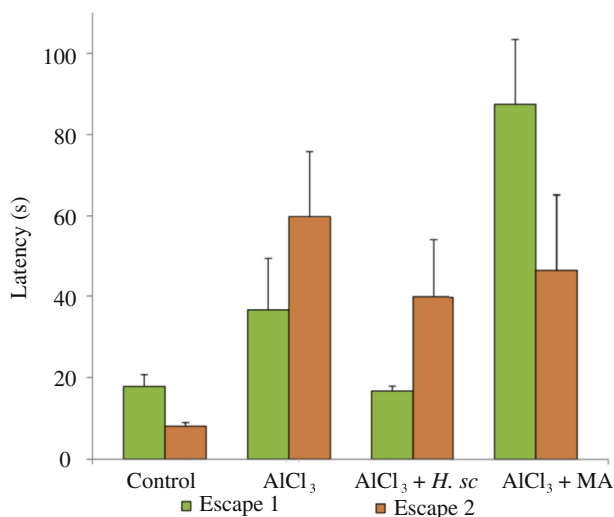

Figure 5. Comparative analysis of escape tasks inside each experimental group (elevated T-maze).

Values are given as mean ± SEM. No significant differences between Escape 1 and Escape 2 were recorded. H. sc: H. scoparia.
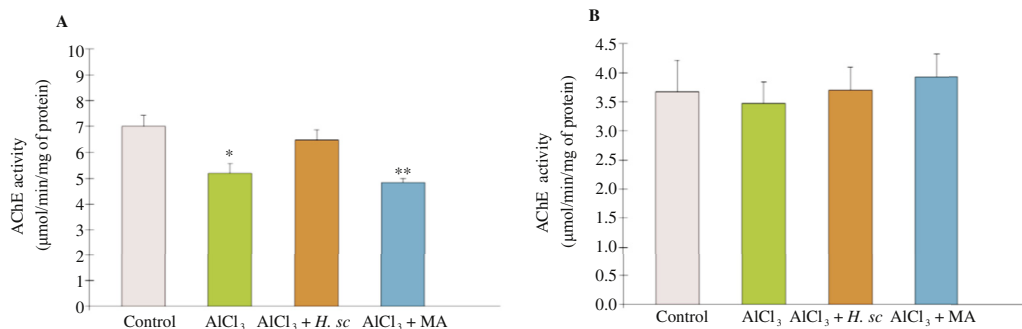
Arthrophytum (100 mg/kg) treatment improved modestly the alteration in AChE activity in the region mentioned above when compared to Al treated rats (Figure 6).

3.4. Effect of treatment on lipid peroxidation and GSH contents in cerebrum and cerebellum

Changes in TBARS and GSH levels were illustrated in Figures 7 and 8, and a significant increase in TBARS levels by 53.43% in cerebrum of intoxicated rats was noted when compared to controls. A highly significant ($P \leq 0.001$) increase was also noted in cerebellum of exposed rats, and these results were accompanied by a reduction in GSH levels in cerebrum and cerebellum of Al treated rats (59.72% and 5.04% respectively) in comparison with those of controls.

The co-administration of *H. scoparia* and Al decreased the TBARS production by a rate of 4.26% in cerebrum and 72.36% in cerebellum. This treatment alleviated significantly GSH levels in brain regions when compared to intoxicated rats. The plant extract showed more efficient results in term of restoring normal values of some altered parameters than the chelation strategy did.

3.5. Effect of treatment on antioxidant enzymes activities in cerebrum and cerebellum

Exposure to Al produced significant changes in the cerebrum and cerebellum redox status. A very significant decrease ($P \leq 0.01$) in CAT levels, GR and GPx activities was recorded in intoxicated group compared to controls (Figures 9–11).

Oral administration of aqueous *H. scoparia* extract during Al exposure showed an amelioration in CAT, GR and GPx by significantly increasing their values (61.79%, 43.74% and 48.47% respectively) in cerebrum when compared to those in Al treated group.

Treatment with MA failed again in establishing normal balance between oxygen reactive species (ROS) generating and antioxidants.

3.6. Effect of treatment on brain histopathological changes

Pathological changes in the cerebrum and cerebellum of Al intoxicated rats were examined under light microscopy. We noted that there were a neuronal loss, a spongy degeneration and vacuolated cytoplasm when sections were found to be intact in control group. These changes were minimized in Al + *H. scoparia* and Al + MA treated groups (Figure 12).

Figure 6. Effects of treatment with *H. scoparia* and MA on AChE (μmol/min/mg protein) activity in cerebrum (A) and cerebellum (B) of control and Al intoxicated rats after 90 days of exposure.

Values are given as mean ± SEM. *: $P \leq 0.05$, **: $P \leq 0.01$ compared with control group. H. sc: H. scoparia.
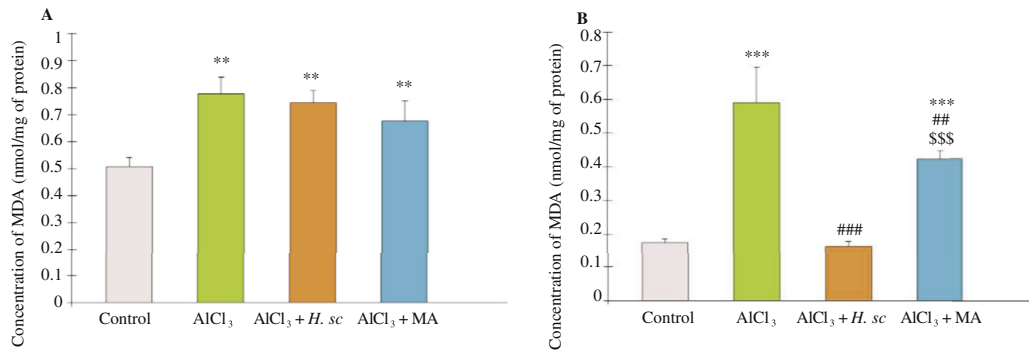
Figure 7. Effects of *H. scoparia* and MA on TBARS (nmol/mg of protein) in cerebrum (A) and cerebellum (B) of control and rats treated with Al after 90 days of treatment.

Values are given as mean ± SEM. **: $P \leq 0.01$, ***: $P \leq 0.001$ compared with control group. ##: $P \leq 0.01$, ###: $P \leq 0.001$ compared with $AlCl_3$ group. $$$: $P \leq 0.001$ compared with $AlCl_3$ + *H. scoparia* group. *H. sc*: *H. scoparia*.
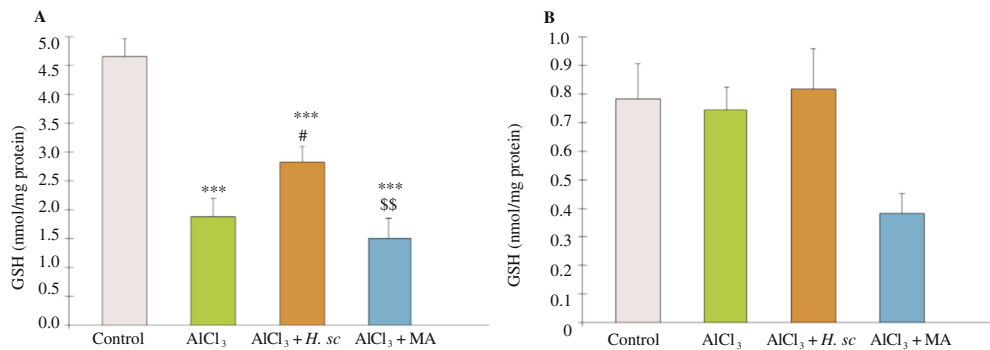

Figure 8. Effects of treatment with *H. scoparia* and MA on GSH levels in cerebrum (A) and cerebellum (B) of control and rats treated with Aluminium after 90 days of exposure.

Values are given as mean ± SEM. ***: $P \leq 0.001$ compared with control group. #: $P \leq 0.05$ compared with $AlCl_3$ group. $$: $P \leq 0.01$ compared with $AlCl_3$ + *H. scoparia* group. *H. sc*: *H. scoparia*.
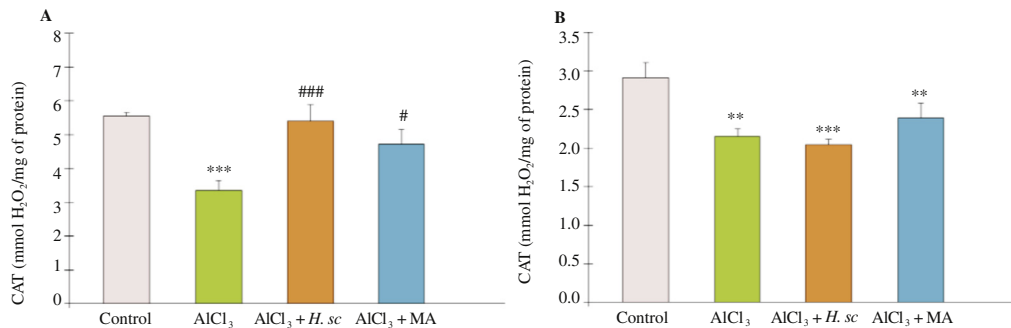

Figure 9. Effects of *H. scoparia* and MA on CAT level (mmol H_2O_2/mg of protein) in cerebrum (A) and cerebellum (B) of control and rats treated with Al after 90 days of exposure.

Values are given as mean ± SEM. **: $P \leq 0.01$, ***: $P \leq 0.001$ compared with control group. #: $P \leq 0.05$, ###: $P \leq 0.001$ compared with $AlCl_3$ group. *H. sc*: *H. scoparia*.
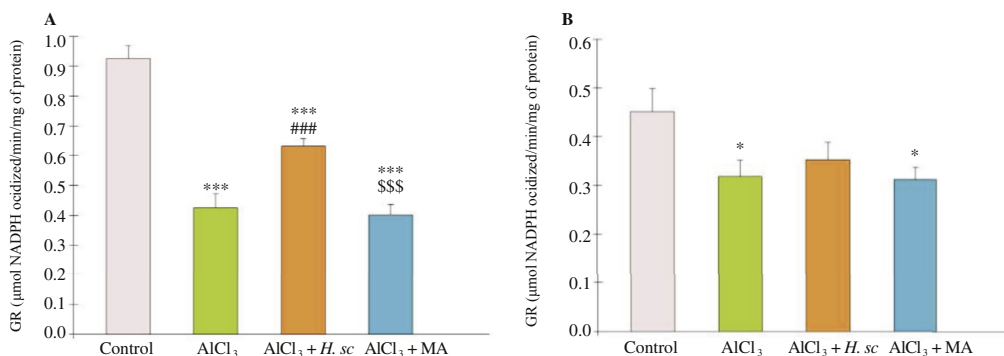
Figure 10. Effects of *H. scoparia* and MA on GR (μmol NADPH oxidized/min/mg of protein) activity in cerebrum (A) and cerebellum (B) of control and rats treated with Al after 90 days.

Values are given as mean ± SEM. *: $P \leq 0.05$, ***: $P \leq 0.001$ compared with control group. ###: $P \leq 0.001$ compared with $AlCl_3$ group. $$$: $P \leq 0.001$ compared with $AlCl_3$ + *H. scoparia* group. *H. sc*: *H. scoparia*.

Figure 11. Effects of *H. scoparia* and MA on GPx (nmol/min/mg of protein) activity in cerebrum (A) and cerebellum (B) of control and rats treated with Al after 90 days of exposure.

Values are given as mean ± SEM. *: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$ compared with control group. ###: $P \leq 0.001$ compared with AlCl$_3$ group. *H. sc*: *H. scoparia*.
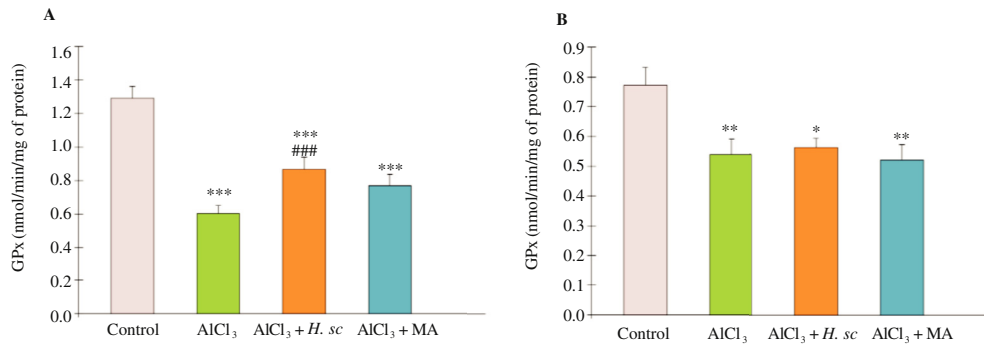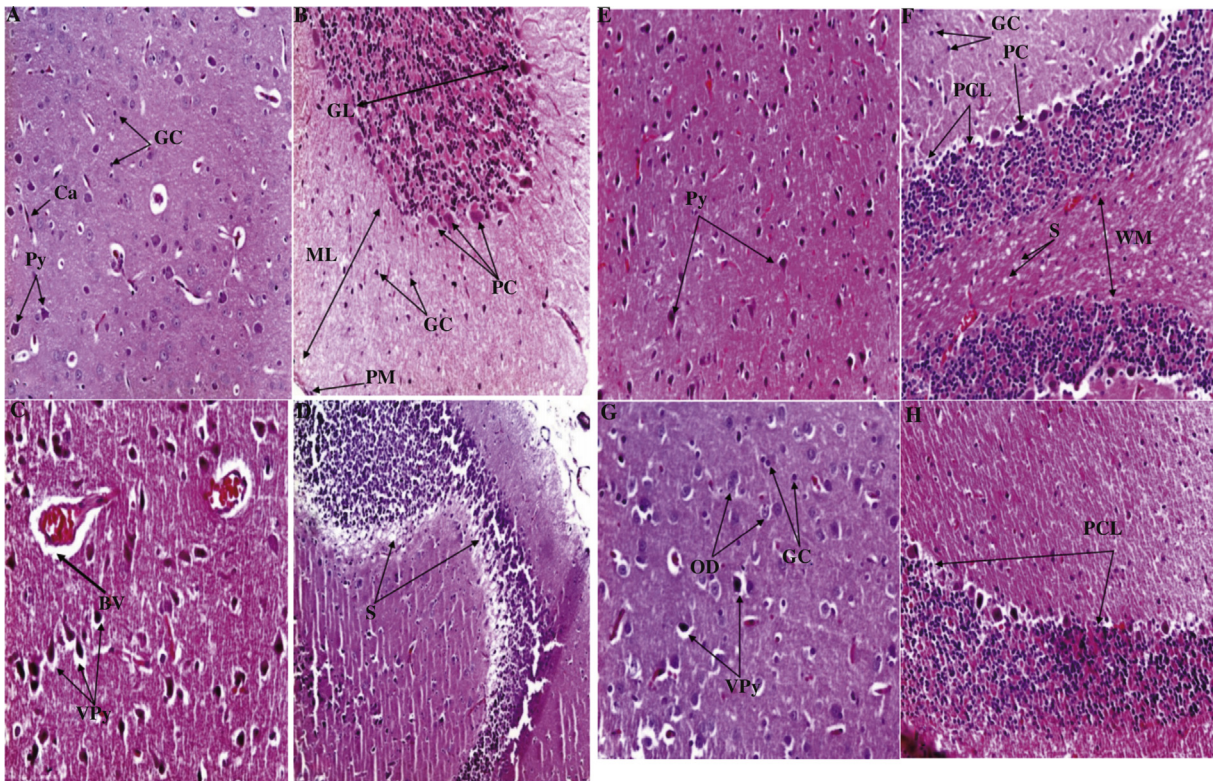

Figure 12. Effects of *H. scoparia* on Al-induced histological changes in cerebral cortex and cerebellum of control and experimental rats.

A and B (control): Sections of cerebral cortex (A) and cerebellum (B) showing normal histo-architecture (H&E, 20×); C and D (Al: 50 mg/kg BW): Sections of cerebral cortex (C) and cerebrum (D) showing neuronal spongiosis, gliosis with apparent vacuoles in both regions in addition to disorganization in cerebellum layers (H&E, higher magnification, C: 40×; B: 10×); E and F (Al + *H. scoparia* 100 mg/kg BW): Sections of cerebrum (E) and cerebellum (F) showing very reduced vacuolar spaces around the pyramidal cells in cerebral cortex, a modest loss in Purkinje's cells and a minimized spongiosis in cerebellum (H&E, 20×); G and H (Al + MA 100 mg/kg BW): Cerebrum and cerebellum section showing less alterations in the histoarchitecture compared to Al group especially in cerebellum, some vacuolated neuronal cells still exist in cerebrum (H&E, 20×). GL: Granular layer; ML: Molecular layer; GC: Glial cell; PM: Pia mater; PC: Purkinje's cell; Py: Pyramidal cells; VPy: Vacuolated pyramidal cell; PCL: Purkinje's cell loss; WM: White matter; S: Spongiosis; OD: Oligo dendrocyte.

4. Discussion

The results from the present research indicate that Al exposure has changed the BW and relative weights of the whole brain and cerebrum, which reveal a possible detrimental effect of Al on the body and brain weight as compared to the control (Table 1). These results concur with many previous researches. Julka *et al*.[61] reported that sub-acute Al exposure of rats generated a loss of about 27.8 g in animals BW. Tripathi *et al*.[62] also noticed a reduction in terminal body weights of animals administered Al during 90 days while Sharma *et al*.[35] noticed

a significant reduction of about 50.44% gain in weight in Al treated rats. The loss of brain weight after sub-acute Al treatment could be a result of the spongiosis of the neuropil resulting in retarded development of the animals[61]. This agrees with our results about histology of studied organs.

In this study, we have investigated the behavioural and the potential neuropathological effect of acute/sub-chronic experimental exposition of rats to Al chloride, and tested animals presented neurological disorders including learning impairments and memory deficits as well as neuronal loss when compared to controls.

In the present study, Al chloride was found to decrease the crossing scores according to the results obtained after performing in the open field apparatus. Even secondary parameters evaluated in this test were altered including rearing, grooming and sniffing. Similar observations have also been reported by Lal et al.[63] who noticed significant reduction in the spontaneous locomotor activity after daily Al treatment of rats (500 mg Al/L in drinking water) for 180 days. Sharma et al.[35] made the same observations after an intragastrically administration of Al lactate to rats for 12 weeks. Yellamma et al.[64] also reported an hypokinesia (reduced locomotor activity) in rats administered with sub lethal dose of Al once a day for 25 days continuously. Elsewhere, Abd-Elhady et al.[13] did not find any distinct effects of Al on crossing or rearing done by the animals.

The elevated T-maze apparatus which reflects learning and memory abilities by measuring inhibitory avoidance revealed that intoxicated animals presented differences in IAL compared to normal control animals. This refers to possible learning process failures and memory deficits caused by the neurotoxicant.

These results are supported by those described in previous works when peripheral and oral administrations of Al to rodents lead to learning and memory deficits[65–67], and the same result was found in studies using other forms of Al which showed that administration of Al citrate to rats decreased IAL values[53]. Similar results that indicate impairments of rats receiving Al in drinking water in the passive avoidance task have been reported[68]. Abd-Elhady et al.[13] also demonstrated that Al caused a deterioration in learning and memory functions in passive avoidance task after Al treatment. The latencies of animals in the closed arms of the previously described apparatus seem to be higher when compared the values of inhibitory avoidance of the intoxicated group to their baseline latency and the IAL 1 values of control group. This matches with anterior works reporting that Al citrate administration impaired inhibitory avoidance performance[53]. While others have indicated that the stay of animals is normally longer in the closed arms in attempts following the first trial because they prefer to explore more open space than confine and protect ones[50,53].

In Arthrophytum treated group and MA treated group, we found that IAL 1 is particularly closed to IAL of control group, which reflects an effective action of plant molecules and chelating elements on restoring normal state.

In the present work, short-term memory was seen to be significantly affected by Al exposure referring to values of Avoidance 2. The amnestic effect of Al was also consolidated by performances done 72 h after avoidance 2 when tested IAL3 in AlCl$_3$ group was reduced in comparison with control group suggesting a long-term memory alterations.

Obtained results showed that one-way escape from the open arms of the T-maze was not affected by Al intoxication as it was with IAL trials when compared intoxicated group to control group. Additionally it was shown that memory in this task was not impaired. Thus, different types of memory seem to exist, each having specific underlying brain mechanisms. This can be explained by the fact that some brain structures (like amygdala complex) lesions attenuate expression of emotion, behaviour and memory whereas their integrity is not required for other types of memory[50,69]. Based on our results, Al-induced neurodegeneration seems to be verified, and this is in accordance with the findings that memory impairments along with compromised learning behaviour are the major neurodegeneration disorders[70] such as Alzheimer's disease affecting particularly some brain structures like hippocampus[71] and amygdala[72].

Al exposure is known to produce neurotransmission disruption and cholinotoxicity[73–75], and acetylcholine is usually related to short-term memory. Our finding demonstrated that Al causes disturbances in cholinergic neurotransmission, and H. scoparia extract co-administrated with Al revealed a better effect on learning in animals since passive avoidance in this group were improved in comparison with intoxicated animals. These results concur with previously reported data indicating that a co-administration of some plant preparation like Vitis vinifera extract with Al showed a recovery from amnestic troubles[76].

AChE is usually located in membranes (erythrocytes) of vertebrates and non-vertebrates. The enzyme controls ionic current in excitable membranes and plays an essential role in nerve conduction process at the neuromuscular junction[77] and motor function[78]. That's why some previous studies reported that Al altered the muscular-locomotion activities by decreasing them, which can explain our result about behaviour (crossing task values) since high levels of Al not only interfered with the memory but also attenuated the motor functions and led to decreased motor activities and grip strength in mice[79]. However, giving H. scoparia antioxidant extract could restore altered motor function and acquisition-memory process (closed to normal) by modulating AChE activity.

Although AChE enzyme always receives a big attention in the study of Al neurotoxicity, the elevated acetylcholine levels are known to improve learning and memory[80] and AChE plays an essential role in cognitive functions[81] by two mechanisms including elevating acetylcholine levels and promoting the cholinergic neurogenesis[82]. Results of previous studies showed that Al has biphasic effect on AChE activity (increased at 4 and 14 days and decreased at 60 days of intoxication)[83]. Lakshmi et al.[76] have also reported an decrease in AChE activity in brain as a response to Al intoxication.

AChE activity measured in the present study was observed to be decreased after Al exposure. This could be explained by an accumulation of the metal in rat brain. Authors mentioned that exposure of rats to Al chloride by intubation for 60 days presented accumulation of the neurotoxicant at different levels in the brain[83] affecting even serotoninergic neurotransmission. Similar results suggested that the decrease of 5-HT level in hippocampus while causing cholinergic hypofunction by administration of neurotoxin AF64A is a direct result of losing cholinergic input[73]. Also, it has been observed that Al influences the metabolism of acetyl-CoA which leads to a possible reduction in the formation of acetylcholine and hence the substrate for AChE enzyme[84]. These results were also described by Ravi et al.[85].

Other works highlighted the relation between Al exposure and glucose since production of acetyl-CoA is widely linked to pyruvate, a key molecule in glycolysis process[86]. Our results about AChE activity disagree with some others indicating that Al exposure led to an increase in AChE activity in brain of rats[87–89]. This could be explained by the hypothesis of the biphasic effect of Al related to the metal exposure duration established by Kumar[83].

H. scoparia extract administered in parallel with Al improved modestly the decreased enzyme activity in brain regions. Similar

reports have been noted by Kakad et al.[90] and Lakshmi et al.[76] when administration of Vitis vinfera, a black grapes fruit from India, to Al exposed rats alleviated AChE activity. Also Kumar et al.[25] have found that chronic oral treatment with curcumin (30 mg/kg and 60 mg/kg) significantly ameliorated the reduction in AChE activity compared to Al chloride treated group.

The positive response of cholinergic neurons in term of system reactivation has been explained by two hypothesis: antioxidant plant extract/flavonoids administration changed the configuration of AChE or corrected the impaired metabolism of glucose[76,91].

Since it was reported that the manifestation of oxidative stress generation in brain was a response to sub-chronic exposure to Al[32,74,89], we undertook the present study based on measuring LPO levels and quantitating endogenous antioxidants (CAT, GR, GSH, GPx).

Now, it is well documented that Al-induced oxidative stress in neurons involves an imbalance between generation of ROS and antioxidants[92,93].

Lipid oxidation products are one of the main consequences associated with oxidative stress and brain is considered to be the most sensitive target to be damaged due to the high level of lipid content and tissue oxygen consumption[32].

The significant increased cerebrum and cerebellum levels of MDA found in our study reflect the efficiency of Al in activation of lipid production process. These results corroborated the previous findings that Al exposure enhanced iron-dependant LPO in rat brain[94,95]. While administration of Al through intraperitoneal injection increased LPO in cerebrum and cerebellum.

GSH is an important intracellular non-enzymatic antioxidant, and it is considered as the most important scavenger of free radicals and cofactor of many detoxifying enzymes against oxidative stress like GPx, GR and others. It is able to regenerate the most important antioxidants, vitamins C and E, back to their active forms[96,97].

In our case, we noted decreased GSH levels in cerebrum and cerebellum of intoxicated rats compared to those found in controls. Antioxidant enzymes are the first cellular molecules required for defence against ROS generation. Thus, in the present work, increased oxidative stress and brain injury were evident by decreased GPx, GR activities and CAT level in cerebrum and cerebellum. All of these records are in agreement with the fact that Al reduced the total antioxidant parameter levels enhancing imbalance between pro-oxidant and antioxidant potentials. Results similar to ours made it clear that Al chloride intraperitoneally administered at dosages of 0.7 and 35 mg/kg BW for 14 days resulted in higher Al concentration in hippocampus and cerebellum in the Al treated group compared to the control[26]. Also, Azadeh and Abdollahi[98] reported that most studies on Al toxicity demonstrated a decrease in both the activity of GPx and the concentration of GSH. Nayak et al.[99] found the same results with GR and GSH when co-exposure of rats to both ethanol and Al favoured the development of Al-induced oxidative stress in cerebrum.

H. scoparia administration to Al treated rats was found to significantly re-equilibrate antioxidant parameters back to normal values. This positive effect of Algerian Arthrophytum on oxidative stress defence is probably due to its secondary metabolites composition including alkaloids, polyphenols and flavonoids.

Benkrief et al.[100] reported that H. scoparia from Algeria contained the alkaloids carnegine, and N-methylisosalsoline as major tetrahydroisoquinoline alkaloids in addition to isosalsoline, N-methylcoryaldine, dehydrosalsolidine, isosalsolidine and N-methyltryptamine as minor alkaloids, while others reported that H. scoparia from Algeria Nabors regions contained quercetin-galactose-rhamnose commonly called rutin as the major and most active flavonoid[42].

Similarly, recent studies highlighted the determinant role of some specified isolated secondary metabolites such as resveratrol[101] and quercetin[35] in protection and remission from respectively Al-induced brain neuroinflammation and cognitive impairments/neurotransmission dysfunction related to oxidative damage. Similar studies using bioflavonoids from plant and fruit extracts such as pomegranate peel showed decreased Al accumulation and stimulated anti-apoptotic proteins against Al exposure in rat brain[102]. Prakash et al.[103] also found that fisetin, a natural flavonol, can attenuate increased LPO and reduced GSH levels in brains of Al chloride treated mices. Similar findings were recorded when curcumin supplementation helped to normalize the levels of some oxidative stress parameters including reduced GSH following chronic administration of Al to rats[104]. In the other hand, some authors claim that the supplementation of rodents by some trace element metals as antioxidants like selenium is suitable for removing Al toxicity[105].

The examination of H&E stained sections revealed that Al can cause marked histopathological abnormalities in brain tissues (cerebrum and cerebellum) including neuronal vacuolization, spongiosis, gliosis and cellular rarefaction in cerebral cortex. These results are correlated to those claimed by many authors. Bhadauria[9], Prakash et al.[103], Bihaqi et al.[106], Matyja[107] and Sumathi et al.[108] all reported the same modifications induced by Al on cerebral cortex histoarchitecture. Therefore, these alterations are associated with learning-memory impairments[109]. Others reported that vacuolated cells are a striking feature in both ageing and Al-treated brain parenchyma[110], and they may be considered as the initial stages of dying cells producing a swollen appearance with indistinct boundaries[110,111].

In Al + H. scoparia group, spongiosis and alterations were really minimized and loss of cell degeneration appeared clearly.

Histological observations of control cerebellum H&E stained sections showed typical cell characteristics and organizations including pia mater, molecular and granular cell layer and Purkinje's cell layer. In Al-treated group, spongiosis was also noticed in addition to disorganization in layers and some Purkinje's cells loss. It was documented that these cells are responsible for motor co-ordination through their projections until cerebral cortex and any damage in the cell layer may change the motor co-ordination[112]. This could explain the decreased locomotor activity found in Al-teated rats in the present study.

The co-administration of Al and H. scoparia showed better improvement in cerebrum and cerebellum histology than that in Al + MA group.

In conclusion, the results of the present study indicate that H. scoparia extract is a potential formulation which can be used for treatment of Al neurotoxicity. It shows more efficient recovery from the toxicant-induced oxidative damage, histopathological changes and AChE activity inhibition than MA.

Conflict of interest statement

Acknowledgments

References

[1] Walton JR. Evidence that ingested aluminum additives contained in processed foods and alum-treated drinking water are a major risk factor for Alzheimers disease. *Curr Inorg Chem* 2012; **2**: 19-39.

[2] Yokel RA, McNamara PJ. Aluminum toxicokinetics: an updated minireview. *Pharmacol Toxicol* 2001; **88**(4): 159-67.

[3] Sahin G, Varol I, Temizer A, Benli K, Demirdamar R, Duru S. Determination of aluminum levels in the kidney, liver, and brain of mice treated with aluminum hydroxide. *Biol Trace Elem Res* 1994; **41**(1–2): 129-35.

[4] Duce JA, Bush AI. Biological metals and Alzheimer's disease: implications for therapeutics and diagnostics. *Prog Neurobiol* 2010; **92**(1): 1-18.

[5] Exley C, House ER. Aluminium in the human brain. *Monatsh Chem* 2011; **142**: 357-63.

[6] Walton JR. Aluminum disruption of calcium homeostasis and signal transduction resembles change that occurs in aging and Alzheimer's disease. *J Alzheimers Dis* 2012; **29**(2): 255-73.

[7] Shaw CA, Tomljenovic L. Aluminum in the central nervous system (CNS): toxicity in humans and animals, vaccine adjuvants and autoimmunity. *Immunol Res* 2013; **56**(2–3): 304-16.

[8] Sánchez-Iglesias S, Soto-Otero R, Iglesias-González J, Barciela-Alonso MC, Bermejo-Barrera P, Méndez-Alvarez E. Analysis of brain regional distribution of aluminium in rats via oral and intraperitoneal administration. *J Trace Elem Med Biol* 2007; **21**(Suppl 1): 31-4.

[9] Bhadauria M. Combined treatment of HEDTA and propolis prevents aluminum induced toxicity in rats. *Food Chem Toxicol* 2012; **50**(7): 2487-95.

[10] Al-Kahtani MA. Renal damage mediated by oxidative stress in mice treated with aluminium chloride: protective effect of taurine. *J Biol Sci* 2010; **10**: 584-95.

[11] Belyaeva EA, Sokolova TV, Emelyanova LV, Zakharova IO. Mitochondrial electron transport chain in heavy metal-induced neurotoxicity: effects of cadmium, mercury, and copper. *ScientificWorldJournal* 2012; **2012**: 136063.

[12] Pohl HR, Roney N, Abadin HG. Metal ions affecting the neurological system. *Met Ions Life Sci* 2011; **8**: 247-62.

[13] Abd-Elhady RM, Elsheikh AM, Khalifa AE. Anti-amnestic properties of ginkgo biloba extract on impaired memory function induced by aluminum in rats. *Int J Dev Neurosci* 2013; **31**(7): 598-607.

[14] Hashmi AN, Yaqinuddin A, Ahmed T. Pharmacological effects of ibuprofen on learning and memory, muscarinic receptors gene expression and APP isoforms level in pre-frontal cortex of AlCl$_3$-induced toxicity mouse model. *Int J Neurosci* 2015; **125**(4): 277-87.

[15] Jamil A, Mahboob A, Ahmed T. Ibuprofen targets neuronal pentraxins expression and improves cognitive function in mouse model of AlCl$_3$-induced neurotoxicity. *Exp Ther Med* 2016; **11**(2): 601-6.

[16] Mahboob A, Farhat SM, Iqbal G, Babar MM, Zaidi NU, Nabavi SM, et al. Alpha-lipoic acid-mediated activation of muscarinic receptors improves hippocampus- and amygdala-dependent memory. *Brain Res Bull* 2016; **122**: 19-28.

[17] Kawahara M, Kato-Negishi M. Link between aluminum and the pathogenesis of Alzheimer's disease: the integration of the aluminum and amyloid cascade hypotheses. *Int J Alzheimers Dis* 2011; **2011**: 276393.

[18] Walton JR. Chronic aluminum intake causes Alzheimer's disease: applying sir Austin Bradford Hill's causality criteria. *J Alzheimers Dis* 2014; **40**(4): 765-838.

[19] Kumar A, Singh A, Ekavali. A review on Alzheimer's disease pathophysiology and its management: an update. *Pharmacol Rep* 2015; **67**: 195-203.

[20] Garcia T, Esparza JL, Nogués MR, Romeu M, Domingo JL, Gómez M. Oxidative stress status and RNA expression in hippocampus of an animal model of Alzheimer's disease after chronic exposure to aluminum. *Hippocampus* 2010; **20**(1): 218-25.

[21] Ramírez-Altamirano MDJ, Fenton-Navarro P, Sivet-Chiñas E, Harp-Iturribarria FDM, Martínez-Cruz R, Cruz PH, et al. The relationship of aluminium and silver to neural tube defects; a case control. *Iran J Pediatr* 2012; **22**(3): 369-74.

[22] Colomina MT, Roig JL, Sánchez DJ, Domingo JL. Influence of age on aluminum-induced neurobehavioral effects and morphological changes in rat brain. *Neurotoxicology* 2002; **23**: 775-81.

[23] Niu PY, Niu Q, Zhang QL, Wang LP, He SE, Wu TC, et al. Aluminum impairs rat neural cell mitochondria *in vitro. Int J Immunopathol Pharmacol* 2005; **18**(4): 683-9.

[24] Kaur A, Joshi K, Minz RW, Gill KD. Neurofilament phosphorylation and disruption: a possible mechanism of chronic aluminium toxicity in Wistar rats. *Toxicology* 2006; **219**(1–3): 1-10.

[25] Kumar A, Dogra S, Prakash A. Protective effect of curcumin (*Curcuma longa*), against aluminium toxicity: possible behavioral and biochemical alterations in rats. *Behav Brain Res* 2009; **205**: 384-90.

[26] Yuan CY, Lee YJ, Hsu GS. Aluminum overload increases oxidative stress in four functional brain areas of neonatal rats. *J Biomed Sci* 2012; **19**: 51.

[27] Walton JR. Aluminum involvement in the progression of Alzheimer's disease. *J Alzheimers Dis* 2013; **35**(1): 7-43.

[28] Giorgianni CM, D'Arrigo G, Brecciaroli R, Abbate A, Spatari G, Tringali MA, et al. Neurocognitive effects in welders exposed to aluminium. *Toxicol Ind Health* 2014; **30**(4): 347-56.

[29] Chaitanya TV, Mallipeddi K, Bondili JS, Nayak P. Effect of aluminum exposure on superoxide and peroxide handling capacities by liver, kidney, testis and temporal cortex in rat. *Indian J Biochem Biophys* 2012; **49**(5): 395-8.

[30] Kumar A, Prakash A, Dogra S. Neuroprotective effect of carvedilol against aluminium induced toxicity: possible behavioral and biochemical alterations in rats. *Pharmacol Rep* 2011; **63**(4): 915-23.

[31] Lu XT, Liang RF, Jia ZJ, Wang H, Song WF, Li QY, et al. [Effect of aluminum exposure on cognitive function in electrolytic workers and its influential factors]. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 2013; **31**(2): 113-6. Chinese.

[32] Kumar V, Gill KD. Oxidative stress and mitochondrial dysfunction in aluminium neurotoxicity and its amelioration: a review. *Neurotoxicology* 2014; **41**: 154-66.

[33] Nunomura A, Perry G, Hirai K, Aliev G, Takeda A, Chiba S, et al. Neuronal RNA oxidation in Alzheimer's disease and Down's syndrome. *Ann N Y Acad Sci* 1999; **893**: 362-4.

[34] Yin Z, Lee E, Ni M, Jiang H, Milatovic D, Rongzhu L, et al. Methylmercury-induced alterations in astrocyte functions are attenuated by ebselen. *Neurotoxicology* 2011; **32**(3): 291-9.

[35] Sharma DR, Wani WY, Sunkaria A, Kandimalla RJ, Verma D, Cameotra SS, et al. Quercetin protects against chronic aluminum-induced oxidative stress and ensuing biochemical, cholinergic, and neurobehavioral impairments in rats. *Neurotox Res* 2013; **23**(4): 336-57.

[36] Asada T, Takaya S, Takayama Y, Yamauchi H, Hashikawa K, Fukuyama H. Reversible alcohol-related dementia: a five-year follow-up study using FDG-PET and neuropsychological tests. *Intern Med* 2010; **49**(4): 283-7.

[37] Bourogaa E, Nciri R, Mezghani-Jarraya R, Racaud-Sultan C, Damak M, El Feki A. Antioxidant activity and hepatoprotective potential of *Hammada scoparia* against ethanol-induced liver injury in rats. *J Physiol Biochem* 2013; **69**: 227-37.

[38] El-Shazly A, Wink M. Tetrahydroisoquinoline and beta-carboline alkaloids from *Haloxylon articulatum* (Cav.) Bunge (Chenopodiaceae). *Z Naturforsch C* 2003; **58**: 477-80.

[39] Saidi SA, Bourogâa E, Bouaziz A, Mongi S, Chaaben R, Jamoussi K, et al. Protective effects of *Hammada scoparia* flavonoid-enriched fraction on liver injury induced by warm ischemia/reperfusion. *Pharm Biol* 2015; **53**(12): 1810-7.

[40] Mezghani-Jarraya R, Hammami H, Ayadi A, Damak M. Molluscicidal activity of *Hammada scoparia* (Pomel) lljin leaf extracts and the principal alkaloids isolated from them against *Galba truncatula*. *Mem Inst Oswaldo Cruz* 2009; **104**: 1035-8.

[41] Bourogaa E, Bertrand J, Despeaux M, Jarraya R, Fabre N, Payrastre L, et al. *Hammada scoparia* flavonoids and rutin kill adherent and chemoresistant leukemic cells. *Leuk Res* 2011; **35**: 1093-101.

[42] Abraham GE, Flechas JD. Management of fibromyalgia: rationale for the use of magnesium and malic acid. *J Nutr Med* 1992; **3**: 49-59.

[43] Russell IJ, Michalek JE, Flechas JD, Abraham GE. Treatment of fibromyalgia syndrome with Super Malic: a randomized, double blind, placebo controlled, crossover pilot study. *J Rheumatol* 1995; **22**(5): 953-8.

[44] Llobet JM, Domingo JL, Gómez M, Tomás JM, Corbella J. Acute toxicity studies of aluminium compounds: antidotal efficacy of several chelating agents. *Pharmacol Toxicol* 1987; **60**(4): 280-3.

[45] Täckholm V. *Students' flora of Egypt*. 2nd ed. Beirut: Cairo University and Cooperative Printing Company; 1974, p.127.

[46] Boulos L. *Flora of Egypt*. Azollaceae-Oxalidaceae, Vol. 1. Cairo: Al Hadara Publishing; 1999, p. 123.

[47] Millan MJ. The neurobiology and control of anxious states. *Prog Neurobiol* 2003; **70**: 83-244.

[48] Cauli O, Morelli M. Subchronic caffeine administration sensitizes rats to the motor-activating effects of dopamine D(1) and D(2) receptor agonists. *Psychopharmacology (Berl)* 2002; **162**: 246-54.

[49] Viana MB, Tomaz C, Graeff FG. The elevated T-maze: a new animal model of anxiety and memory. *Pharmacol Biochem Behav* 1994; **49**: 549-54.

[50] Graeff FG, Netto CF, Zangrossi H Jr. The elevated T-maze as an experimental model of anxiety. *Neurosci Biobehav Rev* 1998; **23**: 237-46.

[51] Dhingra D, Parle M, Kulkarni SK. β-Alanine protects mice from memory deficits induced by ageing, scopolamine, diazepam and ethanol. *Indian J Pharm Sci* 2006; **68**: 216-21.

[52] Kulkarni KS, Kasture SB, Mengi SA. Efficacy study of *Prunus amygdalus* (almond) nuts in scopolamine-induced amnesia in rats. *Indian J Pharmacol* 2010; **42**: 168-73.

[53] Silva AF, Aguiar MS, Carvalho OS, Santana Lde N, Franco EC, Lima RR, et al. Hippocampal neuronal loss, decreased GFAP immunoreactivity and cognitive impairment following experimental intoxication of rats with aluminum citrate. *Brain Res* 2013; **1491**: 23-33.

[54] Okhawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; **95**(2): 351-8.

[55] Aebi H. Catalase. In: Bergmeyer HU, editor. *Methods of enzymatic analysis*. Vol. 2. 2nd ed. Weiheim: Verlag Chemie; 1974, p. 673-84.

[56] Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem* 1968; **25**(1): 192-205.

[57] David H, Richard JS. *Methods of enzymatic analysis*. 1st ed. Bergmeyer J, Grab M, editors. Beach Floride: Verlag Chemie Weinheim Deer Field; 1983, p. 358.

[58] Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. *Science* 1973; **179**: 588-90.

[59] Ellman GL, Courtney KD, Andres V Jr, Feather-stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961; **7**: 88-95.

[60] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**(1): 265-75.

[61] Julka D, Vasishta RK, Gill KD. Distribution of aluminum in different brain regions and body organs of rat. *Biol Trace Elem Res* 1996; **52**(2): 181-92.

[62] Tripathi S, Mahdi AA, Hasan M, Mitra K, Mahdi F. Protective potential of *Bacopa monniera* (Brahmi) extract on aluminum induced cerebellar toxicity and associated neuromuscular status in aged rats. *Cell Mol Biol (Noisy-le-grand)* 2011; **57**(1): 3-15.

[63] Lal B, Gupta A, Gupta A, Murthy RC, Ali MM, Chandra SV. Aluminum ingestion alters behaviour and some neurochemicals in rats. *Indian J Exp Biol* 1993; **31**(1): 30-5.

[64] Yellamma K, Saraswathamma S, Kumari BN. Cholinergic system under aluminium toxicity in rat brain. *Toxicol Int* 2010; **17**(2): 106-12.

[65] Sethi P, Jyoti A, Singh R, Hussain E, Sharma D. Aluminium-induced electrophysiological, biochemical and cognitive modifications in the hippocampus of aging rats. *Neurotoxicology* 2008; **29**: 1069-79.

[66] Ribes D, Colomina MT, Vicens P, Domingo JL. Impaired spatial learning and unaltered neurogenesis in a transgenic model of Alzheimer's disease after oral aluminum exposure. *Curr Alzheimer Res* 2010; **7**(5): 401-8.

[67] Linardaki ZI, Orkoula MG, Kokkosis AG, Lamari FN, Margarity M. Investigation of the neuroprotective action of saffron (*Crocus sativus* L.) in aluminum-exposed adult mice through behavioral and neurobiochemical assessment. *Food Chem Toxicol* 2013; **52**: 163-70.

[68] Connor DJ, Harrell LE, Jope RS. Reversal of an aluminum-induced behavioral deficit by administration of deferoxamine. *Behav Neurosci* 1989; **103**(4): 779-83.

[69] Antunes M, Biala G. The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn Process* 2012; **13**(2): 93-110.

[70] Shimizu S, Mizuguchi Y, Sobue A, Fujiwara M, Morimoto T, Ohno Y. Interaction between anti-Alzheimer and antipsychotic drugs in modulating extrapyramidal motor disorders in mice. *J Pharmacol Sci* 2015; **127**(4): 439-45.

[71] Moodley KK, Chan D. The hippocampus in neurodegenerative disease. *Front Neurol Neurosci* 2014; **34**: 95-108.

[72] Klein-Koerkamp Y, Heckemann RA, Ramdeen KT, Moreaud O, Keignart S, Krainik A, et al. Amygdalar atrophy in early Alzheimer's disease. *Curr Alzheimer Res* 2014; **11**(3): 239-52.

[73] Kumar S. Aluminium-induced changes in the rat brain serotonin system. *Food Chem Toxicol* 2002; **40**(12): 1875-80.

[74] Abu-Taweel GM, Ajarem JS, Ahmad M. Neurobehavioral toxic effects of perinatal oral exposure to aluminum on the developmental motor reflexes, learning, memory and brain neurotransmitters of mice offspring. *Pharmacol Biochem Behav* 2012; **101**(1): 49-56.

[75] Singla N, Dhawan DK. Regulatory role of zinc during aluminium-induced altered carbohydrate metabolism in rat brain. *J Neurosci Res* 2012; **90**(3): 698-705.

[76] Lakshmi BV, Sudhakar M, Aniska M. Neuroprotective role of hydroalcoholic extract of *Vitis vinifera* against aluminium-induced oxidative stress in rat brain. *Neurotoxicology* 2014; **41**: 73-9.

[77] Nachmansohn D. *Chemical and molecular basis of nerve activity*. New York: Academic Press; 1975, p. 120.

[78] Poulin B, Butcher A, McWilliams P, Bourgognon JM, Pawlak R, Kong KC, et al. The M3-muscarinic receptor regulates learning and memory in a receptor phosphorylation/arrestin-dependent manner. *Proc Natl Acad Sci U S A* 2010; **107**(20): 9440-5.

[79] Hu H, Yang YJ, Li XP, Chen GH. [Effect of aluminum chloride on motor activity and species-typical behaviors in mice]. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 2005; **23**(2): 132-5. Chinese.

[80] Batool Z, Sadir S, Liaquat L, Tabassum S, Madiha S, Rafiq S, et al. Repeated administration of almonds increases brain acetylcholine levels and enhances memory function in healthy rats while attenuates memory deficits in animal model of amnesia. *Brain Res Bull* 2016; **120**: 63-74.

[81] Baraldi T, Schöwe NM, Balthazar J, Monteiro-Silva KC, Albuquerque MS, Buck HS, et al. Cognitive stimulation during lifetime and in the aged phase improved spatial memory, and altered neuroplasticity and cholinergic markers of mice. *Exp Gerontol* 2013; **48**(8): 831-8.

[82] Park D, Yang YH, Bae DK, Lee SH, Yang G, Kyung J, et al. Improvement of cognitive function and physical activity of aging mice by human neural stem cells over-expressing choline acetyltransferase. *Neurobiol Aging* 2013; **34**(11): 2639-46.

[83] Kumar S. Aluminium-induced biphasic effect. *Med Hypotheses* 1999; **52**(6): 557-9.

[84] Szutowicz A, Bielarczyk H, Kisielevski Y, Jankowska A, Madziar B, Tomaszewicz M. Effects of aluminum and calcium on acetyl-CoA metabolism in rat brain mitochondria. *J Neurochem* 1998; **71**(6): 2447-53.

[85] Ravi SM, Prabhu BM, Raju TR, Bindu PN. Long-term effects of postnatal aluminium exposure on acetylcholinesterase activity and biogenic amine neurotransmitters in rat brain. *Indian J Physiol Pharmacol* 2000; **44**(4): 473-8.

[86] Holtzman D, Hsu JS, Desautel M. Absence of effects of lead feedings and growth-retardation on mitochondrial and microsomal cytochromes in the developing brain. *Toxicol Appl Pharmacol* 1981; **58**: 48-56.

[87] Prakash A, Shur B, Kumar A. Naringin protects memory impairment and mitochondrial oxidative damage against aluminum-induced neurotoxicity in rats. *Int J Neurosci* 2013; **123**(9): 636-45.

[88] Thirunavukkarasu SV, Venkataraman S, Raja S, Upadhyay L. Neuroprotective effect of *Manasamitra vatakam* against aluminium induced cognitive impairment and oxidative damage in the cortex and hippocampus of rat brain. *Drug Chem Toxicol* 2012; **35**(1): 104-15.

[89] Wu ZH, Du YM, Xue H, Wu YS, Zhou B. Aluminum induces neurodegeneration and its toxicity arises from increased iron accumulation and reactive oxygen species (ROS) production. *Neurobiol Aging* 2012; **33**(1): 199.

[90] Kakad VD, Mohan M, Kasture VS, Kasture SB. Effect of *Vitis vinifera* on memory and behaviour mediated by monoamines. *J Nat Remed* 2008; **8**(2): 164-72.

[91] Strunecka A, Grof P. The role of glucose in the pathogenesis of Alzheimer's disease revisited: what does it tell us about the therapeutic use of lithium? *Cent Nerv Syst Agents Med Chem* 2006; **6**(2): 175-92.

[92] Belaïd-Nouira Y, Bakhta H, Bouaziz M, Flehi-Slim I, Haouas Z, Ben Cheikh H. Study of lipid profile and parieto-temporal lipid peroxidation in AlCl₃ mediated neurotoxicity modulatory effect of fenugreek seeds. *Lipids Health Dis* 2012; **11**: 16.

[93] Chen TJ, Hung HS, Wang DC, Chen SS. The protective effect of Rho-associated kinase inhibitor on aluminum-induced neurotoxicity in rat cortical neurons. *Toxicol Sci* 2010; **116**(1): 264-72.

[94] Moumen R, Ait-Oukhatar N, Bureau F, Fleury C, Bouglé D, Arhan P, et al. Aluminium increases xanthine oxidase activity and disturbs antioxidant status in the rat. *J Trace Elem Med Biol* 2001; **15**(2–3): 89-93.

[95] Nehru B, Anand P. Oxidative damage following chronic aluminium exposure in adult and pup rat brains. *J Trace Elem Med Biol* 2005; **19**(2–3): 203-8.

[96] Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* 2006; **160**(1): 1-40.

[97] Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. *Toxicology* 2011; **283**(2–3): 65-87.

[98] Azadeh M, Abdollahi M. A systematic review on oxidant/antioxidant imbalance in aluminium toxicity. *Int J Pharmacol* 2011; **7**(1): 12-21.

[99] Nayak P, Sharma SB, Chowdary NV. Augmentation of aluminum-induced oxidative stress in rat cerebrum by presence of pro-oxidant (graded doses of ethanol) exposure. *Neurochem Res* 2010; **35**: 1681-90.

[100] Benkrief R, Brum-Bousquet M, Tillequin F, Koch M. [Alkaloids and flavonoid from aerial parts of *Hammada articulata* ssp. *scoparia*]. *Ann Pharm Fr* 1990; **48**: 219-24. French.

[101] Zaky A, Mohammad B, Moftah M, Kandeel KM, Bassiouny AR. Apurinic/apyrimidinic endonuclease 1 is a key modulator of aluminum-induced neuroinflammation. *BMC Neurosci* 2013; **14**: 26.

[102] Abdel Moneim AE. Evaluating the potential role of pomegranate peel in aluminum-induced oxidative stress and histopathological alterations in brain of female rats. *Biol Trace Elem Res* 2012; **150**(1–3): 328-36.

[103] Prakash D, Gopinath K, Sudhandiran G. Fisetin enhances behavioral performances and attenuates reactive gliosis and inflammation during aluminum chloride-induced neurotoxicity. *Neuromolecular Med* 2013; **15**: 192-208.

[104] Sood PK, Nahar U, Nehru B. Curcumin attenuates aluminum-induced oxidative stress and mitochondrial dysfunction in rat brain. *Neurotox Res* 2011; **20**(4): 351-61.

[105] Viezeliene D, Beekhof P, Gremmer E, Rodovicius H, Sadauskiene I, Jansen E, et al. Selective induction of IL-6 by aluminum-induced oxidative stress can be prevented by selenium. *J Trace Elem Med Biol* 2013; **27**: 226-9.

[106] Bihaqi SW, Sharma M, Singh AP, Tiwari M. Neuroprotective role of *Convolvulus pluricaulis* on aluminium induced neurotoxicity in rat brain. *J Ethnopharmacol* 2009; **124**(3): 409-15.

[107] Matyja E. Aluminum enhances glutamate-mediated neurotoxicity in organotypic cultures of rat hippocampus. *Folia Neuropathol* 2000; **38**(2): 47-53.

[108] Sumathi T, Shobana C, Mahalakshmi V, Sureka R, Subathra M, Vishali A, et al. Oxidative stress in brain of male rats intoxicated with aluminium and neuromodulating effect of *Celastrus paniculatus* alcoholic seed extract. *Asian J Pharm Clin Res* 2013; **6**(Suppl 3): 80-90.

[109] Brodal P, editor. *The central nervous system: structure and function*. 3rd ed. Oxford: Oxford University Press; 2003.

[110] Ponsar C, Florence A, Gauthier A, Crichton R, van den Bosch de Aguilar P. Degenerative changes induced in the rat brain by administration of aluminium citrate: a model for the study of cerebral ageing involution. *Behav Process* 1993; **29**: 139-40.

[111] Struys-Ponsar C, Florence A, Gauthier R, Crichton RR, van den Bosch de Aguilar P. Ultrastructural changes in brain parenchyma during normal aging and in animal models of aging. *J Neural Transm Suppl* 1994; **44**: 111-32.

[112] Bhalla P, Dhawan DK. Protective role of lithium in ameliorating the aluminium-induced oxidative stress and histological changes in rat brain. *Cell Mol Neurobiol* 2009; **29**: 513-21.