ABSTRACT

Objective: The goal of the current work is to study the possible neuroprotective effect of aqueous extract of clove (Syzygium aromaticum) on ions homeostasis, acetylcholinesterase (AChE) activity and the oxidative status in different brain regions of adult male Wistar albino rats which were intoxicated by aluminium chloride (AlCl₃).

Methods: Rats were divided into four groups, Group I was received normal saline; Group II was administered orally with AlCl₃ (150 mg/kg b. wt.); Group III was received aqueous extract of clove (200 mg/kg b. wt.) and Group IV was received combined treatment with AlCl₃ and clove. All the groups were treated for 14 days.

Results: The treatment with AlCl₃ caused a significant elevation in the concentration of aluminium (Al) and calcium (Ca²⁺) ions, AChE, malondialdehyde (MDA) and nitrite/nitrate levels, while a significant reduction in the level of magnesium (Mg²⁺) and sodium (Na⁺) ions, reduced glutathione (GSH) and glutathione peroxidase (GPx) activity was observed. Meanwhile, the combined treatment with AlCl₃ and clove was found to restore the investigated parameters to be near the normal values.

Conclusion: The results presented here, indicate that the toxic effects of AlCl₃ could be mediated through modifying the intracellular brain ions homeostasis, cholinergic dysfunction and oxidative damage in rat brains which may lead to impaired neuronal function. Taken together the results of this study also showed that clove offers neuroprotection against AlCl₃-induced neurotoxicity.

Keywords: Clove, Aluminium, Ions, Acetylcholinesterase, Oxidative stress and Rats

INTRODUCTION

Al is the third common element in the earth's crust, it represents 8% of total components [1]. Al is available to humans through drinking water, Al vessels, Al foils used in food packaging, and higher levels of Al in food and beverages such as tea [2, 3]. Al ions have the ability to change properties of cellular structure of membranes and affect different enzymes like alkaline phosphatase, AChE, and adenyl cyclase [4, 5]. It has the potential to cause neurological disorders in human and animals, it's accumulation in the brain has been linked to various neurodegenerative diseases [6, 7].

Al has been implicated in Alzheimer's disease (AD), Parkinsonism, Dementia complex and causes extensive damage to the nervous system, but the precise molecular mechanisms responsible for its neurotoxicity is still unknown. Moreover, the chronic exposure to Al may result in mood changes, dysmnesia, convulsions, muscular weakness, and pathological fractures of bones [8, 9]. Several studies have reported that the administration of Al was found to enhance the oxidative stress in the brain of rats [10-12].

Clove is the dried reddish brown flower bud of Syzygium aromaticum (Family: Myrtaceae). It contains volatile oil (14% - 21%), tannin (10% - 13%), phenol, sesquiterpene ester and alcohol [13]. The most important constituent of clove is the phenylpropene eugenol which gives this spice its pungent, distinctive aroma. Eugenol makes up 70 % to 90 % of the essential oil and 15 % of the dry weight of clove buds [14].

Studies have attributed therapeutic properties to clove such as aphrodisiac, stomachic, carminative, antispasmodic, anti-inflammatory, antioxidant, anti-hyperglycemic, anti-stress, anti-mutagenic, and allelopathic, as well as antiseptic and anesthetic to relieve toothache among other pains. It is also reported to be useful in conceiving in high doses and acts as a contraceptive in low doses and useful in cataract [15-17]. But there are few studies about the effect of clove on the neuronal activities; therefore, the main aim of this work is to study the potential neuroprotective effect of aqueous extract of clove on AlCl₃-induced neurotoxicity through the estimation of brain ions homeostasis, AChE activity, the level of MDA, nitrite/nitrate, GSH and GPx activity.

MATERIALS AND METHODS

I- MATERIALS

Aluminum chloride was purchased from Sigma (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade. Double-distilled water was used as the solvent.

Clove flower bud aqueous Extract preparation

Dried flower buds of clove were identified by the Department of Botany, Faculty of Science, Helwan University and powdered (2 mm mesh size). 10 g crude powder was mixed in 50 ml of double-distilled water, and the mixture was left over night. The mixture was then filtered and stored at 4°C till further use [18].

Animals

The experimental animals used in this study were forty adult male Wistar albino rats weighing 150-170g purchased from the breeding unit of Holding Company for Biological Products and Vaccines, "VACSERA", Cairo-Egypt. Rats were bred under specified pathogen-free conditions and fed a standard diet and water ad libitum which were approved by state authorities and followed Egyptian rules for animal protection.

Experimental design

Animals were allocated to four groups of ten rats each. First one was served as a control group (Ctrl) and was received water by oral gavage. The second group was treated with AlCl₃ (Al) (150 mg/kg b.wt.) [19]. The aqueous extract of clove (CV) (200 mg/kg b.wt.) [20] was administered to rats of the 3rd group. Meanwhile, the rats of 4th group were gavaged with AlCl₃ and after one hour, the same group was
administered aqueous extract of dried flower bud of clove (AICV). All groups were treated for 14 consecutive days. Animals were sacrificed by sudden decapitation on the 14th day. Heads of sacrificed animals were immediately dissected after decapitation and brains were rapidly excised from skulls, blotted and chilled. The brain tissue was rapidly wiped dry with filter paper. Dissection was performed on an ice-cooked glass plate. Brains were separated into cerebral cortex, cerebellum, brainstem and hippocampus according to [21].

II- Methods

Brain regions were weighed and wrapped in plastic films then in aluminium foil and quickly frozen in a refrigerator (-70°C) till used for brain ions and biochemical estimations.

Estimation of brain ions

According to Murphy [22], Al, Ca²⁺, Na⁺ and Mg²⁺ ions were estimated in brain regions at room temperature for 24 hours, removed from plastic pieces and transferred to dry tube. Then, 2.5ml of piperidine was added, and the tissue was incubated for 24 hours at 60°C when no tissue was visible.

The tubes were cooled at room temperature then 1ml of perchloric acid was added to precipitate most of the protein. After 10 minutes, 3.5ml of deionized-distilled water was added and mixed. Fifteen minutes later the tubes were centrifuged for 10 minutes at 16000 r.p.m. in an ultra centrifuge. Aliquots of supernatant were used for ion analysis in an atomic absorption spectrometer.

Estimation of AChE and oxidative stress status in brain homogenate

The brain regions were homogenized in ice-cold medium containing 50 mM Tris–HCl and 300 mM sucrose, pH 7.4 [23] for the estimation of AChE activity [24], the level of MDA [25], nitrite/nitrate levels [26], GSH content [27] and the GPx activity [28].

Statistical analysis

The obtained data were presented as means ± standard error. One-way ANOVA was carried out, and the statistical comparisons among the groups were performed with Duncan’s test using a statistical package program (SPSS version 17.0). \( P \leq 0.05 \) was considered as significant for all statistical analysis in this study.
AlCl₃ treatment induced a disturbance in ions homeostasis in rat brains. It caused an increase in the concentration of Al and Ca²⁺ ions in most of the examined brain regions. The disturbance in Ca²⁺ homeostasis was one of the earliest molecular changes which can affect synaptic transmission and hence cognition that occur in AD patients where the Ca²⁺ level were elevated in different brain regions. The increment may be due to Ca²⁺ release from intracellular stores, a process reported to be triggered in the presence of metals. It has been investigated that AlCl₃ has the ability to inhibit the activities of Na⁺ channels in hippocampus, leading to the decrease of Na⁺ influx which may delay the generation and conduction of electrical signals in neurons; this process made the cells persisted in depolarizing state and facilitated energy expenditure. Mg²⁺ deficiency was found to be present in several chronic, age-related diseases and neurodegenerative diseases such as AD, and lead to specific impairments in emotional memory, particularly in the hippocampus and it is associated with high Al incorporation into brain neurons. Therefore, concluded that the increment of brain Mg²⁺ level leads to the enhancement of short term synaptic facilitation, improves learning, memory functions in rats and may have therapeutic potential for treating AD in humans. In the present study, the clove gavage to AlCl₃-intoxicated rats induced a recovery in Al, Ca²⁺, Na⁺ and Mg²⁺ levels in most brain regions under investigation. The disturbance in Ca²⁺ homeostasis was one of the earliest molecular changes which can affect synaptic transmission and hence cognition that occur in AD patients where the Ca²⁺ level were elevated in different brain regions. The increment may be due to Ca²⁺ release from intracellular stores, a process reported to be triggered in the presence of metals.

The disturbance in Ca²⁺ homeostasis was one of the earliest molecular changes which can affect synaptic transmission and hence cognition that occur in AD patients where the Ca²⁺ level were elevated in different brain regions. The increment may be due to Ca²⁺ release from intracellular stores, a process reported to be triggered in the presence of metals.

DISCUSSION
Several studies have reported that the exposure to Al is associated with neurotoxicity. The disturbance in the metal homeostasis has been recorded in many of neurologic dysfunctions. Considering the results of the present study, it seems that Al and Ca²⁺ levels were increased, while the levels of Na⁺ and Mg²⁺ were elevated in all brain regions of AlCl₃-intoxicated rats. Several studies have demonstrated that Al exposure leads to the increase in the levels of AD patients where the Ca²⁺ level were elevated in different brain regions. The increment may be due to Ca²⁺ release from intracellular stores, a process reported to be triggered in the presence of metals. Mg²⁺ deficiency was found to be present in several chronic, age-related diseases and neurodegenerative diseases such as AD, and lead to specific impairments in emotional memory, particularly in the hippocampus and it is associated with high Al incorporation into brain neurons. Therefore, concluded that the increment of brain Mg²⁺ level leads to the enhancement of short term synaptic facilitation, improves learning, memory functions in rats and may have therapeutic potential for treating AD in humans. In the present study, the clove gavage to AlCl₃-intoxicated rats induced a recovery in Al, Ca²⁺, Na⁺ and Mg²⁺ levels in most brain regions under investigation. The disturbance in Ca²⁺ homeostasis was one of the earliest molecular changes which can affect synaptic transmission and hence cognition that occur in AD patients where the Ca²⁺ level were elevated in different brain regions. The increment may be due to Ca²⁺ release from intracellular stores, a process reported to be triggered in the presence of metals.

Table 1: It shows the effect of aqueous extract of clove (200 mg/kg b.wt.) and/or AlCl₃ (150 mg/kg b.wt.) on the level of MDA (nmol/g tissue) in brain homogenates of adult male Wistar albino rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cerebral Cortex</th>
<th>Cerebellum</th>
<th>Brainstem</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl</td>
<td>11.19 ± 0.87</td>
<td>7.23 ± 0.21</td>
<td>10.94 ± 0.27</td>
<td>13.44 ± 1.52</td>
</tr>
<tr>
<td>Al</td>
<td>21.42 ± 1.33 a</td>
<td>15.85 ± 1.08 a</td>
<td>21.59 ± 0.56 a</td>
<td>23.6 ± 2.11 a</td>
</tr>
<tr>
<td>CV</td>
<td>13.71 ± 0.72</td>
<td>8.16 ± 0.55</td>
<td>11.29 ± 0.47</td>
<td>14.23 ± 1.53</td>
</tr>
<tr>
<td>AlCV</td>
<td>16.18 ± 2.37 a</td>
<td>10.76 ± 0.18 a</td>
<td>17.63 ± 0.41 a</td>
<td>17.85 ± 1.75 a</td>
</tr>
</tbody>
</table>

Values are represented as means ± SE, n = 10; a: Significant against control group at P ≤ 0.05; b: Significant against AlCl₃ treated group at P ≤ 0.05.
Moreover, eugenol and isoeugenol attenuated the levels of nitrite/nitrate, MDA and reactive oxygen species in acrylamide-induced oxidative stress in rat’s brain [55, 76]. Moreover, [13] indicated that acute administration of an ethanolic extract of clove enhances the learning and memory recall processes in mice which support the anti-oxidative, anti-amyloid-β peptide activity and cholinomimetic action of its eugenol component. It could be concluded that oral administration of AlCl₃ for 14 days caused a disturbance in ions homeostasis, impaired AlCl₃ activity and it was found to cause oxidative stress in the studied brain regions, on the other hand, the clove aqueous extract alleviated AlCl₃-induced neurotoxicity.

**REFERENCES**

28. Prabhu SD, Salama G. The heavy metals ions Ag+ and Hg2+ trigger Ca2+ release from cardiac sarcoplasmic reticulum. Arch Biochem Biophys. 1990; 277:47–55.


