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Original Article

THE NEUROPROTECTIVE EFFICENCY OF THE AQUEOUS EXTRACT OF CLOVE (*SYZYGIUM AROMATICUM*) IN ALUNINIUM-INDUCED NEUROTOXICITY

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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 ($AlCl_3$).

Methods: Rats were divided into four groups, Group I was received normal saline; Group II was administered orally with $AlCl_3$ (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 $AlCl_3$ and clove. All the groups were treated for 14 days.

Results: The treatment with $AlCl_3$ caused a significant elevation in the concentration of aluminium (Al) and calcium (Ca^{2+}) ions, AChE, malondialdehyde (MDA) and nitrite/nitrate levels, while a significant reduction in the level of magnisium (Mg^{+}) and sodium (Na^{+}) ions, reduced glutathione (GSH) and glutathione peroxidase (GPx) activity was observed. Meanwhile, the combined treatment with $AlCl_3$ 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 $AlCl_3$ 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 $AlCl_3$-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, sesquoterpene 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 neuroprotoctive effect of aqueous extract of clove on $AlCl_3$-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 fourty 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 $AlCl_3$ (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 $AlCl_3$ and after one hour, the same group was

administered aqueous extract of dried flower bud of clove (AlCV). 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 cooled glass plate. Brains were separated into cerebral cortex, cerebellum, brainstem and hippocamppus 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, Ca^{2+}, Na^{+} and Mg^{+} 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.

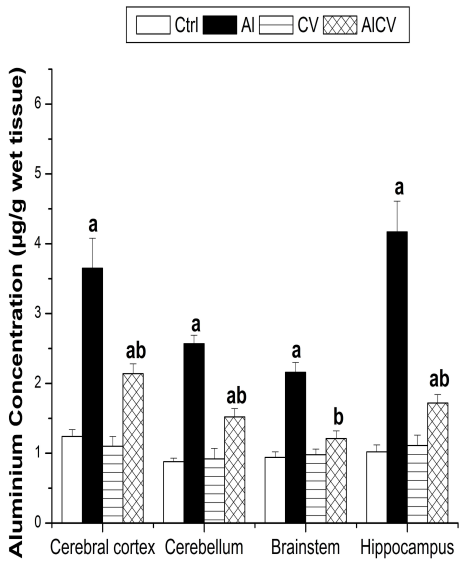

Fig. 1: It shows the aluminium ions levels (µg/g wet tissue) in brain homogenates of adult male Wistar albino rats treated with aqueous extract of clove (200 mg/kg b.wt.) and/ or AlCl3 (150 mg/kg b.wt.) for 14 days. a: Significant against control group at P ≤ 0.05; b: Significant against AlCl3 treated group at P ≤ 0.05.

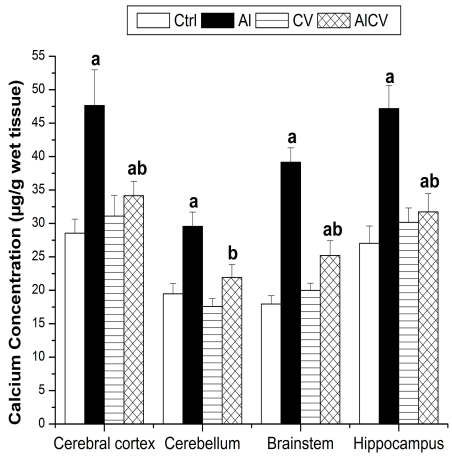

Fig. 2: It shows the changes in calcium ions (Ca2+) level (µg/g wet tissue) in brain homogenates of adult male Wistar albino rats treated with aqueous extract of clove (200 mg/kg b.wt.) and/ or AlCl3 (150 mg/kg b.wt.) for 14 days.a: Significant against control group at P ≤ 0.05; b: Significant against AlCl3 treated group at P ≤ 0.05.

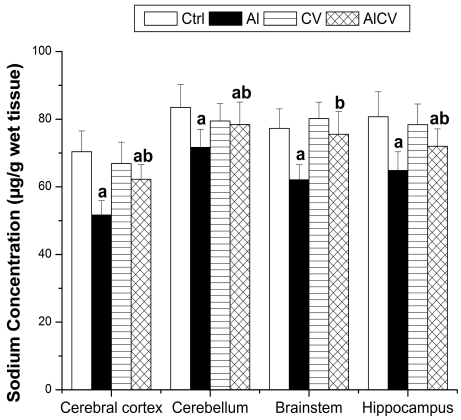

Fig. 3: It shows the clove induced alterations in the levels of sodium ions (Na^{+}) (µg/g wet tissue) in different brain regions of adult male Wistar albino rats treated with $AlCl_3$ for 14 days.a: Significant against control group at *P ≤ 0.05*; b: Significant against $AlCl_3$ treated group at *P ≤ 0.05*.

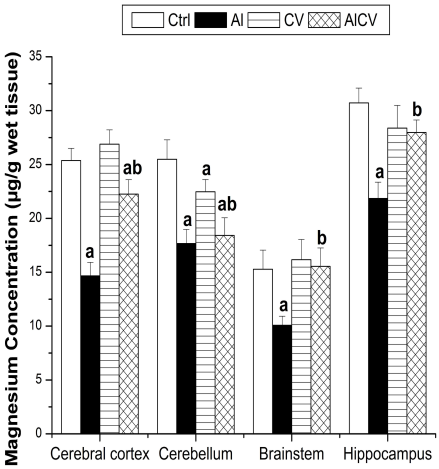

Fig. 4: It shows the the neuroprotective role of clove on magnesium ions (Mg^{2+}) (µg/g wet tissue) level in different brain regions of $AlCl_3$ -intoxicated rats for 14 days. a: Significant against control group at *P ≤ 0.05*; b: Significant against $AlCl_3$ treated group at *P ≤ 0.05*.

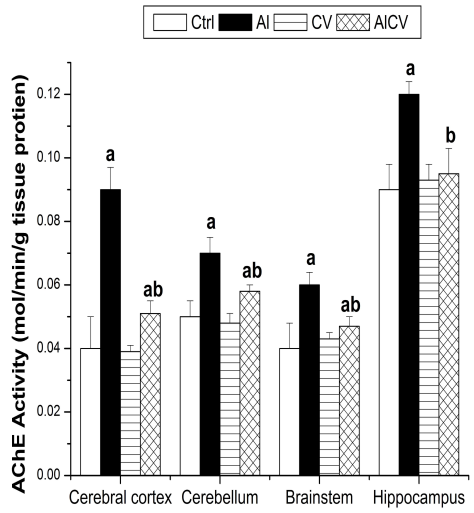

Fig. 5: It shows the acetylcholine esterase (AChE) activity (mol/min/g tissue protein) in different brain regions of adult male Wistar albino rats treated with aqueous extract of clove (200 mg/kg b.wt.) and/ or $AlCl_3$ (150 mg/kg b.wt.) for 14 days. a: Significant against control group at $P \leq 0.05$; b: Significant against $AlCl_3$ treated group at $P \leq 0.05$.

RESULTS

The data represented in Figures (1), (2), (3) and (4) showed that the oral administration of $AlCl_3$ (150 mg/kg b.wt.) for 14 consecutive days induced a disturbance in ions homeostasis in rat brains. It caused an increase in the concentration Al and Ca^{2+} ions, while a significant decrease was recorded in the levels of Na^+ and Mg^+ ions in most of the experimented brain regions at $P<0.05$ when compared to control group. On the other hand, the treated rats with the aqueous extract of clove (200 mg/kg b.wt.) exhibited a non-significant alterations in the concentrations of Al, Ca^{2+}, Na^+ and Mg^+ ions in most of tested brain regions as compared with the normal values.

Moreover, the concentration of the estimated ions in most of examined brain homogenates were reverted back to be near normal level after the treatment with $AlCl_3$ and clove together; this reflect the ameliorative effect of aqueous extract of clove against $AlCl_3$ induced neurotoxicity.

One way ANOVA revealed that, the orally treated rats with $AlCl_3$ showed a significant elevation in the levels of MDA and nitrite/nitrate, while a significant decrease in the content of GSH and GPx activity were found in the selected brain homogenates at $p<0.05$. Meanwhile, a non-significant change was noticed in the investigated parameters in most of the tested brain regions in clove treated group as compared to control group. Furthermore, the concurrent treatment with $AlCl_3$ and clove showed that the levels of the oxidative parameters returned to the normal values; these findings also proves that the aqueous extract of clove can provide the neuroprotection against the oxidative damage induced by $AlCl_3$ in rat brains (Tables 1, 2, 3 & 4).

The treatment with $AlCl_3$ induced cholinergic impairment; this was clear by elevating the activity of AChE in the experimented brain tissues, on contrast, the orally administered rats with aqueous extract of clove exhibited a non-significant variation in the activity of the enzyme as compared to control group, moreover, the activity of AChE was inhibited in the rats brain of $AlCl_3$ and clove combined treated group when compared to $AlCl_3$ group (Figure 5).

DISCUSSION

Several studies have recorded that the exposure to Al is associated with neurotoxicity [29-31]. The disturbance in the metal homeostasis has been recorded in many of neurological dysfunctions. Considering the results of the present study, it seems that Al and Ca^{2+} levels were increased, while the levels of Na^+ and Mg^+ were elevated in all brain regions of $AlCl_3$-intoxicated rats. Several studies have demonstrated that Al exposure leads to the increment of the brain content of Al in different brain regions [32-35]. Al could affect the brain function even at very low dose [36]. Since, Al could be accumulated in the human brain [37]; there are three routes that have been proposed by which Al could enter the brain from systemic circulation: blood-brain barrier by using specific transport systems as transferrin and monocarboxylate, nasalolfactory pathway and cerebrospinal fluid [38, 39].

The disturbance in Ca^{2+} homeostasis was one of the earliest molecular changes which can affect synaptic transmission and hence cognition that occur in AD patients where the Ca^{2+} level were elevated in different brain regions [40-43], these findings are in agreement with the results of the current study. It has been attributed the intracellular Ca^{2+} levels increment to the increase in depolarization-induced Ca^{2+} uptake or failure of Ca^{2+} expelling system which could be a direct result of decreased Ca^{2+} effluxing ability [41]. Moreover, the increment may be due to Ca^{2+} release from intracellular stores, a process reported to be triggered in the presence of metals [44]. It has been investigated that $AlCl_3$ 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 [37, 45]. It has been indicated that Mg^+ is essential for cell functions such as transport of Ca^{2+}, K^+; modulates signal transduction; energy metabolism and cell proliferation [46]. So, Mg^+ 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 [47-49]. Therfore, [50] and [51] concluded that the increment of brain Mg^+ 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 $AlCl_3$-intoxicated rats induced a recovery in Al, Ca^{2+}, Na^+ and Mg^+ levels in most brain regions under investigation. Eugenol, the major active conistituent of clove, inhibits amyloid-β peptide-induced excessive influx of Ca^{2+} into neurons that causes neuronal death and provides strong support for the therapeutic and prophylactic potential of herbs containing eugenol for the management of AD [52-54]. In addition, [55] recorded that the eugenol and isoeugenol inhibited Na^+ currents in the rat dorsal root of ganglion neurons through its interaction with both resting and inactivated Na^+ channels [56].

Table 1: It shows the effect of aqueous extract of clove (200 mg/kg b.wt.) and/ or $AlCl_3$ (150 mg/kg b.wt.) for 14 days on the level of MDA (nmol/g tissue) in brain homogenates of adult male Wistar albino rats.

Groups	Cerebral Cortex	Cerebellum	Brainstem	Hippocampus
Ctrl	11.19 ± 0.87	7.23 ± 0.21	10.94 ± 0.27	13.44 ± 1.52
Al	21.42 ± 1.33 [a]	15.85 ± 1.08 [a]	21.59 ± 0.56 [a]	23.6 ± 2.11 [a]
CV	13.71 ± 0.72	8.16 ± 0.55	11.29 ± 0.47	14.23 ± 1.53
AlCV	16.18 ± 2.37 [ab]	10.76 ± 0.18 [ab]	17.63 ± 0.41 [ab]	17.85 ±1.75 [ab]

Values are represented as means ± SE, n = 10, a: Significant against control group at $P \leq 0.05$, b: Significant against $AlCl_3$ treated group at $P \leq 0.05$

Table 2: It shows the level of nitrite/nitrate (µmole/g tissue) in brain homogenates of adult male Wistar albino rats treated with aqueous extract of clove (200 mg/kg b.wt.) and/ or $AlCl_3$ (150 mg/kg b.wt.) for 14 days.

Groups	Cerebral Cortex	Cerebellum	Brain Stem	Hippocampus
Ctrl	6.74 ± 0.35	14.66 ± 0.50	11.57 ± 0.25	9.26 ± 0.34
Al	10.09 ± 0.22 [a]	23.47 ± 0.95 [a]	19.59 ± 0.29 [a]	15.83 ± 0.29 [a]
CV	6.23 ± 0.29	12.42 ± 0.98	11.76 ± 0.24	8.84 ± 0.30
AlCV	6.19 ± 0.19 [b]	12.23 ± 0.93 [b]	10.99 ± 0.22 [b]	7.11 ± 0.35 [ab]

Values are represented as means ± SE, n = 10, a: Significant against control group at $P \leq 0.05$, b: Significant against $AlCl_3$ treated group at $P \leq 0.05$

Table 3: It shows the changes in the level of the reduced glutathione (µmol/g tissue) in brain homogenates of adult male Wistar albino rats treated with aqueous extract of clove (200 mg/kg b.wt.) and/ or $AlCl_3$ (150 mg/kg b.wt.) for 14 days.

Groups	Cerebral Cortex	Cerebellum	Brain Stem	Hippocampus
Ctrl	0.24 ± 0.03	1.22 ± 0.14	0.58 ± 0.03	9.42 ± 0.25
Al	0.16± 0.02 [a]	0.91± 0.08	0.35 ± 0.02 [a]	1.84 ± 0.06 [a]
CV	0.36 ± 0.02 [a]	1.32 ± 0.15	0.40 ± 0.02	10.28±0.24
AlCV	0.35 ± 0.02 [ab]	1.90± 0.06 [ab]	1.21 ± 0.13 [ab]	10.25± 0.32 [ab]

Values are represented as means ± SE, n = 10, a: Significant against control group at $P \leq 0.05$, b: Significant against $AlCl_3$ treated group at $P \leq 0.05$

Table 4: It shows the changes in the activity of glutathione peroxidase (U/g tissue) in brain homogenates of adult male Wistar albino rats treated with aqueous extract of clove (200 mg/kg b.wt.) and/ or $AlCl_3$ (150 mg/kg b.wt.) for 14 days.

Groups	Cerebral Cortex	Cerebellum	Brain Stem	Hippocampus
Ctrl	57.52± 2.26	30.89 ± 1.33	38.95 ± 2.09	67.32± 1.68
Al	20.25± 1.14 [a]	19.66± 0.73 [a]	25.75 ± 0.77 [a]	34.40± 2.79 [a]
CV	52.94± 2.59	26.54± 2.15	37.08 ± 2.69 [a]	71.46 ± 2.39
AlCV	43.18± 2.22 [ab]	23.41± 1.65 [a]	20.02 ± 3.00 [ab]	52.66± 3.12 [ab]

Values are represented as means ± SE., n = 10, a: Significant against control group at $P \leq 0.05$, b: Significant against $AlCl_3$ treated group at $P \leq 0.05$

Our results showed a significant elevation in AChE activity as a result of $AlCl_3$ gavage in all studied brain regions. These data are in agreement with several reports where attributed the increment of AChE activity *in vivo* and to a lesser extent *in vitro* to Al administration, and concurrently to aggregate amyloid accelerating the gathering of amyloid-β into fibrils [5, 57-60]. It has been suggested that the Al exposure increased AChE activity via allosteric interaction between Al and the peripheral anionic site of the enzyme molecule [61]. Al produces cholinotoxic effects by blocking the supply of acetyl-CoA, which is required for acetyl choline synthesis or by affecting the activities of choline acetyl transferase [62].On contrary, the clove administration induced a significant reduction in AChE activity in $AlCl_3$-intoxicated rats in the present study, indicating to its neuroprotective effect. It has been investigated by [63] that clove extraction inhibit the activity of AChE. In addition, [55] recorded that the eugenol and isoeugenol decreased the elevation of AChE activity and the intracellular Ca^{2+} level in cerebral cortex and cerebellum of acrylamide treated rats.Al is a non-redox active metal that is able to cause damage in the oxidative status in neurons by generating reactive oxygen species through Fe^{+2}, and considered as a factor in different neurological diseases including AD [64, 65]. The Al administration to neonatal rats induced an elevation in nitrite/nitrate level and lipid peroxidation products in different brain regions [66-70].The treatment with Al inhibits the content of GSH and markedly decreases the activity of GPx are in different brain tissues [69, 71-73] which lends support to the present data and this may prove that Al induce oxidative stress in the brain. While, the clove aqueous extract gavage for 14 consecutive days resulted in a significant increase in GSH content, GPx activity and a significant reduction in MDA andnitrite/nitrate levels in different brain regions of $AlCl_3$ treated rats., our findings are in agreement with [74-75].

The clove has the ability to decrease the oxidative stress in diabetic rats [75]. Likewise, [74] deduced that clove has triggered the secretion of antioxidant enzymes in enhanced levels which in turn stopped the oxidative damage by quenching free radicals. So, it has ability to prevent diseases associated with oxidative stress. 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 $AlCl_3$ for 14 days caused a disturbance in ions homeostasis, impaired AChE activity and it was found to cause oxidative stress in the studied brain regions, on the other hand, the clove aqueous extract alleviated $AlCl_3$-induced neurotoxicity.

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