



Research Article

ISSN : 2277-3657  
CODEN(USA) : IJPRPM

## ***Effect of aqueous *Salvia officinalis* extract on Aluminum chloride-induced neurotoxicity in female rats***

**Amina Boussadia<sup>1\*</sup>, Omar Kharoubi<sup>1</sup>, Zakia Lahouel<sup>1</sup>, Abderrezzak Benglia<sup>2</sup>,  
Abdelkader Aoues<sup>1</sup>**

<sup>1</sup> Laboratory of experimental Bio-Toxicology, Bio-Depollution, and Phytoremediation, Department of Biology, Faculty of natural and life sciences, University Oran1 ABB, Oran, Algeria.

<sup>2</sup>Laboratory of Biochemistry, Regional Military University Hospital of Oran, Algeria.

\*Email: Aminaboussadia @ yahoo.fr

---

### **ABSTRACT**

The neurotoxicity of Aluminum has been discovered in several experiments. It is a neurotoxic element involved in the etiology of some neurodegenerative diseases. Hence, the present study was designed to evaluate the neuroprotective effect of *Salvia officinalis* aqueous leaf extract (SAE) on the behavioral, histological, and biochemical alterations caused by Aluminum chloride in female Wistar rats. Chronic aluminum chloride ( $AlCl_3$ ) exposure developed behavioral deficits by decreasing locomotor activity and caused a significant reduction of spontaneous alternation. Besides,  $AlCl_3$  exposure showed a significant decrease in Acetylcholinesterase activity (-54.49%), Catalase activity (-42.48%), and a significant increase in the mean concentration of Malondialdehyde (MDA) compared to control group. Histological alterations were observed in the brain of aluminum chloride exposed group, which explains the neurodegenerative effect of aluminum on rat brain histology. SAE treatment improved behavioral and histological changes in  $AlCl_3$ -induced rats, attenuated biochemical alteration by recording an increase in Acetylcholinesterase activity at 73.68% and enhanced catalase activity by 62.7%, as well as a decrease in the MDA level of -86.81% compared to the  $AlCl_3$  group. The current study demonstrates that *Salvia officinalis* can be administered as a food additive to protect against the neurotoxicity of  $AlCl_3$  and to ameliorate behavioral and oxidative status.

**Key words:** Aluminium, *Salvia officinalis* extract, Brain, Behavior test, Histological study.

---

### **INTRODUCTION**

Aluminum is widely distributed in the ambient environment and used in various fields [1, 2]. It is present in several consumer products, including cooking utensils, drugs, cosmetics, and food additives [3-5]. However, it has no specific biological or physiological function [6] and can even induce neurotoxicity [7, 8].

Aluminum is a neurotoxic element involved in the etiology of some neurodegenerative disorders like Alzheimer's diseases [9, 10], Parkinson's diseases [11] dialysis encephalopathy, amyotrophic lateral sclerosis, and other chronic neurodegenerative diseases [12, 13].

The exposure to aluminum in the long-term could affect the neurocognitive function and dopaminergic metabolism; it could cause dendritic atrophy and behavioral deterioration [14] and may be associated with the neurofibrillary tangles and amyloid plaques of patients with Alzheimer's disease [15]. It is well known that aluminum affects the hippocampus [16] and cortex regions [17] more severely than the other areas of the central nervous system (CNS).

The effects of aluminum on the CNS were largely studied; it can exert a harmful effect on the activity of choline acetyltransferase especially in the hippocampus [18]. Moreover, aluminum was shown as being able to not only cross the hematoencephalic barrier [19] but also increase its permeability [20].

*Salvia officinalis* L., (sage) belongs to family Lamiaceae, herbaceous plants cultivated worldwide. It's well known aromatic and medicinal vegetal species largely employed in flavoring and seasoning food which grows in countries bordering the Mediterranean Sea [21, 22]. Its botanical name refers to its medicinal properties: *Salvia* is derived from the Latin word "salvage" and means "to cure".

*Salvia officinalis* L. is used as a traditional herbal medicine against various diseases, such as gastric disturbances and inflammatory processes [23]. It presents many pharmacological properties, most of them associated with its largest polyphenol content [24-26].

Various extracts of *Salvia* have been evaluated for biological effects, and their neuroprotective [27, 28], antimicrobial [29], anti-inflammatory [30, 31], immunomodulatory, antioxidant [32, 33], spasmolytic, anticancer [34, 35], and cholinergic [21, 36, 37] effects.

Earlier studies demonstrated the presence of carnosol, rosmarinic acid, oleanolic acid, ursolic acid, and in the leaves of *S.officinalis* [38]. Some properties of these compounds have already been described, such as their antioxidant activity [39, 40].

Rosmarinic and carnosic acids, which have antioxidant properties, are present in high concentrations in the sage extract [41]. The inhibition of lipid peroxidation by rosmarinic acid and carnosol [21] as well as the anti-inflammatory and pro-inflammatory activities of ursolic acid [42, 43] have already been described, too.

The present study aimed to determine the neurobiochimic and antioxidant properties of the aqueous extract of *Salvia officinalis* against chronic exposure to aluminum chloride and to investigate its potential against Aluminum-induced neurodegenerative changes in Wistar rats.

## MATERIAL AND METHODS

### Collection and identification of plant materials

The fresh leaves of *Salvia officinalis* were collected from the region of Mostaganem, North-West of Algeria during March; 2016. The taxonomic identity of the plant was confirmed by Professor **HADJAJ** in the Botany Laboratory of Oran1 University, Algeria. The plant materials were washed with distilled water than were dried for one month at room temperature.

### Preparation of aqueous extract

Aqueous extract of sage was obtained by extraction of powder leaves (50 g) with distilled water (500 ml) for 30min (two times) at 75°C. The extract was filtered then lyophilized and it was stored at -20°C before use (The yield of extracts was 9%).

### Experimental animal model and treatments

Thirty-two female Wistar rats with a weight of (65 ± 5) g were used. They were housed in the cages with four per cage and fed ad libitum. They were exposed to a 10h light:14h dark cycle and the room temperature was maintained at (24 ± 2) °C.

Animals were randomly divided into 4 experimental groups, each consisting of 8 rats:

- **Group 1 (Contol):** received intraperitoneal solutions of 0.9% saline solution (NaCl) for 3 months.
- **Group 2 (AlCl<sub>3</sub>):** were injected intraperitoneally with Aluminum chloride (AlCl<sub>3</sub>, 6H<sub>2</sub>O) once a week at a dose of 60mg/kg body weight (BW)/ml dissolved in saline solution for 3 months.
- **Group 3 (intoxicated treated group, AlCl<sub>3</sub> + SAE):** were injected with Aluminum chloride as for group 2 and received an intragastrically aqueous extract of *Salvia officinalis* at a dose of 150mg/kg BW/day for 3 months.
- **Group 4 (treated group, SAE):** received an intragastrically aqueous extract of *Salvia officinalis* dissolved in distilled water at a dose of 150mg/kg BW/day and injected intraperitoneally with saline solution for 3 months.

At the end of the experiments, the rats were sacrificed by intraperitoneal injection of pentobarbital anesthesia (10%). The brains were quickly dissected out, washed with a saline solution under the ice and weighed. Then, each brain was divided into two portions (hemispheres). The right hemispheres were immediately stored at -80°C

until biochemical assay. The second portion was fixed in formalin buffer (10%) for histopathological investigation.

### Preparation of brain homogenate

The right hemispheres of the brain were homogenized into phosphate buffer solution (PBS, 0.1M; pH=7.4) at the rate of 1g/10ml. Rat brain homogenates were centrifuged at 3000r/min for 15 min at 4°C, the resultant supernatant was centrifuged for 10min at 10000r/min and 4°C and the final supernatant obtained was used for the estimation of antioxidant parameters and acetylcholinesterase (AChE) activity.

## 2.5. Behavioural parameters:

### ✓ Open-field

The open-field test is the most popular test of behaviors [44] for the evaluation of motor activity as a reaction to an unknown environment by exploration [45]. It is utilized to provide a quantitative and qualitative measurement of exploratory and locomotor activity in rodents.

The apparatus consists of a wooden box (90cm × 90cm × 25cm) divided into 36 equal squares (15cm × 15cm). Individual animals were placed in the center of the apparatus and allowed to freely explore the maze for 30min. The following behavioral components were measured: rearing (standing upright on the hind legs), locomotion (the total number of square crossing with all four paws), grooming (includes face cleaning, licking and scratching), and sniffing (the nose is held close to the floor).

The field must be cleaned from trial to trial using 70% ethanol and wiped dry.

### ✓ Y-Maze

The Y-maze is used to measure short term memory, and stereotypic behavior [46]. Rodents usually prefer to explore a new arm of the maze rather than returning to one that was visited previously. Spontaneous alternation behavior is a typical manifestation of short-term spatial (work) memory measured in the Y- labyrinth task [47]. Therefore, the Y-maze is composed of 3 equally arms (120°, 15cm high and 41cm long). Each animal was placed into the end of the start arm facing the wall and was allowed to freely explore the three arms labeled A, B and C for 5min.

The sequence of arm entries is recorded. The subject should show a tendency to enter a less recently visited arm and the percentage of alternation is calculated as ((actual alternations / maximum alternation) ×100). The apparatus was cleaned with 70% ethanol and allowed to dry between sessions.

## Biochemical Estimation

### ✓ Catalase (CAT) activity

The determination of catalase (CAT) activity was carried out according to the method of [48]. The mixture of the reaction, containing 0.2 ml brain homogenate, 0.2 ml phosphate buffer (1/1.5M, pH=7), and 0.2 ml H<sub>2</sub>O<sub>2</sub> solution (0.2M) were agitated then incubated at room temperature for 5min, the reaction was stopped by the addition of 0.2 ml solution of acetic acid/potassium dichromate (3V/1V) and the absorbance was directly measured at 570nm. The results were presented as μmol hydrogen peroxide consumed/mg of protein.

### ✓ Lipid peroxidation level

Lipid peroxidation level was measured by the quantification of thiobarbituric acid-reactive substances (TBARS) according to the method of [49]. Briefly, 0.1ml brain homogenate was added to 0.1 ml sodium dodecyl sulfate (8.1%), then completing volume with 0.3ml distilled water. The mixture was incubated at 95°C for 60 min then cooled.

After incubation, 0.5 ml distilled water and 2.5 ml butanol/pyridine (15v/1v) were added, the final solution was centrifuged at 4000r/min for 10min. The absorbance of the supernatant was measured at 535nm and the amount of malondialdehyde (MDA) formed was calculated based on the molar extinction coefficient of MDA (1.56 × 10<sup>5</sup> M<sup>-1</sup>cm<sup>-1</sup>). The results were expressed as μmol of MDA/mg protein.

### ✓ Estimation of tissue AChE

AChE activity was assayed according to the method of [50]. The reaction mixture contained 0.02ml brain homogenates, 3ml phosphate buffer (100 mM, pH=8.0) and 0.1 ml DTNB. The reaction was initiated by adding

0.05 ml acetylthiocholine solution. The absorbance of the complex was recorded each 2min for 10 min at 412nm at 25°C and the enzyme activity was expressed as micromoles of acetylthiocholine hydrolyzed / min/mg protein.

#### ✓ Protein estimation

The protein content was estimated by the method of [51] using bovine serum albumin as the standard.

#### 2.7. Histologie

The left hemisphere of each brain was fixed in the 10% formalin solution and embedded into paraffin. Paraffin blocks were cut at a thickness of 4 µm using the rotary microtome. Finally, the sections were stained with hematoxylin and eosin (H & E) then examined using the light microscope.

#### Statistical analysis

The data were expressed as mean value ± standard error. The differences between the means were analyzed with one-way analysis of variance ANOVA followed by Tukey's post hoc test using the SPSS 23 version. A level of P value less than 0.05 was considered to be significant.

### RESULTS

#### Effect of Aluminum chloride and treatment on body weight, weight gain, and relative brain weight

**Table 1:** Effect of AlCl<sub>3</sub> exposure and *Salvia officinalis* treatment on body weight, body weight gain and relative brain weight

Experimental groups				
	Control	AlCl <sub>3</sub>	AlCl <sub>3</sub> + SAE	SAE
<b>Initial BW (g)</b>	67.49 ± 1.88	67.54 ± 2.15	67.08 ± 2.04	67.54 ± 1.72
<b>Final BW (g)</b>	197.18 ± 6.8	164.97 ± 7.41	187.47 ± 7.48 <sup>###</sup>	197.63 ± 8.03 <sup>###</sup>
<b>Body Weight gain (g)</b>	129.69 ± 6.36	97.44 ± 6.84 <sup>***</sup>	120.4 ± 7.23 <sup>###</sup>	130.08 ± 8.26 <sup>###</sup>
<b>Relative brain weight (g)</b>	0.99 ± 0.04	0.93 ± 0.04 <sup>*</sup>	0.96 ± 0.05	1.00 ± 0.05 <sup>##</sup>

Values are given as mean±SEM each group. (\*: P≤0.05, \*\*\*: P≤0.001) compared with the control group; (##: P≤0.01, ###: P≤0.001) compared with AlCl<sub>3</sub> group.

The results in Table 1 show that rats intoxicated with AlCl<sub>3</sub> induced a significant decrease (P≤0.001) in body weight and weight gain compared to the control rats. Treatment of AlCl<sub>3</sub>-intoxicated rats with *S.officinalis* aqueous extract significantly (P≤0.001) increased body weight with a significant change in weight gain (23.56%) compared to the AlCl<sub>3</sub> group.

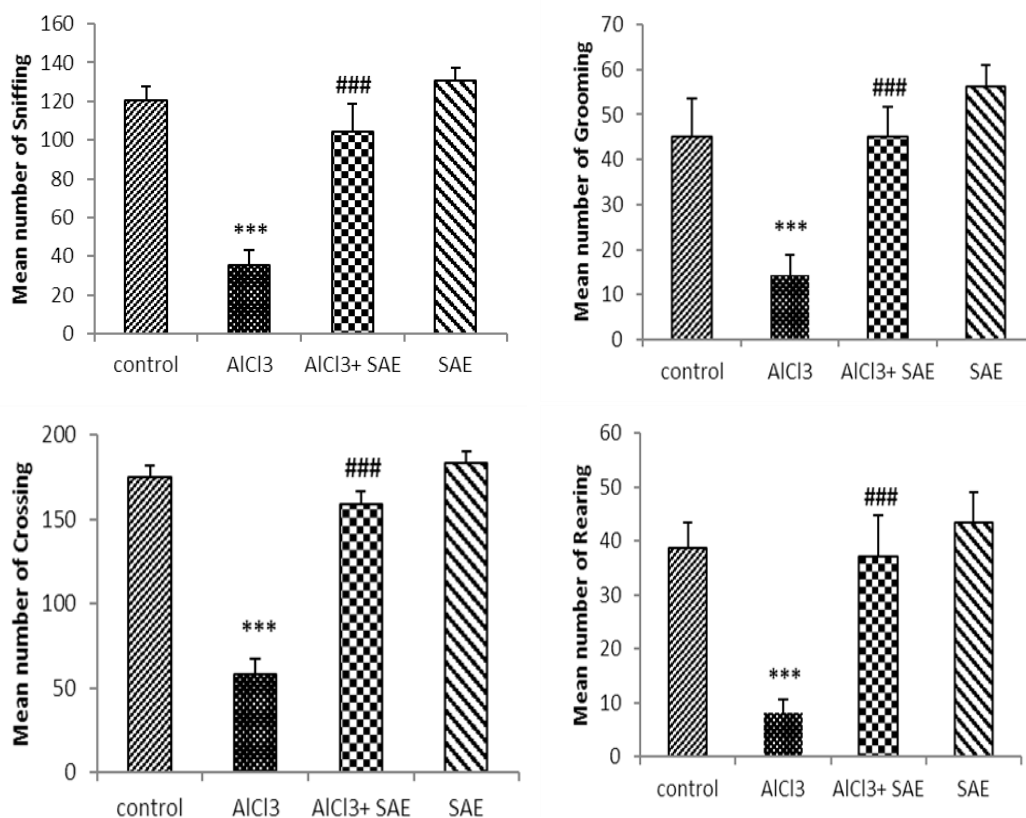
The relative weight of the brain also significantly decreased (P≤0.05) in the AlCl<sub>3</sub> group in comparison to the control one, treatment with *S.officinalis* resulted in the recovery of relative brain weight compared to the AlCl<sub>3</sub> group.

#### Effect of treatment on behavioural parameters

##### ✓ Open field

One-way ANOVA and Tukey analysis of the behavioural parameters in the open field (Fig 1) showed a significant decrease of **66.66%** in crossing, **79.27%** in rearing, **70.64%** in sniffing, and **68.0%** in grooming, while this decrease was lower in intoxicated treated rats (**9.47%**, **4.14%**, **13.26%**, and **0%**, respectively) compared to the control one.

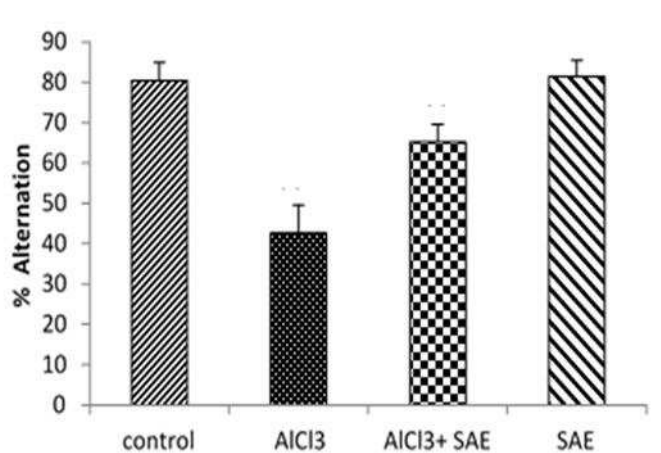
In contrast, the intoxicated rats treated with the aqueous extract of *Salvia officinalis* had a significant (P ≤ 0.001) increase in crossing, rearing, sniffing, and grooming compared to the AlCl<sub>3</sub> group.



**Figure 1:** Effect of AlCl<sub>3</sub> exposure on Open-Field parameters (crossing, rearing, sniffing, and grooming). Values are given as mean  $\pm$  SEM. \*\*\*:  $P \leq 0.001$  compared to the control group, ###:  $P \leq 0.001$  compared to the AlCl<sub>3</sub> group.

#### ✓ Y-maze

The statistical analysis of the results indicated that spontaneous alternation significantly reduced (47.01%) in the AlCl<sub>3</sub> group compared to the control, whereas the *S.officinalis* treatment of AlCl<sub>3</sub>-induced rats (AlCl<sub>3</sub>+SAE) significantly increased spontaneous alternation (53.05%) compared to the AlCl<sub>3</sub> group (Fig 2).



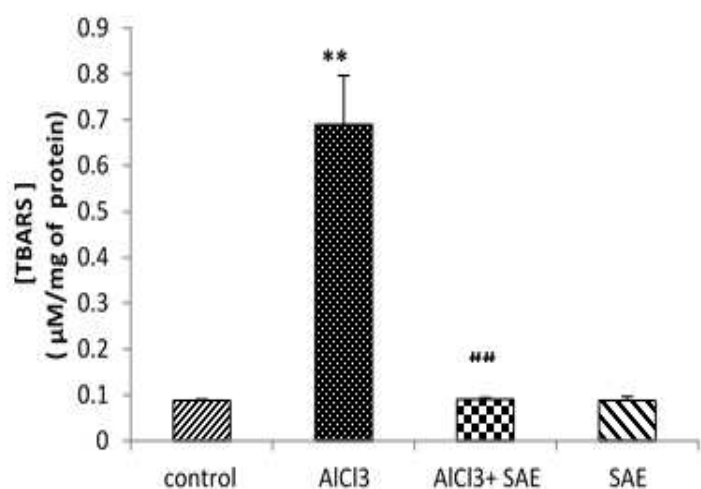
**Figure 2:** Effect of *S.officinalis* on the working memory of control and AlCl<sub>3</sub>-induced rats. Values are given as mean  $\pm$  SEM. \*\*\*:  $P \leq 0.001$  compared to control group, ###:  $P \leq 0.001$  compared to the AlCl<sub>3</sub> group.

#### Estimation of the antioxidant enzyme level in the brain homogenate of rats

##### ✓ Effect of aqueous extract of *Salvia officinalis* on MDA level in the brain

The obtained results in Fig 3 revealed that the brain MDA level significantly ( $P \leq 0.001$ ) increased in the  $AlCl_3$  group compared to the control.

The administration of the aqueous extract of sage to rats intoxicated with  $AlCl_3$  caused a significant reduction ( $P \leq 0.001$ ) in the elevated brain TBARS concentration (-86.81%) compared to the  $AlCl_3$  intoxicated group.

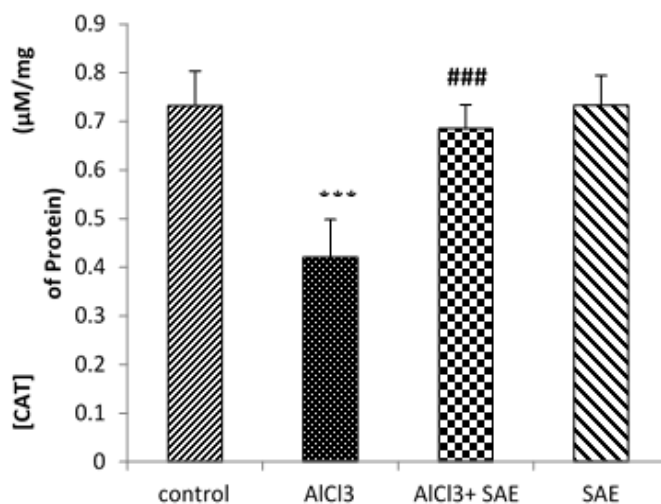


**Figure 3:** Effect of  $AlCl_3$  on TBARS in rat's brains before and after treatment by *Salvia officinalis*. Values are given as mean  $\pm$  SEM. \*\*:  $P \leq 0.001$  compared with the control group, ###:  $P \leq 0.001$  compared with  $AlCl_3$  group.

#### ✓ Effect of *Salvia officinalis* treatment on Catalase activity (CAT)

Fig 4 shows the effect of chronic exposure to  $AlCl_3$  and treatment on Catalase activity.

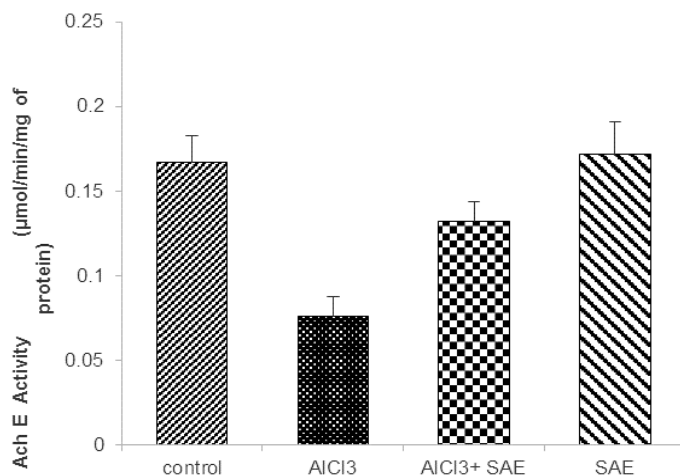
$AlCl_3$  administration induced a significant reduction ( $P \leq 0.001$ ) of Catalase activity as compared to the control. In contrast, the treatment of  $AlCl_3$ -rats with *S.officinalis* ( $AlCl_3+SAE$  group) induced a significant elevation ( $P \leq 0.001$ ) in CAT activity about 62.7% compared to  $AlCl_3$  group.



**Figure 4:** Effect of *S.officinalis* treatment on CAT activity of rats exposed to  $AlCl_3$  for 90days. Values are given as mean  $\pm$  SEM. \*\*\*:  $P \leq 0.001$  compared with the control group; ###:  $P \leq 0.001$  compared to the  $AlCl_3$  group

#### Effect of treatment on AChE activity

The statistical analysis showed that  $AlCl_3$ -exposure in rats significantly decreased the level of AChE ( $P \leq 0.001$ , -54.49%) in comparison to the control group, but significant elevation of AChE level (73.68%) was recorded in the intoxicated treated group compared to the  $AlCl_3$  group (Fig 5).

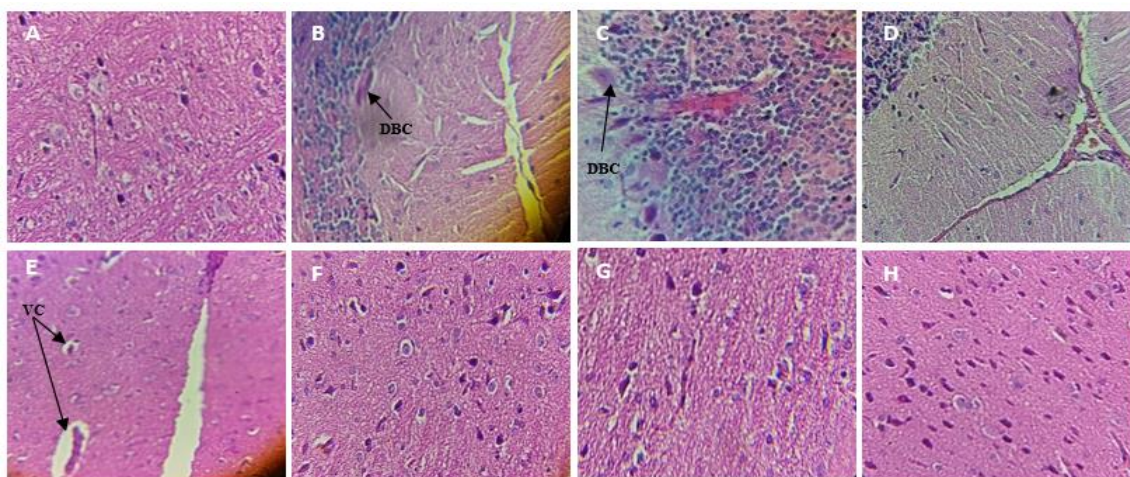


**Figure 5:** Effect of *S.officinalis* on AChE activity on the brain of control and AlCl<sub>3</sub>-exposure rats. Values are given as mean ± SEM. \*\*\*: P<0.001 compared to the control group, #: P<0.05 compared to the AlCl<sub>3</sub> group.

### Effect of treatment on brain histopathological changes

Fig 6 shows the effect of *Salvia officinalis* aqueous extract in aluminum-induced histological changes in the brain of the experimental rats.

The microscopic examination of the brain section of the control and treated groups showed normal morphological structure (Fig 6; A and H). On the other hand, the brain section of AlCl<sub>3</sub> animals showed marked deterioration as visualized by vacuolated cytoplasm, dilatation of blood capillary, reduced pyramidal cells in comparison to the control group (Fig 6; B, C, D, and E).



**Figure 6:** Effect of *Salvia officinalis* aqueous extract on AlCl<sub>3</sub>-induced histological changes in the brain of the experimental rats (H & E, 40×).

A (control); B, C, D, and E (AlCl<sub>3</sub>: 60mg/kg BW); F and G intoxicated treated group (AlCl<sub>3</sub> + AE); H treated group (AE).

VC: Vacuolated Cytoplasm,  
DBC: dilatation of blood capillary

### DISCUSSION

The current study suggests that AlCl<sub>3</sub> exposure caused a significant reduction in the final body, gain body weight and relative brain weight compared to the control group. These results are in agreement with previous studies such as those of **Ogueche et al.** [52] who observed that the bodyweight of rats exposed to aluminum decreased with an increase in the concentration of aluminum. Thus, **Bharathi et al.** [53] concluded that AlCl<sub>3</sub> exposure resulted in significant weight loss. Therefore, this reduction appears to be caused by a decrease in food intake.

In this study, we investigated biochemical, behavioral, and histopathological alterations caused by chronic AlCl<sub>3</sub> exposure and the possible effect of treatment with the aqueous extract of *Salvia officinalis* that has been used traditionally to improve memory and reduce age-related cognitive decline.

The present results demonstrated that female rats exposed to AlCl<sub>3</sub> differ from their control by their locomotor behavior, resulting in a significant decrease in the average number of a cross, rearing, sniffing, and grooming. Our results are consistent with other research showing that AlCl<sub>3</sub> exposure affects the development and behavior of rats [54]. Besides, **Erizi *et al.*** [55] found 47% of reduction in locomotor performance in aluminum-treated rats.

These behavioral changes may result from subtle changes in serotonin levels in brain regions after AlCl<sub>3</sub> exposure. A significant decrease in serotonin and dopamine levels was observed after treatment in the brain and cerebellum compared to the control group [54].

According to the present results, working memory was also affected by AlCl<sub>3</sub> exposure for 3 months by recording a significant reduction of 47.01% in spontaneous alternation; it is likely that Aluminum causes a cognitive impairment characterized by a deficiency of working memory. This is consistent with different studies of memory, in which Aluminum causes cognitive dysfunction and negatively affects the spatial learning and memory capacity of rats such as **Zghari *et al.*** [56] studies who reported that chronic exposure to Aluminum induced a significant decrease of spontaneous alternation in male and female rats. Moreover, spatial memory as measured by the y-maze test depends on the learning function and memory of the hippocampus and is related to the NMDA receptor/Ca<sup>2+</sup> influx signaling pathway [57].

Aluminum may inhibit this receptor. Many authors have demonstrated that these receptors involved in spatial learning [58], working and reference memory [59].

In our current work, the AlCl<sub>3</sub>-intoxicated rats receiving the aqueous *Salvia officinalis* extract exhibited behavior similar to that of the control group with a significant difference compared to the AlCl<sub>3</sub> group. These results indicate that *Salvia officinalis* may be useful in the development of a therapeutic agent for neuroprotection. Thus, **Eidi *et al.*** [60] concluded the effect of *Salvia officinalis* leaves on memory retention and its interaction with the muscarinic and nicotinic cholinergic system in rats. Furthermore, it has been reported that aqueous extract of *Salvia officinalis* leaves possesses a mnemonic impact on rats in Y-maze active test [61].

The present study also showed that oxidative stress was important in the AlCl<sub>3</sub> group, which was indicated by a statistically significant increase in the mean concentration of MDA and a significant decrease in catalase activity. These results are in agreement with previous studies; these data could be explained by an increase in lipid peroxidation of biological membranes, and alteration of the integrity of the membrane, modification of the membrane capacity, improvement of its permeability, and loss of its fluidity; affecting membrane-bound enzymes leading to cell leakage [62]. On the other hand, **Ananda *et al.***, [63] reported a significant decrease in Catalase activity in the brain of Wistar rats exposed to aluminum.

Manipulation of aluminum-intoxicated rats with *Salvia officinalis* extract revealed a significant reduction in MDA level and elevation in Catalase activity compared to the AlCl<sub>3</sub> group. These results confirmed that *Salvia officinalis* can significantly modulate oxidative stress parameters in young female rats causing by AlCl<sub>3</sub> through its antioxidant composition. These data are consistent with in vitro study of **Miura** [64], which showed that the sage is able to neutralize free radicals through the presence of many components, active agents with high antioxidant activity, such as carnosol, carnosic and rosmarinic acids, rosmadial, rosmanol, epirosmanol, methyl carnosate, and luteolin 7-O-β-glucopyranoside. These antioxidant compounds can stimulate endogenous antioxidant defense systems and trap reactive species [65]. Besides; **El-kholy *et al.***, [66] reported that aqueous *Salvia officinalis* extract inhibited the lipid peroxidative malondialdehyde (MDA) product in rats' brains and increase Catalase activity.

The neurotransmission induced by acetylcholine is fundamental for the functioning of the nervous system. Acetylcholine has a significant role in several aspects of cognitive function and behaviors including learning and memory. Its blockage is fatal and its progressive loss, as in Alzheimer's disease [67], multisystemic atrophy [68] and other conditions is associated with progressive deterioration of cognitive.

The results suggest that the AlCl<sub>3</sub>-induced rats showed a significant reduction in AChE when compared to the control group. These data are consistent with previous research in the brain of rats [69, 70] suggested that intraperitoneal administration of AlCl<sub>3</sub> to rats at a dose of 4.2mg/Kg for 28 days led to a significant decrease in AChE activity. Besides, many studies reported that aluminum modifies the properties and structure of the cell membrane, inhibiting enzymes such as acetylcholinesterase [71] causing changes and alterations in cholinergic



function. Amador et al. [72] reported that aluminum interferes with cholinergic neurotransmission, glutamatergic and gamma-aminobutyric [73].

Treatment with *S.officinalis* should moderate aluminum-induced cholinergic deficits by inhibiting the destruction of acetylcholine, which explains the significant increase in AChE activity in the intoxicated treated group of 73.68% in comparison to AlCl<sub>3</sub> group. These results could be explained by the inhibitory capacity of *Salvia officinalis*, which is well documented in the literature; previous studies have suggested its possible action on acetylcholinesterase by reduction of acetylcholine degradation [74].

According to histological findings, histological changes were seen in the brain of AlCl<sub>3</sub>-exposed rats when compared to the control one. Based on these results, we, therefore, concluded that AlCl<sub>3</sub> exposure has neurodegenerative impacts on the brain histology of rats. The pattern of changes seen in this study is similar to those of Buraimoh et al. [75] who showed that the administration of different doses of AlCl<sub>3</sub> for 12 weeks induced neural vacuolation and cortex necrosis cerebral, reduced pyramidal cells on the brain of rats as an indication of neurodegeneration.

The powerful antioxidant activity of the aqueous extract of *Salvia officinalis* and its ability to increase antioxidant enzymes and promote oxygen to the brain could prevent AlCl<sub>3</sub>-induced oxidative damage. Thus, Iuvone et al. [27] demonstrated the cytoprotective effect of sage against the toxicity of A $\beta$  neuronal cells (beta-amyloid plaques).

## CONCLUSION

In conclusion, this study revealed that the treatment with the aqueous extract of *Salvia officinalis* in AlCl<sub>3</sub>-intoxicated rats significantly reduced the oxidative stress status and improved biochemical parameters and caused architectural disorders; this effect has been achieved through its potent anticholinesterase activity and antioxidant capacity. These results demonstrated that *Salvia officinalis* aqueous extract prevented aluminum chloride-induced neurotoxicity in rat brains and provided a good therapeutic approach for intervention against neurological diseases.

## REFERENCES

1. Bondy SC. Prolonged exposure to low levels of aluminum leads to changes associated with brain aging and neurodegeneration. *Toxicology*. 2014;315:1-7.
2. Walton JR, Wang MX. APP expression, distribution and accumulation are altered by aluminum in a rodent model for Alzheimer's disease. *Journal of Inorganic Biochemistry*. 2009;103(11):1548-54.
3. Yokel R.A. The toxicology of aluminum in the brain: a review. *Neurotoxicology*, 2000; 21(5):813-828.
4. Chen T.J, Hung H.S, Wang D.C, Chen S.S. The protective effect of Rho-associated kinase inhibitor on aluminum-induced neurotoxicity in rat cortical neurons. *Toxicol Sci*; 2010; 116(1): 264-272.
5. Awad ZM, Abd AN, Hassan KH. Corrosion Prevention of Aluminum in Acidic Media Using *Cydonia Vulgaris* Leaves Extract as Environmentally Friendly Corrosion Inhibitor. *J. Biochem. Technol*. 2018;9(3):10-6.
6. Walton JR. Cognitive deterioration and associated pathology induced by chronic low-level aluminum ingestion in a translational rat model provides an explanation of Alzheimer's disease, tests for susceptibility and avenues for treatment. *International Journal of Alzheimer's disease*. 2012;2012.
7. Exley C. Why industry propaganda and political interference cannot disguise the inevitable role played by human exposure to aluminum in neurodegenerative diseases, including Alzheimer's disease. *Frontiers in neurology*. 2014;5:212.
8. Wu Z.H, Du Y.M, Wu Y.S and 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.
9. Percy ME, Kruck TP, Pogue AI, Lukiw WJ. Towards the prevention of potential aluminum toxic effects and an effective treatment for Alzheimer's disease. *Journal of inorganic biochemistry*. 2011;105(11):1505-12.
10. Osman AS, Gad MH, Hareedy AA, Mishriki AA, Rasheed EA. Sitagliptin attenuates cognitive impairment in the rat model of Aluminum-induced Alzheimer's disease. *J. Adv. Pharm. Educ. Res*. 2019;9(3):53-61.

11. Oyanagi K. The nature of the parkinsonism-dementia complex and amyotrophic lateral sclerosis of Guam and magnesium deficiency. *Parkinsonism & related disorders*. 2005;11:S17-23.
12. Exley C, House E, Polwart A, Esiri MM. Brain burdens of aluminum, iron, and copper and their relationships with amyloid- $\beta$  pathology in 60 human brains. *Journal of Alzheimer's Disease*. 2012;31(4):725-30.
13. Bondy SC. The neurotoxicity of environmental aluminum is still an issue. *Neurotoxicology*. 2010;31(5):575-81.
14. Petit TL, Biederman GB, Jonas P, LeBoutillier JC. Neurobehavioral development following aluminum administration in infant rabbits. *Experimental neurology*. 1985;88(3):640-51.
15. Khachaturian A.S, Zandi P.P, Lyketsos C.G, Hayden K.M, Skoog I, Norton M.C, Tschanz J.T, Mayer L.S, Welsh-Bohmer K.A, Breitner J.C. Antihypertensive medication use and incident Alzheimer's disease: the Cache County Study. *Arch Neurol*; 2006; 63(5): 686-692.
16. Deloncle R, Guillard O. Mechanism of Alzheimer's disease: arguments for a neurotransmitter-aluminium complex implication. *Neurochemical research*. 1990;15(12):1239-45.
17. Urano S, Asai Y, Makabe S, Matsuo M, Izumiya N, Ohtsubo K, Endo T. Oxidative injury of synapse and alteration of antioxidative defense systems in rats, and its prevention by vitamin E. *European journal of biochemistry*. 1997;245(1):64-70.
18. Julka D, Sandhir R, Gill K.D. Altered cholinergic metabolism in rat CNS following aluminum exposure implications on learning performance. *J Neurochem*; 1995; 65(5):2157-2164.
19. Peng JH, Xu ZC, Xu ZX, Parker JC, Friedlander ER, Tang JP, Melethil S. Aluminum-induced acute cholinergic neurotoxicity in rat. *Molecular and chemical neuropathology*. 1992;17(1):79-89.
20. Favarato M, Zatta P, Perazzolo M, Fontana L, Nicolini M. Aluminum (III) influences the permeability of the blood-brain barrier to [ $^{14}$ C] sucrose in rats. *Brain research*. 1992;569(2):330-5.
21. Cuvelier ME, Berset C, Richard H. Antioxidant constituents in sage (*Salvia officinalis*). *Journal of Agricultural and food chemistry*. 1994;42(3):665-9.
22. Miliauskas G, Venskutonis PR, Van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food chemistry*. 2004;85(2):231-7.
23. Alonso J. Tratado de fitofarmacos y nutracéuticos. Rosário : Corpus Librus ; 2004.
24. Lu Y, Foo LY. Polyphenolics of *Salvia*—a review. *Phytochemistry*. 2002;59(2):117-40.
25. Bauer J, Kuehnl S, Rollinger JM, Scherer O, Northoff H, Stuppner H, Werz O, Koeberle A: Carnosol and carnosic Acids from *Salvia officinalis* inhibit Microsomal Prostaglandin E2 Synthase-1. *The Journal of Pharmacology and Experimental Therapeutics*; 2012; 342(1): 169-176.
26. Generalić I, Skroza D, Šurjak J, Možina SS, Ljubenković I, Katalinić A, Šimat V, Katalinić V. Seasonal variations of phenolic compounds and biological properties in sage (*Salvia officinalis* L.). *Chemistry & biodiversity*. 2012;9(2):441-57.
27. Iuvone T, De Filipis D, Esposito G, D'Amico A, Izzo A. The Spice Sage and Its Active Ingredients Rosmarinic Acid Protect PC 12 Cells from Amyloid-beta peptide- induced neurotoxicity. *J Pharmacol. Exp*; 2006; Ther. 317(3): 143-149.
28. De Oliveira NC, Sarmento MS, Nunes EA, Porto CM, Rosa DP, Bona SR, Rodrigues G, Marroni NP, Pereira P, Picada JN, Ferraz AB. Rosmarinic acid as a protective agent against genotoxicity of ethanol in mice. *Food and chemical toxicology*. 2012;50(5):1208-14.
29. Bawazir AE, Aljarari RM. Protective Effect of *Boswellia carteri* on Aluminium Chloride-Induced Alzheimer's Disease in Male Albino Rat. *Int. J. Pharm. Phytopharm. Res*. 2018;8(6):29-39.
30. Sahu A, Rawal N, Pangburn MK. Inhibition of complement by covalent attachment of rosmarinic acid to activated C3b. *Biochemical pharmacology*. 1999;57(12):1439-46.
31. Al-Musayeb N, Perveen S, Fatima I, Nasir M, Hussain A. Antioxidant, anti-glycation and anti-inflammatory activities of phenolic constituents from *Cordia sinensis*. *Molecules*. 2011;16(12):10214-26.
32. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food chemistry*. 2001;49(11):5165-70.
33. Pérez-Tortosa V, López-Orenes A, Martínez-Pérez A, Ferrer MA, Calderón AA. Antioxidant activity and rosmarinic acid changes in salicylic acid-treated *Thymus membranaceus* shoots. *Food chemistry*. 2012;130(2):362-9.

34. Karmokar A, Marczylo TH, Cai H, Steward WP, Gescher AJ, Brown K. Dietary intake of rosmarinic acid by ApcMin mice, a model of colorectal carcinogenesis: levels of parent agent in the target tissue and effect on adenoma development. *Molecular nutrition & food research*. 2012;56(5):775-83.
35. Sharmila R and Manoharan S. Anti-tumor activity of rosmarinic acid in 7, 12-dimethylbenz (a)anthracene (DMBA) induced skin carcinogenesis in Swiss albino mice. *Indian J Exp Biol*; 2012; 50:187-194.
36. Baricevic D and Bartol T. Pharmacology: The biological/pharmacological activity of the *Salvia* genus. Dans E.K. Spiridon, SAGE: The genus *Salvia*. Athens, Greece: Overseas Publishers Association; 2000; 143-184.
37. Zupkó I, Hohmann J, Rédei D, Falkay G, Janicsák G, Máthé I. Antioxidant activity of leaves of *Salvia* species in enzyme-dependent and enzyme-independent systems of lipid peroxidation and their phenolic constituents. *Planta Medica*. 2001;67(04):366-8.
38. Topçu G. Bioactive triterpenoids from *Salvia* species. *Journal of natural products*. 2006; 69(3):482-7.
39. Lu Y, Foo LY. Antioxidant activities of polyphenols from sage (*Salvia officinalis*). *Food chemistry*. 2001;75(2):197-202.
40. Schwarz K and Ternes W. Antioxidative constituents of *Rosmarinus officinalis* and *Salvia officinalis*. II. Isolation of carnosic acid and formation of other phenolic diterpenes. *Z Lebensm Unters Forsch*; 1992; 195: 99-103.
41. Cuvelier ME, Richard H, Berset C. Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. *Journal of the American Oil Chemists' Society*. 1996;73(5):645-52.
42. Baricevic D, Sosa S, Della Loggia R, Tubaro A, Simonovska B, Krasna A, Zupancic A. Topical anti-inflammatory activity of *Salvia officinalis* L. leaves: the relevance of ursolic acid. *Journal of ethnopharmacology*. 2001;75(2-3):125-32.
43. Ikeda Y, Murakami A, Ohigashi H. Ursolic acid: An anti-and pro-inflammatory triterpenoid. *Molecular nutrition & food research*. 2008;52(1):26-42.
44. Walsh R.N and Cummins R.A. The open-field test: A critical review. *Psychological Bulletin*; 1976; 83 (3): 482-504.
45. Roth KA, Katz RJ. Stress, behavioral arousal, and open field activity--a reexamination of emotionality in the rat. *Rev*; 1979; 3; 247-263.
46. Kokkinidis L, Walsh MD, Lahue R, Anisman H. Tolerance to d-amphetamine: Behavioral specificity. *Life sciences*. 1976;18(9):913-7.
47. Kim YT, Yi YJ, Kim MY, Bu Y, Jin ZH, Choi H, Dore S, Kim H. Neuroprotection and enhancement of spatial memory by herbal mixture HT008-1 in rat global brain ischemia model. *Am J. Clin. Med*; 2008; 36: 287-299.
48. Sinha AK. Colorimetric assay of catalase. *Analytical biochemistry*. 1972;47(2):389-94.
49. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*. 1979;95(2):351-8.
50. Ellman GL, Courtney KD, Andres Jr V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology*. 1961;7(2):88-95.
51. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. *The journal of Biological Chemistry*, 1951; 193(1): 265-275.
52. Ogueche P.N, Ugwu C.E, Ezejindu D.N, Omeje M, Dike C.C, Okonkwo C.O, Maduka H.C.C, Obidoa. Aluminum Intoxication Induced Biochemical and Histopathological Alterations in Male Wistar Albino Rats Hepatocytes, *Journal of Natural Sciences Research*, 2014; ISSN 2224-31786. V 4, N°24.
53. Bharathi MD, Thenmozhi AJ. Attenuation of aluminum-induced neurotoxicity by tannoid principles of *Emblca officinalis* in Wistar rats. *International Journal of Nutrition, Pharmacology, Neurological Diseases*. 2018;8(2):35.
54. Bhalla P, Garg ML, Dhawan DK. Protective role of lithium during aluminium-induced neurotoxicity. *Neurochemistry international*. 2010;56(2):256-62.
55. Erazi H, Sansar W, Ahboucha S, Gamrani H. Aluminum affects glial system and behavior of rats. *Comptes rendus biologiques*. 2010;333(1):23-7.

56. Zghari O, Rezqaoui A, Ouakki S, Lamtai M, Chaibat J, Mesfioui A, El Hessni A, Rifi EH, Essamri A, Ouichou A. Effect of chronic aluminum administration on affective and cognitive behavior in male and female rats. *Journal of Behavioral and Brain Science*. 2018;8(4):179-96.
57. Conrad CD, Grote KA, Hobbs RJ, Ferayorni A. Sex differences in spatial and non-spatial Y-maze performance after chronic stress. *Neurobiology of learning and memory*. 2003;79(1):32-40.
58. Tsien JZ, Huerta PT, Tonegawa S. The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell*. 1996;87(7):1327-38.
59. Levin E.D, Bettogowda C, Weaver T, Christopher N.C. Nicotine-dizocilpine interactions and working and reference memory performance of rats in the radial arm maze. *Pharmacol Biochem Behav*; 1998; 61 (3): 335-340.
60. Eidi M, Eidi A, Bahar M. Effects of *Salvia officinalis* L. (sage) leaves on memory retention and its interaction with the cholinergic system in rats. *Nutrition*; 2006; 22(3): 321-6.
61. Smach MA, Hafsa J, Charfeddine B, Dridi H, Limem K. Effects of sage extract on memory performance in mice and acetylcholinesterase activity. In *Annales pharmaceutiques francaises 2015* (Vol. 73, No. 4, pp. 281-288). Elsevier Masson.
62. Liaquat L, Sadir S, Batool Z, Tabassum S, Shahzad S, Afzal A, Haider S. Acute aluminum chloride toxicity revisited: Study on DNA damage and histopathological, biochemical and neurochemical alterations in rat brain. *Life sciences*. 2019;217:202-11.
63. Anand R, Kumari P, Kaushal A, Bal A, Wani WY, Sunkaria A, Dua R, Singh S, Bhalla A, Gill KD. Effect of acute aluminum phosphide exposure on rats—A biochemical and histological correlation. *Toxicology letters*. 2012;215(1):62-9..
64. Miura K, Kikuzaki H, Nakatani N. Antioxidant activity of chemical components from sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.) measured by the oil stability index method. *J Agric Food Chem*; 2002; 50(7): 18, 45-51.
65. Sa C, Ramos A, Azevedo M, Lima C, Fernaudes-Ferreira M, Pereira-Wilson C. Sage tea drinking improves lipid profile and antioxidant defences in humans. *Int J Mol Sci*; 2009; 10: 39, 37-50.
66. El-Kholy W.M, El-Habibi E.M and Mous A.T (2010). Oxidative stress in brain of male rats intoxicated with aluminum and the neuromodulating effect of forms of sage (*Salvia officinalis*). *Journal of American Science*; 6(12): 1283-1297.
67. Wright CI, Geula C, Mesulam MM. Neuroglial cholinesterases in the normal brain and in Alzheimer's disease: relationship to plaques, tangles, and patterns of selective vulnerability. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*. 1993; 34(3):373-84.
68. Polinsky RJ, Holmes KV, Brown RT, Weise V. CSF acetylcholinesterase levels are reduced in multiple system atrophy with autonomic failure. *Neurology*. 1989;39(1):40-.
69. Shaik AB, Shenoy S, Anupama V, Rao KM, Amuthan A. Antidepressants modulate behavioral, biochemical, and histological alterations induced by chronic aluminum chloride administration in wistar rats. *Journal of Pharmacology and Pharmacotherapeutics*. 2019;10(1):16.
70. Abdelghany AK, Khalil F, Azeem NM, El-Nahass ES, El-Kashlan AM, Emeash HH. Ginseng and moringa olifera ameliorated cognitive impairments induced by aluminium chloride in albino rat. *Adv. Anim. Vet. Sci*. 2019;7(7):557-65.
71. Platt B, Fiddler G, Riedel G, Henderson Z. Aluminium toxicity in the rat brain: histochemical and immunocytochemical evidence. *Brain research bulletin*. 2001;55(2):257-67.
72. Amador FC, Santos MS, Oliveara CR. Lipid peroxidation and aluminum effects on the cholinergic system in nerve terminals. *Neurotox. Res*; 2001; 3(3): 223-333.
73. Cordeiro J.M, Silva V.S, Oliveira C.R, Gonçalves P.P. Aluminum-induced impairment of Ca<sup>2+</sup> modulatory action on GABA transport in brain cortex nerve terminals. *J Inorg Biochem*, 2003; 97(1): 132-142.
74. Akhondzadeh S, Noroozian M, Mohammadi M, Ohadinia S, Jamshidi AH, Khani M. *Salvia officinalis* extract in the treatment of patients with mild to moderate Alzheimer's disease: a double blind, randomized and placebo-controlled trial. *J Clin. Pharm*; 2003; Ther. 28 (1): 53-59.
75. Buraimoh AA, Tayo I, Ojo SA. Effects of Aluminium Chloride on the Histology of Cerebellum of Wistar Rats. *Pharmacology&Toxicology Research*. 2014;1:1-4.