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# Effect of Aluminium and Aqueous extract of Rosmarinus officinalis on rat Brain: Impact on Neurobehavioral and Histological study

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## ABSTRACT

'Rosmarinus officilnalis' is a plant used in Mediterranean diet and traditional medicine, possessing various antioxidant and cytoprotective bioactivities. In this study, we investigated the potential neuroprotective efficacy of aqueous Rosemary extract (AER) against neurotoxicity induced by Aluminum (Al), in terms of behavioral, biochemical and histological aspects in young rats. an intraperitoneal injection of Al, at the weekly dose of 60mg/Kg was given to the animals. A treatment of 150mg/Kg/day of AER was administered by gavage over periods of 6 or 12weeks. Al caused intense changes over time in body and brain weight, increase in neurological disorders such as depression, anxiety, and deficiency in memory skills. Results show also disturbances in locomotors activity, with a significant inhibition of AchE and increase LDH activity compared to control. Additionally, Al induced structural damages in the cerebral cortex, and the CA1 region of hippocampus. However, treatment with AER resulted in improved depression and anxiety state, locomotors activity and restored memory skills. Results show that AER increase the AchE activity and decreased neuronal loss in the cerebral cortex and the CA1 region of hippocampus with the 6weeks treatment but induced disruption and structural modification of brain tissue after the 12 weeks treatment. The Aqueous extract of Rosemary possess a neuroprotector and corrective effect against neurological alterations induced by Aluminum, but when administered over a long period of time, the extract can cause a no beneficial effect and morphologic modifications in cerebral tissue and behavior test.

**Keywords:** Rosmarinus officinalis, Aluminum, neuro-behavior, brain structure.

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## INTRODUCTION

Metal interacting with biological components is receiving an unprecedented level of attention from the field of neuroscience. The accumulating evidence continues to substantiate the essential function of metals in the healthy brain[1]. However, impairment of metal homeostasis has been perceived as one of the key factors in the progression of neurodegeneration. Studies exploring the causative role of metals in the molecular pathogenesis of neurological disorders are rapidly expanding[2]. A metal of such interest is aluminum, which is widely used as an additive in our modern diet, incorporated in certain drugs (antacids, anti-diarrheal), and in cosmetology[3] .

Although Aluminum administered orally is poorly absorbed, it has been shown that some aluminum compounds such as maltolate, ascorbate, succinate, lactate or citrate are much more easily absorbed. For instance, citric acid increases aluminum absorption by 5 to 10 times in humans and animals[3].Aluminum has been associated with many diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and senile dementia of the Alzheimer type[4].

Transition metals, such as Al, can easily cross the blood-brain barrier, leading to significant accumulation in the brain. Once in the central nervous system the metal can form a stable complex with L-glutamic acid and accumulates in different regions of the brain.in the striatum, hippocampus and cerebral cortex,causing neural and glial disorders[5]. And can also enter different parts of the cell, including mitochondria, lysosomes and the nucleus[6]. This is likely to cause morphological changes[7], and participate in neural system failure and disturbance of brain function, like neurotransmitters metabolism between neurons[8]. Such as modifications in serotonin, noradrenaline, GABA, dopamine and glutamate rates[9].

Further, Aluminum being a potent cholinotoxin it interferes with the synthesis of acetylcholine[10], which is involved in regulating several functions in central nervous system such as cognition, memory, consciousness, attention and then the regulation of mood disorders such as depression and anxiety[11],and may cause apoptotic neuronal loss due to changes in acetylcholinesterase (AchE) levels in the brain[12]. According to several studies, chronic exposure to aluminum induces changes in neurological behavior, neuropathological, neuro physical and neurochemical changes[13].

Rosmarinus officinalis L., (family: Lamiaceae) is a dense shrub, native to the Mediterranean basin. The plant is now grown all over the world for its Mediterranean culinary virtues, and its classification by the European Food Safety Authority (EFSA,2008) as a safe natural antioxidant in food preservative[14], and in traditional medicine.

According to numerous studies, Rosemary may have antioxidant, antidiabetic, anti-cancer activity[14] and therapeutic effect against stress-related psychiatric disorders[15]. The hexane-ultrasound rosemary extract can reduce neuropathic hypersensitivity and protect nervous tissues[16], and the hydroalcoholic extract of Rosemary has been shown to reduce the permeability of (BBB), potentially leading to reduction in cerebral edema and intracranial pressure and restoring cerebral blood flow and energy[17].

In this study, we investigated the potential neuroprotective efficacy of Aqueous Extract of Rosemary (AER) against neurotoxicity induced by aluminum in terms of behavioral, biochemical and histological aspects in young rats.

### Theory

A number of studies have pointed out that the compounds bio isolated assets of plant are potentially beneficial against the deleterious effects of the aluminm and on the activity glutamatergic synaptic at the level of different regions of the brain and more particularly at the level of the hippocampus. As well, the orientation of our research toward the identification of new biomolecules potentially cyto-protective, can contribute positively to the reduction of the deleterious effects of aluminum on the brain functions and the appearance of the neurodegenerative disease.

## MATERIALS AND METHODS

### Preparation of the aqueous extract of Rosmarinus Officinalis

The Rosemary plant was grown and dried in the open air in the dark in Es-Senia Oran (Algeria), in October 2016. It was identified and authenticated at the Herbarium of Botany Directorate of the University Es-Senia (Oran). Fifty (50) Grams of the air part of Rosemary were extracted with 500 ml of distilled water by continuous hot extraction at 60°C twice during 30min, and the filtrate was lyophilized. The extraction yielded 7,06g (14,12%). When needed, the extract was dissolved in distilled water.

### Animals and tissue preparation

In this study, sixty-four (64) male Wistar rats aged 5 weeks, weighing approximately 60 ± 10g were used. The rats were housed under normal conditions with free access to food and water (12hours light/dark, Temperature 22 ± 2°C). The study protocol was approved by the University's Scientific Committee. The animals were divided into four groups of 16rats each. The following protocol was used for each group:

**Control:** An intraperitoneal (I.P) injection of 0,9% saline solution (NaCl); **Al**: An I.P injection of 60mg/Kg body weight (B.W) of Aluminum Chloride once a week at 8:00am; the used dose was based on different studies already carried out previously which showed that aluminum intoxication with a dose of 50mg/Kg three times a week or daily to young or adult male wistar rats for 6and 12weeks causes alterations in brain[13,18], **Al+AER**: this group received an I.P injection of 60mg/kg B.W ($AlCl_3$) once a week and, concomitantly, a treatment with aqueous extract of rosemary (AER) at a dose of 150mg/Kg(B.W)/day by gavage at 5pm. **AER:** was used as controls treated with a dose of 150mg/kg(B.W)/Day of aqueous extract of rosemary (AER) by gavage.

All group were treated under the same housing conditions. At the end of the experiment, behavioral tests were performed, and weight of each rat was recorded. Eight (8) rats from each group were sacrificed under anesthesia (I.P, Chloral Solution injection) after 6 weeks; while the remaining eight rats from each group continued the experiment until 12weeks. The brain of each rat was then removed, washed with an isotonic solution (0,9%), weighed, and stored at -80°C until use.

The brain of each rat was divided into two parts (Right and Left), the right part was crushed and homogenized with phosphate buffer (1/10 W/V, pH 7,4) with a homogenizer, and centrifuged at 3000g for 15 min at 4°C. The supernatant was isolated and centrifuged at 10000g for 10min at 4°C. The final supernatant was separated then used for the estimation biochemical parameters.

### Neurobehavioral study

Before starting, the behavioral tests are used in an isolated room with no noise; all rats were pre-trained for 7 days on all the behavioral tests employed. Behavioral tasks were started on the day following pre-training and continued for 15 days. Training was performed during the last treatment month.

Forced swimming test:

Forced swimming were performed according to the technique of Porsolt et al[19] ; this test were used to evaluate the animal's depressive behavior, and consists of subjecting each rat to a forced swimming test inside a cylinder (20,7cm in diameter×39cm in height) filled with water at 22±2°C for 6minutes. The parameters recorded during the test were mobility time [MT], and immobility time [IT].

Dark/Light test:

This test is used against unconditioned anxiety in rodents[20], for 20minutes each rat was placed in a box consisting of two equal part compartments (44×8,5×25), One compartment illuminated by light, and one dark compartment, separated by a door, generally rats hated places with light, hence more the animal is not anxious, more its exploration would be reduced in the dark compartment. During testing the parameters recorded were time passed in the dark [TPDC], and light compartment [TPLC].

The Elevated Plus Maze:

This technique described by Pelow[21], The device is composed of four arms (L=50/L=10cm) that communicate through a central area (5×5cm), two arms closed by (20cm) high walls, placed at height of (50cm) from the ground. Each rat was placed in the central zone, facing a closed arm to explore the labyrinth for 20 minutes, in order to evaluate anxious behavior according to its spontaneous aversion to vacuum, the parameters measured were the time passed in the Open Arms [TPOA], and the Closed Arms [TPCA].

Radial Arm Maze:

To evaluate the working and reference memory of the animals, the radial arm labyrinth consisted of 8arms (20cm) starting from a central area of (30cm), arranged at a height of 50cm from the ground; in short For 4 Days, a food reward system was placed at the end of each arm. Subsequently, each rat was positioned individually in the central area to explore this new environment for 10 minutes each day. Working memory errors [WME], were calculated (Number of repeated entries in the previously explored arms). On the 5th and 6th days the food reward was placed only in 4 arms (Arm n° 2,4,6,8). The reference memory errors [RME] were calculated (Number of repeated entries in the unappetizing arms)[18].

Open field:

The open field test is used to provide a qualitative and quantitative measure of exploratory and locomotors activity in rodents[22]. it is in the form of an open rectangular box of (75cm×40cm×35cm) with a black background with white lines on the ground delimiting the (20) tiles, each rat was placed in one of the four corners of the open field for 15minutes, its locomotors activity will be evaluated according to the number of squares crossed by the animal every 5minutes.

### Biochemical estimation

The activity of acetylcholinesterase was determined by the Elmman's spectrophotometric method[23]. Briefly, an aliquot of brain homogenate (0,05ml) was added to tubes containing (3ml) phosphate buffer, (0,02) acetylcholine solution with (0,1ml) DTNB. The absorbance was measured at 412nm in a UV spectrophotometer and expressed in µmol/min/mg of Protein. Lactate Dehydrogenase (LDH) activity in brain was measured spectrophotometrically, by using commercial reagent Kits. Briefly, an aliquot (100µl) of brain homogenate was mixed with (3ml) of working reagent, incubated for 1 minute and the absorbance at 340nm was measured. The total protein levels in homogenates were determined following the method of Lowry[24]. Briefly, proteins were mixed with copper ions in alkaline medium and reduced by Folin reactive. The absorbance of the blue colored product was evaluated at 500nm.

### The histological study

The left hemisphere of the brain was fixed by the formaldehyde buffer (10%), immersed in alcohol baths (24 Hours), poured into mold containing paraffin melted for inclusion, and then cooled. With a microtome, 3 micron tissue sections were selected, collected on glass slides, rehydrated, and then stained with hematoxylin and eosin as nuclear and cytoplasmic dyes[25]. The sections were analyzed using a microscope. This technique was performed at the west military Hospital of Oran.

### Statistical analysis

Values are represented as mean±Standard deviation (SD). Statistical comparisons were performed using a one-way analysis of variance (ANOVA). If the ANOVA analysis indicated significant differences, Tukey's post-hoc test was performed to compare mean values between treatment groups and controls. A value of P<0,05 was considered as statistically significant, P<0,01 a very significant and P<0,001 a highly significant.

## RESULTS

Table 1 shows that Al induced a significantly decreased final body weight (P<0,05) of -21,79% and -12,79%,after 6 and 12 weeks, respectively. Conversely, the statistical analyses show significantly enhanced relative whole brain weight of +17,68% and +9,31%, after 6 and 12 weeks, compared to the control group (P<0,05).

The Al+AER group exhibited an increase in final body weight (+19,67% and +28,67%; (P<0.05)), after 6 and 12 weeks, compared to the (Al) group ; absolute whole brain weight was significantly higher at 6-weeks (by +12,31%) than the Al group, After 12 weeks, but no difference was noted at 12 weeks. On the other hand, the Al+AER group showed a significant reduction (-18,60%; p<0,05) of the relative whole brain weight after 12-weeks, compared to Al group. AER group show a significant increased value in final body weight (+11,77%; p<0.05) compared to controls, After 12-weeks.

**Table 1:** Body and Brain Weight Changes after Short and Long Term of Treatment

| Experimental Groups | Initial Body weight [g] | Final Body weight [g] | Absolute whole Brain weight [g] |
|---|---|---|---|
| After 6 Weeks | | | |
| Control | 67,30±2,82 | 158,19±4,15 | 1,71±0,07 |
| Al | 69,28±2,10 | 123,71±6,60* | 1,57±0,10* |
| Al+AER | 67,37±1,40 | 148,05±12,55# | 1,77±0,09# |
| AER | 66,87±1,45 | 153,97±15,09 | 1,73±0,05 |
| After 12 Weeks | | | |
| Control | 66,82±2,52 | 202,97±12,99 | 1,85±0,04 |
| Al | 69,53±2,13 | 176,99±3,87* | 1,80±0,06* |
| Al+AER | 68,63±2,72 | 227,69±18,49# | 1,88±0,02# |
| AER | 69,20±3,86 | 230,06±29,89* | 1,84±0,02 |

The parameters: **Final Body weight:** the mean weight of the rats in each group on the last day of the experiment, **Absolute whole Brain weight:** the mean brain weight of the rats in each group. **[g] :** Grammes. Values are represented as mean ± SD each group. *:P<0,05, **:P<0,01, ***:P<0,001 compared with control group; #:P<0,05, ##:P<0,01 ###:P<0,001 compared with (Al) group. (one-way analysis of variance (ANOVA))

**Effect of treatment on behavioral parameters**

**Forced swimming Test**

The forced swimming test, evaluated by measurement of the immobility time (IT), is commonly used to assess depressive behaviour in animals. After 6 and 12 weeks, results show a higher score (-63,99%; p<0,001) in immobility time (IT) in the Al exposed animals compared to controls. There was no significant change in Al+AER group compared to the Al group (Table 2) after the 6-week exposure. However, treatment with AER for 12 weeks showed a significant decrease (p<0,001), relative to Al group. In addition, AER induced a significantly decreased score compared to control (Table 2).

**Dark/Light Test**

This test shows that compared to the control group, the Al group spent a significantly larger period in the dark area, but not the Al+AER or Al groups, after the 6-week study (Table 2).

In the 12-week study, Aluminium caused a highly significant increase in time spent in dark compartments compared to control (+40,23%; p<0.001). Inversely, the AER treated animals spent significantly less time in the dark (-17,89%), relative to Al group (Table 2).

**Elevated Plus Maze Test**

At 6 weeks of treatment, compared to control, the Al group spent significantly less time (P<0,001) in the open arms than in the closed arms (-87,38%), indicating that Aluminum induced an enhanced stress. No significant changes were noted between Al+AER group and the intoxicated group (Table 2).

The results at 12 weeks showed that the time spent in open arms was significant decreased (-83,69%, p<0,001) in Al group compared to control. However, the AER treated group (Al+AER group) exhibited a significant increase in score due to longer period of time (+89,61% ) spent in open arms, compared to Al group by (Table 2).

**Table 2:** Effects of Rosemary Extract on Behavioural Test after Intoxication by Aluminum

| Experimental Groups | Forced Swimming Test IT [S] | Dark/Light Test TPDC [S] | Elevated Plus Maze Test TPOA [S] | Radial Arm maze test | |
|---|---|---|---|---|---|
| | | | | WME [Score] | RME [Score] |
| After 6 Weeks | | | | | |
| Control | 77,50±20,29 | 773,10±49,80 | 168±74,73 | 33±9,59 | 1,33±0,51 |
| Al | 215,20±38,20*** | 967,20±90,50*** | 21,20±20,22*** | 51,87±9,40*** | 3,33±1,03*** |
| Al+AER | 192,30±31,24 | 920,30±62,80### | 33±58,34 | 9,62±4,53### | 1,50±0,83## |
| AER | 147,30±33,60 | 815,70±124,80 | 125,60±38,16 | 15,87±5,98*** | 0,66±0,51 |
| After 12 Weeks | | | | | |
| Control | 169,60±40,2 | 628,20±136 | 191,10±45,83 | 30,33±13,92 | 1±0 |
| Al | 257,10±25,20* | 1046,1±90,50*** | 31,10±11,03*** | 63,66±15,29** | 3,50±0,83*** |
| Al+AER | 194,30±15,20# | 858,80±186,40 | 300±25,32### | 41,16±15,03 | 2,66±0,81 |
| AER | 111,60±30,40** | 729,75±106 | 156,60±27,35 | 44,66±18,09 | 2±0,89 |

The parameters: **IT**: Immobility time, **TPDC**: The time passed in the dark compartment, **TPOA**: The time passed in the open arms, and **WME**: Working Memory errors, **RME**: Reference Memory errors were evaluated respectively in the test. **[S]:** Seconds. Values are represented as mean ± SD each group. *:P<0,05, **:P<0,01, ***:P<0,001 compared with control group; #:P<0,05, ##:P<0,01 ###:P<0,001 compared with (Al) group (one-way analysis of variance (ANOVA))

**Radial Arm Maze Test**

The Radial 8-Arms Maze test is generally used for evaluation of short-term and reference memories. After the 6 and 12-week studies, results (Table 2) show a highly significant increase in scores of working and reference memory errors in Al group compared to controls (P<0,001). Relative to the Al group, the co-administration of AER and Al induced significantly decreased scores of working and reference memory errors after 6 weeks of -81,45% and -54,05%, respectively. No changes in the number of working and reference memory were observed after 12 weeks. Similarly, The AER treatment alone induced a significantly decreased number of working memory (-51,90%) and reference errors (-54%) compared to controls after 6 weeks. However, the number of working memories remained constant after 12 weeks.

**Open field Test**

Table 3shows that the locomotor activity changed at 5 min in the Al group compared to the control group in the 6-week study. These changes were statistically significant at 5min (-35,46%; p<0,05). During the same 5 min period, the group treated with 150mg/Kg (B.W)/day (AER) exhibited a rather significant increase of +30,38% (p<0.05) in the locomotor's activity compared to the Al group. No additional differences were observed during the remainder of experiment.

Results show that chronic administration of Al induced hyperactivity in rats by increasing the locomotors activity significantly at 5min compared to control (P<0.001).

Results also show that AER induced a significant increase of locomotor's activity from 5 to 15min compared to control. However, the co-administration of AER and Al induced a significant decrease (P<0,05) at 5min compared to Al group by -35,09%.

**Table 3:** Effects of Rosemary on Locomotors Activity at the End of 6 And 12 Weeks of Treatment

| Experimental Groups | Locomotion [Score] | | |
|---|---|---|---|
| | 5min | 10min | 15min |
| After 6 Weeks | | | |
| Control | 207,25±36,79 | 104,50±47,73 | 52,12±41,76 |
| Al | 133,75±30,29* | 89,50±37,02 | 32,37±26,13 |
| Al+AER | 192,12±36,37# | 99±42,44 | 44,62±38,65 |
| AER | 216,75±70,77 | 162,25±12,29 | 44,75±40,82 |
| After 12 Weeks | | | |
| Control | 143±20,29 | 114,12±22,66 | 107,12±18,34 |
| Al | 180,37±6,50*** | 117,12±36,29 | 71.00±16,50 |
| Al+AER | 155,37±17,99# | 145,50±23,21 | 62.00±15,22 |
| AER | 197,75±30,55 | 155,87±30,60 | 133,75±61,91 |

Values are represented as mean ± SD each group. **[Score] :** the mean number of squares crossed by the animal every 5minutes. Values are represented as mean ± SD each group. *:P<0,05, **:P<0,01, ***:P<0,001 compared with control group; #:P<0,05, ##:P<0,01 ###:P<0,001 compared with (Al) group (one-way analysis of variance (ANOVA))

**Effect of treatment on AchE Activity**

In this study we investigated the changes in AchE activity after exposure and treatment with Al and AER. Compared to controls, Administration of Al for 6 and 12 weeks induced a highly significant inhibited in AchE activity, -73,35% (p<0,001) and -66,15% (p<0,01), respectively (Fig. 1).

Nonetheless, the Acetylcholinesterase activity was significantly increased, +67,43% and, after the co-administration of 150mg/Kg(B.W)/Day of AER and Al compared to Al only group after 6weeks. But no changes after 12 weeks.

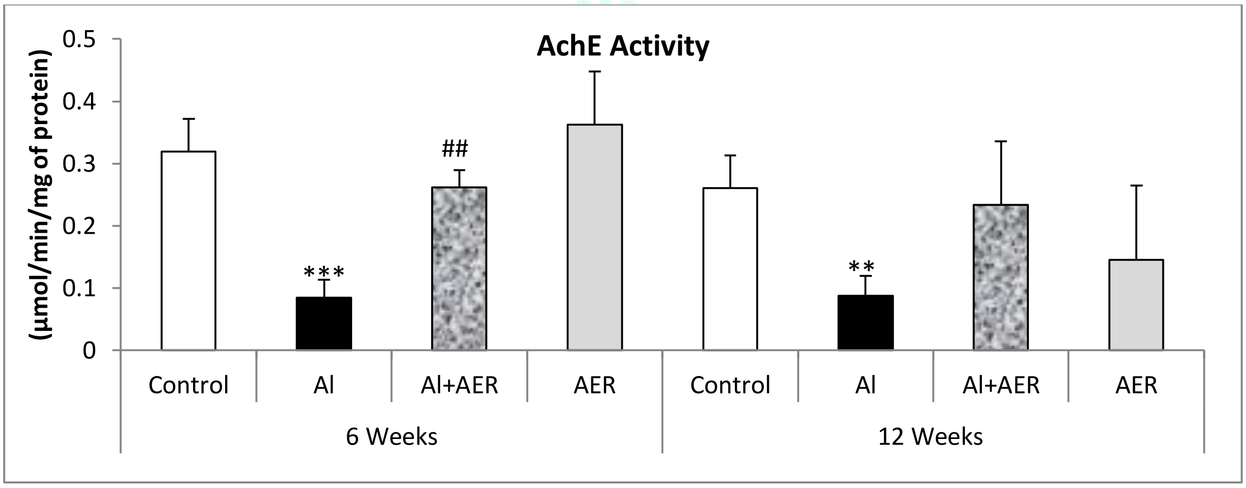


**Figure 1:** The Effects of Rosemary Extract on Acetylcholinesterase Activity after 6 And 12 Weeks of Aluminum Intoxication

Values are represented as mean ± SD each group. *:P<0,05, **:P<0,01, ***:P<0,001 compared with control group; #:P<0,05, ##:P<0,01 ###:P<0,001 compared with (Al) group. (one-way analysis of variance (ANOVA))

**Histopathological study**

Examination the slides of rat cerebral cortex revealed that after 6 and 12 weeks shows that aluminum induced cellular degeneration, necrosis, fibrosis, and vacuolated neuronal cells; In addition, the CA1 region of Hippocampus showed pyknosis of pyramidal cells (Al-B).

However, AER treatment seems to inhibit the effect of aluminium as evident with the increase in number of cellular units, reduced neuronal death, absence of fibrosis and of vacuolated neuronal cells (Al+AER-C). Indeed, in the CA1 region of hippocampus we observed less pyknosis of the pyramidal cells after 6 weeks. After 12 weeks, most hippocampal neurons exhibited pyknosis of pyramidal cells (**PPc**) compared to Al group. Examination of slides of treated group (AER) showed no changes in the structure of cerebral cortex and CA1 region of Hippocampus after 6 and 12weeks compared to control (AER-D).

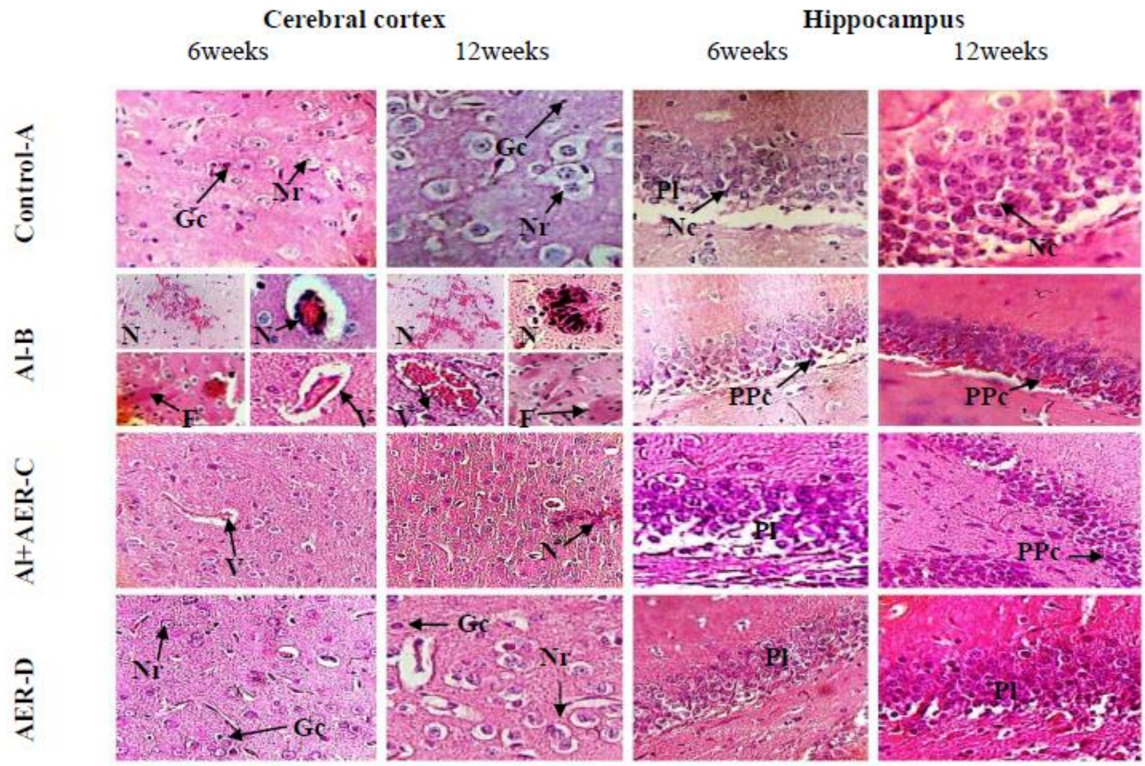


**Figure 2:** Effect of Rosemary Extract on Brain Structure of Cortex and CA1 Region of Rat after Aluminum Intoxication (H&E, ×40)

Showing: **Control-A :** NORMAL HISTO-ARCHITECTURE (**Gc**) GLIAL CELLS, (**Nr**)NEURO, (**Nc**) NEURONAL CELLS, (**Pl**) PYRAMIDAL LAYER.

**Al-B)** IN THE CEREBRAL CORTEX (H&E, ×100): NECROSIS (**N**), FIBROSIS (**F**), VACUOLATED NEURONAL CELLS (**V**); IN THE HIPPOCAMPUS, THE MAJORITY OF HIPPOCAMPAL NEURONS EXHIBITED PYKNOSIS OF PYRAMIDAL CELLS (**PPc**) WITH DEEP STAINING.

**Al+AER-C)** CO-ADMINISTRATION AER+Al TREATMENT SHOWING IN CEREBRAL COTEX: REDUCE NEURONAL DEATH, ABSENCE OF FIBROSIS, WITH PERSISTENT VACUOLATED NEURONAL CELLS. IN THE HIPPOCAMPUS THERE WERE LESS PYKNOSIS OF THE PYRAMIDAL CELLS AFTER 6WEEKS, AND MOST HIPPOCAMPAL NEURONS EXHIBITED PYKNOSIS OF PYRAMIDAL CELLS (**PPc**) AFTER 12 WEEKS.

**AER-D)** AFTER AER TREATMENT SHOWING NORMAL HISTO-ARCHITECTURE.

### Effect of treatment on LDH Levels

The LDH activity was significantly increased in Al group after 6 weeks (+64,19%, p<0.001) and 12weeks (+ 65,20%, p<0,01) of aluminum exposure, compared to controls.

However, the Al+AER treated group showed a significant decrease in LDH activity by -57,90% after 6 weeks (P<0,001) compared to the untreated Al group. There were no significant changes in LDH activity within the 12-week groups (Fig. 3).

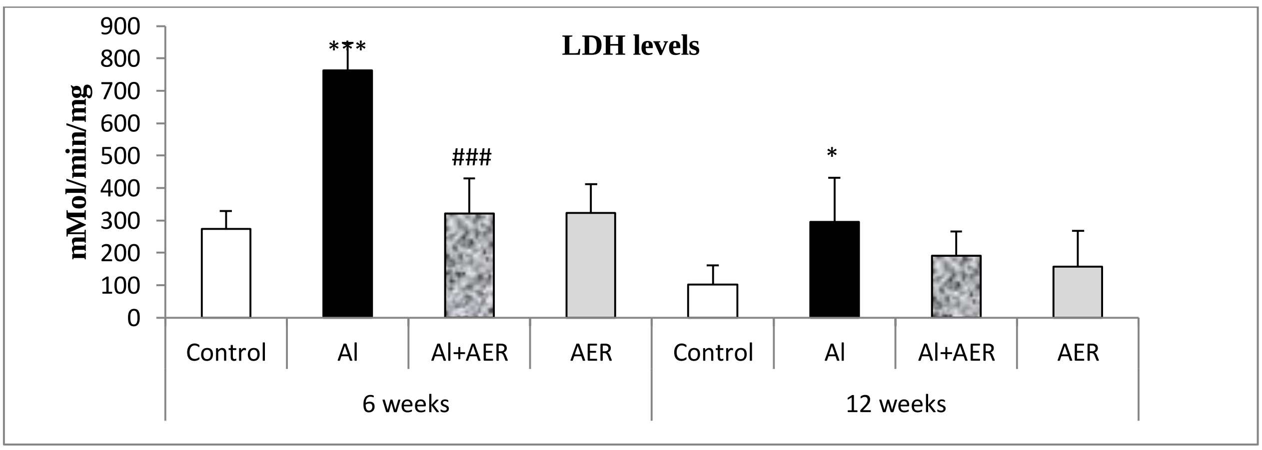


**Figure 3:** Role of Rosemary Extract on LDH Activity after 6 And 12 Weeks of Aluminum Intoxication

Values are represented as mean ± SD each group. *:P<0,05, **:P<0,01, ***:P<0,001 compared with control group; #:P<0,05, ##:P<0,01 ###:P<0,001 compared with (Al) group. (One-way analysis of variance (ANOVA))

## DISCUSSION

In the Algerian tradition Rosmarinus officinalis (Rosemary) plant is widely known for its therapeutic and antioxidant potential in the treatment of neurodegenerative diseases[26]. To examine this,we investigated the neuroprotective efficacy of Aqueous Extract of Rosemary on the behavioral, biochemical, and structural changes induced by the administration of Aluminum in young rats after 6weeks and 12 weeks of treatment.

In our experiment, administration of Al after 6 and 12 weeks resulted in a decrease in the final body weight of the rats comparable to those obtained by El-Shafei and al[27]. Such decreases could be attributed to the interaction of Aluminum with the hormonal status and /or protein synthesis[28].

Additionally, the total whole brain weight was significantly reduced when compared to controls. This supports earlier findings of Bhlla and Dhawan[29], maybe due to deleterious effect of increased lipids peroxidation in oxidative stress[29]. Contrarily,simultaneous administration AER and Al, at both 6- and 12-week treatments,resulted inan increase in the final body weight and the absolute whole brain weight, compared to the (Al) group. This effect is probably due tothebeneficial effect of phenolic compounds in this extract.

The comportmental studies showed that chronic and sub chronic exposure to Aluminum causes an increased immobility time of rats in the forced swimming test; reflecting the depressed state of animal. This state is due to the reduction of serotonin level in central serotoninergic system[30], in cortex[9], hippocampus, striatum, and spinal cord brain regions of rat pups following oral exposure to Al[31].

Administration of AER to Al group (Al+AER) and treated groupe by only the aqueous extract of rosemary (AER) at the dose of 150 mg/Kg(B.W)/day increase the mobility time after 12 weeks, perhaps indicating that the R. officinalis possesses an antidepressant potential that when interacting with the monoaminergic system[32], leads to improved serotonergic functions within the brain[14].

The Dark/light and elevated plus maze tests indicate that Al is inducing an anxiety state, confirming earlier findings[22]. However, the mechanisms responsible for the induction of anxiety are not well defined. Zald and Prdo[33], observed an increase in blood flow to the hippocampus and amygdale, while others histological studies have implicated apoptosis and necrosis in the different part of the brain following aluminum intoxication[34]. There are also reports suggesting that dysfunction of the GABAergic system (noradrenergic, serotonergic and dopaminergic neurons) contribute to the development of anxiety. Finally, aluminum affect the CFR (corticotrophin releasing factor) could have played a role in the development of anxiety[34,35]. After 12-week of study, Al+AER group spent less time in the dark compartment [TPDC] of the Dark/Light test and more time in the open arms [TPOA] in the Elevated Plus Maze test, compared to (Al) group. This observation suggests that Rosemary has an important anxiolytic potential due, perhaps, to increased levels of 5-hydroxy tryptamine and dopamine in rat brain as well as decreased levels of norepinephrine[36].

After chronic and sub-chronic intoxication, the (Al) group presented a deficit of memory performances compared to control, supporting previous finding[37]. A deficit in memory performance after Aluminum exposure could be explained by degeneration of cholinergic terminals in the cortex and hippocampus, as well as deterioration in hippocampal function[38]. Relative to Al group, the co-administration of AER to the intoxicated group attenuated both working and references memory errors and enhanced memory after 6weeks of treatment. However, this benefit appears to be short term only, and not after long period of intoxication by aluminium.

In our results, the sub-chronic and chronic administration to Al affected the locomotors activity in the OFT; sub-chronic exposure the Aluminum induced a decrease locomotors, supporting with previous studies[40], but not others[41], after 6weeks to exposure to Al. On the other hand, after 12weeks of exposition, the Aluminum induce an hyperactivity, a similar increase was observed after administration 50mg/Kg/day of Aluminum for 12 weeks in the drinking water[42], and other works show a significant decreased in locomotors activity[13,34], after chronic Aluminum exposure, this could be explained by the altered function of GABA receptors, which could be responsible of increased excitability[43].

The Co-administration of aqueous extract of rosemary and Al, after both 6 and 12 weeks, induce a reduction of locomotors activity; a similar results were observed in animal model of depression[44]. On the other hand, after 12 weeks the AER induced increase the locomotors activity, this effect maybe due to the antidepressant and anxiolytic potential of AER, but the AER mechanisms involved in behavior effects are not yet clear.

Acetylcholinesterase (AChE) is the primary cholinesterase in the body. It is an enzyme that catalyses the breakdown of acetylcholine and other choline esters that function as neurotransmitters[45]. Cholinergic neurotransmissions play key roles in promoting secretion of the soluble fragment of the ß-amyloid precursor protein, known to affect neurite outgrowth and to promote neuronal survival[46]. The cholinergic system play an important role in the regulation of central nervous system function and cognitive disorders which are often observed in depression, stress and memory[47,48].

Data obtained in this study shows that the administration of Al inhibited of AChE activity after chronic and sub-chronic exposure; This could be explained by the direct neurotoxic effect of Aluminum and by the disturbance of the cell membrane phospholipids associated with an increase in lipid peroxidation[49].Similar results were reported previously when animals were exposed for 4 weeks of 84mg/Kg of aluminum[45]. Other studies[50] showed that after 12 weeks of Al intoxication at a dose of 50mg/Kg, acetylcholinesterase activity decreased in striatum and hypothalamus but decreased in cerebellum, hippocampus and cerebral cortex.

The discrepancy in AChE activity observations may be related to the dose of Al, to the membrane composition, to the presence of different AChE molecular forms,or to a reflection of the biphasic effect of aluminum[51].

Our results show that the Co-treatment with AER induced an increase of AChE activity after 6weeks, in agreement with previous findings[47]. This effect, as well as the partial improvement of memory, may be due to the presence in the extract of polyphenolic and terpenic compounds, such as rosmarinic and carnosic acid[47]. However, the duration of treatment with rosemary aqueous extract gives an opposite suitable effect after long period (12weeks).

This histological study supports previous investigations[52;54] that Aluminum exposure leads to progressive alterations in the rat brain. This is manifested by adecrease in the number of cellular units relative to controls, fibrosis and vacuolation of neuronal cells in the cerebral cortex, as well as necrosis of pyramidal cells in the CA1 region of hippocampus *(Al-B)*. The hippocampus and the cerebral cortex are the key structures of memory formation[55], which could imply that morphologic abnormalities could partially explain the deficits of memory performances caused by Al.

On the other hand, after the administration of 150mg/Kg/day of rosemary aqueous extract by gavages, the severity of tissue damage observed in Al group was considerably reduced in (AER+AL) group. Observation of histological sections at the cerebral cortex shows an increase in the number of neuronal cells, associated with reduced neuronal degeneration and cell death in the cerebral cortex after 6 and 12 weeks of treatment, with an absence of fibrosis but the presence of vacuolated neuronal cells persisting.

In addition, histological examination of sections of the hippocampus showed less apoptotic cells, after 6 weeks; the same observations were inferred by administration at the dose of (20,40,80mg/ml) for 7 days and 100mg/kg/day for 23 days[56]. However, after 12 weeks there was deformation of the granular layer, compared with aluminum exposed group at 6 weeks, which could be explained by the impact of aluminum on brain cells over a long period of time.

This reduction in neuronal degeneration and cell death in the cerebral cortex and hippocampus could be due to the presence of phenolic compounds that inhibit and protect against cell death [57;60],

A key signature for necrotic cells is the permeabilization of the plasma membrane. This event can be quantified in tissue culture settings by measuring the release of the intracellular enzyme lactate dehydrogenase (LDH). To confirm results of our histological study and detect cell damage and or death, we analyzed the level of this enzyme in the brain; Our results showed that after intoxication at short and long period by Al; the LDH levels increase significantly compared to control, indicating damaged membranes and cell necrosis [61-62]. Consistent with other results in this study, the Al+AER treated group induced decrease in LDH activity. But there was no change in LDH activity after 12 weeks; these results could explain the persistence of pyramidal cell necrosis.

The biochemical (AchE and LDH activities) and the histological results of this study strongly indicate that short term AER treatment appears to slow neuronal death, prevent fibrosis and persistent vacuolated neuronal cells in the cerebral cortex in rats exposed to Aluminum.

## CONCLUSIONS

The results of the present study reveal that aluminium mediates progressive alterations in the rat brain. Administration of 150mg/kg (B.W)/day aqueous extract of rosemary could restore and protect the neurological function capacities after 6-weeks. However, administration of plant extract over a long period of time may cause a no beneficial effect on tissue and enzymes activities.

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