

## EXPERIMENTAL RESEARCH CONCERNING THE EFFECT OF ALUMINIUM COMPOUNDS ON ANXIETY IN MICE

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### Abstract

Anxiety disorders are the most common mental illnesses in adults. Anxiety distorts perception, decreases the power of concentration, affects thinking, learning, associative and evocation memory and is usually treated with benzodiazepines - anxiolytic drugs - such as diazepam or oxazepam. Benzodiazepines are clinically used in racemic forms despite the fact that the pharmacological activity of the two enantiomers is different, the activity being restricted only to the enantiomers with (S) configuration. Oxazepam is also the ultimate pharmacologically active metabolite of many benzodiazepines derivatives, and it is metabolized to the inactive glucuronide. Experimental researches show that aluminium inhibits the release of mediators in the synaptosomal fractions with inhibitory effects being produced mainly by decreasing the activity of the Na<sup>+</sup>/K<sup>+</sup>-ATP-ase and to a lesser extent through direct interaction with neurotransmitter transporters. Based on these data from the literature, this study aimed to test the possible anxiolytic effect of 2 aluminium compounds (AlCl<sub>3</sub> and Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>) in mice after single and chronic administration. For testing the anxiolytic effect the suspended cross maze test was applied after different time intervals, after substances administration. The results showed that after 30 minutes both substances presented an anxiolytic effect regardless the administered dose, as compared to the control. After 120 minutes, only the higher doses of aluminium compounds maintained the anxiolytic effect. The anxiolytic effect was present also after chronic administration (14 days) for the higher dose (1 mg/kg bw) of aluminium chloride and for the lower dose (0.1 mg/ kg bw) of aluminium sulphate. Taking into account that both aluminium compounds had an anxiolytic effect, it can be concluded that this effect is mainly due to the aluminium ion.

### Rezumat

Anxietatea reprezintă una dintre cele mai frecvente tulburări psihice întâlnită la adulți, care distorsionează percepția, scade capacitatea de concentrare, afectează gândirea, învățarea și memoria asociativă și evocativă. Anxietatea este de obicei tratată cu medicamente anxiolitice de tipul benzodiazepinelor, cum ar fi diazepamul sau oxazepamul [8]. Benzodiazepinele sunt utilizate în clinică în forme racemice și în ciuda faptului că activitatea farmacologică a celor doi enantiomeri este diferită, activitatea este limitată numai pentru enantiomerii (S). Oxazepamul este metabolitul activ, final, al multor derivați benzodiazepinici, care, la rândul lui, este și el metabolizat într-un glucuronconjugat inactiv. Cercetările experimentale au arătat că aluminiul inhibă eliberarea mediatorilor, efectele sale inhibitorii fiind determinate în principal prin scăderea activității pompei Na<sup>+</sup>/K<sup>+</sup> ATP-dependentă și într-o mai mică măsură prin interacțiunea directă cu neurotransmițătorii. Pe baza acestor date, prezentul studiu și-a propus să evidențieze posibilul efect anxiolitic a 2 săruri de aluminiu (AlCl<sub>3</sub> și Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>) la șoarece după administrare unică sau repetată. Pentru cercetarea efectului anxiolitic, s-a utilizat testul labirintului în cruce suspendat, realizat la diferite intervale de timp, după administrarea celor 2 substanțe. Rezultatele au arătat că la 30 de minute după administrarea celor 2 săruri de aluminiu, ambele substanțe au prezentat un moderat efect anxiolitic, indiferent de doză, comparativ cu lotul martor, în timp ce la 120 de minute doar dozele mari din cele 2 substanțe au crescut semnificativ parametrii utilizați ca indicatori ai anxietății. Efectul anxiolitic a fost prezent și după administrarea cronică (14 zile), atât pentru doza mare (1 mg/kgc) de clorură de aluminiu cât și pentru doza mică (0.1 mg/kgc) de sulfat de aluminiu. Având în vedere că efectul anxiolitic a fost prezent pentru ambele săruri de aluminiu, se poate presupune că acest efect se datorează ionului aluminiu *per se*.

**Keywords:** aluminium chloride, aluminium sulphate, anxiety

### Introduction

Anxiety disorders are the most common psychiatric disturbances in adults, as evidenced by the extensive research in recent years. A study conducted in 2005

showed that 27% of Europe's population has experienced at least one mental illness, the most frequently diagnosed being one of the anxiety disorders [1]. Anxiety disorders have a significant impact on everyday life, causing much suffering of affected

individuals. At the same time they have a significant economic impact with high direct and indirect costs related to reduced quality of life and patient lost opportunities, decreased work abilities and high healthcare costs.

The existing research does not reveal specific mechanisms of action for aluminium, but it is well known that this ion competes in the biological systems with cations, especially with magnesium, despite the difference in oxidative status. Also aluminium binds to transferrin and to citrate in the plasma and it can also influence the secondary transmission systems and availability of calcium. Scientific data shows that aluminium interferes with the main neurotransmitters regarding their synthesis, storage, release, activation or inactivation of their receptors.

Observations and studies show that aluminium intoxication decreases by 40% the level of dopamine in the black substance and causes an imbalance of dopamine metabolites, suggesting alteration of its metabolism. In addition, aluminium inhibits dopamine- $\beta$ -hydroxylase which is responsible for the conversion of dopamine to norepinephrine and which promotes aggregation of  $\alpha$ -synuclein with increased neurodegeneration of the black substance [2].

A study in which aluminium was administered intraperitoneally showed that the metal produces a decrease of density in dopamine D1 and D2 receptors from the cortex and black substance, decrease that was proportional to the dose [3].

Research studies show that aluminium inhibits 40-50% of the release of mediators in the synaptosomal fractions (isolated from different brain areas). Inhibitory effects are produced mainly decreasing the activity of the  $\text{Na}^+/\text{K}^+$ -ATP-ase and to a lesser extent through direct interaction with neurotransmitter transporters. Cordeiro's experiments from 2003 [4] showed that aluminium disturbs the  $\text{Ca}^{2+}$ /calmodulin signal dependent of calcineurin that regulates the process of passing the system *via* the synaptic membrane. Administration of aluminium chloride to rats for 60 days led to decreased levels of serotonin and its metabolite 5-hydroxy-indoleacetic acid in the cortex, hippocampus and cerebellum. These changes are related to the inhibitory effect of aluminium on serotonin by cholinergic input loss in investigated brain regions [5].

Barabasz and collaborators [6] showed that aluminium acts as a potent inhibitor of enzymes that use ATP as a substrate (eg.  $\text{Na}^+/\text{K}^+$ -ATP-ase) or of other enzymes such as hexokinase, alkaline phosphatase, acetylcholine transferase or ferroxidase. Based on these literature data, in this study we aimed to highlight a possible anxiolytic effect of aluminium compounds on laboratory animals, in single dose or long-term administration.

## Materials and Methods

In order to study the anxiolytic effect of aluminium salts, three experiments were performed. The first experiment of the study aimed to assess the anxiety status of mice after 30 minutes of the administration of a single dose of aluminium chloride and aluminium sulphate. The second experiment aimed to assess the anxiolytic effect 120 minutes after administration of the two aluminium salts. Finally, the third experiment studied this effect after administering the test substances chronically, for 2 weeks.

To accurately quantify the anxiolytic effect of the aluminium chloride and sulphate we used the suspended cross maze test. By administering an anxiolytic drug such as diazepam or oxazepam, tigmotaxis is reduced, the mouse thus spending more time in the open arms of the maze compared to the control group.

5 groups of 15 albino male mice were used for each experiment assessing the anxiolytic effect of aluminium salts after acute administration. For the third experiment which evaluated the chronic effects of aluminium salts on anxiety, 5 groups of 25 male albino mice were used. All the animals weighed 25-30 grams and they were provided by the "Carol Davila" University of Medicine and Pharmacy Bucharest bio-base. Mice were brought to the laboratory with 24 hours before the start of the tests and they were kept in standard environmental conditions with *ad libitum* access to food and water. The animals were housed in Plexiglas cages (bed of wood chips). The ambient temperature was between 21°C and 24°C and the relative humidity was maintained between 45-60%.

All the experiments were conducted in accordance with The European Directive 86/609/EEC/24.11.1986 and The Romanian Government Ordinance 37/30.01.2002 regarding the protection of animals used for experimental and other scientific purposes. During the first two experiments the substances administered were: aluminium chloride ( $\text{AlCl}_3$ ) - in a dose of 9 mg/kg bw and 18 mg/kg bw, aluminium sulphate ( $\text{Al}_2(\text{SO}_4)_3$ ) - 3.6 mg/kg bw and 7.2 mg/kg bw and saline solution 0.1 mL / 10 g bw. Both aluminium compounds were provided by Sigma Aldrich and were dissolved in 9 ‰ sodium chloride in such concentration that the amount administered was 0.1 mL / 100 g body weight. All substances were injected intraperitoneally and the tests were conducted after 30 and 120 minutes after administering the substances.

During the chronic administration experiment, the substances used were:  $\text{AlCl}_3$  in a dose of 0.2 mg/kg bw and 1 mg/kg bw,  $\text{Al}_2(\text{SO}_4)_3$  0.1 mg/kg bw and 0.5 mg/kg bw and saline solution 0.1 mL / 10 g bw. This experiment lasted 14 days with substances

being administered twice daily during the first 7 days and only once daily during the last 7 days of the experiment. The substances were administered by gavage and testing was carried in day 14 after 2 hours since administration of the last dose of the substances. Doses were chosen considering the available scientific data concerning the toxicity of these aluminium salts. For the acute administration experiments the chosen doses were 1/20 and 1/45 of LD<sub>50</sub> for aluminium chloride and 1/40 and 1/75 of LD<sub>50</sub> for aluminium sulphate. For the chronic administration, the aluminium salts were lowered much more being 1/5000 and 1/10000 of LD<sub>50</sub> for aluminium chloride and 1/60000 and 1/30000 for aluminium sulphate, knowing that chronic exposure to aluminium can determine an aluminium build-up in the body and brain.

In all stages of this study, testing was done using the suspended cross maze test, a plus shaped device that has 2 closed and 2 open arms (with and without sidewalls) that is suspended at a height of 60 cm above the ground. Mice with normal behaviour show aversion towards open spaces (tigmotaxis), preferring to stay close to walls. During our testing, mice were placed in the centre of the maze and for a period of 280 seconds measurements on the time spent by the mouse in open and closed arms were carried out. The timer was started when all 4 paws of the mouse were in the open arm or the closed arm respectively. The parameters obtained were the time spent in closed arms, time spent in open arms, time spent in the centre of the labyrinth, the number of entries made into the open or closed arms. We then calculated the ratio between the time spent in open arms and total time spent in the maze (denoted by the percentage of time spent in the open arm) and the ratio between the number of open arm visits and the total number of visits.

If a substance increases the percentage of time spent in open arms and the number of visits in open arms, compared to the control, it is considered that this substance has an anxiolytic effect.

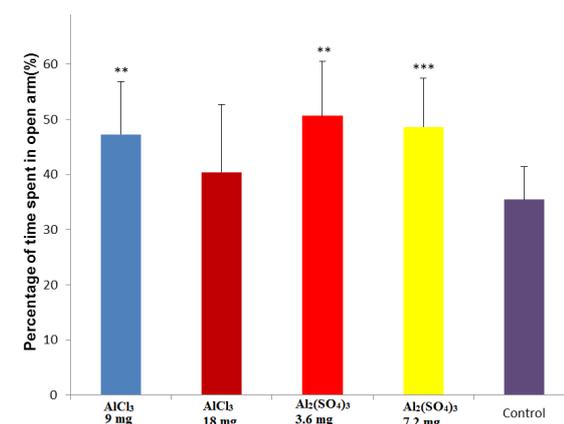
Results were analysed using Microsoft Office Excel. We calculated means and standard deviations for each batch and then applied the Student t-test. Results were considered statistically significant if  $p < 0.05$ .

## Results and Discussion

### *Evaluation of anxiety status 30 minutes after the acute administration of aluminium chloride and aluminium sulphate*

30 minutes after the administration, (Figure 1) the group that received 9 mg/kg bw of AlCl<sub>3</sub> showed an increase in the percentage of time spent in the open arms with an average of 47.27% time spent in the open arms compared with controls which presented an average of 35.45% ( $p < 0.05$ ). Also the dose of

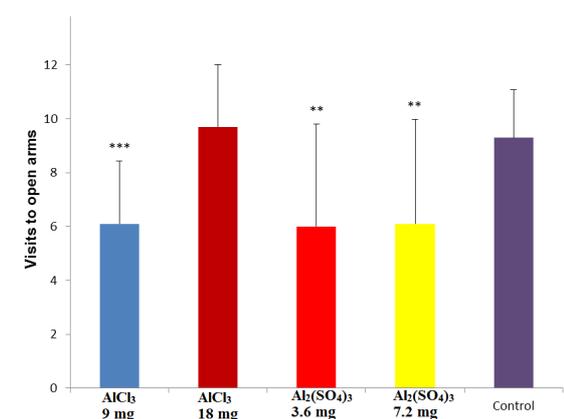
18 mg/kg bw of AlCl<sub>3</sub> showed an increase in the percentage of time spent in the open arms 40.4% but the result was not statistically significant compared to the control group. The percentage of time spent in the open arms registered by the group that received a dose of 3.6 mg/kg bw of Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> was 50.62%, well above the average achieved by the control group of 35.45% ( $p < 0.05$ ). The group that received a dose of 7.2 mg/kg bw of Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> spent in the open arms 48.61% of the total time, higher than the percentage of 35.45% obtained for the control group ( $p < 0.05$ ).



**Figure 1.**

Time spent in the open arms 30 minutes after the acute administration of the aluminium compounds  
 \*\* $p < 0.05$  as compared with the control group;  
 \*\*\* $p < 0.001$  as compared to the control group

Given the results, we can say that the two doses of Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> and the low dose of AlCl<sub>3</sub> had an anxiolytic effect 30 minutes after administration, the effect being statistically significant compared to the control group.



**Figure 2.**

Number of visits in the open arms 30 minutes after the acute administration of the aluminium compounds  
 \*\* $p < 0.05$  as compared with the control group;  
 \*\*\* $p < 0.001$  as compared to the control group

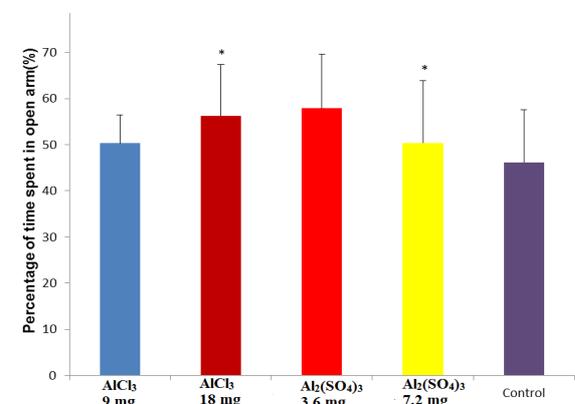
In terms of the number of visits made in the open arms (Figure 2), small doses of AlCl<sub>3</sub> decreased the

number of visits with an average of 6.1 compared to the control group that presented an average of 9.3 ( $p < 0.05$ ). The high dose of  $AlCl_3$  showed an average number of visits in the open arms of 9.4, result statistically insignificant compared to the control group. After 30 minutes the number of visits made in the open arms registered by the group that received the low dose of  $Al_2(SO_4)_3$  was 6 and the one registered by those who received the high dose  $Al_2(SO_4)_3$  was 6.1, lower than the average achieved by the control group of 9.3 ( $p < 0.05$ ).

According to the results, we can state that both doses of  $Al_2(SO_4)_3$  and the low dose of  $AlCl_3$  decreased the number of visits 30 minutes after administration, the effect being statistically significant compared to control.

*Evaluation of anxiety 120 minutes after the acute administration of aluminium chloride and aluminium sulphate*

2 hours after the administration (Figure 3), the low dose of  $AlCl_3$  increased the percentage of time spent in the open arms with an average of 50.27% compared with controls which presented an average of 46.15%, but the result was not statistically significant. Also the high dose of  $AlCl_3$  showed an increase in the percentage of time spent in the open arms 56.26%, result statistically significant compared to the control group.



**Figure 3.**

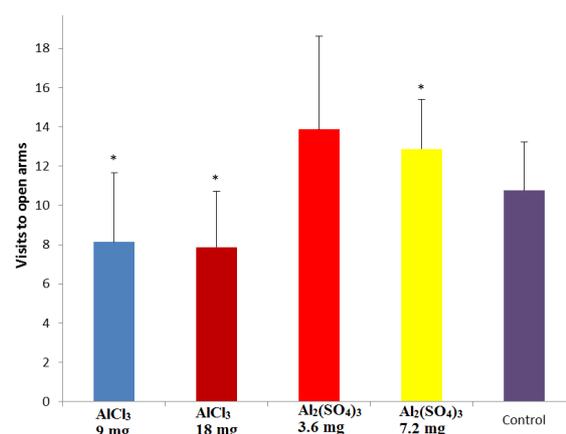
The time spent in the open arms 120 minutes after the acute administration of aluminium compounds

\*\* $p < 0.05$  as compared with the control group;

\*\*\* $p < 0.001$  as compared to the control group

After 120 minutes, the percentage of time spent in the open arms registered by the group that received the small dose of  $Al_2(SO_4)_3$  was 57.84%, above the control average of 46.15%, result at the limit of statistical significance. The group that received the high-dose of  $Al_2(SO_4)_3$  spent in the open arms a percentage of 50.39% of its time, higher than percentage achieved by the control group ( $p < 0.05$ ). According to the results, we can state that only high doses of both compounds had anxiolytic effect at

120 minutes after administration, the effect being statistically significant compared to control.



**Figure 4.**

The number of open arm visits 120 minutes after the acute administration of aluminium compounds

\*\* $p < 0.05$  as compared with the control group;

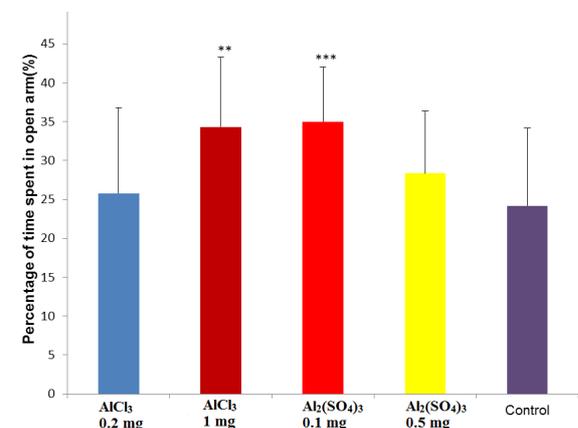
\*\*\* $p < 0.001$  as compared to the control group

In terms of the number of visits made in the open arms, 120 minutes after the administration of the small dose of  $AlCl_3$  (Figure 4) it decreased the number of visits, registering an average of 8.12 compared with controls which presented an average of 10.75 ( $p < 0.05$ ). The high dose  $AlCl_3$  showed an average number of visits in the open arms of 7.87, result that is statistically significant compared to the control. After 120 minutes, the number of visits in the open arms (Figure 4) recorded in the animals receiving  $Al_2(SO_4)_3$  was higher than the average achieved by the control ( $p < 0.05$ ): those receiving low dose recorded a total of 13.87 visits in the open arms, and those receiving the high-dose recorded 12.87 visits. According to the results, we can say that the two doses of  $AlCl_3$  decreased the number of visits 120 minutes after administration. The high dose of  $Al_2(SO_4)_3$  increased significantly the number of visits in the open arms compared with controls. These effects were statistically significant compared to the control group.

*Evaluation of anxiety after chronic administration of aluminium chloride and aluminium sulphate*

After the chronic administration of a dose of 0.2 mg/kg bw  $AlCl_3$  it increased the percentage of time spent in the open arms (Figure 5) recorded an average of 77.3 compared with controls which presented an average of 72.5, but the result was not statistically significant. Also, the dose of 1 mg/kg bw of  $AlCl_3$  showed an increase in the percentage of time spent in the open arms averaging 102.9, result statistically significant compared to control. After 14 days the percentage of time spent in the open arms registered by the group that received a dose of 0.1 mg/kg bw of  $Al_2(SO_4)_3$  was 105, well above the average of 72.5 achieved by the control,

result with statistical significance. The group that received a dose of 0.5 mg/kg bw  $\text{Al}_2(\text{SO}_4)_3$  spent in open arms a percentage of 104.5, higher than the percentage of 72.5 achieved by the controls ( $p < 0.05$ ). According to the results, we can say that only the high dose of  $\text{AlCl}_3$  and the low dose of  $\text{Al}_2(\text{SO}_4)_3$  caused an anxiolytic effect after chronic administration, the effect being statistically significant compared to the control group.



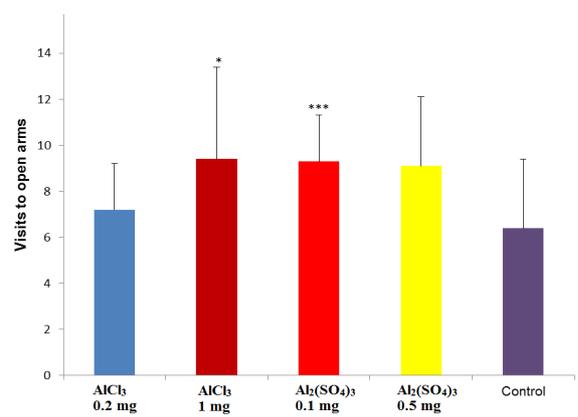
**Figure 5.**

Time spent in the open arms after chronic administration of aluminium compounds

\*\* $p < 0.05$  as compared with the control group;

\*\*\* $p < 0.001$  as compared to the control group

After chronic administration it was observed that the number of visits in the open arms made by the group that received the low dose of  $\text{AlCl}_3$  (Figure 6) increased, with an average of 7.2 compared with controls who presented an average of 6.4. The high dose of  $\text{AlCl}_3$  showed 9.4 average of visits spent in the open arms, result statistically significant compared to controls.



**Figure 6.**

Number of visits in the open arms after chronic administration of aluminium compounds

\*\* $p < 0.05$  as compared with the control group;

\*\*\* $p < 0.001$  as compared to the control group

The number of visits made in the open arms of a group that received the small dose of  $\text{Al}_2(\text{SO}_4)_3$  was

9.3 which increased compared to the average of 6.4 registered in the control group ( $p < 0.05$ ). The group that received the high-dose of  $\text{Al}_2(\text{SO}_4)_3$  performed a total of 9.1 visits in the open arms, above the average of control, but the result is not statistically significant.

According to the results, we can state that the two aluminium compounds increased the number of visits in the open arms in chronic administration.

## Conclusions

30 minutes after the administration of the two aluminium salts, in large doses, increased the percentage of time spent in the open arms thus demonstrating an anxiolytic effect. The anxiolytic effect of  $\text{Al}_2(\text{SO}_4)_3$  was maintained also 2 hours after the administration.

In chronic administration, only the high-dose of  $\text{AlCl}_3$  and the low-dose of  $\text{Al}_2(\text{SO}_4)_3$  lead to an anxiolytic effect.

The anxiolytic effect of studied aluminium compounds may be due to the aluminium ion. This anxiolytic effect caused by administering the high doses of aluminium chloride or aluminium sulphate may be due to the interference with the neuronal transmission systems (possible GABA or serotonergic).

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