Attempt at the determination of aluminum nitrate LD$_{50}$ and the study of its neurotoxicological effect in Wistar rat
Try to see the determination of aluminum nitrate LD$_{50}$ and the study of its neurotoxicological effect in Wistar rat

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Abstract

Values of oral lethal dose of aluminum (Al) nitrate are extremely different and rare in the literature. The study is an attempt to determine the oral LD$_{50}$ of aluminum nitrate in the Wistar rats and to measure the effect of the high doses of this aluminum compound on rats’ different organs, on acetylcholinesterase (AChE) activity, and on acetylcholine (ACH) levels in the hippocampus. Four groups of male Wistar rats are used ($n = 28$). The treated groups receive three doses of aluminum nitrate (Al$_1 = 2,500$ mg/kg, Al$_2 = 3,500$ mg/kg, and Al$_3 = 4,500$ mg/kg) once by gavage, while control rats receive tap water. All rats are examined twice daily for mortality and impairment during the 2-week experiment. The body weight (BW) is measured at the beginning and at the end of the experiment. Dissection is realized for each dyed rat and the dosage of AChE activity and ACh levels is realized at the end of the experiment by colorimetric method. The obtained results show that the higher dose (Al$_3$) kills 30% of the rats under study and causes spleens’ dark discoloration in the dyed rats. Both Al$_2$ and Al$_3$ decrease significantly the spleen weight ($p < 0.01$) and AChE activity ($p < 0.01$), but increase significantly the ACh levels ($p < 0.01$ and $p < 0.001$, respectively), in hippocampus of rats. Even if the lethal dose of aluminum nitrate is not reached, the effect of the high acute doses on viscera and cholinergic system is demonstrated.

Keywords: Aluminum nitrate; spleen; AChE; ACh; rat.

Introduction

Aluminum is the third most abundant element, after oxygen and silicon in the earth’s crust. It is widely distributed and constitutes approximately 8% of the earth’s surface layer (Kabata-Pendias and Pendias, 1993). Due to their several chemical and physical properties, aluminum and its compounds are used in different fields. They are used in many diverse and important industrial applications such as alums in water treatment and alumina in abrasives and furnace linings. They are found in consumption products such as antacids, astringents, buffered aspirin, food additives, vaccines, and antiperspirants (aluminum profiles). Aluminum has several inorganic compounds (aluminum bromide, chloride, acetate, nitrates, and so on) that differ by their physicochemical characteristics and toxicity, identified by their LD$_{50}$ (Llobet et al., 1987; Yellamma et al., 2010).

Aluminum was considered, for a long time, as nontoxic element and completely excreted out of the body by renal way. However, nowadays, it is a well-known fact that Al affects several organs (brain, liver, kidney, etc.) and gets accumulated in them, and it specifically targets the nervous system (Julka et al., 1996; Azzaoui et al., 2008; Rawy et al., 2012). The hippocampus is most affected by aluminum toxicity. Santos et al. (1987) demonstrated a preferential accumulation of Al in the hippocampus in rat and Abd El-Rahman (2003) reported spongiform changes in the neurons of the hippocampus, nuclear deformity, neurofibrillary degeneration, and foci of demyelination in Al-intoxicated rats. Indeed, it is admitted that aluminum impairs the cholinergic system; some studies found that aluminum increases the AChE activity (Blilkei-Gorzo, 1993; Zatta et al., 2002). However, others showed that Al decreases the AChE activity (Kaizer et al., 2008; Yellamma et al., 2010). Gulya et al. (1990) proved that the effect of Al on AChE is biphasic: it increases AChE at low concentrations of Al and decreases it at higher concentrations of Al.

The aim of the present study is to determine an oral LD$_{50}$ of aluminum nitrate from which the rare values found in the literature are extremely different (542 mg Al/kg BW (National Research Council, 1981); 261 mg Al/kg (BW) (Llobet et al., 1987); and 3,671 mg Al/kg (BW) in some material safety data sheet of the same product. By corollary,
another aim is to evaluate the effect of all aluminum doses used on rats’ organs, on AChE activity, and on ACh level in rats’ hippocampus.

Materials and Methods

Animals and treatment
Male Wistar rats, 6 months of age and 197.05 ± 0.66 g in weight (mean ± SEM, n = 28) at the beginning of the treatment, are used in this study. They were reproduced in colony room of Biology Department, Faculty of Sciences, Kenitra, Morocco. The rats are put in propylene cages under standard conditions (20°C, 50–70% humidity, and 12L:12D cycle). They are given free access to food and tap water. The control rats (n = 7) are given tap water and the tested rats (n = 21) receive three different doses: Al1 = 2,500 mg/kg (n = 7), Al2 = 3,500 mg/kg (n = 7), and Al3 = 4,500 mg/kg (n = 7) of aluminum nitrate (Farco Chemical Supplies) diluted in distilled water, once by gavage. Experimental procedures are also examined and approved by the internal ethical committee for animal welfare.

Observations
All rats were examined twice on a daily basis for mortality during the 2-week experiment.

Body and organs’ weight
Bodyweight is recorded at the beginning of the test and at the end of the experiment. The weight of the brain, liver, spleen, and kidneys is also taken at the end of the experiment, after anesthetia by the chloral 7%. All of these organs are subjected to detailed internal examination.

Determination of AChE levels
The specific activity of AChE is determined as described by Ellman et al. (1961). The reaction mixture contained 3.0 ml of 0.1 M phosphate buffer (pH 8.0), 20 ml of 0.075 M acetylthiocholine iodide, and 100 ml of 0.01 M 5,5-dithiobis-2-nitrobenzoic acid. The reaction was initiated with the addition of 100 ml of synaptosomal fraction. The color absorbance was measured at 412 nm in spectrophotometer (Reddy et al., 2007).

Determination of ACh levels
ACh levels were determined as described by Augustinsson (1963). The synaptosomal fractions of hippocampus were placed in boiling water for 5 min to terminate the AChE activity and also to release the bound ACh. To the synaptosomal fractions, 1 ml of alkaline hydroxylamine hydrochloride followed by 1 ml of 50% HCl was added. The contents were mixed thoroughly and centrifuged. To the supernatant 0.5 ml of 0.37 M ferric chloride was added and the intensity of the color developed was read at 540 nm against a reagent blank in a spectrophotometer (Reddy et al., 2007). Both results (of AChE and ACh) are expressed as percentage of control results.

Statistical analysis
The group data are expressed as mean ± SEM. The statistical tests used are analysis of variance (ANOVA1) and the least significant difference (LSD) post-hoc test. Differences between groups are considered significant at p < 0.05, 0.01 and 0.001.

Results
The objective of the experiment is to determine the LD50 of aluminum nitrate; however, the used doses are very high, and the 50% of death is not reached—even at the last dose of 4,500 mg/kg of aluminum nitrate. At this dose, only 30% of rats died.

Gross pathology
In the sacrificed rats, all the organs are normal in the four groups. However, in the rats receiving Al3 (4,500 mg/kg) and that died before the end of the experiment, a dark discoloration of the spleen is observed.

Bodyweight
The obtained results show no significant difference in BW between control and all treated groups, even at the beginning (BW0) or at the end of the experiment (BW1) (Figure 1).

Organ weight
The three administered doses of Al (Al1, Al2, and Al3) had no effect on the weight of the brain, liver, and kidneys. However, they cause a significant decrease of spleen weight (F(3,16) = 7.48; p < 0.01). The post-hoc statistical study demonstrates that the high significant decrease is obtained after the administration of Al2 and Al3 (p < 0.01 and p < 0.001, respectively; Table 1).

AChE levels in the brain
The administration of aluminum nitrate decreases the AChE activity in hippocampus of intoxicated rats compared to the ones under control (F = 4.11; p < 0.01). The LSD post-hoc statistical test demonstrates that the Al2 and Al3 doses decrease significantly (p < 0.01) the AChE activity by 35.3% and 44.17%, respectively (Figure 2).
Figure 1: Effect of Al on bodyweight of intoxicated rats. 

BW0: initial bodyweight, BW14: bodyweight at the end of the study. C: control rats, Al1: rats receiving 2,500 mg/kg of Al nitrate, Al2: rats receiving 3,500 mg/kg of Al nitrate, and Al3: rats receiving 4,500 mg/kg of Al nitrate.

Table 1: Effect of Al on the brain, liver, spleen, and kidney weights (g/100 g b.w.) ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Control (C)</th>
<th>Al1</th>
<th>Al2</th>
<th>Al3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>1.89 ± 0.07a</td>
<td>1.80 ± 0.07a</td>
<td>1.79 ± 0.07a</td>
<td>1.73 ± 0.08a</td>
</tr>
<tr>
<td>Liver</td>
<td>5.16 ± 0.34a</td>
<td>5.14 ± 0.26a</td>
<td>4.84 ± 0.42a</td>
<td>4.83 ± 0.55a</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.44 ± 0.04a</td>
<td>0.35 ± 0.03a</td>
<td>0.30 ± 0.02a</td>
<td>0.22 ± 0.02a</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.81 ± 0.05a</td>
<td>0.79 ± 0.04a</td>
<td>0.82 ± 0.03a</td>
<td>0.91 ± 0.04a</td>
</tr>
</tbody>
</table>

Note: Results are represented as mean ± SEM. Weight is expressed in g/100 g of BW. Values that do not have the same letters (a, b, c) are significantly different from (p < 0.01)**. C: control rats, Al1: rats receiving 2,500 mg/kg of Al nitrate, Al2: rats receiving 3,500 mg/kg of Al nitrate, and Al3: rats receiving 4,500 mg/kg of Al nitrate.

Figure 2: Effect of Al on AChE levels on hippocampus of rats. C: control rats, Al1: rats receiving 2,500 mg/kg of Al nitrate, Al2: rats receiving 3,500 mg/kg of Al nitrate, and Al3: rats receiving 4,500 mg/kg of Al nitrate. Every column represents the means of % of AChE levels compared to control ones. The statistical difference between control (C) and treated (Al2, Al3) is significant at p < 0.01**.

ACh levels in the brain
The administration of aluminum nitrate increases the ACh levels in hippocampus of intoxicated rats compared to the ones under control (F = 9.89; p < 0.001). The LSD post-hoc statistical test demonstrates that the Al2 and Al3 doses increase significantly the ACh levels (p < 0.01 and p < 0.001, respectively) (Figure 3).
Discussion

The experiment focused to determine an LD₅₀ of aluminum nitrate whose data are old, rare, and divergent in the literature. Some old studies reported that the oral LD₅₀ in the rats is 542 mg/kg (National Research Council, 1981), 264 mg/kg (US Coast Guard, 1984), and 261 mg/kg of aluminum nitrate (Llobet et al., 1987). However, most recent LD₅₀ value published in some Material Safety Data Sheet of societies producing aluminum nitrate as Mallinckrodt Chemicals, Sigma Aldrich, and Ficher Scientific, is 3,671 mg/kg of aluminum nitrate by oral route. In this study, the higher dose used (Al₁ = 4,500 mg/kg) caused the death of only 30% of the rats under study. So it is impossible for us, to increase the dose more than 4,500 mg/kg, to determine the LD₅₀ because the OCDE (2001) sets the dose of 5,000 mg/kg as maximum testing dose of acute study tests.

The current findings show that aluminum does not affect the BW of intoxicated rats. In previous study, it was found that aluminum decreased the BW of intoxicated rats, but at the end of the intoxication experiment (90 days; Azzaoui et al., 2008). Other research found that exposition to aluminum salts did not affect the weight gain of rats (Muller et al., 1990; Gonda and Lehotzky, 1996).

Indeed, high acute doses of Al affect significantly the rat organs. The spleen of rats receiving the high dose of Al, and dying before the end of experiment, shows dark discoloration. A significant decrease of spleen weight in group receiving high dose (Al₁ and Al₂) is registered, but the other observed organs show impairments. Few studies related to the effect of aluminum on spleen are published. Those we have come across evoked high aluminum accumulation in this organ. This accumulation could perturb the normal functioning of this organ (Llobet et al., 1987; Julka et al., 1996).

It was reported that the accumulation of Al in the brain, following acute and chronic intoxication by aluminum, causes biochemical changes leading to damage in the cholinergic system (Kosik et al., 1983; Julka et al., 1995; Meyer et al., 1996; Kaizer et al., 2005).

The cholinergic system is essential in mediating cognitive processes. Thus, any dysfunction in this system will induce impairments in all neurocognitive performance especially in learning and memory (Miù et al., 2003; Azzaoui et al., 2008; Voss et al., 2010; Abu-Taweel et al., 2012). The measure of AChE activity and ACh levels in the hippocampus of intoxicated rats shows a significant decrease in the AChE activity and significant increase in ACh levels, in rats receiving the acute high doses of Al (3,500 and 4,500 mg/kg). This result is consistent with others who found that the high concentrations of aluminum inhibit the AChE activity (Marquis and Black, 1984; Gulya et al., 1990). Indeed, Moraes and Leite (1994) report the in vitro inhibitory effect of very low concentrations of aluminum salts (IC₅₀ = 4.1 x 10⁻¹² M) on bovine...
brain AChE. Moreover, acute toxicity of aluminum chloride at 3.7 g/kg BW, administered per o.s. to gerbil, decreases the activity of AChE in the mitochondrial and microsomal fractions of hippocampus (Micic and Petronijevic, 2000). An in vitro study by Jankowska et al. (2000) demonstrates that an excessive AChE release, evoked by Al, is likely to increase acetyl-CoA utilization for resynthesis of the neurotransmitter pool and cause deficit of this metabolite in differentiated cells. Recently, Yellamma et al. (2010) have proved that AChE activity is inhibited by 700 mg/kg (BW) of aluminum acetate in hippocampus of orally intoxicated rats and their results also reveal that while AChE activity is inhibited, ACh level is elevated differentially in the studied area of the brain under aluminum toxicity.

Even several studies about the neurotoxic effect of aluminum are conducted (Jankowska et al., 2000; Kaizer et al., 2005; Nayak, 2006; Azzaoui et al., 2008; Yellamma et al., 2010; Abu-Taweel et al., 2012), its pathway is still discussed. Some studies suggest that Al interferes with the metabolism of glucose leading to the reduction of the synthesis of the precursors of the ACh. Other research shows that it could interact with Na⁺/K⁺ ATPase and Ca²⁺/Mg²⁺ ATPase affecting the system of neurotransmission at the level of the neuronal presynaptic membrane (Nayak, 2002). Also, it is found that Al interferes with iron and magnesium (Crichton et al., 2002) and with calcium in extra and intracellular compartments, leading to an alteration in acetyl-CoA metabolism (Bielarczyk et al., 1998).

In this study, the high doses used (3,500 and 4,500 mg/kg) of aluminum nitrate affect the spleen (the principal organ of immunity), decrease the AChE activity and increase the ACh levels in hippocampus. More investigations are needed to understand well the neurotoxic effect of aluminum nitrate in acute toxicity.

Ethical Approval
The study was approved by the institutional ethical committee of the Department of Biology, Faculty of Sciences, Ibn Tofail University, Kenitra, Morocco.

Conflict of Interests
There is no conflict of interest.

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