Aluminium Induced the Oxidative Stress Modification and a Behavioural Variation in Rat 

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Abstract

Background: Aluminium (Al) poisoning is a potential factor in brain damage at even low and high levels, neurochemical dysfunction and severe behavioral troubles. Considering this effect, our study was carried out to investigate the effects of wormwood extract to restore enzymes activities, lipid peroxidation, carbonyl and behavioral changes induced by Al. Methods: twenty four Wistar rats were divided into four groups: one group was exposed to a mixture containing100 mg/kg body weight of AlCl3 in the drinking with wormwood extract (Al+Pl), another group received wormwood extract (200 mg/kg body weight), other group received water after stopped intoxication for 5 weeks (Al(-)) and a groups as control. Activities of lactate dehydrogenase (LDH), Catalase (Cat), thiobarbituric acid-reactive substances (TBARS) and carbonyl level were determined in the whole brain of rats and the grooming and locomotors activity were defined in all groups. Results: The behavioral test (locomotors, sniffing and grooming test) indicates a significant hyperactivity in the Al+Pl group compared with the control group. After treatment with wormwood extract, the Pl indicates a lower activity compared with control and Al+Pl group. The intoxicated group (Al+Pl) has a significantly increased TBARS and carbonyl value compared to control (P < 0.05) and, after treatment with the wormwood extract, a significant reduction was noted. The LDH activity decreased significantly (P < 0.05) in the Al+Pl group compared with the control, by ~39% and was increased in catalase activity by +196%. After wormwood extract administration, LDH and Cat activity were significantly changed compared to Al+Pl group. Conclusion: These data suggest that aqueous wormwood extract may play a very useful role in reduction of the neurotoxicological damage induced by Aluminium. Administration of wormwood extract may induce a neurochemical change and attenuate the alteration in neurobehavioral activity, observed in the development of age related disorders, such as Alzheimer’s disease.

Keywords 
Aluminium, Behavioral Test, Brain, Catalase, Carbonyl, Lactate Deshydrogenase, Lipid Peroxidation

1. Background

Aluminium (Al) is the third most abundant metal in the earth’s crust. Exposure to Al is almost inevitable, since it is present in air, soil and water [1]. It is a highly neurotoxic element that may be involved in neuronal degeneration in human and experimental animal [2]. It is well established that it induces the production of free radicals in brain. Accumulation of free radicals may cause degenerative events of aging such as alzheimer’s disease (AD) [3]. Indeed, lipid peroxidation is considered as the most prominent form of oxidative damage in neurodegenerative
lesions due to the brain’s relative enrichment in polyunsaturated fatty acids [4]. On the other hand, an increasing number of reviews implicating cholesterol metabolism in the development of Alzheimer’s disease (AD) have been published [5] which suggest cholesterol as a target for treatment. However, an animal model cannot to reproduce all cognitive, behavioral, and biochemical abnormalities observed in AD patients [6], but using Al salts, a partial reproduction of some AD hallmarks, have been achieved [7].

Experimental evidence suggests that cellular damage mediated by free radicals can be involved in the pathology associated with Al intoxication [3]. In fact, the cerebral damage induced by Aluminium occurs preferentially in specific populations of neurons in cortex and hippocampus. [8], the cognitive functions are localized in the cerebral cortex, while the cerebellum regulates the execution of movements, whereas the hippocampus area is the site of memory storage and was implicated in behavioral comportment. Consequently, these anatomical sites are crucial by modulating the emotive answer, memory and behavior, and exposure of brain under development to aluminium, for a long time, can compromise a variety of neurotransmitter systems. [9].

Moreover, in the search for new biomolecule, which may offer neuroprotection by controlling both behavioral and oxidative stress, we have tested Absinthium Artemesia L extract. Wormwood (Artemisia absinthium L.) has a high content of nutrients and phytochemicals such as total phenolic compounds and total flavonoids, suggesting that these compounds contribute to the antioxidative activity [10]. Phenolic substances such as flavonols, cinnamic acids, coumarins and caffeic acids or chlorogenic acids are believed to have antioxidant properties that may play an important role in protecting cells and any organ from oxidative degeneration [11, 12]. However, no study has reported the effects of Artemisia absinthium L. on Aluminium-induced neurotoxicity. The deficits in learning and memory in Al-exposed rodents are accompanied by damage to neurons and changes in some neurotransmitters, such as the cholinergic and catecholamine neurotransmitter system are involved [13, 14]. In the present study, we aimed to investigate the neuroprotective effect of wormwood against aluminium-induced oxidative stress changes in the rat brain. Furthermore, we assessed the ability of wormwood to attenuate the aluminium effects on behavioral changes.

2. Materials and Methods

2.1. Reagents

Aluminium chloride (AlCl₃, 6H₂O) and all used chemicals were of analytical grade and purchased from Sigma-Aldrich Chemical Co. (St. Louis, France).

2.2. Preparation of Wormwood Plant Extracts (A. Ab)

Whole plants of Artemisia Absinthium L. were collected from Boussefer (Oran), Algeria, in June. The plant was identified and authenticated at the Herbarium of Botany Directorate in Es-Senia (Oran) University. Five hundred grams of whole wormwood plants were extracted with 1.5 L of distilled water by continuous hot extraction at 60°C twice for 30 min and the filtrate was lyophilized. The residue collected (yield 75 g) was stored at -20°C. When needed, the extract was dissolved in distilled water.

2.3. Animals and Tissue Preparation

In the experiment, 24 Wistar rats were used. The rats were housed in groups of tree rats per cage and had free access to food and water, except during the tests. They were exposed to a 14–10-h light-dark cycle and the room temperature was controlled at 23±2°C. Animals were exposed to Al when they weighed 70±8.21 g.

Experiments were performed during 9 weeks. 24 Wistar rats were divided into five groups according to:

- Control: Rats (n=6) received normal drinking water during 12 weeks.
- Al+Pl: Rats exposed to Al (at the concentration of 200 ppm, in the form of AlCl₃ in their drinking water ad libitum) for 9 weeks.
- Pl: After 4 weeks of intoxication with AlCl₃, this group received wormwood extracts at the dose of 200 mg/L in drinking water ad libitum for additional 5 weeks.
- Al(-): after 4 weeks of intoxication with AlCl₃, the rats received normal drinking water for 5 weeks.

Animals were sacrificed by cervical decapitation under pentobarbital anesthesia (60 mg/kg). The brain was removed, washed with normal saline and all the extraneous materials were removed before weighing. The brain was kept at ice-cooled conditions all the time. The brain was removed and quickly excised, minced with ice-cold saline, blotted on filter paper and homogenized in 50 mmol/l phosphate buffer (pH 7.4). The supernatant was frozen at -20°C for further determination of enzymatic activities and lipid peroxidation level.

The present work was in strict respect of the ethics regarding the use, the handling and preservation of the animals as specified by the Ethics Committee of the scientific committee of our university (University of Oran 1 Ahmed benbella) (02/2010).
2.4. Biochemical Estimation

The activities of catalase were assayed colorimetrically at 620 nm, in brain and expressed as µmoles of H₂O₂ consumed/min/mg as described by Sinha (1972) [15]. The carbonyl formation was measured using 2,4-dinitrophenylhydrazine (DNPH) as a reagent according to Levine et al. [16] with some modifications. Briefly: 100 µl of homogenate (10%) was incubated with 20µl streptomycin sulphate (10% w/v) solution and the mixture was centrifuged at 3000xg. The supernatant was equally divided in two test tubes and the protein was precipitated by adding equal volumes of 20% trichloroacetic acid (TCA). The tubes were again centrifuged at 3000xg and supernatant was decanted. 1.5 ml DNPH (10 mmol/l) in 2 mol/l HCl was added to one tube and 0.5 ml 2 mol/l HCl was added to another tube. Both the tubes were vortex mixed for 1 hour. To these tubes 1.5 ml 20% TCA was added and kept for 15 minutes at room temperature. Then the mixture was centrifuged at 4000xg. The precipitates were washed three times with ethyl acetate: ethanol mixture (1: 1) to remove the excess of DNPH. The final protein pellet was dissolved in 1.25 ml 6M-guanidine hydrochloride and the absorbance of both solutions (DNPH and HCl) was measured at 370 nm in a UV spectrophotometer. Lipid peroxidation in the brain was estimated colorimetrically by thiobarbituric acid reactive substances (TBARS) by the method of Niewieta and Samuelsson [17]. The activity of LDH in brain was measured using commercial reagent kits (Randox-Antrim, UK), briefly, the tissue was homogenate in 5 mL buffer containing 100 mM potassium phosphate (pH 7.0) and 2 mM EDTA, per gram tissue. Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay. 50µL of samples was mixed with 950µL of working reagent and mixed briefly; the reaction could be monitored by measuring the absorbance at 565nm.

2.5. Behavioural Observations

2.5.1. Post-Weaning Behavioral Tests

At 9 weeks, the behavioral tests were performed in the first half of the light phase of light/dark cycle. All behaviors were scored by a single trained observer unfamiliar with treated animals. Hand operated counters and stopwatches were used to score animals’ behavior.

2.5.2. Motor Activity (Open Field Test, (OFT))

The open field test provides simultaneous measures of locomotion, and anxiety [18]. The open field used was a square wooden arena measured (90 x 90 x 25 cm). The floor was divided by white lines into 36 smaller squares (15 x 15 cm). The open field maze was cleaned between each rat to avoid odor cues. The rats were carried to the test room in their home cages and tested once at a time for 10 min each. Other elements of exploratory activity such rearing, grooming and sniffing were carefully observed and time spent performing each behavior was recorded. These parameters were defined as follows: rearing (standing on hind legs with paws pressed against the wall of the arena); sniffing (continuous placing nose against the floor for at least 2 s); grooming (using paws or tongue to clean/scratch body) [19].

2.5.3. Statistical Analysis

The mean±SEM values were calculated for each group to determine the significance of the intergroup difference. Each parameter was analyzed separately using the one-way analysis of variance (ANOVA) test. To determine the difference between the groups, Student’s “t” - test was used. P<0.05 were considered to be significant.

3. Results

The body weights gain of rats is presented in fig. 1. A significant decreased gain in body weight was observed in all treated groups compared to control group. However, Pl group indicate a significant increase in body weights compared to Al+Pl by 70.5%.

![Figure 1. Effect of aluminium on body weight gain in all groups. Values are mean±SD (n = 6).](image)

*P<0.05, All group were compared vs. control. (Student’s “t”-test)

After stopped intoxication and treatment with wormwood extract, the level of TBARS significantly (P < 0.05) increased in the Al, Al+Pl and Al(-) group compared to control group by +84%, +108% and 87% in whole brain [Figure 2]. Carbonyl level was significantly higher in Al+Pl group by +156 vs. control (P<0.05), no a significant variation was noted between Pl and Al(-) compared to control. The catalase activity was significantly reduced in brain of Al group vs. control after 4 weeks of intoxication (P < 0.05) by -37%. After 5 weeks of stopped intoxication, a maximum
decreased activity was noted in brain of Al+Pl group (+147%) compared to control ($P < 0.05$). No differences were noted between the group Al(-) and control group after 5 weeks of stopped Aluminium intoxication [Figure 2]. We observed a significant decrease ($P < 0.05$) in the LDH activity in whole brain areas in the Al+Pl group compared with control group by -39%. After stopped intoxication, Al(-) group indicated a significant increase ($P < 0.05$) in LDH activity in all brain compared vs. control +38%. (figure 3).

Test (fig. 5), showed that time spent in the dark box was significantly increased in Al+Pl rats ($p<0.05$). Conversely, Al(-) group showed a significant reduction, in time spent in the dark compartment, compared to Al+Pl groups ($p<0.05$).

Values are mean±SD ($n = 6$). *$P<0.05$, All group were compared vs. control. (Student’s “$t$”-test)

Locomotors activity counts and sniffing test count (59.5±6.1 and 5.33±0.91, respectively) was significantly increased in Al+Pl group compared to control and all other group. We noted that the PI groups indicate a lower count compared to control, in the two tests. [figure 4]. After stopped intoxication for 4 weeks, Al(-) group displayed a lightly increased grooming activity counts (25.16±2.51) compared to control rats. There was no significant difference in grooming activity counts between all treated groups as compared to the corresponding control (figure 4). Results from dark-light box
Correlation analysis of all parameters in brain (Table 1) indicate that the TBARS level in brain was correlated positively with Carbonyl and catalase activity with a variable degree of correlation (r=0.612), but LDH activity was correlated negatively with TBARS, catalase and carbonyl level.

Table 1. Correlation coefficients among the biochemical parameters measured in the brain of rat after exposure to aluminium.

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<thead>
<tr>
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<th>TBARS</th>
<th>Catalase</th>
<th>Carbonyl</th>
<th>LDH</th>
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<tbody>
<tr>
<td>TBARS</td>
<td>1</td>
<td>0.521</td>
<td>0.612</td>
<td>-0.231</td>
</tr>
<tr>
<td>Catalase</td>
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<td>0.992</td>
<td>-0.567</td>
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<tr>
<td>LDH</td>
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4. Discussion

Alzheimer disease is a neurodegenerative disorder without an effective treatment. Alzheimer disease is associated with a decline in cognitive abilities. Aluminium is a non redox metal with is thought to be involved in etiology of Alzheimer's disease where it increases brain oxidative damage [20-21] causes inflammation, induces Aβ deposition, Tau phosphorylation and alters cholinergic transmission. Aluminium cause learning and memory deficits [22-23], which are in concordance with our findings, assessment was made using MWM paradigms.

The major effects of aluminium-induced neurotoxicity have been related to lipid peroxidation via free radical production [24]. In our study, chronic administration of aluminium chloride resulted in marked oxidative stress as indicated by increase in lipid peroxidation, carbonyl levels and catalase activity, which confirms the susceptibility of brain to oxidative insult. In other work, it was defined that this could be due to the reduced axonal mitochondria turnover, disruption of Golgi and reduction of synaptic vesicles induced by aluminium treatment which results in release of oxidative products like malondialdehyde, carbonyls, peroxynitrates within the neurons [25].

Furthermore, Nehru and Anand [26] reported a significant increase in brain thiobarbituric acid reactive substances in rats after stimulation by aluminium salts which was known to be bound by the Fe3+ carrying protein transferrin, thus reducing the binding of Fe2+ and increasing free intracellular Fe2+ that causes the peroxidation of membrane lipids and consequently membrane damage. Aluminium, being an inert metal, has been suggested to induce oxidative damage indirectly by potentiating the peroxidative effect of Fe2+. The promoted reactive oxygen species (ROS) formation can subsequently attack almost all cell components including membrane lipids by producing lipid peroxidation [25].

Aluminium neurotoxicity results in a behavioural and neurochemical alteration in neurons as a result of changes and disruption of the main structural components of the blood brain barrier, through primary injury to astrocytes and to secondary damage of the endothelial microvessel [27]. There are numerous studies utilizing experimental animal models on the central nervous system; these studies have mainly been concerned with the possible effects of aluminium on certain performance tasks that might reflect a cognitive function (learning and memory) or sensory-motor function in the infant animal exposed to lead very early in life [28]. Cholinergic neurons are positive markers for the evolution of memory and related disorders affecting acetylcholine and resulting in decreased activity of acetylcholinesterase and choline acetyl transferase [29]. Recent findings suggested that administration of aluminium was found to increase acetylcholinesterase in mouse brain [30]. In this study, the effects of exposure to chronic aluminium administered and it's exposed to wormwood extract on parameters of anxiety and related fear behaviours were investigated in rats. Our result indicated that the chronic aluminium exposure caused a significant increase in the anxiety levels of rats. But Thorne et al., have observed that animals treated with elevated doses of aluminium hydroxide occurred changes in behaviour: memory impairment and change ability of learning in the case of two different trials, associated with the detection of elevated aluminium concentrations at the cerebral level [31]. Interestingly, a significant improvement in the indicated measurement was observed when the rat was treated with wormwood extract (PI). Our results reported in the dark-light box test showed in aluminium-treated rats that time spent in the light compartment is reduced significantly compared to control animals, after stopping intoxication (Al(-)) the rat spent significantly more time in the light chamber compared with Al-Pl group. The data obtained herein in stereotypic tests showed that an obvious anxiety was installed in the
behaviour when the rats were exposed to aluminium. This finding indicates that aluminium-treated rat showed abnormal behaviour when compared to control. Wormwood extract administration has a very beneficial effect no with respect to the state of anxiety. Studies have been reported the role of oxidative stress in anxiety-like behaviour in rodents, and increased anxiety has been found to be positively correlated with increases in reactive oxygen species in granulocytes [32]. Hence, increased anxiety-like behaviours in the current study in aluminium-exposed rats (Al+PL) may be attributed to oxidative stress, which indicated by significantly increased of TBARS level and changes in enzymes activity (P<0.05), that catalyze reversible reactions amine group transfer between glutamic acid and pyruvate [30], in brain of rats. Regarding the cerebral protective effect of wormwood, we found that co-administration of wormwood extract with Al reduced significantly levels of TBARS and carbonyl and modified LDH and catalase activity in brain. [33-34]. Eventually, the decrease of LPO and change in LDH activity in brain after wormwood administration might also be attributed to its oestrogenic constituents (saponines, trigoneosides, flavonoids) [35]. Their action could be direct since phytoestrogens have shown potential neuroprotective properties [36].

5. Conclusion

In conclusion, the epidemiologic and experimental studies reported, there is ample evidence which supports the fact that aluminium plays a pivotal role in the neuropathology of AD. This study validates the fact that chronic exposure to aluminium causes cognitive dysfunction and oxidative damage after few weeks in rat. Moreover, it is establish that the Artemisia absinthium possesses antioxidant, anti-inflammatory and a cognition enhancement activity. In this current study, it has been suggested that the aqueous extract can be used as a regular nutrient to protect brain from chronic aluminium toxicity by amelioration of enzyme activities involved in expression neurotransmitter and protect brain cell from the deleterious effect of ROS generated in neuronal membrane and in the integrity functional of cell.

Competing Interests

The authors declare that they have no competing interests.

References


