Amelioration of aluminium - intake oxidative stress by some antioxidants in male albino rats

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Abstract

Background:
Aluminum is potentially toxic to humans. The Agency for Toxics Substances and Disease Registry (ATSDR) reported that aluminum accumulates mainly in the bone, liver, testes, kidneys and brain. The goal of the present study was to assess in rats the pro-oxidant effects induced by $\text{Al}^{3+}$ exposure, as well as the protective role of exogenous melatonin (M), vitamin E (vit. E) or N-acetylcysteine (NAC). The effect of aluminium (Al) alone or combined with antioxidants (M), (vit. E) or (NAC) on some physiological parameters and antioxidants in male albino rats were studied.

Material and methods:
The animals were assigned to 5 groups: control (group I); $\text{Al}^{3+}$--intake (53.5 mg $\text{AlCl}_3$/litre drinking water, group II); 5 mg melatonin/kg b.wt. plus $\text{AlCl}_3$ (group III); or vitamin E(100 mg/kg b.w.) plus $\text{AlCl}_3$ (group IV) or 100mg N-acetylcysteine plus $\text{AlCl}_3$ (group V). Rats were orally administered their respective doses daily for 30 days. At the end of the treatment period, blood was obtained. Thereafter, brain, liver, kidney and testes were removed. These tissues were processed to examine oxidative stress markers: reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GSHpx) and lipid peroxidation end products {malondialdehyde(MDA) + 4- hydroxynonenal (4-HNE)}. Samples of these tissues were also used to determine $\text{Al}^{3+}$ concentrations.

Results:
In Al-toxicated group , serum glucose and total cholesterol levels, liver enzyme activities (ASAT and ALAT), as well as, lipid peroxidation end products {malondialdehyde (MDA) + 4- hydroxynonenal (4-HNE)} were elevated significantly in the brain, liver, kidney and testes tissues when compared with control group. On the other hand, serum triglycerides and tissue (liver, kidney and testes) intracellular antioxidants glutathione (GSH) and superoxide dismutase (SOD) and liver glutathione peroxidase (GSHpx) activity decreased significantly. Brain GSH also decreased but SOD showed no significant changes. Melatonin, vit. E and NAC improved the levels of the different changed parameters when combined with Al. The most improved correction was recorded when $\text{Al}^{3+}$ combined with vit. E followed by M, then NAC. Serum $\text{Al}^{3+}$ levels were increased in $\text{Al}^{3+}$ treated group as well as groups exposed to $\text{Al}^{3+}$ combined with vit. E, M or NAC when compared with control group. $\text{Al}^{3+}$ could not be detected in tissues by atomic spectrophotometer (aluminium metal concentrations were below the limit of detection by AAS).

Conclusion:
The results show that $\text{Al}^{3+}$ exposure promotes oxidative stress in different tissues while melatonin, vitamin E and N-acetylcysteine exert antioxidant actions in $\text{Al}^{3+}$-treated animals. The protective effects of these antioxidants against cellular damage caused by $\text{Al}^{3+}$-induced oxidative stress, together with its low toxicity, make them worthy of investigation as potential supplements to be included in the treatment of neurological disorders in which the oxidative effects must be minimized as well as protection against liver, kidney and testes damage by Al- exposure. Dietary vitamin E supplementation may offer further protection.

Key words: Aluminium, melatonin, vitamin E, N-acetylcysteine, antioxidants, lipid peroxidation, MDA.
Introduction

Aluminum (Al) is the third most abundant element (8%) in the earth's crust and its compounds are distributed widely in nature (WHO, 2009). It constitutes of all soils, plants and animals (Yokel and McNamara, 2001 and Krewski et al., 2006). Although Al³⁺ is present in trace amounts in the biological material, it does not appear to be an essential element (Goyer, 2004) and usually considered to have harmful effects on general health. In addition to occurring naturally in food and water, Al³⁺ is added to drinking water, many processed foods, cosmetics, toothpaste, antiperspirants and adjuvants in various parenteral preparations and pharmaceutical agents (Becaria et al., 2002 and Pournourmohammadi, et al., 2008). Al³⁺ metal is used widely in different fields (cans, utensils, containers, automobile bodies, pigments, ...). Al³⁺ hydroxide is used as an antacid and has been used in the past to reduce phosphate accumulation in uremia (Yokel, 2000).

Aluminum is potentially toxic to humans. The Agency for Toxic Substances and Disease Registry (ATSDR) reported that aluminum accumulates mainly in the bone, liver, testes, kidneys and brain (ATSDR, 1990). Exposure to Al³⁺ could occur through three principal routes: 1) Inhalation of air contaminated with Al³⁺ compounds. 2) Oral ingestion of Al³⁺ dusts or with food and drinking water (WHO, 2009). The ingestion pathway is the most significant route of transfer of Al³⁺ from the environment to animals and humans, and 3) Dermal route (Akyol et al., 2004). In industrial settings, inhalation is the most important route of Al entry into the body. This leads to absorption of Al into the blood with possible systemic intoxication (Polizzi, 2002). Gastrointestinal absorption is minimal, although accumulation and toxicity were observed after intake of high doses of Al³⁺ in persons with chronic renal failure (Arnich et al., 2004 and Stella et al., 2005). The richest natural dietary sources of Al³⁺ are herbs and tea leaves (Jansen et al., 2002). The consumption of foods containing aluminum-containing food additives are a major source of aluminum in the diet (Saiyed and Yokel, 2005 and Soni et al., 2001).

Aluminum is a neurotoxicant (Sood et al., 2011). Esparza et al.(2003) showed that aluminum exposure promoted oxidative stress in different neural areas of the animals, including those in which aluminum concentrations were not significantly increased. It has been shown to play a role in the etiology of uremia – and dialysis – associated disorders of the brain (dialysis encephalopathy) and bone Al³⁺ associated bone disease. Al³⁺ also has been proposed as an environmental factor that may contribute to some neurodegenerative diseases, including Alzheimer's disease (AD).

It seems that Al³⁺ has varying effects on different organs associated with different exposure routes. However, Al³⁺ has a catalytic activity that produces free radicals stimulating oxidative injury in the brain (Christen, 2000 and Lemire et al., 2011).

In a review article, Mohammadiread and Abdollahi (2011) recorded a significant increase in LPO and inhibition of antioxidant enzymes by Al³⁺ in plasma (Ranjbar et al., 2008), brain (Sood et al., 2011), testes (Yousef and Salama, 2009 and Khattab et al., 2010), kidney, renal cortex, serum, erythrocyte (Farina et al., 2005), hepatocyte, liver (Mailloux et al., 2011).

Hypothetically, since oxidative stress plays a pathogenic role in Al³⁺ toxicity, supplementation with antioxidants should attenuate oxidative stress and improve oxidative stress-mediated damage in Al³⁺ toxicity. Therefore, there is an urgent need to identify effective antioxidants with therapeutic potential to ameliorate Al³⁺ toxicity.

Melatonin (N-acetyl-5-methoxytryptamine) is the major product of the pineal gland in vertebrates. It is a well-known antioxidant and free radical scavenger. Moreover, its solubility in lipid and aqueous media, which allows it to cross morphophysiological barriers and enter subcellular compartments, permit melatonin to function as a highly effective inhibitor of oxidative damage (Esposito and Cuzzocrea, 2010). It is a very potent and efficient endogenous free radical scavenger. It reacts with the highly toxic hydroxide radical and provide on – site protection against oxidative damage to different biomolecules (Reiter, 2000). It is also involved in the regulation of electron transfer, detoxifying reactive radical intermediates and control pre-oxidative processes (Tan et al., 2000).

Vitamin E, a lipid-soluble vitamin with antioxidant properties has an important role in protecting biological systems (Paulis et al., 2011). Vitamin E has a high antioxidant capacity and plays a fundamental biologic role, especially in protecting cells and tissues from...
oxidative damage and prevents the formation of toxic oxidation products such as those formed from unsaturated fatty acids (Quiles et al., 2002 and 2006). Also, vitamin E is effective in scavenging lipid radicals (ROO-) and is recognized as a potent chain-breaking antioxidant, with the particular function of preventing lipid peroxidation in the membrane and lipoproteins (Lorenzoni and Ruiz-Feria, 2006).

N-acetylcysteine (NAC), a cysteine prodrug, has shown promise in numerous pathological conditions involving oxidative stress (Vosters and Neve, 2002 and Kamboj et al., 2006a). As a sulfhydryl donor NAC contributes to the regeneration of glutathione and by directly acting as a free radical scavenger (Aydin et al., 2002). Various studies have shown that NAC administration has a beneficial effect against oxidative stress in neurodegenerative diseases (Pocernich et al., 2001 and Kamboj et al., 2008). Prakash and Kumar (2009) suggested that N-acetyl cysteine has a neuroprotective effect against aluminium-induced cognitive dysfunction and oxidative damage in rats.

So, the objective of this work was to investigate the ability of (M), vit. E or NAC to resist oxidative damage on the rat brain, liver, kidney and testes during exposure to Al<sup>3+</sup>. Also, the study aimed to compare the protective effects of these antioxidants.

Material and methods

Animals and treatment:

The chosen dose of Al<sup>3+</sup> was depended on the U.S. EPA survey of water supplies throughout the U.S., the maximum aluminum concentration reported in finished water where an aluminum compound was used as a coagulant was 5.35 mg/L (ATSDR, 1990) and multiplied by 10 i.e. the used dose was 53.5mg/ L drinking water (this limit is expected in developing countries).

Thirty male albino rats (180 – 200 gm) were used in this experiment. They were randomized and housed six to a cage in Stainless steel cages containing sawdust bedding. They received standard rat chow and water ad libitum. The room conditions were maintained at 22±2 °C and 12/12-h light/dark cycle. The animals were divided into five groups each consists of 6 animals:

1- Control group without any treatment.

2- Aluminium- intake group (53.5 mg AlCl<sub>3</sub>/litre drinking water).

3- Aluminium- intake group (53.5 mg AlCl<sub>3</sub>/litre drinking water) and supplemented with 100mg vitamin E / kg b.wt. by gastric tube.

4- Aluminium- intake group (53.5 mg AlCl<sub>3</sub>/litre drinking water) and supplemented with a daily dose of melatonin (M) 5 mg / kg b.wt. by using gastric tube.

5- Aluminium- intake group (53.5 mg AlCl<sub>3</sub>/litre drinking water, and supplemented with NAC (100mg/kg b. wt.) in distilled water by gastric tube.

Chemicals:

All chemicals, Aluminium chloride (AlCl<sub>3</sub>), Melatonin and N-acetyl cysteine Were purchased from Sigma Co. USA. Vitamin E “α-tocopherol acetate” capsules, supplied by Pharco Pharmaceutical Co., Egypt (each capsule contains 100 mg vitamin E).

After the experimentation period, the animals were fasted for 12 hours, and then sacrificed by sharp razor through jugular vein. The blood was collected; serum was separated and used for different analysis. The collected tissues (brain, liver, kidney and testes) of each animal were removed quickly, dried by filter paper, weighed and homogenized and kept at -20°C for analysis.

Methods:

The concentrations of glucose and total cholesterol in serum were estimated by kits obtained from Stanbio, Texas, USA according to Tietz (1995) and triglycerides was measured by the method of Bucolo and David (1973). ASAT and ALAT activities in sera samples were estimated according to the method of Schumann et al. (2002). GSH contents, SOD activity in the tested organs (brain, liver, kidney and testes) were determined by the methods of Prince and loose (1969) and Nishikimi et al. (1972) respectively. Hepatic GSHpx was measured according to the method of Ammerman, et. al. (1980) and Lipid peroxidation in the different tissues was estimated by colorimetric assay of malondialdehyde (MDA) + 4- hydroxynonenal (4- HDNE) as described by Esterbauer et. al. (1991), using kits from Wak-Chem Medical GMBH, Germany.
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Al\textsuperscript{3+} was determined in all samples using Atomic Absorption Spectrophotometer (AAS), at AAS unit, Chemistry department, Faculty of Science, Mansoura University.

Data are expressed as mean ± S.D. Statistical analysis of the results was performed by ANOVA (SPSS program) followed by Post Hoc tests. A difference was considered significant when p ≤ 0.05.

Results

The effect of Al\textsuperscript{3+} alone or combined with different antioxidants on serum glucose , total cholesterol, and triglycerides levels as well as ASAT and ALAT activities are presented in table (1). The obtained data revealed significant increases in glucose, total cholesterol levels and ASAT and ALAT activities in Al\textsuperscript{3+}- exposed group when compared with control group, while serum triglycerides were decreased. Different antioxidants (vit. E, M and NAC) combined with Al\textsuperscript{3+} corrected these changes to nearly the control level.

Serum Al\textsuperscript{3+} concentrations are represented in table (1): It elevated in all Al\textsuperscript{3+}- exposed rats alone or combined with antioxidants. Atomic absorption spectrophotometer could not detect Al\textsuperscript{3+} in the different tested tissues (aluminium metal measurements were below the limit of detection by AAS).

Table (1): some serum parameters in Al\textsuperscript{3+}- treated rats and antioxidants (M, vit.E or NAC).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>control</th>
<th>Al\textsuperscript{3+}</th>
<th>Al\textsuperscript{3+} + M</th>
<th>Al\textsuperscript{3+} + vit. E</th>
<th>Al\textsuperscript{3+} + NAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/100ml)</td>
<td>77.038±0.928</td>
<td>86.558±7.532\textsuperscript{a}</td>
<td>74.998±5.467\textsuperscript{b}</td>
<td>78.963±7.143</td>
<td>77.460±6.086</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/100ml)</td>
<td>92.985±12.597</td>
<td>105.938±19.799\textsuperscript{a}</td>
<td>92.813±13.025</td>
<td>93.112±11.628</td>
<td>93.530±9.203</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/100ml)</td>
<td>56.980±4.696</td>
<td>47.643±2.022\textsuperscript{a}</td>
<td>54.497±3.469\textsuperscript{b}</td>
<td>56.387±3.653 \textsuperscript{b}</td>
<td>56.985±4.698\textsuperscript{b}</td>
<td></td>
</tr>
<tr>
<td>ASAT (U/liter)</td>
<td>51.730±10.509</td>
<td>143.627±24.626\textsuperscript{a}</td>
<td>57.503±6.226 \textsuperscript{b}</td>
<td>54.188±5.920 \textsuperscript{b}</td>
<td>51.730±10.509\textsuperscript{b}</td>
<td></td>
</tr>
<tr>
<td>ALAT (U/liter)</td>
<td>40.807±6.546</td>
<td>63.592±7.695\textsuperscript{a}</td>
<td>46.720±5.997 \textsuperscript{b}</td>
<td>43.213±6.607 \textsuperscript{b}</td>
<td>45.922±6.250\textsuperscript{b}</td>
<td></td>
</tr>
<tr>
<td>Aluminium (Pg/liter)</td>
<td>0.232±0.066</td>
<td>8.443±1.219 \textsuperscript{a}</td>
<td>7.232±0.814 \textsuperscript{a}</td>
<td>8.332±1.517 \textsuperscript{a}</td>
<td>7.172±0.686 \textsuperscript{a}</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SD of 6 animals in each group.

a Significant at P≤0.05 when compared with control group.

b Significant at P≤0.05 when compared with Al\textsuperscript{3+}- intake group.

In table (2): Brain GSH, SOD and \{malondialdehyde(MDA)+4- hydroxynonenal (4-HNE)}in different groups are represented. Brain GSH decreased significantly and SOD insignificantly while (MDA)+4- HNE elevated significantly.

Table (2): some antioxidant parameters and \{malondialdehyde(MDA)+ 4- hydroxynonenal (4- HNE)}in the brain of Al\textsuperscript{3+}- treated rats and antioxidants (M, vit.E or ANAC).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Control</th>
<th>Al\textsuperscript{3+}</th>
<th>Al\textsuperscript{3+} + M</th>
<th>Al\textsuperscript{3+} + vit. E</th>
<th>Al\textsuperscript{3+} + NAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (µmol/g)</td>
<td>7.15± 0.49</td>
<td>5.15± 0.52a</td>
<td>6.36± 0.57</td>
<td>6.34± 0.47</td>
<td>6.40± 0.55</td>
<td></td>
</tr>
<tr>
<td>SOD (U/g)</td>
<td>103.00± 7.28</td>
<td>94.33± 5.74</td>
<td>100.67± 4.99</td>
<td>100.00± 3.61</td>
<td>106.00± 7.87</td>
<td></td>
</tr>
<tr>
<td>(MDA)+4- HNE (µmol/g)</td>
<td>1.55± 0.15</td>
<td>4.30± 0.28 a</td>
<td>2.34± 0.10 b</td>
<td>1.57± 0.39 b</td>
<td>2.56± 0.03 ab</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SD of 6 animals in each group.

a Significant at P≤0.05 when compared with control group.

b Significant at P≤0.05 when compared with Al\textsuperscript{3+}- intake group.
As shown in table (3): significant decreases of natural liver antioxidants GSH content; GSHpx and SOD activities in Al$^{3+}$- treated group. On the other hand, (MDA) + 4-HNE increased significantly in the same group when compared with control group. Rats treated with Al$^{3+}$ and antioxidants showed improvements in the different tested parameters. Vitamin E had more antioxidant effect than M and NAC.

Table (3): some antioxidant parameters and \{malondialdehyde(MDA) + 4- hydroxynonenal (4- HNE)\} in the liver of Al$^{3+}$- treated rats and antioxidants (M, vit.E or NAC).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>control</th>
<th>Al$^{3+}$</th>
<th>Al$^{3+}$+ M</th>
<th>Al$^{3+}$+vit E</th>
<th>Al$^{3+}$+NAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH</td>
<td>21.94±1.90</td>
<td>9.39±13.9a</td>
<td>16.64±1.49b</td>
<td>37.49±2.08ab</td>
<td>15.95±2.12b</td>
</tr>
<tr>
<td>(µmol/g)</td>
<td>21.94±1.90</td>
<td>9.39±13.9a</td>
<td>16.64±1.49b</td>
<td>37.49±2.08ab</td>
<td>15.95±2.12b</td>
</tr>
<tr>
<td>GSHpx</td>
<td>581.983± 54.822</td>
<td>344.310± 51.371a</td>
<td>524.497± 72.770b</td>
<td>594.278± 53.272b</td>
<td>566.818± 65.268b</td>
</tr>
<tr>
<td>(U/g)</td>
<td>46.143± 3.517</td>
<td>28.627± 5.065a</td>
<td>35.170± 4.771ab</td>
<td>46.188± 4.964b</td>
<td>40.063± 4.571b</td>
</tr>
<tr>
<td>SOD</td>
<td>2.29± .22</td>
<td>4.24± 0.32 a</td>
<td>2.32± 0.16 b</td>
<td>1.29± 0.18 ab</td>
<td>2.49±0.07 b</td>
</tr>
<tr>
<td>(U/g)</td>
<td>2.88±0.17 ab</td>
<td>1.25±0.03 b</td>
<td>2.88±0.17 ab</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SD of 6 animals in each group.

Kidney GSH content and SOD activity decreased significantly while (MDA)+ 4- HNE level increased significantly (table :4).

Table (4): some antioxidant parameters and \{malondialdehyde(MDA) + 4- hydroxynonenal (4- HNE)\} in the kidney of Al$^{3+}$- treated rats and antioxidants (M, vit.E or ANAC).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>control</th>
<th>Al$^{3+}$</th>
<th>Al$^{3+}$+ M</th>
<th>Al$^{3+}$+vit E</th>
<th>Al$^{3+}$+NAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH</td>
<td>23.66± 2.51</td>
<td>9.08± 0.96 a</td>
<td>15.66± 1.45 ab</td>
<td>29.89± 2.73 b</td>
<td>19.52± 2.53 b</td>
</tr>
<tr>
<td>(µmol/g)</td>
<td>23.66± 2.51</td>
<td>9.08± 0.96 a</td>
<td>15.66± 1.45 ab</td>
<td>29.89± 2.73 b</td>
<td>19.52± 2.53 b</td>
</tr>
<tr>
<td>SOD</td>
<td>61.33± 3.65</td>
<td>30.68± 3.10 a</td>
<td>67.67± 1.96 b</td>
<td>72.00± 8.00 b</td>
<td>45.00± 3.17 ab</td>
</tr>
<tr>
<td>(U/g)</td>
<td>61.33± 3.65</td>
<td>30.68± 3.10 a</td>
<td>67.67± 1.96 b</td>
<td>72.00± 8.00 b</td>
<td>45.00± 3.17 ab</td>
</tr>
<tr>
<td>(MDA)+ 4- HNE</td>
<td>1.57± 0.12</td>
<td>3.33± 0.09 a</td>
<td>2.79± 0.21 ab</td>
<td>1.25± 0.03 b</td>
<td>2.88± 0.17 ab</td>
</tr>
<tr>
<td>(µmol/g)</td>
<td>1.57± 0.12</td>
<td>3.33± 0.09 a</td>
<td>2.79± 0.21 ab</td>
<td>1.25± 0.03 b</td>
<td>2.88± 0.17 ab</td>
</tr>
</tbody>
</table>

Mean ± SD of 6 animals in each group.

In the present study, testes GSH slightly decreased while SOD significantly decreased with a concomitant increase in the lipid end products in Al$^{3+}$- intake group. The use of external antioxidant vit, E , M and NAC combined with Al$^{3+}$ enhanced these changes to nearly that of control group( table: 5).
Table (5): some antioxidant parameters and {malondialdehyde(MDA) + 4- hydroxynonenal (4- HNE)} in the testes of Al³⁺- treated rats and Al³⁺ plus antioxidants (M, vit.E or ANAC).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>control</th>
<th>Al³⁺</th>
<th>Al³⁺ + M</th>
<th>Al³⁺ + vit E</th>
<th>Al³⁺ + NAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (µmol/g)</td>
<td>27.38± 1.09</td>
<td>25.33± 0.38</td>
<td>27.27± 1.45</td>
<td>26.48± 0.74</td>
<td>26.41± 0.97</td>
</tr>
<tr>
<td>SOD (U/g)</td>
<td>28.00± 2.70</td>
<td>17.17± 1.40 a</td>
<td>26.33± 3.77b</td>
<td>31.33± 3.24 b</td>
<td>21.83± 1.42ab</td>
</tr>
<tr>
<td>(MDA)+4- HNE (µmol/g)</td>
<td>0.81± 0.04</td>
<td>1.92± 0.44 a</td>
<td>1.08± 0.06 b</td>
<td>0.69± 0.02 b</td>
<td>0.99± 0.09 b</td>
</tr>
</tbody>
</table>

Mean ± SD of 6 animals in each group.
a Significant at P≤0.05 when compared with control group.
b Significant at P≤0.05 when compared with Al³⁺- intake group.

Discussion

Aluminium is present in several manufactured foods and medicines and is also used in water purification (WHO, 2009). Aluminium is generally poorly absorbed by the gastrointestinal tract, much less than 1 percent in humans (Arnich et al., 2004 and Stella et al., 2005).

Aluminum has not been shown to have a definite biological function. Therefore, the present experiment was undertaken to determine the effectiveness of some antioxidants (M, vit. E or NAC) in modulating the aluminium chloride (AlCl₃) induced brain, liver, kidney and testes toxicity of rats.

Our results, recorded higher serum glucose and cholesterol levels in Al³⁺- intake group when compared with control group. The increased level of serum cholesterol may be due to the increased lipid peroxidation and membrane fluidity which previously recorded by Silva et al. (2002). Many authors recorded similar results (El-Demerdash,2004 and Fyiad, 2007) who reported high levels of glucose and cholesterol in rats exposed to Al³⁺. The antioxidants ( M,vit.E or NAC) corrected the bad effects of Al³⁺ on serum glucose, cholesterol and triglycerides. These changes were returned to approximately normal levels by vit. E, melatonin, or NAC treatments which can be attributed to their antioxidant activity. Franzini et al.(2008) recorded lowered glucose levels by various antioxidants and attributed this results to their antioxidant actions.

In this study, highly significant increases of ASAT and ALAT were recorded. This agree with many authors who used Al³⁺ – oral administration (El-Demerdash, 2004), interperitonial or in drinking water (Nedzvetsky et al., 2006). This elevations may be due to damage of cell membranes and release of its enzymes to the blood, since, Rajash and Latha, 2004) stated that elevation activities of these enzymes are indicative of cellular leakage and loss of the functional integrity of liver cell membrane. Also, Silva et al. (2002) and Stevanovic et al. (2008) suggested that the mechanism of Al³⁺ pro-oxidant action may be produced through its interaction with the membranes, subtle changes in the rearrangement of lipids which could attack and facilitate the propagation of lipid peroxidation leads to loss of membrane integrity, decrease its fluidity, disrupt the functioning membrane bound enzymes receptors and ion channels, which leads finally to cell death.

Oral administration of aluminium resulted in a significant increase in serum Al³⁺ of all Al³⁺- exposed rats but not in tissues (brain, liver, kidney and testes) Aluminium contents of the studied tissues could not be detected in this study may be due to the different rout of administration and the small dose available for every rat or may be the detected limit of AAS system was high. Also, aluminium serum concentration in our study is only 8.443µg Al³⁺ / liter, Since antioxidants (vit E, M and NAC) have no effects on serum Al³⁺- contents when combined with Al- exposure, it means that these antioxidants have no effect on aluminium excretion.

Antioxidants are generally categorized to non-enzymatic and enzymatic. Non-enzymatic antioxidants include dietary compounds (vitamins C and E), minerals (selenium and zinc), glutathione, uric acid and
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ubiquinol. Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx) are the main enzymatic antioxidants (Abdollahi et al., 2004 and Rezaie et al., 2007).

In tissues such as liver and brain, GSH is oxidized to GSSG in the presence of ROS resulting in a shift of GSH. In the present study aluminium worthy acts as a pro-oxidant. Similarly, Esparza et al. (2003) suggested that aluminium might facilitate membrane peroxidation by increasing their susceptibility to free radicals induced damage.

Lipid peroxidation is one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity and carcinogenicity of many xenobiotics (Anane and Creppy, 2001). Al$^{3+}$ has been reported to induce lipid peroxidation, and to alter physiological and biochemical characteristics of biological systems. Experimental animal models and cell culture studies reveal that aluminium affects the expression of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase and glutathione (GSH) possibly leading to membrane fragility as a consequence (Abubakar et al., 2003).

Elevation of lipid peroxidation in brain, liver, kidney, and testes as evidenced by the increased production of malondialdehyde (MDA) + 4- hydroxynonenal (4- HDNE) in the present study (tables:2,3, 4and 5), suggests participation of free – radical induced oxidative cell injury in mediating the toxicity of Al$^{3+}$ as previously recorded by Anane and Creppy (2001) and Dua and Gill (2001). The aluminium-induced group had an increase in malondialdehyde (MDA) + 4- hydroxynonenal (4- HDNE) associated with a significant reduction (P≤ 0.05) in liver reduced glutathione levels and also a reduction of hepatic GSHpx and SOD activities. Furthermore, neurons appear to be particularly vulnerable to free radicals as the important natural antioxidant glutathione content is low, they have higher membrane content of polyunsaturated fatty acids and brain requires substantial quantities of oxygen for metabolism (Gupta et al., 2004).These changes were significantly attenuated in the Al$^{3+}$ -exposed rats combined with antioxidants ( M, vit. E or NAC). Since M, vit. E and NAC play important roles as antioxidants and are consequently expected to protect tissues from damage caused by reactive oxygen metabolites (El- Demerdash,2004, Prakash and Kumar (2009) and Esposito and Cuzzocrea (2010 ).Aydin et al. (2002) suggested that NAC decreased lipid peroxidation by direct scavenging of free radicals or by increasing GSH levels. In addition, Pocernich et al. (2001) and Kamboj et al. (2008) have also shown that NAC has an inhibitory effect on brain lipid peroxidation and has a protective role in membrane stabilization as a free radical scavenger.

Mohammarad and Abdollahi (2011) reported that coadministration of α-tocopherol (Vitamin E) at 500 µg/g diet significantly preserved the GSH content of the brain and decreased the rate of lipid peroxidation. Brain had elevated lipid peroxidation end products {malondialdehyde (MDA) + 4- hydroxynonenal (4- HNE)} and reduction in GSH but not SOD activity. However, the lack of significant changes in brain cortex SOD activity (table, 2) after aluminium exposure is supported by the work of Abubakar et al. (2004b). These results may be due to the brain potent defenses against superoxide including dietary free-radical scavengers (ascorbate, α-tocopherol), the endogenous tripeptide glutathione, and enzymatic antioxidants (David et al. 2004).

Testicular oxidative stress appears to be a common feature in much of what underlies male infertility, which suggests that there may be benefits to develop better antioxidant therapies for relevant cases of hypospermatogenesis (Yousef and Salama, 2009 and Khattab et al., 2010). Yousef et al. (2005) found that aluminium enhanced lipid peroxidation in plasma, testes and liver.

In conclusion, the data presented in this paper using experimental animals, demonstrated that the toxic effects of Al$^{3+}$ such as neuro -, hepato-, nephron- and testicular toxicity, as a result of oxygen free radical generation, can be alleviated by administration of antioxidants M, vit. E and NAC.

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تحسين الشدة التأكسدية الناتجة من التسمم بالألومينيوم في ذكور الجرذان باستخدام بعض مضادات الأكسدة

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ملخص

يستخدم الألومنيوم بكثرة في أواني الطهي والتخليص وتنقية المياه وبعض العقاقير، كما قد يدخل الجسم عن طريق الجهاز التنفسي والجلد. وقد تولد شوارد حرة نتيجة للتجسم به. ولذلك تهدف هذه الدراسة لحماية الأنسجة (المخ والكبد والكلى والخصية) من التسمم بالألومنيوم باستخدام بعض مضادات الأكسدة (الميلاتونين وفيتامين هـ و ن- أسيتيل سيستين).

تم إعطاء نماذج من الذكور البيضاء 53.5 مجم كلوريد الألومنيوم كل لتر من ماء الشرب لمدة 30 يوماً؛ بينما عوملت باقي المجموعات بكلوريد الألومنيوم في ماء الشرب بالإضافة إلى مضادات الأكسدة فيتامين هـ 100 مجم/كجم والميلاتونين 5 مجم/كجم أو ن- أسيتيل سيستين 100 مجم/كجم عن طريق أنبوب المعدة وقورنت النتائج بالمجموعة الضابطة.

ارتفاع مستوى الجلوكوز والكوليسترول و إنزيمات الكبد ناقلات الأمين (ASAT & ALAT) ارتفاعاً ذو دلالة إحصائيةً في مصل الدم؛ بينما انخفضت الدهون الثلاثية انخفاضاً ذو دلالة إحصائيةً في المجموعة المسممة بالألومينيوم بالمقارنة بالمجموعة الضابطة. وتحسن هذا الانخفاض باستخدام مضادات الأكسدة: فيتامين هـ والميلاتونين و ن- أسيتيل سيستين.

واكتشف أن الجلوتاثيون المختزل في المخ والكبد والكلى والخصية نقصاً ذو دلالة إحصائيةً في ذكور الجرذان المسممة بالألومينيوم عند مقارنتها بالمجموعة الضابطة، وصاحبها نفس في الإكسيميات المضادة للأكسدة (جلوتاثيون بيروكسيدوزي في الكبد و فوق أكسيد الديسبروسيز في الكبد والكلى فقط) ولم يغير في نسب المخ؛ وارتفاعاً ذو دلالة إحصائيةً في أكسيد الدهون الفوقية في جميع الأنسجة المختبرة. لعبت مضادات الأكسدة المستخدمة دوراً هاماً في تعديل هذه التغيرات وتحسنها إلى مستويات تقترب مما هو مسجل للمجموعة الضابطة. و كانت أفضل النتائج المسجلة لفيتامين هـ ثم الميلاتونين ون- أسيتيل سيستين.

ويستخلص من هذه الدراسة أهمية مضادات الأكسدة الطبيعية المتاحة في الغذاء كفيتامين هـ، والعلاجية كالميلاتونين ون- أسيتيل سيستين لمنع حدوث مضاعفات التسمم بالألومنيوم الذي يستخدم بكثرة كأواني للطهي وتعبئة غذائية و في بعض العقاقير.

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