The Protective Role of Omega-3 and Melatonin Against AlCl₃

Induced some Biochemical Changes in Albino Rats

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Abstract

The aim of the present study was to demonstrate the protective role of omega-3, melatonin and their combination against AlCl₃ induced some biochemical changes especially (OS, lipid peroxidation and AD) markers in serum of experimental rats. Forty adult female rats were used in this present study. They were divided randomly into five groups, each group with eight rats. G1: considered as a control group. G2: (1000mg/L drinking water) AlCl₃. G3: (1000mg/L drinking water) AlCl₃ + omega-3 oil (4000mg/kg diet). G4: (1000mg/L drinking water) AlCl₃ + melatonin (50mg/kg diet). G5: (1000mg/L drinking water) AlCl₃ + omega-3 oil (4000mg/kg diet) + melatonin (50mg/kg diet). All the above groups left for 40 days. Administration of AlCl₃ (1000mg/L) caused decreased rats brain weight, while there were no statistical changes in body weight, water intake and diet consumption among the studied groups. The results of biochemical study revealed that AlCl₃ decreased SOD level while MDA and Aβ (1-42) peptide level increased. On the other hand, melatonin, omega-3 and their combination effectively increased SOD level except for omega-3 treated group in which no such effect detected on SOD level, while not affected on MDA level but decreased Aβ (1-42) peptide level. As well as CK activity increased in observed in AlCl₃ group while treatment with melatonin, omega-3 and their combination ameliorate this effect. Additionally, AlCl₃ unexpectedly decreased urea concentration, only combination of omega-3 and melatonin normalized urea concentration. Sub-acute dose of AlCl₃ did not cause any significant effect on serum ALP, AST, ALT creatinine, LDH and glucose levels.

In conclusion AlCl₃ caused many biochemical changes in of albino rats, while omega-3, melatonin either alone or together showed a protective role against AlCl₃ toxicity. This protection could be through the antioxidant and anti-amyloidal role of omega-3 and melatonin.

Keywords: AlCl₃ Toxicity, Omega-3, melatonin

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Introduction

Aluminum (Al) is a potent neurotoxin that plays a pivotal role in the neuropathology of Alzheimer's disease (AD), prolonged Al exposure induces cognitive dysfunction, oxidative damage and increases in deposition of beta amyloid (Aβ) in vivo (Kumar et al., 2009).

The toxic effects of Al may be due to the generation of reactive oxygen species (ROS), this metal is absorbed through the skin, gastrointestinal tract, lung and nasal mucosa, then accumulate in kidney, liver, brain and bone (Anand et al., 2002; EL-Demerdash, 2004).

On the other hand, fish fats are the major dietary source of docosahexaenoic acid (DHA), and reduced fish DHA intake increases risk for AD, it is a key component of all cell membranes which is found in abundance in the brain and retina (Tully et al., 2003; Maclean, 2005). Regarding OS, it is possible that chronic administration of poly unsaturated fatty acids (PUFAs) may make the brain more vulnerable to lipid peroxidation, thus inducing antioxidative defense capacity and leading to elevated tolerance and protection against FR induced injury (Cao et al., 2008).

As well as, melatonin is a secretary product of the pineal gland and capable of preventing OS (Reiter et al., 1995; Vazan et al., 2004). Paulis and Simko, (2007) reported that the potent antioxidant ability of melatonin is explained by the potential to scavenge hydroxyl FR (·OH), hydrogen peroxide (H₂O₂), peroxinitrite anion (ONOO⁻), singlet oxygen (1O₂), superoxide radical (O₂⁻) and peroxyl radical (LOO⁻).

Aluminum is added to drinking water for purification, and accelerates oxidative damage to biomolecules like lipids, proteins, nucleic acids. It accumulates specially in the brain which is more vulnerable to FR damage than any other organs, therefore, this study designed to demonstrate the protective role of omega-3, melatonin and their combination against AlCl₃ induced some biochemical changes especially (OS, lipid peroxidation and AD) markers in serum and brain supernatant of experimental rats.

Materials and Methods

Animals and housing:

Forty adult female albino rats (Rattus norvegicus) of about 190-240g B.W. and 10-12 weeks old were used. Animals were housed in plastic cages bedded with wooden chips. They were housed under standard laboratory conditions, about 12:12 light/dark photoperiod (LD) at 22 ± 4 °C (Coskun et al., 2004). Regular 12-hours diurnal cycles were kept using an automated light-switching devise. The animals were given standard rat pellets and tap water ad libitum.
Experimental Design:

The experimental rats were divided randomly to five groups (each of eight animals. This experiment was carried out for 40 days as explained here: **Group 1: Control rats**: Rats were supplied with standard chow plus tap water *ad libitum*. **Group 2: AlCl₃ treated rats**: Rats were supplied with standard chow plus AlCl₃ (1000mg/L drinking water *ad libitum*). **Group 3: AlCl₃ treated rats plus omega-3**: Rats were supplied with standard chow plus AlCl₃ (1000mg/L drinking water *ad libitum*) plus omega-3 (4000mg/ kg diet). **Group 4: AlCl₃ treated rats plus melatonin**: Rats were supplied with standard chow plus AlCl₃ (1000mg/L drinking water *ad libitum*) plus melatonin (50 mg/kg diet). **Group 5: AlCl₃ treated rats plus omega-3 plus melatonin**: Rats were supplied with standard diet plus AlCl₃ (1000mg/L drinking water *ad libitum*) plus omega-3 (4000mg/ kg diet) plus melatonin (50 mg/kg diet). Dose selection depended on the literatures review.

Anesthesia, Dissection and Removal of Brain:

All animals were anesthetized with ketamine (35mg/kg B.W.) and xylazine (5mg/kg B.W.) (Laird et al., 1996; (Krinke, 2000), sacrificed at the end of experiment then all brains had been weighed.

Body weight, water intake and diet consumption:

Body weight recorded before, middle and after the experiment, water intake daily recorded while diet consumption recorded twice a week.

Serum preparation:

Blood samples were taken by cardiac puncture into chilled tubes without ethylene diamine trichloro acetic acid EDTA centrifuged at 3000 rpm for 15 minutes for biochemical estimation (Cheng, 2002).

Biochemical determination:

Serum superoxide dismutase (SOD), malondialdehyde (MDA) and Aβ (1-42) level determined by using enzyme linked immune sorbent assay kit (ELISA) (Sunlongbiotic, China), while serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, urea and glucose determined by using biochemistry autoanalyzer (Cobas C111- Roche/Germany) Biolabo reagent kit/France. Serum creatine kinase NAC and LDH measured by using kit obtained from Cobas /USA.

Statistical analysis:

All data are expressed as mean ± standard error (mean ± S.E.) and statistical analysis was carried out using statistically available software statistical package for the social sciences (SPSS version 20). Statistical differences were determined by Duncan
test for multiple comparisons after analysis of variance (ANOVA). P<0.05 was considered statistically significant.

Results and Discussion

Brain weight, body weight water intake and diet consumption:

As illustrated in Table 1, administration of AlCl$_3$ in a dose (1000mg/L) caused significant decrease (p ≤ 0.05) of brain weights in comparison to rats control group, this result is in agreement with the finding of other researchers (Zatt et al., 2003; Kawahara, 2005), they concluded that Al contributes to neuronal cell death by disrupting the integrity of neurotrophic support and induces neuronal apoptosis both in vivo as well as in vitro, subsequently leading to brain atrophy. Since shrinkage of the neurons is a characteristic feature of apoptotic mode of cell death appeared in the current study, as well as gliosis (inflammation) which is always associated with necrosis (Kumar, 2012), also detected, so based on the correlation between Al and ageing such decrease in the brain weight related to AlCl$_3$ inducing both forms of cell death.

On the other hand, significant increase in the brain weights observed in all treated groups in comparison to AlCl$_3$ group indicating that high neuroprotective ability of omega-3 and melatonin alone or together through their metal chelating, antioxidant and anti-apoptotic activity. No significant differences (p ≤ 0.05) in body weight, water intake and diet consumption among all these groups well be recorded.

Oxidative stress markers:

Serum SOD level significantly decreased (p ≤ 0.05) in AlCl$_3$ group with comparison to control group, while dietary supplementation of omega-3 with AlCl$_3$ caused non-significant increase in the level of it, unlike melatonin supplementation alone, or in combination with omega-3 along with AlCl$_3$ in which SOD level significantly increased in the normal rate when compared to AlCl$_3$ group, (Table 1), it means that melatonin as antioxidant better than omega-3, since melatonin as it has a small size, it can easily diffuses through membranes of tissues and it does not need specific receptors to carry out its antioxidant activity (Noyan et al., 2004), as well as, it regulates the activity and gene expression of antioxidant and pro-oxidant enzymes (Reiter et al., 1997; Sahin et al., 2004).

The production of endogenous antioxidants enzymes is increased in the body cells when omega-3 FAs are included in the diet. Such increase in endogenous antioxidative enzymes could protect normal cells from oxidative damage (Hardman et al., 2005). In addition, melatonin forms complexes with Al, cadmium (Cd$^{+2}$), Cu$^{+2}$, Fe$^{+3}$ and Zn$^{+2}$, thus conferring the role of melatonin in metal detoxification (Gulcin et al., 2003), the metal chelating ability of melatonin has been shown to increase with
increasing concentration of the latter. It reduces the absorption and translocation of toxic metal ions in brain and also prevent cellular damage that arise from FR generation, lipid peroxidation and PUFA, protein and DNA oxidation, diminishing energy metabolism, advanced glycation end products (AGE), MDA and SOD-1 in senile plaques (Pappolla et al., 1997a; Pappolla et al., 1997b; Karbonik et al., 2001).

The highest serum SOD level in the current study obtained by combination group, no such study performed in order to compare it with the present finding, since they considered as antioxidants; therefore, their combination strongly support the antioxidant system of the body through metal chelating ability which in turn reduced FR generation, so less SOD consumed by cells.

Serum MDA concentration significantly increased \( (p \leq 0.05) \) in AlCl\(_3\) group in comparison with control group, while non significant differences detected when rats treated with omega-3, melatonin and their combination as compared to AlCl\(_3\) group, (Table 1).

Aluminum caused marked oxidative damage by increasing lipid peroxidation and decreasing SOD level. This could be due to the reduced axonal mitochondria turnover, disruption of Golgi and reduction of synaptic vesicles induced by Al exposure which results in release of oxidative products like MDA and carbonyls within the neurons (Bharathi et al., 2006), this is already supported by the present study.

The mechanism that explains the beneficial effects of omega-3 on lipid peroxidation has not been understood. However, it has been demonstrated that an increase in monosaturated FAs or a reduction in PUFA in the membrane lipids decrease the susceptibility of membranes to oxidation attack, while DHA reduce OS in the brain (Suresh, 1992; Hashimoto, 2006). As well as, melatonin has a phenol group that provides a proton to detoxify ‘OH or H\(_2\)O\(_2\) and thus can reduce lipid peroxidation induced by Al in Alzheimer’s patients (Allegra et al., 2003). Higher doses of melatonin and omega-3 needed to increase SOD level and other antioxidants in the serum in order to lowering serum MDA levels in order to maintain the balance between antioxidants and lipid peroxidation products.

**Beta amyloid (1-42) peptide level:**

Serum Aβ (1-42) peptide level in AlCl\(_3\) group showed a significant increase \( (p \leq 0.05) \) when compared with control group, may be related to the fact that Al may attacks the nucleus and may cause nuclear vacuolation as subsequently leading to the formation of this peptide which is strongly bind with Al and deposited there. This result is supported by other researchers (Hardy and Higgins, 1992; Castronia et al., 2010), they reported that excessive intake of Al may cause the deposition of amyloids in the neurons and defects memory as well as learning disorders in rats while all treated
groups showed significant decrease in the levels of this peptide when compared with AlCl$_3$ group (Table 2).

Johansson et al. (2007) demonstrated that DHA stabilizes Aβ (1-42) oligomers, thereby hindering their conversion (maturation) into insoluble fibrils, it decreases the level of Aβ in detergent insoluble membrane fractions (DIFs) and reduces the amyloid burden in hippocampus and the parietal cortex of transgenic AD model mice (Lim et al., 2005).

Omega-3 FAs facilitate α-secretase interaction with APP to produce nontoxic fragments and prevent Aβ formation, shield the essential recognition sequence and intramembranous cleavage site for γ-secretase, serve as a local sink for FRs that reduce γ-secretase activity, that can be induced by FR damage to the protein complex and directly inhibit fibrillation as well as formation of toxic oligomeric species of Aβ, they are central components of glial and neuronal membrane phospholipids, and take part in brain membrane remodeling, synthesis and in signal transduction (Rapoport, 2001).

Since Al attack the cell membrane as well as the nucleic acid, so this oil decreased the level of this peptide both in serum of rats by maintaining membrane stability of the cells and preventing gene mutation induced by Al. Because melatonin disrupt the imidazole-carboxylate salt bridges of Aβ (Huang et al., 1997; Masilamoni, J., 2008).

so it prevents further deposition of this peptide in serum and brain supernatant in response to Al attack. Millan-Plano et al. (2003), concluded that melatonin inhibit Al induced formation of Aβ protein and oxidative end products in the synaptosomal membranes, by binding with Al such binding may shed light into the role of this element in the etiology of AD (Lack et al., 2001).

Since Al attack the cell membrane and increases production of Aβ, while omega-3 as well as melatonin have a strong antioxidant and antiamyloid activity, that’s why their combination greatly inhibits the progressive formation of amyloid fibril.

Liver, renal function parameters, creatine kinase, lactate dehydrogenase and glucose level:

Statistical analysis revealed non-significant difference (p ≤ 0.05) in serum ALP, AST, ALT, creatinine and LDH level between AlCl$_3$ and control group. A significant decrease (p ≤ 0.05) recorded in serum urea concentration of AlCl$_3$ group as compared to control group (Table 2), only combination group of omega-3 and melatonin significantly normalized urea concentration as compared to AlCl$_3$ group finding is similar to the results obtained by Nouira et al. (2013), they concluded that AlCl$_3$ decreased urea concentration in the plasma, furthermore, urea is the chief nitrogenous end product of amino acids and thus protein catabolism, is elaborated in the liver, filtered by the glomerulus, reabsorbed in part by the tubules and excreted in urine. Therefore, urea concentration depends not only on the glomerular filtration, but also on dietary protein intake, organism protein catabolism and liver production aptitude (Whelton et al., 1994), while combination of omega-3 and melatonin
ameliorate these effects due to great antioxidant role of this combination against harmful effect of Al.

Additionally, significant decrease (p≤0.05) in CK activity in all groups as compared to AlCl$_3$ group (Table 2) these results somewhat similar to the study of Mair, (1999) who concluded that release of CK and LDH requires a leaky plasma membrane and degradation of subcellular structure; thus, quantitatively greater amounts of CK and LDH are associated with increased myocardial and neuronal damage.

On the other hand, fish oil supplementation with food in a dose of (4000mg/kg diet) caused a significant reduction in serum CK activity while not showed significant effect on LDH activity in comparison with all groups of the experiment. The present finding is somewhat in a good accordance with the results obtained by Gopal et al. (2011), they reported that fish oil supplementation with diet protects myocardium against OS through its antioxidant defense mechanism, anti-thrombotic activity and thereby restores the structural and functional integrity of myocardium.

Furthermore, dietary supplementation of melatonin (50 mg/kg diet) decreased CK activity by maintaining membrane integrity as well as strong metal chelating ability restricting the leakage of this enzyme, this result is similar to the data obtained by Attia et al. (2009), they reported that CK activity decreased in the serum of rats in response to oxidative stress induced by sodium nitrate (NaNO$_3$) that disrupt the membrane permeability.

Table 1. Shows (Mean ± S.E.) Effects of AlCl$_3$, Omega-3, Melatonin and their Combination on Brain and some biochemical parameters in Albino Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>*Serum MDA (µ mol/L)</th>
<th>*Serum SOD (IU/ml)</th>
<th>* Brain weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.709 ± 0.104 *</td>
<td>32.480 ± 0.895</td>
<td>169.200 ± 5.161 b</td>
</tr>
<tr>
<td>AlCl$_3$</td>
<td>1.510 ± 0.149 b</td>
<td>16.570 ± 2.965 a</td>
<td>153.400 ± 2.521 a</td>
</tr>
<tr>
<td>AlCl$_3$ + omega-3</td>
<td>1.788± 0.134 b</td>
<td>20.560 ± 3.515 ab</td>
<td>169.600 ± 5.491 b</td>
</tr>
<tr>
<td>AlCl$_3$ + melatonin</td>
<td>1.541 ± 0.368 b</td>
<td>25.660 ± 2.210 bc</td>
<td>172.000 ± 6.633 b</td>
</tr>
<tr>
<td>AlCl$_3$ + omega-3 + melatonin</td>
<td>1.695± 0.109 b</td>
<td>37.060 ± 2.825 d</td>
<td>175.600 ± 5.036 b</td>
</tr>
</tbody>
</table>

- Data presented as mean ± S.E.
- The same letters mean no statistical differences
- The different letters mean statistical difference
- * = P<0.05
- n=8 for each group

In fact, the best results obtained by co administration of omega-3/melatonin along with AlCl$_3$ decreased the activity of CK, since this combination strongly maintain
the membrane stability, and strongly prevent oxidative damage due to their FR scavengering ability; therefore, prevent the leakage of CK as supported by the current study.

Table 2. Shows (Mean ± S.E.) Effects of AlCl$_3$, Omega-3, Melatonin and their Combination on Brain and some biochemical parameters in Albino Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>*Serum Creatine Kinase (IU/L)</th>
<th>*Serum urea (mg/dl)</th>
<th>*Serum β-amyloid (µg/L)</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>539.300 ±17.990$^{ab}$</td>
<td>50.100 ±2.251$^b$</td>
<td>24.850 ± 3.185$^a$</td>
<td>Control</td>
</tr>
<tr>
<td>AlCl$_3$</td>
<td>1075.000 ±46.040 $^c$</td>
<td>40.460 ± 2.956$^a$</td>
<td>44.970 ± 3.305$^b$</td>
<td>AlCl$_3$</td>
</tr>
<tr>
<td>AlCl$_3$+ omega-3</td>
<td>586.900 ±97.490$^{ab}$</td>
<td>47.140 ± 2.111$^a$</td>
<td>32.380 ± 5.911$^a$</td>
<td>AlCl$_3$+ omega-3</td>
</tr>
<tr>
<td>AlCl$_3$+ melatonin</td>
<td>449.000 ±9.610 $^a$</td>
<td>45.730 ±1.725$^a$</td>
<td>24.740 ± 3.650$^a$</td>
<td>AlCl$_3$+ melatonin</td>
</tr>
<tr>
<td>AlCl$_3$+ omega-3+ melatonin</td>
<td>673.600 ±8.991$^{b}$</td>
<td>48.910 ±2.129$^b$</td>
<td>27.610 ± 4.315$^a$</td>
<td>AlCl$_3$+ omega-3+ melatonin</td>
</tr>
</tbody>
</table>

- Data presented as mean ± S.E.
- The same letters mean no statistical differences
- The different letters mean statistical differences* =P<0.05
- n=8 for each group

Conclusions

1. Oxidative stress responsible for brain atrophy but melatonin, omega-3 either alone or together protects against brain weight loss by protecting large number of healthy neurons against oxidative damage through their antioxidant role, while sub-acute dose of AlCl$_3$ (1000mg/L drinking water) has no effect on appetite hence not effect on body weight.

2. Aluminum chloride cause biochemical alterations in the serum of female rats, while melatonin, omega-3 either alone or together could protect against OS through their antioxidant and anti-amyloidal role in response to AlCl$_3$ toxicity.

3. Aluminum chloride causes myocardial and neuronal damage by enhancing FR generation leading to inactivate NO level subsequently leads to vasoconstriction and increases CK activity. while melatonin, omega-3 and their combination ameliorate these effects through their FR scavenging ability.
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