THE POSSIBLE PROTECTIVE ROLE OF VITAMIN E ON THE JOINED NEUROBEHAVIORAL EFFECTS OF LEAD TOXICITY AND NOISE STRESS IN RATS

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ABSTRACT

Lead and noise exposure are common pollutants affecting human health specially mental health. The aim of this study was to clarify the role of vitamin E on the possible joined neuro-behavioral influence of lead and Noise stresses on Sprague Dawely rats. Lead acetate (15 mg/kg) was injected i.p. alone or after noise exposure (110 db for 30 min. /day). Vitamin E ( 200 mg /kg/day) was administrated orally before either lead or noise or both. Neurotransmitters, acetyl-cholinesterase activity and brain antioxidants were performed in addition to Open field and Passive avoidance tests Brain areas were examined Histopathologically. Results indicated increased Malondialdehyde and decreased Norepinephrine and Acetyl cholinesterase activity in cerebral cortex with both lead and noise which returned to normal level by vit E. In open field test, Lead and noise group showed increased latency and decreased ambulation, grooming and rearing records. In passive avoidance test, latency was decreased. Histopathological examination revealed cellular toxicity with lead. In Conclusion, administration of vitamin E protects brain from the additive oxidative stress induced by both pollutants.

KEYWORDS: Lead Acetate; Noise; Vitamin E; Neurotransmitters, Oxidative Stress, Open Field Test

INTRODUCTION

Lead (Pb) neurotoxicity include behavioral abnormalities, learning disabilities and impaired cognitive functions in experimental animals and humans (Ruff et al. 1996). Pb exposure mainly affects cholinergic system by reducing acetylcholine release, uptake and turnover rates (Prasanthi et al. 2006). Experimental studies showed that treatment of rats with Pb acetate 100 mg/kg for seven days resulted in significant decrease in the level of noradrenaline, dopamine, serotonin and 5-hydroxyindol acetic acid in different brain regions (Waggas, 2012). Rats intoxicated with Pb acetate showed decreased levels of the oxidative stress biomarkers in the brain tissue of exposed animals (Yun, 2011). Noise stress in rats
influence the hypothalamic pituitary adrenal axis and causing an elevation of brain biogenic amines according to Pignatelli and coworkers (2000). Children exposed to noise had tasks affected involving central processing and language such as reading comprehension, memory and attention (Haines et al., 2001). Noise exposure lead to oxidative stress and hearing impairment (Al-Naemi & Abdal, 2012). Vitamin E was found to be excellent for strengthening the antioxidative defense system, and maintaining membrane fluidity in the brain of diabetes-induced rats (Hong et al. 2004). Vitamin E was reported to delay the onset of cognitive decline and loss of neurons in rats (Kabay et al. 2009). The aim of the present study is to screen the effects of either Pb or noise and both on brain antioxidants, neurotransmitters and behavior of rats and the possible protective effect of vitamin E.

MATERIAL AND METHODS

Animals:

Male adult Sprague–Dawley rats (150–200 g) were obtained from the breeding colony of the National Organization for Drug Control and Research and maintained in the animal house at relative humidity, with 12-h light–dark cycle, temperature 21–24°C with free access to food and water ad libitum. Experimental procedures were conducted in accordance with the ethical guidelines for investigations in laboratory animals and were approved by the Research Ethical Committee of Faculty of Pharmacy, Cairo University (Egypt) to comply with the Guide for the Care and Use of Laboratory Animals (ILAR,1996)

Experimental Design and Sampling: Table (1)

<table>
<thead>
<tr>
<th>Groups of rats (n: 12–14/group)</th>
<th>Treatment (for 14 days)</th>
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<tbody>
<tr>
<td>group (1)</td>
<td>Control, received 0.9% saline.</td>
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<tr>
<td>group (2)</td>
<td>Vehicle, received corn oil and 0.9% saline.</td>
</tr>
<tr>
<td>group (3)</td>
<td>Received Lead acetate (Loba-Chemie, India)(15 mg/kg/day, i.p.).</td>
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<tr>
<td>group (4)</td>
<td>Exposed to noise (110 db for 30 min./day).</td>
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<tr>
<td>group (5)</td>
<td>Received Vit. E in corn oil (Glaxo-Smith Kline pharmaceuticals)(200 mg/kg/day, P.O.) two hours before lead acetate dosage (15mg/kg/day, i.p.).</td>
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<tr>
<td>group (6)</td>
<td>Received lead acetate (15 mg/kg/day, P.O.) before exposed to noise (110 db, 30 min./day).</td>
</tr>
<tr>
<td>group (7)</td>
<td>Received Vit. E (200 mg/kg/day, P.O.) before exposed to noise (110 db, for 30 min./day).</td>
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<tr>
<td>group (8)</td>
<td>Received Vit. E (200 mg/kg/day, P.O.) two hours before lead acetate dosage (15 mg/kg/day, i.p.), then exposed to noise (110 db for 30 min./day).</td>
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</table>

At the end of experimental period, the brain was dissected out and divided into three regions (cortex-mid brain- Pons & medulla) in order to assess neurotransmitters and acetyl-
cholinesterase activity. The other set of brains was washed in saline, dried and weighed to assess their relative weights (brain weight / body weight × 100) than divided into two equal groups. The first group was kept in –80°C refrigerator in order to prepare tissue homogenates to assess GSH and MDA levels, while the second group was preserved in 10% formalin solution for histological assessment.

**Behavioral testing:**

- **Passive Avoidance Test (Apparatus: fig 1):** was done by the method of Bures *et al.* (1983) with modifications made by Narayanan *et al.* (2010).
- **Open Field Test:** Apparatus used was a wooden box (90×90×50 cm) with red walls and white polished bottom (Looser 1982), divided into 36 equal squares (15×15 cm each) using permanent paint. The arena was also virtually divided into a central square and peripheral margin (Weiss 2004). The open field was placed in a quiet room under normal illumination of white fluorescent lighting. Each rat was weighted and placed gently in the middle of the arena and videotaped for 3 minutes (Lo Pomo 2006) using a video camera (Sony Handycam Vision, CCD-TRV408, Sony Corporation, Japan). The animal was then returned to the home cage. The open field was thoroughly wiped using 10% isopropyl alcohol and dried before placement of a new subject in order to obviate possible biasing effects due to odor clues left by previous rats (Lazarini 2001). Behavioral sessions were then analyzed according to (Cunha and Masur 1978) for the following parameters: Latency, is the time in seconds elapsed from placement of the subject in the arena till it makes the first move. Ambulation, is the number of squares the animal entered with all four paws during the test session (Volosin *et al.* 1988). Grooming, represents the time in seconds the animal was scratching face, licking, paws, fur or genitals. Rearing, is the number of times the rat stands on the hind limb with or without forelimb support (Van den Buuse and de Jong 1989)

![FIG 1:Passive avoidance apparatus](image)
Estimation of Antioxidants in Brain Tissue:

Brain Reduced Glutathione (GSH) content was determined according to Prins and Loose (1969). Brain Malondialdehyde (MDA) content in tissue homogenate was determined according to Buege and Aust (1978) with a slight modification in the incubation period according to Deniz et al. (1997). All procedures were performed according to manufacturers’ instructions.

Evaluation of Brain Monoamines Levels:

Dopamine (DA), Norepinephrine (NE) and Serotonin (5-HT) were evaluated in various brain regions using a standardized flourimetric assay according to Ciarlone (1978) and Khalifa et al. (1997) using spectro-photofluorometer RF-5000 Shimadzu, Japan. All procedures were performed according to the manufacturers’ instructions.

Evaluation of Brain Acetyl Cholinesterase Activity:

The procedure used in the present study of the determination of AChE activity in the brain samples is a modification of the method of Ellman et al. (1961) as described by Gorun et al. (1978).

Histological Examination:

Samples from brain from different groups were removed, rinsed in formalin, dehydrated, cleared, impregnated and embedded in paraffin to facilitate ease of cutting for histological assessment of tissue damage using haematoxylin and eosin according to Banchroft et al. (1996).

Statistical Analysis:

Results were expressed as mean ± SEM. Statistical Analysis was performed using the SPSS version 16 (Chicago, IL), whereas the graphs were drawn using a prism computer program (GraphPad software Inc. V5, San Diego, CA). Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey–Kramer Multiple Comparison Test. Probability values of less than 0.05 were considered statistically significant (Armitage P, Berry 1987).
RESULTS

Total body weight and relative brain weight of rats were shown in table 2 & figure 2

- Behavioral testing (open field and passive avoidance tests) results were shown in table 3
- Antioxidant in brain areas were shown in table 4
- Neurotransmitters in brain areas were shown in figure 2
- Histopathological studies of brain tissue were shown in figure 3

**FIG 2:** Relative brain weight of all groups of rats:

![Relative brain weight of all groups of rats](image)

- Significant difference from control (P<0.05).
- Significant difference from noise (P<0.05).

Table (2): Effect of treatment with Pb (15mg/kg), exposure to noise (110 db, 30 min/day) and pretreatment with vitamin E (200mg/kg) on total body weight change. (Results are represented as mean ±S.E.M in grams and percent change).

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Mean wt 1st day</td>
<td>106.4 ± 2.76</td>
<td>100.9 ± 2.96</td>
<td>121.0 ± 2.64</td>
<td>109.6 ± 3.67</td>
<td>123.0 ± 2.41</td>
<td>109.3 ± 4.06</td>
<td>111.1 ± 3.4</td>
<td>116.2 ± 3.58</td>
</tr>
<tr>
<td>Mean wt %change 1st week</td>
<td>115.6 ± 3.18</td>
<td>121.0 ± 3.59</td>
<td>117.29 ± 1.99</td>
<td>125.93 ± 3.61</td>
<td>120 ± 2.68</td>
<td>125.07 ± 4.4</td>
<td>115.21 ± 2.68</td>
<td>115.71 ± 5.05</td>
</tr>
<tr>
<td>+8.65 %</td>
<td>+19.97 %</td>
<td>-3.07 %</td>
<td>14.93 %</td>
<td>-2.44 %</td>
<td>+14.44 %</td>
<td>+3.66 %c</td>
<td>-0.43 %</td>
<td></td>
</tr>
<tr>
<td>Mean wt %change 2nd week</td>
<td>128.2 ± 3.88</td>
<td>133.6 ± 3.83</td>
<td>112.1 ± 1.98</td>
<td>142.4 ± 3.74</td>
<td>121.8 ± 3.56</td>
<td>140.9 ± 5.04</td>
<td>119.3 ± 2.98</td>
<td>111.7 ± 5.65</td>
</tr>
<tr>
<td>+20.49 %</td>
<td>+32.5 %</td>
<td>-7.38 %a</td>
<td>+29.93 %a</td>
<td>+0.95 %a</td>
<td>+28.89 %a</td>
<td>+7.33 %ac</td>
<td>-3.87 %a</td>
<td></td>
</tr>
</tbody>
</table>

- Significant difference from control (P<0.05).
- Significant difference from lead (P<0.05).
- Significant difference from noise (P<0.05).
- Significant difference from lead+noise (P<0.05).
Table (3): Effect of treatment with Pb (15mg/kg), exposure to noise (110 db, 30 min/day) and pretreatment with vitamin E (200mg/kg) on Passive avoidance and Open field parameters. Results are represented as mean ±S.E.M (in seconds).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Vehicle</th>
<th>Lead</th>
<th>Noise</th>
<th>Lead+Vit.E</th>
<th>Lead+Noise</th>
<th>Noise+Vit E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passive avoidance 24hrs after training</td>
<td>180±0.0</td>
<td>180±0.0</td>
<td>165±11.26</td>
<td>145.3±22.3 a</td>
<td>179.9±0.12</td>
<td>157.9±14.8</td>
<td>179.8±0.13 c</td>
</tr>
<tr>
<td>Passive avoidance 48hrs after training</td>
<td>180±0.0</td>
<td>179.7±0.21</td>
<td>168.89±11.11</td>
<td>148.6±20.93 a</td>
<td>179.9±0.11</td>
<td>167±8.70</td>
<td>179.9±0.10 c</td>
</tr>
<tr>
<td>Latency</td>
<td>1.6±0.54</td>
<td>1.9±0.90</td>
<td>5.2±1.58</td>
<td>1.4±0.50</td>
<td>2.1±0.70</td>
<td>10.2±1.53 a</td>
<td>1.1±0.46 c</td>
</tr>
<tr>
<td>Ambulation</td>
<td>16.9±2.77</td>
<td>17.5±3.77</td>
<td>5.0±1.06 a</td>
<td>12.0±2.26</td>
<td>11.56±3.15 a</td>
<td>8.9±2.59 a</td>
<td>12.67±3.03</td>
</tr>
<tr>
<td>Grooming</td>
<td>2.1±0.62</td>
<td>2.0±0.54</td>
<td>0.7±0.26 a</td>
<td>1.6±0.56</td>
<td>1.2±0.63</td>
<td>0.6±0.31 a</td>
<td>1.33±0.33</td>
</tr>
<tr>
<td>Rearing</td>
<td>4.1±1.11</td>
<td>4.3±0.83</td>
<td>1.3±0.26 a</td>
<td>3.5±0.37</td>
<td>3.6±0.73 b</td>
<td>2.3±0.97 b</td>
<td>4.1±1.10 a</td>
</tr>
</tbody>
</table>

a  Significant difference from control (P<0.05).
b  Significant difference from lead (P<0.05).
c  Significant difference from noise (P<0.05).
d  Significant difference from lead+noise (P<0.05).

Table (4): Effect of treatment with Pb (15mg/kg), exposure to noise (110 db, 30 min/day) and their combination, pretreatment with vitamin E (200mg/kg) on brain antioxidants (GSH and MDA)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Vehicle</th>
<th>Lead</th>
<th>Noise</th>
<th>Lead+Vit E</th>
<th>Lead+Noise</th>
<th>Noise+Vit E</th>
<th>Lead+Noise+Vit E</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH in Brain (mg/g)</td>
<td>5.69±0.15</td>
<td>5.65±0.44</td>
<td>5.23±0.17</td>
<td>5.09±0.39</td>
<td>6.12±0.27 b</td>
<td>5.73±0.39</td>
<td>6.16±0.34 c</td>
<td>5.77±0.23 a c</td>
</tr>
<tr>
<td>MDA in Brain (nmol/g)</td>
<td>7.51±0.71</td>
<td>7.23±0.61</td>
<td>9.975±0.22 a</td>
<td>7.40±0.38</td>
<td>7.18±0.27 b</td>
<td>9.37±0.52 a c</td>
<td>6.41±0.59 a</td>
<td>6.71±0.66 d</td>
</tr>
</tbody>
</table>

a  Significant difference from control (P<0.05).
b  Significant difference from lead (P<0.05).
c  Significant difference from noise (P<0.05).
d  Significant difference from lead+noise (P<0.05).
FIG( 2) : Effect of administration of Pb (15 mg/kg, i.p.), exposure to noise (110 db for 30 min./day) and the effect of pretreatment with vitamin E (200mg/kg) on 5-HT, NE, DA and AChE levels in Cortex, Mid brain and Pons-Medulla regions in male rats:
a  Significant difference from control (P<0.05).
b  Significant difference from lead (P<0.05).
c  Significant difference from noise (P<0.05).
d  Significant difference from lead+noise (P<0.05).

FIG 3): HISTOPATHOLY OF BRAIN TISSUE OF VARIABLE GROUPS (A,B,C,D,E,F,G)
DISCUSSION

In this study, Lead decreased the mean percent change in body weight of rats but noise increased it. Exposure to noise significantly decreased brain relative weight, by co-administration of lead it was increased and returned to normal level with vit E. The decrease in body weight was explained by Ghanem et al. (2009) by a decrease in food intake and the increase in relative brain weight explained by Yun et al. (2011) due to oxidative stress induced by lead. Vit E increased body weight (Osfor et al. 2010).

In this study lead and noise in rats caused neurotoxicity manifested in abnormalities in behavioral tests, antioxidants and neurotransmitters release in brain as well as brain histopathological abnormalities but Vit. E normalizes these records.

Regarding the behavioral tests, the Passive avoidance task is a fear-aggravated test used to evaluate learning and memory in rodent models of CNS disorders. In this test, subjects learn to avoid an environment in which an aversive stimulus (such as a foot-shock) was previously delivered (Lee et al. 2006). The open-field test measured exploratory and anxiety-related behaviors in rodents and access general locomotor activity levels. Latency period is an index of responsiveness and decision, ambulation frequency is a marker of exploration and locomotor activity meanwhile rearing and grooming are indices of exploratory and vertical activities (Belzung and Griebel 2001).

In this study, In open field test, latency period increased meanwhile ambulation, grooming and rearing records decreased with Pb. Lead pass through the blood brain barrier due to its substitution for calcium ions and back-transport via the Ca- ATPase pump (Bradbury and Deane 1993). Hypoactivity and reduction in exploratory behaviors with lead was reported by NourEddine et al. (2005) and was confirmed by Pachauri et al. (2009). Previous studies by Rodrigues (1996) detected hyperactivity in perinatal lead in rats indicating less habituation. Mansouri et al. (2013) found hyperactivity in the open field only in male rats and this was accompanied by an increase in acetylcholine in the prefrontal cortex, dopamine was unaffected whereas serotonin was decreased.

In passive avoidance test, Pb caused decrease in the time spent to enter the dark room 24 and 48 hours after training and vitamin E increased it. Anita et al. (2012) found that animals have a much shorter latency period and neurotransmitter alterations with lead.

In this study, although noise exposure decreased the latency time to cross to the dark room in the passive avoidance test, it did not cause any significant alteration in the different indices of the open field test. These findings indicated altered learning and memory ability with no apparent effect on the motor and exploratory activities. However, Pan et al. (2006)
found that two weeks of noise stress (2 hours/day, 85 dB) increased square crossing and vertical movement. Naqvi et al. (2012) showed that 15 days sub-chronic exposure to noise stress induced anxiety and depression like behavior in male rats. In this study, Vit. E modulated parameters in open field and passive avoidance tests. Vit. E improved performance in the radial arm and Morris water mazes in rodent (Zaidi and Banu, 2004) but Halagappa et al. (2007) revealed impairment in cognition and sensorimotor skills with vit. E. Rucklidge et al. (2014) found efficacy for micronutrients in the treatment of attention deficit syndrome in adults.

In this study, Pb caused changes in 5-HT and DA levels in cortex, mid brain and pons-medulla regions and decreased AChE level in cortex. The regional variations in neurotransmitters and acetyl choline may be due to different affinity of brain areas to lead or synaptic variability. Vit. E returned neurotransmitters to nearly normal range but further decrease occurred in AChE.

In this study, MDA was increased in brain by lead. Abd El-Kader et al. (2012) showed that Pb increased peroxidative reactions. Pb has an affinity to sulfhydryl group thus inhibiting functional –SH groups in enzymes (Patra et al. 2011). Pb is antagonist to selenium (Ait Hamadouche et al. 2012).

Noise stress did not show significant alterations in the levels of MDA and GSH assessed in brain tissue. Manikandan et al., (2005) found increased MDA levels in different areas of the brain after 30 days of 100 dB white noise exposure in addition, Cheng et al.,(2011) showed evidence of oxidative damage in the critical region by moderate-intensity white noise exposure and this has influence on learning and memory in mice.

In this study vit E normalize GSH and MDA in brain and significantly increased GSH levels, as compared to noise group. Vitamin E can cross the blood-brain barrier (Niki, 1987) and decreased oxidative stress (Hong et al. 2004).

In this study, Exposure to noise caused significant decrease in NE, DA and AChE in the cerebral cortex meanwhile in Pons-medulla region, DA was significantly increased along with non-significant increase in NE and AChE levels.

Pignatelli et al. (2000), showed that the adrenal function and the entire hypothalamic-pituitary adrenal axis were severely affected, under the influence of noise stress in rats, resulting in the elevation of brain biogenic amines. Ravindran et al., (2005) reported alterations in brain DA levels and increased monoamine turnover ratios following noise
stress. Different studies explained the changes in neurotransmitters level during noise exposure by the tendency of noise to induce a state of oxidative stress.

In this study, Histology of the brain of Pb treated animals showed mild changes in the form of scattered shrunken of neuronal cells with deeply stained cytoplasm and pyknotic nuclei in addition to neuronal chromolysis, revealing some degenerative changes. Sharifi et al. (2002) reported apoptotic cell death in hippocampus in rats intoxicated with Pb. Amal and Mona (2009) reported that administration of Pb in different dose levels caused pyknosis of neurons associated with focal gliosis in addition to focal cerebral hemorrhage, furthermore, vitamin E, reduced brain tissue damage. Kabay et al. (2009) revealed neuroprotective effects of vitamin E as reported by reducing ischemic neuronal alterations in diabetic-rats. In a study by Wu et al. (2010), vitamin E showed good protective potential against oxidative damage and learning disability after mild traumatic brain injury in rats.

In conclusion, both lead and noise altered the proper function of the brain by enhancement of oxidative stress which is ameliorated by coadministration of Vitamin E.

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REFERENCES