Abstract. – Background and Objectives: Alzheimer’s disease (AD) is a neurological disorder associated with aging. The neuropathologic features of this disease are senile plaques and neurofibrillary tangles, which are present in memory structures and cerebral cortex. This study focuses on evaluation of estradiol administration in the management of neurological changes accompanied with AD in ovariec
tomized rats intoxicated with aluminum.

Materials and Methods: Fifty adult female rats were classified into 5 main groups as follows: Group (1): gonad intact control, Group (2): ovariectomized control group, Group (3): ovariectomized rats were injected subcutaneously with estradiol for 18 weeks, Group (4): ovariectomized rats were injected intraperitoneally with aluminum chloride (AlCl₃) daily for three months, Group (5): ovariectomized rats were injected intraperitoneally with AlCl₃ for three months, then, they were injected subcutaneously with estradiol three times weekly for 18 weeks. Hydrogen peroxide (H₂O₂), malondialdehyde (MDA), total antioxidant capacity (TAC), superoxide dismutase (SOD), catalase (CAT), B cell lymphoma 2 (Bcl-2), brain derived neurotrophic factor (BDNF), acetylcholinesterase (AchE) and acetylcholine (Ach) were determined in brain tissue of all studied groups.

Results: The results showed that brain TAC, SOD activity, Bcl-2 and BDNF levels were decreased significantly due to ovariectomy. Brain H₂O₂, MDA levels and AchE activity were increased significantly after AlCl₃ administration to ovariectomized rats. Brain TAC, Bcl-2, BDNF and Ach levels were decreased significantly as a result of AlCl₃ supplementation to ovariectomized rats. Treatment of rats with estradiol produced marked improvement in the most studied biomarkers and this finding was well documented by histological investigation of the brain which showed remarkable improvement in the feature of the neurons in addition to the disappearance of plaques.

Conclusions: We could conclude that estradiol administration significantly ameliorates the neurodegeneration characteristic of Alzheimer’s disease in experimental rat model. This may be attributed to its powerful antioxidant, ant apoptotic, neurotrophic as well as its anti amyloidogenic activities.

Key Words: Alzheimer’s disease, Ovariectomy, Aluminum, Plaques, Estradiol.

Introduction

Today many countries have rising aging populations and are facing an increased prevalence of age related diseases, such as Alzheimer’s disease. Alzheimer’s disease (AD) is a neurodegenerative disorder characterized clinically by progressive memory loss and subsequent dementia, and neuropathologically by senile plaques, neurofibrillary tangles and synapses loss. The abnormal accumulation of extracellular amyloid-beta peptide (Aβ) and the intracellular neurofibri
tary tangles (NFTs) are believed to be responsible for the neuronal loss and the degeneration of the cholinergic system. Other essential abnormalities are gliosis, chronic inflammation, exci
totoxicity and oxidative stress.

In recent years, a number of studies have in
dicated the potential role of various metal ions in the pathogenesis of Alzheimer’s disease (AD). Aluminum could be implicated in the formation of neurofibrillary tangles and Aβ peptides aggregation in the AD brains. Researchers suggest that decrease in estradiol levels during aging may increase the risk of Alzheimer’s disease, the most common type of dementiain. The classic view of estradiol actions in the brain was confined to regulation of ovulation and reproductive behavior in the female of all mammalian species studied, including humans.
ing evidence now documents profound effects of estradiols on learning, memory and mood as well as neurodevelopmental and neurodegenerative processes. Multifaceted effects of estradiols include improvement of cerebral metabolic profile and the reduction of oxidative stress through actions on the mitochondria. Additionally, estradiols have antiinflammatory actions and antiapoptotic activities to prevent the death of various cell types suggesting that a woman’s endogenous and exogenous estradiol exposures during midlife and in the late postmenopause might favorably influence Alzheimer risk and symptoms. Moreover, estradiols may decrease the production of amyloid-beta peptide which is central peptide for the formation of senile plaques in Alzheimer’s disease.

Materials and Methods

17β-Estradiol was purchased from Sigma Co. (St Louis, MO, USA) and aluminum chloride was supplied from BDH Laboratory Supplies Poole, Dorset, U.K.

Experimental Animals

Fifty young adult female Sprague-Dawley rats weighing 100-120 g were obtained from the Animal House Colony of the National Research Centre (NRC), Cairo and acclimated in a specific pathogen free (SPF) barrier area at 25±1°C and humidity (55%) and controlled constantly with a 12 h light/dark cycle. The rats were ovariec-
tomized surgically in Hormones Dept, N.R.C., and were housed with ad libitum access standard laboratory diet consisting of casein 10%, salts mixture 4%, vitamins mixture 1%, corn oil 10% and cellulose 5% completed to 100g with corn starch. Animals cared for according to the guidelines for animal experiments by the Ethical Committee of NRC, Cairo (Egypt).

The animals were classified into five main groups each with 10 rats.

**Group one:** Gonad intact control (nonovariectomized) group-treated with vehicle [(Di- methylsulfoxide (DMSO) 5% in saline] three times weekly for 18 weeks after six months of starting the experiment.

**Group two:** Ovariectomized control group-treated with vehicle (DMSO 5% in saline) three times weekly for 18 weeks after six months of surgical operation.

**Group three:** Ovariectomized rats were injected subcutaneously with estradiol three times weekly in a dose of 80 µg/kg b.wt. dissolved in DMSO 5% in saline for 18 weeks, after six months of surgical operation.

**Group four:** Ovariectomized rats were injected intraperitoneally (i.p.) with aluminum chloride (AlCl₃) dissolved in distilled water daily for three months in a dose of 4.2 mg/kg b.wt. after three months of surgical operation and served as Al-intoxicated control group.

**Group five:** Ovariectomized rats in this group were injected i.p. with AlCl₃ (4.2 mg/kg b.wt.) daily for three months, after three months of surgical operation. Then, they were injected subcutaneously with estradiol in a dose of 80 µg/kg b.wt. three times weekly for 18 weeks.

Brain Tissue Sampling and Preparation

At the end of the experiment, the rats fasted overnight, were subjected to anesthesia by diethyl ether and sacrificed. The whole brain of each rat was rapidly dissected and washed with isotonic saline and dried on a filter paper. Each brain was sagitally divided into two portions. The first portion of each brain was weighed and homogenized to give 10% (w/v) homogenate in ice cold medium containing 50 mM Tris-HCl and 300 mM sucrose at pH: 7.4. The homogenate was centrifuged at 3000 rpm for 10 min in cooling centrifuge at 4°C. The supernatant (10%) was stored at –80°C and used for the biochemical analysis including oxidative stress biomarkers (H₂O₂ and MDA), antioxidant status (TAC), SOD and catalase (CAT), antiapoptotic marker [B cell lymphoma 2 (Bcl-2)], neurotrophic factor [brain derived neurotrophic factor (BDNF)] and cholinergic markers [acetylcholine-esterase (AchE) and acetylcholine (Ach)]. Also, brain total protein concentration was measured to express the concentration of different brain parameters per mg protein. The second portion was fixed in formalin buffer (10%) for histological investigation.

Biochemical Analyses

Brain hydrogen peroxide level was determined by colorimetric method using hydrogen peroxide assay kit purchased from Biodiagnostic Co., Cairo, Egypt, according to the method of Aebi. Brain malondialdehyde level was evaluated by colorimetric method using lipid peroxide (malondialdehyde) assay kit purchased from Biodiagnostic Co., Cairo, Egypt, according to the method described by Satoh. Brain total antioxidant capacity was as-
sayed by colorimetric method using total antioxidant capacity assay kit purchased from Biodiagnostic Co., Cairo, Egypt, according to the method of Koracevic et al\textsuperscript{18}. Brain superoxide dismutase activity was determined by colorimetric method using superoxide dismutase assay kit purchased from Biodiagnostic Co., Cairo, Egypt, according to the method described by Nishikimi et al\textsuperscript{19}. Brain catalase activity was determined by colorimetric method using catalase assay kit purchased from Biodiagnostic Co., Cairo, Egypt, according to the method described by Aebi\textsuperscript{16}. Brain Bcl-2 level was detected by enzyme linked immunosorbent assay (ELISA) technique using Bcl-2 assay kit purchased from Bender Med Systems Co., Vienna, Austria, according to the method described by Barbareschi et al\textsuperscript{20}. Brain BDNF level was detected by ELISA technique using BDNF immunoassay kit purchased from R&D System Co., Abingdon, U.K., according to the method described by Barakat-Walter\textsuperscript{21}. Acetylcholinesterase activity was determined by colorimetric method using acetylcholinesterase assay kit purchased from Quimica Clinica Aplicada S.A Co., Amposta, Spain, according to the method of Den Blaauwen et al\textsuperscript{22}. Acetylcholine level was measured by colorimetric method using choline/acyetylcholine assay kit purchased from Biovision Research Products Co., Linda Vista Avenue, Mountain View, CA, USA, according to the method of Oswald et al\textsuperscript{23}. Quantitative estimation of brain total protein homogenate was carried out according to the method of Lowry et al\textsuperscript{24}.

**Histological Examination**

The brain tissues fixed in formalin buffer for one week, were washed in running tap water for 24 hours and dehydrated in ascending series of ethyl alcohol (50-90), then in absolute alcohol. The samples were cleared in xylol and immersed in a mixture of xylol and paraffin in the oven at 60°C. The tissues were transported to pure paraffin wax with melting point 58°C in the oven, and then mounted in blocks and left at 4°C. The paraffin blocks were sectioned on the microtome at thickness of 5 µm and mounted on clean glass slides and left in the oven at 40°C for dryness. The slides were deparafinized in xylol and then immersed in descending series of ethyl alcohol (100-50). The ordinary haematoxylin and eosin stain was used to stain the slides\textsuperscript{25}.

**Statistical Analysis**

The results were expressed as means ± SE of the mean. Data were analyzed by one-way analysis of variance (ANOVA) and was performed using the Statistical Package for the Social Science (SPSS, Chicago, IL, USA) program, version 11 followed by least significant difference (LSD) to compare significance between groups\textsuperscript{26}. Difference was considered significant when \( p \) value was <0.05.

\[
\text{% difference} = \frac{\text{Treated value} - \text{control value} \times 100}{\text{Control value}}
\]

**Results**

Table I shows that ovariectomized (OVX) control group revealed insignificant increase in brain H\textsubscript{2}O\textsubscript{2} and MDA levels in comparison with those in gonad intact control group. Treatment of ovariectomized rats with estradiol hormone caused insignificant reduction in brain H\textsubscript{2}O\textsubscript{2} and MDA levels in comparison with those in ovariectomized control group.

**Table I.** Effect of treatment with estradiol on brain oxidative stress biomarkers in ovariectomized and Al-intoxicated ovariectomized rats. Data are represented as mean±SE of 10 rats/group.

<table>
<thead>
<tr>
<th>Parameters groups</th>
<th>MDA (nmol/mg protein) mean ± SE</th>
<th>H\textsubscript{2}O\textsubscript{2} (nmol/mg protein) mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonad intact control</td>
<td>5.60 ± 0.30</td>
<td>6.90 x 10\textsuperscript{-3} ± 3.23 x 10\textsuperscript{-4}</td>
</tr>
<tr>
<td>Ovx control</td>
<td>6.40 ± 0.34 (14.28%)</td>
<td>9.30 x 10\textsuperscript{-3} ± 4.00 x 10\textsuperscript{-4} (34.78%)</td>
</tr>
<tr>
<td>Ovx + estradiol</td>
<td>5.87 ± 0.34 (-8.20%)</td>
<td>8.06 x 10\textsuperscript{-3} ± 4.47 x 10\textsuperscript{-4} (-13.33%)</td>
</tr>
<tr>
<td>Al-intoxicated control</td>
<td>9.60 ± 0.40\textsuperscript{b} (50%)</td>
<td>3.40 x 10\textsuperscript{-2} ± 1.86 x 10\textsuperscript{-3}b (265.59%)</td>
</tr>
<tr>
<td>Ovx + Al + estradiol</td>
<td>6.85 ± 0.31\textsuperscript{c} (-28.64%)</td>
<td>2.20 x 10\textsuperscript{-2} ± 1.37 x 10\textsuperscript{-3}c (-35.29%)</td>
</tr>
</tbody>
</table>

Ovx: ovariectomized. b: Significant change at \( p < 0.05 \) in comparison with ovariectomized control group. c: Significant change at \( p < 0.05 \) in comparison with Al-intoxicated control group. (%): Percent of difference with respect to corresponding control value.
Daily administration of AlCl₃ in Ovx rats resulted in significant elevation in brain H₂O₂ (265.59%) and MDA (50%) when compared to Ovx control group. Treatment of Al-intoxicated Ovx rats with estradiol hormone produced significant reduction in brain H₂O₂ (~35.29%) and brain MDA (~28.64%) levels when compared with Al-intoxicated control rats.

Table II shows that ovariectomy induced significant reduction in brain TAC (~37.62%) and SOD activity (~26.6%) in comparison with gonad intact control group. Brain catalase activity was decreased insignificantly due to ovariectomy as compared to gonad intact control group. On the other hand, treatment of Ovx rats with estradiol hormone induced insignificant increase in brain SOD and CAT activities when compared to those in Ovx control rats. Insignificant elevation in brain TAC was also detected after administration of estradiol hormone in Ovx rats when compared to that in Ovx control rats.

Daily administration of AlCl₃ to Ovx rats induced insignificant inhibition in brain CAT activity (~15.95%) and SOD activity (~12.72%), and significant reduction in brain TAC (~28.55%) as compared to Ovx control group. Treatment of Al-intoxicated Ovx rats with estradiol hormone produced significant elevation in brain TAC (42.76%) and CAT activity (23.02%) as compared to Al-intoxicated control rats. While, treatment of Al-intoxicated Ovx rats with this hormone induced insignificant increase in brain SOD activity in comparison with Al-intoxicated control rats.

Table III shows that ovariectomy resulted in significant decrease in brain Bcl-2 levels (~40%) and brain BDNF (~29.32%) in comparison with gonad intact control group. Treatment of Ovx rats with estradiol hormone produced significant increase in brain Bcl-2 level (15.94%) and insignificant increase in brain BDNF level as compared to those levels in ovariectomized control group.

Administration of AlCl₃ in Ovx rats led to significant reduction in brain Bcl-2 (~15.57%) as well as brain BDNF (~27.05%) levels as compared with those in Ovx control rats. Treatment of Al-intoxicated Ovx rats with estradiol hormone produced insignificant elevation in brain Bcl-2 level, while it caused significant increase in brain BDNF level (~23.87%) in comparison with Al-intoxicated control group.

Table IV shows that ovariectomy caused insignificant increase in brain AchE activity, while it showed insignificant decrease in brain Ach level in comparison with gonad intact control group. Treatment of Ovx rats with estradiol hormone revealed insignificant decrease in brain AchE activity accompanied with insignificant increase in brain Ach level in comparison with Ovx control group.

Aluminum administration in Ovx rats induced significant elevation in brain AchE activity (41.04%) and significant reduction in brain Ach level (~25.92%) as compared with Ovx control rats. Treatment of Al-intoxicated Ovx rats with estradiol hormone produced significant decrease in brain AchE activity (~13.56%) accompanied with significant increase in brain Ach level (20%) in comparison with Al-intoxicated control group.

**Histological Investigations**

Microscopic examination of brain sections of gonad intact control rats (Figure 1) showed normal morphological structure of the hippocampus. Microscopic examination of brain of ovariectomized control rats (Figure 2) showed normal morphological structure of the hippocampus. Also

---

**Table II.** Effect of treatment with estradiol on brain antioxidant status in ovariectomized and Al-intoxicated ovariectomized rats. Data are represented as mean ± SE of 10 rats/group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CAT (U/mg protein) mean ± SE</th>
<th>SOD (U/mg protein) mean ± SE</th>
<th>TAC (mmol/mg protein) mean ± SE</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonad intact</td>
<td>5.79 ± 0.31</td>
<td>2.81 ± 0.18</td>
<td>14.20 ± 0.81</td>
<td>Ovx control</td>
</tr>
<tr>
<td>Ovx control</td>
<td>4.95 ± 0.29 (-14.55%)</td>
<td>2.06 ± 0.19 (-26.60%)</td>
<td>8.86 ± 0.43 (-37.62%)</td>
<td>Ovx + estradiol</td>
</tr>
<tr>
<td>Ovx + estradiol</td>
<td>5.34 ± 0.31 (7.95%)</td>
<td>2.30 ± 0.13 (11.51%)</td>
<td>9.37 ± 0.43 (5.81%)</td>
<td>Ovx + estradiol</td>
</tr>
<tr>
<td>Ovx + Al + estradiol</td>
<td>4.16 ± 0.26 (-15.95%)</td>
<td>1.80 ± 0.17 (-12.72%)</td>
<td>6.33 ± 0.35 (-28.55%)</td>
<td>Ovx + Al + estradiol</td>
</tr>
<tr>
<td>Ovx + Al + estradiol</td>
<td>5.11 ± 0.34 c (23.02%)</td>
<td>2.00 ± 0.18 (11.11%)</td>
<td>9.03 ± 0.51 c (42.76%)</td>
<td>Ovx + Al + estradiol</td>
</tr>
</tbody>
</table>

Ovx: ovariectomized. a: Significant change at p < 0.05 in comparison with gonad intact control group. b: Significant change at p < 0.05 in comparison with ovariectomized control group. c: Significant change at p < 0.05 in comparison with Al-intoxicated control group. (%): Percent of difference with respect to corresponding control value.
microscopic examination of hippocampus of ovariectomized rats administrated with estradiol hormone showed normal morphological structure of the hippocampus (Figure 3). Microscopic investigation for brain section of Al-intoxicated ovariectomized rats demonstrated various sizes of amyloid plaques formation in the cerebral cortex and in the hippocampus (Figure 4). Histological investigation of brain section of Al-intoxicated ovariectomized rats-treated with estradiol hormone revealed more or less normal structure in the hippocampus i.e. all amyloid plaques that were formed under the influence of ovariectomy as well as AlCl₃ administration disappeared by the treatment with this hormone (Figure 5).

**Discussion**

There is a growing evidence that oxidative stress and estradiol deprivation after menopause or ovariectomy are two main risk factors which are closely related to the pathological development of Alzheimer’s disease (AD). Also, aluminum has been implicated in aging-related changes and particularly in neurodegenerative diseases as it promotes the formation of amyloid-β (Aβ) plaques²⁷.

The data in Table I show that each of H₂O₂ and MDA in the brain of either Ovx rats or Al-intoxicated Ovx rats has significantly increased as compared to gonad intact control group. Tuneva et al²⁸ reported that in vitro and in vivo studies demonstrated increased ROS including H₂O₂ production in different brain areas due to Al exposure. Aluminum has a strong prooxidant activity in spite of its nonredox status. Kumar et al²⁹ observed that Al exposure is associated with impairment of antioxidant defense system that may lead to oxidative stress. Also, Al could increase the activity of monoamine oxidase enzyme (MAO) in the brain which leads to increased generation of H₂O₂³⁰. Aluminum can induce lipid peroxidation and to alter physiological and biochemical behavior of living organism a matter implicated in increased brain MDA level. MDA

<table>
<thead>
<tr>
<th>BDNF (pg/mg protein) mean ± SE</th>
<th>Bcl-2 (ng/mg protein) mean ± SE</th>
<th>Parameters groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>88.26 ± 3.31</td>
<td>1.15 × 10⁻¹ ± 5.74 × 10⁻¹</td>
<td>Gonad intact control</td>
</tr>
<tr>
<td>62.38 ± 3.15⁺ (-29.32%)</td>
<td>6.90 × 10⁻² ± 3.00 × 10⁻³ (-40.00%)</td>
<td>Ovx control</td>
</tr>
<tr>
<td>70.76 ± 3.10 (13.43%)</td>
<td>8.00 × 10⁻² ± 4.00 × 10⁻³ (15.94%)</td>
<td>Ovx + estradiol</td>
</tr>
<tr>
<td>45.50 ± 2.49⁻ (-27.05%)</td>
<td>5.82 × 10⁻² ± 3.70 × 10⁻³ (-15.57%)</td>
<td>Al-intoxicated control</td>
</tr>
<tr>
<td>56.36 ± 2.76⁻ (23.87%)</td>
<td>6.60 × 10⁻² ± 3.08 × 10⁻³ (13.30%)</td>
<td>Ovx + Al + estradiol</td>
</tr>
</tbody>
</table>

Table III. Effect of treatment with estradiol on brain antiapoptotic marker (Bcl-2) and neurotrophic factor (BDNF) in ovariectomized and Al-intoxicated ovariectomized rats. Data are represented as mean ± SE of 10 rats/group.

Ovx: ovariectomized. a: significant change at p < 0.05 in comparison with gonad intact control group. b: Significant change at p < 0.05 in comparison with ovariectomized control group. c: Significant change at p < 0.05 in comparison with Al-intoxicated control group. (%): Percent of difference with respect to corresponding control value.

<table>
<thead>
<tr>
<th>Ach (nmol/mg protein) mean ± SE</th>
<th>AchE (U/mg protein) mean ± SE</th>
<th>Parameters groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.50 × 10⁻² ± 3.42 × 10⁻³</td>
<td>568.96 ± 26.11</td>
<td>Gonad intact control</td>
</tr>
<tr>
<td>8.10 × 10⁻² ± 3.25 × 10⁻³ (-4.70%)</td>
<td>608.55 ± 33.75 (6.95%)</td>
<td>Ovx control</td>
</tr>
<tr>
<td>8.30 × 10⁻² ± 2.52 × 10⁻³ (2.46%)</td>
<td>595.40 ± 28.49 (-2.16%)</td>
<td>Ovx + estradiol</td>
</tr>
<tr>
<td>6.00 × 10⁻² ± 2.55 × 10⁻³ (-25.92%)</td>
<td>858.30 ± 43.82⁺ (41.04%)</td>
<td>Al-intoxicated control</td>
</tr>
<tr>
<td>7.20 × 10⁻² ± 3.83 × 10⁻³ (20.00%)</td>
<td>741.85 ± 36.56⁻ (-13.56%)</td>
<td>Ovx + Al + estradiol</td>
</tr>
</tbody>
</table>

Table IV. Effect of treatment with estradiol hormone on brain acetylcholinesterase (AchE) and acetylcholine (Ach) in ovariectomized and Al-intoxicated ovariectomized rats. Data are represented as mean± SE of 10 rats/group.

Ovx: ovariectomized. b: Significant change at p < 0.05 in comparison with ovariectomized control group. c: Significant change at p < 0.05 in comparison with Al-intoxicated control group. (%): Percent of difference with respect to corresponding control value.
is the major aldehyde formed after breakdown of lipid hydroperoxides\textsuperscript{31}.

Estradiol supplementation in Al-intoxicated Ovx rats caused significant decrease in brain H\textsubscript{2}O\textsubscript{2} and MDA levels. Bednarek-Tupikowaska et al\textsuperscript{32} showed that estradiol decreased the production of ROS \textit{in vitro} and \textit{in vivo} and protected neural tissue against oxidative stress as it serves as a free radical scavenger\textsuperscript{33}. Estradiol donates the hydrogen atom from its phenolic OH to hydroxyl and lipid peroxy radicals, resulting in the termination of cell membrane phospholipid peroxidation chain reaction that causes cell damage\textsuperscript{34} and reduces MDA level.

The data in Table II show marked decrease in each of brain TAC, SOD and CAT activities in both of Ovx rats and in Al-intoxicated ovx rats. Munoz-Castaneda et al\textsuperscript{35} showed that the lack of estradiol by ovariectomy induced a reduction of antioxidant status (GSH, SOD and GPx) and elevated lipid peroxides in rats. It has been demonstrated that aluminum altered the cellular redox state of the brain by inhibiting the enzymes involved in antioxidant defense, such as SOD and catalase, which function as blockers of free radical processes\textsuperscript{36}. Long term exposure to oxidative stress due to Al exposure leads to exhaustion of antioxidative enzymes\textsuperscript{37}. Aluminum causes brain damage via ROS more than any other organ because of its high lipid content, high oxygen turnover, low mitotic rate as well as low antioxidant concentration\textsuperscript{38}.
Windows into estradiol effects in Alzheimer's disease therapy

Treatment of Ovx rats and Al-intoxicated Ovx rats with estradiol resulted in marked increase in each of brain TAC, SOD and CAT activities. One of the well documented effects of estradiol is its antioxidant properties; estradiol protects neurons and neuronal cell line against oxidative stress. Estradiol binds to a specific estradiol response element (ERE) in the promoter region of target genes, leading to transcriptional activation of various antioxidant enzymes such as SOD and GPX. Thus, the antioxidant effect of estradiol is unlikely to be due to the direct interaction of estradiol and free radicals. Rather, estradiol appears to strengthen the free radical enzyme defense system by increasing gene expression of these enzymes.

The data in Table III show that significant decrease in both of antiapoptotic marker (Bcl-2) and brain derived neurotrophic factor (BDNF) levels in the brain of Ovx rats and in Al-intoxicated Ovx rats. Sharma and Mehra stated that ovariectomy decreased Bcl-2 expression and increased pro apoptotic marker (Bax) expression in the rat hippocampus. Altered Bax/Bcl-2 ratio is critical to Al-induced apoptosis leading to activation of caspase-3 and release of cytochrome . Kumar et al. reported that aluminum induces oxidative stress on the neuronal cells and increases p53 protein expression by activating p38 MAPK to initiate apoptosis and this is accompanied by marked inhibition of Bcl-2 and increased Bax expression. Ohyagi et al. showed that amyloid could activate p53 by direct interaction with the p53 promoter which led to Bax and caspase-6 activation with subsequent reduction of Bcl-2 and execution of the cell death pathway.

Takuma et al. showed marked decrease in the BDNF mRNA level in the hippocampus due to ovariectomy in mice. Campbell et al. implicated astrocytes as the principal target for A1 toxic action, thus blocking the release of neurotrophic factor. Disruption of the proinflammatory cytokine/neurotrophin balance by A1 plays an important role in the neurodegenerative disease. Aluminum intoxication results in increased tumor necrosis factor (TNF-α) and macrophage inflammatory protein-1α (MIP-1α), with consequent decrease in nerve growth factor (NGF) and BDNF. BDNF is one of the target genes of phosphorylated cAMP-responsive element binding protein (CREB) so that its mutation or blocking resulted in a dramatic loss of BDNF transcription. Decrease in brain BDNF level could be associated with AD pathogenesis.

Treatment of both Ovx rats and in Al-intoxicated Ovx rats with estradiol resulted in remarkable elevation in both brain Bcl-2 and BDNF. Nilsen and Brinton demonstrated that estradiol increased antiapoptotic proteins, Bcl-2 and Bcl-xL, which prevented activation of the permeability transition pore and thereby protects against the mitochondrial Ca accumulation. Estradiol can directly upregulate this survival factor through receptor-mediated interactions with regions of the bcl-2 promoter, which contains several putative estradiol responsive sites. Further estradiol regulated the expression of BDNF and increased levels of its mRNA after long term estradiol treatment in forebrain region. Therefore, estradiol modulates the expression of BDNF as a consequence of activation of CREB via protein kinases, such as PKA, CaMK IV and MAPK.

Table IV shows appreciable increase in brain AchE activity and concomitant decrease in Ach level in both Ovx rats and Al-intoxicated Ovx rats. Gulya et al. showed that aluminum caused cholinergic system dysfunction that may contribute to learning and memory deficits observed in Alzheimer’s dementia. Zhang et al. reported increased AchE activity in Al overload in rats. Kaizer et al. suggested that Al exposure increased AchE activity via allosteric interaction between Al and the peripheral anionic site of the enzyme molecule, leading to the etiology of AD pathological deterioration.

Aluminum exerts cholinotoxic effects by blocking the provision of acetyl-CoA which is required for Ach synthesis or by impairing the activities of choline acetyl transferase (ChAT) enzyme itself. Aluminum promoted the formation of amyloid plaques (Aβ 1-42) which significantly reduced brain Ach level leading to greater hippocampal Ach reduction accompanied by more memory impairment. The effect of Aβ1-42 increased when Aβ1-42 was combined with Ovx.

Treatment of both Ovx rats and Al-intoxicated Ovx rats with estradiol resulted in remarkable decrease in brain AchE activity and increase in Ach level. A growing evidence indicated that estradiol may interact with the cholinergic function in the hippocampus to modulate learning and memory. Estradiolic compounds have shown antiinflammatory activity, decreased production of proinflammatory cytokines such as IL-6, IL-1β, and TNF-α which promoted activity and expression of AchE. Bohacek et al. showed that estradiol treatment can enhance
basal forebrain cholinergic function in rats. Cholinergic neurotransmission could be enhanced by estradiol at multiple levels (a) at the synthetic level, by increasing the synthesis of Ach⁶⁷(b) at the reuptake level, through influencing the transport and/or packaging of acetylcholine into vesicles; (c) through structural effects that would enhance synaptic connectivity; and (d) in aging and neurodegeneration, through facilitating the survival of otherwise degenerating cholinergic neurons, perhaps through action on growth factors and growth factor receptors⁶⁸.

Microscopic examination of the brain of ovariectomized rats showed that ovariectomy didn’t produce any histological changes in the hippocampus and this finding is in agreement with that of Van Groen and Kadish⁶⁹ who demonstrated that estradiol depletion by ovariectomy didn’t affect amyloid-beta deposition in hippocampus.

Microscopic examination of the brain of ovariectomized rats-treated with estradiol showed normal structure of the hippocampus.

Microscopic investigation for the brain of Al-intoxicated ovariectomized rats revealed the presence of amyloid plaques formation in the cerebral cortex as well as in the hippocampus in addition to the observable regression in the number of neuronal cells in the hippocampus. In accordance of our results Abd El-Rahman⁷⁰ demonstrated that aluminum administration causes the presence of neuritic plaques that appeared with dark center, neuronal damage and degeneration in the cerebral cortex and the hippocampus.

Treatment of Al-intoxicated ovariectomized rats with estradiol revealed more or less normal structure in the hippocampus, i.e. all amyloid plaques that were formed under the effect of AlCl₃ administration disappeared under the influence of this hormone. These findings are in agreement with Morinaga et al⁷¹ who demonstrated that estradiol exerts antiamyloidogenic activity.

References


6) Chen JO, Camavara PR, Barnes CP, Yager JD. Regulation of mitochondrial respiratory chain biogenesis by estradiol/estradiol receptors and physiological, pathological and pharmacological implications. Biochim Biophys Acta 2009; 1793: 1540-1570.


Windows into estradiol effects in Alzheimer’s disease therapy

33) CHEN JQ, CAMMARATA PR, BAINES CP, YAGER JD. Regulation of mitochondrial respiratory chain biogenesis by estradiols/estriol receptors and physiological, pathological and pharmacological implications. Biochim Biophys Acta 2009; 1793: 1540-1570.
34) HENDERSON VW, BRINTON RD. Menopause and mitochondrial superoxide dismutase to aluminum-induced oxidative damage. Toxicology 2009; 255: 117-123.
36) KUMAR V, BAL A, GILL KD. Susceptibility of mitochondrial super-oxide dismutase to aluminum-induced oxidative damage. Toxicology 2009; 255: 117-123.
37) MENG XM. Mechanism of damage to erythrocytes after burn injury in rat changes in lipid peroxidation, antioxidant function and sulfhydryl groups. Chung Hua Cheng Hsing Shao Shang Wai Ko Tsu Chih 1991; 7: 205-207.
42) SHARMA K, MEHRA RD. Long term administration of estradiol or tamoxifen to ovariectomized rats affects neuroprotection to hippocampal neurons by modulating the expression of Bcl-2 and Bax. Brain Res 2008; 1204: 1-15.
45) KUMAR V, BAL A, GILL KD. Aluminum-induced oxidative DNA damage recognition and cell cycle disruption in different regions of rat brain. Toxicology 2009b; 264: 137-144.


54) NILSEN J, MOR G, NAFTOLIN F. Estradiol regulated developmental neuronal apoptosis is determined by estradiol receptor subtype and the Fas/Fas ligand system. J Neurobiol 2000; 43: 64-78.


63) FARR SA, BANKS WA, MORLEY JE. Estradiol potentiates acetylcholine and glutamate-mediated posttrial memory processing in the hippocampus. Brain Res 2000; 864: 263-269.


67) BOHACEK J, BEARL AM, DANIEL JM. Long term ovarian hormone deprivation alters the ability of subsequent oestradiol replacement to regulate choline acetyltransferase protein levels in the hippocampus and prefrontal cortex of middle aged rats. J Neuroendocrinol 2008; 20: 1023-1027.


