Original Research Article

Assessment Role of Dehydroepiandrosterone (DHEA) and Melatonin or their mixture on Physiological and Biochemical Parameters in Alzheimer's rats induced by aluminium chloride

Abstract

Alzheimer's disease is progressing neuronal degeneration disease leads to impairment of memory and cognitive ability. It is the most common cause of dementia and its increases with age. the present study was to investigate whether the Dehydroepiandrosterone (DHEA) and Melatonin or their mixture could potentially prevent aluminium-induced neurotoxicity in the cerebral cortex, hippocampus and cerebellum of the rat brain. The results showed that malondialdehyde (MDA) and total nitric oxide (TNO) showed significant (P< 0.001) increase in the mean values of Al intoxicated rats. However, the cortex and hippocampus SOD, CAT activities and GSH contents were significantly decreased in rats intoxicated with AlCl3.Additionally, choline acetyl transferase (ChAT) was a significant increase. The administration of Dehydroepiandrosterone (DHEA) and Melatonin corrected all these alterations and the maximum ameliorating effects were exhibited in the rats treated with the mixture of Dehydroepiandrosterone (DHEA) and Melatonin.

Keywords: Dehydroepiandrosterone - Melatonin - aluminium chloride – rats.

Introduction

Despite the genetic and environmental factors and the ageing process itself, multiple evidence from experimental models and postmortem studies in Alzheimer's disease (AD) brain tissue demonstrate that neuro-degeneration is associated with morphological and biochemical features. Considerable evidence suggests a role for oxidative stress/damage (amyloid beta peptide, iron/hydrogen peroxide) or neurotoxic by-products of lipid peroxidation (4-hydroxy-2-nominal, acrolein) and inflammation, in the pathogenesis of neuron degeneration, which, in turns, are known to cause cell death (***Querfurth and LaFerla 2010).***

Recently, several reports indicate that, among factors, metal ions (Al, Zn, Cu, Fe, etc) could specifically impair protein aggregation and their oligomeric toxicity. Also, metal-induced (direct) and metal-amyloid-β (indirect) linked neuronal cell death through the formation of reactive oxygen species (ROS) being critical to the understanding of the mechanisms which metal-induced cell death, and thus its role in neurodegenerative disorders (***Starke et al. 2013)***.

Some metals are essential for humans and for all forms of life. Even though metals are necessary for biological systems, they are usually required only in trace amounts; in excess, it can be toxic, if not fatal. Environmental metal exposure has been suggested to be a risk factor for the AD. High-term exposure to certain metals like manganese (Mn), iron (Fe), aluminum (Al) and many others like copper (Cu), mercury (Hg), zinc (Zn), lead (Pb), arsenic (As), alone or in combination, can increase neurodegenerative process, especially to Alzheimer's disease (AD).Aluminium (Al) is the most widely distributed metal in the environment and is extensively used in a wide variety of products: cans, foils and kitchen utensils, as well as parts of aeroplanes, rockets and other items that require a strong, light material. It can be deposited on the surface of the glass to make mirrors and also to make

synthetic rubies and sapphires for lasers. Aluminium (Al) is found in the environment in its natural forms or as a source of human contamination resulting from mining and smelting, activities that increase their distribution throughout the environment. Al occurs naturally only in compounds, never as a pure metal. Because of its strong affinity to oxygen, it is almost never found in the elemental state; instead, it is found in oxides or silicates ***(Nayak, 2002).***

In nature, this trace element is found in its oxidized state Al^{3+} (a soluble toxic form of Al), which binds to others molecules like chloride, forming Aluminum chloride ($AlCl_3$). Aluminium chloride ($AlCl_3$) is an important coagulant used in water treatment and purification being another source ofe exposure. Two of the most common compounds are potassium aluminium sulfate ($Kale(SO_4)^2 \cdot 12H_2O$), and aluminium oxide ($Al_2O_3$). Although aluminium is a widespread element, almost all metallic aluminium is produced from the ore bauxite ($AlOx(OH)_3$-2x). Bauxite is a complicated mixture of compounds consisting of 55% of aluminium, oxygen, and other elements ***(Nayak, 2002).***

Abnormal production or clearance of a small peptide, the amyloid β-peptide (Aβ), which is the major constituent of the senile plaques, is a widely accepted causative agent in degenerative disorders like an AD ***(Rauk, 2009)***. Aβ is a 39- to 43-residue peptide cleaved from the C-terminal region of a much larger protein, the amyloid precursor protein (APP), where the most abundant fragments are Aβ (1–40) and Aβ (1–42), being the latter the most neurotoxic ***(Rauk, 2009).***

Several studies have shown that Aβ exerts its toxicity by generating reactive oxidative stress (ROS) molecules, leading to peroxidation of membrane lipids and lipoproteins, induction of H_2O_2 and hydroxynonenal (HNE) in neurons, damages DNA and transport enzymes inactivation ***(Xu et al., 2001***).In addition to a high metabolically levels of ROS, there are other sources that are thought to play an important role in the AD progression. Among them, mitochondrial and metal abnormalities are the major sources of oxidative stress ***(Su et al., 2008).*** There is some experimental evidence that Al exposure can adversely affect the dopaminergic system. Extended exposure to 100mM Al lactate increased striatal levels of the dopamine metabolite, what, in turns, suggests that exposure to Al may cause an increased turnover of dopamine ***(Li et al., 2008).***

Being involved in the production of reactive oxygen species (ROS), aluminium may cause impairments in mitochondrial bioenergetics and may lead to the generation of oxidative stress which may lead to a gradual accumulation of oxidatively modified cellular proteins, lipids and affects endogenous antioxidant enzyme activity, leading to degeneration of neuronal cells. In this way, aluminium is a strong candidate for consideration as a subtle promoter of events typically associated with brain ageing and neurodegenerative disorders ***(Wu et al., 2010).***The present study aimed to investigate the role of oxidative stress and the status of the antioxidant system in the management of aluminium chloride (AlCl3) induced brain toxicity in rats and further to elucidate the potential role of Dehydroepiandrosterone (DHEA) and Melatonin or their mixture in alleviating such negative effects.

Materials and Methods

Animals:

Sixty male albino rats (Sprague Dawley), weighing 225±10 and obtained from The Nile Co. for Pharmaceuticals and Chemical Industries, Cairo, Egypt were used. They were still in stainless cages,

two to three per cage, at a temperature of 25±1°C with alternatively 12 hour light and dark cycles. Water was given for rats ad-libitum and they were kept under the same controlled conditions and provided with their daily dietary requirements of standard diet pellets according to ***NRC (1977)*** contained 20% protein, 5% fibre, 3.5% fat, 6.5% ash and a vitamin mixture. The study was conducted in accordance with the ethical guidelines of Faculty of women's.Ain Shams University, Egypt.

Drugs and chemicals:

Aluminium chloride - hydrated ($AlCl_3.6H_2O$), was purchased from Sigma Chemical Co. (St. Louis, MO, USA). It was immediately dissolved in distilled water. All other chemicals and solvents were the most commonly available.

Experimental design:

The study included two experiments; the first experiment was carried out to compare the disturbance in the oxidative stress of the brain, as a result of administration aluminum chloride to achieve this purpose, a comparison was made between a group of five control rats and other five rats were received 100mg aluminum chloride/kg body weight in the drinking water for three months as described by ***Sethi et al. (2009).***

In the other experiment, five comparisons were done between normal control rats (n= 10 rats) and four groups of rats administration aluminium chloride. The first experimentally functional neurotoxicity group was served as recovery group. The second functional neurotoxicity group rats were treated with oro-gastric tube which dissolved in 5% DMSO in saline at a dose of 250mg DHEA/Kg b.w./day for one month ***(Kurata et al., 2004).*** The third functional neurotoxicity group rats were treated orally with 7mg melatonin /kg b.w./days which dissolved in 5% DMSO in the aid of oro-gastric tube for one month according to ***Esparza et al. (2003)*** and stated with ***Sushma et al. (2011).*** The fourth functional neurotoxicity group rat as received both melatonin and DHEA for the same previous period. All animal groups were divided into two intervals (2 & 4 weeks and five rats in each group).

At the end of each experimental, rats were sacrificed 24 h after the last test and the brain tissues were dissected and washed with ice-cold saline. The brain tissues were either subjected to biochemical analysis immediately or kept frozen at -80°C until the time of analysis where they were homogenized in saline. The brain tissue homogenates were used to measure oxidative/antioxidant profile; lipid peroxides expressed as malondialdehyde (MDA) and total nitric oxide (TNO) as well as antioxidant status expressed as, dismutase (SOD) and glutathione (GSH & GSSG) superoxide.

Brain acetylcholine (Ach) level, acetylcholine esterase (ACHE) activity, brain-derived neuro-trophic factor (BDNF) concentration and amyloidβ-peptide (Aβ) content were also used for all groups.

Brain tissue sampling and preparation:

At the end oftheexperiment, the ratsrat'swere fastedovernight, subjected toanesthesia with diethyldiethylether andsacrificed. The whole brainof eachrat was rapidlydissected, washed withwithisotonic saline andanddried on filterfilterpaper. Each brain was divided sagittally into two portions.

The first part wasweighed and homogenizedhomogenizedin ice-cold medium containing50 mMTris/ HCl and300 mM sucrose atatpH7.4 to givegivea10 % (w/v) homogenate ***(Tsakiris etal., 2004).*** This homogenate was centrifuged at 1400×g for 10 min at 4°C. The supernatant was storedat

–80 °C and used for biochemical analyses that included oxidative stress biomarker (MDA), antioxidant status (GSH, GSSG, SOD and, CAT, anti-apoptotic marker (Bcl-2), brain neurotrophic factor (BDNF) and cholinergic markers (AchE and Ach). Also, brain totaltotal protein concentrationwas measured to express the concentrationconcentrationof different brain parameters per mg protein.

Biochemical analyses:

Lipid peroxidation products represented by malondialdehyde (MDA) were measuredmeasured byby the method of***Satoh (1978)*** using thiobarbituricthiobarbituricacid (TBA) andmeasuring the reaction product spectrophotometrically at 534 nm.

Statistical Analysis:

Results were expressed as the mean ± standard error (SE) for all animals in each group. Statistical analysis was carried out using oneway analysis of variance (ANOVA) followed by Duncan's multiple range tests according to ***(Duncan,1955) and (Snedecor and Cochran,1982).*** Results were considered significantly different if were $P < 0.001$ and $P < 0.05$.

RESULTS

Acetylcholine (Ach) level and -derived neuro-trophic factor (BDNF) concentration in brains shown in **table (1)**, in rats administrationaluminum chloride, there were significant ($p<0.05$) reductions in serum compared to normal control ones, On the other hand, there were significant increasing ($p<0.05$) in the cortex and choline acetyl transferase AChE activities, and amyloidβ-peptide (Aβ) content.

Table (1): Effect of aluminium administration on brain marker profile in male albino rats (Mean ± SE).

	Control group	Al- toxicity group
Ach (µmol/mg protein)	93.11± 1.68	63.72 ± 1.79*
AchE (U/mg protein)	592.28 ± 8.67	901.13 ± 11.84*
BDNF (pg/mg protein)	110.52 ± 1.93	71.55 ± 1.32*
Amyloidβ-peptide (pg/mg protein)	14.06 ±0.42	28.16 ±0.81*

Values were expressed as means ±SE. P<0.05 (significant)

In the current study, malondialdehyde (MDA) and total nitric oxide (TNO) showed significant (P< 0.001) increase in the mean values of Al intoxicated rats. On the opposite, administration to Al intoxicated animals daily showed decreases in the cortex and hippocampus SOD, CAT activities and GSH contents compared to control group **(Table 2).**

Table (2): A comparison between normal and Al intoxicated rats groups on brain antioxidant & oxidative status profile in male albino rats (Mean ± SE).

	Control group	Al-toxicity group

GSH (U/mg protein)	40.71±1.113	22.53±0.692*
GSSG (µM/mg protein)	0.62±0.007	0.95±0.013*
SOD (U/mg protein)	3.43±0.079	2.38±0.047*
CAT (U/mg protein)	7.08±0.131	5.41±0.086*
MDA (nmol/mg protein)	3.67 ± 0.009	5.82 ± 0.018*
TNO (pg/ mg protein)	38.53 ± 1.026	67.09 ± 1.872*

Values were expressed as means ±SE. P<0.05 (significant)

In the current investigation, amelioration effect occurred in the brain marker profile of rats group that were treated with Dehydroepiandrosterone (DHEA) and Melatonin or their mixture for 15 & 30day depending on the time of treatment **(Table 3).**

Table (3): Amelioration effects of dehydroepiandrosterone (DHEA) or Melatonin or their mixture on brain marker profile in male albino rats (Mean ± SE).

Groups	Control	Al-toxicity groups			
		Without treatment	Al+ DHEA	Al+ Melatonin	Al+ Mixture
Ach (µmol/mg protein)					
15 days(N = 5)	$92.67\pm 2.72^{A}_{a}$	$62.39\pm 2.29^{B}_{a}$	$71.48\pm2.35^{C}_{a}$	$77.01\pm2.82^{D}_{a}$	$81.19\pm3.14^{E}_{a}$
30 days (N = 5)	$93.02\pm 2.81^{A}_{a}$	$54.53\pm 1.75^{B}_{b}$	$75.24\pm2.69^{C}_{b}$	$84.56\pm3.53^{F}_{b}$	$89.78\pm3.91^{G}_{b}$
AchE (U/mg protein)					
15 days(N = 5)	$598.79\pm10.39^{A}_{a}$	$932.55\pm16.02^{B}_{a}$	$813.23\pm17.28^{C}_{a}$	$759.87\pm14.94^{D}_{a}$	$700.61\pm14.55^{E}_{a}$
30 days (N = 5)	$603.12\pm10.61^{A}_{a}$	$1217.63\pm19.21^{B}_{b}$	$744.52\pm14.76^{C}_{b}$	$674.52\pm13.69^{D}_{b}$	$623.88\pm11.78^{E}_{b}$
BDNF (pg/mg protein)					
15 days(N = 5)	$103.42\pm2.81^{A}_{a}$	$72.65\pm2.33^{B}_{a}$	$80.95\pm2.49^{C}_{a}$	$85.22\pm2.74^{D}_{a}$	$89.55\pm2.91^{E}_{a}$
30 days (N = 5)	$104.56\pm2.86^{A}_{a}$	$66.59\pm2.09^{B}_{b}$	$86.89\pm2.85^{C}_{b}$	$93.87\pm3.23^{D}_{b}$	$100.26\pm3.67^{E}_{b}$
Amyloidβ-peptide (pg/mg protein)					
15 days(N = 5)	$12.64\pm0.51^{A}_{a}$	$34.57\pm1.27^{B}_{a}$	$26.09\pm1.07^{C}_{a}$	$23.21\pm0.97^{D}_{a}$	$20.65\pm0.85^{E}_{a}$
30 days (N = 5)	$12.61\pm0.49^{A}_{a}$	$39.16\pm1.49^{B}_{b}$	$22.05\pm0.91^{C}_{b}$	$19.37\pm0.79^{D}_{b}$	$15.12\pm0.68^{E}_{b}$

A, B, C, D, E Means with a common superscript within a row are significantly different at (P<0.05).
a, b Means with a common subscript within a column are significantly different at (P<0.05).

On detecting total nitric oxide (TNO), glutathione disulfide(SGGS)and serum MDA from the data in the **table (4),** it was denoted that marked depletion in rats groups treated with antioxidant depending on the duration of treatment

Table (4): Amelioration effects of dehydroepiandrosterone (DHEA) or Melatonin or their mixture of brain antioxidant & oxidative profile in male albino rats (Mean ± SE)

Groups	Control	Al-toxicity groups			
		Without treatment	Al+ DHEA	Al+ Melatonin	Al+ Mixture
GSH (U/mg protein)					
15 days(N = 5)	$40.52\pm1.379^{A}_{a}$	$24.59\pm0.892^{B}_{a}$	$29.89\pm0.976^{C}_{a}$	$34.41\pm1.081^{D}_{a}$	$35.92\pm1.124^{E}_{a}$
30 days (N = 5)	$40.43\pm1.366^{A}_{a}$	$22.67\pm0.813^{B}_{b}$	$33.79\pm1.065^{C}_{b}$	$37.06\pm1.163^{D}_{b}$	$39.54\pm1.228^{A}_{b}$
GSSG (µM/mg protein)					
15 days(N = 5)	$0.57\pm0.006^{A}_{a}$	$1.16\pm0.023^{B}_{a}$	$0.76\pm0.018^{C}_{a}$	$0.72\pm0.018^{D}_{a}$	$0.68\pm0.015^{E}_{a}$
30 days (N = 5)	$0.58\pm0.007^{A}_{a}$	$1.41\pm0.031^{B}_{b}$	$0.72\pm0.016^{C}_{b}$	$0.66\pm0.015^{D}_{b}$	$0.57\pm0.012^{A}_{b}$
SOD (U/mg protein)					
15 days(N = 5)	$3.21\pm0.081^{A}_{a}$	$2.17\pm0.048^{B}_{a}$	$2.49\pm0.059^{C}_{a}$	$2.79\pm0.071^{D}_{a}$	$2.95\pm0.082^{E}_{a}$
30 days (N = 5)	$3.19\pm0.079^{A}_{a}$	$1.88\pm0.041^{B}_{b}$	$2.67\pm0.068^{C}_{b}$	$3.03\pm0.085^{D}_{b}$	$3.21\pm0.087^{A}_{b}$

CAT (U/mg protein)					
15 days(N = 5)	$7.23\pm0.232^{A}{}_{a}$	$5.19\pm0.133^{B}{}_{a}$	$5.42\pm0.139^{C}{}_{a}$	$6.32\pm0.168^{D}{}_{a}$	$6.78\pm0.185^{E}{}_{a}$
30 days (N = 5)	$7.21\pm0.231^{A}{}_{a}$	$4.77\pm0.126^{B}{}_{b}$	$6.19\pm0.162^{C}{}_{b}$	$6.94\pm0.207^{D}{}_{b}$	$7.19\pm0.229^{A}{}_{b}$
MDA (nmol/mg protein)					
15 days(N = 5)	$3.40 \pm 0.045^{A}{}_{a}$	$6.62 \pm 0.154^{B}{}_{a}$	$4.94 \pm 0.119^{C}{}_{a}$	$4.49 \pm 0.096^{D}{}_{a}$	$4.01 \pm 0.084^{E}{}_{a}$
30 days (N = 5)	$3.41 \pm 0.045^{A}{}_{a}$	$7.19 \pm 0.161^{B}{}_{b}$	$4.62 \pm 0.107^{C}{}_{b}$	$3.83 \pm 0.074^{D}{}_{b}$	$3.37 \pm 0.059^{A}{}_{b}$
TNO (µmol\mg protein)					
15 days(N = 5)	$39.12 \pm 1.98^{A}{}_{a}$	$74.17 \pm 2.52^{B}{}_{a}$	$68.92 \pm 2.41^{C}{}_{a}$	$57.44 \pm 2.32^{D}{}_{a}$	$52.66 \pm 2.29^{E}{}_{a}$
30 days (N = 5)	$38.88 \pm 1.95^{A}{}_{a}$	$74.36 \pm 2.57^{B}{}_{b}$	$63.27 \pm 2.35^{C}{}_{b}$	$46.62 \pm 2.11^{D}{}_{b}$	$41.02 \pm 2.01^{E}{}_{b}$

A, B, C, D, E Means with a common superscript within a row are significantly different at (P<0.05).
a, b Means with a common subscript within a column are significantly different at (P<0.05).

DISCUSSION

Aluminium has an association with Alzheimer's disease mainly characterized by deterioration of memory, is a chronic and progressing degenerative disease of a central nervous system that was induced by multiple complicated factors ***(Salomone et al.,2012)***. The effect of the nervous system, causing loss of memory, imbalance and paralysis ***(Drago et al.,2008)***. By this reason, the present study aimed to study the effect of the antioxidant potential on rats administrationaluminum chloride against Al neurotoxicity.

In this study, there were significant increases in the oxidative stress markers lipid peroxidation following aluminium administration. Such results are in harmony with those obtained by***Johnson et al. (2005)*** who stated that the neurotoxicity of aluminium may be a result of LPO. Furthermore, ***Nehru and Anand (2005)*** reported a significant increase in brain thiobarbituric acid reactive substances in rats after stimulation by aluminum administration which was known to be bound by the Fe3+ carrying protein transferrin, thus reduction of the binding of Fe2+ and elevate free intracellular Fe2+ that causes the peroxidation of membrane lipids and consequently damage in brain. Aluminium, being an inert metal, has been reported that, induce oxidative stress by the peroxidative effect of Fe2+. It promotes reactive oxygen species (ROS) formation. ROS attack nearly all cell components including membrane lipids thus producing lipid peroxidation (***Christen, 2000).***

The present results demonstrate that there was a significant elevation in brain H_2O_2, NO and MDA levels of rats administration aluminium. ***Tuneva et al. (2006)*** explained an increase in ROS, including H2O2 production in different brain areas due to administered with AlCl3. Also, Aluminum could increase brain MDA level ***(Kumar et al., 2008).*** The present of significant increase of brain NO level after AlCl3administration in rats is in harmony with the former studies of the ***Guixetal. (2005).*** The NO elevation in brain tissue may be related to Al-induced nitric oxide synthase (NOS) activity with consequent elevation in NO production in rat brain tissue and microglial cells ***(Guix et al., 2005).*** The authors found that cerebellar levels of inducible NOS (iNOS) protein in rats w significantly increased following both short and long-term Al administration. On the contrary, the results of this study explained that rats administration aluminium led to AChE inhibition.

In the present study, there w a significant elevation in the oxidative stress markers acetylcholine (Ach) level and the brain-derived neurotrophic factor *(BDNF)* concentration for one month following aluminium administration in rats. The same results are in agree with those obtained by***Atack et al., 1983*** who reported that a relevant loss of membrane integrity, and consequently a strong effect of Al on the activity/functionality of various membrane-bound enzymes, including AChE. The finding may thus have loss of short-term memory in Alzheimer's disease. ***Nehru and Anand (2005)*** reported a significant elevation in brain thiobarbituric acid reactive substances in rats after stimulation by Al salts which was known to be bound by the Fe3+ carrying protein transferrin, thus reducing the binding of Fe2+ and increasing free intracellular Fe2+ that causes neuro-behaviour changes.

The present results demonstrate that there was a significant elevation in brain NO and MDA levels of rats administered with AlCl3. ***Tuneva et al. (2006)*** demonstrated an increase in ROS, including H2O2 production in different brain areas due to Al exposure. Also, Al could increase the activity of monoamine oxidase (MAO) in the brain, which leads to increased generation of H2O2 (Huh et al., 2005). Aluminium could induce lipid peroxidation and alter the physiological and biochemical behaviour of the living organism, a matter implicated in the increased brain MDA level ***(Kumar et al., 2008).*** The finding of significant elevation of brain NO level after AlCl3 administration in ovariectomized rats is in agreement with the previous studies of the ***Guix et al. (2005).*** The NO elevation in brain tissue may be related to Al-induced nitric oxide synthase (NOS) activity with a consequent increase in NO production in rat brain tissue and microglial cells ***(Guix et al., 2005).***

The findings of the present study, also, showed a decrease in the activity of antioxidant enzymes (superoxide dismutase (SOD) glutathione (GSH), catalase (CAT), in the cortex and hippocampus tissues compared with the control rats. These findings are harmony with studies of ***Savory et al. (2003) and Johnson et al. (2005)*** who reported that increased levels of oxidative stress and products of lipid peroxidation in the brain are the major contributing factors in the development of neurodegenerative diseases***.*** Similar data recorded a decrease in the antioxidants such as GSH) and SOD activity ***(Moumen et al., 2001)*** in the brain of Al exposed rats ***(Chainy et al., 1996)*** and human. Al-induced an increment in lipid peroxidation (LPO) levels with depletion of glutathione and reduction of activity of glutathione peroxidase (GSH-Px), glutathione–S-transferase (GST) and Catalase (CAT) (Mahieu et al., 2005).

However, the increased Al concentration could deleteriously affect the neurons, leading to depletion of antioxidants and metal ions ***(Kumar et al., 2008)*** through the induction of free radicals, that exhausting SOD and CAT which function as blockers free radical processes. These results are in accordance with ***(Nehru and Anand, 2005)***who recorded a significant decrease in the activities ofSOD and CAT in the brain of rats after Al treatment.Alternatively, the decreased enzyme activities could be related to a reduced synthesis of the enzyme proteins as a result of higher intracellularconcentrations of Al ***(Albendea et al., 2007).***

It is obvious that treatment of Al-intoxicated rats with DHEA produced a significant decrease in brain MDA levels. These remarkable effects of DHEA may be related to DHEAinhibiting the monoamine oxidase (MAO) activity in the brain. Considering the important role attributed to MAO activity in the generation of H2O2 ***(Marklund et al., 1982),*** the inhibitory effect of DHEA on MAO activity can be regarded as a mechanism by which DHEA could reduce oxidative stress, production of H_2O_2 and lipid peroxidation ***(Kumar et al., 2008).***

The present results also revealed a marked decrease in brain NO level as a result of DHEA administration in Al-intoxicated rats. DHEA has been found to inhibit NMDA-induced NO production and NO synthase (NOS) activity in hippocampus cell culture ***(Kurata et al., 2004).*** Considering total antioxidant activity (TAC) and antioxidant enzyme activities.). Long-term exposure to oxidative stress due to Al exposure leads to exhaustion of antioxidative enzymes. DHEA exhibits antioxidant properties in experimental systems ***(Aragno et al., 1999).*** Several explanations have been put forward for multitargeted antioxidant effects of DHEA, including its up-regulating effect on catalase expression and activity ***(Schwartz et al., 1988),*** as well as its activating action on the thioredoxin system ***(Gao et al., 2005).*** DHEA could also suppress superoxide anion production ***(Mohan & Jacobson, 1993).***

The remarkable decrease was recorded in brain GSH, GPx, GR, SOD and CAT activities in Al-intoxicated rats. ***Munoz-Castaneda et al. (2006)*** showed that reduction of the antioxidant status

(GSH, SOD and GPx) accompanied by elevated lipid peroxides in rats. A drastic depletion of brain GSH may be due to the increased cytotoxicity of H2O2 in endothelial cells as a result of inhibition of glutathione reductase***(El- Rigal et al., 2006).*** The significant depletion of, GSH in the brain of rats indicates the damage of the second line of the antioxidant defence system. This probably further exacerbates oxidative damage via an adverse effect on critical GSH-related processes. Reduced antioxidant status as a result of increased ROS production in experimental has been reported previously ***(Li et al., 2008).***

Aluminium exposure causes impairment of the antioxidant defence system that may lead to oxidative stress ***(Kumar et al., 2009).*** Aluminium 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 ***(Di et al., 2006).*** The study of ***Di et al. (2006)*** suggested that lower SOD activity in the brain due to Al exposure may be due to the altered conformation of SOD molecule as a result of Al-SOD complex formation.

The remarkable decrease was recorded in brain GSH, SOD and CAT activities in Al-intoxicated rats. ***Munoz-Castaneda et al. (2006)*** showed that the lack of estrogens by ovariectomy-induced reduction of the antioxidant status (GSH, SOD and GPx) accompanied by elevated lipid peroxides in rats. A drastic depletion of brain GSH may be due to the increased cytotoxicity of H2O2 in endothelial cells as a result of inhibition of glutathione reductase. Aluminium exposure causes impairment of the antioxidant defence system that may lead to oxidative stress ***(Kumar et al., 2009).*** Aluminium 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 (***Di et al., 2006).*** The study of ***Di et al. (2006)*** suggested that lower SOD activity in the brain due to Al exposure may be due to the altered conformation of SOD molecule as a result of Al-SOD complex formation.

Regarding the brain-derived neurotrophic factor (BDNF) level, the present data showed a significant decrease in brain levels BDNF Al-intoxicated rats. ***Takuma et al. (2007)*** showed marked decrease in the BDNF mRNA level in the hippocampus due to in mice. Disruption of the proinflammatory cytokine/ neurotrophin balance by Al plays a serious role in the neurodegenerative disease ***(Nagatsu et al., 2000).*** DHEA administration in Alintoxicated rats resulted in significant increase in BDNF levels. The mechanism by which DHEA could stimulate Bcl-2 expression is that DHEA binds to and activates G-protein coupled membrane receptor alpha inhibitory subunit (Gαi) that, in turn, activates protooncogenic tyrosine kinase c (Src), protein kinase C (PKC) and MAPK/ERK pathway. Therefore, DHEA could increase the Bcl-2 level and stimulate Bcl-2 function. Several transcription factors contributing to the regulation of BDNF promoters have been characterized and CREB is one of them (***Tabuchi et al., 2002***) The data in the current study revealed that DHEA administration produced a significant decrease in brain AchE activity associated with significant increase in brain Ach level in Al-intoxicated ovariectomized rats. It has been demonstrated that DHEAS significantly increases Ach release in the hippocampus.Thus, the promoting effect of DHEAS on Ach release in the hippocampus may be one mechanism for its memory enhancing effect ***(Zheng, 2009).***

The present study showed that melatonin defends cells from a variety of oxidative stress events and it is effective in preventing different types of degenerative disorders in which free radical generation is involved (Kilic et al.,2004) reduces the harmful effects of lipid peroxidation in the brain. This finding is relevant because increased levels of oxidative stress and products of lipid peroxidation in the brain are the major contributing factors in the development of neurodegenerative diseases. There is evidence that Al is able to produce free radicals that cause lipid peroxidation, there by damaging neuronal membranes and increasing blood–brain barrier permeability. The present results indicate that a pattern of AD similar to that obtained by ***(Sushma et al., 2007).***

Melatonin has a potent scavenger of reactive oxygen species, in the alterations induced by Al on the oxidative stress parameters. Mel (N-acetyl 5 methoxytryptamine) is naturally produced by the pineal gland from serotonin by a process catalysed by enzymes: arylalkylamine-N-acetyltransferase and hydroxindazole-O-methyltransferase. The synthesis and release of Mel in normal conditions are stimulated by darkness and inhibited by light in response to signals originated in the suprachiasmatic nucleus ***(Cardinali, 2007).***

Melatonin antioxidant properties result mainly from electron donation and unrestricted crossing of morpho-physiological barriers and its easy access to subcellular compartments facilitates the ROS scavenger effect ***(Tan et al., 2007***). Melatonin can act as an indirect antioxidant through the activation of the major antioxidant enzymes including superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase ***(Rodriguez et al.,2000).***

Melatonin can act as an indirect antioxidant through the activation of the major antioxidant enzymes including superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase (Rodriguez et al.,2000). Melatonin supplementation significantly reversed the Al-induced cell injury in the cerebellum.(Sushma et al., 2011). Thus, the antioxidant properties of Mel have been extensively studied, as well as the use of this molecule as a cell protector and as a potential disease preventing agent (Bandopadyay and Chattopadyay, 2006).

Conclusion:

The current investigation demonstrates that combination of DHEA and Melatonin possesses a good potential to inhibit oxidative stress and able to improve the antioxidant status and particularly by inhibiting the acetylcholinesterase activity

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