Original Research Article

Modulatory effects of Dehydroepiandrosterone (DHEA) and Melatonin or their mixture on Alzheimer's rats induced by aluminum chloride

Abstract

The present study was designed to investigate the role of oxidative stress and the status of antioxidant system in the management of aluminum chloride ($AlCl_3$) 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. The results revealed that the levels of melondayaldehide (MDA) , total nitric oxide (TNO) and glutathione disulfide (SGGS)were significantly increased, however, the activities of superoxide dismutase (SOD) and catalase (CAT) as well as the reduced glutathione (GSH) content were significantly decreased in rats intoxicated with AlCl3 .Additionally, serum and brain regions Acetylcholinesterase (AChE) activities were significantly increased. On the other hand, the results exhibited that, sage when given in any form along with AlCl3 was able to regulate the mentioned parameters and the values returned close to the normal ones. It can be concluded that Al-induced neuronal oxidative stress and inhibition of the antioxidant system, accompanied with disturbed lipid profile, total protein and enzyme activities could be the cause of $AlCl_3$ neurotoxicity. In addition Dehydroepiandrosterone (DHEA) and Melatonin or their mixture by their antioxidant constituents, could be able to antagonize Al neurotoxicity perhaps by reducing the oxidative stress and improving the antioxidant status and particularly by inhibiting the Acetylcholinesterase (AChE) activity, thus may improve memory and other brain cognitive activities.

Keywords: Dehydroepiandrosterone - Melatonin - aluminum chloride – rats.

Introduction

Despite the genetic and environmental factors and the aging 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-nonenal, acrolein) and inflammation, in the pathogenesis of neuron degeneration, which, in turns, are known to cause cell death.

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.

Some metals are essential for humans and for all forms of life. Even though metals are necessary in 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 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).

Aluminum is the most widely distributed metal in the environment and is extensively used in daily life that provides easy exposure to human beings. No biological function of the element has been identified, whereas some aspects of its toxicity have been described. It has been suggested that there might be a relationship between high levels of Al and increased risk of a number of pathogenic disorders, such as microcytic anemia, osteomalacia and possibly, neurodegenerative disorders including dialysis encephalopathy, Parkinson's disease (PD) and Alzheimer's disease (AD).

Aluminum (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 airplanes, rockets and other items that require a strong, light material. It can be deposited on the surface of glass to make mirrors and also to make synthetic rubies and sapphires for lasers. Aluminum (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+} (soluble toxic form of Al), which binds to others molecules like chloride, forming Aluminum chloride ($AlCl_3$). Aluminum chloride ($AlCl_3$) is an important coagulant used in water treatment and purification being another source for exposure. Two of the most common compounds are potassium aluminum sulfate ($Kale(SO_4)^{2\cdot}\ 12H_2O$), and aluminum oxide ($Al_2O_3$). Although aluminum is a widespread element, almost all metallic aluminum is produced from the ore bauxite ($AlOx(OH)_3$-2x). Bauxite is a complicated mixture of compounds consisting of 55% of aluminum, 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 AD ***(Qiu & Folstein, 2006; LaFerla et al., 2007 and 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 ***(Kontush et al., 2001*** and ***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 increased turnover of dopamine ***(Li et al., 2008).***

Several metals interact with β-amyloid (Aβ) in senile plaques. It is interesting to note that, compared to other Aβ-metal complexes (Aβ-Fe, Aβ-Zn, Aβ-Cu). Aβ-Al is unique in promoting a specific form of Aβ oligomerization that has marked neurotoxic effects ***(Drago et al., 2008).*** There are a lot of ways which Al can damage neural cells: (i) interfering with glucose metabolism, leading to low amounts of Acetilcholine (Ach) precursors; (ii) interacting to ATPase Na+/K+ and Ca2+/Mg2+ -depending, altering excitatory aminoacid release; (iii) inhibition the binding of Ca++; (iv) incresing the production of AMPc; (v) causing changes in the cytoskeleton protein, leading to phosphorilation, proteolysis, transport and synthesis disruption; (vi) interacting directly to genomic structures, and most importantly (vii) inducing oxidative damage by lipid peroxidation ***(Drago et al., 2008).***

Being involved in the production of reactive oxygen species (ROS), aluminum 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, aluminum is a strong candidate for consideration as a subtle promoter of events typically associated with brain aging and neurodegenerative disorders ***(Sethi et al., 2008; Kumar & Gill, 2009 and Wu et al., 2010).***

Materials and Methods

Animals:

Sixty male albino rats (Sprague Dawley), weighing 250±20g and obtained from The Nile Co. for Pharmaceuticals and Chemical Industries, Cairo, Egypt were used. They were housed in stainless steel cages, three to four per cage, at a temperature of 27±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% fiber, 3.5% fat, 6.5% ash and a vitamin mixture. The study was conducted in accordance with the ethical guidelines of Ain Shams University, Egypt.

Drugs and chemicals:

Aluminum chloride - hydrated ($AlCl_3.6H_2O$), was purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). It was freshly dissolved in distilled water. All other chemicals and solvents were of highest grade-commercially available.

Experimental design:

The study comprised two experiments; the first one was carried out to compare the disturbance in the oxidative stress markers and biochemical parameters of the brain as a result of functional neurotoxicity of aluminum chloride administration, to achieve this purpose, a comparison was done 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 second experiment, five comparisons were made between normal control rats (n= 10 rats) and four groups of rats with experimentally functional neurotoxicity. 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 30 days ***(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 30 days according to ***Esparza et al. (2003)*** and confirmed with ***Sushma et al. (2011).*** The fourth functional neurotoxicity group rat was received both DHEA and melatonin for the same previous period. All animal groups were divided into two intervals (15 & 30 days and five rats in each interval).

At the end of each experimental period, 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 for biochemical analysis immediately or kept frozen at -80°C till the time of analysis where they were homogenized in saline. The brain tissue homogenates were used to assess oxidative/antioxidant profile; lipid peroxides expressed as malondialdehyde (MDA) and total nitric oxide (TNO) as well as antioxidant status expressed as, glutathione (GSH & GSSG) superoxide dismutase (SOD).

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

Brain tissue sampling and preparation:

At the end of the experiment, the rats were fasted overnight, subjected to anesthesia with diethyl ether and sacrificed. The whole brain of each rat was rapidly dissected, washed with isotonic saline and dried on filter paper. Each brain was divided sagitally into two portions.

The first portion was weighed and homogenized in ice-cold medium containing 50 mM Tris/HCl and 300 mM sucrose at pH 7.4 to give a 10 % (w/v) homogenate ***(Tsakiris et al., 2004) .*** This homogenate was centrifuged at 1400×g for 10 min at 4°C. The supernatant was stored at –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 total protein concentration was measured to express the concentration of different brain parameters per mg protein.

Biochemical analyses:

Lipid peroxidation products represented by malondialdehyde (MDA) were evaluated by the method of ***Satoh (1978)*** using thiobarbituric acid (TBA) and measuring 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

Brain acetylcholine (Ach) level and brain-derived neuro-trophic factor (BDNF) concentration as shown in **table (1)**, in Al intoxicated rats, there were significant ($p<0.05$) reductions in serum compared to normal control ones, On the other hand, significant ($p<0.05$) elevations were seen in cortex and hippocampus AChE activities, and amyloidβ-peptide (Aβ) content.

Table (1): A comparison between normal and Al intoxicated rats groups 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 other hand, daily administration to Al intoxicated animals 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±0.49 $^{A}_{a}$	39.16±1.49 $^{B}_{b}$	22.05±0.91 $^{C}_{b}$	19.37±0.79 $^{D}_{b}$	15.12±0.68 $^{E}_{b}$

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 **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 on 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±1.379 $^{A}_{a}$	24.59±0.892 $^{B}_{a}$	29.89±0.976 $^{C}_{a}$	34.41±1.081 $^{D}_{a}$	35.92±1.124 $^{E}_{a}$
30 days (N = 5)	40.43±1.366 $^{A}_{a}$	22.67±0.813 $^{B}_{b}$	33.79±1.065 $^{C}_{b}$	37.06±1.163 $^{D}_{b}$	39.54±1.228 $^{A}_{b}$
GSSG (µM/mg protein)					
15 days(N = 5)	0.57±0.006 $^{A}_{a}$	1.16±0.023 $^{B}_{a}$	0.76±0.018 $^{C}_{a}$	0.72±0.018 $^{D}_{a}$	0.68±0.015 $^{E}_{a}$
30 days (N = 5)	0.58±0.007 $^{A}_{a}$	1.41±0.031 $^{B}_{b}$	0.72±0.016 $^{C}_{b}$	0.66±0.015 $^{D}_{b}$	0.57±0.012 $^{A}_{b}$
SOD (U/mg protein)					
15 days(N = 5)	3.21±0.081 $^{A}_{a}$	2.17±0.048 $^{B}_{a}$	2.49±0.059 $^{C}_{a}$	2.79±0.071 $^{D}_{a}$	2.95±0.082 $^{E}_{a}$
30 days (N = 5)	3.19±0.079 $^{A}_{a}$	1.88±0.041 $^{B}_{b}$	2.67±0.068 $^{C}_{b}$	3.03±0.085 $^{D}_{b}$	3.21±0.087 $^{A}_{b}$
CAT (U/mg protein)					
15 days(N = 5)	7.23±0.232 $^{A}_{a}$	5.19±0.133 $^{B}_{a}$	5.42±0.139 $^{C}_{a}$	6.32±0.168 $^{D}_{a}$	6.78±0.185 $^{E}_{a}$
30 days (N = 5)	7.21±0.231 $^{A}_{a}$	4.77±0.126 $^{B}_{b}$	6.19±0.162 $^{C}_{b}$	6.94±0.207 $^{D}_{b}$	7.19±0.229 $^{A}_{b}$
MDA (nmol/mg protein)					
15 days(N = 5)	3.40 ± 0.045 $^{A}_{a}$	6.62 ± 0.154 $^{B}_{a}$	4.94 ± 0.119 $^{C}_{a}$	4.49 ± 0.096 $^{D}_{a}$	4.01 ± 0.084 $^{E}_{a}$
30 days (N = 5)	3.41 ± 0.045 $^{A}_{a}$	7.19 ± 0.161 $^{B}_{b}$	4.62 ± 0.107 $^{C}_{b}$	3.83 ± 0.074 $^{D}_{b}$	3.37 ± 0.059 $^{A}_{b}$
TNO (µmol\mg protein)					
15 days(N = 5)	39.12 ± 1.98 $^{A}_{a}$	74.17 ± 2.52 $^{B}_{a}$	68.92 ± 2.41 $^{C}_{a}$	57.44 ± 2.32 $^{D}_{a}$	52.66 ± 2.29 $^{E}_{a}$
30 days (N = 5)	38.88 ± 1.95 $^{A}_{a}$	74.36 ± 2.57 $^{B}_{b}$	63.27 ± 2.35 $^{C}_{b}$	46.62 ± 2.11 $^{D}_{b}$	41.02 ± 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 the etiology of Alzheimer's disease and some other neurodegenerative diseases. It exerts its toxic effect on nervous system especially at high concentration, causing loss of memory, speech disturbances, dysparaxia, tremors, jerking movement's impaired muscular coordination and paralysis ***(Drago et al., 2008)***. For that reason, the present study aimed to look into the antioxidant potential of various sage preparations against Al neurotoxicity.

The present results demonstrate that there was significant elevation in brain H_2O_2, 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 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 ***Garrel et al. (1994***) and ***Guix et al. (2005).*** The NO elevation in brain tissue may be related to Al-induced nitric oxide synthase (NOS) activity with nconsequent increase in NO production in rat brain tissue and microglial cells ***(Guix et al., 2005).*** Those authors found that cerebellar levels of inducible NOS (iNOS) protein in rats was significantly elevated following both shortand long-term Al administration.On the

other hand, the results of the present study showed that sage administration of Al intoxicated rats led to AChE inhibition.

In the present study, there were significant increases in the oxidative stress markers acetylcholine (Ach) level and brain-derived neuro-trophic factor (BDNF) concentration following Al exposure for 30 days in rats. Such results are in harmony 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 some bearing on loss of short-term memory in Alzheimer's disease. ***Nehru and Anand (2005)*** reported a significant increase 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 the peroxidation of membrane lipids and consequently membrane damage. Aluminum, being an inert metal, has been suggested to induce oxidative damage indirectly by potentiating the peroxidative effect of Fe2+. It promotes reactive oxygen species (ROS) formation. ROS subsequently attack almost all cell components including membrane lipids thus producing lipid peroxidation ***(Christen, 2000).***

The present results demonstrate that there was 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). Aluminum could induce lipid peroxidation and alter the physiological and biochemical behavior 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 ***Garrel et al. (1994)*** and ***Guix et al. (2005).*** The NO elevation in brain tissue may be related to Al-induced nitric oxide synthase (NOS) activity with consequent increase in NO production in rat brain tissue and microglial cells ***(Guix et al., 2005).***

The findings of the present study, also, showed that Al treated rats was accompanied by concomitant decrease in the activity of some antioxidant enzymes involved in the detoxification of ROS, namely SOD, CAT as well as the level of GSH in the cortex and hippocampus tissues comparing with the control declaring the prooxidant effect of Al. These findings agreed with the antecedent studies of ***Savory et al. (2003) and Johnson et al. (2005)*** whom showed that Al exposure enhanced the neuronal lipid peroxidative damage with concomitant alterations in the enzymatic antioxidant defense status, thus having serious bearing on the functional and structural development of the central nervous system ***(Dua and Gill, 2001).*** Similar data recorded a decrease in the antioxidants such as GSH ***(Wu and Cederbaum, 2003)*** and SOD activity ***(Yousef, 2004)*** in the brain of Al exposed rats ***(Chainy et al., 1996)*** and human **(Dua and Gill, 2001)**. Moreover, such results are consistent with the studies indicated that Al intake produced an oxidative stress-related change, contributed to its neurotoxicity ***(Flora et al., 2003).*** However, in rats, a significant relationship between Al exposure and the presence of oxidative stress was established also by ***Gómez et al. (2005).*** This could be caused by inflicting damage to membrane lipids, proteins and antioxidative enzyme defense system ***(Jyoti et al., 2007).***

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 nexhausting SOD and CAT which function as blockers of free radical processes. These results are in accordance with ***(Nehru and Anand, 2005)*** who recorded a significant decrease in the activities of SOD and CAT in 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 intracellular concentrations of Al ***(Albendea et al., 2007).***

It is obvious that treatment of Al-intoxicated rats with DHEA produced significant decrease in brain MDA levels. These remarkable effects of DHEA may be related to DHEA inhibiting the monoamine oxidase (MAO) activity in 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 ***(Yildirim et al., 2003)*** 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).***

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 ***(Yousif & El-Rigal, 2004 and El- Rigal et al., 2006).*** The significant depletion of, GSH in brain of rats indicates the damage of the second line of antioxidant defense system. This probably further exacerbates oxidative damage via 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).***

Alternatively, since GSH has been reported to be involved in protein and DNA biosynthesis so, the reduction in its content and in the antioxidant enzymes (SOD and CAT) resulted from Al intoxication may partly explain the decline in the total protein content. Additionally, Al induced reactive oxygen species (ROS) formation and promoted oxidative stress ***(Exley, 2004 and Kumar et al., 2009)*** enhancing peroxidative damage to lipids and proteins of the cellular membranes ***(Julka and Jill, 1996)*** is another suggestion for protein decline. Such an explanation, which was confirmed by ***Jyoti et al. (2007),*** indicated that Al exposure caused oxidative stress inflicting damage to membrane lipids, proteins and antioxdative enzyme defense system. Exposure of proteins to free radicals leads to gross structural and functional modifications including protein fragmentation, formation of crosslinks and aggregates, protein peroxides generation, and enzymatic oxidation and degradation or clearance ***(Albendea et al., 2007).***

Aluminum exposure causes impairment of the antioxidant defense system that may lead to oxidative stress ***(Kumar et al., 2009).*** 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 ***(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.

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 ***(Yousif & El-Rigal, 2004)***. Aluminum exposure causes impairment of the antioxidant defense system that may lead to oxidative stress ***(Kumar et al., 2009).*** 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 (***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 brainderived neurotrophic factor (BDNF) level, the present data showed 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 ovariectomy in mice. Disruption of the proinflamatory cytokine/ neurotrophin balance by Al plays an important 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. These kinases activate the prosurvival transcription factors CREB which stimulate the expression of antiapoptotic proteins such as Bcl-2 and Bcl-xl ***(Charalampopoulos et al., 2006).*** Therefore, DHEA could increase 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 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 (***Rhodes et al., 1996***). Thus, the promoting effect of DHEAS on Ach release in the hippocampus may be one mechanism for its memoryenhancing effect ***(Zheng, 2009).***

The present work was performed to examine the effect of melatonin, a potent scavenger of reactive oxygen species, in the alterations induced by Al on the oxidative stress parameters. Mel (N-acetyl 5 methoxy tryptamine) is naturally produced by the pineal gland from serotonin by a process catalysed by enzymes: arylalkylamine-N-acetyltransferase and hydroxindazole-Omethyltransferase. The synthesis and release of Mel in normal conditions is stimulated by darkness and inhibited by light in response to signals originated in the suprachaismatic 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; Reiter et al.,2003***). Melatonin can act as an indirect antioxidant through the activation of the major antioxidant enzymes including superoxide dismutase,catalase, glutathione reductase and glutathione peroxidase ***(Akubulut et al., 2008; Rodriguez et al.,2000)***

Melatonin also 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 ***(Benitez-King et al.,2003 and Kilic et al.,2004).*** Melatonin supplementation significantly reversed the Al induced cell injury in 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 ***(Karbownick and Reiter, 2000; Reiter and Tan, 2003; 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 improving the antioxidant status and particularly by inhibiting the acetylcholinesterase activity, thus may improve memory and other brain cognitive activities.

REFERENCES

Akbulut, K.G.; Gonul, B.& Akbulut, H.(2008): Exogenous melatonin decreases age-induced lipid peroxidation in the brain. Brain Research;;1238:31-35

Albendea, C.D.; Trullen, E.M.G.; Broto, L.E.; Miana-Mena, F.G.; Plano, S.M.; Gonzales, M.C.R.; Balların, E.M. & Garcia, J.J. (2007): Melatonin reduces lipid and protein oxidative damage in synaptosomes due to aluminum. J. Trace Elem. Med. Biol., 21:261–268.

Aragno, M.; Tamagno, E.; Gatto, V.; Brignardello, E.; Parola, S.; Danni, O.& Boccuzzi, G. (1999): Dehydroepiandrosterone protects tissues of streptozotocin-treated rats against oxidative stress. Free Radic Biol Med 26: 1467–1474.

Atack, J.R.; Perry, E.K.; Bonham, J.R.; Perry, R.H.; Tomlinson, B.E.; Blessed, G. & Fairbairn, A. (1983): Molecular forms of acetylcholinesterase in senile dementia of Alzheimer type: Selective loss of intermediate (10S) forms. Neurosci. Lett., 40:199–204.

Bandopadhyay, D.& Chattopadhyay, A.(2006):Reactive oxygen species- induced gastric ulceration: Protection by melatonin. Current Medicinal Chemistry; 1999;13:1187-1202.

Benitez-King, G,;Tunez, I. &Bellon, A.(2003):Melatonin prevents cytoskeletal alterations and oxidative stress induced by okadaic acid in NIE-115 cells. Experimental neurology ;;182:151-159.

Cardinali,D.(2007): NeurociencialAplicada Edit Med Panamericana BsAs Argentina;368-381.

Chainy, G.B.N.; Samanta, L. & Rout, N.B. (1996): Effect of aluminium on superoxide dismutase, catalase and lipid peroxidation of rat liver. Res. Commun. Mol. Pathol. Pharmacol., 94:217–20.

Charalampopoulos, I.(2006): Alexaki VI, Lazaridis I, Dermitzaki E, Avlonitis N, Tsatsanis C, Calogeropoulou T, Margioris AN, Castanas E, Gravanis A, G-protein-associated, specific membrane binding sites mediate the neuroprotective effect of dehydroepiandrosterone. FASEB J 20: 577–579.

Christen, Y. (2000): Oxidative stress and Alzheimer disease. Am. J. Clin. Nutr., 71: 6215- 6295.

Di, J.; Yao, K.;Han, W.& Bi, S; (2006): Study on the interaction of copper zinc superoxide dismutase with aluminum ions by electrochemical and fluorescent method. Spectrochim Acta Mol Biomol Spectr 65: 896– 900.

Di. J.;Zhang ,M.; Yao, K.;& Bi, S. (2006): Direct voltammetery of catalase immobilized on silica sol-gel and cysteine modified gold electrode and its application. Biosen Bioelect 22: 247–252.

Drago, D.; Bettella, M.; Bolognin, S.; Cendron, L.; Scancar, J.; Milacic, R.; Richelli, R.; Casini, A.; Messori, L.;Tagnon, G. & Zatta, P. (2008): Potential pathogenic role of betaamyloid(1-42)-aluminum complex in Alzheimer's disease. The International Journal of Biochemistry & Cell Biology, Vol. 40, No. 4, pp. 731-746

Dua, R. & Gill, K.D. (2001): Aluminum phosphide exposure: implications on rat brain lipid peroxidation and antioxidant defence system. Pharmacol. Toxicol., 89:315–329.

El–Rigal ,N.S.; Aly, S.A.; Rizk, M.Z.& Said,A.A. (2006): Effect of Ailanthus altissima and Ziziphus spina Christi extracts on some hepatic marker enzymes and antioxidants in Shistosoma mansoni infected mice. Pol J Food Nutr Sci 15/56: 199–206.

El–Rigal ,N.S.; Aly, S.A.;, Rizk, M.Z.& Said,A.A. (2006) :Effect of Ailanthus altissima and Ziziphus spina Christi extracts on some hepatic marker enzymes and antioxidants in Shistosoma mansoni infected mice. Pol J Food Nutr Sci 15/56: 199–206.

Esparza ,J.; Gómez, M.; Romeu, M.; Mulero, M.; Sánchez, D.;Mallol, J. & Domingo ,J.(2003): Aluminum-induced pro-oxidant effects in rats: protective role of exogenous melatonin. Journal of Pineal Research; 35 (1) : 32–39.

Exley, C. (2004): The pro-oxidant activity of aluminum. Free Rad. Biol. Med., 36: 3387.

Flora, S.J.S.; Mehta, A.; Satsangi, K.; Kannan, G.M. and Gupta, M. (2003): Aluminum induced oxidative stress in rat brain: response to combined administration of citric acid and HEDTA.Comp. Biochem. Physiol., Part C, 134:319–28.

Gao, J.; Sun, H.Y.; Zhu, Z.R.; Ding, Z.& Zhu, L. (2005): Antioxidant effects of dehydroepiandrosterone are related to upregulation of thioredoxinin SH-SY5Y cells. Acta. Biochim. Biophys Sin 37: 119–125.

Garrel ,C.; Lafond, J.L.; Guiraud, P.; Faure,P.& Favier ,A. (1994): Induction of production of nitric oxide in microglial cells by insoluble form of aluminum. Ann New York Acad. Sci 738: 455–461.

Gomóez, M.; Esparza, J.L.; Nogués, M.R.; Giralt, M.; Cabré, M. & Domingo, J.L. (2005): Prooxidant

Guix ,F.X.; Uribesalgo ,I.; Coma, M.& Munoz, F.J. (2005) :The physiology and pathophysiology of nitric oxide in the brain. Progress in Neurobiol 76: 126–152.

Johnson, V.J.; Kim, S.H. & Sharma, R.P. (2005): Aluminum-maltolate induces apoptosis and necrosis in neuro-2a cells: Potential role for p53 signaling. Toxicol. Sci., 83: 329-339.

Julka, D. & Gill, K.D. (1996): Altered calcium homeostasis: a possible mechanism of aluminium-induced neurotoxicity. Biochim. Biophys. Acta., 1315: 47–54.

Jyoti, A.; Sethi, P. & Sharma, D. (2007): Bacopa monniera prevents from aluminium neurotoxicity in the cerebral cortex of rat brain. J. Ethnopharmacol., 111:56–62.

Karbownick ,M.& Reiter, R.J.(2000):Antioxidative effects of melatonin in protection against cellular damage caused by ionizing radiation. Proc Soc Exp Biol Med;;225:9-22.

Kilic ,E .; Killic, U.; Yulug, B. (2004): .Melatonin reduces disseminate neuronal death after mild focal ischemia in mice via inhibition of Caspase- 3 and its suitable as and add on treatment to tissue plasminogen activator. Journal of Pineal Research;36:171-176.

Kontush, A.; Berndt, C.; Weber, W.; Akopyan, V.; Arlt, S.; Schippling, S. & Beisiegel, U. (2001): Amyloid-beta is an antioxidant for lipoproteins in cerebrospi- nal fluid and plasma. Free Radical Biology & Medicine, Vol. 30, No.1, pp. 119-128

Kumar, P.; Taha, A.; Sharma, D.; Kale, R.K. & Baquer, N.Z. (2008): Effect of dehydroepiandrosterone (DHEA) on monoamine oxidase activity, lipid peroxidation and lipofuscin accumulation in aging rat brain regions. Biogerontol., 4: 283–284

Kumar, V.& Gill, K.D. (2009). Aluminium neurotoxicity: neurobehavioural and oxidative aspects. Archives of Toxicology, Vol. 83, No. 11, (Nov. 2009), pp. 730-735

Kumar, V.; Bal, A. & Gill, K.D. (2009): Susceptibility of mitochondrial superoxide dismutase to aluminium induced oxidative damage. Toxicol., 255: 117–123.

Kumar, V.; Bal, A. & Gill, K.D. (2009): Susceptibility of mitochondrial superoxide dismutase to aluminium induced oxidative damage. Toxicol., 255: 117–123.

Kurata, K.; Takebayashi, M.; Morinobu, S.& Yamawaki, S.; (2004): Beta-estradiol, dehydroepiandrosterone, and dehydroepiandrosterone sulfate protect against N-methyl-D-aspartate-induced neurotoxicity in rat hippocampal neurons by different mechanisms. J Pharmacol Exp Ther 311: 237–245.

LaFerla, F. M.; Green, K. N.& Oddo, S. (2007): Intracellular amyloid-β in Alzheimer's Disease. Nature Reviews Neuroscience,. 7(8):499-509

Li, H.; Campbell, A.; Ali, S.F.; Cong, P.& Bondy, S.C. (2008): Chronic exposure to low levels of aluminum alters cerebral cell signaling in response to acute MPTP treatment. Toxicology and Industrial Health, Vol. 23, No. 2, (Jan. 2008), pp. 515-524.

Li, W.; Ota, K.; Nakamura, J.; Naruse, K.; Nakashima, E.; Oisa ,Y.&Hamada,Y. (2008): Antiglycation effect of gliclazide on in vitro AGE formation from glucose and methylglyoxal. Exp Biol Med 23: 176–179.

Marklund, S.L.; Westman, N.G.; Lundgren, E.& Roos, G. (1982) :Copper– and zinc-containing superoxide dismutase, manganese-containing superoxide dismutase, catalase, and glutathione peroxidase in normal and neoplastic human cell lines and normal human tissues. Cancer Res 42: 1955–1961.

Mohan ,P.F.&Jacobson ,M.S. (1993) :Inhibition of macrophage superoxide generation by dehydroepiandrosterone. Am J Med. Sci 306: 10–15.

Munoz–Castaneda ,J.R.; Muntane ,J.; Munoz, M.C.; Bujalance ,I.; Montilla, P.& Tunez ,I. (2006) :Estradiol and catecholestrogens protect against adriamycin- induced oxidative stress in erythrocytes of ovariectomized rats. Toxicol Lett 160: 196–203.

Nagatsu ,T.; Mogi, M.; Ichinose, H.& Togari, A. (2000): Changes in cytokines and neurotrophins in Parkinson's disease. J Neural Transm 20: 277– 290.

National Research Council (NRC). Guide for the Care and Use of Laboratory Animals: 8th Ed, 2011. [cited 2013 Apr 3]. Available from: https://grants.nih. gov/grants/olaw/Guide-for-the-Careand-use-of- laboratory-animals.pdf.

Nayak, P. (2002): Aluminum: Impacts and Disease. Environmental Research, 89(2):101-115.

Nehru, B. & Anand, P. (2005): Oxidative damage following chronic aluminium exposure in adult and pup rat brains. J. Tra. Elem. Med. Biol. 19:203–208

Qiu, W.O.& Folstein. M. (2006): Insulin, insulin-degrading enzyme and amyloid-β peptide in Alzheimer's disease: review and hypothesis. Neurobiology of Aging,27(2):190–198

Rauk, A. (2009): The chemistry of Alzheimer's disease (AD). Chemical Society Reviews,38(9):2698-2715

Reiter, R.J.&Tan, D.X.(2003): Melatonin a novel protective agent against oxidative injury of the ischemic/reperfused heart. Cardiovascular Research ;;58:10-19.

Rhodes ME, Li PK, Flood JF, Johnson DA (1996) Enhancement of hippocampal acetylcholine release by the neurosteroid dehydroepiandrosterone sulfate: an in vivo microdialysis study. Brain Res 733: 284–286

Rodriguez, C.;Mayo, J.C.;Sainz, R.M.(2000): Regulation of anti-oxidant enzymes: a significant for melatonin. Journal of Pineal Research;;436:1-9.

Rodriguez, C.;Mayo, J.C.;Sainz, R.M.(2000): Regulation of anti-oxidant enzymes: a significant for melatonin. Journal of Pineal Research; 436:1-9.

Satoh ,K. (1978): Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta 90: 37–43.

Savory, J.; Herman, M.M. & Ghribi, O. (2003): Intracellular mechanisms underlying aluminuminduced apoptosis in rabbit brain. J. Inorg. Biochem., 97: 151-154.

Schwartz, A.G.; Whitcomb, J.M.; Nyce, J.W.; Lewbart, M.L.& Pashko, L.L; (1988): Dehydroepiandrosterone and structural analogs: a new class of cancer chemopreventive agents. Adv Cancer Res 51: 391–425.

Sethi, P.;Jyoti, A.; Singh, R.; Hussain, E.& Sharma, D. (2008): Aluminium-induced electrophysiological, biochemical and cognitive modifications in the hippocampus of aging rats. NeuroToxicology, Vol. 29, No.2, pp. 1069-1079

Sethi, P.A.; Jyoti, E. Hussain, & Sharma, D. (2009): Curcumin attenuates aluminium-induced functional neurotoxicity in rats. Pharmacology, Biochemistry and Behavior 93: 31–39.

Su, B.;Wang, X.; Nunomura, A.;Moreira, P.I.; Lee, H.-gon, Perry, G.; Smith, M.A. & Zhu., X. (2008): Oxidative Stress Signaling in Alzheimer's Disease. Current Alzheimer Research, Vol. 5, No. 6, pp.525-532, ISSN 1567-2050.

Sushma, J.N.; Priyanka, M. & Rao, K. (2011): Neuroprotective role of Melatonin against aluminum-induced oxidative stress in the hippocampus of mouse brain. Journal of Applied Pharmaceutical Science 01 (10); 2011: 126-133

Tabuchi ,A.; Sakaya, H.;Kisukeda ,T.; Fushiki, H.&Tsuda, M. (2002) Involvement of an upstream stimulatory factor as well as cAMP responsive element binding protein in the activation of brain derived neurotrophic factor gene promoter. J. Biol Chem 277: 35920–35931.

Takuma ,K.;Matsuo, A.; Himeno, Y.; Hoshina, Y.; Ohno, Y.; Funatsu, Y.; Arai ,S.; Kamei ,H.;Mizoguchi ,H.;Nagai, T.; Koike, K.;Inoue, M.& Yamada,L.(2007):One molecule ,many derivatives:a never-ending interaction of melatonin with reactive oxygen and nitrogen species? Journal of Pineal Research;42(1),28-42.

Tsakiris ,S.;Schulpis, K.H.; Marinou, K.& Behrakis ,P. (2004): Protective effect of L-cysteine and glutathione on the modulated suckling rat brain Na+,K+-ATPase and Mg2+-ATPase activities induced by the in vitro galactosaemia. Pharmacol Res 49 : 475–479.

Tuneva ,J.; Chittur ,S.; Boldyrev, A.A.; Birman, I.& Carpenter, D.O. (2006): Cerebellar granule cell death induced by aluminum. Neurotoxicol Res9: 297–304.

Wu, D. & Cederbaum, I. (2003): Alcohol, Oxidative Stress, and Free Radical Damage. Alcohol Res. Health, 27: 277-284.

Wu, Z.; Du, Y.; Xue, H.; Wu, Y. & Zhou, B. (2010): Aluminum induces neurodegeneration and its toxicity arises from increased iron accumulation and reactive oxygen species (ROS) production. Neurobiol of Aging. Jul 29.

Xu ,J.; Chen ,S.; Ahmed, S.H.; Chen, H, Ku ,G.;Goldberg, M.P.& Hsu, C.Y. (2001): Amyloid-beta peptides are cytotoxic to oligodendrocytes. J. Neuroscience, 21(1):1-6.

Yildirim ,A.; Gumus, M.; Dalga ,S.;Sahin ,Y.N.& Akcay, F. (2003) : Dehydroepiandrosterone improves hepatic antioxidant systems after renal ischemia–reperfusion injury in rabbits. Ann Clin Lab Sci 33: 459–464.

Yousef, M.I. (2004): Aluminum induced changes in hemato-biochemical parameters, lipid peroxidation and enzyme activities of male rabbits: protective role of ascorbic acid. Toxicol., 199:47–57.

Yousif ,M.F.& El-Rigal, N.S. (2004) :C-glycolsyl flavones O-glycosides of clerodendrum splendens G.Don. and antioxidants activity in schistosome–infected mice. Egypt J Biomed Sci 14: 128–137

Zheng ,P. (2009) :Neuroactive steroid regulation of neurotransmitter release in the CNS: action, mechanism and possible significance. Progr Neurobiol 89: 134-152.