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*Chapter 3*

# THE RISK OF ALUMINIUM NEUROTOXICITY FOR YOUNG ANIMALS AND HUMANS DUE TO MULTIPLE EXPOSURE OPPORTUNITIES, PARTICULARLY PERINATAL

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## ABSTRACT

Aluminium (Al) is a well-known neurotoxicant in adults as well as in developing stages of animals and humans. Al is reported in the etiology of several neurodegenerative disorders like Parkinson's disease, dementia, and Alzheimer's disease. Besides multiple exposure opportunities for early exposure of the fetus to Al, the route or mode of exposure is also critical for the developing offspring. Al-induced behavioral alterations as well as cognitive deficits and rodent brain neurotransmitters level, have been widely reported in literature but exact mechanism in the offspring of perinatally Al exposed dams is not yet understood properly and needs more attention. In the present study, the perinatal oral exposure of the dams to 300 and 600 mg/kg Al (aluminium chloride) resulted in a significant and dose-dependent reduction in postnatal body weight gain, delays in opening of the eyes and appearance in the body hair fuzz, and deficits in the sensory motor reflexes of the mice pups during weaning period (from the day of birth to postnatal day 21). At adolescent and adult ages of the male offspring, a significant and dose-dependent deficit was also observed in their learning capability (at postnatal day-

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PD25), and cognitive behavior (at PD30-36). Furthermore, a significant and dose-dependent disturbance in the levels of neurotransmitters like dopamine (DA) and serotonin (5-HT); non enzymatic oxidative stress (OS) indices like thiobarbituric acid-ractive substances (TBARS) and total reduced glutathione (GSH); and enzymatic OS indices like glutathione S-transferase (GST), catalase (CAT), and superoxide dismutase (SOD) were observed in the forebrain region of the offspring at post-natal day (PD)7, PD14, PD21, PD30, and PD36. Thus, perinatal Al exposure can affect the in utero developing fetus, raising the concerns that during a critical prenatal and postnatal period of brain development, Al exposure has potential neurotoxic hazards and might modify the properties of the dopaminergic system and inflicts OS that changes the threshold of that system or other related systems resulting into a longer lasting cognitive dysfunction. A reduced use of Al during pregnancy is of crucial importance in preventing Al-induced neurotoxicity in the offspring.

**Keywords:** perinatal, aluminium, mice offspring, neurotoxicity, cognitive dysfunction, brain, neurotransmitters, oxidative stress

## INTRODUCTION

Aluminum (Al) neurotoxicity is well known in adults (Golub and Germann, 2001) as well as in developing stages of altricial animals like rats and mice (Domingo, 1995; Golub and Domingo, 1996). Early exposure to Al in life may lead to increased functional impairments and potential developmental neurotoxicities (Reuhl, 1991). Al has been linked with the etiology of several neurodegenerative disorders like Parkinson's disease (Yasui et al., 1992; Sanchez-Iglesias et al., 2009), dementia (Forbes et al., 1995), and Alzheimer's disease (Kawahara, 2005; Mahdi et al., 2006), however, linkages to the findings outlined above do not convincingly demonstrate a causal relationship between Al and neurodegenerative diseases (Massey and Taylor, 1989; Exley, 2001). It is argued that not the increased exposure to Al alone but the elevated level of Al may be the factor in initiating and aggravating the pathogenesis of neurodegenerative diseases (Sethi et al., 2008), but still it is not generally accepted that Al is a contributory factor in the etiology of such neurodegenerative diseases because the precise mechanism of such disease pathogenesis remains unknown, and still this issue is controversial (Kawahara and Kato-Negishi, 2011).

Although multiple exposure opportunities lie for early exposure of the fetus to Al, which may include prenatal, postnatal and perinatal exposures, perinatal period is critically most important from the brain development point of view. Perinatal involves prenatal as well as postnatal periods and a major portion of brain cells (70%) of rodents are known to form postnatally after birth (Patel, 1983). The brain is usually susceptible to disruption by drugs during its development, with drugs potentially affecting a variety of developmental processes, including neuronal migration and differentiation, glia proliferation, along with normal ontogenetic changes in cell adhesion, neural communications, energy utilization, and apoptosis (Goodlett and Horn, 2001). Different portions of the brain develop at different time and hence differ in the timing of their vulnerability to drug-induced disruptions. Thus, consequences of developmental exposures to drugs are to some extent dependent on the timings of the exposure. Perinatal period in rodents is approximately equivalent to the first and second trimesters of human pregnancy, with the first 10 days or so of postnatal life in

rats, approximating the third human trimester (Spear and File, 1996). It is for this reason that we consider here to discuss the deleterious neurotoxic effects of Al in mice offspring that are exposed to Al during perinatal period.

Besides exposure opportunities, the routes or modes of Al exposure also affect the developmental neurotoxicity and many of such perinatal studies have been carried out by exposing the animals through Al injection during early pregnancy; in spite of the fact that oral administration appears to be more readily common in human populations. Furthermore, orally ingested Al consumed through foods, water, freshly-prepared natural sources (mainly fresh fruit, vegetables and meat), and as additive in commercially-processed foods and beverages (Greger, 1993; Walton, 2007), currently comprises the main form of Al exposure for the general public (WHO, 2006). Also, Al gets an easy access to our body through use of cooking utensils, deodorants, antacids, etc. (Yokel, 2000).

Although long-term effects of developmental exposure (perinatal) has not been studied much in this regard in human populations, a recent study detected a delay in neuromaturation in preterm infants exposed to high levels of Al through parenteral fluids (Bishop et al., 1997). Behavioral effects of developmental (perinatal) exposure to Al have been examined to some extent using laboratory rodents (Colomina et al., 1999; Gonda and Lehotzky, 1996; Gonda et al., 1997; Santucci et al., 1994; Abu-Taweel et al., 2012). Maternal stress during pregnancy can have serious negative effects on the learning abilities of the offspring (Nishio et al., 2001; Sternberg and Ridgway, 2003). Exposure to Al during pregnancy along with maternal stress can further have serious interactions and result into learning defects in the offspring (Colomina et al., 2005; Roig et al., 2006). Al has been demonstrated to be capable not only of crossing the blood brain barrier (BBB), but also of increasing its permeability (Zatta et al., 2002). It is well established that Al is toxic to the growth and development of fetuses and suckling in experimental animals (Domingo, 1995; Sharma and Mishra, 2006). Moreover, it has been shown that gestational and lactational exposure to doses of Al that do not produce maternal toxicity can result in persistent neurobehavioral deficits in the offspring of some mammals (Colomina et al., 2005; Abu-Taweel et al., 2012). These Al doses may not correspond to the level of exposure in humans, but its toxic effects reported in animal studies have a significant relevance to Al exposure in humans (Abu-Taweel et al., 2012). Although Al induced behavioral alterations as well as cognitive deficits have been reported in literature, the effects in the offspring of perinatally exposed Al dams are not yet understood properly and need more attention in order to gain information on the longer lasting effects of Al transferred from dams to offspring. Furthermore, Al induced changes in the rodent brain neurotransmitters studies are scanty (Kumar, 2002). Al administration has been reported to induce oxidative stress (OS) by inflicting damage to membrane lipid, proteins and antioxidative enzyme defense system (Jyoti et al., 2007). Furthermore, Al has been associated with the OS in brain of adult rodents which in turn has been linked with neurodegenerative diseases and cognitive dysfunctions (Mahdi et al., 2006; Sanchez-Iglesias et al., 2009; Sumathi et al., 2013). Although OS and neuron alterations in brain tissue of neonatal rats have been reported by direct exposure of the pups to Al (Yuan et al., 2011, 2012), information on such OS in developing brain of the pups whose mothers have been exposed to Al during gestation and lactation periods is lacking.

Thus, the effect of perinatal oral Al-administration to pregnant mice needs to be assessed in the offspring at neurobehavioral, cognitive and biochemical levels (brain neurotransmitters and OS indices) to investigate the possible long term physiological mechanism associated

with Al toxicity related with cognitive dysfunction in the offspring. All data on maternal effects itself, have been excluded from this chapter, however; no effects were noticed on the dams.

## MATERIALS AND METHODS

### Animals

Male and female Swiss–Webster strain mice (10–12 weeks old) were housed in opaque plastic cages (three females to one male in each cage) measuring 30×12×11 cm, in the animal facility of the Zoology Department, King Saud University, Riyadh, Saudi Arabia.

Animals were kept under reversed lighting conditions with white lights on from 22.30 to 10.30 h local time. The ambient temperature was regulated between 18 and 22°C. After pregnancy (appearance of vaginal plug was considered as day one of pregnancy), the males were removed from the cages and the females were subjected to experimental treatments. Food (Pilsbury's Diet) and water were available ad libitum, unless otherwise indicated.

### Experimental Design for Al Treatments

All pregnant mice were divided into 3 groups. The first group served as the control group and received plain tap water only, whereas the second and third groups were exposed to 300 and 600 mg/kg aluminum chloride (Al) per day respectively, dissolved in plain tap water, through oral administration. Our pilot studies have shown that the normal and/or pregnant mice on an average consume 30 ml water per day and thus the Al doses were prepared accordingly. These Al doses formed the sole drinking fluid source for the experimental group of pregnant mice during the required period of the experiment. It is likely that the present high concentrations of Al used in the drinking water might have affected total fluid intake due to its astringent property, but no efforts were made to assess any such differences in total fluid intake specifically. Fresh Al doses were replaced in the drinking bottle every day. The factor for the possibility of presence of Al traces in food and tap water was not taken into account for calculating the daily Al intake. However, this factor was minimized by giving the same source of food and tap water to all experimental groups including the controls. All pregnant mice were housed individually. Treatment started from day 1 of pregnancy, and was continued until postnatal day 15 (PD15) and thereafter the mothers were switched to plain tap water. The pups of each experimental group were culled to only eight per dam on the day of birth (PD0) and were left with their mothers until PD22. During this weaning period, three male pups of each litter were color marked from the others, and were subjected to various behavioral tests (described below) under dim lighting (ca 8 lux). In all, 21 pups belonging to seven litters from each treatment category were considered. All observations were recorded on PD1 and repeated every other day until PD21 in the same cohort of three color marked male pups of each litter. These observations were used to measure the early development of sensory motor coordination reflexes together with morphological development in the pups. For statistical analysis, the mean of all three cohorts (color marked pups) per litter was

considered as a single score. Thus, seven replicates from each treatment category were considered for the following observations.

### *Physical Assessment during Weaning Period*

Physical developmental landmarks like body weight, opening of the eyes and appearance of body hair fuzz, were evaluated in the developing offspring starting from day 1 after birth (PD1) through the entire weaning period until PD21.

#### Body Weight

Weight is a useful indicator of development. Thus, the pups were weighed every alternate day from PD1 until PD21.

#### Eye Opening and Hair Appearance

The day at which the body hair fuzz appeared, and the eyes opened was also recorded. These two parameters are also useful morphological indicators of development.

### *Neuromotor Maturation Assessment during Weaning Period*

#### Righting Reflex

The time taken by a pup placed on its back to turn over and place all four paws on the substrate was recorded. An upper limit of 2 min was set for this test.

#### Cliff Avoidance

Pups were placed on the edge of a table top with the forepaws and face over the edge. The time taken by the pup to back away and turn from the "cliff" was recorded. Again an upper limit of 2 min was chosen. A latency of 2 min was attributed when the animal fell from the "cliff".

#### Rotating Reflex

The surface used to measure the rotating reflex was the same as that used for righting reflex, except that it was inclined at an angle of 30°. The pups were placed on this surface with their heads pointed downward. The time elapsed until the pup rotated its body through 180° geonegatively and faced its head upward, was recorded as the rotating time. The upper limit of this test was also set at 2 min.

### *Cognitive Behavioral Assessment*

The following tests were evaluated in the same cohort of male offspring (bearing in mind to include representatives of each litter) in all the behavioral tests.

#### Active Avoidance Responses

The active avoidance responses were measured in the animals at PD 25, using an automatic reflex conditioner "shuttle box" (Ugo Basile, Comerio, Varese, Italy). The rectangular shaped shuttle-box was divided into two chambers of equal size by a stainless steel partition with a gate providing access to the adjacent compartment. Before starting the trial sessions, each animal was allowed to adapt and acquaint itself with the shuttle box for 2

min without any stimulus. A 6 s duration light (21W) and buzzer (670 Hz and 70 dB) were switched on consecutively and used as a conditioned stimulus (CS). The CS preceded the onset of the unconditioned stimulus (US) by 5 s. The US was an electric scrambler shock (1 mA for 4 s) applied to the grid floor. If the animal avoided the US by running into the other compartment within 5 s after the onset of the CS, the microprocessor recorder unit of the shuttle box recorded an avoidance response and this was considered as conditioned avoidance response to avoid the electric shock. Each animal was given 50 trials with a fixed intertrial interval of 15 s. During the 50 trial session of the individual animal, the total number of avoidance was measured. The total time taken until the animal entered the other compartment to avoid the shock treatment (latency of avoidance response or escape latency in seconds) was also measured for each animal and the results were expressed for each group of animals. The spontaneous migration of the mouse to the other compartment between trials was also assessed by measuring the number of crossings between the chambers when no shock was present during UCS and CS (inter-trial crossing). The recorder unit of the automated shuttle box continuously recorded these parameters during the whole experimental period (50 trials) of each animal.

**Morris Water-Maze Test**

The test has been extensively used to assess cognitive functions in rat (Rutten et al., 2002; Tariq et al., 2008) and mice (Lamberty and Gower, 1991a, 1991b) models. Starting at the age of PD30, the mice offspring were tested for visual–spatial memory using a water-maze (Morris, 1984). The water-maze consisted of a galvanized white circular water tank (90 cm diameter, 50 cm height) filled with clear tap water (22 ± 1°C) to a depth of 15 cm. A 6×6 cm size, stainless steel, adjustable, white, escape platform was placed 1 cm below the water level and 13 cm from the rim. The water was made opaque by addition of 1 l of milk, which prevented visualization of the platform. Four points on the rim of the tank were designated north (N), south (S), east (E) and west (W), thus dividing the pool into four quadrants (NW, NE, SE and SW). On the first day, each offspring (P30) was allowed to swim freely in the pool for 60 s without the platform present in the pool. This free swim enabled the mice to become habituated to the training environment. On days 2–5, offspring (P31 to P35, respectively) were trained for 24 trials (six trials a day, with an inter-trial interval of 30 s) to locate and escape onto the submerged platform. At the start of each trial, the mouse was held facing the perimeter of the water tank and dropped into the pool to ensure immersion. The latency from immersion into the pool to escape onto the hidden platform (maximum duration of trial 120 s) was recorded. If the mouse did not find the platform in 120 s, it was manually guided with the help of a glass rod to mount on the platform and a score of 120 s was recorded for each of such experimenter-terminated trials. The number of such unsuccessful trials was counted and expressed as a percentage of failures on each testing day. On mounting the platform, each mouse was given a 30 s inter-trial interval for rest and for learning and memorizing the spatial cues to reach the platform for escape. To minimize handling, at the end of the trials, the animals were allowed to climb onto a wire mesh grid and transferred to their cage without further handling.

On day 6, P 36 mice were subjected to a 120 s probe trial in which the platform was removed from the pool. The time spent in each quadrant (within 120 s probe test time) was recorded on an electronic time recorder. In this part of probe trials in water-maze test, normal animals typically spend more time in the quadrant where the platform had been previously

located than in the other quadrants. The testing procedures used during the four days of locating the hidden platform provide a measure of hippocampal-dependent spatial reference memory, while the probe trial is a measure of the strength of spatial learning, the closest parallel to episodic memory in humans (Jeltsch et al., 2001; Spiers et al., 2001).

### *Biochemical Studies*

For biochemical studies, a sub-set of developing offspring (n = 8/group) were sacrificed at different ages (PD7, 14, 21, 30 and 36) by decapitation and their brains were dissected on ice. The fore-brain was isolated (including the cerebral areas with hippocampus and striatum) and frozen in liquid nitrogen and stored at −70°C for neurotransmitters and OS indices estimations.

### *Determination of Monoamines*

The monoamines, dopamine (DA) and serotonin or 5-hydroxytryptamine (5-HT) were estimated using the modified method of Patrick et al. (1991). A 10% homogenate of the fore-brain was prepared by homogenizing the tissues for 10 s in 0.1 M HClO4 containing 0.05% EDTA, centrifuged at 17,000 rpm at 4°C for 5 min. The supernatants were filtered using 0.45 μm pore filters and analyzed by high performance liquid chromatography (HPLC). The mobile phase consisted of 32 mM citric acid monohydrate, 12.5 mM disodium hydrogen orthophosphate, 7% methanol, 1 mM octane sulfonic acid and 0.05 mM EDTA. The mobile phase was filtered through 0.22 μm filter and degassed under vacuum before use. μBondpak C18 column was used at a flow rate of 1.2 ml/min and the injection volume of the sample was 20 μl. The levels of DA and 5-HT were calculated using a calibration curve and results were expressed as ng/mg tissue weight.

### *Determination of Nonenzymatic OS Indices*

#### Lipid Peroxides

Lipid peroxides (LP) were determined spectrophotometrically as thiobarbituric acid-reactive substances (TBARS) according to the method of Ohkawa et al. (1979). Tissue lipid peroxide levels were quantified using extinction coefficient of $1.56 \times 10^5$ m−1 cm−1 and expressed as nanomoles of TBARS formed per g tissue weight. The results are expressed as nmol/g wet weight.

#### Glutathione

Reduced glutathione (GSH) level was measured enzymatically in the brain tissue by a slightly modified method (Mangino et al., 1991). The slope of the change in absorbance was used to quantitate total GSH by comparing the slope of the samples with a standard curve prepared with pure glutathione (Sigma). The specific activity is expressed into umol/g tissue weight.

### *Determination of Enzymatic OS Indices*

#### Glutathione-S-Transferase

Glutathione S-transferase (GST) was estimated by the method of Habig et al. (1974) by using 1-chloro-2,4-dinitrochlorobenzene (CDNB) as substrate at 340 nm. The GST activity is expressed as U/g tissue weight.

#### Catalase

Catalase (CAT) activity was measured by the method of Aebi (1972), by tracking the decomposition of hydrogen peroxide by measuring decrease in extinction of $H_2O_2$ at 240 nm. The activity of CAT is expressed as rate constant of first order reaction K per gram tissue weight.

#### Superoxide Dismutase

Superoxide dismutase (SOD) activity was estimated by the method of Misra and Fridovich (1972). Activity is expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50% which is equal to U per gram tissue weight.

### *Statistical Analysis*

All data were analyzed by using the Student–Newman–Keuls multiple comparison test of ANOVA by the INSTAT computer program. Significant effects in locomotory test were further evaluated using Dunn's multiple comparison tests.

## RESULTS

### Physical Assessment during Weaning Period

Physical developmental landmarks were evaluated in the developing offspring starting from the day 1 after birth (PD1) through entire weaning period unto PD21. Although the body weights of pups born to Al-treated mice lagged behind the control group on PD1, the difference was statistically insignificant. However, the Al exposed offspring at both doses, showed a significant decline ($p < 0.05$) in their body weight gain as compared to the controls, from PD3 onwards. From PD5 upto the weaning period (PD21), the Al exposed offspring remained lagging behind the controls in body weight very significantly ($p < 0.001$) in a dose-dependent manner (Figure 1).

Other morphological developments such as the opening of the eyes and appearance of body hair fuzz were also significantly ($p < 0.001$) delayed in the Al exposed offspring in a dose-dependent manner (Figure 2).

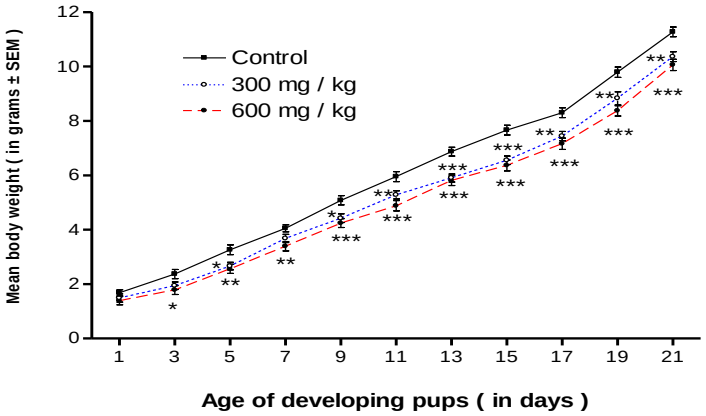


Figure 1. Effect of perinatal aluminium doses (300 and 600 mg/kg body weight) exposure on the body weight gain of mouse pups during the weaning (lactation) period. (*, **and ***) represent statistically significant (*P < 0.05, P < 0.01 and P < 0.001 respectively)* from the control group; see text.
(Taken from: Abu-Taweel et al., 2012 *Pharmacology, Biochemistry and Behavior*, 101: 49 – 56.)

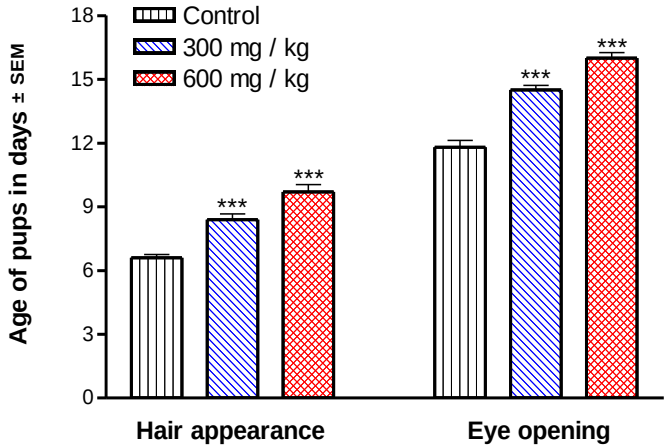


Figure 2. Effect of perinatal aluminium doses (300 and 600 mg/kg body weight) exposure on the body hair appearance and eye opening in the mouse pups. (***) represents statistically significant (*P < 0.001)* from the control group; see text.
(Taken from: Abu-Taweel et al., 2012 *Pharmacology, Biochemistry and Behavior*, 101: 49 – 56.)

## Behavioral Studies

### *Neuromotor Maturation*

The neuromaturation of reflexes during the weaning period of the developing pups was assessed from the day of birth PD1 until PD21. The righting, rotating, and cliff avoidance reflexes in the Al-exposed offspring were found to be significantly and dose-dependently suppressed throughout the weaning period (Figure 3).

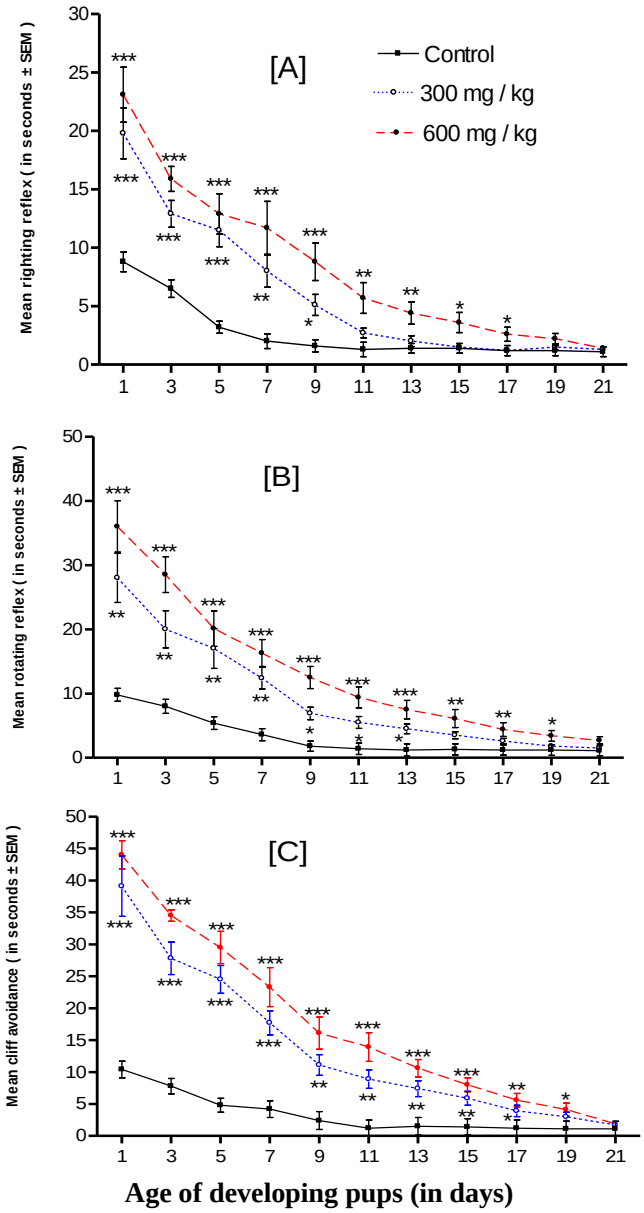


Figure 3 A – C. Effect of perinatal aluminium doses (300 and 600 mg/kg body weight) exposure on the righting reflex (A), rotating reflex (B) and cliff avoidance (C) of mouse pups during the weaning (lactation) period. (*, **and ***) represent statistically significant (*P < 0.05, P < 0.01 and P < 0.001 respectively)* from the control group; see text.
(Taken from: Abu-Taweel et al., 2012 *Pharmacology, Biochemistry and Behavior*, 101: 49 – 56.)

### *Active Avoidance Test*

In the shuttle-box active avoidance test, the Al-exposed offspring (PD25), showed a statistically significant and dose-dependent decrease in the number of avoidances during the trial period as compared to the control group (Figure 4 A). The spontaneous migration of the mouse to the other compartment between trials measuring the number of crossings between the chambers when no shock was present (inter-trial crossing) showed a significant and dose-

dependent decrease in the number of inter-trial crossings as compared to the controls (Figure 4B). The total time taken during the entire trials by the animals to enter the other compartment to avoid the shock treatment (latency of avoidance response in seconds) was also measured for each animal. The results showed that the animals exposed to Al were poor learners in a dose-dependent manner and took significant time in avoiding the shock treatment as compared to the controls (Figure 4 C).

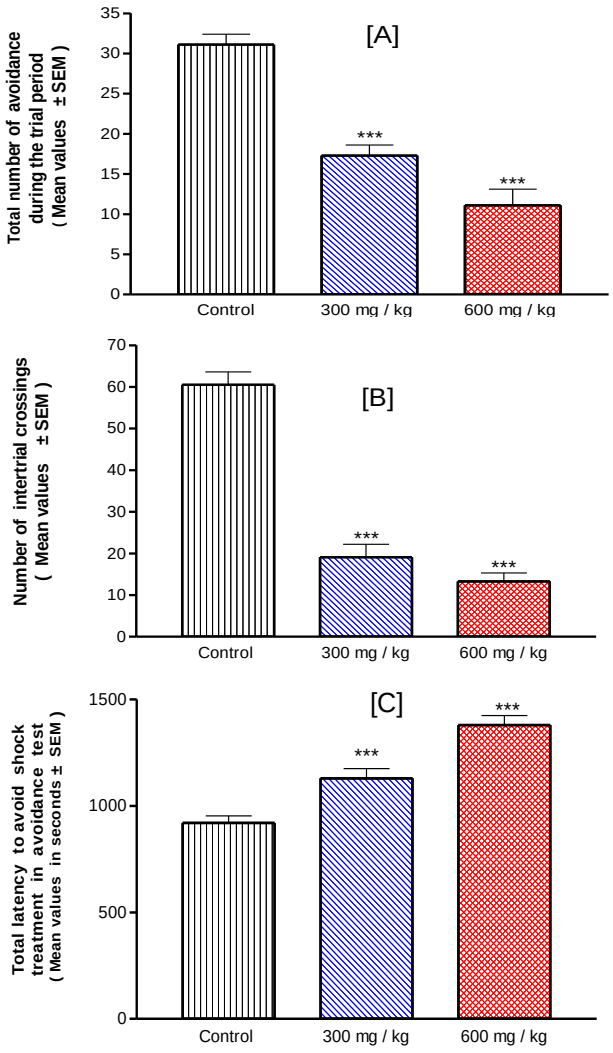


Figure 4 A – C. Effect of perinatal aluminium doses (300 and 600 mg/kg body weight) exposure on the mean performance value of the mice offspring at the age of 25 days postnatal in the active avoidance task. Mice were given a 50 trial session and the total number of times they avoided the shock by moving to the other compartment of the shuttle box (A), the number of inter-trial crossings between the chambers in the absence of current shock (B) and the total time taken by the animals (latency) to avoid the shock (C) were measured. (***) represents statistically significant ($P < 0.001$) from the control group; see text.
(Taken from: Abu-Taweel et al., 2012 *Pharmacology, Biochemistry and Behavior*, 101: 49 – 56.)

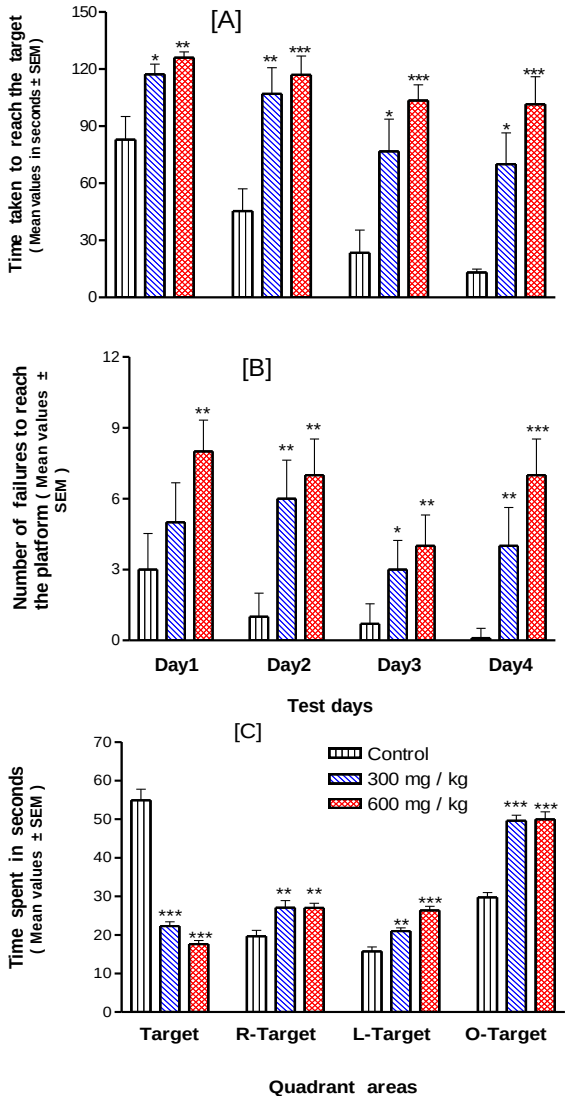


Figure 5 A – C. Performance in Morris water-maze of mice offspring at the age of 30 days postnatal whose mothers were exposed perinatally with aluminium doses of 300 and 600 mg/kg body weight.
A – shows the mean latency to reach the hidden platform (y-axis) on each testing day (x-axis). Animals subjected to aluminium exposure were slower in finding the platform than the controls on all testing days.
B – shows the number of failures or unsuccessful trials (y-axis) on each testing day (x-axis). Aluminium exposed offspring showed maximum number of failures in finding the platform as compared to controls.
C – shows probe test performance in Morris water-maze of mice offspring at the age of 36 days. The aluminium exposed offspring spent less time in the target quadrant than the control group. (*, **and ***) represent statistically significant (*$P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively)* from the control group; see text. R-Target denotes quadrant on the right side of the target quadrant, L-Target denotes the quadrant on the left side of the target quadrant and O-Target represents quadrant opposite to the target quadrant.
(Taken from: Abu-Taweel et al., 2012 *Pharmacology, Biochemistry and Behavior*, 101: 49 – 56.)

### *Morris Water-Maze Task*

Mice offspring with Al treatment, exhibited longer escape latencies to reach the platform as compared with the control group ($p < 0.01$; Figure 5 A), however, all groups displayed a gradual improvement in performance over the 4 days of testing (training) period. The number

of unsuccessful trials (failures) to reach the platform was also significantly higher in Al treated offspring as compared to the control group on all testing days ($p < 0.001$; Figure 5 B).

The probe trial studies showed that Al exposed offspring spent more time in other three quadrants than the target (platform) quadrant as compared to the control group ($p < 0.001$; Figure 5 C), in search of the platform.

## Biochemical Studies

### *Levels of Monoamines in Forebrain*

There was a significant ($p < 0.001$) and dose-dependent inhibition of dopamine level in the forebrain of mice offspring treated with Al as compared to the control group at the ages PD7, PD14, PD21, PD30 and PD36 (Figure 6 A). On the contrary, the level of 5-HT was significantly ($p < .001$) depleted in the offspring at all developing ages, exposed only to higher dose of Al, whereas, the lower dose had no effect at any developing ages (Figure 6 B).

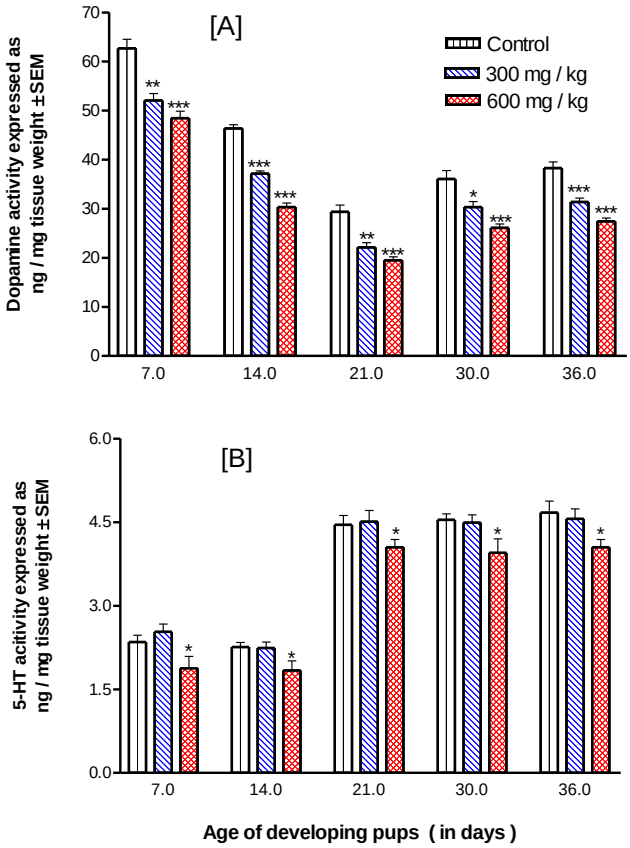


Figure 6 A and B. Effect of perinatal aluminium doses (300 and 600 mg/kg body weight) exposure on the mean levels of dopamine (A) and 5-HT (5-hydroxy-tryptamine or serotonin) (B), in the forebrain of the offspring at various postnatal developing ages (x-axis). (*, **and ***) represent statistically significant (*$P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively)* from the control group; see text.
(Taken from: Abu-Taweel et al., 2012 *Pharmacology, Biochemistry and Behavior*, 101: 49 – 56.)

***Levels of Nonenzymatic OS Indices***

LP determined as TBARS were found to be elevated significantly ($p < 0.001$) due to perinatal Al exposure in the developing forebrain of the offspring throughout the postnatal development period (PD7, PD14 and PD21) and even at adolescent ages PD30 and PD36 in a dose-dependent manner (Figure 7 A). On the contrary, reduced glutathione (GSH) level remained depleted significantly ($p < 0.001$) at all developing ages in a dose-dependent manner (Figure 7 B).

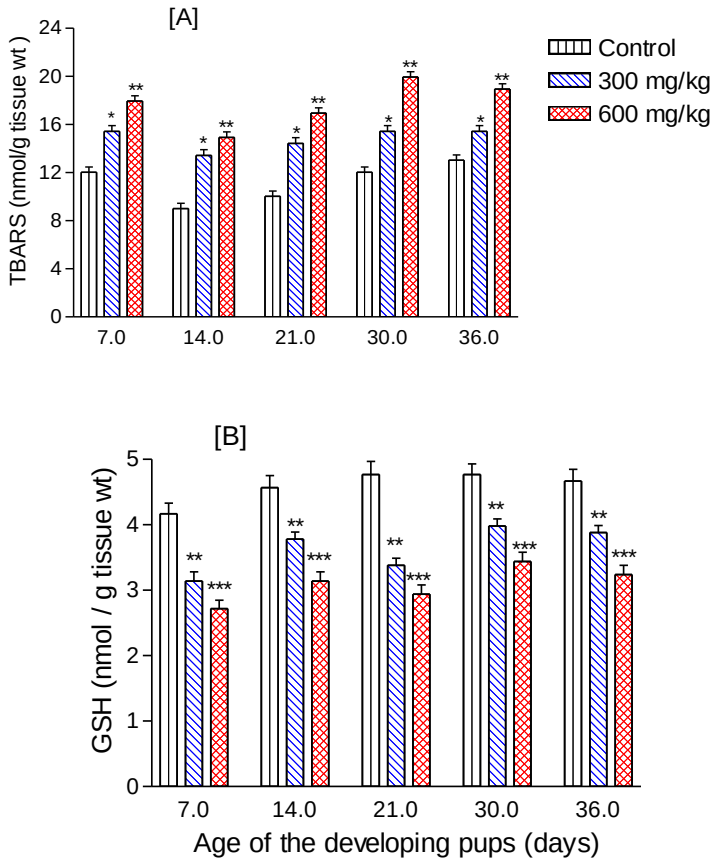


Figure 7 A and B. Effect of perinatal aluminium doses (300 and 600 mg/kg body weight) exposure on the mean levels of nonenzymatic oxidative stress indices like (A) lipid peroxidation content (TBARS), and (B) total glutathione (GSH) level, in the forebrain of the offspring at various postnatal developing ages (x-axis). (*, **and ***) represent statistically significant (*P < 0.05, P < 0.01 and P < 0.001 respectively)* from the control group; see text.

***Levels of Enzymatic OS Indices***

The levels of enzymatic OS indices GST, CAT, and SOD remained significantly ($p < 0.001$) depleted in a dose-dependent manner in the fore-brain of the developing offspring at PD7, PD14, PD21, PD30 and PD36 ages (Figures 8 A, B, and C respectively).

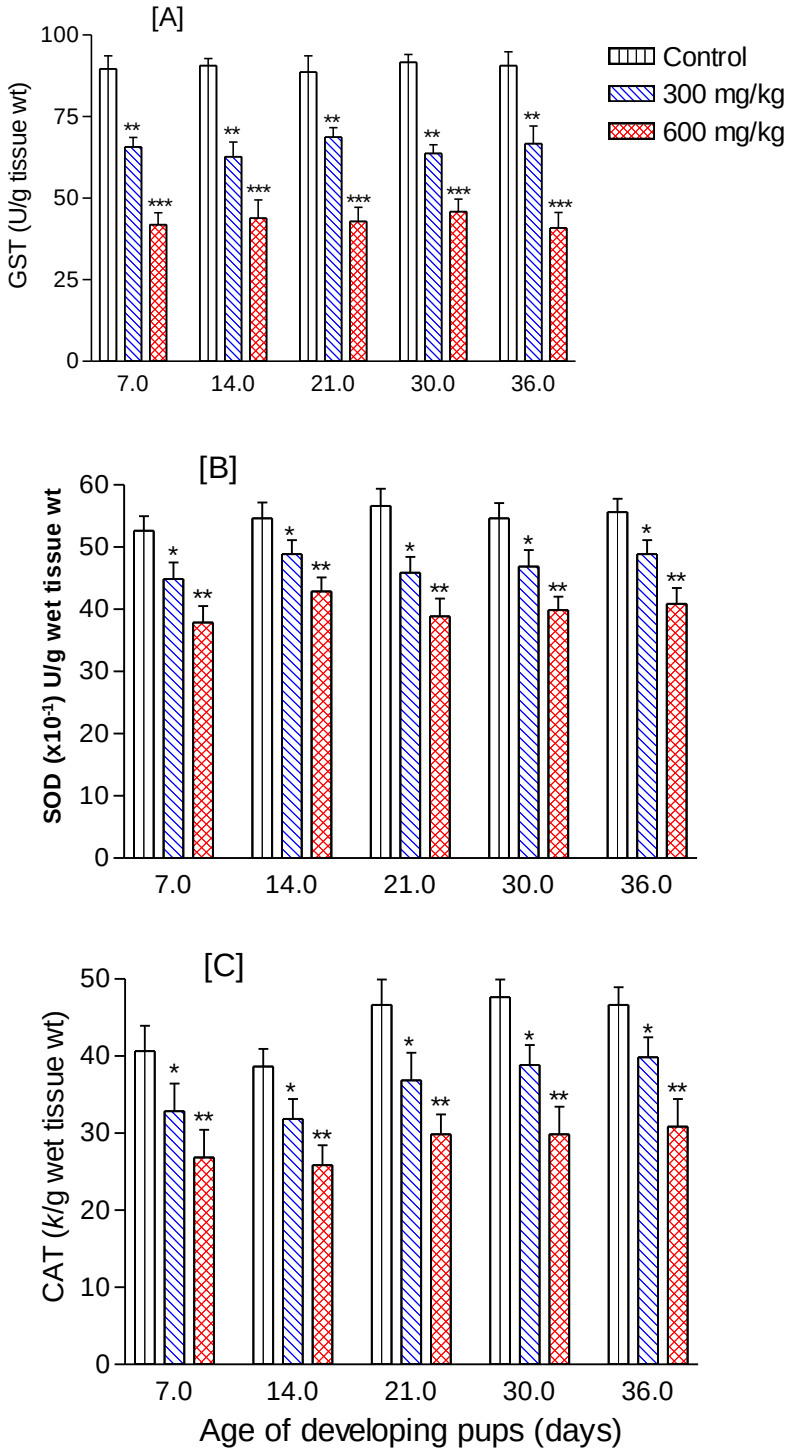


Figure 8 A – C. Effect of perinatal aluminium doses (300 and 600 mg/kg body weight) exposure on the mean levels of enzymatic oxidative stress indices like (A) glutathione S-transferase (GST) level, (B) catalase (CAT) activity, and (C) superoxide dismutase (SOD) activity in the forebrain of the offspring at various postnatal developing ages (x-axis). (*, **and ***) represent statistically significant (*P < 0.05, P < 0.01 and P < 0.001 respectively)* from the control group; see text.

## DISCUSSION

The present results demonstrate that female mice exposed to Al during pregnancy produce pups that markedly differ from their controls in the rate of physical maturation, neuromotor development, cognitive responses in the active avoidance test and Morris water-maze test, in the levels of neurotransmitters and oxidative stress indices in their forebrain region. The postnatal suppression of body weight gain and delay in opening of eyes and the appearance of body hair fuzz in the Al exposed pups, might indicate a lasting effect of the perinatal Al exposure on general growth retardation in mice offspring. Perinatal Al administration has also affected the preweaning reflexes in the mice pups. All reflexes including righting, rotating and avoiding of the cliff by the developing pups were significantly suppressed as compared to the controls. This clearly suggests for a direct Al intervention with the developing pups in utero because Al has been shown to be transferred to offspring through placenta or milk (Abu-Taweel et al., 2012). A recent study using radio isotopic Al showed that considerable amounts of Al administered to pregnant and/or lactating rats, crossed the blood brain barrier and was deposited into the brain of fetuses and sucklings through the transplacental passage and/or maternal milk which remained persistent throughout their lifetime (Yumoto et al., 2001). Thus, Al exposure during fetal life does retard motor development and physical maturation, as have been suggested for other drugs (Brain et al., 1994) and compounds (Ajarem and Ahmad, 1991; 1998).

It is well established that Al is toxic to the growth and development of fetuses and sucklings in humans and experimental animals (Domingo, 1995; Yumoto et al., 2001; Sharma and Mishra, 2006). Gestational and lactational exposure to Al doses can result in persistent neurobehavioural deficits in the offspring of some mammals (Roig et al., 2006). Studies in rodents have also demostrated that during pregnancy, exposure to Al causes long-lasting effects on emotionality and learning capabilities (Nishio et al., 2001; Sternberg and Ridgway, 2003; Shaw and Petrik, 2009; Kumar et al., 2009). Al exposure in animals and humans results in behavioral changes and intellectual impairment and it is possible that the behavioral changes could be the result of subtle changes in the serotonin level of the brain regions following Al exposure (Kumar, 2002).

The results of this study showed that the levels of dopamine was reduced significantly and dose-dependently whereas 5-HT was inhibited only at the higher dose of Al in the forebrain tissue of the Al-exposed mice offspring at all ages. There is evidence of an inhibitory role of DA mediated receptor ($D_2$ type) in depressing the hyperexcitability of hippocampal and striatal neurons (Freitas et al., 2004; Nascimento et al., 2005). A number of 5-HT receptor subtypes have been reported for having different roles in the functions of serotonergic neurotransmission, including the functions connected with learning and memory processes (Petkov et al., 1995). The mice offspring exposed to Al tended to perform badly in water maze parameters and also resulted in decreased number of avoidances in the automatic reflex conditioner as compared to the control offspring. This suggests for a tendency towards decreasing of the memory effect of Al under conditions of reduced functional capacity of serotonergic neurotransmission. Recently, a growing body of research has focused on the participation of serotonin (5-HT) in the neurochemical mechanisms of cognition and especially of learning and memory. Potential toxic mechanisms of action for Al may include disruption in serotonergic neurotransmission through disturbed levels of neurotransmitters in

the brain hippocampus (Richter-Levin and Segal, 1991). Other reported mechanisms of action of Al toxicity may include enhancement of inflammation (i.e., microgliosis) and the interference with cholinergic projections (Platt et al., 2001), reduced glucose utilization (Joshi. 1990), defective phosphorylation-dephosphorylation reactions (Cordeiro et al., 2003), altered rate of transmembrane diffusion and selective changes in saturable transport systems in the blood brain barrier (Kaya et al., 2003), oxidative damage on cellular processes by the inhibition of the glutathione redox cycle (Murakami, 1999) and inflicting damage to membrane lipid, proteins and antioxidative enzyme defense system (Jyoti et al., 2007). Al has been reported to alter blood-brain barrier (Banks and Kastin, 1985; Zatta et al., 2002) and gets deposited in the cortex, cingulate bundles, corpus callosum (Plat et al., 2001) and hippocampus (Struys-Ponsar et al., 1997; Fattoretti et al., 2004). As an important target organ of neurotoxicity, the hippocampus (located in the forebrain region of rodents) is a crucial element of the neurotoxicity basis of higher cognitive function (Savage et al., 2004; Tariq et al., 2008). It is evidently suggested in the present chapter that the brain may be the most susceptible target organ for Al toxicity.

The present chapter indicated a significant and dose-dependent increase in TBARS and decrease in GSH and CAT, GST, and SOD levels in the forebrain region of the mice treated with Al clearly suggesting for a high level of enzymatic (GST, SOD, and CAT) and nonenzymatic (TBARS and GSH) OS in a long lasting manner. It is documented that in infants although their growth rate is rapid, their blood brain barrier, detoxification system (liver), and excretory system (kidney) are not well developed (Snell, 2001; Blackburn and Loper, 1992). Crossing of Al through the blood brain barrier and accumulation in the brain glial and neural cellsg and dose dependent OS in the forebrain of the mice offspring inflicting cognitive dysfunctions.

## CONCLUSION

From the present results it can be concluded that Al exposure during pregnancy can affect the fetus, raising the concerns that during a critical perinatal period of brain development, Al exposure might modify the properties of the dopaminergic system and thus change the threshold of that system or other related systems. Furthermore, Al exposure during perinatal period may disrupt the steady state balance between ROS production (TBARS) and their scavenging by the cellular antioxidant system (GSH). Perinatal Al exposure alters the brain enzymatic (GST, CAT, SOD) and nonenzymatic (TBARS and GSH) antioxidant defense system (AOS) and causes a long lasting and dose dependent effects. Disruption of neuromotor reflexes in the neonatal stage and cognitive dysfunction evidenced through shuttle box and water maze tests in the offspring at adolescent ages further support the possibility that Al exposure during pregnancy (perinatal period) has potential neurotoxic hazards to the in utero developing fetus brain. Reduced use of Al during pregnancy is of crucial importance in preventing Al-induced neurotoxicity in the offspring. In addition, a biogenetical level of investigations in context to perinatal Al exposure in offspring for a longer lasting effects may play a crucial role and may be a potential area for further studies.

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