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Neurobehavioral toxic effects of perinatal oral exposure to aluminum on the developmental motor reflexes, learning, memory and brain neurotransmitters of mice offspring

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ABSTRACT

Aluminum (Al) is a known neurotoxicant and circumstantial evidence has linked this metal with several neurodegenerative disorders like Alzheimer's disease, but no causal relationship has yet been proved. Al-induced behavioral alterations as well as cognitive deficits and rodent brain neurotransmitter level, are well known in adults but the 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/day Al (aluminum chloride) resulted in significant and deleterious effects in the offspring inflicting a dose-dependent reduction in postnatal body weight gain, delays in opening of the eyes and appearance of 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). During adolescent ages of the male offspring, a significant and dose-dependent deficit was also observed in their locomotor activity at postnatal day 22 (PD 22), learning capability (at PD 25), and cognitive behavior (at PD 30–36). Furthermore, a significant and dose-dependent disturbance in the levels of neurotransmitters like dopamine (DA) and serotonin (5-HT) was also observed in the forebrain region of the offspring at PD 7, PD 14, PD 21, PD 30, and PD 36. Thus, perinatal Al exposure, particularly during pregnancy and lactation period, can affect the in utero developing fetus and postnatal developing sucklings, raising the concerns that during a critical perinatal period of brain development, Al exposure has potential and long lasting neurotoxic hazards and might modify the properties of the dopaminergic system and thus can change the threshold of that system or other related systems at later ages. A reduced use of Al during pregnancy is of crucial importance in preventing Al-induced delayed neurotoxicity in the offspring.

1. Introduction

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 aluminum 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).

Al is a known adult neurotoxicant (Golub and Germann, 2001) as well as its neurotoxicity has been reported during developmental stages also (Domingo, 1995; Golub and Domingo, 1996). Al has been linked with the etiology of several neurodegenerative disorders like Parkinson's disease (Yasui et al., 1992), dementia (Forbes et al., 1995), and Alzheimer's disease (Kawahara, 2005), however, overwhelming medical and scientific opinions are that the findings outlined above do not convincingly demonstrate a causal relationship between Al and neurodegenerative diseases (Massey and Taylor, 1989; Exley, 2001). It is possible 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 Alzheimer's disease pathogenesis remains unknown, and still this issue is controversial (Kawahara and Kato-Negishi, 2011). Al administration has been reported to induce oxidative stress by inflicting damage to membrane lipid, proteins and antioxidative enzyme defense system (Jyoti et al., 2007). Long-term effects of developmental exposure has not yet been studied in this regard in human populations, although a recent study detected a delay in neuromaturation in preterm infants exposed to high levels of Al through parenteral fluids (Bishop et al., 1997). Long-term behavioral

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effects of developmental exposure 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). Many of these studies used Al injection during early pregnancy; however, it would be valuable to know if Al effects are seen with oral administration which is more readily common in human populations. Bearing in mind that the general and maternal toxicities to Al are important to consider for variables like pregnancy completion, pregnancy duration, litter size, neonatal and postnatal mortality and maternal body weights during pregnancy and lactation, these maternal data are not included in the present study and the Al toxicity has been evaluated in the offspring only. However, it may be worth mentioning here that Al consumption by the dams had no significant deleterious effects in the dams itself, such as their normal activity, body weight or general health.

Maternal stress during pregnancy can have serious negative effects on the learning abilities of the offspring (Nishio et al., 2001; Sternberg and Ridgway, 2003). Furthermore, 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 (for references, see 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). However, it may be noted here that many experimental studies on animals and on isolated cells have shown that Al has toxic effects on the nervous system, but in almost all cases the doses of Al used were much higher than those occurring naturally in tissues (Gitelman, 1988). The doses used in the present study are also of fairly higher magnitude and have been selected on the basis of our pilot studies and also in conjunction with a reported study (Colomina et al., 1999). Thus the Al doses used herein 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.

Although Al induced behavioral alterations as well as cognitive deficits have been reported in literature, the effects in the offspring of perinatally Al exposed dams are not yet understood properly and need more attention in order to gain information on the longer lasting effects of Al transfer from dams to offspring. Furthermore, Al induced changes in the rodent brain neurotransmitter studies are scanty (Kumar, 2002). In the present study, the effect of perinatal oral Al-administration to pregnant mice was assessed in the offspring at neurobehavioral, cognitive and biochemical levels (brain neurotransmitters) to investigate the possible long term physiological mechanism associated with Al toxicity in the offspring. The data on maternal effects itself, has been excluded and shall form a part in a separate communication.

2. Materials and methods

2.1. 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 (PD 1) 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.

2.2. Experimental design and aluminum 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 (PD 1) and was continued until postnatal day 15 (PD 15) and thereafter the mothers were switched to plain tap water. The pups of each experimental group were culled to only eight per dam on PD 0 and were left with their mothers until PD 22. 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 PD 1 and repeated every other day until PD 21 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.

2.3. 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 (PD 1) through the entire weaning period until PD 21.

2.3.1. Body weight

Weight is a useful indicator of development. Thus, the pups were weighed every alternate day from PD 1 until PD 21.

2.3.2. 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.

2.4. Neuromotor maturation assessment during weaning period

2.4.1. 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.

2.4.2. 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”.

2.4.3. 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.

2.5. Behavioral assessment during post-weaning period

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.

2.5.1. Locomotory activity test

After weaning at PD 22, 10 male offspring from each treated groups were subjected to 'Locomotor Activity' tests in an experimental wooden arena of square shaped measuring 80×80×30 cm and the floor was divided into 64 squares of equal size and various elements of behavior like numbers of squares crossed, wall rears, rears and washes, and durations of locomotion and immobility were observed (Ajarem, 1987; Ajarem and Ahmad, 1998). The visual observations in the arena lasted 300 s for each animal.

2.5.2. 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 (21 W) 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.

2.5.3. 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 PD 30, 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 (P 30) 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 (P 31 to P 35, 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).

2.6. Biochemical studies

For biochemical studies, a sub-set of developing offspring ($n=8$/*group*) were sacrificed at different ages (PD 7, 14, 21, 30 and 36) and the level of neurotransmitters 5-hydroxytryptamine or serotonin (5-HT) and dopamine (DA) was estimated in their fore-brain. The animals were killed by decapitation and the 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 determination of neurotransmitters.

2.6.1. 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 $HClO_4$ containing 0.05% EDTA, centrifuged at 17,000 rpm at 4 °C for 5 min. The supernatants were filtered using 0.45 m 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 m filter and degassed under vacuum before use. Bondpak C18 column was used at a flow rate of 1.2 ml/min and the injection volume of the sample was 20 l. The levels of DA and 5-HT were calculated using a calibration curve and results were expressed as ng/mg tissue weight.

2.7. 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.

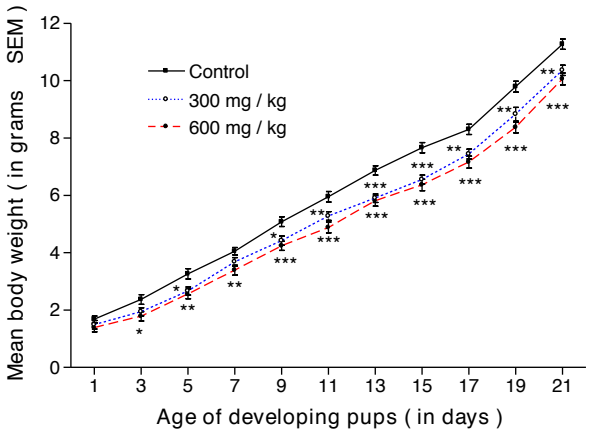

Fig. 1. Effect of perinatal aluminum dose (300 and 600 mg/kg body weight) exposure on the body weight gain of mouse pups during the weaning (lactation) period. *, ** and *** represent statistical significance (pb0.05, pb0.01 and pb0.001 respectively) from the control group; see text.

3. Results

3.1. Physical assessment during weaning period

Although the body weights of pups born to Al-treated mice lagged behind the control group on PD 1, the difference was statistically insignificant. However, the Al exposed offspring at both doses, showed a significant decline (pb0.05) in their body weight gain as compared to the controls, from PD 3 onward. From PD 5 up to the weaning period (PD 21), the Al exposed offspring remained lagging behind the controls in body weight very significantly (pb0.001) in a dose-dependent manner (Fig. 1).

Other morphological developments such as the opening of the eyes and appearance of body hair fuzz were also significantly (pb0.001) delayed in the Al exposed offspring in a dose-dependent manner (Fig. 2).

3.2. Behavioral studies

3.2.1. Neuromotor maturation

The neuromaturation of reflexes during the weaning period of the developing pups was assessed from the day of birth PD 1 until PD 21. 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 (Fig. 3).

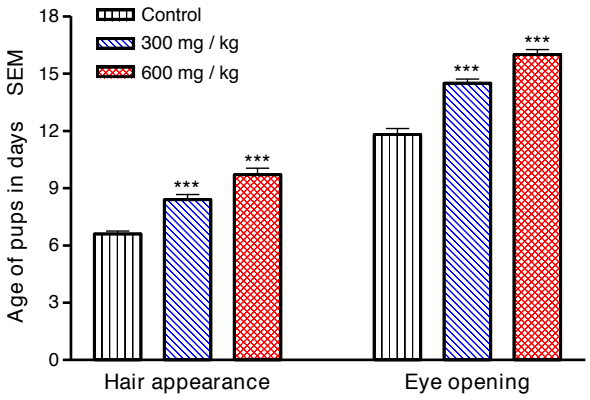

Fig. 2. Effect of perinatal aluminum dose (300 and 600 mg/kg body weight) exposure on the body hair appearance and eye opening in the mouse pups. *** represents statistical significance (pb0.001) from the control group; see text.

3.2.2. Locomotory test

The locomotor activity test (Table 1) showed that perinatal Al-exposure had a significant and dose-dependent suppressive effect on the numbers of squares crossed, wall rears and rears, and on the locomotion duration in the weaned animals (PD 22). On the other hand, the number of wash and duration of immobility was significantly and dose-dependently increased as compared to the controls.

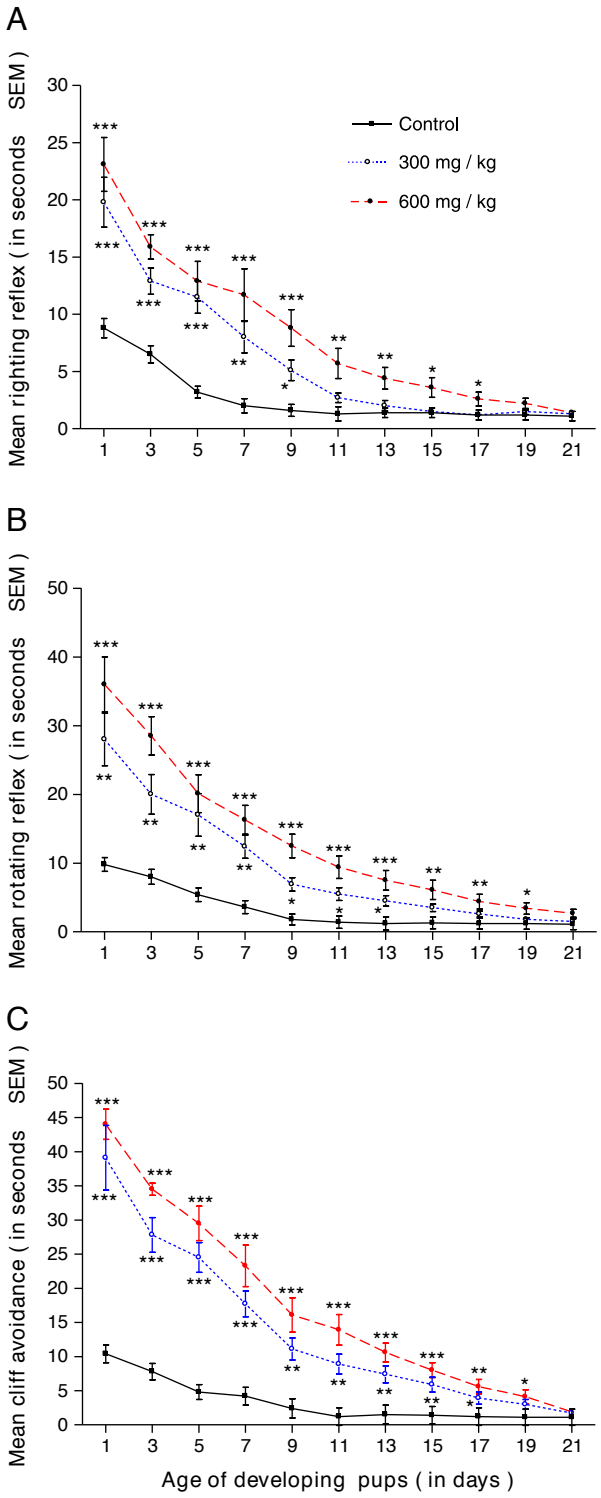

Fig. 3. A–C. Effect of perinatal aluminum dose (300 and 600 mg/kg body weight) exposure on the righting reflex (A), rotating reflex (B) and cliff avoidance (C) of mouse pups during the weaning (lactation) period. *, ** and *** represent statistical significance (pb0.05, pb0.01 and pb0.001 respectively) from the control group; see text.

Table 1
Effect of perinatal aluminum exposure on the locomotor activity of male offspring at adolescent age (post natal day 22).

Treatment group	Median number (with ranges) of acts and postures					
	Number of squares crossed	Wall rears	Rears	Wash	Locomotion duration (s)	Immobility duration (s)
Control	385 (357–425)	38 (30–44)	19 (13–24)	7 (2–11)	190.6 (146.5–250.5)	110.5 (55.2–162.1)
300 mg/kg	285** (240–350)	24** (14–29)	10** (1–15)	10* (9–22)	104.4*** (81.2–150.4)	198.1*** (150.6–220)
600 mg/kg	245*** (200–330)	20*** (10–33)	9*** (2–20)	12** (6–17)	96*** (70.3–120.1)	204*** (180–230.1)

* Significantly different ($p<0.05$) from the control by Dunn's multiple comparison test.
** Significantly different ($p<0.01$) from the control by Dunn's multiple comparison test.
*** Significantly different ($p<0.001$) from the control by Dunn's multiple comparison test.

3.2.3. Active avoidance test

In the shuttle-box active avoidance test, the Al-exposed offspring (PD 25), showed a statistically significant and dose-dependent decrease in the number of avoidances during the trial period as compared to the control group (Fig. 4A). 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 (UCS and CS inter-trial crossings) showed a significant and dose-dependent decrease in the number of inter-trial crossings as compared to the controls (Fig. 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 or escape latency 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 a significant time in avoiding the shock treatment as compared to the controls (Fig. 4C).

3.2.4. Morris water-maze task

Mice offspring with Al treatment, exhibited longer escape latencies to reach the platform as compared with the control group ($p<0.01$; Fig. 5A), 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 ($p<0.001$; Fig. 5B). The probe trial studies showed that Al exposed offspring spent more time in the other three quadrants than the target (platform) quadrant as compared to the control group ($p<0.001$; Fig. 5C), in search of the platform.

3.3. Biochemical studies

3.3.1. Concentration of monoamines in forebrain

There was a significant ($p<0.001$) and dose-dependent inhibition of DA level in the forebrain of mice offspring treated with Al as compared to the control group at ages PD 7, PD 14, PD 21, PD 30 and PD 36 (Fig. 6A). On the contrary, the level of 5-HT was significantly ($p<0.001$) 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 (Fig. 6B).

4. Discussion

The present results demonstrate that female mice exposed to Al during pregnancy and lactation, produce pups that markedly differ from their controls in the rate of physical maturation, motor development, locomotory behavior, cognitive and active avoidance responses and in the levels of neurotransmitters in their forebrain region. Moreover, the postnatal suppression of body weight gain and the 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. Such gestational and lactational exposure to Al has been reported for resulting into Al accumulation in the cerebellum and cerebrum of rats (Yumoto et al., 2001, 2003). Furthermore, Al during fetal development has been shown to impair the ability of nursing mouse pups to retain absorbed iron (Fe) and (Mn) (Golub and Domingo, 1996). Thus, it is more than likely that both consequences (i.e., Al accumulation and/or imbalance in essential elements) induce neurobehavioral alterations in the offspring as suggested earlier (Golub et al., 1989; Golub and Domingo, 1996). Perinatal Al administration has also affected the preweaning reflexes in the mice pups. All reflexes including righting, rotating and avoidance 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 as well as during lactation period, because Al has been shown to be transferred to offspring through placenta and/or milk. A major portion of brain cells (70%) of the closely related rats are known to be formed after birth (Patel, 1983). Furthermore, a recent study using radio isotopic Al showed that considerable amounts of Al administered to pregnant and/or lactating rats, crossed the BBB and were 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., 2000, 2001). Thus, Al exposure during fetal and suckling 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). Significant suppression of all reflexes including righting, rotating and avoidance of the cliff, in the weaning pups during the postnatal weaning period, clearly suggests for a direct Al interaction with the developing pups in utero (prenatal exposure) as well as after birth through their mother's milk (postnatal exposure). Thus, the continuous exposure to Al doses during prenatal (fetal development period in utero) as well as postnatal (early developmental period of the pups after birth) periods, has a cumulative exposure in the developing brain tissue, inflicting a longer lasting and delayed effect on the offspring's deficits in their neurobehavior and cognitive functions at adolescent and/or adult stages as evidenced in the present study. However, further studies are warranted in support of these longer lasting effects of perinatal Al exposure in the offspring. Besides maternal exposure to Al during such periods, a concomitant exertion of the dams to stress has a serious interaction and can ultimately result into persistent neurobehavioral and postnatal developmental deficits in the rat offspring (Colomina et al., 2005; Roig et al., 2006). Furthermore, studies in rodents have also demonstrated that during pregnancy, exposure to Al causes long-lasting effects on emotionality and learning capabilities (Kumar et al., 2009; Shaw and Petrik, 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 DA were reduced significantly and dose-dependently whereas 5-HT was inhibited only at the higher dose of Al in the forebrain (cerebral part containing hippocampus and striatum) 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

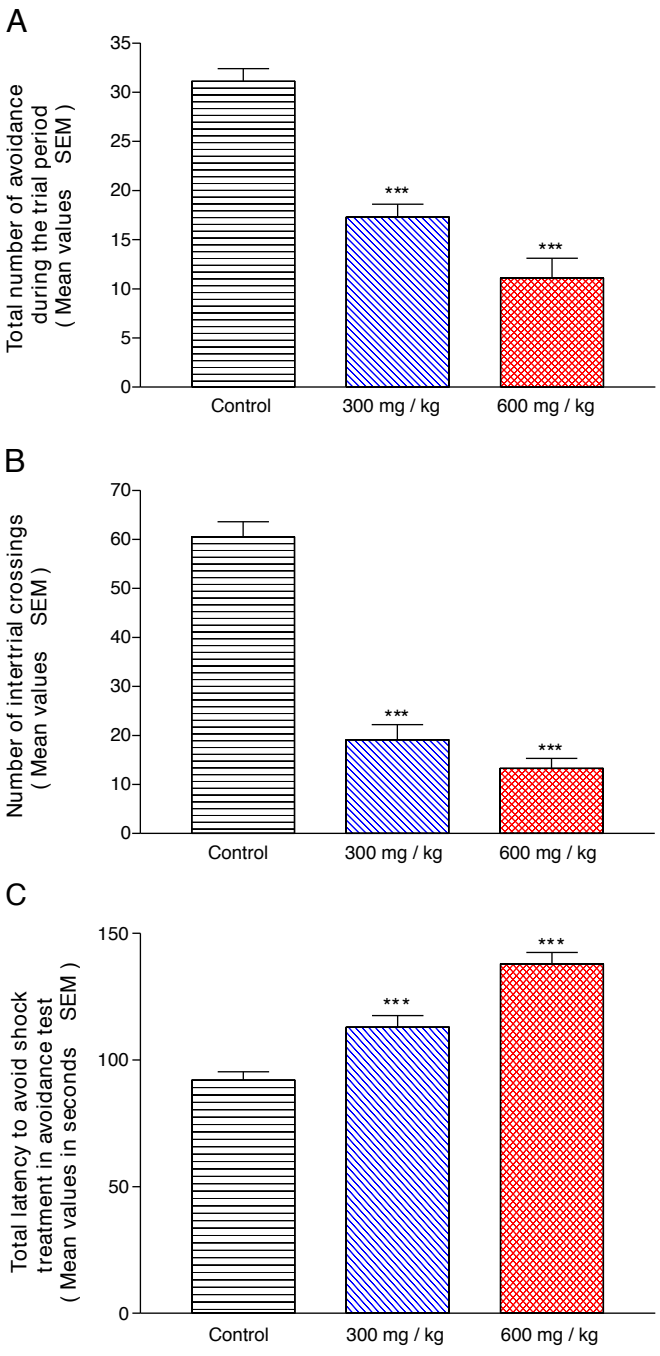

Fig. 4. A–C. Effect of perinatal aluminum dose (300 and 600 mg/kg body weight) exposure on the mean performance value of the mice offspring at the age of 25 days postnatal in the active avoidance task. Mice were given a 50 trial session and the total number of times they avoided the shock by moving to 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 or the escape latency (C) were measured. *** represents statistical significance ($p \leq 0.001$) from the control group; see text.

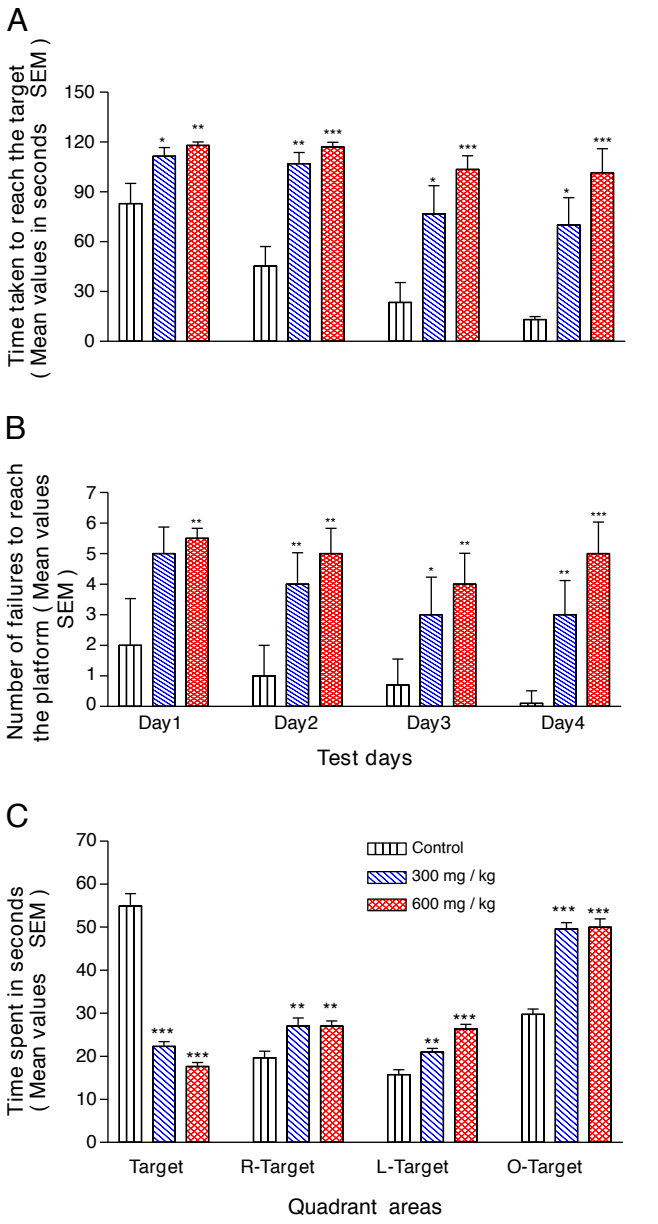

Fig. 5. A–C. Performance in Morris water-maze of mice offspring at the age of 30 days postnatal whose mothers were exposed perinatally with aluminum doses of 300 and 600 mg/kg body weight. A — the mean latency to reach the hidden platform (y-axis) on each testing day (x-axis). Animals subjected to aluminum exposure were slower in finding the platform than the controls on all testing days. B — the number of failures or unsuccessful trials (y-axis) on each testing day (x-axis). Aluminum exposed offspring showed maximum number of failures in finding the platform as compared to controls. C — probe test performance. The aluminum exposed offspring spent less time in the target quadrant than the control group. 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 the quadrant opposite the target quadrant. *, ** and *** represent statistical significance ($p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ respectively) from the control group; see text.

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 (escapes) in the automatic reflex conditioner as compared to the control offspring. This suggests for a tendency toward 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

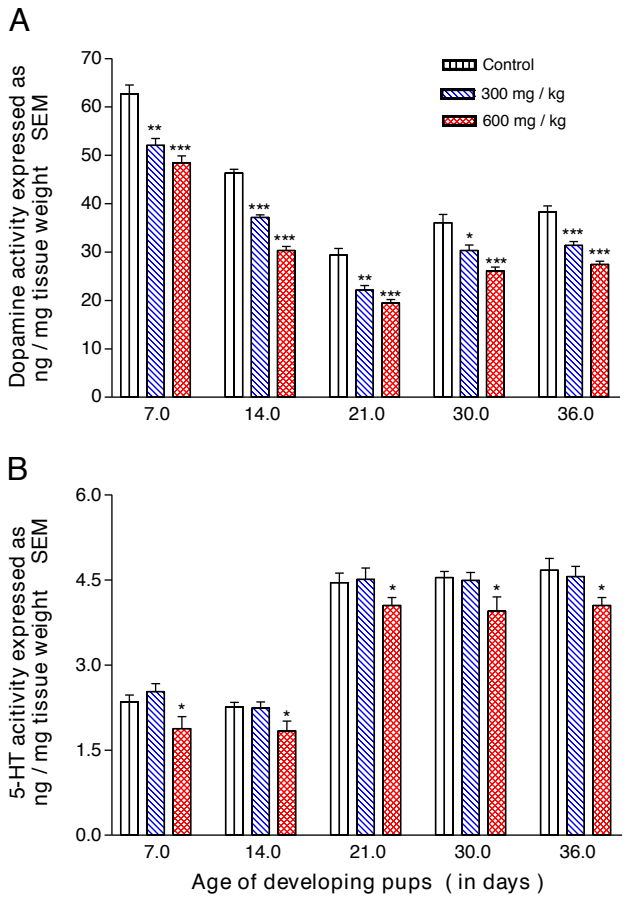

Fig. 6. A and B. Effect of perinatal aluminum dose (300 and 600 mg/kg body weight) exposure on the mean levels of dopamine (A) and 5-HT (5-hydroxy-tryptamine or serotonin) (B), in the forebrain (including the cerebral areas with hippocampus and striatum) of the offspring at various postnatal developing ages (x-axis). *, ** and *** represent statistical significance (p Þ 0.05, p Þ 0.01 and p Þ 0.001 respectively) from the control group; see text.

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 (BBB) (Kaya et al., 2003), oxidative damage in brain cells (Rui and Yongjian, 2010) and inflicting damage to membrane lipid, proteins and antioxidative enzyme defense system (Jyoti et al., 2007). Al has been reported for not only crossing the BBB (Banks and Kastin, 1985; Yumoto et al., 2001) but also it increases its permeability (Favarato et al., 1992; Banks et al., 1996; Exley, 2001) and gets deposited in various regions of the cerebrum and cerebellum (Platt et al., 2001; Yumoto et al., 2001, 2003) including the hippocampus (Struys-Ponsar et al., 1997; Fattoretti et al., 2004; Shi-Lei et al., 2005). As an important target organ of neurotoxicity, the hippocampus (located in the forebrain cerebral region of rodents) is a crucial element of the neurotoxicity basis of higher cognitive function (Savage et al., 2004; Tariq et al., 2008). Based upon the earlier reports for the persistence of considerable amounts of Al administered to pregnant and/or lactating rats into the brain of fetuses and sucklings throughout their lifetime (Yumoto et al., 2001), it is evidently suggested in the present study that the brain may be the most susceptible target organ for Al toxicity during early perinatal period since such toxicity may be expressed later also at adolescent/adult stages in a delayed manner in the form of altered neurobehavior and cognitive functions.

5. Conclusions

From the present results it can be concluded that Al exposure during pregnancy can affect the fetus, raising the concerns that during a critical prenatal period of brain development in utero through placenta and also during postnatal development through milk, Al exposure might modify the properties of the dopaminergic system and thus change the threshold of that system or other related systems. This might further be supported by the present findings in the offspring both at neonatal and adolescent stages providing evidence that Al exposure during pregnancy has potential neurotoxic hazards to the in utero developing fetus brain and also through milk during postnatal developing pup brain. Reduced use of Al during perinatal period is of crucial importance in preventing a delayed and longer lasting Al-induced neurotoxicity in the offspring at later adolescent/adult stages. In addition, further studies in context to perinatal Al exposure in offspring may play a crucial role and may be an important area for future research.

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