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Ethological Problems and Learning Disability due to Aluminium Toxicity in Rats

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A total of 35 Sprague-Dawley adult rats were used to investigate the effect of aluminium toxicity on behavioural patterns of adult female rats and learning ability of offspring. Rats were allotted into 4 groups, group one received 2g/l anhydrous aluminium chloride (n=10), group two received 3g/l anhydrous aluminium chloride (n=10), group three received 3.5g/l anhydrous aluminium chloride in drinking water (n=10) and control group did not receive anhydrous aluminium chloride (n=5) from 8th day of pregnancy till weaning of pups.

The obtained results showed that feeding time increased significantly in 2g/l and 3.5g/l anhydrous aluminium chloride groups than control one, while, litter licking frequency and nursing time increased significantly in 2g/l anhydrous aluminium chloride than other groups. On contrary lying time decreased significantly in rats treated with 2g/l anhydrous aluminium chloride than other groups, licking and scratching decreased in 3g/l and 3.5g/l anhydrous aluminium chloride groups. In considering, the time spent in closed arms by offspring pups exhibited much times significantly than control group, while, time spent in open arms of elevated plus maze decreased significantly in all treated groups than control group. On the other hand, number of entries in open arms significantly decreased in treated groups than control one.

Livestock subjected to wide variety of stressors (e.g. thermal, nutritional, environmental and productive) but there have been a significant increase in interest in psychological or behavioural stressors due to concern over animal wellbeing. The existence of behavioural needs or instinctive derives (e.g. for exercise, social interaction or control over environmental stimuli) have become important issues (Friend, 1991). Colomina *et al.,* (2005) observed significant alterations in feed and water consumption during gestation in dams exposed to aluminium, also Mahieu *et al.,* (2009) demonstrated that melatonin reduced oxidative damage induced by aluminium in rat kidney and differences in feed and water intake of aluminium treated rats from controls. A single oral dose of aluminium nitrate nonahydrate 1327 mg/kg given to mice on gestation day 12 and found that aluminium induced maternal toxicity by reduction in feed consumption (Domingo *et al.,* 2000). Furthermore, Kowalczyk *et al.,* (2004) when assed the influence of long term aluminium chloride intake on biochemical parameters by administration of aluminium chloride in drinking water at a dose of 80 mg/l for three months found that feed and water intake decreased. Abdel-Aal *et al.,* (2011) stated that exposure to aluminium was associated with significant reductions in spontaneous locomotor behaviour in open field test and not caused any significant alterations of the animal`s performance in Rota-rod test, while there were significant reductions in exploratory activity in open field test.

Walton (2007) reported that four out of six rats consumed aluminium from 16 months of age to the conclusion of their lifespan (average 29.8 months) in an amount (1.5 mg/kg body weight) continued to perform the memory task in old age without significant deficit and the remaining two obtained significantly lower mean memory scores in old age than in middle age. Also, there are considerable interest generated in prenatal influence of environmental and nutritional factors on physical and mental development of offspring where, prenatal exposure of rat pups to aluminium and lead produced neurobehavioral impairments and learning deficits characterized by learning disabilities, hypoactivites and high emotionality. In addition, experimental designs and models of laboratory animals are one of great chance to stimulate human maternal environment and exploring the potential risk of such agents (Kaoud *et al.,* 2008). The objectives of this study are to investigate the effect of anhydrous aluminium chloride toxicity on welfare of rats by measuring behaviour of adult female rats and learning ability of their offspring.

Material and methods

This investigation was carried out at Department of Animal Husbandry and animal Wealth Development, Faculty of Veterinary Medicine, Alexandria University, 2013.

Animals. A total of 35 Sprague-Dawley pregnant female rats (3 months and 110-130g) were allotted into four groups, group one received 2g/l anhydrous aluminum chloride in drinking water (n=10) (Gromysz-Kalkowska *et al.,* 2004), group two received 3g/l anhydrous aluminum chloride in drinking water (n=10) (Schetinger *et al.,* 1999), group three received 3.5g/l anhydrous aluminum chloride in drinking water (n=10) (Misawa and Shigeta., 1992) and group four received water without anhydrous aluminum chloride (n=5) daily from day 8 pregnancy till weaning (Delville, 1999).

Management. Rats were fed ration containing 16.3% crude protein, 6.8 % fat and 3.5 % crude fibre, housed in 35 individual breeding plastic cages (37x30x14cm) with 1-2 cm wood shaving bedding replaced two times per week. Rats were kept under natural light-dark cycle without any alteration in lighting program.

Vaginal smears were done to detect the time of positive oestrus according to Harkness and Wagner (1983) then mating took place by monogamous system where one female was regularly mated by one male introduced to females in breeding cages (Fatma, 2005) and then persists with pups till weaning. Pregnancy was detected by vaginal smears on the next morning and the day of finding sperms was called day zero of gestation (Luna, 1968).

Behavioural observations. Rats were observed three times weekly and four hours daily within periods of late morning and early afternoon. Focal sample observation was carried out according to Morimoto *et al.,* (1993).

The observed patterns were ingestive, body care, resting, locomotor, investigatory behaviour, while, maternal behaviour carried out according to Ferreira *et al.,* (2002) including licking (grooming), nursing, nest building and retrieving.

Learning ability was measured by using the elevated plus maze according to (Pellow *et al.,* 1985), where the elevated plus –maze apparatus was made of smooth brown opaque platforms with two open arms (50x10 cm) and two closed arms of the same size, the wall of this chamber was 40 cm high and the whole apparatus was elevated 50 cm above floor. Each rat was placed in the central square (10x10cm) facing closed arm. At the end of each trial lasting 5 min, the arms was cleaned and dried to remove excreta. The recorded parameters were time spent in open or closed arms, time of latency in seconds that rats took to enter open or closed arm, number of entries into open and closed arms and ratio spent in open and/or closed arms expressed as percentage.

Statistical analysis. The behavioural data was analysed by two way analysis of variance and learning ability was analysed by one way analysis of variance by SAS (2004), using proc GLM.

Results and Discussion

The data presented in Table (1) showed that feeding time increased significantly ($p<0.05$) in rats treated with 2g/l (9.62±0.66 min/hr) and 3.5g/l anhydrous aluminium chloride (7.87±0.61 min/hr) than control group (5.65±0.59 min/hr), while, standing time decreased significantly ($P<0.05$) in rats treated with 3g/l anhydrous aluminium chloride (0.20±0.05 min/hr) than control group (0.62±0.18 min/hr), moreover, lying time decreased significantly ($P<0.05$) in rats treated with 2g/l anhydrous aluminium chloride (28.89±1.83 min/hr) than other groups.

During last two weeks of pregnancy and during lactation period feeding time and drinking frequency increased significantly (11.27±0.90 min/hr and 0.94±0.10 freq/hr $p<0.05$) while, standing time decreased gradually during experimental period especially during last week of lactation, while rats exhibited much lying time during last two weeks of pregnancy than during lactation period with a gradual decrease during lactation period. These results are in close accordance with Colomina *et al.,* (2005) who found no significant alterations in feed consumption or water consumption during gestation in dams exposed to aluminium and Mahieu *et al.,* (2009) who demonstrated that melatonin reduces oxidative damage induced by aluminium in rat kidney and found that feed and water intake of aluminium treated rats were not different from controls. While, Domingo *et al.,* (2000) found that single oral dose of aluminium nitrate nonahydrate 1327 mg/kg given to mice on gestation day 12 induced maternal toxicity by reduction in feed consumption. Furthermore, Kowalczyk *et al.,* (2004) when assed the influence of long term aluminium chloride intake on biochemical parameters by administration of aluminium chloride in drinking water at a dose of 80 mg/l for three months found that feed and water intake decreased.

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Table 1: Least square means and their standard error of the effect of aluminium chloride (Alcl3) and period of pregnancy and lactation on ingestive and resting behaviour of rats.

		Ingestive behaviour		Resting behaviour	
		Feeding (Min/hr)	**Drinking (Freq/hr)**	**Standing (Min/hr)**	**Lying (Min/hr)**
Treatment	**Control**	5.65±0.59[c]	0.66±0.08[a]	0.62±0.18[a]	36.78±2.05[a]
	2g/l $Alcl_3$	9.62±0.66[a]	0.73±0.06[a]	0.39±0.09[ab]	28.89±1.83[b]
	3g/l $Alcl_3$	6.16±0.39[c]	0.54±0.07[a]	0.20±0.05[b]	36.91±1.61[a]
	3.5 g/l $Alcl_3$	7.87±0.61[b]	0.69±0.07[a]	0.43±0.08[ab]	36.93±1.42[a]
Period	**2nd Week pregnancy**	4.57±0.56[c]	0.45±0.07[c]	0.74±0.15[a]	54.98±0.65[a]
	3rd Week pregnancy	4.70±0.51[c]	0.52±0.07[bc]	0.40±0.10[b]	55.51±0.58[a]
	1st Week lactation	5.93±0.49[c]	0.68±0.07[b]	0.36±0.08[b]	22.82±1.44[b]
	2nd Week lactation	8.48±0.65[b]	0.68±0.07[b]	0.25±0.07[b]	19.92±1.16[c]
	3rd Week lactation	11.27±0.90[a]	0.94±0.10[a]	0.15±0.07[b]	19.81±1.09[c]

Means within the same column under the same category carry different superscripts are significantly different.

Table 2: Least square means and their standard error of the effect of aluminium chloride ($Alcl_3$) and period of pregnancy and lactation on movement activities, body care and investigatory behaviour of rats.

		Body care behaviour		Investigatory behaviour		
		Licking (Freq/hr)	**Scratching (Freq/hr)**	**Cage (Freq/hr)**	**Trough (Freq/hr)**	**Movement (Freq/hr)**
Treatment	**Control**	3.12±0.51[a]	3.08±0.54[a]	1.51±0.27[a]	0.07±0.02[a]	1.53±0.18[a]
	2g/l $Alcl_3$	3.01±0.22[a]	3.48±0.25[a]	1.49±0.16[a]	0.06±0.01[a]	1.35±0.08[a]
	3g/l $Alcl_3$	2.10±0.19[b]	2.37±0.20[b]	1.19±0.13[a]	0.11±0.02[a]	1.58±0.13[a]
	3.5 g/l $Alcl_3$	1.98±0.19[b]	2.11±0.14[b]	1.45±0.14[a]	0.07±0.01[a]	1.82±0.21[a]
Period	**2nd Week pregnancy**	3.60±0.40[a]	4.20±0.45[a]	1.81±0.23[a]	0.10±0.02[a]	1.15±0.14[c]
	3rd Week pregnancy	2.62±0.26[b]	2.45±0.24[c]	1.37±0.17[ab]	0.07±0.02[a]	1.29±0.14[bc]
	1st Week lactation	2.80±0.21[b]	3.17±0.24[b]	1.38±0.18[ab]	0.08±0.02[a]	1.76±0.14[ab]
	2nd Week lactation	1.75±0.19[c]	2.04±0.16[c]	0.99±0.14[b]	0.05±0.01[a]	1.71±0.22[ab]
	3rd Week lactation	1.58±0.22[c]	1.71±0.18[c]	1.42±0.18[ab]	0.09±0.02[a]	1.98±0.20[a]

Means within the same column under the same category carry different superscripts are significantly different.

Licking and scratching frequencies (Table 2) decreased significantly (P<0.05) in rats treated with 3g/l and 3.5g/l anhydrous aluminium chloride than control group (2.10±0.19 and 1.98±0.19 vs. 3.12±0.51 freq/hr), while treatment had no significant effect on movement activities and investigation of cage and trough frequencies. However, there were gradual increase in the movement activities and trough exploration throughout pregnancy and lactation period with highest increment during last week of lactation (1.98±0.20 and 0.09±0.02 freq/hr), while, dams exhibited significantly (P<0.05) and gradual decline in licking frequency and cage investigation from 2^{nd} week of pregnancy (3.60±0.40 and 1.81±0.23 freq/hr) till 3^{rd} week of lactation in licking (1.58±0.22 freq/hr) and 2^{nd} week of lactation for cage exploration (0.99±0.14 freq/hr). The influence of treatment on movement activities agreed with Colomina *et al.,* (2002) who reported that exposure of rats to aluminium nitrate nonahydrate had no significant effect on locomotor activity in an open field test. Furthermore, Colomina *et al.,* (2005) found no alterations in the motor activity in the open field test for aluminium treated rats. Also, Roig *et al.,* (2006) reported that exposing rats to aluminium in drinking water did not alter significantly motor activity in the open field test. while, Abdel-Aal *et al.,* (2011) stated that exposure to aluminium was associated with significant reductions in spontaneous locomotor behaviour in open field test and not caused any significant alterations of the animal`s performance in Rota-rod test, while there were significant reductions in exploratory activity in open field test.

Among the four types of treatments the maternal behaviour of rats including licking frequency of pups and nursing time were significantly higher (P<0.05) in dams treated with 2g/l anhydrous aluminium chloride (3.48±0.37 freq/hr and 37.49±1.40 min/hr, than other groups and non-significantly higher in nest building and retrieving frequencies (0.75±0.15 and 0.27±0.07freq/hr, respectively). Alterations in behaviour of mother are known to affect infant development and several drugs have been shown to disrupt elements of maternal behaviour thus any disturbance to maternal care or the delicate mother-pup relationship may explain differential patterns of behaviour in the offspring rather than direct effects of prenatal exposure to a toxicant and the result of a pilot study suggested that differences exist in the pattern of maternal behaviour displayed by control and aluminium exposed mothers as control mothers spent more time involved in the pup-directed behaviours of nursing and licking and less time in nest building during the first two postnatal weeks than dams treated with aluminium during gestation while treated mothers had a longer latency to retrieve on postnatal day 3 although this difference did not reach significance (Laviola *et al.,*1990).

During lactation period, dams exhibited significantly (P<0.05) gradually decline in licking frequency of pups and nursing time from 1^{st} week (3.49±0.33 freq/hr and 37.15±1.33 min/hr) till 3^{rd} week (1.40±0.18freq/hr and 32.97±0.98 min/hr). However, nest building and retrieving frequencies were higher during 2^{nd} week lactation (0.83±0.16 and 0.32±0.06 freq/hr) and lower during last week of lactation (0.33±0.07 and 0.02 freq/hr). The rapid onset of maternal behaviour in newly parturient rat is stimulated in parturition by the hormonal changes accompanying pregnancy, the parturient female displays full complement of maternal behaviour at birth and in some cases before parturition then decline with growth of pups (Bridges and Ronsheim 1990). Bernuzzi *et al.,* (1986) reported that aluminium treated mothers had no significant effect on nest building, retrieval of pups to nest and time spent with young following exposure to $ALCL_3$ in the dam`s diet from day 8 of gestation till parturition.

Pups which parentally exposed to aluminium had a greater effect on all neurobehavioural parameters and suffered from learning deficits in which the time needed for correct choice in maze leaning test was significantly higher as compared to control ones (Kaoud *et al.,* 2008). Moreover, the amount aluminium chloride ingested by rats had significantly longer (P<0.05) time spent in latency to closed arms and time spent in closed arm for 3g/l group (6.80±1.32 and 255.41±8.25 sec) and shorter time in offspring of rats treated with 2g/l aluminium chloride (4.59±0.96 and 254.43±7.51 sec) and very short time in pups from dam fed no aluminium (1.79±0.93 and 170.75±22.23 sec). However, the number of entries to closed arms was significantly higher in offspring from dam ingested 3.5g/l aluminium (3.48±0.28) than those ingested 3g /l aluminium (2.75±0.28) and 2g/l aluminium (2.54±0.22).

The presented data in Table (4) deducted that pups from dams that did not receive any aluminium spent very short time to enter open arms in elevated plus maze (1.07±0.49 sec) and spent very long time in open arms (127.39±22.48

sec) with much number of entries into open arms (2.07±0.26) and highly open/closed arm ratio (99.38±0.50). On contrary dams consumed 2g/l aluminium chloride spent significantly ($P<0.05$) longer time to enter open arms (1.69±0.55 sec), spent short time in it (36.56±7.65 sec) and less number of entries (1.31±0.21) and low level of open/closed ration (97.32±0.9). This could be attributed to several Mg^{+2} dependent enzyme systems which could be disturbed as a result of its substitution with aluminium; moreover, infants might be at particular risk of aluminium toxicity because of the immaturity of the blood-brain barrier, the gut and renal system. Furthermore, the hippocampus and the cerebral cortex are the key structures of memory formation because the hippocampus is especially indispensable in the integration of spatial formation, a decline in learning ability may be induced by the deterioration of hippocampal function and however the frontal cortex plays a critical role in both spatial and nonspatial working memory. In addition, there may be domain-specific subdivisions within dorsal and ventral regions of the lateral prefrontal cortex which sub serve working memory for spatial and nonspatial information (Bimonte-Nelson *et al.,* 2003).

Moreover, vital processes involving rapid $Ca^{\pm 2}$ exchanges between extracellular and intracellular calcium compartments would be impaired by substitution of $Al^{\pm 3}$ (Bonnie and Carson 2000). In this regard, the impairment of neural exchange of $Ca^{\pm 2}$ ions by aluminium would result in vitiation and spoiling of dopaminergic pathways (DA) since the accumulation of dopaminergic pathways (DA) is mainly mediated through its effects on potassium and calcium channels (Waxman, 1997).

From these results it could be concluded that administration of aluminium chloride to rats in drinking water resulted in deviations in the normal behaviour of adult female rats and this deviation extend to the offspring of the dam drink water containing aluminium chloride which was obvious in the form of increment in the time spent in closed arms of elevated plus maze and avoidance of open arms of the maze.

Table 3: Least square means and their standard error of the effect of aluminium chloride ($Alcl_3$) and period of lactation on maternal behaviour of rats.

		Licking (Freq/hr)	Nursing (Min/hr)	Nest building (Freq/hr)	Retrieving (Freq/hr)
Treatment	**Control**	2.30±0.34^{b}	33.38±4.98^{b}	0.39±0.06^{a}	0.10±0.01^{a}
	2g/l $Alcl_3$	3.48±0.37^{a}	37.49±1.40^{a}	0.75±0.15^{a}	0.27±0.07^{a}
	3g/l $Alcl_3$	2.08±0.21^{b}	34.81±1.10^{ab}	0.64±0.17^{a}	0.19±0.04^{a}
	3.5g/l $Alcl_3$	1.63±0.17^{b}	32.72±1.31^{b}	0.51±0.09^{a}	0.19±0.04^{a}
Period	**1st Week lactation**	3.49±0.33^{a}	37.15±1.33^{a}	0.64±0.13^{ab}	0.23±0.06^{a}
	2nd Week lactation	2.26±0.17^{b}	34.19±1.14^{ab}	0.83±0.16^{a}	0.32±0.06^{a}
	3rd Week lactation	1.40±0.18^{c}	32.97±0.98^{b}	0.33±0.07^{b}	0.05±0.02^{b}

Means within the same column under the same category carry different superscripts are significantly different.

Table 4: Least square means and their standard error of the effect of aluminium chloride ($Alcl_3$) on learning ability of rat's offspring.

Treatment	Latency to closed arm (Sec)	Time spent in closed arm (Sec)	No of entries in closed arm	Latency to open arm (Sec)	Time spent in open arm (Sec)	No of entries in open arm	Ratio of open/closed arms
Control	1.79±0.93^{b}	170.75±22.23^{b}	3.36±0.49^{ab}	1.07±0.49^{a}	127.39±22.48^{a}	2.07±0.26^{a}	99.38±0.50^{a}
2g/l $Alcl_3$	4.59±0.96^{ab}	254.43±7.51^{a}	2.54±0.22^{b}	3.28±0.90^{a}	38.20±6.68^{b}	1.41±0.21^{b}	97.54±0.52^{b}
3g/l $Alcl_3$	6.80±1.32^{a}	255.41±8.25^{a}	2.75±0.28^{ab}	1.69±0.55^{a}	36.56±7.65^{b}	1.31±0.21^{b}	97.32±0.49^{b}
3.5g/l $Alcl_3$	6.75±1.34^{a}	248.48±7.93^{a}	3.48±0.28^{a}	1.92±0.71^{a}	42.85±7.97^{b}	1.40±0.16^{b}	97.11±0.53^{b}

Means within the same column under the same category carry different superscripts are significantly different.

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