

## Effects of Aluminium Chloride on Anxiety-Related Behaviour

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**Abstract: Problem statement:** Aluminium is presents in many manufactured foods, medicines and is also added to drinking water for purification purposes. It has the potential to be neurotoxic in human and animals but its contribution in Alzheimer's disease remains contradictory. Anxiety affects one-eighth of the total population world-wide and has become an important area of research interest in psychopharmacology. **Approach:** The present study was aimed at evaluating the effects that Aluminium chloride could have on anxiety-related Behaviour of Adult wistar Rats. A total of Ten (10) Adult wistar rats were used for this experiment. The wistar rats were divided into five groups: Group I was the control that received distil water, Group II received 475 mg Kg<sup>-1</sup>, Group III received 950 mg kg<sup>-1</sup>, Group IV received 1,425 mg kg<sup>-1</sup> and Group V received 1,900 mg kg<sup>-1</sup> through oral intubation for duration of Eight weeks. Elevated plus maze was used to assay for anxiety-related behaviour of the wistar rats. The wistar rats were placed at the junction of the four arms of the maze, facing a closed arm, allowed to freely explore the maze and their behavior was recorded simultaneously for 5 m by means of a video camera. **Results:** Wistar rats treated with Aluminium Chloride had, increased faecal boli, increased number of time crossing close arm entries and increased average time spent in close arms; but decreased time (lesser time) spent in the open arm of the maze when compared with the control group. This in turn implies that the Aluminium treated groups were more anxious than the control groups in exploration of their activities on the Elevated plus maze. **Conclusion:** It was concluded that Aluminium Chloride exposure has negative effects on anxiety-related behaviour of wistar rats as indicated by increased rate of anxiety in aluminium treated rats.

**Key words:** Elevated Plus Maze (EPM), manufactured foods, Aluminum (Al), aluminium containing compounds, oppositely positioned, aluminium treated rats

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### INTRODUCTION

Aluminum (Al) has the potential to be neurotoxic in human and animals. It presents in many manufactured foods and medicines and is also added to drinking water for purification purposes (Newairy *et al.*, 2009). Al is widely used in antacid drugs, as well as in food additives and tooth paste (Malekshah *et al.*, 2005). Environmental pollution with different aluminium containing compounds, especially those in industrial waste expose people to higher than normal levels of Al (Kloppel *et al.*, 1997). Particulate matters distributed by cement-producing factories contain, high amount of Al and animals and populations residing in the vicinity are exposed to the pollution (Fatima *et al.*, 2001). Al is a possible contributing factor in Alzheimer's disease (Campbell, 2002). Evidence for the contribution of Al to Alzheimer's disease (AD, remains

contradictory (Flaten, 2001; Gupta *et al.*, 2005). However, epidemiological studies have indicated a link between Al in drinking water and AD and a variety of human and animal studies have implicated learning and memory deficits after Al exposure (Buraimoh *et al.*, 2011a; Exley, 2005; Schmidt *et al.*, 2001; Yokel, 2000). It has been shown clearly that aluminium accumulates in various mammalian tissues such as brain, bone, liver and kidney (Wills *et al.*, 1993; Sahin *et al.*, 1994) and is accompanied by renal failure (Alfrey, 1980) or associated with age (Gomez *et al.*, 1997).

Although aluminum has been implicated in Alzheimer's disease, Parkinsonism, Dementia complex and causes extensive damage to the nervous system, to date the mechanism of Al neurotoxicity has not been fully elucidated (Niu *et al.*, 2007). In recent researches, aluminum has been reported to accelerate oxidative damage to bio molecules like lipid, protein and nucleic

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acids (Jyoti *et al.*, 2007). It was also reported that aluminium chloride could induce brain damage which may impair memory and learning as seen in Alzheimer disease (Buraimoh *et al.*, 2011a). This element (Al) appears mainly in food products and in drinking water from both natural sources and treatment methods (Yokel and McNamara, 2001).

Anxiety is a general term for several disorders that cause nervousness, fear, apprehension and worrying. These disorders affect how we feel and behave and they can manifest real physical symptoms. Mild anxiety is vague and unsettling, while severe anxiety can be extremely debilitating, having a serious impact on daily life. Anxiety affects one-eighth of the total population world-wide and has become an important area of research interest in psychopharmacology during this decade. Anxiety is considered a problem when symptoms interfere with a person's ability to sleep or otherwise function. Generally speaking, anxiety occurs when a reaction is out of proportion with what might be normally expected in a situation. Benzodiazepines are the major class of compounds used in anxiety and they have remained the most commonly prescribed treatment for anxiety. However, the realization that benzodiazepines present a narrow safety margin between the anxiolytic effect and those causing unwanted side effects has prompted many researchers to evaluate new compounds in the hope that other anxiolytic drugs will have less undesirable effects (Grundman *et al.*, 2007). Basic research of complex behaviour has led to a dramatic increase in the use of rodent behaviour tests (Crawley, 2007). The Elevated Plus Maze (EPM) has been described as a widely used behavioural assay for anxiety behaviour responses of rodents, mice or rats (Pellow *et al.*, 1985). The aim of this experiment was to evaluate the effects that aluminium chloride could have on anxiety-related behaviour of Adult wistar rats.

## MATERIALS AND METHODS

This research work was conducted in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

**Experimental animals:** Ten (10) Adult wistar rats were used for this experiment. The wistar rats were housed in a stainless steel cages maintained at standard environmental conditions (12-12h light-dark cycle with light on at AM) with sufficient food, water and under good ventilation. The wistar rats were kept for two weeks (14days) before commencement of administration. This was to enable them to acclimatize.

**Experimental design:** The wistar rats were divided into five groups: Control group was given distil water

while the four Aluminium exposed groups were given various concentrations of aluminium chloride as follows: Group I was the Control:

- Group II received 475 mg Kg<sup>-1</sup>
- Group III received 950 mg kg<sup>-1</sup>
- Group IV received 1,425 mg kg<sup>-1</sup>
- Group V received 1,900 mg kg<sup>-1</sup> (LD25)

The LD50 was 3,800 mg kg<sup>-1</sup>. The duration of administration was Eight weeks and the route of administration was through oral intubation.

**The elevated plus maze:** The Elevated Plus Maze (EPM) is a piece of equipment and protocol used by behavioural scientists to measure anxiety levels in laboratory rodents, Mice, rats. Different-sized mazes can be used for either rats or mice, but the same principles apply to each experiment. The elevated plus maze has four arms, arranged in a + shape; two of these arms have sides, so providing some shelter and a more secure feeling for the animal and the other two arms are open. The EPM apparatus consists of a plus shaped (“+”) maze elevated above the floor with two oppositely positioned closed arms, two oppositely positioned open arms and a center area. The two opposite arms, 50×50 cm, crossed with two enclosed arms of the same dimension but having 40 cm high walls. The arms were connected with a central square, 10×10cm, giving the apparatus shape of a plus sign. The maze was kept in a room and elevated 50 cm above the floor. Wistar rats were placed individually in the centre of the maze, facing an enclosed arm. Thereafter, number of entries and time spent on the open and enclosed arms were recorded during the next 5 min. An arm entry was defined when all four paws of the wistar rats were in the arm. The premise behind the elevated plus maze is that more anxious rats will spend less time in the uncovered, exposed arms of the maze and less anxious rats will explore the open arms more freely (Shepherd *et al.*, 1994).

**Experimental procedure:** After oral administration of various concentrations of Aluminium chloride to each group of the Wistar rats except group I (control) that received distill water, the wistar rats were placed at the junction of the four arms of the maze, facing an enclosed arm, allowed to freely explore the maze and their behaviour was recorded simultaneously for 5 min by means of a video camera mounted above the maze and the records analyzed. The preference for being in open arms over closed arms was calculated to measure anxiety-like behaviour and an increase in open arm activity (duration and/or entries) reflected anti-anxiety behaviour.

**Statistical analysis:** SPSS for Windows Version 10.0 was used to perform one-factor Analysis of Variance (ANOVA) in order to compare the results of the treated with the control and to check whether the differences observed was statistically significant or not. The probability level was set at  $p < 0.05$  (i.e., 95% confidence interval).

## RESULTS AND DISCUSSION

Aluminium (Al) is one of the most abundant metals in the earth's crust. Human exposure to Al has been increasing over the last decades. This element appears mainly in food products and in drinking water derived from both natural sources and treatment methods (Yokel and McNamara, 2001). Drinking water contribution has been estimated to be ranging from 3-8% (WHO, 2002) Health Canada, 1997. Role of Al intoxication in different organs in neurodegenerative diseases has been recently emphasized (Somova *et al.*, 1997; Exley, 1999). Buraimoh *et al.*, 2011b stated that Aluminium chloride exposure has negative effects on behavioral endpoints of wistar rats (i.e., alters behaviour) and can impair learning and memory. In this study, the effects of Aluminum chloride on anxiety-like behavior in wistar rats were shown in Fig. 1-5. The locomotor activity of wistar rats was assessed by the number of crossing squares noted as scores per time of 5 min for each wistar rats. The numbers of times of entries of wistar rats into both opened and closed arms of the elevated plus maze significantly increased across the treated groups (Fig. 4-5 and Table 4-5). There was increase in defecation which may be due to heightened autonomic activity in the treated groups when compared with the control group (Fig. 3-5 and Table 3-5). There was also increased number of time crossing close arm entries (Fig. 4 and Table 4) and increased average time spent in close arms (Fig. 2 and Table 2); but decreased time (lesser time) spent in the open arm of the maze when compared with the control group (Fig. 1 and Table 1).

The preference for being in open arms over closed arms was calculated to measure anxiety-like behavior and an increase in open arm activity in control group (duration and/or entries) reflected anti-anxiety behaviour (Fig. 1 and Table 1). There was gradual significant decrease in open arm activity (duration/time spent in open arms) of the elevated plus maze from groups I to V. (Fig.1 and Table 1). This was eminent in the control group I, that exhibited an anti-anxiety behaviour (Increased open arms activity) when compared with the treated groups II to V (decreased open arms activity).

Table 1: Analysis of Variance of the time spent in open arms of the elevated plus maze

Groups	Count	Sum (sec)	Average (sec)	Variance
X1	2	570	285	50
X2	2	530	265	50
X3	2	390	195	50
X4	2	340	170	200
X5	2	260	130	200

p-value = 0.000113

Table 2: Analysis of variance of the time spent in close arms of the elevated plus maze

Groups	Count	Sum (sec)	Average (sec)	Variance
X1	2	30	15	50
X2	2	70	35	50
X3	2	210	105	50
X4	2	260	130	200
X5	2	340	170	200

p-value = 0.000113

Table 3: Analysis of variance of the number of faecal boli

Groups	Count	Sum	Average	Variance
X1	2	0	0.0	0.0
X2	2	3	1.5	0.5
X3	2	4	2.0	0.0
X4	2	6	3.0	0.0
X5	2	7	3.5	0.5

P-value = 0.0003265

Table 4: The number of time on close Arm entries

Groups	Count	Sum (sec)	Average (sec)	Variance
X1	2	8	4.0	0.0
X2	2	13	6.5	0.5
X3	2	17	8.5	0.5
X4	2	19	9.5	0.5
X5	2	22	11.0	0.0

P-value-0.000327

Table 5: The number of time on open Arm entries

Groups	Count	Sum (sec)	Average (sec)	Variance
X1	2	4	2.0	0.0
X2	2	8	4.0	0.0
X3	2	11	5.5	0.5
X4	2	16	8.0	0.0
X5	2	18	9.0	0.0

P-value = 1.73 E-05

The preference of wistar rats for staying in closed arms over open arms observed in the treated groups was used as a measure of anxiety-like behaviour and hence Aluminium chloride in this study was implicated in anxiety-like behaviour unlike the control that showed preference for more exploration of open arms, showed by statistically significant increased time spent in open arms and less exploration of closed arms in control group when compared with the treated groups (Fig. 1 and 2; Table 1 and 2) and hence Aluminium may affect their learning ability as shown in Fig. 1 and Table 1. This agreed with the study of (Berlyne *et al.*, 1972; Deloncle and Guillard, 1990) that stated that long-term administration of soluble salt of Aluminium to rats worsens their learning ability together with diminished cholinergic function and the rats become lethargic.

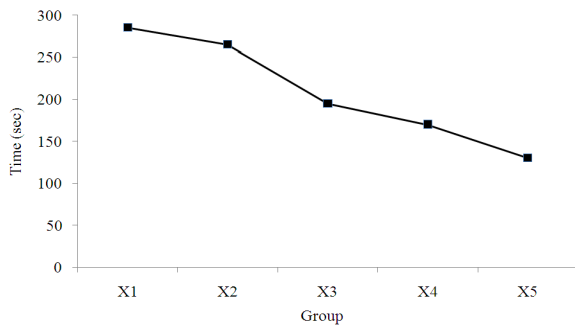


Fig. 1: Graph of average time spent in open Arms of the elevated plus maze

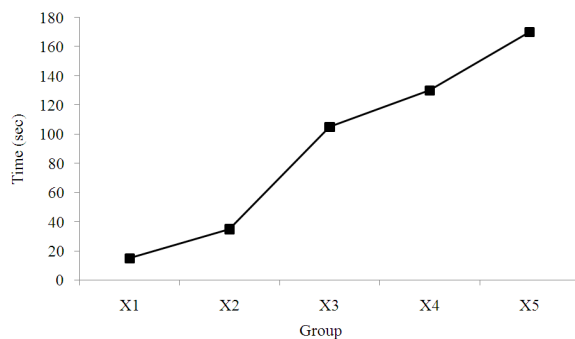


Fig. 2: Graph of average time spent in close Arms of the elevated plus maze

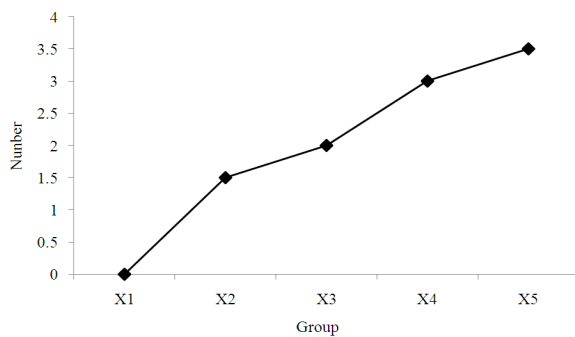


Fig. 3: Graph of the number of faecal boli

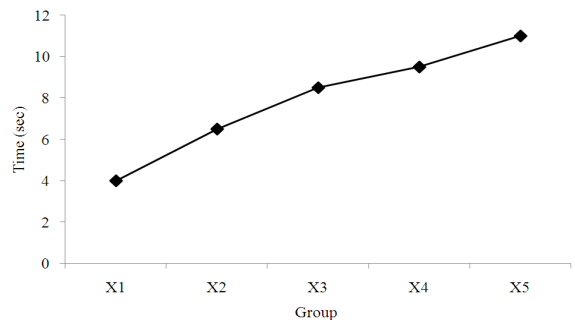


Fig. 4: Graph of number of time on close Arm entries

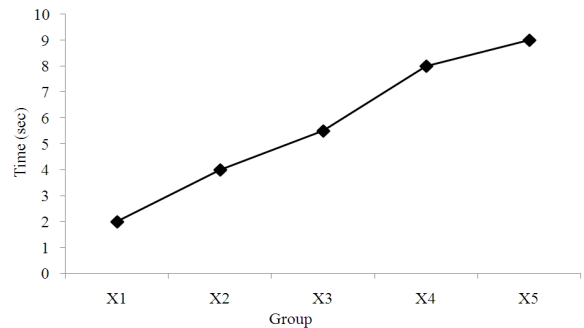


Fig. 5: Graph of the number of time on open Arm entries

Our observations revealed that the wistar rats treated with Aluminium Chloride had increased faecal boli across group, spent lesser time in the open arm of the maze and more time in closed arm than the control group and it was statistically significant at  $p < 0.05$  (Table 1-3 and Fig. 1-3). This in turn indicated that the Aluminium treated groups were more anxious than the control groups in exploration of their activities on the maze (Fig. 1 and Table 1). Based on our observations, we therefore conclude that Aluminium Chloride exposure has negative effects on anxiety-related behaviour of wistar rats as indicated by increased rate of anxiety in aluminium treated rats.

### CONCLUSION

It was concluded that Aluminium Chloride exposure has negative effects on anxiety-related behaviour of wistar rats as indicated by increased rate of anxiety in aluminium treated rats.

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