Neurobehavioural dysfunction in experimental fluorosis

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Abstract:
To assess the toxic effect of sodium fluoride on neurobehavior the present study was performed. Young and healthy Wistar albino rats weighing 100-200 g were randomly grouped in four separate cages. Group I was given 1 ml double distilled deionized water/kg body weight/day for forty days and kept as control. The remaining groups II, III and IV were treated with 100, 200 and 300 ppm of sodium fluoride/kg body weight/day via oral gavage for the same period. After treatment period rats were assessed for behavioral parameters exploratory and non-exploratory motor activity by open field habituation test. A significant (p<0.05) influence on exploratory motor activity assessed on sodium fluoride administered to rats was noted on first occurrence to the open field test. The rats exposed to different sodium fluoride concentrations demonstrated a significant (p<0.05) difference in anxiogenic profile of behavioral changes, as measured by time spent freezing and vegetative behaviors. There was a significant(p<0.05) session factor diminishing effect due to habituation over the course of three successive test sessions in open field test.

Key words: Neurobehavioral toxicity, Open field test, Sodium fluoride, Wistar albino rats

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INTRODUCTION

Fluoride is essential and has beneficial effects on bones and teeth if present in drinking water in the appropriate range of 0.7–1.5 mg/L (WHO, 2011). Beyond this limit, exposure to fluoride in drinking water can cause a number of adverse effects. Toxic effects of excessive intake of fluoride are matters of serious international concern (Verma et al., 2006). Since fluoride has evidenced to cross blood brain barrier, a link between excessive exposure to fluoride and dysfunction of the central nervous system has established (Blaylock, 2004; Ge et al., 2005). Prolonged exposure to sodium fluoride has been reported to induce deleterious effects on soft tissues and changes in behaviour through locomotor activity impairment (Ekambaram and Paul, 2001, 2003). The severity of the adverse effects of fluoride on the behavior of rats is directly correlated with the concentrations of fluoride ion in the plasma and in specific regions of the brain (Mullenix et al., 1995). There is also evidence that subchronic (30 days) and chronic (90 days) sodium fluoride administration (100 mg sodium fluoride per liter) impaired step-down inhibitory avoidance (Wu et al., 2006). Moreover, Wang et al. reported that 30-day-old offspring rats that had been exposed to high fluoride levels since conception presented lower scores in the step-down inhibitory avoidance task.

Non-associative behavioral habituation affords one of the most essential forms of learning, both in animals and in humans. Open field is a potentially useful model for simultaneous assessment of anxiety and memory (Weiss and Greenberg, 1996). The open field has been long established as an appropriate test for measuring situational anxiety in rodents (Millan, 2003). Rats submitted for the first time to an open field display higher spatial exploration, a form of information storage (Eichenbaum, 1996), than in successive exposures. Thus, the decrement in response to successive exposures is
taken as an index of memory of habituation (Izquierdo et al., 2001; Winograd and Viola, 2004). A deteriorating effect has been reported for sodium fluoride on performance in some memory tasks such as open field habituation in rats (Pereira and Dombrowski, 2009).

The involvement of various arrays of measurements to properly evaluate both associative and non-associative neurobehavior in rats is not well implemented. Thus, the objectives of the current study were to find out the influence of exposure to different concentrations of sodium fluoride in rats on neurobehavior.

MATERIALS AND METHODS

**Animals and housing:** Young and healthy Wistar albino rats weighing 100-200 g were housed in standard polypropylene cages with stainless steel grill tops and bedded with paddy husk. Animals were maintained on a 12 hours light and dark cycle at a room temperature of 25±2°C with free access to feed (standard laboratory pellets) and water throughout the study. The animals were acclimatized to the laboratory conditions for two week prior to the experimentation. The experimental protocol was approved by Institutional Animal Ethics Committee, Punjabi University, Patiala (Approval no.107/99/CPCSEA-2012-10).

**Experimental design:** Rats were weighed and randomly divided in four groups with six rats per group. The administration lasted for forty consecutive days which was done via oral gavage. Group I was given 1 ml double distilled water/kg body weight/day and was kept as control group, while the remaining group II, III and IV was treated with 100, 200 and 300 ppm respectively.
Open Field habituation Test: The locomotor activity and habituation were measured in the open field test after completion of sodium fluoride treatment by methods of Walsh and Cummins(1976). The open field used was a square wooden arena measured (90 x 90 x 25cm). The floor was divided by black lines into 36 small squares (15 x 15cm). Behavioral test was recorded with a video camera adjusted above the center of the arena. Rats were gently placed into center of the arena and allowed to explore the apparatus for 3 minutes. During the three minutes of exploration, the time spent freezing (no movement) was quantified. Exploratory motor activity measures included horizontal locomotion (the number of squares crossed) as well as vertical activity (rearing). Non-exploratory measurements comprised only the vegetative behaviour (numbers of urination episodes and defecated fecal boli). After the 3 minutes test session, the rat was returned to its home cage and the open field was cleaned using 70% ethyl alcohol. To assess the process of habituation to the novelty of arena rats were exposed to the apparatus for a 3 minutes test session, on three consecutive days.

Statistical Analysis: Behavioral test parameters were analyzed using the general linear models procedure in SPSS® statistical software (SPSS, 2016). When ANOVA was significant, post hoc Bonferroni test was conducted for individual comparisons. A probability of p<0.05 was considered significant for all evaluations. All data are expressed as mean ± SEM.

RESULT

A repeated measures ANOVA with a Sphericity Assumed correction determined that mean of dose effect differed statistically significantly between different dose group. A significant (p<0.05) influence on exploratory motor activity
assessed on sodium fluoride administered to rats was noted on first occurrence to the open field test. There was a significant ($p<0.05$) dose difference for horizontal activity (numbers of crossed squares: $F_{(3, 48)}=8.416; p=0.0001$) and vertical activity (numbers of rearing; $F_{(3, 48)}=7.170; p=0.0001$). The rats exposed to different sodium fluoride concentrations demonstrated a significant ($p<0.05$) difference in anxiogenic profile of behavioral changes, as measured by time spent freezing and vegetative behaviors. There was a significant ($p<0.05$) dose effect in duration of freezing, with rats administered 300 ppm sodium fluoride spent more time freezing than 200 ppm, 100 ppm and their counterparts in control group ($F_{(3, 48)}=3.725; p=0.017$). For vegetative behaviors non-significance differences were recorded for urination episodes ($F_{(3, 48)}=1.543; p=0.215$), defecation (numbers of fecal boli: $F_{(3, 48)}=1.120; p=0.350$) and numbers of grooming time ($F_{(3, 48)}=0.226; p=0.878$) in fluoride treated groups and control rat.

There was a significant ($p<0.05$) session factor diminishing effect due to habituation over the course of three successive test sessions of open field on the following parameters: horizontal activity (numbers of crossed squares: $F_{(2, 40)}=41.364; p=0.0001$), vertical activity (numbers of rearing: $F_{(2, 40)}=18.615; p=0.0001$) and freezing time ($F_{(2, 40)}=26.071; p=0.0001$). There was non-significant effect on defecation (numbers of fecal boli: $F_{(2, 40)}=0.155; p=0.857$), numbers of urination episodes ($F_{(2, 40)}=0.011; p=0.989$) and numbers of grooming time ($F_{(2, 40)}=2.354; p=0.103$).

At the first occurrence in open field, statistical analysis revealed a significant ($p<0.05$) dose influence for locomotor activity, where 300 ppm sodium fluoride group displayed more horizontal locomotion (Fig.1) ($F_{(3, 20)}=6.009; p=0.004$) and higher vertical activity (Fig. 2) ($F_{(3, 20)}=9.049; p=0.001$) as compared to control group. In addition, 300 ppm dose groups were the most anxious group as indicated by duration of freezing time (Fig.3) ($F_{(3, 20)}=5.284; p=0.008$), and numbers of urination episodes...
(Fig.4) \( F(3, 20) = 5.037; p = 0.009 \) while numbers of defecated fecal boli (Fig.5) \( F(3, 20) = 3.041; p = 0.053 \) and numbers of grooming time (Fig.6) \( F(3, 20) = 0.192; p = 0.901 \) had non-significant change.

**DISCUSSION**

At present, numerous experiments have already demonstrated that behavior changes can occur in an organism exposed to doses less than that which would cause obvious structural irregularities. In open field test, spontaneous activity and habituation are measured for detecting both ambulation and rearing. For assessment of emotionality, the open field is one of the simplest and most popular tests currently in use (Weiss and Greenberg, 1996). The test is employed to assess anxiety-related conflict arising between the drive to explore by venturing into the center of the arena and safety by remaining in a corner or along a wall (Weisstaub et al., 2006).

Our study found that there was a significant decrease in horizontal activity (number of squares crossed) in 300 ppm sodium fluoride treated group on second and third day of exposure to open field as compared to control and is in agreement with the results of Sarkozi et al. (2015), who found marginal decrease in ambulation and increase in immobility and local activity in the open field in the third and sixth week. Reduced open field habituation is comparable with findings of Pereira et al. (2011), in which they found fluoride intake impaired the habituation of the rats to the open field. However El-letheyet et al. (2010) and El-lethey&Kamel (2011) found no significant differences in horizontal activity (numbers of line crossings) in open field. A significant effect of sodium fluoride regarding the number of crossed squares in the test session was observed. Both the 50 and 100 ppm NaF groups exhibited higher locomotor activity compared to the 1.54 ppm group (Chioca et al., 2008)
In this study and as a trial to dissociate between “general activity” and “exploration”, ambulation was only related to horizontal locomotion (amount of distance traveled) than vertical activity which is more sensitive to anxiety state of the individual (Lapin et al., 1995; Brown et al., 1999). In the present result there is a significant decrease in vertical (rearing) activity in rat exposed to 300 ppm sodium fluoride than 100 ppm and 200 ppm sodium fluoride and control group rats. This is corroborated by results of Kamal et al. (2010), Ellethey et al. (2010) in rats. During the three months study period, (Sandeep et al., 2013) the sodium fluoride exposed mice (10ppm and 5ppm) were more active and restless compared to the control group. With regard to crossings, rearings, sniffings, groomings of open field behavior in the NaF exposed group have shown significant decrease.

In present study 300 ppm sodium fluoride treated rats spent more time being immotile than 200ppm, 100 ppm sodium fluoride and control group. Our finding is in accordance with the results of earlier study of Kamal et al. (2010), Ellethey et al. (2010), which resulted that rats exposed to sodium fluoride spent more time freezing on the first exposure to open field test. Increased immobility in the open field is characteristic for increased levels of anxiety. An inhibition of some of these body positions and activities were impaired by high fluoride exposure. The results in the high fluoride group were consistent with animal data reported earlier (Paul et al., 1998 and Ekambaram and Paul, 2001).

Elevated number of urination episodes, defecation (numbers of fecal boli) and grooming time were recorded for 300 ppm dose than 200 ppm 100 ppm sodium fluoride and control group rats on whole test session. This is incompliance with results of El-lethey et al. (2010). Kivrak, (2012) reported in open field test, the number of defecations was higher in the study group, and transitions, rearing, and grooming activities were less frequent. Under these conditions, fluoride appeared to have
no antidepressant effect in mice but rather an anxiety stimulating effect.

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CONFLICT OF INTERESTS
The authors declare that they have no conflicts of interests.

REFERENCES
Female Mice with Special Reference to Behavioral Studies and ACh and AChE Levels. *p<0.001 as compared to control group.

Figure 1. Effect of different doses of sodium fluoride on horizontal activity in open field test on albino rats. Data are represented as mean of six rats per group. * p<0.0001 as compared to control group.

Figure 2. Effect of different doses of sodium fluoride on vertical activity in open field test on albino rats. Data are represented as mean of six rats per group. * p<0.0001 as compared to control group.

Figure 3. Effect of different doses of sodium fluoride on freezing time in open field test on albino rats. Data are represented as mean of six rats per group.
Figure 4. Effect of different doses of sodium fluoride on urination episodes in open field test on albino rats. Data are represented as mean of six rats per group.

Figure 5. Effect of different doses of sodium fluoride on defecation activity in open field test on albino rats. Data are represented as mean of six rats per group.

Figure 6. Effect of different doses of sodium fluoride on grooming time in open field test on albino rats. Data are represented as mean of six rats per group.