EFFECTS OF FLUORIDE ON ANXIETY AND DEPRESSION IN MICE

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SUMMARY: The aim of this study was to evaluate the effects of fluoride on anxiety and depression in mice. Control and study groups were formed with ten 4-month-old male Swiss mice in each group. For 90 days, mice in the control group were given drinking water containing 0.3 ppm fluoride while the study group was given water with 40 ppm fluoride. Tail suspension was not significantly different between the study and the control groups. In an open field test, however, 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.

Keywords: Anxiety effect; Depression effect; Fluoride in mice; Open field test; Tail suspension test; Locomotor activity.

INTRODUCTION

Although fluorosis is known for its negative effects on teeth and bones,[1-3] in recent years many studies have found that fluoride (F) can cause impairment of brain function[4-6] and other organ systems.[7-12] While F has been shown to affect cognitive skills, its effect on anxiety and depression remains unclear. The aim of this study was to evaluate the effects of F on anxiety and depression in adult male mice.

MATERIALS, PROTOCOLS, AND METHODS

The study was conducted with 20 healthy, 4-month-old male Swiss mice weighing 28.8±1.18 g. The mice were randomly divided into two equal groups and kept in an environment with constant temperature (20–21ºC) and were exposed to light and dark on a 12-hr cycle. The first group was given drinking water containing 0.3 ppm F *ad libitum* for 90 days. The second group was given 40 ppm F with the same standard mouse diet. After 3 days of pause, the mice were tested in a random order in an open field test and a tail suspension test conducted between 9:00 am and 3:00 pm. Both tests were conducted once for each subject.

Open field test: The open field test for unconditional anxiety, first reported by Hall in 1936,[13] was used for the assessment of locomotor activity and indirect testing of normal anxiety. As one of the most popular tests of animal physiology, it has been used for studies on rats, mice, pigs, sheep, rabbits, and hens. Horizontal transitions (movement from one square to another), vertical transitions (rearing on hind limbs), grooming frequency, and the number of defecations are recorded over a certain period of time. There is a linear relationship between the locomotor activity and the number of transitions, the behaviour of exploring the environment, and rearing. Grooming and defecation are accepted as the indicators for autonomous functions.[14] Loss in numerical parameters indicates anxiety[14] and has been used in anxiety studies.[15-21]

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In our study, the open field test consisted of a plexiglass arena of 64 squares (80×80×40 cm) illuminated by fluorescent lighting. The mice were placed in the open field in the same corner square. They were observed for 6 min, and the number of transitions, frequency of rearing, amount of grooming, and number of defecations were recorded. Transition of 4 limbs from one square to the other was accepted as one square. Standing on 2 limbs was counted as rearing.[22-24] As in the literature, the arena was cleaned with 70% alcohol after every test, and the amount of grooming and defecation was recorded.[23]

Tail suspension test: This immobilization test is often used to assess behaviours related to depression.[25,26] In it the mice were hung from the last cm of their tails, and, for 6 min, inactivity time was measured in seconds. Results were recorded as ± SEM standard error of the mean. For statistical analysis the t test for two independent values was used. A value of $p<0.05$ was accepted as statistically significant.

RESULTS

As seen in the Table, in the tail suspension test, there was no statistically significant difference between the two groups regarding the time of immobilization. In the open field test, although the number of defecations was higher in the study group, the number of transitions, rearing, and grooming in the study group were less frequent than in the control group.

Table. Mean number (±SEM) of behavioral measures of 10 mice in each group[a]

Drinking water F (mg/L)	Rearing	Grooming	No. of squares	Defecation	Immobilization (seconds)
Control (0.3)	35.2±1.98	3.3±0.33	258.3±15.3	2.8±0.44	234.20±15
Study (40)	26.3±1.48*	2.1±0.23*	184.4±18.85*	4.7±0.47*	236.10±19

[a]Rearing, grooming, number of squares and defecation were counted within six minutes for each mouse. Immobilization test was conducted for each mouse in a separate six-minute period. All tests were conducted once.
*Compared with the control $p<0.01$.

DISCUSSION

Depending on what it is associated with (e.g., aluminium), F can pass through the brain-blood barrier and accumulate in the brain and cause damage in neuronal development and function by decreasing neural synapses, neurotransmitter synthesis, and the number of receptors.[27-29] It also impairs the central neural system, leading to fatigue, malaise, insomnia, headache, lethargy, daze, mental impairment, and memory dysfunction,[30] It has been suggested that F has a

negative effect on cognition and memory by affecting protein and enzyme systems.[31] In some villages in India with high fluoride water, the number of people suffering from headache, insomnia, and lethargy was higher than in low F water villages.[32] Psychological interactions occur in these patients due to biochemical changes in their enzyme systems.[30] According to Emsley et al., F can disrupt N-H bonds in proteins by causing N-H-F bonds to form.[33] F also disrupts the structure of cytochrome P peroxidase,[34] thereby affecting basic cell energy and the structure of G proteins and causing behavioral changes.

As seen here, the study group of mice showed a significant decrease in horizontal and vertical activity and grooming in agreement with increased anxiety. The number of defecations was also higher in the study group. In female Wistar rats, Paul et al. found a decrease in locomotor activity from F intake.[35] Similarly, Mullenix et al. observed diminished locomotor activity in their study of rats on high F intake and found that the decrease was proportional to increased F concentrations in the brain.[36] In agreement with our findings, Ekambaram et al. also reported decreased motor activity in rats with high F intake.[37,38] Likewise, Niu et al. reported finding a decrease in locomotor activity in rats receiving 150 mg NaF/L in their drinking water.[39]

On the other hand, in the study by El-lethey et al., although the number of transitions, defecations, and rearings was higher in the rat group with exposure to 100 ppm NaF, the results were not statistically significant.[40] Bera et al. reported no change in locomotor activity in rats exposed to F perinataly.[41] Likewise, Chioca et al. concluded that locomotor impairment was not observed in rats given 50–100 ppm NaF in their drinking water.[42] The conflicting results of these studies may be due to methodological differences such as the difference in open field arena, the conduction time of the test, and the F dosage.

Depression is an important disorder that affects individuals and groups.[43,44] As seen in a recent editorial review, there are clearly neurotoxic effects of fluoride.[45] Even so, although the literature does not appear to report any animal studies revealing a F-depression relationship, the findings recorded here are important in showing that F has no antidepressant effects. In view of its restriction to only two experimental conditions, the present study has obvious limitations. Before these results can be generalized, further studies on anxiety and depression models are needed.

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