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Effects of Fluoride Exposure on Motor Performance, Depression and Memory in Elderly Mice

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Yaşlı Farelerde Florür Maruziyetinin Motor Performans, Depresyon ve Hafıza Üzerine Etkileri

Amaç: Florürün yaşlanma döneminde motor fonksiyonlar, davranışsal ve bilişsel işlevler üzerine etkilerini gösteren yeterli kanıt yoktur. Bu çalışmada yaşlı farelere oral gavaj yolu ile verilen sodyum florürün motor fonksiyonları, davranışsal ve bilişsel işlevleri etkileyip etkilemediğinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Balb-C fareleri rastgele seçilmiş ve kontrol, düşük ve yüksek doz (sırası ile 3 ve 9 mg / L sodyum florür) olma üzere 3 gruba ayrılmıştır. Tüm gruplara 28 gün süreyle sodyum florür maruziyeti sonrasında lokomotor aktivite testi, zorlu yüzme testi ve yeni nesne tanıma testi uygulanmıştır.

Bulgular: Hem düşük hem de yüksek doz sodyum florür gruplarında yaşlı farelerin, lokomotor aktivitelerinde azalma meydana gelmiş ancak anlamlı bir fark gözlenememiştir. Gruplar arasında, zorunlu yüzme testinde hareketsiz kalma süresinde meydana gelen artış, istatistiksel olarak anlamlı bulunmamıştır. Ancak, yeni obje tanıma testi sonuçları, yüksek doz (9 mg / L) grubunda, düşük doz (3 mg / L) ve kontrol grubuna kıyasla, fark indeksine göre istatistiksel olarak anlamlı bir fark olduğunu göstermiştir.

Sonuç: Bulgularımız, yaşlı farelerde yüksek doz sodyum florür maruziyetinin yeni obje tanıma testi sonuçlarına göre bilişsel işlev bozukluklarını artırabileceğini göstermektedir.

Anahtar Kelimeler: Florür, yaşlı, hafıza, kognitif fonksiyon, depresyon

Effects of Fluoride Exposure on Motor Performance, Depression and Memory in Elderly Mice

Aim: There is insufficient evidence to support the effect of sodium fluoride on motor, behavioral and cognitive functions in the aging period. We aimed to investigate whether sodium fluoride exposure by oral gavage affected the locomotor activity, behavioral and memory functions in elderly mice.

Material and Methods: Balb-C mice were randomly divided into three groups: control, low and high (3 and 9 mg/L sodium fluoride, respectively) dose groups. Locomotor activity test, forced swim test and novel object recognition test were performed after 28 days of feeding in all groups.

Results: Older mice in both low and high sodium fluoride dose groups exhibited decreased locomotor activity but no statistically significant difference was observed. The increase in the immobility time in the forced swim test was also not statistically significant among the groups. However, the novel object recognition test results showed significant difference in high dose (9 mg/L) group when compared to low dose (3 mg/L) and control groups regarding to discrimination index.

Conclusion: Our findings indicate that high dose sodium fluoride may induce impairments in cognitive function based on the object recognition test results in elderly mice.

Keywords: Fluoride, geriatric, memory, cognitive function, depression

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INTRODUCTION

Fluoride (F), an essential trace element for human body, is very widely distributed in the natural environment and it is a normal constituent of soft tissues, body fluids, teeth and bones (1). The principal sources of F are fluoridated water and F containing dental products. The protective effects of F on dental health were first observed in 1930 as there was less tooth decay in communities consuming naturally fluoridated water compared to non-fluoridated areas. Because of beneficial effects of F, it was begun to use in dentistry in 1940 and since then, it is being added to various consumer products such as toothpastes. Fluoride is consumed commonly through the hygiene of oral cavity and absorbed through the gastrointestinal tract (2, 3).

When consumed in adequate quantity, fluoride prevents dental caries, assists in the formation of dental enamels, and prevents deficiencies in bone mineralization. However there are many different exposure routes for human in last years, and ingestion of fluoride at excessive exposure levels causes dental fluorosis skeletal fluorosis, and manifestations such as gastrointestinal, neurological, and urinary problems (4). Despite these problems, fluoride sources have been increased by several products such as fluoridated foodstuffs, insecticides, groundwater, toothpaste, drugs, vapors released from industries using fluoride containing compounds and dentifrices (5). Fluoride toxicity targets not only the bone and teeth, but also soft tissues including kidney (6), brain (7), and blood (8) and it has a toxicity on cells, genes and immune system (9). As known, F is one of the most reactive elements. In the case of a toxic amount of fluoride in the body, fluoride attacks oxygen and disrupt the metabolism resulting in the production of hydrogen peroxide as a product. F induced free radical generation consequently results in oxidative stress due to its high electronegativity. In addition, fluoride results in excessive production of free radicles that disrupt the antioxidant formation (10).

Consumption of drinking water contaminated with fluoride is known to lead to neurotoxicity. Fluoride-induced psychiatric manifestations include memory impairment, lethargy and thinking difficulties. Excessive exposure to fluoride leads to loss of neuronal cell bodies, synaptic structures and inhibition of acetylcholinesterase enzyme activity, this may induce cognitive and neurobehavioral deficits common in patients with neurodegenerative diseases. Therefore, it is important to provide suitable antioxidants that have neuroprotective properties capable of suppressing the oxidative stress, neuroinflammation and apoptosis (11).

The researches related with fluoride is largely focused on dysfunction of the central nervous system or potential neurotoxicity. Fluoride is known to cross the blood–brain barrier, accumulate within various parts of the brain, and especially in the hippocampus altering its structure and function. Some animal and cell culture studies demonstrated that fluoride has harmful effects to the hippocampus and causes nonreversible neuronal damage leading to the

loss of neurons. Maladaptive changes of neural networks resulting in abnormal regulation of neuronal plasticity underlies the pathophysiology of major depressive disorders. Hence, these results suggest that the accumulation of F in brain tissue can disrupt the synthesis of certain neurotransmitters and receptors in neuronal cells of brain and may also provoke neural dysplasia or other damage. Recently, Yan-Jie et al. have reported that the levels of nicotinic acetylcholine receptors (nAChRs) are significantly declined in the brains of rats with fluorosis and in PC12 cells exposed to high concentrations of fluoride (12, 13, 14, 15). The nAChRs are transmitter-gated ion channels composed of α and β subunits. In human and animal brains, 42 and 7 nAChRs are the major types of the receptors, demonstrating physiological and pharmacological functions and involving in learning and memory-related behaviors and in neuroprotective effects (14,15). On the other hand, in experimental animal studies, the levels of acetylcholinesterase enzyme were seen to be increased significantly by given a dose of 5,15 or 50 ppm of F in their drinking water. The increased acetylcholinesterase might lower ACh levels and given that said enzyme degrades the ACh neurotransmitters, it has important impact of brain cognitive and other functions during aging (16). ACh plays an important role in regulating several different functions, such as the transition from sleep to wakefulness and processes of cognitive function, among others. Gao Q et al. have been reported to be altered the cognitive functions in mice treated with fluorinated water. The ability to learn has been found to be decreased in subjects who drink water with high concentrations of F in comparison with those who drink water containing a lower concentration of this element (17).

Excessive ingestion of fluoride may cause toxic and harmful effects on cognitive function during aging period. According to fluoride poisoning data collected by the American Association of Poison Control (AAPC), tooth paste ingestion remains the main source of toxicity followed by fluoride containing mouth washes and supplements (18). However, there are no evidence that show the effects of fluoride on cognitive functions and neurodegeneration aging period. The effect of F on learning and memory functions during aging is unknown or, the role of F consumption not known clearly in the ageing period. Therefore, we investigated the effects of low and high dose F on learning and memory functions, depression and motor activity in old mice.

MATERIALS AND METHODS

Animals

Total 17 male Balb-C (27±2 weeks old, 20-30 g) older mice were provided by the Experimental Animal Center of Yeditepe University. All mice were housed under controlled 12-h/12-h light–dark cycle at a room temperature of 22 ± 1 °C. Mice were given free access to water and food. Experiments were performed following 28 days of feeding. Before each test, the animals were placed in the laboratory for 30 min to be acclimated to

the test environment. The study protocols and tasks was approved by the Animal Ethical Comittee of Yeditepe University. All behavioral testing occurred during the light phase between 9 am and 9 pm and were conducted in compliance with animal cares guidelines and with protocols.

Fluoride administration

Based on fluoride (Sigma, Germany) concentrations, 17 mice were randomly divided into tree different group, as control group (water), Group L (low fluoride, 3 mg/L NaF) and Group H (high fluoride, 9 mg/L NaF). There were no significant differences in body weight among groups. During the test, the mice were continually treated with their respective NaF concentrations via oral gavage. Control animals received drinking water during the entire experiment.

Locomotor Activity Test

Locomotor activities were measured with an open-field activity monitoring system (MAY 9908 & 0107 model – Activity Monitoring System – Commat Ltd., TR). This system had one Plexiglas cage (42 cm × 42 cm × 30 cm) equipped with infrared photocells. Mice were placed into activity chamber one by one with the purpose of behavioral analysis for 10 minutes habituation period. Measurements of locomotor activity will be performed after 30 minutes from fluoride administration. Locomotor activity was recorded as a total of ambulatory, horizontal and vertical activity. The chamber was cleaned after each measurement and left to dry before each session. Experiments were performed in a sound-proof room (19, 20).

Porsolt Forced Swim Test

The Porsolt Forced Swim Test (FST) was applied to all groups on the 28th day. A transparent glass tank having height of 25 cm and width of 15 cm which contained water (22-23°C) at the height of 20 cm was use for the forced swim test. In this glass tank, each mouse was individually placed into the water and forced to swim for 10 minutes. When the animal is tired or despair, the animal eventually stops trying and he gives up swimming and begins to stay immobile. The immobility times (s) were monitored and recorded. An increase in the immobility time indicates depression-like behavior. After mice were placed one by one in the tank which was filled with water, each mouse's swimming and immobile behaviors observed for 10 minutes. When the animal stops swimming and floats on the surface of the water it is considered to have "given up". At the end of each session, each mouse was removed from the water, carefully dried with paper towels, returned to its home cage and left under the heater until it was dry (21).

Novel Object Recognition Test (NOR)

The Novel Object Recognition (NOR) test is used to evaluate cognition, learning and memory in rodent. This test is based on the spontaneous tendency of rodents to spend more time exploring a novel object than a familiar one. The NOR test is completed over 3 days: habituation day, training day, and testing day.

The choice to explore the novel object reflects the use of learning and recognition memory. Test is conducted in an open field plexiglass box (40 × 40 × 45 cm) with two different kinds of objects. The novel objects were different in shape and color but similar in size. The mice were considered to be exploring when they faced, touched, or sniffed the objects, and time spent for each object was recorded over a 10-min period. During habituation, the animals are allowed to explore an empty arena. Twenty-four hours after habituation, the animals are exposed to the familiar arena with two identical objects placed at an equal distance. The next day, the mice are allowed to explore the open field in the presence of the familiar object and a novel object. The time spent exploring each object was recorded and the discrimination index (DI) was calculated (DI = (novel − sample) / (novel + sample) (22, 23).

Statistical Analysis

The data were presented as a means ± standard error of mean. The group differences between low dose (3 mg/L), high dose (9 mg/L) and the locomotor activities in the sodium fluoride groups were analyzed using the two-way and one-way analysis of variance (ANOVA). The one-way ANOVA followed by Turkey's Post Hoc Test for multiple group comparison. Differences and correlations were considered significant at $p<0.05$. All the data were analyzed by SPSS for Windows 15.0

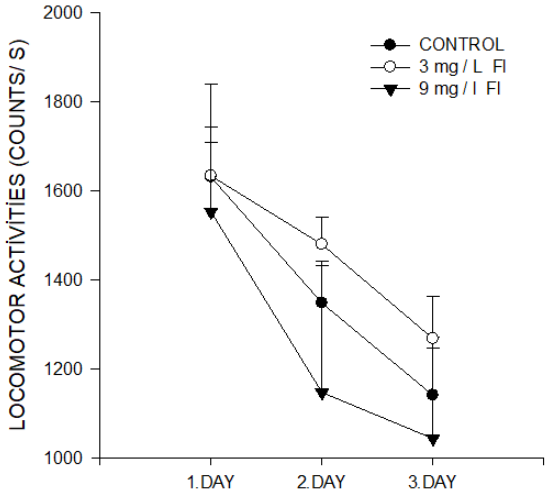
RESULTS

In order to evaluate the effects of fluoride on cognitive functions or dysfunction of cognitive performance in older mice, total locomotor activity measures, immobility performances in the forced swim test (FST) and novel object recognition test (NOR) were performed for all groups.

The Effects of Sodium Fluoride Exposure on Locomotor Activity Test

At the end of 4 weeks sodium fluoride consumption, older mice in all treatment groups (both low and high sodium fluoride dose groups) exhibited normal activity in the first 10 min (Fig. 1). No statistically significant differences were observed in all groups, but locomotor activity measures was decreased in high dose sodium fluoride administrated groups.

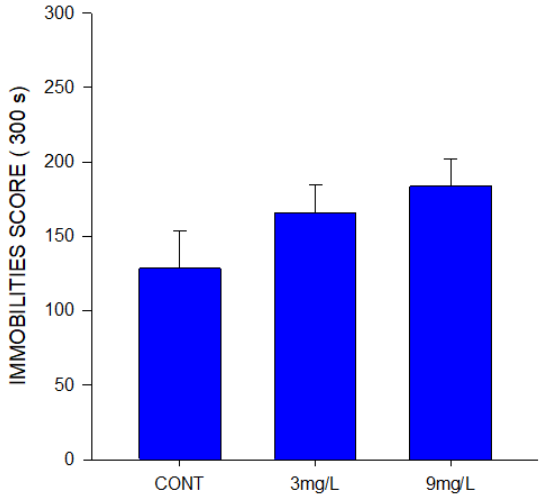
Figure 1. Effects of sodium fluoride on locomotor activity. Time course changes in LMA of sodium fluoride treated mice for 10 min.



The Effects of Sodium Fluoride Exposure on immobility time in the FST

The groups treated with high dose (9 mg/L) and low dose (3 mg/L) sodium fluoride resulted an increase in the immobility time when compared to control group (Fig. 2). However, there was no significant difference when compared to control group (F=1,796, p < 0.208).

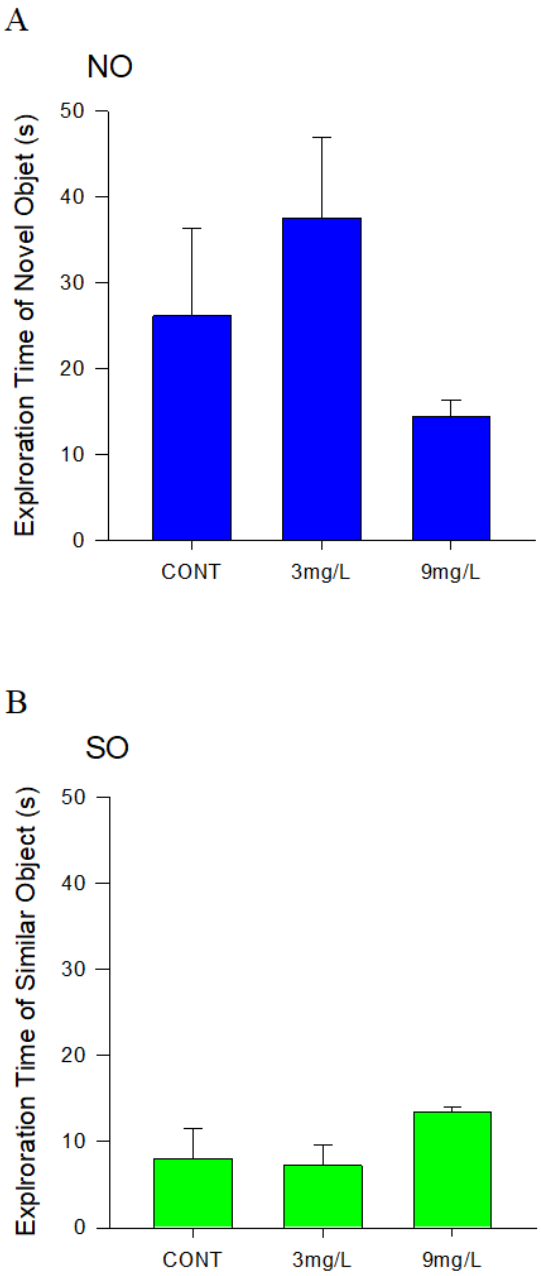
Figure 2. The effects of sodium fluoride exposure on the depression-like behaviors (n = 6 each). No significant differences when compared to control group for last 4 min: F(2, 17) = 1,796, P = 0.208).

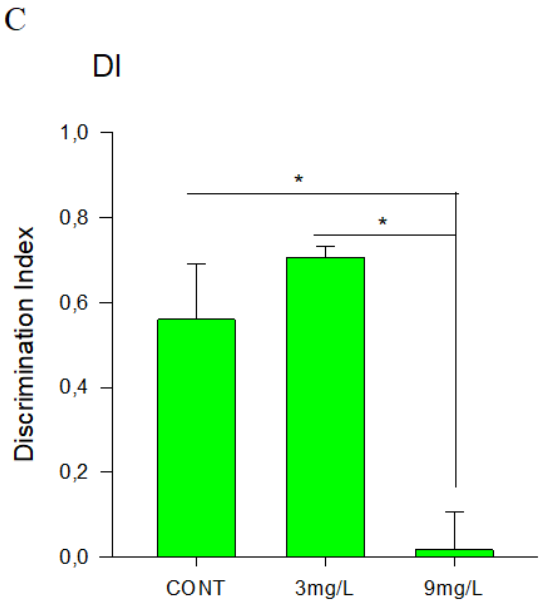


The Effects of Sodium Fluoride Exposure on Novel Object Recognition Test (NOR)

The novel object recognition test results showed that the exploration time of novel object decreased in high dose (9 mg/L) compared to low dose (3 mg/L) and control group (Fig.3A and B). On the other hand, there is statistically significant difference between high dose (9 mg/L), low dose (3 mg/L) and control group regarding to discrimination index (F=13,626, p < 0.01) (Fig. 3C).

Figure 3 (A, B and C). Effect of sodium fluoride on novel object recognition test. Data are the mean exploration time (Mean ± SEM) of sample object and the novel object. **(C)** Discrimination index was calculated using the time spent exploring the novel object and the same object (*p < 0.01 significantly different 9 mg/L versus 3mg/L and control).





DISCUSSION

Our findings demonstrated that F exposure contributed to impairment of memory according to the novel objet recognition test results. Additionally, locomotion and depression behavioral performances did not change by F administration.

Aging particularly affects the physiological mechanisms including brain functioning, and associated with a decline in memory (24). Age-related structural and functional changes in the hippocampus (decreased hippocampal neurogenesis, alternations in neurotransmitter levels and synaptic transmission), may cause an impairment in cognitive performance (25). Physical mobility is highly important to maintain a healthy living in elderly. Ability to walk, balance in standing and rising from a chair could be challenging tasks for old age, since mobility impairments are commonly seen in the ageing population. A systematic review of Demnitz and his friends (2016), concluded a positive correlation between mobility and cognitive function in healthy older adults (26). Depression is characterized by persistent sadness and a loss of interest in activities that a person normally enjoy, and also accompanied by an inability to carry out daily activities according to WHO definition (27). Risk of depression is expected to be increased specifically among older populations. The association between cognitive impairment and depression was demonstrated in several studies, and mobility limitations in elderly, was found to be related with higher risk of depressive symptoms (27, 29).

According to the American Association of Poison Control Centers'(AAPCC) 34th Annual Report, number of reported cases regarding fluoride toothpaste exposure was 17.337, 32 of these cases were classified as moderate severity and 300 patients were treated in a health care facility (30). Toothpastes and other fluoride containing dental products (mouth rinses etc.) are commonly used by geriatric population and appears to be an important source of F exposure. Many studies revealed that F can cross the blood brain barrier, accumulate in brain tissue and exposure to F may cause central nervous system dysfunction including cognitive impairment and mood disorders (31).

Regarding the toxic effects of excessive fluoride ingestion, we investigated the effects of F on CNS in aged mice, and the results of our present study revealed some unwanted effects like mood changes, decrease in

locomotor activities and altered cognitive performances. Our data showed that high dose sodium fluoride (9 mg/L) exposure decreased the locomotor activity when compared the low dose sodium fluoride (3 mg/L) group and control group. However, no statistically significant differences were observed, and this can be related with the exposure time.

It is well known that a decrease in the neurotransmitter levels (eg. serotonin and dopamine) are strongly related with mood and behavioral changes (32). Also, the concentration of the 5-hydroxyindoleacetic acid (metabolite of 5-hydroxytryptamine), was decreased in the brains of sodium fluoride-treated animals, and may be responsible from the behavioral problems (33). The study results of emotional and cognitive changes by F, suggests that; developmental fluoride exposure leads to cognitive decline and depression-like behaviors (34). Similarly, in our study, the immobility time in the FST, which is considered as an indicator of depressive behavior, was higher in mice treated with high dose (9 mg/L) sodium fluoride when compared to the other groups, however, the difference between groups was not statistically significant.

The study, demonstrated both locomotor and cognitive deficits in aging rat brain, suggesting the increased oxidative stress and brain atrophy may be one of the causative factors of aging (24). Bhatnagar et al (2006) investigated the effects of chronic F intoxication on the antioxidant defense mechanism. According to the results of his study, decreased free radical scavenging enzymes in neuronal cell bodies revealed hippocampal neuron loss and significant reduction in AchE activity (35). Paul et al (1998) investigated the effects of sodium fluoride on locomotor activity and in accordance with our results, there was a neurobehavioral deficit resulting in an inhibition of locomotor activity (33).

α7- nAChRs, are localized in the hippocampus and cerebral cortex, critical regions for the synaptic plasticity, learning and memory. Shan et al (2004) showed that nAChR levels decreased in an animal model with fluoride toxicity. To investigate the relationship between fluorosis associated decrease in nAChRs and oxidative stress, they pretreated cultural PC12 rat pheochromocytoma cells with vitamin E (antioxidant and inhibitor of lipid peroxidation) before F exposure, and the decrease in nAChR subunit proteins was mostly prevented (36). As known, F induced free radical generation consequently results in oxidative stress due to its high electronegativity. So, chronic fluorosis can cause abnormalities in the nerve function and therefore effect synaptic plasticity necessary for learning and memory. In our study F exposure contributed to impairment of memory according to the behavioral test results. High dose (9 mg/L NaF) fluoride treated group showed decreased performance on NOR test, compared to the control group. The results were assigned by comparing the time spent with the sample and the novel objects and a decrease in NOR test performance refers

cognitive impairment. There was a statistically significant decrease in the time difference spent with the novel and sample objects, expressed as discrimination index, and the exploration time spent with the novel object was also decreased.

CONCLUSION

Excessive F exposure may contribute to an impairment on memory performance in the ageing population. Since the geriatric population is more prone to present with cognitive decline, more comprehensive and clinical studies are needed to confirm our findings and to investigate the mechanisms of F neurotoxicity and exposure.

Declaration of Interest

The authors report no conflicts of interest.

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