BIOCHEMICAL CHANGES IN BRAIN AND OTHER TISSUES OF YOUNG ADULT FEMALE MICE FROM FLUORIDE IN THEIR DRINKING WATER

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SUMMARY: One-month old female Swiss albino mice were given 60 ppm and 120 ppm F^- (from NaF) in their drinking water for 30 days to study effects of fluoride on neurotransmitter enzymes (AchE, BchE), anti-oxidant enzymes (SOD, CAT), and lipid peroxidation (MDA) in brain (hippocampus), liver, and gastrocnemius muscle. Activities of AchE and BchE showed a concentration-dependent decrease in all the tissues studied, which was highly significant in liver and muscles. Superoxide dismutase (SOD) and catalase (CAT) also showed a significant concentration dependent decrease in all the tissues, which was highly significant for CAT at 120 ppm in liver and muscles. Malondialdehyde (MDA), however, showed a significant concentration dependent increase in gastrocnemius muscle, but in brain and liver it had an initial decrease at 60 ppm F^- that changed to a significant increase at 120 ppm. Ascorbic acid exhibited significant concentration-dependent increases in all the tissues examined. Total protein showed a concentration dependent decrease in brain and muscles but an increase in liver. The results of the study indicate that elevated fluoride in drinking water affects not only mammalian neurotransmitter functions but also antioxidant systems.

Keywords: Acetylcholinesterase; Ascorbic acid; Catalase; Cholinesterase; Female mice; Liver; Malondialdehyde; Mouse brain; Muscles; Superoxide dismutase.

INTRODUCTION

Evidence that fluoride (F) crosses the blood brain barrier[1] raises the possibility that F can affect the structure and functions of the central or peripheral nervous system. Earlier reports on the effects of NaF on rat brain[2-15] and other tissues,[16-20] and on neuronal cell bodies in the hippocampus[21] suggest that excess fluoride intake has CNS effects. Other reports also show changes in levels of trace metals in brain[22] and antioxidant defense in brain of rats.[23-29] Significantly impaired learning and memory, shown in rats,[30-33] reduced motor coordination, and behavior symptoms like nervousness, depression, tingling sensations in fingers and toes, excessive thirst, and tendency to urinate frequently in human patients,[34] after excess intake of fluoridated water suggest that not only the structure but functions of the central nervous system are also affected.

The present investigation was undertaken to study the effects of two different F concentrations in drinking water on neurotransmitter enzymes, antioxidant enzymes, and lipid peroxidation in mice.

MATERIALS AND METHODS

Animals: Healthy young adult (one-month old) female Swiss albino mice (n=40, bw 25±5 g) were used. The animals were obtained from Animal house at Bhupal Nobels College of Pharmacy, Udaipur, approved under the Ministry of Social Justice and Empowerment, Government of India for animal experimentation

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(Approval No. 870/ac/05/CPCSEA). All the protocols and methodology were approved by the institutional ethical committee. The mice were given low-F food and water *ad libitum* and were housed in an animal house at 26±5ºC with a 12-hr light/dark cycle.

Treatment: The mice were divided into three groups: control (n=10), experimental group E1 (n=15), and experimental group E2 (n=15). Each mouse was caged separately. Control group mice were given deionized, defluoridated water. Mice in the E1 group were given 60 ppm F (from 132.6 mg NaF/L) in their drinking water while those in the E2 group were given 120 ppm F (from 265.2 mg NaF/L) in their drinking water.

After 30 days all the mice were weighed and sacrificed by cervical decapitation. The brain, liver, and gastrocnemius muscles were dissected out and placed on chilled glass plates, dried, and weighed. Brains were cut into two sagittal pieces with the help of surgical blade, and the hippocampus was dissected out under a stereomicroscope. Tissues of at least three animals were pooled to prepare enough sample for biochemical estimation. Similarly, liver and gastrocnemius muscle were dissected out, minced, and then homogenized in cold 100 mM pH 7.2 phosphate buffer using a Teflon mechanical homogenizer. Samples were then diluted tenfold and the homogenate was spun at 10,000 rpm for 15 min and the supernatant was used for enzymatic assay.

Biochemical estimation by standard methods were conducted for AchE (E.C. 3.1.1.7),[35] BchE (E.C. 3.1.1.8),[35] SOD (E.C.1.1.15.11),[36] CAT (E.C.1.11.1.6),[37] MDA,[38] ascorbic acid,[39] and total protein[40] in brain (hippocampus), liver, and gastrocnemius muscle of control and F-treated animals.

Statistical analysis: The data were statistically analyzed by Student's t test.

RESULTS

Results of the various treatments are shown in Tables 1–3. Activities of AchE and BchE showed a concentration-dependent decrease in all the tissues studied (Tables 1–3). But in liver (Table 2) and muscles (Table 3) the decrease was highly significant at 120 ppm F.

Table 1. Effects of F in drinking water on various parameters in the hippocampus of mice

Group	Control	60 ppm (E1)	120 ppm (E2)
AchE (ΔOD)	0.11± 0.01	0.062±0.01*	0.022±0.004§
BchE (ΔOD)	0.09± 0.009	0.027±0.011‡	0.017±0.023
SOD (% inhibition of NBT reduction)	56.55± 1.30	49.99±0.91§	38.10±0.862§
CAT (μmoles H_2O_2 utilized/min/mg of protein)	53.57± 6.26	27.38±4.06§	10.71±2.27§
MDA (nmoles/mL)	1.85± 0.32	1.21±0.28	4.67±0.68‡
Ascorbic acid (mg/100mL)	0.87± 0.20	1.83±0.17	2.24±0.22‡
Total protein (mg/mL)	0.10± 0.00	0.09±0.00	0.032±0.005§

Data represented as mean ±S.E. *P<0.05; †P<0.02; ‡ P<0.01; §P<0.001; no sign = nonsignificant.
Comparison between: Control with E1 group and E2 group; E1 group with E2 group.

Table 2. Effects of F in drinking water on various parameters in the liver of mice

Group	Control	60 ppm (E1)	120 ppm (E2)
AchE (ΔOD)	0.105±0.013	0.077±0.44	0.01±0.026$^{\#}$
BchE (ΔOD)	0.125±0.012	0.035±0.009‡	0.01±0.006‡
SOD (% inhibition of NBT reduction)	45.68±0.90	39.13±1.23§	30.34±0.89‡
CAT (μmoles H_2O_2 utilized/min/mg of protein)	27.38±4.06	23.80±10.28	4.16±0.73‡
MDA (nmoles/mL)	1.98±0.32	1.72±0.65	2.04±0.22*
Ascorbic acid (mg/100mL)	0.708±0.14	1.45±0.14§	2.04±0.22*
Total protein (mg/mL)	0.125±0.03	0.137±0.04	0.29±0.56

Data represented as mean ±S.E. *P<0.05; †P<0.02; ‡ P<0.01; §P<0.001; no sign = nonsignificant.
Comparison between: Control with E1 group and E2 group; E1 group with E2 group.

Table 3. Effects of F in drinking water on various parameters in the gastrocnemius muscle of mice

Group	Control	60 ppm (E1)	120 ppm (E2)
AchE (ΔOD)	0.87±0.008	0.057±0.011	0.02±0.004§
BchE (ΔOD)	0.05±0.009	0.032±0.008	0.01±0.006‡
SOD (% inhibition of NBT reduction)	49.99±0.912	39.13±0.763§	31.03±0.745§
CAT (μmoles H_2O_2 utilized/min/mg of protein)	29.76±4.900	11.90±4.120*	7.41±3.072‡
MDA (nmoles/ mL)	1.92±0.397	3.08±0.726	4.22±0.764*
Ascorbic acid (mg/100mL)	0.541±0.142	1.29±0.141‡	1.62±0.078§
Total protein (mg/mL)	0.35±0.077	0.317±0.130	0.13±0.02

Data represented as mean ±S.E. *P<0.05; †P<0.02; ‡ P<0.01; §P<0.001; no sign = nonsignificant.
Comparison between: Control with E1 group and E2 group; E1 group with E2 group.

Superoxide dismutase (SOD) and catalase (CAT) showed significant concentration-dependent decreases in all the tissues (Tables 1–3). CAT showed a highly significant decrease at 120 ppm F in liver (Table 2) and gastrocnemius muscle (Table 3).

Malondialdehyde (MDA) showed significant concentration-dependent increase in gastrocnemius muscle (Table 3) but in brain (Table 1) and liver (Table 2), however, an initial decrease occurred at 60 ppm F and a significant increase at 120 ppm F.

Ascorbic acid also exhibited a significant concentration-dependent increase in all the tissues examined (Tables1–3). Total protein showed concentration-dependent decrease in brain (Table 1) and gastrocnemius muscle (Table 3) but an increase in liver (Table 2).

DISCUSSION

Excessive intake of F from NaF in drinking water significantly reduces AchE and BchE activity in the hippocampus. This decrease could be due to loss of neuron cell bodies in the hippocampus,[3,4,6,8,9,21] loss of synaptic structures,[15,31] or inhibition of enzyme activity.[41,42] These effects could be corroborated with cognitive dysfunctions observed in experimental animals[30-33,43,44] as well as in fluorosis patients.[43,46] A similar decrease in the activity of the cholinesterases in liver and muscles could be due to inhibition of the enzyme activity or loss of synaptic structures. In fact, in a study recently conducted in this laboratory, symptoms of severe memory loss, suppressive behavior manifestations, numbness in hands and legs, low pain threshold, disturbed gait, and reduced metabolism

were observed in fluorosis patients.[44] These changes suggest that excess F intake impairs neurotransmission functions.

Significant decrease in free radical scavenging enzymes and a concomitant increase in lipid peroxidation (MDA) in all the tissues studied suggests an increase in oxidative stress after excessive F intake. Such increase in free radicals in neuronal cell bodies could be correlated with loss of neurons in hippocampus and synaptic structures in neuromuscular junctions and in liver.

A parallel increase in ascorbic acid content, an important antioxidant in brain and other organs after F intake, is difficult to explain. An increase in ascorbic acid content suggests its role in amelioration of F-induced stress. Therefore the increase observed in our study may be related to a compromised activity of the tissue against F intake. A significant decrease in total protein supports our observation of deceased activity of various enzymes.[47]

In conclusion, the present study suggests that chronic F intoxication markedly affects antioxidant defense, which may result in dysfunctions of neurotransmission in brain, liver, and muscles.

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