

MITIGATION BY BLACK TEA EXTRACT OF SODIUM FLUORIDE-INDUCED HISTOPATHOLOGICAL CHANGES IN BRAIN OF MICE

MH Trivedi,^a RJ Verma,^b NP Sangai,^b NJ Chinoy[†]

Gujarat, India

SUMMARY: Sixty young adult inbred Swiss strain male albino mice (*Mus musculus*) were randomly divided into six equal groups. Groups I and II served as control and antidote groups, respectively. Groups III and IV were orally administered 0.2 and 0.4 mg sodium fluoride (NaF) in 0.2 mL of deionized water/animal/day (= 6 mg and 12 mg NaF/kg bw/day, respectively) for 30 days and served as low dose (LD) and high dose (HD) groups, respectively. Groups V and VI were administered NaF as in groups III and IV but were also given 2% black tea extract (BTE) infusion instead of drinking water for 30 days. The mice in each group were weighed individually, and mean weights and percent gain in body weight were recorded. An average of 33.9% weight gain was found in Groups I and II, whereas the gain was only 6.24% in Groups III and IV, which was clearly and significantly lower than in the two control groups. BTE with NaF in Groups V and VI showed a 29.49% average weight gain that was not only significantly higher than that of the NaF-treated Groups III and IV but also close to that of the two control groups. After 30 days of treatment, brains of control and treated groups of animals were quickly isolated. Cerebral hemisphere (CH), cerebellum (CB), and medulla oblongata (MO) regions of brain were separated carefully, weighed to the nearest mg and studied for their histopathology. NaF treatment (Groups III and IV) caused significant dose-dependent reduction in absolute organ weight whereas BTE together with NaF (Groups V and VI) resulted in amelioration of weight loss of CH, CB, and MO. Normal features of histology were observed in control group slides of CH, which consisted of outer folded cortex of gray matter covering the inner white matter. CB showed a well-developed molecular layer, Purkinje cell layer, granular layer, and core of white matter. Similarly, MO showed a well-developed molecular layer, Purkinje cell layer, granular layer, and blood vessel structure. NaF treatment induced degenerative changes, pycnotic nuclei, fatty infiltration, and chromatolysis in CH, CB, and MO with a more severe effect with HD than LD. With BTE in combination with NaF treatment, normal histological features similar to control slides were observed.

Keywords: Brain histopathology; Cerebral Hemisphere; Cerebellum; Fluoride toxicity; Male albino mice; Medulla oblongata; Neurotoxicity.

INTRODUCTION

Excessive intake of fluoride (F) causes fluorosis, which is a progressive degenerative disorder that predominantly affects the skeletal system, teeth, and also the structure and function of skeletal muscle,¹ brain,² and spinal cord³ in experimental animals. Li et al.⁴ observed adverse neurological effects in the brain of humans with exposure to F. In the last several decades, effects of F on the brain and its activity have become the subject of considerable interest in the field of F toxicity. Some researchers have also found a correlation between F exposure and the IQ of schoolchildren⁵ and have also suggested that children with dental fluorosis are at greater risk of decreased mental acuity.

^aFor correspondence: Dr MH Trivedi, Department of Earth and Environmental Science, KSKV Kachchh University, Mundra Road, Bhuj – 370 001, Kachchh, Gujarat, India. E-mail: drmrugesh.trivedi@gmail.com. ^bDepartment of Zoology, School of Sciences, Gujarat University, Ahmedabad – 370 009, Gujarat, India. [†]Deceased 8 May 2006.

Tea has one of the highest total flavonoid contents of all plants, at 15% of the leaf by dry weight.⁶ Black tea constitutes about 80% of the tea manufactured in the world and is mainly consumed in Western and some Asian countries. Black tea contains several polyphenols such as biflavonols, theaflavins (TF), and thearubigins.⁷ It has been reported that tea extracts have antioxidative,⁸ antitumor,⁹ antimutagenic, and anticarcinogenic activities.¹⁰ Tea polyphenols have also been noted to induce apoptosis and cell cycle arrest in a wide array of cell lines.¹¹ The present investigation was designed to examine the effects of black tea extract on various neuropathological changes induced by NaF in different parts of the brain of male mice.

MATERIALS AND METHODS

Animals: Sixty young adult inbred Swiss strain male albino mice (*Mus musculus*), weighing approximately 30–35 g, were obtained from Cadila Pharmaceutical Pvt. Ltd. Ahmedabad, India. The mice were maintained in a 12-hr light/dark cycle at 26±2°C and were provided certified pelleted rodent feed supplied by Amrut Feeds, Pranav Agro Industries Limited, Pune, India, and water or tea infusion *ad libitum* throughout the experiment. *Guidelines for Care and Use of Animals in Scientific Research 1991*, published by the Indian National Science Academy, New Delhi, India, were followed. The research protocols were reviewed and approved by the Committee for the Purpose of Control and Supervision of Experiments for Animals (Reg-167/1999/CPCSEA), New Delhi, India.

Chemicals: All chemicals used in the present study were of analytical grade procured from HiMedia Laboratories Pvt. Ltd., Thomas Baker (chemicals) Ltd., Qualigens Fine Chemicals, and S.D. Fine Chemicals Pvt. Ltd., Mumbai, India.

Plan of study: As shown in Table 1, 60 adult male albino mice (*Mus musculus*) were used in the experiment.

Table 1. Experimental protocol

Group	Treatment	No. of animals	Duration of treatment (days)	Day of autopsy
I	Control	10	30	31 st
II	Black tea extracts (2%)	10	30	31 st
III	NaF Treatment (6 mg/kg bw/day)	10	30	31 st
IV	NaF Treatment (12 mg/kg bw/day)	10	30	31 st
V	As in Group III + Black tea extracts (2%)	10	30	31 st
VI	As in Group IV + Black tea extracts (2%)	10	30	31 st

The mice were divided into six equal groups with two per cage and five cages per group. Group I (control) animals were maintained without any treatment. Group II animals received black tea extract (BTE, 2% instead of drinking water) for 30 days and served as antidote control group. Group III and IV animals were orally administered 0.2 and 0.4 mg NaF in 0.2 ml of deionized water/animal/day

(= 6 mg and 12 mg NaF/kg body weight, respectively) for 30 days and served as low dose (LD) and high dose (HD) groups, respectively. Group V and VI animals were administered NaF as in Groups III and IV and were given 2% black tea extract (BTE) infusion instead of drinking water for 30 days.

Eighty grams of black tea solids (Lipton Yellow Label of Hindustan Lever Limited, Mumbai, India) and 4 L of deionised water were used to produce 2% tea infusion.¹² All the NaF treatments were given orally for 30 days using a feeding tube attached to a hypodermic syringe. The dose of NaF was based on LD₅₀ value in mice.¹³ Oral administration was used since it is the most common route of F exposure.

To study the effect of feeding NaF alone and NaF plus BTE on body weight, each mouse in each group was weighed individually at the end of the 30-day experiment and mean group weights were calculated along with the percent gain in body weight. Also after 30 days, the animals were sacrificed by cervical dislocation, and the brains of control and all treated groups of animals were quickly isolated and blotted free of blood. The CH, CB, and MO regions of the brain were separated carefully, weighed to the nearest mg on an analytical balance, and fixed for 18 hr in alcoholic Bouin's fixative. A pinch of lithium carbonate was added to remove excess picric acid in the fixative.

Histological studies: Histological studies were carried out using the standard technique of hematoxylin and eosin staining. The brain tissues were dehydrated by passing ascending grades of alcohol through them, clearing them in xylene, and finally embedding them in paraffin wax (mp 58–60° C). Transverse sections of 5- μ m thickness were cut on a rotary microtome. These sections were stained with Ehrlich's hematoxylin and eosin in alcohol, dehydrated in alcohol, cleared in xylene, mounted in DPX, and examined microscopically.

Statistical analysis: For each parameter at least 10 replicates were made. The results were statistically analysed using one-way Analysis of Variance (ANOVA) followed by Tukey Test by SPSS–17th version. For significance $p < 0.05$ was used. Comparisons of p -values between different groups were performed.

RESULTS

BODY AND ORGAN WEIGHT CHANGES:

Body weight: Changes in body weight of controls and various treated groups of mice are shown in Figure 1. A comparable weight gain with no significant differences was found in control Group I (34.13%) and control Group II (33.67%). However, the percent of weight gain was significantly lower ($p < 0.05$) in the NaF-treated Groups III and IV (LD: 6.31%; HD: 6.16%; mean: 6.24%). On the other hand, administration of BTE infusion together with NaF resulted in a significant amelioration in NaF-induced improvement in body weight gain, with 29.56% in Group V and 29.41% in Group VI (mean: 29.49%).

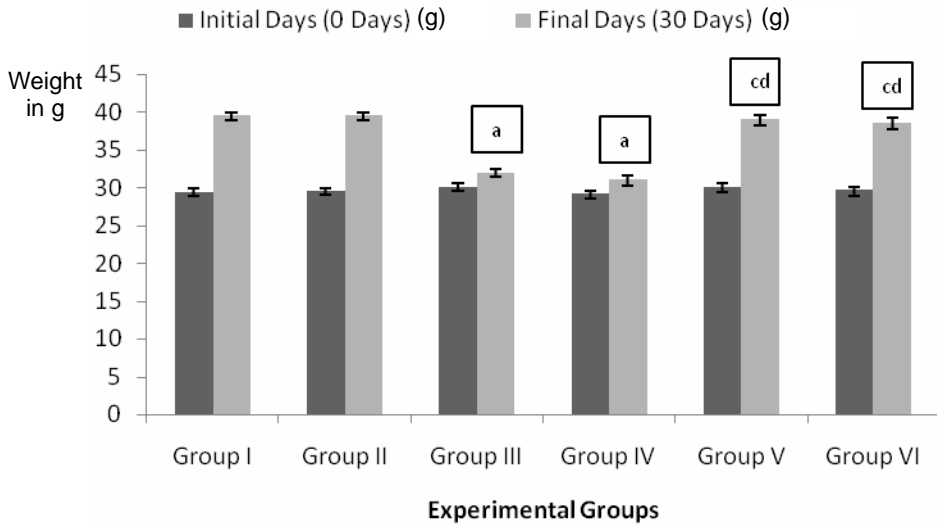


Figure 1. Effect of black tea extract on NaF-induced changes in body weight of mice. Values are mean \pm SEM, n = 10. ^aAs compared to Group I; ^bAs compared to Group II; ^cAs compared to Group III; ^dAs compared to Group VI; ^eAs compared to Group V; ^fAs compared to Group IV.

Organ weight: Figures 2, 3, and 4 show the effect of NaF and NaF plus BTE on the absolute weights of CH, CB, and MO, respectively.

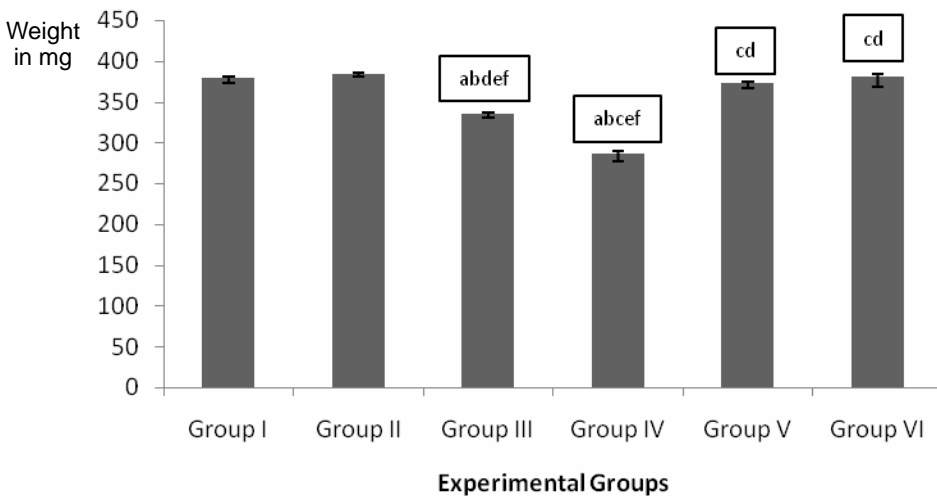


Figure 2. Effect of black tea extract on NaF-induced changes in weight of cerebral hemisphere. Values are mean \pm SEM, n = 10. ^aAs compared to Group I; ^bAs compared to Group II; ^cAs compared to Group III; ^dAs compared to Group VI; ^eAs compared to Group V; ^fAs compared to Group IV.

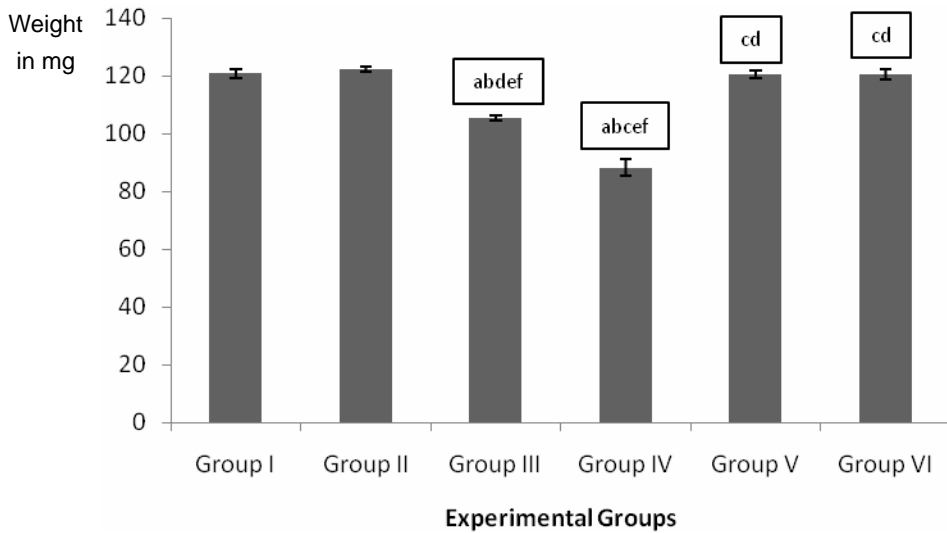


Figure 3. Effect of black tea extract on NaF-induced changes in weight of cerebellum. Values are mean \pm SEM, n = 10. ^aAs compared to Group I; ^bAs compared to Group II; ^cAs compared to Group III; ^dAs compared to Group VI; ^eAs compared to Group V; ^fAs compared to Group IV.

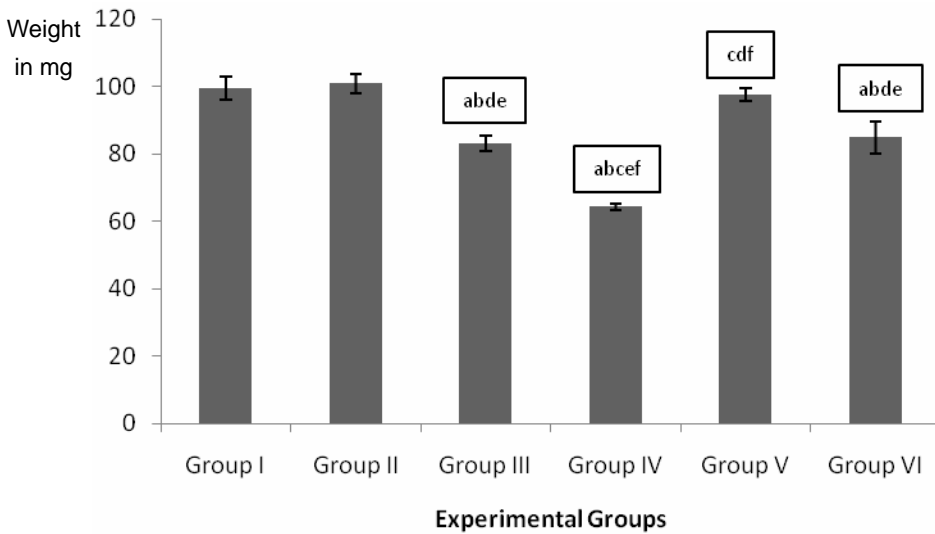


Figure 4. Effect of black tea extract on NaF-induced changes in weight of medulla oblongata. Values are mean \pm SEM, n = 10. ^aAs compared to Group I; ^bAs compared to Group II; ^cAs compared to Group III; ^dAs compared to Group VI; ^eAs compared to Group V; ^fAs compared to Group IV.

No significant effect was observed in the absolute weights of CH, CB, and MO in the two control Groups I and II. As compared with the untreated control Group I, oral administration of NaF to the animals of Group III and IV for 30 days caused significant ($p < 0.05$), dose-dependent reduction in the absolute weights of CH (LD: -11.55%; HD: -24.49%), CB (LD: -12.68%; HD: -26.95%) and MO (LD: -15.56%; HD: -34.68%). However, BTE treatment significantly ($p < 0.05$) mitigated NaF-induced change in the absolute weights of all three parts of the brain (Groups

V and VI), as compared to NaF alone treated Groups III and IV. The amelioration was almost complete for the LD and HD in Groups V and VI treated with BTE in combination with NaF.

HISTOPATHOLOGICAL STUDY:

Cerebral hemisphere: The transverse CH section of control mice showed well-developed cerebral cortex. The CH consisted of outer folded cortex of gray matter covering the inner white matter. Its characteristic neurons with particular functions and unique connections showed normal variations in the arrangements of cells in the different parts of the six layers of CH. Underneath the outermost molecular layer were four layers of pyramidal cells plus and the multiform sixth layer composed of fusiform cells (Figure 5).

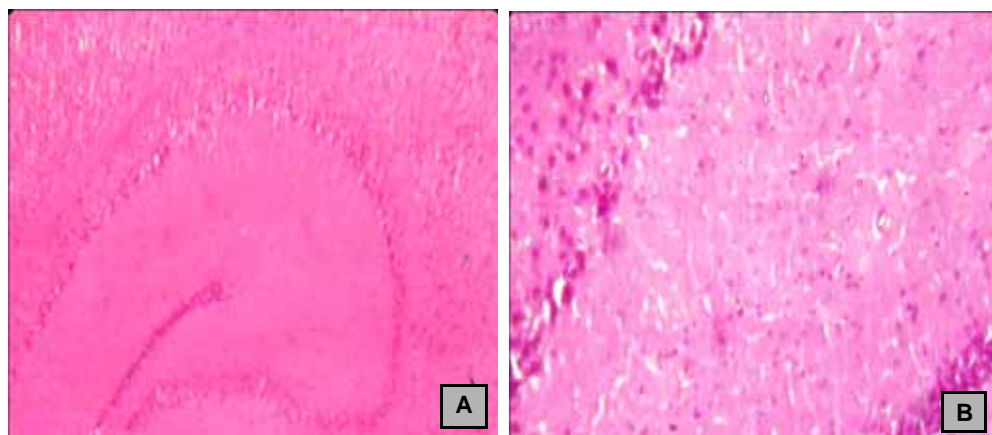


Figure 5. Transverse section (TS) of CH of untreated control mice.
Magnification A: $\times 225$; B: $\times 900$.

No apparent histological changes were observed in BTE alone treated mice (Figure 6).

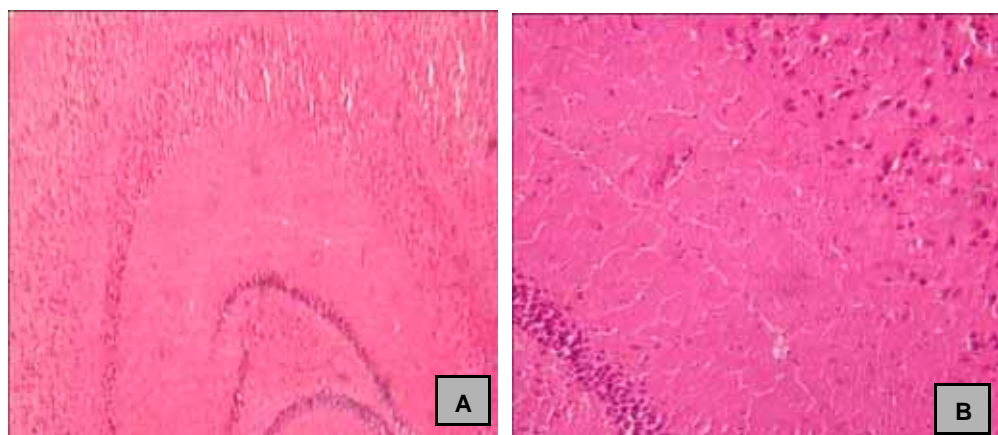


Figure 6. TS of CH of BTE alone treated mice. Magnification A: $\times 225$; B: $\times 900$.

Oral administration of NaF for 30 days caused alterations in CH histology involving degenerative changes, pycnotic nuclei, fatty infiltration, and chromatolysis in the LD (Figure 7) and HD (Figure 8) groups. The effect was greater for HD than for LD.

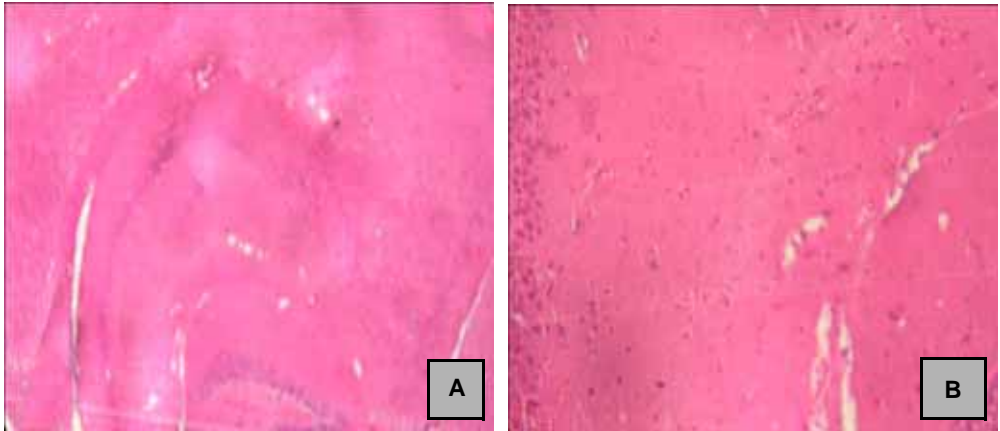


Figure 7. TS of CH of LD-NaF treated (6 mg/kg/b.w/day) mice. Magnification A: $\times 225$; B: $\times 900$.

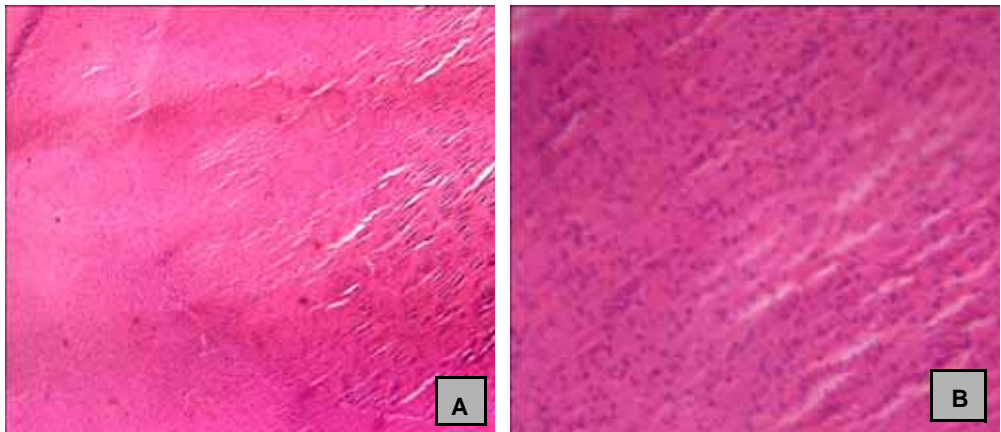


Figure 8. TS of CH of HD-NaF treated (12 mg/kg/b.w/day) mice.
Magnification A: $\times 225$; B: $\times 900$.

Treatment with BTE for 30 days along with NaF caused significant amelioration in NaF-induced effects in both LD (Figure 9) and HD (Figure 10) of Groups V and VI. Degenerative changes, pycnotic nuclei, fatty infiltration, and chromatolysis were not observed.

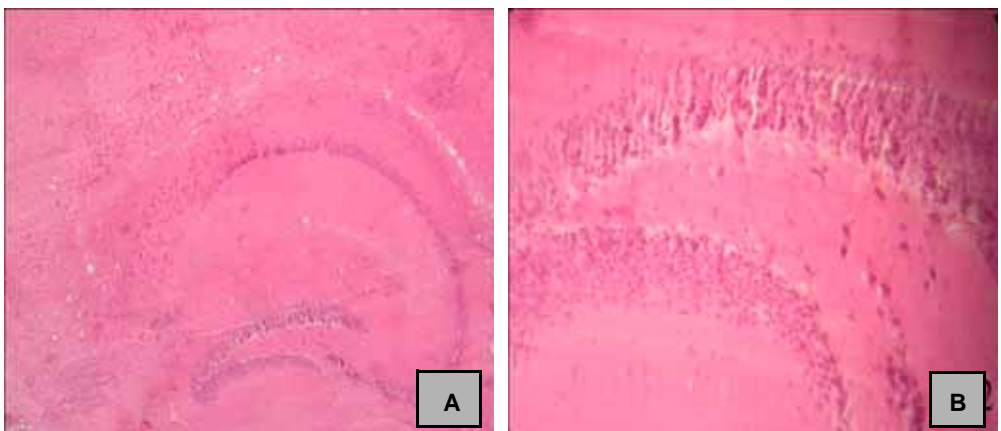


Figure 9. TS of CH of LD-NaF plus BTE treated mice. Magnification A: $\times 225$; B: $\times 900$.

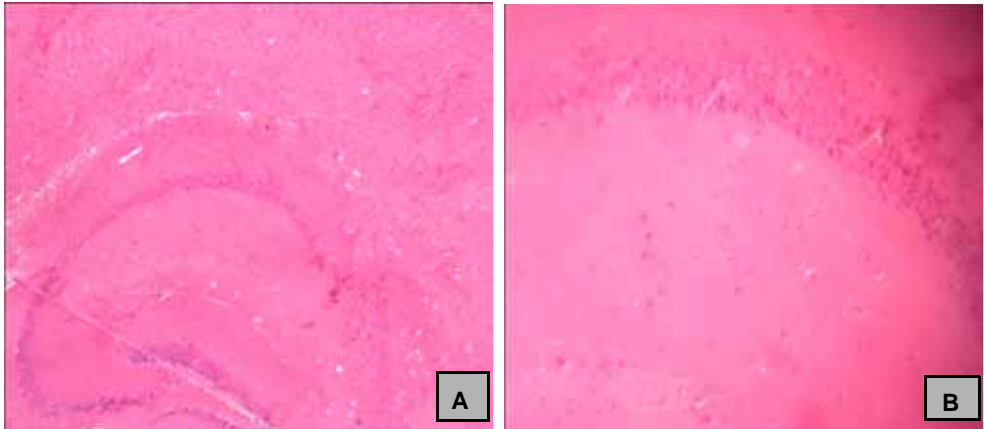


Figure 10. TS of CH of HD-NaF plus BTE treated mice. Magnification A: $\times 225$; B: $\times 900$.

Cerebellum: The transverse section of CB of control mice showed a well-developed molecular layer, Purkinje cell layer, granular layer, and core of white matter (Figure 11).

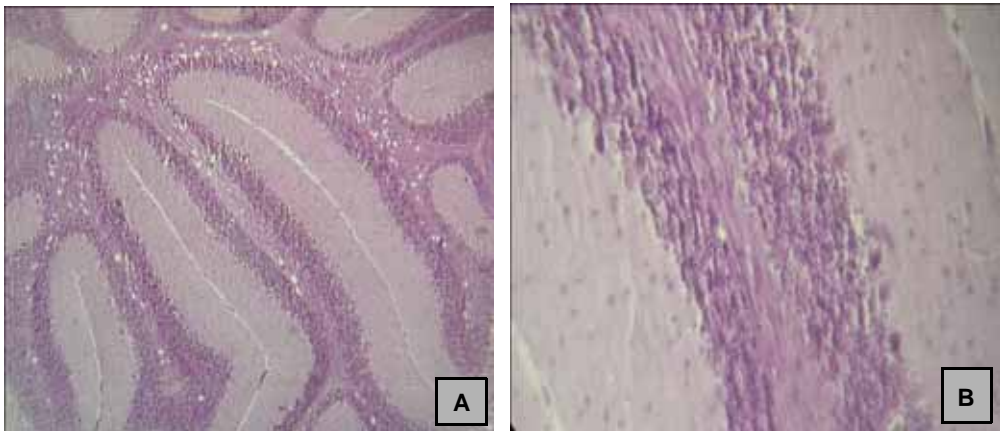


Figure 11. TS of CB of untreated control mice. Magnification A: $\times 225$; B: $\times 900$.

No apparent histological changes were observed in BTE alone treated mice (Figure 12).

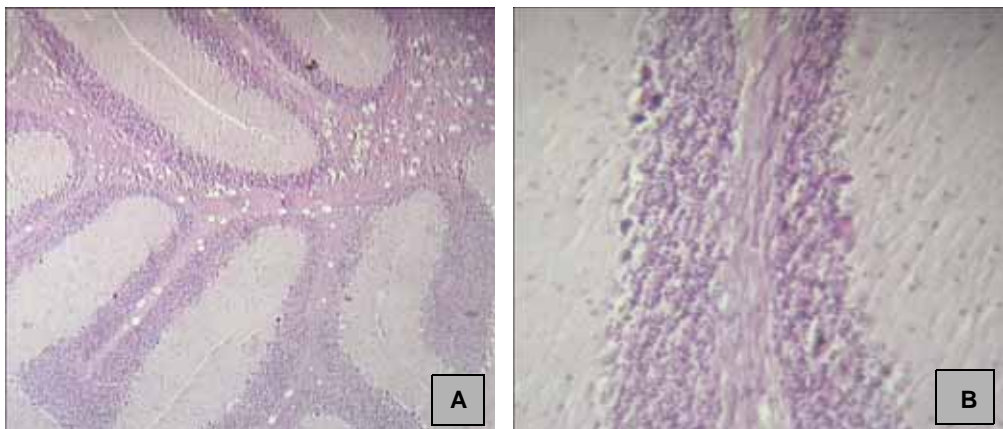


Figure 12. TS of CB of BTE alone treated mice. Magnification A: $\times 225$; B: $\times 900$.

NaF treatment for 30 days caused alteration in its histology involving, degenerative changes, pycnotic nuclei, fatty infiltration, and chromatolysis with LD and HD (Figures 13 and 14, respectively). The effect was greater with HD than with LD.

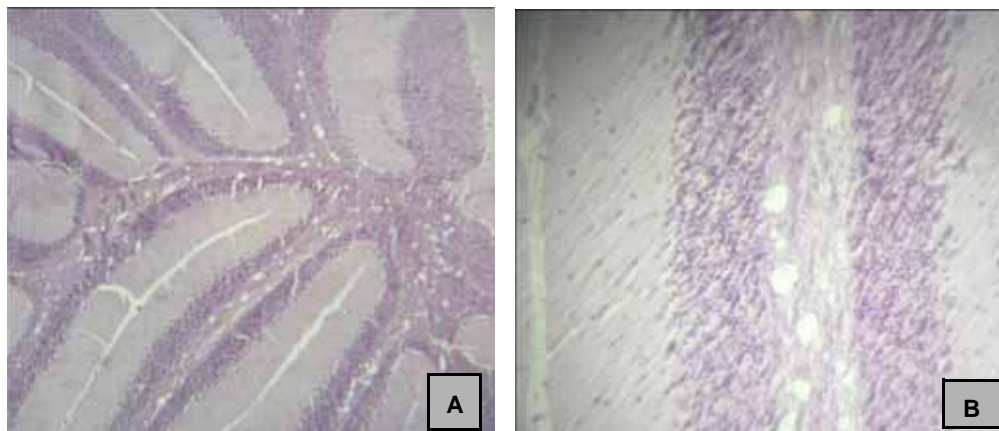


Figure 13. TS of CB of LD-NaF (6 mg/kg bw/day) treated mice. Magnification A: $\times 225$; B: $\times 900$.

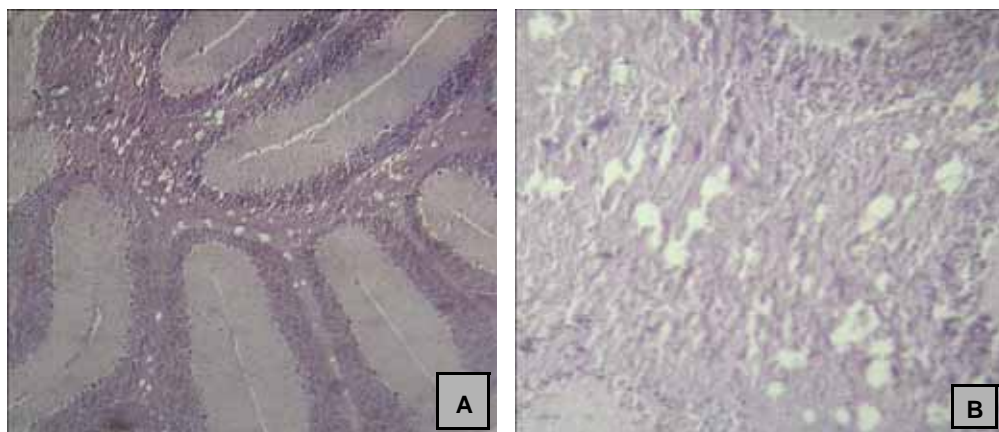


Figure 14. TS of CB of HD-NaF (12 mg/kg bw/day) treated mice. Magnification A: $\times 225$; B: $\times 900$.

Treatment with BTE for 30 days along with NaF at both LD and HD showed normal histological features and no degenerative changes, pycnotic nuclei, fatty infiltration, and chromatolysis (Figures 15 and 16, respectively).

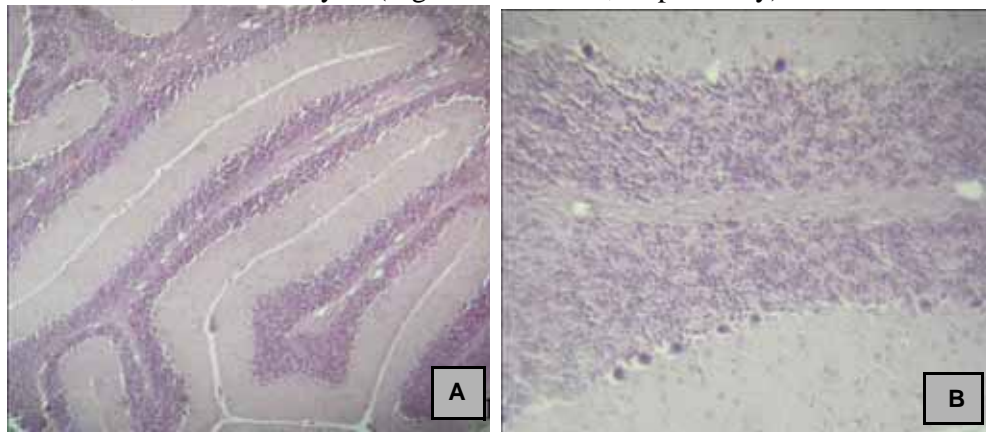


Figure 15. TS of CB of LD-NaF plus BTE treated mice. Magnification A: $\times 225$; B: $\times 900$.

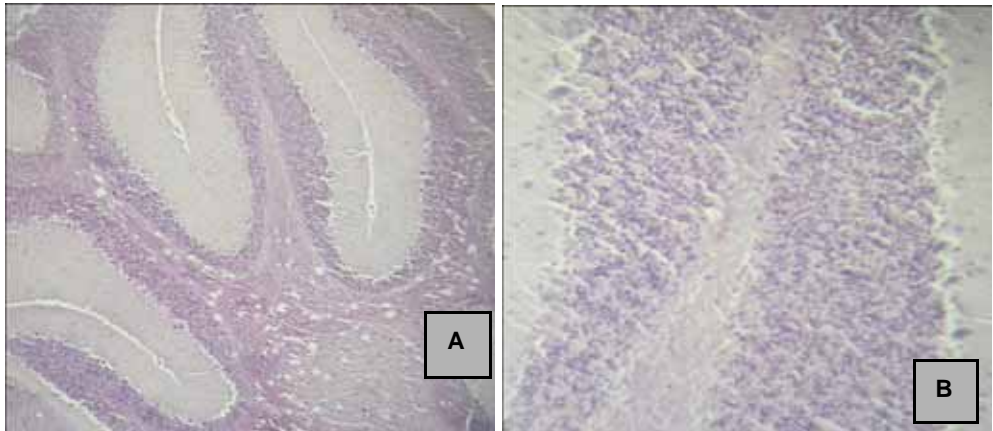


Figure 16. TS of CB of HD-NaF plus BTE treated mice. Magnification A: $\times 225$; B: $\times 900$.

Medulla oblongata: The transverse section of MO of untreated control mice showed well-developed molecular layer, Purkinje cell layer, granular layer, and blood vessel (Figure 17).

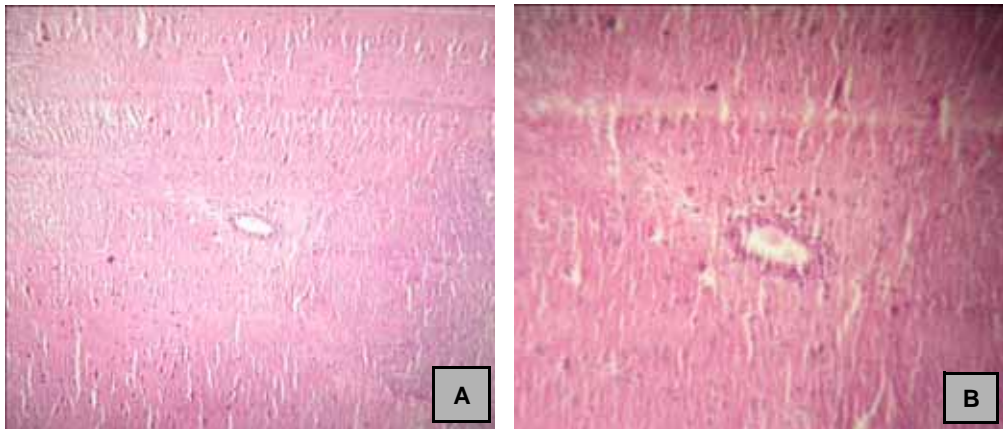


Figure 17. TS of MO of untreated control mice. Magnification A: $\times 225$; B: $\times 900$.

No apparent histological changes were observed in BTE alone treated mice (Figure 18).

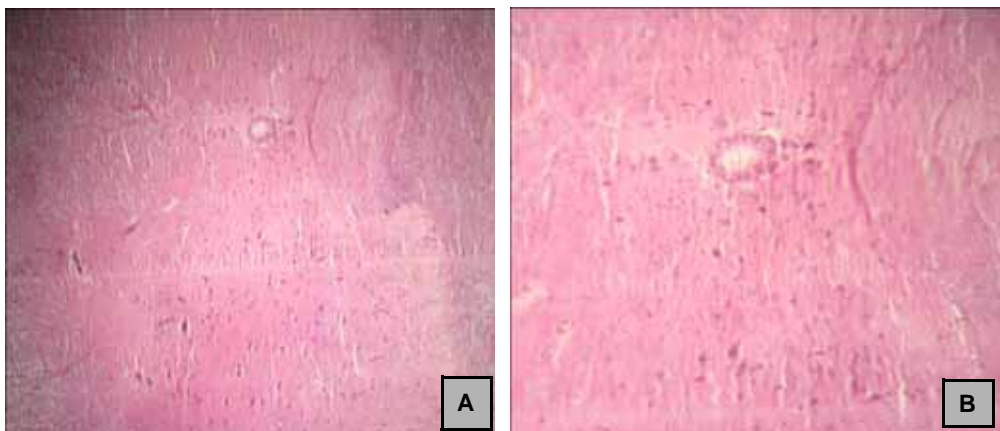


Figure 18. TS of MO of BTE alone untreated control mice. Magnification A: $\times 225$; B: $\times 900$.

NaF treatment for 30 days caused some alteration in MO histology involving degenerative changes, pycnotic nuclei, fatty infiltration, and chromatolysis with LD and HD (Figures 19 and 20, respectively). The effect was greater with HD than LD.

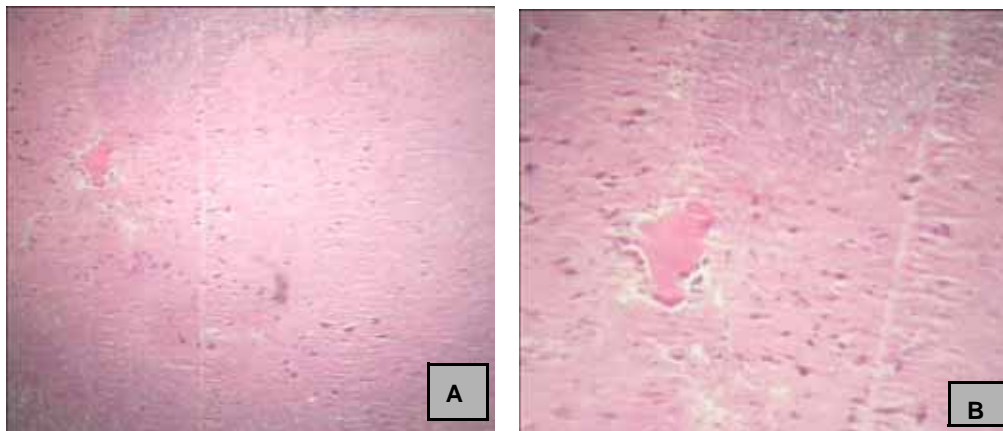


Figure 19. TS of MO of LD-NaF (6 mg/kg bw/day) treated mice. Magnification A: $\times 225$; B: $\times 900$.

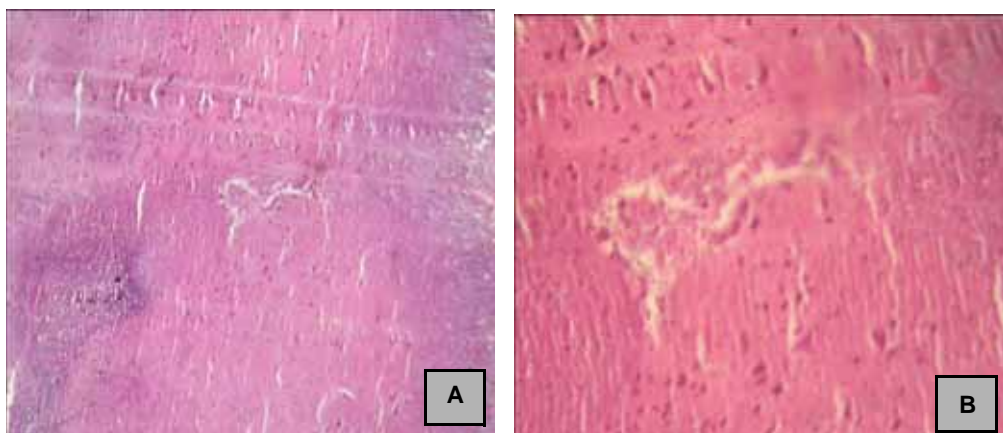


Figure 20. TS of MO of HD-NaF (12 mg/kg bw/day) treated mice. Magnification A: $\times 225$; B: $\times 900$.

Treatment with BTE for 30 days along with NaF caused significant amelioration in NaF-induced effects with both LD and HD (Figures 21 and 22).

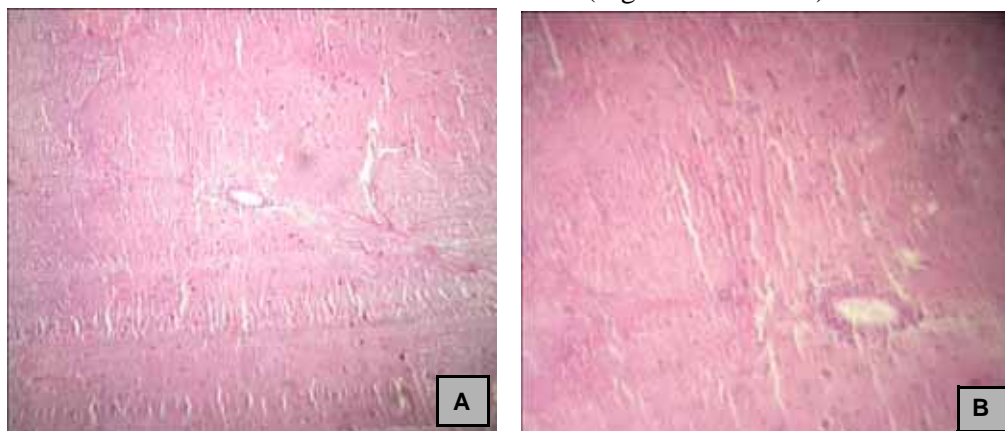


Figure 21. TS of MO of LD-NaF plus BTE treated mice. Magnification A: $\times 225$; B: $\times 900$.

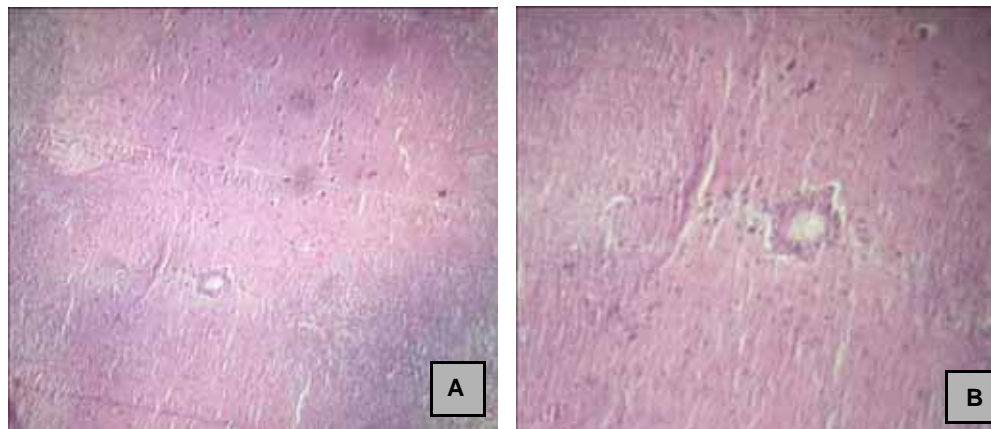


Figure 22. TS of MO of HD-NaF plus BTE treated mice. Magnification A: $\times 225$; B: $\times 900$.

Degenerative changes, pycnotic nuclei, fatty infiltration, and chromatolysis were not observed.

DISCUSSION

Oral administration of NaF for 30 days caused significant reduction in body weight (Figure 1) of mice that can be attributed to lower food consumption resulting in decreased protein synthesis and lower energy metabolism. A consistent reduction in body weight in mice by 5.2 mg F/kg body weight for 35 days has been reported by Pillai et al.¹⁴ Similar results from F were also reported in rats¹⁵ and in mice,¹⁶ and studies in our laboratory have likewise found a decline in body weight of rat pups, and dams by F treatment.¹⁷

NaF treatment for 30 days caused a significant decrease in absolute weights of CH, CB, and MO regions of brain in Group III and IV animals (Figures 2–4). This might be due to inhibition by F of protein synthesis and/or breakdown of protein. Other investigators have also reported reduction in absolute weight of CH, hippocampus, CB, and other regions of the brain in experimental animals exposed to F.¹⁸ Trabelsi et al.¹⁹ has reported reduction in weight of cerebral hemisphere and cerebellum which might be due to effect of F on cell proliferation in the external germinal layer of CH and CB. All these factors could account for reduction in body weight and organ absolute weight.

Treatment with 20% BTE alone in Group II did not have any significant effect on morphological changes, change in body weight gain as well as absolute weight of CH, CB, and MO regions of brain as compared to untreated control Group I. However, administration of 2% BTE with LD and HD NaF in Groups V and VI resulted in a significant improvement in body weight gain as well as absolute weights of CH, CB, and MO regions of brain, as compared to NaF alone treated Groups III and IV. This might be due to the antioxidative effect of tea extract. Kurihara et al.²⁰ suggested that tea infusion has beneficial effects on health related to its antioxidative action. Eid et al.²¹ have shown that body weight gain was

lower when broiler chickens were treated with corticosterone (CTC). High dosage with polyphenols, however, tended to reduce the body-weight gain effect of CTC.

Histopathological studies clearly indicate occurrence of neuron degenerative changes, chromatolysis, pycnotic nuclei, fatty infiltration, and vacuolization in CH (Figures 7 and 8), CB (figures 13 and 14), and MO (Figures 19 and 20). It may be inferred, therefore, that during NaF intoxication, F crossed the blood brain barrier and damaged the nerve cells. Neurological changes associated with skeletal fluorosis have been attributed to compression radioculomyelopathy.²² Axonal degeneration with secondary demyelination in myelinated fibres in the sural nerves in patients with skeletal fluorosis has also been reported.²³ Central and peripheral nerves were damaged directly by F, and the damaged function of motor nerves was imputed to osteoproliferation of vertebrae.²⁴ Earlier, Shivarajashankara et al.²⁵ have reported neurodegenerative changes in the brain of rats exposed to F in early stages of life. Zhavoronkov²⁶ has also reported foci of demyelination in the cortex and subcortical areas, a decrease in the number of Purkinje cells in the CB, swelling and irregular staining of the Nissl substance, and pycnosis of neurons in experimental animals subjected to F intoxication.

As seen here, oral administration of NaF and BTE together resulted in almost complete amelioration in all histopathological changes observed from NaF in CH (Figures 9 and 10), CB (Figures 15 and 16), and MO (Figures 21 and 22) regions of mice brain. This effect might be due to antioxidative properties of tea polyphenols. Tea flavonols are a group of polyphenols that possess strong electrophilic centers at two positions,²⁷ a property that provides an opportunity for them to react with nucleophiles like F, thus reducing the toxic effects of F.

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