BLACK TEA EXTRACT MITIGATION OF NaF-INDUCED LIPID PEROXIDATION IN DIFFERENT REGIONS OF MICE BRAIN

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SUMMARY: As part of our investigation of fluoride toxicity effects in a group of 80 Swiss albino adult male mice, we examined the mitigating effects of black tea extract (BTE) on the F-induced enzymatic and non-enzymatic parameters of oxidative stress in the cerebral hemisphere (CH), cerebellum (CB), and medulla oblongata (MO) of the brains of these mice. Oral administration of 6 and 12 mg NaF/kg bw/day to the mice for 30 days resulted in a significant increase in level of lipid peroxidation (LPO) and dehydroascorbic (DAA) acid as well as a decrease in glutathione (GSH), total ascorbic acid (TAA), and reduced ascorbic acid. In addition, the activities of the enzymatic antioxidants catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GSH-Pr), and glutathione peroxidase (GSH-Px) as well as cholinesterase (ChE) also decreased. No significant recovery in any of these parameters was observed upon withdrawal of the NaF treatment for 30 days. However, administration of BTE along with the NaF during the experiment resulted in significant mitigation of all the NaF-induced effects that were examined.

Keywords: Cerebellum; Cerebral hemisphere; Enzymatic and non-enzymatic parameters; Fluoride toxicity; Male albino mice; Medulla oblongata; Neurotoxicity; Oxidative stress.

INTRODUCTION

Fluorine (as fluoride ion) is the thirteenth most abundant element, present to the extent of 0.06–0.9% in the Earth's crust, according to a 1984 report of the World Health Organization.[1] A 1991 report titled "The effectiveness of water fluoridation" issued by the National Health and Medical Research Council[2] states that fluoridation of drinking water supplies at a level of 1 ppm F protects against dental caries and is not associated with any known adverse health effects. However, anything in excess is dangerous and can cause toxicity. Intake of high levels of fluoride is known to cause structural changes,[3] altered activities of enzymes,[4-5] and metabolic lesions[6-7] in the brain of experimental animals. Increased free radical generation and lipid peroxidation are proposed to mediate the toxic effects of fluoride on soft tissues.[8-9] Some studies also revealed changes in levels of trace metals in the brain of mice[10] and antioxidant defence in the brain of mice and rats.[11-16]

Significantly impaired learning and memory, shown in mice and rats,[17-20] as well as reduced motor coordination, and behaviour symptoms like nervousness, depression, tingling sensations in fingers and toes, excessive thirst, and tendency to urinate frequently in human patients[21] after excess intake of fluoridated water suggest that not only the structure but functions of the central nervous system is also affected.

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Tea (*Camellia sinensis*) is the most popular beverage worldwide. Of the approximately 2.5 million metric pounds of dried tea manufactured annually, about 80% is consumed as black tea.[22] Oxidation and partial polymerization of polyphenols and their esters, such as, catechins and their gallates, to theaflavin and thearubigin, lead to the brisk and characteristic colour of black tea. Simultaneous release of volatile aromatic aglycones by various glycosidases is responsible for the typical flavour of black tea. It has been shown that black tea has protective effects to arrest cancer progression and heart diseases.[23-24] Nagasawa et al.[25] reported that Sprague–Dawley rats stressed with electrical stimulus exhibited decrease in protein carbonyl content of muscles with EGCG (Epigallocatechin gallate). Oral administration of an aqueous extract of black tea along with aflatoxin caused amelioration of aflatoxin-induced lipid peroxidation by increasing the anti-oxidant enzymatic activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) as well as the non-enzymatic antioxidants reduced glutathione (GSH) and ascorbic acid in the liver of mice as compared to those given aflatoxin alone.[26] Mitigation by black tea extract of sodium fluoride (NaF)-induced toxicity on mice brain *in* vivo[27-29] and on human erythrocytes *in vitro*[30] has been previously studied in our laboratory.

In view of these findings, the present investigation was conducted to study the mitigating effect of black tea extract (BTE) on NaF-induced changes in enzymatic and non-enzymatic antioxidants in the cerebral hemisphere (CH), cerebellum (CB), and medulla oblongata (MO) regions in mice brain.

MATERIALS AND METHODS

The same eighty young adult inbred Swiss strain male albino mice (*Mus musculus*) employed in our previous laboratory work[27] were used with approval by committee for the purpose of control and supervision of experiments for animals (Reg-167/1999/CPCSEA).

Eighty g 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, similar to the procedure we reported earlier.[30] All the treatments were given orally for 30 days using a feeding tube attached to a hypodermic syringe (Table 1). After 30 days of treatment, the animals were sacrificed by cervical dislocation, brain of control and all treated groups of animals were quickly isolated and blotted free of blood. The CH (cerebral hemisphere), CB (cerebellum), and MO (medulla oblongata) regions of brain were separated carefully at low temperature and stored immediately in frozen condition to preserve enzyme activity until the following tests were performed.

Cholinesterase (E.C.3.1.1.7): The activity of cholinesterase (ChE) in the CH, CB, and MO brain regions of the control and all treated mice was assayed by the method of De La Huerga et al.[32] The basis of the assay is the enzymatic hydrolysis of acetylcholine; the unhydrolyzed acetylcholine is converted into acetyl hydroxamic acid in presence of hydroxylamine which gives a brown ferric complex with ferric chloride. The O.D. (optical density) of the samples was plotted in the graph of a standard curve to obtain enzyme activity in units/mL,

which was further divided by the amount of enzyme protein. The result was expressed as ChE activity/mg protein.

Table 1. Experimental protocol

Groups	Treatment	No. of mice	Duration of treatment (days)	Day of autopsy
I	Control	10	30	31st
II	Black tea extracts (2%)	10	30	31st
III	Low Dose NaF (6 mg/kg body wt/day)	10	30	31st
IV	High Dose NaF (12 mg/kg body wt/day)	10	30	31st
V	As in Group III + Withdrawal for 30 days	10	30	61st
VI	As in Group IV + Withdrawal for 30 days	10	30	61st
VII	As in Group III + Black tea extracts (2%)	10	30	31st
VIII	As in Group IV + Black tea extracts (2%)	10	30	31st

Superoxide Dismutase (E.C. 1.16.1.1): The activity of superoxide dismutase (SOD) in the above-noted brain regions was assayed by the modified spectrophotometric method of Kakkar et al.[33] In this method, the formazon formed at the end of the reaction indicates presence of the enzyme. One unit of the enzyme activity is defined as the enzyme concentration required to lower the optical density by 50% at 560 nm of chromogen formed in one min under the assay conditions. The activity was calculated and expressed as units/mg protein.

Catalase (E.C. 1.11.1.6): Catalase (CAT) activity in the same three brain regions was assayed by the modified method of Luck.[34] The assay mixture consisted of 0.5 mL of 50 mM phosphate buffer (pH 7.0), 1 mL aliquot, and 0.5 mL of 10 mM H_2O_2 was added to initiate the reaction. The tube for the blank contained the complete reaction mixture without the aliquot. The decrease in absorbance was noted every 5 seconds for 30 seconds at 240 nm on a Systronics UV-Visible Spectrophotometer (Model No.118). The enzyme activity was expressed as µM of H_2O_2 consumed/mg protein/min.

Glutathione reductase activity (E.C. 1.6.4.2): Glutathione reductase (GSH-Rx) activity in the three brain regions was assayed by a modified method of Mavis and Stellwagen.[35] This enzyme catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH). The Δ340 nm/min using the maximum linear rate for both the test and blank was obtained. The enzyme activity was calculated and expressed as nM of NADPH consumed/mg protein/min.

Glutathione peroxidase activity (E.C.1.11.1.9): Glutathione peroxidase (GSH-Px) activity in the three brain regions was assayed by the modified method of Pagila and Valentine.[36] The enzyme activity was calculated and was expressed as units/mg protein/min, where 1 unit of GSH-Px corresponds to nM of NADPH consumed/mg protein/min.

Lipid peroxidation (LPO): Lipid peroxidation (LPO) levels in the three brain regions were determined by the method of Okhawa et al.[37] The method is based on the formation of a red chromophore that absorbs at 532 nm following the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA) and other breakdown products of peroxidized lipids collectively called as thiobarbituric acid reactive substances (TBARS). The protein content in homogenates was analyzed by the method of Lowry et al.[38] as described earlier. The results were expressed as nM of MDA/mg protein/60 min.

Glutathione (GSH): The concentration of glutathione (GSH) in the three brain regions was assayed by the method of Grünert and Philips.[39] GSH present in the tissue reacts with sodium nitroprusside to give a red coloured complex in saturated alkaline medium. The concentration of GSH was calculated and expressed as µg/ 100 mg tissue weight.

Total, dehydro, and reduced ascorbic acid: Levels of total ascorbic acid (TAA), dehydroascorbic acid (DAA) and reduced ascorbic acids (RAA) were estimated in the three brain regions by the method of Roe and Kuether.[40] Total ascorbic acid was oxidized to dehydroascorbic acid by Norit reagent in the presence of trichloroacetic acid. This involves reaction with 2,4-dinitrophenylhydrazine in the presence of sulphuric acid to yield a red coloured 2,4-dinitrophenylhydrazone, which was measured colorimetrically. The dehydro form (DAA) was estimated by using 6% trichloroacetic acid. The difference between total and dehydroascorbic acid gave the value of reduced ascorbic acid. The concentration of ascorbic acid was calculated and expressed as mg/g tissue weight.

Statistical analysis: For each parameter at least 10 replicate tests were conducted. Results are expressed as standard error of the mean (± S.E.M). The results were statistically analysed using one-way Analysis of Variance (ANOVA) followed by Tukey Test by SPSS – 17 software. Levels of significance were accepted with $p<0.05$. Comparisons of p-values between different groups were also performed.

RESULTS

Acetylcholineesterase activity: Oral administration of NaF caused a significant ($p< 0.05$) decline in cholinesterase (ChE) activity in the mice brain in CH at low dose (LD): –15.95%; in CB at high dose (HD: –40.92%), in CB at LD: –20.08% and at HD: –37.52%; and in MO at LD: –24.32% and at HD: –42.16% compared to the untreated control Group I. There was a diminutive improvement in the activity of cholinesterase upon withdrawal of the NaF treatment for 30 days but it was not significant compared to the combined NaF and BTE treated Groups VII and VIII). Oral administration of BTE for 30 days along with NaF caused significant amelioration in the activity of cholinesterase in Groups VII and VIII compared to Groups III and IV that received only NaF. Improvement in enzyme activity as compared to treated Groups III and IV was significant in CH (LD: +15.80%; HD: +36.32%), CB (LD: +19.20%; HD: +31.73%), and MO (LD: +23.36%; HD: +30.10%) (Tables 2–4)

Table 2. Effect of sodium fluoride and its amelioration by black tea extract on cerebral hemisphere in mice brain

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
ChE	17.30 ± 0.87	17.94 ±0.64	14.54 ± 0.61 abf	10.22 ± 0.9 abcegh	16.32 ± 0.88 df	11.35 ±0.85 abcegh	17.27 ± 0.61 df	16.05 ± 0.53 df
LPO	27.83 ± 1.02	26.37 ± 0.71	40.45 ±0.71 abdfgh	72.46 ±1.33 abcegh	39.04 ± 0.71 abdfg	70.43 ± 0.10 abcegh	28.83 ± 0.95 cdefh	37.75 ± .24 abcdfg
Catalase	35.24 ± 0.54	35.29 ± 0.43	20.10 ± .41 abdfgh	14.25 ± 0.15 abcegh	20.95 ± 0.42 abdfgh	14.87 ± 0.37 abcegh	34.60 ± 0.53 cdefh	24.82 ± 0.58 abcdefg
SOD	3.66 ± 0.58	3.65 ± 0.48	0.95 ± 0.09 abdfgh	0.16 ± 0.04 abcegh	0.96 ± 0.02 abdfgh	0.17 ± 0.01 abcegh	3.47 ± 0.31 cdefh	2.41 ± 0.30 abcdefg
Glutathione	30.43 ± 0.55	30.24 ± 0.30	18.38 ± 0.30 abdfgh	15.20 ± 0.21 abcegh	18.50 ± 0.62 abdfgh	15.97 ± 0.67 abcegh	29.86 ± 0.28 cdefh	22.49 ± 0.25 abcdefg
Dehydro AA	1.28 ± 0.03	1.24 ± 0.04	2.50 ± 0.05 abdfgh	2.94 ± 0.06 abcegh	2.42 ± 0.06 aabdfgh	2.84 ± 0.04abcegh	1.34 ± 0.03 cdefh	1.69 ± 0.05 abcdefg
Reduced AA	4.05 ± 0.17	4.06 ± 0.18	1.46 ± 0.10 abdfgh	0.39 ± 0.09 abcegh	1.47 ± 0.14a bdfgh	0.55 ± 0.11abcegh	4.01 ± 0.14 cdefh	2.54 ± 0.16 abcdefg
Total AA	5.33 ± 0.16	5.30 ± 0.18	3.97 ± 0.10 abdfgh	3.34 ± 0.10 abcegh	3.89 ± 0.10 abdfgh	3.38 ± 0.12 abcegh	5.31 ± 0.15 cdefh	4.43 ± 0.16 abcefg
GSH-Rx	1.08 ± 0.06	1.06 ± 0.03	0.65 ± 0.02 abdfg	0.49 ± 0.04 abcegh	0.65 ± 0.03 abdfg	0.50 ± 0.03 abcegh	1.01 ± 0.01 cdefh	0.72 ± 0.03 abdfg
GSH-Px	0.98 ± 0.04	0.98 ± 0.04	0.64 ± 0.07 abdfg	0.38 ± 0.03 abcegh	0.64 ± 0.02 abdfg	0.38 ± 0.02 abcegh	0.97 ± 0.05 cdefh	0.61 ± 0.02 abdfg

Values are mean ± S.E.M; n=10.

[a]As compared to group I: p<0.05. [b]As compared to group II: p<0.05. [c]As compared to group III: p<0.05. [d]As compared to group IV: p<0.05.

[e]As compared to group V: p<0.05. [f]As compared to group VI: p<0.05. [g]As compared to group VII: p<0.05. [h]As compared to group VIII: p<0.05.

Table 3. Effect of sodium fluoride and its amelioration by black tea extract on cerebellum in mice brain

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
ChE	16.63 ± 0.66	16.54 ± 0.55	13.29 ± 0.81 abdefg	10.39 ± 0.79 abcegh	15.73 ± 0.7 cdf	11.10 ± 1.06 abcegh	16.45 ± 0.79 cdf	15.22 ± 0.82 df
LPO	27.91 ± 0.69	28.22 ± 0.72	47.08 ± 1.15 abdfgh	69.13 ± 0.77 abcegh	45.14 ± 1.17 abdfgh	67.30 ± 0.46 abcegh	28.01 ± 0.51	31.93 ± 0.61 abcdefg
Catalase	32.83 ± 1.04	32.50 ± 0.73	22.54 ± 0.97 abdfgh	16.20 ± 0.18 abcegh	22.99 ± 0.59 abdfgh	16.38 ± 0.60 abcegh	31.47 ± 0.72 cdefh	28.49 ± 1.06 aabcdefg
SOD	3.15 ± 0.43	2.84 ± 0.22	0.96 ± 0.11 abdfg	0.13 ± 0.03 abcegh	1.00 ± 0.32 abdfg	0.13 ± 0.01 abcegh	2.82 ± 0.19 cdefh	1.21 ± 0.11 abdfg
Glutathione	33.50 ± 0.85	33.50 ± 0.75	18.53 ± 0.21 abdfgh	14.18 ± 0.44 abcegh	19.12 ± 0.70 abdfgh	14.61 ± 0.43 abcegh	32.11 ± 0.28 cdefh	26.8 3 ± 0.37 abcdefg
Dehydro AA	1.28 ± 0.04	1.27 ± 0.05	2.23 ± 0.07 abdfgh	2.72 ± 0.11 abcegh	2.13 ± 0.08 abdfgh	2.61 ± 0.12 abcegh	1.29 ± 0.04 cdefh	1.84 ± 0.13 abcdefg
Reduced AA	3.54 ± 0.13	3.53 ± 0.11	1.26 ± 0.16 abdfgh	0.23 ± 0.02 abcegh	1.37 ± 0.14 abdfgh	0.42 ± 0.10 abcegh	3.44 ± 0.15 cdefh	2.29 ± 0.24 abcdefg
Total AA	4.82 ± 0.15	4.80 ± 0.08	3.49 ± 0.15 abdfgh	2.96 ± 0.13 abcegh	3.50 ± 0.08 abdfgh	3.04 ± 0.06 abcegh	4.73 ± 0.12 cdefh	4.13 ± 0.15 abcdefg
GSH-Rx	0.86 ± 0.03	0.83 ± 0.03	0.50 ± 0.01 abdfgh	0.40 ± 0.02 abcegh	0.50 ± 0.02 abdfgh	0.40 ± 0.03 abcegh	0.81 ± 0.04 cdefh	0.67 ± 0.02 abcdefg
GSH-Px	0.95 ± 0.04	0.94 ± 0.04	0.60 ± 0.07 abdfg	0.34 ± 0.03 abcegh	0.61 ± 0.03 abdfg	0.34 ± 0.02 abcegh	0.94 ± 0.05 cdefh	0.61 ± 0.04 abdfg

Values are mean ± S.E.M; n=10.

[a]As compared to group I: p<0.05. [b]As compared to group II: p<0.05. [c]As compared to group III: p<0.05. [d]As compared to group IV: p<0.05.

[e]As compared to group V: p<0.05. [f]As compared to group VI: p<0.05. [g]As compared to group VII: p<0.05. [h]As compared to group VIII: p<0.05.

Table 4. Effect of sodium fluoride and its amelioration by black tea extract on medulla oblongata in mice brain

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
ChE	16.08 ± 0.52	15.80 ± 0.43	12.17 ± 0.72 abdfg	9.30 ± 0.76 abcegh	13.07 ± 0.83 abdfg	9.65 ± 0.97 abcegh	15.88 ± 0.83 cdefh	13.30 ± 0.43 abdfg
LPO	25.56 ± 0.71	25.88 ±0.73	44.74 ± 1.20 abdfgh	66.79 ±0.77 abcegh	43.40 ±1.12 abdfgh	65.39 ± 0.65 abegh	25.67 ± 0.53 cdefh	29.59 ± 0.62 abcdefg
Catalase	29.14 ± 0.46	28.55 ± 0.57	22.80 ± 0.71 abdfgh	19.61 ± 0.30 abcegh	23.68 ± 0.66 abdfgh	20.30 ± 0.50 abcegh	29.04 ± 0.56 cdefh	26.56 ± 0.59 abcdefg
SOD	3.02 ± 0.31	2.95 ± 0.16	1.13 ± 0.14 abdfgh	0.16 ± 0.05 abcegh	1.13 ± 0.15 abdfgh	0.16 ± 0.03 abcegh	2.77 ± 0.22 cdefh	1.67 ± 0.08 abcdefg
Glutathione	31.04 ± 0.84	30.76 ± 0.74	15.79 ± 0.16 abdefgh	11.44 ± 0.53 abcegh	16.38 ± 0.47 abdefgh	11.54 ± 0.53 abcegh	30.37 ± 0.29 cdefh	22.91 ± 0.32 abcdefg
Dehydro AA	1.12 ± 0.04	1.11 ± 0.05	1.95 ± 0.09 abdfgh	2.57 ± 0.12 abcegh	1.88 ± 0.04 abdfg	2.52 ± 0.07 abcegh	1.13 ± 0.04 cdefh	1.68 ± 0.12 abcdfg
Reduced AA	3.54 ± 0.13	3.51 ± 0.10	1.38 ± 0.15 abdfgh	0.29 ± 0.07 abcegh	1.47 ± 0.09 abdfgh	0.34 ± 0.09 abcegh	3.47 ± 0.15 cdefh	2.25 ± 0.25 abcdefg
Total AA	4.66 ± 0.15	4.62 ± 0.08	3.33 ± 0.15 abdfgh	2.86 ± 0.16 abcegh	3.35 ± 0.08 abdfgh	2.86 ± 0.05 abcegh	4.61 ± 0.13 cdefh	3.94 ± 0.16 abcdefg
GSH-Rx	0.67 ± 0.01	0.67 ± 0.02	0.5 ± 0.03 abdfg	0.43 ± 0.02 abcegh	0.55 ± 0.02 abdfg	0.44 ± 0.02 abcegh	0.67 ± 0.01 cdefh	0.56 ± 0.01 abdfg
GSH-Px	0.91 ± 0.04	0.90 ± 0.04	0.59 ± 0.06 abdfg	0.23 ± 0.01 abcegh	0.60 ± 0.03 abdfg	0.23 ± 0.01 abcegh	0.91 ± 0.05 cdefh	0.57 ± 0.03 abdfg

Values are mean ± S.E.M; n=10.

[a]As compared to group I: p<0.05. [b]As compared to group II: p<0.05. [c]As compared to group III: p<0.05. [d]As compared to group IV: p<0.05.

[e]As compared to group V: p<0.05. [f]As compared to group VI: p<0.05. [g]As compared to group VII: p<0.05. [h]As compared to group VIII: p<0.05.

Lipid peroxidation: Lipid peroxides (LPO) are products of free radical modification of fatty acid residues of lipids. As shown in Tables 2–4, malondialdehyde (MDA), a main indicator of lipid peroxidation, was significantly ($p< 0.05$) increased in NaF-treated mice, as compared to untreated Group I controls. The effect was dose-dependent in Groups III and IV) CB (LD: +68.69%; HD: +147.69%) and MO (LD: +75.04%; HD: +161.31%). Withdrawal of NaF for 30 days caused partial but significant recovery in Groups V and VI. Administration of BTE along with NaF in Groups VII and VIII showed significant recovery in the level of MDA as compared to the groups treated with NaF alone (Tables 2–4).

GSH, TAA, RAA, and DAA contents: As shown in Tables 2–4, NaF treatment caused a significant ($p<0.05$) reduction in the level of GSH in CH (LD: –39.60%; HD: –50.05%), CB (LD: –44.69%; HD: –57.67%), and MO (LD: –49.13%; HD: –63.14%), TAA in CH (LD: –25.52%; HD: –37.34%) CB (LD: –27.59%; HD: –38.59%) and MO (LD: –28.54%; HD: –38.63%), and RAA in CH (LD: –63.95%; HD: –90.37%) CB (LD: –64.41%; HD: –93.50%) and MO (LD: –61.02%; HD: –91.81%). It also increased the level of DAA in CH (LD: +95.31%; HD: +29.61%) CB (LD: +74.22%; HD: +2.50%) and MO (LD: +74.11%; HD: +29.46%) as compared to untreated control Group I. Withdrawal of NaF (Groups V and VI) for 30 days caused partial but non-significant improvement compared to NaF and BTE treatment (Groups VII and VIII). Co-administration of BTE along with NaF in Groups VII and VIII showed significant recovery in all parameters examined compared to animals treated with NaF alone.

CAT, SOD, GSH-Rx, and GSH-Px activity: Compared to the untreated control Group I, NaF treatment for 30 days caused significant ($p<0.05$) decrease in the activities of CAT in CH (LD: –42.96%; HD: –59.56%), CB (LD: –31.34%; HD: –50.65%) and MO (LD: –21.76%; HD: –32.70%); SOD in CH (LD: –74.04%; HD: –95.63%), CB (LD: –69.52%; HD: –95.87%) and MO (LD: –62.58%; HD: –94.70%); GSH-Rx in CH (LD: –39.81%; HD: –54.63%), CB (LD: –41.86%; HD: –53.49%), and MO (LD: –17.91%; HD: –35.82%); GSH-Px in CH (LD: –34.69%; HD: –61.22%), CB (LD: –36.84%; HD: –64.21%), and MO (LD: –35.16%; HD: –74.73%). Upon withdrawal of NaF for 30 days (Groups V and VI), there was no improvement in the activity of SOD, GSH-Rx, and GSH-Px in CH, CB, and MO regions of mice brain, whereas the activity of CAT showed diminutive improvement, but it was partial and non-significant compared to that of the BTE plus NaF treated groups. Oral administration of BTE for 30 days along with NaF caused significant amelioration in CH, CB, and MO regions of mice brain (Groups VII and VIII) compared to recovery from groups treated with NaF alone (Tables 2–4).

DISCUSSION

Cholinesterases are enzymes that hydrolyze esters of choline. Cholinesterase is linked to cholinergic nerve function and plays a key role in deacetylating acetylcholine.[41] Reduced enzyme activity in CH, CB, and MO brain regions was observed with NaF treatments in the present study. Inhibition of cholinesterase

activity in the brain of NaF-treated mice has already been reported in an earlier study.[5] Gastrocnemius muscle cholinesterase also decreases after NaF, $AlCl_3$, and their combined treatment.[42-43] This toxic effect may lead to altered utilization of acetylcholine, thus affecting the transmission of nerve impulses in brain.[44] The exact mechanism of action is not clearly understood, but it might be due to increase in oxidative stress and decrease in antioxidative defence of fluoride intoxicated animals. The ameliorative effect of BTE might be due to its strong antioxidative property.[45] Acetylcholine is also known to play a very important role in controlling brain inflammation.

Lipid peroxidation is regarded as one of the primary key events in cellular damage. Peroxidation of lipid molecules invariably changes or damages their molecular structure with an increase in cellular permeability, resulting in an influx of Ca^{+2} and causing further mitochondrial damage. Dismutation of superoxide anion to H_2O_2 and O_2 by superoxide dismutase (SOD) is often called the primary defence against oxidative stress. The major route involves decomposition of hydrogen peroxide to water by catalase and glutathione peroxidase. Glutathione peroxidase is one of the body's principal means of protecting against oxidative damage. It catalyses the reduction of hydrogen peroxide and lipid peroxide by glutathione (GSH), which serves as an electron donor.[43]

Increase in lipid peroxidation and inhibition of the activities of some antioxidant enzymes such as superoxide dismutase (SOD), glutathione reductase (GSH-Rx), glutathione peroxidase (GSH-Px) and catalase (CAT) as well as decreased levels of glutathione in the brain in the present study are in agreement with similar findings in kidney, liver, bones and brain[14] of fluoride treated rats. Liu et al.[47] found that arsenic, fluoride, and their combination affected the activities of SOD and GSH-Px in liver, kidney, and blood of rats as well as in their offspring.[48] Decrease in activity of free radical scavenging enzymes (SOD, GSH-Px, and GSH-Rx) has also been found in people living in areas of endemic fluorosis in China.[49] Fluoride has been demonstrated *in vivo* and *in vitro* to cause increased lipid peroxidation in human erythrocytes[50] and in brain, RBC, and liver of 100-ppm fluoride-treated rats.[14]

Ascorbic acid is a powerful reducing reagent known to help activate several enzymes and to act as an antioxidant for detoxifying various toxic substances.[51] In our mice, NaF caused a significant decline in total ascorbic acid and GSH levels in CH, CB, and MO brain regions, thus indicating increased stress in the animals leading to rapid utilization of ascorbic acid. This finding suggests that stored ascorbic acid is rapidly oxidized in these tissues under fluoride-induced stress and converted into its dehydro form, which consequently increased, as in the present study.

Significant depletion of GSH in different areas of the brain observed in the present study as well as in earlier studies[11] indicates that fluoride depends upon GSH for detoxification. This is due to the fact that intracellular GSH is an important factor in the cytotoxic effect of a large number of compounds due to its reducing action. During free radical scavenging action, ascorbic acid is

transformed into L-dehydro ascorbate**.** Reduced glutathione is required for the conversion of L-dehydroascorbate back to ascorbate.[51] The resulting fall in the level of GSH (reduced glutathione) decreases the conversion of L-dehydro ascorbate to ascorbate and probably explains the lowered level of reduced ascorbic acid in NaF-treated animals. Agents that ameliorate NaF-induced lipid peroxidation increase the antioxidative activity of cells. Black tea reduces oxidative stress and oxidation-promoting activities by its important chemopreventive action attributable to its antioxidative polyphenolic constituents.

Sengupta et al.[52] have shown significant reduction in the number of aberrant crypt foci and levels of lipid peroxidation in the colon of tea-treated rats. Maity et al.[53] found that thearubigin, the most predominant polyphenol of black tea, ameliorated the occurrence of diarrhoea and the disruption of colonic architecture by reducing lipid peroxidation in the inflamed colon of rats. Tea catechins have also been shown to have protective effects on the neuronal injury of cerebral ischemia in rats. Tea catechin could decrease the concentration of the MDA in brain tissue and serum and it could alleviate or prevent the injury to the blood brain barrier.[54]

In conclusion, the present investigation has shown that a 2% black tea extract has significant mitigating effects on F-induced oxidative stress in the cerebral hemisphere, cerebellum, and medulla oblongata regions in the brains of mice.

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