BLACK TEA AMELIORATION OF SODIUM FLUORIDE-INDUCED ALTERATIONS OF DNA, RNA, AND PROTEIN CONTENTS IN THE CEREBRAL HEMISPHERE, CEREBELLUM, AND MEDULLA OBLONGATA REGIONS OF MOUSE BRAIN

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Summary: Oral administration of sodium fluoride (NaF, 6 and 12 mg/kg body weight/ day) to Swiss male albino mice for 30 days caused significant, dose-dependent reduction in DNA, RNA, and protein contents in cerebral hemisphere, cerebellum, and medulla oblongata of the brain. After 30 days of NaF treatment, followed by withdrawal of treatment for 30 days, partial but significant amelioration occurred. Administration of 2% black tea extract alone for 30 days did not cause any significant effect. However, concurrent administrations of NaF and black tea extract for 30 days cause significant amelioration in all parameters studied.

Keywords: Amelioration of fluoride toxicity; Black tea; Cerebellum; Cerebral Hemisphere; DNA; Medulla oblongata; Mouse brain; Protein; RNA.

INTRODUCTION

Fluorosis caused by excess intake of fluoride is a slow, progressive degenerative disorder, known to affect predominantly the skeletal system, teeth, and also the structure and functions of skeletal muscle,[1-3] brain,[4] and spinal cord.[5] Recent studies have shown accumulation of fluoride in the hippocampus of the brain[6] causing degeneration of neurons and decreased aerobic metabolism.[7]

Epidemiological investigations have revealed that the intelligent quotient (IQ) of children living in high fluoride areas of Tianjin, Guizhou, and other provinces of China is 8-12% lower than in children living in low fluoride areas.[8-9] As fluoride is an archoplasm intoxicant,[10] it can induce chromosomal aberrations,[11] sister chromatid exchanges,[11-12] and DNA damage[10,13] in different tissues. Guan et al.[14] found that high fluoride can penetrate the blood brain barrier and inhibit the synthesis of DNA and RNA in the brain of offspring rats. Furthermore, the incidence of the Down syndrome, a congenital chromosomal abnormality affecting the brains of children, is higher among births to younger mothers in high fluoride areas.[15] However, the effect of fluoride on DNA, RNA, and protein contents in different regions of the brain is far from clear.

In a developing country like India, tea is one of the most common beverages consumed by a large population. It contains a large number of antioxidative polyphenols; typically 93% of total tea phenolic compounds are flavonoids.[16-17] Earlier investigations have revealed that one or two cups of tea have the same "radical scavenging capacity" as five portions of fruits and vegetables or 400 mg vitamin C equivalents.[18-19]

Our previous experiments revealed that black tea has a significant ameliorative effect on NaF-induced toxicity in human red blood corpuscles (*in vitro*)[20] and protein content of liver and kidney in mice.[21] The present study was undertaken to

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evaluate the possible ameliorative effect of black tea extract on NaF-induced changes in the DNA, RNA, and protein contents of different regions of the brain in mice.

MATERIALS AND METHODS

The eighty young adult inbred Swiss-strain male albino mice (*Mus musculus*) used here were the same mice employed in our previous study.[21] They were provided with laboratory animal feed and water *ad libitum* and maintained in a 12-hr light/dark cycles at 26±2°C. The animal feed was prepared as per the formulation given by the National Institute of Occupational Health, Ahmedabad, India. *Guidelines for Care and Use of Animals in Scientific Research 1991* published by the Indian National Science Academy, New Delhi, India, were followed.

As shown in Table 1, the mice were divided into eight equal groups and caged separately. Group I (control) animals were maintained without any treatment. Group II received black tea (2% in drinking water) for 30 days and served as antidote control group. Groups III and IV 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. Groups V and VI were administered NaF as in groups III and IV; thereafter the treatment was withdrawn for another 30 days. Groups VII and VIII were administered NaF as in groups III and IV and also given 2% black tea infusion instead of drinking water for 30 days.

Table 1. Experimental protocol

Group	Treatment	No. of animals	Duration of treatment (days)	Duration of withdrawal (days)	Day of autopsy
I	Control	10	30	--	31st
II	Black tea extract (2%)	10	30	--	31st
III	NaF (6 mg/kg body wt./ day)	10	30	--	31st
IV	NaF (12 mg/kg body wt./ day)	10	30	--	31st
V	Low dose withdrawal from day 31	10	30	30	61st
VI	High dose withdrawal from day 31	10	30	30	61st
VII	Low dose (as in Group III) + Black tea extract (2%)	10	30	--	31st
VIII	High dose (as in Group IV) + Black tea extract (2%)	10	30	--	31st

Twenty grams of black tea solids (Lipton Yellow label of Hindustan Lever Limited, Mumbai, India) and 1000 mL deionized water were used to produce a 2% tea infusion.[21-22] Stock solutions of analytical grade NaF (Sisco Research Laboratory Pvt. Ltd., Mumbai, India) were prepared by dissolving 1 and 2 mg

NaF/mL in deionized water and used as low dose and high dose, respectively. The effective dose of black tea was based on earlier work[21-22] on these same male mice. All treatments were given orally for 30 days using a feeding tube attached to a hypodermic syringe.

On completion of the treatment periods, the animals were sacrificed by cervical dislocation. The cerebral hemisphere, cerebellum, and medulla oblongata regions of brain were dissected carefully, blotted free of blood, weighed to the nearest mg and utilized for study. The DNA, RNA, and protein measurement were done as follows.

The DNA content was estimated by the method of Giles and Meyer.[23] in which the DNA in the supernatant reacts with diphenylamine to give a blue coloured complex, the optical density of which was measured colorimetrically. The concentration of RNA was estimated by the method of Swift et al.[24] in which the RNA in the supernatant reacts with orcinol reagent to give a green coloured complex, which was also measured colorimetrically. The concentration of protein was measured by the method of Lowry et al.[25] using bovine serum albumin as the standard.

Statistical Analysis: The results were expressed as the standard error of the mean (± SEM). The data were statistically analyzed using one-way Analysis of Variance (ANOVA) followed by the Tukey test. The level of significance was taken as $p < 0.05$. Comparisons of p-values between different groups were also performed. Percent change between control and low dose Group III and high dose Group IV NaF-treated mice were calculated. In addition, the percent changes between low dose NaF-treated Group III and Groups V and VII (low dose + withdrawal and low dose + black tea extract, respectively) as well as between Group IV (high dose NaF-treated) and Groups VI and VIII (high dose + withdrawal and high dose + black tea extract, respectively) were also calculated.

RESULTS

As seen in Tables 2 and 3, oral administration of NaF (6 and 12 mg/kg body weight/day) for 30 days caused significant, dose-dependent reduction in the contents of DNA, RNA, and protein contents in three regions of brain (cerebral hemisphere, cerebellum, and medulla oblongata). Withdrawal of NaF treatment for 30 days resulted in significant partial recovery in all parameters studied. Fluoride treatment also caused changes in DNA/RNA, DNA/protein, and RNA/protein ratios, indicating alteration in transcription and translation process.

Administration of 2% black tea extract alone for 30 days did not cause significant changes in the DNA, RNA, and protein content in different regions of brain (Table 2). However, administration of black tea extract along with NaF significantly ameliorated F-induced changes in DNA, RNA, and protein content in different regions of brain (Table 3). The amelioration was almost complete in the low dose NaF-treated Group VII but was only partial but significant in the high

dose Group VIII. Administration of black tea extract also ameliorated fluoride-induced changes in DNA/RNA, DNA/protein, and RNA/protein ratios.

Table 2. Effect of NaF dose and black tea extract on brain DNA (μmoles/100 mg fresh tissue weight), RNA (μmoles/100 mg fresh tissue weight) and protein (mg/100 mg fresh tissue weight) contents in mice. Values are mean ± SEM (n = 10 per group).

Parameters	Control		Treated	
	Group I	Group II	Group III	Group IV
	Control	Black tea extract	Low dose NaF	High dose NaF
		Cerebral Hemisphere		
DNA	379.58±9.78	376.89±12.04	300.19±11.25^{abdefgh}	206.50±14.60^{abcefgh}
RNA	132.16±5.58	133.50±5.19	65.77±2.54^{abdegh}	51.90±1.61^{abcefgh}
Protein	17.33±0.48	17.29±0.53	9.71±0.40^{abdefgh}	4.40±0.22^{abcefgh}
		Cerebellum		
DNA	364.15±9.78	364.60±10.96	285.11±12.03^{abdefg}	193.80±15.78 ^{abcefgh}
RNA	130.12±5.53	132.76±5.26	64.49±2.39 ^{abdegh}	50.56±1.54 ^{abcefgh}
Protein	16.57±0.54	16.52±0.56	7.70±0.46 ^{abdefgh}	4.65±0.33 ^{abcefgh}
		Medulla		
DNA	351.88±9.84	350.07±8.97	295.56±8.55^{abdefgh}	184.72±14.52^{abcefgh}
RNA	128.68±5.44	131.29±5.27	62.68±2.24^{abdegh}	50.09±1.62^{abcefgh}
Protein	13.84±0.49	13.79 ±0.65	6.55±0.37^{abdefgh}	3.58±0.75^{abcefgh}

[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 NaF dose on brain DNA (μmoles/100 mg fresh tissue weight), RNA (μmoles/100 mg fresh tissue weight) and protein (mg/100 mg fresh tissue weight) contents in mice and its amelioration by black tea extract. Values are mean ± SEM (n = 10 per group).

Parameters	Withdrawal		Treated + Antidote	
	Group V	Group VI	Group VII	Group VIII
	Low dose NaF + Withdrawal	High dose NaF + Withdrawal	Low dose NaF + Antidote	High dose NaF + Antidote
		Cerebral Hemisphere		
DNA	339.69±5.83^{abcdfgh}	266.2±15.37^{abcdeg}	376.43±11.93 ^{cdefh}	259.32±13.29 ^{abcdeg}
RNA	77.83±2.03^{abcdfgh}	63.09 ±1.57^{abdegh}	127.34±4.98 ^{cdefh}	114.61±5.06 ^{abcdefg}
Protein	11.74±0.33^{abcdfgh}	6.69±0.27^{abcdegh}	17.02±0.50 ^{cdefh}	10.96±0.36 ^{abcdefg}
		Cerebellum		
DNA	323.72±6.63^{abcdfgh}	252.40±15.41^{abcdegh}	365.06±10.53 ^{cdefh}	286.02±6.16 ^{abdefg}
RNA	81.64± 2.16^{abcdfgh}	63.02±1.48^{abdegh}	125.93±4.96^{cdefh}	113.60±5.12 ^{abcdefg}
Protein	8.68±0.32^{abcdfgh}	5.85±0.31^{abcdegh}	16.26±0.66 ^{cdefh}	12.07±0.49 ^{abcdefg}
		Medulla		
DNA	322.36±4.59^{abcdfgh}	241.05±14.52^{abcdegh}	349.16±9.98 ^{cdefh}	268.76±7.38 ^{abcdefg}
RNA	80.64±2.14^{abcdfgh}	62.35±1.69^{abdegh}	124.99±5.03 ^{cdefh}	112.73±5.18 ^{abcdefg}
Protein	7.44±0.38^{abcdfgh}	4.53±0.39^{abcdegh}	13.48±0.36 ^{cdefh}	7.91±0.45 ^{abcdefg}

[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.

DISCUSSION

Fluoride has been reported to cause a depression in DNA and RNA synthesis in cultured cells.[26] Fluoride inhibits nucleic acid synthesis and attachment of m-RNA to ribosomes. The decrease in RNA content of rabbit brain observed during acute and chronic fluoride intoxication seems to be due to fluoride-induced

inhibition of protein synthesis.[4] In the present study, too, the DNA, RNA, and protein contents in cerebral hemisphere, cerebellum, and medulla oblongata regions in the brain significantly declined from NaF treatment for 30 days in mice (Tables 2 and 3). Fluoride-induced alterations in DNA/RNA, DNA/protein, and RNA/protein ratios were also observed. These might be due to the inhibitory action of fluoride on DNA synthesis or to alteration in the synthesis of RNA. Fluoride has been reported to cause a depression in DNA and RNA synthesis as well as DNA/RNA and DNA/protein ratios, which is indicative of the probable disturbances in the process of transcription and translation, as well as mitotic cycles and chromosomal aberrations.[27-28] It is also reported that fluoride enhances lipid peroxidation and inhibits antioxidative enzymes in brain, liver, kidney, heart, and blood of fluoridated mice.[29] The oxygen-derived free radicals are also a major source for DNA damage, which can cause strand breaks and base alteration in the DNA

Our findings suggest a profound ameliorative effect of black tea extract on NaF-induced reduction in DNA, RNA, and protein contents of cerebral hemisphere, cerebellum, and medulla oblongata regions of brain in mice. Thirty days after withdrawal of the 30-day NaF treatment, partial recovery occurred without black tea extract. In comparison with the combined administration of 2% black tea extract and NaF, however, it was not nearly so significant. Administration of black tea extract along with NaF significantly ameliorated NaF-induced DNA, RNA, and protein content alterations and DNA/RNA, DNA/protein, and RNA/protein ratios.

The ameliorative effect of black tea extract against NaF toxicity may be due to the presence of monomeric catechins that affect plasma antioxidant biomarkers and energy metabolism.[30] Polyphenols are well known for their ability to reduce membrane lipid peroxidation and increase malondialdehyde levels that can prevent oxidative damage caused by NaF.

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