Acta Poloniae Pharmaceutica – Drug Research, Vol. 64 No. 3 pp. 221–225, 2007 ISSN 0001-6837
Polish Pharmaceutical Society

AMELIORATION BY BLACK TEA OF SODIUM FLUORIDE-INDUCED CHANGES IN PROTEIN CONTENT OF CEREBRAL HEMISPHERE, CEREBELLUM AND MEDULLA OBLONGATA IN BRAIN REGION OF MICE

MRUGESH H. TRIVEDI, RAMTEJ J. VERMA*
and NILOUFER J. CHINOY

Department of Zoology, University School of Science, Gujarat University, Ahmedabad 390 009, India

Abstract: Oral administration of sodium fluoride (NaF, 6 and 12 mg/kg body weight/day) to Swiss strain male albino mice for 30 days caused significant dose-dependant reduction in the content of acidic, basic, neutral, and total protein in cerebral hemisphere, cerebellum and medulla oblongata region of 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 administration of NaF and black tea extract for 30 days caused significant amelioration in all parameters studied.

Keywords: ameliorative, black tea, sodium fluoride, cerebral hemisphere, cerebellum, medulla oblongata, protein

Extensive contamination of ground water by fluoride has been reported in China (1) and India (2). Fluorosis, caused by long-term intake of high level of fluoride, is characterized by clinical manifestation in bones and teeth (3). However, detrimental effects of high fluoride intake are also observed in soft tissues (4, 5). In advanced stages of fluorosis, neurological manifestation such as paralysis of limbs, vertigo, spasticity in extremities, and impaired mental activity are observed in human beings (6). However, there are disagreements about the toxic effect of fluoride on internal organs. The kidney is known to be a target organ of fluoride among internal organs (7) but the effect of fluoride on liver and brain is not clear.

Manocha et al. (8) administered fluoridated water to the squirrel monkeys for 18 months at the concentration of 0, 1 and 5 ppm of fluoride. Significant cytochemical changes were observed in the kidneys of monkeys treated with 5 ppm fluoride in drinking water. For the liver, the activities of Krebs cycle enzymes were slightly enhanced in the groups administered fluoride. The nervous system appeared to be unaffected. On the other hand, Mullenix et al. (9) demonstrated that the exposure to fluoride via drinking water significantly altered the behavior of female rats compared to the controls. It is of interest, therefore, to know whether neurological effects can be induced in mice by oral exposure to fluoride.

Various epidemiological investigations in China have reported a relationship between fluoride and intelligence showing an intelligence quotient (IQ) lowering by 8 to 10 points in children living in villages with high fluoride in food or drinking water (10-12). However, for such evaluation adequate index is required, e.g. alteration in protein content of brain due to fluoride intoxication.

In many parts of the world, tea is one of the most widely consumed beverages, second only to water. Tea flavanoids exhibit antioxidant activity (13), and while tea is not a replacement for fruits and vegetables, its antioxidant acitivity has been found in several studies to be comparable to that of fruits and vegetables. One or two cups of tea have the same "radical scavenging capacity" as five portions of fruits and vegetables or 400 mg vit. C equivalent (14). Our previous experiments revealed that black tea have significant amelioration on NaF induced toxicity on red blood cells (*in vitro*) (15) and protein content of liver and kidney in mice (16).

The purpose of this study was to determine the changes in protein content (acidic, basic, neutral and total protein) in different regions of brain (cerebral hemisphere, cerebellum and medulla oblongata) of Swiss strain male albino mice (*Mus musculus*) after

* Corresponding author: phone: 91-079-26302362, e-mail: ramtejverma2000@yahoo.com

exposure to NaF via oral route for thirty days. Moreover, it is important to study antioxidant brain defense and effect of antioxidants like catechine, gallocatechine etc. present in black tea extract to evaluate its ameliorative effect against NaF intoxication.

EXPERIMENTAL

Eighty young adult inbred Swiss strain male albino mice (*Mus musculus*) weighing approximately 30 to 35 g were obtained from Zydus Research Centre, Ahmedabad, India. They were provided with laboratory animal feed and water *ad libitum* and maintained in a 12-h light/dark cycle 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. In 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.

Twenty grams of black tea solids (Lipton Yellow Label of Hindustan Lever Limited, Mumbai, India) and 1000 mL of deionized water were used to produce a 2% tea infusion (16, 17). 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 (16). The effective dose of black tea was based on earlier work in male mice (16, 17). 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 protein fraction and measurement were done as follows.

The acidic, basic, neutral, and total proteins were extracted separately (16, 18). The tissue was homogenized in ice-cold 10% trichloroacetic acid (TCA) to precipitate the proteins. The homogenates were incubated at 70°C for 20 min, cooled, and centrifuged. The supernatant was discarded and the residue taken as the total protein. Lipids and nucleic acids were removed by washing with ethanol. The residue was then treated with known volume of 0.2 M HCl and incubated at 100°C for 30 min and centrifuged. The resulting supernatant was taken as the extract of basic proteins. The residue was treated with a known volume of 0.1 M NaOH and kept overnight at room temperature and centrifuged. The supernatant served as the extract of acidic proteins. Neutral proteins were calculated by subtracting the sum of basic and acidic proteins from total proteins.

Determination of acidic, basic, neutral, and total proteins was done spectrophotometrically by the method of Lowry et al. (19) using bovine serum albumin as standard.

Table 1. Experimental protocol.

Group	Treatment	No. of animals	Duration of treatment	Duration of withdrawal	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

Statistical analysis: The results were expressed as mean ± 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 – 4, oral administration of NaF (6 and 12 mg/kg body weight/day) as compared with control (Group I) for 30 days caused significant ($p < 0.05$), dose-dependent reduction in the content of acidic, basic, neutral, and total protein content in the cerebral hemisphere, cerebellum and medulla oblongata regions of mice brain. Withdrawal of NaF fluoride treatment for 30 days resulted in significant ($p < 0.05$) but partial recovery in all proteins, as compared with respective low (Group III) and high (Group IV) dose NaF-treated groups.

Administration of 2% black tea extract alone for 30 days did not cause significant ($p < 0.05$) change in cerebral hemisphere, cerebellum and medulla oblongata regions of brain in mice (Tables 2 – 4). However, administration of black tea extract along with NaF significantly ($p < 0.05$) ameliorated NaF-induced changes in cerebral hemisphere, cerebellum and medulla oblongata regions of mice brain. The amelioration was almost complete in the low dose NaF + antidote-treated (Group VII) but was only partial in the high dose (Group VIII).

DISCUSSION AND CONCLUSION

The NaF-induced reduction in protein content of cerebral hemisphere, cerebellum and medulla oblongata regions of mice brain observed here might be due to either increased proteolysis or decreased protein synthesis. Many investigators (16, 18, 20, 21) have reported protein degradation in skeletal muscles of rabbits during experimental fluorosis. Also, F is known to affect the rate of cellular protein synthesis, which is mainly due to impairment of peptide chain initiation (22, 23). The reduction in

Table 2. Effect of sodium fluoride and its amelioration by black tea extract on protein content (mg%) in cerebral hemisphere of mice brain.

Type of protein	CONTROL		NaF-TREATED		NaF-TREATED + WITHDRAWN		NaF-TREATED + ANTIDOTE	
	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V	GROUP VI	GROUP VII	GROUP VIII
	Control	Black tea extract	L.D.	H.D.	L.D. + withdrawal	H.D. + withdrawal	L.D. + antidote	H.D. + antidote
Acidic protein	11.29 ± 0.22	11.29 ± 0.23	6.21 ± 0.25 ^{abdefgh}	2.55 ± 0.15 ^{abcefgh}	7.66 ± 0.27 ^{abcdfgh}	4.08 ± 0.21 ^{abcdegh}	11.13 ± 0.34 ^{cdefh}	6.97 ± 0.27 ^{abcdefg}
Basic protein	4.41 ± 0.28	4.38 ± 0.255	2.56 ± 0.32 ^{abdefgh}	1.17 ± 0.15 ^{abcefgh}	3.00 ± 0.05 ^{abcdfg}	1.80 ± 0.19 ^{abcdegh}	4.30 ± 0.28 ^{cdefh}	3.01 ± 0.20 ^{abcdfg}
Neutral protein	1.62 ± 0.22	1.57 ± 0.34	0.93 ± 0.04 ^{abdeg}	0.67 ± 0.02 ^{abcegh}	1.07 ± 0.053 ^{abcdfg}	0.79 ± 0.06 ^{abcgh}	1.58 ± 0.35 ^{cdefh}	0.97 ± 0.06 ^{abdfg}
Total protein	17.33 ± 0.48	17.29 ± 0.53	9.71 ± 0.40 ^{abdefgh}	4.40 ± 0.22 ^{abcefgh}	11.74 ± 0.33 ^{abcdfgh}	6.69 ± 0.27 ^{abcdegh}	17.02 ± 0.50 ^{cdefh}	10.96 ± 0.36 ^{abcdefg}

Values are mean ± S.E.M; n = 10.
a – As compared to group 1: $p < 0.05$.
b – As compared to group 2: $p < 0.05$.
c – As compared to group 3: $p < 0.05$.
d – As compared to group 4: $p < 0.05$.
e – As compared to group 5: $p < 0.05$.
f – As compared to group 6: $p < 0.05$.
g – As compared to group 7: $p < 0.05$.
h – As compared to group 8: $p < 0.05$.

Table 3. Effect of sodium fluoride and its amelioration by black tea extract on protein content (mg%) in cerebellum of mice brain.

Type of protein	CONTROL		NaF-TREATED		NaF-TREATED + WITHDRAWN		NaF-TREATED + ANTIDOTE	
	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V	GROUP VI	GROUP VII	GROUP VIII
	Control	Black tea extract	L.D.	H.D.	L.D. + withdrawal	H.D. + withdrawal	L.D. + antidote	H.D. + antidote
Acidic protein	11.56 ± 0.41	11.76 ± 0.28	5.28 ± 0.34 ^{abdefgh}	3.28 ± 0.36 ^{abcefgh}	5.82 ± 0.30 ^{abcdfgh}	3.79 ± 0.25 ^{abcdegh}	11.57 ± 0.36 ^{cdefh}	9.00 ± 0.39 ^{abcdefg}
Basic protein	3.81 ± 0.15	3.70 ± 0.33	1.79 ± 0.37 ^{abdegh}	1.03 ± 0.08 ^{abcefgh}	2.11 ± 0.13 ^{abcdfgh}	1.67 ± 0.11 ^{abdegh}	3.66 ± 0.42 ^{cdefh}	2.37 ± 0.34 ^{abcdefg}
Neutral protein	1.19 ± 0.17	1.18 ± 0.14	0.62 ± 0.03 ^{abdefg}	0.32 ± 0.74 ^{abcegh}	0.74 ± 0.03 ^{abcdfg}	0.38 ± 0.06 ^{abcegh}	1.03 ± 0.10 ^{abcdefh}	0.70 ± 0.04 ^{abdfg}
Total protein	16.57 ± 0.54	16.52 ± 0.56	7.70 ± 0.46 ^{abdefgh}	4.65 ± 0.33 ^{abcefgh}	8.68 ± 0.32 ^{abcdfgh}	5.85 ± 0.31 ^{abcdegh}	16.26 ± 0.66 ^{cdefh}	12.07 ± 0.49 ^{abcdefg}

Values are mean ± S.E.M; n = 10. Notations a – h like in Table 2.

Table 4. Effect of sodium fluoride and its amelioration by black tea extract on protein content (mg%) in medulla oblongata of mice brain.

Type of protein	CONTROL		NaF-TREATED		NaF-TREATED + WITHDRAWN		NaF-TREATED + ANTIDOTE	
	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V	GROUP VI	GROUP VII	GROUP VIII
	Control	Black tea extract	L.D.	H.D.	L.D. + withdrawal	H.D. + withdrawal	L.D. + antidote	H.D. + antidote
Acidic protein	9.37 ± 0.31	9.36 ± 0.45	4.80 ± 0.39 ^{abdfgh}	2.61 ± 0.17 ^{abcefgh}	5.04 ± 0.31 ^{abdfgh}	3.03 ± 0.40 ^{abcdegh}	9.12 ± 0.13 ^{cdefh}	5.81 ± 0.41 ^{abcdefg}
Basic protein	3.07 ± 0.37	3.06 ± 0.39	1.01 ± 0.10 ^{abdegh}	0.55 ± 0.02 ^{abcefgh}	1.51 ± 0.19 ^{abcdfgh}	0.90 ± 0.05 ^{abdegh}	2.94 ± 0.18 ^{cdefh}	1.37 ± 0.11 ^{abcdefg}
Neutral protein	1.49 ± 0.19	1.49 ± 0.14	0.74 ± 0.03 ^{abdg}	0.41 ± 0.60 ^{abcegh}	0.88 ± 0.05 ^{abdfgh}	0.59 ± 0.04 ^{abeg}	1.42 ± 0.21 ^{cdefh}	0.71 ± 0.07 ^{abdeg}
Total protein	13.84 ± 0.49	13.79 ± 0.65	6.55 ± 0.37 ^{abdefgh}	3.58 ± 0.75 ^{abcefgh}	7.44 ± 0.38 ^{abcdfgh}	4.53 ± 0.39 ^{abcdegh}	13.48 ± 0.36 ^{cdefh}	7.91 ± 0.45 ^{abcdefg}

Values are mean ± S.E.M; n = 10. Notations a – h like in Table 2.

protein content of NaF-treated animals supports the view that F inhibits oxidative decarboxylation of branched chain amino acids and simultaneously promotes protein breakdown (24).

The disturbance of protein synthesizing systems in fluorosis has been attributed to a decrease in activity of a group of enzymes catalyzing the key process of cellular metabolism. The enzymes are glutamine synthetase catalyzing certain stages of amino acid biosynthesis and methionine activating enzymes of the liver (25). Kathpalia and Susheela (26) have observed that administration of large doses of fluorine to rabbits caused a 10 to 46 percent reduction in protein content in most body tissues.

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 (15, 16, 27). It is reported that quercetin, a unique flavanol present in black tea extract, can reduce free radicals and delay in myoglobin release (28), which can be correlated with the absence of pale colored tissue in control animals. Polyphenols are well known for their ability to reduce membrane lipid peroxidation and increase malondialdehyde levels that can prevent oxidative damage caused by NaF.

Our findings suggest a profound ameliorative effect of black tea extract on NaF-induced reduction in protein content of cerebral hemisphere, cerebellum and medulla oblongata regions of mice brain. Thirty days after withdrawal of the 30-day NaF treatment, partial recovery occurred. In comparison with the combined administration of 2% black tea extract and NaF, however, it was not nearly so significant.

Acknowledgment

Financial assistance under Gujarat Government Research Scholarship 2005-06 from the Gujarat Government, Gandhinagar, India, is gratefully acknowledged.

REFERENCES

1. Wang L.F., Hung J.H.: Soc. Sci. Med. 41, 1191 (1995).
2. Susheela A.K.: Curr. Sci. 77, 1250 (1999).
3. Bhussry B.R., Demole V., Hodge H.C., Jolly S.S., Singh A., Taves D.R.: Toxic effects of larger doses of fluoride. in Fluorides and human health. p. 255, WHO, Geneva 1970.
4. Zhavoronkov A.A.: Arch. Pathol. 39, 83 (1977).
5. Monosour P.A., Kruger B.J.: Fluoride 18, 53 (1985).
6. Waldott G.L., Burgstahler A.W., Mckinney H.L.: Fluoridation: the great dilemma. Coronado Press, Lawrence, Kansas 1978.
7. Tsunoda H.: Chronic fluoride poisoning. in Pediatric Toxicology, Seki T., Hayakawa H., Yamashita F., Yoshida R. Eds., p. 255, Nakayama Shoten, Tokyo 1981.
8. Manocha S.L., Warner H., Olkowaski Z.L.: Histochem. J. 7, 343 (1975).
9. Mullexin P.J., Denbesten P. K., Schunior A., Kernan W.J.: Neurotoxicol. Teratol. 17, 169 (1995).
10. Lu Y., Sun Z.R., Wu L.N., Wang X., Lu W., Liu S.S.: Fluoride 33, 74 (2000).
11. Li X.S., Zhi J.L., Gao R.O.: Fluoride 28, 189 (1995).
12. Zhao L.B., Liang G.H., Zhang D.N., Wu X.R.: Fluoride 29, 190 (1996).
13. Leung L.K., Su Y., Chen R., Zhang Z., Huang Y., Chen Z.Y.: J. Nutr. 131, 2248 (2001).
14. Du Toit R., Volsteedt Y., Apostolides Z.: Toxicology 166, 63 (2001).
15. Verma R.J., Trivedi M.H., Chinoy N.J.: Fluoride 39, 269 (2006).
16. Trivedi M.H., Verma R.J., Chinoy N.J.: Fluoride 39, 311 (2006).
17. Landau J.M., Wang Z.Y., Young G.Y., Ding W., Yang C.S.: Carcinogenesis, 19, 501 (1998).
18. Shashi A., Singh J.P., Thapar S.P.: Fluoride 25, 155 (1992).
19. Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J.: J. Biol. Chem. 193, 265 (1951).
20. Renner K.A., Howard J.B.: Biochemistry 35, 5353 (1996).
21. Floyd G.A., Traugh J.A.: Eur. J. Biochem. 117, 257 (1981).
22. Horez W., Mc Carty K.S.: Biochim. Biophys. Acta 228, 526 (1971).
23. Godehaux W., Atwood K.C.: J. Biol. Chem. 251, 292 (1976).
24. Chang T.W., Globder A.L.: J. Biol. Chem. 253, 3677 (1978).
25. Zahvaronkov A.A., Strochkova L. S.: Fluoride 14, 182 (1981).
26. Kathpalia A., Susheela A.K.: Fluoride. 12, 125 (1978).
27. Williamson G., Manach C.: Am. J. Clin. Nutr. 81, 243S (Suppl.) (2005).
28. Pietri S., Seguin J.R., Arbigny D., Drieuk A., Culcasi M.: Cardiovasc. Drug Ther. 11, 121 (1997).

Received: 21.11.2006