AMELIORATION BY BLACK TEA OF SODIUM FLUORIDE-INDUCED EFFECTS ON DNA, RNA, AND PROTEIN CONTENTS OF LIVER AND KIDNEY AND ON SERUM TRANSAMINASE ACTIVITIES IN SWISS ALBINO MICE

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SUMMARY: In an extension of previous work on fluoride-induced toxicity in a group of 80 Swiss albino male mice, a study was made of the ameliorative effects of polyphenols in black tea on the DNA, RNA, and protein contents of the liver and kidneys and the activity levels of SGPT (serum glutamate pyruvate transaminase) and SGOT (serum glutamate oxaloacetate transaminase) in the blood serum. Oral administration of sodium fluoride (NaF, 6 and 12 mg/kg-bw/day) for 30 days resulted in significant dose-dependent increases in SGPT and SGOT activity levels and reduction in the DNA, RNA, and protein contents of the liver and kidneys. Withdrawal of treatment for 30 days caused significant but partial recovery in all these parameters. Administration of 2% black tea extract alone for 30 days did not cause any significant alteration in them. However, concurrent administration of NaF and black tea extract for 30 days caused significant amelioration in all parameters studied.

Keywords**:** Amelioration of fluoride toxicity; Black tea; DNA; Kidney; Liver; Protein; RNA; Serum glutamate oxaloacetate transaminase; Serum glutamate pyruvate transaminase.

INTRODUCTION

Tea is one of the most common beverages consumed by large numbers of people worldwide. In a recent review, therapeutic effects of black tea for treating coronary heart disease, cancer, and dental problems were given a prominent role.[1] Antioxidative properties of different types of tea have been found to be comparable to those of vitamin C and green vegetables.[2,3] Previous experiments performed in our laboratory indicate black tea has ameliorative effects against fluoride toxicity and aflatoxicosis.[4-7]

Occurring especially in drinking water, fluoride (F) is a potent toxicant. Epidemiological and experimental studies have revealed zonal necrosis, hyalinization of hepatic lobules with loss of cells, vacuolization in cytoplasm, necrosis of renal tubular cells, and dilation of tubules during fluorosis.[8-10] Enhancement of lipid peroxidation has also been shown to play an important role in fluorosis.[11,12] Fluoride can induce chromosomal aberrations, sister chromatid exchanges,[13,14] and DNA damage[15-16] in different tissues.

The aim of the present study was to evaluate ameliorative effects of black tea extract on NaF-induced changes in DNA, RNA, and protein contents in the liver and kidneys of mice, along with changes in the activity levels of two serum transaminases.

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MATERIALS AND METHODS

The same eighty young adult inbred Swiss strain male albino mice (*Mus musculus*) employed in our earlier work[4,6] were used here. 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.[17]

On completion of the treatment periods, the animals were sacrificed by cervical dislocation. Blood was collected by cardiac puncture and serum was separated by centrifugation. Liver and kidneys were dissected carefully, blotted free of blood, weighed to the nearest mg, and utilized for study. The DNA, RNA, and protein measurements were done as reported earlier.[6]

Photometric determination of SGPT (serum glutamate pyruvate transaminase) and SGOT (serum glutamate oxaloacetate transaminase) activities in control and all treated groups of animals was conducted by the method of Reitman and Frankel.[18] In this procedure for the estimation of SGPT, blood serum was allowed to react with alanine, and the quantity of lactate formed was measured photometrically. Estimation of SGOT was done by using aspartate and photometric measurement of the color intensity.

Statistical analysis: Results are expressed as standard error of the mean (± SEM). Data were analyzed statistically as in our previous investigations.[4,6]

RESULTS

Oral administration of NaF (6 and 12 mg/kg body weight/day) to the mice for 30 days caused, as compared with control (Group I) significant ($p<0.05$), dose-dependent increase in SGPT (Figure 1) and SGOT (Figure 2) in blood and reduction in the contents of DNA, RNA, and protein in liver (Table 1) and kidney (Table 2). Withdrawal of NaF treatment for 30 days resulted in significant ($p<0.05$) but partial recovery in all parameters studied, as compared with respective low (Group III) and high (Group IV) dose NaF-treated groups. Fluoride treatment also caused changes in the DNA/RNA, DNA/protein, and RNA/protein ratios, indicating alteration in transcription and translation process.

Administration of 2% black tea extract alone to the mice for 30 days did not cause significant ($p<0.05$) changes in SGPT (Figure 1) and SGOT (Figure 2) in serum and DNA, RNA, and protein contents in liver (Table 1) and kidney (Table 2). However, administration of black tea extract along with NaF significantly ($p<0.05$) ameliorated F-induced changes in SGPT and SGOT in blood and DNA, RNA, and protein content in liver and kidney. The amelioration was almost the same in the low-dose NaF + Antidote-treated (Group VII) and high-dose NaF + Antidote-treated (Group VIII) groups. Administration of black tea extract also ameliorated F-induced changes in the DNA/RNA, DNA/protein, and RNA/protein ratios.

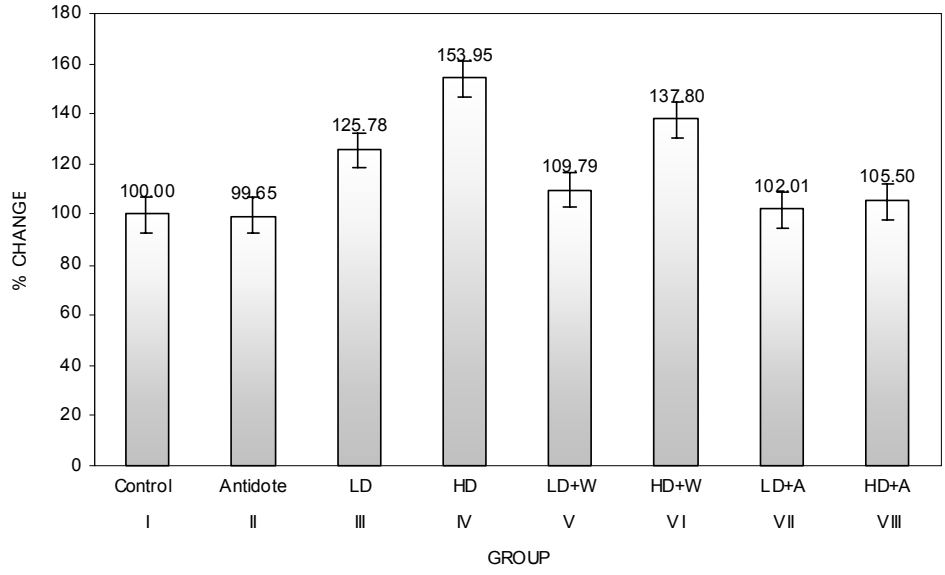

Figure 1. Effect of NaF on SGPT in mice after 30 days.

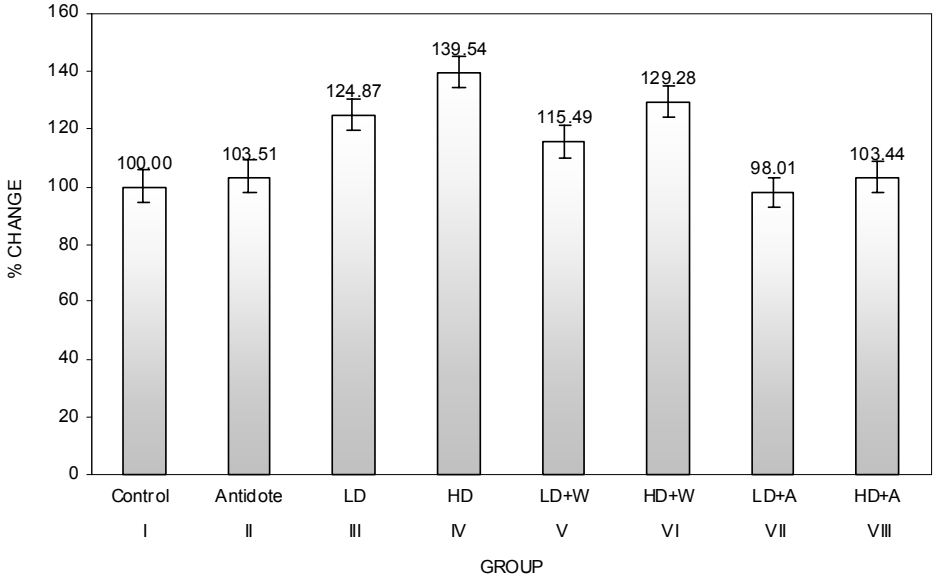

Figure 2. Effect of NaF on SGOT in mice after 30 days.

Table 1. Effect of NaF dose on liver 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	Control		NaF-Treated		NaF-Treated + Withdrawal		NaF-Treated + Antidote	
	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
	Control	Black tea extract	Low dose NaF	High dose NaF	Low dose NaF + Withdrawal	High dose NaF + Withdrawal	Low dose NaF + Antidote	High dose NaF + Antidote
DNA	683.04 ±15.11	682.75 ±11.35	559.2 ±16.44 abdefg	436.13 ±8.24 abcefgh	599.17 ±26.65 abcdfg	511.45 ± 12.03 abcdegh	670.35 ±13.64 cdefh	576.2 ±12.02 abdfg
RNA	431.38 ±12.21	425.11 ±7.74	300.19 ±11.25 abdefg	197.32 ±13.58 abcefgh	339.69 ± 5.83 abcdfgh	266.20 ±15.37 abcdegh	420.97 ±8.01 cdefh	306.62 ±10.85 abdefg
Protein	28.81 ±0.12	28.29 ±0.11	14.25 ±0.07 abdefgh	9.46 ±0.12 abcefgh	19.94 ±0.12 abcdfgh	14.74 ±0.1 abefgh	28.43 ±0.22 cdefh	18.57 ±0.11 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.

Table 2. Effect of NaF dose on kidney 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	Control		NaF-Treated		NaF-Treated + Withdrawal		NaF-Treated + Antidote	
	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
	Control	Black tea extract	Low dose NaF	High dose NaF	Low dose NaF + Withdrawal	High dose NaF + Withdrawal	Low dose NaF + Antidote	High dose NaF + Antidote
DNA	465.90 ±5.03	464.99 ±7.81	313.73 ±7.11 abdefg	238.32 ± 0.18 abcefgh	363.69 ±10.71 abcdfgh	277.39 ±6.58 abcdegh	445.92 ±6.93 cdefh	307.82 ±11.32 abdefg
RNA	380.50 ±14.65	380.56 ±8.35	281.82 ±11.48 abdefg	176.65 ±13.92 abcefgh	317.16 ±6.05 abcdfgh	242.78 ±15.30 abcdegh	376.43 ±10.38 cdefh	284.12 ±11.21 abdefg
Protein	27.29 ±0.25	26.69 ±0.28	13.28 ±0.18 abdefgh	8.65 ±0.09 abcefgh	15.68 ±0.13 abcdfgh	11.00 ±0.11 abcdegh	26.64 ±0.22 cdefh	18.94 ±0.13 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

The increased activities of SGPT and SGOT observed here in blood serum of mice indicate fluoride toxicity. Fluoride has been reported to cause a depression in DNA and RNA synthesis in cultured cells.[19] Fluoride inhibits nucleic acid synthesis and attachment of m-RNA to ribosome. 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.[20] Fluoride has been reported to cause reduction in DNA and RNA synthesis as well as DNA/RNA and DNA/protein ratio, indicating probable disturbances in the process of transcription, translation, as well as mitotic cycles and chromosomal aberrations.[21-22] It is also reported that fluoride enhances lipid peroxidation and inhibits antioxidative enzymes in liver, kidney, heart, brain, and blood of

fluoridated mice.[23-24] The oxygen-derived free radicals are also a major source for DNA damage, which can cause strand breaks and base alteration in the DNA. Therefore the reduction in protein content may be due to either direct effect of fluoride on protein synthesis or indirectly through DNA and RNA damage.

Our findings suggest a profound ameliorative effect of black tea extract on NaF-induced reduction in DNA, RNA, and protein contents in liver and kidney of mice and SGPT and SGOT activities in blood. 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.[1] Quercetin, a unique flavanol present in black tea extract, has been shown to affect antioxidant biomarkers by enhancing resistance of lymphocyte DNA to strand breakage, increasing plasma antioxidant capacity, decreasing tissue inhibitor of matrix metalloproteinase-1 expression, improving renal function, and promoting oxidative resistance to LDL (Low Density Lipoprotein).[25] Polyphenols are well known for their ability to reduce membrane lipid peroxidation and increase malondialdehyde levels that can prevent oxidative damage caused by NaF.

In conclusion, this work has shown that NaF induced significant adverse alterations in SGPT and SGOT activity and decreased levels of DNA, RNA, and protein in liver and kidney of mice. Withdrawal of NaF resulted in partial recovery, but concurrent administration of black tea extract and NaF markedly reduced the extent of fluoride intoxication, apparently because of the powerful ameliorative effects of polyphenols in tea. Further studies are needed, however, before final conclusions can be reached on the therapeutic value of black tea to help alleviate fluoride intoxication.

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