**EFFECTS OF SODIUM FLUORIDE ON DNA, RNA AND PROTEIN CONTENTS IN LIVER OF MICE AND ITS AMELIORATION BY CAMELLIA SINENSIS**

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Fluorosis is the phenomenon caused by excessive fluoride ingestion for a prolonged period of time. Fluoride intoxication can damage skeleton, teeth, liver, kidney and brain mainly due to its ability to induce oxidative stress and initiate lipid peroxidation (1). Numerous studies have shown that fluorosis may induce nucleotide damage, inhibition of protein synthesis and alters mitochondrial functions resulting in energy deficient state of the cell (2). Fluorine compounds also act on the organic part of supporting tissues, including proteins. Fluoride increases the mass of non-collagen proteins such as proteoglycans and glucosaminoglycans, accelerating skin aging even though protein biosynthesis is generally suppressed. The final outcome includes progressive vascular lesions (3). Excessive exposure to fluoride may lead to acute poisoning, hyperemia, cerebral edema, and degeneration of the liver. Fluoride can increase the rate of DNA damage, and induce apoptosis and expression of p53 in human embryo hepatocytes. Furthermore, for both apoptosis and the level of p53 expression, there exists a rise tendency with the increase concentration of fluoride (4).

Tea is the second most commonly drank liquid on earth after water. Tea is an infusion of the leaves of the *Camellia sinensis* plant. Tea leaves contain many compounds, such as polysaccharides, volatile oils, vitamins, minerals, purines, alkaloids (e.g., caffeine) and polyphenols (catechins and flavonoids). Catechins include gallocatechin, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) (5). Proven medicinal properties include antioxidant, anti-inflammatory, anti-allergic, antibacterial and antiviral effects. Long term black tea supplementation is capable of protecting plasma proteins from oxidative injury, and demonstrates that chronic black tea administration protects liver tissues against oxidation (6). The polyphenol mainly responsible for the prevention of cancer formation is epigallocatechin-3-gallate (EGCG). When applied to mouse skin, EGCG prevents UVB-induced oxidative stress and suppression of the immune system. In animal models of liver cancer, tea and tea polyphenol administration inhibit carcinogen-induced decrease in the oxidized DNA base, 8-hydroxy-2'-deoxyguanosine. Results from studies in rats and mice showed that tea consumption protects against liver cancer induced by chemical carcinogens (7). Tea polyphenols can be a useful supplement in the treatment of liver disease and should be considered for liver conditions in which pro-inflammatory and oxidant stress responses are dominant (8).

Present study focuses on the sodium fluoride induced toxicological changes in nucleic acids (DNA and RNA) and protein content in mice liver and its possible reversal by black tea infusion.

**MATERIALS AND METHODS**

**Chemicals**

Analytical grade sodium fluoride was purchased from Loba Chemie, Mumbai, India. All the other chemicals used were of AR grade.

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Experimental animals
Young adult inbred Swiss strain male albino mice (*Mus musculus*) weighing approximately 30–35 g were obtained from Serum Institute of India Pvt. Ltd, Hadapsar, Pune, India. Animals were provided with certified pelleted rodent feed supplied by Amrut Feeds, Pranav Agro Industries Ltd., Pune, India and water *ad libitum* and maintained under 12 h dark and light cycles at 26 ± 2°C. Guidelines for Care and Use of Animals in Scientific Research 1991 published by Indian National Science Academy, New Delhi, India, was followed. All the experimental protocols were approved by the Committee for the Purpose of Control and Supervision of Experiment on Animals (Reg-167/1999/CPCSEA), New Delhi, India.

Experimental design
Forty animals were divided into four groups and caged separately. Group I (untreated control) animals were maintained without any treatment. Animals of group II received black tea extract (2% in drinking water) for 30 days and serve as pre-treatment controls. Animals of group III were orally administrated with sodium fluoride in 0.2 mL of water at a dose of 10 mg/kg body weight for 30 days. Group IV were orally treated with sodium fluoride as mentioned for group III animals and given 2% black tea infusion instead of drinking water for 30 days.

Two percent tea infusion (Lipton Yellow Label of Hindustan Lever Ltd., Mumbai, India) was prepared in deionized water. The effective dose of black tea was based on earlier work on male mice (9). The dose of sodium fluoride was based on LD₅₀ value of sodium fluoride i.e., 54.4 mg/kg body weight for male mice. All the treatments were given orally for 30 days using a feeding tube attached to a hypodermal syringe.

On completion of the treatment the animals were sacrificed by vertical dislocation. Selected organs such as liver of all the controls and treated groups of animals were quickly isolated, blotted free of blood and weighed.

Biochemical analysis
Protein content
Protein content in liver of controls and treated groups of animals were estimated by the method of Lowry et al. (10). Protein containing preparation reacts with the Folin-Ciocalteu’s phenol reagent to give a colored complex. This color development was due to two reactions occurring simultaneously i.e., the reaction of alkaline copper sulfate solution with peptide bonds and reduction of phosphomolybdic and phosphotungstic acids by aromatic amino acids like tyrosine and tryptophan present in the protein. The blue color developed was quantitatively proportional to the total protein, which is measured spectrophotometrically. A known weight of tissue was homogenized in a definite volume of glass distilled water. In the sample tube, 0.2 mL of tissue homogenate, 0.6 mL of distilled water, 4 mL of alkaline copper sulfate solution was added. In the blank tube, instead of the sample 0.2 mL of distilled water was taken. The tubes were incubated at room temperature for 15 min. Then, 0.4 mL of Folin-Ciocalteu phenol reagent (diluted 1:1 with distilled water) was added to each tube and was thoroughly mixed. The tubes were allowed to stand at room temperature for 30 min. The optical density of the blue color developed was read at 540 nm on ELICO double beam UV-VIS spectrophotometer model SL-164.

Extraction of nucleic acids
A known weight of fresh tissue was homogenized in 5 mL of cold 5% trichloroacetic acid (TCA) in a cooled pestle and mortar and the homogenate was kept at 0–4°C for 30 min. The precipitates obtained after centrifugation for 10 min at 1000 × g were dissolved again in 5 mL of TCA and left for 30 min at 0–4°C. Thereafter, centrifugation was carried out and the precipitates obtained were dissolved in 3:1 alcohol: diethyl ether mixture and left for 30 min at 50°C. This process was repeated once again, and the supernatants were discarded. The precipitate was dissolved in 5 mL of 0.1 M KOH and incubated at 37°C for 16–18 h. Then, 0.17 mL of 6 M HCl and 5 mL of 10% TCA was added to the incubated suspension and precipitates were allowed to be formed at 4°C for 30 min. After centrifugation, the supernatant and pellet were separated. The supernatant was used for RNA estimation. The pellet containing DNA and protein was then separated by centrifugation after cooling at 4°C for 30 min and used for DNA estimation.

Estimation of DNA
The estimation of DNA in the liver of controls and all treated groups of animals was carried out by the method of Giles and Meyer (11). The DNA in the supernatant reacts with diphenylamine to give a blue colored complex whose optical density was measured spectrophotometrically. One milliliter of supernatant was added to 2 mL of diphenylamine reagent in a test tube, vortexed and incubated at 37°C for 16–18 h. The blank was run with 1 mL of
Effects of sodium fluoride on DNA, RNA and protein contents in liver of mice...

double distilled water instead of supernatant. The optical density (O.D.) of the resultant blue color was read at 620 nm on ELICO double beam UV-VIS spectrophotometer model SL-164.

Estimation of RNA

The estimation of RNA in the liver of controls and all treated groups of animals were carried out by the method of Mejboum (12). The RNA in the supernatant reacts with orcinol reagent to give a greenish color which was measured spectrophotometrically. To 1 mL of supernatant, 3 mL of working orcinol reagent were added. The tubes were kept in boiling water bath for 20 min. The blank was prepared with 1 mL of double distilled water instead of supernatant. After cooling, the O.D. of the green color developed was read at 670 nm on ELICO double beam UV-VIS spectrophotometer model SL-164.

Measurement of fluoride from black tea was done using ion selective electrode (Orion Research, USA, Model no. 96-09).

Statistical analysis

The results were expressed as 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.

RESULTS

Protein content: As seen in Figure 1, oral administration of NaF (10 mg/kg body weight/day) for 30 days caused significant (p < 0.05) reduction in the content of protein in the liver of mice. Administration of black tea extract along with NaF significantly (p < 0.05) ameliorated fluoride-induced changes in the liver protein content. No significant change was noted between untreated and black tea control group animal protein content.

DNA content: In comparison with the controls and the fourth group, the content of DNA was significantly (p < 0.05) lowered in the NaF treated group (Group III). On treating mice with a combination of black tea extract along with NaF it was seen in

RNA content: In comparison with the controls and all treated groups of animals were carried out by the method of Mejboum (12). The RNA in the supernatant reacts with orcinol reagent to give a greenish color which was measured spectrophotometrically. To 1 mL of supernatant, 3 mL of working orcinol reagent were added. The tubes were kept in boiling water bath for 20 min. The blank was prepared with 1 mL of double distilled water instead of supernatant. After cooling, the O.D. of the green color developed was read at 670 nm on ELICO double beam UV-VIS spectrophotometer model SL-164.

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that the concentration of DNA significantly (p < 0.05) increased as compared to the group treated with NaF only (Fig. 2). DNA content of the black tea control group was almost equal to untreated control group.

**RNA content:** The analysis of RNA content in the control groups showed that there was no significant change in the level of RNA (Fig. 3). In NaF treated mice, however, there was a significant (p < 0.05) decrease in the RNA content in comparison to treated mice, however, there was a significant (p < 0.05) decrease in the RNA content in comparison to the control (Group I) as well as group treated with combination of NaF and black tea (Group IV).

**Fluoride content:** A solution of the black tea contained 0.58 ppm of fluoride.

## DISCUSSION

Manifestation of fluoride toxicity is closely associated with its ability to induce oxidative stress (13–15). The reduction in liver protein content in mice induced by NaF observed here might be due to either increased proteolysis or decreased protein synthesis or fluoride induced osmotic imbalance caused by lipid peroxidation. The reduction in protein content of NaF-treated animals supports the view that fluoride inhibits oxidative decarboxylation of branched chain amino acids and simultaneously promotes protein breakdown (16). Many investigators have reported protein degradation in liver of rabbits during experimental fluorosis. Also, fluoride is known to affect the rate of cellular protein synthesis, which is mainly due to impairment of peptide chain initiation (17). The disturbance of protein synthesizing systems in fluorosis has been attributed to a decrease in activity of a group of enzymes catalyzing the key processes of cellular metabolism. This reduction or loss of enzymatic activity could be due to fluoride generated free radicals ultimately causing inactivation of enzymes. Kathpalia and Susheela (18) have observed that administration of large doses of NaF to rabbits caused a 10 to 46% reduction in protein content in most body tissues.

In comparison with the controls, the levels of nucleic acids were significantly lowered in the liver of NaF treated animals. On treating mice with a combination of black tea extract along with NaF it was seen that the concentration of nucleic acids increased as compared to the group treated with NaF. Fluoride intoxication produces specific metabolic alterations in nucleic acid synthesis in the liver of experimental rabbits. An inhibitory effect of fluoride on DNA synthesis in liver cells has been reported (19). Wang et al. (14) have shown the high degree of correlation between lipid peroxidation and reduced DNA content in the fluoride intoxicated hepatocytes cultured cells. This is in accordance with our earlier studies that fluoride administration to mice induced lipid peroxidation and altered the levels of enzymatic and non-enzymatic antioxidants in brain (Trivedi and Verma – unpublished data). Studies reported that free radicals can lead to alteration in DNA double helical structure (20). Fluoride can also cause an increase in cAMP production by stimulating adenylate cyclase (21) and could contribute to the reduced level of DNA. Reduced RNA content of the fluoride treated animals could be due to reduced protein synthesis as indicated by altered RNA/protein ratio. Reactive oxygen species (ROS) generated due to fluoride intoxication could lead to inactivation of several enzymatic proteins involved in biosynthesis and metabolism. Fluoride-induced reduction in DNA, RNA and protein content might be due to direct and/or indirect effect of fluorosis. Fluoride is known to reduce absorption of calcium and magnesium from gut (22, 23). Both of these metal ions are needed by metalloenzymes involved in transcription, translation and enzymatic cascade mechanisms, acting as secondary messengers. Depletion of calcium and magnesium might be the reason for a decrease in synthesis of DNA, RNA and protein.

Plant polyphenols possess strong antioxidative potency responsible for their protective effect on various toxicants. Our findings suggest a profound ameliorative effect of black tea extract on NaF-induced reduction in protein, DNA and RNA contents in liver of mice. 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 (24). Vidjaya et al. (25) showed that black tea polyphenols polyphenon-B and BTF-35 can effectively protect liver DNA from oxidative damage. Moreover, tea theaflavins showed inhibitory effect on cellular oxidative stress and DNA damage in liver cell lines (26). Studies reported that consumption of black tea can improve the levels of antioxidants neutralizing free radicals such as superoxide dismutase, catalase, glutathione S-transferase and reduced glutathione thus protecting from oxidative stress induced damage (27).

From the present findings conclusion can be drawn that sodium fluoride can induce damage to the nucleic acids and protein content in mice liver, which can be effectively reversed by black tea infusion.
REFERENCES


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