THERAPEUTIC EFFICACY OF *TAMARINDUS INDICA* (L) TO PROTECT AGAINST FLUORIDE-INDUCED OXIDATIVE STRESS IN THE LIVER OF FEMALE RATS

P Ekambaram,[a] T Namitha, S Bhuvaneswari, S Aruljothi, D Vasanth, M Saravanakumar

Coimbatore, India

SUMMARY: To evaluate the protective effect of tamarind pulp against fluoride (F)-induced oxidative stress in the liver, adult female Wistar rats were treated daily for 45 days with sodium fluoride (300 ppm NaF = 136.7 ppm fluoride ion) in drinking water, alone or in combination with tamarind pulp (20 mg/kg bw by oral intubation). Malondialdehyde (MDA), antioxidant enzyme activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and ascorbic acid level in the liver, and levels of calcium and F, plus activities of aspartate transaminase (AST) and alanine transaminase (ALT) in serum were determined 24 hr after the last treatment. In the NaF-treated animals, a significant increase in MDA content and a concomitant decrease in antioxidant enzyme activities of SOD, CAT, GSH-Px, and the ascorbic acid level in liver and increased activities of AST and ALT, and increased calcium and F concentrations in serum were observed. Administration of tamarind pulp together with NaF produced significant amelioration in all parameters studied, indicating that tamarind pulp is able to prevent free radical induced oxidative stress by F, attributable to its antioxidant property. It is concluded that tamarind pulp may be useful to prevent the oxidative damage caused by consumption of excessive amounts of F.

Keywords: Antioxidant activity; Fluoride toxicity; Liver enzymes; Oxidative stress; Rat liver; Tamarind pulp.

INTRODUCTION

Endemic fluorosis is caused by excessive fluoride (F) ingestion, especially in drinking water above 1 ppm over a prolonged period.[1] During the last several years, numerous reports on animals and humans from India, China, and other countries indicate that F in varying concentration generates increased levels of free radicals.[2-6] As a consequence, the balance between the oxidative system and the antioxidant system is broken and oxidative stress is augmented during F exposure.[7-9] Oxidative stress can be effectively prevented by supplementing with natural antioxidants,[10-16] among which tamarind pulp has been reported to scavenge reactive oxygen species (ROS)[17] with beneficial effects in dogs, humans, and rabbits.[18-20] In the present work, the possibility of preventing F-induced oxidative stress in rats with tamarind pulp has been investigated by analyzing various biochemical parameters such as the malondialdehyde (MDA) content, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) activities, ascorbic acid in the liver, along with activities of alanine transaminase (ALT) and aspartate transaminase (AST) in serum, and the bioavailability of F and calcium in serum.

MATERIALS AND METHODS

Animals: Colony bred adult 4–5 month old female Wistar rats weighing 130–150 g were used. Six animals were chosen randomly for each test and each control

[a]For correspondence: Dr P Ekambaram, Assistant Professor, Molecular Toxicology Lab, Department of Biotechnology, Bharathiar University, Coimbatore – 641 046, Tamilnadu, India. E-mail: ekas3001@rediffmail.com

group. Animals were caged in groups (3 per cage) and were maintained at room temperature (22–26ºC) with a normal 12-hr light/dark cycle. The animals were fed a balanced commercially available pelleted rat chow (Sai Durga Feeds Pvt Ltd Bangalore, India), and the tap water was supplied *ad libitum*. The experiments were conducted in accordance with ethical norms approved by Ministry of Social Justice and Empowerment, Government of India, and Institutional Animal Ethical Committee guidelines.

Chemicals and treatment: Sodium fluoride (NaF, Merck, Mumbai, India) was administered *ad libitum* in the drinking (tap) water at a concentration of 300 ppm (= 135.7 ppm F ion) for 45 days. Another two groups of animals received tamarind fruit pulp, alone and in combination with NaF, for 45 days. Tamarind pulp was prepared by soaking 1 g of tamarind fruit pulp (collected from single tamarind tree, Kalveerampalayam, Coimbatore, India) in 50 mL of tap water. Thirty minutes later the soaked fruits were squeezed and the pulp was strained. This pulp was given to the rats by oral intubation in a volume of 0.1 mL/100 g bw, at a level of 20 mg/kg bw daily for 45 days. The dosage of *Tamarindus indica* was chosen based on a dose response study[21] and taking into account human consumption levels.[22] The control groups received tap water *ad libitum* for 45 days.

Sample collection and biochemical study: After the 45th day of treatment, the rats were sacrificed by cervical decapitation. The blood samples were collected without anticoagulant and were used for serum preparation for F, calcium estimation, and measurement of AST and ALT activities. The liver was dissected out and placed on chilled glass plates, weighed, washed in ice-cold saline and then homogenized in Tris-HCl buffer (pH 7.4). After homogenization, the tissues were centrifuged at 10,000 rpm at 5ºC for 15 min and the supernatant was used for biochemical estimations.

In liver tissues, biochemical estimation by standard methods was conducted for protein,[23] MDA,[24] SOD (E.C.1.1.15.11),[25] CAT (E.C.1.11.1.6),[26] GSH-Px (E.C.1.11.1.9),[27] activities, and ascorbic acid,[28] and in serum for AST (EC 2.6.1.1),[29] ALT (EC 2.6.1.2)[29] activities. The F level[30] in the serum was determined with an Orion Fluoride Ion Analyser, model 9409, and calcium[31] by atomic emission spectrophotometry, ARL, Model 2410.

Statistical analysis: The data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test.

RESULTS

Effect of NaF: In the liver of NaF-treated animals, the total protein concentration was decreased significantly by 44.1% (Figure 1A), whereas the MDA content was increased by 87.9% (Figure 1B).

Decreased activities of SOD (63.3%), CAT (47.6%), GSH-Px (30.4%) and ascorbic acid level (66.4%) were also observed in the liver tissue of these animals (Figures 2A, 2B, 2C, and 2D).

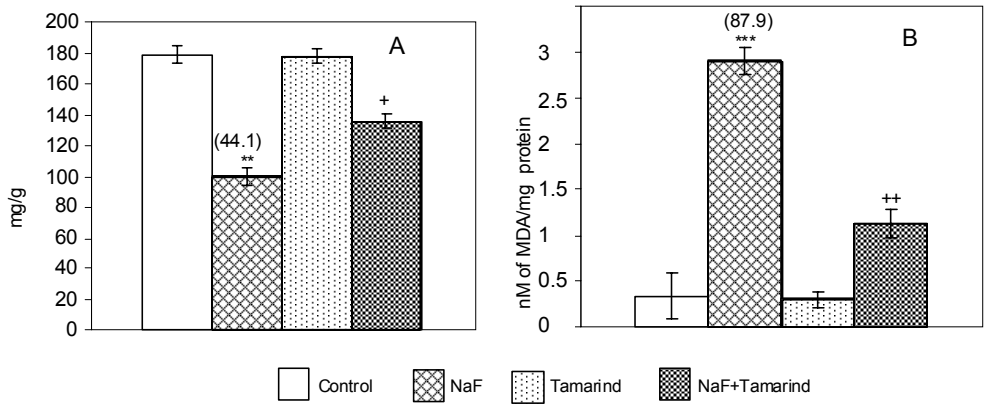

Figure 1. Protein (A), MDA content (B), in the liver of control and test animals. Each bar represents mean±SEM of 6 animals. % change from control value in parenthesis. **p<0.01, ***p<0.001 compared to control. +p<0.05, ++p<0.01 compared to NaF treated group (one way ANOVA followed by Tukey's multiple comparison test).

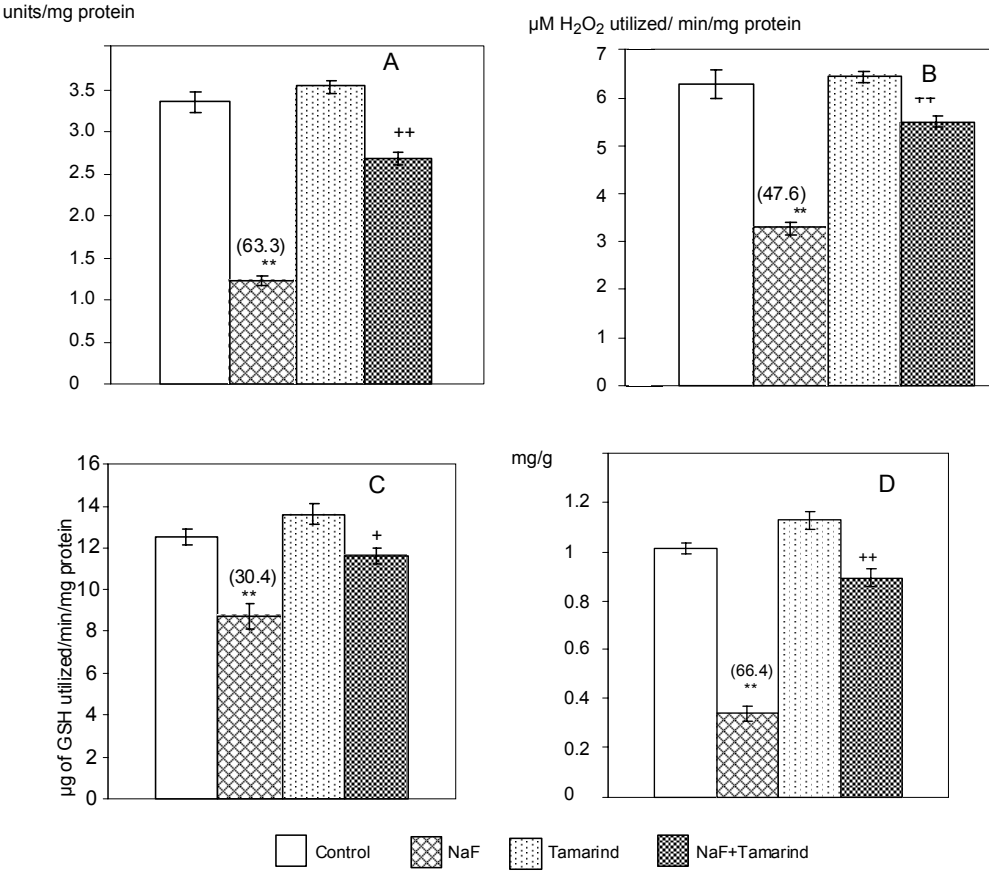

Figure 2. SOD (A), catalase (B), glutathione peroxidase (C) activities and ascorbic acid level (D) in the liver of control and test animals. Each bar represents mean±SEM of 6 animals. % change from control value in parenthesis. **p<0.01 compared to control. +p<0.05, ++p<0.01 compared to NaF treated group (one way ANOVA followed by Tukey's multiple comparison test).

Moreover, NaF treatment also produced a marked increase (795%) in the concentration of F in the serum from 0.216 mg/L to 1.933 mg/L (Figure 3A). The serum F in the tamarind group was 0.147 mg/L and in the NaF + tamarind group it was 1.071 mg/L. The serum calcium concentration was 67.3% lower in the NaF group of animals (Figure 3B). In the serum the activities of AST and ALT were increased by 24.9% and 28%, respectively, when compared with control animals (Figures 3C and 3D).

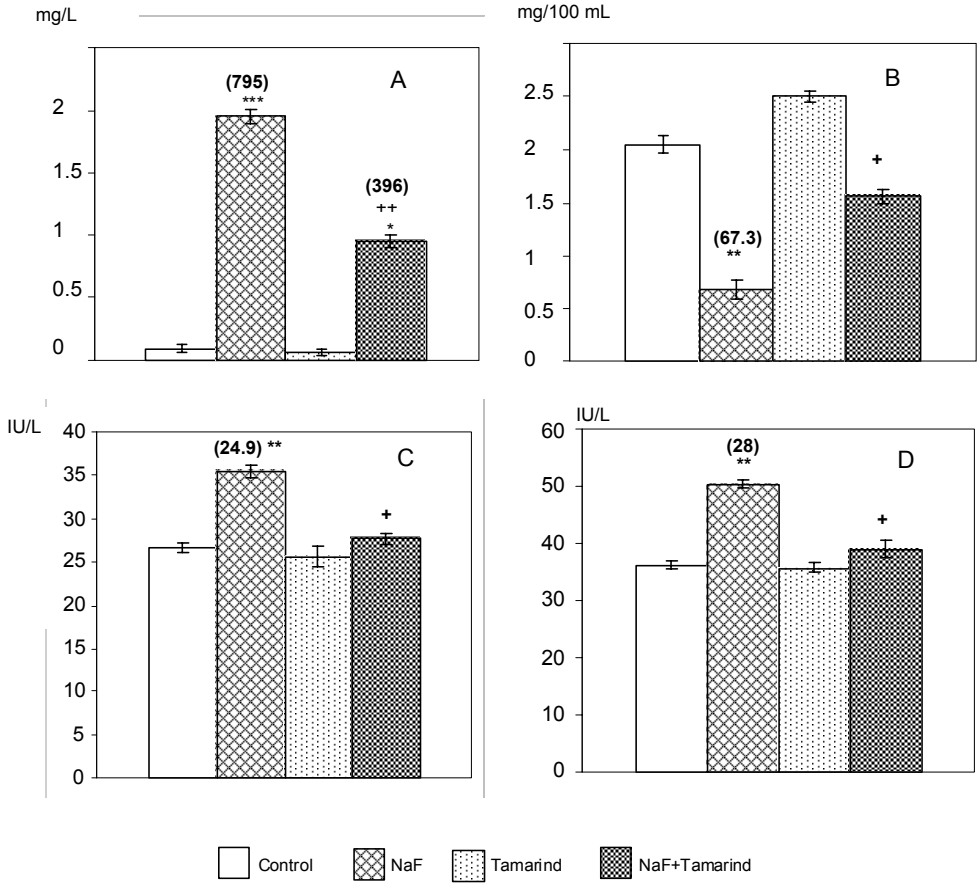

Figure 3. Fluoride (A) and calcium (B) levels, AST (C) and ALT (D) activities in the serum of control and test animals. Each bar represents mean±SEM of 6 animals. % change from control value in parenthesis. *p<0.05, **p<0.01, ***p<0.001 compared to control. +p<0.05, ++p<0.01 compared to NaF treated group (one way ANOVA followed by Tukey's multiple comparison test).

Effect of tamarind: When compared to control animals, administration of tamarind alone did not produce any significant changes in total protein or MDA content in liver (Figures 1A and 1B) and AST, ALT activities in serum (Figures 3C, and 3D). Similarly, activities of SOD, CAT, GSH-Px and ascorbic acid level in the liver (Figures 2A, 2B, 2C, and 2D), were also not altered significantly in these animals. The concentration of serum F was also not altered significantly in these animals (Figure 3A). However, a slight increase in serum calcium level (Figure 3B) was observed in these animals, but it was statistically not significant.

Effect of NaF + tamarind: As seen in Figures 1A, 1B, 3C, and 3D, tamarind counteracted the toxic effects of NaF by increasing the protein content and decreasing the MDA content in the liver and the activities of AST and ALT in the serum. As seen in Figures 2A, 2B, 2C, and 2D, the lowered activities of SOD, CAT, and GSH-Px, and the ascorbic acid level in the liver were enhanced significantly in NaF + tamarind treated animals. The concentration of F was decreased markedly ($p<0.01$) in the serum of these animals compared to that in the NaF-treated animals (Figure 3A). Nevertheless, the F concentration was higher ($p<0.05$) than in the control, but the NaF-induced hypocalcemia was prevented by tamarind (Figure 3B).

DISCUSSION

As a potent protoplasmic poison, F is toxic to any living cell and is known to cause various biochemical alterations, including oxidative stress when certain limited levels are exceeded.[7,9] A likely mechanism by which F induces oxidative stress is through generation of ROS (reactive oxygen species).[9] Therefore, the present study is focused on the role of oxidative stress induced by F and to investigate whether tamarind pulp has a protective effect or not.

The data obtained here show clearly that intake of water containing 300 ppm NaF (= 135.7 ppm F ion) for 45 days resulted in a marked increase in the concentration of F in the serum of adult female rats, in agreement with a previous report from our laboratory.[32] Impairment of renal function contributes to elevated F in the serum of these animals.[20,33] Maruthamuthu[34] found that tamarind pulp has the ability to bind with F. Hence, animals receiving tamarind pulp along with NaF have shown decreased F ion in serum. Later, Khandare et al.[18,19] confirmed that tamarind pulp decreases the bioavailability of F in dogs and humans. The same results were also observed in rabbits.[20]

In support of the present results, hypocalcemia has been found in rats treated chronically with NaF.[32] The property of F to interact with calcium in the saliva to form insoluble calcium fluoride has been proposed to account, at least in part, for hypocalcemia in these animals.[35] In the present study it is evident that an elevation of calcium in the serum is produced by co-administration of tamarind pulp.[20]

In order to rule out a possible damage to the liver from by treatment with tamarind extract or with tamarind + F, serum transaminase activities were evaluated. In accord with previous work,[17,20,36] the results show that while treatment with NaF was hepatotoxic and caused liver damage, treatment with tamarind pulp extracts alone or tamarind + F did not alter serum AST and ALT activities.[20]

In the NaF treated animals, an increase in MDA content and decrease in the activities of the antioxidant enzymes SOD, CAT, GSH-Px, and ascorbic acid in the liver was found in agreement with previous work.[9,37-39] Thus, F obviously stimulates the respiratory burst and produces superoxide and hydroxyl radicals by the Haber-Weiss reaction and impairs these antioxidant enzymes, possibly by forming a strong hydrogen bond with the amide group.[40] On the other hand, the

NaF + tamarind group shows a decrease in MDA content and increase in antioxidant enzymes due to a protective antioxidant effect and/or significant lipid lowering effect of tamarind plant constituents present in the pulp extracts.[17]

Because of their free radical scavenging ability, antioxidants have an important role in ameliorating F toxicity.[12-16,41] As seen in the experiments here, the free radical scavenging effect of tamarind pulp is authenticated by the decreased MDA content in the liver and by the increased levels of polyphenols (34.02±2.11 nM/mL) and flavonoids (35.51±5.61 µg/mL).[17]

People living in South Asian countries add tamarind pulp to their cooking with cooked curries to increase the taste. In the present study the rats that received only tamarind pulp exhibited no differences from the control animals in any of the parameters tested, revealing that oral administration of tamarind pulp is nontoxic. Administration of tamarind along with NaF resulted in prevention of the toxic effects of F. The high level of calcium in tamarind pulp may have a major role in its protective effect.[20] The antioxidant property of tamarind[17] also seems to play a vital role in its protective effect since it significantly decreases the oxidative stress effects of F intoxication as seen here with rats.

The findings of the present study lead to the conclusion that fluorosis caused by water containing F can be effectively prevented by dietary inclusion of tamarind pulp. However, further studies using the other biomarkers are desirable in order to determine the extent of oxidative stress induced by F and the beneficial effects of tamarind pulp to ameliorate that stress.

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