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Mitigation Effect of Tamarind Pulp on Serum Biochemical Factors And Tooth of Fluoride Intoxicated Albino Rat

D.Sakthika
Department of Zoology
A.P.C.Mahalaxmi College for Women, Thoothukudi

Abstract

High fluoride exposure affects human beings and animals health through oxidative stress, immune suppression, apoptosis, and affecting nutrient utilization. Hence, ameliorative measures are important to prevent their endemicity and disease progress. The present study was aimed to investigate the toxic effect of sodium fluoride (NaF) on selected serum biochemical factors (protein, glucose, cholesterol, biliubin, SGOT and SGPT) and tooth and its possible ameliorative effect by Tamarind fruit pulp. Twelve weeks old albino rats (*Rattus norvegicus*) of average weight of 100 g were randomly distributed into three groups of five animals each. The first group was kept as control. The second group was given 5ml drinking water with 0.1514mg NaF /kg- bw/day and the third group was fed the same dose of NaF and 5ml water with 100mg/kg-bw/day tamarind pulp. After 45 days of experimental period protein, glucose and cholesterol was significantly ($P<0.05$) decreased and Bilirubin, SGOT and SGPT were significantly ($P<0.05$) increased in the NaF treated rat than the control group. However rats treated with Tamarind pulp showed significant improvement in all these parameters. Dental fluorosis was developed in group I and Group II showed less dental fluorosis than Group I. From the study, it could be concluded that $1/10^{th}$ LC50 dose of fluoride caused alterations in serum biochemical factors and tooth and these effects could be ameliorated by Tamarind pulp.

Key words: Rat, NaF, Biochemicals, Tooth, Toxicity, Tamarind pulp, Mitigation.

Introduction

Fluoride is ubiquitously present in soil, water, plants and air. In the animal body, F makes its presence through water and food. But, some of the recent studies indicate that, most of the F comes from pharmaceutical drugs (20%) and through agrochemicals (30-40%) (WHO, 2006). According to World Health Organization F exposure to animals above the 1.5 ppm, set at chronic fluoride toxicity. Through water exposure, this type of toxicity is endemic in most of the countries across the world (WHO, 2003).

Fluoride crosses the blood brain barrier (BBB) easily and induces neural cell degeneration. Several studies indicated that hippocampus of rat brain can lead to the degenerate due to the imbalance between oxidant– antioxidant balance when rat is exposed to fluoride. (Bharti and Srivastava, 2009). Skeletal fluorosis and impairment of soft tissue function are the earliest toxic effects of fluoride as it could cross cell membranes, and enter soft tissues. The long term high fluoride intake disturbs the antioxidant defense, suggesting increased oxidative stress as one of the mediating factors in the pathogenesis caused by fluoride (Trivedi *et al*., 2008).

Excess fluoride ingestion results in dental fluorosis. The mechanisms affected by long term chronic exposure to low levels of fluoride are likely to differ from those affected by acute exposures to high levels of fluoride (Giambro *et al*, 1995). Some mechanisms affected by lower chronic fluoride levels, resulting in enamel fluorosis, are likely to be specific to this uniquely mineralizing tissue, while others may also affect other cells and tissues. Enamel fluorosis refers to fluoride-related alterations in enamel, which occur during enamel

development. These alterations become more severe with increasing fluoride intake, and time of exposure. The severity of fluorosis is related to the concentration of fluoride in the plasma, considered to be in equilibrium with the tissue fluid that bathes the enamel organ (Angmar-Mansson and Whitford, 1984.

Tamarindus indica is a tree native to Africa, but also widely cultivated throughout India, Sudan, Indonesia, Pakistan, Philippines, Java, Spain and Mexico (Matinello, *et al*., 2006). Its fresh and dried fruits are used as sour flavoring agent in various Indian cuisines. Anti-inflamatory and antioxidant activity of tamarind fruit pulp have been reported (Rimbau *et al*., 1999). Khandare, *et al*., 2004 reported that tamarind (*Tamarindus indica*) fruit intake reduces its burden in dog and human, by increasing F excretion. Maruthamuthu *et al*., (1987) reported the binding of fluoride by tamarind in vitro.

Hence the aim of this study is to investigate the changes in the Serum Biochemical factors and tooth of the albino rat *Rattus norvegicus* intoxicated by fluoride and the role of tamarind pulp as a mitigation agent.

Materials and methods

Chemicals

Analytical grade Sodium fluoride was purchased from Fischer inorganics and aromatics Ltd, Madras.

Dosage of fluoride

LC_{50} value of fluoride for albino rat was 51.45mg/Kg Bw (Vijaybaskara Rao, 1994). The experimental rat was orally administered with $1/10^{th}$ LC_{50} dose (0.514mg/100g B.W) of fluoride. Oral administration was preferred in view of water being the main source of fluoride among the human population in endemic areas.

Preparation of Tamarind pulp

Tamarindus indica fruit pulp (100g) was mixed with 200ml distilled water in a round bottom flask and left overnight at room temperature. The mixture was shaken vigorously several times in between next day, the mixture was filtered with Whatman filter paper no 1. The filtrate was evaporated in vacuum using a rotary film evaporator. About 10g of semi-solid paste was diluted with 100 ml of distilled water and used for the experiment.

Experimental animals

Albino Lab rat (*Rattus norvegicus*) of 100±10gm weight was obtained from an inbred colony maintained in the animal house of Sankaralingam Bhuvaneshwari College of Pharmacy, Sivakasi.

Rats were maintained according to the guidelines set by Institutional Animal Ethical committee (IAEC, India), maintained under controlled conditions of temperature (23±2ºC), humidity (50±5%) and a 12-hours light- dark cycle. Animals were given standard mice feed and water *ad libitum*. The present study was approved by the Institutional Animal Ethics Committee (Ref. No. SBCP/ 2012-2013/IAEC/CPCSEA/05) and conducted as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India, New Delhi, India. Blood was collected from the tail vein of the rats after 45 days of experimental period. Blood samples were immediately brought to the laboratory for analysis.

Experimental design

Rats were divided into 3 groups. Stock solution of NaF was prepared by dissolving 5.14 mg NaF in 100 ml distilled water. The experimental design of the study is shown in Table-1.

Methods of estimation

Serum protein of control and all treated animals was estimated by the method of Lowry *et al* (1951) at 540 nm on a Spectronic-88 Bausch and Bomb Spectrophotometer. Protein was expressed as mg/dl blood. **Cholesterol** was estimated by enzymatic colorimetric test as described by (Tietz, 1979) and expressed as mg/dl blood. **Glucose** was estimated by Glucose Oxidase/Peroxidase method as described by Trinder, (1969) and expressed as mg/dl blood. **Bilirubin** was estimated by modified Jendrassik and Grof's (1938) method and expressed as µg/dl blood.

SGOT and SGPT were estimated by method recommended by German Society for Clinical Chemistry using the Enzopak Diagnostic Kit (Reckon Diagnostics, India).

Statistica analysis

Experimental data were expressed as mean ± SD. Differences between groups were evaluated by one way analysis of variance using Sigma Plot Software build version 2014.0. Post ANOVA analysis was carried out by Duncan's test and Values of $P<0.05$ were considered statistically significant.

List of tables, figures and plates

Table.1 Experimental Design

S.No	Sample	Treatment	No. of Rat	Days
1	Control	Standard diet + Water *ad libitum*	5	45
2.	Fluoride treatment	$1/10^{th}$ LC_{50} dose of F + Standard diet and Distilled water *ad libitum*	5	45
3.	Fluoride & Tamarind treatment	$1/10^{th}$ LC_{50} dose of F and 100mg/kg-bw/day Tamarind pulp, standard diet + Distilled water water *ad libitum*	5	45

Table-2 Serum biochemical factors of rat

S.No	Sample	Protein mg/dl	Cholesterol mg/dl	Glucose mg/dl	Bilirubin µg/dl	SGOT µg/dl	SGPT µ/dl
1	Control	5.65±0.3	112.00±1.5	86.7±10.2	0.16±0.6	53±3.56	77.67 ±11.7
2.	Group I	$4.45±0.3^{a}$	$62.3± 1.3^{a}$	$59.3±7.5^{a}$	$0.87 ±0.4^{a}$	$87.67±11.2^{a}$	$104.34 ±1.7^{a}$

3.	Group II	5.43±0.4[a]	97.6±1.6[b]	72.0±9.0[b]	0.38 ±0.6[a]	76.66±4.6 [a]	85.34 ±12.9[a]
4	F-Values	9.864	92.3	26.79	46.32	66.52	54.11

Comparison of Group I and Group II with control, a shows significant at (P<0.005) and b shows significant at (P<0.001).

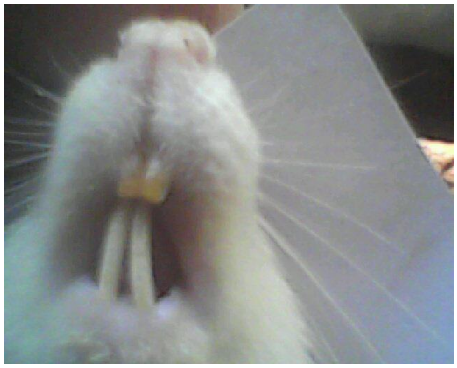

Plate-1 Control rat tooth with non fluorotic tooth

Plate-2 Group I rat with yellow pigmentation in the tooth

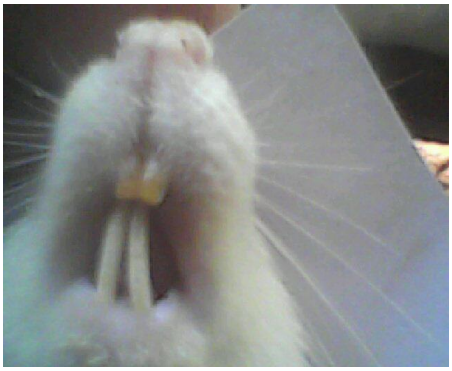

Plate-3 Group II rat with less yellow pigmentation in the tooth

Results and Discussion

The present investigation was carried out to explore the effects of potential toxicity of fluoride on the serum biochemical factors and tooth of albino rats and its possible mitigation upon treatment of *Tamarindus indica* pulp extract.

The amount of protein in the serum of rat decreased significantly **(P<0.005)** after 45 days of fluoride administration; however the protein value was significantly **(P<0.005)** normalized in the rat treated with tamarind pulp. Fluoride decreased the cell number (23%), total protein content (30%) and increased LDH (+236%) release in human and rabbit collecting duct and Henle's loop (Cittanova *et al*., 2002). These evidences support the decrease of protein in the serum of fluoride intoxicated rats in present study.

The cholesterol and glucose value of the rat significantly **(P<0.005)** decreased in the fluoride administered rat and it was elevated nearly **(P<0.001)** to the control group of rat in the tamarind pulp administered rat. Fluoride decreases the absorption of cholesterol and bile salts from plasma and intestine which could result in an increased conversion of bile acids in the liver and bile acids are known to inhibit cholesterol synthesis. This may be an indicative of hepato biliary disturbances in fluoride intoxication (Bennis *et al*., 1993). The improvement in carbohydrate, lipid and antioxidant metabolisms was observed in the fluoride intoxicated rats by Rupal Vasant and Narasimhacharya, (2012) after administration of tamarind leaves extract. They explained that it could be due to the multi-factorial effects of secondary metabolites present in tamarind leaves. On the other hand, these metabolites could also have acted individually or synergistically to reduce the oxidative stress caused by consumption of fluoride.

In the experimental rats bilirubin, serum glutamate pyruvate transaminase (SGPT) and glutamate oxalate transaminase (SGOT) are increased significantly **(P<0.005)** in group I and decreased significantly **(P<0.005)** in group II from the control group of rat. The

increased level of bilirubin also confirms the increased level of red blood cell destruction associated with fluoride administration, while the increase in the total bilirubin can be accounted for by the haem degradation that follows red blood cell destruction (Krucken *et al*., 2005). Significant elevation in enzyme activity of SGOT and SGPT following sodium fluoride treatment was also observed by (Bandyopathyay *et al*., 2012). Nahid Tabasum and Agrawal., (2003) in their study reported that elevated SGOT and SGPT levels in mice were significantly reduced after administration of *Embelia ribes* fruit extract. In the present study also decreasing concentration of SGOT and SGPT were observed after administration of tamarindus fruit pulp in the fluoride treated rats.

Dental fluorosis was observed in the experimental rat after 45 days. The control rats showed normal tooth (Plate-1). Yellow pigmentation on mandibular incisor teeth was visible in the rat treated with fluoride (Plate-2). Less deposit of yellow pigmentation was observed in the rat treated with tamarind pulp (Plate-3). Edible parts, leaves, pods and small fruits of the plants *Ziziphus jujube, Z. mauritiana, Z. nummularia, Prosopis cinearia, Acacia nilotica, Acacia senegal*, *Pithecellobium dulce, Ficus carica, F. bengalensis, F. religiosa, Euphorbia cadusifolia*, *Tamarindus indicus*, are very rich in calcium (Ca) and ascorbic acid (vitamin C) nutrients (Rathore, 2009). These nutrients interfere with the fluoride metabolism and ultimately reduce the fluoride toxicity (Swarup and Dwivedi, 2002). Similarly, in camel fluoride toxicity is also less due to high content of Ca and vitamin C nutrients in their natural foods or food chains (Choubisa, 2010). This evidence support the ameliorative effect of tamarind fruit pulp in reducing dental fluorosis in the present study.

Conclusion

From the results of the present study it is concluded that short term administration of fluoride induced toxicity in the blood biochemical parameters and tooth. Furthermore, these effects were mitigated by administration of tamarind pulp as it is a rich source of vitamin C. So, it is recommended to use dietary supplements of vitamin C for amelioration of toxic manifestations in fluoride exposed populations. Also, presence of fluoride in the environment and drinking water should be monitored regularly to prevent fatal consequences.

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