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Research Article

PROTECTIVE EFFECT OF TAMARINDUS INDICA FRUIT PULP EXTRACT ON EXPRESSION OF TYPE 1 COLLAGEN IN TEETH OF FLUORIDE EXPOSED RATS

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Abstract

Purpose: The present study investigated the beneficial effect of Tamarindus indica fruit pulp extract on the expression of type 1 collagen gene in dentin of incisor teeth of fluoride exposed rats.

Design/methodology/approach: Eighteen rats were randomly divided into three groups of six each. Group I served as control and received only tap water; group II received 200 ppm sodium fluoride in their drinking water and group III received fluorinated drinking water along with hydro-methanolic extract of T. indica fruit pulp (200 mg/kg body weight) daily by gavage for a period of 90 days. Dentin from incisor teeth was harvested at the end of the experiment for total RNA extraction.

Findings: Fluoride exposed rats showed the down-regulation of expression of type 1 collagen (Col1a1) gene as compared to control. Co-administration of T. indica fruit pulp extract during exposure to fluoride through drinking water increases the expression of Col1a1 gene in dentin as compared to untreated fluoride exposed rats.

Conclusion: It is concluded that daily oral administration T. indica fruit pulp extract had the protective effect on fluoride induced down regulation of type 1 collagen mRNA in dentin of incisor teeth of rats.

Key words: *Fluoride, Tamarindus indica, Dentin, Type 1collagen.*

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INTRODUCTION

Teeth are composed of three calcified tissues, enamel, dentin, and cementum, and one delicate specialized connective tissue, the pulp. Of the three calcified tissues, the dentin forms the bulk of the tooth substance and gives the basic shape to each tooth. Dentin can be considered a mineralized connective tissue, because the extracellular matrix largely dictates its characteristics[1]. The major structural protein of the extracellular matrix of dentin is type I collagen, making up 90% of organic matrix proteins. These fibres not only provide a matrix for the deposition of hydroxyapatite crystals, but also limit the quantity of mineral that can be deposited in the dentin[2].

Many studies have shown that fluoride can negatively affect collagen metabolism and leads to the breakdown of collagen in different tissues experimental animals[3-6]. Earlier experiments have shown the toxic effect of fluoride on down-regulation of expression of type I collagen (Col1a1) gene in the ribs of rabbits[3], teeth of sheep, guinea pigs and rats[5,7,8] and skeletal muscles of rats[4].

Herbal medicines have been used traditionally worldwide for the prevention and treatment of various diseases. In India medicinal plants are widely used in the ayurvedic medicines for the treatment of

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various health problems[9]. Previous studies reported that medicinal herbs like tamarind fruit pulp, fruit and seeds of Moringa oleifera, bark extract of Terminalia arjuna and blackberry juice play a protective role against fluoride induced toxic effects[10-14].

Tamarind commonly known as ambli or imli (Tamarindus indica L.) belonging to Leguminosae family have been used for various medicinal purposes[15]. In the last few years, a number of reports documented the beneficial effect of tamarind in fluoride toxicity[13,16-19]. However, the ameliorative potential of tamarind on fluoride induced collagen degradation in dentin has never been assessed. Therefore, the present study was conducted to evaluate the ameliorative effect of extract of tamarind fruit pulp on the expression of type I collagen gene in dentin of incisor teeth of rats, continually exposed to 200 ppm of sodium fluoride in drinking water.

MATERIALS AND METHODS

Experimental animals: Female albino Wistar rats weighing 100-120 g of 8 weeks of age bred in the Laboratory Animal Resource Section of Indian Veterinary Research Institute were used. The animals received a normal laboratory pellet diet (composition: wheat bran-12%, maize- 87%, salt-1%, fluoride-4.20 ppm) and water ad libitum. The animals used in the present study were maintained in polypropylene cages in 12 h dark/12 h light cycles with temperature of the laboratory animal house ranging from 18 to 25°C and humidity between 55 and 60%. The study was approved by the Institute Animal Ethics Committee (IAEC, IVRI).

Plant material: Tamarind fruits were purchased from the local market in Bareilly, Uttar Pradesh, India. The plant material was identified and authenticated from Botanical Survey of India, Central National Herbarium, Howrah, India (Voucher specimen no. CNH/1-1/2007 Tech11) where voucher reference specimens were deposited. The extract was prepared as described previously[18].

Experimental procedure: Eighteen rats were randomly divided into three groups of six rats each. Group I served as control and received only tap water; group II received sodium fluoride (NaF, MW 41.99, 99% pure, Qualigens Chemicals, Mumbai, India) @ 200 mg/L of drinking water and group III received fluorinated drinking water (200 mg/L added NaF) along with hydro-methanolic extract of T. Indica fruit pulp (200 mg/kg body weight) daily by gavage for a period of 90 days. The dose of sodium fluoride to induce toxicity was selected based on published literature and earlier studies conducted in our laboratory[3,4,6]. The dose of T. indica was selected based on previous studies in our laboratory[16-18].

After 90 days, the animals were deprived of food overnight and sacrificed by decapitation. Incisor teeth were harvested and dentin was quickly removed using a scalpel. The dentin was snap frozen in liquid nitrogen and stored at -80°C for total RNA extraction.

Reverse transcriptase and real-time polymerase chain reaction (PCR) for collagen: Two pairs of specific primers (Table 1) for Col1a1 and Gapdh were designed according to the alignments of the published cDNA sequences. The total RNA from the dentin was isolated as described previously[5]. Reverse transcriptase (RT) was performed using the first strand cDNA synthesis kit (Fermentas, Life Sciences) as described in a previous report[5]. Quantitative real time PCR conditions and analysis of the Col1a1 gene expression level were the same as described earlier[5]. The results of real time PCR were depicted as the fold change of the Col1a1 mRNA level in the dentin of the experimental rats compared with the normal rats.

Table 1 : Primer sequences with their corresponding PCR product size and position

Gene	Primers	Primer locations	Product (bp)	Genbank accession No.
Col1a1	5'-CTTCGTGTAAACTCCCTCCATCC-3' (sense) 5'-AAGTCCATGTGAAATTGTCTCCCA-3' (antisense)	4454-4599	136	NM_053304
Gapdh	5'-ACATCATCCCTGCATCCACT-3' (sense) 5'-TTTCTCCAGGCGGCATGTCA-3' (antisense)	684-823	140	NM_017008.3

Results and Discussion

The expression level of Col1a1 gene calculated by the $\Delta\Delta C_T$ method in the rats of different groups is reported in Table 2. The result showed down-regulation of expression of Col1a1 gene in dentine of fluoride exposed groups, which increased significantly with concomitant use of T. indica fruit pulp extract. The aim of the present study was to assess the ameliorative potential of T. Indica fruit

Table 2 : Fold change of Col1a1 gene expression level in rats of different groups relative to healthy control

Group	ΔCT (Avg Col1a1 CT - Avg Gapdh CT)	ΔΔCT (ΔCT Gr II - ΔCT Gr I)	Fold difference in Col1a1 relative to Group I (2^-ΔΔCT)
I	2 71±0 11	0±0 11	1
II	3 75±0 09	1 04±0 09	0 49
III	3 21±0 11	0 49±0 10	0 71

Group I: Control; Group II: Fluoride exposed rats; Group III: Fluoride + T. indica fruit pulp extarct. Data are reported as mean ± S.E. for 6 rats in each group

pulp extract on fluoride induced collagen degradation in dentin of incisor teeth of rats. The ameliorative potential of extract was judged in terms of their ability to up-regulate the expression level of type 1 collagen gene in dentin of incisor teeth of rats, continually exposed to 200 ppm of sodium fluoride in drinking water.

Tamarind has long been used as traditional medicines for the treatment of a wide variety of ailments and diseases. Components of Tamarindus indica have been used as spice, food components, digestive, carminative, laxative, expectorant and blood tonic[20]. Pods (fruits) are the most valuable part, which has often been reported as curative in several pharmacopoeias. The fruits have the highest levels of protein (2–3 g/100 g) and carbohydrate (41.1–61.4 g/100 g) of any fruit[21] and serve a good source of important minerals i.e., zinc, iron, sodium, potassium, magnesium, phosphorus, and calcium and vitamins i.e., ascorbic acid, riboflavin, thiamin and niacin[21-23].

Food rich in protein, calcium and ascorbic acid has been suggested to increase the expression level of Col1a1 gene in fluoride exposed rabbits[3,24]. Proanthocyanidins (catechin and epicatichin) stabilize and increase the cross-linkage of type 1 collagen fibrils by hydroxylation of proline which is an essential step of collagen biosynthesis[25]. The present study revealed the down regulation of expression level of the Col1a1 gene of dentin by 51% on the 90th day in fluoride exposed rats as compared with the normal rats. Co-administration of tamarind fruit extract with fluoride prevents the down-regulation of the expression of Col1a1 gene in dentin as compared to fluoride exposed rats, indicating the beneficial effect of tamarind fruit extract against fluoride toxicity. The ameliorative potential on expression of type 1 collagen gene in dentin might be due to the presence of high concentration of protein, calcium, ascorbic acid and proanthocyanidins in the extract of tamarind fruit pulp.

CONCLUSIONS

The present study indicated the protective effect of T. Indica fruit pulp extract on fluoride induced down regulation of type 1 collagen mRNA in dentin of incisor teeth of rats. Further investigations to identify the active principle(s) are obviously needed together with a detailed evaluation on the mechanisms involved in the observed activity.

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