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Combined Effect of *Tamarindus indica* and *Emblica officinalis* on enzyme profile and lipid profile after fluoride intoxication in albino rats

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Abstract: *The present investigation was carried out to evaluate the combined effect of Tamarindus indica and Emblica officinalis on serum enzyme profile and lipid profile after fluoride intoxication in albino rats. Both these medicinal plants have been used as a valuable ingredient of various medicines in India. Three groups of ten adult male albino rats were administered 14.29 mg/L fluoride drinking water (collected from the fluoride zone in Agra region) for 30, 45 and 60 days and another three groups of ten rats were treated first with fluoride water in the same way as mentioned above and then treated with combined T. indica (10gm/Kg body weight) and E. officinalis (500 mg/Kg body weight) fruit extract (mixed in ratio of 1:2) orally for 15, 30 and 45 days, respectively. Results showed a very highly significant ($p<0.001$) increase in serum AST, highly significant ($p<0.01$) in ALT and non-significant ($p>0.05$) increase in LDH after fluoride water intoxication; while a very highly significant ($p<0.001$) decrease in serum AST, highly significant ($p<0.01$) decrease in ALT and non-significant ($p>0.05$) decrease in LDH after combined T. indica and E. officinalis treatment, respectively. There were observed highly significant ($p<0.01$) increase in serum total cholesterol, LDL, VLDL, very highly significant ($p<0.001$) increase in TG and a very highly significant ($p<0.001$) decrease in HDL after fluoride intoxication; while a very highly significant ($p<0.001$) decrease in serum total cholesterol, LDL, VLDL, TG and a very highly significant ($p<0.001$) increase in HDL after combined T. indica and E. officinalis treatment, respectively. T. indica reduces the serum fluoride concentration through chelation bio-mechanism and provides nutrients; while E. officinalis acts as a strong antioxidant. We concluded that T. indica and E. officinalis have compensated the fluoride toxicity that affects the body metabolism.*

Key Words: *Enzyme profile, Lipid profile, Albino rat, Fluoride water, Tamarindus indica and Emblica officinalis.*

Introduction

Medicinal plants form the backbone of traditional system of medicine in India. Medicinal plants are rich source of novel drugs that form the ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs. WHO pointed out that more than 80% of world's population depends on plants to meet their primary health care needs. *Tamarindus indica* is a slow-growing, long-lived massive tropical tree of the family Fabaceae. The fruits are pods oblong, curved, or straight with rounded ends, somewhat compressed and indehiscent although brittle. The flesh of the fruit consists of dry, sticky, dark brown pulp, and inside the pulp are shiny black seeds. The fruits of *T.indica* contain a number of different chemical substances including volatile constituents, lectins, triterpenoids, reducing sugars, organic acids, free amino acids, minerals, vitamins, phenolics, saponins, and tannins (Ishola *et al.*, 1990). The medicinal properties of the pulp are antibacterial, antifungal, hypoglycemic, cholesterolemic, cytotoxic, anti-inflammatory, gastrointestinal, laxative, hypolipomic and antioxidative (Ferrara, 2005, Martinello *et al.*, 2006 and Librandi *et al.*, 2007). *Emblica officinalis* is a small to medium sized deciduous tree with globose, fleshy, pale yellow fruits of the family Euphorbiaceae. *Emblica officinalis* has

many phytocemicals like phenolics, saponins, tannins, flavonoids, organic acids, sugars, free amino acids, minerals and vitamins (Rehman, 2007). Pharmacological investigations on Amla have reported them to have antibacterial, antiviral, antimicrobial, antioxidative, anti-inflammatory and hypolipidemic properties (Saeed and Tariq, 2007, Tarwadi and Agte, 2007; Mathur *et al.*, 1996). Amla is useful in cancer, age-related renal disease, diabetes and liver dysfunction (Qureshi *et al.*, 2009). The antioxidant activity of fruits of *E. officinalis* has been traced to its tannoid principles both *in vitro* and *in vivo* (Bhattacharya *et al.*, 2002).

Ground water is one of the most important sources of drinking water and contamination of ground water with fluoride is increasingly becoming a matter of grave concern as 17 states in India have been declared endemic for fluorosis, and of these 5 states have indicated hyperendemicity for fluorosis (Choubsia, 2001). In thousands of villages across Uttar Pradesh it is causing death and severe illness. Although fluorosis has been regarded in the past as disease affecting only bone, and teeth, recent studies have given ample evidence that fluoride toxicity adversely affects most of the soft organs (Neurath, 2005; Cicek *et al.*, 2005). Acute oral exposure to fluoride may produce effects including nausea, vomiting, abdominal pain, diarrhoea, fatigue, drowsiness, coma, convulsions, cardiac arrest, and death. Chronic fluoride ingestion may cause skeletal fluorosis, clinical fluorosis, dental fluorosis, non-skeletal manifestations, or any combination of the above and in final stage it causes premature aging (Ahmad *et al.*, 2000).

The current therapeutic approach to fluoride poisoning is to increase the excretion of fluoride by chelation. Co-administration of antioxidants such as vitamins C and E or N-acetyl cysteine during chelation therapy has been found to be beneficial in increasing fluoride mobilization and assisting the recovery of altered biochemical variables (Susheela and Bhatnagar, 2002).

Table 1. Biochemical changes in serum enzyme profile in albino rats after fluoride water ingestion and combined *T. indica* and *E.officinalis* treatment.

	Experimental Period								
Parameters	30 days Control	30 days F.W.I.	15 days combined T.indica and E.officinalis treatment after 30 days	45 days Control	45 days F.W.I.	30 days combined T. indica and E.officinalis treatment after 45 days	60 days Control	60 days F.W.I.	45 days combined T.indica and E.officinalis treatment after 60 days
AST (U/L)	64.7 ± 2.3335	80.4 ± 3.5314***	65.8 ± 2.3607***	65.9 ± 2.3211	81.3 ± 2.8869***	67.8 ± 2.3132***	66.5 ± 2.5177	105.6 ± 7.1898***	71.8 ± 2.6865***
ALT (U/L)	41.000 ± 5.0354	55.9 ± 5.1130*	42.4 ± 5.1363*	43.6 ± 5.4041	62.2 ± 5.2994*	46.00 ± 5.3562*	47.00 ± 4.7679	68.8 ± 5.4422**	49.2 ± 4.6994**
LDH (U/L)	446.2 ± 43.8251	456.2 ± 44.0340 N.S	449.1 ± 44.2112 N.S	445.1 ± 43.8011	464.4 ± 44.1155 N.S	447.7 ± 43.7635 N.S	444.0 ± 43.2093	465.3 ± 43.6188 N.S	442.1 ± 43.1443 N.S

F.W.I.- Fluoride Water Ingestion, NS-Non significant ($p>0.05$), *-Significant ($p<0.05$), ** Highly significant ($p<0.01$), ***- Very highly significant ($p<0.001$)

Table 2. Biochemical changes in serum lipid profile in albino rats after fluoride water ingestion and combined *T. indica* and *E. officinalis* treatment.

Parameters	Experimental Period								
	30 days Control	30 days F.W.I.	15 days combined T.indica and E.officinalis treatment after 30 days F.W.I.	45 days Control	45 days F.W.I.	30 days combined T. indica and E.officinalis treatment after 45 days F.W.I.	60 days Control	60 days F.W.I.	45 days combined T.indica and E.officinalis treatment after 60 days F.W.I.
Total Cholesterol (mg/dl)	70.6 ± 1.5216	75.1 ± 1.3203*	66.1 ± 1.636***	73.7 ± 1.2741	79.1 ± 1.1200**	66.7 ± 1.4907	74.0 ± 1.4907	79.4 ± 1.3515**	61.9 ± 1.4410***
Triglyceride (mg/dl)	34.6 ± 1.0132	38.3 ± 0.8950**	32.5 ± 0.9574***	38.5 ± 0.9574	43.2 ± 1.0728**	33.5 ± 1.0461***	41.5 ± 0.9574	47.3 ± 0.8306***	34.4 ± 0.9092***
Low density lipoprotein (mg/dl)	37.5 ± 0.9574	42.2 ± 1.3316**	35.5 ± 0.9574***	39.5 ± 0.9574	44.1 ± 1.3370**	34.5 ± 0.9574***	43.5 ± 0.9574	50.0 ± 1.6193**	37.1 ± 1.1***
Very low denisity lipoprotein (mg/dl)	22.5 ± 0.9574	26.9 ± 0.9	21.5 ± 0.9574***	26.5 ± 0.9574	31.1 ± 0.9**	22.1 ± 1.1100***	29.5 ± 0.9574	35.1 ± 1.2423**	22.6 ± 0.9092***
High density lipoprotein (mg/dl)	46.5 ± 0.9574	42.8 ± 0.8273**	48.5 ± 0.9574***	47.5 ± 0.9574	43.6 ± 0.9092**	50.3 ± 1.0440***	44.5 ± 0.5	29.2 ± 0.3265***	49.5 ± 0.4013***

F.W.I.- Fluoride Water Ingestion, Ns- Non significant (P>0.05) *-Significant (p<0.05), **-Highly significant (p<0.01),***-Very highly significant (p<0.001)

A number of very recent investigations revealed that protein molecules from various plant sources possess antioxidant and hepatoprotective activities (Oh *et al.*, 2006; Lee *et al.*, 2006). *Tamarindus indica* and *Emblica officinalis* possesses antioxidant properties and protects organs against toxin-induced oxidative stress (Mathur *et al.*, 1996; Chatterjee *et al.*, 2006; Bhattacharjee and Sil, 2007). Therefore, the present investigation evaluated the beneficial effects of *T.indica* and *E.officinalis* on the serum enzyme profile and lipid profile after fluoride intoxication in albino rats.

Materials and Methods

Ninety male albino rats (*Rattus norvegicus*) of wistar strain weighing 120 ± 25 gm and eight-weeks old were randomly divided into nine groups of ten animals. Each group was kept in a separate polypropylene cage, and maintained in controlled temperature (25 ± 2°C), humidity (65 ± 10%) and proper circadian rhythm. The animals were acclimatized for 20 days before starting the experiment. During this period animals had free access to normal diet and the water given *ad libitum*.

The fluoride water was collected from fluoride zone in Agra region from as usual water sources like hand pumps in polypropylene bottles. The fruits of *Tamarindus indica* and *Emblica officinalis* were procured from local market and taxonomically identified by Department of Botany, School of Life Sciences, Khandari campus, Dr. B.R.A. University, Agra. The fruit pulp was macerated with the help of pistil and mortar. This macerated pulp was dissolved in 1 litre of distilled water, stirred intermittently, and then left overnight. Finally, this solution was filtered by muslin cloth. The filtered extract was stored in refrigerator at a temperature of 2- 3 °C.

The selected dose of *Tamarindus indica* for entire research was 10 gm/kg body weight given to albino rats (Khandare *et al.*, 2000, 2002). The therapeutic dose of *Emblica officinalis* was selected at 500 mg/kg body weight (Thangaraj, *et al.*, 2007).The combined extract was formed by mixing the *Tamarindus indica* and *Emblica officinalis* (jn ratio of 1:2) 15 min before the dosing.

The three groups of albino rats were treated as control for 30, 45 and 60 days. Another three groups were treated with fluoride water for 30, 45 and 60 days. Remaining three groups were treated first with fluoride water in the same way as described above and then treated with combined *T. indica* and *E. officinalis* fruit extract for 15, 30, and 45 days, respectively. The aqueous fruit extract was given orally to the rats by gavage tube. At the end of the respective treatment period, the rats were sacrificed under chloroform anesthesia by 24 hours after the last dosing.

The concentration of fluoride ions in water sample was measured by the method of Harwood (1969). Serum AST and ALT were estimated by the method of Reitman and Frankel (1957) and serum LDH by the method of UV Kinetics (IFCC/SFBC, 1982) prescribed by Oba and Uriteni (1982). Serum total cholesterol was estimated by the method of Wybenga (1970), HDL by the method of Warnic *et al.* (1985), LDL and VLDL by the method of Friedwald *et al.* (1972) and serum triglyceride by the method of McGowan (1983).All the data were statistically analyzed by using Student's 't' test and ANOVA. All the results were expressed as mean ± S.E.

Results and Discussion

The serum AST increases and is very highly significant ($p<0.001$), ALT increases and is highly significant ($p<0.01$) and LDH increases non-significant ($p>0.05$) after fluoride water ingestion for 30, 45 and 60 days; while serum AST decreases and is very highly significantly ($p<0.001$), ALT decreases and is highly significantly ($p<0.01$) and serum LDH decreases non-significantly ($p>0.05$) after combined *Tamarindus indica* and *Emblica officinalis* treatment for 15, 30 and 45 days with fluoride water ingestion treated groups, respectively (Table 1 and 2 and Fig. 1-8).

The adverse toxic effects of fluoride arise due to enzyme inhibition, collagen break down, gastric damage and disruption of the immune system (Ahmad *et al.*, 2000). Along with the other toxic effects, fluoride treatment induces oxidative stress causing significant depletion of the activities of the antioxidant enzymes and enhancement of lipid peroxidation (Shanthakumari *et al.*, 2004; Manna *et al.*, 2006, 2007). The exact mechanism by which tamarind ingestion decreases fluoride retention in bones is not properly known, yet. Tamarind appears to mobilize deposited fluoride from bone by enhancing urinary excretion of fluoride due to its active component that interacts with fluoride (Khandare *et al.*, 2000, 2002, 2004). Sriramachari (1983) and Maruthamuthu and Reddy (1987) have also shown that fluoride is bound by Tamarind. The aqueous extract of *E. officinalis* fruit increases cardiac glycogen levels and decreases serum GOT, GPT and LDL in rats having induced myocardial necrosis (Tariq *et al.*, 1977). In rat, the flavonoids from *E. officinalis* effectively reduced lipid levels in serum and tissues and exerted a significant inhibitory effect of hepatic HMG CoA reductase enzyme activity (Anila and Vijayalakshmi, 2002). *In vitro* and animal studies have indicated that amla have

potent anti-oxidant effect against several test systems such as superoxide radicals, lipid peroxide formation induction by Fe+++/ADP ascorbate system, hydroxyl radical scavenging action and in systemic augmentation of antioxidant enzymes in the brain of laboratory animals (Mathur *et al.*, 1996).

In the present study, an increased aspartate transaminase (AST) level in the serum after fluoride water ingestion is due to fluoride water intoxication; while decreased AST level in the serum after combined *Tamarindus indica* and *Emblica officinalis* treatment in same group of fluoride intoxication may be due to chelating nature of Tamarind and antioxidant characteristic of Amla. It can also be correlated with an increase and decrease in serum ALT and LDH in the fluoride and combined *T.indica* and *E.officinalis* treated groups respectively. Similar findings have also been reported by Guo *et al.*, (2003), Rzeuski *et al.*,(1998) and Shivarajshankara *et al.*, (2001) in rats and human due to side effect of fluoride intoxication on the lipid peroxidation and antioxidant system. Similar results have also been reported by Khandare *et al.*, (2002) and Saeed and Tariq, (2007) due to supplementation of Tamarind and Amla as nutritional antioxidants which reduced fluoride-caused oxidative stress.

In the present study, an increased alanine transaminase (ALT) level in the serum after fluoride water ingestion is due to fluoride water intoxication; while decreased ALT level in the serum after combined *Tamarindus indica* and *Emblica officinalis* treatment in same group of fluoride intoxication may be due to chelating nature of Tamarind and antioxidant characteristic of Amla. It can also be correlated with an increased and decreased serum AST and LDH in the fluoride and combined *T.indica* and *E.officinalis* treated groups, respectively. Similar findings have also been reported by Guo *et al.*, (2003), Rzeuski *et al.*, (1998) and Shivarajshankara *et al.*, (2001) in rats and human due to side effect of fluoride intoxication on the lipid peroxidation and antioxidant system. Similar results have also been reported by Khandare *et al.*, (2002) and Saeed and Tariq (2007) due to supplementation of Tamarind and Amla as nutritional antioxidants which reduced fluoride-caused oxidative stress.

In the present study, non-significant increase in lactate dehydrogenase (LDH) level in the serum after fluoride water ingestion is due to fluoride water intoxication; while non-significant decrease in LDH level in the serum after combined Tamarindus indica and Emblica *officinalis* treatment in same group of fluoride intoxication may be due to chelating nature of Tamarind and antioxidant characteristic of Amla. Elevated levels of LDH may be seen with toxicants and pollutants (Cobben, 1997).

In the present study, total cholesterol increased due to adverse effects of fluoride toxicity on body metabolism; while decrease in cholestrol level was seen due to beneficial effects of Tamarind and Amla. Another possibility is that the fruit extracts reduced cholesterol level in serum by inhibiting hepatic 3-hydroxy-3-methylglutaryl-Co-A (HMG CoA) reductase activity (Anila and Vijayalakshmi, 2002; Iftekhar *et. al.*, 2006; Martinello *et al.*, 2006; Librandi *et al.*, (2007). Serum low density lipoprotein (LDL) increased due to adverse effects of fluoride toxicity on body metabolism; while decrease due to beneficial effects of Tamarind and Amla. Similar findings have also been reported by (Mathur *et. al., 1*996) and (Iftekhar *et. al.,* 2006) in hypercholesterolaemic rats due to ability of *Emblica officinalis* to prevent low-density lipoprotein oxidation.Serum high density lipoprotein (HDL) decreased due to adverse effects of fluoride toxicity on body metabolism; while HDL increased due to beneficial effects of Tamarind and Amla. Similar findings have been reported by Antony *et. al.*, (2006) due to enhanced reverse cholesterol transport by *E. officinalis.* Serum triglycerides (TG) and serum very low density protein (VLDL) also increased due to adverse effects of fluoride toxicity on body metabolism; while both the parameters decreased due to beneficial effects of Tamarind and Amla. Similar findings have been reported by Antony *et. al.*, (2006) due to interference in cholesterol synthesis and transport by *E.*

officinalis.

Thus, it was concluded that there is reduced oxidative stress exhibited by decreased serum AST, ALT and LDH level after combined treatment with *T. indica* and *E. officinalis* in fluoride intoxicated rats. There is also reduction in harmful fats *viz* total cholesterol, LDL, VLDL and triglycerides; while enhancement of the beneficial HDL cholesterol occurred after the treatment. Thus, Tamarind with Amla exerts beneficial effects on body metabolism and altered biochemical indices in fluoride toxicity. Application of this study will be useful in minimizing the side effects of fluoride intoxication in body in fluoride-rich areas.

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