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Original Research Article

Biochemistry for better diagnosis and therapy

Ameliorative Effect of Tamarindus indica and Vitamin D Supplementation On Fluoride Induced Biochemical Parameters in Albino Rat

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Abstract: Fluoride water problem is very common in all areas of world including India. The deleterious effects of fluoride on bones causes fluorosis. This problem arises mainly due to gradual lowering of ground water level, which is merely responsible for degrading water quality. The hand pumps of many areas are restricted for use due to excess fluoride in their water. In a country like India, it is very tough to provide pure drinking water to all. Hence this study is designed to study the effect of fluoride on biochemical parameters in albino rats, the mammalian model. The aim and objective of this study is to evaluate combination of two antioxidants viz. *Tamarindus indica* (rich source of vitamin C) and vitamin D against fluoride toxicity in mammals for which albino rat is used as model. Tamarindus have significant amount of antioxidants and vitamin D also have immunity and healing properties, hence this can be a reason behind their cumulative ameliorative effect against sodium fluoride intoxication. Further, the effect of fluoride can be minimized or ameliorated using some antioxidants and vitamins. For this purpose, the fluoride intoxicated rats were observed for possible amelioration by *Tamarindus indica* and vitamin D supplementation. The fluoride intoxication showed altered biochemical parameters like serum protein, SGOT, SGPT, ACP, ALP and LDH.

Key words: Fluoride, *Tamarindus Indica*, Vitamin D, Serum Glutamic-Oxaloacetic Transaminase, Serum Glutamic Pyruvic Transaminase, Acid Phosphatase, Alkaline Phosphatase, Lactate Dehydrogenase..

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1. INTRODUCTION

Fluoride intoxication is a serious problem in many countries including India. However, fluoride can also enter public water systems from natural sources, including runoff from weathering of fluoride, containing rocks and soils and leaching from soil into groundwater. Fluoride pollution from various industrial discharges and emissions can also contaminate water supplies. Environmental Protection Agency (EPA) sets 3 types of standards for environmental contaminants. The maximum contaminant level goal (MCLG) is a health goal set at a concentration at which no adverse health effects are expected to occur and margins of safety are judged "Adequate". The maximum contaminant level (MCL) is the enforceable standards that is set as close to MCLG as possible, taking into consideration other factors such as treatment technology and costs. For fluoride, the MCLG and MCL are both 4 mg/l. For some contaminants, EPA also establishes a secondary maximum contaminant level (SMCL) to manage drinking water for aesthetic or cosmetic effects. The SMCL for fluoride is 2mg/l[1]. The major sources of fluoride in ground water are fluoride bearing rocks like fluorspar, cryolite, fluorapatite and hydroxyl apatite. The presence of fluoride in ground water is governed by several factors, like igneous rock formation, metamorphic rocks and weathering processes. 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 great concern, as 17 states in India have been declared endemic for fluorosis. In thousands of villages across Uttar Pradesh, it causes death and severe illness. Although fluorosis has been regarded as disease affecting only bone and teeth, recent studies showed ample evidences to have effect on soft tissues including vital organs[2]. Fluoride in varying concentration induce free radical toxicity in both animals and people living in areas of endemic fluorosis[3]. People in Indian villages have been long exposed to fluoride through drinking water, air and food. Fluoride is also known to cross the cell membrane and to enter tissues. Generation of free radicals, lipid peroxidation and altered antioxidant defense system are considered to play an important role in the toxic effects of fluoride[4]. Medicinal plants form the backbone of traditional system of medicine in India. Medicinal plants are rich sources of oral drugs that form the traditional system of medicine, modern medicines, neutraceuticals, food supplements, bioactive compounds that lead to synthetic drugs. *Tamarindus indica* is a slow growing long lived massive tropical tree of the family Fabaceae. The pharmacological early investigations revealed that, the *Tamarindus indica* pulp is an antibacterial, antifungal, hypoglycemic, anti-inflammatory and antioxidative in its activities. The Tamarindus intake appears to have additional beneficial effect on mobilization of deposited fluoride from bone by enhancing urinary excretion of fluoride[2]. The aim and objective of this study is to evaluate combination of two antioxidants viz. *Tamarindus indica* (rich source of vitamin C) and vitamin D against fluoride toxicity for which albino rat is used as model.

2. MATERIALS AND METHODS

The male albino rats (*Rattus norvegicus*) of wistar strain weighing between 120±25g were obtained from the colony of albino rats bred in the animal house of Zoology Department, School of Life Sciences, Khandari Campus, Agra and were used for experimental purpose. Animal study was approved by IAEC/Upation of the Dr. B. R. Ambedkar University, Agra, Uttar Pradesh (Reg.No.1608/CPCSEA). The albino rats were housed in polypropylene cages measuring 45x25x15cm and maintained at controlled temperature of 25±2^{0}C, humidity 65±10% and proper circadian rhythm. The acclimatized animals were divided into different groups as per protocol for 7, 15, 30, 45 and 60 days experiment including control set, sodium fluoride treated set, sodium fluoride + *Tamarindus indica* + Vit. D supplemented set and caged separately. They were maintained on standard diet- Goldmohar brand feed and water *ad libitum*. The fluoride was given to albino rats in form of sodium fluoride (NaF). The dose of NaF was 10mg/Kg body weight[5]. The fruits of *Tamarindus indica* were procured from local market. All the materials were taxonomically identified by Department of Botany, School of Life Sciences, Khandari Campus, Agra. The aqueous pulp extract of *Tamarindus indica* was prepared by extraction with hot water in order to simulate the local procedure. The selected therapeutic dose of *Tamarindus indica* was 10g/kg body weight[6]. 52% of RDI vitamin C is available in *Tamarindus indica*. The vitamin D powder was procured from a medical store and the dose given was 5mg/kg body weight[7]. All treatments were given orally using a syringe and a bent tip canula. The doses were given for 7, 15, 30, 45 and 60 days respectively. The albino rats of all the groups were sacrificed under light anesthesia. The biochemical parameters were estimated through standard procedures and protocols viz. Protein profile through Duma method[8]; SGOT, SGPT through Reitman and Frankel method[9]; Acid and Alkaline phosphatase through Kind and King method[10,11]; LDH through UV kinetic method[12].

3. STATISTICAL ANALYSIS

All the data were subjected to statistical analysis through software (Ky Plot version 3.0) using ANOVA[13].

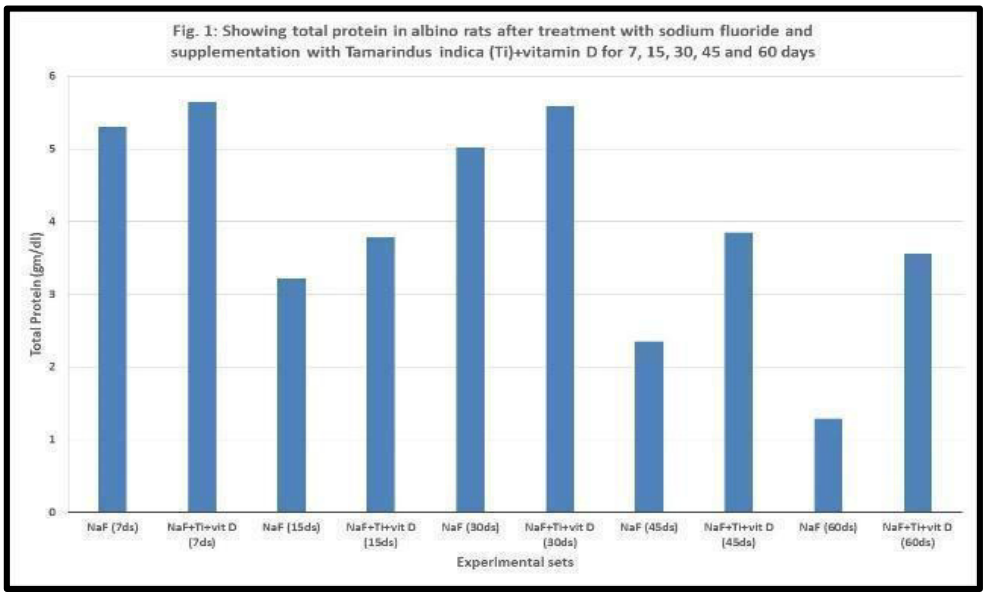
4. RESULTS AND DISCUSSION

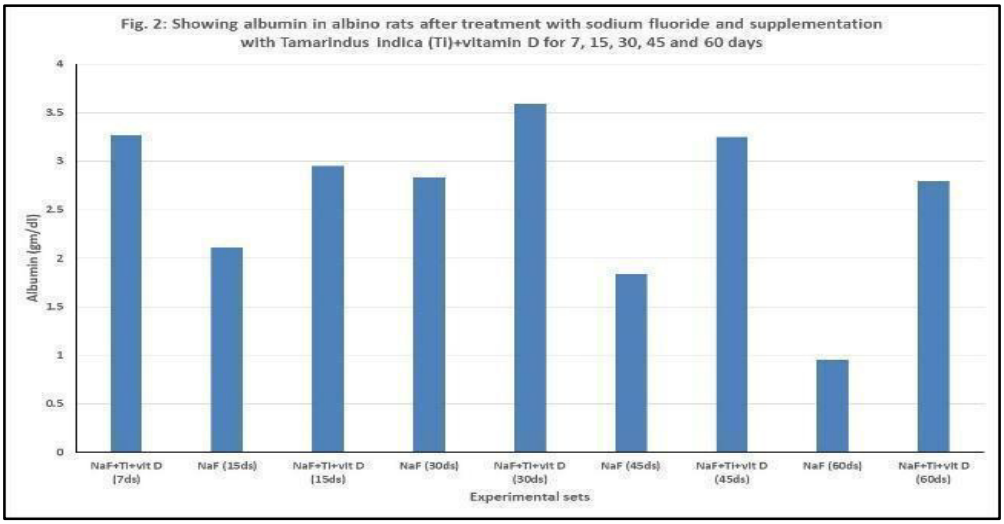
The biochemical parameters showed altered level after the treatment with sodium fluoride and also showed amelioration by supplementation of *Tamarindus indica* extract and vitamin D. The protein profile includes total protein, albumin and globulin, show decrease after NaF treatment and increase after supplementation with *Tamarindus indica* extract and vitamin D. The enzyme profile includes SGOT, SGPT, ACP, ALP and LDH, showed increase after NaF treatment and decreasing trend after supplementation with *Tamarindus indica* extract and vitamin D. The results were significant in magnitude after applying ANOVA. The results are shown in Table-1 and figure 1-8.

Table-1: Showing various biochemical parameters in albino rats after treatment with sodium fluoride and supplementation with Tamarindus indica (Ti)+vitamin D for 7, 15, 30, 45 and 60 days

Experimental Sets	Parameters							
	Total Protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	ACP (U/L)	LDH (U/L)
Control	5.58±0.30	3.2±0.26	2.38±0.31	70±3.35	45.1±4.89	185±10.38	46.3±2.87	389.7±27.57
NaF (7days)	5.3±0.26*	2.83±0.25*	2.47±0.32^{NS}	76.9±4.71*	53.4±5.34*	206.7±10.31*	54.7±3.58*	408.3±28.42*
NaF+Ti+Vit D (7days)	5.65±0.25*	3.27±0.28*	2.38±0.31^{NS}	74.9±4.17^{NS}	48.6±4.48*	195.7±6.40*	48±3.81*	384.2±26.66*
NaF (15days)	3.21±0.16**	2.11±0.24**	1.1±0.21***	121.3±4.76***	93.7±4.23***	255.1±8.11***	84.8±3.68***	481±12.22***
NaF+Ti+Vit D (15days)	3.78±0.18*	2.95±0.24*	0.83±0.13**	81.5±3.87***	55.9±2.79***	174.6±5.26***	47.9±4.01***	377±9.33***
NaF (30days)	5.02±0.17*	2.83±0.27*	2.19±0.19**	74.4±3.27^{NS}	69.9±3.25***	205.6±6.29**	68±4.14**	358.5±11.03*
NaF+Ti+Vit D (30days)	5.59±0.25*	3.59±0.26*	2±0.21*	46.6±3.12***	56.1±4.15**	183±5.34**	56.7±3.21*	286.4±7.86**
NaF (45days)	2.35±0.19***	1.84±0.12***	0.51±0.01***	168.1±6.52***	118.3±6.66***	270.3±4.71***	113.8±5.27***	567.8±11.88***
NaF+Ti+Vit D (45days)	3.85±0.16**	3.25±0.18***	0.6±0.13*	98.2±3.17***	58±4.95***	231.8±3.89**	78.2±2.14***	355.3±13.48***
NaF (60days)	1.29±0.07***	0.95±0.01***	0.36±0.07***	217.5±3.98***	160±4.88***	315.4±6.82***	155±6.2***	534.2±9.93***
NaF+Ti+Vit D (60days)	3.56±0.18***	2.79±0.16***	0.77±0.14***	139.8±4.96***	97.5±3.95***	217.7±4.22***	96.7±3.73***	329.4±7.34***

Significance level: $^{NS}p>0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$





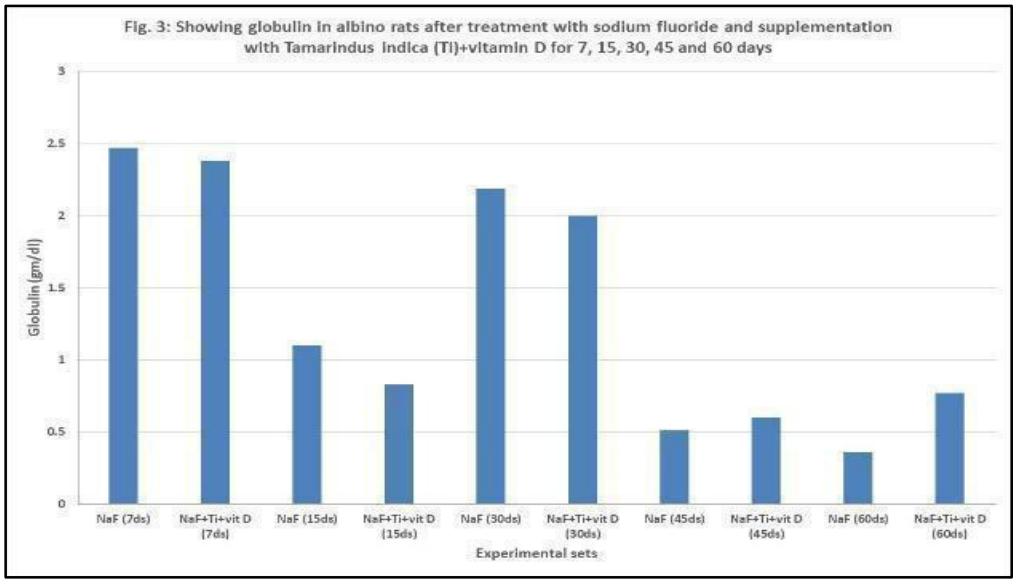


Fig. 3: Showing globulin in albino rats after treatment with sodium fluoride and supplementation with Tamarindus indica (Ti)+vitamin D for 7, 15, 30, 45 and 60 days
Globulin (gm/dl)
3
2.5
2
1.5
1
0.5
0
NaF (7ds)
NaF+Ti+vit D (7ds)
NaF (15ds)
NaF+Ti+vit D (15ds)
NaF (30ds)
NaF+Ti+vit D (30ds)
NaF (45ds)
NaF+Ti+vit D (45ds)
NaF (60ds)
NaF+Ti+vit D (60ds)
Experimental sets

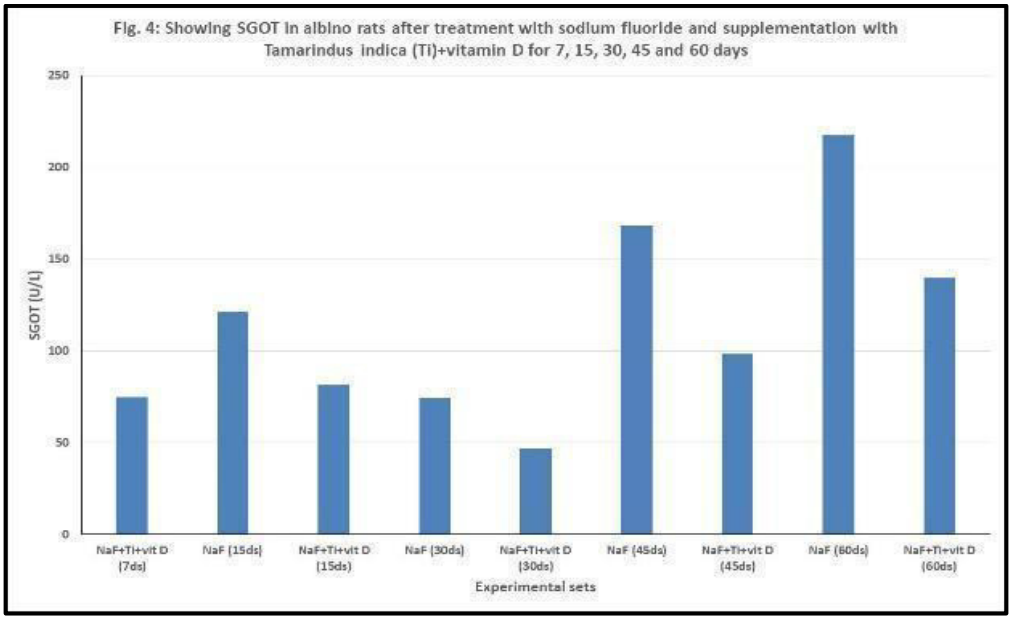


Fig. 4: Showing SGOT in albino rats after treatment with sodium fluoride and supplementation with Tamarindus indica (Ti)+vitamin D for 7, 15, 30, 45 and 60 days
SGOT (U/L)
250
200
150
100
50
0
NaF+Ti+vit D (7ds)
NaF (15ds)
NaF+Ti+vit D (15ds)
NaF (30ds)
NaF+Ti+vit D (30ds)
NaF (45ds)
NaF+Ti+vit D (45ds)
NaF (60ds)
NaF+Ti+vit D (60ds)
Experimental sets

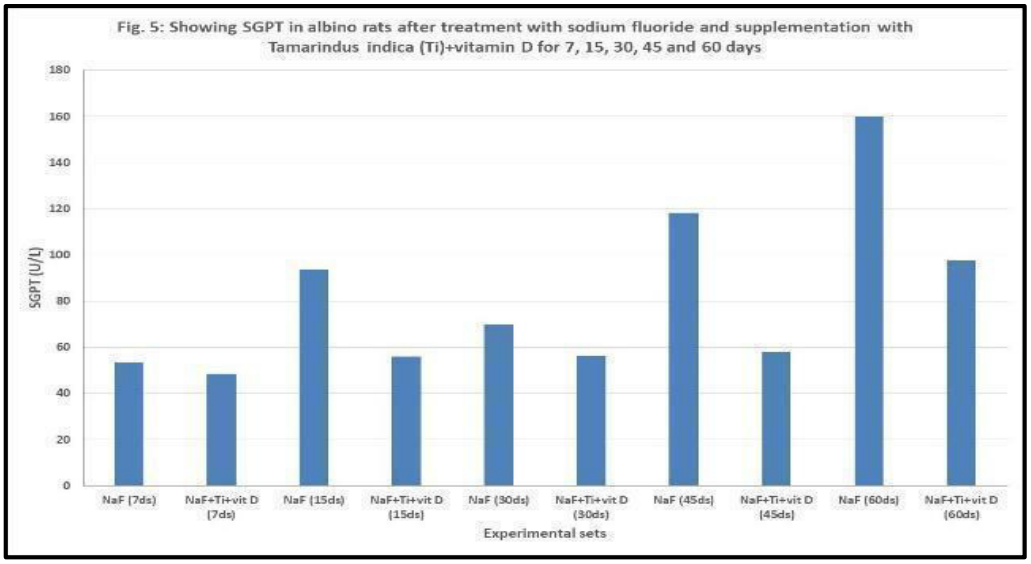


Fig. 5: Showing SGPT in albino rats after treatment with sodium fluoride and supplementation with Tamarindus indica (Ti)+vitamin D for 7, 15, 30, 45 and 60 days
SGPT (U/L)
180
160
140
120
100
80
60
40
20
0
NaF (7ds)
NaF+Ti+vit D (7ds)
NaF (15ds)
NaF+Ti+vit D (15ds)
NaF (30ds)
NaF+Ti+vit D (30ds)
NaF (45ds)
NaF+Ti+vit D (45ds)
NaF (60ds)
NaF+Ti+vit D (60ds)
Experimental sets

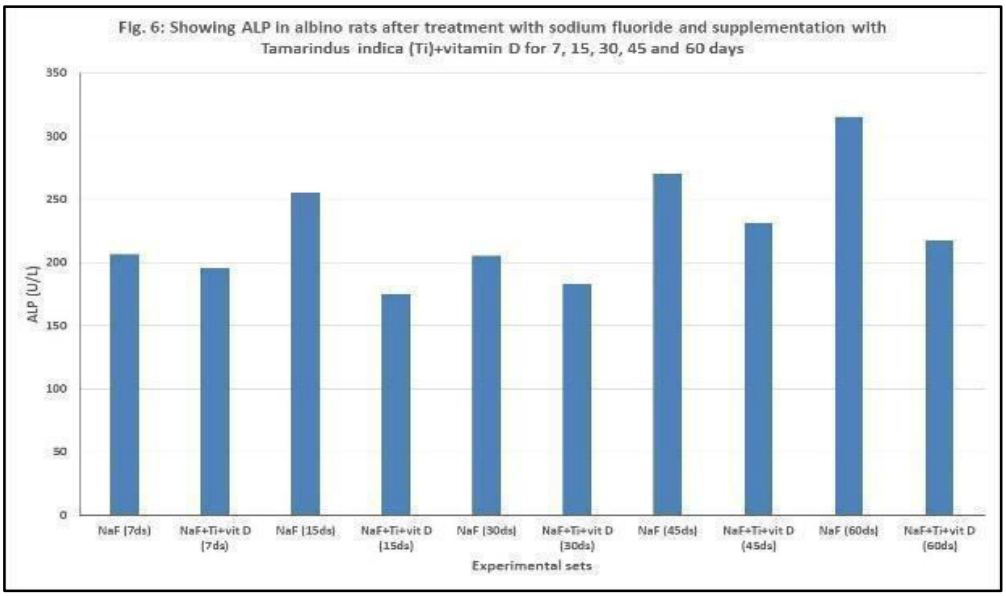


Fig. 6: Showing ALP in albino rats after treatment with sodium fluoride and supplementation with Tamarindus indica (Ti)+vitamin D for 7, 15, 30, 45 and 60 days
ALP (U/L)
350
300
250
200
150
100
50
0
NaF (7ds)
NaF+Ti+vit D (7ds)
NaF (15ds)
NaF+Ti+vit D (15ds)
NaF (30ds)
NaF+Ti+vit D (30ds)
NaF (45ds)
NaF+Ti+vit D (45ds)
NaF (60ds)
NaF+Ti+vit D (60ds)
Experimental sets

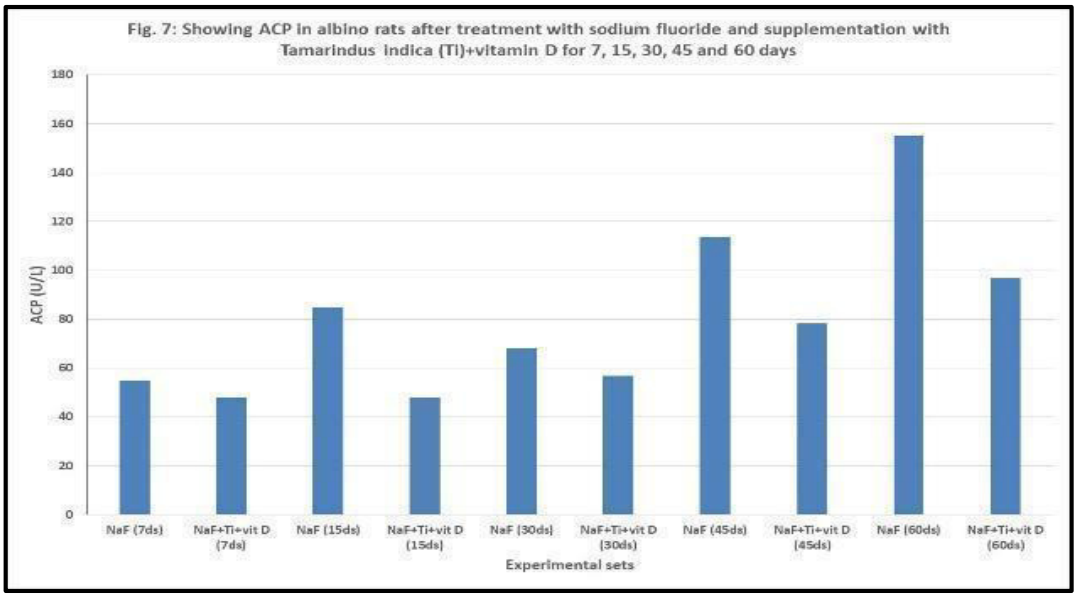


Fig. 7: Showing ACP in albino rats after treatment with sodium fluoride and supplementation with Tamarindus indica (Ti)+vitamin D for 7, 15, 30, 45 and 60 days
ACP (U/L)
180
160
140
120
100
80
60
40
20
0
NaF (7ds)
NaF+Ti+vit D (7ds)
NaF (15ds)
NaF+Ti+vit D (15ds)
NaF (30ds)
NaF+Ti+vit D (30ds)
NaF (45ds)
NaF+Ti+vit D (45ds)
NaF (60ds)
NaF+Ti+vit D (60ds)
Experimental sets

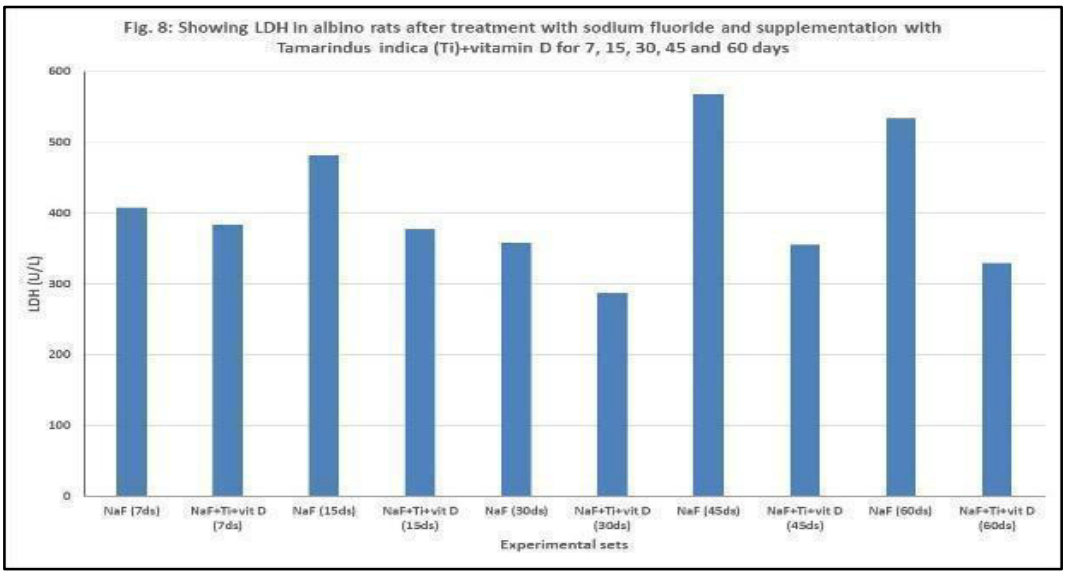


Fig. 8: Showing LDH in albino rats after treatment with sodium fluoride and supplementation with Tamarindus indica (Ti)+vitamin D for 7, 15, 30, 45 and 60 days
LDH (U/L)
600
500
400
300
200
100
0
NaF (7ds)
NaF+Ti+vit D (7ds)
NaF (15ds)
NaF+Ti+vit D (15ds)
NaF (30ds)
NaF+Ti+vit D (30ds)
NaF (45ds)
NaF+Ti+vit D (45ds)
NaF (60ds)
NaF+Ti+vit D (60ds)
Experimental sets

The altered biochemical parameters in albino rats may be due to the toxic effect of fluoride[2]. A significant decrease in total protein, albumin, and globulin is due to interrupted synthesis of protein. The alteration in enzyme activity is due to toxic stress of fluoride on liver which enhances the enzyme secretion in serum and showed damage to tissue. This can also be correlated to change in ionic calcium which is one of the important ion for the initiation and maintenance activity of soft vital organs[3]. Fluoride decreases absorption of bile salts, hence increased enzymatic activity in liver to a limit of damage. It is known that, fluoride enhances lipid peroxidation and inhibits the antioxidant enzymes leads to liver damage[14]. Ingestion of fluoride causes decrease in ionized calcium was reported by Anfal and Samir[15] and Sharma *et al.*[16] The adverse toxic effects of fluoride arise due to enzyme inhibition, collagen breakdown and disruption of immune system. Fluoride treatment induces oxidative stress and produces free radical, that can damage soft tissue and cell membrane. Alteration by *Tamarindus indica* and vitamin D reduces free radicals by acting as antioxidant instead of natural endogenous antioxidant enzymes[4,17] which were inhibited during fluoride intoxication[18]. Among the wide variety of fluoride toxic effects, its effect on the activity of transport of proteins is particularly important. As mentioned in some research, fluoride affects secretion and vesicular traffic via transport proteins, that are synthesized in ER and subsequently transported to the Golgi and plasma membrane[19]. In the present study, increased SGOT, SGPT, ACP, ALP and LDH level after fluoride treatment is due to fluoride intoxication, while decreased level of SGOT, SGPT, ACP, ALP and LDH after combined *Tamarindus indica* and vitamin D treatment in same group may be due to chelating nature of Tamarindus and antioxidant nature of vitamin D. Similar findings have also been reported by Guo *et al.*[20], Chaudhary *et al.*[21], Barbier *et al.*[22] Similar results have also been reported by Khandare *et al.*[23-24] with supplementation of Tamarindus and Amla as nutritional antioxidant which reduces fluoride caused oxidative stress. Fluoride has toxic effects on various body organs. The balance between the oxidative system and the antioxidant system is broken and oxidative stress is augmented during fluoride exposure[25]. Oxidative stress can be effectively prevented by supplementing with natural antioxidant among which *Tamarindus indica* and vitamin D are supplemented in present study, have been reported to scavenge the free radicals and ROS[26]. In the present study, fluoride exposed rats exhibited elevated levels of marker enzymes with significant decline after *Tamarindus indica* and vitamin D supplementation. The antioxidants reverse the lipid peroxidation and oxidative effects of fluoride. Polyphenols and flavonoids are reported important antioxidants in *Tamarindus indica* extract[27].

5. CONCLUSION

From this investigation, it could be concluded that the *Tamarindus indica* and vitamin D in combination is beneficial in the medicinal perspective against the potent toxic stress of fluoride by ameliorating its free radical nature and neutralizing ROS. This study will help the society to know about the fluoride toxicity and how to minimize or ameliorate with vitamin C containing *Tamarindus indica* extract.

6. ACKNOWLEDGEMENTS

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7. AUTHORS CONTRIBUTION STATEMENT

First author Ms. Shalini Yadav is the main author who conducted experimental work in laboratory under supervision of second author Dr. P.K. Singh (supervisor). The third author Mr. Prem Sagar helped in arrangement of data and graphical presentation.

8. FUNDING SOURCE

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9. CONFLICT OF INTEREST

Conflict of interest declared none.

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