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Ameliorative effect of *Tamarindus indica* l. leaf powder on haemato-biochemical and oxidative stress parameters in Fluorotic cattle

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Abstract
The present experiment was conducted for the evaluation of the ameliorative effect of tamarind leaf powder (*Tamarindus indica* L.) in fluorotic cattle reared in vicinity of aluminium smelter plant. Thirty cattle with body weight around 250kg exhibiting chronic signs of fluorosis and ten healthy cattle from non fluorotic area were incorporated in the study. Fluorotic cattle were divided into three groups consisting of 10 cattle each. Group I from fluoride free area served as healthy control. The group II received no treatment and served as disease control. Group III and group IV was supplemented with tamarind leaf powder @15g and 30 g/day along with feed for a period of 60 days. Haemato-biochemical parameters along with erythrocytic oxidative stress parameters like catalase (CAT), superoxide dismutase (SOD) and lipid peroxidation (LPO) were evaluated in cattle of different groups. Significant alterations in haemato-biochemical parameters were observed in fluorotic cattle as compared to healthier ones. Marked increase in lipid peroxidation and decrease in catalase and superoxide dismutase activities was noted in fluorotic cattle as compared to healthy cattle. Supplementation of tamarind leaf powder @ 15 mg and 30 mg per kg per animal restored the haemato-biochemical parameters and reduced oxidative load after 60 days of experimental period. In conclusion the present study found that dried powder of *Tamarindus indica* leaf has ameliorative potential on management of fluorosis in cattle.

Keywords: Fluorosis, tamarind leaf, oxidative stress, haemato-biochemical parameters, Cattle

1. Introduction
Fluorine is a highly reactive halogen, which forms stable fluoride complexes after binding with almost all cations, is mostly found in the form of fluorides. Accumulation of fluoride in water, vegetables, grasses etc. may results both acute and chronic forms of toxicity in human and animals [1]. Fluoride is a major generator of free radicals like superoxides and hydrogen peroxides that potentiate the lipid peroxidation and imparts oxidative stress[2]. Oxidative stress is a biochemical disequilibrium which indicates imbalance between the pre-oxidants and antioxidants which ultimately leads to destabilise the delicate balance among biomolecules like lipids, proteins and DNA at cellular level. It plays a predominant role in disease manifestations like cardiovascular dysfunction, neurodegenerative disorder, hypercholesterolemia, diabetes, etc. [3].

Management of fluoride toxicity in animals is a difficult task, as the effect of fluorosis is irreversible. A number of chemical ameliorative agents like calcium [4], aluminium [5], copper [6] and boron [7] have been tried, with varying degree of success. But some of them also have toxic effects when administered in higher doses or for a prolonged period of time e.g. toxicity of aluminium is amplified in the presence of fluoride ions [8].

India possesses several medicinal plants and some of them have been used by farmers for several purposes [9]. The fruit pulp of *T. indica* has been documented to act as an ameliorative agent in case of experimental and natural cases of fluorosis [10, 11]. More recently the dried leaf powder of *T. indica* has been used to reduce the experimentally induced fluorosis in rabbits [12].

However, protective effect of tamarind on fluoride-induced oxidative stress has been evaluated in experimental animals [2, 13]. but no report on the effects of tamarind leaf powder on oxidative stress in fluorotic cattle is published. Therefore, taking into account of the above facts, the present study was conducted to evaluate theameliorative potential of dried powder of tamarind leaf in endemic fluorosis in cattle.

2. Materials and Methods

2.1 Plant materials

Tender leaves of *Tamarindus indica* were collected from in and around the Bhubaneswar city. The leaves were air dried, grounded to powder with the help of electronic grinder and stored in air tight container. Dried *Tamarindus indica* leaf powder (15g and 30g) was poured into separate zip polythene packet.

2.2 Study site

This study was carried out in five villages located within 2 km radius of the aluminium smelter plant and 3 km radius of the captive power plant in Talcher-Angul industrial complex of Odisha. The present study site is located 133 km away from Bhubaneswar city at latitude 20.83°N and longitude 85.15°.

2.3 Animals and experimental design

Thirty cattle exhibiting chronic sign of fluorosis and ten healthy cattle from non fluorotic area were incorporated in the present study. Fluorotic cattle were divided into three equal groups consisting of 10 cattle each. Group I from fluoride free area served as healthy control. The group II received no treatment and served as disease control. Group III and group IV was supplemented with tamarind leaf powder @15g and 30 g/day with feed for a period of 60 days.

2.4 Collection of blood

Around 10 ml blood from all animals was collected in morning hours by jugular venipuncture. 2 ml of blood was stored in heparinized glass vial (Hi-Media, Mumbai) for preparation of RBC hemolysate. Another 3 ml of blood was stored in EDTA vial (BD Franklin Lakes NJ, USA) for hematological estimation and 5 ml was stored in heparinized glass vial (Hi-Media, Mumbai) for extraction of plasma. The tubes were marked properly and transported to laboratory in an ice box.

2.5 Separation of plasma

Plasma samples were separated from the heparinized blood samples after centrifugation at 3000 rpm for 15 minutes in thermostable refrigerated centrifuge machine (Model 5417R, Eppendorf, Germany) and stored at -20 °C for estimation of biochemical parameters.

2.6 Haemato-biochemical analysis

Haematological and biochemical parameters were estimated as described in previous report [14].

2.7 Oxidative stress indices

Ten percent RBC hemolysate was used for estimation of lipid peroxidase (LPO), superoxide dismultase (SOD) and catalase (CAT) as described previously [14].

2.8 Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) with post-hoc analysis by Duncan's multiple comparison tests using SPSS 16 software. Results were expressed as mean±SE with $p<0.05$ considered statistically significant.

3. Results and Discussion

The haematological parameters of cattle of different experimental groups are shown in the Table 1. Significant decrease ($P\leq0.05$) in haemoglobin percentage, total erythrocyte count, total leukocyte count and packed cell volume was observed in fluorotic cattle (Group II, III and IV) as compared to the healthy cattle (GroupI) on 1st day of the experiment. Significant ($p<0.05$) increase in values of above parameters was observed after supplementation of tamarind leaf powder on 30th and 60th day of the experimental period. Differential leukocyte picture revealed significant ($P\leq0.05$) decrease in neutrophil count whereas there was an increase in eosinophil, monocyte and lymphocyte percentage in cattle of group II, III and IV as compared to the cattle of group I. Significant correction was observed in group III and IV on day 30 and 60 of the experiment.

Biochemical parameters of fluorotic and healthy cattle are given in Table 2. Group II, III and IV cattle revealed significantly higher ($P\leq0.05$) levels of ALP, AST, ALT, blood urea nitrogen and creatinine level as compared to healthy cattle on day 0 of the experiment. Significant amelioration was observed in cattle supplemented with tamarind leaf powder on day 30 and day 60 of the experimental period.

Low level of plasma calcium was observed on day 0 in cattle of group II, III and IV as compared to healthy cattle. Significant correction in plasma calcium was observed in group III and IV on 30 and 60 day of experimental period.

Erythrocytic oxidative stress indices in cattle of different experimental groups are presented in Figure 1-3. Fluoride produces huge oxidative stress over the cells of the affected animal. On the 1st day of the experiment, significant increase ($P\leq0.05$) in lipid peroxidation while decline in catalase and superoxide dismutase activities were observed in cattle of group II, III and IV as compared to healthy cattle. The increase in the activities of catalase and superoxide dismutase was significant whereas that for lipid peroxidation was reduced significantly after supplementation of tamarind leaf to group III and IV at the end of the experiment.

A significantly alterations in haematological parameters viz. Hb, TEC, TLC DC and PCV in fluorotic cattle may be due to reduced erythropoiesis as fluoride intoxication depresses the bone marrow activity [15]. Fluoride-induced disturbances in hematopoietic system in mice and human have been recorded [16]. Decline in heamoglobin percentage might be due to toxic effect of fluoride on the serum level of iron and poor retention of iron [17]. Significant PCV changes in this study might be due to toxic effects of fluoride on the RBC membrane and further shrinkage of cell [18]. In the present investigation, there was increase in Hb, TEC, TLC and PCV value in the treatment groups after 60 days of supplementation of tamarind leaf powder. This might be due to high Fe and antioxidant like polyphenols and flavonoids present in tamarind leaf [19].

Elevated ALP value was observed in fluorotic cattle as compared to cattle from non fluorotic area. This finding was in agreement with many other observations reported in fluorotic animals [18]. Increased fluoride concentration in bones is directly related with increase in serum alkaline phosphatase activity [20]. Since fluoride stimulates osteoblastic activity [21]. the increased activity of alkaline phosphatase can be related to abnormal bone development [20]. Supplementation of dried powder of tamarind leaves to the fluorotic cattle significantly reduced the activity of alkaline phosphatase. The beneficial effect of tamarind on reduction of alkaline phosphatase activity might be due to presence of high calcium content in the dried pulp [22]. The significant increase in plasma ALT and AST might be due to toxic effect of Fluoride compounds [18]. Restoration of enzyme activities in cattle supplemented with tamarind leaf powder is due to

presence of flavonoids, β-carotenes and ascorbic acid in tamarind which have hepato-protective effect due to their anti-oxidant property [23].

Significant rise in the level of urea and creatinine in the fluorotic cattle than non fluorotic cattle was recorded in the present study, which was also reported by Singh and Swarup [24]. Swarup *et al*. [25], and Maiti and Das [18]. Kidneys play an important role as far as the regulation of total body fluoride is concerned. However, if it rises to toxic level then there will be renal dysfunction as various enzyme systems in the kidneys fail to perform. Increased levels of BUN and creatinine level in the affected animals are indicative of degenerative changes in the kidney [24]. This increased level might also be due to catabolism of protein because of partial starvation in affected animals [25].

A significantly lower concentration of calcium was noted in fluorotic cows as compared to healthy animals. This might be attributed to the decrease in absorption as well as higher excretion of calcium in urine [26]. As fluoride is a highly electronegative element with a strong affinity towards electropositive elements, fluoride binds with calcium in the gastro-intestinal tract causing reduction in their absorption resulting in hypocalcaemia [27]. These findings corroborates with earlier workers [18-26] who also reported a decline in serum calcium in fluorotic animals. However, supplementations of tamarind leaf powder to the fluorotic cattle produced a significant increase in calcium concentration. The beneficial effect of tamarind might be due to reduced absorption or an increase in excretion of fluoride from the body.

Fluoride is a major generator of free radicals like superoxides which potentiate the lipid peroxidation and inhibit the anti-oxidative enzymes in liver, kidney, heart, ovary etc. [28]. Among various players that are associated with cellular defence mechanism, super oxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) are important ones which act against the detrimental action of oxygen reactive species. SOD is an enzyme primarily responsible for the conversion of O^{2-} into O2. SOD is also a metallo-protein that enhances the elimination of the primary toxicity of O^{2-} and the secondary toxicity of OH^{-} and H_2O_2 by decreasing the concentration of O^{2-} [28]. Catalase is a haeme containing enzyme which catalyzes the disproportion of H_2O_2 into water and oxygen. This enzyme plays an important role in the removal of H_2O_2 generated by SOD. Stress condition severely affects the concentration of catalase in the body [28]. In this study, a reduced catalase, SOD and increased LPO activity suggested that oxidative stress was produced by fluoride intoxication. Our findings corroborate those of earlier reports of Kant *et al.* [29].

Supplementation of tamarind leaf to fluorotic cattle significantly reduced the erythrocytic lipid peroxidation and increase in activity of SOD and catalase, suggests restoration of oxidative and anti-oxidative balance. There are several conflicting reports with respect to use of different formulations in altering oxidative stress indices [13]. However it could be concluded that the use of *Tamarindus indica* leaf powder could help in restoring haemato-biochemical parameters and maintaining oxidative and anti-oxidative balance.

The results of the present study suggested that dried powder of *Tamarindus indica* leaf has ameliorative potential on the management of fluorosis and fluorosis induced oxidative damage in cattle. However, further study is warranted aimed at the fractionation and identification of the active ingredients of tamarind leaf responsible for amelioration of fluorosis.

Table 1: Haematological parameters of cattle of different groups at different observation period

Parameter	Group	Day 0	Day 30	Day 60
Heamoglobin concentration (g %)	I	11.27 ± 0.04^{B}	11.33 ± 0.07^{C}	11.13 ± 0.17^{B}
	II	9.23 ± 0.20^{A}	9.01 ± 0.14^{A}	9.53 ± 0.14^{A}
	III	9.23 ± 0.11^{aA}	10.10 ± 0.37^{abA}	11.12 ± 0.38^{bB}
	IV	9.11 ± 0.05^{aA}	11.24 ± 0.56^{bC}	11.92 ± 0.53^{bB}
Total leukocyte count (10^3 / mm^3)	I	9668.62 ± 4.37^{B}	9742.61 ± 12.58^{C}	9807.81 ± 47.06^{C}
	II	6776.02 ± 12.42^{A}	6850.01 ± 156.97^{A}	6828.02 ± 123.91^{A}
	III	6230.01 ± 17.00^{aA}	7852.60 ± 175.92^{bB}	8580.20 ± 175.18^{bB}
	IV	6200.01 ± 221.36^{aA}	7434.00 ± 449.29^{bAB}	8142.40 ± 543.93^{bB}
Total erythrocyte count (10^6 / mm^3)	I	5.64 ± 0.01^{B}	5.64 ± 0.07^{D}	5.68 ± 0.03^{C}
	II	3.16 ± 0.03^{A}	3.12 ± 0.04^{A}	3.14 ± 0.05^{A}
	III	3.08 ± 0.03^{aA}	4.23 ± 0.03^{bC}	4.72 ± 0.02^{cB}
	IV	3.13 ± 0.03^{aA}	4.01 ± 0.11^{bB}	4.51 ± 0.12^{cB}
Packed cell volume (%)	I	33.85 ± 0.11^{B}	34.01 ± 0.22^{C}	33.35 ± 0.51^{C}
	II	25.77 ± 0.59^{A}	25.61 ± 0.43^{A}	26.36 ± 0.41^{A}
	III	25.89 ± 0.62^{aA}	31.12 ± 1.18^{bB}	33.96 ± 1.05^{cC}
	IV	24.85 ± 0.72^{aA}	29.28 ± 1.26^{bB}	29.60 ± 0.26^{bB}
Neutrophil (%)	I	38.7 ± 0.98^{B}	37.3 ± 1.39^{C}	38.1 ± 1.36^{C}
	II	16.5 ± 0.51^{A}	16.3 ± 0.97^{A}	16.5 ± 0.75^{A}
	III	16.5 ± 00.4^{aA}	22.6 ± 1.20^{bB}	26.8 ± 1.46^{cB}
	IV	17.3 ± 0.37^{aA}	23.4 ± 0.93^{bB}	28.2 ± 1.07^{cB}
Eosinophil (%)	I	6.5 ± 0.87^{A}	7.5 ± 0.81^{A}	7.3 ± 0.24^{A}
	II	17.1 ± 0.58^{B}	17.1 ± 0.54^{B}	17.3 ± 0.68^{B}
	III	17.3 ± 0.86^{B}	16.4 ± 0.60^{B}	15.8 ± 0.58^{B}
	IV	17.3 ± 0.40^{B}	16.2 ± 0.97^{B}	16.2 ± 0.86^{B}
Monocyte (%)	I	0.5 ± 0.40^{A}	0.5 ± 0.40^{A}	0.5 ± 0.40^{A}
	II	2.3 ± 0.37^{B}	2.3 ± 0.66^{B}	1.9 ± 0.58^{B}
	III	2.3 ± 0.58^{bB}	0.2 ± 0.2^{aA}	0.0 ± 0.0^{aA}
	IV	2.5 ± 0.68^{bB}	0.6 ± 0.4^{aA}	0.2 ± 0.2^{aA}
Lymphocyte (%)	I	54.7 ± 1.20^{A}	55.1 ± 0.70^{A}	54.1 ± 1.30^{A}
	II	64.1 ± 0.548^{B}	64.5 ± 1.16^{C}	64.3 ± 1.02^{C}
	III	64.3 ± 0.51^{bB}	60.8 ± 1.56^{abBC}	57.4 ± 1.33^{aB}
	IV	63.1 ± 0.32^{bB}	59.8 ± 1.16^{bB}	55.4 ± 0.51^{aB}

Group I: Healthy control; Group II: Disease control; Group III: Tamarind treatment 15gm; and Group IV: tamarind treatment 30gm. Values (mean ±S.E.) having no common superscript (small letter in a row and capital letter in a column) differ significantly at $p<0.05$.

Table 2: Biochemical parameters of cattle of different groups at different observation period

Parameter	Group	Day 0	Day 30	Day 60
Alkaline Phosphatase (IU/L)	I	127.58 ± 1.27^{A}	127.78 ± 0.63^{A}	127.58 ± 1.33^{B}
	II	325.95 ± 1.44^{B}	326.48 ± 0.55^{C}	327.05 ± 1.14^{D}
	III	321.62 ± 1.01^{cB}	197.51 ± 2.69^{bB}	139.14 ± 2.09^{aC}
	IV	325.75 ± 1.76^{cB}	182.97 ± 2.16^{bB}	119.21 ± 0.59^{aA}
Aspartate aminotransferease (IU/L)	I	55.26 ± 0.20^{A}	55.038 ± 0.21^{A}	54.88 ± 0.40^{A}
	II	93.48 ± 1.73^{B}	93.57 ± 0.92^{C}	97.24 ± 1.45^{D}
	III	95.95 ± 2.12^{B}	88.04 ± 5.30^{C}	87.10 ± 5.23^{C}
	IV	94.03 ± 3.60^{bB}	76.97 ± 3.13^{aB}	75.51 ± 3.06^{aB}
Blood urea nitrogen (mg/dl)	I	24.17 ± 0.14^{A}	23.76 ± 0.12^{A}	24.65 ± 0.10^{A}
	II	64.57 ± 0.76^{B}	65.36 ± 0.58^{C}	66.78 ± 0.98^{D}
	III	63.65 ± 1.40^{cB}	54.09 ± 1.23^{bB}	48.86 ± 1.14^{aC}
	IV	64.73 ± 1.59^{cB}	53.14 ± 1.29^{bB}	45.09 ± 1.16^{aB}
Creatinine (mg/dl)	I	0.42 ± 0.01^{A}	0.45 ± 0.02^{A}	0.45 ± 0.02^{A}
	II	1.51 ± 0.19^{B}	1.57 ± 0.10^{C}	1.58 ± 0.10^{C}
	III	1.32 ± 0.15^{bB}	0.94 ± 0.003^{aB}	0.87 ± 0.003^{aB}
	IV	1.39 ± 0.10^{bB}	0.88 ± 0.06^{aB}	0.78 ± 0.05^{aB}
Plasma Calcium (mg/dl)	I	10.16 ± 0.24^{C}	10.01 ± 0.13^{D}	10.22 ± 0.40^{D}
	II	6.31 ± 0.81^{A}	7.17 ± 0.74^{A}	6.75 ± 0.57^{A}
	III	$7.1230.64^{aAB}$	7.39 ± 0.66^{bB}	8.09 ± 0.72^{bB}
	IV	7.42 ± 1.03^{aB}	8.45 ± 0.75^{bC}	9.17 ± 0.92^{bC}

Group I: Healthy control; Group II: Disease control; Group III: Tamarind treatment 15gm; and Group IV: tamarind treatment 30gm. Values (mean ±S.E.) having no common superscript (small letter in a row and capital letter in a column) differ significantly at $p<0.05$.

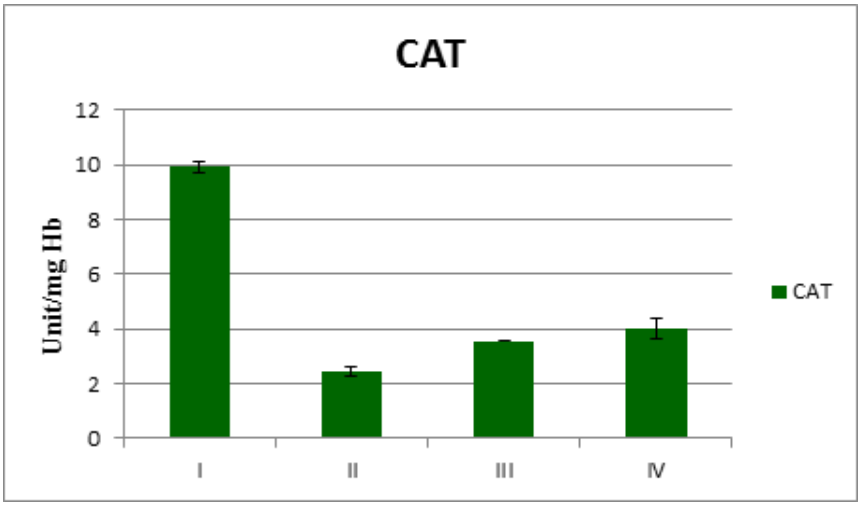

Fig 1: Catalase activity (Units/mg Hb) of different experimental groups on 60 days of the experimental period. Group I: Healthy control; Group II: Disease control; Group III: Tamarind treatment 15gm; and Group IV: tamarind treatment 30gm

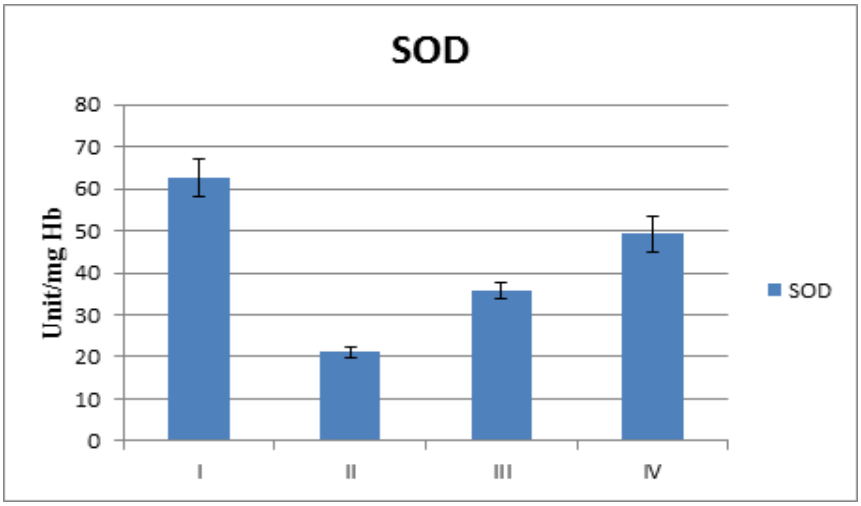

Fig 2: Superoxide Dismultase activity (Units/mg Hb) of different experimental groups on 60 days of the experimental period. Group I: Healthy control; Group II: Disease control; Group III: Tamarind treatment 15gm; and Group IV: tamarind treatment 30gm

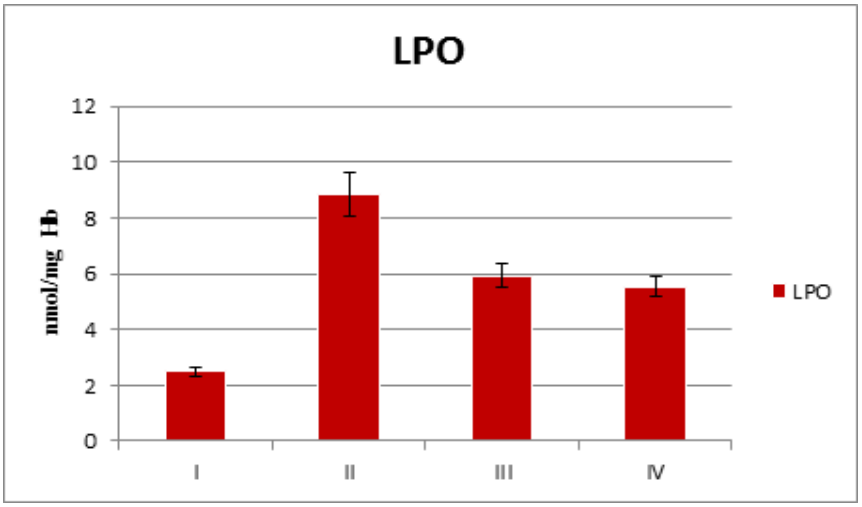

Fig 3: Lipid peroxidase activity (nmol/mg Hb) of different experimental groups on 60 days of the experimental period. Group I: Healthy control; Group II: Disease control; Group III: Tamarind treatment 15gm; and Group IV: tamarind treatment 30gm

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