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Research Article

Protective Effects of Aqueous Extract of Fruit Pulp of *Tamarindus indica* on Motor Activity and Metabolism of the Gastrocnemius Muscle of Rats Treated with Fluoride.

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ABSTRACT

This study reports the protective effects of tamarind ingestion against fluoride induced changes on motor activity and metabolism of Gastrocnemius muscle (GM) of rats. The male Wistar rats were divided into four groups - control, fluoride treated i.p. (20 mg NaF/Kg bw) and Tamarind (Tamarindus indicus III -150mg/kg bw and IV- 300mg/kg bw) + i.p.fluoride (20 mg NaF/Kg bw) groups. The body weight, organo somatic index, motor activity and free radical scavenging enzymes activity in GM-(Superoxide dismutase-SOD, Catalase-CAT, Glutathione transferase-GST, Glutathione peroxidase-GPx,) decreased and Lipid peroxidation-LPO, Xanthine oxidase-XOD levels increased in fluoride treated rats whereas in tamarind+ fluoride treated rats (III and IV) the fluoride toxic effects were reversed to control values. Similarly beneficial effect was observed on metabolic and membrane bound enzymes like, Succinate dehydrogenase (SDH), Lactate dehydrogenase (LDH), Alanine aminotransferase (ALAT), Sodium Potassium ATPase (Na+K+ ATPase) and Acetylcholinesterase (AchE) in gastrocnemius muscle tissue of tamarind ingested rats compared to fluoride treated rats.

This study therefore showed the inhibitory effect of fluoride on motor activity and some enzymes associated with free radical scavenging, metabolism, energy production and membrane transport in GM. The toxic effect was found to be reduced more in the higher dose of tamarind (IV group -300mg/kgbw) than lower dose (III group - 150mg/kgbw). These findings indicate the protective role of tamarind on the gastrocnemius muscle against fluoride toxicity. The efficacy of tamarind fruit pulp could be due to its ability to bind to fluoride making it less available to the organ systems, as well as improve protein synthesis in the body which in turn could be associated to its composition- rich in amino acids and minerals.

Keyword: Tamrindus indica, Oxidative stress, Fluoride (NaF), Gastrocnemius muscle, Motor activity.

INTRODUCTION

Fluoride in small doses (0.5 to 1.0 mg/L) is therapeutic in preventing dental caries[1]. But on prolonged exposure at higher levels through water, food and environment, it tends to accumulate in all the tissues especially those which have affinity towards calcium retention like teeth[2] bones[3] Pineal gland[4] and muscles[5,6]. Accumulated fluoride is reported to cause morphological[7], physiological[8] and biochemical[6] changes in the tissues leading to altered metabolism in them due to the malfunctioning of the enzymes[6], ion channels[9] and receptors[10] associated with them evident by manifestation of changes in physical parameters like deformities of bones and teeth, loss of motor coordination, muscle weakness[8] etc.

Efforts to eliminate or minimize the detrimental effects of fluoride at both invitro and invivo levels have shown positive results. The *invitro* methods involved, membrane processes[11], Coagulation-Precipitation[12], adsorption [13] to mention a few. These methods met with less success as they are pH dependent and also economically not viable. The *invivo* methods involved administration of proteins,[14] amino acids,[15] minerals, vitamins,[16] and plant products,[17] which ameliorated the fluoride effect on the organ systems. One such plant product is tamarind fruit pulp whose administration was reported to reduce the fluoride content in the body by increasing the fluoride excretion in urine[18]. The efficacy of tamarind in the alleviation of oxidative stress in the liver of female rats is reported[19]. But there is no comprehensive study on the efficacy of the aqueous extract of tamarind fruit pulp on locomotory behaviour, metabolic and oxidative stress in the gastrocnemius muscle of fluoride treated rats. Therefore, this study reports the protective effect of aqueous extract of tamarind fruit pulp on motor activity and metabolism of gastrocnemius muscle.

MATERIAL AND METHODS

Tamarindus indica (Tamarind) extract – Tamarind fruits are collected from the farms of Ranga Reddy district of Telangana state. The fruits pods are cleaned off its fibre, seeds, pericarp and washed lightly with distill water. The cleaned fruits are soaked in warm water (double distilled

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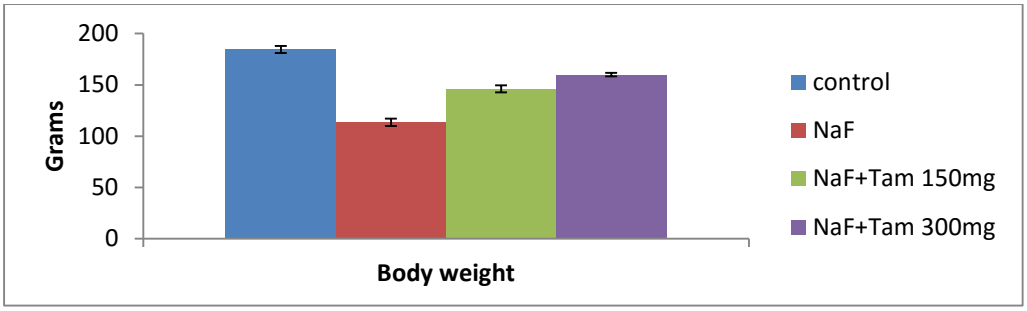

Figure 1: Effect of aqueous extract of tamarind on Bodyweights of fluoride treated rats. Values are mean±S.D. of six animals per group. The values of multiple comparison test were significant ($p<0.05$) among groups I-Control, II-NaF (20mg/kgbw), III-NaF (20mg/kgbw) +Tam (150mg/kgbw) and IV- NaF (20mg/kgbw)+Tam (300mg/kgbw).

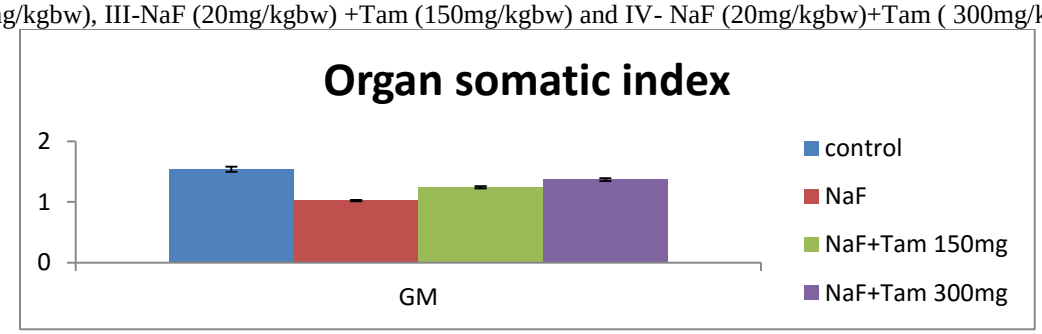

Figure 2: Effect of aqueous extract of tamarind on Organ weights of fluoride treated rats. Values are mean±S.E. of six animals per group. The values of multiple comparison test were significant ($p<0.05$) among groups I-Control, II-NaF (20mg/kgbw), III-NaF (20mg/kgbw) +Tam (150mg/kgbw) and IV- NaF (20mg/kgbw)+Tam (300mg/kgbw).

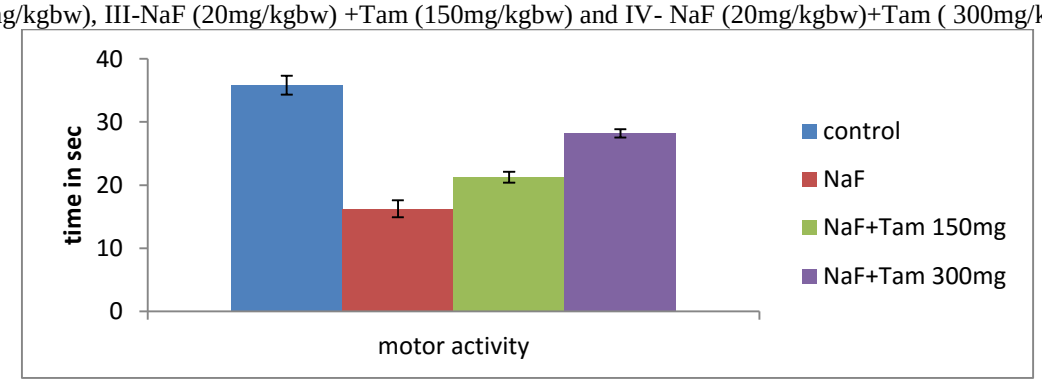

Figure 3: Effect of aqueous extract of tamarind on Motor activity of fluoride treated rats. Values are mean±S.E. of six animals per group. The values of multiple comparison test were significant ($p<0.05$) among groups I-Control, II-NaF (20mg/kgbw), III-NaF (20mg/kgbw) +Tam (150mg/kgbw) and IV- NaF (20mg/kgbw)+Tam (300mg/kgbw).

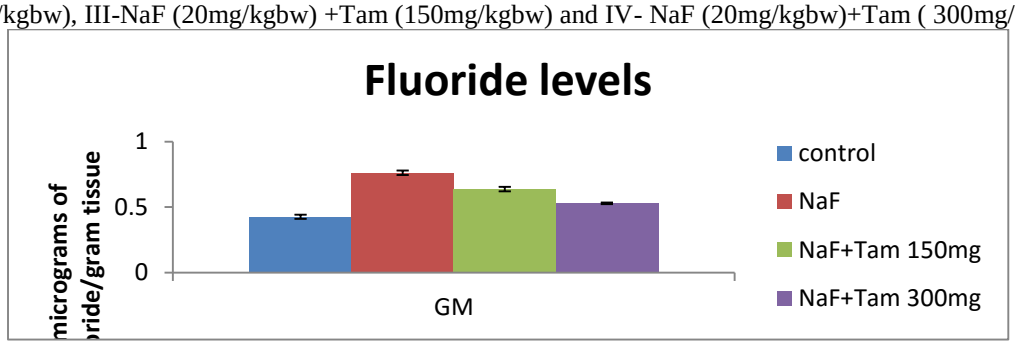

Figure 4: Effect of aqueous extract of tamarind on Fluoride levels of fluoride treated rats. Values are mean±S.E. of six animals per group. The values of multiple comparison test were significant ($p<0.05$) among groups I-Control, II-NaF (20mg/kgbw), III-NaF (20mg/kgbw) +Tam (150mg/kgbw) and IV- NaF (20mg/kgbw)+Tam (300mg/kgbw).

water) and later pulp is extracted which is passed through fine sieve.

Design of experiment

Twenty four male Wistar rats, weighing 185-200gm were obtained from the NIN, Hyderabad, India. The rats were housed in polypropylene cages and were fed a standard pellet diet (Hindustan Lever Ltd, Lipton India, Bangalore) and water supplied ad libitum till the duration of experiment.

The animals were allowed to acclimatize to the laboratory conditions before the commencement of experiment. The rats were divided into 4 groups with 6 animals in each. The

Table 1: Protective effect of aqueous extract of *Tamarindus indica* fruit pulp on antioxidant, metabolic and membrane bound enzymes of gastrocnemius muscle of fluoride treated rats.

Free radical scavenging, metabolic and membrane bound enzymes	Control	NaF(20mg/kgbw)	NaF(20mg/kgbw) + Tam 150mg/kgbw	NaF(20mg/kgbw) + Tam (300mg/kgbw)
LPO[a]	3.75 ± 0.126	6.27 ± 0.100	5.71 ± 0.056	4.32 ± 0.134
XOD[b]	0.403 ± 0.015	0.649 ± 0.003	0.511 ± 0.015	0.452 ± 0.001
SOD[c]	12.19 ± 0.086	6.11 ± 0.179	7.86 ± 0.111	11.07 ± 0.095
CAT[c]	11.87 ± 0.071	6.13 ± 0.066	8.46 ± 0.051	10.55 ± 0.049
GPx[d]	8.87 ± 0.081	3.59 ± 0.117	5.02 ± 0.092	7.76 ± 0.063
GST[e]	32.15 ± 0.180	19.93 ± 0.115	25.21 ± 0.269	28.93 ± 0.093
Ache[f]	1.316 ± 0.0133	0.692 ± 0.013	0.755 ± 0.0131	1.138 ± 0.008
SDH[b]	1.358 ± 0.015	0.509 ± 0.008	0.713 ± 0.006	1.178 ± 0.039
LDH[b]	1.103 ± 0.003	0.529 ± 0.015	0.705 ± 0.004	0.964 ± 0.011
ALAT[b]	13.36 ± 0.197	8.83 ± 0.217	9.78 ± 0.077	11.9 ± 0.248
Na+K+ATPase[g]	133.45 ± 0.69	57.18 ± 1.468	73.79 ± 3.829	114.4 ± 1.516

first group is treated as control in which the animals were injected intraperitoneally (i.p.) with mammalian physiological saline, the second group was administered sodium fluoride (NaF) - (20 mg NaF/Kg bw) through intraperitonial injection and the third and fourth groups were injected sodium fluoride (NaF) i.p. (20 mg NaF/Kg bw) and concomitantly crude aqueous tamarind fruit pulp extract (150mg and 300mg/kg bw respectively) was orally fed by oral intubation. (The doses of NaF 20mg/Kg bw is well below the LD_{50} for rodents, which is reported to be 51.6 mg F/Kg bw/day)[20]. Similarly the dose of tamarind is chosen on the basis of earlier studies by different workers [18,21]. The rats were maintained for 14 days. After 14 days animals were sacrificed by cervical decapitation, Gastrocnemius muscle was dissected out, blotted free of blood and immediately transferred to ice cold conditions and used as per the requirement for each parameter to be studied.

Body weight and Organo-somatic index

The bodyweight of each animal was recorded on every day till day 14 and organ somatic index was also recorded after animal dissection. From these values the Organo-somatic index was calculated by the following formula:

O.S.I = Weight of Tissue (g) / Weight of the body (g) x 100

Estimation of fluoride: Fluoride levels in the tissues of four groups were determined by the Birkel method,[22] with required modifications and are expressed as µg F/g dry tissue. In this method the tissues are homogenized, dried for 24hrs at 105^{o}C in a closed compartment, a weighed 200 mg dry sample was dissolved in 2 mL of a 1 : 1 mixture of 11.6 M perchloric acid and 14.3 M nitric acid and neutralized with citrate buffer to a pH 5.5 (7.8 M sodium hydroxide and 1.0 M trisodium citrate). The sample thus obtained was used after appropriate dilutions for recording the fluoride content on a fluorimeter (Orion R 94-09).

Rota rod performance

Rota rod performance test for motor activity is determined by the method of Jones and Robert,[23] the time of coordination between control and experimental groups (Model Rota rod 4 compartments Dolphin^{TM} instruments) is recorded.

Assay of enzymes

The lipid peroxidation (LPO) level as malodialdehyde (MDA) was estimated by the method of Bhuyan[24] et al., (1981) and expressed as micromoles of MDA/g wet wt of tissue. Xanthine oxidase (XOD) activity was assayed by the method of Govindappa and swami[25], (1965) expressed as micromoles of formazan formed/mg protein/hr. Superoxide dismutase (SOD) activity was assayed according to the method of Marklund and Marklund[26] (1974) and expressed as Units/mg protein/min. Catalase (CAT) activity was measured by the method of Chance[27] et al., (1982) and calculated as Units/mg protein/min. Glutathione peroxidase (GPX) activity was measured by the method of Martinez [28]et al., (1979) and the activity expressed as nanaomoles of NADPH oxidized/mg protein/min. Glutathione transferase (GST) activity was estimated by using the substrate, 1- chloro-2,4-dinitrobenzene (CDNB) by the method of Habig[29] et al., (1974) and the activity is expressed as nanomoles of GS-CDNB formed/mg protein/min.

Acetylcholinesterase activity was estimated by the method of Ellman[30] et al., (1961) and Ache activity was expressed as nanomoles of acetylcholine hydrolyzed/mg protein/min. Succinate dehydrogenase (SDH) enzyme activity and Lactate dehydrogenase (LDH) activity was determined by the method of Nachlas[31] et al., (1960) and the activity expressed as micromoles of formazan formed/mg protein/hr. The activity of alanine amino transferase (ALAT) was assayed by the method of Reitman and Frankel,[32] (1957) and the enzyme activity was expressed as Units/mg protein/min. The $Na^{+}K^{+}$ ATPase activity was assayed according to Kaplay[33] (1978) and the inorganic phosphate was estimated by the method of Taussky and Shorr,[34] (1953) and the enzyme activity was expressed as nanomoles of Pi liberated/mg protein/min.

Statistical analysis was carried out using the one-way ANOVA followed by Duncan and Dunett's multiple comparison test with significance set at $p<0.05$. The analytical data are presented as means ± S.E. of six animals per group.

RESULTS AND DISCUSSION

Fig 1 and 2 ($p<0.05$) show that the animals in the groups II (NaF), III (NaF+Tam-150mg) and IV (NaF+Tam-300mg) showed a significant ($p<0.05$) decrease in bodyweight and Organosomatic index which is in accordance with the earlier studies[6,35] when compared to control group animals. This decrease in weight can be attributed to lower food intake[6,35], degeneration of structure of organs[7] and decreased protein levels[36]. In both the above parameters the NaF + Tam groups (III and IV) showed less reduction in bodyweight and OSI dose dependently in accordance with the earlier studies than NaF group in which administration of minerals and protein rich diet reduced the fluoride toxicity. In the present study the same has been supplied by tamarind pulp which is a rich source of proteins and minerals[37,38].

Fluoride decreased the Rota rod endurance time as seen in Fig 3 ($p<0.05$) in the fluoride treated rats when compared to control group rats. This can be related to the fluoride accumulation in GM leading to the depletion of proteins as was reported in the previous studies[7,36]. The Rota rod endurance time improved in tamarind supplemented groups dose dependently indicating the protective role of tamarind. This could be due to the presence of minerals and vitamins[37,38], in the pulp whose administration individually in earlier reports showed amelioration. Tamarind is reported to have capability of binding the fluoride,[39] making its concentration less in serum and thus making its bioavailability less in the body.

The fluoride levels as seen in fig 4 increased significantly ($p<0.05$) in GM tissues in the NaF group when compared to control and NaF + Tam group (III and IV) of rats. This can be attributed to the fluoride affinity for the calcium[40] which is comparatively more in muscle. The calcium content present in the tamarind supplement might have lead to the binding of fluoride making its bioavailability less in the third and fourth groups as seen in the previous studies where calcium administered lead to the decrease in the fluoride accumulation in the tissues.

The enzyme activities are expressed as: a:Lipid peroxidation (LPO) (MDA-malondialdehyde)-(micromoles of MDA/gm wet wt of tissue), b:Xanthine oxidase (XOD)-Succinate Dehydrogenase (SDH)-Lactate dehydrogenase (LDH)-Alanine amino Transferase(ALAT)-micromoles of formazan formed/mg protein/hr, c: Superoxide dismutase (SOD)-Catalase (CAT)- Units/mg protein/min, d: Glutathione peroxidase (GPx)-nanomoles of NADPH oxidized/mg protein /min, e: Glutathione transferase (GST)-nanomoles of GS-CDNB formed/mg protein/min, f: Acetylcholinesterase (AchE)-nanomoles of acetylcholine hydrolyzed/mg protein/min, g: Sodium potassium ATPase (Na^+K^+ATPase)-nanomoles of Pi liberated/mg protein/min.

The table 1 shows significant ($p<0.05$) increase in the LPO and XOD levels in GM tissues of the NaF group in the present study indicating the damage to the tissues and generation of free radicals (ROS) as reported by earlier studies[41]. The tamarind supplemented groups showed remarkable recovery in both the parameters (LPO and XOD). This could be attributed to the binding capacity of fluoride to tamarind and anti lipoperoxidative property of the tamarind[42,43,44].

A significant ($p<0.05$) decrease in the free radical scavenging or antioxidant enzymes (SOD, CAT, GPx, GST) is seen table 1 in the NaF group as reported in previous studies[6,35,41], indicating the exhaustive attempt of the enzymes to decrease the free radicals generated by fluoride. The tamarind fruit pulp seems to decrease the intensity of fluoride toxicity by increasing the nutritional status of the body which is one of the determining factor in the manifestation of fluorosis[45] (Susheela and Bhatnagar, 2002). The antioxidant enzymes showed significant improvement dose dependently in their levels in the tamarind treated groups (III and IV) which can be correlated with the antioxidant property of the tamarind [19,43,44].

Acetylcholinesterase activity decreased drastically as seen in **table 1** ($p<0.05$) in the gastrocnemius muscle of NaF group corroborating with the fact of fluoride accumulation leading to the reduction in the activity of AchE in the neuromuscular junction as reported in the earlier studies[46,47]. This decrease of AchE can be related to the decreased Rota rod activity observed in the present study. The reason can be attributed to the alterations developed in the histology of the muscle[7] due to fluoride accumulation leading to the reduction in the motor activity along with AchE inhibition in accordance with the previous studies[6,8]. The AchE activity was not affected to the extent found in the NaF group in the tamarind supplemented groups. This could be due to the nutrient rich composition of the tamarind pulp which supplies the amino acids, minerals as well as proteins whose supplementation,[14,15,16] proved to ameliorate fluoride toxicity by improving protein levels in the body.

As seen in table 1 a significant ($p<0.05$) reduction in the SDH activity was observed in the GM of NaF group in accordance with the earlier studies[6,46]. This may be due to the inhibitory effect of fluoride on the respiratory chain enzymes[48]. The results suggest reduction of energy production and this may lead the cell to utilize alternative pathway for energy. Tamarind supplemented groups (III and IV) showed significant recovery in the functioning of the kreb cycle enzymes. The minerals, amino acids, flavanoids and polyphenols of the tamarind pulp seem to extend a protective role to the kreb cycle enzymes as reported in earlier studies[15,16].

LDH activity in the GM of NaF group rats decreased significantly ($p<0.05$) in the present study as reported in previous study[6]. The reduction of LDH level may be due to the inhibitory effect of fluoride on the glycolytic enzymes[49]. The tamarind administered groups of rats showed remarkable improvement in the LDH activity. This could be attributed to the radical free environment in the cell by the antioxidant enzymes which regained their potency in the presence of nutrient rich pulp of tamarind[19,43,44] The Na^+K^+ATPase activity reduced drastically in the GM tissues of NaF group of rats as observed in table 1 ($p<0.05$). It has been reported that fluoride inhibits the Na^+K^+ATPase enzyme[9,46] . This can be corroborated with the fluoride causing lipid

peroxidation leading to the damage of the cell membranes. Thus the enzymes, ion channels and receptors associated with the membranes are also affected[6,9,10]. The significant inhibition of Na^+K^+ATPase by NaF might be another causative factor for the change in neuromuscular metabolism leading to its dysfunction in NaF rats along with inhibition of AchE which is observed in the present study. The Tamarind administered groups (III and IV) along with NaF shows marked improvement in the Na^+K^+ATPase activity indicating the maintenance of the cell membrane's integrity, fluidity and enzymes. This can be attributed to the anti lipoperoxidant property of the tamarind[46,47].

Table 1 ($p<0.05$) signifies a marked decrease in the ALAT activity in the GM of NaF group in accordance with the earlier findings[6,36]. This can be related with protein breakdown and damage to the cell membrane and thus it signifies loss of the cytosolic enzymes leading to their decrease in the cell. This is in correlation with the concomitant increase in the levels of ALAT in the serum as was observed in the previous study[6]. The ALAT activity improved in the tamarind supplemented group of rats (III and IV), indicating its protective role against fluoride toxicity[19]. This may be due to tamarind fruit pulps rich composition of nutrients as well as its antilipoperoxidant and antioxidant property[42,43,44]

CONCLUSION

The results obtained in the tamarind supplemented (NaF+Tam) III and IV groups indicate that the tamarind possesses the potential of amelioration against the metabolic and oxidative stress caused by the fluoride toxicity. A marked improvement was also exhibited in the locomotory behavior in the tamarind administered rats indicating its protective role on the gastrocnemius muscle tissue. The benefit observed against the fluoride toxicity in the gastrocnemius muscle was more pronounced in the IV-fourth group (Tam-300 mg/kgbw) than the III-third group (Tam-150 mg/kgbw). This property
exhibited by the tamarind may be attributed to fruit pulps' rich composition of proteins, minerals, amino acids and flavanoids and also may be due to the binding capacity of fluoride to the tamarind.

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