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Triphala-An Excellent Antioxidant in Mitigating Fluoride Endocrine Toxicity

Zoology

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Dhara D. Vyas	Department of Zoology, University School of Sciences, Gujarat University, Ahmedabad 380 009, India.
Rajendra N. Bhatt	Department of Zoology, University School of Sciences, Gujarat University, Ahmedabad 380 009, India.
Raveendra B. Meda	Department of Zoology, University School of Sciences, Gujarat University, Ahmedabad 380 009, India.
Mandava V. Rao	Department of Zoology, University School of Sciences, Gujarat University, Ahmedabad 380 009, India.

ABSTRACT

Sodium fluoride (NaF) at a dose of 10 mg/kg body weight was administered orally to female rats daily for 30 days to evaluate its affect on thyroid functions in relation to oxidative stress in rats. The parameters studied were gravimetric and biochemical indices in endocrine tissue of fluoride fed rats. Treatment brought about an alteration in body and organ weights followed by biochemical indices. Alterations in the antioxidant indices in the thyroid tissue were confirmed by increased lipid peroxidation (LPO) along with decrements in other antioxidant indices such as superoxide dismutase (SOD) and catalase (CAT) levels affecting its internal milieu in fluoride intoxicated rats. Thyroid hormone levels Tri-iodothyronine (T3), Thyroxine (T4) and TSH were also affected. Supplementation of antioxidant, triphala (30 mg/kg body weight) to treated animals, revealed recovery in these endocrine organ functions due to its probable protective role. Thus, triphala mitigated NaF induced endocrine toxicity in a rat model, as it has good antioxidant properties.

INTRODUCTION

An increase in the concentration of toxic pollutants in the biosphere and their ultimate entry into the biological systems will pose grave problems on human, natural resources and also on the ecological balance. Jacks et al.1 revealed that the presence of fluorine in ground water is mainly a natural phenomenon and is mainly influenced by local and regional conditions. In short, fluorine is the most reactive element known to science. It is ninth element of periodic table, belongs to the group VII B with atomic weight 18.9984, which was isolated by Henri Moisson. It is widely dispersed in the environment accounting for 0.3 g/kg of the earth's crust.2 Investigations have shown that fluoride affects not only bones and the skeleton, but also the muscles,3 gastro-intestinal systems,4 erythrocytes,5,6 endocrine glands7,8 and vital organs.9-12 Fluoride toxicity is not confined to the bone and dental tissues alone, but involves more than one endocrine organ and is evident in adults as well as children. Alterations in hormonal profiles are now believed to be related to chronic exposure to environmental fluoride. As antioxidant Triphala has affinity to increase the antioxidant status significantly of animals which might have contributed to the chemoprevention.13 Triphala is a traditional Ayurvedic herbal formulation, gallic acid as a major ingredient. Sabu and Kuttan14 reported that methanolic extract (75%) of Terminalia chebula, Terminalia belerica, Emblica officinalis and their combination named 'Triphala' are being used extensively in Indian system of medicine. They were found to inhibit lipid peroxide formation and to scavenge hydroxyl and superoxide radicals in vitro. The objective of the study is to investigate the effect of fluoride and anti oxidative activity of triphala on NaF induced toxicity in thyroid of adult rats.

METHODS AND MATERIALS

Animals*:* Healthy adult female Wistar rats (*Rattus norvegicus*) weighing between 230-250 g were obtained from zydus Life sciences, Ahmedabad, India, under the Animal Maintenance and Registration No. 167/PO/C/99/ CPCSEA, from the Ministry of Social Justice and Empowerment, Government of India Committee for the purpose of Control and Supervision of Experiments on Animals, Chennai, India. The Rats were acclimatized for fifteen days prior to the commencement of the treatment and were housed in an air-conditioned animal house at 26±2oC with exposure to 10–12 hr of daylight at a relative humidity of 30–70%. They were fed a standard rat chow and were given water (0.6–1.0 ppm F) *ad libitum*.

Experimental design: After a 15-day adaptation period, the animals were divided into five different groups (Table 1) of 15 each and caged separately. Based on our earlier studies,12 the following doses were given for 30 days. Group I (control) rats were maintained on standard diet. Group II was treated with *Triphala* alone (30mg/kg bw) orally. Group III was administered a dose of NaF (10mg/kg bw) orally. Group IV was given 10 mg/kg bw dose of NaF along with *Triphala* 30mg/kg bw orally.

At the end of the 30-day treatments, on the 31 day the rats were weighed on an animal weighing balance (Ohaus, USA) and sacrificed by cervical dislocation. The thyroid gland was dissected out carefully, blotted free of blood, weighed to the nearest milligram, and used for the estimation of Lipid peroxidation, Superoxide Dismutase (SOD, E.C.1.1.15.11) and Catalase (CAT, E.C.1.11.1.6), by using the method of Ohkawa et al.,15 Kakkar et al.,16 Sinha,17 respectively. For estimation of T3, T4, TSH in serum, blood was collected by cardiac puncture, and the serum was separated and used. Activities of serum T3, T4, TSH were assayed by the method of Rongen et al.18

Table 1. Experimental Protocol

Group	Treatment and daily dose (15 rats in each group	Duration (days)	Day of autopsy
I	Untreated control	-	Sacrificed with treated
II	Triphala alone (30 mg/kg bw, orally)	30	31st
III	NaF (10 mg/kg bw, orally)	30	31st
IV	NaF treated (10 mg/kg bw, orally) + Triphala (30 mg/kg bw, orally)	30	31st

Statistical analysis: For all biochemical parameters, a minimum of 6–8 replicates were performed. Data are presented as mean ± SEM. One-way analysis of variance (ANOVA) with Tukey's significant difference post hoc test was used to compare differences among groups. Data were analyzed statistically by Graph Pad Prism 5.0 statistical software. P values <0.05 were considered significant.

RESULTS

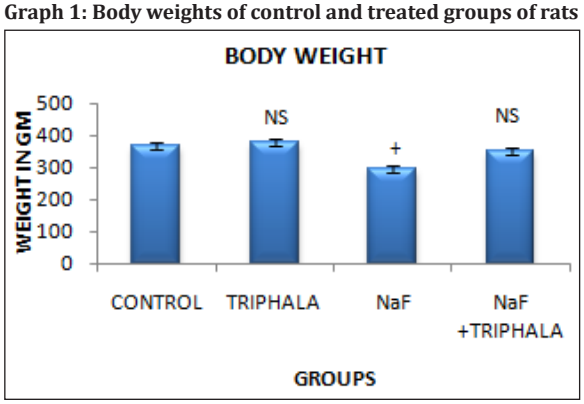
Body and organ weights: Body and Organ weights of the rats treated with NaF (Group III) was significantly ($p<0.001$) decreased as compared to the control animals (Group I) and the

animals administered Triphala alone (Group II). Combined group (NaF + Tiphala, Group IV) did not show any significant changes (Graph 1, 2).

Antioxidant indices: Antioxidant indices in thyroid fall extensively in NaF treated group animals. Antioxidant enzymes like SOD and CAT activities were declined significantly ($p<0.001$) in group III as compared to control group I. Moreover, NaF treatment also produced a marked elevated levels of lipid peroxidation as compared to the control group (I). Administration of Triphala along with NaF-treated (Group IV) rats expressed no differences in anti-oxidant indices as compared to control, and no change in Triphala alone treated groups. Serum T3, T4 levels were decresed ($p<0.001$) significantly whereas serum TSH levels were increased following NaF treatment as compared to the control group (Table 2).

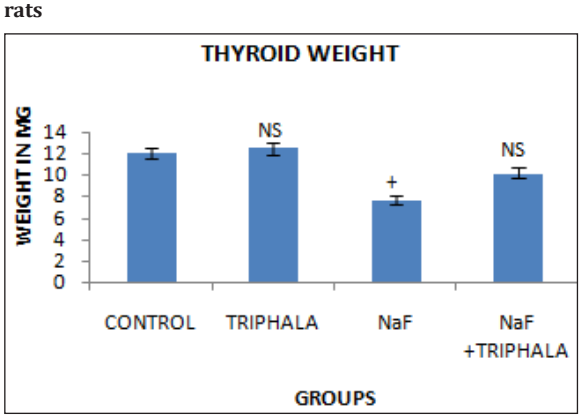
As with the body and organ weights, the above-mentioned parameters were essentially unchanged in the Group II rats treated with triphala alone. Similarly, pretreatment with NaF+Triphala treated Group IV rats revealed no significant changes in these indices compared to that of control Group I.

Graph 1: Body weights of control and treated groups of rats



Values are Mean± S.E., NS = Non significant;
* P<0.05; ** P<0.01; + P<0.001

Graph 2: Thyroid weights of control and treated groups of rats



Values are Mean± S.E., NS = Non significant;
* P<0.05; ** P<0.01; + P<0.001

Table 2. Biochemical parameters of control and experimental groups.

Parameter	Control (G-I)	Triphala (G-Ii)	Naf (G-Iii)	Naf +Triphala (G-Iv)
TBARSa	17.36 ±0.38	17.66 ±0.51NS	32.86 ±0.67+	17.14 ±0.72NS
Catalasec	7.30 ±0.29	7.52 ±0.59NS	4.889 ±0.43+	6.824 ±0.68NS
Superoxide dismutaseb	1.657 ±0.05	1.863 ±0.07NS	0.761 ±0.031+	1.624 ±0.22NS
T3d	115.4 ±5.0	121.0 ±3.84NS	87.75 ±2.34+	109.2 ±5.18NS
T4e	4.80 ±0.13	4.94 ±0.27NS	3.18 ±0.16+	4.59 ±0.21NS
TSHf	1.10 ±0.02	1.11 ±0.04NS	2.09 ±0.07+	1.51 ±0.11NS

NS = Non Significant, *= P<0.05, **=P<0.01, += P<0.001
(Groups II toIV compared with control group I), values are Mean ± S.E, a=n moles of MDA formed/100mg tissue weight, b= units/ mg protein, c=µmoles of H_2O2 consumed/min/mg protein, d= µmoles/ 100mg tissue weight, e=pg/ml, f=ng/dl , g=mIU.

DISCUSSION

The body and organ weight were showing significant reduction in the NaF treated rats. In support of our findings other researchers also reported decreased body weight in the animals treated with different doses of fluoride, which is attributed to decreased food intake and reduction in protein levels.[19]

The antioxidant enzyme plays an important role in protecting biological tissues from the harmful effects of reactive oxygen species (ROS).20 These enzymes and non-enzyme components are mutually supportive team of defense against these ROS. In the present study, oxidative stress induced by fluoride as revealed a significant decline in levels of SOD, CAT followed by elevated level of lipid peroxidation, affected thyroid function. Triphala is known to protect against extensive oxidative damage in the case of a harmful action of some metals on the thyroid, this might be due to the free radical and hydroxyl scavenging activity. Moreover, Triphala has powerful antioxidant vitamin C, acts not only to prevent toxicity but also works as a detoxification medicine**21,22** Its presence in excess *in vivo* could scavenge the electrophilic moieties produced by toxic chemicals and conjugate them to less toxic products.**23**

The principal hormones are being T_3 and T_4 in serum. Serum T_3, T_4 levels were decreased significantly following NaF treatment and in contrast serum TSH levels were increased as compared to the control group, which are in support with the earlier results of Zhan et al.,24 who reported reduced levels of serum T_3 and T_4 in young pigs fed with 100, 250, and 400 mg fluoride/ Kg diet. Trabelsi et al.25 also reported a significant decrease in the plasma free T_4 level in 14-day-old mice whose mothers had been treated with 0.5 g NaF/L in drinking water. Kahl and Bobek,26 found a significant reduction of protein bound iodine as well as an overall reduction of iodine and a reduction of iodine uptake by the thyroid gland in fluoride treated rats. Other reports in the literature suggest that tyrosine and its metabolite levels were also influenced by fluoride. In fact, increased urinary loss of tyrosine is known to occur in men living in high-fluoride areas and in monkeys receiving low daily doses of fluoride.27 Thyroid hormones control the body's entire oxidant/antioxidant system and fluoride may deiodinase directly as a thyroid stimulating hormone (TSH) analogue since TSH levels directly correlate with malondialdehyde activity.25 Perhaps, changes in the thyroid hormone levels in the present study might have imbalanced the oxidant/antioxidant system.28

Triphala has been reported to contain several active ingredients like gallic acid, chebulagic acid, and chebulinic acid and several compounds that have been proposed to be responsible for its claimed health benefits.29 The synergistic activity of these reported antioxidants may be responsible for the protective effects shown against fluoride-induced reduction in antioxidant indices.30 The protection offered by triphala may be attributed to the combined effects of various constituents rather than to any single component.

In conclusion, this study has showed that the polyphenolic compound. Triphala, exert impressive protection against thyroid dysfunction in rats induced by F- in their drinking water. By implication, these results indicate that these polyphenolic compounds might have therapeutic value in human clinical studies. In any event, further studies are clearly desirable.

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