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In Vivo Ameliorative Effect of Amla Extract on Fluoride Induced Oxidative Stress on Thyroid Gland in Rats

KEYWORDS: Ameliorative effect, Amla extract, Fluoride, Thyroid dysfunction, Rats

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ABSTRACT *The present study was an attempt to investigate beneficial role of Amla fruit extract on fluoride (F-) induced thyrotoxicity in the rats. The adult male rats were administered 5 (L.D) and 10 (H.D) mg/kg b.w of fluoride orally for 60 days with and without aqueous extract of Amla at a dosage of 20 mg/kg b.w. Amla effectively countered toxic effect of Sodium fluoride (NaF). Compared to NaF alone, combine treatment prevented the NaF induced reduction in body and organ weights as well as decreased antioxidative indices and energy metabolites and mitigation in these thyroid effects was observed in the Amla+NaF fed groups. Thus, excessive F- administration induced thyroid dysfunction in rats; which is mitigated by Amla extract supplementation due to its anti-stress effect.*

INTRODUCTION

Modern drugs can be life savers but they often have too many side effects to warrant their use for every little sniffle. Herbal remedies tend to heal without suppressing symptoms, and used in the correct dosage are perfectly safe and have no side effects. Amla is normally known as Indian gooseberry, has been used extensively in the ancient Indian Ayurveda as a potent rasayana i.e. a herbal formulation that helps attain longevity and rejuvenation.[1,2]

As a potent protoplasmic poison, Fluoride is toxic to living cells, generating reactive free radicals and causing destructive biochemical alterations including oxidative stress in a variety of animal species.[3,4,5,6,7] In animals, various changes occur after chronic administration of F^- including inhibition of pineal function, membrane-bound ion transport, neurotransmission, and adverse changes in enzymatic activities of different organs and balance of blood electrolytes.[8,9] Excess intake of F^- causes fluorosis, a slow progressive degenerative disorder.[10]

The thyroid gland produces hormones which control our metabolism-the rate at which we burn our fuel and its hormones are necessary for maturation in the postnatal animal, particularly for the central and peripheral nervous systems[11,12] and the skeleton.[13,14] Reports are available that certain antioxidants are able to ameliorate F-induced oxidative stress in rats.[15,16,17,18] However, the effects of Embilica officinalis in ameliorating F-induced biochemical changes remained to be determined in thyroid gland, and here we report the results of our investigation of their effects on thyroid histology and biochemical parameters in rats.

MATERIALS AND METHODS

Animals: Healthy adult male Wistar rats (*Rattus norvegicus*) weighing between 200-250 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 15 days prior to the commencement of the treatment and were housed in an air-conditioned animal house at 26±2°C 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*.

Exposure: The rats were divided into five groups of 10 each with a 60-day treatment period for each group. Group I served as control; Group II rats were given Amla aqueous extract at a dose of 20 mg/ kg bw/day. Sodium fluoride, NaF (Qualigens Fine Chemical, Mumbai, 99% purity) was administered orally (5 and 10 mg/kg bw/day) with a feeding tube attached to a hypodermic syringe to the rats in Group III and IV. Group V rats were treated Amla aqueous extract (20 mg/kg bw/day), and NaF (10 mg/kg bw/day) was administered orally. At the end of the 60-day treatments, on the 61 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 Total Protein, Lipid peroxidation (LPO), Superoxide Dismutase (SOD, E.C.1.1.15.11), Catalase (CAT, E.C.1.11.1.6), and Glutathione (GSH) by using the method of Lowry et al.,[19] Ohkawa et al.,[20] Kakkar et al.,[21] Sinha, [22] Ellman,[23] respectively.

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, IV) were significantly ($p<0.001$) decreased as compared to the control animals (Group I) and the animals administered Amla alone (Group II). Combined group (Amla + NaF, Group V) did not show any significant changes.

Antioxidant indices: Antioxidant indices in thyroid fall extensively in NaF treated animals. Antioxidant enzymes i.e. SOD and CAT activity were declined in these groups. Decrements were also seen in non-enzymatic antioxidants, like GSH levels by NaF treatment. Moreover, NaF treatment also produced a marked elevated levels of lipid peroxidation and glutathione (GSH) as compared to the control group (I). Administration of Amla along with NaF-treated (Group V) rats expressed no differences in anti-oxidant indices as compared to control, and Amla alone treated groups.

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As with the body and organ weights, the above-mentioned parameters were essentially unchanged in the Group II rat treated with Amla alone. Similarly, pretreatment with Amla of the Group V Amla+NaF treated rats revealed no significant changes in these indices as compared to the controls in Group I.

Table 1: Body and organ weights of control and experimental groups.

PARAMETER	CONTROL (G-I)	AMLA (G-II)	NaF		AMLA+ H.D. (G-V)
			L.D. (G-III)	H.D. (G-IV)	
Total Body Weight (gm)	367± 12.27	379± 10.20^{NS}	306± 8.41^{4**}	284± 9.67^{+}	353± 8.91^{NS}
Thyroid (mg)	11.68± 0.54	12.43± 0.67^{NS}	9.58± 0.39^{+}	8.56± 0.46^{+}	11.65± 0.71^{NS}

NS = Non Significant, *= P<0.05, **= P<0.01, += P<0.001 (Groups II to V compared with control group I), values are Mean ± S.E, G=Group

Table 2: Antioxidant indices of control and experimental groups.

PARAMETER	CONTROL (G-I)	AMLA (G-II)	NaF		AMLA+ H.D. (G-V)
			L.D. (G-III)	H.D. (G-IV)	
LPO[a]	19.57 ±0.46	19.89 ±0.62^{NS}	30.47 ±0.84^{+}	35.47 ±0.98^{+}	19.05 ±0.84^{NS}
Superoxide dismutase[b]	1.884 ±0.06	2.063 ±0.09^{NS}	1.575 ±0.12^{+}	0.8767 ±0.042^{+}	1.635 ±0.11^{NS}
Catalase[c]	8.297 ±0.36	8.640 ±0.70^{NS}	5.115 ±0.44**	3.968 ±0.38^{+}	7.067 ±0.38*
Glutathione[d]	32.80 ±0.58	33.23 ±0.71^{NS}	29.01 ±0.82^{+}	20.83 ±1.0^{+}	30.28 ±0.31^{NS}

NS = Non Significant, *= P<0.05, **=P<0.01, += P<0.001 (Groups II to V 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, G=Group

DISCUSSION

The significant reduction observed in the body and organ weight of the NaF treated rats in this study is consistent with earlier results.[24,25] Bharti and Shrivastava[26] also reported decreased body weight in the animals treated with different doses of fluoride, it is attributed to decreased food intake and reduction in protein levels. These results further demonstrated a significant decline in the total protein levels in the thyroid after NaF exposure, this reduction that could be due to impaired protein synthesis caused by fluoride.[27]

The antioxidant indicators plays an important role in protecting biological tissues from the harmful effects of reactive oxygen species (ROS).[28] These enzymes and non-enzymes components are mutually supportive team of defense against these ROS. Oxidative stress results due to loss of the balance between antioxidant system and ROS. In our investigation, F^- induced oxidative stress as revealed a significant decline in levels of CAT, SOD, and GSH levels followed by elevated level of LPO, affecting thyroid function. Amla is known as powerful antioxidant to protect against oxidative damage in the case of a harmful action of some metals on the thyroid,[29] this might be due to its free radical and hydroxyl scavenging activity. Furthermore, Sharma et al.[30] and Linder[31] also documented that amla acts as to prevent toxicity as well as a detoxification drug. Glutathione peroxidase is one of the key enzymes of antioxidant defense and its activity in cells of various organs is stimulated by Amla. It increases hepatic GSH, GPX and ascorbic acid contents. They represent an important defense mechanism in protecting cells against oxygen free radicals. This substance has further been demonstrated to activate the antioxidative enzymes such as CAT and GPx.[32,33]

Amla has been reported to contain several active ingredients. Amla contains ascorbic acid, and on dehydration it was reduced by 40%. The presence of tannins, trigallolyl glucose, flavonoids, polyphenols like Embilicanin A and Embilicanin B, ellagic acid, and phyllemblic acid has been reported in Amla fruit.[34] The synergistic activity of these reported antioxidants may be responsible for the protective effects shown against fluoride-induced amelioration in antioxidant indices.

In conclusion, this study has showed that the polyphenolic compound, Amla exert notable shield against thyroid dysfunction in rats induced by F^- in their drinking water.[35] These results indicate an infiltration that these polyphenolic compounds might have therapeutic value in clinical trails to mitigate fluoride toxicity.

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