ALLEVIATORY EFFECTS OF HYDROALCOHOLIC EXTRACT OF CAULIFLOWER (*BRASSICA OLERACEA* VAR. *BOTRYTIS*) ON THYROID FUNCTION IN FLUORIDE INTOXICATED RATS

Mitta Raghavendra,[a,*] Reddy Kandula Ravindra,[b] Yadav Pulala Raghuveer,[c]
Jayaveera Korlakunta Narasimha,[d] Maheswara Rao Vattikuti Uma,[a]
Polu Navakishor[a]

Hyderabad, Kurnool, and Ananthapuramu, India

ABSTRACT: Fluorosis is a progressive degenerative disorder. Excess intake of fluoride (F) affects the functioning of the thyroid gland. The present study was conducted to evaluate the alleviatory effect of a hydroalcoholic extract of leaves of the cauliflower plant on thyroid function in sodium fluoride (NaF) intoxicated rats. Thirty-six male Wistar albino rats were divided into six groups of six animals in each: Group I served as the normal control; Group II served as the toxic control; Groups III, IV, and V served as treatment groups and received extract at three doses 100 mg, 200 mg, and 400 mg per kg body weight respectively; and Group VI served as the plant control and received a hydroalcoholic extract of leaves of cauliflower plant in a dose of 400 mg per kg body weight. All groups except I and VI received NaF (100 ppm) through drinking water for 30 days. After completion of the study, the animals were fasted overnight, blood was collected by retro-orbital puncture, and the serum levels of thyroxine (T_4) and triiodothyronine (T_3) were measured by radioimmunoassay. Compared to the toxic control group, the administration of the 100 and 200 mg doses of the hydroalcoholic extract of cauliflower leaves resulted significant alleviatory reductions in the levels of total T_4 and the administration of the 200 and 400 mg doses of the extract resulted in significant alleviatory reductions in the levels of total T_3.

Keywords: Cauliflower plant (*Brassica oleracea* var. *botrytis*); Sodium fluoride; Thyroid hormones.

INTRODUCTION

The thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4) are tyrosine-based hormones produced by the follicular cells of the thyroid gland and are regulated by thyroid-stimulating hormone (TSH) made by the thyrotropes of the anterior pituitary gland (adenohypophysis). Both the hormones are essential for the proper development and differentiation of all the cells of the human body, and the regulation of protein, fat, and carbohydrate metabolism. Thyroid hormones increase heart rate, cardiac contractility, and cardiac output. They also promote vasodilation, which leads to enhanced blood flow to many organs. In addition, thyroid also produces calcitonin, which plays a vital role in calcium (Ca^{2+}), and phosphorus metabolism.[1]

Fluorine is a highly reactive element which does not exist in a free form in nature but instead is commonly present as the fluoride ion (F). The main sources of F intake include drinking water, soil, food, dental products, industrial dust, and smoke. It is not an essential trace element and is not required for the healthy development of teeth or bones. Since the late 1940s the use of fluorides for the

[a]Department of Pharmacology, Ch Malla Reddy College of Pharmacy, Hyderabad–501401, India; [b]Creative Educational Society's College of Pharmacy, Kurnool–518218, India; [c]Department of Biotechnology, Rayalaseema University, Kurnool-518002, India; [d]Department of Chemistry, Jawaharlal Nehru Technological University Anantapur, Ananthapuramu–515002, India; *For correspondence: M Raghavendra, Assistant Professor, Department of Pharmacology, Ch Malla Reddy College of Pharmacy, Hyderabad–501401, India; E-mail: mittargv@gmail.com;

prevention of dental caries—especially the adjustment of the fluoride content of drinking water—has been a subject of considerable controversy.[2] In higher doses fluoride may cause dental, skeletal, and nonskeletal fluorosis in both human beings [3-6] and domestic animals.[7-10] Experimentally, it has also been observed and reported that chronic exposure to a relatively low level of F causes adverse changes in diverse soft tissues and organs including thyroid dysfunction.[11]

Globally, many plant based nutritional supplements and foods are recognized for having therapeutic effects on some chronic and degenerative diseases with fewer side effects than some synthetic medications. Many nutritionally rich plants have the potential for ameliorating F-induced toxicity.[12-20]

Cauliflower (*Brassica oleracea* var. *botrytis*) is one of the economical, nutritionally rich plants and contains flavonoids and polyphenolic compounds. Its claimed properties include anticancer, antiobesity, antioxidant, anti-inflammatory agent, and hepatoprotective effects.[21] No previous studies have been done of the possible protective effects of cauliflower leaves on NaF-induced toxicity. In the present study, we investigated the effect of a hydroalcoholic extract of cauliflower leaves on F-induced thyroid gland dysfunction.

MATERIALS AND METHODS

Collection and authentication of plant material: Fresh leaves of cauliflower were collected from a local market in Kadapa, Kadapa district, Andhra Pradesh, India, during the month of June, 2014. The plant material was authenticated by the Chief Scientist, Raw Material Herbarium and Museum, Delhi, and a certified specimen was stored in the Department of Pharmacognosy, Ch. Malla Reddy College of Pharmacy, Hyderabad, India.

Preparation of extract: Initially, the freshly collected leaves were cleaned and shade dried. After drying, the leaves were milled into a coarse powder by a mechanical grinder (sieve no: 10/44). The coarse powder was extracted with hydro-alcohol (30:70) by using a maceration process. The solvent was removed under reduced pressure to get a semisolid residue (yield 31.4% w/w). The extract was stored in an airtight container at 4º C for further studies.

Animals: Male Wistar rats weighing 200–250 g were used for the study. The animals were obtained from Sai Tirumala Enterprises Pvt Ltd, Hyderabad, India. The animals were randomly allocated to the treatment groups in polypropylene cages with paddy husk as bedding. The animals were housed at a temperature of 24±2ºC. A 12:12 hr light:dark cycle was followed. They were fed with standard commercial pellets (containing total carbohydrates 54%, crude protein 19.96%, crude fat 3.73%, crude fiber 3.50%, calcium 1.44%, and phosphorus 0.81%) rat chow, and water *ad libitum* during the experiment. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidelines of the IAEC (IAEC no: CPCSSEA/1657/IAEC/CMRCP/PhD-14/35).

Experimental design: The dose of NaF was selected based on the previous studies,[14] and after ten days of adaptation period, thirty-six animals were divided into six groups of six animals each. Group I served as a normal control and

received drinking water. Group II served as a toxic control and received NaF (100 ppm) through drinking water for 30 days. Groups III, IV, and V served as the treatment groups and received the cauliflower leaves extract (100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively) for 30 days and NaF administered through drinking water. Group VI served as a plant control and received cauliflower leaves extract alone (400 mg/kg) once daily for 30 days. After the treatment schedule, animals were fasted overnight and blood, collected by retro-orbital puncture, was allowed to clot for approximately 1 hr at room temperature and then centrifuged at 2,500 rpm for 15 min to obtain the serum.

Estimation of thyroxine and triiodothyronine: The serum thyroxine and triiodothyronine levels were estimated by radioimmunoassay using a commercially available Cis-Bio RIA Kit.

The statistical analysis was done using GraphPad InStat™, version 3.10.[22]

RESULTS

The NaF-intoxicated rats showed increased serum levels of total T_4 and total T_3 compared to the normal control group (Table 1). Compared to the toxic control group, the administration of the 100 and 200 mg doses of the hydroalcoholic extract of cauliflower leaves resulted significant reductions in the levels of total T_4 and the administration of the 200 and 400 mg doses of the extract resulted in significant reductions in the levels of total T_3. (Figures 1 and 2). Compared to the normal control group, the administration of 400 mg of the hydroalcoholic extract of cauliflower leaves alone resulted in a significant increase in the level of T_4 but no significant change in the T_3 level The results clearly showed that the hydroalcoholic extract of cauliflower leaves stimulated thyroid function.

Table 1. Effects of hydroalcoholic extract of cauliflower leaves on total serum thyroxine and triiodothyronine in sodium fluoride intoxicated rats

	Group	Total serum thyroxine±SD (µg/mL)	Total serum triiodothyronine±SD (ng/mL)
I	Normal control	2.52±0.06	0.42±0.01
II	Toxic control,100 ppm NaF	3.60±0.12†	0.53±0.03*
III	100 mg cauliflower leaves extract + 100 ppm NaF	3.27±0.08‡	0.5±0.03
IV	200 mg cauliflower leaves extract + 100 ppm NaF	2.76±0.17§	0.39±0.02§
V	400 mg cauliflower leaves extract + 100 ppm NaF	3.49±0.04	0.44±0.01‡
VI	Plant control, 400 mg cauliflower leaves extract	3.15±0.11†,§	0.43±0.01§

Compared to the normal control group: *p<0.001, †p<0.0001; compared to toxic control group: ‡p<0.001, §p<0.0001

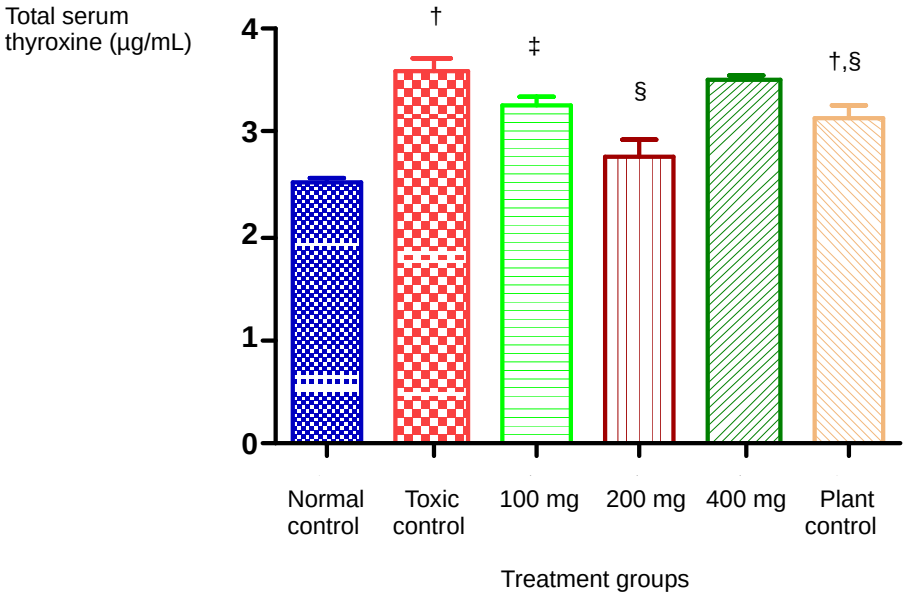

Figure 1. Effects of hydroalcoholic extract of cauliflower leaves on total serum thyroxine level in sodium fluoride intoxicated rats. Compared to the normal control group: *p<0.001, †p<0.0001; compared to toxic control group: ‡p<0.001, §p<0.0001.

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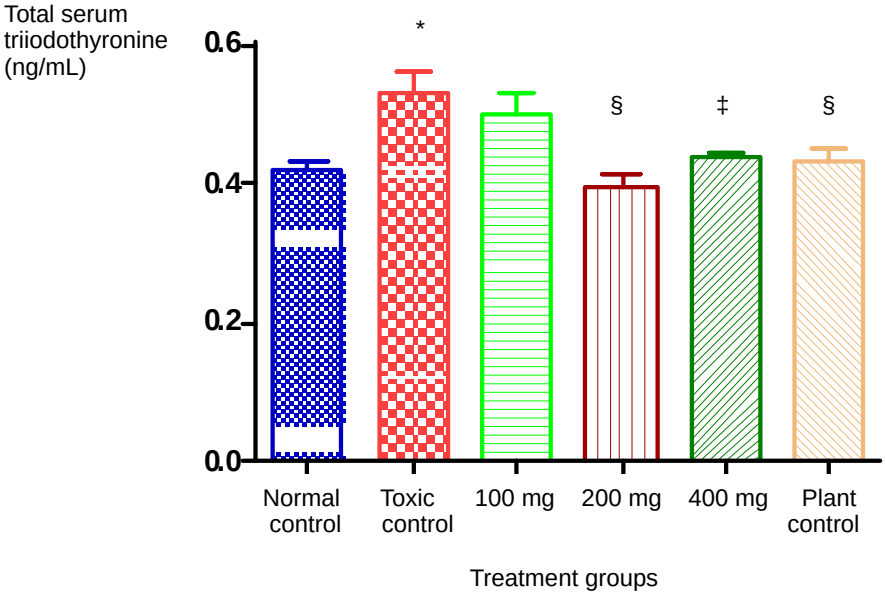

Figure 2. Effects of hydroalcoholic extract of cauliflower leaves on total serum triiodothyronine level in sodium fluoride intoxicated rats. Compared to the normal control group: *p<0.001, †p<0.0001; compared to toxic control group: ‡p<0.001, §p<0.0001.

DISCUSSION

Fluorine is a halogen chemically related to iodine but is chemically more active than iodine. The fluoride ion is a cumulative poison and can interfere with iodine metabolism in the thyroid gland by having an antagonistic effect. F accumulates in the thyroid gland and can directly injure the structure of thyroid follicle, provoke cytoplasm reduction and karyopyknosis of follicular epithelial cells, diminish the count of microvilli on the cristae of epithelial cells, and lead to bulging of the vacuoles in the follicular epithelial cells.[23] Earlier studies conducted in both animals and human beings clearly indicated that the thyroid gland is sensitive to the F.[24-29]

A possible mechanism for the increased serum level of total T_4 and total T_3 might be an activation by F of the pituitary gland resulting in the release of more T_4 from follicular cells. In turn, the T_4 is then deiodinated and changed into active T_3 by deiodinase in order to supply T_3 in the blood circulation.[30]

Many effects primarily attributed to fluoride are caused by the synergistic action of fluoride plus aluminum.[31] Soluble aluminofluoride complexes, fluoroaluminate (AlF_4), are formed in water solutions containing fluoride and traces of aluminum.[32] These complexes are able to simulate phosphate groups in many biochemical reactions.[32] $AlF_\times$ are used in many laboratory investigations of guanine nucleotide proteins (G proteins).[32] They affect various enzyme activities and cell signalling cascades.[32] Fluoride in a daily dose of 5–10 mg/day has been used to treat hyperthyroidism.[33] It has been hypothesized that fluoride mimics the thyroid stimulating hormone (TSH) and although this would stimulate thyroid function rather than lower it the overproduction of cyclic adenosine monophosphate (cAMP) results in a desensitization of the TSH receptor leading ultimately to reduced activity of the thyroid gland.[32,34]

Susheela et al. found thyroid hormone derangements in 49 of 90 children (54.4%) living in fluoride endemic, non-iodine deficient areas with five patterns of disturbance: (i) high TSH with normal free T_4 ($FT_{4)}$ and free T_3 (FT_3) (46.9%); (ii) normal TSH and FT_4 but low FT_3 (32.7%); (iii) high TSH and FT_3 with normal FT_4 (14.3%); (iv) high TSH with normal FT_3 but low FT_4 (4.1%); and (v) high TSH with normal FT_4 but low FT_3 (2.0%).[28] They noted that a negative feedback mechanism is involved in the production of thyroid hormones so that when the pituitary gland senses a drop in circulating FT_3 levels it releases more TSH to stimulate the production of the “pro-hormone” T_4 which then undergoes peripheral deiodination to produce the active hormone T_3.[28] Three iodothyronine deiodinase enzymes, D_1, D_2, and D_3, catalyse the deiodination. D_1, expressed in the thyroid gland, liver, and kidney, converts T_4 to T_3 in peripheral tissues, particularly in the liver, and is reflected in plasma T_3 levels. D_2, expressed in brain, pituitary gland, and skeletal muscle, converts T_4 to T_3 in local tissues, and through its presence in skeletal muscle makes some contribution to the plasma T_3.[28] D_3, expressed in brain, placenta, and fetal tissues, is an inactivating enzyme producing inactive metabolites by converting T_4 to reverse T_3 (rT_3) and T_3 to 3,3’-T_2.[28]

The release of TSH is controlled by positive input from the hypothalamic hormone thyrotropin-releasing hormone (TRH) and by negative input from T_4 and T_3, with the T_3 rather the T_4 being responsible for the feedback response.[35] TSH binds to G-protein-coupled receptors in the surface membranes of thyroid follicular cells which leads to increases in both cAMP and diacylglycerol/inositol triphosphate second messenger pathways.[35] Some T_3, the active form of thyroid hormone, is secreted directly by the thyroid along with T_4, but most T_3 is produced from T_4 by one of two deiodinases (D_1 and D_2) in the peripheral tissue. T_3 enters the nucleus of the target cells and binds to specific receptors, which activate specific genes.[35] Fluoride does not compete with iodide for transport into the thyroid.[35] The administration of fluoride in drinking water to rats results in variable effects on the T4 and T3.[35] Hara found elevated T_3 and T_4 at the lowest dose of approximately 0.1 mg/kg/day.[35,36] With intermediate doses (3–4 mg/day) the T_3 was decreased and the T_4 was normal.[35,36] At the highest doses (10–20 mg/kg/day), TSH and growth hormone were decreased indicating possible effects on pituitary function.[35,36]

CONCLUSION

In conclusion, this study has demonstrated that a hydroalcoholic extract of cauliflower leaves showed a significant alleviatory effect at doses of 100–400 mg/kg against NaF-induced thyroid gland dysfunction. However, further studies are required to understand the exact pathway or mechanism for the thyroregulatory role of the extract.

ACKNOWLEDGEMENTS

The authors are thankful to the Secretary and Correspondent of the CMR Group of Institutions, Mr Ch Gopal Reddy, for his support which was necessary for the successful completion of the research work. We are also grateful to Dr Sunita Garg, Chief Scientist, for the authentication of the plant material.

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