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Possible amelioration of hyperthyroidism by the leaf extract of *Annona squamosa*

Excess secretion of thyroid hormones (hyperthyroidism) is often considered as a causative factor for some of the common health problems including diabetes mellitus and heart disease[1]. Although several scientific studies have been made considering plant extracts in relation to different health problems, investigations on the herbal regulation of hyperthyroidism are meagre[2,3]. In our endeavour to find a suitable plant extract, for the regulation of hyperthyroidism, an attempt was made with the leaf extract of *Annona squamosa* to study its relation to the regulation of thyroid function.

Annona squamosa L. (family Annonaceae), commonly known as custard apple, is cultivated throughout India, mainly for its edible fruit. This plant is attributed with some medicinal properties including anti-tumour, diuretic, antifertility activities in mice and rats[4–6]. Its thyroregulatory activity, if any, was hitherto uninvestigated, despite the fact that some of the reported medicinal properties are regulated by thyroid hormones[7]. This paper reports an attempt to investigate the effect of its leaf extract in the regulation of hyperthyroidism considering laboratory mouse as working model.

Sodium dodecyl sulphate, Diethylenetriamine penta-acetic acid (DTPA), *Tris*, L-T_4 and thiobarbituric acid (TBA) were supplied by E. Merck, Mumbai, India. Other chemicals were of reagent grade and were obtained from Loba Chemie, Mumbai, India. Radioimmunoassay (RIA) kits for the estimation of total serum triiodothyronine (T_3) and thyroxine (T_4) were purchased from Bhabha Atomic Research Centre (BARC), Mumbai, India.

Healthy leaves of *Annona squamosa* were collected locally, dried under shade and powdered with the help of an electric grinder. An aqueous extract was prepared by the cold percolation method using 35 g of dried leaf powder in 500 ml doubled distilled water (DDW). After filtration, the extract was concentrated and dried under vacuum. The yield of the extract was 17.4%. The powdered extract was dissolved in double distilled water to prepare the required doses for the final administration.

Colony bred, two and half-month-old adult Swiss albino male mice weighing 28 ± 2 g were acclimatized for 7 days in a light-and-temperature controlled (14 h light : 10 h dark, 27℃ ± 1) animal room with the provision of food (Gold Mohur mice feed, Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. Thirty-five animals were divided into five groups of seven each. Group I animals receiving only 0.1 ml distilled water everyday served as control, Gr. II, Gr. III, IV and V were treated with pre-standardized dose (0.5 mg/kg) of L-thyroxine (L-T_4) initially for 12 days to render them hyperthyroidic[8]. While Gr. II continued to receive only T_4, Gr. III, IV and V animals were orally administered *Annona squamosa* leaf extract at three different doses (37.5, 75.0 and 150 mg/kg respectively) along with an equivalent dose of T_4 for the last 8 days before termination. On the 21st day, overnight fasted animals were sacrificed by cervical dislocation, blood from each one was collected, allowed to clot, centrifuged and serum samples were stored at – 20℃ until assayed for T_3 and T_4 concentrations.

Serum concentrations of T_3 and thyroxine T_4 were estimated by radioimmunoassay following the protocols of the RIA kits as done earlier[2,3]. In brief, RIA was performed using *Tris* hydroxymethyl amino methane (THAM buffer 0.14 M, containing 0.1% gelatin; pH 8.6) as assay buffer. The antisera, hormone standards, radio-labelled hormones (^{125}I-T_4 and ^{125}I-T_3) and the control sera were reconstituted with assay buffer/double-distilled water as mentioned in the kit. Incubation was done at 37℃ (30 min for T_4 and 45 min for T_3), which was terminated by the addition of PEG. The tubes were then centrifuged at 2000 g for 20 min. After decanting the supernatant the tubes were subjected to radioactive counting. Hormones of the samples were calculated from the standard curves of T_3 and T_4.

For the estimation of hepatic LPO, SOD and CAT activities, methods used in our earlier publications were followed[2,3]. Briefly, the liver was homogenized in 10% (w/v) ice-cold phosphate buffer (0.1 M, pH 7.4) and the homogenate was centrifuged at 15,000 × g for 30 min. LPO was determined in the supernatant by the reaction of TBA with malondialdehyde (MDA), a product formed due to the peroxidation of lipids, following the method of Ohkawa *et al.*[9]. The amount of MDA was measured by taking the absorbance at 532 nm (extinction coefficient, $E = 1.56 \times 10^5$), using a Shimadzu UV-160 A spectrophotometer. LPO was expressed as nM of MDA formed/h/mg protein. The hepatic SOD activity was assayed according to a method mentioned elsewhere[10]. This enzyme activity was expressed as units/mg protein and one unit of enzyme is defined as enzyme activity that inhibits autooxidation of pyrogallol by 50%. Catalase activity (μ mol of H_2O_2 decomposed/min/mg protein) was estimated following the method of Aebi[11]. The hepatic protein content was determined by the routine method using bovine serum albumin as standard[12].

Results were expressed as means ± SEM and were analysed by analysis of variance (ANOVA), followed by Student's *t* test.

From the results (Tables 1 and 2) it was evident that 0.5 mg L-thyroxine treatment for 20 days significantly increased the serum T_3 and T_4 concentrations ($P < 0.001$ for both) as observed in our earlier studies[2,3]. Hepatic LPO also increased significantly with a concomitant decrease in SOD and CAT activities ($P < 0.001$ for all). However, all three doses (37.5 mg/kg, 75 mg/kg and 150 mg/kg) of the plant extract could decrease the thyroid hormone concentrations when administered simultaneously with T_4 for the last 8 days. While serum T_3 decreased by 65.74%, 51.21%, 52.9% ($P < 0.001$, $P < 0.05$, $P < 0.05$ at three doses respectively), corresponding decreases in serum T_4 were 60.6%, 68.5% and 65.16% respectively ($P < 0.001$ for all) as compared to Gr. II which received only T_4. It was found while higher-dosed group, hepatic LPO increased significantly ($P < 0.001$), it remained unaltered in both the medium- and lower-dosed group where it was decreased significantly with an increase in CAT activities ($P < 0.001$, for both).

Table 1. Effects of three different doses of *Annona squamosa* leaf extract for 8 days on the alterations in serum concentrations of T_3 (ng/ml) and T_4 (ng/ml) along with their percentage decrease in L-T_4 induced hyperthyroid mice

Groups	T_3 (ng/ml)	% decrease (–) in T_3 conc.	T_4 (ng/ml)	% decrease (–) in T_4 conc.
Control	0.87 ± 0.08		57.57 ± 3.59	
L-T_4	2.89^a ± 0.31		117.71^a ± 5.31	
L-T_4 + A (37.5 mg/kg)	0.99^x ± 0.31	– 65.74	46.37^x ± 5.02	– 60.6
L-T_4 + A (75 mg/kg)	1.41^y ± 0.23	– 51.21	37.07^x ± 2.28	– 68.5
L-T4 + A (150 mg/kg)	1.36^x ± 0.15	– 52.94	41.00^x ± 4.0	– 65.16

Data are mean ± SEM ($n = 7$). *x*, $P < 0.001$, *y*, $P < 0.01$ and as compared to the respective values of T_4 treated animals. *a*, $P < 0.001$ as compared to the respective control values.

Table 2. Effects of three different doses of *Annona squamosa* leaf extract for 8 days in the alterations in hepatic LPO (nM of MDA formed hr^{-1} mg^{-1} protein), SOD (units mg^{-1} protein) and CAT (μmol of H_2O_2 decomposed min^{-1} mg^{-1} protein) activities in mice

Groups	LPO	SOD	CAT
Control	0.612 ± 0.12	5.48 ± 0.305	60.88 ± 5.06
L-T_4	2.48^a ± 0.098	3.10^a ± 0.113	32.16^a ± 1.03
L-T_4 + A (37.5 mg/kg)	1.12^x ± 0.030	4.10 ± 0.210	59.19^x ± 2.05
L-T_4 + A (75 mg/kg)	2.25 ± 0.099	3.98 ± 0.110	40.65 ± 4.02
L-T_4 + A (150 mg/kg)	3.93^x ± 0.022	3.15 ± 0.410	38.13 ± 3.98

Data are mean ± SEM ($n = 7$). *x*, $P < 0.001$, and as compared to the respective values of T_4 treated animals. *a*, $P < 0.001$ as compared to the control values.

These findings clearly reveal that *Annona squamosa* leaf extract exhibits thyroid inhibitory effects in mice, but alters hepatic LPO in a dose-dependent manner. At low concentration it appears to be antithyroidic as well as antiperoxidative, whereas, at higher concentration it is found to be thyroid inhibitory but hepatotoxic as indicated by enhanced LPO, thereby suggesting the unsafe nature of the highest dose.

The prevalent circulating thyroid hormone is T_4 which is secreted from the thyroid gland, whereas T_3, which is considered as the most metabolically active hormone, is synthesized in lesser amount by the gland and is formed mostly in liver by monodeiodination of T_4. As T_4 is synthesized only in thyroid gland and all the three doses of the plant extract decreased the concentrations of this hormone, it appears that the leaf extract-induced decrease in T_4 concentration could either be the result of direct inhibition of T_4 synthesis and/or release at the thyroidal level. However, decrease in serum T_3 concentration by all the three doses could be due to the inhibition in monodeiodination of T_4 in peripheral tissues, which is known to be the major process of its generation. Another possibility of plant extract-induced decrease in thyroid hormone concentrations could be the increased utilization of the hormones by the body.

Although further investigations are required to reveal the exact mechanism of action(s) of thyroregulatory role of *A. squamosa* leaf extract, the present findings clearly indicate that this extract is inhibitory to thyroid functions. However, it must be emphasized that a lower dose of *A. squamosa* leaf extract may prove to be effective and safe in ameliorating hyperthyroidism.

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