

Fruit extract of *Embolica officinalis* ameliorates hyperthyroidism and hepatic lipid peroxidation in mice

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Received March 12, 2003, accepted April 20, 2003

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Pharmazie 58: 753–755 (2003)

The ethanolic extract from the fruits of *Embolica officinalis* Gaertn (Euphorbiaceae) was investigated to evaluate its possible ameliorating effects, on the L-thyroxine (L-T₄) induced hyperthyroidism and on hepatic lipid peroxidation in mice. While an increase in serum T₃ (triiodothyronine) and T₄ (thyroxine) concentrations, and in a thyroid dependent parameter, hepatic glucose 6-phosphatase (glu-6-pase) activity was observed in L-T₄ (0.5 mg/kg/d) treated animals, simultaneous oral administration of the plant extract at a dose of 250 mg/kg/d (p.o.) for 30 days in hyperthyroid mice reduced T₃ and T₄ concentrations by 64 and 70% respectively as compared to a standard antithyroid drug, propyl thiouracil (PTU) that decreased the levels of the thyroid hormones by 59 and 40% respectively. The plant extract also maintained nearly normal value of glu-6-pase activity in hyperthyroid mice. The plant extract also decreased hepatic lipid peroxidation (LPO) and increased the superoxide dismutase (SOD) and catalase (CAT) activities in hyperthyroid mice, exhibiting its hepatoprotective nature. Our findings suggest that the test material may potentially ameliorate the hyperthyroidism with an additional hepatoprotective benefit.

1. Introduction

Elevated or reduced thyroid hormone levels have a wide range of adverse effects on growth, development and metabolism, reproduction, digestion and neural function [1]. Therefore, chronic excess in circulating thyroid hormones (hyperthyroidism) can cause serious health problem including diabetes and heart ailments [2, 3]. Obviously amelioration of hyperthyroidism is always necessary.

Limited literature is available on the regulation of hyperthyroidism by plant extracts [4–7]. Because of the paucity of information, in the present investigation, an attempt has been made to reveal the efficacy, if any, of *Embolica officinalis* fruit extract in the regulation of hyperthyroidism in mice.

E. officinalis, commonly known as Indian gooseberry, is a deciduous tree of the family Euphorbiaceae, found both in the wild and in cultivated states of tropical countries including India. Its fruit extract is considered to be a strong rejuvenating agent, particularly for the blood, bone, liver and heart. It is useful in curing cough, dyspnea, inflammation of eyes, and jaundice [8]. It's anti-tumor, hepatoprotective, anti-ulcer, anti-cholesterolaemic and anti-atherogenic effects were also reported earlier [9–13]. However, nothing was known on its thyroregulatory activity so far and the present investigation is an attempt in this direction.

2. Investigations and results

In this study the effects of the ethanolic fruit extract of *E. officinalis* were investigated in L-T₄ induced hyperthy-

roid mice. Estimation of serum thyroid hormone concentrations and hepatic lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT) and glu-6-pase (glu-6-pase) activities in liver, the major target organ of a drug metabolism were considered as end parameters.

Phytochemical screening of the ethanolic extract of *E. officinalis* fruit was also done using the method of Harborne [14] which exhibited the positive test for tannins, alkaloids, sucrose, & phenols as previously reported [15], ensuring the good quality of the fruits.

Significant results were obtained in almost all parameters. Administration of L-T₄ to normal mice increased the serum T₃, T₄ concentrations, and hepatic glu-6-pase activity significantly ($P < 0.001$ for all) indicating the attainment of hyperthyroidic condition. It also increased the LPO ($P < 0.001$) and decreased SOD and CAT activities ($P < 0.001$ and $P < 0.01$ respectively when compared to the respective control values). Simultaneous plant extract administration in T₄ treated mice significantly decreased the serum concentration of T₃, T₄, and hepatic glu-6-pase activity ($P < 0.001$ for all). In this group, while LPO was decreased significantly, the activity of both SOD and CAT were enhanced ($P < 0.001$ for all three) as compared to the values of hyperthyroidic animals. In euthyroid animals, following the administration of the plant extract only, a significant reduction in serum T₃ and glu-6-pase activity ($P < 0.01$ and < 0.05) was observed. Administration of the standard antithyroid drug, PTU to the hyperthyroidic animals also caused a significant reduction in the levels of both the thyroid hormones ($P < 0.01$ for

both), and in hepatic G-6-pase activity ($P < 0.001$). However, in this group reductions in T_3 and T_4 were only 59 and 40% respectively as against the plant extract where the calculated values were 64 and 70% respectively.

3. Discussion

From these results, it is evident that *E. officinalis* fruit extract may potentially ameliorate hyperthyroidism, as it could decrease the serum concentrations of both the thyroid hormones, T_3 and T_4 in L- T_4 induced hyperthyroid mice. This decrease was found to be 64% and 70% respectively as compared to that of a PTU + T_4 treated group where the percent reduction was comparatively less (59 and 40% respectively), suggesting that the plant extract may prove to be more effective than the standard antithyroid drug, PTU. A parallel decrease in a thyroid dependent parameter, glu-6-pase activity further supported the thyroid inhibitory role of this extract.

Earlier, root extracts of two other plants (*Ranwolfia serpentina* and *Convolvulus pluricaulis*) were reported to inhibit thyroid functions in hyperthyroidic animals [5, 6]. Effects of the present plant appear to be similar to that of *R. serpentina*, where also both T_3 and T_4 concentrations were decreased. However, unlike the earlier reported plants, the present one is easily available and cost effective as well.

The mode of action of an antithyroid drug is mediated either through the gland or through the peripheral organs. Since in the present investigation both hormones were decreased by an *Embllica* extract in hyperthyroid animals, it appears that the plant extract interferes with the synthesis/or release of the hormones at the extra thyroidal level, the primary site of T_3 generation as well as at the glandular level. This is because of the fact that, while thyroxine is produced only in the thyroid gland, the majority of T_3 is generated by peripheral monodeiodination of T_4 , particularly in the liver [16].

Lipid peroxidation is the most common manifestation of generation of reactive oxygen species (ROS) in organisms and a significant increase in LPO in cells is always related to the perturbation in membrane structure and cell function [17]. Therefore, an increase in LPO normally indicates the toxic effect of a compound. On the other hand antioxidants including SOD and CAT are compounds, which scavenge and suppress the formation of ROS and oppose their reactions. In this study, following T_4 administration, an increase in LPO was observed as reported earlier by Pereira

Table 2: Effects on hepatic LPO (n M MDA formed/h (mg/protein)) and CAT (μ M H_2O_2 decomposed/min/mg protein) activity following the administration of test extract (200 mg/kg) for 30 days in normal euthyroid or in T_4 induced hyperthyroid male mice

Groups	LPO	SOD	CAT
Control	1.09 ± 0.08	7.07 ± 0.23	70.77 ± 6.87
T_4	2.39 ^a ± 0.14	3.98 ^a ± 0.51	45.06 ^b ± 3.12
EO + T_4	0.61 ^x ± 0.02	6.78 ^x ± 0.36	73.17 ^x ± 2.89
EO	0.81 ^c ± 0.07	7.67 ± 0.58	69.17 ± 0.39
PTU + T_4	1.01 ^x ± 0.06	6.09 ^x ± 0.33	65.06 ^x ± 6.10

Data are means $\pm$ s.e.m. (n = 7); x, $P < 0.001$ compared to the respective values of T_4 treated group; a: $P < 0.001$ and b: $P < 0.01$ compared to the respective control value

et al. [18]. However, it was significantly decreased in the L- T_4 treated group that received *Embllica* extract, suggesting that the plant extract may reduce the hepatotoxic effects in hyperthyroid animals. On the other hand, antioxidant enzymes, SOD and CAT were enhanced to near normal levels in this group. Decrease in LPO and an increase in enzyme activities in the liver do indicate not only the safe nature of the plant extract but also its antiperoxidative value.

From these results it is evident that administration of *E. officinalis* fruit extract can ameliorate L- T_4 induced hyperthyroidism in mice. Considering the lowering effect on the thyroid hormones and the hepatoprotective nature of the plant extract it is suggested that the test material/fruit extract may be used for regulation of hyperthyroidism in humans.

4. Experimental

4.1. Animals

Swiss adult Albino male mice, weighing 30 ± 2 g were maintained in a standard light and temperature controlled room with the provision of laboratory feed (Gold Mohur feed, Hindustan Lever Ltd., Bombay, India) & water *ad libitum* for a week before experimentation.

4.2. Chemicals

Thiobarbituric acid (TBA), pyrogallol, hydrogen peroxide, diethylene triamine pentaacetic acid (DTPA), L- T_4 , PTU and sodium dodecyl sulphate (SDS) were obtained from E. Merck (India) Ltd., Bombay, India. Radioimmunoassay (RIA) kits for the estimation of total T_3 and T_4 , were supplied by the Bhabha Atomic Research Center (BARC), Mumbai, India. And all other chemicals were of reagent grade and obtained from Loba chemie, Mumbai, India.

4.3. Preparation of plant extract

Fruits of *E. officinalis*, purchased from the local market were identified by taxonomist Prof. A. Seerwani, Indore, India and a voucher specimen was preserved in our laboratory for future reference. Good quality fruits were air-dried and then ground in to fine powder by pulverization. The dried powder was extracted with 95% ethanol at 60°C by soxhlation as described earlier [19]. The extract, (approximately 12% of air dried powder) was suspended with 10% aqueous solution of Tween 80 and was used in the experiment.

4.4. Experimental design and drug administration

Thirty-five healthy animals were divided in to five groups of seven each, and the initial body weight of each one was recorded. While gr. I receiving suspending reagent (0.1 ml/d/animal) served as control, gr. II animals were injected (i.p) every day with 0.5 mg/kg of L- T_4 for inducing hyperthyroidism [20], gr. III received (p.o) the fruit extract at a dose of 250 mg/kg [13] along with an equivalent dose of L- T_4 , gr. IV was treated with the same amount of plant extract only as used in gr. III. and gr. V was treated with the standard antithyroid drug PTU (10 mg/kg) along with T_4 (i.p). Treatments were continued for 30 days. On the last day of the experiment, blood was collected from each animal and serum samples were subjected

Table 1: Effects of *E. officinalis* fruit (EO) extract (200 mg/kg) for 30 days on total serum T_3 , T_4 concentrations (ng/ml), and hepatic glu-6-pase activity (μ M phosphate generated/min/mg/protein) in normal euthyroid or in T_4 induced hyperthyroid male mice

Groups	T_3	T_4	G-6-pase
Control	0.92 ± 0.12	64.00 ± 7.49	0.284 ± 0.084
T_4	2.54 ^a ± 0.36	121.42 ^a ± 10.72	0.590 ^a ± 0.043
T_4 + EO	0.91 ^x ± 0.06	35.42 ^x ± 3.21	0.138 ^x ± 0.037
EO	0.38 ^b ± 0.06	60.57 ± 5.52	0.101 ^c ± 0.014
T_4 + PTU	1.05 ^y ± 0.14	73.45 ^y ± 6.28	0.32 ^x ± 0.02

Data are means $\pm$ s.e.m. (n = 7); a: $P < 0.001$; b: $P < 0.01$ and c: $P < 0.05$ compared to the respective control values and x, $P < 0.001$; y, $P < 0.01$ and z, $P < 0.05$ compared with the respective values of T_4 treated group

to radio-immunoassay (described later) for the estimation of total T₃ and T₄ concentrations, as performed routinely in our laboratory [5]. After exsanguination, the liver was removed quickly and processed for the investigation of LPO, SOD, CAT and glu-6-pase activities as done earlier [3–5].

4.5. Biochemical assay of hepatic LPO, SOD and CAT

For the evaluation of LPO, SOD and CAT activities, 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. The procedures adopted for the assay of LPO, SOD, CAT & glu-6-pase estimations were taken from standard protocols [20–23]. In brief, LPO was determined in the supernatant by the reaction of TBA in which malondialdehyde (MDA), a product formed due to the peroxidation of lipids, was estimated. The amount of MDA was measured by taking the absorbance at 532 nm (extinction coefficient, E = 1.56 × 10³), using a Shimadzu UV-160 A spectrophotometer. LPO was expressed as nM of MDA formed/h/mg protein. The hepatic SOD activity was assayed by auto-oxidation of pyrogallol. The enzyme activity was expressed as units/mg protein and one unit of enzyme is defined as the enzyme activity that inhibits auto oxidation of pyrogallol by 50%. CAT activity was estimated by considering the amount of H₂O₂ decomposed and the enzyme activity is expressed as μmol of H₂O₂ decomposed/min/mg protein. Protein content was determined using bovine serum albumin (BSA) as standard by the method of Lowry et al. [24].

4.6. Radio-immunoassay (RIA) of thyroid hormones

Total circulating T₃ and T₄ were estimated by radio-immunoassay in serum samples following the protocol provided in the RIA kits supplied by Bhabha Atomic Research Centre, Mumbai, India. In brief RIA was performed using Tris hydroxymethyl amino methane (THAM) buffer (0.14 M containing 0.1% gelatin; pH 8.6). The antisera, specific hormone standards, radio labeled hormones (I¹²⁵ T₄ and I¹²⁵ T₃) and the control sera were reconstituted with assay buffer/double distilled water. The reaction mixture comprised of standard/sample, buffer, radio labeled hormone and the respective antibody. The tubes were mixed and incubated at 37 °C (for 30 min for T₄ and 45 min for T₃). The incubation was terminated by the addition of PEG. The tubes were then centrifuged at 2000 × g for 20 min. After decanting the supernatant, the traces of liquid were removed with the help of filter paper wicks without disturbing the precipitate. Finally the tubes were subjected to radioactivity counting for one minute (CPM) using a ¹²⁵I gamma counter. A set of quality control sera was also run with each assay.

4.7. Statistical analysis

Data are expressed as mean ± S.E.M. For statistical evaluation of the data, analysis of variance and the Student's t-test were used.

Acknowledgements: Financial assistance to S. P (Research Associate) from Council of Scientific & Industrial Research (CSIR), New Delhi, India is gratefully acknowledged. We also thank Prof. K.N. Guruprasad (HOD) for some laboratory facilities.

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