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Short Communication

Protective Role of *Beta vulgaris* Peel Extract in T_4-Induced Hyperthyroidism in Male Mice

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ABSTRACT

Objective: The hitherto unknown effects of peel extract of *Beta vulgaris* root in regulating hyperthyroidism was investigated in male albino mice. **Method**: Hyperthyroidism was induced by L-thyroxine(L-T_4) at a pre-standardized dose of 0.5 mg/kg for 12 consecutive days and then the effects of alcoholic extract of the test peel, at two different doses (250 and 500 mg/kg/d for 15 days)were investigated in the hyperthyroid mice considering tissue lipid peroxidation (LPO), super oxide dismutase (SOD), catalase (CAT), glutathione(GSH) and the concentrations of thyroid hormones, glucose and lipids as main parameters.
Result:L-T_4 administration enhanced the levels of serum triiodothyronine (T_3) and T_4 both as well as the hepatic LPO($p<0.001$) with a parallel decrease in the levels of antioxidants(SOD and CAT) and serum lipids. While, after the treatment of both the doses of beet root extract in hyperthyroid animals, LPO was decreased; the values of SOD, CAT and GSH were enhanced significantly in liver tissues with a concomitant decrease in serum thyroid hormone levels, suggesting the antithyroid role of beet root. **Conclusion**:The alcoholic extract of *B.vulgaris*root possesses potential antioxidative and thyrotoxicosis ameliorating activities.

Keywords: *Beta vulgaris,* peel extract, anti- hyperthyroid activity, lipidperoxidation.

1. INTRODUCTION

Thyroid hormones (THs) such as thyroxine (T_4) and tri-iodothyronine (T_3) regulate almost all body functions. Increased level of thyroid hormones in circulation leads to hyperthyroidism, which, if not treated properly, may prove to be a serious health problem[1].Although conventional medicines are available for the treatment of hyperthyroidism, their long term use is believed to produce adverse effects. Therefore, an alternate drug is the need of the hour.

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The continued search among plant secondary metabolites for natural antioxidants has gained importance in recent years because of the increasing awareness of herbal products as potential sources of phenolic oxidants[2]. In fact, the primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. It is also known that the antioxidant content of fruits and vegetables may contribute in the protection from different diseases. In fact, the consumption of fruits and vegetables has been inversely associated with morbidity and mortality from degenerative diseases[3]. Although, it is not known which dietary constituents are responsible for this association, antioxidants appear to play a major role in the protective effect of plant foods[4]. In recent years, the waste materials such as peels and seeds have also been reported to act as rich sources of antioxidants[5,6] and therefore may act as drug /supplement for the amelioration of different diseases including thyrotoxicosis. The present investigation is an attempt on this direction.

Beet root (*B.vulgaris)* of Amaranthaceae family has several varieties with bulb colors ranging from yellow to red. Recent studies provide evidence that beet root ingestion offers beneficial physiological effects that may translate to improved clinical outcomes for several pathological conditions, such as hypertension, atherosclerosis, type-2 diabetes and dementia[7-9].As alterations in thyroid hormones are also associated with these abnormalities, in the present investigation the effects of the peel extract from *B. vulgaris* were evaluated in the T_4-induced hyperthyroid mice to reveal its possible therapeutic use, if any.

2. MATERIALSAND METHODS

Chemicals

Trichloroacetic acid (TCA), thiobarbituric acid (TBA), gallic acid and ascorbic acid were obtained from E. Merck, Mumbai, India and the L-thyroxine was purchased from Sigma Chemical Co. Ltd. St. Louise, USA. While ELISA kits for T_3 and T_4 estimations were supplied by the Rapid Diagnostic PVT. Ltd., Delhi, India; kit for serum glucose estimation was obtained from Span Diagnostic Limited, India. All other chemicals were of reagent grade and were purchased from Hi-Media Research Laboratories Pvt. Ltd., Mumbai, India.

Peel extract preparation

Fresh vegetable of beet roots were purchased from local market and cleaned in water. After the removal of the peels, they were shed dried and ground in to powder form using a kitchen grinding machine and passed through a 60-mesh sieve. Its 70% ethanolic extract was prepared as done earlier[10]. The obtained alcoholic extract was then concentrated under reduced pressure using rota-evaporator till complete drying. The resulted extract was later suspended in distilled water and used for the treatment.

Animals

Healthy colony bred Swiss albino male mice (2 month old, weighing 28± 2 g) were housed under constant temperature (27±1°C) and photo schedule (14 h light and 10 h dark). They were provided with mice feed at *ad libitum* and had free access to drinking water. Standard ethical guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India were followed (Our institutional registration No. is 779/PO/Re/S/03/ CPCSEA).

Experimental design

Four groups, of seven healthy male mice each were established. While group I animals, receiving vehicle (distilled water, 0.1 ml day, per oral) served as control, twenty-one mice in three groups (Gr. II–IV) were administered daily with L-T_4 at a pre-standardized dose of 0.5 mg/kg for 12 consecutive daysto induce hyperthyroid condition[11]. Animals of Gr. II continued to receivethe vehicle in which the test drug was dissolved, while animals of Gr. III and IV were administered with 100 and 250 mg/kg of test peel extract. After 15 days of peel extract treatment, animals were sacrificed, blood was collected from each one, centrifuged and the serum was stored for different estimations. Liver of each animal was also removed, cleaned in phosphate buffer and processed for peroxidation and antioxidant study.

Biochemical estimations

For the evaluation of LPO, SOD, CAT, GSH and GPx activities; the liver tissue was homogenized in PBS (0.1 M, pH 7.4), centrifuged at 15000 ×g for 30min at 4 °C, and the supernatant was used for subsequent analysis. Serum concentration of T_3 and T_4 were estimated by ELISA kits. Protein was estimated following the method of Lowry et al.[12] and for the measurement of serum glucose, the routine laboratory method of Trinder [13] was used.

TBA-reactive substance assay

LPO level in the tissues was measured by the method of Ohkawa et al.[14], which is based on TBA reaction with malondialdehyde (MDA), a product formed as a result of the peroxidation of membrane lipids. The amount of MDA was measured by taking the absorbance at 532 nm (extinction coefficient, E =1.56 × 105), using a Shimadzu UV-160 spectrophotometer. LPO was finally expressed as nM MDA formed/ hour/ mg protein.

SOD activity was determined following the Pyrogallol auto-oxidation inhibition assay method of Marklund and Marklund[15.] The rate of auto-oxidation is calculated from the increase in absorbance at 420 nm. The enzyme activity was expressed as units per mg protein, and 1 unit is defined as the enzyme activity that inhibits auto-oxidation of Pyrogallol by 50%.

CAT assay

CAT activity was estimated following the method of Aebi[16] which is based on the decomposition of H_2O_2 that is measured using a spectrophotometer from the changes in absorbance at 240 nm that was expressed as μM of H_2O_2 decomposed / minute / mg protein.

GSH assay

For the estimation of tissue GSH content, the protocol of Ellman[17] was followed in which the –SH group of GSH reacts with dithionitrobenzoic acid (DTNB) to produce a yellow-colored 2-nitro-5-mercaptobenzoic acid, and the absorbance is taken at 412nm. The GSH content is expressed as µM GSH per mg protein.

3. RESULTS

LPO and antioxidants: L-thyroxine administration significantly increased the hepatic LPO($p<0.001$) as expressed in Table 1 , with a decreased activity of SOD, CAT, GSH and GPx ($p<0.001$, $p<0.001$, $p<0.001$ and $p<0.05$ respectively). While, after the treatment of both the doses of beet root, LPO was decreased, the values of antioxidants were enhanced significantly in liver tissue. The Changes in serum T_3 and T_4 concentrations are illustrated in Figure 1. T_3 was significantly increased in the hyperthyroid mice ($p<0.001$, as compared with that of control ones). As expected, T_4 levels also showed a significant increase ($p<0.001$) in these animals. However, administration of both the doses of beet root peel extract brought a marked decrease in both T_3 and T_4 levels ($p<0.001$, for both, as compared to that of hyperthyroid animals). Both the doses (100 and 250mg/kg) of beet root were found to be effective.

Table 1: Alterations in lipid peroxidation (LPO) and antioxidants following T_4 (0.5 mg/kg), T_4+BV 1(100 mg/kg), T_4+ BV2 (250 mg/kg) administration in the hepatic tissues of mice.

Parameters	CONTROL	L-T_4	L-T_4+BV1	T_4–BV2
LPO (nM MDA/h /mg protein)	1.87 ±0.04	3.45^a ±0.38	1.43^y ±0.22	1.72^x ±0.25
SOD (U/mg protein)	4.28 ±0.25	2.12^a ±0.16	5.34^x ±0.35	6.72^x ±0.22
CAT (µ moles of H_2O_2 decomposed /min/ mg protein)	57.00 ±1.34	35.46^a ±0.71	66.90^x ±10.70	68.00^x ±3.20
GSH (µmoles GSH/mg protein)	2.88 ±0.28	1.56^a ±0.08	3.60^x ±0.38	3.49^x ±0.33
GPx (µ moles of GSH oxidized / mg protein)	3.36 ±0.29	1.11^a ±0.05	3.10^z ±0.87	2.75^a ±0.29

Data are means ± SEM, n=7. a, $p<0.001$, as compared to the respective control values.x, $p<0.001$; y, $p<0.01$, z, $p<0.05$,as compared to the respective value of the T_4- induced mice.

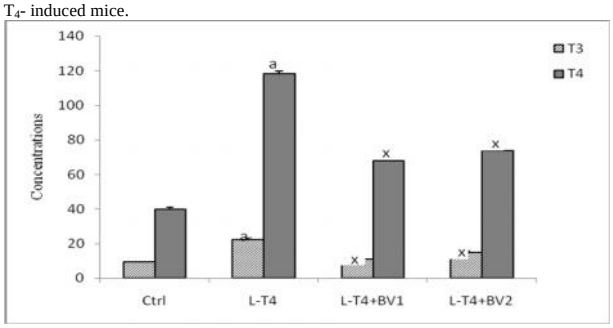

Fig 1: Effects of *Beta vulgaris*(BV) at 100 mg/kg (BV=1) and 250 mg/kg (BV=2) in the alterations in serum concentration of T_4 (ng/ml) and T_3 (ng/ml) in hyperthyroid mice. Data are mean ± S.E.M. (n = 7). a,p<0.001 as compared with the respective control value. x,p<0.001 as compared with the respective value of L-T_4-treated animals

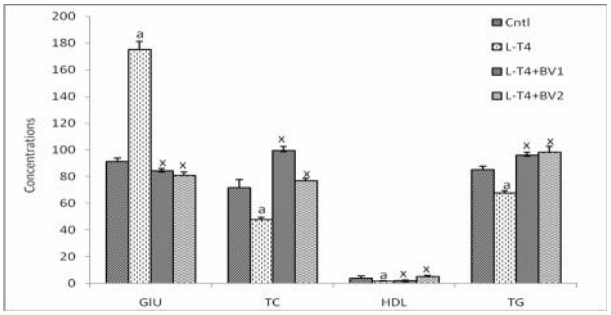

Fig 2: Effects of *Beta vulgaris* (BV) at 100 mg/kg (BV=1) and 250 mg/kg (BV=2) on the serum concentration of total cholesterol (TC, mg/dl), high-density lipoprotein cholesterol (HDL-C, mg/dl), triglyceride(TG, mg/dl), glucose (GLU mg/dl) in hyperthyroid mice. Data are mean ± S.E.M. (n = 7). a, p<0.001 b,p<0.01and c,p<0.05 as compared to the respective control value. x,p<0.001, y,p<0.01 and z,p<0.05 as compared with the respective value of L-T_4-treated animals

4. DISCUSSION

From the results, it is evident that T_4 administration for more than 12 days induced hyperthyroidism in mice as observed earlier[11]. Interestingly, simultaneous administration of T_4 and the test peel extract decreased the level of serum glucose, indicating its glucose lowering effects. Although somewhat similar anti-hyperglycemic activity of *B.vulgaris* extract was reported earlier[18], it was not on peel, but on the whole root, that too not in T_4-induced hyperthyroid animals.

Test peel extract also decreased the level of both the thyroid hormones (T_3 and T_4) in hyper thyroid animals suggesting for the first time its anti-hyper thyroid property.

Development of the hyperthyroid state in vertebrates normally elevates basal metabolic rates with an increased rate of O_2 consumption in target tissues, activating mitochondrial cytochrome oxidase through 3,5-diiodothyronine signaling pathway involving changes in nuclear and mitochondrial gene expression. These conditions determine a higher consumption of cellular antioxidants or the inactivation of antioxidant enzymes, thus inducing oxidative stress[19], often with the concomitant increase in tissue LPO and protein oxidation[20,21]. However, in this study, following the administration of the peel extract in L-T_4-induced hyperthyroid mice, not only hepatic LPO was reduced, but also there was an enhancement in the levels of SOD, CAT and GSH, suggesting the antioxidative potential of the test drug. Somewhat similar observations were made earlier with plant extracts where their free-radical scavenging properties were reported [22]. Of course on the extract of beet root peel, the present report appears to be the first one on the regulation of hyperthyroidism in animals.

5. CONCLUSION

While L-T_4-induced hyperthyroidism enhanced the tissue LPO and decreased the levels of antioxidants, serum cholesterol, triglyceride and HDL; with the simultaneous administration of the test peel extract, all these adverse effects were reversed indicating the regulation of

hyperthyroidism in mice. We suggest that beet root peel has the potential to ameliorate hyperthyroidism and may be considered for therapeutic use after detailed study.

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