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CLITORIA TERNATEA FLOWER EXTRACT MAY HAVE THE POTENTIAL TO REGULATE HYPERTHYROIDISM IN MICE

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ABSTRACT

Background: Hyperthyroidism can prove to be a serious disease, if not treated properly. Although for the treatment of hyperthyroidism some conventional medicines are available, nothing has been reported with respect to the role of flower in this aspect. **Objective:** To investigate the anti-hyperthyroidic effect of *Clitoria ternatea* flower extract on experimentally induced hyperthyroid mice. **Methods:** First of all antioxidative potential of *Clitoria ternatea* flower was studied by an *in vitro* method using DPPH .Then in an *in vivo* study, L-thyroxine (L-T_4) at 0.5 mg/kg/d was administered through intra-peritoneal route for 15 consecutive days to induce hyperthyroidism in which the effects of three different doses (400, 200 and 100 mg/kg body weight) of the *C. ternatea* flower extract for 12 days were investigated on the changes in hepatic lipid peroxidation (LPO), enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathioneperoxidase (GSH). Simultaneously alteration in serum cholesterol was evaluated. **Results:** *In vitro* DPPH assay showed the antioxidant properties of the test extract. In *In-vivo* study, following the administration of L-T_4, serum cholesterol and the tissue LPO were increased with a decrease in anti-oxidative enzymes. However, administration of the *C. ternatea* flower extract to hyperthyroid animals significantly decreased the levels of hepatic LPO and normalized the serum cholesterol and glucose level along with antioxidative enzymes, suggesting it's antihyperglycemic and antiperoxidative potential. **Conclusion:** These findings suggest that *C. ternatea* flower has the potential to correct the hyperthyroidism-induced tissue LPO and hypolipidemia.

KEYWORDS: *Clitoria ternatea* flower, Hyperthyroidism, Hypolipidemia, Lipid peroxidation, Mice.

INTRODUCTION

Thyroid hormones play a crucial role in regulating body metabolism. However, the excess thyroid hormone production and their release in the circulation results in an abnormal condition, known as thyrotoxicosis/ hyperthyroidism that affects different organ systems. Its main symptoms are palpitation, tachycardia, fatigue, anxiety, insomnia, weight loss, heat intolerance, sweating, polydipsia, and tremor of the extremities.[1,2] Thyroid hormones have crucial actions on lipid metabolism as thyroid dysfunctions alter the synthesis and degradation of lipids as well as the function of various enzymes in the lipid metabolism pathway.[3] Hyperthyroidism is usually associated with hypolipidemia through modifications in lipid synthesis, absorption, circulation and metabolism.[4] It may also be a cause of hyperglycemia.[5] Obviously, untreated hyperthyroidism may lead to serious health problems.

Conventional medicines that are being used for the treatment of hyperthyroidism (Carbimazole, Propylthiouracil) are believed to produce adverse effects when used for a long period. Therefore, an alternate therapy is often required. One such option is the use of herbal products that are gaining popularity nowadays.[6- 8] However, on flowers not a single report is found till date.

Clitoria ternateae is a flowering plant native to Southeast Asia, India, and China.[9,10] It is also known as Asian pigeon wings, Butterfly pea, Bluebell-vine, or Cordofan-pea. The plant is known for its blue and white flowers that are used to make natural dyes or as an ornamental plant.[11,12] Its dried flower is popularly known as Blue Tea. Its roots, leaves, and flowers have been used for centuries in traditional medicine to treat a variety of ailments such as fever, headache, anxiety, and inflammation.[13-17] Unfortunately, nothing was known on its regulatory activity on hyperthyroidism till todate.

MATERIALS AND METHODS

Chemicals used

L-thyroxine (L-T_4) was purchased from Sigma-Aldrich chemicals (St. Louis, MO, USA), India[while thiobarbituric acid (TBA), sodium dodecyl sulphate

(SDS) Acetic acid, pyrogallol, DTPA, metaphosphoric acid, sodium chloride, EDTA, Sodium citrate, DPPH (1, 1 Diphenyl-2-picryl hydrazyl),from E Marck, India. For estimations of total cholesterol and glucose, assay kits were obtained from Span diagnostics Pvt. Ltd., Surat, India. All other chemicals (reagent grade) were obtained from Loba Chemie Pvt. Ltd., India.

Processing of flowers

Fresh flowers of *Clitoria ternateae* were procured from various parts of Indore, Madhya Pradesh, India. Procured flowers were washed thoroughly with RO water to make them free from any debris. Cleaned flowers were shade dried at room temperature (RT) for 15-20 days and dried materials were pulverized in the mechanical grinder to powder form.

Preparation of flower extract

Three gm of the powdered flower was added to 20 ml of 70% methanol,[18] and kept for 3 days at room temperature for the extraction process. It was then filtered through Whatman no. 1 filter paper and dried at 37^0C. The flower extract yield was obtained to be 3.5 gm per 100 gm.

Animals

Healthy colony bred Swiss albino male mice (30±2 gm) were used in this study, that were housed in polypropylene cages under standard conditions (14 h light and 10 h dark cycles, at 25 ± 2^0C and 35-50% humidity). Palletized feed and boiled water were provided *ad libitum.* 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).

Determination of total antioxidant activity: An *In vitro study*

Antioxidant compounds like polyphenols, flavonoids, and phenolics could scavenge free radicals and thus can inhibit the oxidative mechanism that leads to degenerative diseases. *In vitro* DPPH radical scavenging activity of *Clitoria ternatea* flower was performed using by using an earlier method,[19] with some modifications. Different concentrations of plant extract (20 µl each) were mixed with 0.4mM DPPH solution in methanol. Finally, 3 ml of methanol was added to the reaction tube and allowed to incubate for 30 minutes in dark at RT. The absorbance was taken at 517 nm against blank. Ascorbic acid was considered as the positive control. Following formula was used for the calculation of percentage inhibition.

DPPH scavenging effect (%) = $(A_{control} - A_{sample})/A_{control} X\ 100$,

Where, A stands for absorbance.

***In vivo* study**

Experimental design

Animals were randomly divided into 6 groups of 5 each. The mice in-group I received saline (0.1 ml/10 g body weight) and served as control group, while that in-group II to VI were administered T_4 (0.5 mg/kg), for 12 consecutive days for induction of hyperthyroidism. Group III also received orally anti thyroid agent, PTU (10 mg/kg), once per day [20]. Animals in Group IV, V and VI were administered orally *C. ternatea* flower extract, at 400, 200 and 100 mg/kg, once per day along with T_4, respectively. After 15 days of flower extract treatment, animals were sacrificed and liver of each one was taken out and stored at -20^0C for the evaluation of LPO and antioxidants. Blood was collected, allowed to clot and centrifuged to get serum that was stored to estimate different parameters.

Oxidative stress biomarkers

Hepatic lipid peroxidation (LPO)

To determine the tissue LPO, thiobarbituric acid substances (TBARS) were measured using an earlier method with some modifications,[21] as routinely done in our laboratory.[22] For this, liver homogenate was prepared in phosphate buffer and centrifuged at 15000 Rpm for 15 min at 4 °C. Its supernatant was mixed with 8.1% SDS, acetic acid (pH 3.5), and thiobarbituric acid. This reaction mixture was heated for 1 h at 95°C and then cooled. After cooling, 4 ml of TCA was added and centrifuged at 3000 rpm for 5 min. The absorbance of the pink colored was measured at 532 nm.

Superoxide dismutase (SOD)

For the study of SOD activity, the method of Marklund and Marklund,[23] as followed and was expressed in units per milligram of protein. Pyrogallol auto-oxidation inhibition assay method is commonly used for the determination of activity of SOD, where the rate of autooxidation was calculated from the increase in absorbance at 420 nm. DTPA was used as a chelator to prevent the interference of Fe++, Ca++, and Mn++. Tris-HCl buffer, tissue supernatant, and pyrogallol were added in a 3-ml cuvette,and the absorbance of the sample was taken at 420 nm at an interval of 30 s. SOD was finally expressed in units per milligram of protein.

Catalase (CAT)

For this, the method as described by Aebi,[24] is based on the decomposition of H_2O_2 was used. In brief, 20µl tissue supernatant, 20µl ml of 50-mM phosphate buffer (pH 7), and 0.1 ml of 30 mM H_2O_2 were added and a decrease in absorbance was measured every 5 sec for 30 sec. at 240 nm. Finally, the CAT activity was expressed as micromoles of H_2O_2 decomposed per minute per milligram of protein.

Reduced glutathione (GSH)

The method of Ellman,[25] is often followed for the estimation of GSH content in which 5, 5-dithio-bis-2-nitrobenzoic acid and DTNB reagents are used. GSH

reacts with DTNB to produce a yellow-colored 2-nitro-5-mercapto-benzoic acid, and the absorbance was taken at 412 nm. Tissue supernatant (0.5 ml) was pipetted out and precipitated with 5% TCA. After centrifugation, the supernatant was taken out and to it was added Ellman's reagent and phosphate buffer. The yellow color so developed was read at 412 nm and the GSH content was expressed in micromoles of GSH per milligram protein.

RESULTS

Results of the *in vitro* study clearly indicated the DPPH free radical (FR) scavenging activity (expressed in % inhibition) of *C. ternatea* flower extract at different concentrations (Table 1). Maximum inhibition of DPPH was observed at the dose of 300µg/ml (84.21%) and with ascorbic acid, that was used as positive control, the inhibition was 87.09%.

Table 1: DPPH radical scavenging activity (% inhibition) of *Clitoria ternatea* flower extracts in comparison to the standard ascorbic acid.

DPPH assay		
Concentration	**Ascorbic acid (%)**	**Flower extract (%)**
400 µg/ml	87.09	77.18
300 µg/ml	87.09	84.21
200 µg/ml	86.37	71.92
100 µg/ml	83.03	43.85
50 µg/ml	79.85	26.31
25 µg/ml	71.01	12.27

Results of *in vivo* studies indicated that after the administration of L-T_4, there was a significant increase ($p<0.0001$) in hepatic LPO. However, there was a significant decrease ($p<0.0001$) in it after treatment with all three different doses (Fig. 1). However, the maximum inhibition was observed at its 400 mg/kg. With respect to antioxidative properties, while in T_4 treated animals, a significant decrease ($p<0.0001$) was observed in SOD, and GSH; in animals treated with flower extract and L-T_4, all three doses normalized the antioxidants. Catalase, however, did not show any significant difference (Fig 1).

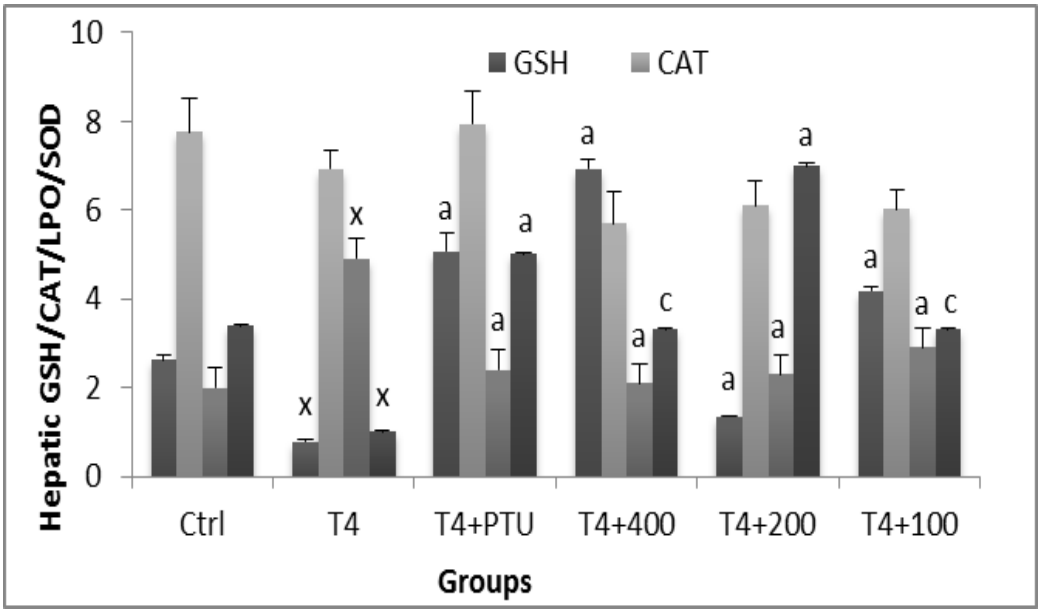

Fig. 1: Alterations in hepatic LPO (nM MDA/h/mg proteinX10), SOD (units/mg proteinX10), CAT (µM H_2O_2 decomposed/min./mg protein), and GSH (µM GSH/mg protein) in L-T_4 and *C. ternatea* flower extract or PTU treated mice. Values are in mean ± S.E.M. $^{x}p<0.0001$ and $^{y}p<0.001$ as compared to respective control value; $^{a}p<0.0001$, $^{b}p<0.05$ and $^{c}p<0.001$ as compared to respective value of the L-T_4 treated group.

Similarly, there was a decrease in serum glucose ($p<0.0001$), and increase in serum cholesterol ($p<0.0001$) concentrations in L-T_4-induced thyrotoxic mice treated with flower extract. While, L-T_4 administration, markedly increase the serum glucose concentration (Fig. 2), 400 and 200 mg/kg of test plant extract not only effectively decreased the serum glucose, but also increased the antioxidants, GSH and SOD. Of course, 200 mg/kg was found to be most effective for serum glucose and cholesterol normalization.

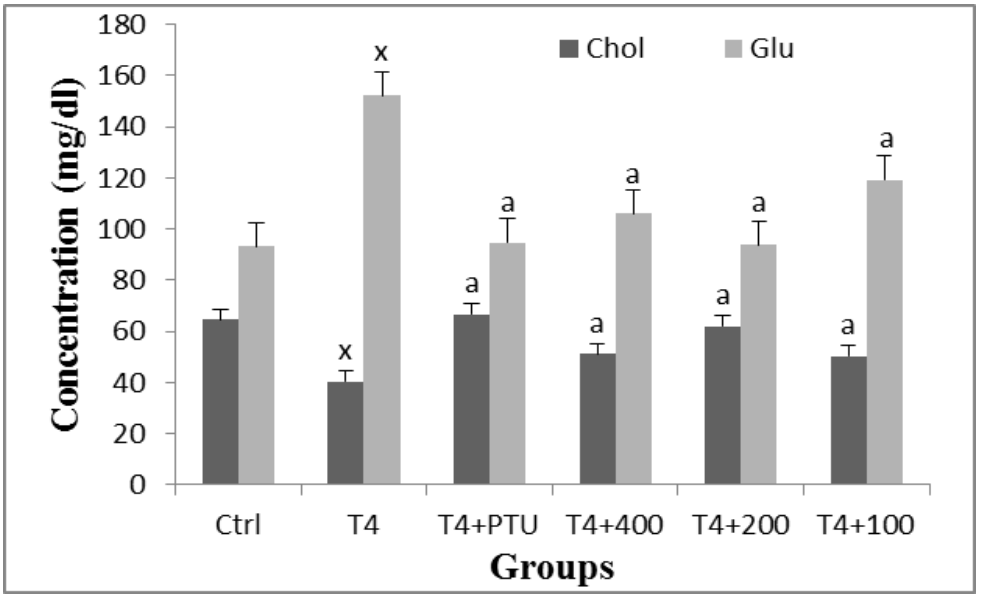

Fig. 2: Effect of *C. ternatea* flower extract or PTU on the alterations in serum cholesterol and glucose in L-T_4 treated mice. Values are in Mean ± S.E.M. $^{x}p<0.0001$, as compared to respective control value; $^{a}p<0.0001$, as compared to respective value of the L-T_4 treated group.

DISCUSSION

From the results, it is clear that *C. ternatea* flower extract has the potential to reduce hyperthyroid-induced physiological changes in mice, as evidenced by reduction in T_4 induced hepatic LPO and enhancement of SOD, CAT and GSH.

Although, similar to these findings, some plant extracts have also been reported to reduce hyperthyroidism[26,27] in animal models, with respect to flower extract practically nothing was known till date with respect to regulation of thyroid function. Therefore, this appears to be the first report on the regulation of thyrotoxicosis/hyperthyroidism, considering a commonly available Indian flower, *C. ternatea*.

With respect to LPO, while exogenous L-T_4 administration significantly decreased the antioxidants and increased the MDA level (the index of LPO), the flower extract in one or the other doses decreased the LPO and increased the antioxidants such as SOD and GSH, clearly revealing the antioxidative properties of *C. ternatea*. This antioxidative potential was also ascertained by *in vitro* DPPH scavenging study. With respect to the changes in serum glucose, there was a condition of hyperglycemia following T_4 administration. This could be due to the fact that, thyroid hormones are also gluconeogenic as well as glycogenolytic in nature. Interestingly the flower extract administration reduced this T_4-induced hyperglycemia. These changes in serum glucose concentrations could be the result of flower extract-induced reduction in thyroid function. Whatever may be the possible mode of action, our findings do suggest the ability of the test flower extract to decrease the hyperglycemic condition in thyrotoxic /hyperthyroid mice. This finding corroborates to the earlier report that indicated the anti-hyperglycemic nature of *C. ternatea flower* in streptozotocin-induced diabetic animals.[28] Thyroid hormones are known to influence lipid metabolism and hyperthyroidism causes hypolipidemia. In this study, also we fund a decrease in serum cholesterol in T_4 induced mice, which was normalized by the co-administration of test flower extract, further confirming the anti-thyroidal role of the test extract. This observation is somewhat similar to an earlier report on another plant extract.[29]

CONCLUSION

Our study suggests that the methanolic extract of *C. ternatea* flower regulates lipid peroxidation and hyperglycemia in T_4-induced thyrotoxic mice. It thus appears to be a promising agent for the regulation of hyperthyroidism, suggesting its use as a medicinal food supplement, particularly for the amelioration of thyrotoxicosis. We assume that the anti-thyroid role of test extract is due to its strong anti-oxidative property.

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