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The effect of *Citrus medica* as antithyroid compound in hyperthyroid female mice induced by L-thyroxine

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ABSTRACT

The aim of the current study was to evaluate the antithyroid property of *Citrus medica* after oral administration for 15 days (200mg/kg) on serum level of thyroid stimulating hormone (TSH) and thyroid hormones (T3 & T4) in hyperthyroid mice induced by L-thyroxine. An alteration was observed in serum T3, T4 and TSH level in L-thyroxine treated mice, simultaneous administration of plant extract reduced the serum level towards normalcy showing the antithyroidic property of *Citrus medica*.

Keywords: - Hyperthyroidism, L-thyroxine, Thyroid hormone, Thyroid stimulatory hormone.

INTRODUCTION

Since thousands of years plants have been utilized as a natural source of medicinal compounds. Scientist and medical professionals have also shown a great interest towards herbal remedies. Adverse effects of allopathic medicines are well documented¹ on the one hand while on the other phytomedicines are gradually increasing in popularity because of their safe and economic nature.

Citrus medica Linn, commonly known as citron in India cultivated in warm moist region in India. Its fruits and leaves are studied to understand its various uses in the treatment of diseases like diabetes and Alzheimer's². Flavonoids present in it also have an anti-inflammatory, antiulcer and diuretic activity³. Apart from these it is also used in constipation, diarrhea and piles⁴.

Despite these use, literature is negligible on the importance of leaves extract in the treatment of thyroid hormone imbalance disorder. Therefore, in the present experiment an attempt has been made to explain the remedial effects of *Citrus medica* leaves extract, if any, in L-thyroxine induced hyperthyroidism considering mice as a working model.

MATERIAL AND METHOD

Chemicals

Thyroxine was purchased from Biodeal Laboratories Ltd, India. Elisa kits for the estimation of thyroid hormones were purchased from DSI s.r.l Italy.

Plant material and preparation of extract

Citrus medica (Rutaceae) plant was collected from Sanjeevani, Bhopal and was identified and authenticated by Department of Botany, Govt MLB College, Bhopal. Plant was dried in shade and powdered. 100 gm of leaf powder was mixed with 1000 ml of distill water and was kept for 72 hrs. The supernatant was collected and evaporated to dryness. This was used as the crude leaf extract to study the antithyroidal effect.

Animals

Swiss albino female mice 25 - 30 gm body weight were procured and housed in separate cages under 12 hours light and 12 hours dark periods.

They were maintained on standard food pellets, water ad libitum and room temperature. The care and handling of mice were in accordance with the protocol approved by Institutional Animal Ethics Committee.

Experimental design

Eighteen female mice were divided into three groups. Group I was treated as control and receiving equivalent amount of distill water. Group II was treated with a dose of Thyroxine (L-T4) (0.5 mg/kg b.w) for 15 days to render them Hyperthyroidic. Group III was treated with L-T4 along with Citrus *medica* extract (250mg/kg b.w) for 15 days. Blood samples were taken on the 15th day for biochemical estimation.

Biochemical estimation

Total triiodothyronine (TT₃), total thyroxine (TT₄) and thyroid Stimulating Hormone (TSH) were estimated by Enzyme linked immunosorbent assay (ELISA) following the protocol provided in the kits, as done earlier in our laboratory⁵. In brief, ELISA was performed using conjugate, standards, buffer, substrate, washing and stop solution. The reaction mixture comprised of standard/sample and conjugate. The tubes were mixed and incubated at 25°C for 90 mins. Wells were washed with washing solution and substrate was added, again incubated for 20 mins. Finally the reaction was terminated by adding stop solution. Absorbance was taken at 450nm.

Statistical analysis

The data were statistically analyzed and presented in the table as Mean ± Sem. comparison among the groups are performed by ANOVA and the level of significance was expressed at P< 0.05

RESULT AND DISCUSSION

The data recorded on the thyroid hormone profile in control and experimental groups are presented in table I. In L-T4 treated animals an increase in serum concentration of T3 and T4 with a decrease in TSH level was observed while reverse results were observed after treatment with CM leaf extract with LT4.

Table I: Effect of Citrus *medica* on TSH (μIU/ml T₃ (ng/ml), and T₄ (n mol/L) in Thyroxine induce Hyperthyroid female mice

Table 1:

GROUP	TSH	T3	T4
CONTROL	1.67 ± 0.32	0.79 ± 0.26	93.00 ± 1.73
L-T4	0.04 ± 0.6*	6.8 ± 1.13*	169 ± 2.08*
L-T4 + CM	0.78 ± 0.18**	1.83 ± 0.30**	77.3 ± 15.07**

Data expressed in Mean ± SEM (n = 6). * when compared to control group, ** when compared to L-thyroxine treated group

The result of the present study shows that the administration of L-T4 increased the serum concentration of T4 and T3 with a decrease in TSH level as compared with control group. The increase in thyroid hormones level may be due to the absorption of L-T4 well by gut, thus increasing the level of thyroxine (T4) in circulation, which is then converted into triiodothyronine (T3) by iodothyronine deiodinases⁶⁻⁷.

However, when 250 mg/kg of Citrus *medica* leaves extract was administered with L-T4, it reversed the concentration as evidenced by a marked decrease in the level of both the thyroid hormones with an increase in TSH, indicating the antithyroidic nature of the extract. Since both the thyroid hormones, T3 and T4 were decreased by the test material, it appears that the Citrus *medica* extract is capable of inhibiting thyroid function both at glandular (major source of T4 synthesis) as well as at the peripheral level of T4 to T3 conversion, the principal pathway of T3 generation.

CONCLUSION

In conclusion our finding reveals that *Citrus medica* leaves extract reduce the serum level of thyroid hormones showing a strong antithyroidal effect. However further investigation is required to delineate its precise mechanism and possible therapeutic values.

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