Evaluation of anti-thyroid activity of ethanolic extract of Thespesia lampas in thyroxine induced hyperthyroid rats

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Abstract: Background: Thyroid disease is common and more prevalent with increasing age. 5%-9% of adults are suffering with subclinical thyroid disease and 0.8%-7.5% are suffering with clinical thyroid disease. The present study is to evaluate the Antithyroid activity of ethanolic extract of Thespesia lampas against Thyroxine induced hyperthyroidism in Rats. Thespesia lampas has been used for centuries to treat gonorrhea, syphilis and its hepatoprotective therapeutic role has been well established.

Method: Hyperthyroidism was induced in experimental rats by administering Thyroxine (600µg/kg/ml) orally for 14 days. While Methimazole (0.04% w/v) administered for 28 days served as the standard drug. Hyperthyroid male albino rats weighing 150-200 g were treated with oral doses of 200 mg/kg and 400 mg/kg of ethanolic extract Thespesia lampas for 28 days.

Result: Results of the study suggested that, the phytochemical screening of extract proved the presence of steroids, flavonoids, terpenoids, glycosides and saponins. The morphological assessment demonstrated that there was a significant increase in the levels of T3, T4 and TSH in thyroxine treated group and also increase in the SGOT, SGPT, VLDL and HDL levels and the body weight also increased. Simultaneous administration of ethanolic extracts of Thespesia lampas lowered the increased levels. This decrease in the levels of T3 and T4 after treating rats with extract was compared with the reference drug Methimazole.

Conclusion: These results served as direct evidence to say that ethanolic extracts of Thespesia lampas have remediating effect on the hyperthyroidism.

Keywords: Hyperthyroidism, Thespesia lampas, TSH, T3, T4.

INTRODUCTION

Hyperthyroidism, a common thyroid disorder, refers to increased synthesis and secretion of thyroid hormones by the thyroid gland. Most common causes of hyperthyroidism are Graves’ disease (GD), toxic multinodular goiter and toxic adenoma. There are three therapeutic choices for hyperthyroidism: thyroid surgery as the oldest modality, radioactive iodine (RAI) and antithyroid drugs (ATDs) (1).

More than 75 years ago, the inhibitory effects of thioureas/thiouracils on thyroid function were documented in animal studies (2, 3), with the first successful use of these agents in hyperthyroid patients being reported 1-2 years later (4, 5). Not too long after, the US FDA approval for thioureylene-containing antithyroid medications was issued for propylthiouracil (PTU), methimazole (MMI), and carbimazole, respectively. Since then, ATDs have consistently been used for treatment of various etiologies of hyperthyroidism. Compared to RAI, ATDs have almost always been the preferred therapeutic modality for GD worldwide (6-8) except in North America where remarkable change of the practice pattern in treatment of GD, i.e. more trend toward ATDs, has recently been documented (9). Considering the widespread use of ATDs and recent data about their long-term efficacy and safety, this review aims to summarize different aspects of these outstanding medications, focusing on the most recent evidence regarding their clinical utility.

The main mechanism of intrathyroidal action of ATDs for inhibition of thyroid hormone synthesis is competition with thyroglobulin tyrosine residues for thyroid peroxidase (TPO)-catalyzed iodination, i.e. iodine organification, thereby decreasing numbers of mono- and di-iodotyrosines; they also interfere with the TPO-catalyzed coupling of iodotyrosines
to form thyroid hormones, 3,5,3′-triiodothyronine (T3) and tetraiodothyronine (T4) (10, 11). Other inhibitory effects of ATDs proposed at the late 1970s (12, 13) need to be investigated further.

It is known that PTU inhibits the type 1 deiodinase-catalyzed T4 to T3 conversion in both the thyroid and peripheral tissues (14); this metabolic pathway contributes to nearly one-fifth of serum T3 in normal physiology and at least half in the setting of hyperthyroidism (11).

An interesting aspect of ATDs properties, not yet fully recognized, is their effects on the immune system with remarkable in-vitro evidence but questionable in-vivo findings regarding the issue whether the observed immunosuppressive effect is the direct ATD property or the consequence of decreased thyroid hormone levels (11, 15). Immunomodulatory effects of ATDs on both humoral and cell-mediated immunity have been investigated (16-20).

Herbal formulation of Leonurus cardiota (commonly known as motherwort herb), rich in flavonoid called quercetin, serves as an adjuvant for overactive thyroid especially against hyperthyroidism. Herbal approach for hyperthyroidism can be safe and efficacious measure for managing the thyroid dysfunctions. Currently, there is no scientific evidence has yet evaluated for the efficacy of anti-thyroid activity of Thespesia lampas. But traditionally the herb Thespesia lampas, is proven to heal thyroid issues. As it contains the chemical constituents like flavonoids, tannins which are used to inhibit the production of thyroid hormone. The purpose of this study is to identify a plant with potential antithyroid properties. An investigation was conducted to compare the effects of ethanolic extract of Thespesia lampas on thyroxine induced hyperthyroidism in rats and the standard drug methimazole in the treatment of hyperthyroidism.

Materials & methods

Plant material collection
The plant of Thespesia lampas were procured from the Tirumala hills of Chittor district, Its botanical identity was authenticated (0399) by prof. k.Madavachetty, Department of Botany, Sri Ventateswara University, Tirupati, Preparation of powder
The plant leaves collected were cleansed by soaking in water, shade dried and grinded to get coarse powder and was sieved by sieve no.22 and preserved in a cleaned container.
Ethanolic extraction Process
Powdered leaves of Thespesia lampas + ethanol (1:3) Soxhlet apparatus, Continuous hot extraction until solvent becomes colorless. Extract was evaporated and concentrated by distillation process. The extract was preserved by addition chloroform few drops.

ACUTE TOXICITY STUDIES
The acute oral toxicity study for Thespesia lampas was performed according to the method described by OECD 423.
Animals: Overnight fasted Albino rats weighing 150-180gms.
Number of animals: Animals are divided into groups and each group containing three animals.
Dosage: Dose levels include increasing doses of Thespesia lampas extract (i.e 5, 50, 300 and 2000 mg/kg).
Observation period: 14 days
Observation parameters: salivation, convulsions, writhing, tremors, loss of hair, biting and scratching behavior, licking of tail, paw grooming behavior and diarrhea.

Pharmacological studies:
Thyroxine induced method
• Selection and acclimatization of animals Adult male Wistar rats weighing 180–220g were used in the experiments after acclimatizing for 15 days.
• The animal house in which the rats were placed is approved by the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA)- IAEC/CESCOP/2023-04. The study was carried out after getting the approval from Institutional Animal Ethics Committee (IAEC)
Induction of hyperthyroidism and treatment design

Animals were divided into five groups each consisting of control group rats were given distilled water 5 ml/kg (p.o.). While the other groups of animals were administered with thyroxine (600 µg/kg) (p.o.) for 14 days to induce hyperthyroidism. After 14 days of the administration, all the animals were screened for biochemical markers like triiodothyronine(T3), total thyroxine(T4), and thyroid stimulating hormone (TSH), and for the next 14 days rats were administered with different treatments as follows:

Treatment design

Group-I: Normal diet (control) (28 days with vehicle)
Group-II: Hyperthyroid induced animals (thyroxine 600 µg/kg/ml) for 14 days +14 days vehicle.
Group-III: Hyperthyroid induced animals treated with standard drug (Methimazole 0.04% w/v) for 28 days. (14 days Thyroxine +14 days Methimazole)
Group-IV: Hyperthyroid induced animals treated with Thespesia lampas extract (200mg/kg) for 28 days. (14 days Thyroxine +14 days low dose)
Group-V: Hyperthyroid induced animals treated with Thespesia lampas (400mg/kg) for 28 days. (14 days Thyroxine +14 days high dose)

- The blood samples were collected from all the groups on the initial day by retro orbital vein and on the 28th day by cardiac puncture. After collecting the blood in centrifuge tubes and centrifuged at 3000 rpm for 15 mins and then the supernatant liquid serum was obtained and the collected serum was analysed for thyroid hormone levels (ELISA METHOD) and various biochemical parameters. Then the animal was sacrificed by euthanasia.
- The thyroid gland was immediately dissected out, washed in ice cold saline to remove the blood and stored in 10% formalin for histopathological studies.

Statistical Analysis

The experimental results were expressed as the mean ± SEM (n=6). Data was analyzed using ANOVA followed by the Dunnett test. P value of ≤0.05 and ≤0.01 was considered statistically significant. Data was processed using graph pad prism version 5.0

Results:

PERCENTAGE OF YIELD

The percentage yield of ethanolic extract of Thespesia lampas was calculated using the above formula and was found to be 11.2% w/w.

Phytochemical studies

Table 1: Preliminary phytochemical analysis of ethanolic extracts of Thespesia lampas

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Test</th>
<th>Ethanolic extract</th>
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<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendorff’s test</td>
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</tr>
<tr>
<td></td>
<td>Wagner’s test</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mayer’s test</td>
<td>-</td>
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<tr>
<td>Tannins</td>
<td>Ferric Chloridetest</td>
<td>+</td>
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Acute toxicity studies

The ethanolic extract of Thespesia lampas did not show any mortality and toxic manifestations up to the dose of 2000 mg/kg.b.w. Based on the OECD 423 guidelines, the 1/10 th dose as 200 mg/kg and double dose 400 mg/kg has been selected as the therapeutic doses.

Figure 1: Effect of ethanolic extracts of Thespesia lampas on serum thyroid hormones
Figure 2: Effect of ethanolic extracts of Thespesia lampas on Lipid Profile (HDL, LDL, Triglycerides, AST, and ALT)
Figure 3: Effect of ethanolic extracts of *Thespesia lampas* on organ weights

**Thespesia lampas** effect on liver gland weight

- Group I
- Group II
- Group III
- Group IV
- Group V

<table>
<thead>
<tr>
<th>Treatment groups</th>
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</thead>
<tbody>
<tr>
<td>Group I</td>
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<tr>
<td>Liver gland weight (g)</td>
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<td>5.0</td>
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</tbody>
</table>

**Thespesia lampas** effect on thyroid gland weight

- Group I
- Group II
- Group III
- Group IV
- Group V

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<thead>
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<th>Treatment groups</th>
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<tbody>
<tr>
<td>Group I</td>
</tr>
<tr>
<td>Thyroid gland weight (mg)</td>
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<tr>
<td>4.0</td>
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</tbody>
</table>

Figure 4: Effect of ethanolic extracts of *Thespesia lampas* on body weights of rats

**Thespesia lampas** effect on body weight of rat

- 0th day
- 14th day
- 28th day

<table>
<thead>
<tr>
<th>Treatment groups</th>
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</thead>
<tbody>
<tr>
<td>Group I</td>
</tr>
<tr>
<td>Body weight (gm)</td>
</tr>
<tr>
<td>300</td>
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0th day
14th day
28th day
DISCUSSION

• A hyperthyroid state is characterized by increased production of triiodothyronine and total thyroxine. This results in the decreased levels of thyroid stimulating hormone in the blood leading to hypermetabolic activity.
• The presence of flavonoids and phenols, which have been said to have anti thyroid and antioxidant action, may account for the effects of ethanol extract of Thespesia lampas against hyperthyroidism.
• The second potential method entails blocking of the TPO-mediated iodination of tyrosine residues in thyroglobulin, a crucial step in the synthesis of triiodothyronine and total thyroxine.
• The components that are directly or tangentially involved in the feedback mechanism, as well as the stimulation of hypothalamic cells to secrete thyrotropin-releasing hormone, may be to blame for the increased concentration of thyroid stimulating hormone.

These enzymes serum activities have been discovered to increase in response oxidative stress that hyperthyroidism causes. Rats' serum AST and ALT increases were prevented by giving them Thespesia lampas extracts. Follicles with an irregular shape and numerous follicular cells lined with basophilic nuclei holding empty luminal colloids in the hyperthyroidism model. On the other hand, it has been noted that methimazole therapy causes variable sized follicles with an abundance of colloid. The findings indicated that thyroid gland follicles with filled colloid and big size were present in the groups treated with the crude extract of ETL. As a result, ETL extract significantly reversed the morphological alterations brought on by thyroxine-induced hyperthyroidism in the thyroid gland.

CONCLUSION

Thyroid dysfunction can always be treated with herbal remedies to avoid hormonal side effects. Among the modern scientific explorations and evaluations of ethno medicine from plants, herbal cure is gaining worldwide acceptance. Based on the restoration of biochemical parameters such as thyroid stimulating hormone, triiodothyronine, and total thyroxine, and the histological studies that revealed glandular tissues filled with follicular colloids, the ethanolic extract of Thespesia lampas has antithyroid potential.

REFERENCES:


