

Evaluation of Ethanol Extract of *Caesalpinia bonducella* L. Seeds on Hyperthyroidism

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Abstract

Aim : The study was conducted to evaluate the role of *Caesalpinia bonducella* seed extract on hyperthyroidism in drug induced animal model. *Caesalpinia bonducella*, seen in topical region of India and different part of the world, being used as a folklore medicine from olden days to treat many human ailments. But the scientific evidence for the use of the plant in hyperthyroidism is remained to be evaluated.

Methods: In the study animals were divided into five group with 6 animal in each and they were made hyperthyroidic with the administration of L- thyroxine for 12 day and followed by the treatment with higher(400mg/kg) and lower dose(200mg/kg) of ethanolic seed extract to evaluate the antithyroid activity of the plant.

Results: The serum analysis of triiodothyroxine, tetra iodothyronine, thyroid stimulating hormone was performed, the histopathological analysis of the thyroid gland also done.

Conclusion: Antithyroid activity is evident from the decreased T3 and T4 levels. It can be said that *Caesalpinia bonducella* significantly by virtue of the presence of tyrosine.

Keywords: *Caesalpinia bonducella* seeds, hyperthyroidism, ethanol extract, L- thyroxine *in vivo* study

INTRODUCTION

Hormone and associated disease is one of the most dealing topic in modern medicine. The imbalance of hormones is mostly associated with development of many disorders. Thyroid hormone one of the endocrine secretion playing an important role in the human and animals changes in the level of this hormone is characterized with the development of different medical condition (1). There are many well established treatment for the diseases, but nowadays the herbal medicine being one of the popular consideration in the treatment and anticipation of many disorders (2). Thyroid glands secrete three types of hormone Triiodothyronine (T3), Thyroxine (T4) and calcitonin. T4 which is a prohormone produced in less amount and T3 being the active hormone (3)(4). Thyroid hormones (THs) action are mediated through thyroid hormone receptors (TR) which belongs to the class of nuclear receptors. The plasma concentration of thyroid hormones are regulated by the classical negative feedback mechanism in the presence of thyrotropin releasing hormone (TRH) and thyroid stimulating hormone (TSH)(5). The major medical condition characterized by disturbance of thyroid hormone are Hypothyroidism and Hyperthyroidism. The alteration in the hormone level linked with divers hemodynamic effects and the action of both the hormones are contrary to each other (6). Hyperthyroidism is the diseases condition characterized when body get exposed to large amount of thyroid hormone while, in case of hypothyroidism body receives only abated amount of thyroid hormone(7). Hyperthyroidism is characterized with development of faster heart rate, increased blood pressure, nervousness, anxiety, loose of scalp hair, weight loss, intolerance to warm temperature(8)(9) on the other hand in Hypothyroidism shows decreased heart rate, pulse rate, weight gain, dry skin, face get puffy(10) Drugs from

plants being one of major source for treatment of many human diseases. The metabolites from plants especially secondary metabolites and their derivatives have being used widely over last half century in many human ailments(11).

Caesalpinia bonducella. L belonging to the family Caesalpiniaceae a prickly shrub distributed along the hoter part of India, Srilanka, Andaman and Nicobar and tropical countries of the world(12). It is one of the medicinal plant used to treat many of human and animal ailments since from olden times. In Ayurveda, Unani different plants of plant like root, leaves, seeds, bark being used from ancient times in the treatment of many diseases like fever, malaria, menstrual complaints, pneumonia, skin diseases, swelling, fever, pulmonary tuberculosis, and edema(13). In the recent years the researches on the medicinal plant are more intrestingly gaining importance. Due to the increased side effects of the drugs, resistance to the medicine, high coast of therapy, the demand of medicinal plants and their active metabolites are increasing. In the study the on effect of *Caesalpinia bonducella* seed in the treatment of hyperthyroidism was carried out.

MATERIALS AND MEIHODS

Collection of plant material

The seeds of *Caesalpinia bonducella* where collected from the local markets of Kottayam, Kerala and was authenticated from CMS, College Kottayam by Dr.Rojimon .from the seeds kernels were removed and shade dried.

Preparation of extract

The kernals were collected from the seed, shade dried and powdered. The powdered seeds are extracted with 90% ethanol using soxhlet apparatus. The crude extract was filtered and evaporated to form a dark brown viscous product (14).

Preliminary phytochemical analysis

The phytochemical studies were conducted to find the chemical constituents in the ethanolic extract. The seed extract were subject to test for Alkaloids, Carbohydrates, Glycosides, Proteins And Amino Acids, Steroids, Saponins, Flavonoids, Terepenoids And Tannins Using Mayer's Test, Felhing's Test, Isoprenid Test, Foam Formation Test, Salkowski test, Ferric chloride test etc(15).

EXPERIMENTAL ANIMALS

In the study, 36 Female *Albino wistar* rats weighing (150 – 200g) were used in the study. The study was approved by the Institutional Animal Ethical Committee, Department Of Pharmaceutical Sciences, Cheruvandoor Campus (IAEC/M.Pharm/DPS/2019-12). The animals were housed in cages, maintained under standard conditions (12 hours light and dark cycles, room temperature and 45 – 55% relative humidity. They were given standard pellet diet (kvasu nutrition dept.) water *ad libitum* during the period of acclimatization for a week.

THYROID INHIBITING ACTIVITY TEST

Procedure

A total of **five groups**, control, positive control, test (2 groups) and standard are selected for the study. Each group contains **six *Albino wistar rats*** (thus a total of 30 animals) weighing 150- 200g. Group I animals received 0.1 ml CMC (the vehicle for extract preparation) every day for the entire study period and served as the normal control group. Rats in Groups II and III were administered (*p.o.*) with a prestandardized dose of L-thyroxine (L-T4; 500 mg/kg per day) for 12 consecutive days to render them hyperthyroidic. From the 13th day of the study, the Group II animals continued to receive only L-T4, whereas animals in Group III were treated with both low dose of extract (200 mg/kg per day, *p.o.*). The animals in Group IV were treated with higher dose of extract (400 mg/kg per day *p.o.*) and group V treated with Propylthiouracil (PTU) as standard drug (10 mg/kg per day *i.p.*) for same duration (17 days)(16). After 17 days of treatment, the experiment was terminated. On the last day, the bodyweight of each animal was again recorded. Animals were then killed by cervical dislocation.

ASSESSMENT OF ACTIVITY

Collection of blood for serum analysis

The samples were collected during the 12th day and

30th day of the experiment. The blood was withdrawn through retro orbital plexus under anesthetic condition on the 12th day and 30th day using cardiac puncture. From the samples of blood the serum being separated and stored at -70^o C before analysis. The serum level of T3, T4, TSH were determined on the 12th day and 30th day by using ELISA kit method.

Histopathological analysis

The thyroid gland of animals being collected and fixed in 10% formalin. After paraffin embedding, the section of the tissue were stained with haematoxylin and eosin (H& E) for an optical microscopy examination. Subsequently, the histological profile of the thyroid gland tissues was observed. the thyroid follicles, colloid and follicular lining epithelium were observed using an automated image analyzing process.

Statistical analysis

All the result were shown as average \pm S.E.M. data was statistically evaluated by one – way analysis of variance (ANOVA) followed by post hoc Tukey's multiple comparison test using Graph pad prism 8 software. $P < 0.05$ was considered as statistically significant.

RESULT AND DISCUSSION

Effect of *Caesalpinia bonducella* extract oh hyperthyroidism

From the data of the blood samples collected on the 12th day and 30th day of the study. the T3, T4 and TSH of the normal control animals was found to be 27.28ng/dl, 4.687 μ g/dl, 0.363 mIU/ml respectively. On the 12th day in all other groups (II, III,IV,V) animals treated with the LT4 shows an increase in the level of T3, T4 and TSH value were decreased this shows all the animals were induced hyperthyroidism. The results for blood samples on the 30th day the animals treated with standard drug PTU and the animals treated with extract the T3, T4 values were increased and the TSH values increase and become comparable to normal control. Group treated with 400mg/kg of ethanolic extract of *Caesalpinia bonducella* showed better result than the other concentration 200mg/kg and was found to be effective as the standard drug propylthiouracil.

THYROID INHIBITING ACTIVITY TEST SERUM ANALYSIS

Table 1: Thyroid function test values on 12th day (induction of hyperthyroidism) of thyroid inhibiting activity test.

Groups	T3 values (ng/dl)	T4 values (μ g/dl)	TSH values (mIU/ml)
Normal control	27.38 \pm 0.4687	4.724 \pm 0.0388	0.363 \pm 0.0002
LT4 induced	120.5 \pm 2.15	18.37 \pm 0.0647	0.0127 \pm 0.0001
CB (200 mg/kg)	163.2 \pm 1.045	17.95 \pm 0.0784	0.0125 \pm 0.0002
CB (400 m/kg)	155.5 \pm 1.231	17.39 \pm 0.0928	0.0133 \pm 0.0002
Standard (PTU treated)	148.1 \pm 1.512	19 \pm 0.0440	0.0148 \pm 0.0001

Table 2: Thyroid function test values on 30th day (at the end of the study) of thyroid inhibiting activity test.

Groups	T3 values (ng/dl)	T4 values (µg/dl)	TSH values (mIU/ml)
Normal control	27.31 ± 0.4917	4.667 ± 0.0424	0.0368 ± 0.0001
LT4 induced	152.5 ± 0.5268 ^a	27.34 ± 0.104 ^a	0.00965 ± 0.0001 ^a
CB (200 mg/kg)	33.3 ± 0.7944 ^{r, x, c}	4.534 ± 0.009 ^{r, x, c}	0.0377 ± 0.0001 ^{ns, x, c}
CB (400 m/kg)	55.05 ± 0.2955 ^{ns, x, b}	5.123 ± 0.01432 ^{ns, x, b}	0.0390 ± 0.0002 ^{r, x, b}
Standard (PTU treated)	24.11 ± 0.4421 ^{x, ns}	4.852 ± 0.05741 ^{x, ns}	0.0375 ± 0.0001 ^{x, ns}

Estimation of serum T₃ level

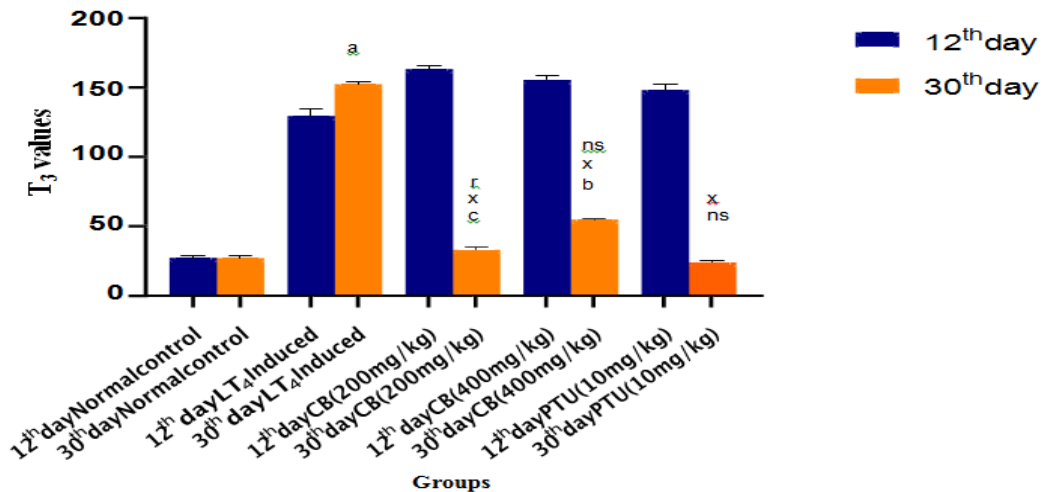


Fig 1: Estimation of serum T₃ on the 12th and 30th day

Values are expressed as mean ± SEM. One way ANOVA followed by Tukey’s multiple comparison, ^a p < 0.001, ^b p < 0.01, ^c p < 0.05 as compared to normal control. ^x p < 0.001, ^y p < 0.01, ^z p < 0.05 as compared to LT4 treated, ^p p < 0.001, ^q p < 0.01, ^r p < 0.05 as compared to PTU treated.

Estimation of serum T₄ level

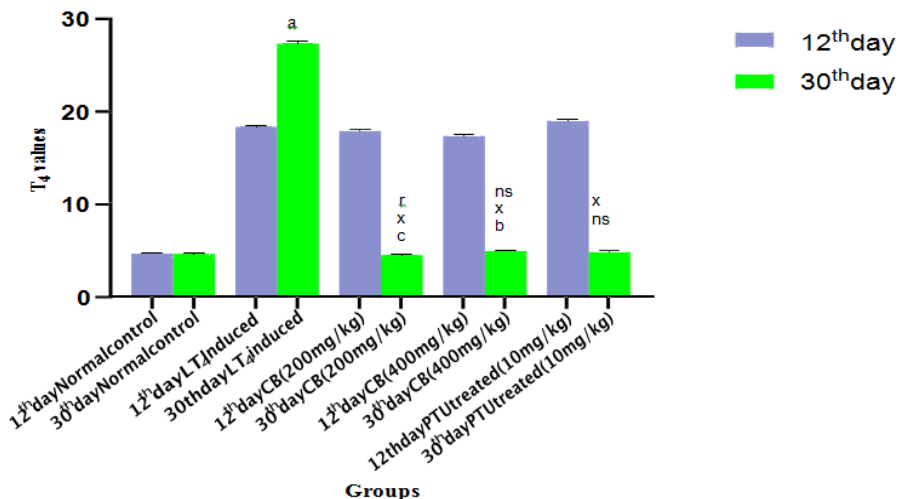


Figure 2: Estimation of serum T₄ on the 12th and 30th day

Values are expressed as mean ± SEM. One way ANOVA followed by Tukey’s multiple comparison, ^a p < 0.001, ^b p < 0.01, ^c p < 0.05 as compared to normal control. ^x p < 0.001, ^y p < 0.01, ^z p < 0.05 as compared to LT4 treated, ^p p < 0.001, ^q p < 0.01, ^r p < 0.05 as compared to PTU treated.

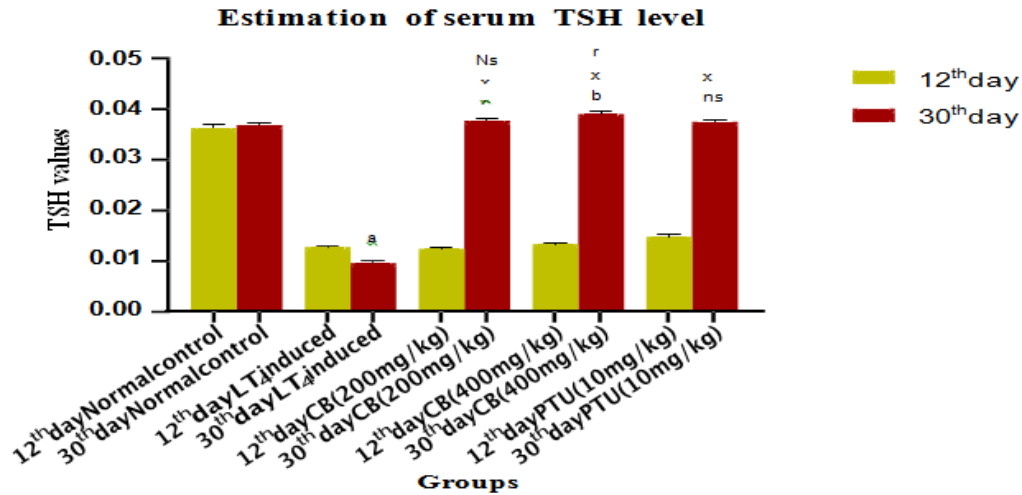


Figure 3: Estimation of serum TSH on the 12th and 30th day

Values are expressed as mean ± SEM. One way ANOVA followed by Tukey's multiple comparison, ^a p < 0.001, ^b p < 0.01, ^c p < 0.05 as compared to normal control. ^x p < 0.001, ^y p < 0.01, ^z p < 0.05 as compared to LT4 treated, ^p p < 0.001, ^q p < 0.01, ^r p < 0.05 as compared to PTU treated.

HISTOPATHOLOGY

From the histopathological data the animals of normal control shows numerous number of follicular cells which are simple cuboidal. The cavity is filled with moderate amount of colloid and show less inward scalloping occasionally and follicular cells have round nuclei with clear cytoplasm. The animals of hyperthyroidic group the follicles are lined by follicular epithelial cells with scanty

colloid and there is no papillary infolding. In the animals treated with standard drug propylthiouracil showed rounded nuclei with clear cytoplasm. In the case of animals treated seed extract the cells are found to be normal filled with follicular cells and many of the follicular cells shows scalloping and there is no papillary infolding of epithelial cells.

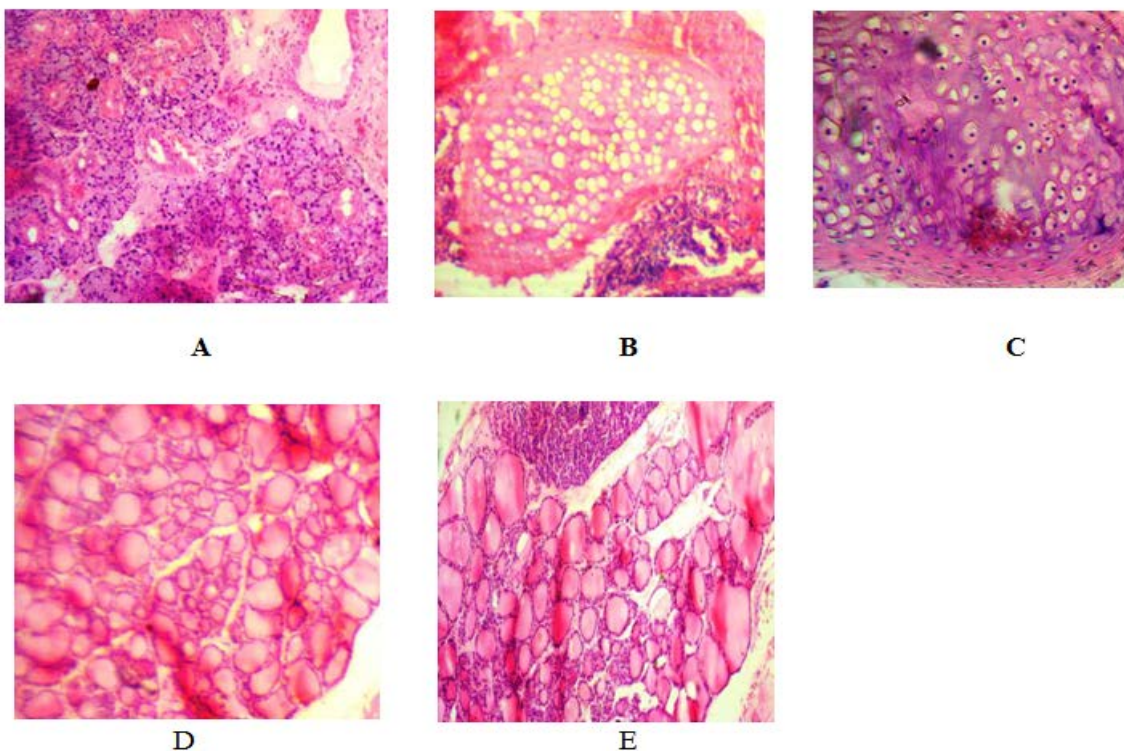


Fig. 3: Histopathology of thyroid glands in different groups A) Normal Control rat B) Hyperthyroid induced rat C) Hyperthyroid induced rat treated with FRBE 200mg/kg D) Hyperthyroid induced rat treated with FRBE 400mg/kg E) Hyperthyroid induced rat treated with Propylthiouracil

DISCUSSION

The last couple of decades have seen a tremendous increase in interest in the biological properties of natural products as a means to identify novel compounds that could have potential in clinical medicine(17). The active components like phenolics, flavonoids and flavonoid-like compounds percolate to the top due to their presence in diet constituents and reported beneficial effects on diverse biological processes and disease conditions. It has previously been reported that the consumption of flavonoids and some phenolic acids by experimental animals induced changes in the thyroid gland. Ethanopharmacological studies on such herb, medicinally important plants continue to interest investigators throughout the world. One such plant, *Caesalpinia bonducella* linn invites attention of the researchers worldwide for its pharmacological activities.

The results of the preliminary phytochemical analysis showed the presence of alkaloids, carbohydrates, phenolic, flavonoids, terpenoids, saponins etc; in which the flavonoids and phenolics are found to be the prominent. These are some of the most important secondary metabolites helps in the inhibition of enzymes like thyroid peroxidase, involved in the thyroid hormone synthesis.

The thyroid inhibiting study was performed by the induction of hyperthyroidism with the administration of LT4, resulting in the increased levels of T3, T4, and decreased levels of TSH and it confirmed the animals were induced with hyperthyroidism. After this the animals were treated with standard drug PTU and EECB (200, 400) upto 30th day and the levels of T3,T4, TSH were again examined. From the results collected on the 30th day it was evident that in the animals treated with the extract T3, T4 levels are decreased and TSH values were increased and which are comparable to normal control, of these the animals treated with a dose of 400 mg/kg show a better result which comparable to the normal and the animals treated with standard drug propylthiouracil. This indicates ethanolic concentrate of CB can possibly cures hyperthyroidism in rats

The histopathological observation of thyroid gland shows change in amount of thyroid follicles and colloid, in the animals of normal control the cells was found to be normal. For the animals treated with CB extract shows the cells are filled with thyroid follicles and moderate amount of colloid. While for the animals treated with the standard drug propylthiouracil the follicular cells was found to be normal and cells were filled with 80- 90% of colloid. Thus it can be concluded that the cells of the thyroid gland shows an improvement with the treatment of extract.

CONCLUSION

The present investigation was conducted to study the effect of the potential antithyroid activity of ethanolic extract of *Caesalpinia bonducella* on L-thyroxine induced

hyperthyroidism in wistar rats. From the findings it can be inferred that the animals treated with the plant concentrate(400mg/kg) shows a great effect as like that of the standard medication and the histopathological studies of the thyroid gland of data also proves the same. Hence, upon all findings and assumption it can be said that the ethanolic extract of *Caesalpinia bonducella* seed can possibly overcome hyperthyroidism in albino rats.

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