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To identify the anti-hypothyroidism activity of phytosomes of isolated compounds from *Phyllanthus amarus* Linn

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Abstract

Hypothyroidism is associated with a deficiency in the production of thyroid hormones viz. T₃ and T₄ which is common in hilly areas. Iodine deficiency is the most common cause of hypothyroidism in many areas of developing countries in Asia, Sub-urban Africa and Latin America. The problem of iodine deficiency disorders (IDD) is hypothesized to be aggravated by malnutrition, environmental goitrogens and microbial pollution in these areas. Worldwide, thyroid dysfunction is a probable risk factor for 1 to 1.5 billion people usually considered due to simple iodine deficiency and presented as goitre (At least 200 million people). Amongst the various thyroid disorders such as hypothyroidism and hyperthyroidism; hypothyroidism is more common. Women are more prone to thyroid disorders especially those having a history of autoimmune disorders. Hypothyroidism is conventionally treated by administering a daily dose of synthetic thyroid hormones e.g. Levothyroxine. However one of the most important adverse effects associated with synthetic thyroid hormone therapy is hyperthyroidism, a higher dose could cause symptoms of nervousness, palpitations or insomnia, typical of hyperthyroidism.

Keywords: Hypothyroidism, phyllanthin, hypophyllanthin, T₃, T₄, TSH, nanoparticle phytosomes

Introduction

In the present studies, the curative activity of Phyllanthin, Hypophyllanthin, combination of Phyllanthin and Hypophyllanthin as well as of 50% Petroleum ether extract of *Phyllanthus amarus* was studied and the results were compared with the marketed drug Levothyroxine (Eltroxin) using the model of Propylthiouracil induced hypothyroidism in *Wistar* albino rats for a period of 22 days^[1, 2]. A study was also carried out to elucidate the TNF-alpha levels in order to evaluate the probable mechanism of action^[3]. Herbal medicines are now in great demand in the developing world for primary health care for better cultural acceptability, better compatibility with the human body and minimal side effects^[4]. So far, no drug is available for the treatment of hypothyroidism or hyperthyroidism preventive or curative for human use in any system of medicine all over the world. *Phyllanthus amarus* (Family: Euphorbiaceae) henceforth referred as PA has been reported to possess thyroxine like activity^[5, 6]. Work was done to explore the thyroxine like activity of Phyllanthin, Hypophyllanthin and combination of both against Propylthiouracil induced hypothyroidism and it was concluded that phyllanthin and hypophyllanthin possess thyroxine like activity and two compounds when administered together were found to possess more activity^[7, 8]. The concentrations of T₃, T₄, TSH and TNF-alpha in the blood serum of the animals were calculated from the standard curves of the same which were constructed by plotting the mean absorbance obtained for each reference standard against its concentration specified in the ELISA kits using ORIGIN software^[9, 10]. The data was subjected to One Way Analysis of Variance (ANOVA) and the significance of the difference between the groups and among the drugs was performed by employing Bonferroni's multiple comparison tests^[11]. Values are expressed as mean ± S.E.M and P values less than 0.05 ($p < 0.05$) were considered significant^[12].

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Materials and Methods

Protocol was approved by an Institutional Ethical committee formed as per the norms of CPCSCA (Committee for Control and Supervision of experimentation on Animals), 2019 and research was conducted in accordance with the internationally accepted principles for laboratory animal use and care. Healthy albino rats (150-250 gm). The animals were housed at a temperature of 20-24 °C and relative humidity of 30-70% with a 12-h light-dark cycle. Food and water were provided *ad libitum*.

Hypothyroidism was induced by oral administration of propylthiouracil (0.75mg/kg) for a period of 30 days. The body weight of each animal was measured weekly. Blood was withdrawn from the retro-orbital plexus on the 1st, 15th, and 30th days using heparin-coated capillary tubes. The serum was separated from the collected blood by refrigerated centrifuge at 4 °C; 4000 rpm for 20 min. The serum was stored at -20 °C for the analysis of various parameters. T₃, T₄, and TSH levels were measured for each serum sample collected using an ELISA reader. Serum cholesterol and total protein were also determined using an autoanalyzer. The method of inducing hypothyroidism was standardized and evaluated by the increased serum TSH levels and decreased T₃ and T₄ levels. The animals in which hypothyroidism was induced were divided into six groups of six animals each and the drugs were administered daily for up to 22 days. The group that was administered standard Eltroxin (30µg/kg) orally served as positive control. Three dose levels i.e., 1, 3, 6 mg /kg of each drug Phyllanthin, Hypophyllanthin, a combination of both and Hydro-alcoholic extract were administered orally to the respective groups for 22 days. The body weight of each animal was measured weekly. Blood was withdrawn from the retro-orbital plexus of the animals on the 15th and 22nd day of treatment using heparin-coated capillary tubes. The serum was separated from the collected blood by refrigerated centrifuge at 4 °C; 4000 rpm for 20 min and was stored at -20 °C for the analysis of various parameters. T₃, T₄, and TSH levels were measured for each serum sample collected using an ELISA reader. Serum cholesterol and total protein were also determined using an autoanalyser. All the rats were sacrificed after 24 hours of the last injection i.e. on the 23rd day, the thyroid gland was isolated and weighed rapidly to avoid loss due to evaporation and preserved in neutral formalin buffer for histopathological studies.

TNF-alpha values were estimated using ELISA in the serum of the curative model on the 1st and 30th days of induction with propylthiouracil. Also, values were estimated on the

22nd day of treatment with phyllanthin, hypophyllanthin, their combination and 50% Hydro-alcoholic extract in order to investigate whether these isolated components are effective against hypothyroidism through its anti-inflammatory activity. It is known that the plant PA possesses anti-inflammatory activity. Aqueous and hexane extracts of PA have demonstrated the ability to inhibit nitric oxide (NO) and prostaglandin E2 (PGE2), attenuation of tumour necrosis factor-alpha (TNF-alpha), endotoxin-induced nitric oxide synthase (iNOS), cyclooxygenase (COX-2) and inhibited NF- kappa- B production *in vitro* as well [7]. The same extracts inhibited the induction of interleukin (IL)-1beta, IL-10, and interferon-gamma in human whole blood and reduced TNF- alpha production *in vivo*.

The drugs used were: Propylthiouracil (Sigma Aldrich Pvt Ltd, India), Eltroxin (GlaxoSmithKline, India) and standards of phyllanthin and hypophyllanthin (Natural Remedies, Bangalore). The drugs phyllanthin, hypophyllanthin, HE, propylthiouracil and Eltroxin were dissolved in 2% gum acacia suspension just before use. Kits used were: ELISA kits for T₃, T₄, TSH (Syntron Bioresearch, Inc., India); Rat TNF-A (Diacclone, India); total protein (RFCL Ltd, India); and total cholesterol (Erza brothers, Mumbai, India).

The concentrations of T₃, T₄, TSH and TNF-alpha in the blood serum of the animals were calculated from the standard curves of the same which were constructed by plotting the mean absorbance obtained for each reference standard against its concentration specified in the ELISA kits using ORIGIN software. The data was subjected to One Way Analysis of Variance (ANOVA) and the significance of the difference between the groups and among the drugs was performed by employing Bonferroni's multiple comparison tests. Values are expressed as mean ± S.E.M and P values less than 0.05 ($p < 0.05$) were considered significant.

Result and Conclusion

Thyroid hormone modulating activity of two components *viz.* phyllanthin and hypophyllanthin isolated from the plant *Phyllanthus amarus* Linn. was evaluated in the form of phytosomes in experimental animals using propylthiouracil induced hypothyroidism in rats.

The activity of hypophyllanthin phytosomes was found to be more efficacious than that of the phyllanthin phytosomes.

Illustrations

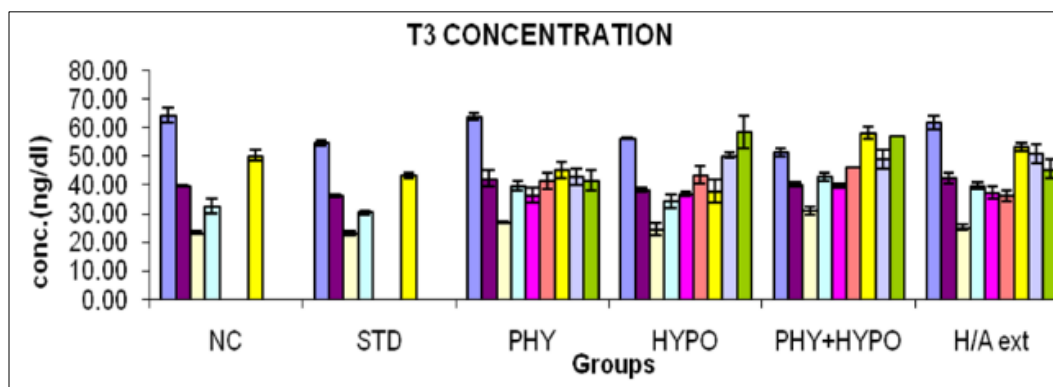


Fig 1: T₃ levels (ng/dl) in different groups on different days of induction and treatment at different doses (Mean ± S.E.M.)

All animals were administered 0.75 mg/kg of propylthiouracil (PTU) for 30 days so as to induce hypothyroidism. Then, the following drugs were administered for 22 days for treatment. NC- negative control group was administered 2% gum acacia, STD- standard drug Eltroxin was administered (30µg/kg), PHY-

phyllanthin was administered at a dose level of 1,3,6 mg/kg, HYPO-hypophyllanthin was administered at a dose level of 1,3,6 mg/kg, PHY+ HYPO- combination of phyllanthin and hypophyllanthin was administered at a dose level of 1,3,6 mg/kg, H/A ext- Hydro-alcoholic extract was administered at a dose level of 1,3,6 mg/kg ($p < 0.05$).

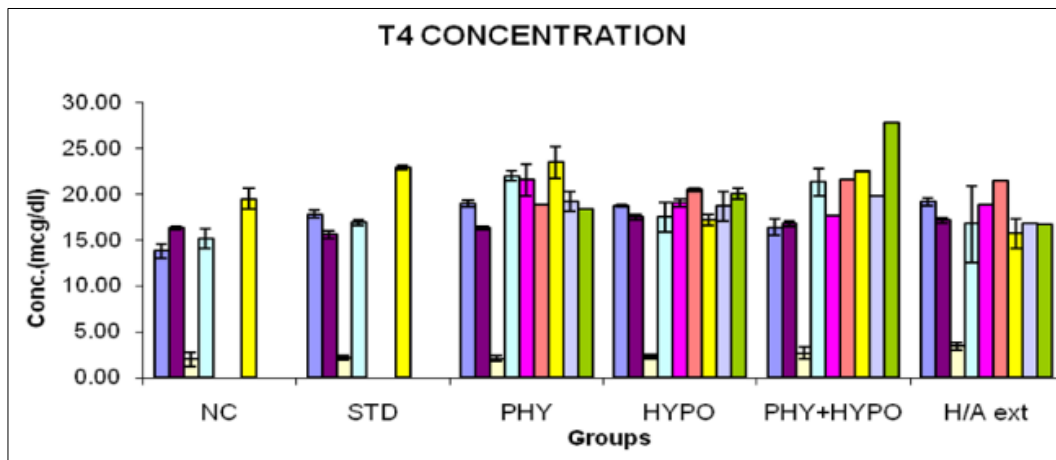


Fig 2: T4 levels (µg /dl) in different groups on different days of induction and treatment at different doses. (Mean ± S.E.M.)

All animals were administered 0.75 mg/kg of propylthiouracil (PTU) for 30 days so as to induce hypothyroidism. Then following drugs were administered for 22 days for treatment. NC- negative control group was administered 2% gum acacia, STD- standard drug Eltroxin was administered (30 µg/kg), PHY- phyllanthin was

administered at a dose level of 1,3,6 mg/kg, HYPO-hypophyllanthin was administered at a dose level of 1,3,6 mg/kg, PHY+ HYPO- combination of phyllanthin and hypophyllanthin was administered at a dose level of 1,3,6 mg/kg, H/A ext- Hydro-alcoholic extract was administered at a dose level of 1,3,6 mg/kg ($p < 0.05$).

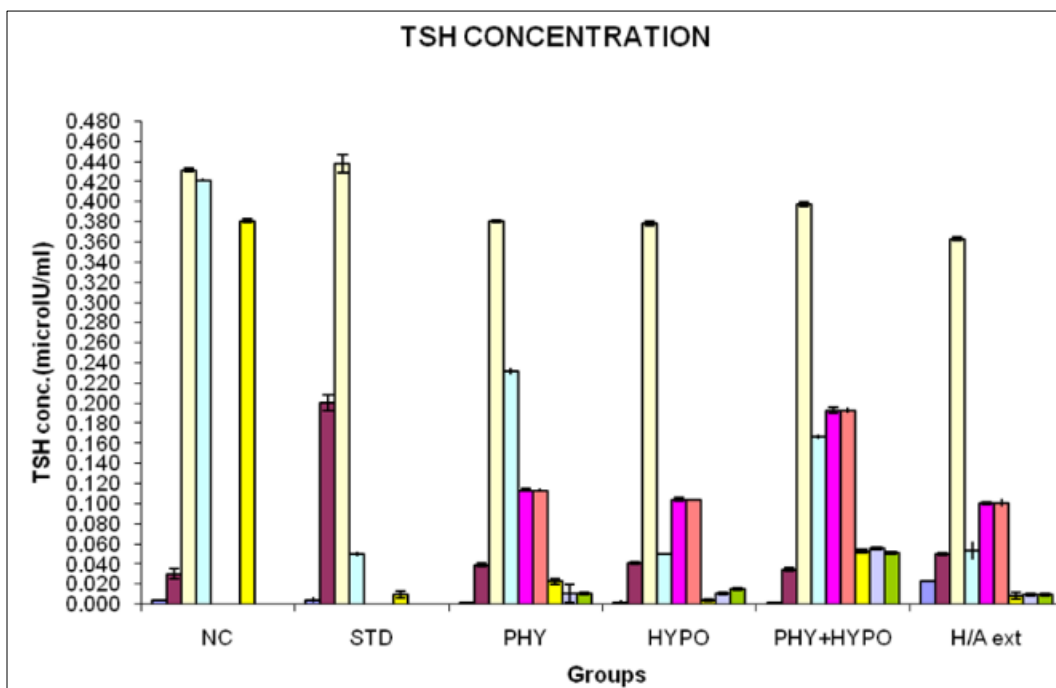


Fig 3: TSH levels (microIU/ml) in different groups on different days of induction and treatment at different doses (Mean ± S.E.M.)

All animals were administered 0.75 mg/kg of propylthiouracil (PTU) for 30 days so as to induce hypothyroidism. Then following drugs were administered for 22 days for treatment. NC- negative control group was administered 2% gum acacia, STD- standard drug Eltroxin was administered (30 µg/kg), PHY- phyllanthin was

administered at a dose level of 1,3,6 mg/kg, HYPO-hypophyllanthin was administered at a dose level of 1,3,6 mg/kg, PHY+ HYPO- combination of phyllanthin and hypophyllanthin was administered at a dose level of 1,3,6 mg/kg, H/A ext- Hydro-alcoholic extract was administered at a dose level of 1,3,6 mg/kg ($p < 0.05$).

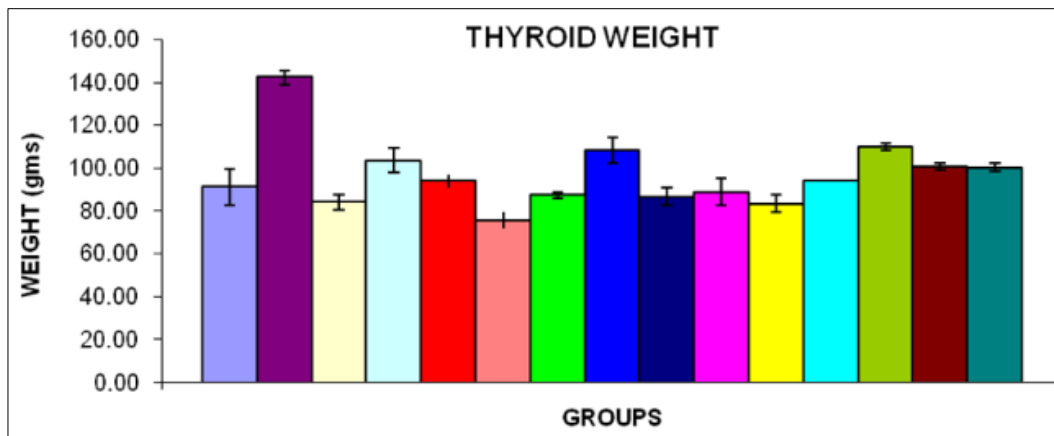


Fig 4: Weight of thyroid gland (mg) in different groups at different doses (mean ± S.E.M.)

All animals were administered 0.75 mg/kg of propylthiouracil (PTU) for 30 days so as to induce hypothyroidism. Then following drugs were administered for 22 days for treatment. NC- negative control group was administered 2% gum acacia, STD- standard drug Eltroxin was administered (30µg/kg), PHY- phyllanthin was administered at a dose level of 1,3,6 mg/kg, HYPO- hypophyllanthin was administered at a dose level of 1,3,6 mg/kg, PHY+ HYPO- combination of phyllanthin and hypophyllanthin was administered at a dose level of 1,3,6 mg/kg, H/A ext- Hydro-alcoholic extract was administered at a dose level of 1,3,6 mg/kg ($p < 0.05$).

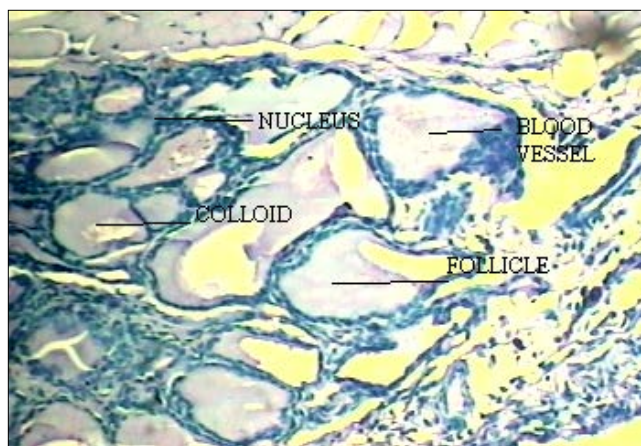


Fig 7: Histology of the rat thyroid gland treated with the standard drug Eltroxin (10X)

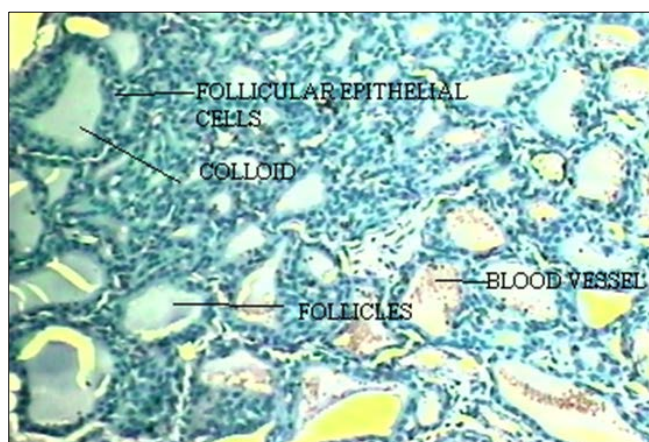


Fig 5: Histology of the normal rat thyroid gland (10X)

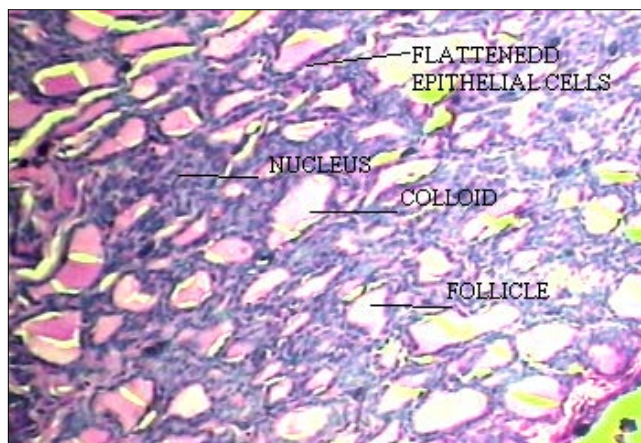


Fig 8: Histology of rat thyroid gland treated with phyllanthin, hypophyllanthin, combination of both and Hydro-alcoholic extract (10X)

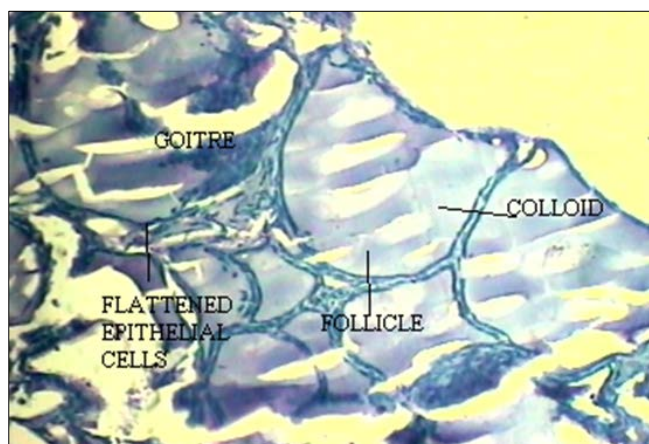


Fig 6: Histology of the rat thyroid gland treated with propylthiouracil and suffering from hypothyroidism or goiter (10X)

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Conflict of interest

The study's authors certify that they have no known financial or personal conflicts of interest that could skew the results.

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