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THYROXINE ACTIVITY OF PHYLLANTHIN & HYPOPHYLLANTHIN ON FEMALE & MALE WISTAR RATS

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ABSTRACT

Hypothyroidism is the disease state in humans and animals caused by insufficient production of thyroid hormone. Hypothyroidism is treated with the levorotatory form of thyroxine (L-T4) and triiodothyronine (L-T_3). But they may cause various side effects and may also be economically costly. Herbal drugs have been used since ancient time for curing various diseases. One such plant is *Pyllanthus amarus*, it is used as an hepatoprotective. Since, the liver metabolises the thyroid hormones T_3 and T4; therefore, in a liver disorder the serum concentrations of both the thyroid hormones may be affected. Encouraging results were obtained when isolated compounds of Phyllanthus amarus viz., Phyllanthin and Hypophyllanthin showed thyroid activity quantified by inducing hypothyroidism in rats using chemical agents like Propythiouracil. Various parameters such as Total protein, total cholesterol, T3 and T4, TSH, daily food and water intake, histopathological parameters, reproductive hormones, weekly body weight, thyroid weight were estimated.

INTRODUCTION

The thyroid gland is a butterfly-shaped endocrine gland located in the base of the neck. It releases hormones that control metabolism, regulating vital functions including: Breathing,

heart rate, body weight, body temperature, cholesterol levels etc. It releases two major hormones T_3 (triiodothyronine) and T4 (tetraiodothyronine).

Almost one-third of the world's population lives in areas of iodine deficiency. In areas where the daily iodine intake is below 50 µg, goitre is usually endemic, and when the daily intake falls below 25 µg, hypothyroidism is seen. The prevalence of goitre in areas of severe iodine deficiency can be as high as 80 %. Populations at particular risk tend to be remote and live in mountainous areas in South-East Asia, Latin America and Central Africa **(Mark *et al*, 2009).** In India too, about 42 million people in India suffer from thyroid diseases. This review will focus on the epidemiology of five common thyroid diseases in India: (1) hypothyroidism, (2) hyperthyroidism, (3) goiter and iodine deficiency disorders, (4) Hashimoto's thyroiditis, and (5) thyroid cancer.

Congenital hypothyroidism is common in India, the disease occurring in 1 out of 2640 neonates, when compared with the worldwide average value of 1 in 3800 subjects. Hypothyroidism is more prevalent in women with about 11.4%, when compared with men, in whom the prevalence was 6.2%. In a hospital-based study of women from Pondicherry, subclinical and overt hyperthyroidism were present in 0.6% and 1.2% of subjects (Ambika *et al*, 2011).

Hypothyroidism is the disease state in humans and animals caused by insufficient production of thyroid hormone. Hypothyroidism is treated with the levorotatory form of thyroxine (L-T4) and triiodothyronine (L-T_3). Compounds like Methimazole, Carbimazole, Thiourea, and Propylthiouracil are used to treat hyperthyroidism, which is a disease state in which there is excessive production of thyroid hormone.

Herbal medicines are found to be more effective for long-standing health issues that do not respond well to available medicines also because of their lower cost and easy availability are preferred. One such herb used since ancient times for curing various diseases is *Pyllanthus amarus*. This plant has been used for its hepatoprotective (Agrawal *et al*, 1985). The hepatoprotective activity of the plant was previously well documented and established in our laboratories. Since, the liver metabolises the thyroid hormones T_3 and T4; therefore, in a liver disorder the serum concentrations of both the thyroid hormones may be affected. Thus, the thyroid levels were determined as one of the study parameters to evaluate the functioning of liver.

Phyllanthus amarus is an erect annual herb 10-50cm high, with a cylindrical stem 1.5-2mm thick with 15-30 leaves. Phyllanthus species are found in countries like India, China, Cuba, Nigeria and Guam. Phyllanthus amarus is a common pantropical weed in moist and shady places.

The plant has been used in Ayurvedic medicine over 2000 years and has a wide number of traditional uses. It is useful in jaundice, hypothyroid, gonorrhoea, frequent menstruation, diabetes, dropsy, diarrhoea, dysentery, intermittent, fevers, diseases of urino-genital system. Used topically as a poultice for skin ulcers, sores, swelling and itchiness, scabies ulcers and wounds. The healing applications are bitter, astringent, deobstruent, cooling, diuretic, stomachic, febrifuge and antiseptic (Aparna et al, 2015).

The effect of Phyllanthin and Hypophyllanthin (isolated constituents of Phyllanthus amarus) for treatment of hypothyroidism was experimented and found that they possess significant thyroxine like activity.

2. MATERIALS AND METHODS

Encouraging results were obtained when isolated compounds of Phyllanthus amarus viz., Phyllanthin and Hypophyllanthin showed thyroid activity quantified by inducing hypothyroidism in rats using chemical agents like propythiouracil. Various parameters such as Total protein, total cholesterol, T_3 and T4, TSH, daily food and water intake, histopathological parameters, reproductive hormones, weekly body weight, thyroid weight were estimated.

2.1. Sample extraction

The dried leaves of phyllanthus amarus (1kg) were thoroughly mixed with sodium carbonate(100g) in water(200ml) and dried for 24 hours. The mixture is the extracted with petroleum ether using a thimble (8 syphonings). A dark green residue was obtained after the removal of solvent by distillation. It was digested in warm alcohol (500ml) and allowed to stand in cold for a day. It was filtered with a vacuum filter and concentrated to one-fourth volume. A waxy solid was obtained which was washed with little alcohol. The alcoholic solution was distilled to recover the solvent. Dark green waxy solid was obtained. Digested in petroleum ether (300cc) andallowedto stand overnight in refrigerator. Crystals wereobtained (1.45g) M.p. 85-90˚C. Crystals were deposited which were filtered and washed with little petroleum ether to render them colourless.

Solid residue containing bitter principle, chlorophyll was subjected to treatment with alkali 1-4% alcoholic potash. It was refluxed for 1 hou. The solvent was the distilled and water was added. Pale yellow solid is separated out which was collected and washed with water and dried later. The dried material was crystallised with diethyl ether: petroleum ether (1:10) mixture. Pale yellow bitter flakes of Phyllanthin were obtained. The petroleum ether solution was concentrated. Waxy crystalline solid was separated out. It is refluxed with alcoholic potash (350ml) for 7 hours. Solvent was removed andwater was added to separate the solid. The solid was washed with water, crystallised from alcohol. Shining prisms of Hypophyllanthin were obtained. The two isolated compounds were validated with standards purchased from Natural Remedies, Bangalore (Phyllanthin and Hypophyllanthin) using HPTLC.

2.2. Experimental animals

Male and Female (150-250g) Albino Wistar rats were used in the study. All animals were kept under identical conditions at DIPSAR animal house at 25±2˚C. The animals were fed standard water and diet.

2.3. Design of the study

Five groups of albino rats weighing (150-250g) were used in groups of 3 males and 3 females each:

Group No.	Treatment	No. of animals
I	Normal (2% gum acacia w/v suspension)	3+3
II	1mg/kg Propylthiouracil in 2% gum acacia w/v suspension	3+3
III	1mg/kg Propylthiouracil in 2% gum acacia w/v suspension along with the standard Thyroxine 40µg/kg in 2% gum acacia w/v suspension	3+3
IV	1mg/kg Propylthiouracil in 2% gum acacia w/v suspension along with 1mg/kg of compound P	3+3
V	1mg/kg Propylthiouracil in 2% gum acacia w/v suspension along with 1mg/kg of compound HP	3+3

They were administered orally for a period of 15 days. In each group blood samples were withdrawn from the tail vein at weekly intervals. All the rats were sacrificed on the 15th day. The thyroid glands were isolated and weighed rapidly to avoid loss due to evaporation. Measuring TSH, T3, T4 monitors the effect of PTU and the inhibition of TSH rise by test compound was used as a parameter for goiter prevention. Also, animal weight, thyroid weight, food and water intake, liver protein levels, blood cholesterol are other few parameters evaluated.

2.4. Procedure

Evaluation Parameters

1. Serum T_3
2. Serum T_4
3. Serum TSH
4. Thyroid weight
5. Animal body weight
6. Daily food intake
7. Daily water intake
8. Total protein
9. Total cholesterol

Body weight was measured using standard weighing balance at the beginning and there after every week of the experiment. Food and water intake was calculated by measuring the amount of rat feed (200g) which was placed in each cage at 9:00am and on the next day the amount left at the same time was measured using an analytical weighing balance. Similarly, water bottles with 450mL of water was filled and was placed in the cages at 10:00 am daily. At the end of the experiment all the animals were sacrificed and the thyroid gland was isolated and then immediately weight. TSH, T3 and T4 was estimated using ELISA kit. The desired number of coated wells were secured. Dispensing of 100µl of standards, specimens, and controls into appropriate wells and then 100µl of Enzyme Conjugate reagent into each well was done. After mixing for 30 secs, incubation at (18-25˚C) for 60 minutes is performed. After dispensing of 100µl of TMB Reagent into each well it is mixed and reaction is stopped by adding 100µl of Stop Solution to each well. All of the blue colour changes completely to yellow and the absorbance at 450nm with a microtiter plate reader is read within 15 minutes. A standard curve was constructed by plotting the mean absorbance obtained for each reference standard against its concentration in µg/dl on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis. Using the mean absorbance value for each sample, determine the corresponding concentration of TSH in µg/dl from the standard curve.

The principle of the protein assay is biuret reaction in alkanline solution, cupric ions react with all compounds with two amide or peptide bonds linked either directly through an

intermediated carbon atom to form violet colour complex. The intensity of this coloured complex is directly proportional to the protein concentration in the sample.

Materials provided with the kit included Biuret Reagent (2 x 150mL) and protein standard (8 g/dl x 5 ml). The blank, standard and the samples were prepared and mixed according to the table above and were incubated at room temperature (22-28˚C) for 20 minutes. The concentration of total protein in gm/dl of the samples and standard were using auto analyser at 546 nm/ green filter against blank within 60 minutes. Total protein in g/dl= Absorbance of Test/ Absorbance of standard x 8**.** Cholestrol esterase hydrolyses esterified cholestrols to free cholesterol. The free cholesterol is oxidized to form hydrogen peroxidase, which further reacts with phenol and 4-aminoantipyrine; by the catalytic action of peroxidase to form a red colour quinonimine dye complex. Intensity of the colour formed is directly proportional to the amount of cholesterol present in the samples. Materials provided with the kit included Buffered enzyme/chromogrn (1x25ml), Buffered enzyme/chromogen (5 x 25ml), Buffered enzyme/chromogen (6x100ml) and Standard (200 mg/dl). The blank, standard and sample were prepared and mixed as per the table and were incubated for 10 minutes at 37˚C for 20 minutes at 20-25˚C. The concentration of cholesterol in mg/dl in the samples and standard were read using auto analyser within 60 minutes against reagent blank.Concentration of cholesterol in Sample (mg/dl): O.D of Sample/ O.D of Standard x Concentration of Standard. Conversion factor 1 g/l X 0.0259= 1mmol/l of Total Cholestrol.

3. OBSERVATION AND RESULTS

3.1. T_3 determination

The studies revealed that the administration of Propyulthiouracil significantly reduce T3 levels on the 15^{th} and 8^{th} day in comparison to day 1^{st} day: 1.14 ± 2.17, 1.53 ± 4.92, 1.72±3.16 for 15^{th}, 8^{th} and 1^{st} day for males and 1.68±2.89, 1.24 ± 3.66, 0.8 ± 1.5 for 15^{th}, 8^{th} and 1^{st} day for females respectively.

The normal group (in 2% gum acacia w/v suspension) showed similar T3 levels (ng/ml) on the 1^{st}, 8^{th} and 15^{th} day. 1.71±2.4, 1.73±1.96, 1.74±0.47 ng/ml for males and 1.65± 2.1, 1.56± 1.43, 1.63± 0.19 ng/ml for females respectively.

The groups treated with Phyllanthin (1mg/kg of body weight) and Hypophyllanthin (1mg/kg of body weight) showed significantly high T3 levels(ng/ml) in the serum on the 8^{th} and 15^{th} day 1.64 ± 5.22, 1.63 ± 4.57 for phyllanthin and 1.70±2.25, 1.52 ± 1.35, 1.31 ± 7.40 ng/ml for

hypophyllanthin for males and for females as 1.64 ± 4.5, 1.51 ± 4.8, 1.58 ± 3.9 and 1.68±1.97, 1.65 ± 0.8, 1.39 ± 5.68 of the study as compared to the group treated with Propylthiouracil alone {P<0.001}.

The activity of Phyllanthin was found to be more than that of Hypophyllanthin. The activity of the standard thyroxine 1.54 ± 0.9, 1.63 ± 0.8 ng/ml on 8th and 15th day for males and 1.66 ± 1.18, 1.65 ± 0.4 ng/ml on 8th and 15th day for females respectively was significantly higher as compared to both Phyllanthin & Hypophyllanthin {P<0.001}.

The T3 levels (ng/ml) in the serum are a measure of thyroid activity. Therefore, the two components Phyllanthin and Hypophyllanthin were found to possess activity against chemically induced hypophyllanthin.

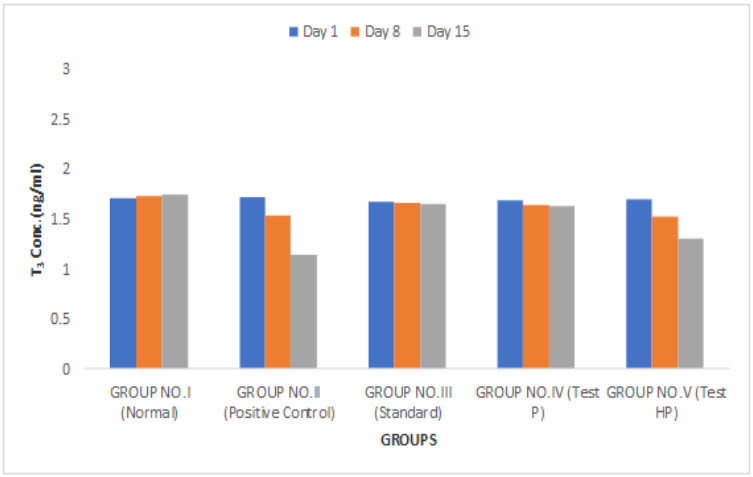

Figure 1A: Shows the T_3 levels (ng/ml) in blood serum for males.

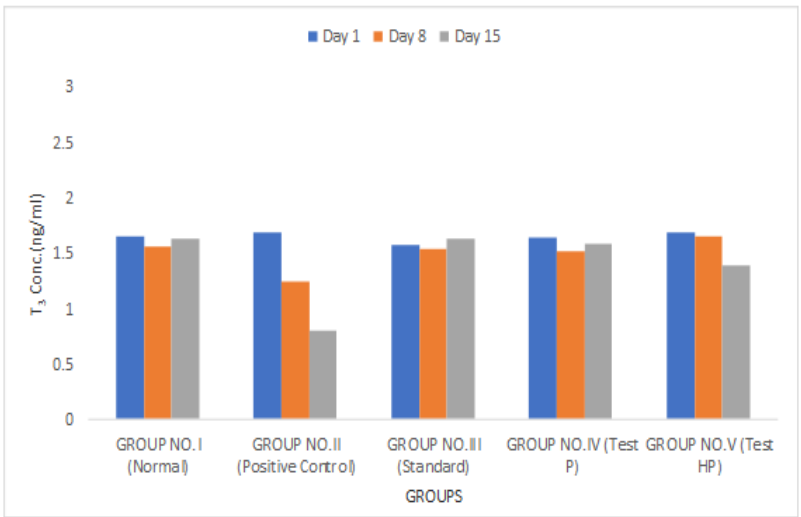

Fig 1B: Shows the T_3 levels (ng/ml) in blood serum for females

3.2. T_4 determination:

The administration of propylthiouracil (1mg/kg of body weight) significantly reduced the T4 levels (µg/dl) in the serum on the 8th and 15th day in comparison to day 1st day as 14.31±0.38, 6.81 ±0.48, 2.35 ± 0.16 µg/dl µg/dl normal group (in 2% gum acacia w/v suspension) showed similar T4 levels (µg/dl) on the 1st, 8th and 15th day as 14.12±0.56, 14.15±0.10, 14.27±0.92 µg/dl for males and 13.8 ±0.4, 13.8 ±0.6, 13.96 ±0.56 µg/dl for females.

The groups treated with Phyllanthin (1mg/kg of body weight) and Hypophyllanthin (1mg/kg of body weight) showed significantly high T4 levels (µg/dl) in the serum on the 8th and 15th day 10.11±0.49, 10.05±2.35 for phyllanthin and 11.36±0.49, 9.76± 0.28 µg/dl for hypophyllanthin for males and 9.88± 0.12, 9.89± 2.24 µg/dl for phyllanthin and 11.1±0.2, 9.53±0.11 µg/dl for hypophyllanthin for females respectively of the study as compared to the group treated with Propylthiouracil alone.

The activity of Phyllanthin was found to be more than that of Hypophyllanthin.

The activity of the standard thyroxine 13.87±0.29, 13.46±0.11 µg/dl on 8th and 15th day for males and 13.4±0.25, 13.15±1.34 µg/dl for females respectively was significantly higher as compared to both Phyllanthin & Hypophyllanthin {P<0.001}.

It has already been discussed that the T4 levels (µg/dl) in the serum as a measure of thyroid activity. So, the two components Phyllanthin and Hypophyllanthin were found to possess activity against chemically induced hypothyroidism.

The administration of Propylthiouracil (1mg/kg of body weight) significantly increased the TSH levels (ng/ml) in the serum on the 8th and 15th day in comparison to the normal levels on the 1st day 16.94±3.8, 48.76±2.71, 49.12±1.11 for 1st, 8th, 15th for males and 17.21±5.1, 51.22±1.91, 52.08±2.3 respectively.

The normal group (in 2% gum acacia w/v suspension) showed similar TSH levels (ng/ml) on the 1st, 8th and 15th day. 14.18±2.30, 14.53±0.68, 14.97±1.24 ng/ml respectively) for males and similarly for females as 14.25±1.90, 14.99±0.23, 15.2±0.85 respectively.

The groups treated with Phyllanthin (1mg/kg of body weight) and Hypophyllanthin (1mg/kg of body weight) showed significantly low TSH levels(ng/ml) in the serum on the 8th and 15th day 27.83±2.4, 29.53±0.86 for phyllanthin and 36.18±2.30, 35.57±2.97 ng/ml for

hypophyllanthin for males. And for females 29.11±3.1, 31.82±1.02 ng/ml and 37.03±2.50, 36.10±2.67 as compared to the group treated with Propylthiouracil alone {P<0.001}.

The activity of Phyllanthin was found to be more than that of Hypophyllanthin.

The activity of the standard thyroxine 19.25±0.67, 22.08±2.57 ng/ml on 8th and 15th day for males and 20.71±0.73, 23.11±3.21 ng/ml for females respectively was significantly lower as compared to both Phyllanthin & Hypophyllanthin.

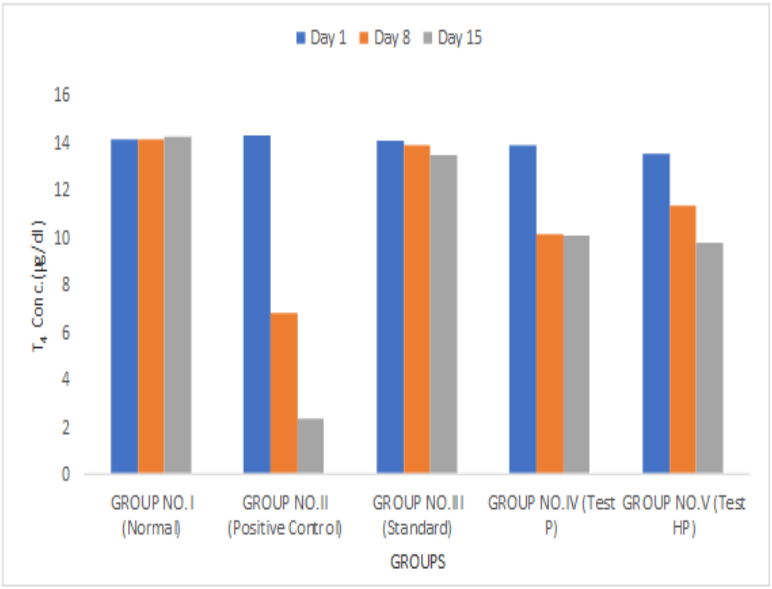

Figure 2A: Shows the T_4 levels (µg/dl) in blood stream for males.

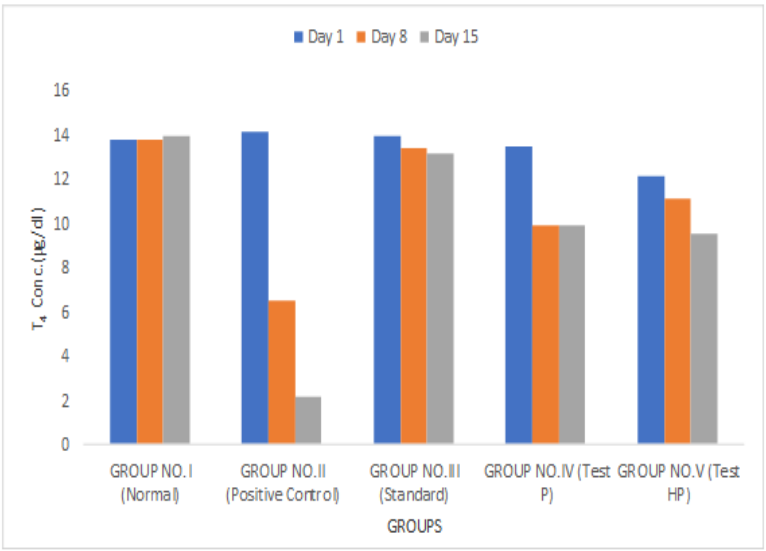

Figure 2B: Shows the T_4 levels (µg/dl) in blood stream for females.

3.3. TSH determination

The TSH levels(ng/ml) in the serum area measure of thyroid activity. As the serum T3 and T4 levels fall down the serum TSH level rises in response to the feedback stimulation of the pituitary gland, which in turn is being stimulated by Thyroid release stimulating hormone, released by the hypothalamus. The TSH levels in serum remain under a feedback mechanism controlled by thyroid hormones. The TSH levels in the animal groups treated with phyllanthin and hypophyllanthin were significantly low which shows that the two components have thyroxine like activity. Therefore, the two components Phyllanthin and Hypophyllanthin were found to possess activity against chemically induced hypothyroidism.
TSH levels (ng/ml) in blood serum in normal group (which received only 2% Gum Acacia Suspension w/v) were 14.18±2.30, 14.53±0.68, 14.97±1.24 ng/ml for males and 14.25±1.90, 14.99±0.23, 15.2±0.85 ng/ml for females at the intervals of 1,8 and 15 days respectively.

When Propylthiouracil (1mg/kg of body weight) was administered in a 2% Gum Acacia Suspension the TSH levels (ng/ml) in blood serum in the positive control group were 16.94±3.8, 48.76±2.71 and 49.12±1.11 ng/ml for males and 17.21±5.1, 51.22±1.91, 52.08±2.3 ng/ml for females at the intervals of 1,8 and 15 days respectively.

Phyllanthin (1mg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration showed maximum TSH levels of 15.01±3.4, 27.83±2.4 and 29.53±0.86 ng/ml for males and 15.89±2.9, 29.11±3.1, 31.82±1.02 ng/ml for females at the intervals of 1, 8 and 15 days respectively.

Hypophyllanthin (1mg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration showed maximum TSH levels of 14.98±0.51, 36.18±2.30 and 35.57±2.97 ng/ml for males and 15.04, ±1.1, 37.03±2.50, 36.10±2.67 ng/l for females at the intervals of 1,8 and 15 days respectively.

The standard Thyroxine (40µg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration showed maximum TSH levels of 14.58±0.87, 19.25±0.67 and 22.08±2.57 ng/ml for males and 15.16±1.41, 20.71±0.73, 23.11±3.21 ng/ml for females at the intervals of 1,8 and 15 days respectively.

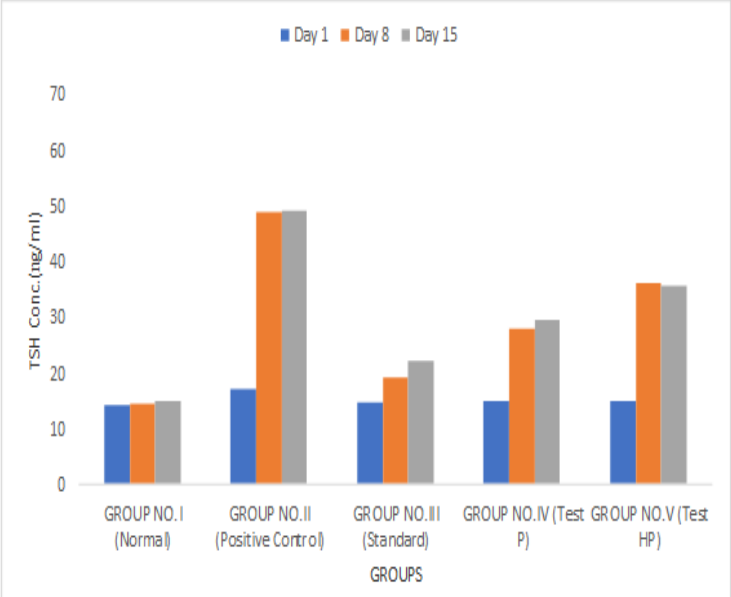

Figure 3A: Shows the TSH levels (ng/ml) in blood serum for males.

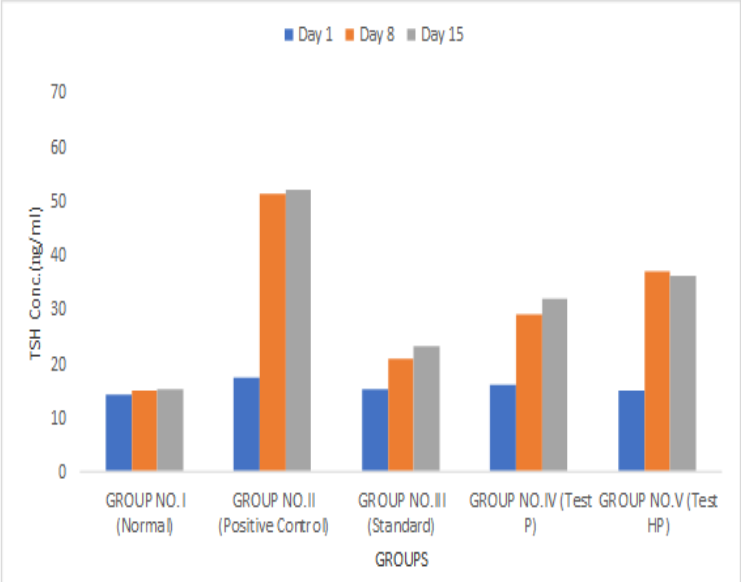

Figure 3B: Shows the TSH levels (ng/ml) in blood serum for females.

3.4. Thyroid weight determination

Thyroid weight (mgs) in the normal group (which received only 2% Gum Acacia Suspension) was 13.6±0.25 mgs for males and 13.1±1.7 for females on the 15th day.

When Propylthiouracil (1mg/kg of body weight) was administered in a 2% Gum Acacia Suspension the thyroid weight in the positive control group were 28.3±0.45 mgs for males and 27.98±0.22 mgs for females on the 15th day.

Phyllanthin (1mg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration showed maximum thyroid weight of 17.4±0.53 mgs for males and 16.9±0.10 mgs for females on the 15th day.

Hypophyllanthin (1mg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration showed maximum thyroid weight of 21.9±0.23 mgs for males and 21.12±1.45 mgs for females on the 15th day.

The standard Thyroxine (40µg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration showed maximum thyroid weight of 14.1±0.71 mgs for males and 13.86±0.58 mgs for females on the 15th day.

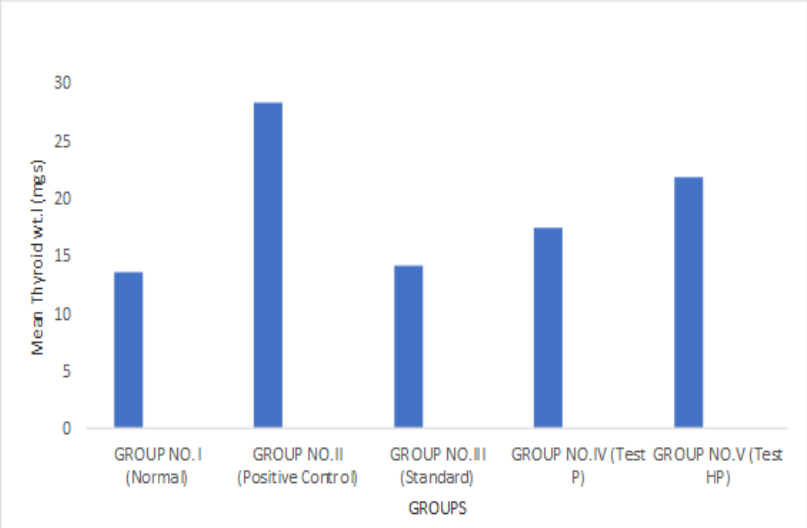

Figure 4A: Shows the thyroid weight (mgs) in different groups for males.

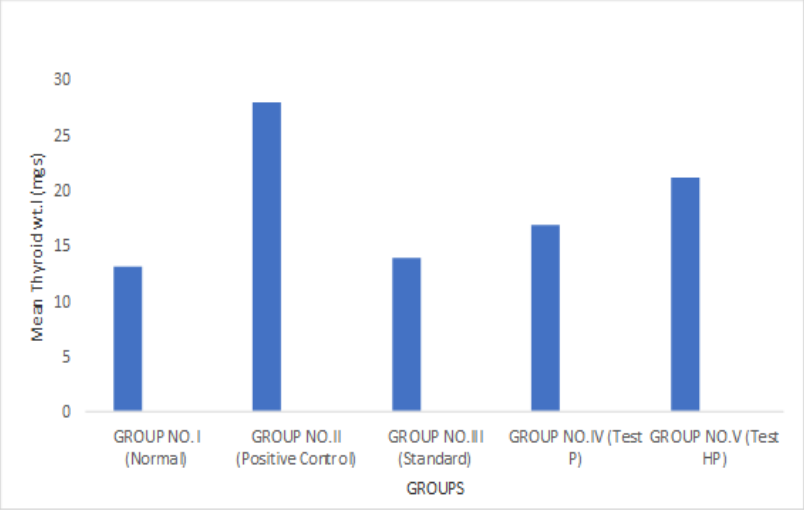

Figure 4B: Shows the thyroid weight (mgs) in different groups for females.

3.5. Determination of weight of the animal

Animal weight (gm) in the normal group (which received only 2% Gum Acacia Suspension w/v) were 200.5± 2.8, 202± 3.02 and 209± 1.92 gm for males and 170.1± 0.41, 172± 0.60 and 174.5± 0.33 gm for females at the intervals of 1,8 and 15 days respectively.

When Propylthiouracil (1mg/kg of body weight) was administered in a 2% Gum Acacia Suspension the animal weight in the positive control group were 200 ± 5.91, 209± 7.05 and 213.5 ± 7.30 gm for males and 182.78 ± 1.28, 196.04 ± 0.47 and 205.2 ± 1.80 gm for females at the intervals of 1,8 and 15 days respectively.

Phyllanthin (1mg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration showed maximum animal weight of 200 ± 9.4, 220 ± 7.1 annd 198.5 ± 9.9 for males and 150.18 ± 3.52, 157.1 ± 4.46 and 158.62 ± 8.98 gm for females at the intervals of 1,8 and 15 days respectively.

Hypophyllanthin (1mg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration showed maximum animal weight of 200 ± 10.5, 216 ± 4.97 and 220 ± 5.03 for males and 157.1 ± 6.8, 169 ± 10.3 and 170 ± 5.76 gm for females at the intervals of 1, 8 and 15 days respectively.

The standard Thyroxine (40µg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration showed maximum animal weight of 187 ± 15.62, 185.54 ± 11.92 and 182.31 ± 7.90 gm for males and 173.4 ± 10.32, 173.6 ± 12.03 and 172.8 ± 7.48 gm for females at the intervals of 1,8 and 15 days respectively.

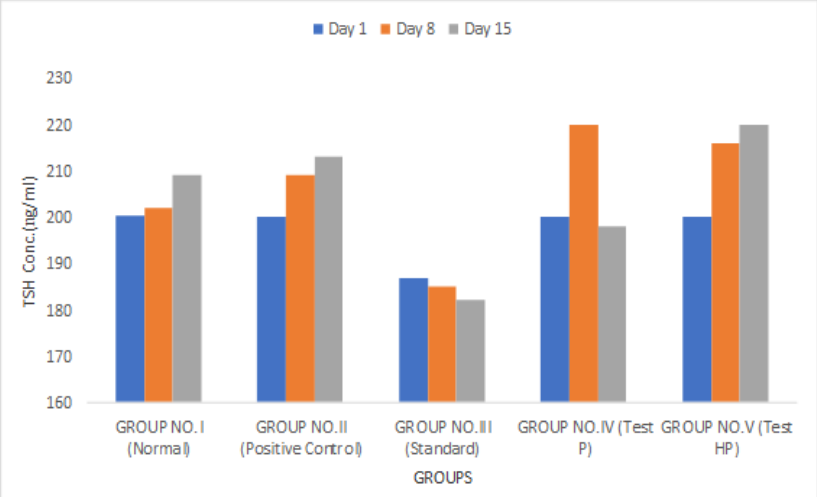

Figure 5A: Shows the animal weight (gm) in different groups for males.

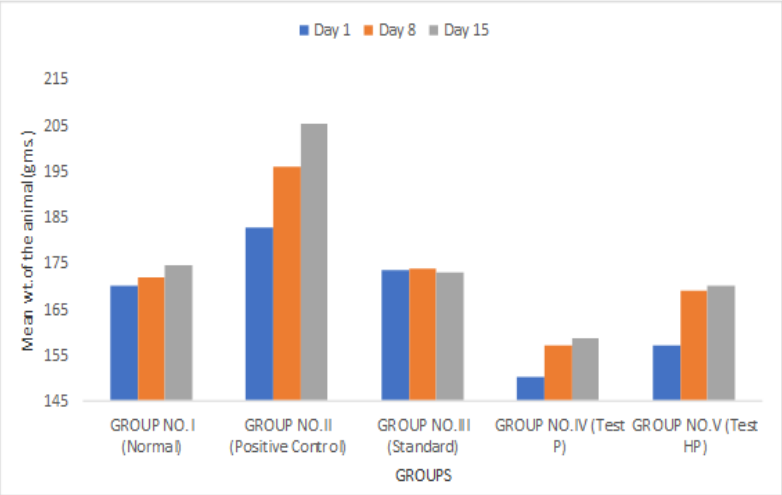

Figure 5B: Shows the animal weight (gm) in different groups for females.

3.6. Estimation of daily food intake

Daily food intake of the normal group (which received only 2% Gum Acacia Suspension w/v) was 10.53±1.2, 10.67± 0.8, 10.93±0.51, 11.4±0.5, 11.8±7.63, 12.4±1.1, 12.65±0.26, 13.2±1.2, 14.1±0.34, 15.82±0.902, 16.9±1.52, 17.3± 3.2, 18.23±0.79, 19.48±2.6, 21.2±0.96 for males and 10.55±0.7, 10.8±0.24, 10.97±0.40, 11.23±0.8, 11.59±1.3, 11.87±0.37, 12.4±0.43, 12.64±1.36, 13.5±3.27, 14.6±1.36, 15.29±3.45, 15.81±0.7, 17.7±0.2, 18.45±0.43, 20.52±0.35 for females at the intervals of 1,2,3,4,5,6,7,8,9,10,11,12,13,14 and 15 days respectively.

When Propylthiouracil (1mg/kg of body weight) was administered in a 2% Gum Acacia Suspension the daily food intake of the positive control was 11.12±1.89, 12.35±0.76, 14.62±0.8, 16.3±1.94, 17.1±3.45, 19.8±0.2, 22.4±1.7, 23.81±0.77, 25.36±3.1, 27.9±0.4, 28.3±2.5, 30.1±0.66, 32.35±1.91, 34.7±2.71, 35±2.8 for males and 11.89±1.45, 12.5±0.66, 13.44±0.2, 14.89±0.84, 16.71±1.5, 17.12±1.97, 18.38±0.8, 20.55±0.11, 20.96±0.94, 24.7±1.89, 25.29±0.45, 27.36±2.51, 30.49±1.77, 31.65±0.37, 32.36±1.45 for females at the intervals of 1,2,3,4,5,6,7,8,9,10,11,12,13,14 and 15 days respectively.

Phyllanthin (1mg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration showed maximum daily food intake of the positive control was 7.95±1.48, 8.32±0.3, 10.9±0.17, 11.46±2.59, 12.36±1.1, 11.12±0.51, 13.96±3.1, 15.1±1.2, 17.7 ±4.9, 19.13±0.65, 20.45±2.51, 18.36±1.34, 18.41±0.65, 21.18±0.3, 20.83±1.20 for males and 7.89±0.2, 9.12±0.81, 10.96±1.51, 11.66±0.7, 11.43±1.5, 12.87±2.5, 13.5±1.34, 14.29±0.15, 15.7±0.28, 15.95±0.4, 16.36±0.26, 16.8±0.35, 17.15±0.96, 18.85± 1.58,

19.56±1.66 for females at the intervals of 1,2,3,4,5,6,7,8,9,10,11,12,13,14 and 15 days respectively.

Hypophyllanthin (1mg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration showed daily food intake of the positive control was 12.68±0.43, 11.35±2.59, 11.11±0.37, 13.2±0.7, 13.45±1.36, 14.7±3.33, 15.1±0.40, 16.8±1.91, 17.54±2.3, 18.21±3.45, 19.45±0.81, 15.4±0.9, 15.56±1.86, 16.45±1.03, 21.1±2.96 for males and 10.85±0.58, 10.88±0.76, 11.56±1.8, 12.14±1.5, 13.81±0.81, 14.56±0.2, 14.81±0.56, 15.5±1.1, 16.12±0.48, 17.6±1.1, 17.85±0.5, 18.22±1.6, 18.71±0.4, 19.16±1.3, 20.5±0.5 for females at the intervals of 1,2,3,4,5,6,7,8,9,10,11,12,13,14 and 15 days respectively.

The standard Thyroxine (40µg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration daily food intake of the positive control was 6.78±1.2, 9.36±0.5, 11.52±0.66, 12.9±3.67, 13.5±1.1, 14.8±3.01, 13.1±2.81, 15.39±0.07, 16.53±2.8, 17.13±1.14, 19.5±2.58, 19.89±3.5, 21.32±1.51, 21.1±0.42, 20.54±0.5 for males and 8.11±0.1, 10.53±0.7, 11.2±1.1, 11.83±0.3, 12.01±0.56, 13.96±1.25, 14.11±0.7, 14.32±0.65, 15.57±0.81, 14.83±0.14, 16.42±1.82, 17.91±0.43, 18.78±0.2, 20.11±1.7, 21.05±0.8 for females at the intervals of 1,2,3,4,5,6,7,8,9,10,11,12,13,14 and 15 days respectively.

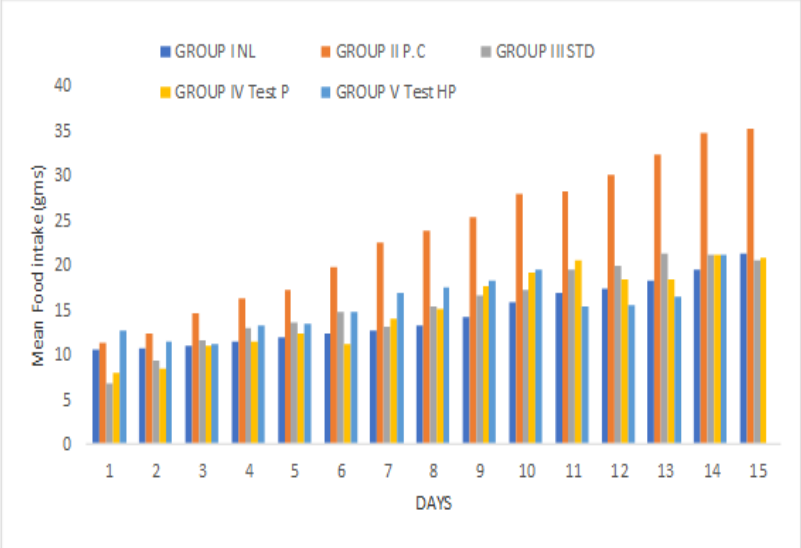

Figure 6A: Shows the Daily Food Intake (gm) in different groups for males.

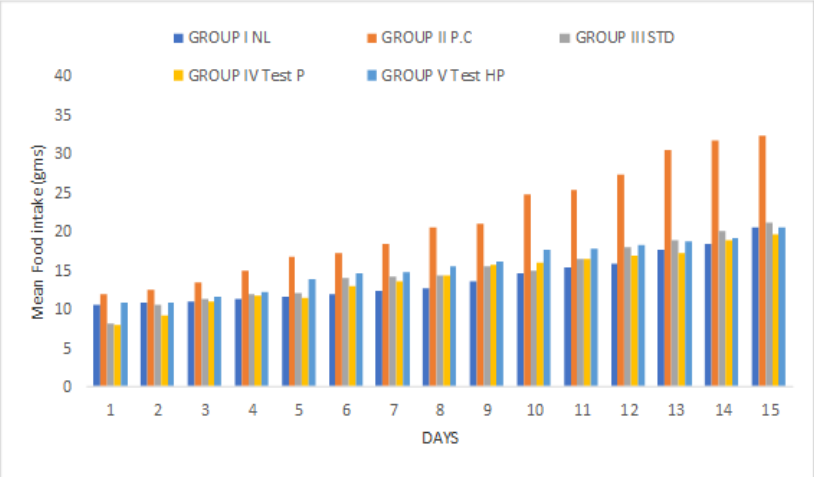

Figure 6B: Shows the Daily Food Intake (gm) in different groups for females.

3.7. Estimation of daily water intake

Daily water intake of the normal group (which received only 2% Gum Acacia Suspension w/v) was 26.2±1.41, 27.31±1.06, 28.4±0.53, 32.5±1.65, 34.62±5.83, 36.3±14.06, 39.61±4.77, 42.87±4.06, 44.15±3.47, 51.7±4.41, 52.2±3.8, 50.19±9.06, 48.64±3.5, 57.75±6.36, 61±2.12 for males and 25.1±1.25, 26.59±1.1, 27.12±0.8, 31,6±0.33, 32.45±1.76, 34.8±12.76, 36.29±11.8, 39.1±0.7, 42.06±3.9, 45.4±2.3, 47.15±2.8, 49.2±1.3, 52.4±2.0, 55.65±5.8, 59.7±1.4 for females at the intervals of 1,2,3,4,5,6,7,8,9,10,11,12,13,14 and 15 days respectively.

When Propylthiouracil (1mg/kg of body weight) was administered in a 2% Gum Acacia Suspension the daily water intake of the positive control was 31.1±0.8, 31.85±0.55, 41.6±1.2, 43.89±0.1, 51.7±8.6, 50.87±4.77, 64.41±1.11, 68.72±0.6, 68.14±0.7, 73.3±1.8, 77.42±4.1, 81.06±2.6, 84.12±0.5, 87.24±1.15, 91.18±0.7 for males and 30.5±0.2, 31.26±0.71, 40.89±1.69, 42.11±1.5, 51.2±3.41, 58.44±2.3, 62.88±7.9, 64.5±1.5, 65.31±0.6, 71.4±2.1, 72.4±3.5, 76.92±1.08, 82.5±2.1, 86.92±1.71, 89.7±0.5 for females at the intervals of 1,2,3,4,5,6,7,8,9,10,11,12,13,14 and 15 days respectively.

Phyllanthin (1mg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration showed maximum daily water intake of the positive control was 35.62±1.76, 37.25±0.2, 38.1±1.11, 41.6±4.6, 44.25±2.29, 45.7±2.81, 43.04±1.41, 44.36±0.2, 55.7±1.06, 48.5±4.1, 51.6±2.12, 50.47±1.76, 52.8±3.8, 54.06±1.96, 56.3±3.53 for males and 34.31±0.3, 36.25±1.76, 37.7±0.88, 39.92±1.11, 42.5±2.6, 44.11±3.47, 44.83±0.53,

46.1±1.7, 47.78±2.5, 48.3±2.4, 48.6±2.1, 49.71±1.8, 50.4±1.9, 53.1±1.65, 55.25±3.7 for females at the intervals of 1,2,3,4,5,6,7,8,9,10,11,12,13,14 and 15 days respectively.

Hypophyllanthin (1mg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration showed daily water intake of the positive control was 27.4±1.06, 33.81±10.41, 37.5±1.59, 42.62±0.88, 44.1±1.71, 48.41±3.8, 49.6±2.5, 51.8±6.5, 57.16±10.2, 59.71±2.4, 61.50±9.5, 62.19±2.42, 64.32±1.79, 64.1±3.7, 66.5±0.6 for males and 26.8±1.41, 33.1±1.65, 36.23±1.71, 39.56±1.2, 41.05±0.3, 42.87±1.8, 45.5±4.1, 49.31±0.5, 54.6±1.06, 57.26±4.6, 56.2±2.4, 59.42±1.5, 60.61±0.25, 62.81±1.41, 67.63±1.75 for females at the intervals of 1,2,3,4,5,6,7,8,9,10,11,12,13,14 and 15 days respectively.

The standard Thyroxine (40µg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration daily water intake of the positive control was 31.35±3.4, 31.9±1.03, 33.15±2.71, 36.8±6.25, 39.25±8.91, 38.33±0.88, 39.2±1.5, 40.4±1.76, 41.4±4.24, 47.6±9.1, 49.31±3.88, 51.1±3.17, 52.5±5.6, 55.35±9.54, 58.4±3.5 for males and 30.21±3.1, 31.45±1.05, 32.8±1.8, 34.31±3.4, 37.19±6.5, 38.53±1.07, 40.1±2.4, 40.97±4.7, 41.6±1.65, 45.0±6.25, 47.11±3.5, 50.8±3.6, 51.2±3.8, 53.54±1.8, 57.6±1.02 for females at the intervals of 1,2,3,4,5,6,7,8,9,10,11,12,13,14 and 15 days respectively.

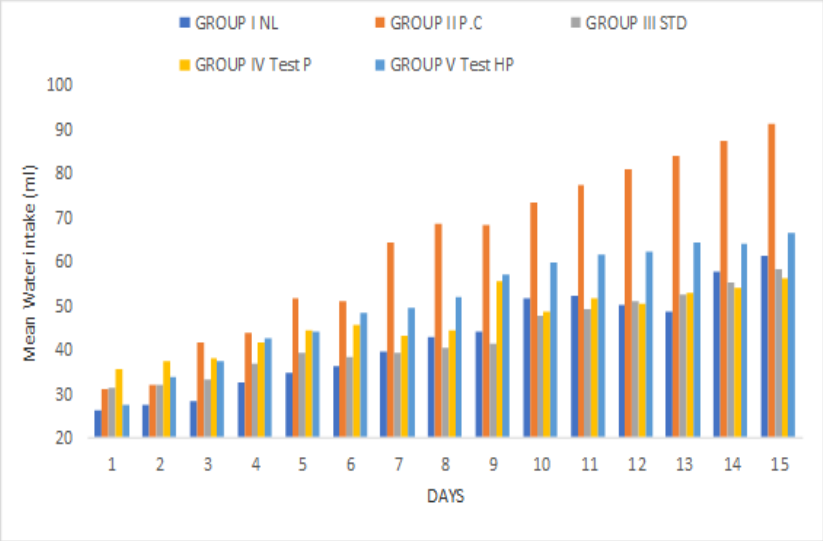

Figure 7A: Shows the Daily Water Intake (ml) in different groups for males.

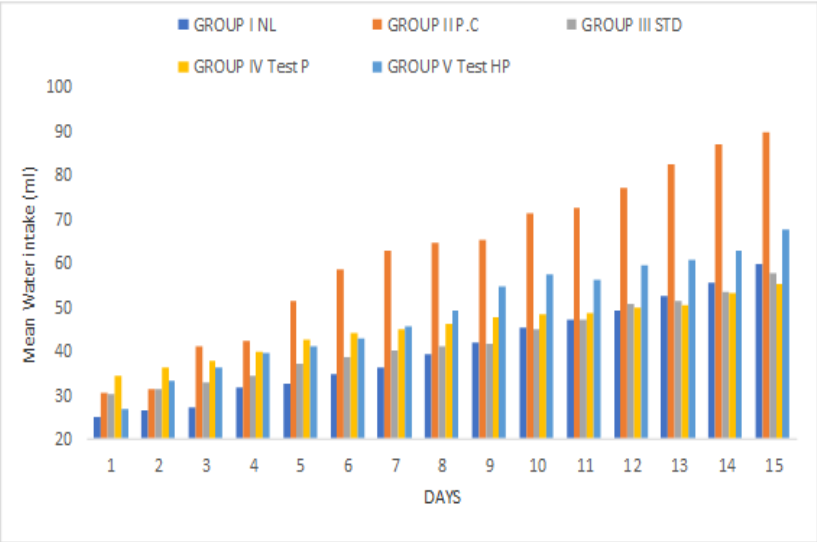

Figure 7B: Shows the Daily Water Intake (ml) in different groups for females.

3.8. Estimation of total protein level

Total serum protein (g/l) levels in the normal group (which received only 2% Gum Acacia Suspension w/v) were 63.2±2.20, 65.9±2.11, 67.1±1.23 g/l for males and 62.9±2.15, 64.6±1.9, 65.8±1.95 for females at the intervals of 1,8 and 15 days respectively.

When Propylthiouracil (1mg/kg of body weight) was administered in a 2% Gum Acacia Suspension the total protein in the positive control group were 64.9±1.77, 67.25±2.9, 71.6±3.35 g/l for males and 64.12±1.45, 66.6±1.8, 70.8±3.1 for females at the intervals of 1,8 and 15 days respectively.

Phyllanthin (1mg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration showed maximum total protein levels of 62.4±3.44, 61.8±2.69, 66.02±1.95 for males and 63.74±1.5, 63.86±2.2, 66.3±0.54 g/l for females at the intervals of 1,8 and 15 days respectively.

Hypophyllanthin (1mg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration showed maximum total protein levels of 63.2±0.43, 66.8±2.77, 69.8±1.65 for males and 63.9±2.3, 64.52±1.11, 68.4±1.23 g/l for females at the intervals of 1,8 and 15 days respectively.

The standard Thyroxine (40µg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration showed maximum total protein of

62.12±2.1, 64.59±2.36, 65.92±0.12 g/l for males and 62.8±3.56, 62.76±1.7, 65.39±1.83 g/l for females at the intervals of 1,8 and 15 days respectively.

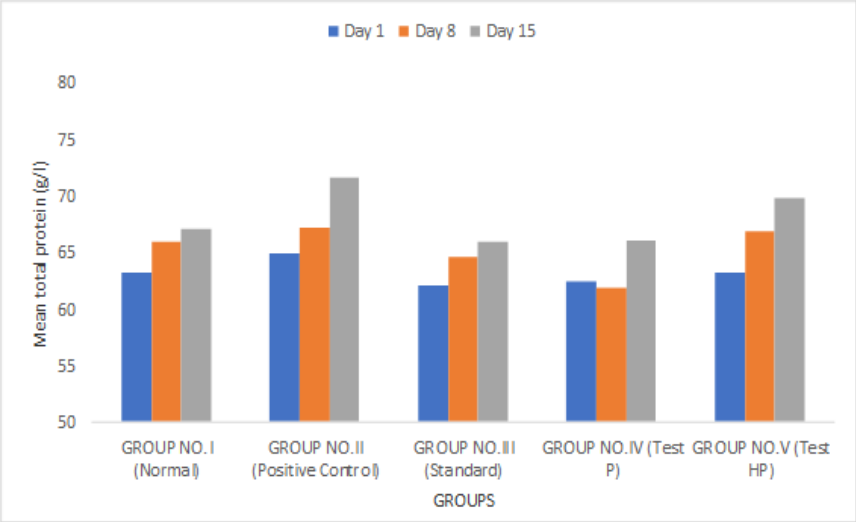

Figure 8A: Shows the total Protein (g/l) in different groups for males.

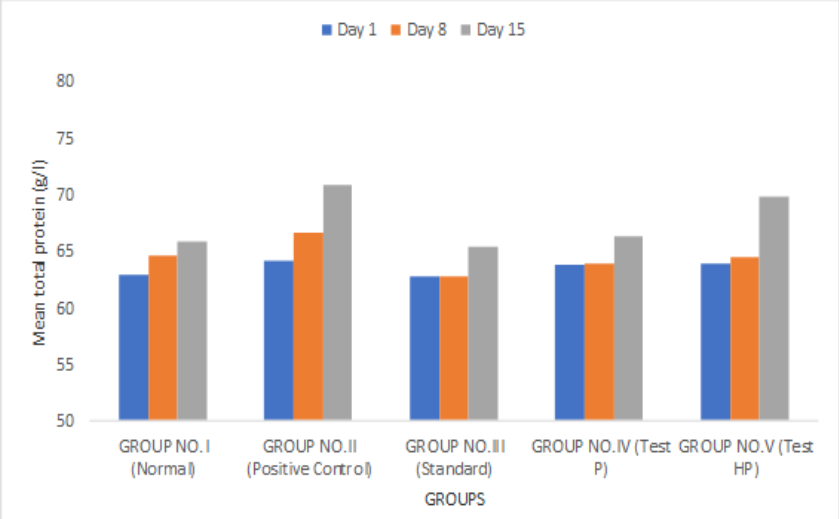

Figure 8B: Shows the total Protein (g/l) in different groups for females.

3.9. Estimation of total cholesterol level

Total serum cholesterol (mmol/l) in normal group (which received only 2% Gum Acacia Suspension w/v) were 2.57±0.12, 2.81±0.4, 2.9±0.2 mmol/l for males and 2.64±0.86, 3.01±0.27, 2.76±0.04 mmol/l for females at the intervals of 1,8 and 15 days respectively.

When Propylthiouracil (1mg/kg of body weight) was administered in a 2% Gum Acacia Suspension the total cholesterol levels in the positive control group were 2.6±0.15,

5.32±0.45, 8.36±0.61 mmol/l for males and 2.86±1.69, 4.7±0.77, 3.66±0.2 mmol/l for females at the intervals of 1,8 and 15 days respectively.

Phyllanthin (1mg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration showed total cholesterol levels of 2.66±0.41, 2.93±0.46, 3.57±0.17 mmol/l for males and 2.81±1.35, 4.53±0.8, 3.32±0.75 mmol/l for females at the intervals of 1,8 and 15 days respectively.

Hypophyllanthin (1mg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration showed total cholesterol levels of 2.45±0.55, 2.97±0.76, 4.88±0.19 mmol/l for males and 2.79±0.84, 4.48±0.5, 3.91±1.7 mmol/l for females at the intervals of 1,8 and 15 days respectively.

The standard Thyroxine (40µg/kg of body weight) administered after 1 hour of Propylthiouracil (1mg/kg) of body weight administration showed total cholesterol levels of 2.61±0.89, 2.7±0.77, 2.58±0.21 mmol/l for males and 2.73±2.46, 4.45±0.61, 3.41±1.21 mmol/l for females at the intervals of 1,8 and 15 days respectively.

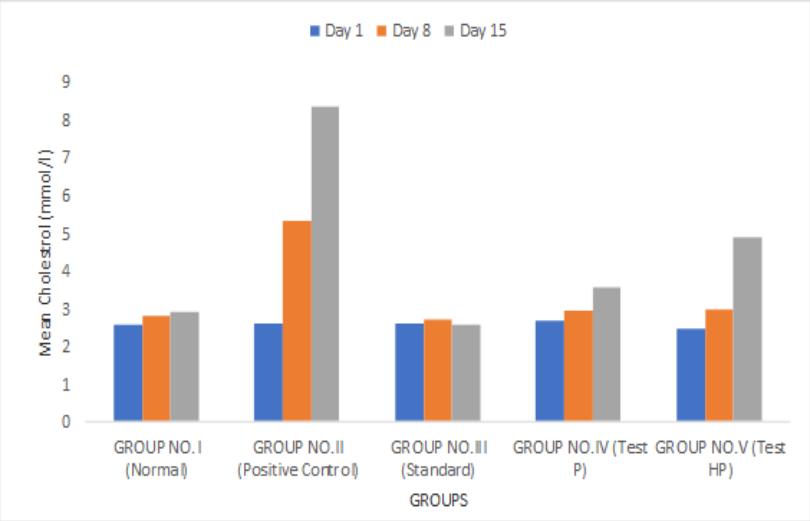

Figure 9A: Shows the Total Cholestrol levels (mmol/l) in different groups for males.

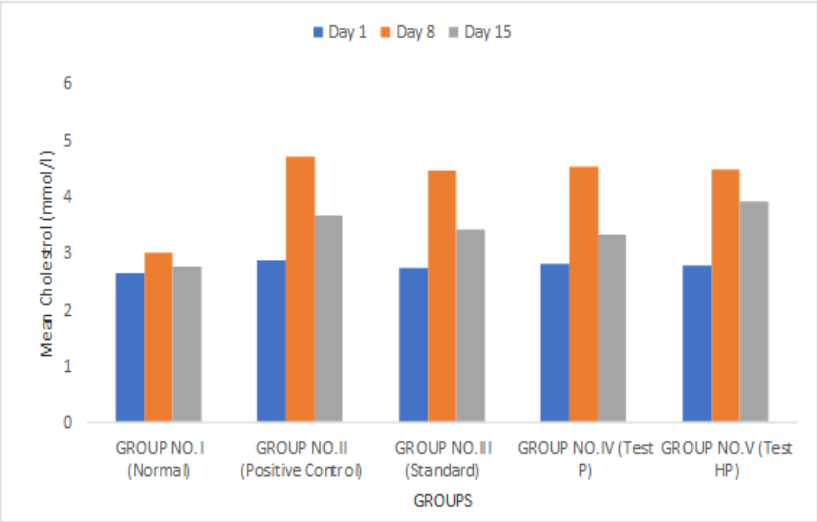

Figure 9B: Shows the Total Cholestrol levels (mmol/l) in different groups for females.

4. DISCUSSIONS

Hypothyroidism is a common endocrine disorder resulting from deficiency of thyroid hormone. It is characterised by insufficient T_3 thyroid hormone at the cellular level due to lack of thyroxin or T4 or from a decreased state of conversion of T4 into T3. According to different research, women are having problems with thyroid gland 3-10 times more, than men. This also applies to toxic goiter, and insufficiency of thyroid hormone.

In 2008, the highest incidence rate of thyroid cancer was estimated to be in France, where the female rate was five times higher than the rate of the lowest ranking country, Greece (18.6 versus 3.3 per 100,000 females). Males developing thyroid cancer, on the other hand, tend to present later than females and have a worse prognosis. Similarly, autoimmune diseases occur predominantly in women. Hormonal fluctuation is the main reason to why women are more effected by thyroid hormone. Surge in estrogen and decline of progesterone shows an effect on reproductive systems and hence attacks the immune system.

The preferred treatment for hypothyroidism is the Levothyroxine ($L\text{-}T_4$). The starting dose should be 1.6 µg/kg of body weight. In elderly patients and patients with cardiac disease starting at a lower dose (25-50 µg/day) and increased by 25 µg/d until the patient is clinically and biochemically euthyroid. The dose should be adjusted about 6 to 8 weeks after treatment is initiated (unless symptoms of overtreatment occur). Once the patient is euthyroid and TSH is stable, an annual follow-up with TSH measurement should suffice. Disadvantages of synthetic preparations are that they may lead to excess of thyroid hormone in the body,

resulting in hyperthyroidism. Also, some people do not have sufficient amounts of the enzyme thyroxine peroxidase needed to convert T4 to T3.

Herbal plants are used as folk medicine by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. The chemicals constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with human body. Ancient literature also mention herbal medicine for age related diseases namely memory loss, osteoporosis, diabetic wound, immune and liver disorder, etc for which no modern medicine or only palliative therapy is available. These drugs are made from renewable resources of raw materials by eco-friendly and will bring economic prosperity to the masses growing these raw materials. (WHO, March 1991).

The herb *Phyllanthus amarus* commonly known as Bhuiamla, possess hepatoprotective activity that has been previously established in our laboratories **(Agrawal et.al,1986)**. The same plant was earlier studied for liver regenerating potential. Since the thyroid hormones T3 and T4 are metabolized by the liver thus the liver disorder directly affects the serum concentrations of both the hormones. Thus, the thyroid levels were measured as one of the parameters to evaluate the activity of liver in alcohol induced damage.

In earlier studies **(Agrawal et.al, 2003)** done on the whole leaves extract petroleum ether the T3 levels were found to be increased from the initial 1.96±0.09 ng/ml on day 1 t0 2.99±0.16 ng/ml on the 7^{th} day and the T4 levels were found to be increased from the initial 10.68±0.39µg/ml on day 1 to 18.68±0.54µg/dl on the 7^{th} day.

In the subsequent study, **(Agrawal et.al, 2004)** in which the two compounds Phyllanthin and Hypophyllanthin were isolated, it was found that in the Phyllanthin treated group the T3 levels increased from the initial 1.45±0.30ng/ml on day 1 to 2.64±0.58ng/ml on the 7^{th} day and the T4 levels were found to be increased from the initial 11.42±0.67µg/dl on the day 1 to 20.68±0.47µ/dl on the 7^{th} day and for the Hypophyllanthin the T3 levels were found to be increased from the initial 1.64±0.70ng/ml on day 1 to 2.50±0.77ng/ml on the 7^{th} day and the T4 levels were found to be increased from the initial 12.22±0.14µg/dl on day 1 to 19.68±0.14µg/dl on the 7^{th} day.

In Study **(Anand et al,2005**) it was concluded that Phyllanthin and Hypophyllanthin (1mg/kg of body weight) showed significant increase in the serum T3 and T4 levels and decrease in the weight of the thyroid gland against both the animal models as compared to the groups treated with the hypothyroid inducing agent alone. When phyllanthin was given to males the level of T3 on 1st day was 1.70±0.67 and 1.46±0.87 on 15th day and for hypophyllanthin it was 1.60±0.891 on day 1 and 1.30±0.29 on day 15th.

T4 levels after treatment with phyllanthin was 13.54±0.36 on day 1 and 12.61±0.28 on 15th day, and with hypophyllanthin was 13.74±0.50 on day 1 and 10.43±0.30 on 15th day.

TSH concentration was decreased in Phyllanthin and Hypophyllanthin treated animals, which indicated that the drugs were acting through anterior pituitary. The decrease in the TSH levels occurs due to the increase in the serum T3 and T4 levels. It was 15.24±3.0 on 1st day and 28.94±3.0 on 15th day for phyllanthin and 13±2.4 for 1st day and 37.03±4.32 ng/ml on 15th day for hypophyllanthin.

The above studies concluded that Phyllanthus amarus possess some pharmacological action over the thyroid gland. In order to evaluate the same two active compounds of the plant Phyllanthin and Hypophyllanthin were isolated and then tested over the chemically induced hypothyroidism using Propylthiouracil as inducers in rats.

The present study is an attempt to evaluate the thyroid hormone modulating activity of Phyllanthin and Hypophyllanthin extracted from Phyllanthus amarus Linn. It is done to compare the effects in males and female rats. Propylthiouracil induced hypothyroidism is one of the commonly reported method to induce hypothyroidism in Wistar albino rats (**Imada M et.al, 1986**). Propylthiouracil hypothyroidism by inhibiting the thyroid gland to uptake the I_2 and release thyroid hormone. Hence, a condition of hypothyroidism is produced. Using this model an anticipated drug having thyroid hormone modulating activity can be screened.

The TSH levels(ng/ml) in the serum area measure of thyroid activity. As the serum T3 and T4 levels fall down the serum TSH level rises in response to the feedback stimulation of the pituitary gland, which in turn is being stimulated by Thyroid release stimulating hormone, released by the hypothalamus. The TSH levels in serum remain under a feedback mechanism controlled by thyroid hormones. The TSH levels in the animal groups treated with phyllanthin and hypophyllanthin were significantly low which shows that the two

components have thyroxine like activity. Therefore, the two components Phyllanthin and Hypophyllanthin were found to possess activity against chemically induced hypothyroidism. Hypothyroidism leads to an increase in the weight of the thyroid gland. The thyroid weight in the phyllanthin and hypophyllanthin treated groups were more toward the normal values. The thyroid weight in Phyllanthin treated group was less as compared to that of the thyroid weight of Hypophyllanthin.

So, the two components Phyllanthin and Hypophyllanthin were found to possess activity against chemically induced hypothyroidism.

The animal weight increases in hypothyroidism and can be taken as a measure of the thyroid activity.

The activity of Phyllanthin was found to be more than that of Hypophyllanthin as the daily food intake of the hypophyllanthin treated group was more than that of the phyllanthin treated group.

The daily food intake in the standard thyroxine treated group was significantly lower as compared to both Phyllanthin and Hypophyllanthin ($P<0.001$) The daily food intake was similar to that of the normal group that received 2% gum acacia w/v suspension only.

The daily food intake increases in hypothyroidism and therefore it can be taken as a measure of the thyroid activity. So, the two components Phyllanthin and Hypophyllanthin were found to possess activity against chemically induced hypothyroidism.

The daily water intake increases in hypothyroidism and therefore it can be taken as a measure of the thyroid activity. So, the two components Phyllanthin and Hypophyllanthin were found to possess activity against chemically induced hypothyroidism.

Hypothyroidism leads to an increase in the Total protein levels (g/l) in the serum. The test compounds Phyllanthin and Hypophyllanthin both reduced the total protein levels in rats. Therefore, the two components Phyllanthin and Hypophyllanthin were found to possess activity against chemically induced hypothyroidism.

Hypothyroidism leads to increase in total cholestrol levels in the serum. The test compounds reduced the cholesterol levels (mmol/l) in rats. Therefore, the two components Phyllanthin

and Hypophyllanthin were found to possess activity against chemically induced hypothyroidism.

5. CONCLUSIONS

The two isolated components Phyllanthin & Hypophyllanthin from Phyllanthus amarus were used to evaluated their thyroxine like activity using Propylthiouracil induced hypothyroidism in male and female rats. The effect of hypothyroidism on female v/s male rats was differentiated. Phyllanthin and Hypophyllanthin significantly increases the serum T3 and T4 levels and decrease in the weight of the thyroid gland against both the animal models as compared to the groups treated with the hypothyroid inducing agent alone. The TSH concentration was decreased in the Phyllanthin & Hypophyllannthin treated animals for male and female rats. Hypothyroidism leads to increase in TSH levels for females more as compared to males which may be due to the sexual dimorphism of thyroid gland and fluctuating hormones and the immune system in females. The two compounds reduced the total protein and total cholesterol in the treated animals. Also, the daily food and water intake, weekly animal weight was reduced in the propylthiouracil treated groups. The activity of phyllanthus was found to be more than that of the Hypophyllanthin in all the parameters studied in the animal model. Therefore, we conclude that the effect of hypothyroidism is more prominent in females than in males. Phyllanthin & Hypophyllanthin both have thyroid hormone modulating activity and the compounds maybe studied in human beings for their efficacy and safety.

6. REFERENCES

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