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HYPOTHYROIDISM AND HISTOLOGICAL ALTERATION IN INDUCED PTU AND PROTECTIVE ROLE OF PHFEXT

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Abstract
Hypothyroidism is one of the most frequent challenging disorders, with around 1.6 billion people globally at risk. Hypothyroidism affects 9.4% of Pakistan's population, according to research. In this study protective role of poly-herbs (PHFext) again induce hypothyroidism was evaluated. *Commiphora wightii, Wathania somnifera, Moringa oleifera, Trigonella foenum graecum*, was used in polyherbal formulation. Ethanolic extracts of Guggul (*Commiphora wightii*), Ashwagandha (*Wathania somnifera*), Moringa (*Moringa oleifera*), Fenugreek (*Trigonella foenum graecum*) was prepared. A total of 18 rabbits was divided into three groups and each group contained 6 rabbits (n=6). Group A was a control group that received a normal diet and mineral water, while Experimental Group Rabbits were divided into two Groups (C and D experimental groups) Experimental group induced with a Propylthiouracil (PTU) (50 mg/kg/day) for 21 days, Group C was given 500 mg/day/oral PHFext and Group C was given Thyroxine 50 mcg/daily for 45 days. Thyroid profile was assessed by using the Mini-VIDAS system. The Thyroid profile was assessed before starting the study, after the induce 21 days, and experimental after 45 days. The result was analyzed using one-way analysis of variance (ANOVA). The T3 (triiodothyronine), T4 (free thyroxine) was very significantly increase ($P<0.001$) in the hypothyroidism treatment group (PTU+PHFext) and also showed that the TSH (thyroid stimulating hormone) level was very significantly decreased ($P<0.001$) in the hypothyroidism treatment group (PTU+PHFext) as compared to the treated group=D (Thyroxine). Histological result showed significant improved in induced animal treated Group=C (PHFext) in Loss of Follicular epithelium, Loss of Colloid, and Congestion of vessels improvement (83.3%).

Keywords: Induce hypothyroidism, Polyherbal formulation, Thyroxine.

Introduction:

Thyroid is one of the most important parts of the endocrine system, and it is also known as the gland of vital hormones. It regulates metabolism, growth, and development through the influence of thyroid hormones, and it plays an important role in brain development during childhood. Thyroid also regulates body thermogenesis, heartbeat, reproductive activity, emotions, and gastrointestinal motility.(1). Thyroid hormones include triiodothyronine, tetraiodothyronine (T3 & T4), and

calcitonin. Imbalances in these hormones can cause a variety of thyroid illnesses, including hypothyroidism, hyperthyroidism, goitre, Graves' disease, and thyroid cancer. (2). Hypothyroidism is caused by a variety of circumstances, including a lack of iodine in the diet, autoimmune disorders, thyroid gland lesions, and is most commonly connected with a pituitary gland issue.(1).
Due to their efficacy and safety, herbal medicines are widely recommended for controlling and curing health-related concerns and disease situations around the world. (3). The World Health Organization lists around 21000 medicinal herbs that are employed in the health-care system. Geographically, Pakistan boasts a large variety of herbal medicinal plants. There are around 6000 medicinal herb species on the list. (4) For therapy and healing of ill conditions, the majority of Pakistani people favor medicinal herbs. (5). Since the 1940s, hepatic adverse effects have been reported with PTU use, and in 2010 the FDA added a black box warning to the prescription insert. Hepatotoxicity occurs in 1 in 10,000 adult patients and 1 in 20,000 pediatric patients who are administered PTU.(6)

MATERIAL AND METHODS

Animals Protocol:

All experimental animals were kept in clean plastic cages in a temperature-controlled setting (28-32°C) with a normal diet and a 12-hour light/dark cycle. (7).

Herbal plants material: (Plant Components)

All four plant (*Commiphora wightii, Wathina somnifera, Moringa oleifera, Trigonella foenum-graecum*) components were obtained at a neighborhood market in Hyderabad. The Department of Pharmacognosy, FOPHS (Faculty of Pharmacy & Health Sciences), Balochistan University, Quetta, identified plant components and created a voucher specimen (Voucher # P037-) was for future reference stored in the herbarium.

Extraction:

For this study, extract was prepared from different parts of the plants. Ethanolic extracts was created by macerating *Commiphora wightii* gum powder in ethanol for seven days, filtering under reduced pressure, and drying in a vacuum desiccator. The ratio of herbs to products was 5:1. (8). The *Moringa oleifera* leaves were harvested and dried in the shade in a room. Leaves were crushed and sieved with a 40# sieve after ten days of drying. The Soxhlet device was used to extract *Moringa oleifera* powder from methanol. The extract was dried at a low temperature in a vacuum evaporator, sterilised using a Whatmann filter no. 42, and reconstituted in saline and condensed under reduced pressure to obtain 5:1 ratio. (9). *Withania somnifera* root was dried, crushed, and submerged in a variety of solvents (Ethanol). The methanolic filtrates were rotary evaporated after filtration, while the aqueous filtrates were lyophilized with a freeze drier. (10). *Trigonella foenum-graecum* Dry powdered Trigonella foenum-graecum (Fenugreek) 100 gram of seeds was continuously extracted for 48 hours with aqueous with 80% ethanol. The extract will be stored at 0-4 ^{o}C until required. At 60 degrees Celsius, the plant extract was pool and evaporate to dry. (11).

Polyherbal formulation composition:

The composition of polyherbal formulation for the 500 mg (*Commiphora wightii* = 125mg, *Moringa oleifera* = 125mg, *Withania somnifera*= 125mg, and *Trigonella foenum-graecum* = 125mg,) packed in hard gelatin capsule. The components are processed and prepared using Ayurvedic and Unani principles with the goal of increasing efficacy. (12)

Acute oral toxicity study:

The acute toxicity experiments were carried out in compliance with OECD guideline no 425 (Organization for Economic Co-operation and Development) (up and down procedure).(13). The plants extract was given at three different doses (100, 250, and 500 mg/kg of body weight). In addition, the plant ethanolic extract from (CW+MO+WS+TF) were given together to see how well they worked together. The combined extracts were also tested at various concentrations of each extract, including

100, 250, and 500 mg/kg of body weight. For each rabbit, the supplied volume was adjusted to 8 ml/Kg. At the commencement of the trial, the vehicle and extract was given only once day. For 14 days, the rabbits were observed for death, convulsions, salivation, sleep, and coma. (14).

Experimental Study:

The Experimental study was done in Sindh Agricultural University Tandojam's Animal Husbandry Sciences Department Animal House. Rabbits with weighed 1.5-2.5 kg and were 2-3 months old. A total of 18 rabbits (6 control and 16 experimental) were employed in this investigation, with the experimental group being separated into two groups of n=6 each: Group A was a control group that was given a normal diet and mineral water, while Experimental Group Rabbits divided into two sub groups (B, C, and D experimental group with six rabbits in each) experimental group induced with a PTU (50 mg/kg/day) was given for 21 days to induce hypothyroidism. After three weeks, a blood sample was drawn from the central ear artery, which was collected in a clean, dry test tube and allowed to clot before the serum was separated. Hormonal level of triiodothyronine (T3), free thyroxine (T4), and thyroid stimulating hormone (TSH) was measured using a Mini-VIDAS system and a technique to link radiation enzymatic by Enzyme Linked Fluorescent Asymmetry (ELFA). (7). After induced all rabbits experimental group C was given 500 mg/day/oral PHFext. of (extracts of CW+MO+WS+TF), and Group D was given Thyroxine 50 mcg/daily for 45 days. (15) After 45 days, blood samples was collected and serum thyroid profile was assessed.(15).

Serum Liver and renal Enzymes Analysis:

Spectrophotometric analysis was used to measure blood urea nitrogen (BUN) and serum creatinine. as well as the levels of the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in the blood were measured by liquiform method kit supplied by Randox laboratories Ltd. (BT29 4QY; Crumlin, County Antrim, UK) was used. The AST kit had a measurement range of 7.20 to 1039 U/L. According to the vender, the ALT measurement range was 9.7–603 U/L. When the sample's absorbance was less than 0.5 and the CV was lower than 10%, linearity was maintained.(16)

Histological investigation:

Thyroid gland tissue samples were collected, and as soon as they were removed from the body, they were preserved in 10% buffered formalin. Sections were cut at 6 microns thickness after tissue preparation and embedding in paraffin, and the slides were stained with hematoxylin and eosin stain. (17).

Statistical Analysis

The results were analysed using one-way analysis of variance (ANOVA) and Dunnett's test, and values with p=0.05 were considered significantly different. (18).

Results:

Thyroid profile results:

The T3 (triiodothyronine) levels in the various groups. the mean T 3 concentration was significantly higher ($p \leq 0.05$) in the group-B (PHF) When compared to the group-D (thyroxin) which show positive result of group-B see Table 1-2. The various groups' levels of (thyroxine) are assessed. When comparing group B (PHF) to group C, the mean T4 concentration was considerably greater ($p \leq 0.05$). in group-C. (thyroxin). Using the Polyherbal formulation to treat hypothyroidism results in a higher T4 level, according to the results. show at Table. 1-2. The TSH (thyroid stimulating hormone) levels in the various groups. the mean TSH concentration was significantly ($p \leq 0.05$) in the PHF group to the CON (control group) and Induce group, but TSH (thyroid stimulating hormone) levels was not significant in group-B(PHF) group when compared to the group-D (thyroxin) see Table 1-2.

Reno-Hepatic protective effects of PHF:

There were definite positive connections between the average levels of serum urea (SU). The control group had the lowest levels of serum urea and creatinine, followed by group B (PHF) no significantly ($P \geq 0.05$), whereas group C (thyroxine) and PTU (Induce) had the greatest levels of both substances very significantly ($P \leq 0.001$) at 45 days after PTU treatment. See graph.1:

Serum Creatinine levels in the various groups. the serum Creatinine concentration was very significantly ($P \leq 0.001$) in the group-B (PHF) when compare to group-C (thyroxine) and Induce group, but no significantly ($P \geq 0.05$) in the group-B (PHF) group to the group-A (control) data shows at Graph.2.

The levels of ALT (alanine aminotransferase) in the various groups. When compared to group-C (thyroxine) and the induce group, the ALT (alanine aminotransferase) concentration was considerably higher than group-B (PHF) very significantly ($P \leq 0.001$), but not significantly ($p > 0.05$) when compare to the group-A. (control) see Graph.3.

Aspartate aminotransferase (AST) levels are displayed in Graph.7 for each group. The alanine aminotransferase (ALT) concentration was significantly greater ($P>0.001$) in group B (PHF) than in group A, and significantly higher (P 0.05) in group B (PHF) than in group C (thyroxine). However, there was no significant difference ($p> 0.05$) between group B (PHF) and group A. (control).

Histological results:

A histological investigation the normal appearance of thyroid tissue in (Picture:1,) control group. The evaluate the revealed deformation of the arteries, follicles, and normal architecture. In most thyroid tissue from the induce group compared to the control, there were less Follicular epithelium cells along with a significant drop in or absence of colloidal and Hyper-congestion of vessels. (Picture:2 & Picture: 3). Thyroid tissue in rabbits with hypothyroidism restored to more-or-less normal size after receiving PHF therapy for 45 days, very Significant improvement in the thyroid tissue appeared normal. High cubical cells line the inside of the follicles. Thyroid tissue had more follicles normal-looking colloidal epithelium and normal-looking vascularity content. (Picture:4 and 5). Histological result shows that restore and rebuild the thyroid tissue very highly by using the polyherbal formulation.

Discussion:

One of the most important components of the endocrine system is the thyroid gland, also referred to as the gland of vital hormones. Thyroxine and triiodothyronine, sometimes known as T4 and T3, are the two main hormones secreted by the thyroid gland. (19). hypothyroidism causes less T3 and T4 to be secreted into the bloodstream. Due to the thyroid gland's improper functioning,(20). The most common medical condition in the world is hypothyroidism. Among endocrine illnesses, primary hypothyroidism is one of the most prevalent. In the developed world, hypothyroidism affects roughly 4.6% of people. (21). The thyroid controls body temperature, heart rate, sexual function, development, emotions, and gastrointestinal motility. (1). It has been proposed that supplement and drug regimens with anti-hypothyroidism qualities and reno-hepato protecting potential could lower the risk for these diseases and disorders that are connected to them. In this study, a polyherbal formulation containing extract from *Commiphora wightii, Wathina somnifera, Moringa oleifera,* and *Trigonella foenum-graecum* was tested in a rabbit model of hypothyroidism to determine its effectiveness in regulating the thyroid gland to treat hypothyroidism and to have renal-hepatoprotective effects. Researchers have studied the effects of *Commiphora wightii* (Resin) have anti-inflammatory, laxative, concoctive, diuretic, expectorant, detergent, emollien, carminative, and antihelminthic properties. (22). Other studies have demonstrated that *C. wightii* significantly affects the cardiac cycle, controlling Systolic and Diastolic arterial pressures, heart rate, and protecting against ischemia by raising lactate dehydrogenase levels and reducing the loss of heart protein. (23). According to a different study, administering *C. wightii* ethanolic extract increase T4 to T3 conversion efficiency. (24). Z-guggulsterone in *C. wightii* its ability to stimulate the thyroid. The administration of isolated Z-guggulsterone to rats resulted in a significant increase in all thyroid function parameters, including

the thyroid's ability to absorb iodine, the activity of the enzymes responsible for producing thyroid hormones, and the ability of tissues to absorb oxygen, indicating a thyroid-stimulating effect.(25).

Wathina somnifera (Root) has been shown in studies to have analgesic, anti-stress, sedative, anti-aging, immune-supportive, arthritis neuroprotective, immunomodulatory, and memory-enhancing qualities.(26). Another study has demonstrated that using a methanolic extract of *W. somnifera* can enhance thyroid hormones and reduce oxidative stress, hence improving thyroid function.(10). *W. somnifera i*s also used to treat diabetes, hypercholesterolemia, and the brain condition known as cerebellar ataxia. It also has the ability to treat hypothyroidism by regulating T3 and T4 and lowering TSH in serum. Antipsychotic medications, which are used to treat schizophrenia but also have the potential to cause side effects, include those that cause irregular serum levels of sugar and fat. (27). Animal studies have demonstrated that *W. somnifera* significantly raises serum T4 and stimulates thyroid activity, suggesting that it may be an effective herbal remedy for treating hypothyroidism. (28).

Research study show that *Moringa oleifera* have significant nutritional value with anti-diabetic, antioxidant, antibacterial, anti-fibrotic, anti-cancer, anti-inflammatory, and anti-hyperglycemic properties. (29). Another study on animals demonstrates the effectiveness of *M. oleifera* in lowering TSH levels, which are strongly inversely linked with T3 and T4 levels. (30). Another animal research confirms that *M. oleifera* and *spinach* have increased effectiveness in regulating T3 and T4 while lowering blood TSH levels. (31).

The use of *Trigonella foenum-graecum* as a spice, herb, nutritional supplement, or medicinal agent for a variety of illnesses, such as antidiabetic, metabolic syndrome, anti-cancer, anti-oxidant, hyperlipidemia, anti-toxic, hepatoprotective, anti-atherogenic, antifungal, and antibacterial properties, has been documented in the literature since antiquity. (32). Some literature documented in diabetic control effects of *T. foenum-graecum* can enhance the TSH level. (33). Another study shows that *T. foenum-graecum* regulate the thyroid hormones. (34) (35).

This study show that all four herbs combine in (polyherbal formulation) have very significant results in Histological as well as thyroid hormonal regulation in induced hypothyroidism, and as compare between the experimental Group=B (Induce+PHFext) and Experimental Group=C (Induce+Thyroxine). Graph:1 show that very significant ($p<0.001$) improvement in the T3 (triiodothyronine), T4 (free thyroxine) and very significant decrease ($p<0.001$) TSH (thyroid stimulating hormone) level in experimental Group=B while Graph:2 show that significant ($p<0.01$) improvement in the T3 (triiodothyronine), T4 (free thyroxine) and very significant decrease ($p<0.001$) TSH (thyroid stimulating hormone) level in experimental Group=C. on the basis of results Group=B (PHFext=500mg) is very potent and effective and Group=C(Thyroxine 50 mcg) in induce Hypothyroidism, and according histological improvement that picture 4 and 5 the experimental Group=B (Induce+PHFext) show very significant improvement in Colloid with follicular epithelium and vascular hyper-congestion as compare to picture 6 and 7 of Group=C (Induce+Thyroxine).

Conclusion:

It's possible that the combination of the most active extracts from all four plants can help to regulate the thyroid hormonal levels as well as repairing the histology of thyroid tissue. The most effective dosage is 500mg per kg/oral/day. Furthermore, more research is needed to isolate, purify, and characterized active component(s) from most active extracts that could be used as a stand-alone or supplementary treatment for hypothyroidism.

References:

1. Negm SHJASEJ. Effect of Soybean, Virgin Coconut and Moringa oleifera Seed Oils on the Propylthiouracil Induced Hypothyroidism in Rats. 2019;40(OCTOBER-DECEMBER):649-59.

2. Naz N, Rizvi S, Sadiq ZJPjops. Assessment of thyroid hormone levels and thyroid disorders: A case study from Gujranwala, Pakistan. 2017;30(4).
3. Mujeeb MI, Abbasi PJRJoP, Sciences P. Comparative Study of Effectiveness and Safety of Herbal Compound Medicine (Constinil) and Allopathic Medicine (Sodium Picosulfate) in Managing Constipation. 2018;6(1):67-75.
4. Shinwari ZK, Qaiser MJPJB. Efforts on conservation and sustainable use of medicinal plants of Pakistan. 2011;43(1):5-10.
5. Khattak SR, Hussain A, Ali AA, Ahmad T, Ayaz S, Akram M, et al. Comparative studies of leaves and bark of Moringa oleifera originated from Khyber Pakhtoon Khwa and Punjab, Pakistan. 2020;9(3):1505-9.
6. Piantanida E, Ippolito S, Gallo D, Masiello E, Premoli P, Cusini C, et al. The interplay between thyroid and liver: implications for clinical practice. 2020;43:885-99.
7. Zahra'a AJJMSCR. Assessment of the impacts of 830 nm Low Power Laser on Triiodothyronine (T3), Thyroxine (T4) and the Thyroid Stimulating Hormone (TSH) in the Rabbits. 2014;2(11):2902-10.
8. Ramesh B, Saralakumari DJJop, biochemistry. Antihyperglycemic, hypolipidemic and antioxidant activities of ethanolic extract of Commiphora mukul gum resin in fructose-fed male Wistar rats. 2012;68:573-82.
9. Gupta A, Gautam MK, Singh RK, Kumar MV, Rao CV, Goel R, et al. Immunomodulatory effect of Moringa oleifera Lam. extract on cyclophosphamide induced toxicity in mice. 2010.
10. Abdel-Wahhab KG, Mourad HH, Mannaa FA, Morsy FA, Hassan LK, Taher RFJMbr. Role of ashwagandha methanolic extract in the regulation of thyroid profile in hypothyroidism modeled rats. 2019;46(4):3637-49.
11. Plants M. Proceeding of. 2010.
12. Devi J, Rajkumar JJAJPP. Antihyperlipidemic effect of ambrex, a polyherbal formulation against experimentally induced hypercholesterolemia in rats. 2013;7:1737-43.
13. Oecd OJAotatcmtn-OfEC-o, Development. Guidelines for the Testing of Chemicals. 2001.
14. Singha MB, Sarma T, Lahkar MJJoP, Phytochemistry. Anti-hypothyroid potential of Bauhinia variegate and Commiphora mukul extracts in albino wistar rats: A histopathological investigation. 2019;8(1):1736-40.
15. Chen W-J, Lin K-H, Lai Y-J, Yang S-H, Pang J-HSJC. Protective effect of propylthiouracil independent of its hypothyroid effect on atherogenesis in cholesterol-fed rabbits: PTEN induction and inhibition of vascular smooth muscle cell proliferation and migration. 2004;110(10):1313-9.
16. S Farrag O, Salman D, Ali FAZ, S Sayed A, Mahmoud ME, A Abd-El Raheem A-ERJJoAVS. Impact of continuous treatment with propylthiouracil on renal and hepatic functions in rabbits. 2020;5(2):49-60.
17. Hossain AOJPRS. Carbimazole and its effects on thyroid gland of female rabbits. 2019;13(3):310.
18. Montgomery DC. Design and analysis of experiments: John wiley & sons; 2017.
19. Saifi N, Jamal YJWJPPS. An analytical study of thyroid hormones in different temperaments (Mizaj). 2016;5:1612-8.
20. Khadtale DD, Vaidya MS. EFFECT OF AYURVEDIC DRUG COMBINATION IN MANAGEMENT OF HYPOTHYROIDISM–A CASE STUDY. 2020.
21. Panthi S, Gao TJIJCEM. Diagnosis and management of primary hypothyroidism in Traditional Chinese medicine (TCM) and Traditional Indian Medicine (Ayurveda). 2015;12(009).
22. Bhat SA, Rather SAJJoDD, Therapeutics. Medicinal benefits and scientific justification of Commiphora mukul (Muqil): A review. 2021;11(1-s):170-2.
23. Ojha SK, Nandave M, Arora S, Mehra RD, Joshi S, Narang R, et al. Effect of Commiphora mukul extract on cardiac dysfunction and ventricular function in isoproterenol-induced myocardial infarction. 2008.
24. Stansbury J, Saunders P, Winston DJJoRM. Promoting healthy thyroid function with iodine, bladderwrack, guggul and iris. 2012;1(1):83-90.

25. Jaiswal S, Bara J, Soni R, Saksena PJIJNHS. Medical uses of Commiphora wightii. 2016;5:76-81.
26. Mukherjee PK, Banerjee S, Biswas S, Das B, Kar A, Katiyar CJJoE. Withania somnifera (L.) Dunal-Modern perspectives of an ancient Rasayana from Ayurveda. 2021;264:113157.
27. Sailaja AKJdo. B. Pranaya ragini. Biomedical Applications of Withania Somnifera''. J Pharmacy and Drug Innovations, 1 (1); DOI: http. 2020.
28. Singh G, Sharma P, Dudhe R, Singh SJABR. Biological activities of Withania somnifera. 2010;1(3):56-63.
29. Kumar R, Nikitha M, Meera V, Umakanth B, Mahesh C, Bharathi D, et al. A pharmacological approaches on moringa oleifera plant-a brief review. 2022:80-5.
30. Alam MA, Quamri MA, Haider NJDm, therapy p. Efficacy and safety of Barg-e-Sahajna (Moringa olifera Lam.) in primary hypothyroidism. 2021.
31. Mondal C, Chandra AKJBJoPS. Goitrogenic/antithyroidal potential of moringa leaves (Moringa oleifera) and spinach (Spinacia oleracea) of Indian origin on thyroid status in male albino rats. 2019;55.
32. Yadav UC, Baquer NZJPb. Pharmacological effects of Trigonella foenum-graecum L. in health and disease. 2014;52(2):243-54.
33. Majumdar J, Chakraborty P, Mitra A, kumar Sarkar N, Sarkar SJE, Endocrinology C, et al. Fenugreek, a potent hypoglycaemic herb can cause central hypothyroidism via leptin–a threat to diabetes phytotherapy. 2017;125(07):441-8.
34. Kiss R, Pesti-Asbóth G, Szarvas MM, Stündl L, Cziáky Z, Hegedűs C, et al. Diosgenin and its fenugreek based biological matrix affect insulin resistance and anabolic hormones in a rat based insulin resistance model. 2019;2019.
35. Al-Zyadi AJJTIJoVM. The therapeutic role of alcoholic extract of fenugreek seeds on hypothyroidism state induced by thiourea and some blood parameters in adult male rabbits: Atyaf JH Al-Zyadi and Jawad K. Arrak. 2015;39(1):1-7.

Table 1 Variable of thyroid hormones between Group A (control) and Experimental Group C (PHF) before and, after induce, and after experimental intake (PHF) in rabbits Experimental group C. show there are a Significant Rise ($p > 0.01$) in T3, T4 production and decrease TSH thyroid hormones after induce (PTU) between the group A (Control) and experimental group C (PHF 500 mg), and the result shows after the treatment a Very Marked Improvement ($p > 0.001$) to reduce T3, T4 and normal TSH in Group-C (PHF).

Table 1: Variable of thyroid hormones before induce Group A (Control), and Group C (PHF) after induce and after experimental supplementation (PHF) in rabbits Experimental group C.

	At induce each group (n=6)			After experimental study each group (n=6)		
Thyroid profile.	Group A (Mean ± SD)	Group C (Mean ± SD)	*P*-Value	IC (Mean ± SD)	Group C (Mean ± SD)	*P*-Value
T3 (triiodothyronine)	1.3733±0.340	0.2383±0.0541	.001	0.2383±0.0541	1.835±0.2172	0.001
T4 (free thyroxine)	4.1283±0.2667	0.715±0.1663	0.001	0.715±0.1663	4.510±0.6587	0.001
TSH (thyroid stimulating hormone)	1.3750±0.5000	5.325±0.4151	0.001	5.325±0.4151	1.083±0.490	0.035

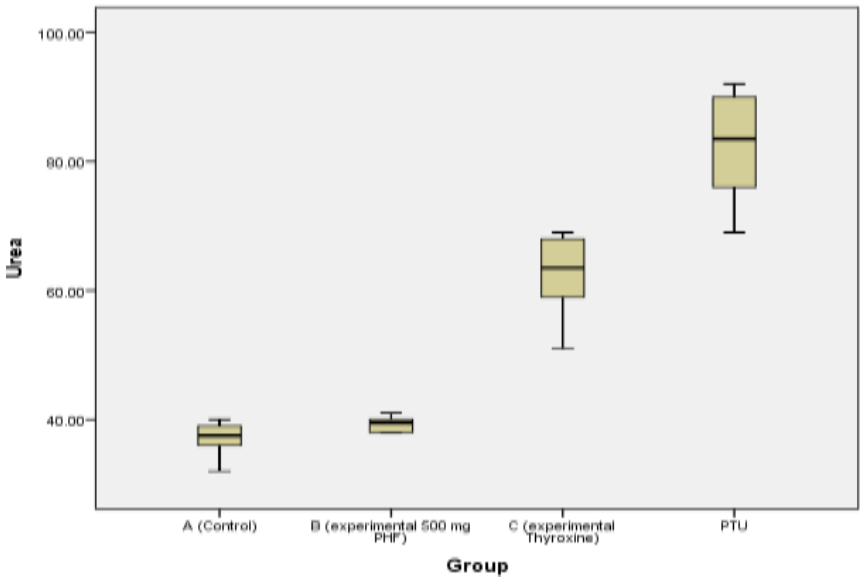
Note: -p >0.05 =not significant, p<0.05 = just significant, p < 0.01 = significant, p < 0.001 =very significant
IC = indued control (after 21 days) after the indue PTU
Group= B is experimental PHF (500mg)

Table 2: Variable of thyroid hormones between Group A (control) and Experimental Group D (Thyroxine) before and after induce, and after experimental intake (Thyroxine) in Experimental group D. show a Significant Rising trend ($p > 0.01$) in T3, T4 production and lower production of TSH thyroid hormones after induce (PTU) between the group A (Control) and experimental group C (Thyroxine), and the result shows a Massive Improvement ($p > 0.001$) to reduce T3, T4 (Thyroxine) and regulate to TSH production in Group D.

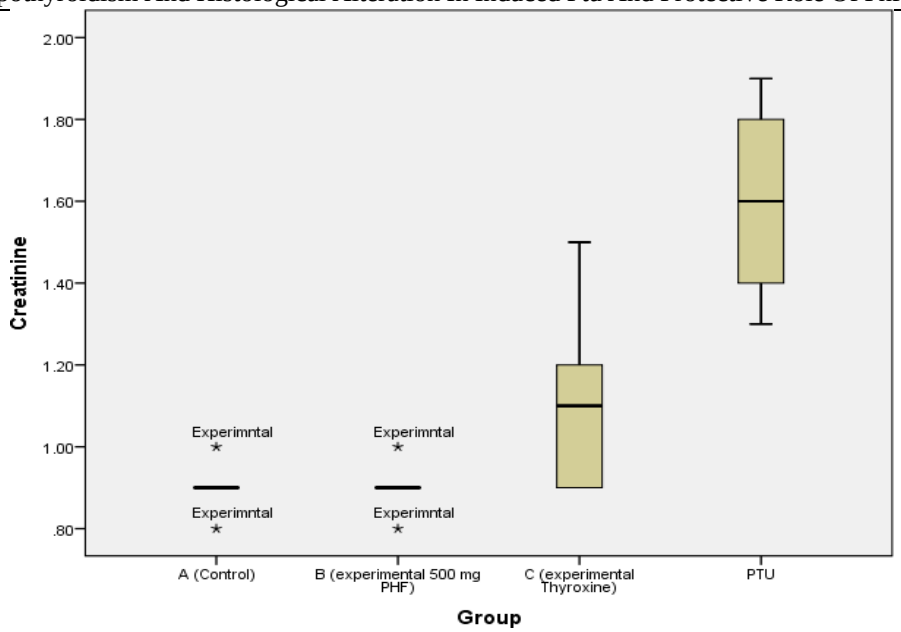
Table2: Variable of thyroid hormones before and after induce and after experimental supplementation (PHF) in rabbits Experimental group D (Thyroxine) .

	At induce each group (n=6)				After experimental study each group (n=6)		
Thyroid profile.	Group A (Mean ± SD)	Group D (Mean ± SD)	*P*-Value		IC (Mean ± SD)	Group D (Mean ± SD)	*P*-Value
T3 (triiodothyronine)	1.3733±0.340	0.3566±0.0542	.001		0.3566±0.0542	0.9967±0.2861	.001
T4 (free thyroxine)	4.1283±0.2667	0.4683±0.3570	.001		0.4683±0.3570	3.2533±3.0356	.015
TSH (thyroid stimulating hormone)	1.3750±0.5000	5.335±0.3928	.001		5.335±0.3928	1.3583±0.5763	.010

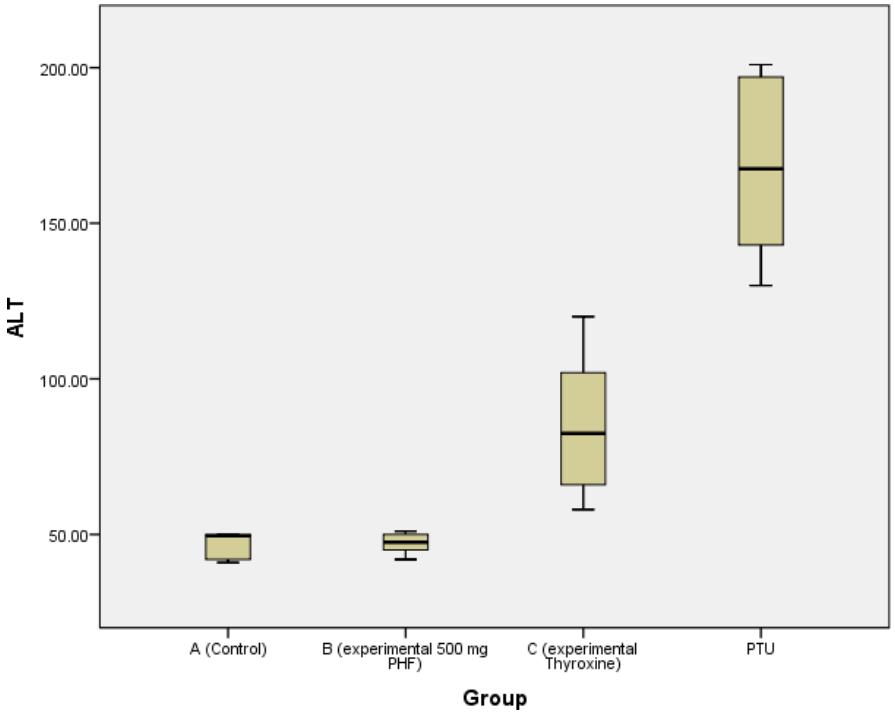
Note: -p >0.05 =not significant, p<0.05 = just significant, p < 0.01 = significant, p < 0.001 =very significant
IC = indued control (after 21 days) after the indue
PTU
Group= D is experimental Thyroxine tablet (0.5 mg)



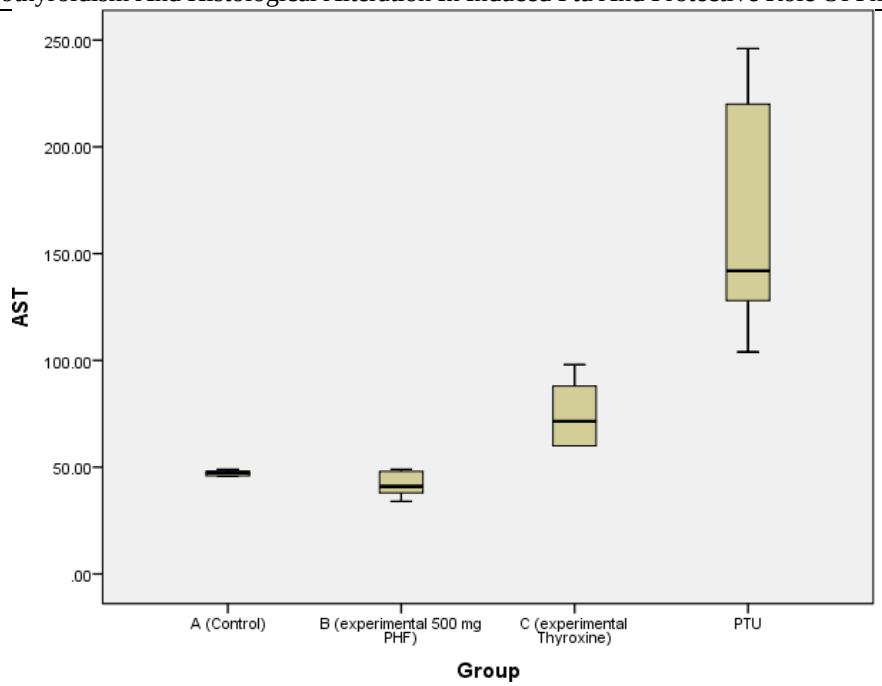
Graph.1: This graph compares the levels of serum urea and serum creatinine in each group. In the graph, after 45 days of trial, Group B greatly outperformed Group C (thyroxine).



Graph.2: This graph show Serum creatinine levels are depicted in this graph for each group, with group B significantly improving (p>0.01) over group C. (thyroxine).

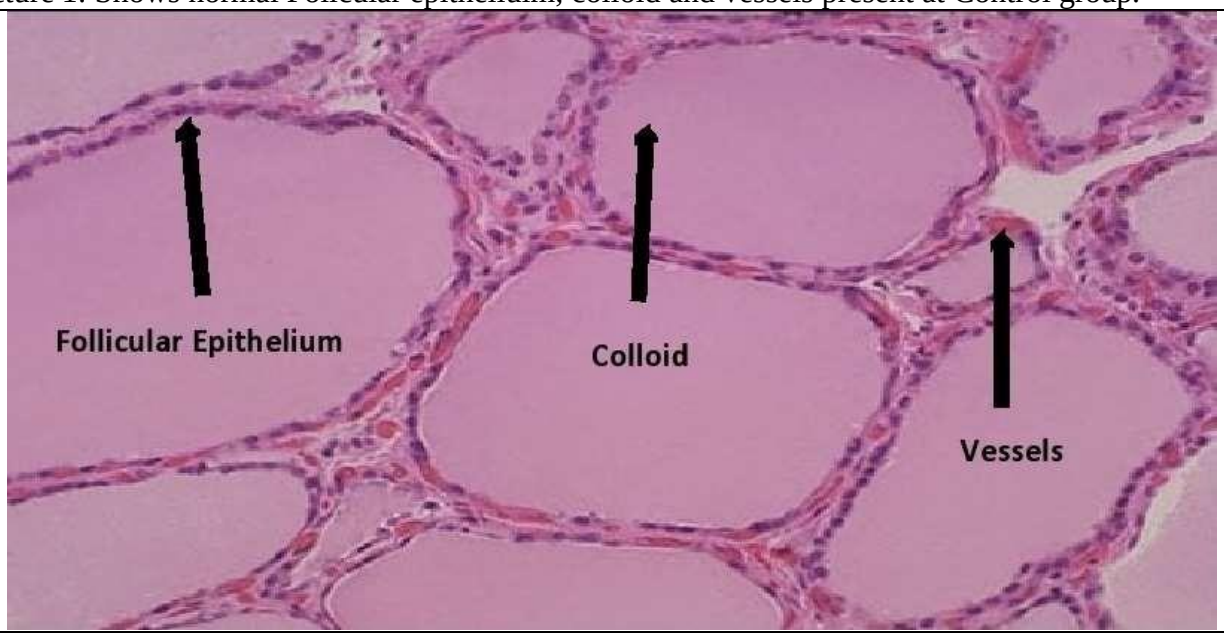


Graph.3: This graph demonstrates the compression of the ALT (alanine aminotransferase) in each group, with group B being significantly more significant (p>0.001) than group C.

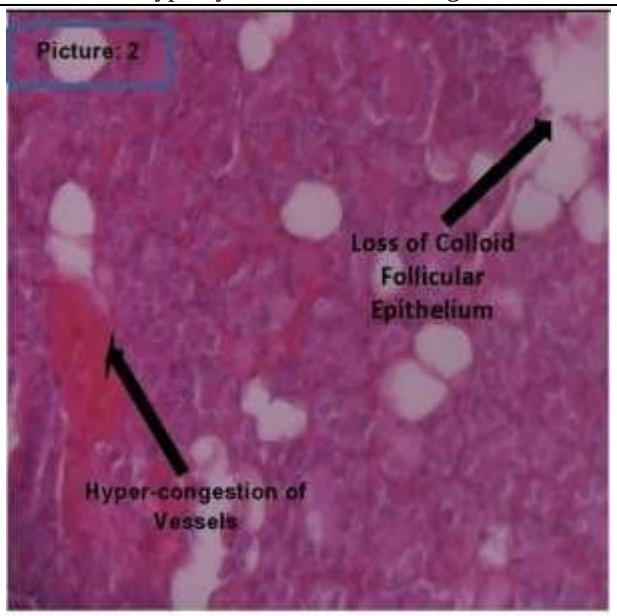


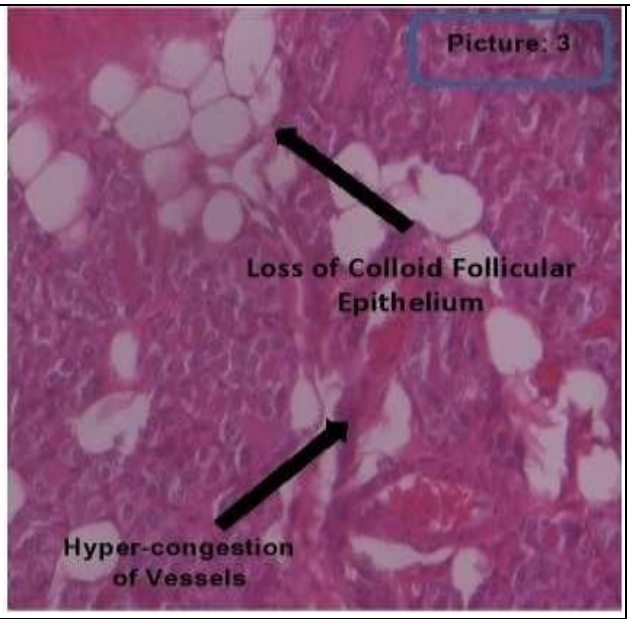
Graph.4: In this graph, all of the groups' aspartate aminotransferase levels are compressed; group B is clearly more significant ($p>0.001$) than group C.

Picture 1: Shows normal Folicular epitheliuim, colloid and vessels present at Control group.



Picture 2 & 3 show Hyper-congestion of vessels and Loss of colloid follicular epithelium at induced Group





Picture 4 and 5 show that After undergoing PHF administration, the thyroid tissue in rabbits with hypothyroidism was recovered to more-or-less normal size.