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Evaluation of Antithyroid Activity of Rosemary in Rodents

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ABSTRACT

Aim of present work was to evaluate antithyroid activity of rosemary. Ethanolic Extract of Rosemary was used as dosing material and experiment was done on the rodents. Rosemary extracts 400 and 200 mg/kg showed comparable effects on the LT4-induced rat hyperthyroidism as compared with PTU 10mg/kg. These effects of rosemary may help the improvement of hyperthyroidisms and accompanied various organ damages, but active compound searches should be proceeding in future.

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1. INTRODUCTION:

The aim of the current study was to evaluate property of antithyroid on herbal plants as well as natural products by using previous events.

The prevalence of thyroid disease rises with age, and it is widespread. In the general population, 5%–9% of adults have subclinical thyroid illness, while 0.8%–7.5% have clinical thyroid disease types [1–3].

The only known iodine-containing substances with biological activity, thyroid hormones serve two crucial purposes. They are key regulators of healthy growth in growing animals and people, particularly in the central nervous system (CNS). Thyroid hormones influence the functioning of almost all organ systems in adults by helping to maintain metabolic homeostasis. The thyroid gland has significant produced hormone reserves to address these needs.

Although local metabolism also takes place in target tissues like the brain, the liver is where thyroid hormones are largely metabolised. In a conventional negative-feedback system, the pituitary hormone thyrotropin accurately controls the quantities of thyroid hormones in the blood. Thyroid hormones mainly work by binding to nuclear thyroid hormone receptors (TRs) and controlling the transcription of particular genes. Thyroid hormones and the nuclear receptors that make up the superfamily of steroid, vitamin D, and retinoid receptors share a similar mechanism of action in this regard[4].

2. Materials and Experimental design

For the study of anti-thyroid activity of Rosemary, an experiment model is selected in such a way that it would satisfy the following:

- The animal should develop hyperthyroidism rapidly.
- Pathological changes in the site of induction should result from damage to the follicular cells of thyroid.
- The symptoms should be ameliorated or prevented by a drug treatment effective in humans.

In the present study rats have been used because, the thyroid hormone production and metabolism of rat resembles to that of humans which is believed to contribute to hyperthyroidism studies.

Animals: Albino Wistar Rats (190-240gm)

Drugs: Ethanolic Extract of Rosemary leaves, L-Thyroxine (T4) (Sigma, USA),

Propylthiouracil (MacLeod's Pharmaceuticals Ltd).

2.1 Selection and Acclimatization of Animals

Adult male wistar rats (6week old) weighing 190–240 g were used in the experiments after allowing 15 days acclimatization. The animals were allocated four per polycarbonate cage in a temperature (20–25^{0}C) and humidity (40–45%) controlled room. The light: dark cycle was 12 hr: 12 hr and normal rodent pellet diet and water were supplied during acclimatization, free to access.

2.2 Induction of Hyperthyroidism

After acclimatization, hyperthyroidism was achieved by daily oral administration of L-Thyroxine (T4) (Sigma, USA) at a dose of 600µg/kg for 12 consecutive days according to the previous established method.

2.3 Preparation of Drugs:

• Ethanolic extract of Rosemary leaves is dissolved in sterile water.

• Propyl thiouracil tablets were weighed, powdered and triturated with saline.

• L-thyroxine tablets were weighed and dissolved in distilled water.

2.4 Treatment Protocol

Animals were randomly divided into 5 groups of 6 rats each after 12 days L-thyroxine (T4) treatments as follows:

Group 1: Served as normal control received 10ml/kg of normal saline.

Group 2: Served as hyperthyroidism control received distilled water orally for 15 days.

Group3: Served as treatment control received 10mg/kg of propyl thiouracil (PTU), orally for

15 days.

Group 4: Served as treatment control received 200mg/kg of Ethanolic extract of rosemary orally for 15 days.

Group 5: Served as treatment control received 400mg/kg of Ethanolic extract orally for 15 days.

After 12 days of L- thyroxine treatment, propylthiouracil was injected intraperitoneally to G3 group in a volume of 5ml\kg dissolved in saline for 15 days to G3 group and plant extract of low dose and high dose administered to G4 and G5 group for 15 days. The dosages of rosemary extracts used in this study were selected based on the previous report, in which 400 mg/kg of Rosemarry extracts showed enough *in vivo* pharmacological effects in rats and propylthiouracil 10 mg/kg was also selected based on the previous *in vivo* efficacy test on the L-Thyroxine (T4) induced hyperthyroidisms in rodents. Equal volume of saline was subcutaneously treated in intact control rats instead of L-Thyroxine (T4), and equal volume of distilled water was oral administered in intact and L-Thyroxine (T4) control rats, instead of rosemary extracts or propylthiouracil.

3. Methodology

After 15 days of treatment, the blood was collected from the retro-orbital plexus puncture of all groups of overnight fasted rats using micro capillary. The serum was separated for the estimation of thyroid hormones (TSH, T3, and T4) and liver enzymes (AST, ALT). Then the animal was sacrificed by decapitation. The thyroid gland was immediately dissected out, washed in ice cold saline to remove the blood and stored in 10% formalin for histopathological studies. The liver was also separated and homogenised for the estimation of lipid peroxidation and antioxidant defence system.

4. Evaluation of Pharmacological parameters

4.1 Estimation of Biochemical Parameters

Serum Thyroid Hormones

6mL of blood samples were collected into evacuated tubes, and serum was separated by centrifugation at 3000 rpm for 10 min at 4^0C. Separated serum was stored at -70^0C before

analysis. Serum levels of T3, T4, and thyroid-stimulating hormone (TSH) were analysed by colorimetric competitive enzyme immunoassay using individual ELISA kit according to Subuddhi *et al*, respectively.

- **Serum Aspartate Aminotransferase (AST) and Alanine** Aminotransferase (ALT) Serum AST and ALT concentrations were measured by automated blood analyser.

- **Liver Lipid Peroxidation (LPO)**

Separated liver tissues were weighed and homogenized in ice-cold 0.01M Tris- HCl (pH 7.4), and then centrifuged for 15 min as described by Kavutcu *et al.* The concentrations of liver LPO were determined by estimating malondialdehyde (MDA) using the thiobarbituric acid test at absorbance 525 nm, as nm of MDA/mg protein.

- **Liver Antioxidant Defence Systems**

Prepared homogenates were mixed with 0.1mL of 25% trichloroacetic acid (Merck, CA, USA), and then centrifuged at 4,200 rpm for 40 min at 4^0C. Glutathione (GSH) contents were measured at absorbance 412nm using 2-nitrobenzoic acid (Sigma, MO, USA). Decomposition of H_2O_2 in the presence of catalase was followed at 240nm.Catalase activity was defined as the amount of enzyme required to decompose 1nM of H_2O_2 per minute, at 25^0C and pH 7.8. Results were expressed as U/mg protein. Measurements of SOD activities were made according to Sun *et al*. SOD estimation was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with nitro tetrazolium blue to form formazan dye. SOD activity was then measured at 560nm by the degree of inhibition of this reaction, and was expressed as U/mg protein.

One unit of SOD enzymatic activity is equal to the amount of enzyme that diminishes the initial absorbance of nitro blue tetrazolium by 50% during 1 min.

4.2 Histology

The sampled thyroid gland tissues were fixed in 10% neutral buffered formalin. After paraffin embedding, 3–4 μm serial sections were prepared. Representative sections were stained with haematoxylin and eosin (H&E) for an optical microscopy examination. Subsequently, the histological profiles of the organs were observed. The mean cross thickness of thyroid gland, thyroid follicle, and follicular lining epithelium were measured using an automated image analysis process.

4.3 Statistical Analysis

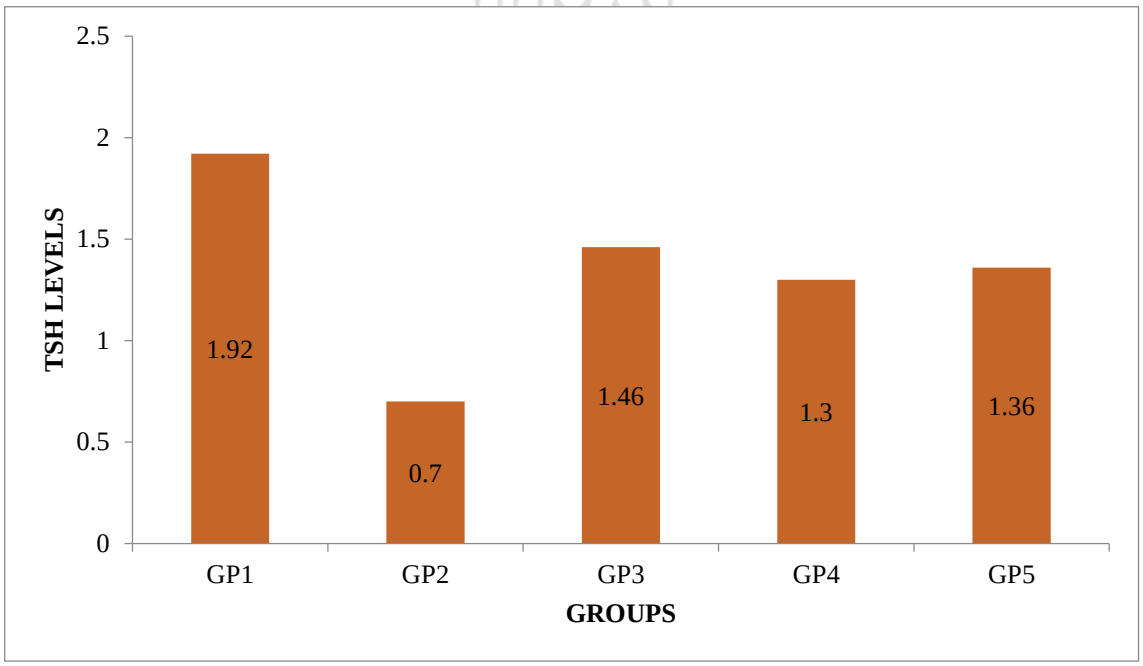
Numerical data are presented as mean± S.E.M. of six rats, the obtain data was analysed using a one-way ANOVA test followed by Newman Keuls multiple range tests. Statistical analyses were conducted using P values < 0.05 were considered significantly different.

Table No. 1: Serum Thyroid Hormone Levels in the L-Thyroxine (T4) and Rosemary Treated Rats

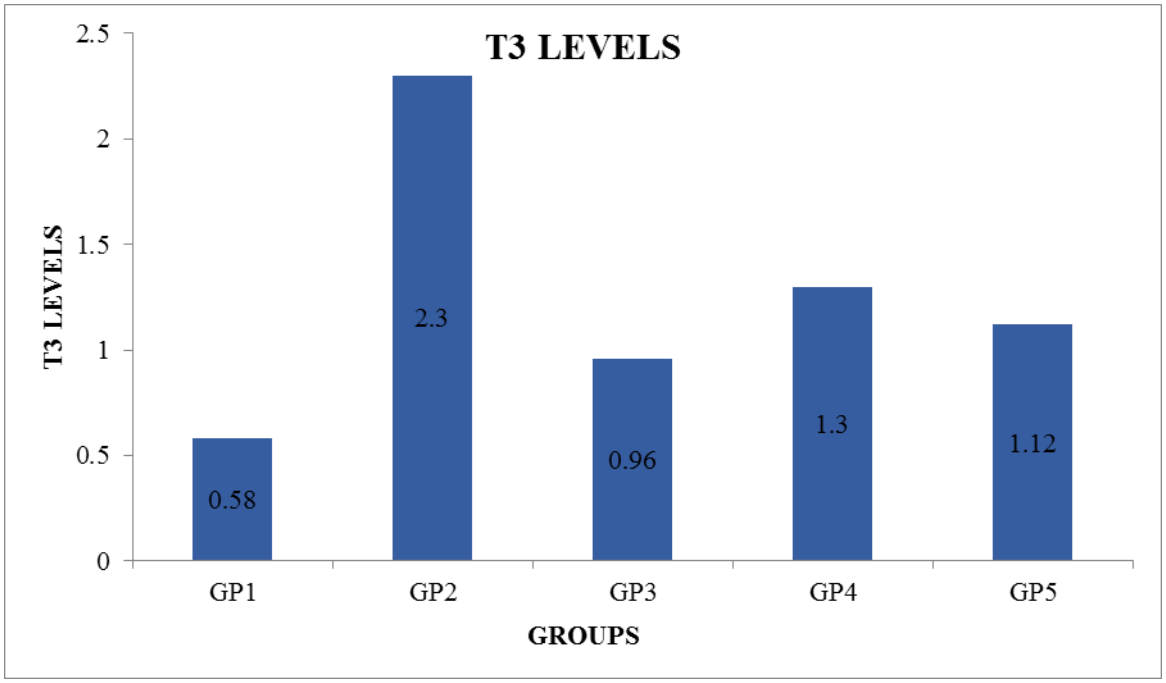
Groups	TSH	T3	T4
1	1.92 ±0.16	0.58 ±0.14	53.72 ±5.90
2	0.70 ±0.07 *a	2.30 ±0.20 *a	171.84 ±4.80 *a
3	1.46 ±0.12 * b	0.96 ±0.12 *b	72.30 ±5.35 *b
4	1.30 ±0.10 * b	1.30 ±0.30 * b	86.12 ±3.10 * b
5	1.36 ±0.11 *b	1.12 ±0.22 *b	78.30 ±4.15 * b

Group 1: Normal; Group 2: Hyper Control; GP3: Standard Control (PTU 10mg/kg); Group 4: rosemary (200mg/kg); Group 5: rosemary (400mg/kg)

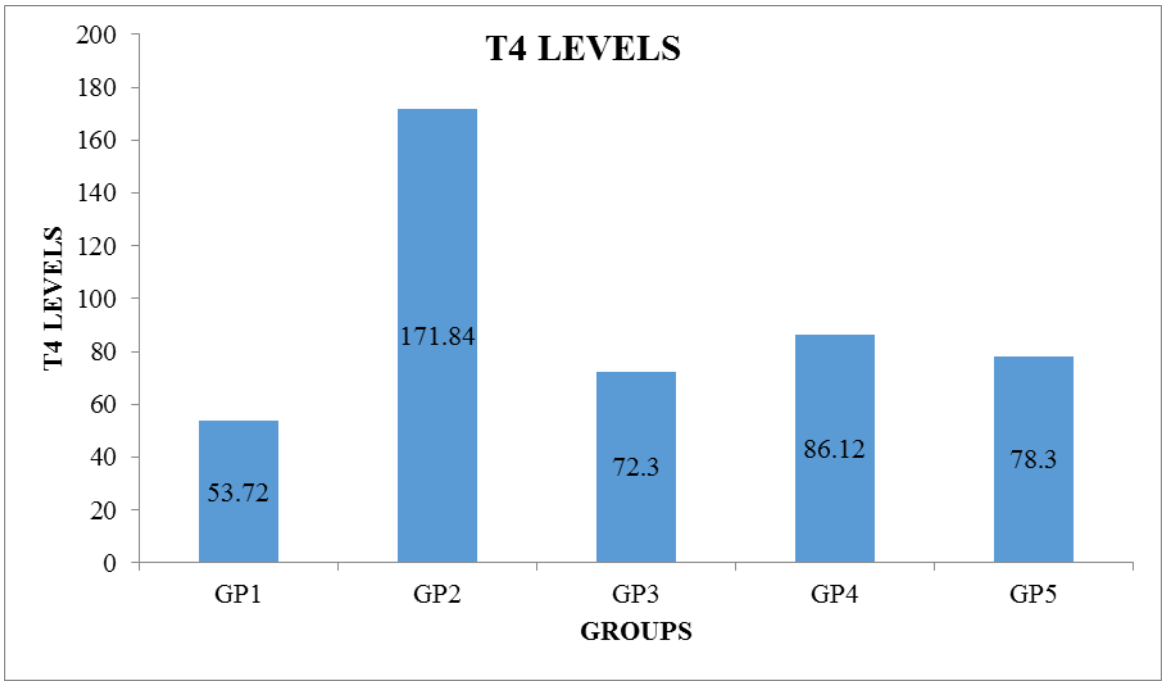
All values are expressed as mean ± SEM□ **a – Values are significantly different from□for 6 animals in each group. Normal control (G1) at P <**b – Values are significantly different□0.01 from hyperthyroid control (G2) at P < 0.01.



Graph 1: Serum thyroid hormone levels in the l-thyroxine (TSH) and rosemary treated rats



Graph 2: Serum thyroid hormone level in the T3 and rosemary treated rats

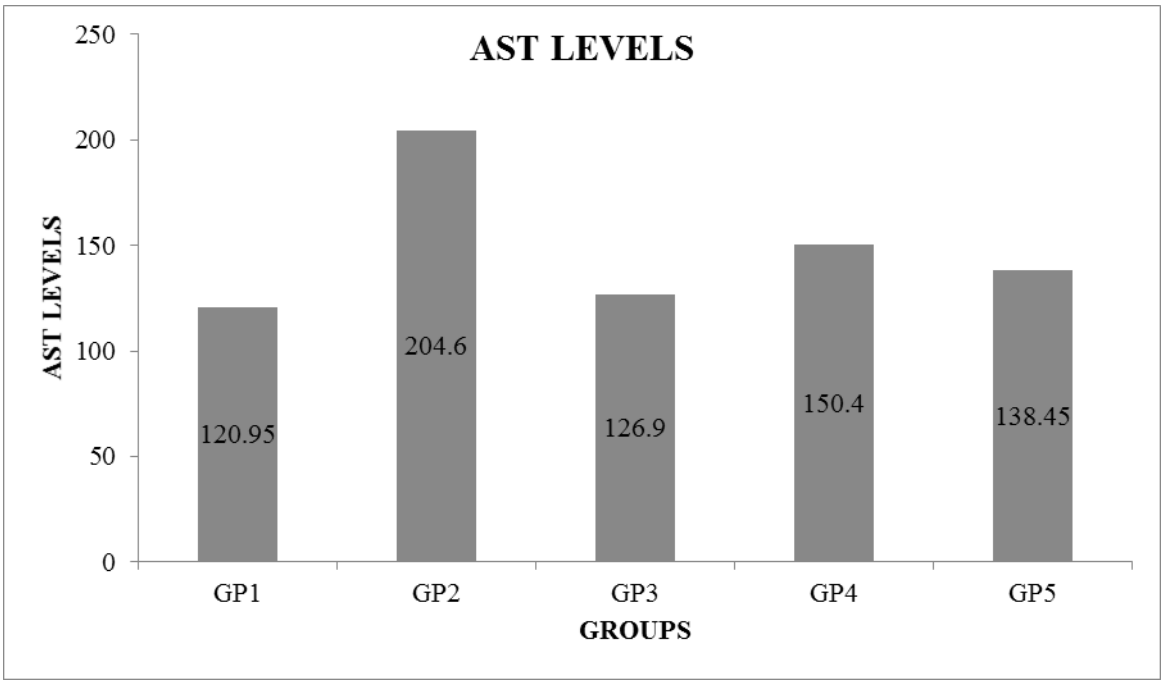


Graph 3: Serum thyroid hormone levels in the L-Thyroxine (T4) and rosemary treated rats

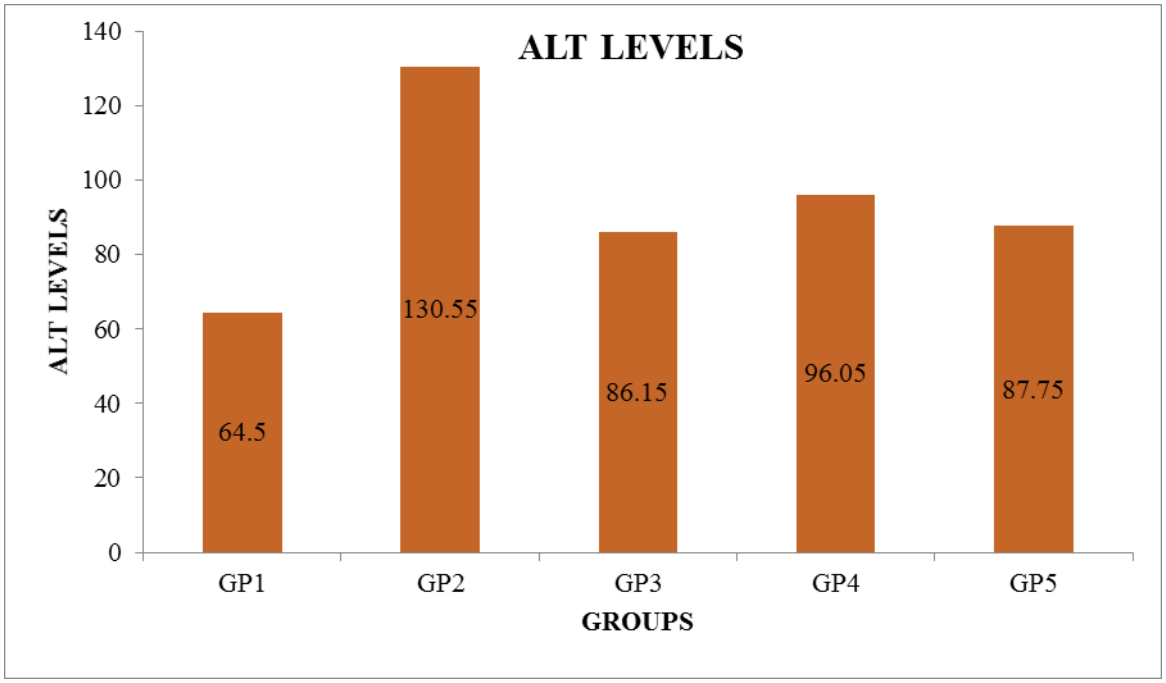
Table No. 2: Effect of rosemary on serum liver enzymes levels in the L-Thyroxine (T4) and rosemary treated animals

Groups	AST(IU/L)	ALT(IU/L)
GP1	120.95±5.20	64.5±3.28
GP2	204.60±8.80*a	130.55±3.75*b
GP3	126.90±5.45*b	86.15±.50*b
GP4	150.40±3.60*b	96.05±4.85*b
GP5	138.45±3.68*b	87.75±3.90*b

GP1- Normal; GP2- Hyper Control; GP3- Standard Control (PTU 10mg/kg); GP4- ROSEMARRY. All values are expressed as mean ± SEM□(200mg/kg); GP5- ROSEMARRY (400mg/kg) for 6 animals in each group. **a – Values are significantly□ different from Normal control (G1) at P < **b – Values are significantly different from hyperthyroid control□0.01 (G2) at P < 0.01.



Graph 4: Effect of rosemary on serum liver enzymes levels in the L-thyroxine (AST) and rosemary treated animals.



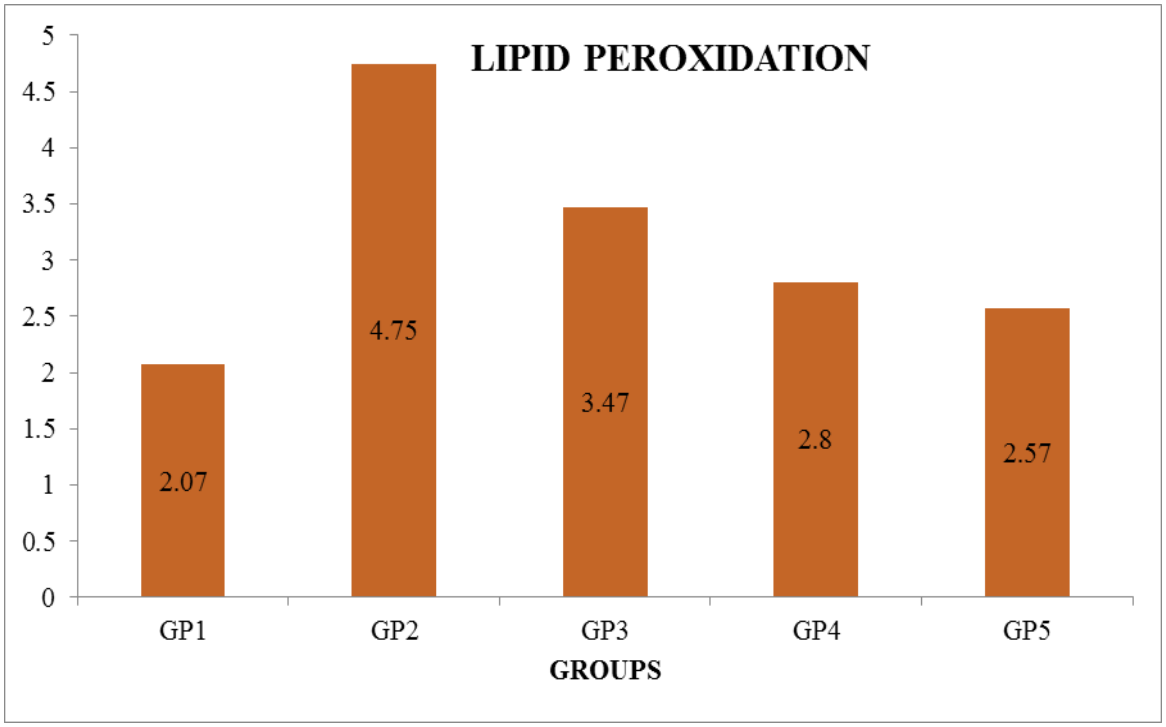
Graph 5: Effect of rosemary on serum liver enzymes levels in the L-Thyroxine (ALT) and rosemary treated animals.

Table No. 3: Liver lipid peroxidation and in the L-thyroxine (T4) and rosemary treated rats

Groups	**MDA**
GP1	2.07±0.12
GP2	4.75±0.30*a
GP3	3.47±0.40*b
GP4	2.8±0.30*b
GP5	2.57±0.16*b

GP1- Normal; GP2- Hyper Control; GP3- Standard Control (PTU 10mg/kg); GP4- ROSEMARRY (200mg/kg);GP5- ROSEMARRY (400mg/kg)

All values are expressed as mean ± SEM□ **a – Values are significantly different from□for 6 animals in each group. Normal control (G1) at P < **b – Values are significantly different□0.01 from hyperthyroid control (G2) at P < 0.01.



Graph 6: Liver Lipid Peroxidation in the L-Thyroxine and Rosemary Treated Rats.

4.4 Histopathological Studies

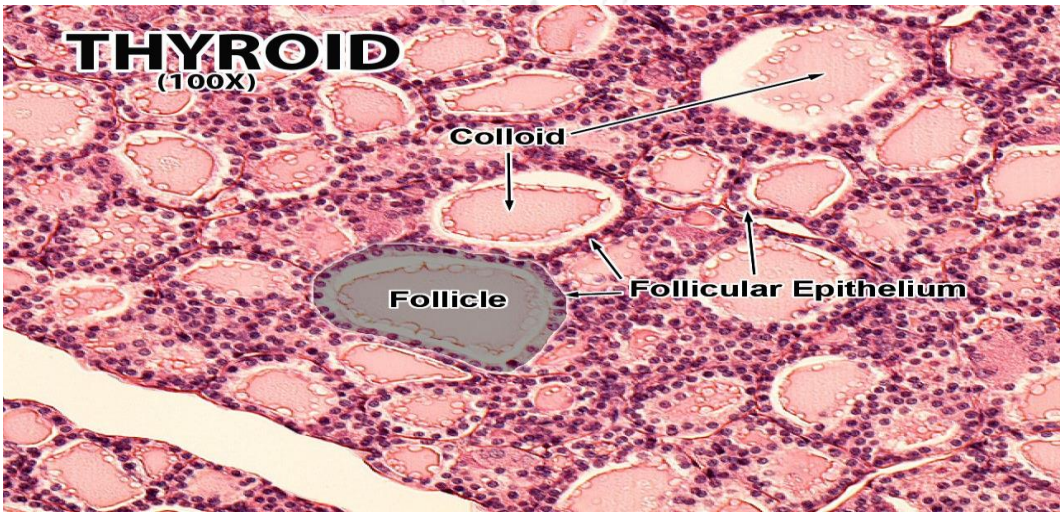
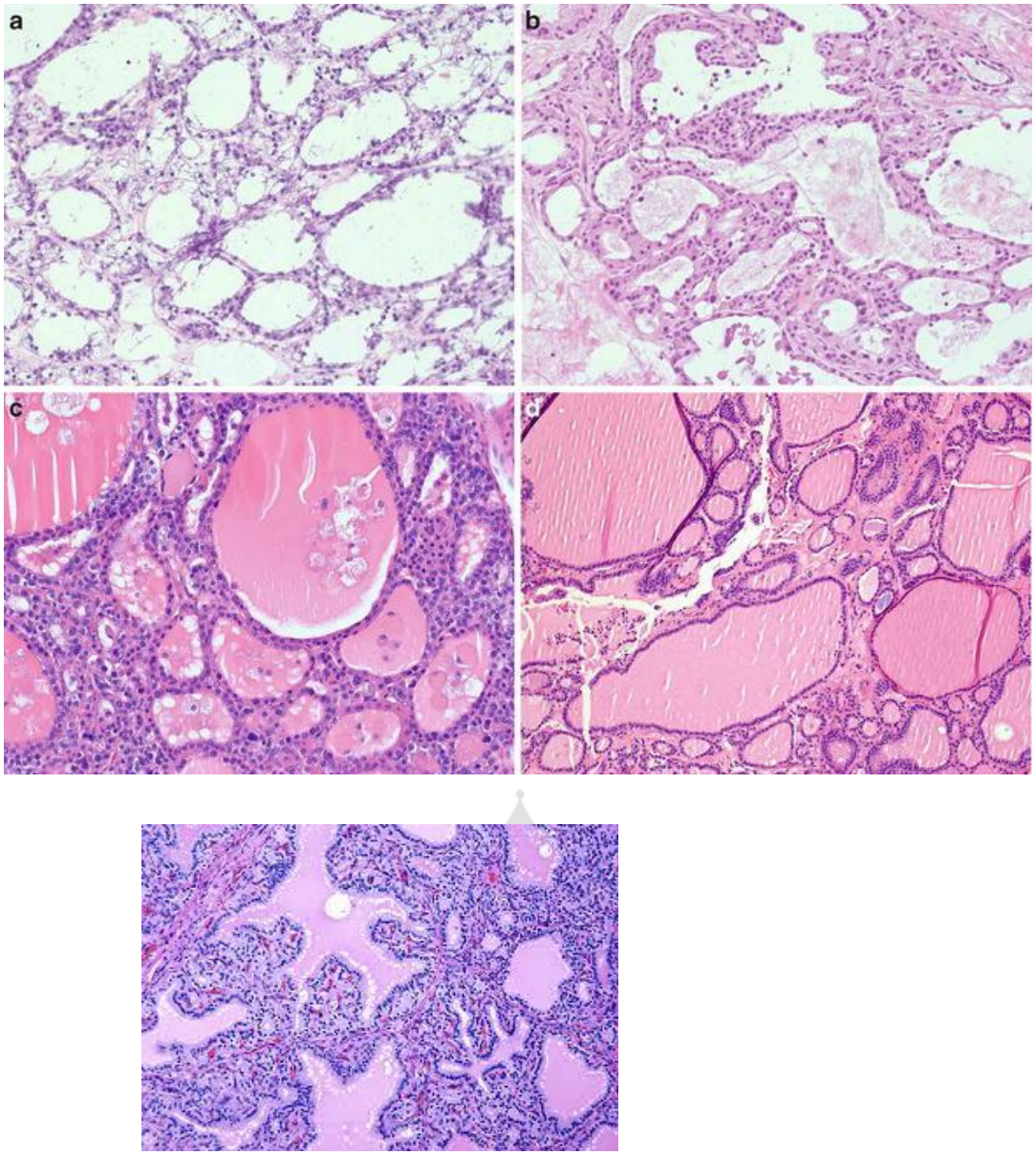


Fig. No. 1: Including Follicle; Follicular Epithelium; Colloid.



Group1: (Control)

Group 2: (Hyperthyroid control)

Group 3: (Standard Control at a Dose of 10mg/Kg.)

Group 4: (Rosemarry Extract at Dose of 200mg/Kg)

Group 5: (Rosemarry Extract at aof Dose 400MG/KG)

5. RESULTS AND DISCUSSION

5.1 Effects on the Serum Thyroid Hormones

L-THYROXINE (T4) treatment induced significant ($P < 0.01$) increase of the serum T3 and T4 levels and decrease of the serum TSH contents. But 400 and 200mg/kg of Rosemary extracts significantly ($P < 0.01$) and dose-dependently normalized the changes on the serum T3, T4, and TSH concentrations induced by L-THYROXINE (T4) as compared with L-THYROXINE (T4) control. PTU 10 mg/kg also normalized the serum thyroid hormone levels, as similar as rosemary extracts 200 and 400 mg/kg, in the present study (Table 1).

5.2 Effects on the Serum AST and ALT

Significant ($P <0.01$) increases of serum AST and ALT levels were detected in L-THYROXINE(T4) control rats as compared with intact control rats, controversially, AST and ALT concentrations in serum of PTU and both two different dosages of rosemary extracts treated rats were significantly ($P < 0.01$) decreased as compared with L-THYROXINE (T4) control rats, respectively (Table 2).

5.3 Effects on the Liver LPO

Continuous subcutaneous L-THYROXINE (T4) injection induced significant ($P < 0.01$) increase of the liver LPO. But 400 and 200mg/kg of rosemary extracts significantly ($P <0.01$) and dose-dependently normalized the changes on the liver LPO induced by L-THYROXINE(T4) as compared with L-THYROXINE(T4) control.PTU 10mg/kg also normalized the liver LPO comparable as rosemary extracts 200 and 400 mg/kg (Table 3).

5.4 Effects on the Organ Histopathology

In histomorphometrically analysis, significant ($P < 0.01$) decreases of the mean thicknesses of cross thyroid glands and follicular lining epithelium were detected in L-THYROXINE (T4) control as compared with intact control. These L-THYROXINE (T4) treatment related histopathological changes of thyroid gland, were dramatically inhibited by treatment of both dosages of rosemary extracts or PTU 10mg/kg.

Nowadays there is considerable interest in the potential health benefits of natural remedies such as medicinal plants and their extracts. One of these extracts is rosemary which has different medicinal properties including anti-inflammatory, anticarcinogenic, platelet

aggregation inhibiting, and metal chelating properties, etc. However, to date, there is no study on rosemary effects against hyperthyroidism. Therefore, in the present study, we investigated the effects of rosemary extracts on LT4-induced hyperthyroidisms and organ damages in comparison with those of PTU in rats.

It has been believed that hyperthyroidism leads to oxidative damage of various organs and antioxidants have been reliable and favourable effects on hyperthyroidism. It is also expected that rosemary extracts also may be showed beneficial effects on hyperthyroidisms and related organ damages. LT4-induced hypothyroidism and related liver damages were normalized by 15 days continuous oral treatment of rosemary extracts 400 and 200mg/kg from 12 days after first LT-4 treatment. Especially rosemary extracts enhanced the liver antioxidant defence systems—they dose-dependently inhibited LT4-induced increases of LPO and changes on the GSH contents, SOD, and catalase activities. These findings are considered as direct evidences that they have favourable ameliorating effect on the hyperthyroidisms and related organ damages induced by LT4 through antioxidant effects. The overall effects of rosemary extracts 400 and 200 mg/kg were similar to that of PTU 10 mg/kg, in the present study.

In the present study, LT4 induced increases of serum T3 and T4 levels, and decreases of serum TSH concentrations were significantly and dose-dependently inhibited by treatment of rosemary extracts. In addition, rosemary extracts significantly ($P < 0.01$) inhibited the LT4-induced histopathological changes on the thyroid glands, the atrophic changes including decreases of mean thicknesses of follicular lining epithelium. These results are considered as direct evidences that GS extracts controlled the hyperthyroid states.400 and 200mg/kg of rosemary extracts showed comparable effects as compared with PTU 10mg/kg in this study.

In the present study, we only focused on the in vivo protective effects to hyperthyroidism of crud extract itself not on the active compounds. Thus, these active compound searches should be proceeding in future.

6. CONCLUSION

In the present study, LT4-induced hypothyroidism and related liver damages were inhibited by oral treatment of Rosemary extracts 400and 200mg/kg. In addition, activities reported gives direct evidences that rosemary extracts have favourable ameliorating effect on the hyperthyroidisms and related organ damages induced by LT4 through effects. Rosemarry extracts 400 and200 mg/kg showed comparable effects on the LT4-induced rat

hyperthyroidism as compared with PTU 10mg/kg. These effects of rosemary may help the improvement of hyperthyroidisms and accompanied various organ damages, but active compound searches should be proceeding in future.

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