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Anti Thyroid and AntiOxidant Effects of Grape Skin Extract on L-Thyroxine Induced Hyperthyroidism in Rats

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ABSTRACT

Hyperthyroidism is a major endocrine disorder that greatly interferes with growth, metabolic activity, and thermoregulation in adults. This research aimed to evaluate the effect of hydroalcoholic grape skin extract (GSE) on hyperthyroidism induced by L-thyroxine (T4) in rats. A total of 30 Wistar rats were randomly divided into five groups (n=6). Group 1 (normal control) received distilled water. Group 2 (hyperthyroid control) received L-thyroxine (600 µg/kg, orally) for 12 consecutive days. Group 3 (positive control) received L-thyroxine and propylthiouracil (PTU, 10 mg/kg, i.p.) for 12 days. Groups 4 and 5 (treatment groups) received L-thyroxine along with GSE at doses of 200 mg/kg and 400 mg/kg orally for 12 days, respectively. The effects of GSE were measured using serum thyroid hormones (T3, T4, and TSH), liver function tests (AST and ALT), liver lipid peroxidation (LPO), glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) antioxidant levels, along with a histopathological examination of the liver. L-thyroxine treatment led to significantly higher T3 and T4 levels in the serum and lower TSH levels, which are signs of hyperthyroidism. Treatment with GSE significantly reduced T3 and T4 levels while increasing TSH levels in a dose-dependent way. The levels of AST and ALT in the hyperthyroid group were significantly decreased in the GSE-treated groups.

Introduction

Hyperthyroidism is a hypermetabolic disorder characterized by thyrotoxicosis, which is caused by excess production of thyroid hormones from the thyroid gland.[1] The two most common pathologic disorders associated with hyperthyroidism are Graves' disease and thyroid carcinoma. Thyroid hormones are vital for growth and development, as well as metabolic function, for almost every organ and tissue. Thyrotoxicosis can affect multiple organ systems causing a wide variety of presentations that include palpitations, tiredness, anxiety, sleep disturbance, weight loss, heat intolerance, excessive sweating, polydipsia, tachycardia, and tremors of extremities.[2-4] Experimental and epidemiological studies show that hyperthyroidism is associated with elevated oxidative stress, including increased reactive oxygen species (ROS) and impaired antioxidant systems.[5-7] Generally, clinical evaluation confirms increased triiodothyronine (T3) and/or thyroxine (T4), and suppressed levels of thyroid-stimulating hormone (TSH).[8] If hyperthyroidism is not properly treated, there can be serious complications. Treatment of hyperthyroidism remains a challenge for endocrinologists across the world.[9]

There has been increasing interest in the use of natural treatments for managing thyroid disorders in recent years. One example is grape skin extract (GSE), which has potential beneficial effects to offer, including anti-inflammatory, anticarcinogenic, platelet aggregation inhibitor, and metal-chelating effects.[10] Numerous studies have confirmed the antioxidant capacity of GSE.[11-13] There have also been numerous human randomized clinical studies that have evaluated the potential beneficial effects of GSE,[14-16] and even some experimental studies that illustrate antidiabetic properties.[17-18] However, to our knowledge no study has examined the potential effects of GSE in the treatment of hyperthyroidism. As such, this study aims to investigate the potential protective effects of grape skin extract against levothyroxine (LT4) induced hyperthyroidism and resultant organ damage in rats, compared to propylthiouracil (PTU), and particularly its potential antioxidant mechanisms

Plant extract:

Five hundred grams of coarsely grounded, dried skin of *Vitis vinifera* Linn. was extracted with 2 L of 70% ethanol for 1 h at 70°C in a 20 L round-bottom flask fitted with a reflux condenser. After filtration, the extract was collected, and the marc was re-extracted with another 2 L of 70% ethanol under the same conditions. The ethanolic extracts were combined after filtration. Then, the marc was extracted with 2 L of distilled water, filtered, and the aqueous extract was combined with the already collected ethanolic extracts. The combined extract was reduced in volume under reduced pressure using a Büchi rotary evaporator (Switzerland) at 65°C and yielded a brownish

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residue. The dried extract was used for subsequent experimental studies.

Experimental Animals

The experiments were performed on 30 wistar rats weighing 180-220 g after allowing 15 days acclimatization. The animals were allocated four per polycarbonate cage in a temperature (20–25°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.The experimental protocol was approved by Ethics Committee and Animals Research.(IAEC/TNMGRU/KMCP/65/2013)

Preparation of Extract and Drugs:

Hydro-alcoholic extract of grape skin was dissolved in sterile water. Propyl thiouracil tablets were weighed, powdered and triturated with saline.L-thyroxine was dissolved in sterile water.

Methodology:

Animals were randomized into five groups of six animals each. Hyperthyroidism was induced by the oral administration of L-Thyroxine (T4) (Sigma, USA) at a daily dose of 600 µg/kg for 12 days.[19] G1 was the normal control and was administered normal saline (10 ml/kg) orally. G2 was the hyperthyroidism control group that orally received distilled water for 12 days.[20] G3 was the standard control under this design; animals received propylthiouracil (PTU) intraperitoneally at 10 mg/kg for 12 days.[21] G4 and G5 were treatment groups, and they received Grape Skins Extract (GSE) orally at doses of 200 mg/kg and 400 mg/kg respectively for 12 days. At the end of the treatment period, blood samples were taken from the retro-orbital plexus of overnight-fasted animals using microcapillary tubes. The serum was separated for assessment of thyroid hormones (TSH, T3 and T4) and liver function enzymes (AST and ALT). Afterward, the animals were humanely killed. The thyroid glands were removed, rinsed in ice-cold saline to remove blood, and placed in 10% formalin for histopathological study. The liver tissues were collected, elution process and pooled for analysis of lipid peroxidation and antioxidant defense markers.

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 ◦ C. Separated serum was stored at −70°C before analysis. Serum levels of T3, T4, and thyroid-stimulating hormone (TSH) were analyzed by colorimetric competitive enzyme immunoassay using individual ELISA kit according to Subudhi *et al*., [22] respectively.

Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)

Serum AST and ALT concentrations were measured by automated blood analyzer (Toshiba200 FR, Toshiba, Japan).[23]

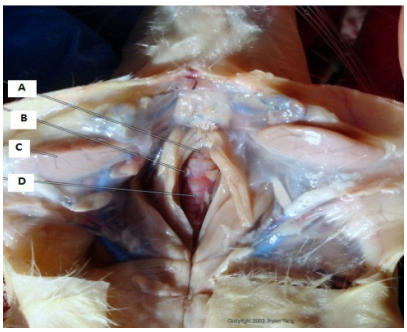

Fig:1 Location of Thyroid gland on rats

A-Larynx, B-Thyroid Gland, C-Sub Mandibular Gland, D- Trachea

Liver Lipid Peroxidation (LPO)

Separated liver tissues were weighed and homogenized in ice-cold 0.01M Tris- HCl (pH 7.4), and then centrifuged, at 12,000 g for 15 min as described by Kavutcu *et al*.[24] 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.[25]

Liver Antioxidant Defense Systems

Tissue homogenates were made and vortexed with 0.1 mL of 25% trichloroacetic acid (Merck, CA, USA), and were then centrifuged at 4200 rpm for 40 min at 4 °C. The supernatant was obtained and used for estimating glutathione (GSH) levels. GSH levels were measured spectrophotometrically at 412 nm with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB; Sigma, MO, USA) as previously described.[26] Catalase (CAT) activity was determined by the rate of decomposition of hydrogen peroxide (H_2O_2) as measured at 240 nm.[27] One unit of catalase activity is defined as the amount of enzyme decomposing 1 nM of H_2O_2 per minute at 25 °C and pH 7.8, and the results are reported as U/mg protein. Superoxide dismutase (SOD) activity was determined following the method of Sun *et al.*[28] In the assay, superoxide radicals are generated by the xanthine–xanthine oxidase system, and react with nitroblue tetrazolium (NBT) to produce a formazan dye; The inhibition of formazan formation was spectrophotometrically measured at 560 nm. One unit of SOD activity is defined as the amount of enzyme inhibiting NBT reduction by 50% under assay conditions; results are expressed as U/mg protein.

Histology

The sampled thyroid gland tissue was fixed in 10% neutral buffered formalin. After paraffin embedding, tissue blocks were cut into serial sections of 3–4 µm thickness. Representative sections were then stained with hematoxylin and eosin (H&E) and examined in an optical microscope. The histological organization of the thyroid gland was assessed. The mean thicknesses of the thyroid gland, thyroid follicles, and follicular epithelial lining of the thyroid gland were evaluated using an automated image analysis system.

Statistical Analysis

Numerical data are presented as mean±S.E.M. of six rats, the obtain data was analyzed using a one way ANOVA test followed by Newmann Keuls multiple range tests. Statistical analyses were conducted using graphpad version 3.1. P values <0.05 were considered significantly different.

Results and Discussion

Effects on the Serum Thyroid Hormones.

L-Thyroxine (T4) treatment induced showed significant (P <0.01) increase in serum T3, T4 levels and decrease in TSH contents. But treatment with 200 and 400mg/kg of GS extracts significantly (P <0.01) normalized the changes on serum T3, T4, and TSH levels in a dose dependent concentration as compared with L-Thyroxine (T4) control. PTU at 10 mg/kg also normalized the serum thyroid hormone levels, as similar to G4 & G5.(Table No:1)

Table:1 Serum thyroid hormone levels in the L-Thyroxine (T4) and GSE treated rats

Groups	TSH(ng/ml)	T_3(ng/ml)	T_4 (µg/ml)
GP1	1.95±0.22	0.57±0.14	53.80±1.70
GP2	0.78±0.11*a	2.28±0.35*a	174.20±3.80*a
GP3	1.45±0.19*b	0.95±0.17*b	71.30±2.04*b
GP4	1.31±0.14*b	1.28±0.23*b	83.10±2.20*b
GP5	1.37±0.17*b	1.12±0.20*b	76.20±2.15*b

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

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

Effects on the Serum AST and ALT

The findings showed a significant (P <0.01) increase in serum AST and ALT levels detected in L-Thyroxine (T4) control rats compared with normal control rats, but AST and ALT levels in sera of PTU and both dosages of GS extracts treatment rats were significantly (P < 0.01) lower compared with L-Thyroxine (T4) control rats respectively (Table No:2).

Effects on the Liver LPO

Long-term L-thyroxine (T4) ingestion induced LPO in rat livers.However treatment groups with GS extract defined above (200 and 400mg/kg) and PTU10mg/kg (G3) significantly (P < 0.01) reduced the changes in liver LPO compared to L-thyroxine (T4) control.(Table No:3).

Table:2 Effect of GSE on serum liver enzyme levels in the L-Thyroxine (T4) and GSE treated rat

Group	AST(IU/L)	ALT(IU/L)
GP1	120.95±2.60	64.3±1.25
GP2	205.60±3.55*a	126.88±3.45*a
GP3	127.90±2.66*b	80.20±2.40*b
GP4	145.38±2.85*b	92.10±2.65*b
GP5	136.45±2.74*b	85.70±2.52*b

GP1- Normal; GP2- Hyper Thyroid Control; GP3- Standard Control (PTU 10mg/kg);

GP4- GSE(200mg/kg);GP5- GSE (400mg/kg)
All values are expressed as mean ± SEM for 6 animals in each group.
**a – Values are significantly different from Normal control (G1) at P < 0.01*
**b – Values are significantly different from hyperthyroid control (G2) at P <0*

Table no: 3 Effect of liver lipid peroxidation and anti oxidant defence systems in the l-thyroxine (T4) and GSE treated rats

Groups	Lipid peroxidation	Anti oxidant defence system		
	MDA(nM/mg protein)	GSH(nµ/mg protein)	SOD (U/mg) protein	CATALASE (U/mg protein)
GP1	2.10±0.15	35.30±1.88	22.65±1.05	30.28±1.22
GP2	4.80±0.38*a	12.15±1.02*a	52.60±2.10*a	48.25±1.70*a
GP3	2.48±0.23*b	26.70±1.64*b	26.05±1.26*b	33.15±1.35*b
GP4	2.85±0.32*b	21.30±1.22*b	31.10±1.45*b	39.50±1.44*b
GP5	2.60±0.27*b	24.25±1.48*b	28.15±1.33*b	36.40±1.40*b

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

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

Effects on the Liver Antioxidant Defense Systems

When comparing the L-Thyroxine (T4) control group to the intact control, the GSH contents were significantly (p<0.01) decreased and SOD and catalase activities were increased. Both dosages of GS extract (200mg and 400mg) significantly and dose-dependently (p<0.01) inhibited changes on the GSH, SOD and catalase. In addition, PTU significantly (p<0.01) inhibited the L-Thyroxine (T4) treatment-related changes on the antioxidant defense systems when compared to the L-Thyroxine (T4) control (Table No:3).

Effects on the Organ Histopathology

There were significant (P < 0.01) reductions of the mean thicknesses of the cross thyroid glands and thickness of follicular lining epithelium in L-Thyroxine (T4) control compared to the intact control, in histomorphometrical

analyses. The histopathological changes of thyroid gland as a result of T4 treatment, were evaluated with treatments with both dosages of GS extracts and PTU 10mg/kg (Fig 2-6).

Histological Studies:

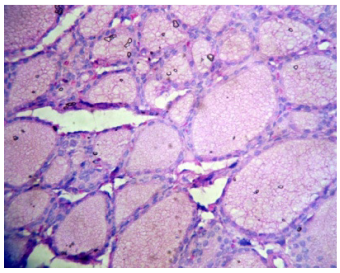

Fig No: 2
Thyroid gland section of GP1 rats (normal control) showing follicles lined by cuboidal epithelial cells filled with 60-70% colloid.

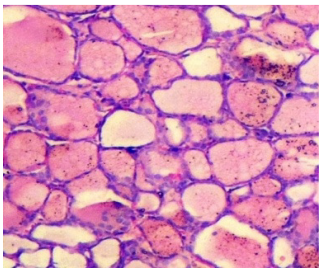

Fig No: 3
Thyroid gland section of GP2 rats(hyperthyroid control) showing follicles Lined by cuboidal epithelial cells filled with 40-50% colloid.

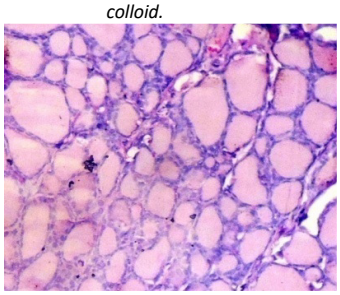

Fig No: 4
Thyroid gland section of GP3 rats (standard control) showing follicles lined by cuboidal epithelial cells filled with 80-90% colloid.

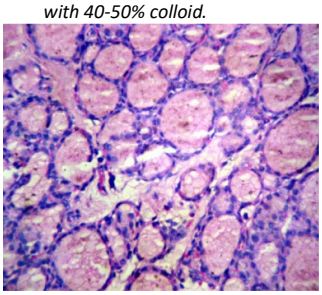

Fig No: 5
Thyroid gland section of GP4 rats(grape skin extract at a dose of 200mg/kg) follicles lined by cuboidal epithelial cells filled with 50-60% colloid

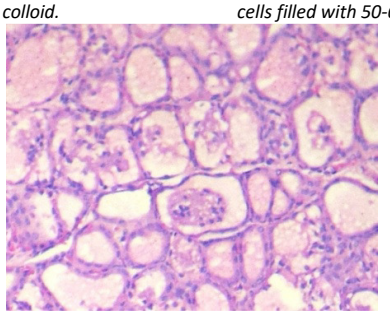

Fig No: 6
Thyroid gland section of GP5 (grape skins extract at a dose of 400mg/kg/rat)rats showing follicles lined by cuboidal epithelial cells filled with 70-80%colloid

Discussion

Thyroid hormones control the basal metabolic rate of target tissues, such as liver, heart, kidney and brain. Prolonged stimulation of the thyroid with exogenous levothyroxine (LT4) may inhibit the activity of the thyroid by interfering with the synthesis of endogenous thyroid hormones,[29,30] thereby producing a hyperthyroid state which produces oxidative damage to multiple organs.[31]

In the present study, LT4 administration showed a marked increase in serum T3 and T4 levels along with a decrease in serum TSH levels. However, grape skin (GS) extracts administered as treatment to LT4 significantly mitigated the changes dose-dependently indicating a regulatory effect on thyroid hormone activity.

Reactive oxygen species (ROS) are known to be highly reactive causing oxidative damage to essential cellular macromolecules like proteins, lipids and DNA.[32,33] LT4 mediated production of ROS lead to oxidative stress in the liver because it is the primary tissue responsive to thyroid hormones with significant biologic reporting and clinical implications.[34] Thyroid hormonal excess and imbalance from hyperthyroidism can significantly impair the structural integrity of the liver, as shown by increased serum elevations of liver enzymes AST and ALT,[35] which are often proportional to lethal oxidative liver damage.[36] GS extracts markedly mitigate the elevation of elevated AST and ALT indicating a hepatoprotective effect as shown by the presence of AST and ALT.

Hyperthyroidism is known to increase lipid peroxidation (LPO) reactions and ROS production. LPO reactions can propagate itself since it causes oxidative Lipid peroxidation (LPO) is a self-sustaining process that results in oxidative damage to cellular membranes, which ultimately results in cell death and the formation of hazardous metabolites.[37,38] Malondialdehyde (MDA), the end product of LPO, is a useful marker for measuring oxidative damage. MDA levels in the livers of hyperthyroid animals were severely elevated, and the GSE treatment reduced the MDA levels.[39,40]

Glutathione (GSH) is an important intracellular thiol involved in detoxification and antioxidant defense. A lack of GSH can increase oxidative insult leading to cellular damage. Similarly, antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, play vital roles in preventing oxidative stress by neutralizing reactive oxygen species (ROS). GSH levels are decreased in hyperthyroidism, which may indicate less reserve capacity of antioxidant defenses, but SOD and catalase levels are increased more as a response to increased ROS.[41]

Conclusion

Herbs can be employed in the management of hyperthyroidism that minimizes the side effects from conventional hormonal therapies. Grape skin extract has been shown to regulate increased lipid peroxidation, increased level of thyroxine, increased levels of liver enzymes, and decreased activity of enzymatic antioxidants. Histopathological studies showed that treatment with grape skin extract leads to the repair of glandular tissue filled with follicular colloids. Therefore, grape skin extract appeared to be a prospective therapeutic agent for the management of thyrotoxicosis. In conclusion, this study demonstrates that grape skin extract has a significant protective effect against thyroxine-induced hyperthyroidism, possibly due to polyphenols and flavonoids present. However, while these preliminary results are exciting, further molecular studies are necessary to determine the exact mode of action of the grape skin extract in relation to its antithyroid activity.

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Conflicts of Interest:

The authors have no conflicts of interest.

Ethical Approval and Consent to Participate:

The study was reviewed in accordance with ethical standards and animal experimentation was approved by the Institutional Animal Ethical Committee, of K.M. College of Pharmacy, Uthangudi, Madurai (Approval No.: IAEC/TNMGRU/KMCP/65/2023).

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