



Thyroid regulating and hepatobiliary protective actions of *Thymus vulgaris*. L in rat model

KEYWORDS

Thyme, Thyroid regulation, hepatobiliary, apoptosis

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ABSTRACT The present investigation was designed to investigate the curative and protective effect of thyme supplementation on thyroid and liver status in propylthiouracil (PTU) -induced hypothyroidism. Rats were divided into 4 groups; group I control euthyroid, group II rendered hypothyroid by administration of 0.1% (w/v) PTU in their drinking water for 90 days, group III received thyme extract orally 0.5% (w/v) for 90 days after hypothyroidism induction. Group IV, thyme extract supplemented prior hypothyroidism induction for 30 days and continue after hypothyroidism induction for 90 days. Hypothyroidism-induced elevation in serum TSH and reduction in T3, T4 level. An elevation in liver enzyme leakage, hepatic oxidative stress, apoptosis, and inflammatory markers were observed. Nevertheless, thyme supplementation only after hypothyroidism induction can alleviate thyroid function and hepatic oxidative damage completely. In contrary, thyme administration before and after hypothyroidism induction regulated thyroid function and restored hepatic antioxidant capacity toward the normal. The present study suggests that supplementation of thyme extract displays a prophylactic role against hypothyroidism and hepatobiliary protective effects.

1. Introduction

Thyroid hormones regulate oxidative metabolism, protein, vitamin and antioxidant enzyme synthesis and degradation (Pereira et al, 1994). Triiodothyronine (T3) regulates the basal metabolic rate of all cells, including hepatocytes through thyroid hormone receptor and thereby modulate hepatic function (Ramadoss et al, 2013; Wu et al, 2013). With this background, liver is a major target organ for thyroid hormone with important biological and medical implications (Subudhi et al, 2009).

Hypothyroidism (HPO) is a clinical syndrome caused due to deficiency of thyroid hormones i.e, characterized by decrease in serum T3 and T4 and increase in serum TSH concentration (Lakshmi et al, 2013). Recent studies have shown that HPO accompanied by an increased production of reactive oxygen species (Toplan et al, 2013). Many investigations have suggested that hypothyroidism may have features that mimic liver disease (pseudo-liver disease): examples include myalgias, fatigue and muscle cramps in the presence of an elevated aspartate aminotransferase from a myopathy as well as coma associated with hyperammonaemia in myxoedema coma and myxoedema ascites. The liver biopsy findings revealed central congestive fibrosis in a number of patients (Laycock and Pascuzzi, 1991; Thobe et al, 2000).

Increased use of synthetic drug therapy leads to many side effects and undesirable hazards. Therefore, there is a worldwide trend to return to natural resources, which are culturally acceptable and economically viable. Thyme (*Thymus vulgaris* L.) belongs to the Lamiaceae family and aromatic native herbs in the Mediterranean region. The therapeutic and antioxidant potential of thyme rests on its contents of flavonoids, thymol, carvacrol, eugenol, aliphatic phenols as well as saponins, luteolin and tetramethoxylated flavones (Dorman and Deans, 2000; Amarowicz et al, 2008; Cerda et al, 2013). *Thymus vulgaris* is a medicinal plant used widely for infections and inflammatory diseases in folk medicine. It has beneficial immunomodulatory effects in infections and immune-related diseases whereas it decreased the proliferation of mitogen-stimulated lymphocytes (Amirghofran et al, 2013).

Thymus vulgaris has essential oils possess wide range spectrum of fungicidal, antiviral and antibacterial activities (Bozin et al, 2006). Thyme is an excellent source of iron, calcium, manganese and vitamin K. (Sasaki et al, 2005). Moreover, thyme promotes blood circulation and functions as an exciting stimulant for the entire system. The stimulating action on the nervous system makes the herb a brilliant remedy for physical as well as mental fatigue, alleviating tension, anxiety and sleeplessness (Höferl et al, 2006). Aromatic plants and their essential oils can use as hepatic-protective supplement in the developing countries towards the development of new therapeutic agents (Sylvestre et al, 2006). Besides, thyme as a natural product is effective in inhibiting tumor growth in mice and damaging effect in all cell lines (Barek et al, 2007). However, information on the oral supplementation of exogenous antioxidants in hypothyroidism induced hepatic oxidative stress is wanting. The present study designed to highlights the protective role of thyme in conquer hepatic oxidative stress associated with hypothyroidism.

2. Materials and methods

2.1. Animal model

Adult male Wistar rats weighing 150-230 gm obtained from Animal House in Faculty of Veterinary Medicine, Zagazig University. They maintained under standard laboratory conditions with free access to the standard diet and water ad libitum and were acclimated for 2 weeks. The experiment performed in accordance with the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, 1996).

2.2. Drugs

Thyrocil (Propyl thiouracil, 50mg) used for hypothyroidism induction. It obtained from Amoun pharmaceutical Co., Cairo, Egypt.

2.3. Plant

Thyme (*Thymus vulgaris* L.) obtained from Isis Company for industries, Cairo, Egypt.

Plant material and extraction procedure

Dried leaves of thyme grounded into powder. 500 gm of

fine-powder subjected to extraction with 1000 ml boiling water in covered flask and left for 30 min. After that, the extract cooled and filtered using filter paper to remove particulate matter (Kandil et al, 1994).

2.4. Animals grouping

Rats divided into four groups, six rats each **Group I:** Rats kept without treatment as control (euthyroid) until end of experiment. **Group II:** Rats rendered hypothyroid by giving 0.1% (w/v) of propyl thiouracil (PTU) in drinking water (Grattagliano et al, 2003) for 90 days. **Group III:** Rats received thyme extract 0.5% (w/v) for 90 days after hypothyroidism induction by PTU. **Group IV:** Rats received thyme for 30 days prior to the hypothyroidism induction and 90 days after.

2.5. Handling of blood and tissue samples

At the end of the experimental period, blood samples individually collected from the inferior vena cava of each rat and serum separated from non-heparinized blood by centrifugation at 3000 rpm for 15 minutes. Immediately after animal decapitation, liver specimens collected and quickly stored at -80°C for assessment of oxidative stress markers.

2.5.1. Hormonal analysis

Total Triiodothyronine (T3) and Thyroxine (T4) measured in serum using Mouse/Rat ELISA Kit of MyBioSource.

Thyroid Stimulating Hormone (TSH) estimated according to Mouse/Rat ELISA Kit of ALPCO.

2.5.2. Assessment of liver function tests

For assessment of liver function tests, AspAT (GOT), AlaAT (GPT) and Alkaline Phosphatase (ALP) activity carried out according to the method of (Reitman and Frankel, 1957) and (Belfield and Goldberg, 1971) respectively. Estimation of total, direct and indirect bilirubin conducted using the procedure of (Walter and Gerade, 1970).

2.5.3. Determination of hepatic oxidative stress markers

Frozen liver tissue homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The homogenates were centrifuged at 5000 rpm for 10 min at 4 °C. The supernatant used for the following analysis.

GSH, GSH-px, SOD & GST were done according to the procedure of (Beutler et al, 1963), (Paglia and Valentine, 1967), (Nishikimi et al, 1972) and (Habig et al, 1974) respectively, while assessment of MDA level was done as described by (Satoh, 1978 and Ohkawa et al, 1979) according to the pamphlet kit of Biodiagnostic Company, Egypt.

The level of pro-inflammatory cytokine tumor necrosis factor- alpha (TNF- α) in liver homogenates determined by aid of ELISA using rat TNF- α immunoassay kit according to the recommendations of the manufacturer. (BD Biosciences Pharmingen, San Diego, CA, USA).

2.5.4. Molecular-Genetic Studies

Part of frozen liver used for DNA extraction, quantification and gel electrophoresis according to the method of (Laird et al, 1991; Perandones et al, 1993; Stamm and Berka, 2006).

2.6. Statistical analysis

Data performed using SPSS statistical version 22 expressed and expressed as mean \pm SE. Statistical analysis done using one-way analysis of variance (ANOVA) followed by the Duncan analysis to assess significant differences among treatment groups. The criterion for statistical significance

was set at $P < 0.05$.

3. Results

3.1. Effect of hypothyroidism on thyroid status

The results of the current study confirmed that the significant reduced levels of serum T3, T4 and higher TSH in PTU treated group as compared with the control euthyroid group (table 1) indicated that hypothyrogenic effect successfully induced by propyl thiouracil. Thyme pretreatment in-group IV exerted significant thyroid regulating action.

3.2. Effect of hypothyroidism on liver function enzymes

We found that serum AlaAT, AspAT, ALP activities and bilirubin (total, direct, indirect) levels were significantly ($P < 0.05$) higher in PTU- treated rats as compared with the control group. Co- administration of Thyme in-group III significantly decreased serum ALP activity, total, direct bilirubin (table 2).

3.3. Effect of hypothyroidism on liver oxidative marker and DNA

In hepatic tissue of the hypothyroid rats, hepatic oxidative stress marker (SOD, GST, GSH-px activities and GSH, DNA content) significantly decreased from euthyroid values. These accompanied by increased hepatic MDA level (table 3). An intense and diffuse smear in lane 1 (group II) was observed indicating fragmentation to the nuclear DNA (Figure 1) indicating hepatic apoptosis.

Thyme supplementation in-group III significantly attenuated over production hepatic MDA and increased hepatic SOD, GST, GSH-px activities as compared with the hypothyroid rats. On the other hand, statistical comparison between group II and group III in our study, regarding serum liver function test (AlaAT, AspAT, indirect bilirubin) and hepatic GSH, TNF- α , DNA content revealed no significant difference ($P < 0.05$). With this line, figure 1 shows a distinct smear in lane 3 (group III). Administration of thyme prior and after hypothyroidism induction can ameliorate the alterations in thyroid function and hepatic antioxidant status. Whereas a slight smear in lane 4 (group IV) was detected reflecting less nuclear DNA fragmentation pattern (Figure 1) and geno-protection influence.

Table (1): Effect of thyme extract supplementation on serum TSH (ng/ml), T3 (ng/dl) and T4 (μ g/dl) hormone levels in hypothyroid rats

	Group I	Group II	Group III	Group IV
TSH(ng/ml)	2.13 \pm 0.08	4.69 \pm 0.16*	4.82 \pm 0.10*	2.76 \pm 0.17†
T3(ng/dl)	145.50 \pm 2.76	91.09 \pm 1.82*	93.60 \pm 3.23*	127.20 \pm 2.11†
T4(μ g/dl)	6.93 \pm 0.24	2.22 \pm 0.10*	2.15 \pm 0.13*	3.93 \pm 0.16†

Table (2): Effect of thyme extract supplementation on serum AlaAT (units/ml), AspAT (units/ml), ALP (IU/L) activity, total, direct and indirect bilirubin (mg/dl) in hypothyroid rats.

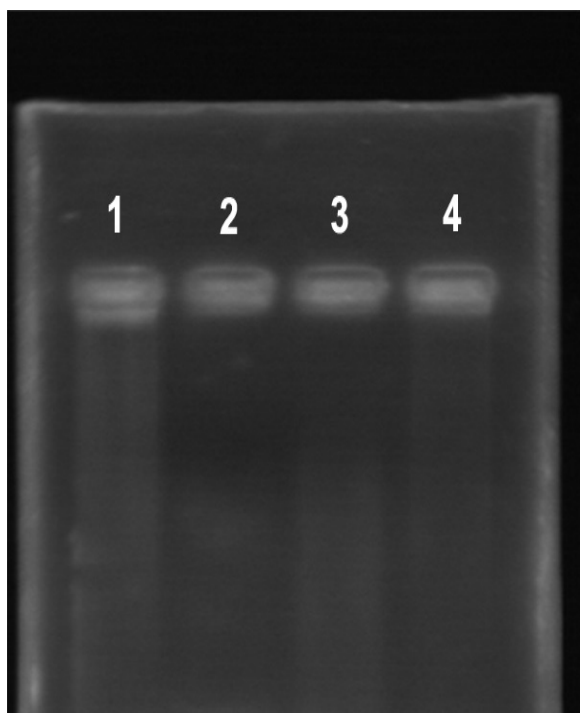
	Group I	Group II	Group III	Group IV
AlaAT (units/ml)	5.74 \pm 0.42	10.78 \pm 0.60*	9.73 \pm 0.52*	7.26 \pm 0.49†
AspAT (units/ml)	2.87 \pm 0.19	7.10 \pm 0.39*	6.88 \pm 0.37*	4.28 \pm 0.28†
ALP (IU/L)	15.52 \pm 0.79	30.52 \pm 0.89*	24.75 \pm 1.17*	20.77 \pm 1.45†
Total bilirubin (mg/dl)	0.75 \pm 0.03	2.41 \pm 0.02*	1.88 \pm 0.02*	1.09 \pm 0.01†
Direct bilirubin (mg/dl)	0.44 \pm 0.03	1.53 \pm 0.01*	1.05 \pm 0.03*	0.66 \pm 0.01†

Indirect bilirubin (mg/dl)	0.32±0.02	0.88±0.02*	0.83±0.03*	0.43±0.02†
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Table (3): Effect of thyme extract supplementation on liver GSH content (mg/ gm tissue), GSH-px (U/ gm tissue), GST (U/ gm tissue), SOD (U/ gm tissue) activity, MDA (nmol/ gm tissue), ,TNF- α (pg/100mg tissue) level and DNA content (μ g/ mg tissue) in hypothyroid rats.

	Group I	Group II	Group III	Group IV
GSH (mg/ gm tissue)	54.88±1.66	28.30±1.22*	30.89±1.40*	38.79±0.88†
GSH-px (U/ gm tissue)	169.24±3.98	101.35±4.25*	125.47±5.5†	131.89±3.29†
GST (U/ gm tissue)	213.23± 4.13	121.56± 5.16*	139.47±4.89*	169.56± 6.25†
SOD (U/ gm tissue)	74.57±2.38	39.00±2.84*	49.46±1.26*	58.84±3.39†
MDA (nmol/ gm tissue)	19.56±0.39	48.30± 1.84*	39.66± 1.03*	27.19±0.61†
TNF- α (pg/100mg tissue)	34.80±2.32	99.55±3.70*	95.40±1.45*	63.90±2.91†
DNA (μ g/ mg tissue)	4.47± 0.03	2.22 ± 0.10*	2.32±0.09*	3.33 ±0.07†

Figure (1). Effect of thyme extract supplementation on agarose gel electrophoretic pattern of liver DNA fragmentation in hypothyroid rats.



Analysis of DNA fragmentation by agarose gel electrophoresis. Lane 1 (group I), Lane 2 (group II), Lane 3 (group III) and Lane 4 (group IV).

4. Discussion

In the present report, we provide an investigation about the role of thyroid hormone on rat liver. In order to achieve this target, we made a deficient state of thyroid hormones by PTU and thereby reduce serum T3, T4 and elevate TSH concentration compared to control euthyroid rats. These results coincide with studies of **Tousson et al, 2012** and **Massoud et al, 2012** whom reported that, TSH level was significantly higher, while T3 and T4 levels were significantly lower in PTU-induced hypothyroid rats when compared to control euthyroid rats. It also known that the activity of thyroid gland predominantly regulated by pituitary TSH. When T4 concentration drops, TSH concentration increases (**Allen and Rana, 2007**).

The present study revealed an elevation in liver enzyme

leakage serum and bilirubin (total, direct, indirect) levels were significantly higher in PTU- treated rats as compared with the control group. **Jaeschke et al 2002** explain that the enzymes produced within hepatocytes constantly leak through the cell membrane, which gave the normal serum level of these enzymes. There is also evidence that hypothyroidism may directly affect the liver structure and function

through increased reactive oxygen species production causing liver cell injury rendering the membranes more permeable (**Sarandol et al, 2005** and **Erdamar et al, 2008**).

Regarding the increase in serum bilirubin in PTU- treated rats. The previous publications of (**Laker and Mayes, 1981; Wu et al, 2013**) revealed that thyroid hormone has shown to regulate diverse hepatic functions such as serum protein synthesis, bile flow and composition, lipid metabolism and trans-membrane sodium transport. Additionally, hypothyroidism has been associated in a few case reports with cholestatic jaundice attributed to reduced bilirubin and bile excretion. In experimental hypothyroidism, the activity of bilirubin UDP-glucuronyltransferase decreased, resulting in a reduction in bilirubin excretion. The reduction in bile flow may be in part due to an increase in membrane cholesterol-phospholipid ratio and diminished membrane fluidity which may affect a number of canalicular membrane transporters and enzymes, including the Na⁺, K⁺-ATPase (**Van- Steenberg et al, 1989**). Besides, it assumed that T3 specifically modulates the activities and expressions of some UDP-glucuronosyltransferases isoforms (UGT1A) (**Goudonnet et al, 1990; Masmoudi et al, 1996**).

Hypothyroid rats evoked hepatic oxidative stress, apoptosis, and inflammatory markers. Since the previous publications supported our finding where thyroid hormone down-regulates hepatic and mitochondrial GSH synthesis via the phosphorylation of g-glutamylcysteine synthetase resulting in impairment of intracellular redox status, DNA synthesis and liver regeneration capacity (**Lu et al, 1991; Gredilla et al, 2001; Grattagliano et al, 2003; Sarandol et al, 2005**). In fact, while the total GSH concentration represents a critical determinant for mitochondrial GSH status and redox environment seem to work also as modulating factors in cell proliferation by interfering with intracellular signals expression (**Chiba et al, 1996; Schafer and Buettner, 2001**). Additionally, hypothyroidism associated with low oxygen utilization and low tissue proliferation rate causing tissue injury, nuclear pyknosis and decrease in hepatocyte nuclei (**Moro et al, 2004; Massoud et al, 2012**). As well as, hepatocyte proliferation suppressed by hypothyroidism probably due to low concentration of T3, which exert their influence at a nuclear level. This process initiated when T3 binds to thyroid hormone receptor increasing the expression of cyclin D and transcription factor E2F (**Pibiri et al, 2001; Allen and Rana, 2007**).

There have been great efforts to find safe and potent natural antioxidants from various plant sources. Aqueous extract of thyme is rich in the total phenolic content and have radical scavenging activity (**Amarowicz et al, 2008**).

It is important to highlight that thyme supplementation to hypothyroid rats can prevent the hypothyroidism occurrence. This agrees with the results of **Lima et al, 2013** whom reported that, Flavonoids in thyme are able to increase iodide uptake and sodium-iodide symporter expression and thyroperoxidase, the key enzyme in thyroid hormones biosynthesis.

Administration of thyme can ameliorate the disrupted liver function enzymes and bile system. These may relate to thyme enhancing SOD, GST, GSH-px activities and replenishing GSH storage (**Youdim et al, 1999; Amarowicz et al, 2008; Grosso et al, 2010**). As well as, Plant volatile compounds appear to accumulate in the cell membrane inhibiting the chain reaction of lipid peroxidation, stabilizing membrane activity (**Seung et al, 2005; Tsai et al, 2007**). Likewise, recent research now indicates that quercetin in thyme attenuated the overproduction the pro-inflammatory cytokine TNF- α at the gene expression level. Moreover, quercetin increases sportive performance, endurance capacity, delays fatigue during training and maintaining mitochondrial biogenesis for the sedentary persons (**Kowalski et al, 2005; Nair et al, 2006; Davis et al, 2009; Hazar**

and Alpay, 2011). Regarding the protective role of thyme at the molecular level, it was claimed that the thyme ability for scavenging free radicals in mitochondria, subsequently restoring mitochondrial GSH and protecting DNA against oxidative damage (**Kaledaite et al, 2011**). Specifically, **Horvathova et al, 2006** noticed the DNA-protective effects of essential oils in thyme on hepatoma HepG2.

This further signifies the curative nature of thyme extract against oxidative damage during hypothyroidism and its ability to restoring normal liver potential.

5. Conclusions

In conclusion, it can suggest that *Thymus vulgaris*. L conveniently exploited to be good thyroid- regulator, hepatobiliary protective and had the potential to apply in the hypothyroidism therapy. These findings may add some information to the literature in this field, in which a definite conclusion has yet to reach. These results revealing an individuality of antioxidant status in relation to tissue responsiveness and duration of the exposure.

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