Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

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Original Paper

Ameliorative effect of lemon balm on eltroxin induced-hyperthyroidism in rats

Ragaa S.M. Kawara[1], Fatma S.M. Moawed[2], Hussein Abd Elmaksoud[1], Yakout Elsenosi[1], Omayma A.R. Abo-Zaid[1]

[1] *Biochemistry and Molecular Biology Department, Faculty of Vet. Med. Benha University, Egypt.*

[2]*Health Radiation Research, National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority, Cairo, Egypt.*

ARTICLE INFO

Keywords

Lemon balm

Hyperthyroidism

Thyroid hormones

Oxidative biomarkers.

Received 09/03/2024
Accepted 17/04/2024
Available On-Line 01/07/2024

ABSTRACT

Lemon balm has many therapeutic effects including antimicrobial activity (anti-parasitic, antibacterial, antiviral, antifungal, etc.). Various studies have shown that Lemon balm possesses a high amount of antioxidant activity through its chemical components including high amount of flavonoids, rosmaric acid, gallic acid and phenolic contents. This study was designed to evaluate the therapeutic effect of Lemon balm extract against hyperthyroidism induced by Eltroxin. Hyperthyroidism was induced by injecting rats with Eltroxin (100 µg/kg/ day) for 14 days. The hyperthyroid rats were orally treated with Lemon balm extract (75 mg/kg/day) at the beginning of the second week of the Eltroxin injection and continued for another week. The levels of TSH , T3 ,T4 and oxidative biomarkers (NO and CAT) were measured using commercial kits. Thyroid specimens were taken for histopathological examination. Lemon balm extract reversed the effect of Eltroxin on rats and attenuated the thyroid hormones Additionally, Lemon balm extract alleviated the inflammatory response by suppressing the NO and increasing CAT when compared to hyperthyroid group. Accordingly, these results might strengthen the protective effect of Lemon balm extract in a rat model of hyperthyroidism.

1. INTRODUCTION

The thyroid gland is a crucial part of the endocrine system, which controls several physiological processes, including the use of oxygen, growth, development, and cellular metabolism. The gland, which is situated near the front of the neck, secretes Tri-iodothyronine (T3) and Thyroxine (T4), which enter the bloodstream and regulate growth, development, and basal metabolic rates. The symptoms of thyroid issues can include fatigue, anxiety, constipation, abnormal weight gain or loss, tremors, diarrhea, irritability, depression, tachycardia, and a sensitivity to cold temperatures. There are an estimated 42 million cases of thyroid diseases in India, making it one of the largest and most prevalent health disorders affecting people worldwide (Unnikrishnan & Menon, 2011). It is well recognized that natural (found in food and water) and/or synthetic (found in medications) substances can alter thyroid function Hackney et al. (1995). Thyroid disruptors are substances that alter thyroid homeostasis and have an impact on hormone synthesis, metabolism, signaling, transport, and/or carcinogenesis, which can initiate an autoimmune response. Depending on the kind of disease, management options include hormone replacement therapy, iodine therapy, surgery, and/or anti-thyroid medication. Certain negative effects of the current treatment modalities include hair loss, appetite loss, and physical weakness. (Sabrina & Di Cristofano, 2019). Moreover, certain drugs interfere with thyroid function, while others may exacerbate the signs and symptoms of hypo- or hyperthyroidism; these problems necessitate a systematic approach to patient care. These days, there is a growing push in research for alternative medical approaches with fewer side effects. In human populations, medicinal plants have played a significant role in both disease prevention and health care (Changizi Ashtiyani et al., 2013a; Changizi Ashtiyani et al., 2013b). Secondary metabolites discovered in medicinal herbs are abundant and have a significant physiological impact on how mammalian tissues operate in both healthy and diseased states (Taheri et al., 2012; Zarei et al., 2012). One of these well-known herbs, lemon balm (LB), has been used for a very long time to cure a variety of ailments, including rheumatoid arthritis, neurological disorders, gastrointestinal disorders, and headaches (Wichtl, 2004; Jun et al., 2012). LB, a perennial herbaceous plant belonging to the Lamiaceae family, is also known by several common names such as garden balm, bee balm, melissa, and melissengeist. Its range of growth extends from central and southern Europe to Iran and central Asia. Due to its culinary qualities, it is also grown all over the world (Chen et al., 2006; Behnam Rassouli et al., 2010; Rasmussen, 2011). This herb has a long history dating back over 2,000 years, and it is widely utilized in traditional medicine. Many applications of the plant have been reported (NourEddine et al., 2005; Rasmussen, 2011). These applications include sedative and mild hypnotic drugs, heart rate reduction, antibacterial, anti-inflammatory, antivirus, antispasmodic, antioxidant, neurotherapeutic, peripheral analgesic, and binding agent to cholinergic receptors (Naghibi et al., 2005; Behnam Rassouli et al., 2010). The purpose of this essay is to shed light on the advantages of lemon balm extract for thyroid function.

2. MATERIAL AND METHODS

2.1. Chemicals and drugs:

Unless otherwise stated, all compounds were obtained from Sigma Aldrich Chemical Co., St. Louis, USA. Etroxin

* Correspondence to: ragaa.salah22@gmail.com

(levothyroxine sodium; 100 µg, 100 tablets) was obtained from GlaxoSmithKline Co., Cairo, Egypt. Abd El-Rahman Harraz (Bab El-Khalk Zone, Cairo, Egypt) provided the lemon balm (LB). According to Abdel-Aziz (2018), lemon balm has been stated to include significant levels of phenolic compounds, gallic acid, rosmaric acid, and flavonoids.

2.2. Experimental Animals

24 male Wister albino rats, weighing between 120 and 150 grams, were purchased from the Nile Company in Egypt. Rats were kept for one week before the commencement of the experiment as an acclimation phase, under typical conditions of 50±5% humidity and a 12:12-h light-dark cycle. Water and starter poultry pellets were provided to the animals without restriction.

2.2.1. Induction of Hyperthyroidism

Depending on Carageorgiou et al. (2007) approach, a daily subcutaneous injection of Eltroxin (100 µg/kg) was used to induce hyperthyroidism for 14 days (Bolkiny et al., 2019).

2.2.2. Experimental groups

Rats were split into three groups (n = 8) following the acclimation phase as follows:
Group I (Control): Rats were administered 0.5 ml of physiological saline subcutaneously (s.c.).
Group II (hyperthyroid): Rats with hyperthyroidism caused by Eltroxin (75 mg/kg/day) for two weeks (Abdel-Aziz, 2018) were included in the untreated group.
Group III (hyperthyroid + Extract): included the hyperthyroid rats that were treated with LB extract (75 mg/kg/day) for two weeks (Abdel-Aziz, 2018) at the beginning of the second week of the Eltroxin injection and continued for another week. The Animal Care and Use Committee, Faculty of Veterinary Medicine, Benha University, Egypt, approved the experimental protocols (BUFVTM 05-04-22).

2.2.3. Sample collection

The animals were fasted for a whole night and then slaughtered using urethane under anesthesia after the experiment. Serum was extracted from each rat by taking blood samples, which were then centrifuged for 15 minutes at 3000 rpm. Following that, thyroid tissues were removed, cleaned in saline, dried, and kept for histological analysis in a 10% buffered formalin solution.

2.2.4. Biochemical determinations

Rat ELISA kits from My BioSource Co. (San Diego, California, USA) were used to measure the levels of serum thyroid stimulating hormone (TSH), total triiodothyronine (T3), and total thyroxine (T4). A commercial kit from Bio-diagnostic Company (Cairo, Egypt) was used to assess the nitric oxide level (NO) and catalase activity (CAT) calorimetrically.

2.2.5. Histopathological Examination

Samples of thyroid tissue were gathered and preserved for histopathology in a 10% neutral buffered formalin solution. Hematoxylin and eosin staining were applied to tissue specimens, which were then sectioned at a thickness of five microns and seen under a light digital microscope (Bancroft & Gamble, 2013).

2.3. Statistical Analysis

Using the SPSS software package (SPSS 20, SPSS Inc, USA), the data were statistically analyzed using one-way ANOVA and the Bonferroni post hoc test to determine group differences at a $p<0.05$. We present the results as mean ± SD (n = 6/group). Additionally, GraphPad Prism 8 (GraphPad, CA, USA) was used to graph the plots.

3. RESULTS

3.1. Effect of LB Extract on the Thyroidal hormones

Compared to the control group, the hyperthyroidism group showed a noteworthy decrease in TSH levels together with a considerable increase in T3 and T4 levels. Conversely, using LB Extract supplements increased TSH levels and decreased T3 and T4 levels, which counteracted the effects of Eltroxin on the thyroid gland (Fig. 1).

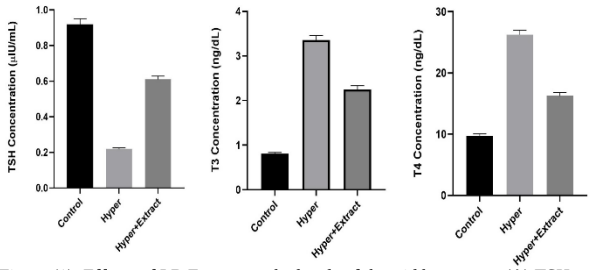

Figure (1): Effects of LB Extract on the levels of thyroid hormones. (A) TSH, (B) T3, and (C) T4. Data are presented as mean ± SD. (A): TSH; (B): T3 and (C): T4 hormone.

3.2. Effect of LB Extract on the Thyroidal gland tissue

Thyroid gland tissue section of E1toxin (hyperthyroidism) demonstrates thyroid follicles of variable sizes. Thyroid follicles were lined by cubical follicular cells with rounded basophilic nuclei. Also,many follicle lumens were empty from colloid. Inthese groups, multiple follicular cells exhibited palenuclei and vacuolated cytoplasm. Atrophied of some thyroidgland follicles and minute blood capillaries were alsorecorded fig.2. Moreover, E1toxin & Extract showed the same histological alterations. Thyroid follicles werevariable in size and were partially filled with colloid. Mostof the thyroid follicles were lined by cubical cells; a few of them showed hyperplasia of follicular epithelial lining.Also, the cytoplasm follicular cells revealed, clear vacuoles with pyknotic or karyolitic nuclei. In additional, the degenerated lining of cells in some of thefollicles were detected.The amount of interfollicular connective tissue was increased, and capillaries were collapsed fig.2.

3.3. Effect of LB Extract on oxidative stress biomarker

When comparing the thyroid tissues of Eltroxin rats to those of control rats, the present results (Fig. 3) showed a sharp decline in CAT activity together with an increasing in NO levels, indicating oxidative damage to the thyroid tissues. On the other hand, there was a noticeable change in the levels of CAT and NO after treatment with LB Extract.

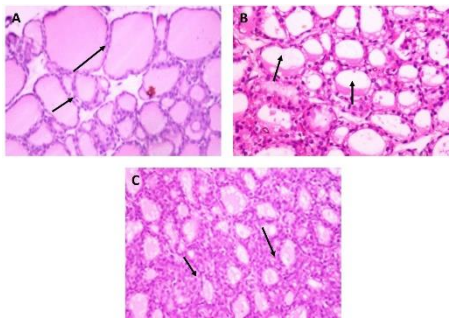

Figure (2): Photomicrograph of thyroid gland tissue section showing normal histological follicles which contained eosinophilic colloid (A). Hyper. Group: decrease in follicle size and peripheral colloidal vacuolations, atrophy of follicles and empty from colloid secretion (B). Hyper + Extract group: variable size follicles and partially filled with colloid and hyperplasia of follicular epithelial lining (C)

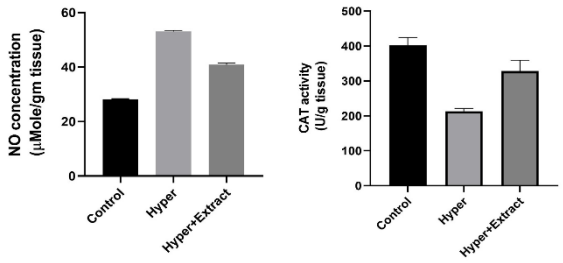

Figure (3): Effects of LB Extract on oxidative stress markers. NO and CAT. Data are presented as mean ± SD.

4- DISCUSSION

The current study's findings, which agree with those of Asker et al. (2015), demonstrated that inducing hyperthyroidism led to a significant rise in blood total T3 and total T4 levels as well as a drop in serum TSH. Following LB treatment, the animals with models of hyperthyroidism showed a considerable improvement in thyroid hormone levels, indicating that LB may have the ability to counteract T3 and T4 levels and prevent hyperthyroidism.

Hyperthyroidism is associated with increased oxidative stress, which can be quantified by measuring NO, an indicator of lipid peroxidation. Baskol et al. (2007) Our results, which show a considerable increase in both NO levels in hyperthyroid model rats, are in line with the findings of Dardano et al. (2006) and Coria et al. (2009). It has been demonstrated that several cellular locations, including the cytoplasm, endoplasmic reticulum, mitochondria, plasma membrane, and peroxisomes, have unique defense systems that prevent the harm that free radicals can inflict. In Littarru et al. (1994) Because enzymes prevent the production of dormant free radicals, hyperthyroidism is linked to decreased CAT activity. The results of Baltaci et al. (2014) and the current study, which shows an increase in NO level, are in agreement. The increase could be attributed to oxidative stress resulting from hyperthyroidism and a decrease in CAT levels, which function as scavengers of free radicals or antioxidants, in comparison to the control group. The hyperthyroidism rat model used in this investigation showed that NO levels had increased and antioxidant CAT had decreased. Hypermetabolic states brought on by thyroid hormone increase mitochondrial electron transport, which raises the quantity of free radicals Mohamed et al. (2014). The active free radicals in biologic membranes can remove hydrogen atoms.

Oxidative stress was decreased by LB treatment. Because LB contains antioxidants like phenolic chemicals, which can stabilize cell membranes, suppress lipid peroxidation, and stop membrane lipids from oxidizing Miraj et al. (2016). In the current investigation, the induction of hyperthyroidism increased NO. The reduction of high NO levels toward the equivalent values of the normal group and the notable enhancement of the antioxidant defense system suggested that LB administration may be beneficial in enhancing the antioxidant status.

5. CONCLUSIONS

The results obtained demonstrated that oxidative stress increased in hyperthyroidism. Besides, it is seen that the treatment with LB counteracted thyroid hormones. The beneficial effect of this extract in correcting hyperthyroidism could be attributed to its high contents of antioxidant components.

AKNOWLEDGMENT

We thank Prof. Dr. Ahmed Osman (Professor of Pathology, Faculty of Veterinary Medicine, Cairo University) for his assistance in the histopathological examination.

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