مجلة الدراسات التربوية والعلمية - كلية التربية - الجامعة العراقية
العدد الثالث والعشرون - المجلد التاسع - علوم الحياة - آب 2024 م
doi.org/10.52866/esj.2023.09.23.27

Effect of Rosemary and Curcumin on Thyroid Hormones in Female Rats

Farah Mahmood Shteeb[1] , Luma Qasim Ali[2] , Zainab Ismail Ibrahim[3]
[1,2] Department of Biology, College of science, Mustansiriyah University, Baghdad, Iraq
[3] College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq
*Corresponding author: farahmahmood@uomustansiriyah.edu.iq.

Abstract :

This study aims to investigate the plant extracts of rosemary and curcumin, which may be of great importance in regulating thyroid hormones after inducing hyperthyroidism and hypothyroidism by levothyroxine and carbimazole, respectively, in female rats. the present study showed there is significant different ($p<0.05$) between effect of low and high of levothyroxine and carbimazole concentrations at 21 and 35 days on TSH, T3, T4 levels, where it found the low and high concentration of levothyroxine lead to decrease level of TSH after 35 and 21 days than control, respectively, and increase levels of T3 after 21 days than control, compared to 35 days than controls. No significant different ($p>0.05$) on levels of TSH by levothyroxine compared to significant different ($P<0.05$) on levels of T3 at 21 days. The concentrations of T4 significantly decreased at 21 days and 35 days versus low concentration 21 days and 35 days, and significant decrease of T3 at 35 days only, versus low concentration at 35 days. Finally, we found there is significant different ($p<0.05$) effect of low and high concentration of carbimazol on levels of TSH, T4, and T3 at 21 days and 35 days , where the high concentration of carbimazole on scored highest effect on levels of TSH, T4, and T3. The rosemary 2 and curcumin 2 extracts scored the highest increase in levels of T4 in 4 weeks than Hypothyroidism, and 8 weeks than Hypothyroidism, T3 in 4 weeks than hyperthyroidism, and 8 weeks than Hypothyroidism, and decrease in levels of TSH, in 4 weeks than Hypothyroidism, and 8 weeks than Hypothyroidism. In contrast, no significant differences ($p>0.05$) appeared between levels of TSH, T4, and T3 at different times (4-8 weeks) for all extracts.

Conclusion: It can be concluded that different concentrations of rosemary and curcumin extracts are beneficial in treating patients with both hypothyroidism and hyperthyroidism during regulation levels of TSH, T3, and T4 during several biological pathways such as; anti-inflammatory, antioxidant, anti-proliferative, apoptosis, angiogenesis, cell cycle and metastasis.

Keywords: Thyroid gland, Hyperthyroidism, Hypothyroidism, Levothyroxine, Carbimazol, Curcumin, Rosemary.

تأثير مستخلص نباتي إكليل الجبل و الكركمين على هرمونات الغدة الدرقية في إناث الجرذان

فرح محمود شتيب[1] , لمى قاسم علي[2] , زينب اسماعيل ابراهيم3
كلية العلوم / الجامعة المستنصرية, بغداد, العراق [2,1]
كلية الطب البيطري, جامعة بغداد, بغداد, العراق[3]
*الباحث الرئيسي : farahmahmood@uomustansiriyah.edu.iq

مستخلص

تهدف هذه الدراسة إلى دراسة المستخلصات النباتية لإكليل الجبل والكركمين والتي قد تكون ذات أهمية كبيرة في تنظيم هرمونات الغدة الدرقية بعد إحداث فرط نشاط الغدة الدرقية وقصور الغدة الدرقية عن طريق الليفوثيروكسين والكاربيمازول على التوالي في إناث الجرذان. أظهرت الدراسة الحالية وجود اختلاف معنوي ($P<0.05$) بين تأثير التراكيز المنخفضة والعالية لليفوثيروكسين والكاربيمازول عند 21 و 35 يوماً على مستويات TSH، T3 و T4، حيث وجد أن التراكيز المنخفضة والمرتفعة لليفوثيروكسين تؤدي إلى انخفاض مستوى TSH بعد 35 و21 يومًا مقارنة بالسيطرة، على التوالي، وزيادة مستويات T3 بعد 21 يومًا اكثر من السيطرة بالمقارنة مع35 يوما اكثر من السيطرة. لا يوجد اختلاف معنوي ($P>0.05$) في مستويات TSH بواسطة الليفوثيروكسين مقارنة بفرق معنوي ($P<0.05$) بمستويات T3 عند21 يومًا. انخفضت معنوياً تراكيز T4 عند 21 يوماً و35 يوماً مقابل التركيز المنخفض في 21 يوماً و35 يوماً، كما انخفض معنوياً T3 عند 35 يوماً فقط مقابل التركيز المنخفض عند 35 يوماً. وأخيراً وجد أن هناك تأثيراً معنوياً ($p<0.05$) للتركيز المنخفض والعالي للكاربيمازول على مستويات TSH وT4 وT3 عند 21 يوماً و35 يوماً، حيث سجل التركيز العالي للكاربيمازول أعلى تأثير على مستويات هرمون TSH، T4، وT3. سجلت مستخلصات إكليل الجبل 2 والكركمين 2 أعلى زيادة في مستويات T4 في 4 أسابيع من قصور الغدة الدرقية، و8 أسابيع من قصور الغدة الدرقية، و T3 في 4 أسابيع من فرط نشاط الغدة الدرقية، و8 أسابيع من قصور الغدة الدرقية، وانخفاض في مستويات TSH في 4 أسابيع من قصور الغدة الدرقية و 8 أسابيع من قصور الغدة الدرقية. في المقابل، لم تظهر فروق معنوية ($p>0.05$) بين مستويات TSH، T4، وT3 في أوقات مختلفة (8-4 أسابيع) لجميع المستخلصات.

الاستنتاج: يمكن الاستنتاج أن التراكيز المختلفة من مستخلصات إكليل الجبل والكركمين مفيدة في علاج المرضى الذين يعانون من قصور الغدة الدرقية وفرط نشاط الغدة الدرقية خلال تنظيم مستويات TSH وT3 و T4 خلال عدة مسارات بيولوجية مثل: مضاد للالتهابات، ومضاد للأكسدة، ومضاد للتكاثر، وموت الخلايا المبرمج، وتولد الأوعية، ودورة الخلية والانبثاث.

الكلمات المفتاحية: الغدة الدرقية، فرط نشاط الغدة الدرقية، قصور الغدة الدرقية، ليفوثيروكسين، كاربيمازول، الكركمين، اكليل الجبل.

Introduction

Thyroid gland is one of major endocrine glands of the body responsible of creating the hormones, thyroxin and triiodothyronine which are essential for the proper organism development in particular for the nervous system and heart, normal growth and skeletal maturation (Luabi *et al*, 2019). Excess thyroid hormone causes hyperthyroidism, which promotes ahypermetabolic state (Brent, 2008). Conversely, reduced thyroid hormone levels causes hypothyroidism, which is associated with hypometabolism (Brent, 2012).

Levothyroxine (LT4), synthetic thyroxine administrated orally, is a favorable treatment for patients with hypothyroidism. Moreover, it could be administered to repress pituitary production of thyroid-stimulating hormone in cases of not poisonous multinodular goiter and diverse forms of neoplasia in the thyroid (Helfand *et al.,*1990). However, Levothyroxine has a limited curative index and subjects patients to the risk of iatrogenic hyper- or hypothyroidism at a dosage just 25% more than usual, or that is more than the most favorable dose, depending on the patient's serum level of TSH (Carr *et al.,*1988). Data from many investigations showed that Levothyroxine induces hyperthyroidism, a "high level of thyroxine" in male rats at 0.5mg/kg intra-peritoneal injection or subcutaneously for successive 12 days (Panda *et al.,* 2007), (Panda *et al.,* 2014).

Carbimazole is an anti-thyroid medication that is commonly used in patients with hyperthyroidism (Kota *et al.,* 2013). Carbimazole is a pro-drug as after absorption it is converted to the active form, methimazole which prevents the thyroid peroxidase enzyme from iodinating and coupling the tyrosine residues on thyroglobulin, hence reducing the production of the thyroid hormones T3 and T4 (Jasim, 2017).

Rosemary (Rosmarinus officinalis) is a common aromatic evergreen shrub in many parts of the world. It has therapeutic properties and has been used in folk medicine as an oral preparation (AI-Sereiti, et. al.,1999), (Ribeiro et. al., 2015).

Several medicinal applications for R. officinalis have been identified, such as treating disorders associated with nervous, endocrine, gastrointestinal, menstrual, hepatic, respiratory, and skin conditions (Begum et. al., 2013).

Rosemary has antifungal, antiviral, antibacterial, anti-inflammatory, antitumor, antithrombotic, antinociceptive, antidepressant, antiulcerogenic, and antioxidant activities (Ribeiro et. al., 2015), (Ojeda et. al., 2013). Owing to its diverse properties, rosemary has also been used widely in the food and cosmetics industries (Stefanovits *et. al.,*2003).

Turmeric (the common name of curcumin) is cultivated in India and other parts of Southeast Asia. Curcumin shows anti-inflammatory, antioxidant, antimutagenic, and antimicrobial activity. Many research investigations have been carried out with curcumin, the main active ingredient of turmeric, given its remarkable anti-inflammatory, antioxidant, anticancer, and anti-aging qualities (Morteza et al., 2017). In addition, curcumin's effect on several biological pathways, such as the apoptotic, anti-oxidant, anti-inflammatory, anti-proliferative, and metastatic pathways, has demonstrated its relevance in thyroid gland problems. (Sahebkar et al., 2022)

Material and method

Plants collection

Rosmarinus officinalis L., commonly known as rosemary, belongs to the Lamiaceae family. The genus Rosmarinus has been merged into the genus Salvia in a recent phylogenetic analysis (UniProt. Taxonomy, 2020). Dry leaves of *Rosmarinus officinalis* were collected from local markets in Baghdad, Iraq.

The plants identified by Dr. Sukeyna Abaas Aliwy Department of Biology/ College of Science /University of Baghdad. Each sample was air-dried in the shade and ground in a blender to produce small pieces, which were then saved in glass containers at room temperature in a dry and dark location.

Curcumin was used in this study as a ready-made extract (NOW Supplements, turmeric curcumin, derived from turmeric root extract, 95% Curcuminoids, herbal supplement, 60 Veg Capsules) was purchased from a local Pharmacy in Baghdad, Iraq.

Preparation of an Aqueous Plants Extracts

The Soxhlet apparatus ISOLAB NS29-32 (Merck KGaA, Darmstadt, Germany) was utilized for the extrac-

tion process. Water, 30% aqueous methanol (SE 30% MeOH), 50% aqueous methanol (SE 50% MeOH), and 50% aqueous ethanol (SE 50% EtOH) were the solutions used as the solvent. (SE H2O). Each plant's aqueous extract was prepared differently depending on the parts of the plant (Harborne, 1984). The extracts were kept in the fridge until they were needed. After the sample and solvent are added to the device, the solvent is heated to remove the solid particles from the material. The liquid that is produced is filtered and absorbed. The solvent's heating does not harm the substance, producing a more concentrated sample (Al-Naqqash, 2013).

Laboratory animals

One hundred and eighty (180) adult female albino rats weighing 165-185 gm and aged 8-10 weeks were obtained from the animal house laboratory of the Biotechnology Research Center, AL- Nahrain University. They were kept for one week for adaptation before starting the treatment under the controlled temperature conditions (25 ± 2ºC). Animals were provided with water and a standard pellet diet daily.

Study Design

In this experimental study, after an adaptation period of one week, A total of 180 healthy albino adult female rats were divided into three main groups each of 60 rats, according to the periods of exposure, which were (21 and 35) days as follows:

Group 1 (control) received distilled water only.

Group 2 (levothyroxine group) received levothyroxine in two different concentrations (2, 4 mg\ kg) (Ferreira *et al.,*2007), respectively in two periods of 21, 35 days to create hyperthyroidism.

Group 3 (carbimazole group) received Carbimazole (2.5, 5 mg\ kg) (El-Bakry *et al.,*2010), respectively in two periods of 21, 35 days to create hypothyroidism.

Animals of groups 2 and 3 follow up the concentration of thyroid hormones (T3, T4, and TSH) through different periods. At the end of the period, scarified half the number (n=30) of animals to examine the physiological changes in the thyroid gland.

The other half number (n=30) of the animals were divided into subgroups to be treated with plant extraction of rosemary and curcumin with two different concentrations (150,200 mg/kg) (100,150 mg/Kg) respectively in

two periods (4, 8) weeks as follows:

Group A (n=15) received levothyroxine and rosemary with low and high concentration in periods of 4 and 8 weeks.

Group B (n=15) received levothyroxine and curcumin with low and high concentrations in periods of 4 and 8 weeks.

Group C (n=15) received carbimazole and rosemary with low and high concentrations in periods of 4 and 8 weeks.

Group D (n=15) received carbimazole and curcumin with low and high concentrations periods of 4 and 8 weeks.

Collection of Blood Samples

Using a medical syringe, five milliliters (ml) of blood was collected directly from each rat's heart by a cardiac puncture. To collect serum (0.5–1.0 ml), each blood sample was placed in a tube containing a gel-free anticoagulant. The tubes were then centrifuged at 3000 rpm for 15 minutes. The serum was then kept at −20 °C until it was needed (Cheng, 2002).

Biochemical Analysis

The ELISA technique was used to measure the levels of thyroid-stimulating hormone (TSH) (use Elabscience kit, USA), triiodothyronine (T3) (O'Neil, 2001), thyroxine (T4) (Wagner et al., 2008) (use AccuBind kit, USA).

Statistical Analysis

The Statistical Analysis System SAS (2012) program was used to affect different factors in study parameters (ANOVA). The least significant difference test was used to significantly compare the means in this study. Results were expressed in terms of mean ± SE or percentage (%) of case frequency. The data were examined for multiple comparisons after one-way analysis of variance (ANOVA), using the Fisher test or t-test. Regression analysis was then performed using analysis of combined variance (ANCOVA). Stat view 5.0 was used to conduct all of the experiments. When $p < 0.05$ was reached, the differences were considered significant.

Results and discussion

Effect of levothyroxine on thyroid hormones

Results of the present study showed there is a significant difference ($p<0.05$) between the effect of low and high levothyroxine concentrations at different times (21 and 35 days) on TSH

and T3 levels, where it found the low and high concentrations of levothyroxine lead to little decrease level of TSH after 21 days (6.6±0.3 and 4.9±0.4) than control (9.8±0.4), compared to 35 days (5.3±0.3 and 3.2±0.4) than controls (9.5±0.4). However, there were little increase levels of T3 after 21 days (3.1±0.2 and 3.8±0.3) than control (2.5±0.1), compared to 35 days (4.1±0.3 and 5.3±0.4) than controls (2.7±0.1). In contrast, did not find a significant difference ($p>0.05$) effect of low and high concentrations of levothyroxine on levels of T4, but there is a significant difference ($P<0.05$) effect between low and high concentrations of levothyroxine on levels of T3 (3.1±0.2 and 3.8±0.3) at 21 days **(table 1).**

Table 1; Effect of different concentrations of levothyroxine on levels of thyroid function tests during different times in rats.

Groups	TSH			T4			T3		
	21 days	**35 days**	**P value**	**21 days**	**35 days**	**P value**	**21 days**	**35 days**	**P value**
Control	9.8±0.4a	9.5±0.4a	*P>0.05*	4.3±0.3b	4.6±0.3b	*P>0.05*	2.5±0.1b	2.7±0.1b	*P>0.05*
levothyroxine Low	6.6±0.3b	5.3±0.3b	***P<0.05****	5.6±0.4a	6.0±0.4a	*P>0.05*	3.1±0.2b	4.1±0.3a	***P<0.05****
levothyroxine -High	4.9±0.4b	3.2±0.4b	***P<0.05****	6.7±0.4a	7.2±0.4a	*P>0.05*	3.8±0.3a	5.3±0.4a	***P<0.05****

Small different letters refer to significant different ($p<0.05$).

The main indication of thyroid hormone insufficiency, hypothyroidism, is the amount of blood TSH in the circulation. The pituitary secretes more TSH when the thyroid hormone result is unusually low. It's important to note that this relationship is nonlinear; for example, a half drop in circulating free T4 (FT4) might cause a 100-fold rise in TSH secretion (Kahaly and Gottwald-Hostalek, 2022). Because of this, the primary diagnostic tool for hypothyroidism is the serum TSH level, with the addition of additional thyroid hormone values for confirmation (Hegedüs et al., 2022). Serum TSH values over an assay-specific reference range found in a group thought to be free of thyroid disease are suggestive of the existence of a population thought to be free of thyroid dysfunction has a level of serum TSH above a reference range specific to the assay, which indicates the presence of hypothyroidism; a condition known as "subclinical hypothyroidism" occurs when thyroid hormone levels are normal but serum TSH is elevated (Wilson et al., 2021).

A previous study showed levothyroxine (LT4), a synthetic form of T4, is the basis of hormone replacement therapy for the treatment of overt hypothyroidism, it was shown that most patients with hypothyroidism can sufficiently restore their thyroid function by carefully adjusting the dose of LT4 over time to bring TSH, T3, and T4 back within the reference range. (Kahaly and Gottwald-Hostalek, 2022). These findings were similar to conducted research that showed the regulated doses of levothyroxine (LT4) play a major role in regulation levels of TSH, T3, and T4 in hypothyroidism patients within normal ranges.

Chaker et al., (2022) mentioned about 90% of hypothyroidism patients were discovered after treatment with levothyroxine (LT4) at different doses and during various times. This drug kept the levels of TSH, T3, and T4 at normal levels.

Salas-Lucia and Bianco, (2022) showed In most tissues (except the brain and pituitary gland), the triiodothyronine (T3) level accurately describes thyroid hormone (TH) signaling and reflects circulating T3 levels. The worrying potential that TH signaling in levothyroxine (LT4)-treated individuals may not be normalized in most tissues is raised because blood T3 levels may not be fully restored in these patients. Physicians may think about how ben-

eficial it could be to measure blood T3 levels to track how well LT4 treatment is working. The goal of the next clinical trials should be to correlate serum T3 levels with clinical outcomes.

The current standard of care for the treatment of hypothyroidism is levothyroxine (LT4) monotherapy, titrated to normalize the circulating level of TSH, T3, and T4. Future studies will certainly improve this thyroid therapy is certain to be improved by future study.

Effect of carbimazole on Hyperthyroidism

Results of the present study showed there is a significant difference ($p<0.05$) between the effect of low and high carbimazole concentrations at different times (21 and 35 days) on TSH levels. Where it found the low and high concentrations of carbimazole lead to little decrease level of TSH after 21 days (15.2±0.6 and 20.4±0.7) than control (9.8±0.4), compared to 35 days (18.5±0.5 and 26.3±1.1) than controls (9.5±0.4). Additionally, the high concentration of carbimazole led to a significant increase in TSH at 21 days (20.4±0.7) and 35 days (26.3±1.1) versus low concentration 21 days (15.2±0.6) and 35 days (18.5±0.5), and significant decrease of T4 at 21 days (2.9±0.2) and 35 days (2.4±0.1) versus low concentration 21 days (3.8±0.3) and 35 days (3.4±0.2)), and significant decrease of T3 at 35 days only (1.4±0.05), versus low concentration at 35 days (1.6±0.07). Finally, there is a significantly different ($p<0.05$) effect of low and high concentrations of carbimazole on levels of TSH, T4, and T3 at 21 days and 35 days, where the high concentration of carbimazole scored the highest effect on levels of TSH, T4, and T3 (table 2).

Table 2; Effect of different concentrations of carbimazole levels of thyroid function tests during different times in rats.

Groups	TSH			T4			T3		
	21 days	35 days	p.value	21 days	35 days	p.value	21 days	35 days	p.value
Control	9.8±0.4 c	9.5±0.4 c	***P>0.05***	4.3±0.3 a	4.6±0.3 a	***P>0.05***	2.5±0.1a	2.7±0.1a	***P>0.05***
carbimazole Low	15.2±0.6 b	18.5±0.5 b	***P<0.05****	3.8±0.3 a	3.4±0.2 b	***P>0.05***	1.99±0.9b	1.6±0.07b	***P>0.05***
carbimazole High	20.4±0.7 a	26.3±1.1 a	***P<0.05****	2.9±0.2 b	2.4±0.1 c	***P>0.05***	1.6±0.06b	1.4±0.05b	***P>0.05***

Small different letters refer to significant different ($p<0.05$)

Docrat et al., (2021) showed that carbimazole plays an important role in treating patients with hyperthyroidism during increased levels of TSH and decreased levels of T3 and T4 and put within normal range. These results were matched with the conducted study. Sultana et al., (2022) showed that carbimazole leads to regulation levels of TSH, thyroid hormones, and anti-oxidants (eg; SOD, MDA, GPx) in patients with hyperthyroidism and put it with normal values.

Usman et al., (2021) mentioned a single dose of carbimazole is considered efficient in treating hyperthyroidism than several doses. These results were not compatible with to present findings that showed the different concentrations and various doses of carbimazole are preferred in hyperthyroidism therapy. Therefore, we suggest using two doses with different concentrations and times of carbimazole to increase the efficiency drug in increasing levels of TSH in patients with hyperthyroidism.

According to the previous study, treating primary hypothyroid female patients with carbimazole in addition to levothyroxine (LT4) raises serum T3, improves LT4 tolerance, and lessens sadness. To validate these results and establish the right dosages for this regimen in various scenarios, larger randomized trials are need-

ed. The target tissues of the hormones produced by the hypothyroidism gland are also included in the pathophysiology of the condition. Therefore, expecting patient satisfaction with merely serum TSH normalization is insufficient. (Elfayoumy et al., 2017).

Effect of rosemary and curcumin extractson levels of thyroid function tests during different times in rats with Hypothyroidism

Results of the present study showed there are significant differences ($p<0.05$) between TSH, T3, and T4 levels among different concentrations of rosemary and curcumin extracts during 4 and 8 weeks in rats with hyperthyroidism. We noticed the rosemary 2 and curcumin 2 extracts scored the highest increase in levels of T4 in 4 weeks (3.3±0.21 and 4.3±0.32) than Hypothyroidism (2.3±0.12), and 8 weeks (3.8±0.27 and 4.6±0.36) than Hypothyroidism (1.9±0.09), T3 in 4 weeks (1.8±0.1and 1.9±0.11) than hyperthyroidism (1.5±0.07), and 8 weeks (1.9±0.11 and 2.1±0.13) than Hypothyroidism (1.5±0.8), and decrease in levels of TSH, in 4 weeks (13.9±0.7 and 12.1±0.7) than Hypothyroidism (25.4±0.5), and 8 weeks (12.6±0.7 and 10.4±0.6) than Hypothyroidism (28.5±0.5)). In contrast, no significant differences ($p>0.05$) appeared between levels of TSH, T4, and T3 at different times (4-8 weeks) for all extracts (table 3).

Table 3; Effect of rosemary and curcumin extractson levels of thyroid function tests during different times in rats with Hypothyroidism

Groups	TSH			T4			T3		
	4-week	8-week	P.value	4-week	8-week	P.value	4-week	8-week	P.value
Control	10.0±0.5d	10.4±0.7d	*P>0.05*	4.5±0.22a	4.6±0.23a	*P>0.05*	2.9±0.08a	3.0±0.09a	*P>0.05*
Hypothyroidism	25.4±0.5a	28.5±0.5a	*P>0.05*	2.3±0.12c	1.9±0.09c	*P>0.05*	1.5±0.07c	1.5±0.8c	*P>0.05*
Rose Conc.1	16.9±0.6b	14.5±0.6b	*P>0.05*	3.0±0.18b	3.5±0.23b	*P>0.05*	1.7±0.09c	1.8±0.1c	*P>0.05*
Rose Conc.2	13.9±0.7c	12.6±0.7c	*P>0.05*	3.3±0.21b	3.8±0.27b	*P>0.05*	1.8±0.1b	1.9±0.11b	*P>0.05*
Cur Conc.1	14.1±0.6c	12.2±0.6c	*P>0.05*	3.3±0.21b	3.6±0.25b	*P>0.05*	1.8±0.09b	1.9±0.11b	*P>0.05*
Cur Conc.2	12.1±0.7c	10.4±0.6d	*P>0.05*	4.3±0.32a	4.6±0.36a	*P>0.05*	1.9±0.11b	2.1±0.13b	*P>0.05*

Small different letters refer to significant different ($p<0.05$).

Shakeri et al., (2022) mentioned that rosemary and curcumin extractions play important roles in the regulation hormones of the thyroid gland, where it found the progression concentrations of curcumin lead to decreased levels of TSH and increased T3 and T4 (treat hypothyroidism) compared to control. Another study by Mohamed and Mogeda, (2019) that revealed curcumin with different concentrations leads to treating hypothyroidism by decreasing TSH and increasing T3 and T4 levels. The above results were compatible with current outcomes.

Previous research showed that the antioxidant effect of curcumin causes hypothyroidism in rats. Curcumin in turmeric, which is mostly found in rhizomes, is thought to be a potent antioxidant that protects against oxidative tissue damage (Memarzia et al., 2021). Additionally, curcumin reduces oxygen to scavenge or neutralize free radicals and reduce their availability for oxidative reactions. Curcumin, however, has been shown to in a dose-dependent manner reduce cell viability and induce cell death in papillary thyroid cancer cells (Zhang et al., 2022). Polyphenolic substances such as curcumin can ameliorate oxidative damage to reactive materials, thus preventing the stimulation of the thyroid gland in female rats (Hameed et al., 2020).

Mansour and Mousa, (2022) found the developed concentrations of rosemary extract lead to treating hypothyroidism during decreased levels of TSH and increased T3 and T4 compared to control, and these findings were matched with present results. The previous study mentioned serum levels of T3 and T4 were significantly increased in rats with rosemary. This could be due to oxidative stress caused by rosemary. Oxidative stress appears to be a key factor in the progression of inflammation (Sathyabhama et al., 2022). Thyroid hormones can protect the body by controlling antioxidant levels. There is a correlation between oxidative stress and hormonal imbalance. Thyroid hormones have an important role in the balance of antioxidants, and it has been found that there is a link between hyperthyroidism and hypothyroidism with oxidative stress in animals and humans (Arcos, 2022).

Table 4; Effect of rosemary and curcumin extracts on levels of thyroid function tests during different times in rats with Hyperthyroidism

Groups	TSH			T4			T3		
	4-week	8-week	P.value	4-week	8-week	P.value	4-week	8-week	P.value
Control	10.0±0.4a	10.4±0.7a	***P>0.05***	4.5±0.22a	4.6±0.23a	***P>0.05***	2.9±0.08c	3.0±0.09c	***P>0.05***
Hyperthyroidism	4.6±0.38a	3.6±0.44d	***P>0.05***	2.3±0.12c	1.9±0.09c	***P>0.05***	5.2±0.31a	5.4±0.3a	***P>0.05***
Rose Conc.1	5.9±0.33d	7.0±0.4c	***P>0.05***	3.0±0.18b	3.5±0.23b	***P>0.05***	4.3±0.24b	4.5±0.24b	***P>0.05***
Rose Conc.2	7.4±0.33c	8.6±0.39b	***P>0.05***	3.3±0.21b	3.8±0.27b	***P>0.05***	3.9±0.2b	4.1±0.21b	***P>0.05***
Cur Conc.1	6.9±0.37c	8.8±0.39b	***P>0.05***	3.3±0.21b	3.6±0.25b	***P>0.05***	4.1±0.21b	4.4±0.21b	***P>0.05***
Cur Conc.2	8.7±0.4b	10.3±0.4a	***P>0.05***	4.3±0.32a	4.6±0.36a	***P>0.05***	3.6±0.17b	3.4±0.17c	***P>0.05***

Small different letters refer to significant different ($p<0.05$)

Effect of rosemary and curcumin extracts on levels of thyroid function tests during different times in rats with Hyperthyroidism

The present study showed significant differences ($p<0.05$) between TSH, T4, and T3 levels among different rosemary and curcumin extract concentrations during 4 and 8 weeks in rats with hypothyroidism. We noticed the rosemary 2 and curcumin 2 extracts scored the highest increase in levels of TSH in 4 weeks (7.4±0.33 and 8.7±0.4) than Hyperthyroidism (4.6±0.38), and 8 weeks (8.6±0.3 and 10.3±0.) than Hyperthyroidism (3.6±0.44), T4 in 4 weeks (3.3±0.21 and 4.3±0.32) than Hyperthyroidism (2.3±0.12), and 8 weeks (3.8±0.27 and 4.6±0.36) than Hyperthyroidism (1.9±0.09), And decrease levels of T3, in 4 weeks (3.9±0.2 and 3.6±0.17) than Hyperthyroidism (5.2±0.31), and 8 week (4.1±0.21 and 3.4±0.17) than Hyperthyroidism (5.4±0.3)). In contrast, no significant variation ($p>0.05$) between levels of TSH, T4, and T3 at different times (4-8 weeks) for all extracts (table 4).

Tsai and Leung, (2021) showed decreased levels of TSH and increased T3 and T4 in patients with hyperthyroidism than controls, and these outcomes were matched with conducted study. Hyperthyroidism, is defined as high levels of triiodothyronine (T3) and/or free thyroxine (FT4) and suppressed thyrotropin (also referred to as thyroid-stimulating hormone), affects 0.2% to 1.4% of the worldwide population. Subclinical hyperthyroidism, characterized by low thyrotropin levels and normal T3 and FT4 levels, affects 0.7% to 1.4% of the worldwide population. Osteoporosis, heart failure, cardiac arrhythmias, and adverse pregnancy outcomes can all result from untreated hyperthyroidism. It is linked to a higher risk of premature death and may cause inadvertent weight loss. (Lee and Pearce, 2023).

Authors revealed that curcumin extract plays a role in treating patients' hyperthyroidism induced by lithium, where it found that curcumin leads to increased levels of TSH and decreased hormones T3 and T4 (Abd El-Twab and Abdul-Hamid, 2016). These findings were compatible with to present results. Curcumin has been shown to play a role in thyroid gland disorder by its effects on several biological processes, such as the cell cycle, metastasis, apoptosis, antioxidant, anti-inflammatory, and anti-proliferative pathways (Shakeri et al., 2022).

One of the problems facing the world today is still hyperthyroidism and other thyroid disorders. Consequently, a prior study identifies turmeric powder as a crucial supplement that, when used in conjunction with the antithyroid medication carbimazole, considerably raises the TSH and thyroid peroxidase antibody (TPOAb) levels of hyperthyroid individuals. When used carefully, turmeric powder is a cheap, readily available herbal treatment for hyperthyroidism that has no known side effects, unlike chemotherapy, which in certain cases can cause severe allergic reactions and other unfavorable effects (Hassan and Aliyu, 2014).

High quantities of rosmarinic acid, which is used to treat hyperthyroidism, have been identified in the chromatography HPLC data. Previous studies have shown that TSH is influenced by rosmarinic acid at the receptor location. It also decreases peripheral T3 conversion inhibits immunoglobins' effects on the TSH receptor and is helpful in the management of Grave's disease

rosmarinic acid. (Hameed et al., 2020).

Rererences

1. A. Al-Naqqash. Evaluation of Three Plant Extracts Activity to the Stopping of Bleeding in Albino Mice. M.Sc. thesis. 2013.
2. Abd El-Twab, S. M., & Abdul-Hamid, M. (2016). Curcumin mitigates lithium-induced thyroid dysfunction by modulating antioxidant status, apoptosis and inflammatory cytokines. The Journal of Basic & Applied Zoology, 76, 7-19.
3. Abraham A, Samuel S, Mathew L. Pharmacognostic evaluation of Curcuma longa L. Rhizome and standardization of its formulation by HPLC using curcumin as marker. Int J Pharm Phytochem Res 2018;10:38-42.
4. AI-Sereiti, M.R.; Abu-Amerb, K.M.; Sena, P. Pharmacology of rosemary (Rosmarinus oificinalis Linn.) and its therapeutic potentials. Indian J. Exp. Biol. 1999, 37, 124–130. [Google Scholar]
5. Ammon HP, Wahl MA. Pharmacology of curcuma longa. Planta Med 1991;57:1-7.
6. Arcos, M. L. B. (2022). Role of thyroid hormones-induced oxidative stress on cardiovascular physiology. Biochimica et Biophysica Acta (BBA)-General Subjects, 130239.
7. Begum, A.; Sandhya, S.; Vinod, K.R.; Reddy, S.; Banji, D. An in-depth review on the medicinal flora Rosmarinus officinalis (Lamiaceae). Acta Sci. Pol. Technol. Aliment. 2013, 12, 61–74. [Google Scholar]
8. Behal R, Mali AM, Gilda SS, Paradkar AR. Evaluation of local drugdelivery system containing 2% whole turmeric gel used as an adjunct to scaling and root planing in chronic periodontitis: A clinical and microbiological study. J Indian Soc Periodontol 2011;15:35-8.
9. Brent, G.A.(2008). Clinical practice. Graves' disease. The New England Journal of Medicine 358: 2594–2605.
10. Brent, G.A.(2012). Hypothyroidism and thyroiditis. In: Williams Textbook of Endocrinology,edited by Melmed SP, Larsen PR, and Kronenberg HM. Philadelphia, PA: Elsevier.
11. Carr D, McLeod DT, Parry G, Thomes HM. Fine adjustment of thyroxine replacement dosage: Comparison of the thyrotropin releasing hormone test using a sensitive thyrotropin assay with measurement of free thyroid hormones and clinical assessment. Clin En-

docrinol. 1988;28(3):325-333. Doi: 10.1111/j.1365-2265.1988.tb01219.x

12. Chaker, L., Razvi, S., Bensenor, I. M., Azizi, F., Pearce, E. N., & Peeters, R. P. (2022). Hypothyroidism (Primer). Nature Reviews: Disease Primers, 8(1).
13. Cheng. Angiotensin Π induced inflammation and vascular dysfunction: Role of oxidative stress and cyclooxygenase. Academic dissertation. University of Helsinki. (2002).
14. Cheng. Angiotensin Π induced inflammation and vascular dysfunction: Role of oxidative stress and cyclooxygenase. Academic dissertation. University of Helsinki. (2002).
15. Çıkrıkçı S, Mozioğlu E, Yılmaz H. Biological activity of curcuminoids isolated from Curcuma longa. Rec Nat Prod 2008;2:19-24
16. Docrat, F., Mokoena, T., Karusseit, V. O. L., & Ankrah, A. O. (2021). The adjunctive use of carbimazole during radioactive iodine treatment reduces the cure rate of Graves' disease. South African Medical Journal, 111(2), 176-179.
17. El-Bakry AM, El-Gareib AW, Ahmed RG (2010). Comparative Study of the Effects of Experimentally Induced Hypothyroidism and Hyperthyroidism in Some Brain Regions in Albino Rats. International Journal of Developmental Neuroscience, 28(5):371–89.
18. El-Bolkiny, Y. E., Salem, M. S., El-Naggar, S. A., & El-Sharkawy, F. R. (2022). Rosemary and Costus Reduces Oxidative Stress and Normalize Pituitary-Thyroid Hormones in Ehrlich-Bearing Mice Treated with Cisplatin. Pediatrics Case Reports, 1(1), 2835-2971.
19. Elfayoumy, K. N., Elgazzar, U. B., Aboalabbas, M., & Al-Adl, A. S. (2017). Adding carbimazole to levothyroxine increases triiodothyronine and improves outcome in patients with primary hypothyroidism: a preliminary study from Egypt. Electronic Physician, 9(2), 3706.
20. Ferreira E, Silva A, Serakides R, Gomes A, Cassalil G. Model of induction of thyroid dysfunctions in adult female mice. Arq Bras Med Vet Zootec. 2007; 59(5):1245-1249.
21. Hameed, S. I., Al-Shahwany, A. W., & Salih, S. J. (2020). Evaluation of the Activity of Some Plants Extracts on Thyroid Gland Regulation in Female Albino Rats. Iraqi Journal of Science, 254-265.

22. Hameed, S. I., Al-Shahwany, A. W., & Salih, S. J. (2020). Evaluation of the Activity of Some Plants Extracts on Thyroid Gland Regulation in Female Albino Rats. Iraqi Journal of Science, 254-265.
23. Harborne. Phytochemical methods. Chapman and Hall. New York 2nd ed. Pp: 288 (1984).
24. Hassan, A. A., & Aliyu, M. M. (2014). Effect of Combined Carbimazole and Curcuma longa Powder in Human Thyroid-Stimulating Hormone and Thyroperoxidase Antibody in Hyperthyroidism. International Journal of Pharmacological and Pharmaceutical Sciences, 15(1), 80-87.
25. Hegedüs, L., Bianco, A. C., Jonklaas, J., Pearce, S. H., Weetman, A. P., & Perros, P. (2022). Primary hypothyroidism and quality of life. Nature Reviews Endocrinology, 18(4), 230-242.
26. Helfand M, Crapo LM. Monitoring therapy in patients taking levothyroxine. Ann Intern Med. 1990;113(6):450-454. Doi: 10.7326/0003-4819-113-6-450
27. Jasim W K (2017) Protective effects of dehydroepiandrosterone (DHEA) against Carbimazol-induced toxicity in male mice. J. int. Sci Pharm. 281-277,(3)8.
28. Kahaly, G. J., & Gottwald-Hostalek, U. (2022). Use of levothyroxine in the management of hypothyroidism: A historical perspective. Frontiers in Endocrinology, 13, 1054983.
29. Kota S K, Meher L K, Kota S K, Jammula S , Modi K D (2013) Carbimazole-induced cholestatic hepatitis in Graves' disease. Indian journal of Endocrinology and metabolism, 17(2), 326.
30. Lee, S. Y., & Pearce, E. N. (2023). Hyperthyroidism: A Review. JAMA, 330(15), 1472-1483.
31. M. S. Wagner, S. M. Wajner and A. L. Maia. The Role of Thyroid Hormone in testicular Development and Function. J. Endocrinol., 199(3): 351-365 (2008).
32. Mansour, D. S., & Mousa, A. M. (2022). Ameliorative potential role of Rosmarinus officinalis extract on toxicity induced by etoposide in male albino rats. Brazilian Journal of Biology, 84.
33. Memarzia, A., Khazdair, M. R., Behrouz, S., Gholamnezhad, Z., Jafarnezhad, M., Saadat, S., & Boskabady, M. H. (2021). Experimental and clinical reports on anti-inflammatory, antioxidant, and immunomodulatory effects of Curcuma longa and curcumin, an updated and comprehensive review. BioFactors,

47(3), 311-350.

34. MJ. O'Neil. The Merck Index. 13A ed. Whitehouse Station, NJ: Merck & Co. Inc.; 987-988 (2001).
35. Mohamed, F., & MOGEDA, M. N. (2019). Possible Ameliorating Effect of Curcumin on the Pancreas of Experimentally Induced Hypothyroidism in Rat: Histological, Immunohistochemical and Biochemical Study. The Medical Journal of Cairo University, 87(December), 4815-4823.
36. Mortaza. & Sahebkar, A. (2017). Curcumin: An effective inhibitor of interleukin-6. *Current Pharmaceutical Design*, *23*(6), 921-931.
37. N M Luabi, N A Zayed, LQ Ali. "Zinc oxide nanoparticles effect on thyroid and testosterone hormones in male rats" , Journal of Physics: Conference Series, 2019.
38. Ojeda-Sana, A.M.; van Baren, C.M.; Elechosa, M.A.; Juárez, M.A.; Moreno, S. New insights into antibacterial and antioxidant activities of rosemary essential oils and their main components. Food Control 2013, 31, 189–195. [Google Scholar] [CrossRef]
39. Panda S and Kar A. Annona squamosa seed extract in the regulation of hyperthyroidism and lipid-peroxidation in mice: possible involvement of quercetin. Phytomed. 2007;14(12):799-805. Doi:10.1016/j.phymed.2006.12.001
40. Panda S and Kar A. Antithyroid effects of naringin, hesperidin and rutin in l-T4 induced hyperthyroid rats: Possible mediation through 5′ DI activity. Pharmacol Report. 2014;66(6):1092-1099. Doi: 10.1016/j.pharep.2014.07.002
41. Ribeiro-Santos, R.; Carvalho-Costa, D.; Cavaleiro, C.; Costa, H.S.; Albuquerque, T.G.; Castilho, M.C.; Ramos, F.; Melo, N.R.; Sanches-Silva, A. A novel insight on an ancient aromatic plant: The rosemary (Rosmarinus officinalis L.). Trends Food Sci. Technol. 2015, 45, 355–368. [Google Scholar] [CrossRef]
42. Ruggeri, R., Minuti, A., Aliquo, F., Campenni, A., Avenoso, A., Giani, F., ... & Angela, D. (2022, May). Curcumin attenuates the pro-inflammatory response induced by hyaluronan oligosaccharides in human thyroid fibroblasts and thyrocytes. In Endocrine Abstracts (Vol. 81). Bioscientifica.
43. Salas-Lucia, F., & Bianco, A. C. (2022). T3 levels and thyroid hormone signaling. Frontiers in Endocrinology, 13, 1044691.

44. Sathyabhama, M., Priya Dharshini, L. C., Karthikeyan, A., Kalaiselvi, S., & Min, T. (2022). The Credible Role of Curcumin in Oxidative Stress-Mediated Mitochondrial Dysfunction in Mammals. Biomolecules, 12(10), 1405.
45. Shakeri, F., Bibak, B., Safdari, M. R., Keshavarzi, Z., Jamialahmadi, T., Sathyapalan, T., & Sahebkar, A. (2022). Cellular and molecular mechanisms of curcumin in thyroid gland disorders. Current Medicinal Chemistry, 29(16), 2878-2890.
46. Shakeri, F., Bibak, B., Safdari, M. R., Keshavarzi, Z., Jamialahmadi, T., Sathyapalan, T., & Sahebkar, A. (2022). Cellular and molecular mechanisms of curcumin in thyroid gland disorders. Current Medicinal Chemistry, 29(16), 2878-2890.
47. Stefanovits-Bányai, É.; Tulok, M.; Hegedus, A.; Renner, C.; Szollosi Varga, I. Antioxidant effect of various rosemary (Rosmarinus officinalis L.) clones. Acta Biol. Szeged. 2003, 47, 111–113. [Google Scholar]
48. Sultana, R., Shahin, A. D., & Jawadul, H. M. (2022). Measurement of oxidative stress and total antioxidant capacity in hyperthyroid patients following treatment with carbimazole and antioxidant. Heliyon, 8(1).
49. Thies, R.T. (2012). Physiology : An Illustrated Review, Thiemc Medical Publishers, Inc. New York.
50. Tsai, K., & Leung, A. M. (2021). Subclinical hyperthyroidism: a review of the clinical literature. Endocrine Practice, 27(3), 254-260.
51. UniProt. Taxonomy—Rosmarinus Officinalis (Rosemary) (Salvia Rosmarinus). Available online: https://www.uniprot.org/taxonomy/39367 (accessed on 6 March 2020).
52. Usman, R., Ismail, S., Tahir, R., & Jamil, M. (2021). Efficacy Of Different Dosage Regimens Of Carbimazole In The Treatment Of Primary Hyperthyroidism. Journal of Ayub Medical College Abbottabad-Pakistan, 33(2).
53. Wilson, S. A., Stem, L. A., & Bruehlman, R. D. (2021). Hypothyroidism: Diagnosis and treatment. American family physician, 103(10), 605-613.
54. Zhang, L., Xu, S., Cheng, X., Wu, J., Wu, L., Wang, Y., ... & Yu, H. (2022). Curcumin induces autophagic cell death in human thyroid cancer cells. Toxicology in Vitro, 78, 105254.