Effects of Rosemary and curcumin leaves extract in Histophysiological aspects of rats with Hypothyroidism.

Farah M. Shteeb[1] , Luma Q. Ali[1] , Zainab I. Ibrahim[2]

[1] Department of Biology, College of Science, AL-Mustansiriyah University, Baghdad, Iraq.
[2] College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.
*Corresponding author: farahmahmood@uomustansiriyah.edu.iq

Received: 09/05/2024
Accepted: 23/07/2024
Online: 18/12/2025

ABSTRACT

Background: Hypothyroidism is a common disease characterized by low thyroid hormone levels. Carbimazole is an anti-thyroid medication that is commonly used in patients with hyperthyroidism. Carbimazole is a prodrug; after absorption, it is converted to the active form, methimazole, which prevents the thyroid peroxidase enzyme from iodinating and coupling tyrosine residues on thyroglobulin, hence reducing the production of the thyroid hormones T3 and T4. **Aim of study:** This study aims to identify the effects of aqueous extracts of rosemary leaves and curcumin in regulating thyroid hormones T3, T4, and TSH after inducing hypothyroidism with carbimazole in female rats. **Methodology:** In an experimental study, a total of 90 healthy adult female rats were assigned randomly to three main groups (30 rats per group) according to the type of treatment and periods. Examined the physiological changes in the thyroid gland of several animals by serological analysis of thyroid hormones after the end of the period (7 weeks) to ensure the induction of hypothyroidism in rats. The remaining animals were then treated with aqueous extracts of rosemary leaves and curcumin at concentrations of 200 mg/kg and 150 mg/kg, respectively, for a period of 8 weeks. **Results:** The present study showed significant differences ($p<0.05$) between the two carbimazole concentrations (2.5 mg/kg and 5mg/kg) in their effects on levels of TSH, T3, and T4 at 7 weeks, where the higher concentration of carbimazole recorded the highest effect on T3, T4, and TSH levels. The rosemary (200 mg/kg) and curcumin (150mg/kg) extracts recorded the highest increase in levels of T3 and T4 in 8 weeks compared to the positive control (hypothyroidism), and decreased the levels of TSH in 8 weeks than hypothyroidism. **Conclusions:** It can be concluded that different concentrations of rosemary and curcumin aqueous extraction are beneficial in treating patients with hypothyroidism by regulating levels of TSH, T3, and T4 that were conducted with microscopic examination of thyroid tissues revealed mostly moderate to marked histopathological changes from epithelial degeneration comparison with normal histology of thyroid gland ranged from cellular to vacuolar degeneration and necrosis of follicles and colloid material filled their lumens of follicles after treatment with rosemary and curcumin.

Keywords: Hypothyroidism, Carbimazol, Curcumin, Rosemary, Rats.
https://doi.org/10.24126/jobrc.2025.19.3.851

INTRODUCTION

The thyroid gland, is one of the most important endocrine glands in the body, as its hormones (thyroxine T4 and Triiodothyronine T3) are essential for normal growth and development; it has an important role in regulating the body's basic metabolic rate (1), the thyroid gland consists of many secretory parts called follicles, the wall of each follicle is lined with a single layer of simple cuboidal epithelial cells called follicular cells which surround a central lumen, is

filled with a clear, viscous protein-rich fluid called colloid. Surface-layer cells of the follicles of the thyroid are responsible for the synthesis and release of thyroid hormones (T4 and T3). They bind to the glycoprotein thyroglobulin, either stored in the colloid or released into the blood in nearby capillaries (2). The term "thyroid disorder" refers to several conditions affecting the thyroid hormones (3). Thyroid hormone production is impaired owing to thyroid abnormalities or iodine deficiency, but it can also be due to pituitary or hypothalamic problems. weight gain, cold sensitivity, and constipation(4). Hypothyroidism is characterized by low thyroid hormone levels, whereas hyperthyroidism is brought on by increased thyroid function. (3) Carbimazole is an anti-thyroid medication that is commonly used in patients with hyperthyroidism (5). Carbimazole is a prodrug, 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 (6). Hypothyroidism is a common disease caused by a thyroid hormone (TH) deficit. It can have major negative effects on the heart, kidney, liver, and brain if left untreated (7). Patients have the choice to use herbal medications either on their own or in combination with more conventional medication. Due to challenges in regulating the levels of thyroid hormone by drugs, the current medical treatments for hypothyroidism are often regarded as inadequate (8). Therefore, it is necessary to develop novel substances derived from plants that, in the case of hypothyroidism, enhance thyroid hormone levels with fewer side effects. A variety of herbal plants, including curcumin (*Curcuma longa*) and rosemary (*Rosmarinus officinalis*), are used to treat hypothyroidism. So, the goal of the current study was to determine how different herbal extracts affect thyroid gland histological morphology and hormone levels.

METHODOLOGY

Materials:

Laboratory animals: Ninety (90) adult female albino rats weighing 165-185 gm and aged 10-12 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 treatment under controlled-temperature conditions (25 ± 2 ºC). Animals were provided with water and a standard pellet diet daily. The Helsinki guidelines were followed in all procedures involving laboratory animals.

Herbal medications include the following plants:

1. *Rosmarinus officinalis L.,* commonly known as rosemary, is a herbal plant that had been identified by Dr. Sukeyna Abaas Al Wagy from the Department of Biology/ College of Science / University of Baghdad. The plant's leaves were purchased at local markets in Al-Bnook Street, Baghdad, Iraq.

2. Curcumin: as a ready-made extract named is (NOW Supplements), turmeric curcumin, derived from turmeric root extract, 95% Curcuminoids, herbal supplement, 60 Veg Capsules) was purchased from a local Pharmacy (Al Bnook Pharmacy) which is located in/ Baghdad, in Baghdad, Iraq.

Methods:

1. Preparation of an Aqueous Plant Extract

The Soxhlet apparatus ISOLAB NS29-32 (Merck KGaA, Darmstadt, Germany) was used for the extraction process. Water (SE H_2O), 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. Each plant's aqueous extract was prepared differently depending on the plant part. 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 affect the substance, producing a more concentrated sample (9).

2. Study Design

In this experimental study, after an adaptation period of two weeks, a total of 90 healthy adult female rats were assigned randomly to 3 main groups as follows:

Group 1 (n=30, control) received distilled water only. Group 2 (n=30, Carbimazole group) received Carbimazole (2.5 mg\ kg). Group 3 (n=30, Carbimazole group) received Carbimazole (5 mg\ kg) for 7 weeks to create hypothyroidism. Animals in groups 2 and 3 were tested for the concentration of thyroid hormones (T3, T4, and TSH) at the end of the

period, and half the number of animals was sacrificed to examine physiological changes in the thyroid gland. The other half of the animals were divided into subgroups to be treated with aqueous extraction of rosemary leaves at a concentration of 200 mg/ kg or curcumin 150 mg/Kg for 8 weeks, as follows: Group A received carbimazole in 5mg/kg and rosemary with (200 mg\kg) while Group B received carbimazole and curcumin (150 mg/kg) for 8 weeks and a group of positive control (hypothyroid rats which continue to receive Carbimazole (5 mg\ kg) for more 8 weeks.

3. Serum samples

Using a medical syringe, five milliliters (mL) of blood was collected directly from a cardiac puncture. To collect serum (0.5–1.0 mL), each blood sample was placed in a tube containing a gel-free anticoagulant. After that, the tubes were centrifuged for 15 minutes at 3000 rpm. The serum was then stored at −20 °C until needed (10). Animals were dissected, and their thyroid was removed, except for the meticulous process of washing. Our technique involves splicing with 2% normal buffer (NaCl) and storing the samples in a refrigerator with a fixation solution (neutral buffer).

Histopathological examination:

The postmortem examination was performed on rats in all experimental groups, including the control group; then, thyroid glands were collected and preserved in 10% neutral buffered formalin for 72 hours before further tissue processing. Tissue slices were stained with hematoxylin and eosin for microscopic examination by light microscope (11).

4. Biochemical Analysis

The Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure the levels of Thyroid-stimulating hormone (TSH) by using the Elabscience kit; (USA), Thyroxine (T4) by using the AccuBind kit; (USA) and Triiodothyronine (T3) (12).

5. Statistical Analysis

The Statistical Analysis System (SAS) was used in this study; the means were statistically compared using the least significant difference test. 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 a combined variance analysis (ANOVA). Stat View 5.0 was used to conduct all of the experiments. When $p< 0.05$ was reached, the differences were considered significant.

RESULTS

The results of the present study showed a significant difference ($p\leq0.05$) in TSH levels between carbimazole doses of 2.5 and 5 mg/kg after 7 weeks of treatment, with levels 18.5±0.5 and 26.3±1.1, respectively, compared to controls (9.5±0.4), showing that the higher effect was achieved with 5 mg/kg carbimazole. A significant decrease in T4 levels after 7 weeks of treatment with 2.5 mg/kg carbimazole (3.4±0.2) was observed versus (2.4±0.1) when the doses were elevated to 5 mg/kg carbimazole. On the other hand, a significant decrease in T3 level was also observed after the same treatment and duration, with (1.6±0.07) for 2.5mg/kg and (1.4±0.05) for the other treatment. Finally, it was observed that the highest carbimazole concentration had the greatest effect on TSH, T4, and T3 levels (Table 1).

Table (1): Effect of Carbimazole levels on Thyroid hormones in female rats after 7 weeks of treatment

Groups	TSH µIU/ml (Mean±SD)	T4 ng/dl (Mean±SD)	T3 ng/dl (Mean±SD)
Control	9.5±0.4 c	4.6±0.3 a	2.7±0.1a
Carbimazole 2.5 mg\kg	18.5±0.5 b	3.4±0.2 b	1.6±0.07b
Carbimazole 5 mg\kg	26.3±1.1 a	2.4±0.1 c	1.4±0.05b

Small different letters refer to significant differences ($p\leq0.05$)

Table (2): Effects of Rosemary and Curcumin extracts on levels of thyroid function tests after 8 weeks of treatment in rats with Hypothyroidism

Groups	TSH µIU/ml (Mean±SD)	T4 ng/dl (Mean±SD)	T3 ng/dl (Mean±SD)
Control	9.5±0.4 d	4.6±0.3 a	2.7±0.1a
Hypothyroidism (Positive control)	28.5±0.5 a	1.9±0.09 c	1.5±0.8 c
Rosemary (200mg/kg)	12.6±0.7 c	3.8±0.27 b	1.9±0.11 b
Curcumin (150 mg/kg)	10.4±0.6 d	4.6±0.36 a	2.1±0.13b

Small different letters refer to significant differences ($p \leq 0.05$).

Table (2) shows the elevating effects of rosemary (200 mg/kg) and curcumin (150 mg/kg) extracts on T4 levels after 8 weeks of treatment (3.8±0.27 and 4.6±0.36) compared to the hypothyroidism (positive control group) (1.9±0.09), as well as T3 levels were elevated to record (1.9±0.11 and 2.1±0.13) compared to the positive control (1.5±0.8), while TSH levels were decreased to (12.6±0.7 and 10.4±0.6) compared to hypothyroidism (28.5±0.5) for the same duration.

The present results of thyroid function tests represented by levels of their hormones TSH, T3, and T4, with histopathological examination of the thyroid gland in treated groups compared with the control group; the latter shows normal cuboidal epithelium-lined follicles filled with colloid (Figure.1). In rats induced hypothyroidism by carbimazole (200 mg/kg) after 7 weeks of daily treatment, atrophy of thyroid tissues marked by hypoplasia of follicles which appeared small in size and lined with low cuboidal even flattened epithelium was seen (Figure. 2 and 3).In the group of rosemary treatment for 8 weeks, there was hyperplasia and regular small follicles containing the full amount of colloidal material (Figure 4), the small size of follicles with a small amount of colloidal material seen (Figure. 5 and 6), also in other section there was large-dilated and hyperchromatic nuclei of lining epithelium and filled with colloid (Figure.7 and 8).

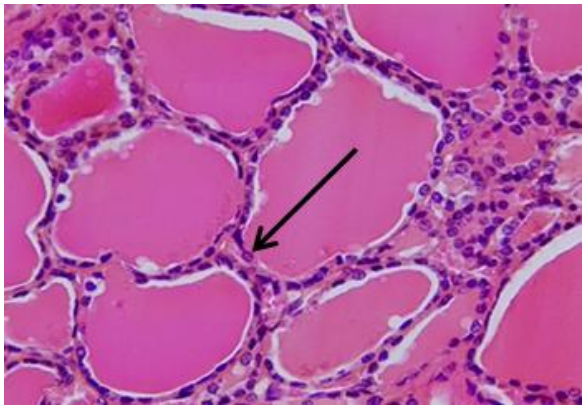

Figure (1): The histological section of the thyroid gland shows normal cuboidal epithelium-lined follicles filled with colloid (arrow). (H&E stain, 400X).

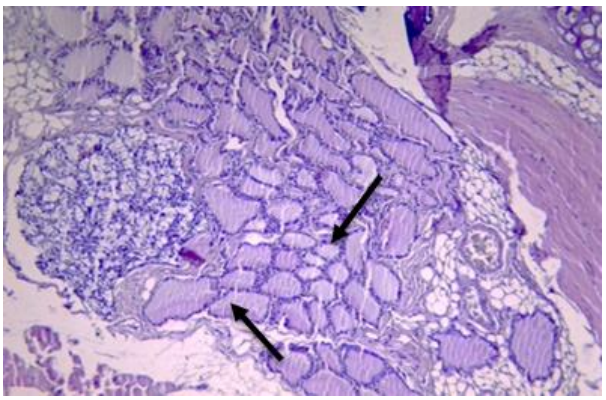

Figure (2): Histological section of the thyroid gland from the group of carbimazole 2.5 mg/kg after 7 weeks; shows different sizes of follicles (arrow). (H&E stain, 40X).

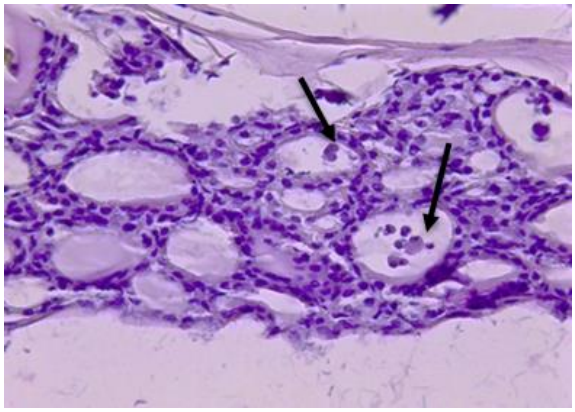

Figure (3): Histological section of the thyroid gland from the group of carbimazole 200 mg/kg after 7 weeks; shows small bodies of degenerated epithelium in the lumen of follicles and a small amount of colloid. (H&E stain, 400X).

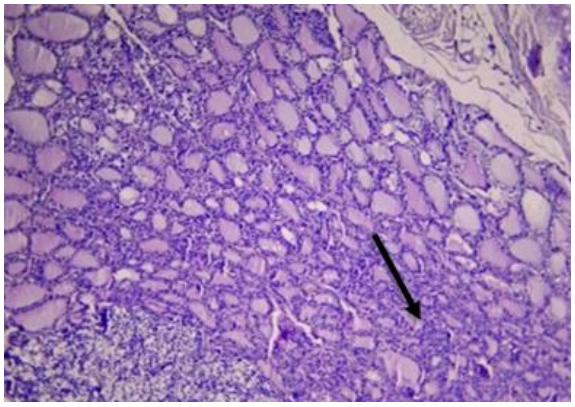

Figure (4): Histological section of the thyroid gland from a group of rosemary treatments after 8 weeks; shows hyperplasia (arrow) and regular small follicles containing the full amount of colloidal material. (H&E stain, 40X).

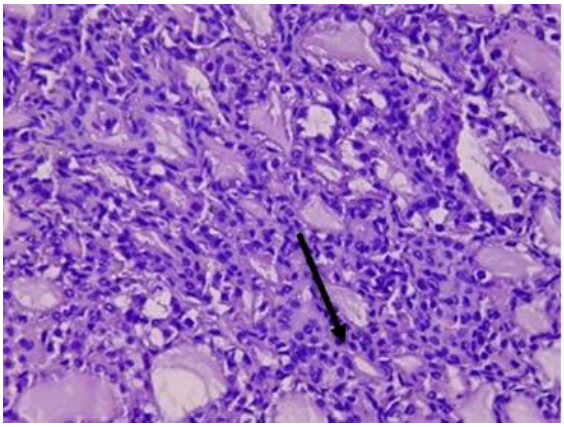

Figure (5): Histological section of the thyroid gland from the group of rosemary treatment after 8 weeks; shows the small size of follicles with a little amount of colloidal material. (H&E stain, 400X).

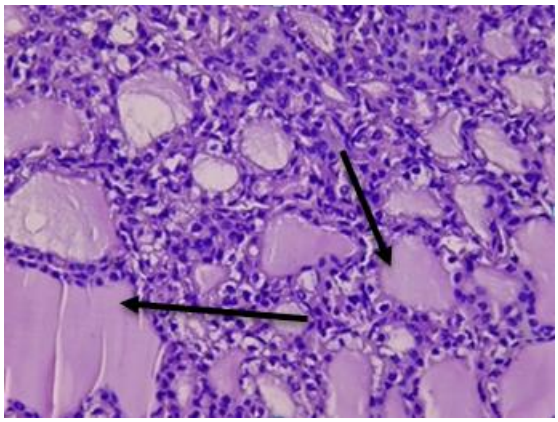

Figure (6): Histological section of the thyroid gland from the group of rosemary treatment after 8 weeks, showing large, dilated, and hyperchromatic nuclei of the lining epithelium, filled with colloid. (H&E stain, 400X).

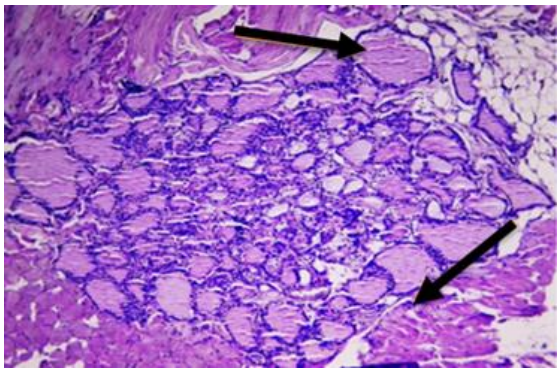

Figure (7): Histological section of the thyroid gland from the group of curcumin treatment after 8 weeks, showing many dilated follicles and small follicles (arrow) filled with colloidal material. (H&E stain, 40X).

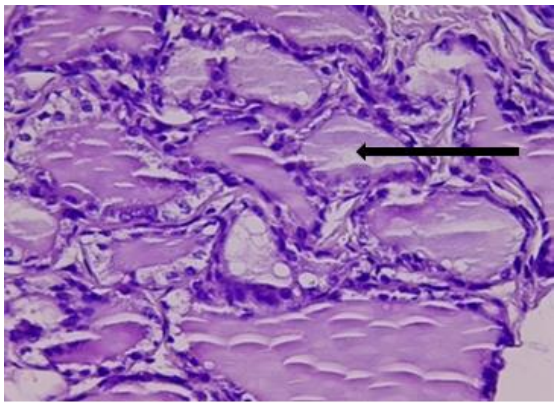

Figure (8): Histological section of the thyroid gland from the group of curcumin treatment after 8 weeks; high magnification from Figure 7 shows thick-irregular colloid-filled follicles lined by degenerated cuboidal epithelium (arrow). (H&E stain, 400X).

DISCUSSION

Docrat *et al*.,(14) suggested that carbimazole plays an important role in treating patients with hyperthyroidism by increasing TSH levels and decreasing T3 and T4 levels to the normal ranges. These results were matched with the conducted study according to the elevation of TSH after 7 weeks of daily administration of a high concentration of carbimazole orally. Sultana and their colleagues showed that carbimazole leads to regulation levels of TSH, thyroid hormones, and anti-oxidants eg; superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GPx) in patients with hypothyroidism and put it with normal values (15). A single dose of carbimazole is considered more efficient in treating hypothyroidism than several doses as mentioned by (16). These results were not compatible with present findings which revealed that different concentrations and various doses of carbimazole are preferred in hypothyroidism therapy. Therefore, in current experiment suggests two doses with different concentrations of long times of carbimazole to increase the efficiency of the drug and return to normal function of the thyroid gland. The current results were compatible with current outcomes suggested by other researchers like (17) who 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 also (18) they revealed that curcumin with different concentrations leads to treating hypothyroidism by decreasing TSH and increasing T3 and T4 levels.

The high concentration of rosemary extract in the present results led to treatment hypothyroidism which decreased levels of TSH and increased T3 and T4 hormones when compared to control negative, these findings were matched with a study of (19), they noted that rats given rosemary have significantly higher serum levels of T3 and T4, which may have been a result of rosemary's antioxidant properties. Abdelaleem and their colleagues described the microscopic changes of hypothyroidism in thyroid tissues as flattened epithelium of follicles with several follicular cell degenerations, cytoplasmic vacuolation, decreased colloid and vascular congestion compared to the control group (20), in the present experiment the lining epithelial of follicles were low cuboidal also appeared degenerative changes from cellular swelling and vacuolation. The present results of histopathology were conducted or explain the physiological results of hypothyroidism on levels of thyroid hormones was TSH high but both T4 and T3 decreased in concentration after treatment with rosemary and curcumin in high dose for 7 weeks kept the levels of T3 and T4 and put within normal range, that was markedly from the returned healthy follicles in thyroid tissue sections when compared to the control group and hypothyroidism rats.

The treatment of hypothyroidism with curcumin showed that curcumin possesses protective effects against toxicity as a non-enzymatic antioxidant (21,22). It acts through mechanisms and has been observed to inhibit or block inflammatory-causing enzymes, such as cyclooxygenase-2 and 5-lipooxygenase. (23), which explains the present microscopic examination and absence of inflammatory process in thyroid glands after 35 days from induction of hypothyroidism. The decreases in indications observed during this investigation were consistent with the results (24). The cells' change from cuboid to squamous height signified a decrease in follicular cell activity. (25).

Rosemary extract can transfer electrons to reactive radicals, changing them into more stable and less reactive forms. This prevents the radicals from attaching themselves to biomolecules in sensitive biological systems, including polyunsaturated fatty acids, lipoproteins, amino acids, DNA, proteins, and carbohydrates. (26), which may explain the mild histological changes of thyroid glands when compared with curcumin in the treatment of hypothyroidism.

CONCLUSION

The induction of hypothyroidism in rats by carbimazole had an injurious effect on follicular cells after four and increased in week eight after oral administration, the treatment with rosemary returns the normal function of thyroid exclusively with high dose that appeared in normal histological morphology of thyroid follicles and their saturation with colloid.

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تأثير مستخلص اوراق اكليل الجبل والكركمين في الجوانب النسيجية والفسيولوجية للجرذان المصابة بقصور الغدة الدرقية

فرح محمود شتيب [1] **لمى قاسم علي** [1] **زينب اسماعيل ابراهيم** [2]

[1] الجامعة المستنصرية ، كلية العلوم ، قسم علوم الحياة
[2] كلية الطب البيطري ، جامعة بغداد

الخلاصة

خلفية عن الموضوع: قصور الغدة الدرقية مرض شائع يتميز بانخفاض مستويات هرمون الغدة الدرقية. كاربيمازول دواء مضاد للغدة الدرقية يُستخدم عادةً لدى مرضى فرط نشاط الغدة الدرقية. يُعد كاربيمازول دواءً مُسبقًا، إذ يتحول بعد الامتصاص إلى الشكل النشط، ميثيمازول، الذي يمنع إنزيم بيروكسيديز الغدة الدرقية من إضافة اليود وربط بقايا التيروزين بالثيروجلوبولين، مما يقلل من إنتاج هرموني الغدة الدرقية **T3** و**T4**. **الهدف من الدراسة:** تهدف هذه الدراسة إلى التعرف على تأثير المستخلصات المائية لأوراق إكليل الجبل والكركمين في تنظيم هرمونات الغدة الدرقية **T3** و **T4**و **TSH** بعد إحداث قصور الغدة الدرقية بواسطة الكاربيمازول في إناث الجرذان. **المواد و طرق العمل:** تم توزيع إجمالي 90 فأرًا بالغًا سليمًا بشكل عشوائي على ثلاث مجموعات رئيسية (30 فأرًا لكل منهما) وفقًا لنوع العلاج والفترات.تم فحص التغيرات الفسيولوجية في الغدة الدرقية لعدة حيوانات عن طريق التحليل المصلي لهرمونات الغدة الدرقية بعد انتهاء المدة (7 اسابيع) للتأكد من تحفيز قصور الغدة الدرقية في الجرذان. ثم عولجت بقية الحيوانات بالمستخلص المائي لأوراق اكليل الجبل والكركمين بتركيزين (200 ملغم/كغم و 150 ملغم/كغم) على التوالي لمدة (8 أسابيع). **النتائج**: أظهرت الدراسة الحالية وجود فروق معنوية **($p<0.05$)** بين تأثير تركيزي الكاربيمازول (2.5 ملغم/كغم و 5 ملغم/كغم) على مستوياتTSH ، **T3**، **T4** عند 7 اسابيع، حيث سجل التركيز العالي للكاربيمازول أعلى مستوياته. التأثير على مستويات **T3** و **T4**و **TSH.** سجلت مستخلصات اكليل الجبل (200 ملغم/كغم) والكركمين (150 ملغم/كغم) أعلى زيادة في مستويات **T3** و **T4**خلال 8 أسابيع مقارنة بالتحكم الإيجابي (قصور الغدة الدرقية)، وانخفاض في مستويات **TSH** في 8 أسابيع مقارنة بقصور الغدة الدرقية.**الاستنتاجات:** يمكن أن نستنتج أن التراكيز المختلفة من المستخلص المائي لإكليل الجبل والكركمين مفيدة في علاج المرضى الذين يعانون من قصور الغدة الدرقية عن طريق تنظيم مستويات **TSH**، **T3**، **T4** التي أجريت مع الفحص المجهري لأنسجة الغدة الدرقية التي كشفت في الغالب عن تغيرات نسيجية معتدلة إلى ملحوظة من تنكس الظهارة مقارنة. مع الأنسجة الطبيعية للغدة الدرقية تراوحت بين الضمور الخلوي إلى التنكس الفراغي ونخر الجريبات والمواد الغروية التي ملأت تجويف البصيلات بعد العلاج بإكليل الجبل والكركمين.

الكلمات المفتاحية: قصور الغدة الدرقية، الكاربيمازول، الكركمين، اكليل الجبل، الجرذان.