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Impact of Alcoholic Cabbage Extract on T3, T4 & TSH Hormones Levels and Histological Architecture of the Thyroid Gland in Adult Male Rats.

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ABSTRACT

The present study sought to investigate the effects of alcoholic white cabbage extract on the thyroid gland by analyzing its morphological and functional status in experimental rats. The adult rats at the age of three months were obtained from the Animal House of the College of Veterinary Medicine, Tikrit University in one month (9/9/2024 to 2024/10/9). Twelve mature male albino rats (weighing between 250 and 300 g) were randomly assigned to three equal groups. Each group included four rats. The first group (G1) served as the control group, the second group (G2) was given cabbage extract at a dose of (100 mg per kg of body weight orally) three days a week for a month, and the third group (G3) received the same dose daily orally for a month. The study found no significant increase ($p \leq 0.05$) in serum TSH concentrations in group (G2). However, group (G3) rats showed a significant increase compared to the control group (G1). Blood concentrations of T3 and T4 significantly decreased (at $p \leq 0.05$) in groups (G2) and (G3) compared to the (G1) group. Histological data for the groups (G2 and G3) show a detectable alteration. Follicle size revealed aberrant, non-homogeneous colloid with a lack of vacuoles, vacuolar degeneration in follicular cells, loss of follicular cells in certain follicles, and follicles of various sizes packed with colloid and enclosed by columnar to flat epithelium. Group (G3) had enlarged or dilated follicles, no peripheral vacuoles, scalloped colloid in some follicles with flattened epithelium, degraded follicular epithelial cells, and bleeding. According to the study, cabbage extract administration causes significant histological changes in albino rats' thyroid glands, such as vacuolar degeneration, collagen fiber deposition, sloughing of follicular cells, scalloping of colloid, flat epithelium, and reduced serum concentrations of T3 and T4, potentially leading to an increase in TSH levels in the blood.

Introduction

The thyroid gland is an endocrine organ that produces and secretes thyroid hormones [1]. The thyroid gland has two kinds of cells: follicular cells and para follicular cells. The follicular cells produce thyroid hormones and surround a region known as the colloid, which stores thyroglobulin, a glycoprotein that includes the precursors T3 and T4. The hypothalamus, pituitary gland, and target gland (the thyroid) form a feedback loop that controls the thyroid gland. The HPT axis describes the interaction between the hypothalamus, pituitary gland, and thyroid gland [2]. The hypothalamus produces thyrotropin-releasing hormone (TRH), a tripeptide secreted into the venous system and directed to the pituitary gland. TRH binds to receptors on thyrotroph cells in the pituitary gland, causing thyroid stimulating hormone (TSH), or thyrotropin, to be produced and secreted [3].

As shown in the diagram (1-1)

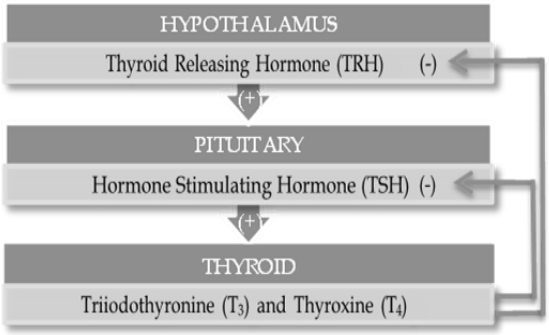

Diagram (1-1): Show effect of hormones on Thyroid gland [3].

Cabbage, a biennial plant indigenous to the Mediterranean and Europe, is recognized for its medicinal benefits. It is a member of the Brassicaceae family, and the cabbage plant, Brassica oleracea, is abundant in active chemicals that are advantageous for human health. These substances are categorized into simple phenols, polyphenols, flavonoids, phenolic acids, hydroxycinnamic acids, carotenoids, and sulfur-containing compounds known as glucosinolates [4]. The glucosinolates present in cabbage are converted to thiocyanate in the digestive system by hydrolysis. This drug inhibits iodine transport in the gastrointestinal tract, resulting in a reduction of thyroid hormone levels while concurrently elevating TSH concentration [5]. Goitrogens cause goitre, and their name comes from the phrase 'goitre,' which refers to the enlargement of the thyroid. Goitrogens reduce the thyroid gland's ability to generate hormones, causing further thyroid illnesses such as hypothyroidism and cretinism in both humans and animals. Isolation and identification of 1-5-vinyl-2-thiooxazolidine, a goitrogen found in various Cruciferae diets [6].

Material and Methods:

Collect plant samples:

White cabbage was collected from the Local market in the Kirkuk city and it was protected well from dirt and dust. It was cut into small pieces. The dried plant was kept into a tightly closed box in conditions free of moisture until use in the extraction process.

preparation of alcoholic extract of cabbage:

The cabbage leaves were desiccated at ambient temperature for 10 days. The desiccated leaves were pulverized using a grinder. The extract was made with a saxolite apparatus by using 50 g of powder addition of 500 ml of 95% ethanol alcohol. The procedure was executed eight successive times. The extraction solution was collected and subjected to a rotary evaporator to remove the alcohol from the extract and enhance its concentration. The extract was then transferred to a freeze-drying apparatus (lupholyzer) to desiccate the solution and procure the extract. It was preserved in glass containers until used [7].

Laboratory animals and Experimental design:

The adult rats at the age of 3 months were acquired from the Animal House of the College of Veterinary Medicine. The research was performed at the animal facility. Faculty of Veterinary Medicine, Tikrit University in one month (9/9/ 2024 to 2024/10/9) It was taken care of in ideal laboratory conditions then subjected to the experimentation and implementation of work. Twelve adult male albino rat weighing (250-300g) were used in this study. They were maintained on a conventional pellet diet. Food and drink were provided ad libitum. All animals were euthanized at the conclusion of the research. Rats were categorized into three groups randomly, each consisting of four rats. Animals from each group were housed in distinct sanitary cages and maintained in a clean, well-ventilated room in accordance with the directives of the Animal Ethics Committee 9 [8].

Group (G1): The control group included four rats. They were administered 0.5 ml of saline solution orally once daily for a duration of one month.

Group (G2): The four rats in this place group were executed vegetable extract verbally at a portion of drug or other consumable of 100 mg/kg, three opportunities per temporal length of event or entity's existence. The extract was dissolve in distilled water, as assign to source in study. For a event of individual temporal length of event or entity's existence, the portion of drug or other consumable was executed verbally utilizing a able to be thrown away syringe.

Group (G3): This group had four rats that were administered cabbage extract daily for one month at the same dosage as in Group (G2). [9].

Collection of blood specimens:

Five milliliters (ml) of blood were collected from the animals' hearts via a heart stab using a medical syringe. Each blood sample was placed in a tube containing a gel-free anticoagulant and centrifuged at 3000 rpm for 15 minutes to produce a sufficient amount of serum free of red blood cells. The blood was then collected and transferred to a designated plastic tube, which was kept sterile and stored at -20 °C until utilized for thyroid function testing: T3, T4, and TSH [10].

Anesthesia and Dissection:

At the conclusion of the research period, the animal was killed by inhalation of an overdose of chloroform Aguwa et al., [11]. The thyroid gland was accessed through a skin incision in the midline on the ventral side of the neck, and the thyroid gland was removed along with the larynx and part of the trachea [12].

Hormonal Assay: Assessment of Serum Thyroid Hormones:

The quantitative analysis of rat T3 hormone was conducted using the rat triiodothyronine hormone, T3 ELISA Kit Catalogue No: ER 1720. Revision: V4.0, Size: 48T/96T, Company: Elabscience, USA.

The primary assay of T3 utilizes the Competitive-ELISA detection technique. The micro titer plate included in this kit has been pre-coated with an antibody specific to T3 [13].

Total Thyroxin (T4) Enzyme Immunoassay:

The quantitative analysis of rat T4 hormone was conducted using the rat thyroxine hormone, T4 ELISA Kit Catalogue No: ER 1721. Revision: V4.0, Size: 48T/96, Company: Elabscience, USA. The principal assay for T4 utilizes a competitive ELISA detection technique. An antibody that is specific to T4 has been pre-coated on the micro titer plate that is included in this kit [14].

Thyroid-Stimulating Hormone (TSH) Assay:

The test for Thyroid Stimulating Hormone is predicated on the premise of a solid phase enzyme-linked immunosorbent assay (ELISA). The quantitative measurement of rat thyroid stimulating hormone was conducted using a rat TSH ELISA Kit.
Catalog Number: E-EL-R0976. Product dimensions:
6T/48T/24T/96T*5, Company: Elab Science, USA The primary assay of TSH utilizes the Sandwich-ELISA detection technique. Additionally, this kit contains with a micro-ELISA plate that has already been pre-coated with an antibody that is specific to rat TSH [15].

Histomorphological analysis:

The thyroid tissues were fixed in neutral buffered 10% formalin and dried using a graded alcohol series (50-100%), cleaned with xylene, infiltrated, and embedded in paraffin wax. The paraffin blocks were sectioned at 4-6µ thickness and stained with Hematoxylin and Eosin (H&E) for histological investigation [16].

Results and Discussions:

The thyroid profile test findings after administration of cabbage extract, as shown in Table-1, indicate an elevation in TSH levels, although T3 and T4 levels were reduced in comparison to the control group (G1). Our study showed that the TSH level increased in group (G2) and (G3) if compared with control group (G1), anyway, control group showed no significance with group (G2) but the analysis showed significant difference with group (G3) as well as there was a significant difference between group (G2) and group (G3) at $p \leq 0.05$. Concerning T4 and T3, the results showed decrease level of the two hormones compared with control group with significant difference among all groups at $p \leq 0.05$ as shown in Table1 and Figure 1

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Table 1: Effect Alcoholic Cabbage Extract on T3, T4 & TSH Hormones Levels			
Hormones / Groups	Control (G1)	(G2)	(G3)
(T3)	13.46±0.25 a	14.75±0.7 a	21.67±0.6 b
T4	10.38±0.77 a	8.20±0.44 b	6.14±0.07 c
(TSH)	2.30±0.11 a	2.08±0.05 b	1.53±0.04 c
Similar letters in rows means absence of significant difference (P<0.05) among groups, whereas different letters in rows means significant difference (P<0.05)			

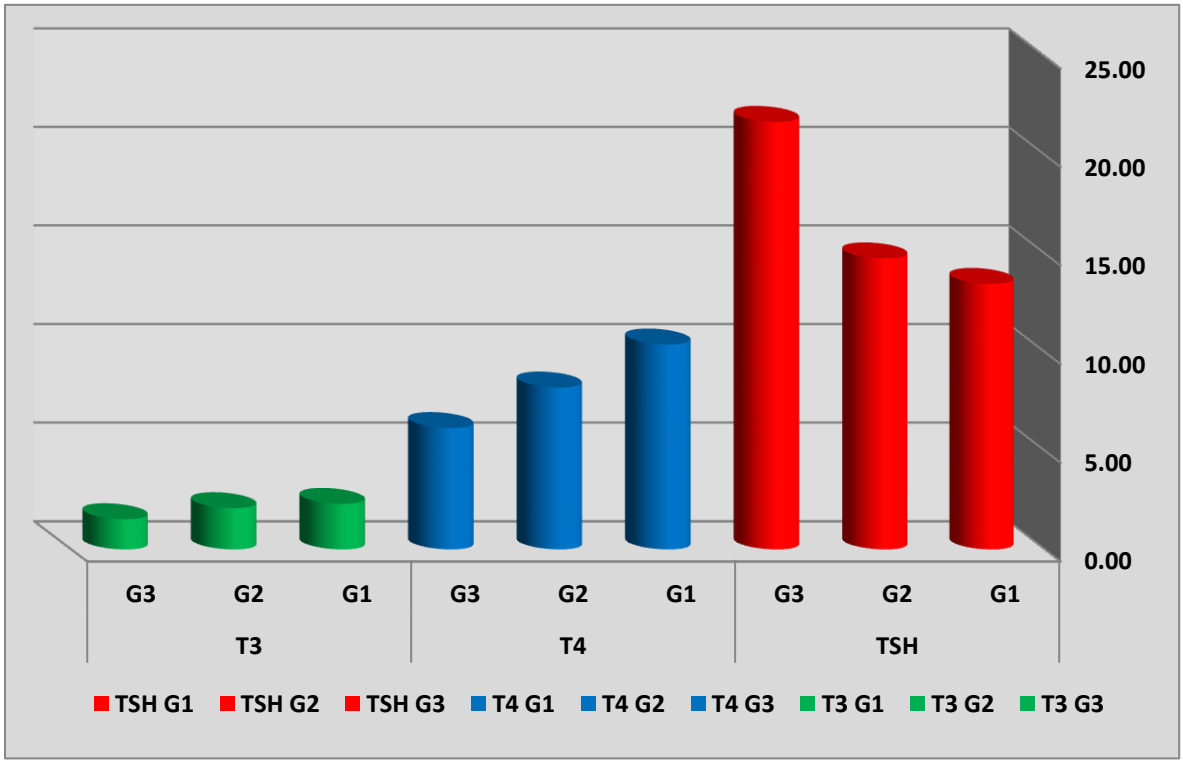

Figure 1: Effect Alcoholic Cabbage Extract on T3, T4 & TSH Hormones Levels

In group G1, thyroid sections exhibited follicles of varying sizes, with their cavities containing acidophilic colloid and peripheral vacuolations. The distribution of follicles is observed in two distinct zones: the outer zone (indicated by the blue line) predominantly contains large and medium-sized follicles, while the inner zone (represented by the black line) is characterized by a higher concentration of small follicles. Thyroid follicles were lined by cuboidal follicular cells situated on a thin basement membrane, with minute blood capillaries extending between the follicles. The follicular epithelium displayed cuboidal cells. Each follicle was encased by a basement membrane and delineated from adjacent follicles by a delicate network of interfollicular connective tissue, abundant in fine collagen fibers and sinusoids. Parafollicular cells, also known as C cells, are located among and between the follicular cells. The cells were larger and exhibited a paler cytoplasm compared to the follicular cells, occurring both singly and in groups, as illustrated in Figures 1 and 2.

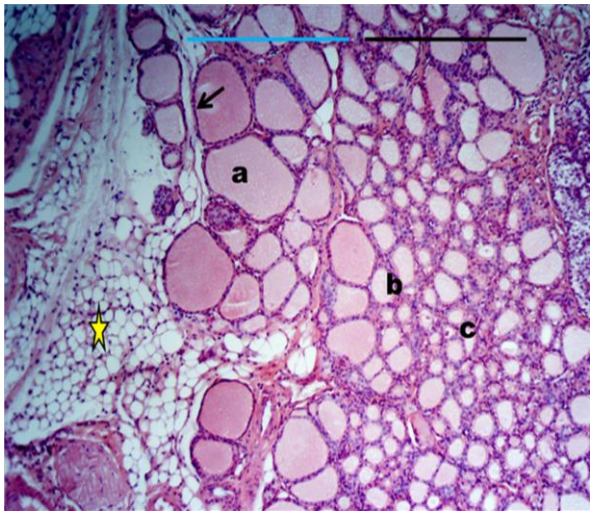

Figure 1: photomicrograph of thyroid gland of group (G1) shows: large follicle (a), moderate size follicle (b), small size follicle (c), cuboidal

follicular cells (black arrow), adipose tissue (asterisk). 10x, H&E stain. Note the distribution of follicles in two zones: the outer zone (blue line) contains most of the large and medium size follicles whereas the inner zone (black line) contains most of the small follicles.

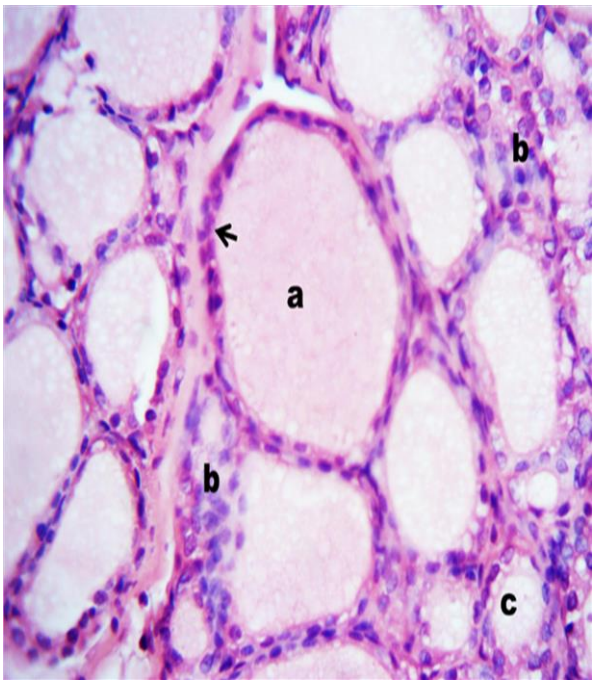

Figure 2: Photomicrograph of the thyroid gland of the (G1) group reveals: cuboidal follicular cells (black arrow), para follicular cells (b), small size follicle (c), and big follicle loaded with colloid with peripheral vacuolation of colloid (a). H&E stain, 40x.

The histology findings of Group (G2) in adult rats exhibited alterations when compared to the Group (G1). These alterations indicate a noticeable enlargement of follicle size, characterized by aberrant colloid that is non-homogeneous and devoid of vacuoles. The colloid inside these thyroid follicles had a foamy appearance and considerably occupied the follicles of rats in group (G2)., vacuolar degeneration in follicular cells, deposition of collagen fibers, sloughing of follicular cells in some follicles, and congested blood vessels. This interlobular connective tissue has many follicles of varying diameters. It was saturated with colloid. The larger follicles located at the periphery were filled with colloid and encased by columnar to flat epithelium. The gland had parafollicular cells (C cells) as a minor population among the follicles, which featured larger follicles located peripherally, along with the follicular cells identified in this group, as shown in figures 3, 4 and 5.

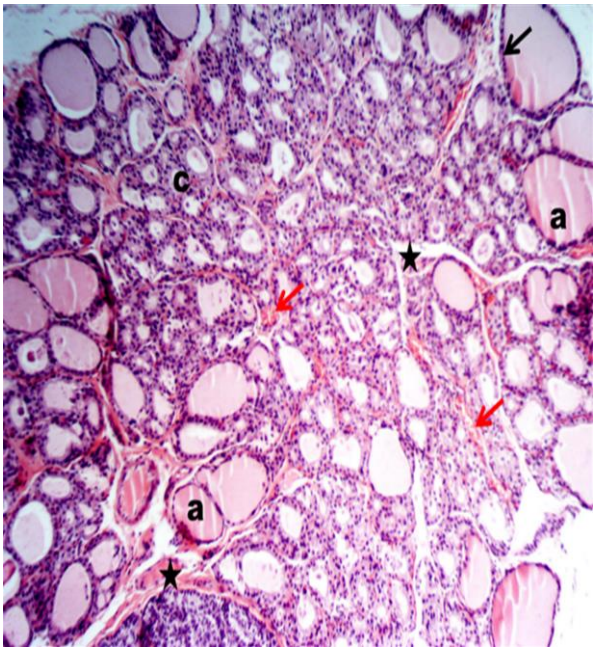

Figure 3: photomicrograph of thyroid gland of group – (G2)- show: numerous follicles contain abnormal colloid non-homogenous with absence of vacuoles (a) flattened follicular cells (black arrow), blood vessels (red arrows), increase the deposition of collagen fibers (asterisk). 10x, H&E stain.

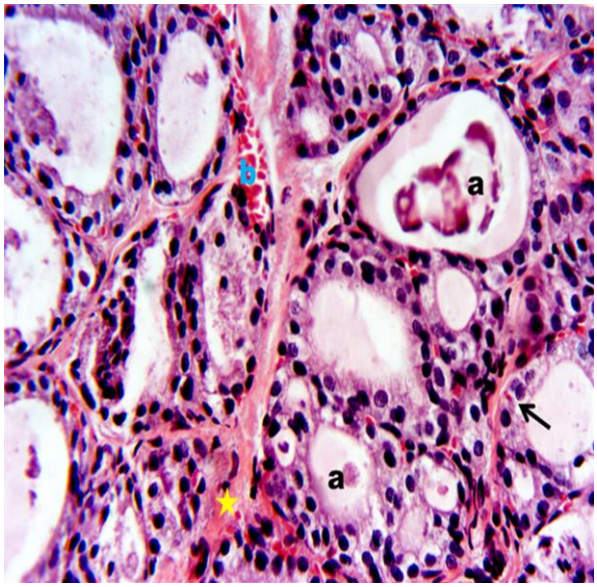

Figure 4: photomicrograph of thyroid gland of group – (G2) show –: numerous follicles contain abnormal colloid non-homogenous with absence of vacuoles (a), vacuolar degeneration in follicular cells (black arrow), congested blood vessels (b), increase the deposition of collagen fibers (asterisk). 40x, H&E stain.

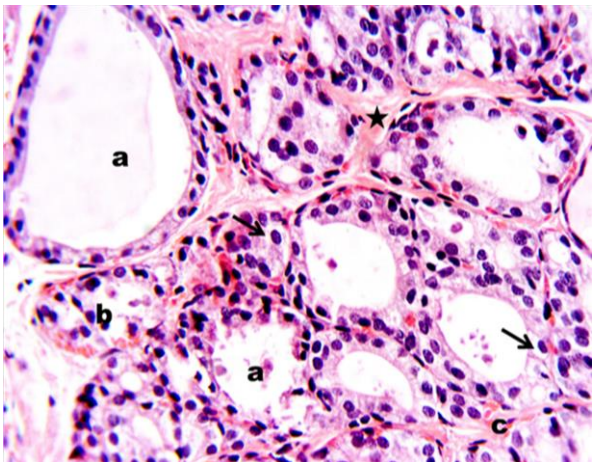

Figure 5: photomicrograph of thyroid gland of group – (G2)- show: numerous follicles contain abnormal colloid non-homogenous with absence of vacuoles (a), slffing of follicular cells in some follicles (b), vacuolar degeneration in follicular cells (black arrow), congested blood vessels (c), increase the deposition of collagen fiber (asterisk) s. 40x, H&E stain.

Thyroid sections in group (G3) exhibited enlarged or dilated follicles, with significant variability in follicular morphology. Numerous follicles were filled with dense colloid, lacking peripheral vacuoles, and some displayed scalloping of colloid accompanied by flattened epithelium. Additionally, certain follicles contained exfoliated cells within their lumens, scalloping of colloid in some follicles. Observations included the presence of dilated, congested blood vessels and atrophy of thyroid follicles, as seen in figures 6 and 7

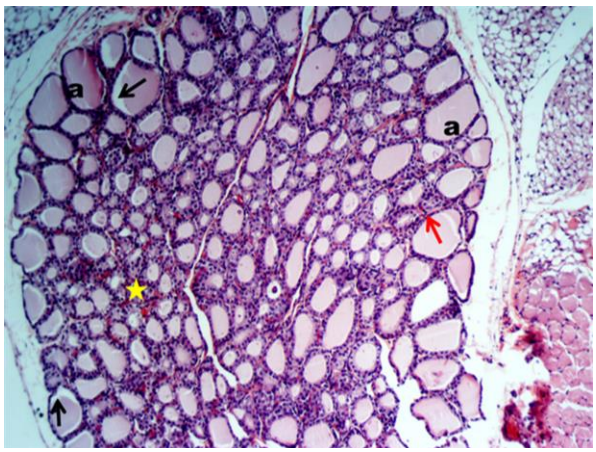

Figure 6: photomicrograph of thyroid gland of group (G2)- show: Note that most of follicles are large and moderate size and few small follicles numerous follicles filled with colloid with absence of vacuoles in the periphery (a) and scalloping of colloid in some follicles (black arrows) flattened follicular cells (red arrow), congested BV (asterisk). 10x, H&E stain.

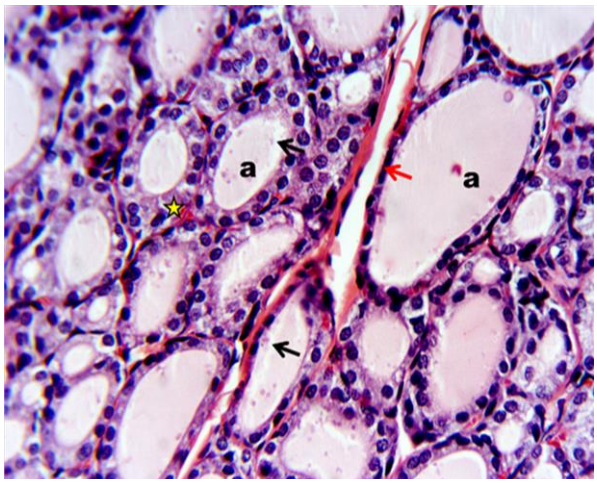

Figure 7: photomicrograph: of thyroid gland of group – (G3) - show: Note that most of follicles are large and moderate size and few small follicles numerous follicles filled with colloid with absence of vacuoles in the periphery (a) and scalloping of colloid in some follicles (black arrows), flattened follicular cells (red arrow), congested BV (asterisk). 40x, H&E stain.

The statistical analysis of the effects of cabbage extract on thyroid hormones in the current research (Table 1) indicates that the administration of the alcoholic extract of cabbage led to a significant reduction in the levels of hormones T3 and T4 in groups G2 and G3 compared to the control group G1. The cabbage extract significantly reduced T3 levels in groups G2 (2.09) and G3 (1.53), and T4 levels in groups G2 (8.2) and G3 (6.15) compared to group G1 (2.31, 10.38), with $P<0.05$ indicating statistical significance. The findings align with prior research [17, 18, 19]. The reduction in thyroid hormones T3 and T4 is attributed to the inhibition of thyroperoxidase (TPO). The mean TSH levels increased to 14.75 in group (G2) and 21.68 in group (G3), in contrast to the level of 13.46 in group (G1), supporting the researchers' findings [20,21]. Cabbage and other cruciferous vegetables, such as broccoli, Brussels sprouts, and cauliflower, contain goitrogenic compounds, notably glucosinolates. These chemicals can interfere with thyroid function by inhibiting iodine absorption, a critical component in the synthesis of thyroid hormones. Prior studies in both animal and human subjects have clearly demonstrated that the thyroid gland responds to cabbage and other cruciferous vegetables, cabbage extract has been demonstrated to restrict iodide absorption, subsequently affecting thyroid hormone production [22]. In alignment with the histological findings, the treated rats receiving cabbage extract demonstrated a reduction in thyroid hormones T3

and T4, alongside follicular hypertrophy and vacuolar degeneration of follicular cells. The findings suggested that the thyroid gland was impaired and may have exhibited reduced activity in its production. This is similar to the findings presented in [23]. The occurrence of atypical thyroid hormone patterns accompanied by hyperplasia in rats fed a millet-based diet was noted by researchers. In male white rats, the elevated TSH output resulted in abnormal thyroid growth and goiter formation [28].

Histological Discussion:

The present histological observations validate that the thyroid lobules in both the right and left lobes of Albino Rats include clusters of thyroid follicles of varying sizes (big, medium, and tiny). The exterior lobular region often contains several medium-sized follicles, while the internal region mostly consists of smaller follicles. These finding is in agreement with other researchers [24] that reviewed the findings of 123 papers including in vitro, animal, and human studies, including adult female rats, all of which pertain to the well-known heterogeneous follicular study of the thyroid gland in animals. In the histological sections, the majority of the thyroid follicles had a high size, while medium and small sizes were also seen (Figure 2). The results concurred with the findings of [22], which demonstrated that the follicles had a highly varied diameter.

Our studies revealed that the lumen of the thyroid follicles was entirely occupied with a clear pink homogeneous substance (colloid) (Figures 1, 2). As per [4]. The follicular colloid contains thyroglobulin, an iodinated glycoprotein that serves as the storage form of thyroxin, which is why it was stained red in these preparations.

The histopathological findings of groups (G2) and (G3) in adult rats exhibited alterations when compared to the control group (G1). These alterations, however, were characterized by many follicles filled with colloid, devoid of vacuoles at the periphery. The epithelial cells in groups (G2) and (G3) got flattened compared to group (G1). Figures 4, 6, and 7. Concerning these data, which were reached in the groups that were administered the extract [25] indicated that the inactive thyroid epithelial cells ranged from low columnar to cuboidal morphology. When the cell becomes active (during the active phase), the epithelial cells transform into tall columnar cells.

Our results indicated that the colloid inside the thyroid follicles had a frothy appearance and considerably occupied the follicles of the adult rat in group (G2). In group (G3), the majority of the thyroid follicles were filled with colloid, with some exhibiting peripheral uneven borders, resulting in a scalloped appearance (Figures 6, 7). The occurrences were elucidated by [26], who indicated that the colloid was prevalent during periods of gland inactivity. When the gland became active, the borders of the colloid became scalloped, generating many tiny reabsorption lacunae.

In this research, the group treated with cabbage extract follicular lumen had desquamated follicular cells. Consequently, several researches [27]. indicated that the deteriorated follicular epithelial cells are prone to detachment. These alterations may be ascribed to cellular distension due to accumulating colloid, leading to cellular disruption [23].

Conclusion:

The current study concludes that the administration of cabbage extract results in significant histological in the thyroid gland of albino rats, characterized by vacuolar degeneration in follicular cells, collagen fiber deposition, sloughing of follicular cells in certain follicles, scalloping of colloid in some follicles accompanied by flat epithelium, and a reduction in serum concentrations of T3 and T4 compared to the control group. can elevate blood levels of TSH.

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تأثير مستخلص اللهانة الكحولي على مستويات هرمونات الغده الدرقية والهرمون المحفز للغده الدرقية والبنية النسيجية الغدية في ذكور الجرذان البالغة

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الملخص

يهدف البحث الحالي دراسة تأثيرات مستخلص اللهانة البيضاء على الغدة الدرقية من خلال تقييم حالتها المورفولوجيا والوظيفية في الجرذان التجريبية. تم توزيع اثني عشر من ذكور الجرذان البالغة بعمر ثلاثة أشهر عشوائيا وكانت اوزانها تتراوح بين (غم) على ثلاث مجاميع متساوية تتكون كل مجموعة من اربعة جرذان. تم اعتبار المجموعة (G1) مجموعة السيطرة ومجموعة (G2) تجرع مستخلص اللهانة عن طريق الفم بجرعة (100ملغم / كغم) من وزن الجسم لمدة ثلاث ايام في الاسبوع واستمرت الجرعة لمدة شهر. بينما تلقت المجموعة الثالثة (G3) نفس الجرعة يوميا واستمرت الجرعة مدة شهر.. اشارت نتائج البحث الى عدم وجود ارتفاع معنوي في تركيز (TSH) عند ($p\leq0.05$) في مصل مجموعة (G2) ومع ذلك اظهرت المجموعة (G3) ارتفاع معنوي مقارنة بمجموعة السيطرةG1. بالإضافة الى ذلك كان هناك انخفاض معنوي في تركيزات T3 وT4 في المصل عند ($p\leq0.05$) مقارنة بمجموعة السيطرة. فضلا عن ذلك تشير النتائج النسيجية للمجموعتين (G2,G3) الى تغير ملحوظ حيث اظهر المجموعتين احتواء الجريبات على مادة غروانية غير متجانسة مع نقص في الحويصلات الغروانية، وانحلال في فجوات الخلايا الجريبية وتساقط الخلايا الجريبية في بعض الجريبات وتميزت الخلايا الجريبية بظهارة عمودية الى مسطحة. بالإضافة الى ذلك اظهرت المجموعة (G3) جريبات متضخمة او متوسعة وتقشر الغوراني في بعض الجريبات مع وجود نزف في الاوعية الدموية. استنتج من الدراسة الحالية ان اعطاء مستخلص اللهانة البيضاء يودي الى تغيرات نسيجية في الغدة الدرقية لدى الفئران البيضاء تتميز بالتنكس الفجوي للخلايا الجريبية وترسب الياف الكولاجين وتقشر الغوراني في بعض الجريبات مصحوبة بضهارة مسطحة وانخفاض في تركيز هرمونات الغده الدرقية وارتفاع مستوى الهرمون المحفز للغدة الدرقية.

الكلمات المفتاحية: مستخلص الكحولي للهانة البيضاء، هرمونات الغدة الدرقية، بنية الغدة الدرقية، ذكور الجرذان البيضاء.