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Histological and endocrine modulatory effects of white cabbage (*Brassica oleracea* var. *capitata*) extract on the thyroid gland in rat

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Abstract

White cabbage (*Brassica oleracea* var *capitata*) is being widely studied for its use in animal feed because it's easy to find and affordable while also being nutritious. However, the presence of glucosinolates in cabbage is a concern as they breakdown into thiocyanates suggestive of possible negative impacts, on the thyroid function of animals when they lack sufficient iodine. This study investigates the effects of ethanolic extract of white cabbage on thyroid gland histology, thyroid hormone profile, and TSH in adult male rats. Twenty-one adult male albino rats were randomly divided into three equal groups (n=7) over a four-months period. Group of control, G2 received 100 mg/kg of cabbage extract three times weekly, and G3 received the same dose daily. T3, T4, and TSH levels were measured using ELISA. Thyroid tissue sections were stained with H&E, PAS, and Masson's trichrome to assess morphology by measuring follicular diameter, thyrocyte height, and epithelial cell number per follicle. The current study concludes that persistent consumption of white cabbage (*Brassica oleracea var. capitata*) extract produces noticeable modifications thyroid morphology and operation.

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Introduction

White cabbage is a cruciferous vegetable that is widely accessible and valued for its nutritional benefits and affordability. Qualities that make it a desirable choice for animal feed in areas with scarce feed supplies. This vegetable is packed with nutrients and phytochemicals including vitamins and fibers that can enhance the well-being and productivity of animals when incorporated into their diets, in moderation. One of its additional elements is glucosinolates that transform into thiocyanates during digestion and are recognized for their goitrogenic properties (1). One research indicates that these substances have the ability to block iodine absorption in the thyroid gland; consequently, affecting the production of thyroid hormones known as T3 and T4 (2). This disruption can result in both tissues related alterations within the thyroid gland. Particularly evident with prolonged consumption or, in situations where dietary iodine levels are insufficient (2). It is important to assess the impact of cabbage as animal feed on animal's health in the long term to guarantee its safety for their well-being and performance levels by monitoring its effects, on the thyroids endocrine function that directly affects growth rate and metabolism. Studies have shown that the presence of glucosinolates in Brassica vegetables can have an impact on thyroid function in animals. The research conducted by Felker *et al.* (3) illustrated how the breakdown of glucosinolates leads to the production of thiocyanates which can inhibit sodium iodide symporter (NIS) a protein for iodine absorption in thyroid cells. This inhibition may result in synthesis of thyroid hormones and consequently contribute to thyroid irregularities. Similar conclusions have been noted by Leung and others in their research work found that Brassica vegetables could have effects on animals if their iodine intake is inadequate (4). Recent research has also brought attention to the significance of controlling the intake of Brassica vegetables like cabbage in animals' diets. For example, a study by Duntas and Doumas (5) highlighted the

impact of prolonged exposure to goitrogens in food on thyroid structure and activity especially, in animals lacking sufficient iodine levels. Additionally, the study conducted by Zhang *et al.* (6) study highlighted the impact of thyroid stimulation caused by low levels of thyroid hormones on thyroid enlargement and the buildup of inactive colloid, in thyroid follicles that can worsen gland damage. Through its release of triiodothyronine (T3) and thyroxine (T4) molecules the thyroid gland performs its essential role of regulating growth metabolism and thermoregulation (7). Under the control of the hypothalamic-pituitary-thyroid axis the hormones function through negative feedback mechanisms to preserve hormonal homeostasis (8). The gland disorder hypothyroidism or hyperthyroidism creates various clinical consequences extending from metabolic difficulties to heart-related effects (9). The current research atmosphere focuses on dietary components that affect thyroid health by investigating goitrogenic compounds which appear in cruciferous vegetables including white cabbage (*Brassica oleracea var. capitata*) (10). White cabbage contains glucosinolates as sulfur-containing compounds that produce isothiocyanates and nitriles as well as thiocyanates through hydrolysis according to Rafiee *et al.* (11). The thiocyanate compound specifically blocks iodide transport into the thyroid gland thus causing a decrease in thyroid hormone production (12,13). As a result, thyroid-stimulating hormone (TSH) levels rise because of hormone production compensation (14). Chronic Brassica vegetable consumption has been shown through laboratory tests on animals to produce four distinct morphological changes affecting the thyroid glands such as follicular hyperplasia with colloid depletion and distorted follicular patterns (15,16). Thyroid enlargement along with hypertrophy occurs in iodine-deficient individuals to whom goitrogens cause these effects (17). Wistar rats in a scientific research setting showed that cabbage extract consumption led to reduced T3 and T4 serum concentrations together with histopathological results like shrunken follicular cells and enlarged follicles according to Aldubayan *et al.* (18). Experimental results from rodent models indicated that rutabaga along with turnip extracts could lead to thyroid suppression patterns similar to another one (19,20). Research by Rana *et al.* (21), established that the thyroid-suppressing properties of cabbage include both hormone blockade and the modification of thyroid tissue inflammatory responses. The thyroid hormone receptor process and peripheral metabolic control are influenced by metabolites derived from cabbage polyphenols alongside flavonoids and secondary compounds (22). Numerous studies demonstrate that *Brassica* vegetables exert minimal thyroid-related effects on iodine-replete subjects yet questions continue about their long-term intake patterns by individuals with restricted iodine intake (23). The biological activity together with glucosinolate content changes when a vegetable is prepared raw instead of cooked such as the findings in Tajiri *et al.* (24).

Therefore, while white cabbage offers nutritional benefits and is a cost-effective feed option, its goitrogenic properties require careful consideration, particularly in terms of iodine levels in the diet, to prevent thyroid dysfunction and ensure the overall health of livestock. The goal of our research is to study of white cabbage *extract (Brassica oleracea* var. *capitata ethanolic)* effects on thyroid gland tissue and levels of T3, T4 and TSH hormones in the adult male albino rat by ELIZA with thyroid tissue morphological measurement to evaluate comprehensive endocrine and structural results.

Materials and methods

Ethical Approval

The Animal Ethics Committee of the College of Veterinary Medicine at University of Tikrit approved all animal experimental procedures following international laboratory animal care guidelines (approval date: September 16, 2024). (Approval no. Tu.Vet. 34).

Plant material and extract preparation

White cabbage (*Brassica oleracea var. capitata*) obtained from Kirkuk's local market proved to be the source of this investigation. A grinder pulverized dried cabbage leaves which went through a cleaning process and followed ten days of air-drying at standard room temperature. Soxhlet method applied for the extraction process. A prepared Soxhlet thimble containing 50 g of dried cabbage powder was exposed to 500 mL of 95% ethanol through eight successive extraction cycles. A rotary evaporator reduced pressure to 40°C as the extract was concentrated and the ethanol was removed through the filtration process. A lyophilize produced freeze-dried semisolid matter from the residue which underwent storage at 4°C in glass vials until it received administration (25).

Experimental animals

A total twenty-one adult albino male rats from University of Tikrit College of Veterinary Medicine Animal House were used throughout the study. The animals received standard rodent food with water accessibility while being maintained inside polypropylene cages within a controlled environment at 22±2°C, 60-70% humidity with 12/12 hours light/dark cycle. The protocol received clearance from the Animal Ethics Committee.

Experimental design

The research involved three separate groups (n=7 each) through random assignment. Group G1 (Control): Received 0.5 mL of normal saline orally every day up to four months. Group G2 (Intermittent Cabbage Extract): Received 100 mg/kg of white cabbage ethanolic extract orally (26). three times per week for four months. Group G3 (Daily Cabbage Extract): Received same dosage as in Group (G2) of white

cabbage ethanolic extract daily for four months. A sterile disposable feeding needle delivered all oral administration of extracts through the gavage process.

Sample collection

Purpose of anesthesia at the experimental endpoint. The vascular procedure for blood extraction involved direct heart puncture through which obtained blood into a 5 mL syringe, by sterile syringe then subject to 3000 RPM centrifugation for fifteen minutes, while placed in plain sample tubes to extract the serum fraction. Serum samples storage at -20 C for hormonal analysis (27).

Hormonal assay

Thyroid hormones existing in blood serum were detected by ELISA test systems provided by Elabscience USA. T3 (Triiodothyronine): Cat No. E-EL-ER1720, Competitive ELISA, T4 (Thyroxine): Cat No. E-EL-ER1721, Competitive ELISA (28). TSH (Thyroid Stimulating Hormone): Cat No. E-EL-R0976, Sandwich ELISA (29). Every procedure followed the defined protocols provided by the manufacturer. The analysis of absorbance took place at a wavelength of 450 nm using an ELISA reader.

Histological examination of thyroid tissue

After obtain the thyroid tissue, put sample in neutral buffered formalin 10% for 72 hours at least to sample then dehydrated by alcohol clearing by xylene, impregnated and embedded in paraffin wax, sectioned at 6 microns thickness. T stained with routine hematoxylin and eosin stain to demonstrated lesions in sectioned tissues in addition to Periodic Acid-Schiff (PAS) for colloid and Masson trichrome stain to detect the presence of and collagen fibers (30). The histological section was examined under the light microscope and photographed using digital camera.

Histomorphometric analysis

The examination of tissue samples under a microscope occurred while recording measurements through Motic Image plus 2.0 software (31). The following variables measured: Follicular diameter (µm), Follicular epithelial (thyrocyte) height (µm) and Number of epithelial cells per follicle. Three randomly selected fields of each section provided measurements which became the basis for computation of average values. we defined small medium and large follicles through specified diameter boundaries.

Statistical analysis

The study displayed data results as standard deviation (SD) along with their mean values. The data analysis occurred through IBM SPSS Statistics version 25 (UK). The research used One-way ANOVA to detect possible variations between the different groups. Duncan's post hoc test became the method for identifying significant variations between groups after initial significance was detected through analysis of variance (ANOVA). The analysis used a p-value threshold of less than 0.05.

Results

The experimental data demonstrated that white cabbage extract in table 1. caused statistically important changes to thyroid hormone levels throughout all treatment groups. The control group (G1) maintained typical T3 measured at 2.31±0.11 while T4 recorded 10.38±0.77 and TSH registered at 13.46±0.29. Members of Group G2 who consumed extract supplementation intermittently experienced a substantial decrease in T3 to 1.82±0.06 and T4 dropped to 6.4±0.58 levels and TSH surged to 21.46±0.31. Trophic hormone levels reached their peak at 23.15±0.12 in Group G3 as this group received daily extract administration while both T3 fell to 1.22±0.03 and T4 declined to 5.64±0.19. The diminished T3 and T4 levels indicate suppressed thyroid hormone production probably because glucosinolate compounds interfere with thyroid iodine intake and hormone creation. The pituitary gland showed a compensatory increase in TSH secretion because it tries to stimulate an underactive thyroid. The treatment caused a decrease in levels of T3 and T4 which depended on the given dosage in both animal groups. The changes in hormone levels verify that cabbage compounds produce negative effects on thyroid activity.

Table 1: Effect of white cabbage extract on thyroid hormone

	T3	T4	TSH
G1	2.31±0.11	10.38±0.77	13.46±0.29
G2	1.82±0.06 *	6.4±0.58 *	21.46±0.31 *
G3	1.22±0.03 *a	5.640.19 *a	23.15±0.12 *a
P value	P<0.05	P<0.05	P<0.05

Note: Values are expressed mean ±standard deviation, * means there was significant with control group of significant difference (P<0.05), whereas different letters in rows means significant difference (P<0.05) within experimented groups.

White cabbage extract treatment affected thyroid follicle diameters in different experimental conditions according to the data presented in the table 2. The control group (G1) showed three distinct follicular diameters which included 19.26 µm for small follicles while medium-sized follicles measured 37.9 µm and large follicles reached 103.16 µm thus indicating typical thyroid follicular structure. The subjects in Group G2 who received intermittent cabbage extract intake displayed expanded thyroid follicles measuring 23.88 µm for small follicles and 67.42 µm for medium follicles and 148.96 µm for large follicles. Follicular diameters expanded the most in Group G3 (daily exposure) to become 28.94 µm (small), 44.98 µm (medium) and 156.3 µm (large). Research data indicates that cabbage extract treatment causes a dose-related expansion of thyroid follicles

especially toward enlarged structures. The thyroid hormone deficiency together with TSH overstimulation leads to increased colloid deposition which causes follicular growth and expansion. The diameter of the follicles increased substantially in the treated groups because of colloid accumulation that caused the follicles to distend. The greatest size changes occurred within the category of large follicles.

Table 2: Effect of white cabbage extract on the diameter of thyroid follicles

Follicles	Diameter of follicles /µm		
	G1	G2	G3
Small	19.26±1.5	23.88±2.6*	28.94±3.3*a
Medium	37.9±6.6	67.42±6*	44.98±5.1*a
Large	103.2±8.6	148.9±16.3*	156.3±14.3*

Note: Values are expressed mean ±standard deviation, * means there was significant with control group of significant difference (P<0.05), whereas different letters in rows means significant difference (P<0.05) within experimented groups.

The informational in table 3 demonstrated the measurement outcome of thyroid follicular epithelial cell (thyrocyte) height within different follicle sizes between experimental test groups treated with white cabbage extract. Research on control animals revealed thyrocytes of normal dimensions with measurement results at 8.78±0.7 µm within small follicles and 8.34±1.1 µm within medium follicles and 8.64±0.7 µm within large follicles. The thyroid follicles of Group G2 consumed intermittent extract showed a substantial reduction in thyrocyte dimension to 6.4±0.27 µm in small follicles and 4.84±0.29 µm in medium follicles with 4.26±0.24 µm in large follicles. Daily consumption of the extract (G3) produced the maximum thyroid cell lowering effect particularly in large follicles where the cells shrank to 4.2±0.12 µm while small follicles measured 7.76±0.54 µm and medium follicles recorded 6.08±0.6 µm. A reduction in epithelial cell flattening serves as the hallmark feature to detect thyroid suppression or hypoactivity since it amounts to underlying problems with hormone synthesis and secretion. The pattern of thyroid tissue alterations collected at the cellular level matches both hormonal data and confirms that regular exposure to cabbage extract promotes thyroid inhibition. Cellular activity. The characterization of hypo-functioning thyroid tissue shows itself through flat follicular epithelium. The thyroid-suppressive effect in G2 was lower compared to what G3 experienced.

The table 4 showed data regarding thyroid follicle cell numbers accompanied by standard deviation (SD)resented as means for different size follicles within three experimental groups. The control group (G1) had 13±0.9 cells in small follicles and 35±2.1 cells and 41±2.4 cells in medium and large follicles. The measurement results from Group G2 (intermittent cabbage extract) showed significant increases across all follicle dimensions which were 18.8±1.1, 38.6±1.8, and 60.6±3.2 for small, medium, and large follicles respectively. Small follicles of Group G3 (daily extract) maintained similar numbers to the control group at 14.6±0.8. However, medium follicles increased to 46.2±3.7 and large follicles rose to 54.6±2.5 compared to G1. The experimental data implies that white cabbage extract promotes thyroid follicular cell growth particularly in medium and large follicle sizes. Follicular hyperplasia appears in the microscopic examination as the defining indicator of initial thyroid gland activity alteration. Small follicle cells in G3 show near-control numbers after long-term exposure possibly because of regulatory feedback mechanisms and degeneration of follicular cells. in G3 The experimental results validate the fact that cabbage extract causes thyroid tissue proliferation by dose and time-dependent mechanisms. The increase of follicular cells in G2 and G3 groups demonstrates hyperplasia. Cellular proliferation in medium and large follicular structures is highly pronounced.

Table 3: Effect of white cabbage extract on thyrocyte height

Follicles	Thyrocyte height / µm		
	G1	G2	G3
Small	8.78±0.7	6.4±0.27 *	7.76±0.54 *a
Medium	8.34±1.1	4.84±0.29 *	6.08±0.6 *a
Large	8.64±0.7	4.26±0.2.4 *	4.2±0.12 *

Note: Values are expressed mean ±standard deviation, * means there was significant with control group of significant difference (P<0.05), whereas different letters in rows means significant difference (P<0.05) within experimented groups

Table 4: Effect of white cabbage extract on the number of thyroid follicular cells per follicle

Follicles	No. of cells/ follicle		
	G1	G2	G3
Small	13±0.9	18.8±1.1 *	14.6±0.8
Medium	35±2.1	38.6±1.8 *	46.2±3.7 *a
Large	41±2.4 c	60.6±3.2 *	54.6±2.5 *a

Note: Values are expressed mean ±standard deviation, * means there was significant with control group of significant difference (P<0.05), whereas different letters in rows means significant difference (P<0.05) within experimented groups.

Thyroid tissue examination showed important structural differences between Groups G1 (control), G2 (intermittent cabbage extract), and G3 (daily cabbage extract). The tissue examination of Group G1 detected well-defined follicular architecture and homogeneous colloid content together with normal cuboidal cellular morphology and the absence of stromal changes (Figure 1). Group G2 members (Figures 2-4) showed the development of worsening architectural impairments throughout their tissue samples. The tissue sections from this group displayed growing variations in

follicle dimensions together with colloid. The epithelial cells in follicles displayed a flat shape together with initial indications of compensatory hyperplasia as the TSH levels rose and appear Distortions of thyroid follicle, Sloughing of cellular follicular debris, hypertrophy of follicular cells with stenosis of lumen, vacuolar degeneration of follicular cells, congested blood vessels. The tissue disruption reached its most severe state in Group 3 (Figures 5-7).

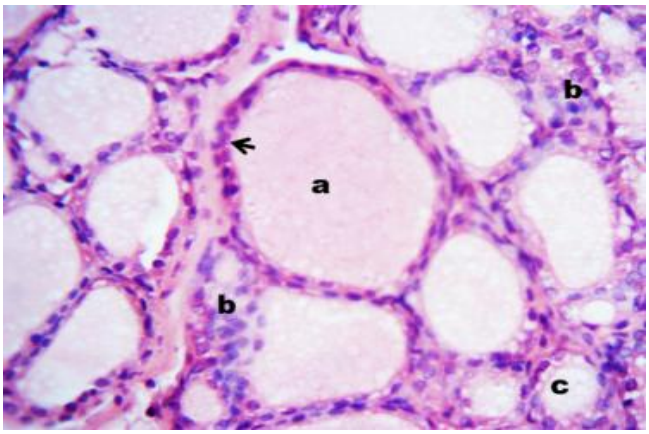

Figure 1: Photomicrograph of thyroid gland of control group (G1) show: large follicle filled with colloid with peripheral vacuolation of colloid (a), Para follicular cells (b), small size follicle (c), cuboidal follicular cells (black arrow). 40x, H&E stain.

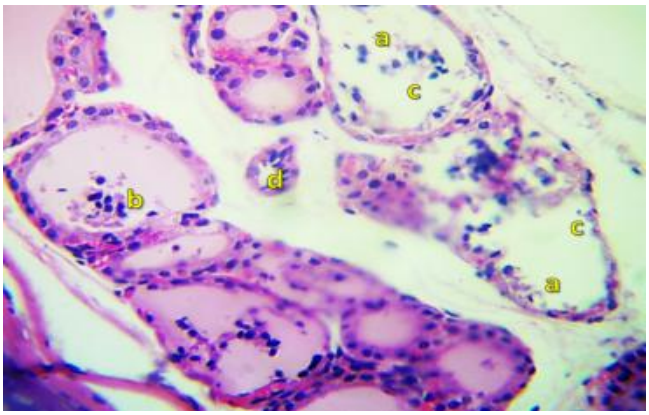

Figure 2: Photomicrograph of thyroid gland of group (G2) shows distortions of thyroid follicle (a), sloughing of cellular follicular debris (b), deficient of colloid (c), and atrophy of follicle (d) 40x, H&E stain.

Histological examination revealed extreme dilation of the follicles combined with cell epithelium thinning or its complete absence alongside irregular distribution of colloid material. The figures demonstrated changes which could be interpreted as early degenerative modification or stress-induced cell deaths, disorganization of thyroid follicles, sever infiltration of inflammatory cells, hemorrhage due to prolonged cabbage exposure containing goitrogenic substances. The severity of pathological tissue changes increased across G1 to G3 based on the dosage received. All changes in thyroid tissue including enlarged follicles, flattened epithelia and colloid loss and enhanced cell division enable clear observation of persistent exposure damage to the thyroid gland's structural organization.

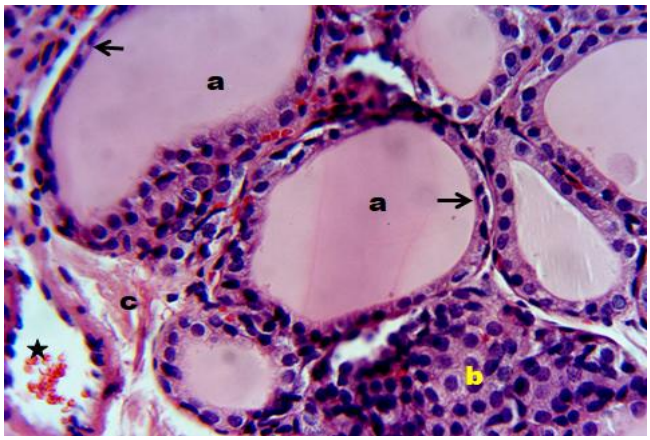

Figure 3: Photomicrograph of thyroid gland of group(G2) shows: numerous thyroid follicles filled with colloid absence of vacuoles in the periphery (a), hyperplasia of Para follicular cells (b), fibrosis between follicles (c), flattened follicular cells (black arrow), congested Blood vessels (asterisk). 40x, H&E stain. Note that most of follicles are large and moderate size and few small follicles.

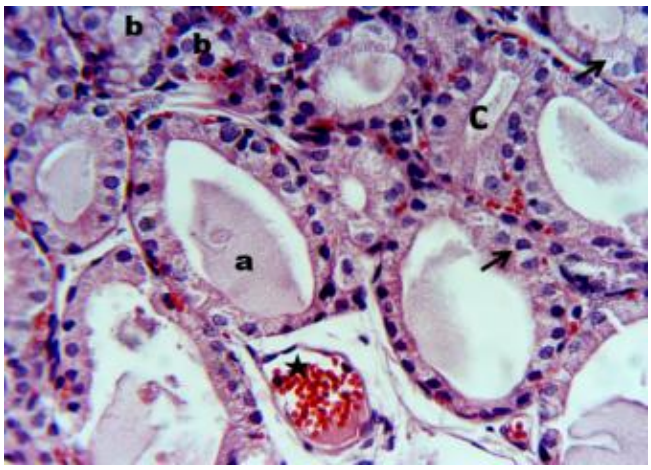

Figure 4: Photomicrograph of thyroid gland of group (G2) shows: numerous follicles filled with abnormal colloid with absence of vacuoles in the periphery (a), vacuolar degeneration of Para follicular cells (b), hypertrophy of follicular cells with stenosis of lumen (c), vacuolar degeneration of follicular cells (black arrow), congested blood vessels (asterisk).40x, H&E stain.

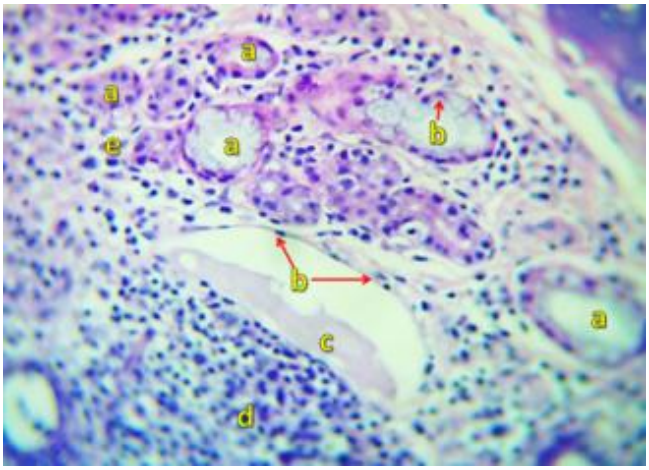

Figure 5: Photomicrograph of thyroid gland of group (G3) shows: disorganization of thyroid follicles and atrophied (a) degeneration of follicular cells (b) shrinkage of colloid (c) Extensive infiltration of WBC (d) Hyperplasia of Para follicular cell (e) 40x, H&E stain.

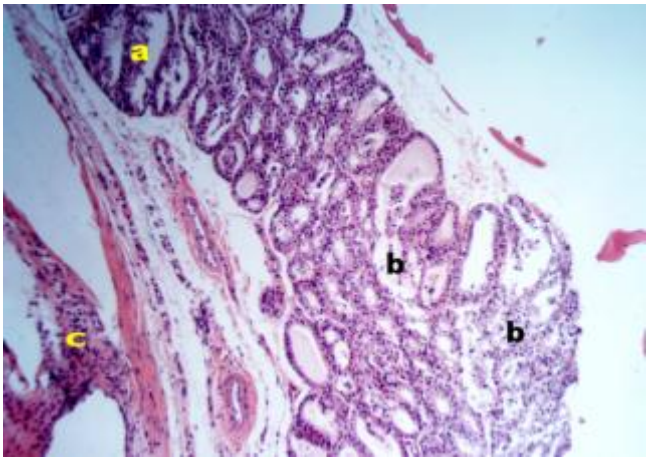

Figure 6: Photomicrograph of thyroid gland of group G3 shows: most of follicles undergo degeneration (a), necrosis (b), sever infiltration of inflammatory cells (c).10x, H&E stain.

The Masson's trichrome staining (Figure 8) staining of G1 individuals revealed healthy thyroid tissue structures that had a normal pattern. The tissue contained low quantities of interstitial connective tissue which structured properly with normal placement of collagen fibers. The follicular epithelial cells displayed normal cuboidal arrangements along with a uniform consistent appearance of the colloid substance (Figure 8). In group 2 (Cabbage Extract - 3 times/week), the tissues take out from the G2 showed minimal damage to their structure. The result category displayed mild architectural disturbances of follicles while the interfollicular stroma contained beginning collagen fiber buildup. The development of fibrotic response seems to occur because cellular stress emerges from suppressed thyroid hormone levels. The figures with "a" scores showed advanced interstitial connective tissue thickening which implies progressive tissue fibrosis. "b" scores detachment of some follicular cells from their original location with their accumulation and death in the center of the follicle, disappearance of some follicular colloid and the center of the follicles filled with hyperplastic follicular cells (Figures 9 and 10). Group G3 (Figure 11) cells from this group consisting of Masson-stained sections received a severe grading in their histopathological evaluation. The interfollicular spaces presented with substantial fibrosis which became visible through dense blue-stained collagen.

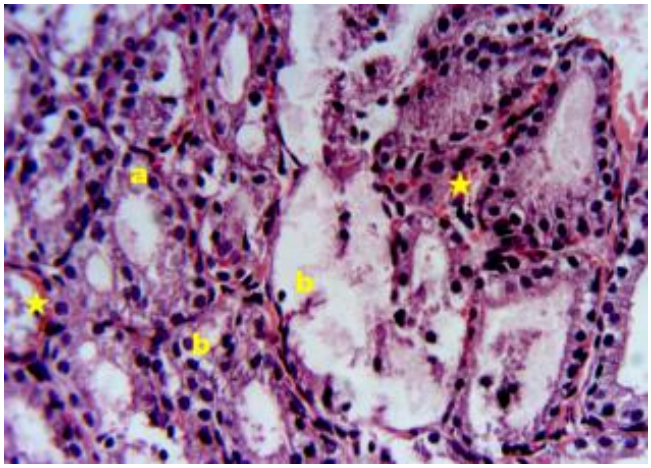

Figure 7: Photomicrograph of thyroid gland of group (G3) shows: vacuolar degeneration of follicular cells (a), necrosis (b) and hemorrhage (asterisk).40x, H&E stain.

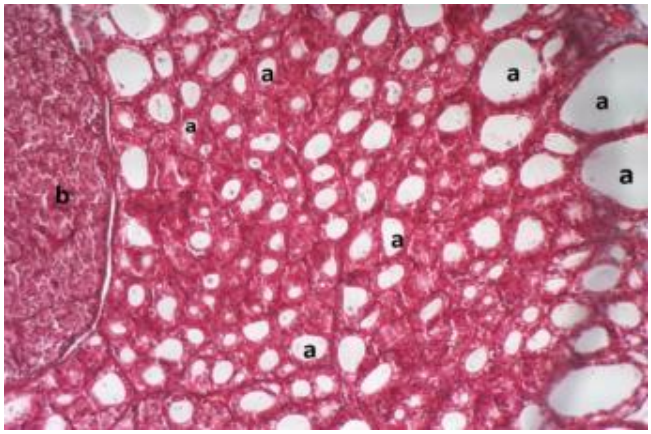

Figure 8: Photomicrograph of thyroid gland of control (G1) group shows normal amount of inter-follicular connective tissue. Different sizes of Thyroid follicles, small, medium and large (a), parathyroid gland (b).10x, Masson's trichrome stain.

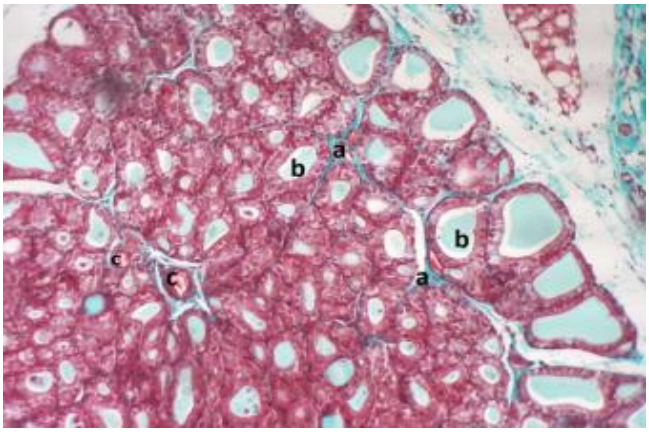

Figure 9: Photomicrograph of thyroid gland of (G2) shows increasing the amount of inter-follicular connective tissue (fibrosis) (a), Thyroid follicles (b), congested blood vessel(c).10x, Masson's trichrome stain.

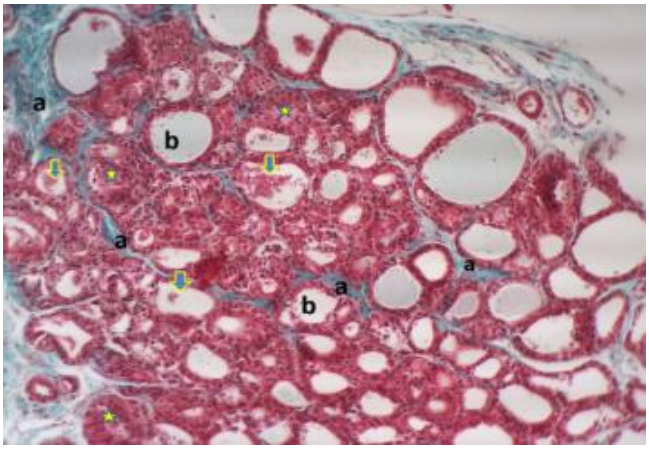

Figure 10: Photomicrograph of thyroid gland of G2 shows increasing the amount of inter-follicular connective tissue (fibrosis) (a). Thyroid follicles (b). Detachment of some follicular cells from their original location with their accumulation and death in the center of the follicle (arrow). Disappearance of some follicular colloid and the center of the follicles filled with hyperplastic follicular cells (star) 10x, Masson's trichrome stain.

Thyroglubin content within the follicular colloid remained abundant in the control group G1 based on their positive PAS staining pattern (Figure 12). The well-defined follicles contained colloid which received positive PAS staining and their epithelial surfaces consisted of cuboidal cells demonstrating typical secretory behavior and storage ability. Group 2 (Cabbage Extract 3times/Week) demonstrated various levels of PAS positivity appeared in thyroid tissue of this group. The thyroid tissue demonstrated average colloid supplies together with limited thyroglobulin storage capacity in some follicles which implied beginning thyroglobulin storage disturbances. The histological results featured incomplete positive PAS staining that revealed collapsed tissue structures affecting portions of the follicles for which colloid distribution was reduced (Figure 13).

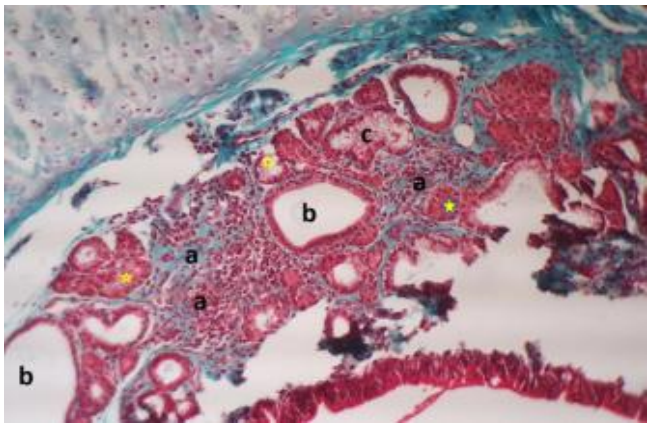

Figure 11: Photomicrograph of thyroid gland of G3 shows increasing the amount of inter-follicular connective tissue (fibrosis) with heavy infiltration of inflammatory cells (a). Large size Thyroid follicles (b), vacuolar degeneration of follicular cells (c), shrinkage and necrosis in some follicles (star).10x, Masson's trichrome stain.

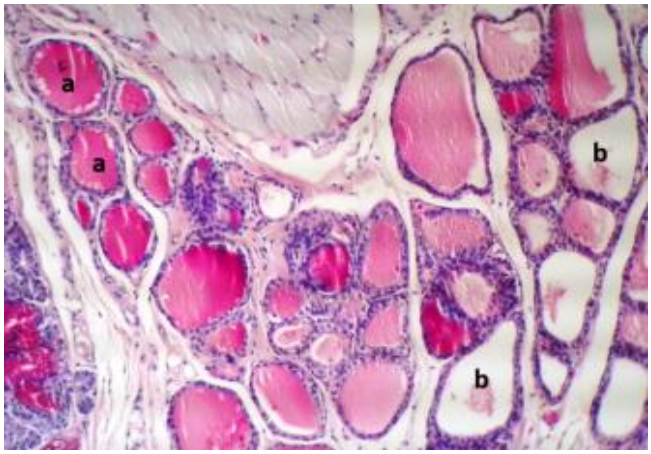

Figure 12: Photomicrograph of thyroid gland of control group (G1) shows normal strong reaction of colloid with PAS stain note that (a) some follicles filled with colloid and presence of peripheral vacuoles indicating active follicles, (b) other follicles are empty.10x, PAS stain.

Group 3 demonstrated the worst alterations of staining patterns. The tissues categorized serious thyroglobulin production suppression through extensive follicular collapse in addition to prominent colloid depletion and minimal PAS positivity. The follicular epithelium might experience destructive changes or full deterioration when tissues showed total PAS staining disappearance in multiple

structures. The extensive PAS-positive material reduction confirms the data showing prolonged hypothyroidism alongside excessive TSH stimulation that leads to glandular exhaustion which destroys tissue architecture (Figure 14).

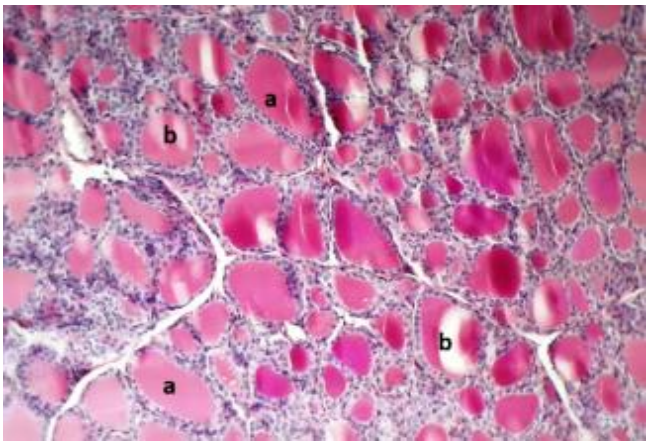

Figure 13: Photomicrograph of thyroid gland of (G2 group)-show: abnormal reaction of colloid with PAS stain, note that most follicles filled with colloid and there are no peripheral vacuoles indicating in-active follicles, (a) other follicles non homogenous colloid(b) 10x, PAS stain.

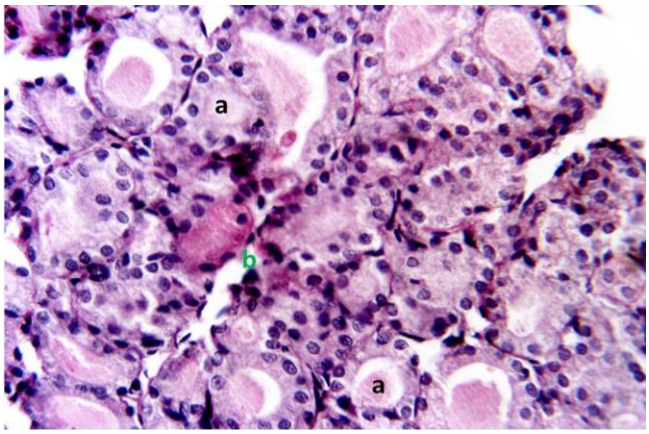

Figure 14: Photomicrograph of thyroid gland of (G3) group show: abnormal reaction of colloid with PAS stain, note that all follicles filled with weakly reacted colloid and there are no peripheral vacuoles indicating in-active follicles, (a) other follicles undergo necrosis (b), 40x, PAS stain.

Discussion

This study was demonstrated the effects of *Brassica oleracea var. capitata* (white cabbage) extract administration on adult male albino rat thyroid gland structure and function. White cabbage consumption within large amounts or extended periods resulted in hormonal impairment together with structural tissue alterations and altered follicle shape representing evidence of cabbage's goitrogenic potential (32). Both treatment groups revealed decreased serum T3 and T4 levels which represents the main features of thyroid hormone blockage, this research indicated that the reduction of thyroid hormones results from glucosinolate metabolites in cabbage which block the sodium/iodide symporter (NIS) operation needed to synthesize thyroid hormones (33). The thyroid hormone production becomes impaired because these substances simulate iodide transport therefore blocking its cell entry thus causing both iodine deficiency inside cells and reduced thyroglobulin iodination (34).

Circulating thyroid hormones decrease because of which the hypothalamic-pituitary-thyroid (HPT) axis activates to increase TSH secretion (23). The treated groups particularly in G3 displayed substantial enlargement in their follicular diameters through histomorphometric assessment, The enlarged follicles indicate colloid accumulation that stems from restricted hormone release of thyroglobulin in follicular lumen storage (35). Thyroid-Stimulating Hormone hyperactivity with reduction in hormone feedback leads to thyroid follicular cell proliferation and increased synthesizing colloid content yet the non-iodinated colloid material remains non-functional (16). The accumulative substance results in distended and abnormal follicle shapes evident in both G2 and G3 groups. The decreased height of thyrocytes seen in treated animals indicates that metabolic activities experience suppression, active follicular cells normally maintain columnar to cuboidal morphology yet they flatten into squamous cells both during hypothyroidism and toxic exposure because secretory activity decreases (8). This research findings showed that the changes occurred to a greater extent in Treatment Group 3 compared to Group 2 pointing to both the intensity of cabbage phytochemicals exposure and the duration of exposure. The results reported by Racine *et al.* (17) show that Brassica vegetables such as kale and turnip have comparable impact on thyroid tissue function, that supporting the findings of this work. The number of follicular cells increased significantly in treated groups especially in medium and large follicles because of TSH-driven hyperplasia according to Chaker *et al.* (9). Long-term thyroid overstimulation transforms into an unhealthy condition which causes thyroid enlargement before leading to tissue fatigue. Research evidence demonstrating goitrogen-treated rats develops larger thyroid glands and denser follicular epithelia (18,19). In the Group 3 displayed fibrosis of a large degree which extended throughout the thyroid gland indicating progressive tissue degradation according to Tajiri *et al.* (2020). The research results confirmed earlier H&E data about how goitrogens affect thyroid tissue which leads to changes in tissue structure and development of fibrosis.

Results from the experimental histopathological analysis across G1, G2 and G3 groups result from goitrogenic compounds found in *Brassica oleracea* cabbage which

release glucosinolates and generate thiocyanate as their hydrolysis products (10). The compounds from cabbage affect thyroid gland iodide uptake and thyroid hormone synthesis while causing TSH levels to rise which modifies thyroid gland structure (36). Breeding rats in Groups G2 and G3 that received cabbage extracts exhibited noticeable follicular size increase with colloid vacancy and thyroid cell thickness alongside cellular number growth. The experimental data shows similar effects to thiocyanate ions by inhibiting sodium-iodide symporter (NIS) transport which leads to reduced T3 and T4 hormone production (37). Once hormone levels decrease the pituitary releases more TSH through a feedback mechanism. The prolonged increase of TSH leads to thyroid follicular cell hypertrophy and hyperplasia thereby explaining the enlarged follicles with higher cell numbers seen in the study (38).

The histological evidence of flattened thyrocytes and distorted colloid content indicates decreased rates of colloid consumption and hormone generation. The examined morphological changes demonstrate classic signs of hypothyroidism that results from goitrogenic exposure (39,40). Studies have established that lengthy Brassicaceae vegetable consumption without sufficient iodine intake causes distortions of thyroid follicle, sloughing of cellular follicular debris, and deficient of colloid in G2 (40,41). Moreover, the increased number of follicular cells per follicle, particularly in medium and large follicles, supports the theory of compensatory hyperplasia due to persistent TSH stimulation (42,34). The increased number of follicular cells found after exposure to the goitrogens demonstrates cellular proliferation for thyroid hormone restoration per rodent cell research (44,45). The thyroid gland in Group 1 operated normally and retained standard follicular structure since cabbage extract treatment was absent resulting in balanced hormone levels (43,46). Scientific findings support the conclusion that morphological abnormalities in G2 and G3 emerge because of the anti-thyroid mechanism of the provided plant extract (22,47). The histological findings of Group 3 displayed fibrosis of a large degree which extended throughout the thyroid gland indicating progressive tissue degradation**,** most of follicles undergo degeneration, necrosis, sever infiltration of inflammatory cells (24,48). The research histological results through H&E staining confirmed how goitrogens affect thyroid tissue which leads to changes in tissue structure and development of fibrosis (40,49).

The collagen accumulation together with fibrotic changes observed in Groups G2 and G3 corresponds to extended thyroid stimulation caused by low T3 and T4 levels. The main reason for thiocyanate ion-blocking of the sodium-iodide symporter (NIS) creates blockade of iodide transport into follicular cells leading to reduced thyroid hormone synthesis (37). Tissue enlargement through hypertrophy and hyperplasia that ultimately transforms the thyroid to fibrotic tissue during stress or compensatory situations (38). Masson-stained fibrosis is visible through the interfollicular deposition of blue-staining collagen fibers. Extensive evidence shows that thyroid fibrosis results from ongoing inflammation or constant hormone stimulation when stress from goitrogens exists. Extended stimulation or cellular harm triggers the thyroid stroma to activate fibroblast cells and boost extracellular matrix generation especially collagen (42). The intermediate grade of fibrosis revealed G2 parallels the more severe scores of G3. Decreasing thyroid hormone levels along with hypothyroidism frequently result in tissue fibrosis because thyroid hormone deficiencies negatively impact tissue metabolism and remodeling procedures. The prolonged deficiency of T3 and T4 hormones leads to dysregulation of immune cellular environments which raises oxidative stress and triggers fibroblast activation (39,50).

Normal thyroglobulin synthesis and storage appeared as strong PAS-positive areas within tissue samples from the control group (G1). The cabbage consumption in groups G2 and G3 led to weakened PAS staining grades which indicated reduced thyroglobulin production together with declining colloid storage, the cells lose their ability to effectively iodinate and store thyroglobulin and as a consequence the colloid becomes depleted and PAS staining diminishes (40,51). Most thyroid glands in the severely affected G3 group developed functional failure when they received daily cabbage exposure as PAS grading revealed characteristics. Studies confirm that persistent exposure to goitrogens results in colloid collapse alongside follicular destruction and suppressed activities of secretory tissue (42,52). Continued metabolic stress alongside oxidative damage will impair the epithelial lining which reduces thyroglobulin secretion (39,53). This research findings confirm previous studies which indicate that consuming high levels of Brassica vegetables without proper iodine supplementation will reduce thyroid performance and lead to hypothyroidism with substantial glandular tissue rearrangement (40,54). G3 exposure as a daily dose resulted in more serious thyroid dysfunction than the intermittent dosage regimen of G2. Galanty *et al.* (32), established in their study that the time span and dosage amount of consumption play a role in creating endocrine threats when consuming goitrogenic substances within iodine-sufficient populations.

Conclusion

The current study concludes that persistent consumption of white cabbage (*Brassica oleracea var. capitata*) extract produces noticeable modifications thyroid morphology and operation. Research findings show that serum concentrations of T3 and T4 decrease while TSH levels rise. The daily extract intake caused histological changes which resulted in follicular enlargement together with epithelial flattening and colloid depletion and interstitial fibrosis, sloughing of follicular cells in certain follicles, scalloping of colloid in

some follicles within all extract group. The study findings also demonstrate most of the follicles undergo degeneration, necrosis, sever infiltration of inflammatory cells, and hemorrhage primarily within the daily extract consuming. Thyroid changes in animals can have an impact on their health and development and may result in increased veterinary expenses and lower productivity in livestock farming operations. It is recommended to be careful when incorporating cabbage into animal feed to prevent any negative financial consequences on animal performance.

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Conflicts of interest

There are no conflicts of interest.

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التأثيرات النسيجية والتعديل الهرموني لمستخلص الكرنب الأبيض على الغدة الدرقية في الجرذان

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فرع الأنسجة والتشريح، كلية الطب البيطري، جامعة تكريت، تكريت، العراق

الخلاصة

يُعتبر الملفوف الأبيض من المواد التي يتم دراستها على نطاق واسع لاستخدامه في أعلاف الحيوانات، وذلك بسبب توفره وسعره المنخفض، إلى جانب قيمته الغذائية الجيدة. ومع ذلك، فإن احتواء الملفوف على مركبات الكلوكوزينولات يشكل مصدر قلق، حيث تتحلل هذه المركبات إلى ثيوسيانات، والتي قد تؤثر سلبا على وظيفة الغدة الدرقية لدى الحيوانات، خاصة في حال نقص اليود الغذائي. تهدف هذه الدراسة إلى تقييم تأثير مستخلص الإيثانول للملفوف الأبيض على البنية النسيجية للغدة الدرقية، وكذلك على مستويات هرمونات الغدة الدرقية الثالث والرابع والهرمون المحفز للدرقية في ذكور الجرذان البالغة. تم تقسيم واحد وعشرين جرذا أبيض بالغا عشوائيا إلى ثلاث مجموعات متساوية على مدى فترة أربعة أشهر. تلقت المجموعة الأولى (مجموعة السيطرة) محلول ملحي عن طريق الفم، بينما تلقت المجموعة الثانية مستخلص الملفوف بجرعة ١٠٠ ملغم/كغم ثلاث مرات أسبوعيا، وتلقت المجموعة الثالثة نفس الجرعة يوميا. تم قياس مستويات هرمونات الغدة الدرقية الثالث والرابع والهرمون المحفز للدرقية باستخدام اختبار الاليزا، كما خضعت أنسجة الغدة الدرقية للفحص النسيجي باستخدام صبغات الهيماتوكسيلين والأيوسين وحامض البريوديك كاشف شيبف وملون ماسون ثلاثي الصبغ، وتم تقييم التغيرات الشكلية من خلال قياس قطر الجريبات، وارتفاع الخلايا الدرقية، وعدد الخلايا الظهارية لكل جريب. أظهرت النتائج انخفاضا في مستويات هرمون الرقية الثالث والرابع مع زيادة تعويضية في الهرمون المحفز للدرقية، وكانت التغيرات أكثر وضوحا في المجموعة الثالثة. استنتجت الدراسة الحالية إلى أن الاستهلاك المستمر لمستخلص الملفوف الأبيض يؤدي إلى تعديلات ملحوظة في مورفولوجيا الغدة الدرقية وطريقة عملها.

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