

**Original article****Ameliorative Role of *Saussurea Lappa* Root Extract "Costus" on Thyroid Tissue Under the toxic effect of Carbimazole induced hypothyroidism.**

Ereny Fekry <sup>1</sup>, Mona M. Awny <sup>2</sup>, George Nagy Refaat <sup>3</sup> & Horeya Arafat <sup>1</sup>

<sup>1</sup> Department of Histology and cell biology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt. <sup>2</sup> Department of Forensic medicine & Clinical Toxicology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt. <sup>3</sup> Department of Internal Medicine, Suez Canal Authority Hospitals, Ismailia, Egypt.

**ABSTRACT****\*Corresponding author**

Mona Mohamed Awny

**E-mail**

mona\_awny@med.suez.edu.eg

**ORCID**

0000-0002-1021-4163

**Introduction:** Carbimazole is an antithyroid medication that is used for the treatment of hyperthyroidism. Carbimazole decreases serum thyroxine, thyroidstimulating hormone, and thyrotropin-binding inhibitory

immunoglobulins. However, carbimazole therapy

can induce hypothyroidism and toxic effects on the thyroid gland tissue itself. *Saussurea lappa* root "Costus" is a traditional treatment that has anti-inflammatory, antioxidant, and immunomodulatory properties. **Materials and methods:** Twenty-four adult male albino rats were equally randomized into four groups and received all treatments orally by intragastric tube: group I served as control. Group II received Costus roots aqueous extract (50mg/kg body weight /2 days). Group III received carbimazole (2mg/100 gm body weight/day). Group IV received carbimazole concomitant with Costus roots aqueous extract. After 4 weeks, the animals were decapitated. Hematoxylin and eosin stain, periodic acid-Schiff reaction, and anti-calcitonin immunostaining were all performed on the prepared sections. **Results:** The Carbimazole group showed follicles lined by squamous epithelium, vacuolated colloid, and fused follicles. Carbimazole caused markedly decreased PASpositive reaction due to colloid vacuolations and increased cytoplasmic immunoreactivity of C cells. In group IV, Costus could restore almost all the histopathological changes and improved C cells and decreased their cytoplasmic immunoreactivity. **Conclusion:** *Saussurea lappa* root "Costus" has a protective effect against carbimazole-induced thyroid damage.

**Keywords:** *Saussurea lappa*, Costus, Carbimazole, hypothyroidism, thyroid, toxicity.

**I. INTRODUCTION**

A bi-lobular endocrine gland, the thyroid is situated in the lower neck. It is a butterfly-shaped organ that is located below the larynx on both sides of the trachea (Shattnan et al., 2017). The structure and functioning component of the thyroid gland is the thyroid follicle. Its wall is made up of parafollicular and follicular cells that surround a central lumen that contains the colloid thyroglobulin (Hossain, 2019). The follicular cells are responsible for secreting the thyroid hormones: tetraiodothyronine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>), while the parafollicular cells (C cells) secrete the polypeptide hormone calcitonin which regulates calcium concentration in the blood (Hossain, 2019). The thyroid hormones have an essential role in most biological processes taking place inside every cell of the body. They are important for growth and different tissue development such as the brain, bone, skeletal muscles, intestine, and auditory system (Visser, 2018). They play important roles in controlling the metabolism of proteins, lipids, and carbohydrates as well as oxygen consumption, basal metabolic rate, thermoregulation, and a host of other essential biological functions (Visser, 2018; Hossain, 2019).

Carbimazole is an antithyroid medication that is used for the treatment of hyperthyroidism (Sakr et al., 2011), and its dose range in humans is 5–40 mg/day orally (Abbara et al., 2020). Methimazole is produced in the liver when carbimazole, a derivative of 3-carbomethoxy methimazole, is metabolised (Sakr et al., 2012). Administration of carbimazole for 2, 4, and 6 weeks effectively decreases serum thyroxine,

thyroid-stimulating hormone, and thyrotropin-binding inhibitory immunoglobulins (Hossain, 2019). After 2–4 weeks, the required decline in free thyroid hormone level is achieved necessitating the initial starting dose of carbimazole to be gradually reduced by 30–50% otherwise hypothyroidism might occur. However, numerous adverse effects and toxicities were reported with carbimazole treatment. Carbimazole treatment in humans induced rash, urticaria, pruritus, arthralgia, fever, agranulocytosis, pancreatitis, and hepatotoxicity associated with significant cholestatic jaundice (Léger & Carel, 2017). Pulmonary hemorrhage and renal toxicities such as necrotizing glomerulonephritis, lupus nephritis, and vasculitis were also reported (Kadhim et al., 2018). Additionally, the thyroid gland tissue itself experienced toxic effects from carbimazole therapy. It increases the risk of thyroid cancer (Sakr et al., 2011). Furthermore, receiving carbimazole during pregnancy and lactation produced microstructural changes in new-born thyroid gland (Sakr et al., 2012). The reported side effects and toxicities of carbimazole mostly occur during the first 3–6 months of its use which restricts the compliance of patients (Léger & Carel, 2017).

Asteraceae family member *Saussurea lappa* root, usually referred to as "Costus," is a medicinal plant with high antioxidant content (Habotta et al., 2021). It is an ancient plant widely distributed in the Himalayan Region of India, and commonly used in traditional Indian medicine (Madhuri et al., 2012). *Saussurea lappa* root "Costus" value as a traditional treatment extends to the Arabic region, especially in Saudi Arabia (Alnahdi,

2017). A wide range of bioactive ingredients was found in *Saussurea lappa* root "Costus" mainly terpenes and other compounds such as alkaloids, flavonoids, and anthraquinones (Zahara et al., 2014). These active constituents have various medicinal actions such as anti-cancerous, anti-inflammatory, antiulcer, antihepatotoxic, antidiabetic, anthelmintic, antifungal, antiepileptic, antihyperlipidemic and immunomodulatory activities (Tousson et al., 2019). Furthermore, it has been observed that the considerable antioxidant activities of Costus extract have a modulatory influence on the thyroid hormones and attenuate the microstructural alterations in the thyroid gland caused by several toxins in rats (Alnahdi, 2017), as well as serum parameters in both hyperthyroid and hypothyroid mice (Mahmoud, 2020). Therefore, the goal of this study was to explore whether the *Saussurea lappa* root extract "Costus" could reduce the harmful toxic changes to the thyroid gland brought on by carbimazole-induced hypothyroidism in adult male albino rats.

## II. MATERIALS AND METHODS

The research was conducted in the Faculty of Medicine, Suez Canal University, Histology and Cell Biology department. Animals were handled in accordance with the institutional animal care ethical committee's requirements at Suez Canal University's Faculty of Medicine (Reference number: 5173).

### II.1. Experimental animals

Twenty-four mature male albino rats of equal age (3 months) and body weight engaged in the experiment (150–200 g). The National Research Centre of Cairo for Experimental

Animals provided the study's animals. Prior to the experiment, rats were housed for a week to allow for adaptation. They had free access to water, a regular diet of animal pellets, and were kept in plastic cages. The animal environment was kept at a constant room temperature (22–24 °C), with 12 hr of daylight and 12 hr of darkness.

### II.2. Drug preparation

- Carbimazole: Obtained from Chemical Industries Development (CID) in the form of tablets. Each contains 5 mg. The selected dose was previously used in induction of hypothyroidism model by using carbimazole in a dose of (2 mg /100 gm) daily for 4 weeks (Abdel-Fatah et al., 2015). The tablet was crushed and dissolved in 1 ml of distilled water. So, each 0.4 ml of the solution contained 2 mg of carbimazole. Accordingly, the administered dose for a rat weighing e.g 200 gm was 4 mg that was dissolved in 0.8 ml of distilled water.
- *Saussurea lappa* absolute, CAS Number: 8023-88-9, Robertet, Inc., France. The required dose was 50 mg/ kg body weight, every 2 days (Abd Eldaim et al., 2019; Bolkin et al., 2019). This dose was prepared as follows: 50 mg of costus roots aqueous extract were dissolved in 50 ml distilled water and thus each 1ml of this aqueous solution contained 1 mg of the costus roots extract. Accordingly, for a rat weighing e.g. 200 gm, the administered dose was 10 ml of the aqueous solution (which contained 10 mg of the costus root extract). This dose (10 ml) was divided into 2 days, as 5 ml each

day and was given orally by intragastric tube as 2.5 ml two times daily.

### ***II.3. Experimental design***

Animals were randomly subdivided into four groups (6 animals each). All treatments were given orally by intragastric tube for 4 weeks.

- Animals in Group I (Control group) received distilled water.
- Animals in Group II (the Costus group) received an aqueous extract of costus roots at a dose of 50 mg/kg body weight every two days (Abd Eldaim et al., 2019; Bolkiny et al., 2019).
- Animals in Group III (Carbimazole group): received carbimazole (2mg/100 gm body weight/day) (Abdel-Fatah et al., 2015).
- Animals in Group IV (Carbimazole + Costus group): received carbimazole concomitant with costus roots aqueous extract with the same previous doses.

### ***II.4. Tissue sample collection***

All animals were ether-anesthetized and sacrificed by decapitation by the end of the 4<sup>th</sup> week. The thyroid gland was removed, preserved for 24 hours in neutral formalin at 10% (v/v), embedded in paraffin sections, and cut at 5µm thickness. The obtained sections were subjected to the following techniques:

- H&E stain: for general histological architecture (Bancroft & Layton, 2019).
- PAS stain: for demonstration of colloid (Layton & Bancroft, 2019).
- Anti-calcitonin immunostaining (DAKO A-567; Dako, Glostrup, Denmark) for demonstration of immunoreactive C cells. Calcitonin antibody-2 (Rabbit

Polyclonal Antibody) was used to stain thyroid sections (Martn-Lacave et al., 2009). A biotinylated antiserum to rabbit/mouse immunoglobulins served as the secondary antibody (Life Trade, Egypt). 3,3-diaminobenzidine tetrahydrochloride served as the chromogen (Sigma). Mayer's hematoxylin was used as a counterstain on tissue sections. Negative and positive controls were run.

Histological, histochemical, and immunohistochemical alterations in the thyroid gland were evaluated qualitatively and quantitatively.

- The five high power fields (X400) in 10 serial sections from each animal of all the analyzed groups were examined to accomplish the qualitative assessment. For each alteration in each of the examined groups, the frequency distribution of the histopathological changes was calculated.
- With a resolution of 10 MP (megapixels) (3656 2740 pixels per image), all images were taken using a calibrated standard digital microscope camera (Tucsen ISH1000 digital microscope camera) with an Olympus® CX21 microscope. For image capture and improvement, "IS Capture" software was done.
- The programme Image J was used to conduct quantitative measurements. The following measurements were assessed in the distinct groups:
  1. Mean of area percentage of PASstained colloid.
  2. Mean of area percentage of calcitonin immunoreactive C cells.

### ***II.5. Statistical assessment:***

The Office 365 software suite from the American company Microsoft Corporation was used to enter data into a Microsoft Excel programme. Then, using Chicago, USA developed IBM Corporation's SPSS version 25 (Statistical Package for Social Sciences), a statistical analysis was conducted. The data were presented as percentages, mean and standard deviation (SD). The results for the histological alterations were contrasted between groups by using one-way analysis of variance (ANOVA) followed by post-hoc Tukey test. The statistical significance was considered when P-value is less than 0.05.

### **III. RESULTS**

#### ***III.1. Hematoxylin and eosin (H&E) staining***

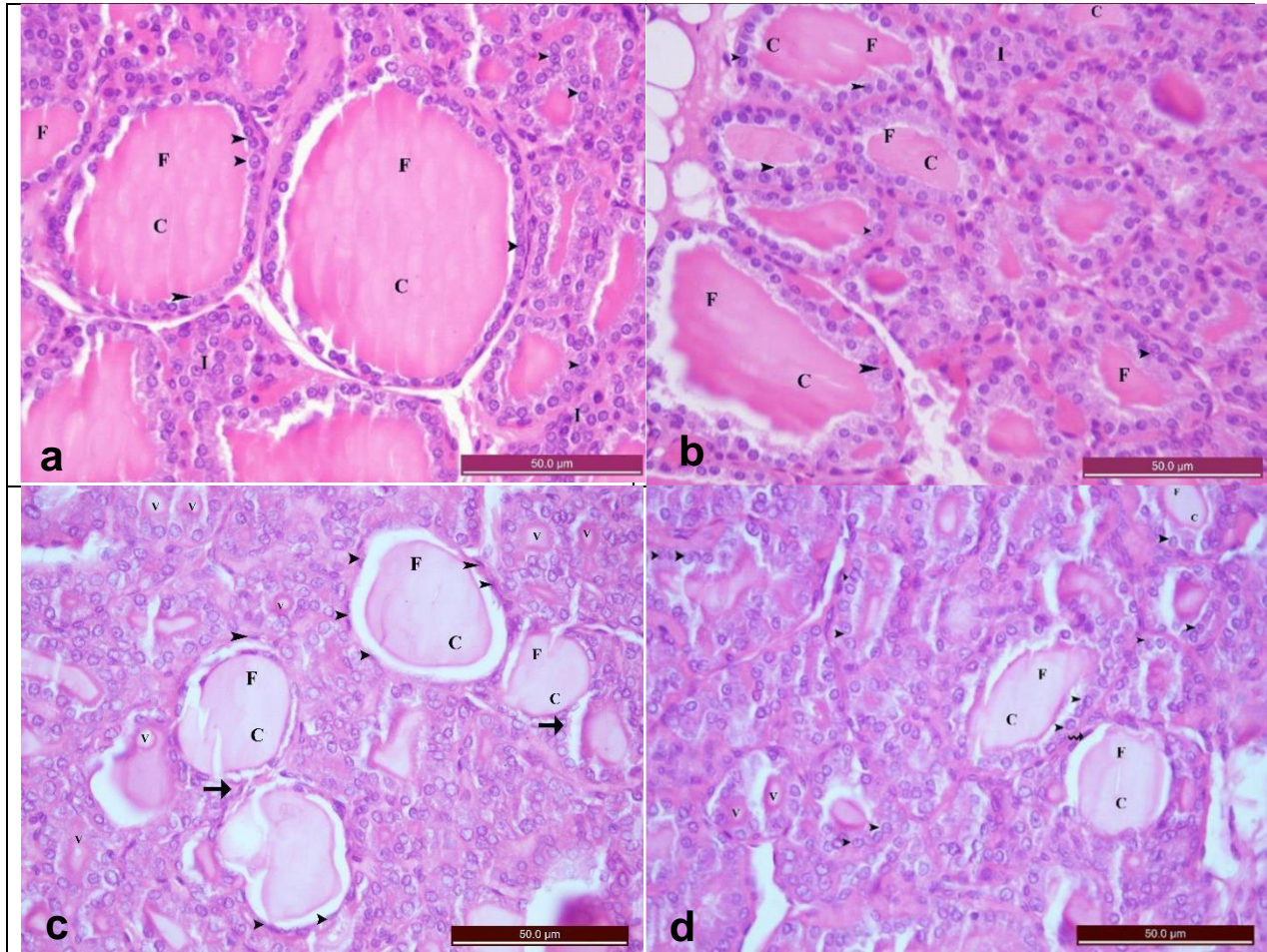
Examination of H&E-stained thyroid sections of the animals of the control group showed normal follicles of varied sizes, lined with cubical epithelium that exhibits rounded vesicular nuclei. The follicular cavities contained acidophilic colloids. The interfollicular cells were seen (Figure 1a). The animals in the costus group's H&E-stained thyroid sections resemble those in the control group (Figure 1b). Carbimazole H&E-stained sections showed marked histopathological changes. In this group, follicles were lined with squamous epithelium in 86% of sections which were statistically significant compared to the control group. All animals showed vacuolations of the colloid in some follicles. Fusion between follicles was also seen in 53% of sections which were statistically significant compared to the control group (Figure 1c). H&E-stained thyroid tissue samples from the

carbimazole and costus groups revealed follicles that were nearly normal and surrounded by cubical epithelium with rounded vesicular nuclei. The follicular cavities contained acidophilic colloids. These changes were statistically significant compared to the Carbimazole group. However, follicles lined with squamous epithelium (30% of sections) and minimal colloidal vacuolations (23% of sections) were still seen (Figure 1d). All histopathological changes of the thyroid in the distinct groups were shown in (Table 1).

#### ***III.2. PAS staining***

Control and costus groups' PAS thyroid stained sections revealed a typical colloid PAS-positive reaction (Figure 2a & 2b). A marked decreased PAS-positive reaction of the colloid was seen in the carbimazole group, which was statistically significant compared to the control group (Figure 2c & Figure 3). However, compared to the carbimazole group, the PAS-positive reaction of colloid in the carbimazole and costus group was similar to that of the control group and statistically significant (Figure 2d & Figure 3).





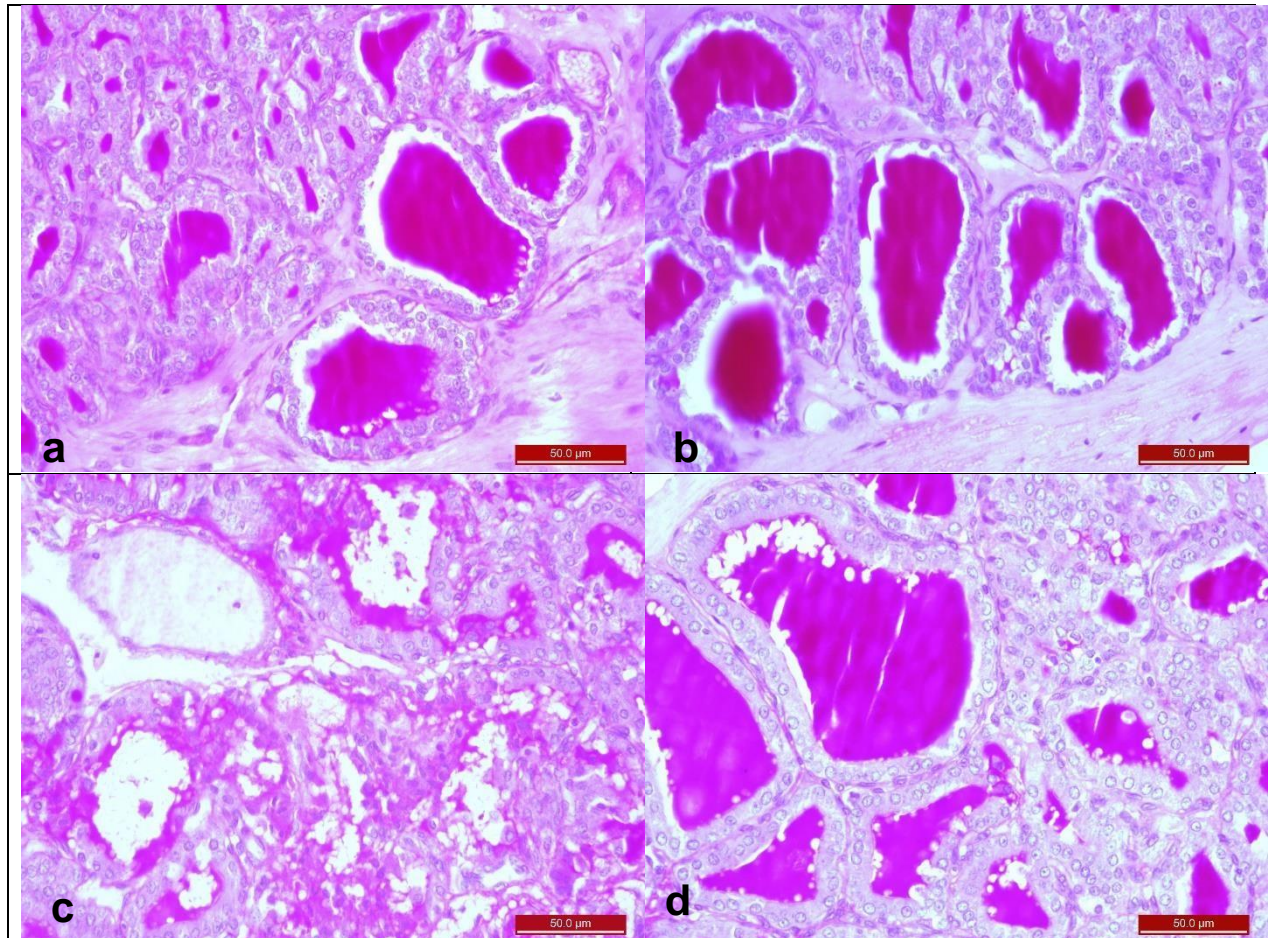
**Figure 1.** Thyroid sections that were stained with H&E X 400. **(A)** The control group's image of follicles of varied sizes (F), lined with cubical epithelium that exhibit rounded vesicular nuclei (arrowheads). The follicular cavities contain acidophilic colloid (C). The interfollicular cells are also shown (I) **(B)** (Costus group). **(C)** (Carbimazole group) showing follicles (F) lined with squamous epithelium (arrowheads). The follicular cavities contain acidophilic colloid (C) which shows vacuolations (V) in some follicles. Fusion between follicles (arrows) is also shown. **(D)** (Carbimazole + Costus group) shows follicles (F) lined with cubical epithelium that exhibits rounded vesicular nuclei (arrowheads). One follicle shows squamous epithelium in its lining (wavy arrow). The follicular cavities contain acidophilic colloid (C). Minimal colloidal vacuolations (v) are still present.

Table 1: The frequency distribution of the different histopathological changes of the thyroid gland of animals in the different groups.

Histo-pathological changes	Control	Costus	Carbimazole	Carbimazole + Costus
	%	%	%	%
Follicles lined with squamous epithelium	2	3	86	30
Follicular fusion	0	0	53	12

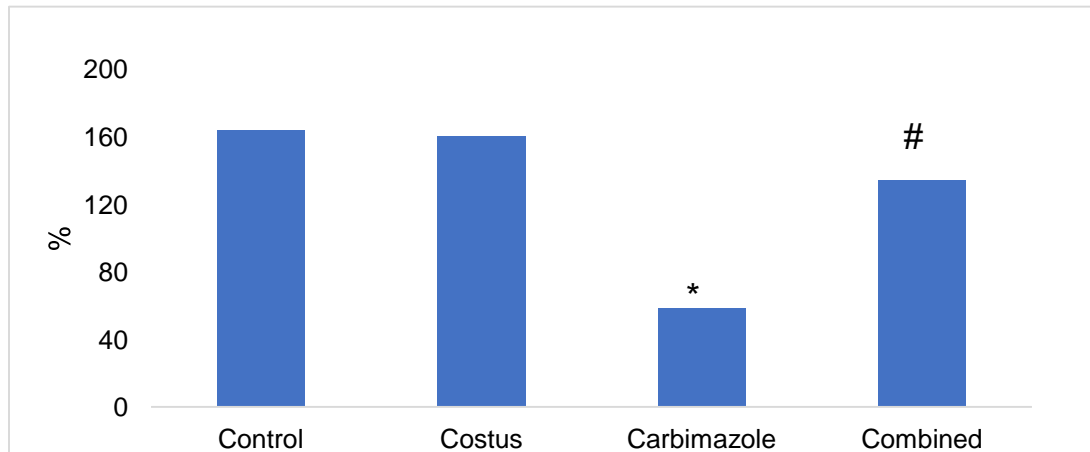
Colloid vacuolation	3	3	100	23
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n= number of animals in each group



**Figure 2.** Thyroid sections from various groups that were stained with PAS X 400 were examined. **(A)** (Control group) shows normal PAS-positive reaction (magenta stained) of colloid inside thyroid follicles. **(B)** (Costus group) is like control. **(C)** (Carbimazole group) showing marked decreased PAS-positive reaction (magenta stained) of colloid inside thyroid follicles. **(D)** (Carbimazole + Costus group) shows a PAS-positive reaction of colloid inside thyroid follicles like the control group/ almost normal.





**Figure 3.** The mean percentage of the colloid's PAS reaction area.

\* significant compared to the control group; # significant compared to the carbimazole group

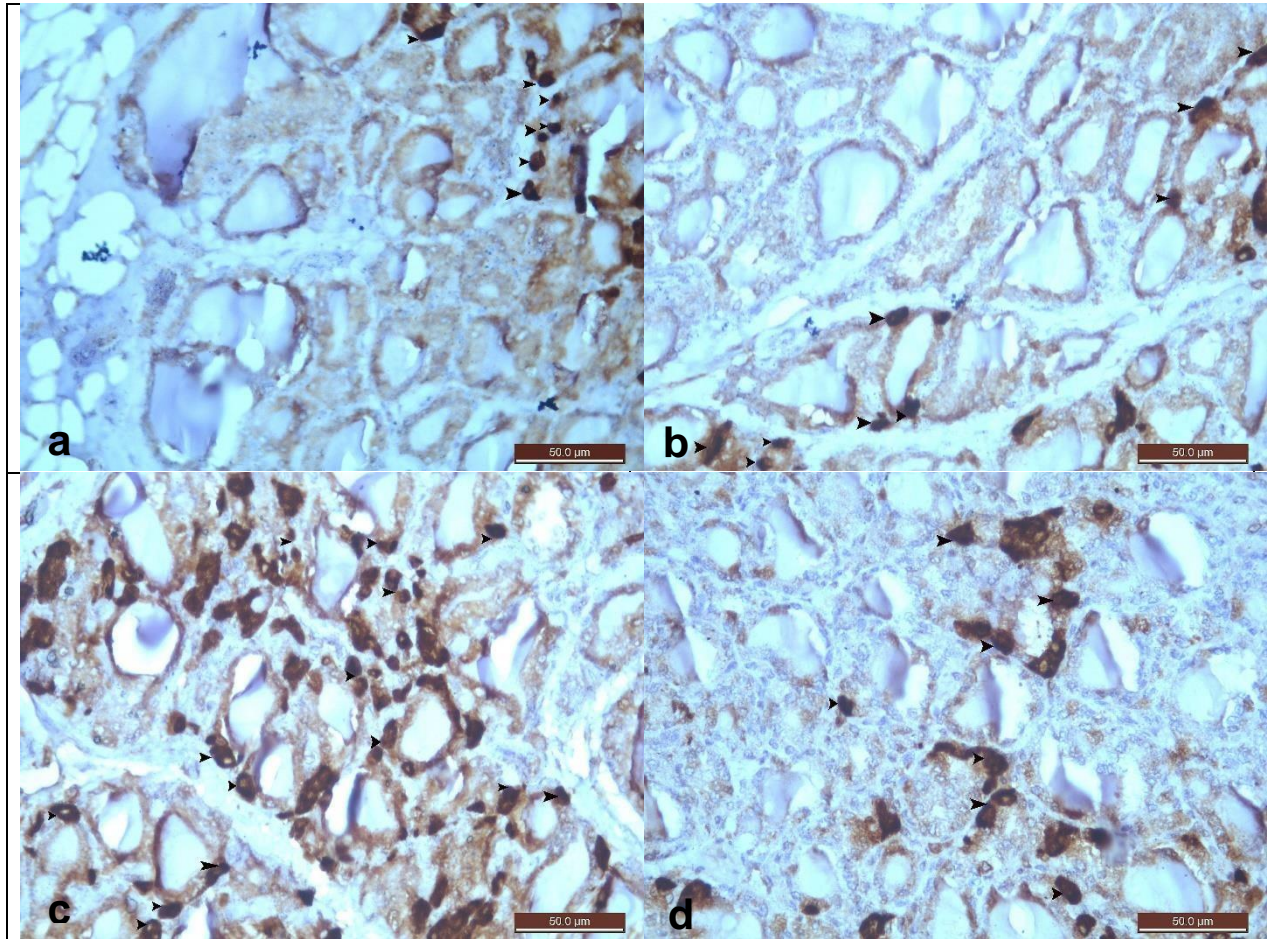
### ***III.3. Anti-calcitonin immunostaining***

Thyroid sections from the control and costus groups displayed nearly negative brownish cytoplasmic reaction of C cells after anticalcitonin immunostaining. (Figures 4a & 4b). Clusters of immunoreactive C cells were seen in the carbimazole group that were statistically significant when compared to the

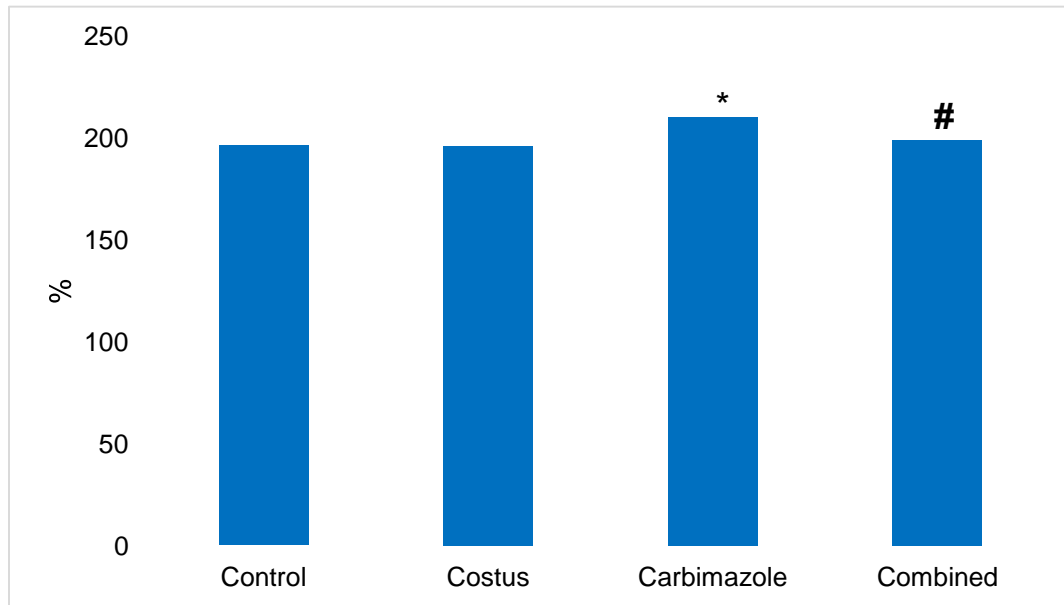
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control group. (Figure 4c & Figure 5). However, decreased brownish cytoplasmic reaction of C cells was seen in the carbimazole and costus group and when compared to the carbimazole group, was statistically significant. (Figure 4d & Figure 5)





**Figure 4.** Thyroid sections from several groups that were immunostained with anti-calcitonin at a magnification of 400. **(A)** (control group) displaying nearly negative brownish cytoplasmic immunoreactive C cells close to the thyroid follicles' basal membrane (arrowheads). **(B)** (Costus group) is like control. **(C)** (Carbimazole group) demonstrating elevated brown cytoplasmic immunoreactive C cells close to the thyroid follicle basal membrane (arrowheads). **(D)** (Carbimazole + Costus group) has diminished brown cytoplasmic immunoreactive C cells close to the thyroid follicles' basal membrane (arrowheads).



**Figure 5.** Mean percentage area of anti-calcitonin immunostaining.

\*Statistically significant compared to the control group:  $P < 0.05$

#Statistically significant compared to the carbimazole group:  $P < 0.05$

#### IV. DISCUSSION

Hypothyroidism, a condition where thyroid hormone production declines due to thyroid gland dysfunction, which interferes with hormone synthesis and secretion, is one of the most common thyroid disorders in people (Elkalawy et al., 2013).

In the current study, we investigated the potential toxicity of experimentally induced hypothyroidism on the thyroid gland's histological structure. Moreover, this work studied the potential therapeutic benefit of "Costus," a root extract from *Saussurea lappa*, on thyroid tissue in hypothyroidism. In the current study, H & E-stained thyroid sections of the carbimazole group showed significant histopathological changes to thyroid tissue as alteration of

lining epithelium of follicles into squamous epithelium, vacuolations of the colloid, and fusion between follicles. These results are explained by the relationship between thyroid follicular cell size and activity. The follicular cells are flat simple squamous when the follicles are inactive. The follicular cells are columnar while the follicles are active. The follicular cells are cuboidal epithelium in their normal state (Petrova et al., 2014). But it is also possible for several cells within the same thyroid tissue to exhibit varying degrees of activity (Khan & Farhana, 2022). Another mechanism attributed these alterations to the thyroid gland's sodium iodide symporter (NIS) being inhibited by reduced thyroglobulin production. The sodium iodide symporter or  $\text{Na}^+/\text{I}^-$  symporter (NIS) is an intrinsic plasma membrane glycoprotein that facilitates the active transport of iodide in the thyroid gland and other nonthyroidal organs, including the

mammary gland during lactation, the stomach, salivary, and lacrimal glands. Iodide, a crucial component of the thyroid hormones T3 and T4, accumulates in the thyroid as a result of functional NIS expression in the thyroid gland (Khan et al., 2005).

The findings of the present study are consistent with those of Shattnan et al. (2017) who investigated the effects of carbimazole-induced hypothyroidism on the thyroid gland, lipid profile, and protective effects of grape seed. Their findings revealed pronounced necrosis in the follicular cells and vacuolations of the colloid, which was present in small amounts. Another experiment into the protective effects of curcumin employed Lithium to cause thyroid failure in rats and revealed thyroid follicles lined with flat cells and having a minimal amount of colloid with most of the follicles having vacuolated cytoplasm in the lithium-treated group of animals (Abd El-Twab & Abdul-Hamid, 2016). Other investigators reported that follicular cells in hypothyroidism usually appear as high cuboidal or even columnar representing hypertrophy and hyperplasia of thyroid follicles which because of the increase in thyroid stimulating hormone (TSH) to compensate for reduced thyroid hormones. However, with the persistence of stress, the thyroid gland becomes exhausted as shown in the present study by the existence of most follicles lined with squamous epithelium that indicates hypoactivity (Aboul-Fotouh et al., 2018).

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Consistent with the previously mentioned histological alterations, animals of the carbimazole group showed markedly

decreased PAS-positive reaction which can be explained by colloid vacuolations. Other researchers reported similar results (AboulFotouh et al., 2018; Shehata et al., 2021). These alterations could take place in a hypothyroid condition as a result of a drop in thyroid hormone levels in the blood. As a result, the follicular cells respond to high TSH by removing thyroglobulin from the follicle lumen more quickly and continuously. This causes more vacuolations in the colloid. Additionally, endocytosis of the colloid often occurs at a rate larger than synthesis, leading to a gradual depletion of the colloid (Aboul-Fotouh et al., 2018).

Concerning the anti-calcitonin immunostaining of the carbimazole group, the present study showed increased cytoplasmic reaction of immunoreactive calcitonin C cells. These results were in agreement with other studies (Elkalawy et al., 2013; Mohammad et al., 2019). Other researchers linked C-cell changes in thyroid status with changes in follicular cells. When TSH rises and T4 falls, three possible pathways for hypothyroid state have been proposed: According to Martn-Lacave et al. (2009), TSH directly regulates C cells, follicular cells regulate C cells, and C cells regulate follicular cells. The first theory is supported by other researchers demonstrating how reactive C-cell hyperplasia manifested in rats when TSH levels were elevated (Nayyar et al., 1989). The second theory was clarified by some researchers who found that follicular cells might control C cells either by locally elevating T3 and T4 or by releasing regulatory substances. For instance, thyroglobulin and other compounds such as fibroblast growth factor are essential in the autocrine regulation of TSH-stimulated follicular cell

proliferation, differentiation, and thyroid hormone synthesis. According to this view, those products might also affect C cells in a paracrine manner (Sawicki, 1995; Eggo et al., 2003). According to the third theory, C cells contributed to the intrathyroidal control of secretion and growth by secreting various regulatory peptides present specifically in C cells. These peptides are typically referred to as "paracrine factors" and are thought to play this role in the thyroid. While gastrin-releasing peptide and helodermin were shown to be thyroid hormone secretion stimulators, other regulatory peptides, including calcitonin, somatostatin, and katacalcin, were found to be thyroid hormone secretion inhibitors (Sawicki, 1995).

Due to the production of free radicals and the weakening of the anti-oxidative defence system, hypothyroidism is a condition associated with increased oxidative stress (Das & Chainy, 2004; Sarandöl et al., 2005; Chakrabarti et al., 2016). Accordingly, it is believed that a key physiological regulator of in vivo cellular oxidative stress is variation in thyroid hormone levels. They are responsible for this because of the effects they have on mitochondrial respiration (Yilmaz et al., 2003). Free radical generation is enhanced in hypothyroidism-related respiratory chain failure in the mitochondria, which ultimately results in oxidative stress (Mancini et al., 2016). Particularly, it has been suggested that an increase in reactive oxygen species brought on by a deficiency in thyroid hormones can cause oxidative stress conditions in the liver, the heart, some skeletal muscles, and the rat cerebellum which then cause a lipid peroxidative response, tissue damage, and cell death (Yilmaz et al., 2003; Bhanja & Chainy,

2010). Oxidative stress can also be exacerbated by metabolic imbalance caused by autoimmune hypothyroidism (Carmeli et al., 2008).

In the current experiment, concomitant treatment of animals with *S. costus* root extract reversed most of the histopathological alterations caused by carbimazole. Other researchers reported similar findings (Alnahdi, 2017; Mahmoud, 2020). Our findings could be explained by the *costus* root extract's antioxidant properties, which protect the thyroid against carbimazole. The antioxidant properties of this compound may be due to its active components, flavonoids, anthraquinone, and many terpenes, including alpha- and betaamyrin, which inhibit NF-k activation (Alnahdi, 2017). *Costus* extract has a high amount of free radical scavenging activity, making it a cytoprotective agent, which causes significant damage to cell components (Bolkiny et al., 2019). Moreover, *costus* extract was also found to have a modulatory effect on the thyroid hormones (Alnahdi, 2017), as well as serum parameters in both hyperthyroid and hypothyroid mice (Mahmoud, 2020).

Our research was in agreement with earlier studies that looked into the *Saussurea costus* plant's antioxidant capacity. Recent studies investigated the antioxidant effect and thyroid gland regulating activities of *S. costus* root extract on thorium-induced brain



damage in adult rats. Thorium disrupted potassium and sodium ions, decreased monoamines in the brain, and induced oxidative stress, which was demonstrated by higher lipid peroxidation and lower glutathione levels. Triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) levels were also raised by thorium. They discovered that *S. costus* root extract taken orally beforehand reduced the effects of thorium, demonstrating its beneficial antioxidant capabilities (Pyun et al., 2018).

The antioxidant and modulatory effect of costus extract on thyroid hormones was investigated by Alnahdi, who proved that pre-treatment with *S. costus* root extract restored thyroid gland morphological alterations and up-regulated thyroid hormones in rats exposed to pesticides, indicating its protective efficacy against toxic effects of the pesticide deltamethrin (Alnahdi, 2017).

It is postulated that C cells and the nearby follicular cells interact, enabling more effectively coordinated actions between the two endocrine populations. The fact that mammalian C cells took a long embryonic route to reach their final destination in the thyroid gland, in the middle of each thyroid lobe, and close to the follicular cells, reinforced this. As a result, C cells may also be affected by major changes in the activity of follicular cells triggered by various substances (Martín-Lacave et al., 2009). Accordingly, the current study's improvement and restoration of the thyroid architecture by *S. costus* root extract also improved the C cells, as evidenced by the brownish cytoplasmic reactivity of C cells in sections from animals of the

carbimazole and costus group were like that of the control group.

## V. CONCLUSION

The present study concluded that *Saussurea lappa* root "Costus" restored the thyroid tissue damage under the hypothyroid condition induced by Carbimazole in adult male albino rats.

## VI. RECOMMENDATIONS

- Regular monitoring of T3, T4, and TSH before and during carbimazole administration.
- Administration of the aqueous extract of the *Saussurea lappa* root "Costus" during Carbimazole treatment to avoid or at least minimize occurrence of hypothyroidism.
- Further research is needed to investigate other protective agents against hypothyroid drugs.

## VII. DECLARATION OF INTEREST

The authors report no conflict of interest.

## VIII. FUNDING SOURCES

This research was not funded by any funding agency.

## IX. REFERENCES

- Abbara, A., Clarke, S. A., Brewster, R.,  
Simonnard, A., Eng, P. C.,  
Phylactou,  
M., Papadopoulou, D.,  
IzziEngbeaya, C., Sam, A. H.,  
Wernig, F., Jonauskite, E.,  
Comminos, A. N.,  
Meeran, K., Kelsey, T. W., & Dhillon,

- W. S. (2020). Pharmacodynamic Response to Anti-thyroid Drugs in Graves' Hyperthyroidism. *Frontiers in Endocrinology*, 11. <https://doi.org/10.3389/fendo.2020.00286>
- Abdel-Fattah, M. E., Mohammed, S. M., & Mohammed, I. H. (2015). Experimentally-induced thyroid dysfunctions on cardiac contractility in adult male albino rats. *Al-Azhar Assuit Medical Journal*, 13(3), 143151.
- Abd El-Twab, S. M., & Abdul-Hamid, M. (2016). Curcumin mitigates lithium-induced thyroid dysfunction by modulating antioxidant status, apoptosis, and inflammatory cytokines. *The Journal of Basic & Applied Zoology*, 76, 7–19. <https://doi.org/10.1016/j.jobaz.2016.10.001>
- Abd Eldaim, M. A., Tousson, E., El Sayed, I. E. T., & Awd, W. M. (2019). Ameliorative effects of *Saussurea lappa* root aqueous extract against Ethephon-induced reproductive toxicity in male rats. *Environmental Toxicology*, 34(2), 150–159. <https://doi.org/10.1002/tox.22669>
- Aboul-Fotouh, G., Abou El-Nour, R., Farag, E., & Boughdady, W. (2018). Histological study on the possible protective effect of curcumin on potassium dichromate induced hypothyroidism in adult male albino rats. *Egyptian Journal of Histology*, 41(2), 220–235. <https://doi.org/10.21608/EJH.2018.13844>
- Alnahdi, H. S. (2017). Injury in Metabolic Gland Induced by Pyrethroid Insecticide Could Be Reduced by Aqueous Extract of *Sassura lappa*. *International Journal of Pharmaceutical Research & Allied Sciences*, 6(2), 86-97.
- Bancroft, J. D., & Layton, C. (2019). 10—The hematoxylin and eosin. In S. K. Suvarna, C. Layton, & J. D. Bancroft (Eds.), *Bancroft's Theory and Practice of Histological Techniques* (Eighth Edition) (pp. 126–138). Elsevier. <https://doi.org/10.1016/B978-0-7020-6864-5.00010-4>
- Bhanja, S., & Chainy, G. B. N. (2010). PTU-induced hypothyroidism modulates antioxidant defense status in the developing cerebellum. *International Journal of Developmental Neuroscience*, 28(3), 251–262. <https://doi.org/10.1016/j.ijdevneu.2010.01.005>
- Bolkiny, Y., Tousson, E., El-Atrsh, A., Akela, M., & Farg, E. (2019). *Costus Root Extract Alleviates Blood Biochemical Derangements of Experimentally Induced Hypo- and Hyperthyroidism in Mice*. *Annual Research & Review in Biology*, 31(5), 1-10. <https://doi.org/10.9734/ARRB/2019/v31i530063>
- Britt, J. L., Powell, R. R., McMahan, C., Bruce, T. F., & Duckett, S. K. (2021). The effect of ergot alkaloid exposure during gestation on the microscopic

- morphology and vasculature of the ovine placenta. *Journal of Histotechnology*, 44(4), 173–181. <https://doi.org/10.1080/01478885.2021.1902670>
- Carmeli, E., Bachar, A., Barchad, S., Morad, M., & Merrick, J. (2008). Antioxidant status in the serum of persons with intellectual disability and hypothyroidism: A pilot study. *Research in Developmental Disabilities*, 29(5), 431–438. <https://doi.org/10.1016/j.ridd.2007.08.001>
- Chakrabarti, S. K., Ghosh, S., Banerjee, S., Mukherjee, S., & Chowdhury, S. (2016). Oxidative stress in hypothyroid patients and the role of antioxidant supplementation. *Indian Journal of Endocrinology and Metabolism*, 20(5), 674–678. <https://doi.org/10.4103/2230-8210.190555>
- Das, K., & Chainy, G. B. N. (2004). Thyroid Hormone Influences Antioxidant Defense System in Adult Rat Brain. *Neurochemical Research*, 29(9), 1755–1766. <https://doi.org/10.1023/B:NERE.0000035812.58200.a9>
- Eggo, M. C., Quiney, V. M., & Campbell, S. (2003). Local factors regulating growth and function of human thyroid cells in vitro and in vivo. *Molecular and Cellular Endocrinology*, 213(1), 47–58. <https://doi.org/10.1016/j.mce.2003.10.034>
- Elkalawy, S. A. M., Abo-Elnour, R. K., El Deeb, D. F., & Yousry, M. M. (2013). Histological and immunohistochemical study of the effect of experimentally induced hypothyroidism on the thyroid gland and bone of male albino rats: The Egyptian Journal of Histology, 36(1), 92–102. <https://doi.org/10.1097/01.EHX.0000424169.63765.ac>
- Habotta, O. A., Ateya, A., Saleh, R. M., & ELAshry, E. S. (2021). Thiamethoxam - induced oxidative stress, lipid peroxidation, and disturbance of steroidogenic genes in male rats: Palliative role of *Saussurea lappa* and *Silybum marianum*. *Environmental Toxicology*, 36(10), 2051–2061. <https://doi.org/10.1002/tox.23322>
- Hossain, A. O. (2019). Carbimazole and its Effects on Thyroid Gland of Female Rabbits. *Indian Journal of Forensic Medicine & Toxicology*, 13(3), 305. <https://doi.org/10.5958/0973-9130.2019.00214.7>
- Kadhim, S. H., Musa, A. U., al-kareem, Z. A., Ubaid, M. M., & Aziz, N. D. (2018). The analysis of the protective feature of *Nigella sativa* in reducing Carbimazole toxicity including liver and kidney parameters on Albino male rats. *Scientific Journal of Medical Research*, 02(05), 14–18. <https://doi.org/10.37623/SJMR.2018.2503>
- Khan, M. A., Fenton, S. E., Swank, A. E., Hester, S. D., Williams, A., & Wolf,

- D. C. (2005). A mixture of ammonium perchlorate and sodium chlorate enhances alterations of the pituitary-thyroid axis caused by the individual chemicals in adult male F344 rats. *Toxicologic Pathology*, 33(7), 776–783.  
<https://doi.org/10.1080/01926230500449832>
- Khan, Y.S., & Farhana, A. (2022). Histology, Thyroid Gland. [Updated 2022 Dec 5]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK551659/>
- Layton, C., & Bancroft, J. D. (2019). 13—Carbohydrates. In S. K. Suvarna, C. Layton, & J. D. Bancroft (Eds.), *Bancroft's Theory and Practice of Histological Techniques* (Eighth Edition) (pp. 176–197). Elsevier. <https://doi.org/10.1016/B978-0-7020-6864-5.00013-X>
- Léger, J., & Carel, J.-C. (2017). MANAGEMENT OF ENDOCRINE DISEASE: Arguments for the prolonged use of antithyroid drugs in children with Graves' disease. *European Journal of Endocrinology*, 177(2), R59–R67. <https://doi.org/10.1530/EJE-16-0938>
- Madhuri, K., Elango, K., & Ponnusankar, S. (2012). *Saussurea lappa* (Kuth root): Review of its traditional uses, phytochemistry and pharmacology. *Oriental Pharmacy and Experimental Medicine*, 12(1), 1–9. <https://doi.org/10.1007/s13596-011-0043-1>
- Mahmoud, M. S. (2020). Costus Root Extract Preserves Thyroid Hormones Levels, Thyroglobulin Expression and Thyroid Tissues in Rats Receiving Valproate Sodium. *The Indonesian Biomedical Journal*, 12(4), 304–312. <https://doi.org/10.18585/inabj.v12i4.1145>
- Mancini, A., Di Segni, C., Raimondo, S., Olivieri, G., Silvestrini, A., Meucci, E., & Currò, D. (2016). Thyroid Hormones, Oxidative Stress, and Inflammation. *Mediators of Inflammation*, 1–12. <https://doi.org/10.1155/2016/6757154>
- Martín-Lacave, I., Borrero, M. J., Utrilla, J. C., Fernández-Santos, J. M., de Miguel, M., Morillo, J., Guerrero, J. M., García-Marín, R., & Conde, E. (2009). C cells evolve at the same rhythm as follicular cells when thyroidal status changes in rats. *Journal of Anatomy*, 214(3), 301–309. <https://doi.org/10.1111/j.14697580.2008.01044.x>
- Mohammad, K. I., Mohammad, S. A., & Elfattah, A. M. A. (2019). Effect of the Possible Role of In vivo Mobilization of Bone Marrow Stem Cells and Sesame Oil on Induced Hypothyroidism in Adult Female Albino Rats. *Middle East Journal of Applied Sciences*, 9(2), 524–547.
- Nayyar, R. P., Oslapas, R., & Paloyan, E. (1989). Age related correlation between serum TSH and



thyroid C cell hyperplasia in Long-Evans rats. *Journal of Experimental Pathology*, 4(2), 87–95.

Petrova, I., Mitevska, E., Gerasimovska, Z., Milenkova, L., & Kostovska, N. (2014). Histological Structure of the Thyroid Gland in Apolipoprotein E Deficient Female Mice After Levothyroxine Application.

- PRILOZI, 35(3), 135–140.  
<https://doi.org/10.1515/prilozi-2015-0017>
- Pyun, H., Kang, U., Seo, E. K., & Lee, K. (2018). Dehydrocostus lactone, a sesquiterpene from *Saussurea lappa* Clarke, suppresses allergic airway inflammation by binding to dimerized translationally controlled tumor protein. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, 43, 46–54. <https://doi.org/10.1016/j.phymed.2018.03.045>
- Sakr, S. A. R., Mahran, H. A., & Nofal, A. E. (2011). Effect of selenium on carbimazole-induced testicular damage and oxidative stress in albino rats. *Journal of Trace Elements in Medicine and Biology*, 25(1), 59–66. <https://doi.org/10.1016/j.jtemb.2010.07.002>
- Sakr, S. A. R., Mahran, H. A., & Nofal, A. E. (2012). Effect of Selenium on Carbimazole-Induced Histopathological and Histochemical Alterations in Prostate of Albino Rats. *American Journal of Medicine and Medical Sciences*, 2(1), 5–11. <https://doi.org/10.5923/j.ajmms.20120201.02>
- Sarandöl, E., Taş, S., Dirican, M., & Serdar, Z. (2005). Oxidative stress and serum paraoxonase activity in experimental hypothyroidism: Effect of vitamin E supplementation. *Cell Biochemistry and Function*, 23(1), 1–8. <https://doi.org/10.1002/cbf.1119>
- Sawicki, B. (1995). Evaluation of the role of mammalian thyroid parafollicular cells. *Acta Histochemica*, 97(4), 389–399. [https://doi.org/10.1016/S0065-1281\(11\)80064-4](https://doi.org/10.1016/S0065-1281(11)80064-4)
- Shattnan, D., Alumeri, J., & Al-Naely, A. (2017). \*Grape seed extract role against carbimazole action on thyroid gland and lipid profile in male rats. 23, 9–16. <https://doi.org/10.29350/jops.2018.23.2.733>
- Shehata, M. M., Mostafa, N. A. M., Metwally, A. M., & Gomaa, A. M. S. (2021). Do resident thyroid stem cells have a role in regeneration of hypophyseal thyroid axis after experimentally induced hypothyroidism in male rats? A histological and immunohistochemical study. *Journal of Current Medical Research and Practice*, 6(3), 247. [https://doi.org/10.4103/jcmrp.jcmrp\\_133\\_20](https://doi.org/10.4103/jcmrp.jcmrp_133_20)
- Tousson, E., El-Atrsh, A., Mansour, M., & Abdallah, A. (2019). Modulatory effects of *Saussurea lappa* root aqueous extract against ethephon - induced kidney toxicity in male rats. *Environmental Toxicology*, 34(12), 1277–1284. <https://doi.org/10.1002/tox.22828>
- Visser, T. J. (2018). Regulation of Thyroid Function, Synthesis and Function of Thyroid Hormones. In P. Vitti & L. Hegedus (Eds.), *Thyroid Diseases* (pp. 1–30). Springer International Publishing.

[https://doi.org/10.1007/978-3-31929195-6\\_1-1](https://doi.org/10.1007/978-3-31929195-6_1-1)

Yilmaz, S., Ozan, S., Benzer, F., & Canatan, H. (2003). Oxidative damage and antioxidant enzyme activities in experimental hypothyroidism. *Cell*

*Biochemistry and Function*, 21(4), 325–330. <https://doi.org/10.1002/cbf.1031>

Zahara, K., Tabassum, S., Sabir, S., Arshad, M., Qureshi, R., Amjad, M. S., & Chaudhari, S. K. (2014). A review of potential of *Saussurea* therapeutic

*lappa*-An endangered plant from Himalaya. *Asian Pacific Journal of Tropical Medicine*, 7, S60–S69. [https://doi.org/10.1016/S1995-7645\(14\)60204-2](https://doi.org/10.1016/S1995-7645(14)60204-2)

## الدور المحسن لمستخلص جذر سوسوريا لآبا "القسط" على أنسجة الغدة الدرقية تحت التأثير السام للكارببمازول المسبب لقصور الغدة الدرقية

إبريني فكري<sup>2</sup>، منى محمد عوني<sup>1</sup>، جورج ناجي رفعت<sup>1</sup>، حورية عرفات<sup>2</sup>

<sup>1</sup> قسم علم الأنسجة وبيولوجيا الخلية، كلية الطب، جامعة قناة السويس، الإسماعيلية، مصر - <sup>2</sup> قسم الطب الشرعي والسموم الإكلينيكية، كلية الطب، جامعة قناة السويس، الإسماعيلية، مصر - <sup>3</sup> قسم الأمراض الباطنة بمستشفيات هيئة قناة السويس، الإسماعيلية، مصر.

**المقدمة:** يعتبر عقار الكارببمازول واحدا من مثبطات الغدة الدرقية ويستخدم لعلاج فرط نشاط الغدة الدرقية. ومع ذلك، يمكن أن يؤدي العلاج بالكارببمازول بدون تقليل جرعة البدء الأولية تدريجياً إلى قصور الغدة الدرقية وتأثيرات سامة على أنسجتها. جذر لآبا سوسوريا "القسط" هو علاج تقليدي يحتوي على خصائص مضادة للالتهابات ومضادة للأكسدة ومعدلة للمناعة. وقد صممت هذه الدراسة لتحديد ما إذا كانت التغيرات السامة في الغدة الدرقية المحدثة بعقار الكارببمازول المسبب لقصور الغدة في ذكور الجرذان البيضاء البالغة يمكن تحسينها عن طريق مستخلص جذر سوسوريا لآبا "القسط".

**المواد والطرق:** أجريت هذه الدراسة على أربعة وعشرين من ذكور الجرذان البيضاء بعد تقسيمهم إلى أربع مجموعات بشكل عشوائي. وقد تلقيت حيوانات البحث جميع العلاجات عن طريق الفم عن طريق أنبوب داخل المعدة. المجموعة الأولى (المجموعة الضابطة). تلقت المجموعة الثانية المستخلص المائي لجذور القسط (05 ملجم / كجم من وزن الجسم / يومين). المجموعة الثالثة تلقت كارببمازول (2 مجم / 155 جرام من وزن الجسم / يوم). تلقت المجموعة الرابعة كارببمازول مصاحباً للمستخلص المائي لجذور القسط. ثم بعد 4 أسابيع، تم قطع رأس الحيوانات. ثم تم إعداد شرائح من الغدة الدرقية وصبغتهم بالهيماتوكسيلين والإيوسين، وكاشف شيف الحامضي، والصبغة الهستوكيميائية المناعية ضد الكالسيونين. ثم أجريت بعض القياسات باستخدام برنامج Image J وهي متوسط النسبة المئوية لتفاعل المادة الغروانية مع كاشف شيف الحامضي، ومتوسط النسبة المئوية لمساحة الخلايا الجار حويصلية المتفاعلة مع الصبغة الهستوكيميائية المناعية ضد الكالسيونين ثم تم تحليل البيانات الناتجة إحصائياً.

**النتائج:** أظهرت مجموعة الكارببمازول تغيرات نسيجية واضحة في الخلايا المبطنة لحويصلات للغدة الدرقية والتحام هذه الحويصلات كما أدى الى نقص في المادة الغروانية داخل الحويصلات والذي يوضح تثبيط في نشاط الغدة الدرقية، كما تسبب الكارببمازول في انخفاض ملحوظ في التفاعل الإيجابي لكاشف شيف الحامضي بسبب الفجوات الغروانية وزيادة النشاط المناعي السيتوبلازمي للخلايا الجار حويصلية. كما أوضحت النتائج



أن

القسط يمكن أن يستعيد جميع التغيرات النسيجية المرضية تقريباً ويحسن الخلايا الجار حويصلية ويقلل من نشاطها المناعي السيتوبلازمي.

**الخلاصة:** يمتلك جذر سوسوريا لبا "القسط" تأثيراً وقائياً ضد تلف الغدة الدرقية الناجم عن الكارببمازول.  
**التوصيات:** هذا ويوصي الباحثين بضرورة متابعة متوى هرمونات الغدة الدرقية بالدم قبل وأثناء العلاج بالكارببمازول، وإعطاء خلاصة جذر سوسوريا لبا "القسط" أثناء العلاج بالكارببمازول وذلك لتجنب أو تقليل قصور الغدة الدرقية، وأخيراً يوصي الباحثون بإجراء أبحاث أخرى لدراسة مواد أخرى تحمي من قصور الغدة الدرقية.