Study of the effect of *Fucus Vesiculosus* algae and relationship to thyroid hormones in rabbit serum dosed with Thiourea.

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Abstract

A biochemical study was conducted to identify the effect of *Fucus Vesiculosus algae* , which is directly related to hypothyroidism in the serum of adult white rabbits dosed with the drug thiourea, by measuring the level of thyroid hormones, antioxidants, and oxidative stress in the blood serum of (28) rabbits. The animals were divided. into four groups, in the following order:

• The positive control group was dosed orally with Distil water.

• The Negative control group: - dosed orally with Thiourea at a concentration of 5 mg/cm3.

• The first group G1 :- was dosed with 5 mg/kg/day of thiourea and then 100 mg/kg/day of crude extract of *Fucus Vesiculosus algae* .

• As for group G2, they were dosed with 5 mg/kg/day of thiourea and then 150 mg/kg/day of the crude extract of *Fucus Vesiculosus algae* . The results showed the following:

The results showed that the level of TSH showed a significant decrease in all groups compared to the positive control group in the fourth week of dosing. Also the T3 hormone level showed a significant increase in all groups C1, G1, and G2 compared to the positive control in the fourth week of dosing. In addition, the T4 hormone showed a significant increase in all groups C2, G1, and G2 in the fourth week of dosing. As well as the glutathione level, it showed a significant decrease in all groups compared to the positive control group in the fourth week of dosing. Also the malondialdehyde showed a significant increase in the serum of the groups compared to the positive control group in the fourth week of dosing.

Keywords: *Fucus Vesiculosus algae* , Thyroid hormones, thiourea, glutathione, malondialdehyde.

Introduction

Medicinal plants were and still are the basis through which the pharmaceutical and pharmaceutical industry has developed. Plants have been exploited as a source of medicines throughout human history

and in all cultures. Scientific progress has allowed us to identify a small portion of the active compounds and their mode of action, as medicinal plants continue to contribute effectively to human health and progress, especially since many voices around the world are calling for a strong return to nature in many fields related to Human health because plants were and still are a source of food and medicine for humanity. Since ancient times, man has known many medicinal plants and herbs that grow wild in his environment. He found that many of them are useful in food and a few work as medicine. He also learned about their properties and determined their benefits by using them. They use traditional medicine to treat and remove diseases [1,2].

Algae and its extracts have also been used in various applications in various fields such as the pharmaceutical industry, environmental treatments, and biotechnology, in addition to their use as powerful naturally occurring anti-cancer compounds that have been proposed to prevent carcinogenesis and to treat many diseases, including diarrhea, lung diseases, and bladder disorders [3, 4]. In addition to the use of many herbs in regulating thyroid hormones, such as Fucus vesiculosus algae, which is rich in iodine, as Hameed and her group indicated that the algae has A role in regulating the level of thyroid hormones and improving its function [5]. Brown algae, widespread on rocky sea coasts in temperate and cold regions, has also been used to treat the thyroid gland because it contains high amounts of iodine [6]. Moss is a genus of brown algae and is characterized by its greenish-brown color as a result of it containing a layer of fucoxaxanthin, and when it dries it turns black [7].

Fucus Vesiculosus algae is used to treat cancerous tumors, especially colon cancer, as a result of it containing high amounts of fibers [8]. It is considered an antioxidant due to its high ability to attack free radicals [9]. Also, algae contains large amounts of iodine that can change the levels of thyroid hormones [10]. The thyroid gland constitutes an important component of the endocrine system that regulates many physiological functions ranging from oxygen use, growth and development to cellular metabolism [11]. Thyroid hormones are considered an essential element in the growth of the body and affect the formation of many enzymatic proteins. These hormones control most of the metabolic processes in the body and any imbalance in these hormones may have a serious clinical impact on the various organs of the body [12].

Glutathione is a non-enzymatic antioxidant that contains vital compounds and defense systems against the danger of free radicals [13]. Glutathione is involved in many metabolic processes, and its deficiency leads to cellular danger as a result of oxidative damage (14). It has multiple functions, most of which are maintaining cellular redox balance, maintaining adequate levels of cystine and removing toxins from foreign substances entering the body [15].

MDA is an indicator of increased oxidative stress and an indicator of oxidation as well as the incidence of some diseases, as the level of MDA increases in cancer patients [16,17]. Fats are one of the most vulnerable biomolecules to oxidative stress, and MDA is the main product of fat oxidation. It is a toxic molecule and must be taken into consideration as it is not only a sign of fat peroxidation, but it can interact with proteins and DNA, leading to the occurrence of oxidative stress (18). 19), so the current research aimed to study the effect of Fucus vesicularis algae and its relationship with thyroid hormones in the serum of adult white rabbits dosed with the drug thiourea.

Materials and methods

• Animals used in the study

(28) adult local white rabbits were used in this study. Their weights ranged from (1-2) kg, and their ages were (4-6) months. The study was conducted for the period from the beginning of April until May of the year 2023, in the animal house at the university. The animals were placed in iron cages covered with metal covers, with a floor covered with sawdust, and the hygiene aspect of the cages was taken into account by replacing the sawdust two to three times a week and sterilizing the cages with disinfectants, and the animals were fed daily and regularly with ready-made feed and water.

• Experiment design

The animals were randomly divided into four groups, with (7) rabbits for each group. The animals were dosed orally with (5 mg/kg/day) of thiourea to induce hypothyroidism, and then they were dosed with the crude extract of Fucus vesica, according to the following groups:

- Positive control group C1: was dosed orally with plain water.

- Negative control group C2: was dosed orally with thiourea at a concentration of 5 mg/kg/day.

- The first group, G1: was dosed orally with (5 mg/kg/day) of thiourea and then dosed with (100 mg/kg/day) of raw algae extract.

- The second group, G2: was dosed orally with (5 mg/kg/day) of thiourea and then dosed with (150 mg/kg/day) of raw algae extract.

• Collect blood samples

After four weeks of dosing, the animals were starved for 12 hours and blood was drawn from them using the heart puncture method. Then the dosing and blood drawing continued in the same way in the fourth week of dosing, as (4) cm3 of blood was collected in the fourth week of dosing. The blood was then emptied into clean, dry plastic tubes (One-time use) Free of anticoagulants. The blood was separated using a centrifuge at a speed of 2500 rpm for 10 minutes. The blood serum was obtained and stored at -20°C after dividing it into three parts in small Eppendrof tubes until the tests were performed. Biochemistry of thyroid hormones, antioxidants, and oxidative stress.

Determination of T_3,T_4 Hormones

It is allowed to concentrate both T3, TSH, and T4 by following the steps attached to the clarity kit prepared by the American company Monobind, and specifying the manufacturer's instructions for the ELISA technology, as they are marked up to T3 according to the method [20]. As for the T4 level, it is determined according to method [21], while TSH is determined according to method [22].

Estimation of glutathione and malondialdehyde levels in blood serum

The concentration of glutathione in serum was estimated using the modified method used by researchers (Sedlak & Lindsay; Tietz) [23]. The concentration of malondihyde in the serum was also estimated using the modified method used by the researchers (Guidet & Shah) [24].

Statistical analysis

The Statistical analysis system - SAS 2012 was used, using Duncun's Multiple Range test to compare between the groups. The Least Significant Differences - LSD was used in the test for the importance of comparing means in this study. Special differences between the groups were also identified in The fourth week of dosing using the T.Test [25].

Results and discussion

Estimation of thyroid hormones for the samples under study:

Table (1) shows the mean ± standard deviation of thyroid hormones for the samples under study

Groups / Parameter	Mean ± SD			
	C1 n =28	C2 n =28	G1 n =28	G2 n =28
TSH (µIU /ml)	0.097±0.036 a	0.023±0.011c	0.047±0.032b	0.036±0.005c
T3 (ng/ml)	3.625±0.649a	2.721±0.437b	2.521±0.427b	2.012±0.283c
T4 (µg/ml)	1.352±0.222c	2.254±0.443b	3.704±1.032a	2.649±0.500b

Thyroid hormones

The protective effect of crude Fucus Vesiculosus algae extract against the effect of thiourea induced hypothyroidism was studied by monitoring the level of thyroid hormones.

Estimation of thyroid-stimulating hormone in blood serum

Table (1) shows that the hormone level for the positive control group was (0.097±0.036) micro-IU/ml, while the negative control group showed (0.023±0.011) micro-IU/ml treated with thiourea. As for groups G1 and G2, it reached (0.047 ±0.032), (0.036±0.005) [micro international units/ml, respectively, and treated with the crude extract of Fucus vesica algae.

The results indicate that the level of thyroid-stimulating hormone showed a significant decrease at the probability level (P≤0.05) in the control group compared to the other groups, as in Figure (1).

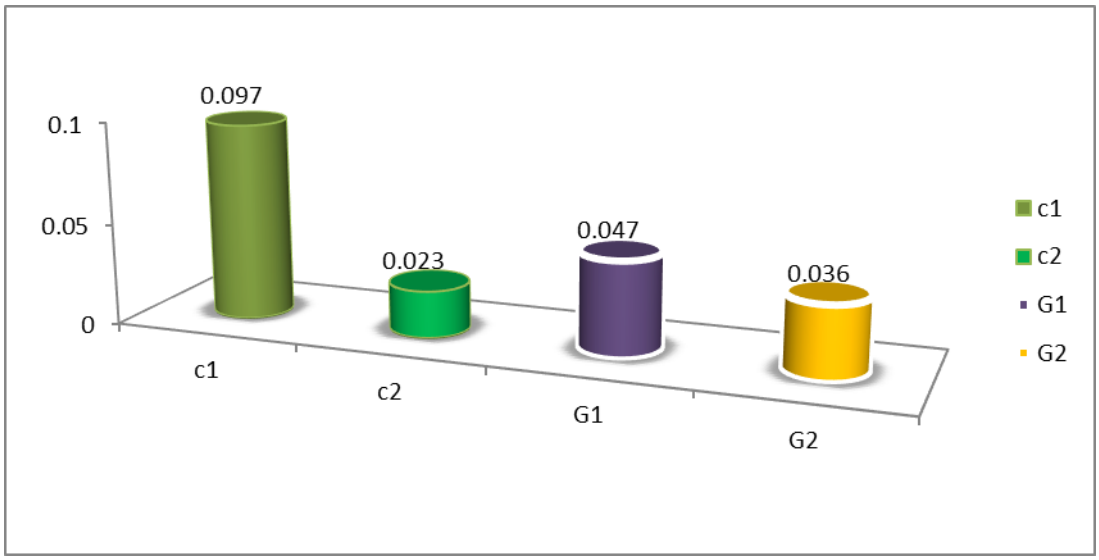

Figure (1): The level of TSH in blood serum.

Research did not indicate a therapeutic attempt for hypothyroidism using Fucus vesicular algae at the hormone level, but Al-Samarrai [9] The effect of raw algae may cause a decrease in the level of thyroid-stimulating hormone in the blood serum of adult white rabbits.

The low level of the hormone secreted by the pituitary gland in group G2 is due to the thyroid gland not responding to the hormone, and thus its level in the blood rises. It may sometimes cause benign hyperplasia of the thyroid gland, and at high concentrations it can cause cancer in rodents. Hyperplasia of the pituitary gland occurs as a result of the hyperfunction required to secrete the hormone TSH [26]. .Also, the decrease in the hormone level in the fourth week of dosing may be due to the role of the raw moss extract in reducing the effect of thiourea, restoring the thyroid gland to its function, and blocking the harmful effect of thiourea. This is due to the properties of the moss as it contains antioxidant compounds that have enhanced the role of the immune system in eliminating toxicity. Thiourea.

Level of T3 hormone

Table (1) shows that the T3 hormone level for the positive control group was (0.649±3.625) ng/ml, while it was (0.437±2.721) ng/ml, and the treatment with thiourea for the negative control group. As for groups G1 and G2, it reached (0.427±2.521). (0.283±2.012) ng/ml respectively and treated with crude extract of *Fucus Vesiculosus algae* .

The results showed that the average level of the hormone decreased significantly in the negative control group compared to the positive control group. The activity of the hormone also decreased at the probability level (P≤0.05) in the blood sera of the two groups treated with the crude extract compared to the negative control group. Here the effective and protective role of the crude extract against the effect of thiourea and its regulation becomes clear. The work of the gland is as shown in Figure (2).

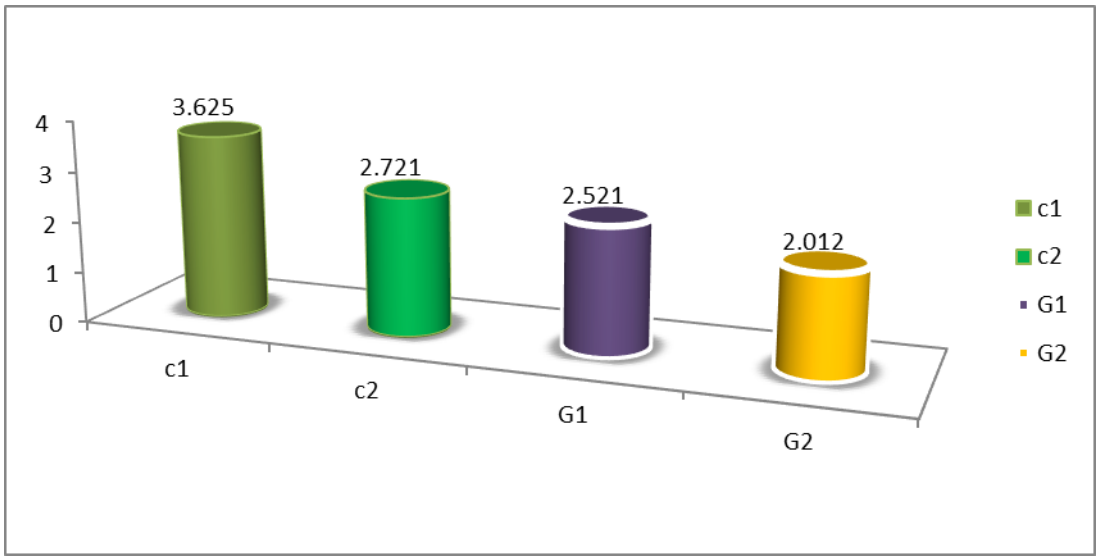

Figure (2): Level of T3 in blood serum

The reason for a decrease in the hormone level in the group treated with thiourea as a negative control group may be due to the interference that occurs due to thiourea in the path of building glandular hormones and its ability to convert iodine to the inorganic form [(27)]. Also, the fucus moss contains iodine, which may obscure the effect of thiourea as an additional source of iodine.

Or the reason for the decrease in the hormone may be explained by an inhibition in the activity of the 5-Monodeiodinase enzyme, which controls the conversion of the T4 hormone to T3 by thiourea, thus causing a decrease in the hormone level and an increase in the level of the T4 hormone [(28)], if this result is consistent with the level of the T4 hormone that was recorded. An increase in the fourth week of dosing, and the reason for the decrease may also be due to the fact that the T3 hormone stimulated the secretion of thyroid-stimulating hormone.

TSH from the pituitary gland through a feedback mechanism, and when this cause is removed, the hormones will return to their normal levels [(29).]

The T3 hormone is 3-5 times more effective in peripheral blood than T4. It also has a low affinity for plasma proteins, which makes it spread outside the vessels more easily than T4. Therefore, T3 is more important at the cellular level than T4. Therefore, it is believed that T3 is the only effective hormone and that T4 is the hormone's generator, as it is transformed in the cell into T3 after the loss of an iodine atom through the deiodenate process, and thus T3 can be considered the biologically active hormone [(30).]

Level of T4 hormone

Table (1) shows that the hormone level for the positive control group was (1.352±0.222) micrograms/cm3, while the negative control group showed (2.254±0.443) micrograms/ml and treated with thiourea. As for groups G1 and G2, it reached (3.704±1.032). (2.649±0.500) respectively and treated with the crude extract of *Fucus vesicular algae.*

The results showed that the average hormone level increased significantly and at the probability level ($P \leq 0.05$) in the blood sera of all groups under study, C2, G1, and G3, compared to the positive control, as in Figure (3).

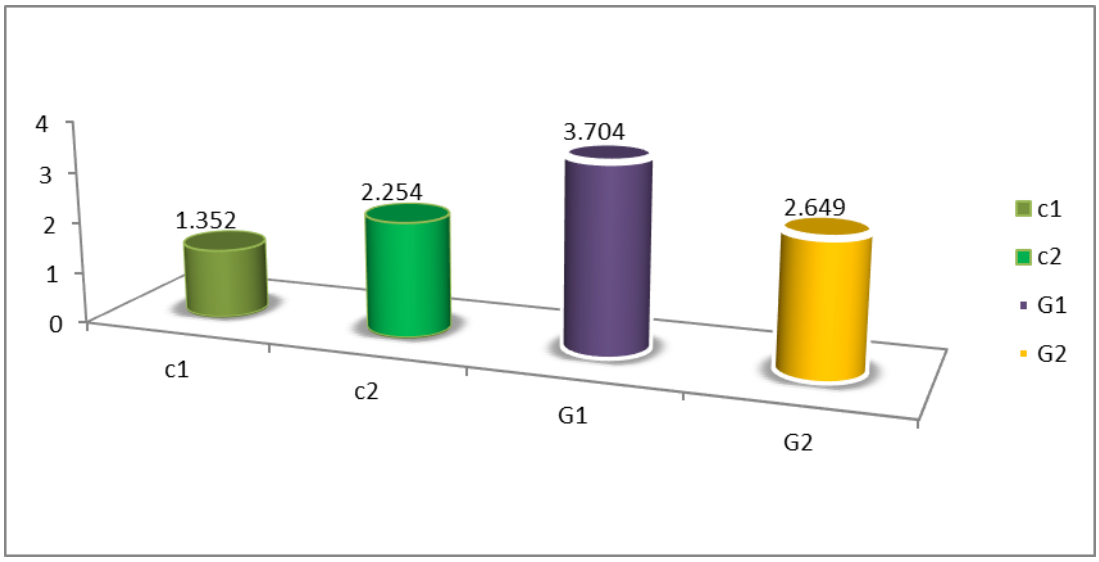

Figure (3): level of T4 in blood serum

Studies have not indicated a therapeutic attempt for hypothyroidism using *Fucus vesicular algae* at the hormone level, but the results of Al-Samarra [9]. It indicated that raw fucus moss shows an increase in the hormone level in healthy groups treated with the moss.

The reason for the increase in the hormone in the negative control group may be due to the cessation of its conversion into T3 hormone within the tissues, which leads to an increase in its concentration in the blood [31]. Or the reason for the high T4 hormone may be due to the ability of raw algae to stimulate the secretion of thyroid hormones [32], This is through the effect of the extracts on the receptors designated for binding to the TSH hormone, which is located on the surface of the basement membrane in epithelial cells, at the level of activation of the adenyl cyclase enzyme located in the cell membrane, which increases the production of cAMP in the cell [30]. Thus, cAMP plays a role as a secondary carrier to activate the basic enzymatic system of thyroid cells, and this process results in a rapid increase in the secretion of thyroid hormones, including T4 [28].

• Measuring the levels of antioxidants and oxidative stress of the samples under study:

Table (2) shows the average ± standard deviation of antioxidants and oxidative stress for the samples under study.

Groups / Parameter	Mean ± SD			
	C1 n =28	C2 n =28	G1 n =28	G2 n =28

GSH (µ mol/l)	**2.408±0.219 a**	**1.510±0.103 b**	**1.880±0.126 c**	**1.756±0.116 b**
MDA (µ mol/l)	**1.560±0.201 c**	**3.474±0.312 a**	**2.282±0.214 b**	**2.768±0.259 a**

Level of glutathione in blood serum

Table (2) shows that the mean ± standard deviation of the glutathione level for the positive control group was (2.408±0.219) micromol/L, while the negative control group showed (1.510±0.103) micromol/L and treated with thiourea. As for groups G1 and G2, they reached (1.880±0.126) and (1.756±0.116) micromol/L, respectively, treated with raw vesicular focus

The results showed that the average level of glutathione decreased significantly in the negative control group compared to the positive control group, and it also decreased significantly in groups G2 and G1 compared to the positive and negative control groups and at the probability level ($P \leq 0.05$) , as in Figure (4).

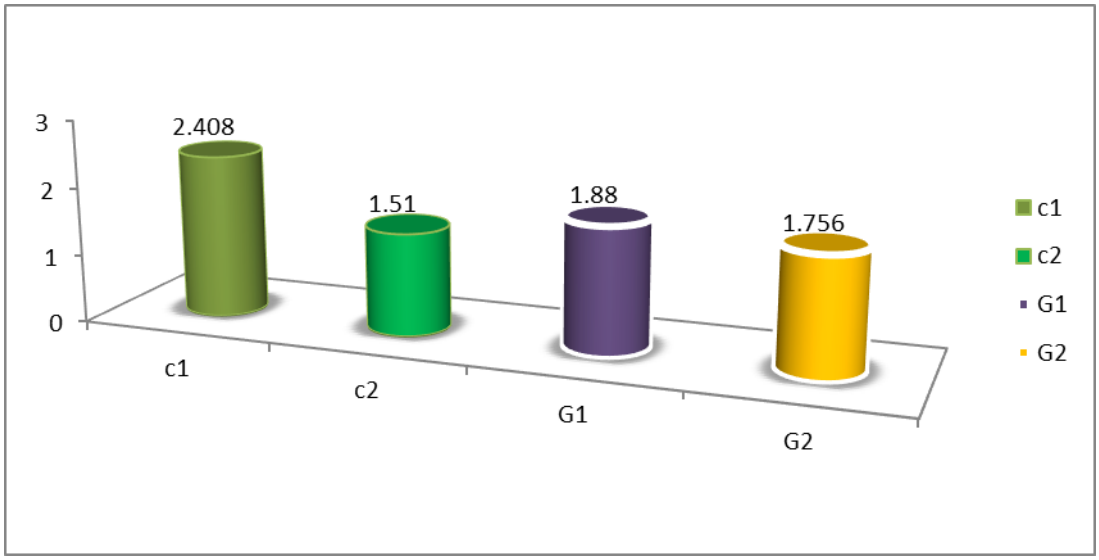

Figure (4): Level of GSH in blood serum

The literature did not indicate the effect of a treatment attempt for hypothyroidism using Fucus vesica algae on the level of glutathione, but the results were inconsistent with the results of [9]. While the results agree with Muhaemin [33], who observed an increase in the level of glutathione as a result of the influence of strains of algae. Chlorella allipsoideq، Scenedsmus acutus، Scenedesmus dimorphus

The reason for the decrease in the level of glutathione in the negative control group may be attributed to the effect of thiourea, as it is a toxic substance to liver cells and other tissues. The reason for the decrease in the level of glutathione in group C2 compared to C1 may be attributed to the fact that thiourea may have caused a deficiency in the raw materials for its construction, especially the coenzyme. (Reduced form) Nicotinamide adenine dinucleotide phosphate (NADPH) produced by the pentose phosphate pathway Which is the catalyst for the action of the glutathione reductase enzyme,

which works to restore the active form of glutathione from the inactive form [34]. Or it may be due to the fact that thiourea led to the consumption of glutathione, which is one of the most important non-enzymatic antioxidants in removing free radicals and their products. Then it is transformed from the active form to the inactive form, glutathione disulfide. The sulfur group in the glutathione synthesis is a good reducing agent, as a hydrogen atom is blown away. Easily due to the weak bond between sulfur and hydrogen (S-H) and the strong bond between carbon and hydrogen (C-H). In free radicals, it protects cell membranes from free radical damage [35]. The reason for this decrease in the concentration of glutathione may also be attributed to the fact that hypothyroidism resulting from thiourea leads to an increase in oxidative stress and a decrease in the GSH-GSSG ratio, as well as a decrease in the effectiveness of the enzymes catalase and superoxide dismutase, as well as its possible effect on the liver, as it can cause damage to the liver cells. Causing hepatic damage, which leads to an increase in free radicals and active oxygen species (ROS) and a decrease in the concentration of glutathione in blood serum [36].

level of malondialdehyde

Table (2) shows that the average ± standard deviation of the MDA level for the positive control group was (1.560±0.201) micromol/L, while the negative control group showed (3.474±0.312) and those treated with thiourea. As for groups G1 and G2, it amounted to (2.282±0.214). Micromol/L, (2.768±0.259) Micromol/L, respectively, treated with raw fucus.

The results showed that the average MDA level increased significantly in group C2 compared to group C1, and groups G2 and G1 recorded a significant increase at the probability level (P≤0.05) compared to the positive and negative control group, as in Figure (5).

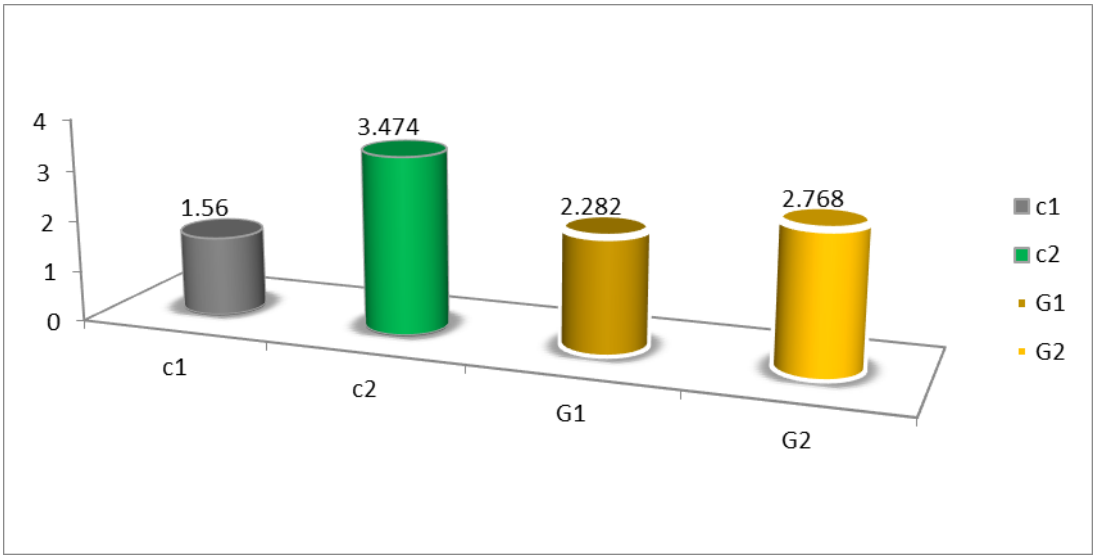

Figure (5): Level of MDA in blood serum

The literature did not indicate the effect of a treatment attempt for hypothyroidism using Fucus vesica moss on the level of malondialdehyde, but the results agreed with the results of Al-Samarrai [9].

The reason for the high concentration of malondialdehyde is attributed to the fact that thiourea may have caused an increase in the speed of consumption of antioxidant defense systems, leading to tissue damage [37]. The increase may also be due to an increase in the activity of free radicals that exceed the

ability of antioxidants to remove or neutralize them, causing an increase in lipid peroxidation and thus leading to an increase in the level of MDA and a loss of the balance between the effectiveness of free radicals and the activity of antioxidants, which leads to a loss in the elasticity of cellular membranes [38]. The reason may also be that the state of oxidative stress may affect the pancreatic β-cells and insulin secretion and thus lead to a decrease in the concentration of insulin in the blood. This stimulates and increases the activity of the enzyme Fatty acyl CoA oxidase, which stimulates

The process of β-oxidation of fatty acids and increased hydrogen peroxide formation and ultimately increased rates of lipid peroxidation and malondialdehyde production [39].

However, the reason for the decrease in the level of malondialdehyde in the groups treated with raw moss may be due to the fact that Fucus vesica contains active and protective compounds that have an important role in reducing oxidative damage and its ability to scavenge free radicals, as increasing the concentration of substances is proportional to the ability to reduce levels of oxidative stress. By reducing the level of malondialdehyde [40].

Histological study

Thyroid gland tissue

The results of the current study showed the effect of thiourea on the tissue of the thyroid gland, as it was observed that necrosis and rupture occurred in the colloidal vesicles of the connective tissue of the gland, with the observation of fibrin deposition in the blood vessel, as shown in Figure (6) compared to Figure (7), in which the gland cells appeared normal, as Connective tissue barriers between the colloidal follicles (normal follicle cells and blood vessels) appear normally, and part of the parathyroid gland tissue appears.

Figure (8) shows for the group treated with raw algae extract at the lower concentration, the appearance of necrosis in the colloidal vesicles and deposition of fibrin, while the cells in Figure (9) appeared almost normal and the necrosis and dehiscence disappeared, with no fibrin deposits observed in the blood vessel when treated with the higher concentration of the extract. Raw algae, which is rich in antioxidants, which work to protect thyroid cells and body cells from the danger of free radicals resulting from treatment with thiourea.

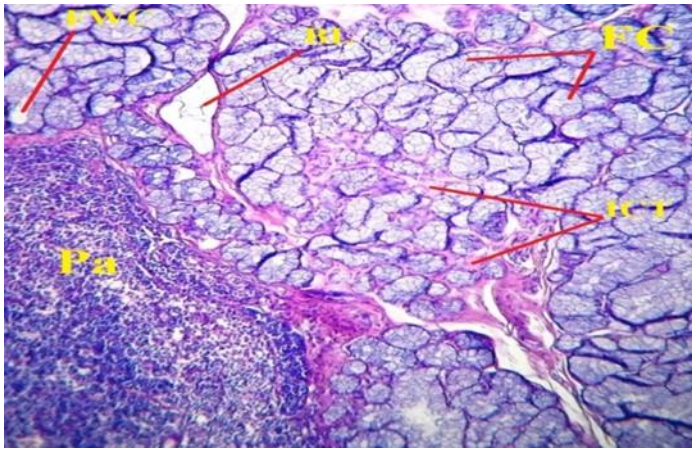

Figure (6): A cross-section of the thyroid gland showing the connective tissue barriers between the colloidal follicles (normal follicular cells) and the blood vessels normally, with part of the gland observed next to the normal thyroid gland for the positive control group (C1) x 200 (HE).

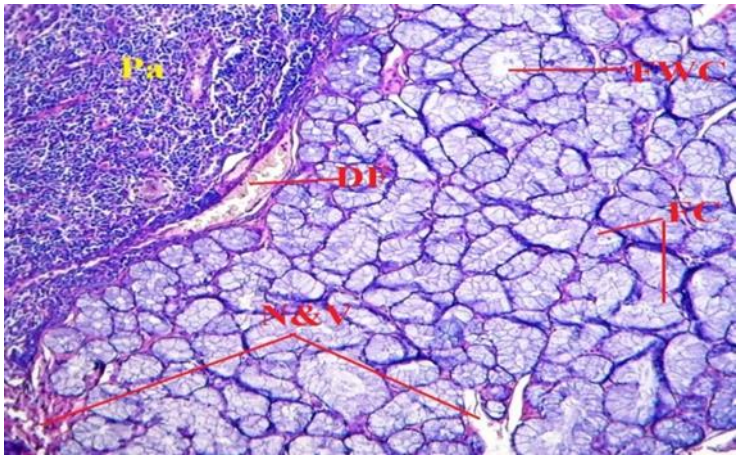

Figure (7): Cross-section showing necrosis and rupture of colloidal vesicles and connective tissue with fibrin deposition in the blood vessel of the negative control group (C2)

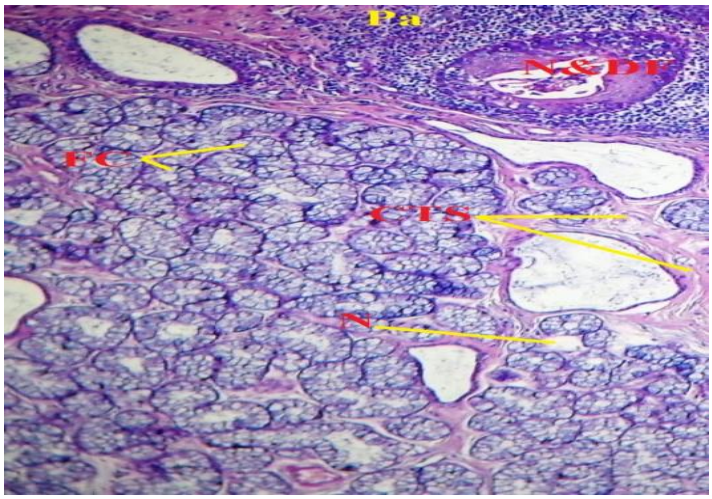

Figure (8): A cross-section showing necrosis in colloidal vesicles, noting necrosis and fibrin deposition next to the thyroid gland for group G1

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