

Role of Fucus vesiculosus L extract in the regulation of thyroid hormones status in adult male rabbits

Hameed, Rafah Razooq; Atya, Kalif Faris and Mohameed, Maha Atya

Department of chemistry, college of education university of Samarra.

Key words: Fucus vesiculosus L., Flavonoids, Thyroid hormones, Antioxidant, malondialdehyde.

(Received :February 2014, Accept : Jun 2014)

Abstract:

Background: The thyroid hormones triiodothyronine (T3) and thyroxine (T4) are formed in a large prohormone molecule. The formation of this hormones depends on an exogenous supply of iodide. So that normal thyroid hormone synthesis requires iodide, *Fucus vesiculosus* (also known by the common name bladder wrack) is a marine algae rich in iodine, which is being used in alternative medicine as a complement (for weight loss and as source of iodine) and in treatment of thyroid disorders, particularly hypothyroidism.

Aim: Evaluation the use of Fucus vesiculosus in alternative medicine as complement and regulator for the thyroid hormones.

Methods: This study presents the effects of aqueous extract and isolated flavonoids from Fucus vesiculosus L algae to the level of thyroid-stimulating hormone-TSH, T3,T4, antioxidant parameters:glutathione-GSH, glutathione peroxidase-GPx, peroxy nitrite and malondialdehyde-MDA in sera of 35 adult male rabbits divided randomly into seven groups (5 animals in each group), in which G₁, G₂ and G₃ treated with (25,50and75) mg/kg/day of aqueous extract of algae respectively, while the G₄, G₅ and G₆ were treated with (25,50and75)mg/kg/day of isolated flavonoids, compared with non-treated as control group.

Results: The results indicate that the level of TSH was significantly decreased ($p \leq 0.05$) in sera of rabbits in G₃ G₄, G₅ and G₆ with no significant change in G₁ and G₂ as compared with control, while the level of T4 were significantly decreased ($p \leq 0.05$) in sera of all six groups G₁, G₂, G₃, G₄, G₅ and G₆ as compared with control. The levels of T3 show significantly increased ($p \leq 0.01$) in sera of G₁, G₂ G₄ and G₆ with no significant change in G₃ as compared with control, the levels of GSH were significantly decreased ($p \leq 0.05$) in sera of G₁, G₂, G₄, G₅ and G₆ with no significant change in G₃ as compared with control. Otherwise the levels of GPx were significantly increased ($p \leq 0.05$) in sera of G₂ and G₆, and decreased in G₅ with no significant change in G₁, G₃ and G₄ as compared with control, while the levels of peroxy nitrate were

significantly decreased ($p \leq 0.05$) in sera of G_1, G_3, G_4, G_5 and G_6 and significantly increased ($p \leq 0.05$) in G_2 as compared with control, while the levels of MDA were significantly increased ($p \leq 0.05$) in sera of G_2, G_3, G_5 and G_6 as compared with control.

Histological examination of thyroid gland sections from different treated groups showed considerable changes as compared with non-treated as control group, the aqueous extract from algae administration stimulates hyperactivity of thyroid gland in all three groups (G_1, G_2 and G_3). Thyroid follicles became larger, containing dense colloidal. Connective tissues in between thyroid follicles along with blood vessels can be observed, with presence of many profollicular cells in groups treated with isolated flavonoids G_4, G_5, G_6 , and the ideal structure of thyroid follicles and moderate colloid material within follicles .

Conclusion: From all the above results we can conclude that the use of algae extracts with suitable antioxidant such as Vit. C (to reduce the oxidative stress) may regulate the thyroid hormones levels and improved the function of thyroid gland in sera of hypothyroidism animals.

دور مستخلص Fucus vesiculosus L في تنظيم هرمونات الغدة الدرقية للارانب الذكور البالغة.

رفاه رزوق حميد ، خلف فارس عطية و مها عطية محمد

قسم الكيمياء، كلية التربية، جامعة سامراء

الكلمة المفتاح: طحلب الفوقس الحويصلي، الفلافونيدات، هرمونات الغدة الدرقية، مضادات الاكسدة، المألون داي الدهايد.

الخلاصة:

تنتج هرمونات الغدة الدرقية ثلاثي ايودوثايرونين T_3 ووالثايروكسين T_4 بكميات كبيرة بشكل جزيئات هرمونية اولية. ان تكوين هذه الهرمونات يعتمد على المصدر الخارجي من الايودايد. لذا فان بناء هرمونات الغدة الدرقية بشكل طبيعي يحتاج الى اليود. وطحلب الفوقس الحويصلي هو طحلب بحري غني باليود، اذ يستخدم في الطب الشعبي كمكمل غذائي لتخفيض الوزن كمصدر لليود اضافة الى استخدامه في علاج امراض الغدة الدرقية (خاصة خمول الغدة الدرقية). لذا تهدف الدراسة الحالية الى تقييم استخدام الطحلب كمكمل غذائي ومنظم لهرمونات الغدة الدرقية من خلال دراسة تأثير المستخلص المائي والفلافونيد المعزول من الطحلب على مستوى هرمونات الغدة الدرقية TSH, T_3, T_4 ، المعطيات المعطيات المضادة للاكسدة مثل الكلوتاثيون GSH ، كلوتاثيون بيروكسيدز، بيروكسي نايتريت والمألون ثنائي الالدهايد MDA في امصال دم 35 من ذكور الارانب البالغة والتي قسمت عشوائيا الى 7 مجاميع (5 في كل مجموعة). عوملت المجاميع الاولى والثانية والثالثة بـ (25 ، 50 ، 75) ملغم/كغم/يوم من المستخلص المائي لطحلب الفوقس الحويصلي في حين عوملت المجاميع الرابعة والخامسة والسادسة بـ (25 ، 50 ، 75) ملغم/كغم/يوم من الفلافونيد المعزول من الطحلب، مقارنة بمجموعة الغير معاملة باعتبارها مجموعة سيطرة.

اظهرت النتائج ان مستوى هرمون TSH انخفض معنويا ($p \leq 0.05$) في امصال دم الارانب في المجاميع الثالثة، الرابعة، الخامسة والسادسة في حين لم تظهر تغيرات معنوية في المجموعتين الاولى والثانية مقارنة بالسيطرة. اما مستوى T4 فقد انخفض معنويا ($p \leq 0.05$) في مصل دم جميع المجاميع الستة مقارنة بالسيطرة. ان مستوى T3 اظهر ارتفاعا معنويا ($p \leq 0.05$) في امصال دم المجاميع الاولى، الثانية، الرابعة والسادسة مقارنة بالسيطرة. وانخفض مستوى الكلوتاثيون معنويا ($p \leq 0.05$) في امصال جميع المجاميع ماعدا المجموعة الثالثة مقارنة بالسيطرة. من ناحية اخرى اظهر انزيم الكلوتاثيون بيروكسيدز ارتفاعا معنويا ($p \leq 0.05$) في امصال دم المجموعتين الثانية والسادسة وانخفض في المجموعة الخامسة مع عدم وجود تغيرات في مستوى نشاط الانزيم في المجموعتين الاولى والثالثة والرابعة مقارنة بالسيطرة، في حين انخفض مستوى البيروكسي نايتريت معنويا ($p \leq 0.05$) في امصال دم المجاميع الاولى، الثالثة، الرابعة، الخامسة والسادسة مع زيادة معنوية في المجموعة الثانية مقارنة بالسيطرة. كما وارتفع مستوى المألون ثنائي الالدهايد معنويا ($p \leq 0.05$) في امصال دم المجاميع الثانية والثالثة والخامسة والسادسة مقارنة بالسيطرة.

اظهرت نتائج الفحوصات النسيجية لمقاطع الغدة الدرقية لذكور الارانب البالغة والمعاملة بالمستخلص المائي والفلافونيدي لطحلب الفوقس الحويصلي تغيرات نسيجية مهمة مقارنة بمجموعة السيطرة غير المعاملة. اذ اظهر المستخلص المائي للطحلب تحفيز وتنشيط للغدة الدرقية في جميع المجاميع الثلاث المعاملة بالمستخلص المائي للطحلب. اذ اصبحت الجريبات الدرقية اكبر حجما، واكثر كثافة للغروان، كما يمكن ملاحظة النسيج الرابط بين الجريبات الدرقية والغني بالاووعية الدموية. مع وجود العديد من خلايا جريبية بنوية (حديثة التكوين) في المجاميع المعاملة بالفلافونيد المعزول (المجاميع الرابعة والخامسة والسادسة)، وتركيب مثالي لجريبات الدرقية مع كميات معتدلة للغروان داخل الجريبات.

نستنتج من هذه الدراسة ان استخدام مستخلصات الطحلب الحويصلي مع مضاد اكسدة مثل فيتامين C (ليقلل الاجهاد التاكسدي) ممكن ان يكون مناسباً لتنظيم مستوى هرمونات الغدة الدرقية وتحسين وظيفتها في امصال دم الحيوانات المصابة بمخمول الغدة الدرقية.

Introduction:

Maintaining body homeostasis is a key factor in human health controlled by various factors and in particular pituitary-thyroid axis⁽¹⁾. Thyroid gland secretes two essential thyroid hormones: triiodothyronine (T3) and thyroxine (T4) which are responsible for regulating cell metabolism in every cell in the human body⁽²⁾. The synthesis and secretion of the two thyroid hormones is influenced by a hormone released by the pituitary gland called thyroid-stimulating hormone (TSH). The synthesis and release of TSH from the pituitary gland is influenced by thyroid hormone levels as well as a hormone released from the hypothalamus called thyrotropin-releasing hormone (TRH)⁽³⁾. The activity of the thyroid gland is regulated by a negative feedback loop, in which thyroid hormones interact with receptors in the pituitary gland to inhibit TSH and at the hypothalamus to inhibit TRH secretion⁽⁴⁾.

The thyroid hormones T3 and T4 are formed in a large prohormone molecule⁽⁴⁾, thyroglobulin, the major component of the thyroid and more precisely of the colloid.

Thyroglobulin is synthesized in the thyroid follicular cells and secreted into the lumen of the follicles. It is an iodinated glycoprotein. It is of special importance because it is necessary for the synthesis of thyroid hormones and represents their form of storage⁽⁴⁾.

The formation of the thyroid hormones depends on an exogenous supply of iodide. The formation of thyroid hormones involves the a complex sequence of events including: (1)Active uptake of iodide by the follicular cells,(2)Oxidation of iodide and formation of iodotyrosyl residues of thyroglobulin,(3)Formation of iodothyronines from iodotyrosines, (4) Proteolysis of thyroglobulin and release of T4 and T3 into blood, and (5) Conversion of T4 to T3^(5,6). So that normal thyroid hormone synthesis requires iodide. *Fucus vesiculosus* (also known by the common name bladder wrack) is a marine alga rich in iodine, which is being used in alternative medicine as a laxative, diuretic, as a complement (for weight loss and as source of iodine), treatment of thyroid disorders, particularly hypothyroidism, rheumatoid arthritis and for its topical effects as a dermatological agent⁽⁷⁾. It is rich in polysaccharides and polyphenolic antioxidants (phlorotannins) ⁽⁸⁾.

In view of these considerations the objective of this work is to evaluation the use of Fucus vesiculosus in alternative medicine as complement and regulator for the thyroid hormones. In parallel the level of T3 and T4, markers of thyroid functions and glutathione(GSH), glutathione peroxidase(GPx), peroxy nitrite, malondialdehyde(MDA) content were estimated also to investigate the antioxidant status.

Material and Methods:

Material: Fucus vesiculosus L. algae were purchased from a local market in Baghdad. Dry algae were powdered by electrical grinder and left for 3 hours in an incubator at 35°C and then kept in refrigerated (4°C) in closed container for used.

Methods:

I-Preparation of extract from Fucus vesiculosus L. algae:

- **Aqueous extract:**100g of dry powder of algae were soaked in 250 ml of hot distilled water for 24 hr, after filtration the precipitate was re-extract with the same volume of hot distilled water. The solution was filtered and centrifugation at 3000 rpm for 15 min. The solvent was evaporated and the aqueous extract was condensed under reduced pressure. The extract was weight, labeled as Ex-Aq and stored at 4°C until used.
- **Extraction of flavonoids:** Extraction of flavonoids was done according to (Chen *et al*, method) with some modification ⁽⁹⁾, in which 100gm of Fucus vesiculosus L. were

extracted with 200ml diethyl ether using soxhlet apparatus for 3 hours to remove fatty contents. The defatted plant material was dried at 35 C° in an air oven, and then extracted twice with 250ml (70%) ethanol solution at 90°C for 2h. The solution was filtered and centrifugation at 3000 rpm for 15 min. The solvent was evaporated and the flavonoids extract was condensed under reduced pressure. The extract was weight, labeled as Ex-F and stored at 4°C until used.

II- Animals: All animals used in this study were local male rabbits purchased from General Company for Drug Industries / Samarra. Male rabbit (1200-1550 g weight) were used at (3-3.5) month average age .Groups of rabbits were housed at room temperature with a lighting schedule of 12 hours light and 12 h dark. Animals had free access to a standard pellet diet and tap water as drinking solution. Different concentration of aqueous and isolated flavonoids from alae were prepared according to the table (1) and the extract orally administrated in daily dose of 1ml /kg/day for 4 weeks:

Table(1):The dosage concentration aqueous and isolated flavonoids from algae

Doses	Concentration mg/ml		
	C ₁	C ₂	C ₃
aqueous	25	50	75
Flavonoids	25	50	75

The experimental study was divided the rabbits randomly into seven groups (5 animals in each group)as described below:

Control-C:Orally administrated daily tap water

Group-G₁:Orally administrated daily dose 1 ml of C₁ of aqueous algae extract.

Group-G₂:Orally administrated daily dose 1 ml of C₂ of aqueous algae extract.

Group-G₃:Orally administrated daily dose 1 ml of C₃ of aqueous algae extract.

Group-G₄:Orally administrated daily dose 1 ml of C₁ of algae flavonoids.

Group-G₅:Orally administrated daily dose 1 ml of C₂ of algae flavonoids.

Group-G₆:Orally administrated daily dose 1 ml of C₃ of algae flavonoids.

III- Collection of blood samples: Before taking the blood samples animals were fasted for 12 hours. The blood serum was collected by centrifuge the blood at 2500 rpm for 15 minutes, then was divided into 3 parts in eppendorf tube and stored at -20°C until analyzed. Determination of plasma levels of thyroid hormones(T3, T4 and TSH) and antioxidant parameters(GPx⁽¹⁰⁾, GSH⁽¹¹⁾, peroxynitrite⁽¹²⁾,MDA⁽¹³⁾,total protein⁽¹⁴⁾ and albumins⁽¹⁵⁾ were carried out.

IV-Histological study: On completion of experiments, animals were anesthetized with chloroform, the thyroid gland was quickly removed, fixed in buffered neutral formalin for 48 hrs. The fixed organs were dehydrated in ascending series of alcohol, cleared in xylene and embedded in paraffin wax, then 4 – 5 μm thick sections were obtained by rotary microtome and stained with Harris hematoxylin and Eosin ⁽¹⁶⁾.

V-Statistical analysis: Results were analyzed statistically using analysis of variance test-ANOVA by using the statistical program Minitab. Averages were compared to calculations of the characteristics of the application Duncan's Multiple Range Test by probability level $P \leq 0.01$ and 0.05.

Results and Discussion:

Table(2) shows the mean \pm SD of thyroid hormones [TSH, T3 and T4] in sera of rabbits treated with aqueous extract and isolated flavonoids from algae and not-treated as control group. The mean \pm SD of TSH in sera of C and three groups G₁, G₂, G₃ treated with aqueous extract were (0.538 \pm 0.274)ng/dl, (0.618 \pm 0.099) ng/dl, (0.657 \pm 0.037) ng/dl and (0.039 \pm 0.021)ng/dl respectively, The results indicate that the level of TSH was significantly decreased ($p \leq 0.05$) in sera of rabbits in G₃ with no significant change in G₁ and G₂ as compared with C, while the level of TSH was significantly lower in G₃ as compared with G₁ and G₂, with no significant change between G₁ and G₂.

The mean \pm SD of TSH in sera of three groups G₄, G₅, G₆ were (0.047 \pm 0.022) ng/dl, (0.071 \pm 0.057) ng/dl and (0.290 \pm 0.072) ng/dl respectively. The results showed that the levels significantly decreased ($p \leq 0.05$) in all three groups G₄, G₅ and G₆ as compared with C, but the level significantly higher in G₆ as compared with G₄ and G₅, Fig.(1).

While the mean \pm SD of T₄ in sera of C and three groups G₁, G₂, G₃ treated with aqueous extract were (10.974 \pm 1.563)ng/dl, (4.294 \pm 0.169) ng/dl, (5.102 \pm 0.529) ng/dl and (5.452 \pm 0.536)ng/dl respectively [Table 2], The results indicate that the levels of T₄ were significantly decreased ($p \leq 0.05$) in sera of all three groups G₁, G₂, G₃ as compared with C, while the level of T₄ was significantly lower in G₁ as compared with G₃, with no significant change between G₁, G₂ and G₂, G₃.

The mean \pm SD of T₄ in sera of three groups G₄, G₅, G₆ were (6.354 \pm 0.709) ng/dl, (7.934 \pm 0.916) ng/dl and (8.608 \pm 0.899) ng/dl respectively. The results showed that the levels significantly decreased ($p \leq 0.05$) in all three groups G₄, G₅ and G₆ as compared with C, but the level significantly lower in G₄ as compared with G₅ and G₆, Fig.(2).

The mean \pm SD of T3 in sera of C and three groups G₁, G₂, G₃ treated with aqueous extract were (0.575 \pm 0.560) μ g/dl, (1.420 \pm 0.658) μ g/dl, (1.460 \pm 0.528) μ g/dl and (0.870 \pm 0.306) μ g/dl respectively[Table(2)].The results indicate that the levels of T3 were significantly increased ($p\leq 0.01$) in sera of G₁ and G₂ with no significant change in G₃ as compared with C, while the level of T3 was significantly lower in G₃ as compared with G₁ and G₂, with no significant change between G₁ and G₂.

The mean \pm SD of T3 in sera of three groups G₄, G₅, G₆ were (1.167 \pm 0.066) μ g/dl, (1.008 \pm 0.265) μ g/dl and (1.507 \pm 0.297) μ g/dl respectively. The results showed that the levels significantly increased ($p\leq 0.01$) in G₄ and G₆ with no significant change in G₅ as compared with C, but the level significantly lower in G₅ as compared with G₆ with no significant change between in G₄, G₅ and G₄, G₆, Fig.(3).

Thyroid hormones (T3 and T4) are derived from tyrosine amino acid. About 95% of thyroid secreting hormones are T4 (thyroxin) whereas T3 plays the main role. Although T3 is secreted by thyroid gland, the majority is synthesized by the metabolism of T4 in peripheral tissues⁽¹⁷⁾. Some tissues like brain and hypophysis can also convert T4 to T3 and cannot be entered to blood but remains there. On the whole, 80% of blood's T3 is made in liver and 20% in thyroid. Secreting TSH controls releasing thyroid hormones. The amount of TSH secreting is also adjusted by level of thyroid hormones in blood. By reduction in these hormones TSH secreting will be increased and then T3 and T4 secreting will be raised^(5,18).

In this study, TSH and T4 were decreased significantly, while T3 increased. No informations were available in the literature about the effect of Fucus vesiculosus L on the serum level of thyroid hormones, but Gupta *et al.*⁽¹⁹⁾ reported that Ficus Carica leaf extract gave the same effect.

The decreased TSH may be due to the feedback inhibition scheme by high level of T3. It appears that the aqueous extract and isolated flavonoids from algae induced increase in T3. However, increase in serum T3 concentration by all the three doses of aqueous extract and isolated flavonoids could be due to the stimulation in monodeiodination of T4 in peripheral tissues, which is known to be the major process of its synthesis. Another possibility of plant extract-induced increase in thyroid hormone concentrations could be the decreased utilization of the hormones by the body⁽¹⁹⁾. Although further investigations are required to reveal the exact mechanism of action of thyroregulatory role of Fucus vesiculosus L extract. The present findings clearly indicate that this extract is stimulant to thyroid functions. However, further studies are required to observe the dose dependant effect of algae extract which might be

effective and safe in ameliorating hypothyroidism. Despite the fact that day-by-day herbal drugs are gaining much importance for their affordable and safe nature, scientific investigations towards the mitigation of thyroid disorders by the plant extracts are meager⁽²⁰⁾.

Table(3) shows the mean±SD of antioxidant parameters [GSH and GPx] in sera of rabbits treated with aqueous extract and isolated flavonoids from algae and not-treated as control group. The mean ± SD of GSH in sera of C and three groups G₁, G₂, G₃ treated with aqueous extract were (3.764 ±0.872)mM/L, (1.923± 0.604) mM/L, (1.278 ± 0.241) mM/L and (3.216 ± 0.182) mM/L respectively, The results indicate that the levels of GSH were significantly decreased (p≤0.05) in sera of rabbits in G₁ and G₂ with no significant change in G₃ as compared with C, while the levels of GSH were significantly lower in G₁ and G₂ as compared with G₃, with no significant change between G₁ and G₂.

But in sera of three groups G₄, G₅,G₆ the mean±SD of GSH were (1.984 ±0.633)mM/L,(2.073 ±0.65) mM/L and (2.648 ± 0.807)mM/L respectively. These results showed that the levels significantly decreased (p≤0.05) in all three groups G₄, G₅ and G₆ as compared with C, but the level significantly higher in G₆ as compared with G₄ and G₅, Fig.(4)

While the mean±SD of GPx in sera of C and three groups G₁, G₂, G₃ treated with aqueous extract were (6.812±1.083) U/L, (7.230±0.852) U/L, (11.770± 3.153) U/L and (7.772±2.472) U/L respectively[Table 3], The results indicate that the level of GPx was significantly increased (p≤0.05) in sera of G₂, with no significant change in G₁ and G₃ as compared with C, while the level of GPx were significantly lower in G₁ and G₃ as compared with G₂, with no significant change between G₁ and G₃.

The mean ±SD of GPx in sera of three groups G₄, G₅,G₆ were (9.375± 1.923) U/L,(3.878±1.878) U/L and (10.874±3.792) U/L respectively. The results showed that the level significantly decreased (p≤0.05) in G₅ and increased in G₆, with no significant change in G₄ as compared with C, but the level significantly lower in G₅ as compared with G₄ and G₆ Fig.(5).

The mean±SD of peroxynitrate in sera of C and three groups G₁, G₂, G₃ treated with aqueous extract were (170.04±38.33)mM/L, (134.85±17.84)mM/L, (224.45±30.1)mM/L and (114.38±15.19) mM/L respectively[Table 3]. These results indicated that the levels of peroxynitrate were significantly decreased (p≤0.05) in sera of G₁ and G₃ and significantly increased (p≤0.05) in G₂ as compared with C, while the levels of peroxynitrate were significantly lower in G₁ and G₃ as compared with G₂, with no significant change between G₁ and G₃.

While the mean \pm SD of peroxy nitrate in sera of three groups G₄, G₅, G₆ were (133.95 \pm 36.17) mM/L, (132.97 \pm 16.64) mM/L and (139.20 \pm 27.26) mM/L respectively. The results showed that the level significantly decreased ($p \leq 0.05$) in all three groups G₄, G₅, G₆ as compared with C, with no significant change between the three groups G₄, G₅, G₆, Fig.(6).

The mean \pm SD of MDA in sera of C and three groups G₁, G₂, G₃ treated with aqueous extract were (6.122 \pm 1.433)mM/L, (5.260 \pm 1.256)mM/L, (8.595 \pm 2.257)mM/L and (10.184 \pm 1.606) mM/L respectively [Table 3]. These results indicated that the levels of MDA were significantly increased ($p \leq 0.05$) in sera of G₂ and G₃ as compared with C, while the level of MDA was significantly lower in G₁ as compared with G₂ and G₃.

The mean \pm SD of MDA in sera of three groups G₄, G₅, G₆ were (7.236 \pm 1.284) mM/L, (9.927 \pm 2.244) mM/L and (9.838 \pm 1.910) mM/L respectively. The results showed that the level significantly increased ($p \leq 0.05$) in G₅ and G₆ as compared with C, while the level of MDA was significantly lower in G₄ as compared with G₅ and G₆, with no significant change between G₅ and G₆, Fig.(7).

The role of thyroid in regulation oxidative stress is recently being explored. Previous reports showed that both hyper and hypothyroidism are associated with increased oxidative stress in human^(21,22). Therefore aqueous extract and isolated flavonoids of Fucus vesiculosus L could be work up as a regulator of hormone, may be due to it contain highly percent of antioxidant and possibly to return balance of thyroid hormone due to remove free radicals.

The antioxidant activity (*in vitro*) of Fucus vesiculosus L was determined by using 1,1-diphenyl-2-picryl-hydrazil-DPPH free radical scavenging method which indicates that the ethyl acetate fraction possess a relatively strong antioxidant against DPPH radicals⁽²³⁾. Glutathione being an important cellular reductant, involved in protection against free radicals, peroxides and toxic compounds⁽²⁴⁾, GSH depletion is one of the chief factors that lead to lipid peroxidation⁽²⁵⁾. In our present study, the GSH levels were decreased in sera of rabbits treated with aqueous extract and isolated flavonoids from algae as compared to control. The decreased GSH level may be due to increase level of lipid oxidation products which may be associated with the less availability of NADPH required for the activity of glutathione reductase (GR) to transform oxidized glutathione to GSH⁽²⁶⁾. Or may be due to the activation of glutathione peroxidase-GPx (GPx catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide)⁽²⁵⁾ by the phytochemical compounds in algae which include alkaloids, flavonoids, phenolic compounds, tannins, terpenoids and sterols⁽²⁷⁾. GPX act as preventive antioxidants which deactivate the

active species such as H_2O_2 , without further generation of free radicals and thereby, reduce the rate of chain initiation. So that the concentration of GSH was reduced, while the concentration of GSSG was elevated⁽²⁸⁾. The decreased GSH level in association with increased GPx activity may support the explanation as evidence.

Otherwise peroxynitrite inhibition of T_3 and T_4 synthesis as well as thyroid synthesis is BH4 (5,6,7,8-tetrahydro-Lbiopterin) dependant. Peroxynitrite is a potent oxidant and nitrating species formed by rapid reaction of two free radicals – nitric oxide and superoxide anion. It can modify variety of biomolecules but possesses high affinity for tyrosine residues in proteins, and 3-nitrotyrosine is a relatively specific marker of peroxynitrite mediated damage to proteins⁽²⁹⁾. In view of numerous reports on detection of significant amount of 3-nitrotyrosine in various pathological conditions of thyroid⁽³⁰⁻³²⁾. So that decreased serum peroxynitrite in all groups treated with aqueous extract and isolated flavonoids from algae may improve the T_3 and T_4 synthesis, thus reduced the formation of toxic 3-nitrotyrosine. While the increase in MDA indicates enhanced peroxidation leading to a failure of the antioxidant defense mechanism to prevent formation of excess free radicals. Fucus vesiculosus algae extract did not have the ability to reduce lipid peroxidation. This elevation may be due to the high dose of extract as compared with our results in lower dose.

Histological examination of thyroid gland sections from different treated groups showed considerable changes as compared with non-treated as control group-C. It is clear from Fig. (8) that thyroid follicles of the control group are filled with colloid and also showed the connective tissue septa and interfollicular connective tissue of euthyroid status and normal thyroid hormones secretion. Aqueous extract from algae administration stimulates hyperactivity of thyroid gland in all three groups (G1, G2 and G3) as shown in Fig. 9, 10 and 11. Thyroid follicles became larger, containing dense colloidal. Connective tissues in between thyroid follicles along with blood vessels can be observed.

The most obvious observation was the presence of many profollicular cells and connective tissue septa in groups treated with isolated flavonoids (Fig. 12, 13 and 14) and the ideal structure of thyroid follicles with their columnar epithelial lining and moderate colloid material within follicles. Thyroid hormones are stored extracellularly in the colloid inside the follicle in the form of iodinated thyroglobulin. Each thyroglobulin molecule contains one to four T_4 molecules. An average of one T_3 molecule is present for fourteen T_4 molecules⁽¹⁾. Accumulation of colloidal materials was due mainly to a direct effect of extract on iodide pump or iodine uptake by thyroid gland, in which the extract may regulate the activity of some

enzymes such as peroxidase and deiodinase⁽³³⁾, thus regulate the thyroid hormones(TSH, T4 and T3) or may be due to the high amounts of iodine in Fucus vesiculosus algae which consider essential for the formation of thyroid hormone^(1,8).

From all the above results we can conclude that the use of algae extracts with suitable antioxidant such as Vit. C (to reduce the oxidative stress) may regulate the thyroid hormones levels and improved the function of thyroid gland in sera of hypothyroidism animals.

Table(2):Mean ± SD of thyroid hormones in sera of rabbits treated with aqueous extract and isolated flavonoids from algae and not-treated as control group

Groups	TSH ng/dl	T ₄ ng/dl	T ₃ µg/dl
C	0.538 ± 0.274	10.974 ± 1.563	0.575 ± 0.560
Aqueous extract			
G1	0.618 ± 0.099	4.294 ± 0.169	1.420 ± 0.658
P<	NS*	0.05	0.01
G2	0.657 ± 0.037	5.102 ± 0.529	1.460 ± 0.528
P<	NS	0.05	0.01
G3	0.039 ± 0.021	5.452 ± 0.536	0.870 ± 0.306
P<	0.05	0.05	0.01
Isolated flavonoids			
G4	0.047 ± 0.022	6.354 ± 0.709	1.167 ± 0.066
P<	0.05	0.05	0.01
G5	0.071 ± 0.057	7.934 ± 0.916	1.008 ± 0.265
P<	0.05	0.05	0.01
G6	0.290 ± 0.072	8.608 ± 0.899	1.507 ± 0.297
P<	0.05	0.05	0.01

*NS=Non significant

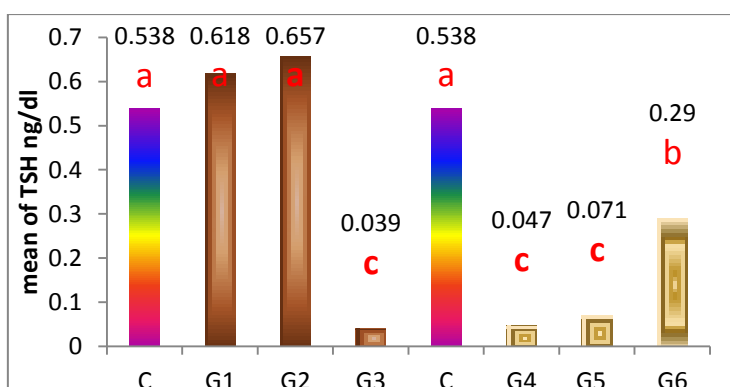


Fig.(1):TSH levels (ng/dl) in sera of adult rabbits treated with aqueous extract and isolated flavonoids from algae and not-treated as control group

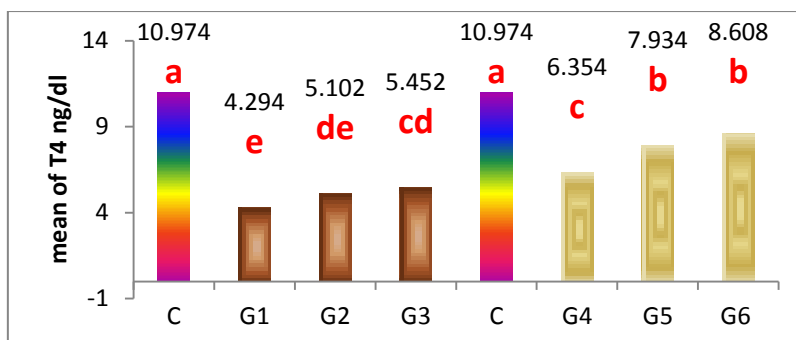


Fig.(2):T4 levels (ng/dl) in sera of adult rabbits treated with aqueous extract and isolated flavonoids from algae and not-treated as control group

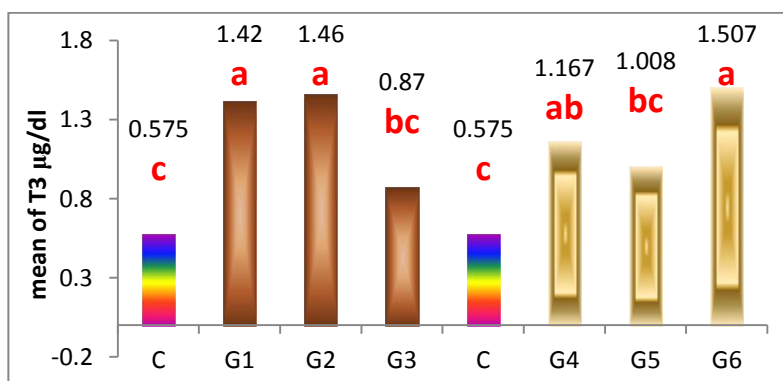


Fig.(3):T₃ levels(µg/dl) in sera of adult rabbits treated with aqueous extract and isolated flavonoids from algae and not-treated as control group.

Table(3):Mean ± SD of GSH, GPx, Peroxynitrit and MDA in sera of rabbits treated with aqueous extract and isolated flavonoids from algae and not-treated as control group

Groups	GSH mM/L	GPX U/L	Peroxynitrit mM/L	MDA mM/L
C	3.764 ±0.872	6.812±1.083	170.04±38.33	6.122±1.433
Aqueous extract				
G1	1.923± 0.604	7.230±0.852	134.85±17.84	5.260±1.256
P<	0.05	NS	0.05	NS
G2	1.278 ± 0.241	11.770± 3.153	224.45±30.1	8.595±2.257
P<	0.05	0.05	0.05	0.05
G3	3.216 ± 0.182	7.772±2.472	114.38±15.19	10.184±1.606
P<	N.S	N.S	0.05	0.05
Isolated flavonoids				
G4	1.984 ±0.633	9.375± 1.923	133.95±36.17	7.236±1.284
P<	0.05	N.S	0.05	N.S
G5	2.073 ±0.65	3.878±1.878	132.97±16.64	9.927±2.244
P<	0.05	0.05	0.05	0.05
G6	2.648 ± 0.807	10.874±3.792	139.20±27.26	9.838±1.910
P<	0.05	0.05	0.05	0.05

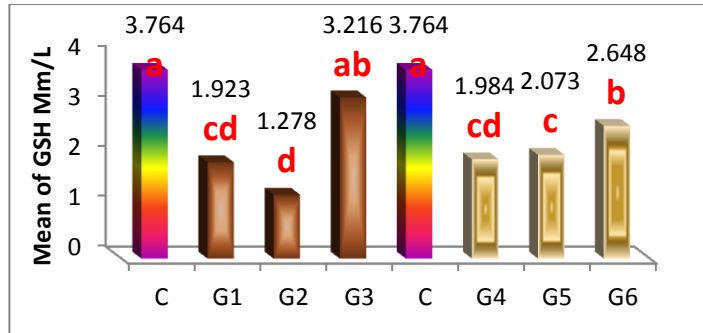


Fig.(4):GSH levels(mM/L) in sera of adult rabbits treated with aqueous extract and isolated flavonoids from algae and not-treated as control group.

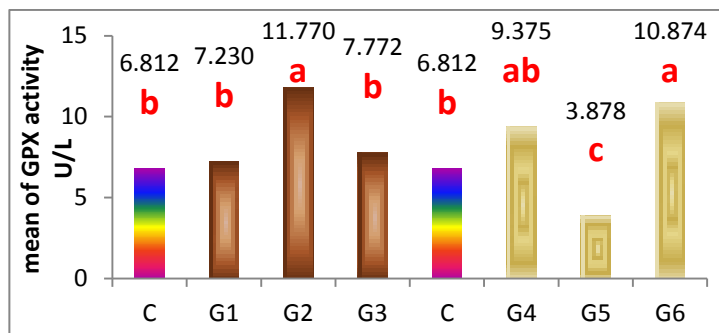


Fig.(5):GPx levels(U/L) in sera of adult rabbits treated with aqueous extract and isolated flavonoids from algae and not-treated as control group.

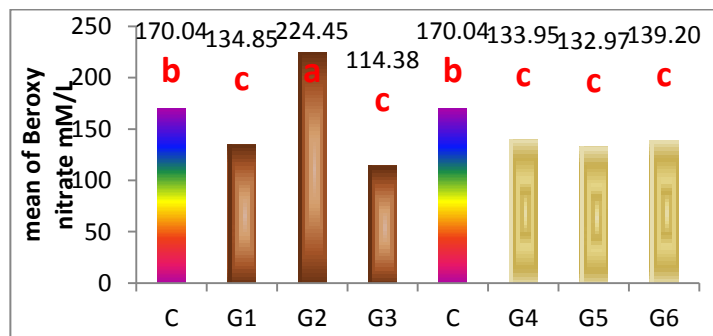


Fig.(6): Peroxynitrate levels(mM/L) in sera of adult rabbits treated with aqueous extract and isolated flavonoids from algae and not-treated as control group.

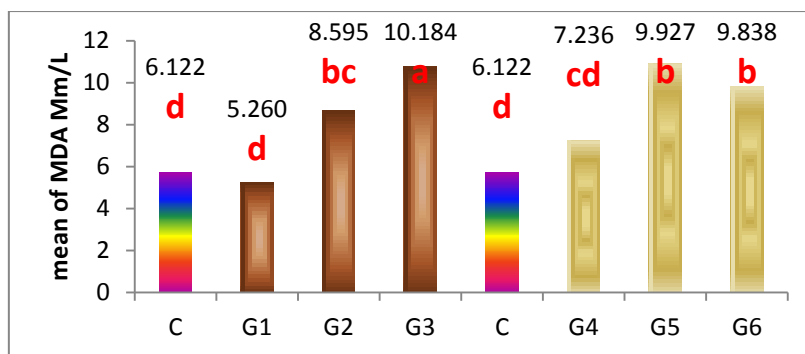


Fig.(7): MDA levels(mM/L) in sera of adult rabbits treated with aqueous extract and isolated flavonoids from algae and not-treated as control group.

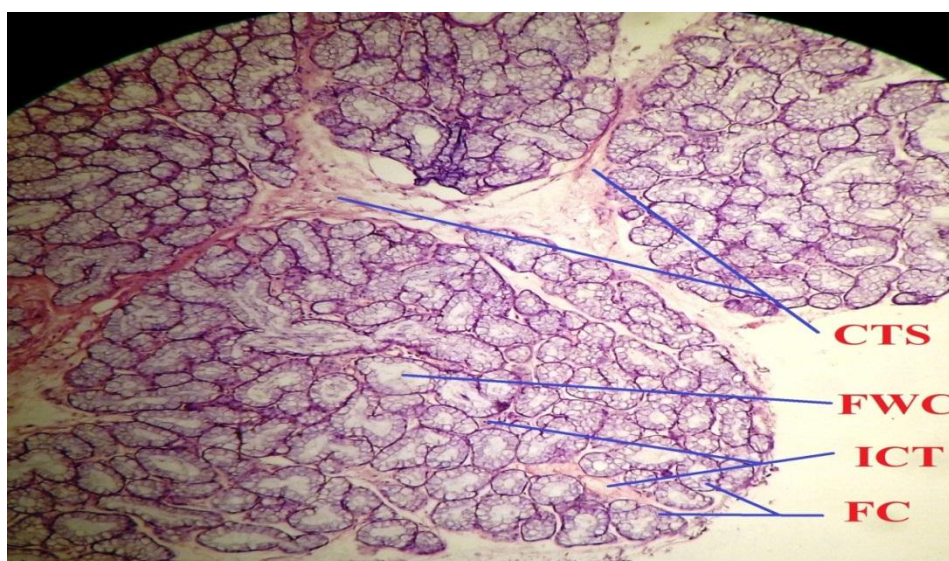


Fig.(8): Cross Section for Normal Rabbits Thyroid tissue, Showed the Connective Tissue Septa, Follicular cells, Follicle with Colloid, Interfollicular Connective Tissue (Control Group-C) (HE)-200X.

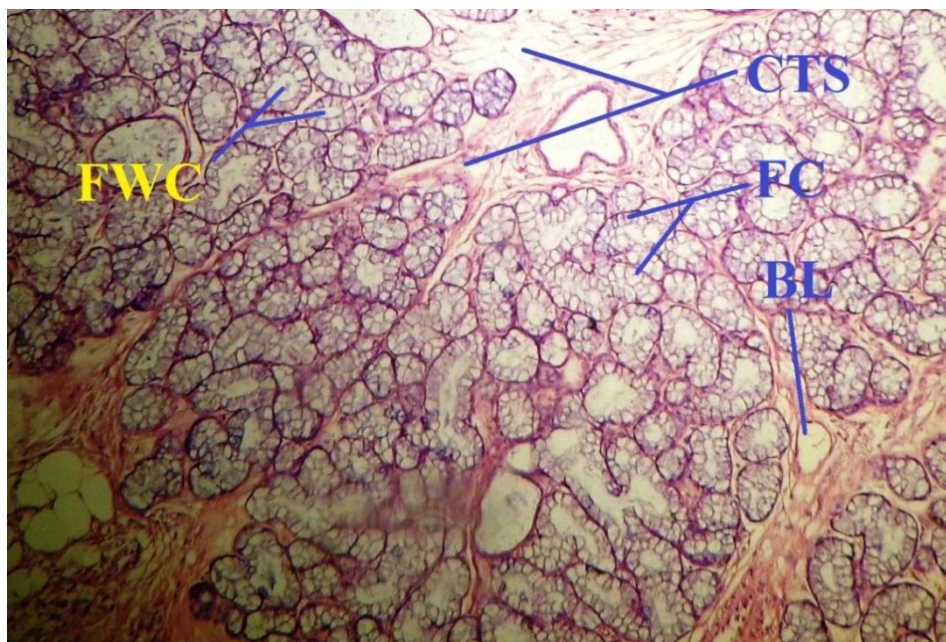


Fig.(9): Cross Section for Rabbits Thyroid tissue, Showed the Blood vessel ;Connective Tissue Septa, Follicular cells and Follicle with Colloid (G1) (HE)-200X.

ICT:Interfollicular Connective Tissue ; **BL:** Blood vessel ; **CTS:**Connective Tissue Septa ; **FC:**Follicular cells ;**FWC:**Follicle with Colloid.

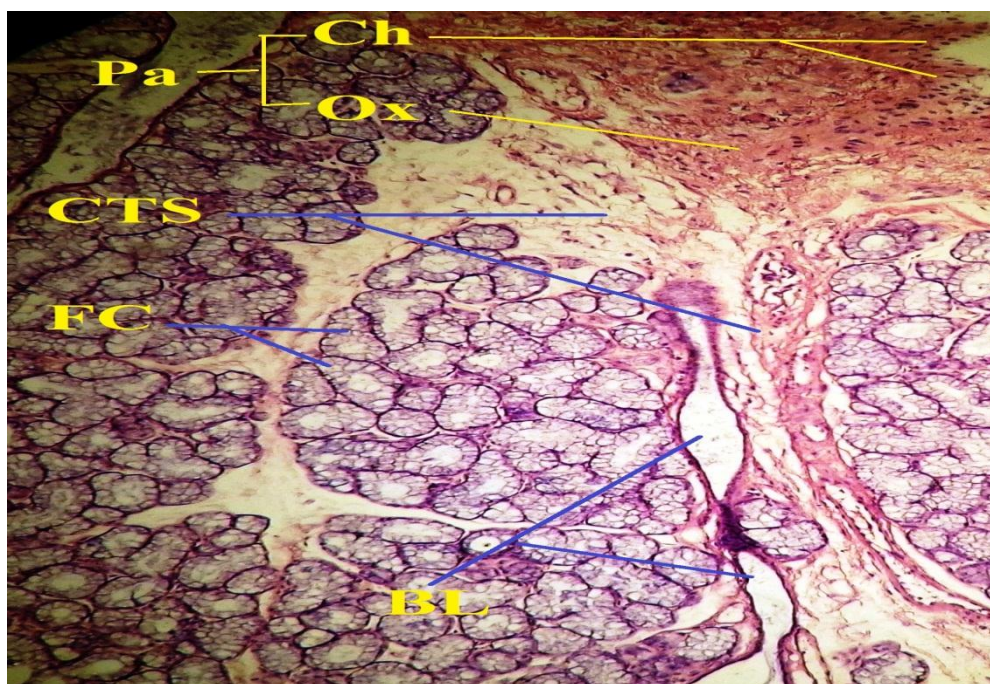


Fig.(10): Cross Section for Rabbits Thyroid tissue, Showed the Connective Tissue Septa, Follicular cells and Parathyroid gland(Ch:Chief cells and Ox: Oxyphil cells) (G2) (HE)-200X.

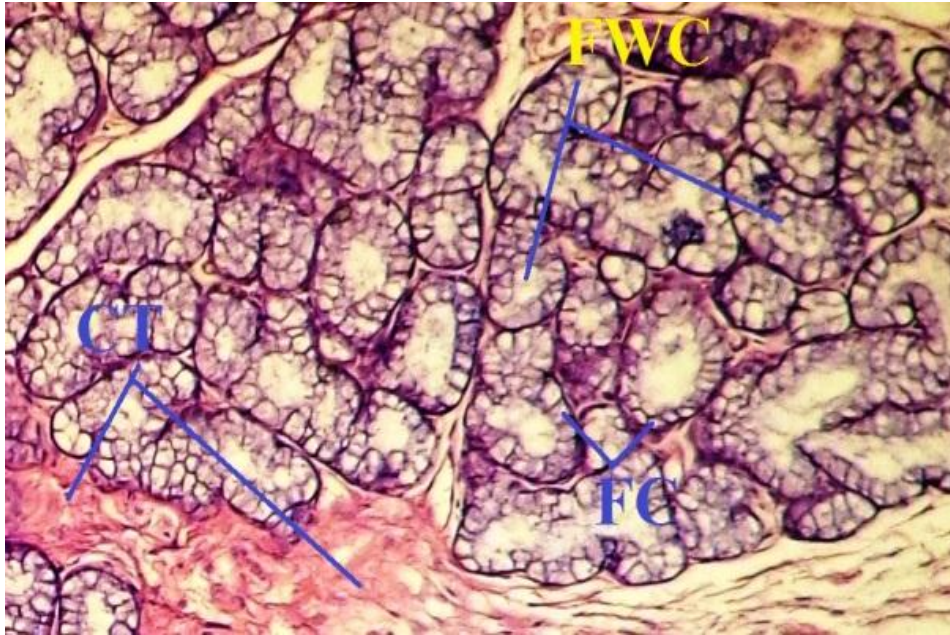


Fig.(11): Cross Section for Rabbits Thyroid tissue, Showed the Connective Tissue, Follicular cells and Follicle with Colloid (G3) (HE)-300X.

BL: Blood vessel ; **CTS:**Connective Tissue Septa ; **FC:**Follicular cells ;**FWC:**Follicle with Colloid.**Pa:**Parathyroid gland; **Ch:**Chief cells and **Ox:** Oxyphil cells.

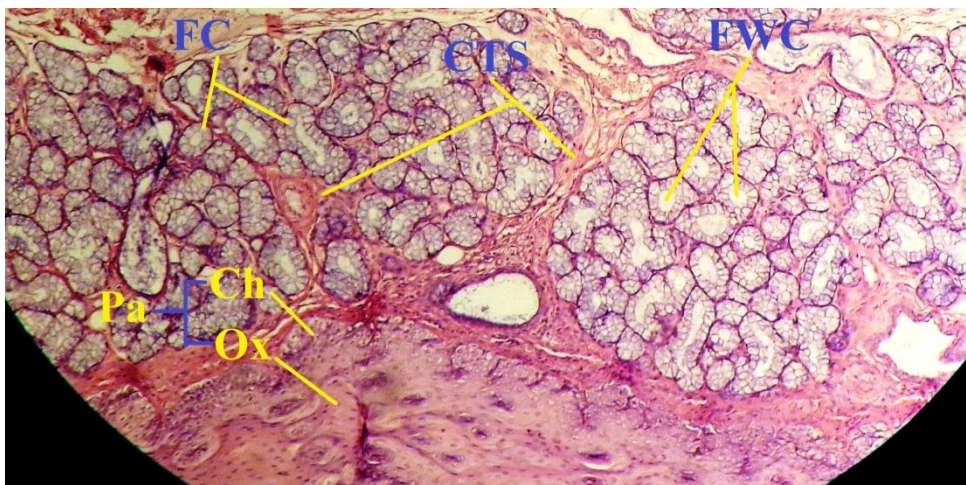


Fig.(12): Cross Section for Rabbits Thyroid tissue, Showed the Connective Tissue Septa, Follicular cells, Follicle with Colloid and Parathyroid gland(Ch:Chief cells and Ox: Oxyphil cells) (G4) (HE)-200X.

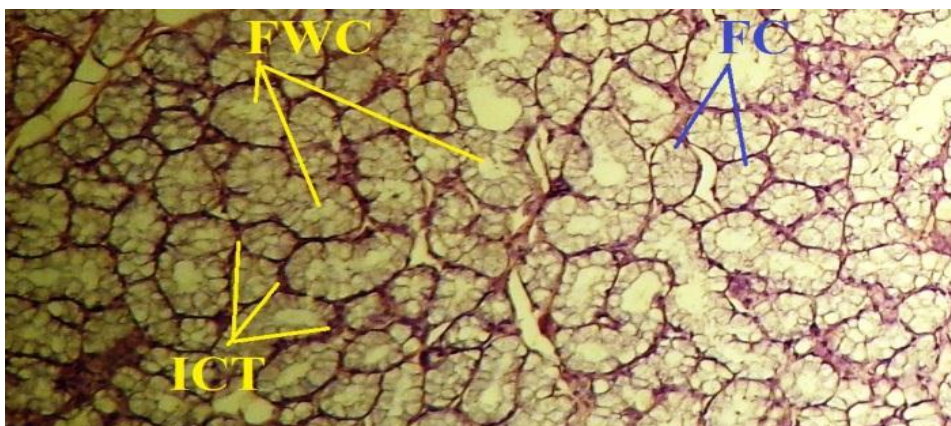


Fig.(13): Cross Section for Rabbits Thyroid tissue, Showed the Interfollicular Connective Tissue, Follicular cells and Follicle with Colloid (G5) (HE)-200X.

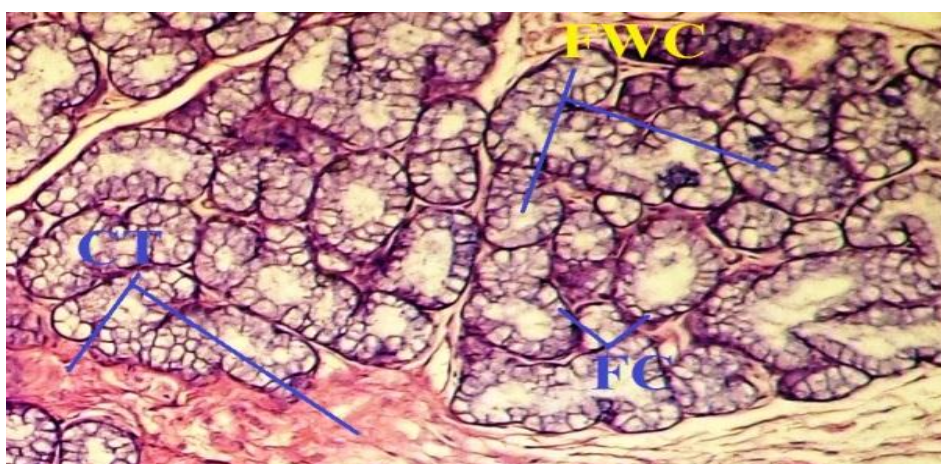


Fig.(14): Cross Section for Rabbits Thyroid tissue, Showed the Connective Tissue, Follicular cells and Follicle with Colloid (G6) (HE)-300X.

CTS:Connective Tissue Septa ; **FC:**Follicular cells ;**FWC:**Follicle with Colloid;
ICT: Follicle with Colloid ;**Pa:**Parathyroid gland; **Ch:**Chief cells and **Ox:** Oxyphil cells.

References:

- 1-Hossini, E.; Sadeghi, H. and Daneshi, A. Armaghane-danesh ;14 (4): 23-30(2010).
- 2- Duntas, L.H. J. Clin Endocrinol. Metab. 95(12):5180–88(2010).
- 3-Iglesias,P. and Díez, J. J. Europ J. of Endocrinol;160(4):503–515(2009)
- 4- Lim, V. S. Ame. J Kid. Dis; 38(4):S80–S84(2001).
- 5-Choksi, N. Y.; Jahnke, G. D. ; Hilaire,C. S. and Shelby,M. Birth Def. Res. (Part B);68:479–91(2003).
- 6-DeRuiter, J. Endocrine Pharmacotherapy Module: Thyroid Section:1-16(2001)
- 7-Arbaizar, B and Llorca, J. Actas Esp Psiquiatr;39(6):401-3(2011).
- 8- Diaz-Rubio M.E., Peez-Jimenez J, Sausa-Calixto F. Int J Food Sci Nut 60 (2): 23-34.(2009).
- 9- Chen, J.J., Li, X.R. and Fang, X. J. Zhejiang Univ. (med. Sci.) 35: 219-223;(2006).

- 10- Green, M.J. and Hill, H.A.O. Chemistry of dioxygen. Methods Enzymol.; 105:15.(1984).
- 11- Sedlak, J. and Lindsay, R. H. "Analytical biochemistry" p.192 (1968). cited by Al-Zamely, O. M.; Al-Nimer, M. S. and Muslih, R. K.Natl. J. Chem .; 4:625-637(2001).
- 12- Vanuffelen,B. E., VanDerzecz,J. and Dekoster,B. M. Biochem. J; 330:719.(1998) (Cited by Al-Zamely et al.,: 2001).
- 13- Schmedes, A. and Hølmer, G. J. Am. Oil Chem Soc.;66(6): 813-817(1989).
- 14-Gornall, A. G.; Bardawill, C. J.and David, M. M. J. Biol. Chem.; 177: 751-66(1949).
- 15-Basil, T.; Doumas, T. and T Peters, J. Clin. Chem.; 55:3 583–584(2009).
- 16- Luna, L.G.; McGraw-Hill Book Company, New York, 3rd Edn. P:258 (1968).
- 17- Ermakova O V. Radiats Bio. Radioecol.; 50 (4):391-7(2010).
- 18-Maleknia, N. Tehran University Publication, Tehran(2004).
- 19- Gupta,R.; Saxena, V and Saraf, S.A. Asian J Pharm Clin Res, 5(2): 44-48(2012).
- 20-A.Kar, S. Panda, S.Bharti,J Ethnopharmacol. 81:281-285(2002)
- 21- Lakshmi,L.J.; Zephy, E. M. and Kumari.S. JARBS;5(1): 63-66(2013).
- 22-Al-Watify,D.G. Journal of Babylon University/Pure and Applied Sciences.2(19):(2011).
- 23-Wang T, Jónsdóttir R, Liu H, Gu L, Kristinsson HG, Raghavan S, Olafsdóttir G. J Agric Food Chem. 5:213-222(2012).
- 24- Mahapatra SK, Das S, Dey SK, Roy S. Al Ameen J Med Sci; 1: 20-31(2008).
- 25-Konukoglu D, Serin O, Kemerli DG, Serin E, Hayiroglu A, Oner B. Clin Chim Acta; 277: 91-98(1998).
- 26-Sarkar S, Yadav P, Trivedi R, Bansal AK, Bhatnagar D. J Trace Elem Med Biol; 9: 144-149(1995).
- 27- Hameed, R.R.; Atea;K.F. and Molameed,M.A. Tikrit J for science. Under Publication(2014)
- 28- Roginsky, V. Arch. Biochem. Biophys; 414: 261-270(2003) .
- 29- Pryor W A, Squandrito G I. Am J Physiol. 268, L699-22(1995),.
- 30- Alturfan, A. A. ; Zengin, E.; Dariyerli, N.*et al* Folia Biologica (Praha) 53, 183-188(2007).
- 31- Franco, M.C.; Antico-Arciuch, V.G.; Peralta, J.G. *et al.*, J. Biol. Chem;281(8):4779-86(2006).
- 32-Fillion,B.B.; Prou,D.; Polydoro,M.; Spielberg,D; Tsika,E. and Wang,Z. The J. Neuroscience, 7, 26(23):6124–6130(2006).
- 33- Kumar ,.V. and Hagler ,H.. Pathologic (Basis of Discase) .6th ed. W.B.Saunders Company ,Philadephia .(1996).