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

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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, peroxynitrite and malondialdehyde-MDA in sera of 35 adult male rabbits divided randomly into seven groups (5 animals in each group), in which G_1, G_2 and G_3 treated with (25,50and75) mg/kg/day of aqueous extract of algae respectively, while the G_4, G_5 and G_6 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 ($p\leq0.05$) in sera of rabbits in G_3 G_4, G_5 and G_6 with no significant change in G_1 and G_2 as compared with control, while the level of T4 were significantly decreased ($p\leq0.05$) in sera of all six groups G_1, G_2, G_3, G_4, G_5 and G_6 as compared with control. The levels of T3 show significantly increased ($p\leq0.01$) in sera of G_1, G_2 G_4 and G_6 with no significant change in G_3 as compared with control, the levels of GSH were significantly decreased ($p\leq0.05$) in sera of G_1 ,G_2, G_4, G_5 and G_6 with no significant change in G_3 as compared with control. Otherwise the levels of GPx were significantly increased ($p\leq0.05$) in sera of G_2 and G_6, and decreased in G_5 with no significant change in G_1,G_3 and G_4 as compared with control, while the levels of peroxynitrate were

significantly decreased ($p\leq0.05$) in sera of G_1,G_3, G_4, G_5 and G_6 and significantly increased ($p\leq0.05$) in G_2 as compared with control, while the levels of MDA were significantly increased ($p\leq0.05$) 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 (G1, G2 and G3). 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 G4,G5,G6, 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.

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

الخلاصة:

تنتج هرمونات الغدة الدرقية ثلاثي ايودوثايرونين T3 ووالثايروكسينT4 بكميات كبيرة بشكل جزيئات هرمونية اولية. ان تكوين هذه الهرمونات يعتمد على المصدر الخارجي من الايوداید. 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 وكمصدر 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 تاثير المستخلص المائي والفلافونيد المعزول من الطحلب على مستوى هرمونات الغدة الدرقية TSH،T3,T4،المعطيات المعطيات المضادة للاكسدة مثل الكلوتاثيون GSH ، كلوتاثيون بيروكسديز،بيروكسي نايتريت والمالون 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 ($p\leq0.05$) في امصال دم الارانب في المجاميع الثالثة، الرابعة، الخامسة والسادسة في حين لم تظهر تغيرات معنوية في المجموعتين الاولى والثانية مقارنة بالسيطرة. اما مستوى T4فقد انخفض معنويا ($p\leq0.05$) في مصل دم جميع المجاميع الستة مقارنة بالسيطرة.ان مستوى T3اظهر ارتفاعا معنويا ($p\leq0.05$) في امصال دم المجاميع الاولى ، الثانية، الرابعة والسادسة مقارنة بالسيطرة. وانخفض مستوى الكلوتاثيون معنويا ($p\leq0.05$) في امصال جميع المجاميع ماعدا المجموعة الثالثة مقارنة بالسيطرة. من ناحية اخرى اظهر انزيم الكلوتاثيون بيروكسديز ارتفاعا معنويا ($p\leq0.05$) 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 ($p\leq0.05$) في امصال دم المجاميع الاولى، الثالثة،الرابعة، الخامسة والسادسة مع زيادة معنوية في المجموعة الثانيةمقارنة بالسيطرة.كما وارتفع مستوى المالون ثنائي الالدهايد معنويا ($p\leq0.05$)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 بالاوعية الدموية. مع وجود العديد من خلايا جريبية بنيوية(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 مناسبا لتنظيم مستوى هرمونات الغدة الدرقية وتحسين وظيفتها في امصال دم الحيوانات المصابة بخمول 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), peroxynitrite, 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-Prepration 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℃ 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℃ 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℃ 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-G_1: Orally administrated daily dose 1 ml of C_1 of aqueous algae extract.

Group-G_2: Orally administrated daily dose 1 ml of C_2 of aqueous algae extract.

Group-G_3: Orally administrated daily dose 1 ml of C_3 of aqueous algae extract.

Group-G_4: Orally administrated daily dose 1 ml of C_1 of algae flavonoids.

Group-G_5: Orally administrated daily dose 1 ml of C_2 of algae flavonoids.

Group-G_6: Orally administrated daily dose 1 ml of C_3 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 ± 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 ± SD of TSH in sera of C and three groups G_1, G_2, G_3 treated with aqueous extract were (0.538 ± 0.274)ng/dl, (0.618 ± 0.099) ng/dl, (0.657 ± 0.037) ng/dl and (0.039 ± 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 G_3 with no significant change in G_1 and G_2 as compared with C, while the level of TSH was significantly lower in G_3 as compared with G_1 and G_2, with no significant change between G_1 and G_2.

The mean ± SD of TSH in sera of three groups G_4, G_5,G_6 were (0.047 ± 0.022) ng/dl,(0.071 ± 0.057) ng/dl and (0.290 ± 0.072) ng/dl respectively. The results showed that the levels significantly decreased ($p \leq 0.05$) in all three groups G_4, G_5 and G_6 as compared with C, but the level significantly higher in G_6 as compared with G_4 and G_5, Fig.(1).

While the mean±SD of T_4 in sera of C and three groups G_1, G_2, G_3 treated with aqueous extract were (10.974 ± 1.563)ng/dl, (4.294 ± 0.169) ng/dl, (5.102 ± 0.529) ng/dl and (5.452 ± 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 G_1, G_2, G_3 as compared with C, while the level of T_4 was significantly lower in G_1 as compared with G_3, with no significant change between G_1,G_2 and G_2, G_3.

The mean ±SD of T4 in sera of three groups G_4, G_5,G_6 were (6.354 ±0.709) ng/dl,(7.934 ±0.916) ng/dl and (8.608 ± 0.899) ng/dl respectively. The results showed that the levels significantly decreased ($p \leq 0.05$) in all three groups G_4, G_5 and G_6 as compared with C, but the level significantly lower in G_4 as compared with G_5 and G_6, Fig.(2).

The mean±SD of T3 in sera of C and three groups G_1, G_2, G_3 treated with aqueous extract were (0.575 ± 0.560)µg/dl, (1.420 ± 0.658)µg/dl, (1.460 ± 0.528)µg/dl and (0.870 ± 0.306)µg/dl respectively[Table(2)].The results indicate that the levels of T3 were significantly increased ($p \leq 0.01$) in sera of G_1 and G_2 with no significant change in G_3 as compared with C, while the level of T3 was significantly lower in G3 as compared with G_1 and G2, with no significant change between G_1 andG_2 .

The mean ±SD of T3 in sera of three groups G_4, G_5,G_6 were (1.167 ± 0.066) µg/dl,(1.008 ± 0.265) µg/dl and (1.507 ± 0.297) µg/dl respectively. The results showed that the levels significantly increased ($p \leq 0.01$) in G_4 and G_6 with no significant change in G_5 as compared with C, but the level significantly lower in G_5 as compared with G_6 with no significant change between in G_4, G_5 and G_4,G_6, 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 G_1, G_2, G_3 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 ($p\leq0.05$) in sera of rabbits in G_1 and G_2 with no significant change in G_3 as compared with C, while the levels of GSH were significantly lower in G_1 and G_2 as compared with G_3, with no significant change between G_1 and G_2.

But in sera of three groups G_4, G_5,G_6 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 ($p\leq0.05$) in all three groups G_4, G_5 and G_6 as compared with C, but the level significantly higher in G_6 as compared with G_4 and G_5, Fig.(4)

While the mean±SD of GPx in sera of C and three groups G_1, G_2, G_3 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 ($p\leq0.05$) in sera of G_2, with no significant change in G_1 and G_3 as compared with C, while the level of GPx were significantly lower in G_1 and G_3 as compared with G_2, with no significant change between G_1 and G_3.

The mean ±SD of GPx in sera of three groups G_4, G_5,G_6 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 ($p\leq0.05$) in G_5 and increased in G_6, with no significant change in G4 as compared with C, but the level significantly lower in G_5 as compared with G_4 and G_6 Fig.(5).

The mean±SD of peroxynitrate in sera of C and three groups G_1, G_2, G_3 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 ($p\leq0.05$) in sera of G_1 and G_3 and significantly increased ($p\leq0.05$) in G_2 as compared with C, while the levels of peroxynitrate were significantly lower in G_1 and G_3 as compared with G_2, with no significant change between G_1 and G_3.

While the mean ±SD of peroxynitrate in sera of three groups G_4, G_5,G_6 were (133.95±36.17) mM/L,(132.97±16.64) mM/L and (139.20±27.26) mM/L respectively. The results showed that the level significantly decreased ($p\leq0.05$) in all three groups G_4, G_5,G_6 as compared with C, with no significant change between the three groups G_4, G_5,G_6 ,Fig.(6).

The mean±SD of MDA in sera of C and three groups G_1, G_2, G_3 treated with aqueous extract were (6.122±1.433)mM/L, (5.260±1.256)mM/L,(8.595±2.257)mM/L and (10.184±1.606) mM/L respectively[Table 3]. These results indicated that the levels of MDA were significantly increased ($p\leq0.05$) in sera of G_2 and G_3 as compared with C, while the level of MDA was significantly lower in G_1 as compared with G_2 and G_3.

The mean ±SD of MDA in sera of three groups G_4, G_5,G_6 were (7.236±1.284) mM/L,(9.927±2.244) mM/L and (9.838±1.910) mM/L respectively. The results showed that the level significantly increased ($p\leq0.05$) in G_5 and G_6 as compared with C, while the level of MDA was significantly lower in G4 as compared with G_5 and G_6, with no significant change between G_5 and G_6,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 peroxynitite 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 T4 molecules. An average of one T3 molecule is present for fourteen T4 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

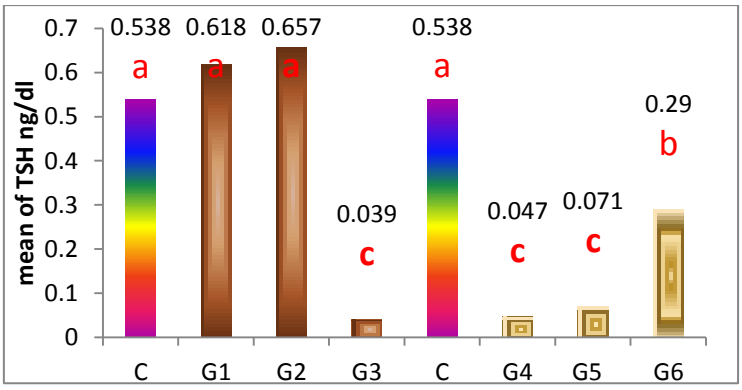

Fig.(1):TSH levels (ng/dl) in sera of adult rabbits treated with aqueous extract and isolated flavonoids from algae and not-treated as control group

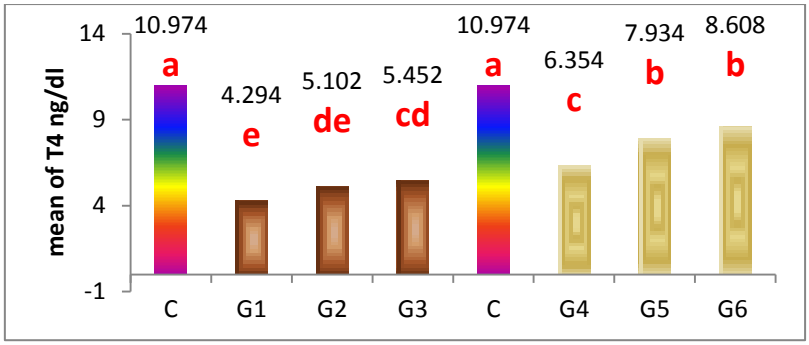

Fig.(2):T4 levels (ng/dl) in sera of adult rabbits treated with aqueous extract and isolated flavonoids from algae and not-treated as control group

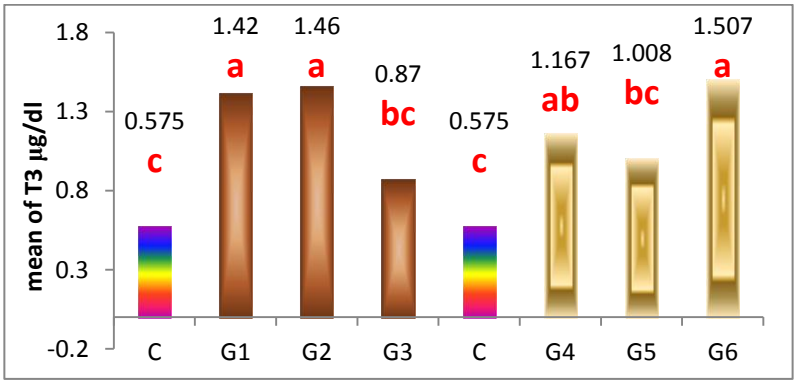

Fig.(3):T_3 levels(µg/dl) in sera of adult rabbits treated with aqueous extract and isolated flavonoids from algae and not-treated as control group.

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

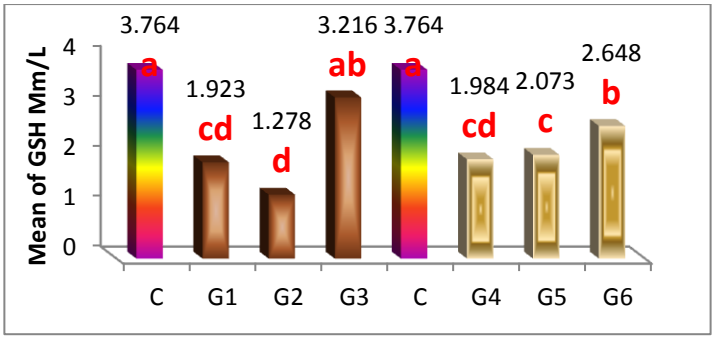

Fig.(4):GSH levels(mM/L) in sera of adult rabbits treated with aqueous extract and isolated flavonoids from algae and not-treated as control group.

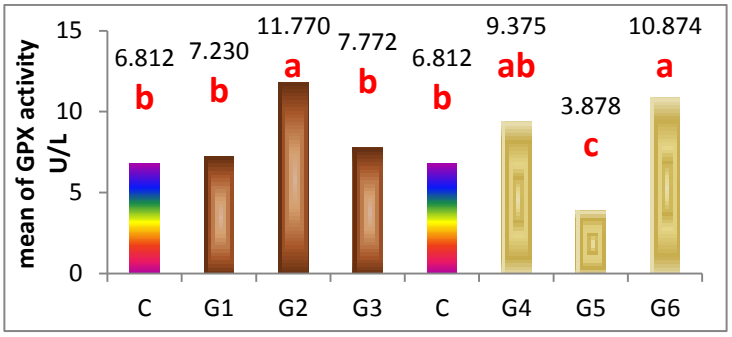

Fig.(5):GPx levels(U/L) in sera of adult rabbits treated with aqueous extract and isolated flavonoids from algae and not-treated as control group.

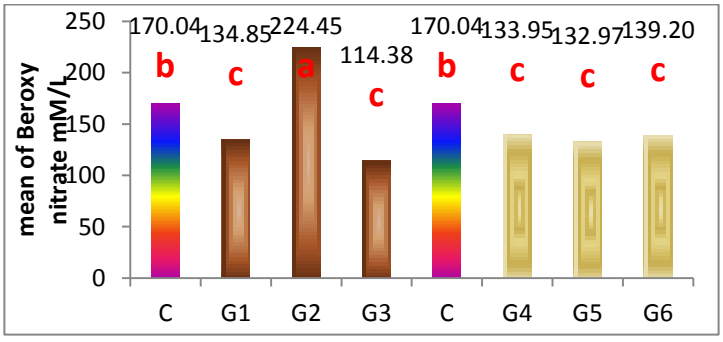

Fig.(6): Peroxynitrate levels(mM/L) in sera of adult rabbits treated with aqueous extract and isolated flavonoids from algae and not-treated as control group.

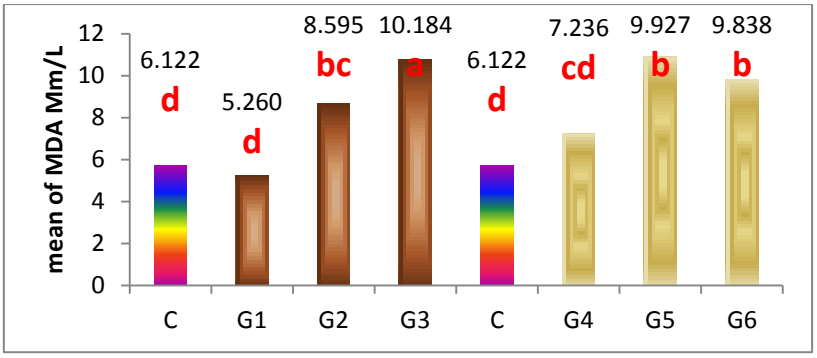

Fig.(7): MDA levels(mM/L) in sera of adult rabbits treated with aqueous extract and isolated flavonoids from algae and not-treated as control group.

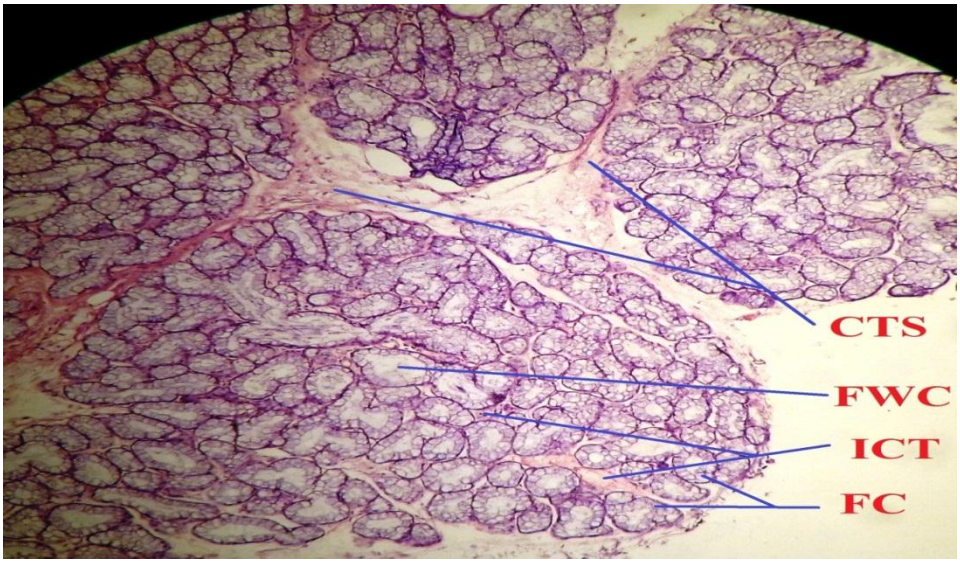

Fig.(8): Cross Section for Normal Rabbits Thyroid tissue, Showed the Connective Tissue Septa, Follicular cells, Follicle with Colloid, Interfollicular Connective Tissu (Control Group-C) (HE)-200X.

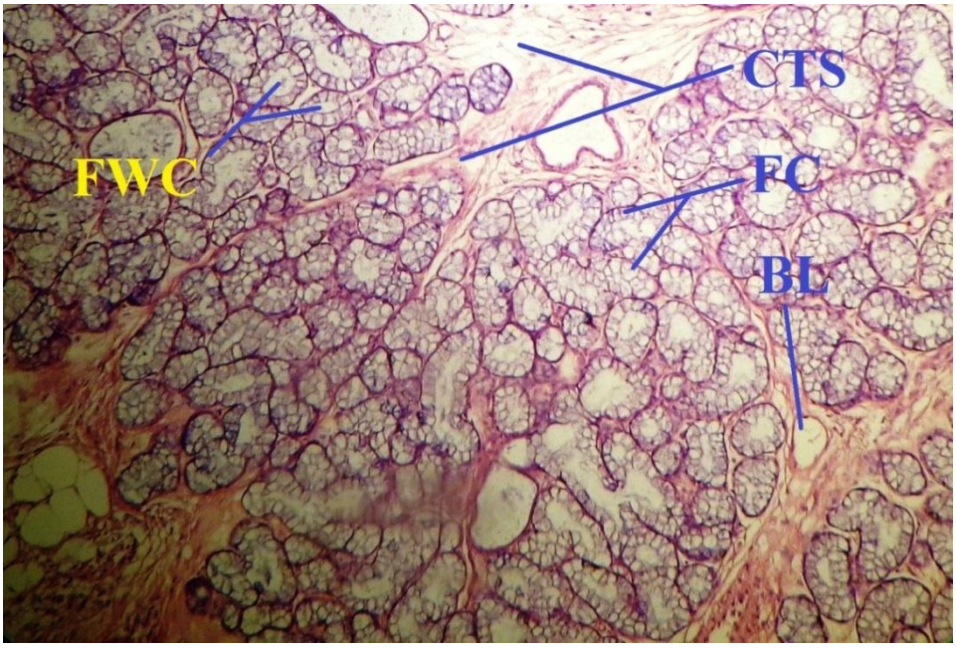

Fig.(9): Cross Section for Rabbits Thyroid tissue, Showed the Blood vessel ;Connective Tissue Septa, Follicular cells and Follicle with Colloid (G1) (HE)-200X.

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

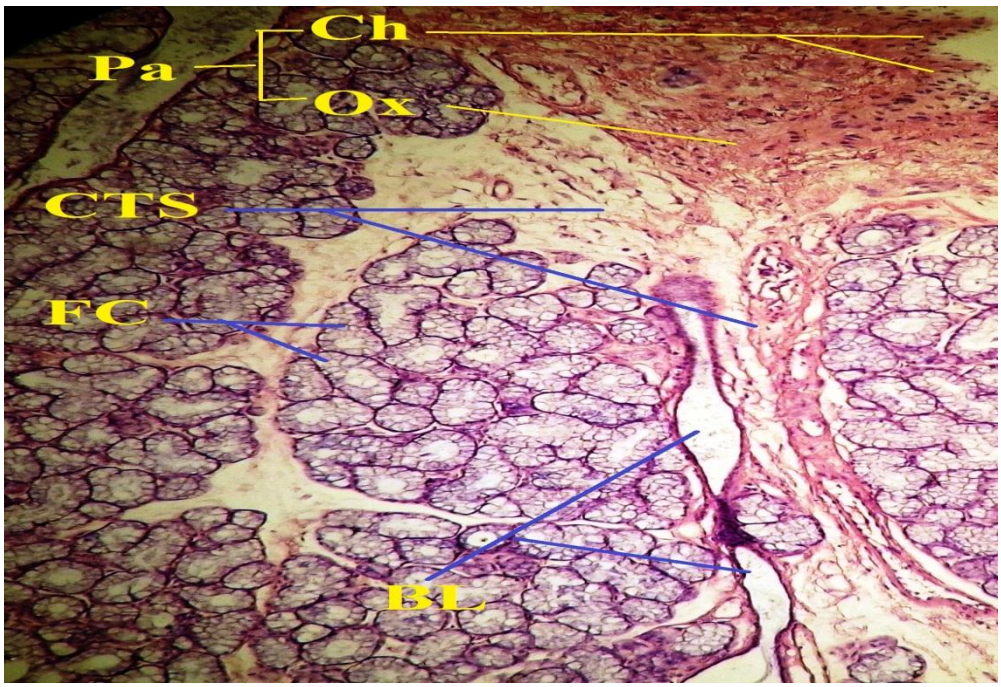

Fig.(10): Cross Section for Rabbits Thyroid tissue, Showed the Connective Tissue Septa, Follicular cells and Parathyroid gland(Ch:Chief cells and Ox: Oxyphil cells) (G2) (HE)-200X.

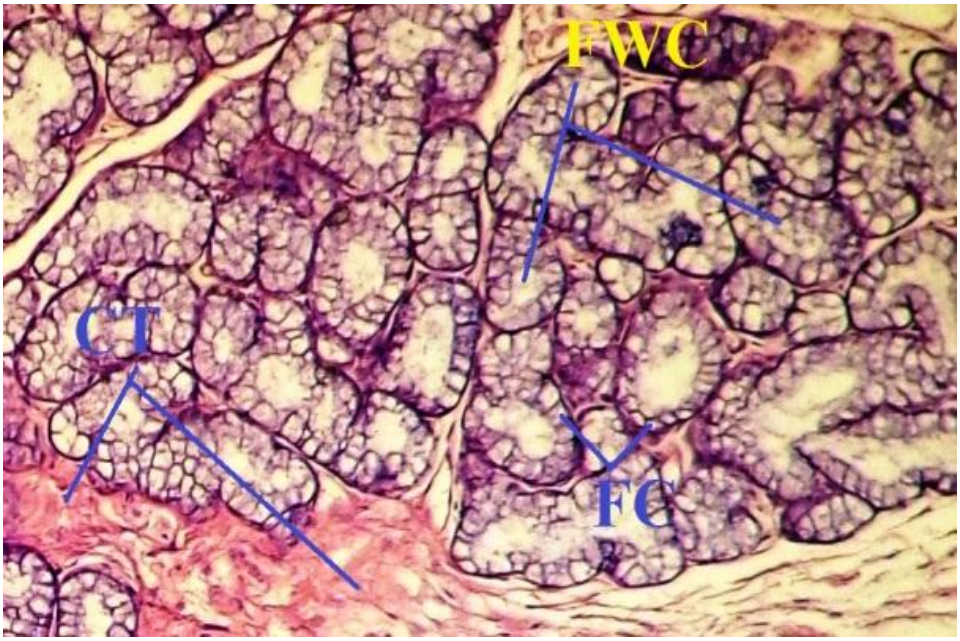

Fig.(11): Cross Section for Rabbits Thyroid tissue, Showed the Connective Tissue, Follicular cells and Follicle with Colloid (G3) (HE)-300X.

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

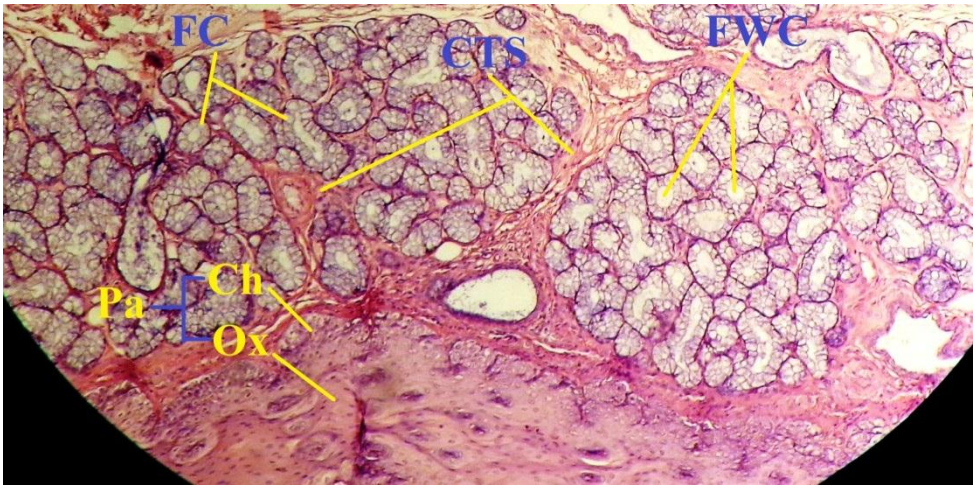

Fig.(12): Cross Section for Rabbits Thyroid tissue, Showed the Connective Tissue Septa, Follicular cells, Follicle with Colloid and Parathyroid gland(Ch:Chief cells and Ox: Oxyphil cells) (G4) (HE)-200X.

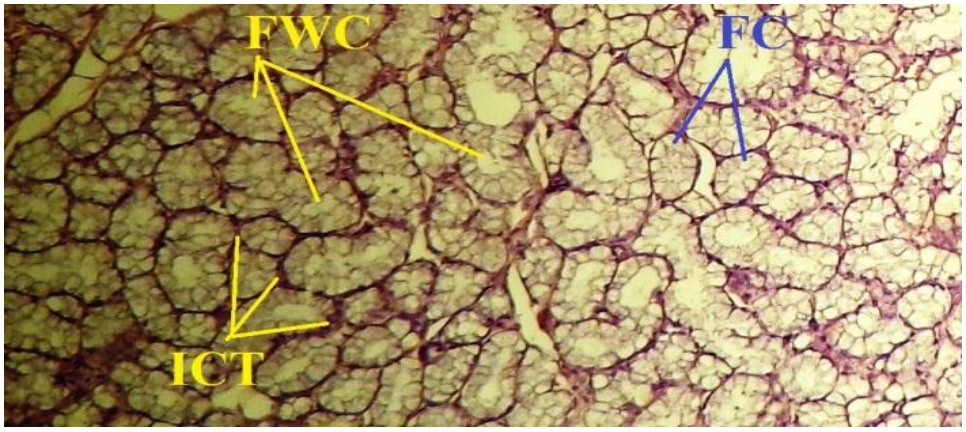

Fig.(13): Cross Section for Rabbits Thyroid tissue, Showed the Interfollicular Connective Tissue, Follicular cells and Follicle with Colloid (G5) (HE)-200X.

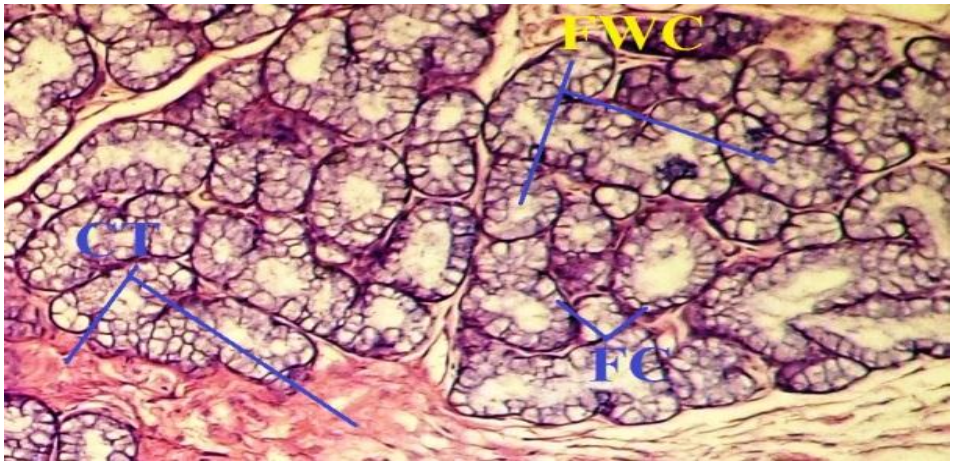

Fig.(14): Cross Section for Rabbits Thyroid tissue, Showed the Connective Tissue, Follicular cells and Follicle with Colloid (G6) (HE)-300X.

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**.**

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