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Histomorphological study of thyroid gland in local adult male squirrel *(Sciurus anomalus)*

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ABSTRACT

The *Caucasian squirrels*, or as called Persian squirrels (*Sciurus anomalus*) are a mammal belongs to order; Rodentia; genus: Sciurus; species: *Sciurus anomalus* in the genus of Sciurus. This study was conducted to study the histomorphological and histochemical structure of thyroid gland in local adult male squirrel(*Sciurus anomalus*). Ten adult healthy male squirrels were used in this study. After euthanasia, six squirrels were used for morphological descriptions. While, the four squirrel used for histological study. Histological sections were examined by light microscope. Morphologically, thyroid gland appeared as small, bright pink structure, consist of right and left lobes, they covered by a thin capsule. The two lobes were entirely separated and not connected by an isthmus. The two lobes were located in the cranial region of trachea on its lateral sides and embedded in the cervical fascia. The histological structure of thyroid gland of squirrel revealed a common pattern of thyroid mammalian histological structure. It is covered by a thin capsule of an inner dense irregular connective tissue made of collagen and elastic fibers, spindle shape fibroblasts and thin outer layer of adipose tissue which display a clear cellular limit interpose with collagenous fibers and few elastic fibers. The lobules in both right and left lobes consisted of an aggregations of different shapes and size follicles which surrounded by a basement membrane, thin connective tissue and collagen fibers, fibroblast and many capillaries. The lining epithelium of the follicles was low simple cuboidal epithelium with spherical nuclei and some follicles were lined by simple squamous epithelium. In conclusion, this study showed that squirrel's thyroid revealed uniformly distributed of variable sized follicles with simple cuboidal epithelium lining.

Keywords: *Caucasian squirrels*, male, Histomorphology, Thyroid gland.

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Introduction

The Caucasian squirrels, or as called Persian squirrels (*Sciurus anomalus*) are a mammal belongs to order; Rodentia; genus: Sciurus; species: *Sciurus anomalus* in the genus of Sciurus. It is inhabit in the forests of Middle East and southwestern Asia. Caucasian squirrel is a medium size rodent with a chestnut gray - grizzled buff dorsum, buff eye rings, and chestnut to buff-yellow under parts. Persian squirrels weighing ranged (200 - 410) g. Color of upper body fur range from greyish brown - pale grey, according to the subspecies, while the under parts is rusty brown - yellowish and of tail is yellow brown to deep red (Abass, 2017).Thyroid gland is an unique gland among the endocrine glands(Braverman and Cooper, 2012). Thyroid gland has similar follicular structure despite there some gross, histological and ultrastructural variations among the species. Thyroid gland responses to environmental and nutritional influences which vary

among the domestic animals (Bhardwaj *et al.,* 2006). Up to date, there are no available studies previously on the thyroid gland on the thyroid gland in local adult male squirrel, therefor this study was conducted to study the histomorphological and histochemical structure of thyroid gland in local adult male squirrel(*Sciurus anomalus*).

Materials and methods

Ten adult healthy male squirrels were used in this study which brought from local supplier in Baghdad- province. All animals were weighed using sensitive digital balance, its weight was ranged between (200 - 202) g and the mean weight was 201 g. After euthanasia, six local male adult squirrels were used for morphological descriptions (shape, color and location and relationships) of thyroid gland, as well as its morphometrical measurements (weight, length, width and thickness) using electronic digital vernier caliper). The photographs of the thyroid gland were taken to depict the gross anatomy by using digital camera Sony cyber-shot (14.2 mega pixel). While four adult male squirrel used for histological and histochemical study. A small pieces (1 cm3) were taken from two lobes of thyroid then proceed with routine histological technique after fixation by (10%) neutral buffered formalin and stained by: Harris Hematoxylin and Eosin , Periodic Acid Schiff Reagent (PAS) and Masson Trichrome Stains(Luna, 1968; Bancroft and Stevens, 2012).The microscopic parameters were recorded and listed in table(Altaay, 2007; Igwenagu *et al.,* 2016). Histological sections were examined by light microscope (Olympus/ Japan) and photographed by(Future Win Joe)camera, with different magnifications. The Statistical Analysis System- SAS (2012) program used to explore the significant differences between right and left lobe of studied parameters (T test) at ($p< 0.050$).

Results

The morphological results showed that the thyroid gland, appeared as small, bright pink structure, consist of right and left lobes, they covered by a thin capsule. The two lobes were entirely separated and not connected by an isthmus (Fig. 1).

Fig. 1: Gross anatomy of thyroid gland in Local male squirrel Shows: Right lobe(R), Left lobe (L), Trachea (T).

The two lobes were located in the cranial region of trachea on it's lateral sides and embedded in the cervical fascia. Right lobe was slightly cranial and slightly shorter than

the left lobe. It was extended between the end of cricoid cartilage and the first tracheal ring (Fig**.** 2), whereas the left lobe extended from the posterior end of cricoid cartilage till the second tracheal ring.

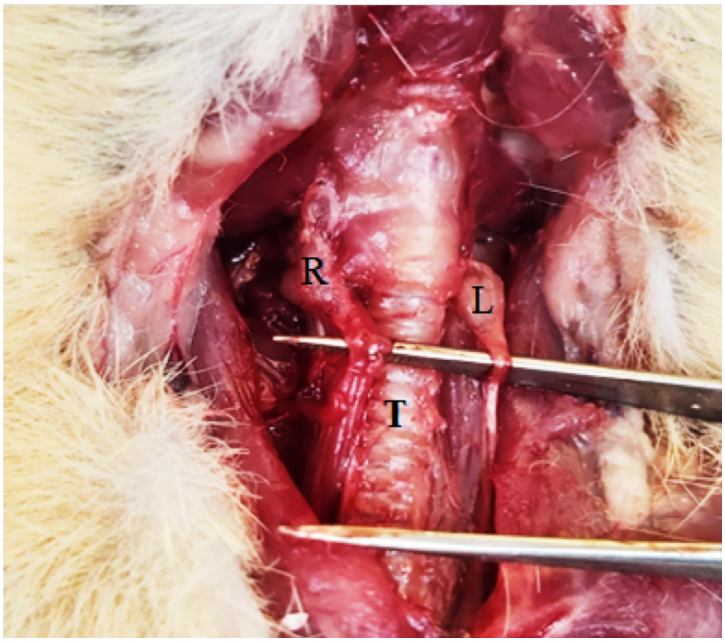

Fig.2 : Gross anatomy of thyroid gland in Local male squirrel Shows: Right lobe(R), Left lobe (L), Trachea (T).

Both lobes appeared as a very small compact mass, the right lobe appeared as rounded to triangle structure, while the left appeared as elongated oval with rounded cranial end and narrower caudal end (Fig**.** 2**,** 3)**.**

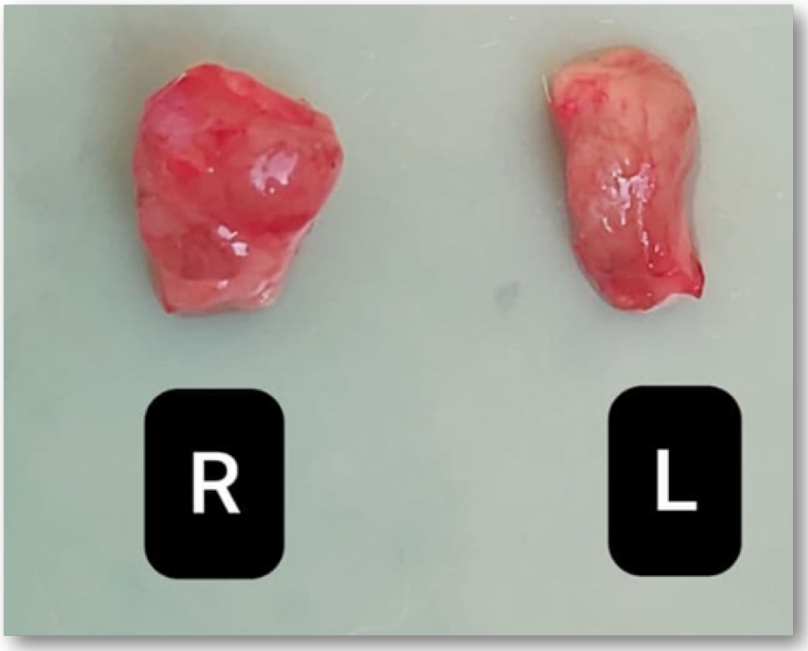

Fig.3: Gross anatomy of thyroid gland in Local male squirrel Shows: Right lobe (R), Left lobe (L).

Lateral surface of each lobe was convex related to numerous structures such as common carotid artery**,** vaso sympathetic trunk, jugular vein**,** and sternomastoideus that crossed

the ventral borders of both lobes**.** Medial surface was flat and related to the tracheal rings (Fig**.** 2, 3)**,** and has two borders (dorsal and ventral), the ventral border was thinner than the dorsal one and covered by sternothyroideus**.** The isthmus was absent, therefore the two lobes were completely separated (Fig**.** 1, 2, 3).

The mean weight of entire thyroid gland, relative weight, mean weight**,** length**,** width**,** thickness of right and left lobes were listed in(Table 1)**.** No significant differences of these parameters between right and left lobes at p< 0**.**05.

Table1: Anatomical Parameters of the Right and Left Lobes of the Thyroid glands in male Local Squirrel.

Morphometrical Parameters	Right Lobe	Left Lobe
Weight (g)	0.34±0.1	0.32±0.2
Length (mm)	3.1± 0.1	4.0± 0.2
Width (mm)	3.6± 0.1	3± 0.1
Thickness (mm)	2.6± 0.02	2± 0.01

Generally the histological structure of thyroid gland in adult male squirrel revealed a common pattern of thyroid mammalian histological structure**.** Its covered by a thin capsule of an inner dense irregular connective tissue made of collagen and elastic fibers**,** spindle shape fibroblasts and thin outer layer of adipose tissue which display a clear cellular limit interpose with collagenous fibers and few elastic fibers (Fig.4, 5).

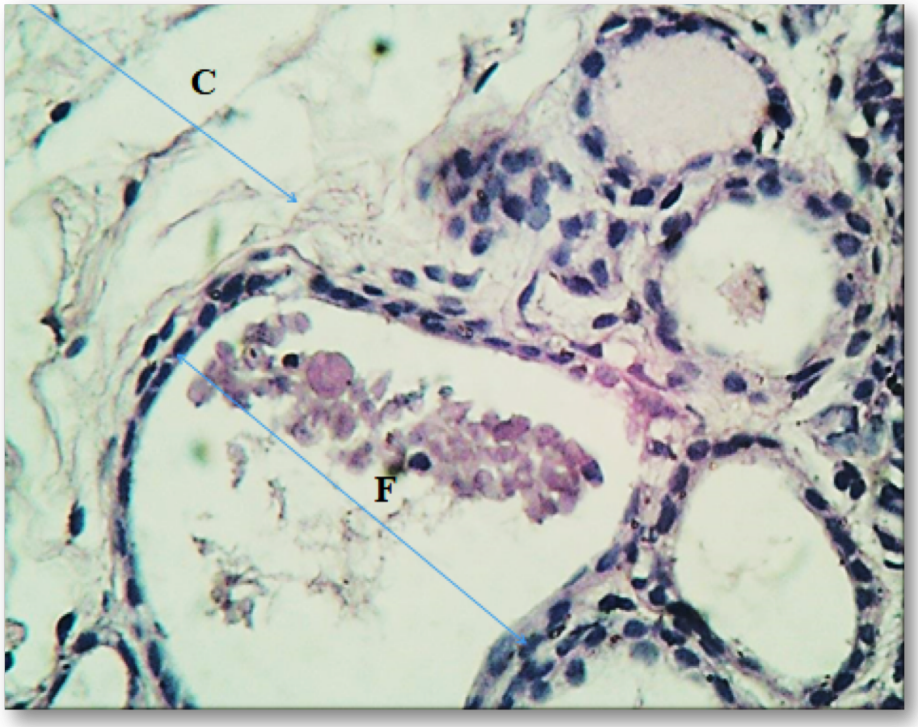

Fig. 4: Histological Section of Left lobe of thyroid gland in local male squirrel Shows: Capsule (C),Follicles (F) X4 (H&E Stain)

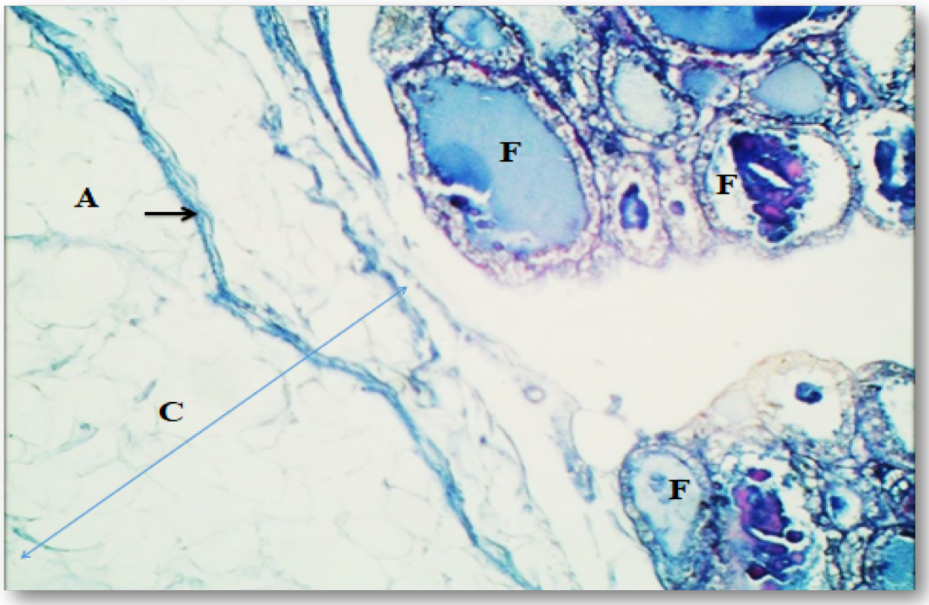

Fig.5 : Histological Section of thyroid right lobein Local male squirrel Shows: Capsule (C), Collagen fibers (black arrow), Adipose tissue (A), Follicles (F), X10 (Masson Trichrome Stain).

Thin trabeculae extend from the capsule that display a small blood vessels into the glandular parenchyma and separating it into clear and different size lobules (Fig.6).

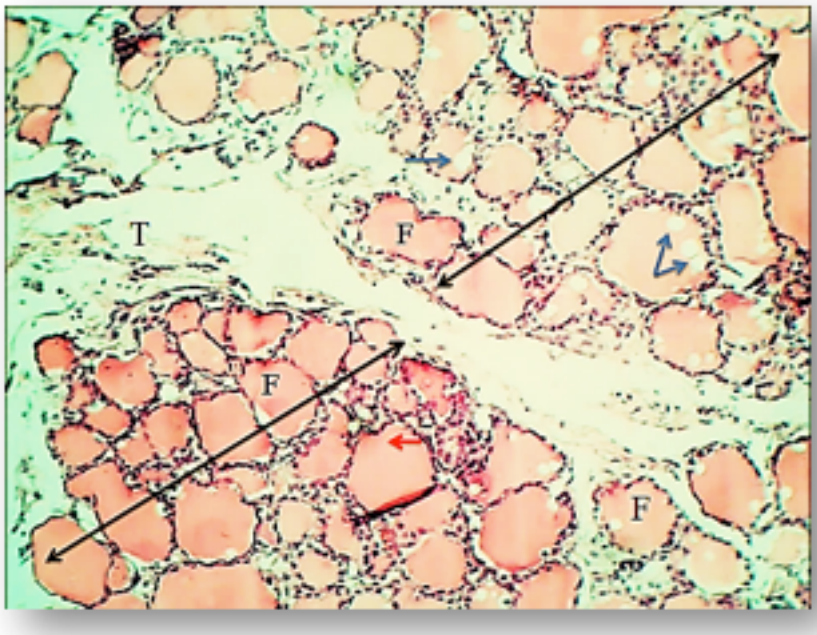

Fig.6 : Histological Section of right lobe of thyroid gland in Local male squirrel Shows: Lobule (black arrows), trabeculae (T)Follicles (F), colloid (red arrow), vacuoles blue arrows. X10 (H&E Stain)

Mean capsule thickness of right and left lobes were1 listed in (Table 2). Statistically, there was no significant differences between right and left lobes at p< 0.05 (Table 2).

Table2: Histological Parameters of the Right and Left Lobes of the Thyroid glands in male Local Squirrel.

Table2: Histological Parameters of the Right and Left Lobes of the Thyroid glands in male Local Squirrel.		
Parameters(µm)	**Right Lobe**	**Left Lobe**
Thickness of capsule	**132.2 ± 0.01**	**132.6± 0.04**
Diameter of small Follicles	**18.1± 3.56**	**18.3± 3.49**
Diameter of Medium Follicles	**49.6± 3.20**	**49.2±3.29**
Diameter of Large Follicles	**64.3±2.1**	**64.10± 2.3**
Height of Follicular Cells	**4.3 ± 0.2**	**4.5± 0.5**

The lobules in both right and left lobes consisted of an aggregations of different shapes and size follicles which surrounded by a basement membrane**,** thin connective tissue and collagen fibers**,** fibroblast and many capillaries(Fig.6, 7).

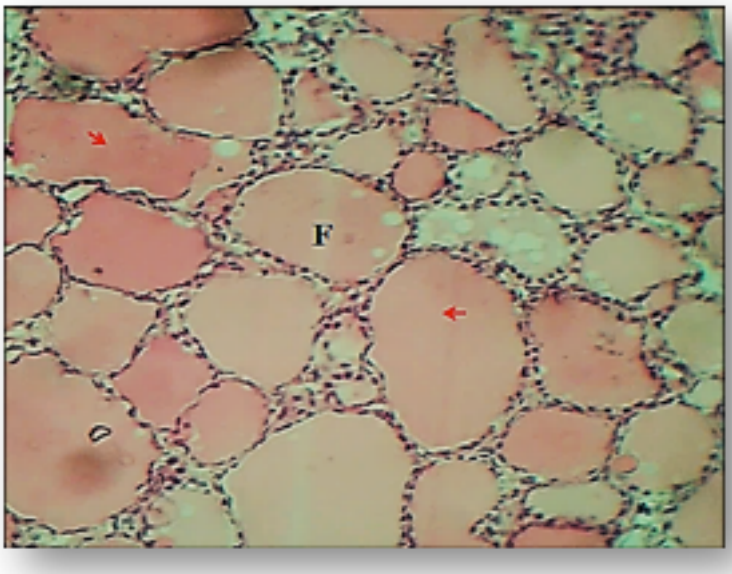

Fig.7: Histological section of right lobe of thyroid gland in male local male squirrel shows: Lobule (black arrow), Follicles (F), colloid (red arrows) X10 H&E Stain X400 (H&E Stain)

Different shapes of follicles were observed as rounded**,** oval**,** elongated, polygonal, and irregular shape follicles**.** The rounded and oval shape follicles were the predominated follicles (Fig.6, 7). There were three sized follicles were reported include; small**,** medium and large -sized follicles**.** Also thyroid gland consisted of a thin network of inter follicular connective tissue rich in blood capillaries which surround each follicles**.** These follicles were spread through right and left lobes and contains colloid substance (Fig**.** 6, 7). In the right and left lobes, the average diameters of small, medium and large size follicles were listed in (Table 2). There were no significant differences between right and left lobes at p< 0**.**05 (Table 2)**.** The lining epithelium of the follicles was low simple cuboidal epithelium with spherical nuclei and some follicles were lined by simple squamous epithelium (Fig**.** 8, 9).

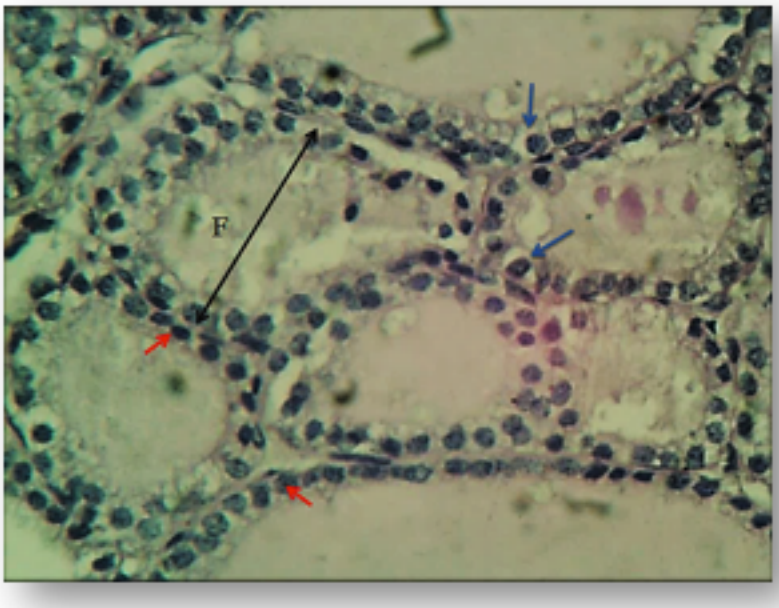

Fig. 8: Histological section of right lobe of thyroid gland in Local male squirrel shows: Follicles(F), simple cuboidal epithelium (red arrows), Parafollicular cells (blue arrows) X100 (PAS Stain).

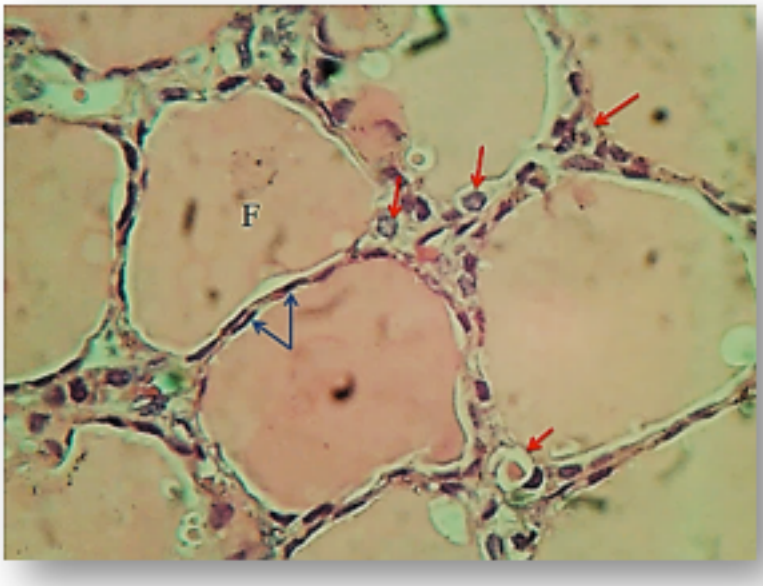

Fig.9: Histological section of right lobe of thyroid gland in male local squirrel shows: Follicles(F), simple squamous epithelium (blue arrows) Parafollicular cells (red arrows) X100 (H&E Stain).

The average height of follicular epithelium were listed in (Table. 2). There were no significant difference between right and left lobes at ($p < 0.05$) (Table2).

The para follicular cells appeared as a large oval to rounded cells, more lightly stained cytoplasm than follicular cells and dense stained nuclei. These cells observed among the follicular cells. The base of follicular and the para follicular cells were rested on the

basement membrane, while few other cells found in between the follicles and organized in groups (Fig. 9, 10).

Thyroid follicles were contained a variable amount of homogenous eosinophilic colloid substance in the intra follicular spaces (Fig**.7,** 8), and none uniformly stained colloid in which many or few peripheral visible empty vacuoles present in the stored colloid **.** Some follicles observed without colloid (Fig. 7, 11). The colloid substance showed a different degree of density**;** some follicles were contains densely stained eosinophilic colloid substance which react strong positive with PAS and showed magenta color**.** Few follicles contains faintly stained colloid which showed weak positive reaction towards the PAS stain (Fig**.** 11).

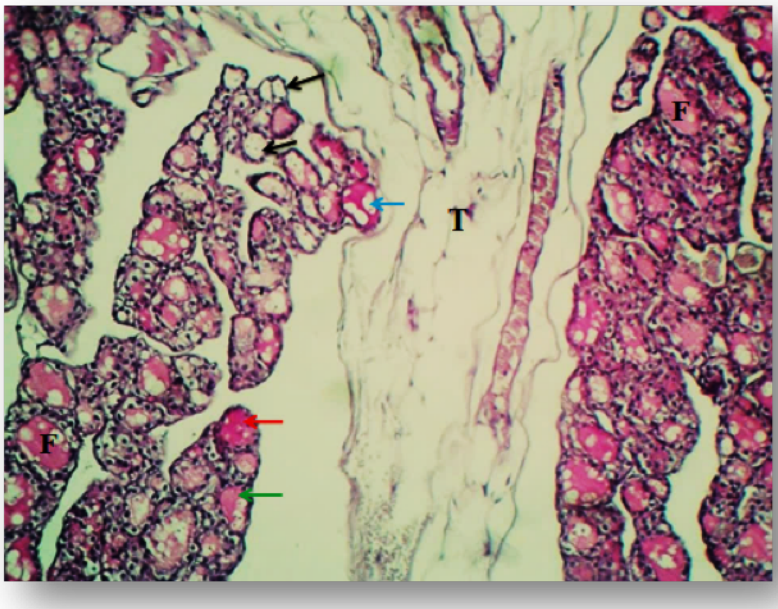

Fig.10: Histological section of Right lobe of thyroid gland in male local squirrel shows: Follicles (F), vacuoles (blue arrow), empty follicles (black arrows), strong positive reaction (red arrow), moderate(green arrow), Trabeculae (T), X100 Positive reaction for PAS Stain

Discussion

Thyroid gland in local male squirrel showed it was composed of two lobes and its location was similar to that reported in other domestic animals (Dawood, 2014). However the situation of thyroid is variable, in majority of animals (Kausar and Shahid, 2006). In goats, thyroid lobes extended between the 4th - 8 th tracheal ring (Dawood, 2014). Currently, the two lobes were separated and no isthmus, as reported by (Mark *et al*.,1984) in cats, but disagree with Dawood (2014) in goats in which the two lobes were connected by a thin strand isthmus.

The general shape of the thyroid gland in male squirrel was similar to those recorded in domestic animals such as in buffalo by (Altaay, 2007). The right lobe appeared as rounded to triangular shape, this was disagree with that reported in sheep, goat, dog and camel (Ali,1987; Habel,1989).In squirrel, statistically, no significant differences between two lobes in gross parameters, similar findings by Dawood (2014) in goat.

Generally, the histological structure of thyroid gland in squirrel was similar to those found in other domestic animals. It was surrounded by a thin capsule of dense irregular

connective tissue structured of collagenous fibers, similar to that reported by Hamad (2008) in goats and Aughey and Frye (2010) in dog.

The thickness of capsule in both lobes was thinner than that reported by (Salih, 2017) in sheep, this may be due to species variation. A vascularized septa extend from capsule to the parenchyma dividing it into distinct lobules that made from of an aggregation of follicles which encircled by a basement membrane. Inter follicular connective tissue contains a network of capillaries which provides a numerous blood supply for follicular cells. Same as reported by (Ali, 2015) in sheep.

The thyroid parenchymal tissue of squirrel was represented by a various follicles, thin inter folllicular connective tissue contained collagen fibers and fibroblast, extensive peri-follicular capillaries and sinusoids, as observed by Igbokwe and Ezeasor (2015) in pigs. Each lobule was made of an aggregation of different shapes and sizes of follicles. Three main size of follicles were found and randomly distributed, this result was agree with Prasanth *et al*. (2012) in cats. Statistically, there was no significant differences in the mean follicular diameters between right and left lobes in studied squirrel, this result may be due to that two lobes considered the main functional structures of the thyroid gland.

The histological examination of thyroid in squirrel showed uniformly distributed of variable sized follicles, as recorded by (Ali *et al.,* 2014).The follicular lining epithelium in squirrel was simple cuboidal epithelium, same as reported by Shehan ,(2017) in goats. Statistically there were no significant differences between two lobes, this result may indicate the two lobes in the same stage of activity. As observed in goats by (Dawood, 2014). The para follicular cells observed as oval to rounded larger and lighter than follicular cells with dense stain nuclei. They were occupied two locations; the inter follicular cells and para follicular positions, as reported by (Seham *et al.*, 2012) in rats. In most mammals, the role of para follicular cells in calcium metabolism through calcitonin hormon (Igbokwe *et al*., 2015). Thyroid follicles in squirrel contained different amounts of homogenous colloid substance which reacted positive for PAS stain, due to its thyroglobulin an iodinated glycoprotein substance (Santos *et al*., 2013). Similar findings by Nadhim, (2017)in Iraqian goats. The functional activity of the follicles can be evaluated based on the color of colloid inside them, According to (Moghanlo and Mohammadpour, 2018).

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