

Histological Study of Effects of Saccharin on Thyroid Gland in Male White Mice

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Abstract:

The present study aimed to evaluate the histological and physiological changes induced by administration of saccharin on the thyroid gland of mice. Thirty adult male mice used in this study were divided into two groups. The first group used as a control; the second group used as the treated group which treated with 1 ml daily of saccharin solution orally administered for 30 days. After ending the treatment period, blood samples were collected to assessment of T3 and T4 levels, then the animals were sacrificed for histological study. The results showed the thyroid gland of male after oral administration with saccharin have significant histological differences in most tissue field of thyroid gland, when compared with control group. The physiological results showed a significant difference of hormonal secretions (T3 and T4). Therefore, it can be concluded that treatment of mice with saccharin caused histological and physiological changes in the thyroid gland. So, we should be reducing the use of saccharin as sweeteners and increase public awareness regarding artificial sweetener consumption.

Keywords: Saccharin, Sweeteners, Thyroid gland

1.Introduction:

In recent years in the world, market demand for low-calorie goods in the forms of widely different sugar-free food items such as drinks powder, sweets, jams, carbonated beverages, jellies, dairy products and canned foods has been seen observed. All sweeteners that known as a different name like low calorie or artificial or alternative sweeteners have taste similar to sugar but it contains no calories [1].

Throughout recent decades, increasing quality and health of life issues have motivated people to reduce the consumption of products that are high in sugar. Food products that contain calorie-free alternatives have become increasingly common. Alternative sweeteners are hundreds to thousands of times sweeter than saccharose. Each sweetener has unique features of intensity of sweetness, teeth coating, persistence of sweet taste and aftertaste result [2]

Sweeteners are food additives that are used to improve the taste of everyday foods. Natural sweeteners are sweet-tasting substances that have a certain nutrient value, monoor disaccharides are the major component in natural sweeteners. In contrast, artificial sweeteners are compounds with very little to no nutritional worth. Artificial sweeteners are recent and are being tested and expanded every day for their uses [3]. Artificial sweeteners which are still of concern despite common use [4]. Recently, the common assumption was that non-nutritive sweeteners (NNS) were safe replacements for sugar, because they offer sweet taste without calories or glycemic impacts. However, evidence from several epidemiologic studies have shown that intake of NNS is associated with increased risk of developing obesity, metabolic syndrome and type 2 diabetes, particularly in diet sodas [5]. The Food and Drug Administration Authority of the United States has approved saccharine, acesulfame-K, aspartame, neotame, sucralose, and stevia for human use within the appropriate daily intake limit

[6].Animal studies note the impact of artificial sweeteners on the immune system. Sugar substitutes were the culprit in the development of Hashimoto thyroiditis in patient [7].Non-nutritious sweeteners are most widely used worldwide. Furthermore, their metabolic outcomes are still uncertain, and their effect on the thyroid action, a main metabolism regulator, has not been studied before [8].Although few studies are suggesting that a high dose, prolonged use of sweeteners might increase the risk of development of certain malignancies, we have found an increased risk of thyroid cancer with artificial sweetener consumption [9].Saccharin (benzoic sulfimide) is a very stable organic acid with chemical formula $C_7H_5NO_3S$. Saccharin is one of the oldest artificial sweeteners [10].Saccharin is used as a tabletop sweetener in drinks, soft drinks, canned fruit, baked products, chewing gum, confectionery, salad dressings and also in cosmetic and pharmaceutical products with considering to acceptable daily intake (ADI) [1].Artificial sweeteners, when used in moderation are an acceptable substitute to natural sweeteners. They appear to be a healthy and pleasant way to enrich food sweetness, taste, and texture while keeping major health risks low [10].

The thyroid gland and its hormones play multifaceted roles in organ development and in the homeostatic control of fundamental physiological mechanisms such as body growth and energy expenditure in all vertebrates [11].Thyroid hormone production is tightly regulated and clinical manifestations of thyroid system dysfunction are well known. T3 is an important regulator [12]

2.Materials and Methods:

2.1. Experimental Animals:

Thirty matured male albino mice with weighing range 27-30gm (three-month-old) were bought from Drug and Healthy center in Baghdad, Iraq. Mice were kept in plastic mice cages, all cages put in the animal house of the college of science in Al- Muthanna University under controlling of temperature 25-28°C, controlled humidity situation (65%) with feeding by using standard pellets for about 15 days to adapt to their environment.

2.2. Chemical: Saccharin

2.3. Exposure dose: The dose level is 1 ml saccharin orally administered for a period of 30 days. According to [1].

2.4. Experimental design:

Thirty male mice were divided into two (A and B) groups, Group A as a control group and group B as treated group. 2.4.1. Control group: There were 15 mice which feed without any substance in their food. Plenty of water was supplied using a feeding bottle. 2.4.2. Treated group: There were 15 mice which orally administered with saccharin at a dose level of 1ml daily for 30 days.

2.5. Preparation of histological slide:

After the end of the treatment period, the animals were anesthetized by using chloroform then sacrificed by bleeding from common carotid artery. Thyroid gland was collected carefully from the mice by using the standard procedure. The samples washed with normal saline to remove any contest of blood. Fixation: the tissue samples were fixated with formalin 10% for a period of (48) hour, then washed under running tap water for one hour until completely removed of most of the formalin odder from the tissue samples. Followed by washing, dehydration of the tissues was conducted by immersing the tissue in a series of gradually increasing concentrations of alcohol (50%, 70%, 80%,

90%, and absolute alcohol). Clearing it is obligatory because the used alcohol for dehydration will not dissolve or mix with molten paraffin. Blocking means the samples embedded into paraffin wax for making blocks. The block was to be trimmed by removing of wax from the surface of the block to expose the tissue, cutting of the tissue was performed by using a microtome. The microtome was pre-set to cut the tissue as thicknesses with 5 μm . Blocks Small ribbons of tissue sections were placed on a microscopic slide with help of warm distil water containing few drops of Mayer's albumen and deparaffinized with xylene solution, the slide was put on the hot plate with (40°C) for overnight. Hematoxylin and eosin yellow solution were used to stain the tissue for preparing permanent slide [13]. Histopathological changes were observed under a light microscope and snaps were taken.

3. The Histological Results of thyroid:

3.1 The Histological Results of male Control Group:

The histological results of thyroid gland in the male showed the parenchyma characterized have few numbers of large follicles which have diameter ($137.23 \pm 23.70 \mu\text{m}$), (Table 1) which distributed among the thyroid parenchyma; while medium and small follicles present in high aggregation among parenchyma ($88.84 \pm 8.79 \mu\text{m}$), ($45.43 \pm 4.57 \mu\text{m}$), (Table 1) respectively. The tissue section of control thyroid gland showed the wall of follicles which lined by simple cuboidal epithelial with thickness was ($10.69 \pm 2.28 \mu\text{m}$), the diameters of follicular cell nuclei was ($41.71 \pm 8.75 \mu\text{m}$) (Table 1) fig (1).

The thyroid gland was surrounded by connective tissue capsules, which composed of two layers; the external layer which consist of loose collagen fibers mixed with large amount of adipose tissue with the presence of a few amount of elastic fibers. The inner layer consists of bundles of collagenous and elastic fibers, with few smooth muscles' cells. The capsule of the thyroid gland was highly vascularized with clear blood vessels supply fig (2). The connective tissue capsule sends thin trabecular in to the parenchyma around the different types of follicles and divided the thyroid parenchyma into poorly defined lobules.

The thyroid parenchyma contained on the different types of follicles which consider the structural and functional unit of thyroid gland. Three types of the thyroid follicles (large, medium, small) with different in shape. The tissue section of male thyroid gland showed high numbers of small follicles more than other type were peripherally distribution. The internal lumen of follicles was lining by simple cuboidal epithelial. Two types of cells located in two locations, one of them located inside the internal surface of thyroid follicles wall, the type one cell have dark nuclei acidophilic cytoplasm, and second type have ovoid dark nuclei centrally location with acidophilic cytoplasm fig (1).

3.2 The Histological Result of Treated Group.

Thyroid tissue sections of treated male mice showed some histological changes. The thyroid gland was surrounded by prominent connective tissue capsule, also showed the wall thickness of thyroid follicles was ($9.46 \pm 1.88 \mu\text{m}$) which have significant difference compared with the control group (Table 1). Also, the result showed the nuclei diameters of follicular cell was ($33.23 \pm 6.20 \mu\text{m}$) which have significant difference when compared with the control group (Table 1).

The diameter of the large follicles was $(151.04 \pm 27.74 \mu\text{m})$, (Table 1) which has significant difference compared with the control group. The diameter of medium follicles was $(79.93 \pm 17.55 \mu\text{m})$ which have significant difference compared with the control group (Table 1), the diameter of small follicles was $(49.42 \pm 4.93 \mu\text{m})$ which has significant difference with control group (Table 1) respectively. This result agreement with [14] which said that the histological result of infected thyroid gland showed the large follicles present in the most prevalent that have significantly increased the diameter $(78.8 \pm 1.9 \mu\text{m})$ compared with large follicles in control group.

The tissue section of treated thyroid gland have abnormal cellular proliferation in the peripheral zone of thyroid loop and between the small follicles of thyroid gland. The tissue field noted blood hemorrhage also noted the large follicles have dominant and wide distributed among the loops of treated thyroid gland may be due to follicles rich in colloid (fig3). This result agreed with [15] which said that negative result from artificial sweetener use was observed. The histological result of thyroid gland after treated time showed lobule of thyroid also showed blood congestion between different types of thyroid follicles, noted necrotic lesion in connective tissue between lobules as shown in (fig 4).

Tissue section of treated thyroid gland have acute necrosis and inflammatory cells between the deferent follicles types, also showed prominent damage in wall of large follicle also noted some abnormal aggregation of parafollicular cells, the tissue section noted disappeared the colloid secretion from the follicles, these result agreed with [16] hypothesized that intake of artificial sweeteners may play a role in thyroiditis (fig 5).

The histological result appeared large follicle filled with thick colloid, the follicular epithelia that lining the internal surface of the follicles was prominent in three types of follicles, the follicular epithelial cells have prominent dark nuclei with acidophilic cytoplasm, also showed connective tissue capsule, cellular proliferation between small follicles (fig 6) these result agreed with [17] which showed in the hyperthyroid group, thyroids presented follicles of several sizes containing a large quantity of colloid, being coated by flattened epithelium and visibly small.

Tissue section of thyroid noted small aggregation of inflammatory cells exactly between small follicles may be due to immune response, the tissue section showed prominent damage in the wall of large follicles may be due to connective tissue damage on the other hand noted cellular proliferation between small follicles and cystic dilation (fig 7).

Tissue section of treated thyroid gland showed lobule of thyroid, abnormal cellular proliferation and inflammatory cells aggregation agreed with [9] which suggested that the association between the autoimmune Hashimoto's thyroiditis with hypothyroidism and excessive consumption of beverages containing artificial sweeteners also they found an increased risk of thyroid cancer with artificial

sweetener consumption (fig 8). The tissue section showed damage in follicle wall and prominent vacuoles in the lumen large follicle also showed irregular space in connective tissue between the follicle, so showed parafollicular cells proliferation (fig 9) these result agreed with [18] which said that sweeteners have induce histological abnormalities in the spleen and bladder and adverse effects on the immune system in mice.

The tissue section showed prominent empty cystic dilation between the follicles also showed blood congestion between the follicles may be because of some tissue degeneration after treated time, so noted abnormal cellular proliferation in different location beside the blood hemorrhage in thyroid gland, also showed tissue degeneration (fig 10). This agreed with the study of [19] which showed the histological examination of sweeteners-treated group was loss of normal architecture of the thyroid gland. Morphometrical measurement showed many follicles were small in size and others had disrupted wall and detached cells in their lumens, there was significant increase in the height of cells and number of follicles with decrease in width of the cells.

Table (1) Effect of Saccharin on Thyroid gland (mean \pm standard deviation).

Treatments parameters	Control	Saccharin	Sig.
wall thickness of thyroid follicles	10.69 \pm 2.28 ^a	9.46 \pm 1.88 ^b	0.01
Follicular cell nucleidiameter	41.71 \pm 8.75 ^a	33.23 \pm 6.20 ^b	0.01
large follicle diameter	137.23 \pm 23.70 ^b	151.04 \pm 27.74 ^a	0.01
medium follicle diameter	88.84 \pm 8.79 ^a	79.93 \pm 17.55 ^b	0.01
small follicle diameter	45.43 \pm 4.57 ^b	49.42 \pm 4.93 ^a	0.01

*Different small letters indicate the existence of significant differences between saccharin and control at the possibility of (0.01).

4. The physiological Results of T3 and T4

A- Control group:

The hormonal secretions of thyroid gland in control group noted in table (2) that showed the level of T3 in serum of male control group was (2.12 \pm 0.287 nmol/l). Table (2) showed the level of T4 in serum of male control group was (43.71 \pm 3.83 nmol/l).

B- Saccharin Treated Group:

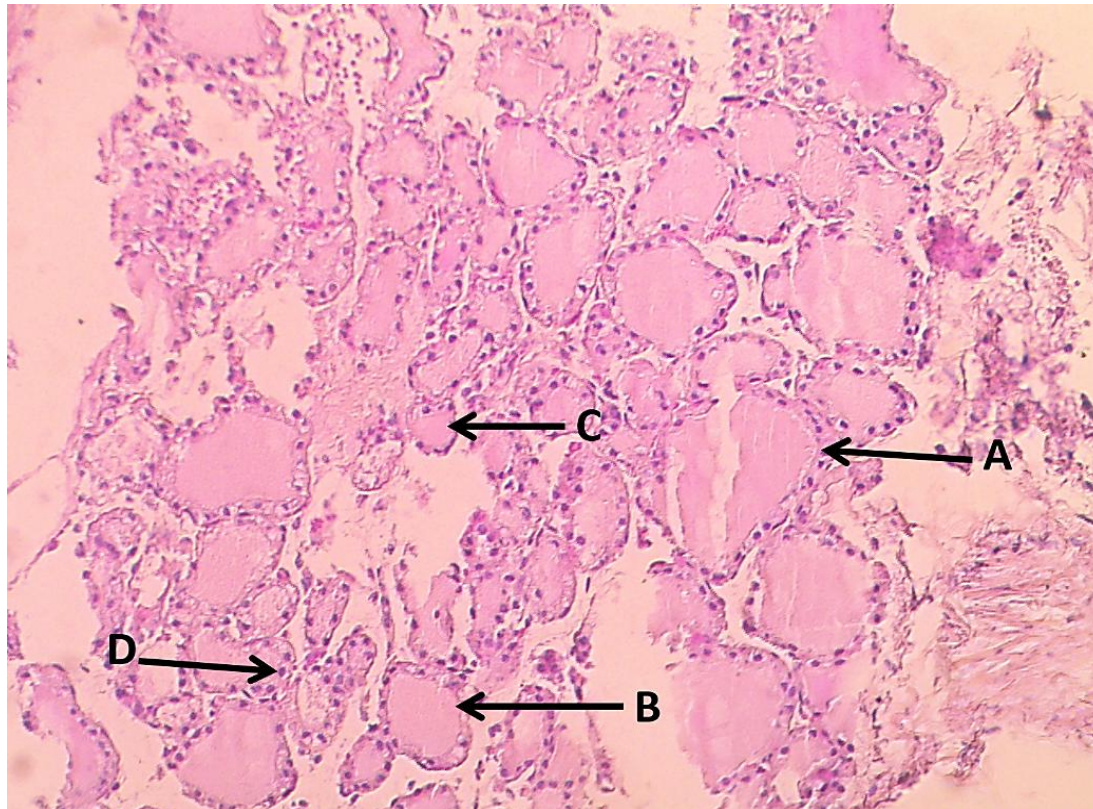
The physiological result showed the level of T3 in treated group noted in table (2) which have the saccharin level in male treated group was (1.49 \pm 0.257 nmol/l). This result appeared the treated group with saccharin have significant decreased in the level of T3 compared with control group. These

significant difference in the level of T3 was may be due loss of food intake that effect on the metabolism rate which lead to negative feedback mechanism on thyroid gland. This biochemical change of T3 after treated with saccharin was agreement with [20] which demonstrated that there was a significant decrease in T3 hormone of rats received sweeteners. This ultimately leads to the failure of the thyroid gland. Table (2) showed the level of T4 in serum of group after treated with saccharin. The level of T4 in male group was $(34.60 \pm 4.88 \text{ nmol/l})$. This result noted the treatedgroup with saccharin have significant difference compared with control group. This biochemical change of T4 after treated with saccharin was agreement with [20] which demonstrated that there was a significant decrease in T4 hormone of rats received sweeteners.

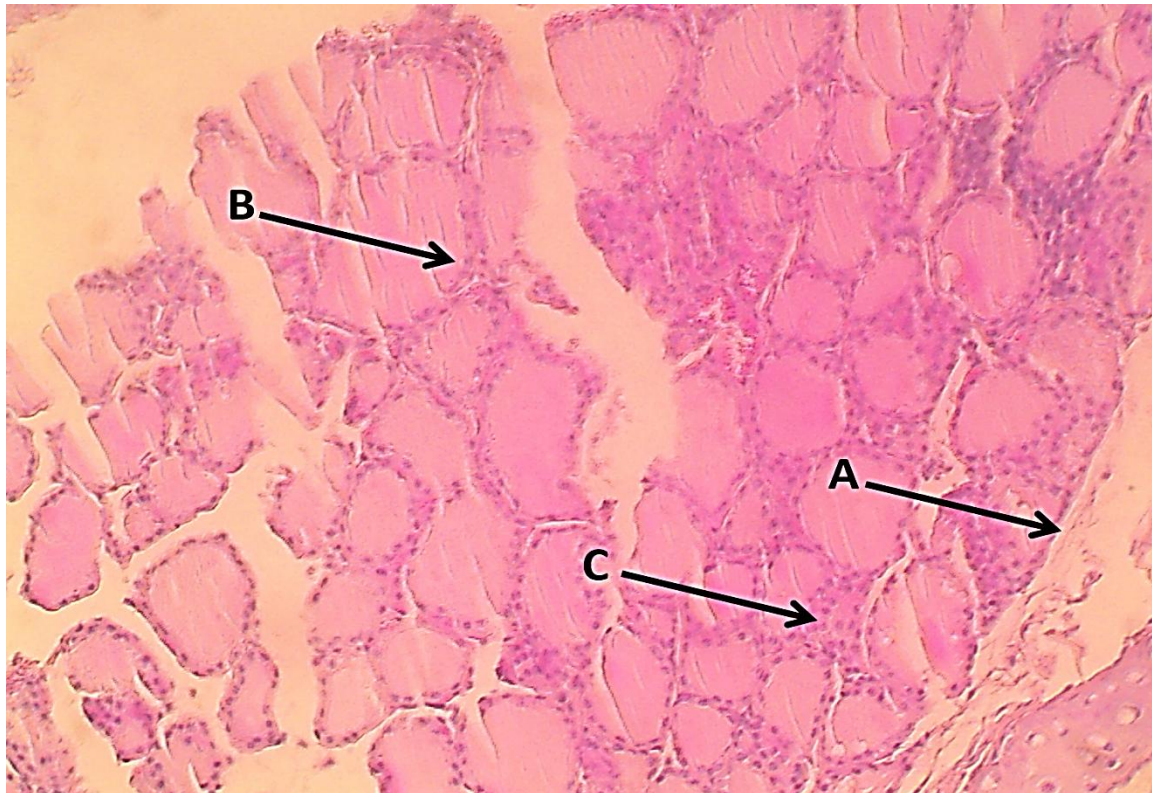
Treatments Parameters	Control	Saccharin	Sig.
T3	2.12 ± 0.287^a	1.49 ± 0.257^b	0.01
T4	43.71 ± 3.83^a	34.60 ± 4.88^b	0.01

Table (2) Effect of Saccharin on T3 and T4 in blood plasma of mice (mean \pm standard deviation).

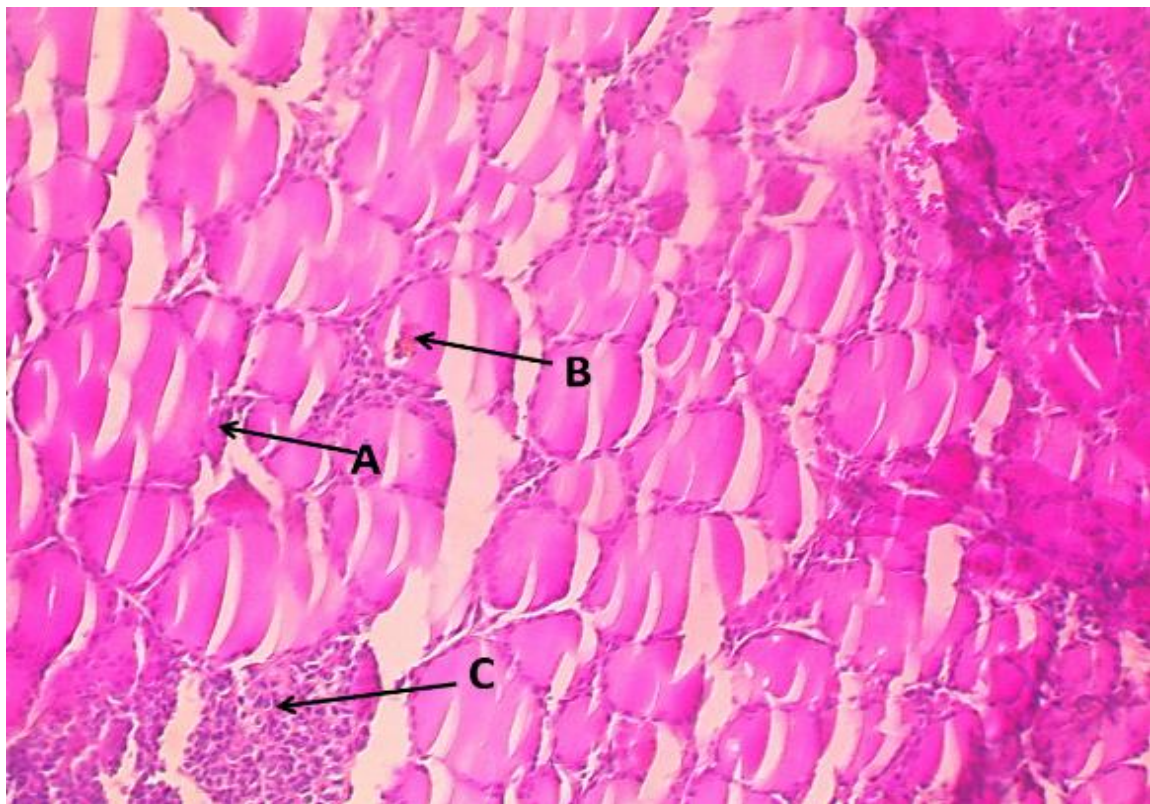
*Different small letters indicate the existence of significant differences between saccharin and control at the possibility of (0.01).



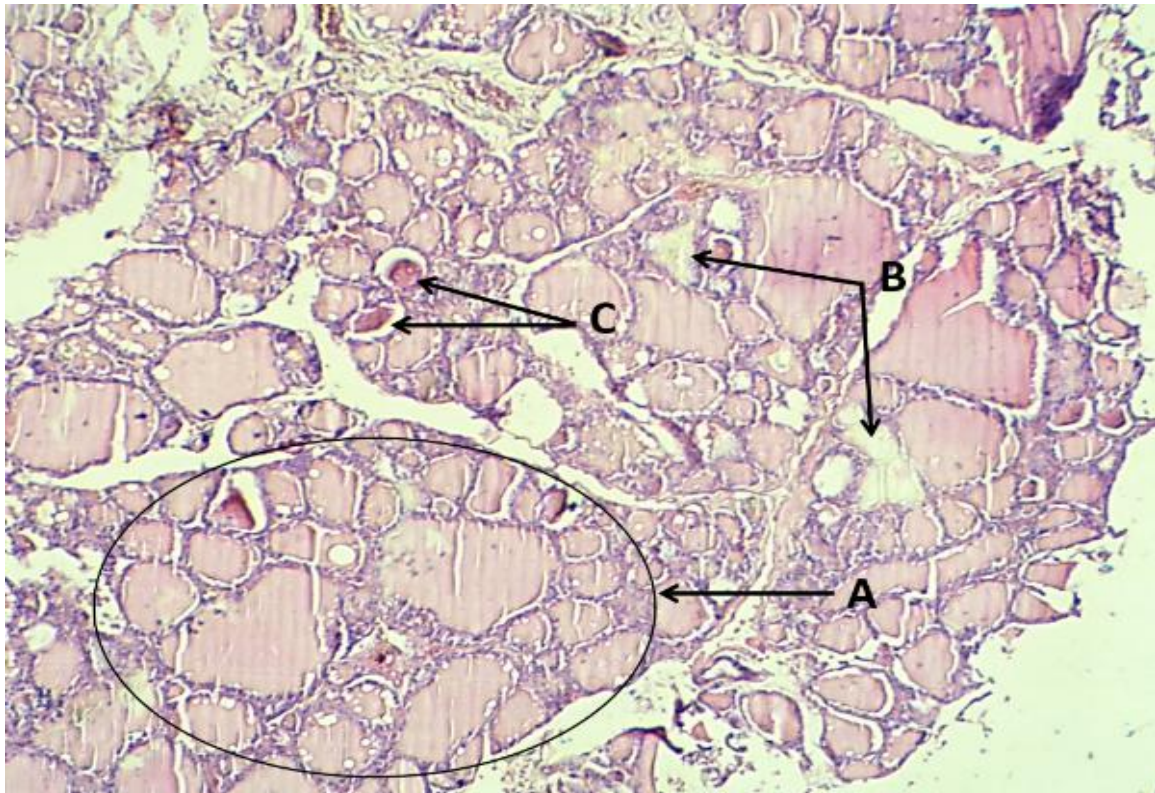
(Figure1): Tissue section of Thyroid in Control group which showed A-large follicle, B-medium follicle, C-small follicle, D- follicular cells. H&E stain 20X



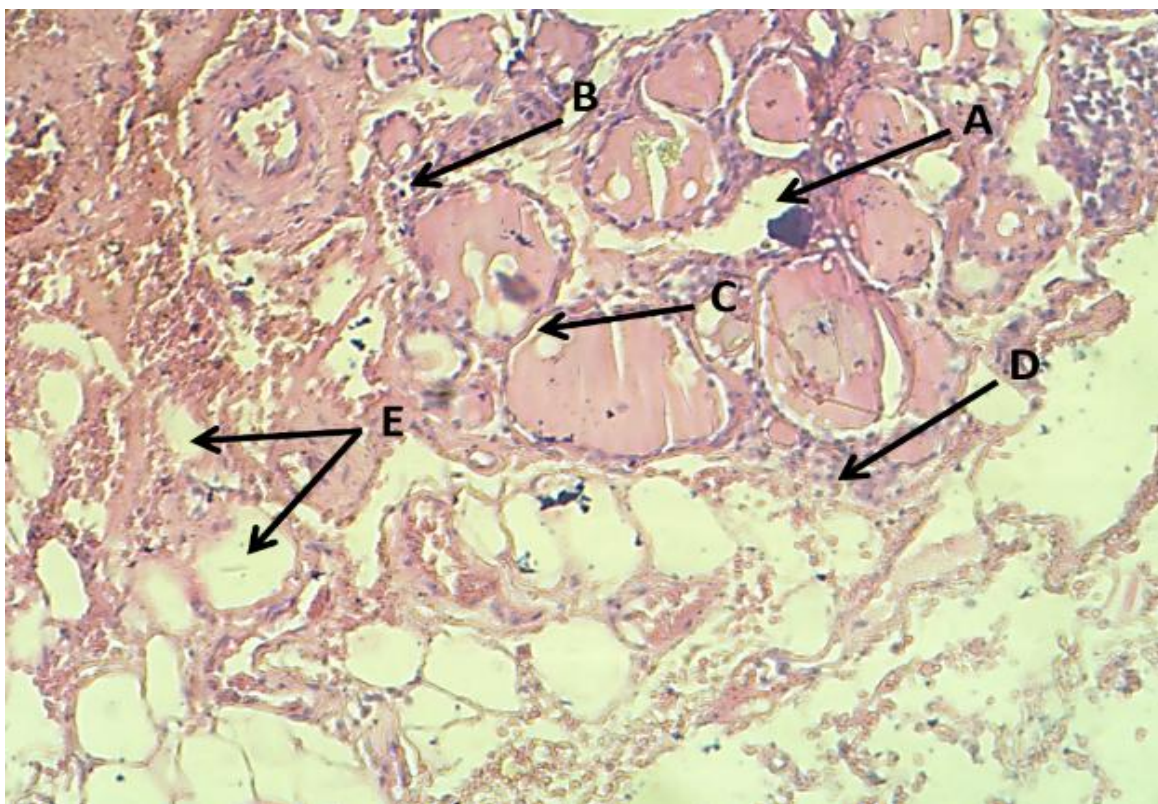
(Figure 2): Tissue section of Thyroid in Control group which showed A- Connective tissue capsule, B- Follicular cells, C- Parafollicular cells. H&E stain 10X



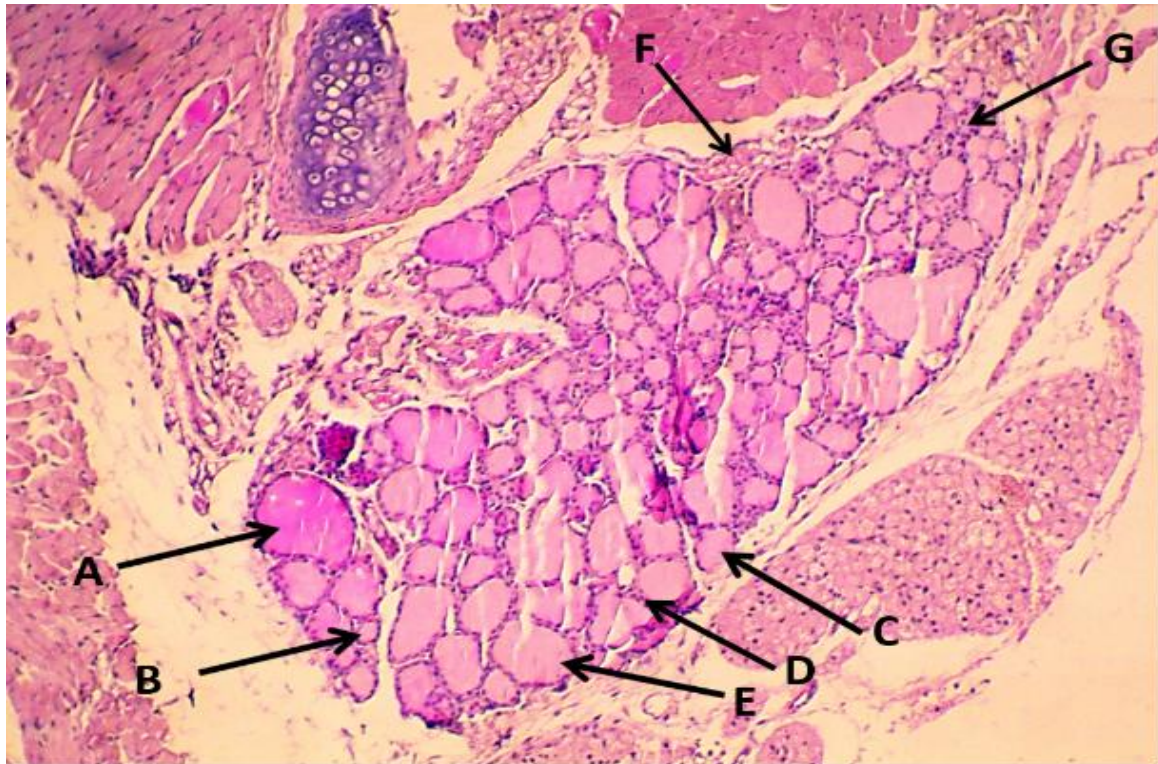
(Figure 3): Tissue section of Thyroid in treated group with saccharin which showed A-large follicle, B- Blood hemorrhage, C-abnormal cellular proliferation. H&E stain



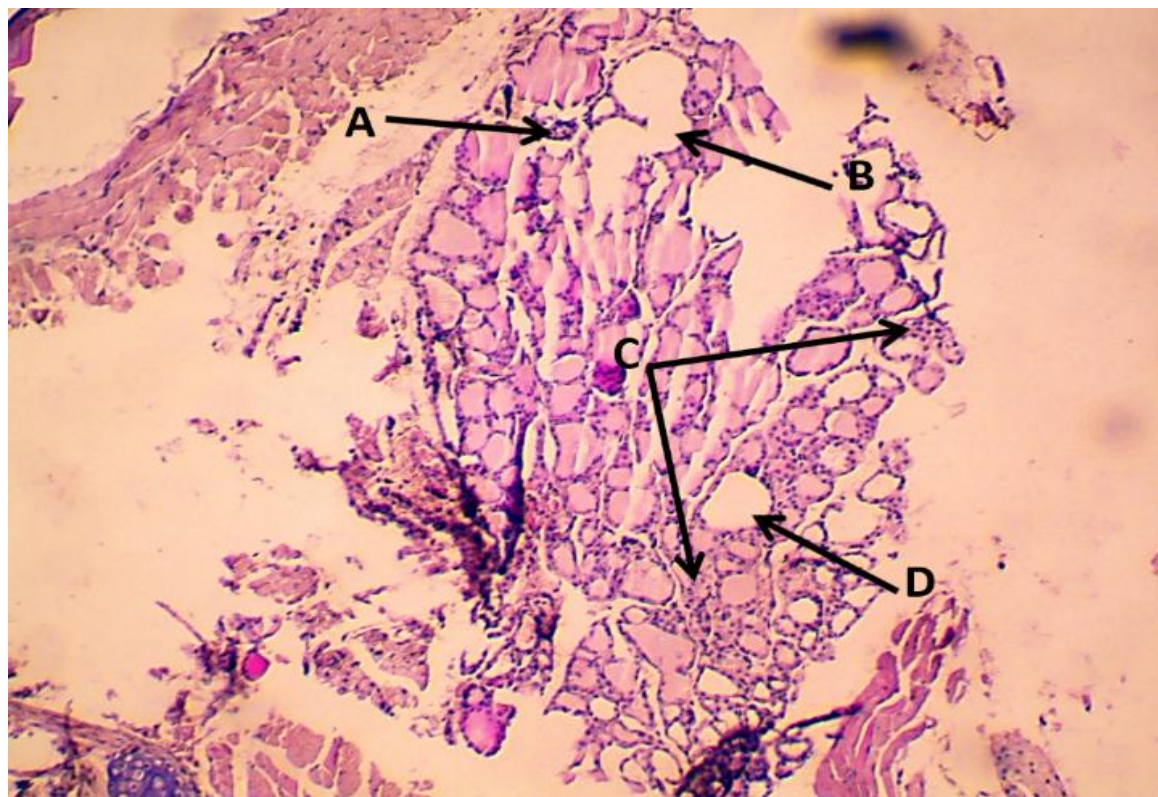
(Figure.4): Tissue section of Thyroid in treated group with Saccharin which showed A- lobule, B- necrotic lesion in connective tissue between lobules, C- blood congestion. H&E



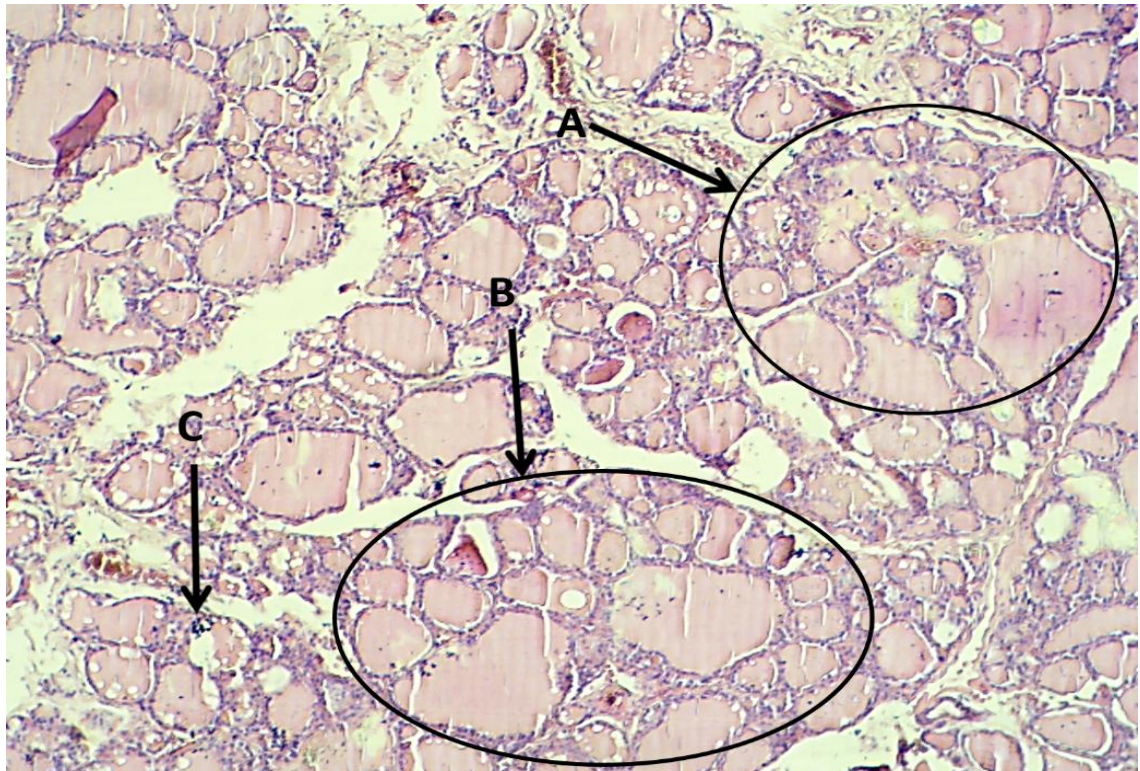
(Fig 5): Tissue section of male Thyroid in treated group with Saccharin which showed A- acute necrosis, B- inflammatory cells, C- prominent damage in wall, D- parafollicular cells, E- disappeared colloid. H&E stain (20)X



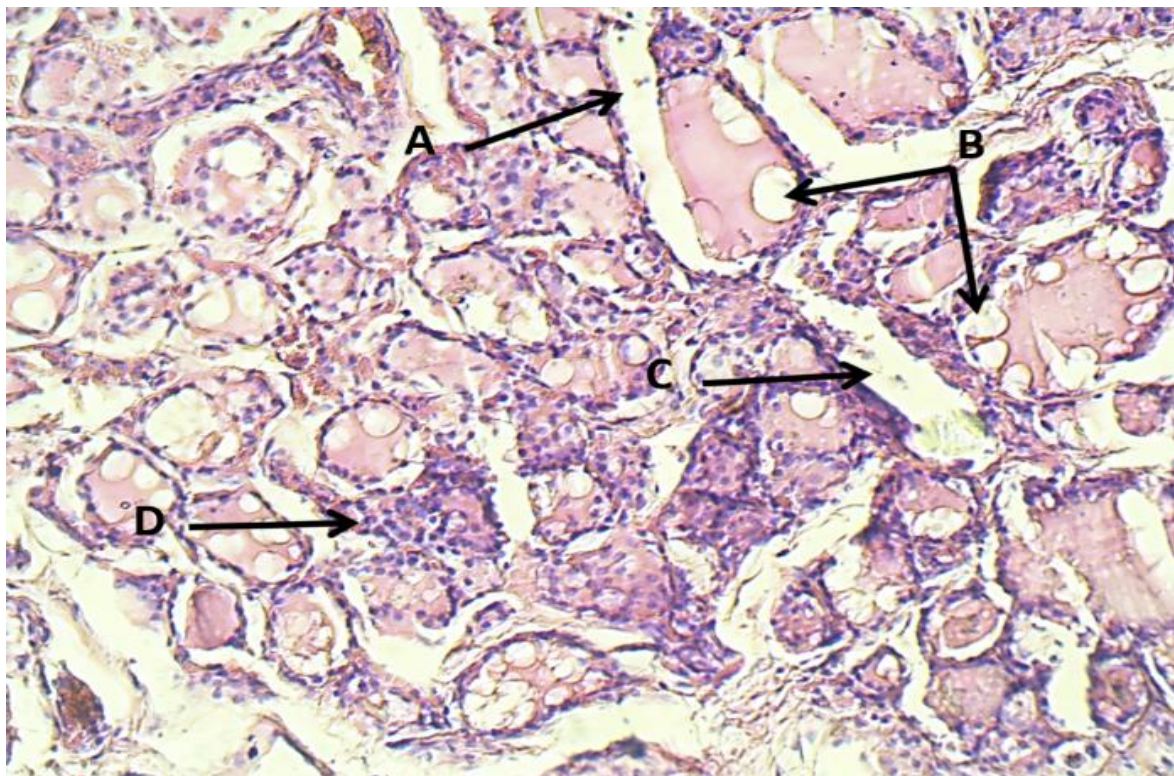
(Fig 6): Tissue section of male Thyroid in treated group with Saccharin which showed A- thick fluid colloid, B- small follicle, C- medium follicle, D- follicular epithelia, E- Large follicle, F- connective tissue capsule, G- cellular proliferation. H&E stain 10X



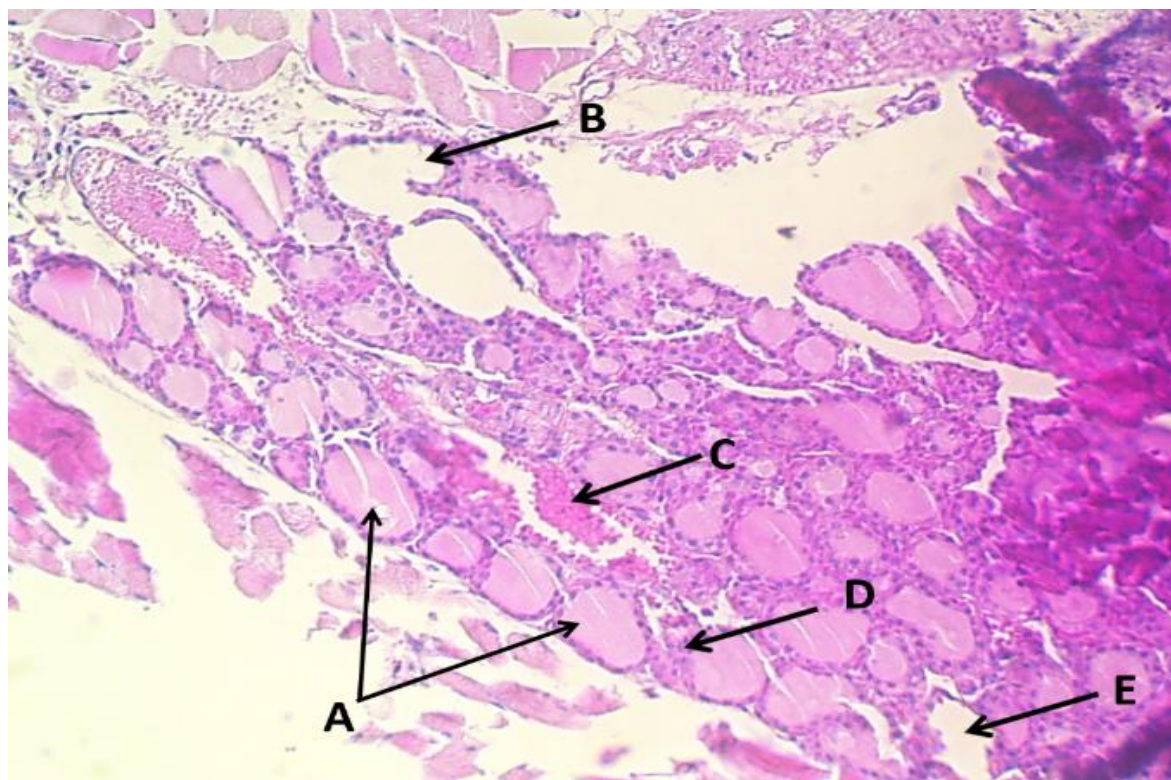
(Fig 7): Tissue section of male Thyroid in treated group with Saccharin which showed A- inflammatory cells, B- prominent damage in follicle wall, C- cellular proliferation, D- cystic dilation. H&E stain 10X



(Fig.8): Tissue section of male Thyroid in treated group with Saccharin which showed A-lobule, B- abnormal cellular proliferation, C- inflammatory cells aggregation. H&E stain (20)X



(Fig 9): Tissue section of male Thyroid in treated group with Saccharin which showed A- follicle wall damage, B- vacuoles, C- irregular space, D- parafollicular cells proliferation. H&E stain (20)X



(Fig 10): Tissue section of male Thyroid in treated group with Saccharin which showed A- Large follicle, B- Empty cystic dilation, C- blood congestion, D- abnormal aggregation of cells, E- tissue degeneration. H&E stain 10X

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