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Effect of High Dose of Soybean Meal on the Histology and Function of the Thyroid Gland in Albino Rat at Pre- and Postpuberty

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Abstract

The study aimed to determine the effect of soybean meal on the histological structure and some functional parameters of the thyroid gland was investigated in this study. For this purpose, 24 white male rats were divided into four groups each of six rats, as following (G1): normal control rats, at the prepubertal life stage, were dissected after 40 days from the weaning (i.e., from Postnatal Day "PND" 21), (G2): prepubertal rats, were treated with high dose of soybean meal "40% of daily diet" for 40 days after the weaning, (G3): normal control rats, at the postpubertal stage, were dissected after 40 days from the puberty (i.e., from PND 60), (G4): postpubertal rats, were treated with high dose of soybean meal (40%) for 40 days after the puberty. Phenotypic, histological and physiological changes were examined. The results reported that, consumption of soybean at prepuberty decreased the thyroid activity by the increasing of the cold "inactive" follicles, while at postpuberty, soybean caused hyperactivity of the thyroid which led to the hyperplasia and hypertrophy, then to the goiter,significant increase in the ratio of cold follicles in the (G2) and significant decrease in the (G4), significant decrease in the height of follicular cell in the (G2) and significant increase in the (G4), significant increase in the diameter of follicular lumen in the (G2) and significant decrease in the (G4). Also, soybean meal caused significant increase in the body weight and significant decline in (T3 and T4) hormones levels.From the results of the present study, it is concluded that the high doses of the soybean affect adversely the function and tissue of the thyroid at both life stages, pre and postpuberty.

Keywords: Soybean, Histology, Function, Thyroid, Puberty.

تأثير الجرعة العالية من كسبة فول الصويا على التركيب النسجي ووظيفة الغدة الدرقية في الجرذ الابيض بمرحلتي ماقبل وبعد البلوغ

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الخلاصة:

هدفت الدراسة الى معرفة تأثير كسبة فول الصويا على التركيب النسجي وبعض المعايير الوظيفية للغدة الدرقية. استخدم ٢٤ جرذ ابيض ذكر لهذا الغرض ، قسمت الى اربعة مجاميع بمعدل ستة جرذان لكل مجموعة وكالاتي: مجموعة (١): مجموعة سيطرة لجرذان غير بالغة شرحت بعد ٤٠ يوم ابتداءا من الفطام (أي من عمر ٢١ يوم) ،مجموعة (٢) : مجموعة جرذان غير بالغة عوملت بجرعة عالية من كسبة فول

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الصويا (أي بنسبة ٤٠% من الغذاء اليومي) لمدة ٤٠ يوم بعد الفطام، مجموعة (٣) : مجموعة سيطرة لجرذان بالغة ، شرحت بعد ٤٠ يوم من البلوغ (أي من عمر ٦٠ يوم) ، مجموعة (٤) : مجموعة جرذان بالغة عوملت بجرعة عالية من كسبة فول الصويا (أي بنسبة ٤٠% من الغذاء اليومي) لمدة ٤٠ يوم بعد البلوغ ، درست لتغيرات المظهرية والنسجية للغدة الدرقية ، كما تم دراسة التغيرات في تركيز الهرمونات ، اشارت النتائج الى ان استهلاك كسبة فول الصويا بجرع عالية تسبب بإنخفاض فعاليةالغدة الدرقية بزيادة الجريبات الخاملة بمرحلة ماقبل البلوغ ، بينما بمرحلة مابعد البلوغ فأن كسبة فول الصويا بجرعها العالية تسببت بفعالية مفرطة للغدة الدرقية مما أدى الى الزيادة في حجم خلاياها وزيادة عددها واخيرا الى تضخم الغدة. زيادة معنوية بنسبة الجريبات الخاملة للمجموعة (٢) وانخفاض معنوي للمجموعة (٤) ،انخفاض معنوي بارتفاع الخلية الجريبية الدرقية للمجموعة (٢) وزيادة معنوية للمجموعة (٤) ، زيادة معنوية بقطر التجويف الجريبي للمجموعة (٢) وانخفاض معنوي للمجموعة (٤)، زيادة معنوية في وزن الجسم وانخفاض معنوي في مستوى الهرمونات الدرقية. وعليه يمكن الاستنتاج ان الجرع العالية من كسبة فول الصويا يؤثر سلبا على وظيفة ونسيج الغدة الدرقية بكلا المرحلتين ماقبل وبعد البلوغ.

Introduction:

Soybean [*Glycine max* (L.) Merrill], a miracle crop of 20th century [1, 2], is a traditional staple of Asian diet but because of its supposed health benefits it has become popular in recent years among non-Asians, especially postmenopausal women [3]. It is consumed as many forms; either solely or in combination with other food products by human beings and as animal feed [4]. It represents an excellent source of high quality protein [5], which comprises 35–40% dry weight of the soybean[6,7]. These proteins contains all amino acids essential to human nutrition, which make it unique among plant-based proteins [7] and make soy products almost equivalent to animal sources in protein quality but with less saturated fat and no cholesterol [8].

Soybean contains on average 15–20% oil by weight[9] which characterized by its high content of polyunsaturated fats, less saturated fat and no cholesterol. Soybean contains macronutrients such as carbohydrates, which are either soluble or insoluble , the latter are called dietary fibers that comprises a great amount approximately 10% dry weight of the soybean [5].

Soybean also contains a wide range of micronutrients and phytochemicals including phytic acid 1.0–2.2%, sterols 0.23–0.46%, and saponins 0.17– 6.16% dry weight of the soybean [10], and the rest of the soybean components is vitamins, minerals [5], moister, ashand no sodium. So , it can be said that soybean contain all the nutrients needed in food [11].

Healthy importance of soybean comes from some of its chemical compounds. One of these chemical compounds is the isoflavone which is the most healthy important component of soybean due to the its many estrogenic, antioxidative, antiosteoporotic, anticarcinogenic [12], antidiabetic activities, both in an animal model [14] and in a human epidemiological study [13]

Concerns have been expressed that soy isoflavone intake adversely affects several organs, one of them is the thyroid [3].The size of the thyroid follicles varies from species to species, for example, it is larger in man than in rat, it is generally quite small in newborn and increases progressively with age. The height of the follicular epithelium depends on its activity and is often inversely proportional to the diameter of follicular lumen. In a resting (cold) [15] inactive gland, the follicle becomes large, the surrounding cells become flattened with abundant and dense colloid [15,16], while in a hot (active) gland, under TSH stimulation the epithelial height increases and becomes columnar with a few, faint colloid and small size follicle [15].

The first description of goitrogenic effects of soybean was byMcCarisson[17] who indicated that several investigators have reported induction of goiter in iodine-deficiency rats maintained on soybean diet, such as with [18] whom reported induction of thyroid carcinomas in rats fed an iodine-deficiency diet containing 40% defatted soybean. Goiter and hypothyroidism were reported in infants fed with adapted soy formula without adequate iodine supply [19]. This adverse effect was eliminated by supplementing commercial soy infant formulas with iodine, or by switching to cow milk [20].

It was showed that both genistein, and to a lesser extent daidzein (the most healthy important forms in soybean) were shown to strongly inhibit the activity of thyroid peroxidase (TPO) [21,22]. Also, It was indicated that isoflavones lead to immune dysfunction by causing potent stimulation of T and B cells mediated immunity due to induced structural changes in thyroid peroxidase [23], this result agreed with findings of Xiao, *et al*. [24] which revealed that isoflavones suppress the binding ability of hepatic thyroid hormone receptor to the thyroid hormone response element of the target genes. Similarly, It was revealed that both genistein and daidzein impaired thyroglobulin (Tg) and thyroid hormones synthesis of Orx middle-aged rats by decrease the expression of Tg and TPO genes [16].

Materials and Methods:

Preparation of Soybean Pellet:

Defatted soybean crushed were purchased from the local fodder markets in the Al-Sinak Street/ Baghdad, powdered by seed grinder. The soybean pellet was prepared according to the study [25] which prepared it as following: 50% bran, 25% white flour, 20% soybean powder and 5% sugar, vitamin, soybean oil and salt, but in this study, the soybean dose was increased to the (40%) proportionally with the other components. These components were mixed, kneaded to make a cylinder blocks similar to the normal rodent pellet and dried under the sun temperature for a few hours. The resulted pellet represented the soybean pellet.

Animals and Experimental Design:

Twenty four Sprague-Dawley (*Rattusnorvegicus*) albino male rats were used in the present study as a mammalian model, purchased from the College of Medicine/ Baghdad University and housed in the animal house of the College of Science for the same University, kept in plastic cages with a metal network cover under climate controlled conditions of the animal house with temperature 25±2°C and 12:12 light and dark cycle.

They were divided into four groups each of six rats, groups (1 and 2) were pelleted in a daily consumption average 12–15 gram at prepubertal life stage from the weaning (i.e., from Postnatal Day "PND" 21) with weight 33–40 g, while the remainder (groups 3 and 4) were pelleted in a consumption average 18–20 gram from the daily diet at the postpubertal life stage from the puberty (i.e., from PND 60) with weight 160–190 g . All the experimental animals pelleted for 40 days as following: groups (1 and 3), normal control rats, were provided with water and food *ad libitum*, while the groups (2 and 4) treated with high dose of soybean meal (40%) from the daily diet [18].

Collection of Blood Samples and Dissection the Animals:

At the end of the experiment the animals were weighed, fully anaesthetized by diethyl ether for several minutes and the blood samples were obtained by heart puncture. Serawere separated by centrifugation 3000 rpm for 15 min, then they were kept in −20°C until using them. The animals were dissected and the thyroid gland was excised, washed and fixed in neutral buffered 10% formalin for the histological preparation.

Histopathological Study:

The fixed tissues of the thyroid in the neutral buffered 10% formalin were dehydrated through graded alcohol series (50−100%), cleared with xylene, infiltrated and embedded in paraffin wax. The paraffin blocks were sectioned in thickness 4–5 µ and stained with Hematoxylin and Eosin stain (H&E) and Toluidine Blue stain (T.B.) [26], then the sections were examined histologically and morphometrically usingMotic Image Plus version 2.0 software program [27].

Physiological Study:

It was represented by the enzyme immunoassay tests (ELISA) for the quantitative determination of concentrations of thyroid gland hormones (T3 and T4), Measurement the Level of T3 Concentration was performed according to the TRIIODOTHYRONINE (T3) ENZYME IMMUNOASSAY TEST KIT, catalog number: BC- 1005, from BioCheck, Inc ,323 Vintage Park Dr., Foster City, CA 94404, and the measurement the Level of T4 Concentrationwas performed according to the TOTAL

THYROXINE (T4) ENZYME IMMUNOASSAY TEST KIT, catalog number: BC- 1007, from BioCheck, Inc ,323 Vintage Park Dr., Foster City, CA 94404.

Statistical Analysis

It was performed by using Statistical Package of Social Sciences (SPSS), version 2.0, (ITS, Los Angeles) computer software. Differences between groups were analyzed by using an analysis of variance (ANOVA), $P>0.05$ was regarded as statistically significant [28].

Results and Discussion:

Weight Measurement:

The statistical analysis of the present study (Tables 1) revealed that the soybean consumption caused significantincrease ($P<0.05$) in body weight in the experimental treated groups compared to the control groups at both life stages, pre and postpuberty. This result agreed with the results of another previous studies [29- 31], and it referred to the high soybean content of protein which comprises 35–40% from its dry weight, and its amino acids are important to muscle synthesis and consequently increase the weight. Also, it was indicated that soybean proteins caused a reduction of the proteolysis of myofibrillar protein in skeletal muscles, this may lead into increased the weight [32].

The result of weight increment was accomplished with the findings from the histopathological study that found hypertrophy in the studied organ (thyroid gland) which caused increase in size and volume of the organ and in turn may participated in increase the weight generally.

Measurement of Thyroid Hormones Concentration:

The statistical analysis of the present study (Table 1) showed that the soybean caused significantdecline ($P<0.05$) in T3 and T4 levels compared to the control groups at both life stages pre and postpuberty. These results agreed with the results of another previous studies [33,34,16]. The reduction in thyroid hormones T3 and T4 was caused by inhibition of thyroperoxidase- (TPO) catalyzed reactions which essential to thyroid hormone synthesis by the soybean compounds and consequently decrease thyroid hormones levels. In the presence of iodide ion, genistein and daidzein blocked TPO-catalyzed tyrosine iodination by acting as alternate substrates, yielding mono-, di-, and triiodoisoflavones [35]. This suggestion was improved by the study [16] which showed decrease in expression of thyroglobulin (Tg) and thyroperoxidase (TPO) genes at level ($P<0.05$) due to soybean consumption. On the other hand, It was indicated that soybean inhibited iodide absorption and consequently affected thyroid hormone synthesis [3]. Coordinating with the morphmetrical and histopathological findings, the treated rats with soybean which revealed the reduction in thyroid hormones T3 and T4 showed either increase in the cold (inactive) follicles ratio or hypertrophy of the follicular epithelium (Table 1), Figure-1 (b and d)) and (Figure-2 (b and d)), both cases indicted that the thyroid gland was impaired and consequently may had depressed activity of this gland to produce T3 and T4.

Table 1- The means and (SE ±**)** of the body weight, thyroid hormones levels and the morphometrical measurements of the experimental groups of rats at pre and postpuberty

Parameters	Means ± SE			
	Prepuberty		Postpuberty	
	Control (G1)	40% Soybean (G2)	Control (G3)	40% Soybean (G4)
Body weight (g)	122.67 ± 4.62 a	188.33 ± 8.51 b	269 ± 10.67 a	332.67± 14.17 b
T3 level (ng/ml)	3.836 ± 0.26 a	1.842 ± 0.32 b	3.608 ± 0.27 a	1.821 ± 0.43 b
T4 level (µg/dl)	11.33 ± 0.31 a	7.12 ± 1.81 b	11.79 ± 0.25 a	7.21 ± 0.75 b
Ratio of cold follicles	16.23 ± 0.689 a	50.31 ± 4.19 b	21.40 ± 1.89 a	2.22 ± 0.22 b
Height of follicular cell (µm)	123.28 ± 4.75 a	72.48 ± 2.75 b	129.62 ± 3.86 a	199.37 ± 2.72 b
Diameter of follicular lumen (µm)	322.93 ± 31.07 a	534.30 ± 34.57 b	500.98 ± 32.86 a	285.24 ± 28.48 b

SD= standard error
Different letters (a and b) are significant at ($P< 0.05$)

Histopathological Examination:

Statistical morphmetrical analysis (Table 1)showed Significant decrease ($P<0.05$) in the height of follicular cell and significant increase ($P<0.05$) in the diameter of follicular lumen in the group of rats which treated with 40% soybean at prepuberty compared to the control group, while the treated group at postpuberty showed significant increase ($P<0.05$) in the height of follicular cell and significantdecrease ($P<0.05$) in the diameter of follicular lumen compared to the control group.

Histologically Figures-(1b and 2b) and coordinating with the morphometry, at prepuberty, exposure to soybean showed its inhibitor effect, which was cleared by several histological findings such as, less folliculogenesis, decrease in the height of follicular cell (flattening of epithelial cell), increase in the diameter of follicular lumen, increase in the ratio of cold follicles and accumulation of colloid material. At postpubertyFigures-(1d and 2d), and due to the feedback mechanism more secretion of TSH which led to hyperactivity, soybean consumption caused increase in the height of follicular cell "cuboidal to columnar epithelial cell", more decrease in the diameter of follicular lumen, rare of the ratio of cold follicles, decrease in the amount of colloid material, congesting of the blood vesseles and increase in the cytoplasmic extendings, consequently caused hypertrophy (an increase in the size of a cell and organ led to goiter) and hyperplasia (an increase in the number of cells in the organ due to the increase in the proliferation).

It was indicated that the inhibition of thyroid hormone synthesis can induce goiter in rodents, it was agreement with the result of the present study [35].

Also, the present study result agreed with the result of Šošić-Jurjević,*et al*. [34] which revealed that the epithelial height and index of activation rate increased, while the luminal colloid and the thyroid hormones decreased after treating the Wistar male rats with 10 mg/kg of either genistein or daidzein for three weeks.

This inhibitor effect was varied in the prepuberty from the postpuberty. At prepuberty, the endocrine gland (pituitary gland) not well formed and has a minor effect [36] and not functional established, therefore, no more TSH secretion led to decrease the activity rate of the thyroid gland Figures-(1b and 2b). While at postpuberty, TSH secretion increased due to the feedback mechanism causing hyperactivity of the thyroid led to the hypertrophy and hyperplasia Figures –(1d and 2d), this

excessive TSH secretion stimulated abnormal thyroid growth and the development of the goiter [37,38].

It is concluded that the high doses of the soybean increased body weight, reduced thyroid hormones levels at both life stages, pre and postpuberty, reduced the activity of the thyroid and impaired its tissue at prepuberty and caused hyperactivity of the gland at postpuberty represented by the penury in the cold follicles ratio, hyperplasia and hypertrophy of thyroid led to the goiter.

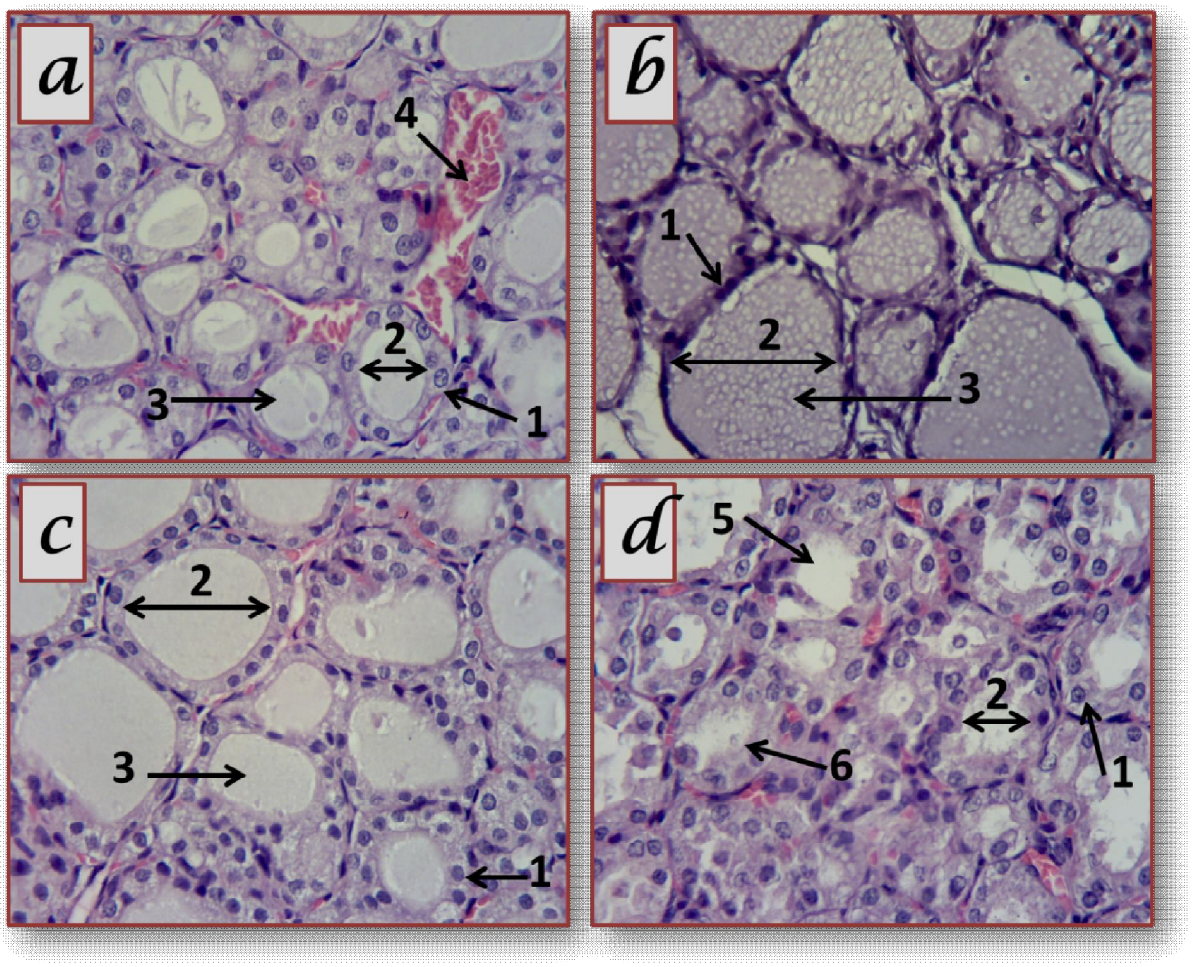

Figure 1- Sections of thyroid gland from the groups (a): control at prepuberty, in which showing normal follicles structure lined by cuboidal epithelium with normal amount of colloid material, (b): treated with 40% soybean at prepuberty, in which showing accumulation of colloid with flattening of epithelial cells (more cold follicles due to the rare secretion of TSH), (c): control at postpuberty, in which showing normal follicles structure lined by cuboidal epithelium with normal amount of colloid material and (d): treated rats with 40% soybean at postpuberty, in which showing increase in the height of epithelium, decrease in the follicular lumen, no colloid material, increase in the cytoplasmic extendings, congesting of the blood vesseles, hypertrophy, hyperplasia and no cold follicles(H&E) 400x

1- Follicular epithelium 2- Follicular lumen 3- Colloid material 4- blood vessels 5- Consumed colloid 6- Cytoplasmic extendings

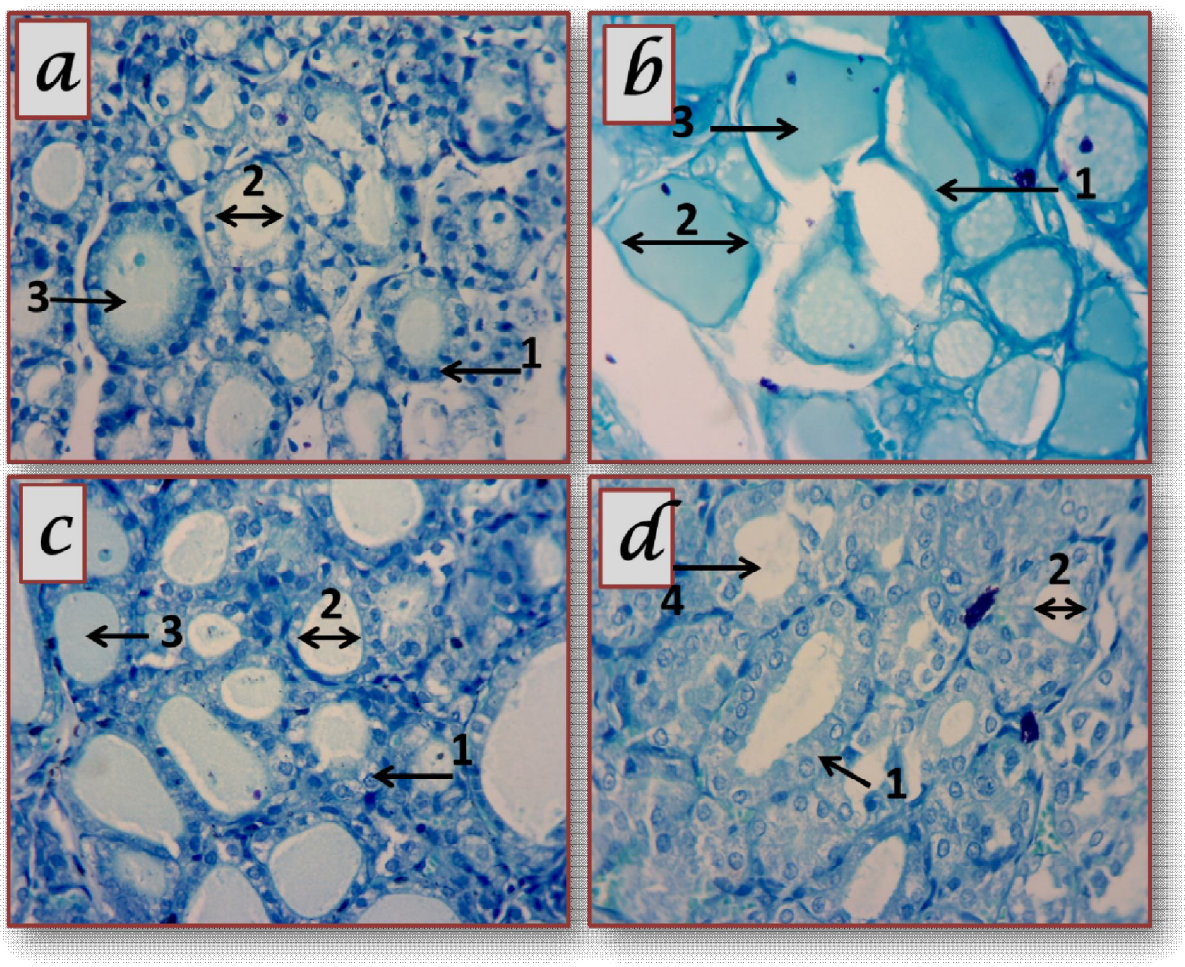

Figure 2- Sections of thyroid gland from the groups (a): control at prepuberty, in which showing normal follicles structure with normal amount of colloid material, (b): treated with 40% soybean at prepuberty, in which showing accumulation of colloid and more cold follicles, (c): control at postpuberty, in which showing normal follicles structure with normal amount of colloid material and (d): treated rats with 40% soybean at postpuberty, in which showing no colloid material and no cold follicles(T.B.) 400x

1- Follicular epithelium 2- Follicular lumen 3- Colloid material 4- Consumed colloid

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