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Effect of prepubertal and postpubertal long-term exposure to the mild dose of soybean seeds on the tissue and function of thyroid gland in albino rat

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Abstract

The aim of this study is to investigate the effect of long-term exposure to the mild dose of soybean seeds on the tissue and some physiological parameters of the thyroid gland in the prepubertal and postpubertal life stages. Twenty four Sprague-Dawley albino male rats were divided into four groups (*n*=6); control rats at the prepubertal life stage; prepubertal rats treated with 20% soybean seeds of daily diet for 40 days after the weaning; normal control rats, at the postpubertal stage; and postpubertal rats, were treated with 20% soybean seeds. Morphmetrical, histological and physiological changes were examined. Consumption of mild dose of soybean seeds along the prepubertal life stage showed significant decrease ($P<0.05$) in the height of follicular cell, significant increase ($P<0.05$) in the diameter of follicular lumen and ratio of cold follicles, slightly non-significant decline in (T3 and T4) hormones levels and significant increment ($P<0.05$) in body weight, while at postpuberty, long-term exposure for the same dose of soybean seeds showed significant increase in the height of follicular cell ($P<0.05$), significant decrease ($P<0.05$) in the diameter of follicular lumen and ratio of cold follicles caused hyperactivity of the thyroid, significant decline ($P<0.05$) in (T3 and T4) hormones levels and slightly non-significant increment ($P<0.05$) in body weight. We concluded that the long-term exposure to the mild dose of the soybean affect adversely the tissue and function of the thyroid at both life stages, pre- and postpuberty.

Keywords: Soybean seeds; Tissue; Function; Thyroid; Puberty

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Introduction

There is growing interest in the potential health threats posed by natural and anthropogenic substances in hum-an's and rodent's food, water and the environment which called endocrine-disrupting chemicals to the endocrine system [1].

From these diet contents and substances: phytoestrogens, which interfere with the absorption of synth-etic thyroid hormone [2]. Soybean [Glycine max (L.) Merrill] is the most important dietary source of isoflavones, an important class of phytoestrogen [1]. The popularity and healthy importance of the soybean, which is the miracle crop of 20th century [3, 4] and the traditional staple of Asian diet, was promoted by positive findings between soy isoflavone consumption and lower incidences of diseases [5], due to the its many estrogenic, antioxidative, anti-osteoporotic, anti-carcinogenic [6], anti-diabetic activities, both in an animal model [7] and in a human epidemiological study [8].

It is consumed just alone or in combination with other food products by human beings and as animal feed [9]. It represents an excellent source of high quality protein [10], which comprises 35-40% dry weight of the soybean [11, 12] and contains all amino acids essential to human nutrition, which make it unique among plant-based proteins [12].

Soybean contains on average 15-20% oil by weight [13] which characterized by its high content of polyunsaturated fats, less saturated fat and no cholesterol. Also, it contains macronutrients such as carbohydrates [10] and 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 [14], and the rest of the soybean components is vitamins, minerals [10], moister, ash and no sodium. So, it can be said that soybean contain all the nutrients needed in food [15].

Concerns have been expressed that soy iso-flavone intake adversely affects several organs, one of these organs is the thyroid [2].The thyroid gland is one of the largest endocrine glands, locates in the neck bellow the larynx. It composed of two lobes, joined by an isthmus, each lobe consists of large numbers of closed spherical follicles [16]. 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) [17] inactive gland, the follicle becomes large, the surrounding cells become flattened with abundant and dense colloid [17 and 18], while in a hot (active) gland, under thyroid stimulating hormone(TSH) stimulation the epithelial height increases and becomes columnar with a few, faint colloid and small size follicle [17].

The first description of goitrogenic effects of soybean was by McCarisson [19] who indicated that several investigators have reported induction of goiter in iodine-deficiency rats main-tained on soybean diet. Goiter and hypothyroidism were reported in infants

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fed with adapted soy formula without adequate iodine supply [20].

It was indicated that both genistein, and to a lesser extent daidzein (the most healthy important forms of isoflavones in soybean) were shown to strongly inhibit the activity of thyroid peroxidase (TPO) [21 and 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 [18].

Method and Material

Preparation of Soybean Pellet

Soybean seeds which were obtained as defatted seeds from the local fodder markets in the Al-Sinak Street/ Baghdad, were powdered by seed grinder, and this soybean powder used in the preparation of soybean pellet according to the study [25] as following: 50% bran, 25% white flour, 20% soybean powder and 5% sugar, vitamin, soybean oil and salt. 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 care and experimental design

Twenty four Sprague-Dawley (Rattus norvegicus) 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, the experimental groups of rats (G1, G2) were treated directly after their weaning (prepubertal stage) in a daily consu-mption average 12–15 g, weighing 33–40 g, while the experimental groups of rats (G3, G4) were treated after their puberty (postpubertal stage) in a daily consumption average 18–20 g, weig-hing 160–190g. All the experimental animals pelleted for 40 days as follo-wing: groups (G1 and G3), normal control rats, were provided with water and food ad libitum, while the groups (G2 and G4) were treated with (20%) soybean seeds from the daily diet [26.[

Collection of blood samples and dissection the animals

At the end of the experiment, the animals were weighed, fully anaesth-etized by diethyl ether for several minutes and the blood samples were obtained by heart puncture. Sera were separated by centrifugation 3000 rpm for 15 min, and then they were kept in −20°C until using them. The animals

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were dissected and the thyroid gland was excised, washed and fixed in neutral buffered 10% formalin for the histological preparation.

Histopathological examination

The fixed tissues of the thyroid in the neutral buffered 10% formalin were washed with running tap water, 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, stained with Hematoxylin and Eosin stain (H&E) and Toluidine Blue stain (T.B.) and finally mounted by Canada Balsam [27], then the sections were examined histologically.

Physiological Examination

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 measurement the level of T4 concentration was 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.

Morphometrical examination

The resulted sections from the histological preparation were examined morphometrically to determination the height of follicular cell and the diameter of follicular lumen using Motic Image Plus version 2.0 software program [28], while the ratio of the cold follicles was calculated according to the study [29.[

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 [30].

Results and Discussion

Weight Measurement

The statistical analysis of the present study **Tab. 1**, indicated that the soybean consumption caused significant increment ($P<0.05$) in body weight in (G2) and slightly non-significant increment ($P<0.05$) in body weight in (G4) compared to the control groups due to the high soybean content of protein which comprises 35-40% from its dry weight, its amino acids are important to muscle synthesis and consequently increase the weight. This result agreed with the results of another previous study [31 and 32]. Also, the study [33] indicated that soybean proteins caused a reduction of the proteolysis of myofibrillar protein in skeletal muscles; this may lead to increase the weight.

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Parameters	Means ± SE			
	Prepuberty		Postpuberty	
	Control **(G1)**	20% soybean **(G2)**	Control **(G3)**	20% soybean **(G4)**
Body weight (g)	**A** 122.67 ± 4.62	**B** 174.17 ± 3.24	**A** 269 ± 10.67	**A** 309.33 ± 6.53
T3 level (ng/ml)	**A** 3.836 ± 0.26	**A** 3.298 ± 0.45	**A** 3.608 ± 0.27	**B** 2.534 ± 0.15
T4 level (µg/dl)	**A** 11.33 ± 0.31	**A** 9.80 ± 1.73	**A** 11.79 ± 0.25	**B** 9.46 ± 0.58
Height of follicular cell (µm)	**A** 123.28 ±4.75	**B** 97.61 ± 5.03	**A** 129.62 ± 3.86	**B** 170.32 ± 7.58
Diameter of follicular lumen (µm)	**A** 322.93±31.07	**B** 460.09 ± 40.49	**A** 500.98 ± 32.86	**B** 376.36 ±33.39
Ratio of cold follicles	**A** 16.23 ± 0.689	**B** 25.10 ± 0.955	**A** 21.40 ± 1.89	**B** 5.96 ± 0.34

SE= standard error
Different letters (A and B) are significant at (P< 0.05)

Physiological Examination

Results of the statistical analysis of the present study **Tab. 1**, revealed a slightly non-significant decline P<0.05 in T3 and T4 levels in the (G2) and significant decline P<0.05 in T3 and T4 levels in the (G4) compared to the control groups. This reduction in thyroid hormones levels may beresulted due to the inhibition of thyroper-oxidase- (TPO) catalyzed reactions which essential to thyroid hormone synthesis by the soybean compounds and consequently decreases 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 [34].

Similarly, the study [18] 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 [2]. The results of the present study agreed with the results of another previous studies [35, 36, 18].

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 **Tab. 1**, **Fig. 1b, d** and **Fig. 2b, d**, both cases indicted that the thyroid gland was impaired and consequently may had depressed activity of this gland to produce the thyroid hormones.

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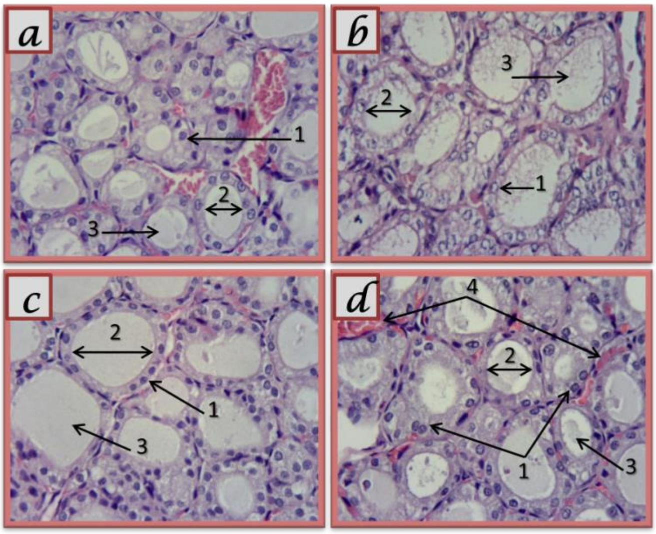

Figure 1.

Sections of thyroid gland from the experimental groups of rats (a): control at prepuberty, in which showing normal follicles structure lined by cuboidal epithelium with normal amount of colloid material, (b): treated with 20% soybean at prepuberty, in which showing follicles lined by cuboidal to flat epithelial cells, (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 20% soybean at postpuberty, in which showing increase in the height of epithelium, decrease in the follicular lumen, less colloid material, congesting of the blood vessels (H&E) 400x

1- Follicular epithelium 2- Follicular lumen 3- Colloid material4- blood vessels

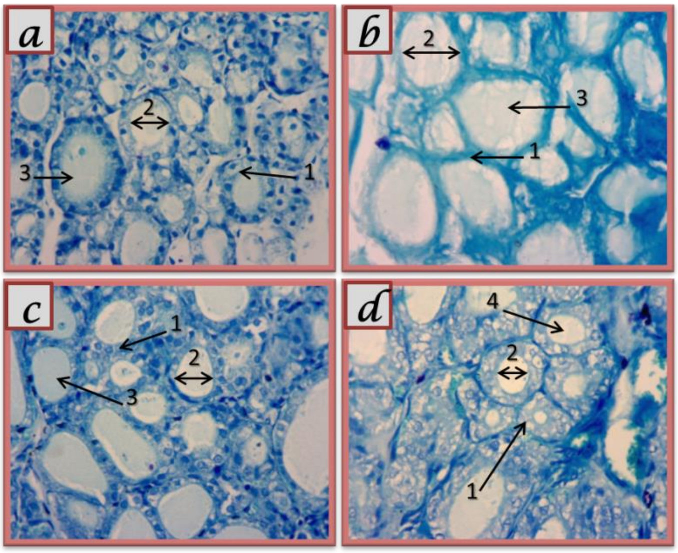

Figure 2.

Sections of thyroid gland from the experimental groups of rats (a): control at prepuberty, in which showing normal follicles structure lined by cuboidal epithelium with normal amount of colloid material, (b): treated with 20% soybean at prepuberty, in which showing follicles lined by flat epithelial cells, (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 20% soybean at postpuberty, in which showing increase in the height of epithelium, decrease in the follicular lumen, consumed colloid material (T.B.) 400x.

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

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Histopathological examination

The statistical analysis of the present study morphometry **Tab. 1**, revealed significant decrease (P<0.05) in the height of follicular cell, significant increase (P<0.05) in the diameter of follicular lumen and significant increase (P<0.05) in the ratio of cold follicles in the treated group of rats at prepuberty (G2) compared to the control group, while the treated group at postpuberty (G4) showed significant increase (P<0.05) in the height of follicular cell, significant decrease (P<0.05) in the diameter of follicular lumen and significant decrease (P<0.05) in the ratio of cold follicles compared to the control group.

Coordinating with the morphometry **Tab. 1**, histologically **Fig. 1b, 2b**, long-term exposure to the mild dose of soybean at prepuberty (G2) showed an inhibitor effect, which represented by several histological findings such as flattening of epithelial cells, increase in the diameter of follicular lumen, increase in the ratio of cold follicles and accumulation of colloid material, while at postpuberty **Fig. 1d, 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", decrease in the diameter of follicular lumen and ratio of cold follicles, less amount of colloid material and congesting of the blood vessels.

Inhibitor effect of soybean seeds varied in the prepuberty from the postpuberty, at prepuberty, the endocrine gland (pituitary gland) is not well formed and has a minor effect [37] and not functional established, therefore, no more TSH secretion led to decrease the activity rate of the thyroid gland **Fig. 1b, 2b**, while at postpuberty, TSH secretion increased due to the feedback mechanism causing hyperactivity of the thyroid led to the hypertrophy of the follicles (figures 1d and 2d), this excessive TSH secretion stimulated abnormal thyroid growth and may had developed the goiter [37].

The study [34] revealed that the inhibition of thyroid hormone synthesis can induce goiter in rodents, which agreed with the result of the present study, as well, the present study result agreed with the result of the study [36] 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.

In conclusions

From this study, it is concluded that the long-term exposure to the mild dose of soybean increased body weight, reduced thyroid hormones levels at postpuberty, reduced the activity of the thyroid and impaired its tissue at prepuberty and caused hyperactivity of the gland at postpuberty.

Competing interests

Authors declare that We have no competing interests.

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Authors' contributions

SA and AB were involved in design of the study, involved in data collection and drafting of the final manuscript. SH involved in conception and design of the study, and offered a critical review of the manuscript for intellectual content. All the authors have read and approved the final version of the manuscript.

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