Basrah journal of veterinary research , vol. 11, 4,2012
Proceedings of the 3rd international Scientific Conference, College of Veterinary Medicine, University of Basrah-Iraq.

DETERMINATION OF SOME TRACE ELEMENTS, REPRODUCTIVE PARAMETERS AND HISTOLOGICAL CHANGES IN HYPOTHYROIDIC FEMALE RABBITS INDUCED BY SOYBEAN ISOFLAVONOID

Muna H. AL-Saeed Alaa Al-Deen H. Jawid Jassim M. A. AL-Kalby

Department of Physiology, Pharmacology and Chemistry, College of Veterinary Medicine, Basrah University, Basrah, Iraq.

ABSTRACT

This study was carried out at the animal house of the College of Veterinary Medicine - Basrah University. An attempt was done to induce hypothyroidism by using isoflavonoid extract from soybean seeds (*Glycin max*.) to investigate the effects of this agent on endocrine axes and as well as on some physiological and histopathological changes in female rabbits. Twenty four virgin female rabbits weighted (١١٢٠-١١٤٧g) and ages (5.5-6m) were divided into 2 equal groups (12 rabbits/group).
First group (control) was drenched 3ml of physiological saline for 2months; second group (SIE) was drenched soybean isoflavonoid extract (200mg/kg B.W.) for 2months. The results of present study revealed that hypothyroidism has been induced by soybean isoflavonoid extract (SIE) which accompanied by significant ($P<0.05$) decrease in serum T_3, T_4 concentrations compared with control. On the other hand, TSH concentration showed a significant ($P<0.05$) increase and a significant decrease in serum concentrations of FSH and LH have been shown in serum of (SIE) treated group. A significant ($P<0.05$) decrease in serum Zn, Na concentrations and a significant ($P<0.05$) increase in Ca concentration were registered in SIE group, Reproductive efficiency was affected in SIE The fertility rates in female rabbits of SIE was 41.66% compared with 100% of control. The histological study revealed that many pathological changes of different degrees in thyroid gland represented by dilated thyroid follicles and flatted thyroicytes, vacuolation in colloid, depletion of parafollicular cells, as well as pituitary gland shows non-clear pituicyte. Adrenal glands showed reduce of zona fasciculata and disturbance of ovarian tissue with high proliferation of dense fibrosis in SIE treated female rabbits compared with control.

INTRODUCTION

Hypothyroidism is a deficiency of thyroid activity, it results in reduced secretion of both T_4 and T_3. The decrease in T_4 and T_3 concentrations lead to hypersecretion of pituitary thyroid stimulating hormone (TSH) and an amplified increase in its concentration in serum [1].
Thyroid gland activity may be affected by natural compound such as phytoestrogen. It operates directly on thyroid tissue through estrogen receptor and causes hypothyroidism. The phytoestrogens such as isoflavonoid from *Glycin max* (soybean) possibly effecting on the secretion of thyroid hormones[2].

The soybean plant is an annual plant native to Southeast Asia. It has oblong pods that contain 2 to 4 seeds or beans. It is one of the oldest cultivated legumes in the world

with a crude protein value of between 38-44% [3]. The crop is cultivated principally for its oil bearing seeds which are widely used for human consumption or as a concentrate feed ingredient for farm animals.

Isoflavonoids (ISF) are synthesized as a branch of the phenylpropanoid pathway. The ISF skeleton originates from the central flavanone intermediate naringenin and liquiritigenin. The physiological effects of ISF include possible antioxidant activity, therefore suggesting a role for isoflavones in the prevention of coronary heart disease, endocrine-responsive cancers and male infertility[4]. Soybean isoflavones compounds mainly possess anti-inflammatory, antiallergic, antiviral, anticarcinogenic, antineoplastic, antimicrobial, antihemninthic, liver protective, antithrombotic, antioxidant, metal chelation and antihormonal effects[5].

Isoflavones also affect enzyme systems such as protein kinase C, tyrosine kinases, ornithine decarboxylase; nucleotide phosphodiesterase, lipooxygenase and glutathione S transferase[6]. These enzymes play a role in the pathogenesis of diseases such as cancer, arthritis, heart disease, inflammatory diseases and in antimicrobial activity[7].

Soybean has been implicated in diet-induce goiter. Therefore ISF reduced thyroid hormone[8]. ISF may influence the thyroid hormone feedback system by interference with their biosynthesis, secretion and metabolism [9].

MATERIAL AND METHODS

Plant Material:

The Soybean (*Glycin max*.) was used in this study. The seeds of soybean were turned to powder with the help of an electric grinder.

Preparation of Soybean Isoflavnoids Extract (SIE)

Fifty grams of dried soybean seeds powder was defatted with (500 ml) n-hexan for 16 hours by soxhlete. The combined n-hexan extract was concentrated below 50°C under reduced pressure in a rotary evaporator to get 10 gm of yellow oily mass. This mass was dried at room temperature and further (40 gm) was refluxed in (500ml) methanol (80%) in water with 3% hydrochloric acid. The sample was refluxed with solvent for one hour then filtered by Buchner funnel and filter paper (Wattman No.185). The filtrate was extracted with an equal volume of chloroform to remove pigments. The alcoholic layer was extracted with an equal volume of ethyl acetate treated with 2% of hydrochloric acid and the ethylacetate layer was concentrated by rotary evaporator at 45˚C and dried at room temperature[10,11]. The resultant extract (3gm) was yellowish and dry material, the percentage was (7.5% w/w).The extract was kept in dark glass container at 4°C.

Experimental Animals

Twenty four healthy adult virgin female domestic rabbits (*Lepus cuniculus domastica*) were brought from the local markets/ Basrah, Iraq of (5.5-6) months age, body weight (1120-1147) gm. Rabbits were kept for an adaptation period 1 month in the animal house of Veterinary Medicine College / Basrah University. Experimental animals were kept in individual cages, provided with ration composed of fodder in addition to green alfalfa (Medicago *sativa*) and tap water *ad libitum* and given a prophylaxis drug against coccidiosis (Amprollium 1g/L of drinking water) and these

animals maintained in air-conditioned quarters (24°C) under standard husbandry condition with alternate 12 hours light /dark period.

Experimental Design

To induce hypothyroidism twenty fourvirgin rabbits were randomly divided into main two equal groups, 12 rabbits / group in an individual cage.

1-The first group (**C**) had been regarded as control each rabbit in the control group was drenched 3ml of normal saline daily by using gastric tube for 2 months.

2-The second group **(SIE)** was drenched (200mg /Kg B.W. single daily dosage) of soybean isoflavonoid extract dissolved in 3ml of normal saline daily for 2 months.

Collection of Blood Samples:

Blood samples (5ml) were collected from each animal before and during experimental weekly by the heart (cardiac puncture). The (5ml) of blood was deposited into tube without anticoagulant and then the blood samples were refrigerated for a maximum of 12h and centrifuged at (5000 rpm) for 15 minutes and serum samples were divided into two parts and stored in polyethylene eppendorff tubes at -20ºC,first part used for hormonal analysis (TSH,T_3,T_4,FSH,LH, cortisol, E_2 and P_4) and other part for biochemical analysis (minerals).

Reproductive Efficiency

Fertility activity

After 4 weeks of treatments female rabbits caged overnight with males (2♀:1♂) of proven fertility. Vaginal smears were examined on the following morning for the presence of spermatozoa in the vaginal smears, this was considered indicative of pregnancy, and this day was counted as day 0 of pregnancy.

Measurement of the Length of Gestation Period

In these experiments, treated pregnant rabbits were carefully noticed until normal delivery occurred. At day 2 or 3 before delivery, the rabbits were caged individually. The time and day of delivery was recorded. In addition, weight of the litters was recorded.

Implantation Site

In these experiments, after delivery of pregnant rabbits were sacrificed immediatly for the notice effect of treatment on implantation site of blastocytes, as described by [12]. The animals were sacrificed and their uteri were exposed and opened by cutting longitudinally to expose the bluish implantation sites, which were counted. Weight of uterus and the left and right ovaries were taken. The ovaries were removed from each animal, placed on a Petri–dish and the number of corpa lutea were counted. The percentage of success of implantation was calculated as:

$$\text{Percentage of success (Ps)} = \frac{\text{No. of implants}}{\text{No. of corpa lutea}} \times 100$$

Histological Techniques

The animals were sacrificed at the end of the experiment (after 8 weeks from the beginning of the experiment) and the organ samples were taken as pituitary gland, thyroid gland, adrenal gland and ovaries. These organs were fixed in 10% buffered formalin, dehydrated progressively in increased ethanol concentrations, treated with xylene and embedded in paraffin. Five microns thickness sections of paraffin-embedded tissue were mounted on glass slides and stained with Haematoxyline and Eosin stain (H & E stain)[13,14].

Statistical Analysis

The results of the present study were analyzed by *t* test between groups and (ANOVA) between periods[15].

RESULTS

1-Effect of SIE on Serum Concentrations of TSH, T_3 and T_4 of Virgin Rabbits

The results of serum TSH, T_3 and T_4 concentrations have been presented in the Table (1). The results indicated that the TSH concentration was significantly ($P \leq 0.05$) increased during studied periods in serum of virgin rabbits treated with SIE compared with control group. The TSH concentration of virgin female rabbits was significantly ($P \leq 0.05$) increased with progressive periods. Serum concentration of T_3 was significantly ($P \leq 0.05$) decreased of treatment with SIE during studied period compared with control. The same pattern was found in the T_4 concentration in studied period.

Table(1)Effect of SIE on Serum Concentrations of TSH, T3 and T_4 of Virgin Rabbits (Mean±SD) (n=12)

Parameters	Treatments	Periods(weeks)		
		0wk	2wk	4wk
TSH (µlU/ml)	Control(Normal saline)	0.40±0.01 Aa	0.41±0.046 Ba	0.41±0.035 Ba
	SIE	0.41±0.05 Ad	0.88±0.021 Ac	1.29±0.011 Aa
T3 (ng/ml)	Control(Normal saline)	1.41±0.010 Aa	1.38±0.01 Aa	1.38±0.012 Aa
	SIE	1.42±0.017 Aa	0.77±0.014 Bc	0.60±0.010 Be
T4 (µg/dl)	Control(Normal saline)	5.04±0.012 Aa	5.13±0.03 Aa	5.11±0.02 Aa
	SIE	4.83±0.04 Aa	3.31±0.024 Bb	2.5±0.019 Bc

SIE=Soybean Isoflavonid Extract,P≤0.05 Capital letters denote differences between SIE and control. P≤0.05 Small letters denote differences within group

2-Effect of SIE on Serum Concentrations of FSH, LH, Cortisol, E2 and P4 Hormones of Virgin Rabbits

The mean values of FSH, LH, cortisol, Estradiol (E_2), Progesterone (P_4) concentrations as presented in the Table (2). The results indicated a significant ($P \leq 0.05$) decrease in

serum FSH and LH concentrations of virgin rabbits treated with SIE group with progressive periods compared with control.

Cortisol concentration was significant (P≤0.05) decreased in serum of virgin rabbits treated with SIE during progressive periods compared with control and within group.

E_2 concentration was significantly (P≤0.05) increased in serum of virgin rabbits treated with SIE compared with control group. Also E_2 concentration was significantly (P≤0.05) increased in serum of virgin rabbits treated with SIE compared within group.

P_4 concentration was significantly (P≤0.05) decreased in serum of virgin rabbits treated with SIE compared with control and within group.

Table(2)Effect of SIE on Serum Concentrations of FSH, LH, Cortisol, Estradiol and Progesterone Hormones of Virgin Rabbits(Mean±SD) (n=12)

Parameters	Treatment	Periods (weeks)		
		0wk	2wk	4wk
FSH (mlU/ml)	Control(Normal saline)	2.73±0.031 Ab	2.78±0.013 Aab	2.86±0.011 Aa
	SIE	2.72±0.022 Aa	2.46±0.034 Bb	2.10±0.017 Bc
LH (mlU/ml)	Control(Normal saline)	2.65±0.05 Ac	4.20±0.002 Ab	5.30±0.006 Aa
	SIE	2.56±0.01 Ac	2.94±0.0012 Bb	4.40±0.0034 Ba
Cortisol (µg/dl)	Control(Normal saline)	4.93±0.013 Aa	5.63±0.019 Aa	5.91±0.032 Aa
	SIE	4.98±0.023 Aa	4.37±0.025 Bb	3.39±0.038 Bc
Estradiol (pg/ml)	Control(Normal saline)	45.95±1.52 Aa	48.8 ± 2.58 Ba	48.58±0.63 Ba
	SIE	46.56± 0.11 Ac	65.37±0.23 Ab	90.74±0.36 Aa
Progesterone (ng/ml)	Control(Normal saline)	0.76±0.024 Ab	0.95±0.013 Aa	1.18±0.027 Aa
	SIE	0.78±0.021 Aa	0.70±0.06 Bab	0.64±0.021 Bb

SIE=Soybean Isoflavonid Extract,P≤0.05 Capital letters denote differences between SIE and control.
P≤0.05 Small letters denote differences within group

3-Effect of SIE on Serum Concentrations of Zn, Ca, P, Na and K of Virgin Rabbits

The results of concentrations of Zn, P, Ca, Na and K in serum of virgin rabbits are represented in the Table (3). The results indicated a significant (P≤0.05) decrease in Zn concentration of virgin rabbits treated with SIE compared with control and within group. Ca and P concentrations were significantly (P≤0.05) increased in serum of virgin rabbits after treated with SIE compared with control and within group. Na concentration was significantly (P≤0.05) decreased after treatment with SIE progressively compared with control and within group. No significant difference was found in K concentration between SIE treated group and control.

Table (3) Effect of SIE on Serum Concentrations of Zn, Ca, P, Na and K of Virgin Rabbits (Mean±SD) (n=12)

Parameters	Treatment	Periods (week)		
		0wk	2wk	4wk
Zinc µg/dl	Control(Normal saline)	120.99±4.17 Aa	122.56±2.89 Aa	127.50±6.69 Aa
	SIE	118.22±4.21 Aa	86.06±2.03 Bb	72.18±0.12 Bc
Calcium mg/dl	Control(Normal saline)	12.42±0.81 Aa	12.74±0.85 Ba	12.86±0.87 Ba
	SIE	12.52±0.88 Ab	17.37±1.52 Aa	17.79±1.59 Aa
phosphorous mmol/L	Control(Normalsaline)	9.64±.96 Aa	9. 54± 0.95 Aa	9.36±0.85 Aa
	SIE	9.78±0.57 Aa	9.87±0.60 Aa	9.97±0.46 Aa
Sodium mmol/L	Control(Normalsaline)	118.15±2.65 Aa	123.59±5.84 Aa	125.01±3.16 Aa
	SIE	121.32±4.67 Aa	90.15±3.27 Bb	72.48±3.24 Bc
potassium mmol/L	Control(Normalsaline)	4.6±0.90 Aa	4.32±0.34 Aab	4.26±0.26 Ab
	SIE	4.5±0.25 Aa	4.65±0.57 Aa	4.57±0.45 Aa

SIE=Soybean Isoflavonid Extract,P≤0.05 Capital letters denote differences between SIE and control.P≤0.05 Small letters denote differences within group

4-Effect of SIE on Reproductive Efficiency of Female Rabbits

The results revealed in Table (4) affected fertility by SIE treatment was found. Not all female rabbits were mated with male and showed positive spermatozoa became pregnant. The results revealed some female rabbits treated with SIE were unable to become pregnant compared with control, 41.66% fertility rate in female rabbits treated with SIE compared with control fertility rate 100%. The results female rabbits treated with SIE revealed a significant (P≤0.05) decrease in newborns weights but the gestation period did not affected and in this study found that those rabbits that had treated with SIE suffered from" partial reproductive failure." These effects were seen in the smaller size of their litter, the number of stillbirth and early fetal deaths.

Table (4) Effect of SIE on Reproductive Efficiency of Female Rabbits (Mean±SD) (n=12)

Treatments / Parameters	Control (normal saline)	SIE
N0. of female pregnancy	12	5
Gestation period	29.5±1.02 A	30±1.05 A
No. of New born	5.08±1.12 A	4.8±1.2 A
No. of New born (Total and range)	61(4-6)	24(4-6)
Weight of New born	35.92±5.35 A	24.96±6.03 B
Fertility Rate %	100%	41.66%
Malformation%	0%	0%
Aborted rabbits %	0%	0%
Dead newborn	0	4
Mortality Rate Of newborn	0%	16.66%

SIE=Soybean Isoflavonid Extract.
Capital letters denote differences between groups, P≤0.05 vs. control.

5-Effect of SIE on Site of Implantation, Number of Corpora Luteum and Successful Implantation % in Sacrificed Female Rabbits

Site of Implantation, number of corpora lutea and successful implantation % are illustrated in the Table (5). The results showed a significant (P≤0.05) decrease of site of implantation in female rabbits in treated group compared with control.

Number of corpora lutea show no significant different in SIE group compared with control group. Successful implantation % was high in control group 79.87 % compared with treated groups SIE 57.74%.

Table (5) Effect of SIE on Site of Implantation , Number of Corpora Luteum and Successful Implantation % in Sacrificed Female Rabbits (Mean±SD) (n=12)

Parameters / Treatments	Site of Implantation	Number of Corpora Luteum	Successful Implantation%	Resorption of fetuses
Control Normal saline	5.12± 1.05 A	6.41±1.03 A	79.87%	0
SIE	4±1.47 B	6.98±1.23 A	57.30%	0

SIE=Soybean Isoflavonid Extract.
Capital letters denote differences between groups, P≤0.05 vs. control.

Result of Histopathological Examination

1- Pituitary gland

The pituitary gland of **control** rabbits showed clear pituicytes with very clear nuclei normally distributed within the nervous tissue. In addition their normal numbers and size of herring bodies as shown in figure (1). While pituitary gland of rabbits treated with **SIE** exhibited histopathological changes included non-clear pituicytes with small nuclei, there fibrous tissue formation distributed within the nervosa tissue and no herring bodies as shown in figure (2).

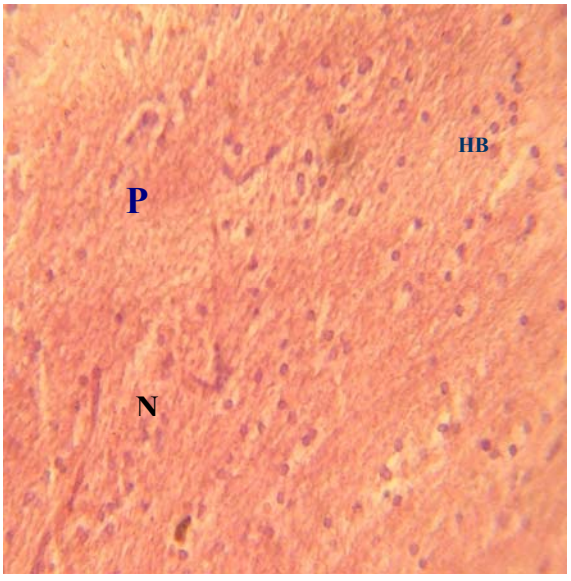

Fig.١ : Pituitary gland of control rabbit. Showing pituicytes (P) with nuclei (N) normally distributed within the nervosa tissue. Normal numbers and size of herring bodies (HB), stain (H&E) 400X.

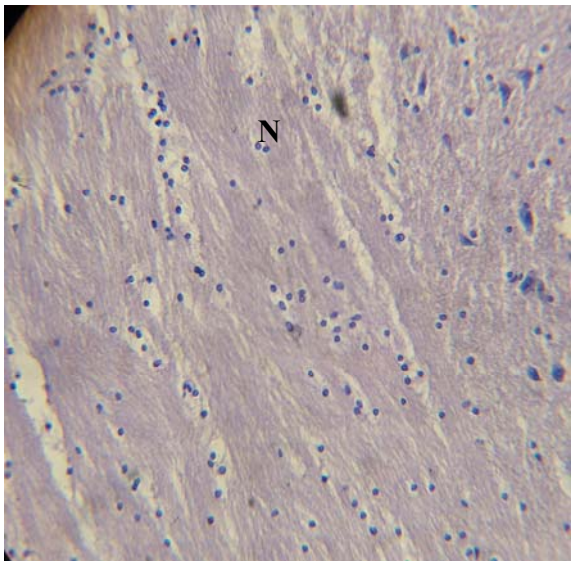

Fig.2: Pituitary gland of rabbit treated with SIE. Showing non-clear pituicytes (P) with small nuclei (N), fibrous tissue formation distributed within the nervosa tissue. No herring bodies, stain (H&E) 400X.

2- Thyroid gland

The thyroid gland of **control** group rabbits appeared normal thyroid tissue composed of thyroid follicles of varies size and filled with colloid and lined by cuboidal thyriocytes, parafollicular cells can be distinguished as shown in figure(3). While thyroid gland of rabbits treated with **SIE** appeared histopathological changes as shown in figure(4).The changes included different size thyroid follicles, dilated thyroid follicles lined by flatted thyriocyte and vacuolation of some follicles depletion of parafollicular cells.

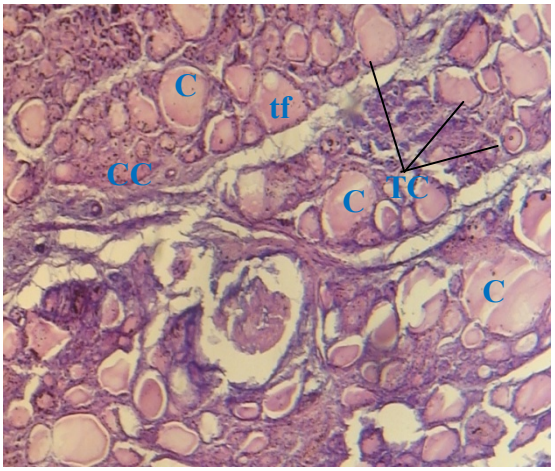

Fig3:Thyroid gland of control rabbit. Showing normal architecture, thyroid follicles (tf), filled with colloid (C) lined by cuboidal thyrocytes (TC) (arrow), parafollicular cells (CC) stain (H&E) 100X.

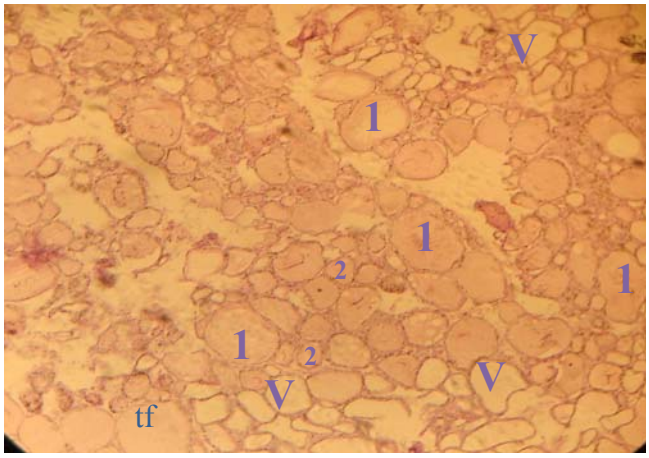

Fig4 : Thyroid gland of rabbit treated with SIE. Showing thyroid follicles of different size (1, 2) dilated and lined by flatted thyrocyte (ft), vacuolation in some follicles(V) and depletion of parafollicular cells, stain (H&E) 100X.

3-Adrenal glands

Adrenal gland of **control** rabbits had normal gland's layers, normal capsule surrounding the first layer of adrenal cortex and to be divided into three zones of adrenal cortex zona glomerulosa, zona fasciculate, zona reticularis and adrenal medulla were shown in figure (5). While the adrenal gland of rabbits treated with **SIE** revealed histopathological changes included degeneration of zona glomerulosa and reduction of zona fasciculate, inspite of normal capsule and normal zona reticularis and adrenal medulla as shown in figure (6).

4- Ovaries

The ovary of **control** rabbits contain normal ovarian cellular tissue with normal Graafine follicles. In addition, there are normal primary follicles and secondary follicles in figure (7).While the ovary of rabbits treated with **SIE** reaveled histopathological changes included very clear disturbance in ovarian tissue or parnchymia, high proliferation of denase fibrous tissue with no primary follicle formation. There is only two Graafine follicle containing dense matrix like tissue structure as shown figure (8).

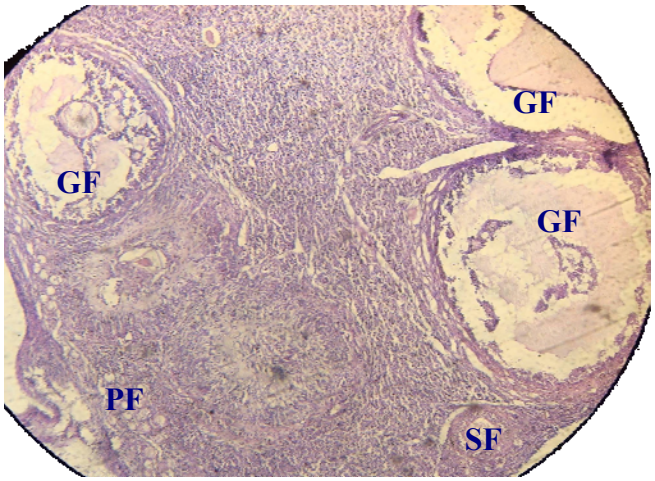

Fig.7 : Ovary of control rabbit.Showing normal ovarian cellular tissue with normal Graafine follicles (GF), normal primary follicles (PF) and secondary follicles(SF), stain (H&E) 40X.

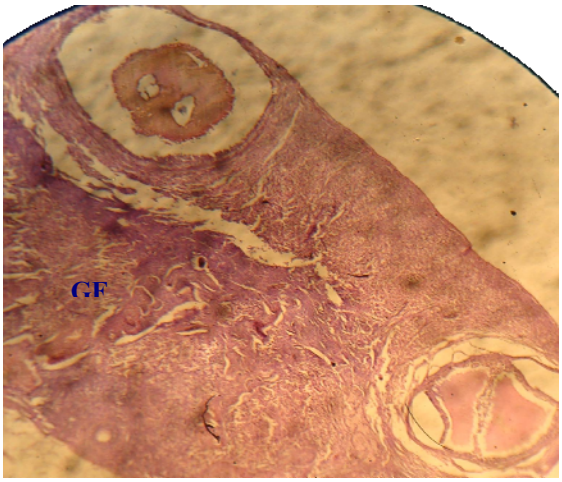

Fig.8: Ovary of rabbit treated with SIE.Showing very clear disturbance ovarian tissue or parnchymia ,high proliferation of denase fibrous tissue with no primary and secondary follicles formation. Graafine follicle (GF) containing dense matrix like tissue structure, stain (H&E) 40X.

DISCUSSION

The obtained results in Table (1) revealed a significant decrease in thyroid hormones concentrations after treatment with SIE with significant increase in TSH after treatment with SIE. This result is supported by histological findings of pituitary and thyroid glands in the present study which indicated markedly suppressed functional status of thyroid gland. Serum concentrations of thyroid hormones(T_3,T_4) and TSH are commonly used as reliable indicators of the thyroid function in humans and experimental animals[16]. Thyroid peroxidase and 5′-deiodinase key enzymes involved in thyroid hormone biosynthesis. All reactions necessary for the formation of T_3 and T_4 are influenced and controlled by pituitary TSH which stimulates follicular cells in the thyroid gland[16,17]. However, because T_3 is the primary determinate of TSH release by the pituitary gland, homeostatic mechanisms should rapidly restore T_3 concentrations nearly to reference rang [10] found a significant increase of TSH. Pituitary TSH secretion is controlled by a negative feedback mechanism modulated by the circulating level of free T_4 and free T_3 and by conversion of T_4 to T_3 in the pituitary thyrotropic cells. T_3 is the metabolically active iodothyroinine. TSH secretion is also influenced by thyrotropin releasing hormone (TRH), a 3-amino acid peptide synthesized in the hypothalamus which stimulates the pituitary to release TSH [16,17 and 18].

The affected thyroid gland by SIE may be attributed to inhibition thyroid peroxidase and 5′-deiodinase key enzymes involved in thyroid hormone biosynthesis[19,20 and 8] found that deiodinase activity were significantly inhibited by isoflavonoid, because antioxidant activity of isoflavonoid [5], antioxidant may affect the peripheral conversion of thyroid hormones by deiodination[21]. The inhibition of these enzymes results in decreased levels of circulating thyroid hormones that leads to increase secretion of TSH by the anterior pituitary. The increased levels of TSH provide a growth stimulus to the thyroid, resulting in goiter. These results are in agreement with [2,22]. But this result disagrees with [23]who found that the thyrotropin, total and free triiodothyronine and thyroxine would not change by tested phytoestrogen in female rats. Also[24] stated that the isoflavonoid mixture did not cause changes in the serum thyroid hormone in rats.

The obtained results in Table (2) revealed that estradiol concentration increased in serum of SIE group but decreased LH, FSH, cortisol and Progesterone concentrations. In addition histological findings of pituitary-adrenal-ovary indicate this alteration. The decreased cortisol concentration which may be attributed to affected HPA axis by SIE thus it lead to decrease cortisol hormone. This result is supported by histological findings in Fig.(6) including degeneration of zona glomerulosa and reduces of zona fasciculata which indicated that treatment with SIE leads to adrenal dysfunction. The result is in accordinated with [25]who found that the isoflavonoid lead to decrease synthesis of cortisol. [26] found that the soy diet leads to decrease weight of adrenal gland and zona fasciculata in monkey. However, the effect of SIE-induced decline in adrenal production of cortisol may be caused higher secretion of ACTH from pituitary gland by feedback mechanism which confirm with [27] and it may contributed to inhibition of LHRH secretion from hypothalamus, possibly mediated by excess ACTH.

The increased estradiol concentration in serum of virgin rabbits treated with SIE attributed to estrogenic effect of SIE and histological findings of ovaries and pituitary in Fig.(8) which including very clear disturbance of ovarian tissue or parnchymia, high proliferation of denase fibrous tissue with no primary and secondary follicles formation. Cystic Graafine follicle (CGF) containing dense matrix like tissue structure indicate imbalance of ovarian hormones in rabbits treated with SIE. The result is in agreement with [28] and this lead to decrease FSH by feedback mechanism and decrease concentration of progesterone[29].

The obtained results present in the Table (3) indicated that a significant decrease serum concentration of Zn in virgin rabbits treated with SIE This decrease in serum concentration of Zn may be attributed to hypothyroidism. [30] also suggested that the hypothyroid plays a role in the pathogenesis of hypozincemia. Hormones have been shown to influence trace metals metabolism at several levels, including excretion and transport of trace metals[31]. For its normal state, thyroid is dependent on the presence of various trace elements both for synthesis and metabolism of thyroid hormone. The role of Zn in thyroid is less well defined but suboptimal or supraoptimal dietary intakes of these elements can adversely affect thyroid metabolism. The involvement of Zn in thyroid function is complex and may include its effect on synthesis and mode of action of hormone[32]. [33] suggested that the Zn is as important as selenium or iodine for thyroid homeostasis. The present study indicated that decreased serum concentration of Zn in hypothyroid rabbits as compared with control group accordainated with [34] [normal range: Zn 75-120 µg/dl [35].

The results of hormones concentrations, reproductive efficiency and histological changes of ovaries indicated that the fertility percentage in female rabbits treated with SIE may be attributed to affect pituitary-thyroid-gonad axis which are indicated by results of hormones patterns and histological findings of these organs of female rabbits.

Many processes involved in growth, development, maturation and metabolism in humans and animals are under the direct or indirect control of the thyroid hormones [36]. Induce hypothyroidism by SIE and continuous administration have sever effect on metabolism and function of various organs and system such as the reproductive system. these caused a significant decline in fertility as evidenced by alterations in hormone patterns, as well as by the profound impact on litter growth and newborn weight Table (5). Hypothyroidism induced by SIE cause reproductive disturbances and reduces fertility percentage might have been caused by the presence of an estrogenic like substance in soy plant (genistein) therefore lead to decrease the number of litters born in rabbits. This

result is in agreement with [37] who have been reported that the reproductive disturbances occur in sheep and rabbits fed the soybean as a large part of the diet.

These modifications to sex hormones may be due to treatment with SIE which is led to change in ovaries tissue and follicuogenesis, which are indicated by histological findings of ovaries. In addition, the effects of the exposure to SIE on steroid hormones may also result from effect of SIE on regulations of steroid synthesis-related enzymes, such as aromatase and 3β-hydroxysteroid dehydrogenases (3β-HSD), which have received the most attention with regarded to enzyme modulation and endocrine disrupters [38].

In the present study, despite the absence of signs of maternal toxicity, of pregnant rabbits treated with SIE that they showed decrease number of pregnant females and a significant reduction in weight of newborn. This result is in agreement with [39] who found that some effects were also noted at the low dose of genistein 20 mg /kg /day including, increased newborns mortality and decreased newborns body weights, however at the mid-dose of 150 mg/kg/ day; no adverse effects on the newborns were noted. In a toxicological assessment approach, agents acting on reproductive parameters may be identified as potentially able to offer risk to the reproductive system [40]. Thus, despite the non-existence of maternal toxicity, these alterations indicate a reproductive injury induced by SIE, probably by a potential embryotoxic action. In the present study, no significant differences in gestation period in pregnant female rabbits treated with SIE in spite of it effect on adrenal gland and decreased cortisol concentration which may be attributed to estrogenic effect of SIE. Similar results was found by [28].

تاثيراستحداث القصور الدرقي باستخدام مستخلص الايزوفلافونيدات لبذورنبات فول الصويا على محاور الغدد الصمية و بعض المعايير الفسلجية والنسجية في إناث الأرانب.

منى حميد السعيد ،علاء الدين حسن جواد ،جاسم محمد احمد

كلية الطب البيطري ،جامعة البصرة ،البصرة ،العراق

الخلاصة

أجريت هذه الدراسة في البيت الحيواني- التابع لكلية الطب البيطري- جامعة البصرة لمحاولة استحداث القصور الدرقي باستخدام مستخلص الايزوفلافونيدات لبذورنبات فول الصويا ولمعرفة ما يعكسه هذا القصور من تأثيرات على محاور الغدد الصمية و بعض المعايير الفسلجية والنسجية في إناث الأرانب.

أستخدم في هذه التجربة (٢٤) أنثى أرنب محلي عذراء تتراوح أوزانها ما بين(١١٢٠-١١٤٧غم) وبمعدل عمر ما بين (٥.٥-٦شهر) قسمت عشوائيا بالتساوي إلى مجموعتين (١٢ أرنب/ مجموعة).

المجموعة الأولى سيطرة تم تجريعها (٣ مل من المحلول الملحي الفسيولوجي)والمجموعة الثانية تم تجريعها (٢٠٠ ملغم /كغم من وزن الجسم مستخلص الايزوفلافونيدات لفول الصويا) واستمرت المعاملة لمدة شهرين (الشهر الأول قبل التزاوج و الشهر الثاني بعد التزاوج).

وتضمنت الدراسة خلال الشهر الأول تحديد بعض القياسات المرتبطة بالقصور الدرقي مثل هرمونات الغدة الدرقية والنخامية والغدة الكظرية وهرمونات القند وبعض المعايير الكيموحيوية كقياس تركيز بعض المعادن(الزنك والكالسيوم والفسفور والصوديوم والبوتاسيم) وفي الشهر الثاني من الدراسة تم مزاوجة الإناث المعاملة بذكور سليمة غير معاملة لغرض دراسة الكفاءة التناسلية وتضمنت تحديد عدد الإناث الحوامل، نسبة الخصوبة ، فترة الحمل، عدد المواليد وأوزانها، عدد الأجنة المجهضة،عدد المواليد الميتة ،عدد المواليد المشوهة ، نسبة التشوه وفي نهاية الفترة المدروسة ذبحت الإناث لدراسة التغييرات النسيجية في الغدد (النخامية والدرقية و الكظرية والمبايض) وكذلك حساب عدد الأجسام الصفراء الموجودة في المبيض ومواقع الانغراس في الرحم. وتوصلت الدراسة إلى النتائج الآتية:

لوحظ انخفاض معنوي ($p\leq0.05$) بتراكيز هرمونات الغدة الدرقية ثلاثي ورباعي اليود لمصل دم الأرانب المعاملة بمستخلص الايزفلافونيدات بينما لوحظ ارتفاع معنوي في تركيز الهرمون المحفز للغدة الدرقية في مصل دم الأرانب. و كذلك انخفضت معنويا ($p\leq0.05$) تراكيز هرمونات الغدة النخامية (LH,FSH) وهرمون الغدة الكظرية (الكورتيزول) في مصل دم الأرانب المعاملة بمستخلص الايزفلافونيدات. وتأثرت هرمونات القند (الاستروجين والبروجستيرون) إذ ارتفع تركيز هرمون الاستروجين وانخفض تركيز هرمون البروجستيرون معنويا ($p\leq0.05$) في مصل دم إناث الأرانب المعاملة بمستخلص الايزفلافونيدات. وبينت الدراسة لمعايير الكيموحيوية وجود انخفاض معنوي ($p\leq0.05$) في تركيز عنصري الزنك والصوديوم وارتفاع معنوي ($p\leq0.05$) بتركيز عنصري الكالسيوم لمصل دم الأرانب المعاملة بمستخلص الايزفلافونيدات
وتأثرت الكفاءة التناسلية كذلك اذ انخفضت نسبة الحمل للإناث المعاملة بمستخلص الايزوفلافونيدات فكانت ٤١.66%
أكدت النتائج وجود تغيرات في نسيج الغدد الدرقية (إذ لوحظ تسطح خلايا الحويصلات الدرقية في مجموعة الايزوفلافونيدات فول الصويا وفقدان الخلايا الفارزة للكالستونين مع وجود فراغات في غروان الجريبات ورافقتها تغيرات في أنسجة المحاور الصمية الأخرى مثل النخامية (عدم وضوح خلايا الغدة النخامية) وتاثرت الغدة الكظرية إذ لوحظ نقصان في المنطقة الحزيمية إما بالنسبة للقند لوحظ اضطراب نسيج المبايض في مجموعة الايزوفلافونيدات فول الصويا.

REFERENCES

1-McDonald,D.V.M.(2003). Veterinary endocrinology and reproduction, 2nd Ed. Lea and Febiger. Philadelphia.

2-Doerge,D.R. and Sheehan,D.M.(2002). Goitrogenic and estrogenic activity of soy isoflavones. *Environ Health Perspect*. 110(3):349-53.

3-Herkelman,K.L.; Cromwell,G.L.; Stahly,T.S.; Pfeiffer,T.W. and Knabe,D.A. (1992). apperent digestibility of amino acids in raw and heated conventional and low-trypsin-inhibitor soybean for pigs. *J Anim Sci*. 70: 818-26.

4-Sierens,J.;Hartley,J.A.; Campbell,M.J.; Leathem,A.J. and Woodside,J.V.(2002). In vitro isoflavone supplementation reduces hydrogen peroxide-induced DNA damage in sperm. *Terato Carcino Muta*. 22:227-34.

5-Mira,L.;Fernandez,M.T.; Santos,M.; Rocha,R.; Florencio,M.H. and Jennings, K.R.(2002). Interactions of flavonoids with iron and copper ions:a mechanism for their antioxidant activity. *Free Radic Res*. 36:1199-208.

6-Middleton,E.J. and Kanadaswami,C.(1993). The impact of plant flavonoids on mammalian biology: Implications for immunity, inflammation and cancer, in The flavonoids: Advences in Research since 1986, Ed by *J B Harbourne* (Chapman& Hall,London). 619-52.

7-Messina,S.M. and Bennink,M.(1998). Soy foods, isoflavones and risk of colonic cancer: Areviw of the *in vitro* and in *vivo* data, *Baillieres Clin Endocrinol Metab.*12:707-28.

8-Divi,R.L.; Chang,H.C. and Doerge,D.R.(1997). Anti-thyroid isoflavones from soybean: isolation, characterization and mechanism of action. *Biochem Pharmacol*. 54:1087-96.

9-Hampl,R.;Ostatnikova,D.;Celec,P.;Putz,Z.;Lapcik,O.andMatucha,P.(2008). Short-term effect of soy consumption on thyroid hormone levels and correlation with phytoestrogen level in healthy subjects.42:53-61.

10-Wandi,J.; Fomum,T.; Tilequin,F.; Segun,E. and Koch,M.(1994). Two isoflavonoes from *Erythrina senegalensis*. *Phytochem*. 35:245-8.

11-John,K.M.M.; Vijayan,D.; Kumar,R.R. and Premkumar,R.(2006). Factors influencing the efficiency of extraction of polyphenols from young tea leaves. *Asian J plant Sci.*5 (1):123-6.

12-El-Tahar,K.E.H.; El-Nasser,M.A.; Ageel,A.M.; El-Obeid,H.A. and Al-Rashood,

K.A.(1991). Effects of N-methyl and N-isobutyl-1,2-diphenyl ethanol amine on the spontaneous and evoked contraction in isolated uterus. *Gen-pharmaco*. 22(4):685-90.

13-Bancroft,J.D.; Stevens,A. and Turner,D.R.(1990). Theory and practice of histological techniques .3rd Ed. Churchill Livingstone. 221-6.

14-Luna,L.G.(1993). Manual of histology staining methods of the Armed Forces Institute of Pathology.3rd ed., New York, Mc grawHill.

15- SPSS Statistical Packages for the Social Sciences. (2001). Statistical soft ware for windows version 13.0 Microsoft. SPSS®, Chicago, IL,USA.

16-Kelly,G.S.(2000). Peripheral metabolism of thyroid hormones. A review. *Alt Med Rev*. 25(4):306-33.

17-Beers,M.H. and Berkow,R.(1999). The Merck manual of diagnosis and therapy. 17th Ed., USA, John Wiley and Sons: 82.

18-Higgins,C.(2007). Understanding laboratory investigations for nurses and health professionals. 2nd Ed. Oxford, Black well Science.142.

19-Cody,V.; Koehrle,J. and Hesch,R.D.(1989). Structure-activity relationships of flavonoids as inhibitors of iodothyronine deiodinase,in environmental goitrogenesis edited by Gaitan,E.(CRC press, Boca Raton,F.L.). 57-69.

20-Divi,R.L. and Doerge,D.R.(1996). Inhibition of thyroid peroxidase by dietary flavonoids. *Chem Res Toxicol*. 9:16-23.

21-Chaurasia,S.S. and Kar,A.(1997). Protective effects of vitamin E against lead-induced deterioration of membrane associated type-Iiodothyronine monodeiodinase(5´D-I) activity in male mice. *Toxycology*. 124:203-209.

22-Udgata,J.R. and Naik,S.N.(2007). Soybean isoflavones: Remdial nutraceuticals in Indian perspective. *J Sci Indu Res*. 66:11-8.

23-Nogowski,L.; Nowicka,E.;Szkudelski,T. and Szkudelska,K.(2007). The effect of Genistein on some hormones and metabolic parameters in the immature, female rats. *J Anim F. Sci*.16:274-82.

24-Ikegami,S.; Tousen,Y.; Ishimi,Y.; UmegaKi,K. and Nakashima,Y. (2006). Possible adverse effect of soy isoflavone mixture on pregnant and lactating rats and their suckling. *J H sci*. 52(5): 558-67.

25-Wong,C.K. and Keung,W.M.(1999). Bovine adrenal 3beta-hydroxy steroid dehydrogenase (E.C.1.1.1.145)/5-ene-4-ene isomerase (E.C.5.3.3.1): characterization its inhibition by isoflavones. *J Steroid Bio chem Mol Biol*. 71:191-202.

26-Wood,C.E.; Cline,J.M.; Anthony,M.S.; Regiter,T.C. and Kaplan,J. (2004). Adrenocortical effects of oral estrogens and soy Isoflavones in female monkeys. *J Clin Endocri Metab*. 89(5):2319-25.

27-Ohno,S.; Nakajima,Y.; Inoue,K.; Nakazawa,H. and Nakajin,S.(2003). Genistein administration decreases serum corticosterone and testosterone levels in rats. Life Sci74:733- 42.

28-Adlercreutz,H.; Goldin,B.; Gorbach,S.; Höckerstedt,K.; Watanabe,S.; Hämäläinen,E.; Markkanen,M.; Mäkelä,T.; Wähälä, K.; Hase,T. and Fotsis,T.(1995). Soybean phytoestrogen intake and cancer risk. *J Nutr.*125: 57-770.

29-Cassidy,A.; Bingham,S. and Setchell,K.(1995). Biological effects of isoflavones in young women: importance of the chemical composition of soyabean products. *Br J Nutr.* 74(4):587-601.

30-Shu-Ming,C.; Cheng-Deng, K.; Low-Tone, H. and Jyh-Fei,L.(2005). Effect of hypothyroidism on intestinal zinc absorption and renal zinc disposal in five-sixths nephrectomized rats. *Japanese J Physi.*55: 211- 9.

31-Henkin,R.I.(1976). Trace metals in endocrinology. *Med Clin North Am.*; 60:779-97.

32-Arthur,J.R. and Beckett,G.J.(1999). Thyroid function. *Br Med Bull*. 55 (3):658-68.
33-Fujimato,S.; Indo,Y.; Higashi,A.; Mastuda,I.; Kashiwabara,N. and Nakashima,I.(1986). Conversion of thyroxin into tri-iodothyronine in zinc-deficient rat liver. *J Pediar Gastroenterol Nutr*. 5:799.
34-Alturfan,A.A.; Zengin,E.; Dariyerli,N.; Alturfan,E.E.;Gumustas, M.K.; Aytac,E.; Aslan,M.; Balkis,N.; Aksu,A.; Yigit,G.; Uslu,E. and Kokoglu,E.(2007). Investigation of zinc and copper levels in methimazole induced hypothyroidism: Relation with the oxidant antioxidant status. *Folia Biologica* (Praha). 53:183-8.
35-Lumsden,J.H.(1983). I units in veterinary medicine. *Can Vet J*. 24:132-3.
36-Oppenheimer,H.L.; Schwartz,H.L.; Mariash,C.N.; Kinlaw,W.B.; Wong,N.C.W. and Freake,H.C.(1987). Advance in our understanding of thyroid hormone action at the cellular levels, *Ender Rev*.8:288.
37-Carter,M.W.; Smart,W.W.G. and Gennaromatrone,J.R.(1954). Estimation of estrogenic activity of genistein obtained from soybean meal. *Proc Soc Exp Biol Med*. 84: 506-7.
38-Whitehead,S.A. and Rice,S.(2006). Endocrine-disrupting chemicals as modulators of sex steroid synthesis. *Best Pract Res Clin Endocri Metab. 20:570*-6.
39-McClain,R.M.; Wolz,E.; Davidovich,A.; Edwards,J. and Bausch,J.(2007). Reproductive safety studies with genistein in rats. *Food Chem Toxicol*, 45:1319-32.
40-Zenick,H. and Clegg,E.D.(1989). Assessment of male reproductive toxicity: a risk assessment approach. *In*: Principles and Methods of Toxicology. New York: Raven Press. 275-309.