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SOYA BEAN AS AN ESTROGENIAL SOURCE FOR IMPROVING FERTILITY OF ALBINO FEMALE MICE

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ABSTRACT
A study was carried out to investigate the effect of using Soya bean as an estrogenial source in a diet on fertility of white females Albino mice through using 20 females' mice, grouped and fed on two different protein source diets for couple months. Animals were killed and slaughtered, samples of blood were taken to measure the levels of sexual hormones (LH, FSH, estradiol and progesterone), mice were overiectomized for histological sections. Results revealed a significant differences ($P < 0.05$) in all mentioned criteria for treated mice as compared with control groups, also histological inspection results indicated an increase in a number and diameters of *corpus luteum* and showed significant difference ($P< 0.05$) in ovarian follicles diameters.

KEY WORDS: Soya bean, fertility, Albino mice

INTRODUCTION

Recently, importances have increased by using a replacement medicine. Scientists and researchers invested their efforts and dedicated in plant of a dual benefits (Nutritional and medical) through its active substances and identifying its medical activities which enters in many popular prescriptions which have a wide importance in medicating many of incurable diseases e.g cancer, sterility…etc, that medicine have failed up to now to overcome it, although owning the more potent chemical antibiotics and the more recent active technologies (Al-Flayh, 1986). Soya bean plant is one of these plants of double benefits that enter in most of nutritional ingredients since its rich in proteins, fats, vitamins, minerals sources, moreover it's cheap. Its considered the main feed for both human and animal, also its free from starch and for that considered as a good food for these who suffering from diabetes (Al-Rawy, 1988) since its inclusion of some active substances such as isoflavones e.g. (Genistine, diaidzen, coumestans) of a large similarity in the chemical composition of estrogen hormone that's able to link with estrogen receptors in target cells (Han et al., 2002). The purpose of this study was to find the effect of Soya beans on hormonal and ovarian activity in albino rates.

MATERIALS AND METHODS

The experiment involved 20 of a white adult albino female with the same age and parity obtained from animal house referred to the biological techniques research center of Al-Jaeria. Experiment was carried out in biology department labs. College of Science for Women, University of Baghdad and were lasted long for 2 months from 12-12-2008 up to 12-12-2009.

TABLE 1: Chemical composition and feedstuffs ingredients of the diets for two groups

Group 1(Treatment)		Group 2 (Control)	
Feedstuffs	Percentage	Feedstuffs	Percentage
Yellow corn	33	Yellow corn	33
Soya bean	20	Protein concentrate	11
Wheat	41	Wheat	41
Barley	4	Barley	4
Fat	1	Meat meal	10
CaCO3	0.7	CaCO3	0.7
NaCl	0.3	NaCl	0.3
AD3 E Vit.	0.1	AD3 E Vit.	0.1
Cholin Chloride	0.05	Cholin Chloride	0.05
Total	100%	Total	100%
Crude protein	19.52%	Crude protein	19.75
ME (kcal/g diet)	3097.70	ME (kcal/g diet)	3102.50

- Groups which its protein percentage and metabolizable energy were calculated in according to National Research Council (NCR), 1994.

All animals were placed in special cages under a certain similar environmental conditions and were divided randomly into 2 groups; the first group was fed on protein source diet (Soya), whereas the second group fed on the

other protein source diet (Table 1). Water offered *ad lib* to both of groups. Mice out of 10 from each group were slaughtered using a dissecting set and general anesthesia after fixation of mice on its back. Blood samples were taken directly from heart by following a method known as heart puncture in order to obtain a large quantity of blood using a medical injection. Blood samples were poured into sterilized test tube, then serum was directly separated using centrifuge apparatus at speed of 2000 cycle for 10 minutes Serum samples were maintained under 25 ºC then isolated and maintained on 4 ºC for hormonal measurements. After slaughtering and dissecting mice, ovaries were removed (overiectomy) and placed in physiological saline solution, all attached adipose and connective tissues were removed using dissecting microscope and dried for a short interval using 2 types of fixatives (10% formalin and bouin solutions), samples were rinsed using tap water, after ovaries. fixation, it transferred into 50% conc. of ethyl alcohol for 1 – 2 hr, then to 10% conc. to ensure removing of yellow color of picric acid. Samples were washed after fixations using aqueous fixatives (10% formalin) and tap water for 3 hr. , then washed with non-equeous fixatives and washed again with 50% conc. ethyl alcohol, all washing and dehydration from tissues have done using a chain of gradient conc. (10, 80, 90, 100%). Clearing have done to remove dehydration in filtration and embedding have done with 2 stages and lasted for 2 – 4 hr. Samples were poured into melted paraffin, after solidation of paraffin, removing of excess embedding substances blocks using rotary microtome to obtain a serial histological sections of 7 mm thickness, in a manner to be suitable for slides (Sztein *et al*., 1998). Many of apparatus, chemical substances, stains were used in the histological inspections such as chloroform, ethanol alcohol, Hcl acid, picric acid, paraffin waxe, heamtoxylin, eosin stains and some fixative substances e.g. formalin, formaldehyde and bouin solutions. Also eyelet, light, table microscopes apparatus were used in histological measurements, slides were prepared and inspected under compound microscope of a different magnification power (Olympus). All histological sections were photographed using a compound light microscope provided with photographic camera. Several microscope powers were used (X40, X100, X400).

Statistical Analysis

The means were tested for differences using t-test at level 0.05.

RESULTS AND DISCUSSIONS

Present study revealed that the differences in ovarian hormones levels between two groups were significant ($P < 0.05$), as shown in table 2 females fed on diet with 20% Soya bean were dominant the females in control group diet in regard to levels of LH, FSH and Estradiol. The highest increasing ratio was in FSH and lowest in LH. These increases in females' hormones may be due to the effect of Soya bean on specialized cells in the anterior lob of pituitary gland which secretes the mentioned hormones (LH and FSH).

TABLE 2: Means of females sexual hormonal levels in blood serum

Group No.	Females sexual hormonal levels (μ gm/mml		
	LH	FSH	Estradiol
1 Control	0.91 ± 0.25^{b}	0.53 ± 0.18^{b}	30.07 ± 2.15^{b}
2 20% Soya	1.77 ± 1.14^{a}	1.37 ± 1.05^{a}	63.33 ± 3.92^{a}
Increasing ratio	0.94	1.58	1.10

Means in the same column with no common superscripts differ significantly ($P < 0.05$)

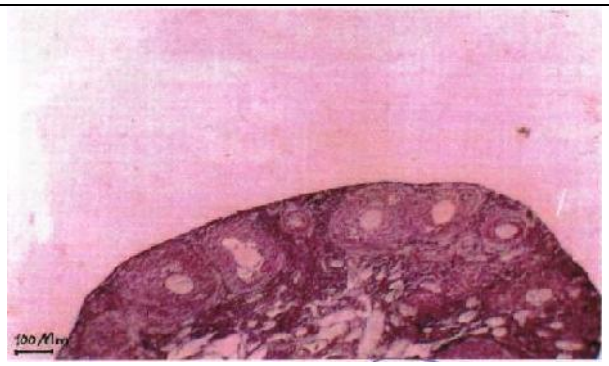

Figure 1: Illustrate growth and development of ovarian follicles in group 1 (20% Soys) microscope power of X40

Figure 2: Growth and development of ovarian follicles in group 2 (Control) microscope power of X100.

Soya bean affects on stimulation of pituitary gland cells to secrete both LH and FSH, this will increase their levels in the blood serum. Which are necessary for estrogen production, FSH will stimulate production and growth of ovarian follicles as shown in figures (1, 2) shows these developments. Similary LH is necessary to stimulate estradiol production from granulosa cells in ovary and causing ovulation (Gugton & Hill, 1996), the above stimulations depend on the production levels of the mentioned hormones, and any factor affecting their production will cause a disturbance in the follicles stimulation and development, on the other hand

development of unique ovarian follicles to Grafian follicle in the 1st stage follicles which performed by 2 processes, these are cells proliferation and differentiations, so any factor affects these two process will affects on follicles (Sirtori, 2001).

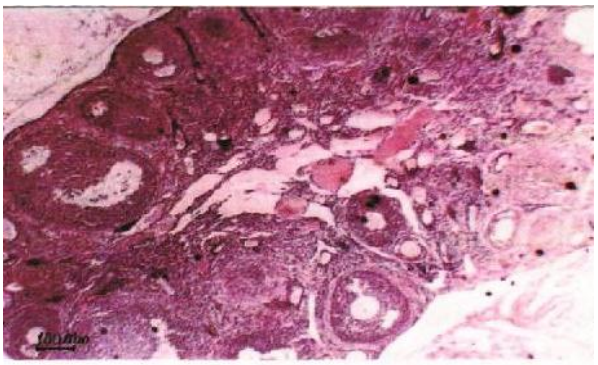

Figure 3: Illustrate the increase in ovary size and an increase in No. of developed ovarian follicles, microscope power of X40

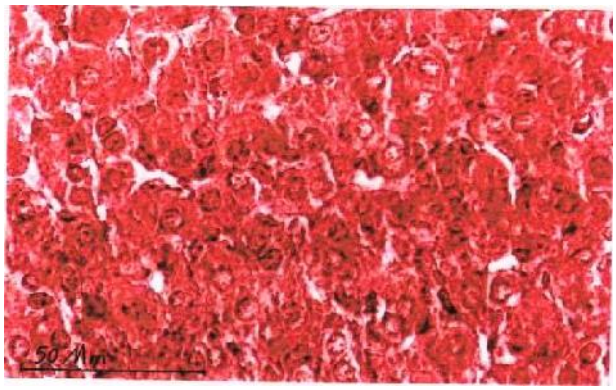

Figure 4: The increasing in Luteuian granulated cells in the CL of group 1 females mice. microscope power of X400

Also the significant increase in the estradiol in females blood serum who fed on Soya bean will cause an increase in ovary size, with the results affects the development of the lining mucous epithelium of fallopian tube and boosting feathering projections activity and increasing its movement toward uterus to assist fertilized ovum to be settled in a proper position inside the uterus (Cordle, 2004), figure 3 illustrate the increase in ovary size and an increase in number of developed ovarian follicles. In case of fail fertilization the size of corpus Luteum (CL) will shrinks and decreases and with result levels of estradiol and progesterone will decrease also, a new ovarian cycle will onset again but if conception occurs the size of CL will increase and maintains fetus (figure 4 indicates the increasing in Luteuian granulated cells in the CL of mice fed Soya beans. Generally, results reveals that the role of Soya in increasing females fertility when its used in a limited concentration in case of sterilized females, since it increases the ovarian activity which stimulate ovulation through its active substance phyto-estrogen (Isoflavone) that stimulates ovulation (Farnsworth, 1975). From above mentioned general changes in this study indicates the success of using Soya in stimulating proliferation and growth of ovarian follicles and ovulation and also increases the ovary size and No. of ovarian follicles and its development and that is appeared in the histological sections inspection of the ovary of group 1 treated with Soya females mice as compared with control group females mice.

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