AL-ZAHRAA UNIVERSITY JOURNAL
Health and Medical Science

*Al-Zahraa Journal for Health and Medical Sciences 2025; 3:*1-10 **Vol. 3, No.4, 2025**
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Phytoestrogenic Effects of Soybeans and Fenugreek Extracts on Sex Hormone and Internal Organs in Ovariectomized Female Wister Rats

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Received: 08 September, Year (2025), Accepted: 04 November. 2025. Published: 31 Dec. 2025

ABSTRACT

The experiment was conducted at a medical research center in the experimental animal unit using female Albino Wistar rats. The number of rats was 50 at 3 months of age, and their weights at the start of the experiment ranged from 200 to 242g. It was divided into five groups: the control group (T1), which was fed the standard casein protein diet. T2 is fed a 50% concentration of soybean seeds extract. T3 is fed a 75% concentration of soybean extract. T4 is fed with fenugreek extract at a concentration of 50%. T5 feeds on fenugreek extract at a concentration of 75% indicates the results to the presence of significant differences in the weight gain of internal organs in mice treated with heart 0.274g, liver 2.294g , uterus 0.074 g, lung 0.676 g, adrenal glands 0.036 and spleen 0.071 at treatments T5 compared treatments (T2,T3,T4), respectively. Based on the results indicate a significant increase in sex hormones Estrogen 123.87, LH 103.98, TSH 0.008 and Prolactin 5.64 µg/mL) in the treated T5 while FSH 103.64 µg/mL indicate a significant decrease in the treated T5.

Keywords: Phytoestrogen , Fenugreek, Soybeans, Ovariectomy.

AL ZAHRAA UNIVERSITY JOURNAL
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Al-Zahraa Journal for Health and Medical Sciences 2025; 3:1-10 **Vol. 3, No.4, 2025**
www.zjhms.alzahraa.edu.iq

Introduction

In females, the body primarily relies on the hormone estrogen, which is secreted by the ovaries. Estrogen levels decline sharply after ovariectomy (OVX), leading to changes in various physiological systems such as metabolism, the immune system, and vital organ functions[1].Soybeans are considered a rich source of isoflavones, such as genistein and daidzein, which are selective estrogen receptor modulators (SERMs) and can partially mimic the effects of estrogen in OVX animals [2]. It has been found that feeding OVX mice a diet rich in isoflavones reduces the relative weight of lymphoid organs, modulates immune indicators, and helps reduce oxidative stress by activating the estrogen receptors (ER) in bones [3]. On the other hand, fenugreek (*Trigonella foenum*-graecum) exhibits clear estrogenic activities in the uterine and vaginal tissues of OVX mice, such as increased dry and wet uterine weight, thickening of the epithelial layer, and high expression of progesterone receptors, which supports its compatibility with cellular estradiol pathways [4] ,Studies comparing the administration of fenugreek to OVX rats have shown that low doses may improve the mechanical properties of bone in some areas, while high doses may disrupt bone mineral formation, especially in the vertebral bone [5]. Regarding the weights of internal organs such as the heart, kidneys, spleen, and adrenal glands, direct studies using both soybeans and fenugreek in the OVX model are still limited [6]. However, the effect of is flavones on activating the hypothalamic-pituitary-adrenal (HPA) axis is supported by a study on orchiectomies male mice, where Adrenocorticotropic Hormone ACTH and corticosterone levels and the size of the adrenal cortex regions increased after treatment with soy extract indicating a clear effect on adrenal gland function [7].As the results of [8] that giving soy to OVX mice reduces the relative weight of lymphoid organs (particularly the spleen), improves immune and metabolic indicators by reducing oxidative stress and increasing ERα receptor activity, which may indirectly affect the size or weight of the spleen and other lymph nodes. This study's goal effects of fenugreek and soybean extracts on sex hormones and internal organs in female Wistar rats with ovariectomies.

Material and method

The experiment was conducted at a medical research center in the experimental animal unit using female Albino Wister rats. The number of rats was 50 at 3 months of age, and their

AL ZAHRAA UNIVERSITY JOURNAL
Health and Medical Science

Al-Zahraa Journal for Health and Medical Sciences 2025; **3**:1-10 **Vol. 3, No.4, 2025**
www.zjhms.alzahraa.edu.iq

weights at the start of the experiment ranged from 200 to 242 g. They were housed in the animal facility at room temperature with 55% humidity, with 12 hours of artificial light and 12 hours of darkness, and with cleanliness maintained. Special plastic cages were used. Research Design was divided into five groups: the control group (T1) fed the standard casein protein diet.T2 is fed a 50% concentration of soybean extract.T3 is fed a 75% concentration of soybean extract.T4 is fed with fenugreek extract at a concentration of 50%. T5 feeds on fenugreek extract at a concentration of 75%. The standard meal components contain 25% casein, 5% corn oil fat, 1% vitamins, 3.5% fiber, and 20% carbohydrates. Removing the ovaries: The operation was carried out under sterile surgical conditions, as animals were drugged using a combination of ketamine and zealzin (40 mg/kg body weight + 5 mg/kg body respectively), and the animal was placed in a backpack position. The animal's abdomen was prepared for the ovary. Two ties around the upper ovarian arteries are placed in the ovarian ovaries and two other ties down the ovaries in the real ovary, using a catgut size of 0/4, and then cut between two ovaries and then cut the ovarian leash to free the ovarian. Next, the surgical site was checked for bleeding is checked for bleeding, then the threads are ducked together, the process is repeated on the right ovaries, the abdomen is sealed with continuous sutures with catgut, and the skin is sealed with intermittent stitches with silk threads, and the thread is removed 10 days later The experimental group, however, had plants extracted using the Sex let method. Soybeans and fenugreek were collected from local markets, washed with distilled water, dried in the shade, and then the sample was ground using an electric blender[9,10]. Weigh out 100 grams of the ground sample and place it in a Thimble . Then, use 70% ether alcohol in a 1000 ml flask. Run the device at 70 degrees for 24 hours. Then the extract was placed in a rotary evaporator to separate the alcohol from the extract [11,12].Secondary metabolism compounds (Alkaloids, Phenols, Glycosides, Tannins, Flavonoids and saponin) screen study According to the method described by [13,14].

Assessing the Health Status of Mice

The mice weight was monitored every two weeks, and the weights of the internal organs of the heart, liver, uterus, kidneys, lungs, adrenal glands, and spleen were estimated. The organs were quickly removed, washed in phosphate solution, thoroughly dried with water, and then weighed in kilograms. The ratio of each organ's weight to the body weight in kilograms was then

AL ZAHRAA UNIVERSITY JOURNAL
Health and Medical Science

Al-Zahraa Journal for Health and Medical Sciences 2025; 3:1-10 **Vol. 3, No.4, 2025**
www.zjhms.alzahraa.edu.iq

calculated [15].After the experiment ended and after fasting for 12 hours, the mice were anesthetized using diethyl ether and blood was collected from the eye vein using special capillary tubes to draw blood. Centrifuge was performed to obtain serum and the samples were analyzed immediately. Estrogen, follicle-stimulating hormone (FSH), prolactin, and thyroid-stimulating hormone (TSH) were measured [16].

Statistical analysis

Use the Tuky test to indicate the difference between the two groups at the level P≤ 0.01 [17].

Results

phytochemical screen study:

The purpose of the photochemical screen study was to identify the active compounds in the extract of fenugreek and soybean plants that exhibit flavonoid s, alkaloids, phenol s, glycosides, and positive results, such as saponin and tannins, as shown in table (1) adverse outcomes.

Table 1. The active compounds screen Fenugreek and Soybean.

Compounds	Fenugreek	Soybean
Flavonoid	+	+
Phenol	+	+
Alkaloid	+	+
Glycoside	+	+
Saponin	-	-
Tannins	_	_

+ found the Compounds , - not found the Compound

Table 2 indicates the presence of significant differences in the weight gain of internal organs in mice treated with heart, liver, uterus, lung , adrenal glands and spleen treatments (T2,T3,T4,T5) with an average weight of heart (0.225±0.06, 0.265±0.07, 0.233±0.05 and 0.274±0.06%) respectively compared to T1, which had an average weight of heart (0.205±0.03%), However, a significant increase in weight was observed in the liver, with an average of (2.234±.0.24, 2.295±0.23, 2.285±0.19 and 2.294±0.17%) respectively compared to T1

AL ZAHRAA UNIVERSITY JOURNAL
Health and Medical Science

Al-Zahraa Journal for Health and Medical Sciences 2025; 3:1-10 **Vol. 3, No.4, 2025**
www.zjhms.alzahraa.edu.iq

(1.587±0.28%) , Significant weight gain in the lungs (0.623±0.09 , 0.623±0.09, 0.072±0.02 and 0.676±0.06 %) respectively compared to T1(0.525±0.12%), accessible values that make biological significance limited Uterus (0.073±0.03, 0.072±0.04, 0.073±0.02 and 0.074±0.05%) respectively, compared to T1 (0.086±0.01%), adrenal glands Significant weight increase (0.035±0.03, 0.039±0.02, 0.038±0.02 and 0.036±0.01%) respectively compared to T1 0.042±0.01 .Spleen Significant weight increase (0.079±0.03, 0.076±0.01, 0.075±0.03 and 0.071±0.02%) respectively compared to T1 (0.084±0.04%).

Table 2 . Effect of Fenugreek and Soybean Extract on the Weights of Internal Organs of Experimental Rats

Mean ±SD						
Treatments	Heart	Liver	Uterus	Lungs	adrenal glands	spleen
T1	0.205±0.03^{a}	1.587±0.28^{a}	0.086±0.01^{a}	0.525±0.12^{a}	0.042±0.01^{ab}	0.084±0.04^{a}
T2	0.225±0.06^{b}	2.234±.0.24^{ab}	0.073±0.03^{b}	0.623±0.09^{b}	0.035±0.03^{a}	0.079±0.03^{b}
T3	0.265±0.07^{a}	2.295±0.23^{b}	0.072±0.04^{ab}	0.686±0.08^{ab}	0.039±0.02^{b}	0.076±0.01^{ab}
T4	0.233±0.05^{ab}	2.285±0.19^{a}	0.073±0.02^{a}	0.654±0.08^{b}	0.038±0.02^{ab}	0.075±0.03^{b}
T5	0.274±0.06^{a}	2.294±0.17^{b}	0.074±0.05^{b}	0.676±0.06^{ab}	0.036±0.01^{a}	0.071±0.02^{a}
**P≤ 0.01	**	**	**	**	**	**

** indicate to the significance effect
Different letters indicate the presence of significant differences.

The results in the table (3) indicate a significant increase in sex hormones Estrogen, FSH,LH, TSH and Prolactin) in the treated groups T2,T3,T4 and T5 for estrogen show significant increase which had an average (118.34±0.76, 121.55, 119.45±0.64 and 123.87±0.43µg/mL) compared to the control group T1 (115.34±0.32 µg/mL). As for the FSH hormone, based on the results, a significant increase was observed in the treatments, with an average of (101.34±.0.37, 102. 43±0.81, 102.13 ±0.75 and 103.64 ±0.37µg/mL) respectively compared to the control group T1 (104.65±0.45µg/mL), LH shows significant increase which had an average (102.56±0.46, 102.97±0.56, 103.98±0.57 and 103.98±0.57µg/mL) respectively compared to the control group T1 (101.65±0.34µg/mL) , show significant increase in TSH which had an average (0.005±0.65, 0.007±0.45, 0.006±0.59 and 0.008±0.76µg/mL) respectively,

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AL ZAHRAA UNIVERSITY JOURNAL
Health and Medical Science

Al-Zahraa Journal for Health and Medical Sciences 2025; 3:1-10 **Vol. 3, No.4, 2025**
www.zjhms.alzahraa.edu.iq

compared to the control group T1 0.003±0.35µg/mL . Prolactin show significant increase which had an average (5.27±0.06, 5.38±0.15, 5.33±0.19 and 5.64±0.18 µg/mL) respectively compared to the control group T1 4.34±0.04µg/mL.

Table 3. Effect of Fenugreek and Soybean Extract on the sex hormones of Experimental Rats

Mean ±SD					
Treatments	Estrogen µg/mL	FSH µg/mL	LH µg/mL	TSH µg/mL	Prolactin µg/mL
T1	115.34±0.32^{a}	104.65±0.45^{a}	101.65±0.34^{a}	0.003±0.35^{a}	4.34±0.04^{ab}
T2	118.34±0.76^{b}	101.34±.0.37^{ab}	102.56±0.46^{b}	0.005±0.65^{b}	5.27±0.06^{a}
T3	121.55±0.45^{a}	102. 43±0.81^{b}	102.97±0.56^{ab}	0.007±0.45^{ab}	5.38±0.15^{b}
T4	119.45±0.64^{ab}	102.13 ±0.75^{a}	103.25±0.76^{a}	0.006±0.59^{b}	5.33±0.19^{ab}
T5	123.87±0.43^{a}	103.64 ±0.37^{b}	103.98±0.57^{b}	0.008±0.76^{ab}	5.64±0.18^{a}
**P≤ 0.01	**	**	**	**	**

** indicate to the significance effect
Different letters indicate the presence of significant differences.

Discussion

Ovariectomy is a common experimental model used to simulate the condition of menopause in humans, which is accompanied by changes in sex hormone levels, particularly estrogen, and widespread physiological effects including the heart, lungs, liver, spleen, and adrenal glands. In this context, the current research aims to evaluate the impact of herbal supplements such as soybeans and fenugreek (*Trigonella foenum-graecum*) as natural sources of flavonoid with estrogen-like activity, in order to compensate for estrogen loss and achieve hormonal and physiological balance [18]. Previous studies have shown that soybeans contain large amounts of isoflavones such as genistein and daidzein, which bind to estrogen receptors and affect the gene expression of target tissues [19]. When administered to ovariectomized mice, the results showed a significant increase in uterine weight compared to the ovariectomized group only, indicating an estrogenic effect of the bean that compensates for the absence of endogenous estrogen. An increase in liver and spleen weights was also observed, which may be associated

AL ZAHRAA UNIVERSITY JOURNAL
Health and Medical Science

Al-Zahraa Journal for Health and Medical Sciences 2025; 3:1-10 **Vol. 3, No.4, 2025**
www.zjhms.alzahraa.edu.iq

with the effect of flavonoids on protein and fat metabolism [20]. As for fenugreek, it is rich in saponins and flavonoids, which have regulatory effects on the endocrine glands. Studies have shown that fenugreek leads to an increase in estradiol hormone concentration in ovariectomized rats, and also increases the weights of the uterus and remaining ovary, indicating a partial restoration of hormonal activity [21]. At the same time, the increased adrenal gland weight in fenugreek-treated mice may be attributed to the activation of the HPA axis as a result of attempting to compensate for the hormonal deficiency by secreting corticosteroids Regarding changes in sex hormones [22]. It is observed that both soy and fenugreek contributed to raising estradiol levels compared to the ovariectomized group, although the increase was more pronounced in the soy group, confirming the effectiveness of isoflavones in this regard [23].On the other hand, the change in liver and spleen weights could also be a result of the antioxidant effect of plant compounds, as phytoestrogen is known for its ability to regulate oxidative stress, thus protecting organs from potential atrophy after removal [24,25].

Conclusion

Soybeans and fenugreek possess estrogen-like properties that affect the relative weights of internal organs and regulate sex hormones in ovariectomized mice. These effects suggest the potential for using these plants as natural alternatives to hormone replacement therapy, but future studies should focus on histologist and clinical evaluation to confirm the long-term efficacy and safety of these compounds.

Acknowledgments

I thank the University of Babylon for giving me the opportunity to complete the laboratory tests and fulfil the research requirements.

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AL ZAHRAA UNIVERSITY JOURNAL
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Al-Zahraa Journal for Health and Medical Sciences 2025; **3**:**1-10** **Vol. 3, No.4, 2025**
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