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The Effect of Fenugreek (*Trigonella foenum-graecum*) Seed and 17-β Estradiol on Serum Apelin, Glucose, Lipids, and Insulin in Ovariectomized Rats

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Background: Menopause, a natural phenomenon, is defined by the fall of ovarian hormones mainly estrogens causing major problems such as insulin resistance. Fenugreek (*Trigonella foenum-graecum*) is known to have some useful properties such as insulin sensitizing effect. Apelin is an adipokine, which has several roles such as regulation of insulin secretion.
Objectives: The objective of the present study was to evaluate the effect of fenugreek seed and 17-β estradiol on serum Apelin along with glucose, lipids and insulin in ovariectomized rats.
Materials and Methods: Forty-nine adult female Wistar rats were randomly divided to seven groups: normal control, ovariectomized control, ovariectomized treated with ethanolic and hexanic extract of fenugreek seed (50 and 150 mg/kg/daily for each), and ovariectomized treated with 17-β estradiol (10 µg/kg/daily) for 42 days. Serum Apelin, glucose, lipids and insulin were measured.
Results: Serum Apelin, glucose, lipids and insulin significantly increased in ovariectomized controls in comparison with normal controls ($P < 0.05$). Serum glucose, lipids and insulin in ovariectomized rats treated with fenugreek seed extract and 17-β estradiol were remarkably lower than ovariectomized controls ($P < 0.05$). Furthermore, 17-β estradiol caused a significant decrease ($P < 0.05$) in serum Apelin in ovariectomized rats.
Conclusions: It appears that fenugreek seed might be effective against hyperglycemia, hyperlipidemia and insulin resistance in ovariectomized rats without impact on serum Apelin. Furthermore, 17-β estradiol could have similar effects along with possible inhibitory effects on serum Apelin. The complicated role of Apelin in menopause needs to be further explored.

Keywords: Apelin-13; Trigonella Foenum-Graecum; 17 Beta-Estradiol; Ovariectomized; Insulin; Lipids

1. Background

Menopause is a natural event characterized by follicular atresia and fall in the secretion of ovarian hormones especially estrogens (1). This condition leads to physiological and biochemical changes in affected females such as increased blood pressure, insulin resistance, and dyslipidemia (2). These changes are associated with increased risk of metabolic syndrome and some chronic diseases such as cardiovascular diseases (3).

Menopause usually occurs in middle-aged women; between 45 and 55 years (4). According to a survey, the number of perimenopausal or postmenopausal females would be about 1.2 billion by the year 2030 (5). Since menopause mostly affects females for about a third of their lives, providing a new effective approach for the prevention of menopause complications or decreasing the risk of associated diseases could be helpful in enhancing health and quality of life among females.

Apelin is an adipokine with a number of active molecular forms, which is expressed in different tissues such as adipocytes (6). Apelin has several important roles such as regulation of insulin secretion through autocrine/paracrine stimulation (7), and inhibition of lipolysis in adipocytes (8). The role of Apelin in lowering blood glucose and insulin sensitivity has been suggested by previous studies (9). Assessment of the possible interaction between Apelin signaling, lipid metabolism, and beta cell function could be worthy of investigation (10). Fenugreek (*Trigonella foenum-graecum*), as an annual medicinal plant, is a member of the Fabaceae family. Fenugreek seed has been used to promote health and as a choice for the management of various human diseases such as diabetes, dyslipidemia and obesity (11, 12). The insulin sensitizing action of fenugreek has been proposed by earlier studies (13).

2. Objectives

For the first time, the present study aimed to evaluate the effect of fenugreek seed and 17-β estradiol on the serum level of Apelin along with glucose, lipids and insulin in ovariectomized (OVX) rats as a model of menopause.

3. Materials and Methods

3.1. Animals

Forty-nine adult female Wistar rats weighing 190 - 220 g, obtained from the Institute of Pasteur, were used. Ethical clearance from this experimental study was approved by the ethical committee at Guilan university of medical sciences and the experiments were done in agreement with internationally accepted principles for laboratory animal use and cares as found in US guidelines (NIH publication #85-23, revised in 1985). Animals were housed in a room maintained at 21 to 23°C with a 12 hours light/dark cycle. All rats had free access to food and water.

3.2. Study Design

After an adaptation period of two weeks, all rats were randomly divided to seven groups (seven rats in each group) as follows:

1) Normal control; receiving daily saline.

The remaining six groups were allocated to undergo bilateral ovariectomy as follows (14):

2) OVX control; receiving daily saline.

3) OVX treated daily with 50 mg/kg of ethanolic extract of fenugreek seed (EEFS).

4) OVX treated daily with 150 mg/kg of EEFS.

5) OVX treated daily with 50 mg/kg of hexanic extract of fenugreek seed (HEFS).

6) OVX treated daily with 150 mg/kg of HEFS.

7) OVX treated daily with 10 µg/kg of estradiol.

Treatment began one day after ovariectomy by Intraperitoneal (IP) injection and all rats were maintained for 42 days on their respective treatments. The weight and fasting blood sugar (FBS) of all rats were measured at the beginning of the research and on the 42^{nd} day of treatment. Blood glucose was measured by a glucometer (Accu chek, Roche, Germany). After 42 days, fasting whole blood samples were obtained from the vein of the tail. The serum of blood samples was separated immediately. The resulting sera were stored at -20°C. The serum levels of Apelin-13 and insulin were determined by using enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Crystal Day Biotech Co., China). The serum levels of cholesterol, triglyceride (TG), low-density Lipoprotein (LDL) and high-density lipoprotein (HDL) were determined by using commercial kits (Pars Azmoon, Iran).

3.3. Plant Material and Extraction

The seed of fenugreek was supplied by a grocery store in Rasht (Guilan province) during years 2013 and 2014, and the species was confirmed by the herbarium department of the agricultural research center at Guilan university. The voucher specimen 11411 was deposited in the Herbarium of the department of botany, faculty of agriculture, Guilan university. Preparation of the ethanolic and hexanic extracts was performed as previously described (11).

3.4. Chemicals

The 17-β estradiol was obtained from Sigma-Aldrich (GmbH, Germany). Ethanol and hexane were purchased from Merck (Germany).

3.5. Statistical Analysis

Data are presented as means ± standard error of the Mean (SEM) and inter-group comparisons were made using the one-way analysis of variance (ANOVA) followed by post hoc Tukey's test. P values of < 0.05 were considered statistically significant. Analysis was performed using the SPSS software version 16.

4. Results

The average body weight of OVX control remarkably increased in comparison to normal control rats ($P < 0.05$). Treatment of the OVX rats with HEFS, EEFS and estradiol resulted in a decrease in body weight (Table 1).

The serum level of cholesterol, TG and LDL remarkably increased while serum HDL significantly decreased in OVX control when compared to normal control rats ($P < 0.05$). However, HEFS, EEFS, and estradiol caused a remarkable decrease in serum level of cholesterol and TG in OVX rats ($P < 0.05$). Following 42 days of treatment with HEFS at a dose of 150 mg/kg/day, EEFS at a dose of 50 mg/kg/day and estradiol, the serum LDL was reduced. Only 17-β estradiol caused remarkable increase in the serum level of HDL (Table 1).

The serum glucose and insulin significantly increased in OVX controls in comparison with normal control rats ($P < 0.05$) (Table 2).

The HEFS, EEFS, and estradiol rats showed a remarkable decrease in the serum levels of glucose and insulin when compared to OVX control rats ($P < 0.05$)(Table 2).

The serum level of Apelin was significantly higher in OVX control rats compared with normal control rats ($P < 0.05$). There were no significant differences in the level of serum Apelin between OVX rats treated with HEFS and EEFS as compared with OVX control rats. The 17-β estradiol caused a significant decrease ($P < 0.05$) in serum Apelin in ovariectomized rats ($P < 0.05$)(Table 2).

Table 1. Effect of Fenugreek Seed and 17-β Estradiol on Body Weight, Serum Cholesterol, Low-Density Lipoprotein, High Density Lipoprotein, and Triglyceride After Six Weeks in Normal Control and Ovariectomized Rats [a,b]

Groups	Body weight, g	Cholesterol, mg/dl	LDL, mg/dl	HDL, mg/dl	Triglyceride, mg/dl
Normal control	233 ± 2 [c]	85 ± 2.6	51.7 ± 4.3	24 ± 1.92	60.1 ± 2.4
OVX control	247 ± 3 [d]	122 ± 9 [c]	83 ± 5.1 [d]	12 ± 2.64 [d]	115 ± 1.1 [d]
OVX + HEFS (50 mg/kg)	235 ± 3 [c]	80 ± 3 [c]	75.1 ± 4 [d]	18 ± 1	84.1 ± 3.9 [c,d]
OVX + HEFS (150 mg/kg)	233 ± 2 [c]	86 ± 4 [c]	60.2 ± 1.6 [c]	20 ± 2.31	71.5 ± 2.9 [c]
OVX + EEFS (50 mg/kg)	229 ± 3 [c]	93 ± 4 [c]	74.4 ± 3.1 [c]	14 ± 1.31	88.2 ± 4.2 [c,d]
OVX + EEFS (150 mg/kg)	231 ± 2 [c]	73 ± 5 [c]	69.8 ± 3.5 [d]	16 ± 2.41	79 ± 3.9 [c,d]
OVX + estradiol (10 µg/kg)	231 ± 1 [c]	100 ± 3 [c,d]	54.2 ± 5.1 [c]	29 ± 1.81 [c]	73.5 ± 4.2 [c]

[a] Values are given as mean ± SEM.
[b] Abbreviations: EEFS: ethanolic extract of fenugreek seed, HDL: high-density lipoprotein, HEFS: hexanic extract of fenugreek seed, LDL: low-density lipoprotein, OVX: ovariectomized.
[c] $P < 0.05$ by comparison with ovariectomized control rats.
[d] $P < 0.05$ by comparison with normal control rats.

Table 2. Effect of Fenugreek Seed and 17-β Estradiol on Serum Glucose, Insulin and Apelin After Six Weeks in Normal Control and Ovariectomized Rats [a,b]

Groups	Glucose, mg/dl	Insulin, U/dl	Apelin, µg/dl
Normal control	97 ± 2.04	489 ± 1.92	75.1 ± 1.5
OVX control	158 ± 1.95 [c]	700 ± 1.64 [c]	88.8 ± 1.9 [c]
OVX + HEFS (50 mg/kg)	132 ± 2.18 [c,d]	627 ± 1.21 [c,d]	86.2 ± 3.5
OVX + HEFS (150 mg/kg)	128 ± 3.18 [c,d]	600 ± 1.81 [c,d]	80 ± 1.9
OVX + EEFS (50 mg/kg)	135 ± 3.38 [c,d]	649 ± 1.01 [c,d]	80.7 ± 2.4
OVX + EEFS (150 mg/kg)	129 ± 2.61 [c,d]	616 ± 0.81 [c,d]	81.4 ± 4
OVX + estradiol (10 µg/kg)	134 ± 6.18 [d]	546 ± 2.81 [c,d]	72.1 ± 0.9 [d]

[a] Values are given as mean ± SEM.
[b] Abbreviations: EEFS: ethanolic extract of fenugreek seed, HEFS: hexanic extract of fenugreek seed, OVX: ovariectomized.
[c] $P < 0.05$ by comparison with normal control rats.
[d] $P < 0.05$ by comparison with ovariectomized control rats.

5. Discussion

The present study was undertaken to find out the effect of fenugreek seed extract and 17-β estradiol on serum Apelin together with some of menopause related parameters such as serum lipids, glucose, and insulin level. Mean level of serum cholesterol, TG, and LDL in OVX control were significantly higher than those of normal control rats, whereas there was a statistically significant decrease of HDL in OVX control in comparison to normal control rats. These findings were similar to that found by the study of Goss et al. (15) in which ovariectomy caused a considerable increase in serum levels of cholesterol and LDL in comparison to normal rats. In another study (16), OVX rats showed no significant changes in TG, cholesterol and HDL levels compared with normal rats. Menopausal statement due to reduced estrogen secretion is associated with hyperlipidemia, which is defined by increased level of cholesterol, TG, LDL, and decreased level of HDL (17). It has been demonstrated that estrogen has a physiological role in lowering cholesterol through up-regulation of hepatic LDL receptor, consequently leading to an enhanced clearance of blood cholesterol (18).

The administration of HEFS and EEFS to OVX rats decreased serum cholesterol and TG compared with ovariectomized control rats. These findings were consistent with those of Sharma et al. (19), where fenugreek seed led to a significant decrease of serum cholesterol and TG. Hypolipidemic effect of fenugreek seed was partly related to the decrease in fat accumulation, up-regulation of LDL receptor and decrease in insulin, which could decrease the activity of pyruvate dehydrogenase and acetyl-CoA carboxylase involved in lipogenesis as well as activity of 3-hydroxy-3-methylglutaryl-CoA reductase involved in cholesterol synthesis (20, 21). The role of fenugreek seed in the management of dyslipidemia and decreased risk of cardiovascular disease during the post menopause period was proposed (22). As compared with normal control rats, ovariectomy caused a considerable increase in the serum level of glucose and insulin. In the study of Mattace Raso et al. (23), no significant differences in serum glucose and insulin between OVX and normal rats were found. Shinmura et al. reported no statistical difference in serum glucose between OVX and normal rats (24). In contrast to the present study, the study of Ahmadi et al. (25) showed significantly decreased serum insulin in OVX rats as compared with control animals. In line with the present study, Posa et al. demonstrated that serum insulin significantly increased in OVX rats (26), which could be regarded as a marker for developing an insulin-resistant state. In addition, an increase in serum insulin may be due to the hyperactivity of the pancreatic islet cells to serum glucose (27). Serum glucose and insulin were considerably lower in OVX rats treated with HEFS and EEFS compared with OVX control animals. Zahedi et al. (28) revealed that plasma glucose in diabetic rats treated with fenugreek seed decreased compared to levels before treatment. It has been suggested that fenugreek seed causes a hypoglycemic effect through several mechanisms such as elevated glucose-stimulated insulin secretion by pancreatic beta cells (29) and activation of insulin receptor by tyrosine phosphorylation (30). It is likely that supplementary therapy of fenugreek seed extract could be beneficial for the improvement of glycemic control and insulin resistance after menopause.

In the present study, there was a significant decrease in the serum level of glucose and insulin in OVX rats exposed to estradiol when compared to OVX control rats. However, Ahmadi and Oryan showed that estradiol was as an enhancer of serum insulin (25). You et al. identified that estradiol in OVX rats remarkably reduced serum glucose and insulin after six weeks (31). In addition, Li et al. showed that estrogen therapy might decrease blood insulin and glucose in menopausal females (32). It appears that estrogen by modulating insulin sensitivity or reducing insulin resistance (33) and lowering lipids (34) could be protective against metabolic abnormalities such as coronary artery disease (CAD) in menopausal females (31).

Ovariectomy led to a remarkable increase in serum Apelin in comparison to normal conditions. Boucher et al. showed that serum level of Apelin was considerably higher in moderately obese humans compared with the control group (6). Increased glucose in OVX rats may cause hyperactivity of pancreatic beta cells and increased insulin (27) and consequently increased Apelin (6). On the other hand, it has been suggested that Apelin has a role in decreasing the amount of glucose via enhanced glucose utilization in muscles (35), and in inhibition of insulin secretion through induction of phosphatidylinositol 3-kinase and phosphodiesterase 3B (36). Therefore, increased serum Apelin in OVX animals appears to act partially as a regulatory mechanism; nevertheless, other possible theories need to be evaluated.

Serum level of Apelin was similar in OVX rats exposed to HEFS or EEFS and OVX control rats. Regarding the potential effects of fenugreek seed on lipids, glucose, and insulin (22, 37, 38), it appears that these effects may not be mediated by serum Apelin. However, more relevant investigations are required to verify this theory.

In OVX animals, serum Apelin remarkably decreased during estradiol treatment compared with the control group. Considering the fact that estradiol has a lowering effect on serum insulin (31, 32) and insulin can elevate Apelin production (6), it appears that reduced serum Apelin under estradiol treatment in OVX rats can be, to some extent, due to decreased insulin. Because of the mitogenic role of Apelin in the epithelial (39) and endothelial cells (40), this effect might be considered in agreement with the beneficial effects of hormone therapy in menopausal females (32). Nevertheless, the complicated role of Apelin during menopause needs to be further explored.

Overall, it appears that fenugreek seed might be effective against hyperglycemia, hyperlipidemia and insulin resistance in OVX rats without impact on serum Apelin. The 17-β estradiol could have similar effects along with possible inhibitory effects on serum Apelin. Since Apelin may affect metabolism via several different mechanisms, more related investigations are needed to determine the potential effect of Apelin on menopausal conditions.

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Authors' Contributions

1- Study concept and design: Mahmood Abedinzade and Korosh Khanaki. 2- Acquisition of data: Sima Nasri. 3- Analysis and interpretation of data: Masome Jamal Omidi. 4- Drafting of the manuscript: Korosh Khanaki. 5- Critical revision of the manuscript for important intellectual content: Korosh Khanaki. 6- Statistical analysis: Bizhan Porramezan. 7- Administrative, technical and material support: Mahmood Abedinzade. 8- Study supervision: Mahmood Abedinzade.

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