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ORIGINAL ARTICLE

Effect of fennel essential oil and flaxseed oil on blood parameters, insulin resistance, and histological structure of ovaries in rats suffered polycystic ovary syndrome

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Abstract

Polycystic ovary syndrome (PCOS) is known as a reproductive disorder in mammalian females. Medical plants are known from ancient times and herbal therapy is common in many countries for preventing and treating different diseases or disorders. The effect of fennel essential oil and/or flaxseed oil on body weight, blood parameters, and histological structure of ovaries and pancreas in rat suffered PCOS, was investigated. Thirty adult Wistar female rats (BW=260±20 g) that 24 of them suffered by PCOS by a single intra-muscular (IM) injection of 4 mg/kg estradiol-valerate (EV) were allocated to the treatments. Fennel essential oil (100 mg/kg BW/day) and flaxseed oil (429 mg/kg BW/day or 240 mg ω-3/kg BW/day) were fed using oral gavage in a 50-day period after PCOS induction. Serum concentration of progesterone, testosterone, insulin, and glucose, and also HOMA-IR, HOMA-β, and QUICKI indices were affected ($P< 0.05$) by the treatments. Fennel essential oil increased ($P <0.05$) blood insulin hormone compared to PCOS and control groups. Our results showed that induction of PCOS in rats increased ($P <0.05$) fasting blood glucose, but consumption fennel essential oil or flaxseed oil decreased its concentration to the normal threshold. Results of this study showed that feeding fennel essential oil or flaxseed oil due to their anti-diabetic effects and anti-oxidative properties improved the body mass index, serum glucose, insulin and progesterone concentration, and HOMA-IR, HOMA-β, and QUICKI indices in rats suffered PCOS.

Keywords Polycystic ovary syndrome · Ovary · Flaxseed oil · Fennel essential oil · HOMA-IR · HOMA-β

Introduction

Polycystic ovary syndrome (PCOS) is known as a reproductive disorder in mammalian females which leads to anovulation and infertility (Jovanovska-Mishevska et al. 2016; Qu et al. 2017). It is well demonstrated that many metabolic disorders including insulin resistance (IR), abnormal glucose tolerance (AGT), dyslipidemia, type2 diabetes mellitus (DM2), and hypertension are all associated with PCOS (Diamanti-Kandarakis and Dunaif 2012; Agapova

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et al. 2014). The condition in which normal quantities of insulin are inadequate for picking glucose up by body cells, is called IR (Le Roith and Zick 2001). Jovanovska-Mishevska et al. (2016) reported that IR has an important role in reproductive disorder pathogenesis.

Medical plants are known from ancient times, and herbal therapy is common in many countries for preventing and treating different diseases or disorders (Aboelsoud 2010). Fennel (*Foeniculum vulgare mill*) is known as a medicinal plant containing many effective compounds. The main content (50–80%) of fennel essential oil is trans-anethole. Moreover, its essential oil has some phenolic components such as hydroxycinnamic acids, tannins, and flavonoids (Carlsen et al. 2010).

Flaxseed oil has about 39% α-linolenic acid and is therefore rich in omega-3 fatty acid. Flaxseed oil has many health benefits and may reduce the risk of diabetes (Goyal et al. 2014). Ouladsahebmadarek et al. (2014) revealed that daily feeding omega-3 (240 mg/kg BW) reduced blood glucose and improved insulin resistance condition in rats suffered PCOS.

Our hypothesis was that essential oil of fennel and flaxseed oil could have positive effects on preventing insulin resistance in PCOS rats. So, the aim of study was to investigate the effects of fennel essential oil and/or flaxseed oil on body weight, blood parameters, and histological structure of ovaries and pancreas in rat suffered PCOS induced by estradiol-valerate.

Material and method

Animal and treatments

This study was conducted in Animal House of Isfahan University of Technology (Isfahan, Iran). The animal care advisory committee of the Isfahan University of Technology approved all experimental procedures, and all institutional and national guidelines for the care and use of laboratory animals were followed (Iranian Council of Animal Care 1995). Thirty adult Wistar female rats (BW=260±20 g) were kindly provided from Razi Vaccine and Serum Research Institute (Karaj, Iran). The animals were maintained under standard animal housing condition with a temperature of 20–23 °C, 12 h light/dark cycle (light on 8 a.m.) and given ad libitum water and standard chow prepared from Behparvar Co. (Tehran, Iran).

All rats had a 10-day adaptation period before the experiment; then, their estrous cycles were examined by vaginal smear at 12:00 to 15:00 h and the rats with abnormal cycle were omitted. The animals were assigned to one of 5 treatments in a completely randomized design with 6 replicates: 1—no supplementation group (control; CON); 2—polycystic syndrome group (PCOS); 3—PCOS rats fed fennel essential oil (FE); 4—PCOS rats fed flaxseed oil (FO); and 5—PCOS rats fed fennel essential oil + flaxseed oil (FE+FO). Fennel essential oil and flaxseed oil were provided from Barij Essence Pharmaceutical Co. (Kashan, Iran). Fennel essential oil (100 mg/kg BW/day) and flaxseed oil (429 mg/kg BW/day or 240 mg ω-3/kg BW/day) (Wang et al. 2020) were fed using oral gavage in a 50-day period after PCOS induction. The fatty acid composition of flaxseed oil was identified by gas chromatography (C16:0=6%, C18:0= 4%, C18:1=15%, C18:2 (ω6)=18%, C18:3 (ω3)=56%).

PCOS induction in rats

A single intra-muscular (IM) injection of 4 mg/kg estradiol-valerate (EV) was done for inducing PCOS in rats. After 30 days, PCOS condition was confirmed by observing the cornified cells in vaginal smears for at least two consecutive estrous cycles.

Body weight measurement

At the time of PCOS confirmation and at the end of study, all rats were weighted after 12 h fasting. Moreover, body length (nose to anus length) was measured at the final day of study and the body mass index (BMI) was calculated using the following equation (Novelli et al. 2007):

$$\mathrm{BMI} = \frac{\text{body wieght (gr)}}{\text{body lenght}^2(\mathrm{cm}^2)}$$

Tissue and serum sampling

At the end of experiment and after 12 h fasting, all rats were anesthetized and their blood samples individually collected by cardiac puncture technique. Then, blood serum was separated by centrifugation at 2500 rpm for 20 min and serum samples were stored at −60 °C before future assays. The ovaries and pancreas were separated from the animal body and then carefully trimmed of adhering fat and connective tissues. These organs were immersed in 10% formaldehyde and transferred to Isfahan University of Medical Sciences for histological and pathological examinations.

Biochemical assays

Concentration of blood glucose (GOD kit; Pars Azmun Co., Karaj, Iran) and insulin (Rat ELISA Kit; Biotech Co., Shanghai, China) were determined by photometric method. In addition, serum estrogen (K208I ELISA Kit; Xema Co Ltd., Moscow, Russia), progesterone (P4) (ELISA Kit; IDEAL Tashkhis Atieh co., Tehran, Iran), and testosterone

(T) (Testosterone AccuBind ELISA Kit; Monobind Inc, Lake Forest, USA) were measured.

Histological assessments of ovaries and pancreas

Formalin-fixed and paraffin-embedded ovaries and pancreas were used for histological studies. Two sections of ovaries were taken from the highest diameter, mounted on a glass slide, stained with hematoxylin and eosin and then follicle count was done as defined by Amini et al. (2016).

Four sections from pancreas tissue blocks were used to study the number of Langerhans islets. Then, the mean values were considered the number of islets in each pancreas. Two diameters of each islet were evaluated with micrometer lens, and the area of islets was calculated based on the following equation (Parsons et al. 1995):

Islet area = 3.141(major axis/2) (minor axis/2)

HOMA-IR, HOMA-β, and QUICKI assessments

Homeostatic model assessment (HOMA) was used as the method for assessing insulin resistance (HOMA-IR) and β-cell function (HOMA-β). Also, the quantitative insulin sensitivity check index (QUICKI) was used for determining insulin sensitivity (Lee et al. 2011):

HOMA-IR= (fasting glucose × fasting insulin/22.5)

HOMA-β = (20 × fasting insulin) / (fasting glucose − 3.5)

QUICKI = 1/ [log (fasting insulin) + log (fasting glucose)]

Statistical analyses

Data were analyzed using a completely randomized design with the GLM procedure of SAS (SAS Institute Inc., Cary, NC). The following model was used:

$$Y_{ij} = \mu + a_{i(j)} + T_j + \beta(xi{-}x) + e_{ij}$$

where Y_{ij} is the dependent variable; μ is the overall mean; $a_{i(j)}$ is the random effect of rat; T_j is fixed effect of treatment; β(xi-x) is the effect of covariate; and e_{ij} is the random residual effect.

The treatment means were compared using a Tukey-Kramer test. Significance level was declared at $P \leq 0.05$, and a tendency toward significance was considered at $0.05 < P \leq 0.1$.

Result and discussion

Body weight change and body mass index

Effects of essential oil and flaxseed oil on rat body weight and BMI are presented in Table 1. Results showed that the treatments had no effect on body weight change. However, BMI increased ($P<0.05$) in PCOS rats compared to control group. The other groups had intermediate BMI values in this regard. The BMI is a tool for monitoring overweight and obesity (Murguía-Romero et al. 2012). It is well known that body fat has negative synergistic effect on insulin sensitivity in patients suffered PCOS (Zweig et al. 2010). Oner and Mudderis (2013) reported that feeding omega-3 in PCOS women decreased their BMI after 2 months. This discrepancy may be related to the supplemental dosage and/or duration of the feeding.

Blood parameters and insulin resistance indices

Data of serum metabolites, and HOMA-IR, HOMA-β, and QUICKI indices are presented in Table 2. Our results showed that PCOS induction and feeding FE and/or FO had no significant effect on serum estrogen concentration. But, the other serum parameters such as progesterone, testosterone, insulin, and glucose were affected ($P< 0.05$) by the treatments. Hazzard et al. (1984) defined that besides insulin resistance, the lipid metabolism in females suffered PCOS could be altered by ovarian or adrenal excretion of sex steroid hormones.

Table 1 Effect of fennel essential oil and flaxseed oil on body weight change and body mass index (BMI) in female Wistar rats suffered PCOS

Trait	Experimental groups[1]				
	CON	PCOS	FE	FO	FE+FO
Body weight change (g)	5.54±7.79	16.58±7.06	12.37±7.01	9.27±7.02	11.33±7.07
Body mass index[3]	0.56^{b}±0.16	0.61^{a}±0.16	0.58^{ab}±0.16	0.58^{ab}±0.16	0.59^{ab}±0.16

[1] CON, no supplementation rats; PCOS, PCOS rats; FE, PCOS rats fed fennel essential oil; FO, PCOS rats fed flaxseed oil; and FE+FO, PCOS rats fed fennel essential and flaxseed oil. The fennel essential oil (100 mg/kg BW/day) and flaxseed oil (429 mg/kg BW/day flaxseed oil which was equal 240 mg ω-3/kg BW/day) were gavaged 7 days past PCO induction for a 50-day period.

[2] The standard error of the mean

[3] Body weight (g)/length^2 (cm^2)

[a,b] Values within a row with different superscripts differ significantly ($P<0.05$)

Table 2 Effect of fennel essential oil and flaxseed oil on serum parameters, and HOMA-IR, HOMA-β, and Quicki indices in female Wistar rats suffered PCOS

Trait	Experimental groups				
	CON	PCOS	FE	FO	FE+FO
Estrogen (ng/ml)	0.049±0.006	0.038±0.006	0.045±0.005	0.041±0.005	0.047±0.007
Progesterone (ng/ml)	18.96^{a}±2.33	6.4^{b}±2.33	18.98^{a}±1.64	16.2^{a}±2.33	13.96^{a}±1.8
Testosterone (ng/ml)	0.27^{bc}±0.034	0.39^{a}±0.04	0.22^{c}±0.035	0.32^{ab}±0.033	0.21^{c}±0.03
Insulin (mIU/ml)	7.98^{b}±0.88	8.18^{b}±0.80	11.25^{a}±0.80	10.35^{ab}±0.80	9.66^{ab}±0.80
Glucose (mg/dl)	133.3^{b}±15.07	188.7^{a}±18.46	125.6^{b}±16.51	116^{b}±16.51	206^{a}±16.51
HOMA-IR[3]	2.52^{c}±0.43	3.98^{ab}±0.49	3.42^{bc}±0.43	2.88^{bc}±0.43	5.16^{a}±0.43
HOMA-β[4]	56.38^{ab}±15.56	28.76^{bc}±17.40	70.94^{ab}±15.56	94.39^{a}±15.56	26.7^{c}±15.56
Quicki[5]	0.166^{a}±0.001	0.160^{bc}±0.001	0.163^{ab}±0.001	0.165^{a}±0.001	0.158^{c}±0.001

[1] CON, no supplementation rats; PCOS, PCOS rats; FE, PCOS rats fed fennel essential oil; FO, PCOS rats fed flaxseed oil, and FE+FO, PCOS rats fed fennel essential and flaxseed oil. The fennel essential oil (100 mg/kg BW/day) and flaxseed oil (429 mg/kg BW/day flaxseed oil which was equal 240 mg ω-3/kg BW/day) were gavaged 7 days past PCO induction for a 50 d period.

[2] The standard error of the mean

[3] Homeostatic model assessment for insulin resistance = fasting glucose × fasting insulin/22.5

[4] Homeostatic model assessment for β-cell function = (20 × fasting insulin) / (fasting glucose − 3.5)

[5] Quantitative insulin sensitivity check index = 1/ [log (fasting insulin) + log (fasting glucose)]

[a-c] Values within a row with different superscripts differ significantly (P <0.05)

The PCOS group had the lowest (P< 0.05) serum progesterone. On the other hand, feeding fennel essential oil and/or flaxseed oil improved serum progesterone in PCOS rats. Therefore, there was no difference between FE, FO, and FE+FO groups with control group from this respect. It seems that fennel essential oil and/or flaxseed oil has been able to improve the conversion of cholesterol to progesterone in theca cells.

The PCOS group showed highest (P <0.05) serum testosterone. However, fennel essential oil reduced the concentration of this hormone to the level of the control group. Although the exact mechanism of PCOS pathogenesis is unclear, the syndrome may be associated with hormonal abnormalities including decreased progesterone and increased testosterone concentrations (Holte et al. 1994). It has been reported that consumption of plant extracts with phytoestrogens content decreases testosterone levels by the negative feedback effects on LH secretion (Malaivijitnond et al. 2004).

In the present study, insulin concentration was affected by experimental treatments, so that fennel essential oil increased (P <0.05) blood insulin hormone compared to PCOS and control groups. On the other hand, our results showed that

Table 3 Effect of fennel essential oil and flaxseed oil on ovarian weight and follicle count in female Wistar rats suffered PCOS

Trait	Experimental groups[1]				
	CON	PCOS	FE	FO	FE+FO
Ovarian weight (mg)					
Right ovary	48.8^{ab}±4.95	62.2^{a}±4.95	40.6^{b}±4.95	43.6^{b}±4.95	35.6^{b}±4.95
Left ovary	52.7^{a}±5.70	42.4^{ab}±5.70	46.2^{ab}±5.70	52^{a}±5.70	35.4^{b}±5.70
Mean	75.1^{ab}±6.34	83.4^{a}±6.34	63.7^{bc}±6.34	69.6^{ab}±6.34	53.3^{c}±6.34
Follicle count (*n*)					
Primary follicle	1.4^{ab}±0.41	0.4^{b}±0.41	2^{a}±0.53	1.2^{ab}±0.41	1^{ab}±0.41
Secondary follicle	5.6±0.79	1.4±0.79	3±1.02	3±0.79	2.2±0.79
Graafian follicle	5.6^{a}±0.62	0.4^{b}±0.62	0.66^{b}±0.81	0.4^{b}±0.62	1.6^{b}±0.62
Cystic follicle	2.9^{b}±2.23	8.9^{a}±2.02	8.0^{ab}±1.98	8.0^{ab}±1.98	6.9^{ab}±2.23

[1] CON, no supplementation rats; PCOS, PCOS rats; FE, PCOS rats fed fennel essential oil; FO, PCOS rats fed flaxseed oil, and FE+FO, PCOS rats fed fennel essential and flaxseed oil. The fennel essential oil (100 mg/kg BW/day) and flaxseed oil (429 mg/kg BW/day flaxseed oil which was equal 240 mg ω-3/kg BW/day) were gavaged 7 days past PCO induction for a 50-day period

[2] The standard error of the mean

[a–c] Values within a row with different superscripts differ significantly (P <0.05)

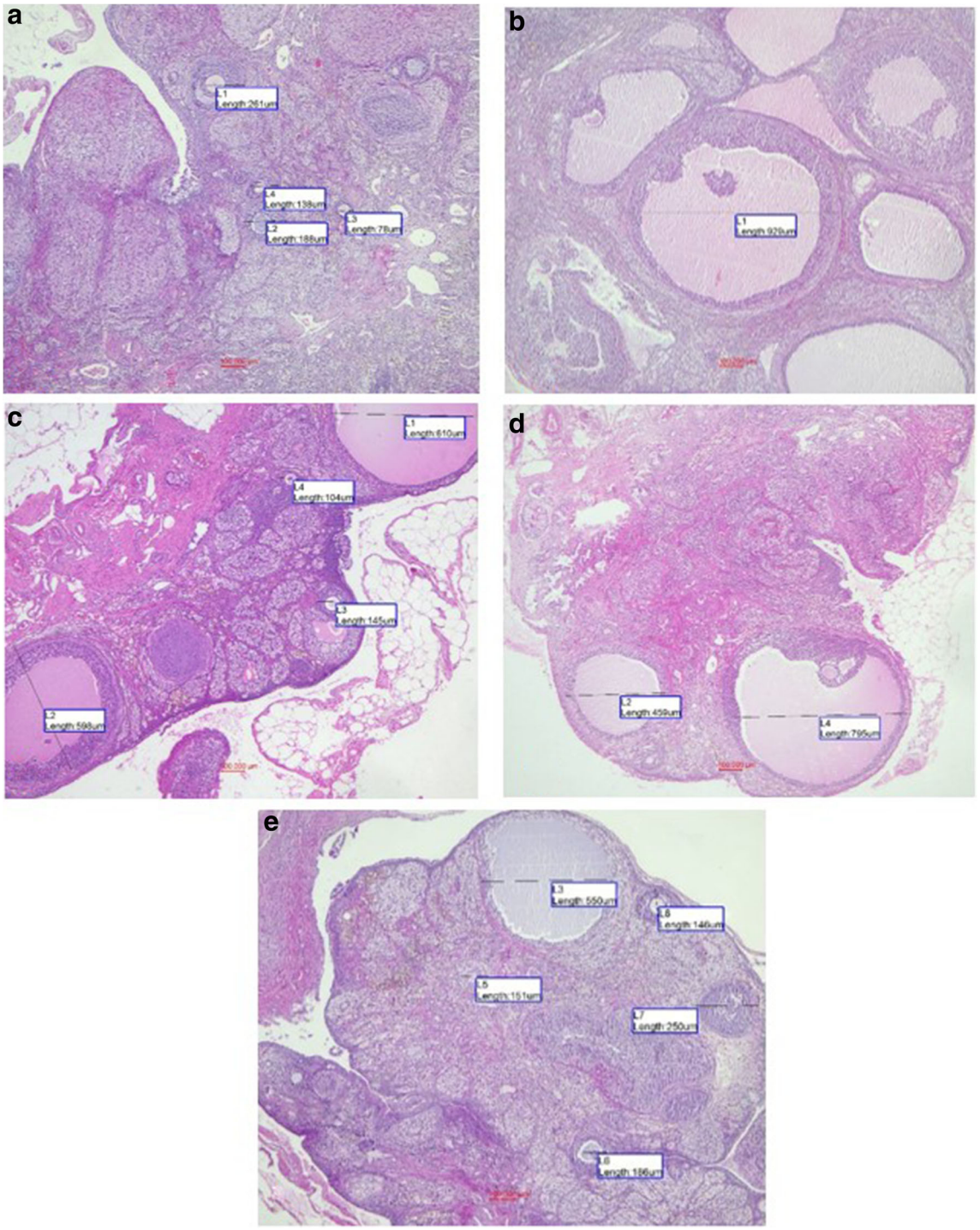

Fig. 1 Ovarian structure in control group (**a**; L1 and L2= secondary follicles and L3 and L4 = primary follicles), PCOS group (**b**; L1, cystic follicles), FE group (**c**; L1 and L2= cystic follicles and L3 and L4= primary follicles), FO group (**d**; L2 and L4= cystic follicles) and FE+ FO group (**e**; L3= cystic follicles, L6 and L7= secondary follicles and L5 and L8= primary follicles), Hematoxylin-eosin (H&E) staining. Optical microscope (magnification × 40)

induction of PCOS in rats increased (P <0.05) fasting blood glucose, but consumption of fennel essential oil or flaxseed oil decreased its concentration to the normal threshold. Nekooeian et al. (2014) reported that feeding pomegranate seed oil increased insulin without affecting blood glucose in rats suffered type 2 diabetes mellitus. They concluded that this effect could be due to the effect of omega-3 and antioxidant contents in pomegranate seed oil. It was surprising that consumption of FE + FO increased rats' blood glucose. The cause of this effect is not well understood and needs further studies.

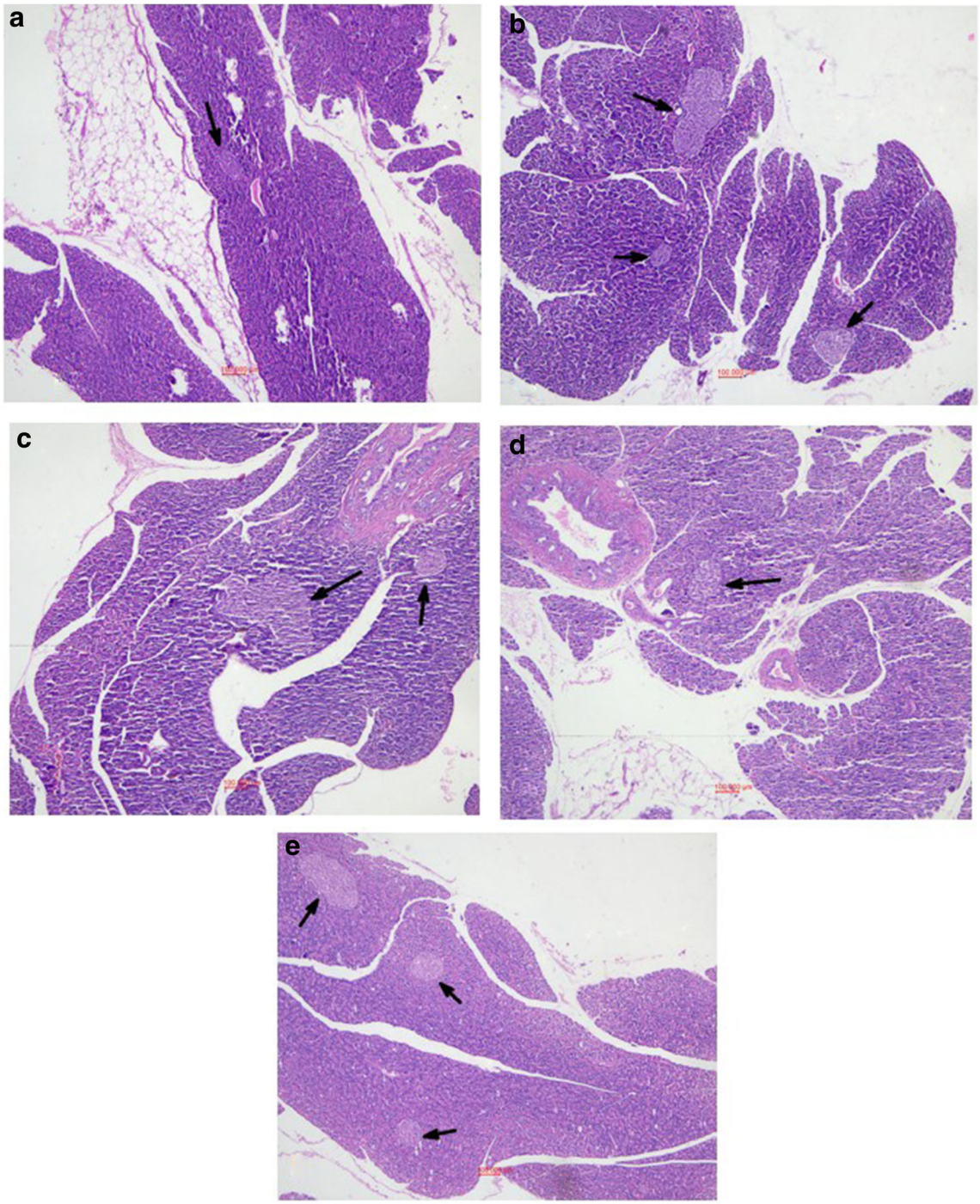

Fig. 2 Pancreas structure in control group (**a**), PCOS group (**b**), FE group (**c**), FO group (**d**) and FE+FO group (**e**). Arrows indicate the position of the Langerhans islets. Hematoxylin-eosin (H&E) staining. Optical microscope (magnification × 40)

Our results showed that HOMA-IR, HOMA-β, and QUICKI were affected ($P<0.05$) by the treatments (Table 2). From this aspect, feeding flaxseed oil has better effects and could reduce the insulin resistance and insulin sensitivity compared to PCOS group. Moreover, rats fed FO had better pancreas β-cell function. Fennel essential oil was also very effective in this respect and had positive effects on insulin sensitivity and pancreatic health. However, HOMA-IR, HOMA-β, and QUICKI indices were not improved in FE+FO group. This finding could be related to the serum glucose and insulin concentration (Lee et al. 2011). Nasri et al. (2017) showed that omega-3 fatty acid supplementation in PCOS women significantly improved gene expression which is involved in the insulin and lipid signaling pathways (PPAR-γ and LDLR).

Table 4 Effect of fennel essential oil and flaxseed oil on number of pancreatic islets and their area in female Wistar rats suffered PCOS

Trait	Experimental groups				
	CON	PCOS	FE	FO	FE+FO
Number of islets	14.5±2.4	9.4±2.4	10.6±2.4	9.5±2.4	12.9±2.4
Islets' area (μm^2)	146.4±22.5	170.3±22.5	192.8±22.5	129.5±22.5	174. 7±22.5

[1] CON, no supplementation rats; PCOS, PCOS rats; FE, PCOS rats fed fennel essential oil; FO, PCOS rats fed flaxseed oil, and FE+FO, PCOS rats fed fennel essential and flaxseed oil. The fennel essential oil (100 mg/kg BW/day) and flaxseed oil (429 mg/kg BW/day flaxseed oil which was equal 240 mg ω-3/kg BW/day) were gavaged 7 days past PCO induction for a 50-day period

[2] The standard error of the mean

Evaluation of ovarian weight and follicle count

Data of ovarian weight and the number of different follicles are presented in Table 3. Result showed that right ovary weight and mean of ovarian weight increased ($P<0.05$) after induction of polycystic ovary syndrome which might be related to the presence of cystic follicles in this group. This finding was in accordance with Ghafurniyan et al. (2015) who showed PCOS induction by injection estradiol valerate significantly increased body weight and ovarian weight of Wistar rats. Feeding fennel essential oil and/or flaxseed oil decreased ($P<0.05$) the weight of right ovary compared to PCOS group. Moreover, mean of ovarian weight was lowest ($P<0.05$) in FE+FO group.

Results of the follicle counts (Table 3) and the ovarian histomorphology (Fig. 1) showed that induction of PCOS syndrome increased ($P<0.05$) cystic follicles compared to control group. The appearance of ovaries in control group was normal, with primary and secondary follicles. On the other hand, appearance of ovaries in PCOS group was changed and the number of cystic follicles increased but number of normal follicles was decreased. This phenomenon could be related to hyperandrogenism which leads to cystic follicle generation (Badawy et al. 2009). Feeding fennel essential oil increased number of primary follicles. However, there were some cystic follicles with lower size compared to cystic follicles in PCOS group. The number of cystic follicles in group FE ($P= 0.06$), FO ($P= 0.07$) and FE+FO ($P= 0.09$) were tended to be lower than the PCOS group. It seems that fennel essential oil and flaxseed oil could ameliorate the PCOS condition, but the higher doses need to be tested to achieve better results. Abtahi-Eivari et al. (2018) reported that feeding 200 and 400 mg/kg of *Galega officinalis* significantly decreased cystic follicles in PCOS rats. Wang et al. (2020) declared that flaxseed oil (1 ml/kg) improved that PCOS condition in rats and they believed that this effect may be via the sex steroid hormones-gut/vaginal microbiota-inflammation axis.

Pancreas structure

Histomorphological study showed that PCOS induction and the treatments (feeding fennel essential oil and/or flaxseed oil) had no effect on pancreatic structure (Fig. 2), and the number and area of Langerhans islets (Table 4).

Conclusion

Results of this study showed that PCOS condition had negative effects on body mass index, blood glucose and hormones, insulin resistance, insulin sensitivity, and pancreatic β cell functions. Feeding fennel essential oil or flaxseed oil due to the anti-diabetic effects and anti-oxidative properties improved the body mass index, serum glucose, insulin and progesterone concentration, and HOMA-IR, HOMA-β, and QUICKI indices in rats suffered PCOS. These supplements had positive effects on follicle count and decreasing the number of cystic follicles. However, the combination of fennel essential oil + flaxseed oil adversely affected the fasting blood glucose and insulin resistance condition in PCOS rats which need to be considered in future studies.

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Declarations

Conflict of interest The authors declare no competing interests.

Research involving human participants and/or animals The animal care advisory committee of the Isfahan University of Technology approved all experimental procedures and all institutional and national guidelines for the care and use of laboratory animals were followed.

Informed consent All institutional and national guidelines for the care and use of laboratory animals were followed.

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