Research Paper: The Association Between Fennel Extract, Serum Lipid Profile, and Leptin Receptor Expression

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ABSTRACT

Introduction: Obesity is among the most severe challenges of our era, with significant health consequences and a high economic burden for health systems. Therefore, many countries have developed political agendas to cope with this ever-rising challenge. Along with chemical medications developed to manage obesity, researchers have focused on some natural ingredients and herbal extracts that are effective in reducing weight. The current study investigated the association between Foeniculum vulgar (fennel) extracts and body weight, lipid profile, and leptin.

Methods: In total, 35 adult male BALB/c mice were investigated in sham, fennel 50 mg/kg, fennel 100 mg/kg, and fennel 200 mg/kg (n=7) groups. The mice were administered fennel extracts for fourteen days while weighted at the intervention's beginning and end. Then, their weight, lipid profile, serum leptin, and expression of leptin protein in the hypothalamus were measured.

Results: After providing the intervention, leptin receptor protein expression was increased in all groups, while serum leptin didn't change significantly. Moreover, a significant decrease was observed in the cholesterol dose of 100 mg/kg/day, triglycerides in 100 and 200 mg/kg/day, and LDL in 50 and 100 mg/kg/day. Serum HDL was increased significantly in a dose of 100 mg/kg/day.

Conclusion: Fennel extract can decrease the lipid profile by changing the expression of the leptin receptor.

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Highlights

- Obesity contributes to many health problems and dyslipidemia.
- Leptin is known for its hunger-blocking can regulate food intake and affects the levels of lipid. circulation.
- Fennel is a plant with strong antioxidant activities [18] that can influence (increase) satiety and (reduce) food intake.
- Fennel contains phytosterols that are known to reduce cholesterol solubilization that in turn decreases its absorption.
- Fennel extract can improve the lipid profile by influencing the leptin receptor expression.

Plain Language Summary

Obesity is one of the most serious challenges of our era, with significant health consequences. Researchers have focused on some natural ingredients and herbal extracts. The current study aimed to investigate the association between Foeniculum vulgar (fennel) extracts and body weight, lipid profile, and leptin. After treatment of fennel, leptin receptor protein expression was increased in all groups, while serum leptin didn't change significantly. A significant decrease was observed in the cholesterol, triglycerides and LDL in some doses of fennel. Serum HDL was increased significantly in a dose of 100 mg/kg/day. So fennel extract can decrease the lipid profile by changing the expression of the leptin receptor.

1. Introduction

Obesity is a serious challenge of our era that is rapidly becoming a leading cause of preventable mortality worldwide. Based on the World Health Organization (WHO) report, in 2016, nearly 650 million adults were obese, and it's projected to rise to 1.12 billion by 2030 (Timper & Bruning, 2017). Therefore, the rising prevalence of obesity and its related diseases soon will turn into a great challenge for health systems (Nejatbakhsh et al., 2017); particularly concerning cardiovascular diseases (Kim, Després, & Koh, 2015), cancer (Calle, Rodriguez, Walker-Thurmond, & Thun, 2003), type 2 diabetes, hyperlipidemia, and hypertension (Maksvytis & Stakisaitis, 2004). Central or abdominal obesity accelerates several major risk factors for our health, including disturbances in plasma glucose, resistance to insulin, cholesterol level, low-density lipoprotein, and apolipoproteins (Freedman et al., 1990). Moreover, abdominal obesity modifies the risk for an atherogenic lipid profile of Triglycerides (TG) (Rakvaag et al., 2019). Maksvytis et al. showed that the lipid profile varied depending on the Body Mass Index (BMI) (Adams et al., 2006).

New information regarding food intake indicates the Central Nervous System (CNS), i.e., influenced by the interaction between genes and environmental factors (Crowley, Yeo, & O'Rahilly, 2002). Hypothalamus heavily influences matching food intake to energy consumption (Crowley et al., 2002). Based on the currently available evidence, Agouti-Related Peptide/Neuropeptide Y (AgRP/NPY) neurons and Proopiomelanocortin (POMC) influence receptors' expression for leptin and insulin (Timper & Bruning, 2017). Fat cells in adipose tissues release leptin, and its concentration regulates based on the metabolism of the body (Morrison, 2009). Therefore, leptin concentration and body fat percentage are associated (Morrison, 2009).

Moreover, leptin is known for its hunger-blocking effects; thus, it heavily influences the consumption of food (Kozłowska et al., 2010). Therefore, the primary impact of leptin is in the hypothalamus, but it also affects other areas of the brain (Harvey, Solovyova, & Irving, 2006). Leptin regulates our food intake and has a crucial role in multiple metabolic pathways (Auwerx, & Staels, 1998). For example, it is well proved that leptin stimulates Triglycerides (TGs) accumulation in tissues (Kavazarakis et al., 2001). Therefore, we can argue that leptin is associated with tissue lipid metabolism and affects the levels of lipid circulation.

Moreover, herbal medicine has positive effects on some metabolism-related disorders (Nejatbakhsh et al., 2017), including regulating the appetite and improving hyperlipidemia (Lemhadri, Hajji, Michel, & Eddouks, 2006), mainly through influencing the expression of in-

sulin and leptin receptors (Handa, Khanuja, Longo, & Rakesh, 2008). One of these herbals is Fennel (Foeniculum vulgar) extract, in which some studies have investigated its effects on metabolic pathways (Du Plessis, Cabler, McAlister, Sabanegh, & Agarwal, 2010).

Fennel is a plant with substantial antioxidant activities (Honardoost, Soleimanjahi, & Rajaei, 2013) that can influence (increase) satiety and (reduce) food intake (Mathern, Raatz, Thomas, & Slavin, 2009). Fennel contains phytosterols reduce cholesterol solubilization, which decreases its absorption (Nejatbakhsh et al., 2017). Capasso, Savino, and Capasso (2007) suggested that fennel could delay gastrointestinal transit and reduce fat and sugar absorption. Few research has studies the relationship between fennel and lipid profile. Afiat et al. reported a minor positive change in the concentrations of serum LDL-C, HDL, and HDL (Afiat et al., 2018). Choi et al. demonstrated that HDL was significantly increased after the administration of fennel (Choi & Hwang, 2004).

However, the exact mechanism of the fennel remains unknown. Thus, the present study intended to analyze the potential effects of fennel extracts on body weight and serum lipid profile and assess whether it is through leptin. Accordingly, we evaluated serum leptin concentration and leptin receptor expression in the hypothalamus of BALB/c mice.

2. Methods

Animals

The study was conducted on 35 male BALB/C mice (20-22 gram). Mice were obtained from the Animal Breeding Center of Iran University of Medical Sciences. Initially, a 12/12 h light/dark cycle was applied for all mice at a temperature of 22±2°C. Before performing the study, all protocols were approved by the Iran University of Medical Sciences (IUMS) Ethics Committee.

Fennel extract

We obtained fennel seeds stored for twelve months from the Isfahan Seed Packers Company (Isfahan, Iran). Then, seeds were grinded. Afterward, we mixed 2 g of powder with 150 ml water and was heated it. In the following, 90 mL of the solution was exposed to sterile gas. The resulting solution was poured equal volume into falcon tubes to purify the extract. Then, the falcons were centrifuged for 15 minutes using a 4400 RPM. This process was repeated daily.

Treatment

Animals have divided 35 mice into five groups (n=7/group):

Control (CO): intact animals that no injections were made on them.

Sham: mice that received solvent of fennel (Distilled water).

Fennel 50 (F50): Mice were treated with fennel (50 mg/kg; IP).

Fennel 100 (F100): Mice were treated with fennel (100 mg/kg; IP).

Fennel 200 (F200): Mice were treated with fennel (200 mg/kg; IP).

The tested animals received daily fennel extracts via Intraperitoneal (IP) injection for 2 weeks. The body weight was measured at the beginning and end of the 2 weeks of the experiment.

Measuring serum leptin & lipid concentration

After two weeks of providing interventions, mice were anesthetized to collect their blood from the heart. Next, blood fractionation was performed using a centrifuge to aliquot the serums. The following serums were prepared for analysis. ELISA kits were used to measure Serum leptin; kits were specially designed for Mouse (EASTBIOPHARM, USA). Values are reported as ng/dL of serum.

GPO-PAP enzymatic and CHOD-PAP enzymatic methods (PARS AZMUN) were used to measure serum triglyceride levels and Total Cholesterol (TC), respectively. Serum HDL level was measured by the Immunoinhibition method, and LDL concentration was derived from the Friedewald equation (Friedewald, Levy, & Fredrickson, 1972).

Preparing tissues for the immunoassays of leptin receptors

To systemically deliver the injected solution, transcardial perfusion was used, initially with PBS, then by 4% Paraformaldehyde (PFA) mixed with PBS (pH 7.4). Dissected brains were post-fixed during the night in 4% PFA in BPS at a temperature of 4oC. After 24 h, brain tissue samples were rinsed using the ice-cold 30% sucrose in BPS. Then, samples were put into optimal cutting tem-

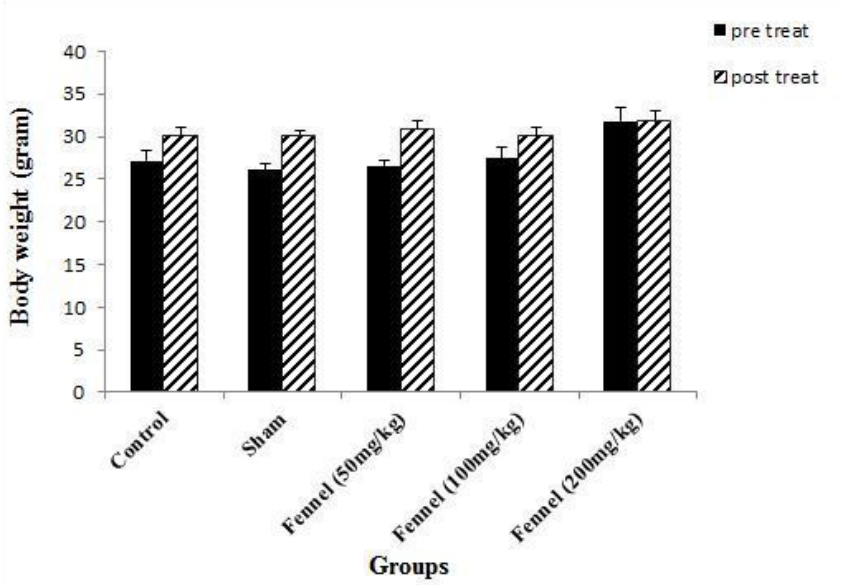

Figure 1. The effect of fennel treatment on bodyweight

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There is no difference between different groups. Data are expressed as Mean±SEM.

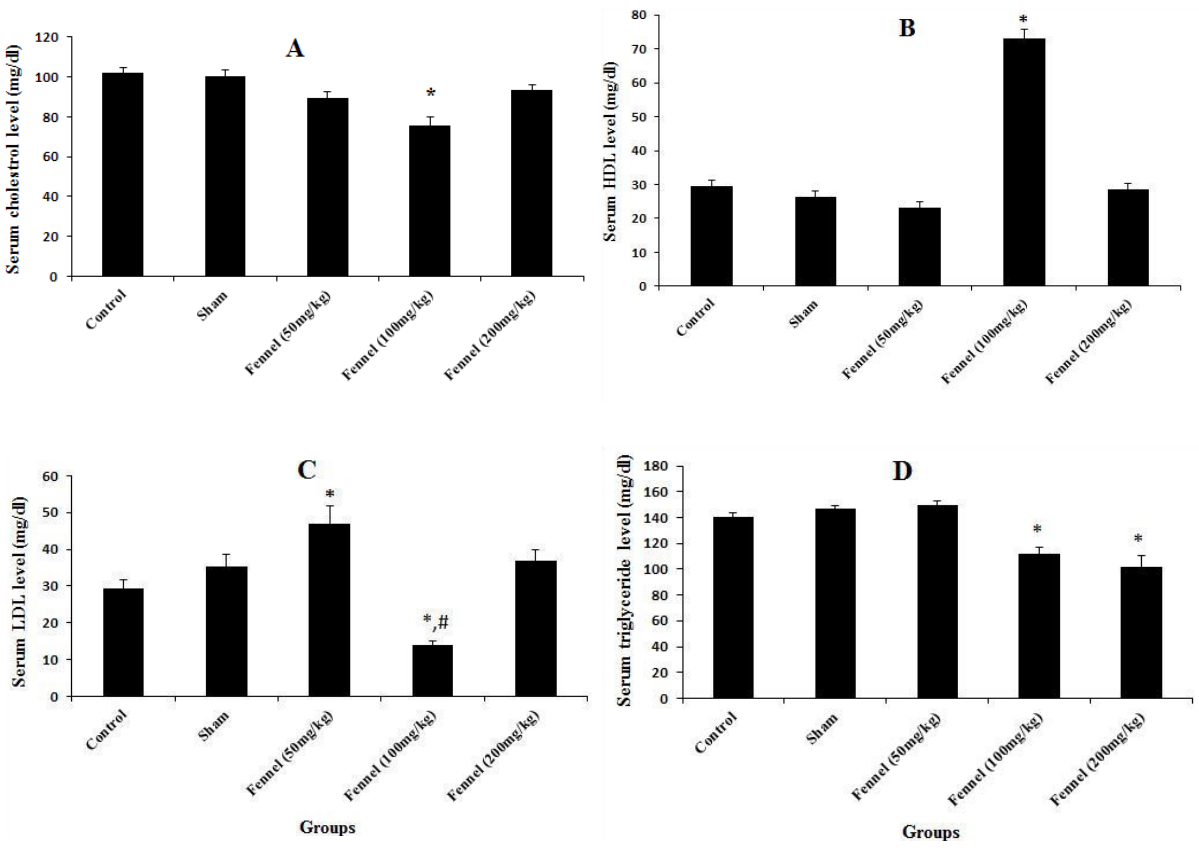

Figure 2. The effects of fennel treatment on the serum lipid profile

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A: Serum cholesterol level; B: Serum HDL level; C: Serum LDL level; D: Serum triglyceride level. Data are expressed as Mean±SEM. *Compared to control group; #compared to 50 mg/kg (P<0.05).

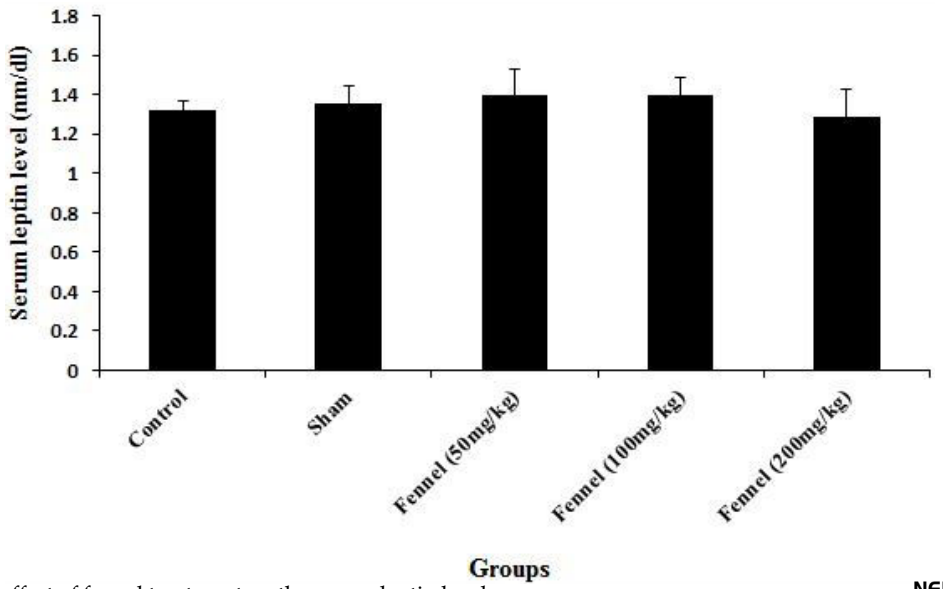

Figure 3. The effect of fennel treatment on the serum leptin level

There is no difference between different groups; Data are expressed as Mean±SEM.

perature compound (OCT, Tissue Tek) before frozen sectioning at -80oC (Webster, 2000).

The analyses of Immunofluorescence (IFS)

Using the floor standing fully automatic cryostat, MNT-SLEE (Mainz GmbH, Germany), the brain samples were sectioned in coronal planes, each with 10 µm thickness. Then, to obtain the hypothalamus, the sections were put into poly-L-lysine coated coverslips. The next step was air-drying, which was performed for 30 min at room temperature. To rehydrate the sections, they were put into 0.1 M PBS twice, each for 10 min. Subsequently, using a blocking solution [0.1 M PBS containing 2% Bovina Serum Albumin (BSA), 1% normal goat serum, & 0.2% Triton X-100] the sections were incubated. Moreover, incubation was performed for 1h using a permeabilization buffer (10% goat serum, 0.1% Triton X-100 in PBS).

To incubate sections, we used a primary antibody at a temperature of 4°C during the night. The sections were rinsed and further incubated using secondary antibodies for snother 2 hours in antibody solution comprised of 5% goat serum and 0.05% Triton X-100 in PBS. Primary antibodies included mouse monoclonal antibodies to the leptin receptor (1:500; bio-rad). The secondary antibody (Santa Cruz Biotechnology) was used to detect leptin receptor immunostaining was FITC conjugated goat anti-mouse IgG (1:200).

All slides were counterstained using the PI (2 µg/ml) to visualize the nuclei. To obtain negative controls, either the primary or secondary antibodies were removed if no signal was observed. A fluorescence microscope (Nokia) was used for analyzing the data. Besides, a digital camera was used to capture images (Zeiss) (Ramroodi et al., 2015; Sarvandi et al., 2015; Sanadgol et al., 2010).

To compare the mean values of various groups, we used the one-way Analysis of Variance (ANOVA) and Tukey's Post Hoc Test. The obtained data were analyzed in SPSS at the significance level of $P<0.05$. The findings are described as Mean±Standard Error (SEM).

3. Results

Bodyweight

There was no significant difference between the research groups (Figure 1).

Serum lipids

The mice in the fennel 100 mg/kg group had the highest decrease in cholesterol level. Besides, the TG level was decreased significantly in both fennel 100 and 200 mg/kg/day groups. Furthermore, LDL was significantly reduced in the fennel 100 mg/kg/day group. Besides, HDL was significantly enhanced in the fennel 100 mg/kg group (Figure 2).

Fennel extract and serum leptin levels

Based on the ELISA test, fennel extract is not associated with significant changes in the serum leptin level (Figure 3).

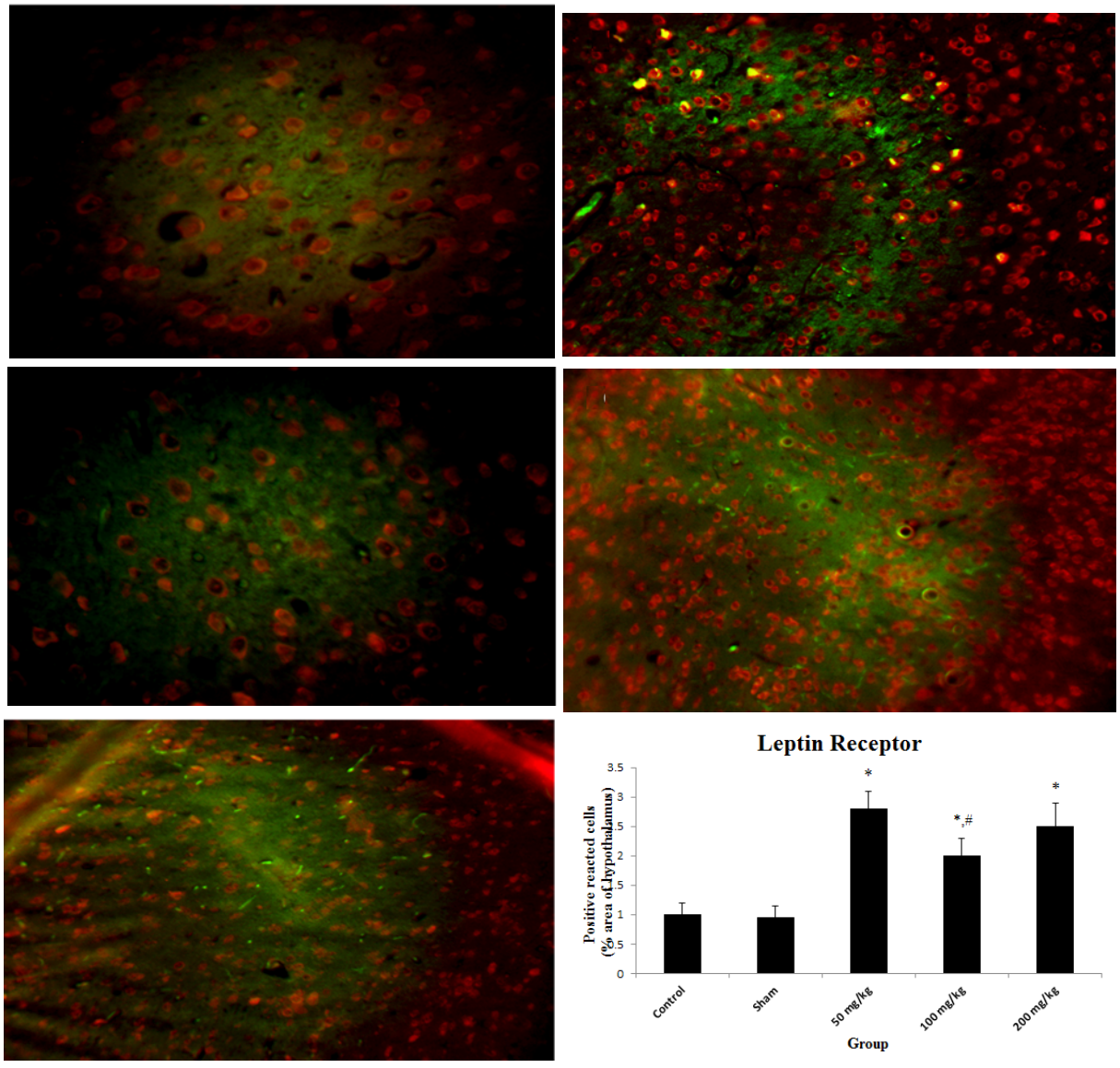

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Figure 4. The effect of fennel treatment on the Leptin receptor protein expression through the hypothalamus in different groups

A: Control group; B: Sham group; C: Fennel 50 mg/kg group; D: Fennel 100 mg/kg group; E: Fennel 200 mg/kg group. Data are expressed as Mean±SEM. *Compared to the control group; #Compared to fennel 50 mg/kg ($P<0.05$).

Effects of fennel extract on leptin receptor protein expression

Based on the collected findings, the expression of leptin receptor protein of all mice was enhanced, particularly in the fennel 50 mg/kg (Figure 4).

4. Discussion

The global prevalence of obesity is rising and is becoming a leading cause of preventable death. Obesity contributes to numerous health problems, such as CVDs, T2D, and dyslipidemia. Moreover, it is usually associated with increased plasma triglycerides, high levels of LDL, low levels of HDL, increased blood glucose levels, insulin resistance, and increased blood pressure (Klop, Elte, & Cabezas, 2013); it increases the risk of various chronic diseases. Moreover, obesity is associated with dyslipidemia, probably through causing insulin resistance in peripheral tissues, increasing the hepatic flux of fatty acids from dietary sources, the hydrolysis of triglycerides, and adipose tissue insulin resistance (Klop et al., 2013).

Recently attention has increased to herbal medicine to manage some disorders (Lemhadri et al., 2006). Fennel or Foeniculum vulgare is from the carrot family and grows widely in the Mediterranean (Arzoo, 2017). By increasing fat and sugar metabolism, fennel helps the body lose weight. In other words, fennel dissolves fat deposits in the bloodstream as an energy source. Based on the present study's findings, fennel could improve blood lipid profile by increasing leptin receptor expression. Fennel contains various ingredients, including phenolic compounds, fatty acids, volatile compounds, flavonoids, amino acids, and phytoestrogens (Arzoo, 2017). Assini et al. believed that flavonoids could effectively reduce lipid and lipoprotein. Besides, the authors reported that due to certain contents, namely naringenin and nobiletin, fennel can decline the accumulation of leptin and prevent the overproduction of lipoprotein (Assini, Mulvihill, & Huff, 2013). A clinical trial on patients with hypercholesterolemia reported that administrating naringin (from the flavonoids family) could significantly reduce LDL of plasma, apoB, and cholesterol by >14% (Jung et al., 2003). Animal studies developed to study metabolic syndrome and CVDs also reported the beneficial effects of flavonoids in reducing plasma lipids such as triglyceride (Park et al., 2013). Moreover, a study has reported that applying flavonoids was associated with significant declines in total cholesterol, LDL-C, and TG (Bao, Hu, Zhang, & Wang, 2016). Samani and Farrokhi (2014) explored the association between cumin extract and the paraoxonase 1 activity and oxLDL. Accordingly, they reported that the flavonoids available in the seed caused decreased levels of both 1 activity and oxLDL. Choi and Hwang (2004) found that the HDL was significantly increased after the administration of fennel. Based on the present study's findings, fennel extract could significantly decline cholesterol, TG, and LDL. Contrarily, it caused an expanding HDL level in adult male BALB/c mice.

Trans-anethole is an active aromatic component isolated from fennel. It composes more than 80% of fennel (Mahmoudi, Soleimani, Saidi, Khamisipour, & Azizsoltani, 2013; Rather, Dar, Sofi, Bhat, & Qurishi, 2016). Its positive effects on appetite are proved by several studies (Bae, Kim, Choue, & Lim, 2015). Besides, it has biologically active molecules with estrogenic activity (Mahmoudi et al., 2013). Based on the available data, estrogens are potent regulators of energy balance. As evidenced in estrogen deficiency, hyperphagia was seen. López and Tena Sempere (2015) believed that it has peripheral and central effects on regulating metabolic homeostasis, glucose, and lipid metabolism. Phytoestrogens are plant-derived compounds (Chrzan & Bradford, 2007) with an extra resemblance to 17β-estradiol. The most well-characterized characteristic of phytoestrogens is binding to estrogen receptors (Kuiper et al., 1998). However, their estrogenic activity is substantially lower than the natural estrogen hormone (Turner, Agatonovic Kustrin, & Glass, 2007). Phytosterols can actively remove cholesterol from intestinal micelles (Nejatbakhsh et al., 2017).

Moreover, fennel can reduce the absorption of fat and glucose levels (Capasso, Savino, & Capasso, 2007). Hayes et al. indicated that phytosterols could effectively reduce serum lipid in high cholesterol gerbils (Hayes, Pronczuk, Wijendran, & Beer, 2002). The present study also revealed that fennel extract could effectively decrease cholesterol, LDL, and TG, increasing serum HDL. Therefore, fennel probably affects the metabolic process by its estrogenic effects.

Hypothalamus heavily influences food intake. Cederroth et al. found that high phytoestrogen levels could change the expression of genes that code the peptides related to appetite hypothalami of mice (Cederroth et al., 2007). Leptin, produced primarily by adipose tissue, has a crucial impact on food intake and energy consumption (Nejatbakhsh et al., 2017). However, its secretion is influenced by estrogen (Kristensen, Pedersen, & Richelsen, 1999). During the estrus cycle, serum leptin was aletered (Tanaka et al., 2001). As reported by numerous studies, estrogen can increase leptin secretion in both humans and rats (Tanaka et al., 2001; Shimizu et al., 1997). Studies reported estrogen's ability to reverse leptin secretion (Yoneda et al., 1998; Henson, & Castracane, 2006). However, O'Neil, Burow, Green, McLachlan, and Henson (2001) found that estrogen could increase the promoter activity of leptin of JEG-3. Hsu and Yen (2007) concluded that flavonoids influence the control of adipogenesis by decreasing intracellular triglyceride levels, mainly by down-regulating expression of transcription factors that control adipogenesis; leptin; C/EBPα; and PPARγ.

As a result, fennel can affect metabolism by influencing leptin secretion. In the present study, the serum level of leptin was constant; however, the expression of its receptor in the hypothalamus was increased, which translates into increased effectiveness of leptin. Research on the effects of fennel on leptin secretion is scarce. In a research on the association between fennel extracts and body weight and leptin concentration, Nejatbakhsh et al. concluded that different doses of fennel, particularly 200 mg/kg, could increase serum leptin and decrease the weight, compared to obese animals (Nejatbakhsh et al., 2017). Hur, Kim, Kim, Ahn, and Ahn (2006) studied the association between fennel and body weight, serum leptin, and Food Efficiency Rate (FER). The authors found a negative association between fennel and FER in

rats. Based on the collected findings, there was no association between fennel and serum leptin concentration; however, it could increase protein expression of leptin receptors in the hypothalamus, which in turn caused improved serum lipid profile. However, further studies are required to examine such association.

5. Conclusion

This study indicated that fennel extract could improve the lipid profile by influencing the leptin receptor expression. Therefore, instead of using chemical drugs that often cause adverse effects, herbal medicine can treat hyperlipidemia. The authors suggest further studies to assess the findings of the present study. Acknowledgments: This research was supported by grant No: "92-01-117-22043" from the Iran University of Medical Sciences, Tehran, Iran.

Ethical Considerations

Compliance with ethical guidelines

All protocols were approved by the Ethics Committee of the Iran University of Medical Sciences (IUMS).

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Authors' contributions

Conceptualization, investigation, writing - original draft, and data collection: Forogh Zakernezhad; Data analysis: Mahmood Barati and Nima Sanadgol; Writing – review & editing: Monireh Movahhedi, Ahmad Majd and Fereshteh Golab.

Conflict of interest

The authors declared no conflicts of interest.

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