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Original Article

The effect of estradiol and selective estrogen receptor modulators on lipid profile in the ovariectomized rat model

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Abstract

Background and purpose: Menopause can increase the risk of cardiovascular diseases, diabetes mellitus, and metabolic syndrome, principally due to estrogen deficiency. In the current experiment, protective effects of selective estrogen receptor modulators (SERMs) and estradiol (E2), alone and combined, were evaluated in a rat model of menopause.
Experimental approach: Forty-eight female Wistar rats underwent ovariectomy to induce a menopause model. Then, the animals were subjected to receive SERMs including tamoxifen (TAM), raloxifene (RAL), and bazedoxifene (BZA) and E2. Finally, serum and liver tissue samples were collected post-treatment for experimental analysis.
Findings/Results: The induction of menopause by ovariectomy reduced the body weight of animals and altered their food intake. TAM, RAL, ethinyl E2 (EE2), and BZA/conjugated estrogens (BZA/CE) improved the ovariectomy-induced elevation of total cholesterol and low-density lipoprotein (LDL) cholesterol significantly. In this regard, the lowering effects of SERMs were significantly greater than EE2. The increased levels of triglycerides were also alleviated by RAL, EE2, and BZA/CE but not TAM. Moreover, the combination of SERMs, especially BZA/CE therapy, had significantly increasing effects on high-density lipoprotein (HDL) cholesterol levels, in a more effective manner than E2 therapy alone. Low-density lipoprotein receptor gene and protein expression levels were also significantly increased by SERMs. The HDL2 subfraction was found to be significantly enhanced in TAM, RAL, and BZA/CE therapy.
Conclusion and implications: The therapy with SERMs, alone or in combination with E2, may be efficiently utilized instead of E2 replacement therapy in post-menopausal conditions.

Keywords: Cardiovascular diseases; Estradiol; Estrogen replacement therapy; Lipids; Menopause.

INTRODUCTION

Menopause is defined as the final menstrual period, occurring around the age of 50. It is a normal process of aging and marks the end of a female's reproductive years (1). Post-menopausal decrease in ovarian hormone secretion can be associated with multiple pathophysiological events, including obesity and related comorbidities, as the hallmark ones (2). The role of estrogens in metabolic, immune, and inflammatory processes has been confirmed in both humans and rodents; however, the exact relationship is not fully elucidated (3). Post-menopausal lack of estrogens has been shown to induce a subcutaneous-to-visceral fat distribution, accompanied by the disruption of insulin sensitivity and predisposition to diabetes, as well as depressive defects (4). Estrogen has long been identified as a critical factor contributing to the regulation of adipose tissue development and fat deposition in females (5).

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Thus, the stoppage of estrogen secretion can change the metabolism of blood lipids, giving rise to an increase in total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels that could enhance the risk of cardiovascular diseases (6). Dyslipidemia observed in post-menopausal women is principally presented as a more atherogenic lipid profile, characterized by an increase in plasma levels of TC, LDL, and triglycerides, and a decrease in high-density lipoprotein cholesterol (HDL-C) content (7). In addition to menopause as a physiological process, ovariectomy, which is considered a common surgical operation among women worldwide, can also result in similar consequences (8).

According to previous investigations, female sexual hormone therapy (i.e., estrogens and progestogens, alone or combined) could delay the onset of diabetes, diminish insulin resistance, prevent dyslipidemia, and decrease blood pressure in healthy women, while in diabetic women, it ameliorates insulin resistance, fasting plasma glucose, and left ventricular hypertrophy (9). Furthermore, estradiol (E2) has been demonstrated to decrease angiotensin II (AngII)-induced hypertension (10). In line, menopause hormone therapy (HT) is the most common pharmacological approach to manage climacteric syndrome; HT helps the body maintain metabolic parameters within the normal ranges (11). The consumption of E2, or estradiol, has been associated with various adverse effects that warrant consideration in clinical practice and research (12). Among the potential adverse effects of E2 consumption, an increased risk of thromboembolic events, particularly in individuals with predisposing factors, is noteworthy. Furthermore, E2 use has been linked to an elevated risk of certain malignancies, including breast and uterine cancer and hypertriglyceridemia. Additionally, individuals undergoing E2 therapy may experience adverse symptoms such as mood disturbances, headaches, nausea, and breast tenderness(13).

Based upon the above-stated evidence, together with considering the side effects of E2 consumption, using selective estrogen receptor modulators (SERMs) as ideal alternatives for E2 is significantly increasing. Since SERMs can act as estrogen agonists/antagonists, they are now being considered for use in breast cancer and osteoporosis therapy, as well as alleviating post-menopausal symptoms (14). Tamoxifen (TAM) and raloxifene (RAL) are considered the most widely used SERMs in clinical settings (15).

Cardiovascular diseases exhibit sensitivity to estrogens. The relative cardio protection in women versus men has prompted debate on the estrogen protective role. Interest in SERMs increased based on the hypothesis that estrogen receptor agonists distinct from estrogens may improve cardiovascular effects (16).

SERMs demonstrated variable impacts on the metabolic and vascular factors that influenced the incidence of coronary artery disease (CAD). SERMs can favorably modulate the lipid and lipoprotein profile (17). It has been shown that TAM and RAL can lower serum cholesterol levels by approximately 40-60% in the ovariectomized rat model (18). Furthermore, the SERMs have been shown to inhibit the oxidation of human LDL or lipid peroxidation in cell membranes in vitro (19). RAL, in particular, appears to have potentially cardioprotective effects, possibly mediated through a mechanism involving an increase in the availability of nitric oxide (NO) on the vascular endothelium (20). Additionally, studies have demonstrated that TAM inhibits cholesterol accumulation in the coronary arteries of monkeys, while RAL similarly inhibits the accumulation of cholesterol in the aorta of rabbits fed a cholesterol-enriched diet (21,22).

The impact of SERMs on the development of atherosclerosis has also been evaluated by measuring their effects on the formation of vascular lesions in animal models. TAM has been shown to significantly inhibit the formation of both diet-induced and spontaneous arterial fatty streak lesions in mice (23).

Paraoxonase 1 (PON1) is an HDL-associated enzyme that hydrolyzes oxidized lipids, contributing to HDL antioxidant activity. PON1 protects both HDL and LDL from lipid peroxidation (24). One study in mice showed that PON1 overexpression reduced atherosclerosis, while PON1 deficiency

accelerated it (25). In humans, higher PON1 activity is inversely associated with cardiovascular disease risk (26). Changes in HDL concentration, composition, and function in post-menopausal women may impact PON1 activity.

Accordingly, the current study was designed to investigate the effect of SERMs, TAM, and raloxifen (RAL), estrogens (ethinyl estradiol (EE2)), and bazedoxifene (BZA)/conjugated estrogen (CE), alone and combined, on lipid profile, PON1 activity, and HDL function in a menopause model simulated by ovariectomized (OVX) rats.

MATERIALS AND METHODS

Animals

Forty-eight female Wistar rats with an age of 4 months (206.8 ± 2.88 g) were purchased from the Central Animal House of Ahvaz Jundishapur University of Medical Sciences (Ahvaz, Iran) and kept in the animal house of Faculty of Veterinary Medicine, Shahid Chamran University (Ahvaz, Iran) under the optimum conditions of moistness and temperature on 12-h light/dark cycles. All experiments were approved by the Ethical Committee of Ahvaz Jundishapur University of Medical Sciences (ethical code: IR.AJUMS.ABHC.1397.050).

Surgical operation of ovariectomy

Animals were first anesthetized with a ketamine/xylazine mixture (60/16 mg/kg, intraperitoneally, Alfasan Co, Woderen-Holland), and then the dorsal mid-lumbar area was shaved bilaterally (27). Skin and the muscle wall were incised under a 2-cm incision to reach the ovaries; the ovarian arteries were then extracted. Eventually, the abdomen was cleansed with 1-2 mL of normal saline, and the skin and muscle were then sutured using 4-0 sterile suture. The ovariectomy of all rats was done 2 weeks before the experiment (28). Rats underwent a 21-day recovery period after the surgical operation.

Experimental procedure and treatments

The rats were divided into 6 groups (n = 8), including 1. sham group, in which the ovariectomy procedure was performed, but no ovaries were removed, indicating that the group maintained a normal estrogen status; 2. a vehicle group, ovariectomized (OVX) rats receiving placebo; 3. OVX + EE2 group, OVX rats receiving EE2 (0.1 mg/kg/day; Aburaihan Pharmaceutical, Tehran, Iran) (29); 4. OVX + TAM group, OVX animals receiving TAM (1 mg/kg/day; Iran Hormone Pharmaceutical Co., Tehran, Iran) (30); 5. OVX + RAL group, OVX animals receiving RAL (3 mg/kg/day; Aburaihan Pharmaceutical, Tehran, Iran) (31); 6. OVX + BZA/CE group, OVX animals receiving 3 mg/kg/day of BZA and 2.5 mg/kg/day of CE (32). The rats orally received the treatments for 6 weeks, and their regular food intake and body weight were recorded daily. All drugs prepared in a powder formulation were dissolved in 1.5% carboxymethyl cellulose suspended in water. The vehicle group received a placebo containing only 1.5% carboxymethyl cellulose. BZA/CE was prepared in the formulae of Duavee tablets (Pfizer, United States) that contained 20 mg of BZA and 0.45 mg of CE.

Serum and tissue preparation

Following the treatments, all rats were euthanized by ketamine-xylazine following an overnight fast. Blood specimens were collected by cardiac puncture and centrifuged at 2500 × *g* for 10 min for further separation of serum fractions. Afterward, the sera were prepared for biochemical analyses. Moreover, multiple sections of the liver tissues were autopsied, washed with saline, frozen in liquid nitrogen, and maintained at -70 °C for further gene expression evaluations.

Lipid profile analysis

Serum levels of triglycerides (TG), total cholesterol (TC), HDL-C, and LDL-C were directly detected using commercially available spectrophotometric kits (Pars Azmoon Co., Iran), based on the manufacturer's procedures. LDL-C levels could also be calculated using the Friedewald equation as follows: LDL-C = TC – [HDL-C + (TG/5)] (33).The serum HDL2 content was measured by the same method used for cholesterol in the supernatant using the Pars Azmoon commercial kit, according to the manufacturer's instructions.

PON1 activity

PON1 activity was analyzed through paraoxon hydrolysis and subsequent release of *p*-nitrophenol, according to the study conducted by Assis *et al*. (34). The activity was then monitored by measuring the light absorbance at 405 nm, over a period of 5 min. The outcomes were calculated using the molar extinction coefficient of *p*-nitrophenol, which was 18,000/M/cm. PON1 activity was expressed in U/L (each U = µmol paraoxon hydrolyzed/min).

Liver homogenates and RNA extract preparation

Total RNA was collected from liver tissue specimens, using an RNA extraction kit (YTA, Yekta Tajhiz Azma, Tehran, Iran). In summary, 1 mL of RL lysing reagent was added to 50 mg of liver tissue, followed by a 5-min homogenization. For an efficient nucleoprotein lysis, the homogenized suspension was incubated at room temperature, and then centrifuged at 12000 rpm for 5 min at 4 ℃; centrifugation led to the elimination of lipids, proteins, and polysaccharides. Two hundred µL of chloroform was added to the supernatant, and centrifuged at 12000 rpm for 10 min at 4 ℃. Half of the supernatant was mixed with ethanol and then transferred to an FARB mini column located in a collection tube. The whole complex was centrifuged at 16000 rpm for 1 min, and the liquid gathered at the bottom was removed. Afterward, 500 µL of wash buffer 1, 600 µL of ethanol, and 750 µL of wash buffer 2 were added to the working column, respectively. The last step (i.e., addition of 750 µL of wash buffer 2) was done twice. Following the steps of adding wash buffer solutions, 1-min centrifugation at 16000 rpm was performed, and the liquid at the bottom of the tubes was removed. Eventually, the FARB mini column was centrifuged at 16000 rpm for 3 min to dry. The dried column was located inside an elution tube and washed with 50 µL of RNase-free distilled water. Total RNA was finally extracted from the column in 1-min centrifugation at 16000 rpm and stored at -80 ℃ for further analyses. Concentration and purity of the extracted RNAs were estimated by determining the light absorbance ratio at 260/280 nm achieved by a Nano-Drop spectrophotometer (Bio-TeK, USA).

Evaluation of the Ldlr gene expression levels by quantitative reverse transcriptase-polymerase chain reaction

Extraction of total RNA from the hepatocytes was carried out using a commercial RNA extraction kit (YTA, Yekta Tajhiz Azma, Tehran, Iran) according to the manufacturer's instructions. Complementary DNA (cDNA) was then synthesized using random hexamer primers and a Revert Aid First Strand cDNA synthesis kit (Thermo Scientific, USA). Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) was performed on the synthesized cDNA using the SYBR Green method (based on the manufacturer's instructions) and the real-time PCR Bio-Rad system (35). The glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) gene was used as the housekeeping gene. Primers were designed by the AlleleID software (version 7.6, Premier Biosoft Corporation, USA); the sequences of primers and their physicochemical characteristics are demonstrated in Table 1.

Table 1. Characteristics of primers used in qRT-PCR analysis of *Ldlr* and *Gapdh* mRNA expression levels.

Gene	Primer	Product size (bp)	Annealing temperature (℃)
Gapdh	**Forward:** CAAGTTCAACGGCACAGTCAAG **Reverse:** CATACTCAGCACCAGCATCACC	122	58.6
Ldlr	**Forward:** GATTGGCTATGAGTGCCTATGTC **Reverse:** GTGAAGAGCAGAAACCCTATGG	182	55

Gapdh, Glyceraldehyde-3-phosphate dehydrogenase; *Ldlr*, low-density lipoprotein receptor; Bp, base pair; qRT-PCR, quantitative reverse transcriptase-polymerase chain reaction.

Protein extraction and Western blotting

Protein extraction from the liver tissue was done by adding 1 mL of cold lysis buffer to 50 mg of liver tissue. The lysis buffer contained detergents, salts, protease inhibitors, and divalent cation chelating compounds, including ethylenediaminetetraacetic acid (EDTA) and ethylene glycol tetraacetic acid (EGTA). All experiments related to protein extraction were performed in cold conditions. The samples were then centrifuged at 16,000 *g* for 20 min at 4 °C. The supernatants were separated for subsequent analyses and stored in -80 °C. The concentration of extracted proteins was then measured by the bicinchoninic acid (BCA) method based on the manufacturer's instructions. Afterward, the protein samples were loaded on a sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE), containing 30% acrylamide solution, 4× resolving buffer, distilled water, 10% ammonium persulfate, and N,N,N',N'-tetramethylethylenediamine (TEMED). SDS-PAGE was done at a voltage of 65V. Then, samples were transferred onto a polyvinylidene difluoride (PVDF) membrane, treated with methanol and transfer buffer. The transferring step was completed at a voltage of 100V for 60 min at 4 ℃. The accuracy of the transferring process was confirmed using a Coomassie blue dye. The membrane pores were then blocked by 3% skim milk. At last, the membrane was washed 3 times with Tris-buffered saline-Tween 20 (TBS-T, 10 mM TBS + 1.0% Tween 20), and then incubated overnight at 4 ℃ with mouse anti-LDL-R antibody dissolved in 1.5% skim milk (1:1000). Following the incubation of the membrane with primary antibody, it was incubated with secondary antibody at room temperature for 1 h. Then, the membrane was again washed with TBS-T (3 times), and protein bands were finally revealed by an enhanced chemiluminescence kit (Bio-Rad, Hercules, USA) using a Bio-Rad ChemiDoc imaging system.

Statistical analysis

All data were reported as mean ± SEM. Using the Image J software, the density of protein bands was calculated, and the LDL-R to GAPDH protein density ratio was quantified. Quantitative analysis of gene expression levels was performed by the $2^{-\Delta\Delta CT}$ method. Statistical analysis was carried out by SPSS version 23.0 using a one-way ANOVA test followed by a Tukey post-hoc test. *P*-value < 0.05 was statistically considered significant.

RESULTS

Food intake and body weight

Food intake and body weight alterations were recorded in all groups (Figs. 1 and 2). Although the ovariectomy could result in food intake alterations, there was no significant difference between the vehicle and sham groups. Moreover, EE2 therapy led to a decline in food intake compared to the vehicle group, which was significant in the third week (Fig. 1). A significant decrease in body weight was observed in EE2, RAL, TAM, and BZA/CE groups compared to the vehicle group, while no significant difference was seen between these groups and the sham group (Fig. 2).

Effect of SERMs on lipid profile

SERMs were further assessed for their possible ameliorative effects on lipid profile in OVX rats. The serum levels of TC, TG, HDL-C, and LDL-C were significantly different between the vehicle and sham groups (Fig. 1).

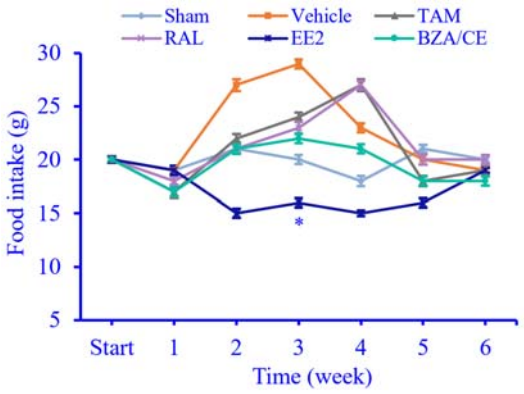

Fig. 1. Food intake for 6 weeks in all experimental groups. The sham group underwent the ovariectomy procedure without ovary removal. The vehicle group received a placebo containing 1.5% carboxymethyl cellulose. EE2, TAM, and RAL were administered at the doses of 0.1, 1, and 3 mg/kg, respectively. BZA/CE was administered as the combination of BZA (3 mg/kg) and CE (2.5 mg/kg). Data were expressed as mean ± SEM. $^{*}P < 0.05$ indicates a significant difference compared with the vehicle group at the respective time point. EE2, Ethinyl estradiol; TAM, tamoxifen; RAL, raloxifene; BZA/CE, bazedoxifene/conjugated estrogen.

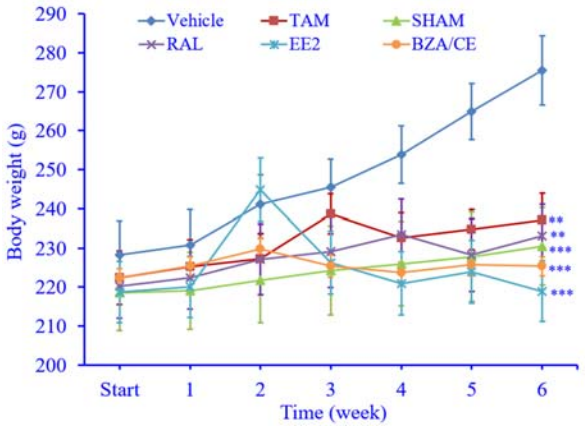

Fig. 2. Body weight of animals for a period of 6 weeks in all experimental groups. The sham group underwent the ovariectomy procedure without ovary removal. The vehicle group received a placebo containing 1.5% carboxymethyl cellulose. EE2, TAM, and RAL were administered at the doses of 0.1, 1, and 3 mg/kg, respectively. BZA/CE was administered as the combination of BZA (3 mg/kg) and CE (2.5 mg/kg). Data were expressed as mean ± SEM. $^{**}P < 0.01$ and $^{***}P < 0.001$ indicate significant differences compared with the vehicle group. EE2, Ethinyl estradiol; TAM, tamoxifen; RAL, raloxifene; BZA/CE, bazedoxifene/conjugated estrogen.

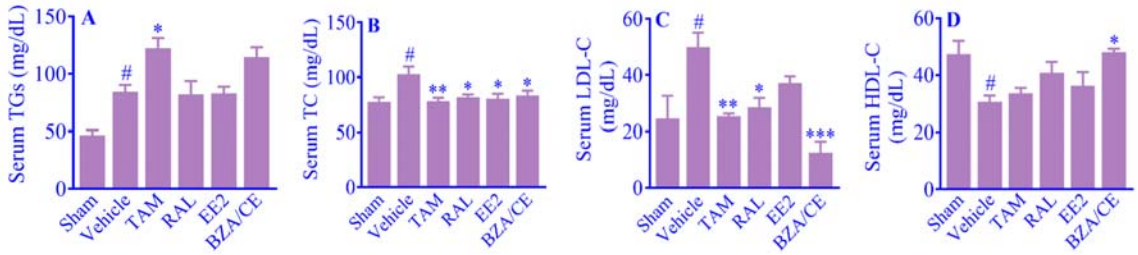

Fig. 3. Effects of SERMs on serum lipid profile in the ovariectomized groups. Serum levels of (A) TGs; (B) TC; (C) LDL-C; (D) HDL-C. The sham group underwent the ovariectomy procedure without ovary removal. The vehicle group received a placebo containing 1.5% carboxymethyl cellulose. EE2, TAM, and RAL were administered at the doses of 0.1, 1, and 3 mg/kg, respectively. BZA/CE was administered as the combination of BZA (3 mg/kg) and CE (2.5 mg/kg). Data were expressed as mean ± SEM. $^{\#}P < 0.05$ indicates significant difference compared with the sham group; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus vehicle group. EE2, Ethinyl estradiol; TAM, tamoxifen; RAL, raloxifene; BZA/CE, bazedoxifene/conjugated estrogen; TGs, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; SERMs, selective estrogen receptor modulators.

The serum levels of TG were significantly higher in OVX rats receiving vehicle than those observed in the sham group. Additionally, the levels of serum TG in the OVX group treated with TAM increased compared to the vehicle group, whereas treating with other medications did not significantly change TG in OVX rats compared to the vehicle group (Fig. 3A).

The serum level of TC was significantly increased in the vehicle group compared to the sham group. On the other hand, there were significant decreases in serum TC levels in OVX animals treated with TAM, RAL, EE2, and BZA/CE compared to the vehicle group (Fig. 3B).

The level of serum LDL-C was significantly increased in the vehicle group compared to the sham group. On the other hand, therapy with SERMs significantly decreased the serum LDL-C levels in TAM, RAL, and BZA/CE groups in comparison to the vehicle group, while the observed decrease was not significant in the EE2 group (Fig. 3C).

Ovariectomy led to a significant decrease in HDL-C levels in the vehicle group versus the sham group. Furthermore, the levels of HDL-C in OVX animals were shown to be increased only in the group treated with BZA/CE compared to the vehicle group. Treatment with other medications did not result in significant changes in HDL-C content in comparison to the vehicle group (Fig. 3D).

PON1 activity

PON1 activity was significantly decreased in the vehicle group compared to the sham group. Whilst therapy with SERMs did not result in significant changes versus the vehicle group (Fig. 4).

Ldlr gene and LDL-R protein expression levels

Figure 5 demonstrates the mRNA expression levels of LDL-R in all groups. Accordingly, a significant decrease was observed in *Ldlr* gene expression in the vehicle group compared to the sham group, demonstrating the effects of ovariectomy. On the other hand, *Ldlr* gene expression significantly increased in the RAL, BZA/CE, TAM, and EE2 groups compared to the vehicle group.

Along with the above-stated findings, LDL-R protein expression levels, detected by Western blotting, indicated a remarkable increase in LDL-R expression in the TAM and BZA/CE groups versus the vehicle group (Fig. 6A). Moreover, the vehicle group showed a significant decrement in the LDL-R expression levels in comparison to the sham group. Whereas the expression levels significantly increased in TAM and BZA/CE groups compared to the vehicle group (Fig. 6B).

HDL2 subfraction

The HDL2 levels of OVX rats in the vehicle group were significantly lower than those in the sham group. Treating the OVX animals with TAM, RAL, and BZE/CE markedly increased HDL2 compared to the vehicle group. However, EE2 therapy did not cause significant changes in HDL2 levels in comparison to the vehicle group (Fig. 7).

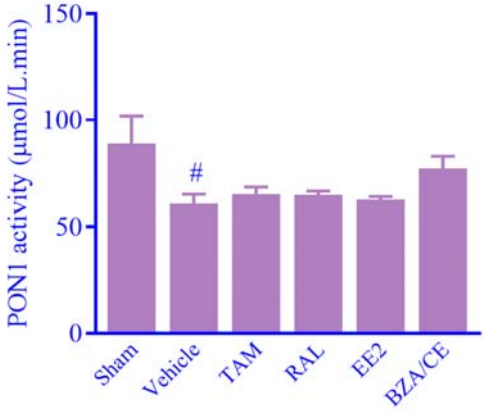

Fig. 4. Serum PON1 activity of ovariectomized rats treated with SERMs. The sham group underwent the ovariectomy procedure without ovary removal. The vehicle group received a placebo containing 1.5% carboxymethyl cellulose. EE2, TAM, and RAL were administered at the doses of 0.1, 1, and 3 mg/kg, respectively. BZA/CE was administered as the combination of BZA (3 mg/kg) and CE (2.5 mg/kg). Data were expressed as mean ± SEM. $^{\#}P < 0.05$ indicates a significant difference compared with the sham group. EE2, Ethinyl estradiol; TAM, tamoxifen; RAL, raloxifene; BZA/CE, bazedoxifene/conjugated estrogen; PON1, paraoxonase 1; SERMs, selective estrogen receptor modulators.

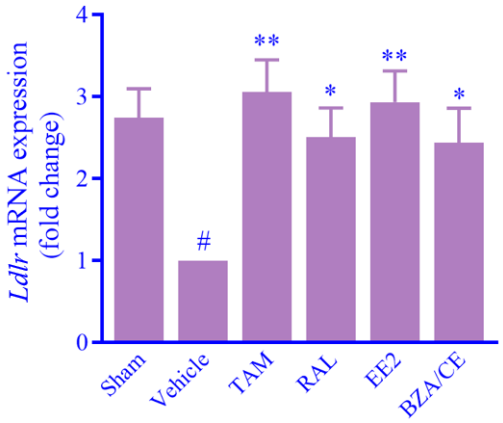

Fig. 5. mRNA expression levels of *Ldlr* in ovariectomized rats treated with SERMs. The sham group underwent the ovariectomy procedure without ovary removal. The vehicle group received a placebo containing 1.5% carboxymethyl cellulose. EE2, TAM, and RAL were administered at the doses of 0.1, 1, and 3 mg/kg, respectively. BZA/CE was administered as the combination of BZA (3 mg/kg) and CE (2.5 mg/kg). Relative expression levels were calculated by the $2^{-\Delta\Delta CT}$ method. Data were expressed as mean ± SEM. $^{\#}P < 0.05$ indicates significant difference compared with the sham group; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus vehicle group. EE2, Ethinyl estradiol; TAM, tamoxifen; RAL, raloxifene; BZA/CE, bazedoxifene/conjugated estrogen; *Ldlr*, low-density lipoprotein receptor; SERMs, selective estrogen receptor modulators.

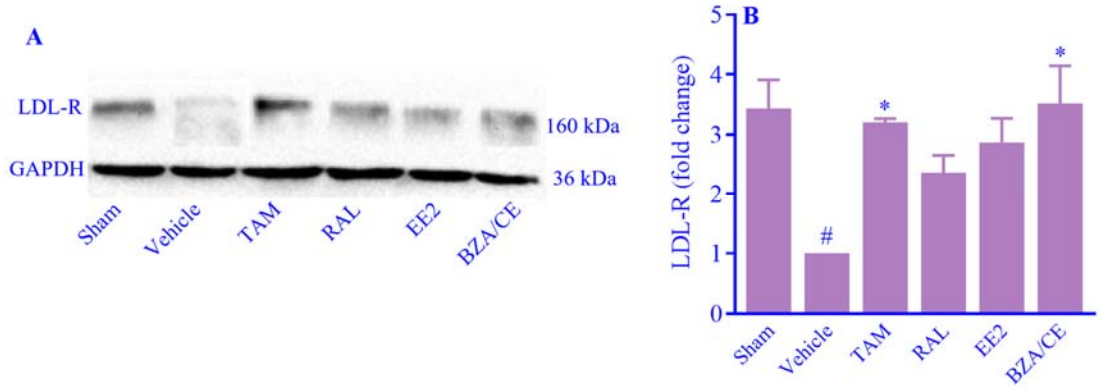

Fig. 6. LDL-R protein expression levels of ovariectomized rats treated with the SERMs. (A) Western blotting and (B) the analysis of relative protein expression across experimental groups. The sham group underwent the ovariectomy procedure without ovary removal. The vehicle group received a placebo containing 1.5% carboxymethyl cellulose. EE2, TAM, and RAL were administered at the doses of 0.1, 1, and 3 mg/kg, respectively. BZA/CE was administered as the combination of BZA (3 mg/kg) and CE (2.5 mg/kg). Data were expressed as mean ± SEM. $^{\#}P < 0.05$ indicates significant difference compared with the sham group; $^{*}P < 0.05$ versus vehicle group. EE2, Ethinyl estradiol; TAM, tamoxifen; RAL, raloxifene; BZA/CE, bazedoxifene/conjugated estrogen; LDL-R, low-density lipoprotein receptor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SERMs, selective estrogen receptor modulators.

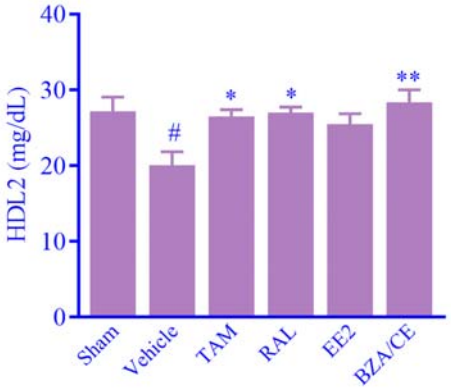

Fig. 7. Serum HDL2 levels of ovariectomized rats treated with SERMs. The sham group underwent the ovariectomy procedure without ovary removal. The vehicle group received a placebo containing 1.5% carboxymethyl cellulose. EE2, TAM, and RAL were administered at the doses of 0.1, 1, and 3 mg/kg, respectively. BZA/CE was administered as the combination of BZA (3 mg/kg) and CE (2.5 mg/kg). Data were expressed as mean ± SEM. $^{\#}P < 0.05$ indicates significant difference compared with the sham group; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus vehicle group. EE2, Ethinyl estradiol; TAM, tamoxifen; RAL, raloxifene; BZA/CE, bazedoxifene/conjugated estrogen; HDL2, high-density lipoprotein 2; SERMs, selective estrogen receptor modulators.

DISCUSSION

Menopause-induced suppression of estrogen synthesis and secretion can lead to a wide variety of clinical complications, in which cardiovascular diseases are of great importance. Additionally, a lack of estrogen contributes to the progression of visceral obesity, metabolic syndrome, and type 2 diabetes mellitus. Considering the pathological role of estrogen deficiency in post-menopausal women and the beneficial effects of estrogen replacement therapy (ERT) in decreasing LDL-C levels, and thus reducing the risk of cardiovascular diseases, a new therapeutic challenge has been created in this field: side effects of ERT could be threatening, and thus should be avoided (36). In this context, SERMs have been introduced as ideal alternatives to E2. SERMs are known as medications that induce the estrogen receptor, with a broad spectrum of effects in different tissues (15). Possible mechanisms of action attributed to SERMs are increasing the expression of LDL receptors (37), as well as the stimulation of lipoprotein lipase activity (38). Therefore, in the current study, the effects of administering TAM, RAL, EE2, and BZA/CE were investigated on the lipid profile and atherogenic indices in OVX rats.

According to the findings, ovariectomized rats treated with TAM, RAL, estrogen (EE2), and BZA/CE demonstrated reduced body weight and altered food intake. The corresponding SERMs were shown to ameliorate increased TC and LDL-C in OVX animals, and the inhibitory effects of SERMs on LDL-C were greater than EE2. Besides, the additive effects of the combined formulae on

HDL were more effective than E2 therapy. The elevation of TG in OVX rats was controlled by RAL, EE2, and BZA/CE, whilst TG levels were increased in the TAM group. Moreover, the additive effects of the BZA/CE combined therapy on HDL were more effective than other formulae. Both SERMs and E2 were shown to be able to improve lipid profile through decreasing the levels of LDL-C, TC, and TG, and increasing the HDL-C levels that had been altered under the ovariectomy. Multiple studies have also revealed valuable findings in line with the current results, including increased concentrations of LDL-C, TC, and TG in OVX rats (39), TAM-induced decrease in LDL-C and TC levels along with an increase in HDL-C content (27), RAL-induced elevation of HDL-C levels (40), E2-induced reduction of both TC and LDL-C, along with HDL elevation in post-menopausal women (41), and decreased TC in OVX rats achieved by BZA/CE (42). The comparison of serum TG levels in under-treatment OVX animals with the vehicle group demonstrated an increase in TG content only in the group treated with TAM; indeed, this effect has been referred to as a side effect of TAM consumption (43).TAM has been observed to stimulate the hepatic synthesis and secretion of very low-density lipoprotein (VLDL) (44). VLDL is the primary lipoprotein responsible for the transport of TGs in the circulation. Additionally, TAM has been reported to decrease the catabolism of both VLDL and intermediate-density lipoprotein (IDL)(45). This effect is attributed to the ability of TAM to reduce the activities of lipoprotein lipase and hepatic lipase, which are key enzymes involved in the clearance of VLDL and IDL particles (44, 45).

Decrease in *Ldlr* mRNA expression levels in the vehicle group was consistent with a previous study indicating the stimulatory role of estrogen on the expression levels of this receptor (46). Moreover, TAM and RAL have been reported to increase the expression of LDL-R in peripheral blood lymphocytes (47). It has been proposed that estrogen and the aforementioned SERMs might increase LDL-R expression through transcriptional and post-transcriptional regulatory mechanisms; for instance, sterol regulatory element-binding protein 2 (SREBP2) can transcriptionally mediate the gene of interest (48). One of the possible mechanisms through which estrogens and SERM drugs may increase LDL-R, in addition to cholesterol synthesis, is the induction of the expression of this receptor by transcriptional and post-transcriptional mechanisms (49). SREBP2 normally mediates transcription of this gene, while post-transcriptional regulation of LDL-R is mediated by PCSK9 (50).

In females, unlike the pre-menopausal period, menopause gives rise to a decrease in HDL-C; ovariectomy also mimics similar conditions in female rats. The results of measuring the serum HDL-C in the vehicle group compared to the sham group revealed a significant decrease in the levels of the corresponding lipoprotein. Also, the levels of HDL-C were shown to be significantly increased in OVX rats treated with BZA/CE compared to the vehicle group, while treating with EE2, RAL, and TAM did not result in significant changes in HDL-C levels compared to the vehicle group. However, in intervention studies on laboratory animal models of menopause, either a decrease or an increase has been reported in HDL-C content (51).

The activity of PON1 is another parameter that is decreased during the menopause, principally due to the estradiol deficiency (52). Depending on the findings, serum PON1 activity was lower in the OVX animals versus the sham group. PON1 is an enzyme with antioxidant effects, whose binding to HDL particles is stabilized by apolipoprotein AI (53). PON1 can associate with HDL to prevent its copper ion-induced oxidation, as well as mitigate its peroxide and aldehyde content. This association can also decrease the LDL atherogenic potential, potentially reducing the risk of atherosclerosis progression (54). In this work, HDL2 sub-fractionation and PON1 activity measurement were carried out to investigate the functional characteristics of HDL, as well as its quantity in groups receiving SERMs. Literature has reported that HDL2 is the major sub-fraction of HDL, linking to coronary or peripheral artery diseases (55). In this context, Gräser *et al*. reported a significant decrease in HDL3 levels following the oral combined HRT; they also reported no changes in HDL2 levels after receiving the HRT regimen (56). Notwithstanding, an increase in HDL-C has been reported after the HRT (57).

The anti-atherogenic effects of HDL are primarily attributed to its potential in removing the lipids (mostly cholesterol) from the vascular wall and a wide range of antioxidant and anti-inflammatory functions. All these beneficial potentials are reported to be dependent on the presence of PON1, as a specific structural constituent of the HDL particles. PON1 is responsible for breaking down the lipid peroxides prior to their accumulation in LDL particles. With its lactonase function, PON1 is physiologically involved in the metabolism of lipid particles through the oxidation of unsaturated fatty acids (25,58). In the current experiment, the observed increase in the content of larger HDL particles, as well as the decrease in HDL2 concentration, along with no changes seen in PON1 activity, can be justified as the tendency of PON1 to be inclined towards denser and smaller HDL particles (59).

Although SERMs, EE2, and BZA/CE were demonstrated to have significant effects on lipoprotein metabolism, the findings suggested that the effects of BZA/CE on reducing the levels of TC and LDL-C against increasing the HDL were greater than the effects of other SERMs. Given the significance of LDL reduction and HDL elevation in the prevention of cardiovascular diseases, the therapeutic potential of BZA/CE and TAM in post-menopausal women can be suggested as an alternative to estrogen therapy. SERMs are believed to exert their protective effects on the cardiovascular system through multiple mechanisms, such as the inhibition of collagen type I and IV (60), blocking the protein kinase C (PKC) (61), increasing the plasma nitric oxide levels, down-regulating the atherosclerosis-related inflammatory mediators (62), and decreasing the plasma LDL-C and TC levels (63). In line with the results of the present experiment, the beneficial effects of SERMs in combination with estrogen have also been reported for other maladies, such as post-menopausal Alzheimer's disease-related cognitive deficits (64) and vasomotor symptoms (65).

CONCLUSION

The administration of SERMs (especially TAM and RAL) is recommended as a therapeutic strategy to ameliorate the post-menopausal situation. In the case of lipid profile, the effects of combination therapies were shown to be more beneficial than the effects of therapy with E2 or SERMs alone.

Importantly, the combination therapy BZA/CE demonstrated the most comprehensive improvement in the lipid profile, suggesting a strong potential for cardiovascular benefit. It means that combination therapies could be effective for cardiovascular function through modifying the lipid profile over time, which requires further investigation. Evaluations on cellular mechanisms and post-receptor signaling cascades involved in the action of SERMs would further clarify the ambiguous aspects of therapy with SERMs and their effects on post-menopausal complications.

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Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contributions

H. Yaghooti and S. Aiiashi contributed to conceptualization and design; S. Aiiashi participated in investigation and performed the laboratory tests, data collection, and draft preparation and writing; A. Kheirollah participated in reviewing and editing; H. Babaahmadi-Rezaei contributed to data analysis, supervision, and validation. All authors have read and approved the finalized article. Each author has fulfilled the authorship criteria and affirmed that this article represents honest and original work.

AI declaration

The authors hereby declared that no generative AI tools were used in the preparation, writing, or analysis of this manuscript. The work was entirely the original product of the authors' own research and intellectual effort. The authors did not use any AI-assisted technologies in the preparation of this manuscript.

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