

Black Cohosh (*Actaea racemosa* L.) Improves Serum Lipid Profiles and Vasomotor Responses in Ovariectomized Rats

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Abstract We investigated the effects of long-term administration of black cohosh extract (BcEx) on serum lipid profiles and vasomotor responses in ovariectomized (OVX) rats and compared them with those of rats administered 17 β -estradiol (E2) or raloxifene, a selective estrogen receptor modulator. Vehicle (OVX- or sham-control), BcEx (0.5 or 3.0 mg/kg/day), E2 (0.5 mg/kg/day), or raloxifene (2.5 mg/kg/day) were injected subcutaneously for 5 weeks, and serum lipid profiles and vasomotor responses were measured at the end of the treatment. BcEx lowered total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels, but did not affect high-density lipoprotein cholesterol or triglyceride (TG) levels. Raloxifene showed a similar effect to that of BcEx, while E2 attenuated the increase in TC and LDL-C levels and significantly increased TG levels. The vascular relaxation induced by carbachol increased significantly in norepinephrine-precontracted aortic rings isolated from BcEx-, E2-, or raloxifene-treated rats. No change in uterine weight was observed in the BcEx-treated group. The raloxifene-treated group showed a similar trend as that of the BcEx-treated group, but E2 significantly increased uterine weight. These results suggest that long-term administration of BcEx behaves similar to the selective estrogen receptor modulator raloxifene.

Keywords: black cohosh, ovariectomy, lipid profiles, vasomotor responses, selective estrogen receptor modulators

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1. Introduction

Estrogen deficiency after menopause causes numerous changes in estrogen-receptive tissues, such as the brain, bones, and the cardiovascular system [1]. Estrogens are believed to be important in preventing cardiovascular diseases [2]; therefore, hormone replacement therapy (HRT) has been used to alleviate postmenopausal symptoms and prevent associated diseases [3]. Because of the controversy over the adverse effects and risks of HRT, alternative and complementary medicines are now used commonly. Selective estrogen receptor modulators (SERMs), which function as either estrogen receptor agonists or antagonists, have become popular as alternatives to HRT [4]. SERMs have beneficial estrogenic effects on the cardiovascular system [5]. Cardioprotective effects of SERMs, including hypolipidemic and vasorelaxant effects, have also been reported [6,7].

The rhizomes of *Actaea racemosa* L. or *Cimicifuga racemosa* (L.) Nutt. (Ranunculaceae), commonly known

as black cohosh, have been used conventionally as a remedy for menstrual cramps, pain during parturition, and climacteric complaints, including hot flashes, irritability, mood swings, and sleeping disorders [8]. A number of clinical trials have examined the efficacy and safety of black cohosh [9,10] and reported estrogenic effects in bones and fat [11], as well as attenuated body weight gain and intra-abdominal fat accumulation in ovariectomized (OVX) rats [12]. However, the mechanism underlying the effectiveness of black cohosh in treating menopausal symptoms is unclear, and its effects remain controversial. We reported previously that extracts of black cohosh (BcEx) elicit endothelium-dependent vasorelaxation in rat aorta by enhancing activity of the nitric oxide (NO)/cGMP system [13]. NO is a key regulator of endothelial function and mediator of the vascular benefits of estrogen [14]. Moreover, raloxifene (RLX), a second-generation SERM, activates endothelial NO synthase [15]. This study investigated the effects of BcEx on serum lipid profiles and vasomotor responses to carbachol in isolated aortic rings from OVX rats precontracted with norepinephrine (NE). We compared the effects to those of 17 β -estradiol (E2) and RLX.

2. Materials and Methods

2.1. Reagents

Carbachol, DMSO, E2, NE, PEG 400, and RLX were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ketamine (Yuhan Co., Seoul, Korea) and xylazine (Bayer Korea Ltd., Gyeonggi-do, Korea) were kindly supplied by Prof. Dr. Bo-Kyung Kim, Department of Physiology, School of Medicine, Konkuk University (Seoul, Korea).

2.2. Plant Extract

The powdered BcEx used in this study was a standard one utilized as the raw material of a dietary supplement. The sample was supplied from the NIH Center for Botanical Dietary Supplements Research at the University of Illinois at Chicago (UIC) and prepared and characterized as previously described [13,16]. Briefly, black cohosh rhizomes/roots were provided by Pure World Botanicals, Inc. (South Hackensack, NJ; lot 9-1744) [17], and were botanically verified by the UIC/NIH Center for Botanical Dietary Supplements Research and characterized by PCR [18]. A voucher specimen (BC001) has been deposited at the Program for Collaborative Research in the Pharmaceutical Sciences (PCRPS), University of Illinois at Chicago. Milled roots/rhizomes of black cohosh was extracted by percolation with 75% ethanol and dried in vacuo. BcEx was stored in a desiccator at room temperature and dissolved in 1.25% DMSO in PEG 400.

2.3. Animal Treatment and Aortic Rings Preparation

Twelve-week-old female Sprague-Dawley (SD) rats (260 to 280 g) were obtained from Hanlim Laboratory Animal Co. (Gyeonggi-do, Korea). Animals were fed a standard laboratory rat chow (PICO-LAB Rodent Diet 20-5053, PMI Feeds, Richmond, IN, USA) for a 24 h, 1-week period of acclimatization with tap water. Diet and water were administered *ad libitum*. The air-conditioned animal room was maintained at $22 \pm 2^\circ\text{C}$, with a relative humidity of $59 \pm 1\%$ and a 12 h light/dark cycle.

One week after acclimatization, 48 SD rats were randomly assigned to one of the six groups. Five groups were surgically ovariectomized, and one group was sham-operated under anesthesia using ketamine (70 mg/kg) and xylazine (10 mg/kg) and allowed to recover for 2 weeks. The OVX SD rats were randomly assigned to one of the five treatment groups. The first group received subcutaneous injections of the vehicle (OVX-control; 100 μL of 1.25% DMSO in PEG 400) daily; the second group received daily injections of E2 (0.5 mg/kg/day in 100 μL of a vehicle); the third group received RLX (2.5 mg/kg/day in 100 μL of a vehicle); the fourth and fifth groups received BcEx (0.5 and 3.0 mg/kg/day in 100 μL of a vehicle, respectively) for 5 weeks. Sham-operated rats (sham-control) received vehicle alone. The body weight of the SD rats was recorded once a week. At the end of the treatment, bloods were taken, rats were sacrificed, and tissue samples were obtained after overnight fasting. The care and use of the animals followed out institutional and

national guidelines, and the protocol was approved by the committee on the Ethics of Animal Experiments of the Korea Food Research Institute (Permit Number: KFRI-M-12028).

2.4. Measurement of Serum Lipid Profiles

For the measurement of serum lipid profiles, bloods were drawn from the orbital sinus vein of rats. The blood samples were centrifuged at 3,000 rpm for 10 min to collect the serum, and were stored at -20°C until further study. Uteri were dissected free of fat, and wet weights were measured. Total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) levels in the serum were determined using automatic dry chemistry analyzer for veterinary (Fuji Photo Film Co., Japan). The concentration of low-density lipoprotein cholesterol (LDL-C) was calculated using the formula of Freidwald et al. [19].

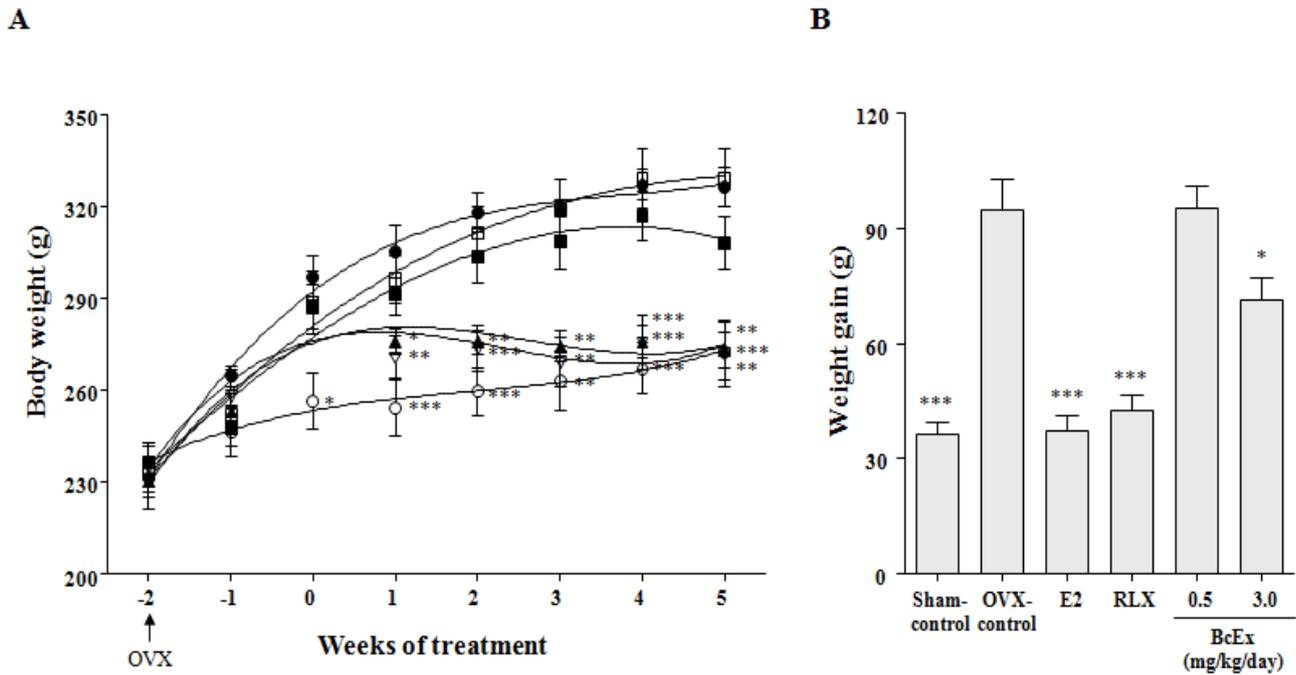
2.5. Vasomotor Responses to Carbachol in NE-precontracted Isolated Aortic Rings

The thoracic segment of aorta was dissected from the surrounding connective tissues and cut into rings 2–3 mm in length. The rings were then transferred to 3-mL horizontal-type muscle chambers, and bathed in physiological salt solution (PSS) containing (mM) NaCl (115), KCl (5), CaCl_2 (2.1), MgSO_4 (1.2), NaHCO_3 (25), glucose (11), and KH_2PO_4 (1.2) at 37°C , in an atmosphere of 95% O_2 and 5% CO_2 . The rings were mounted on stainless steel hooks, connected to a force-displacement transducer (FT03; Grass, West Warwick, RI, USA), which was connected to a polygraph system (RPS 212; Grass) and a computer analyzer (Power Laboratory 400, MacLab; AD Instruments, Castle Hill, Australia). A basal tension of 1 g was applied and each experiment was performed on rings prepared from different rats.

All rings were equilibrated for 60 min under a resting tension of 1 g and then 72 mM KCl PSS until the responses stabilized. A control contraction was then produced by the addition of 300 nM NE. After sustained tension was obtained, carbachol was added cumulatively (10^{-9} – 10^{-5} M) to bathing solution in 2-min intervals (relaxation-response curve to carbachol). The Vasodilator effect of increasing concentrations of carbachol was expressed as percent decrease of the peak NE concentration. The pD_2 was calculated as the negative logarithm of the dilator concentration that induced 50% of the maximal relaxation.

2.6. Statistical Analysis

All results are expressed as means \pm standard error of mean (SEM) for all groups. In the vasomotor response experiment, the number of rings obtained from different rats is represented by n. Relaxation is expressed in terms of the percentage decrease in maximal contraction caused by NE (300 nM). The Prism 5 software (Graph Pad Software, San Diego, CA, USA) was used for the statistical analysis. The differences were analyzed using one-way ANOVA followed by a Dunnett's test. Resulting *P* values less than 0.05 were regarded as significant.



(A) Changes in body weight during experimental periods. Sham-control (○, sham-operated group), OVX-control (●, vehicle injected OVX group), E2 (∇, 0.5 mg/kg/day E2-injected OVX group), RLX (▲, 2.5 mg/kg/day RLX-injected OVX group), or BcEx (□ or ■, 0.5 or 3.0 mg/kg/day BcEx-injected OVX group). (B) Body weight gain after 5 weeks of treatments. *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$ vs. the OVX-control group by one-way ANOVA followed by Dunnett's test ($n = 6-7$, respectively).

Figure 1. Effect of subcutaneously injected BcEx, 17 β -estradiol (E2), and raloxifene (RLX) on body weight in ovariectomized rats

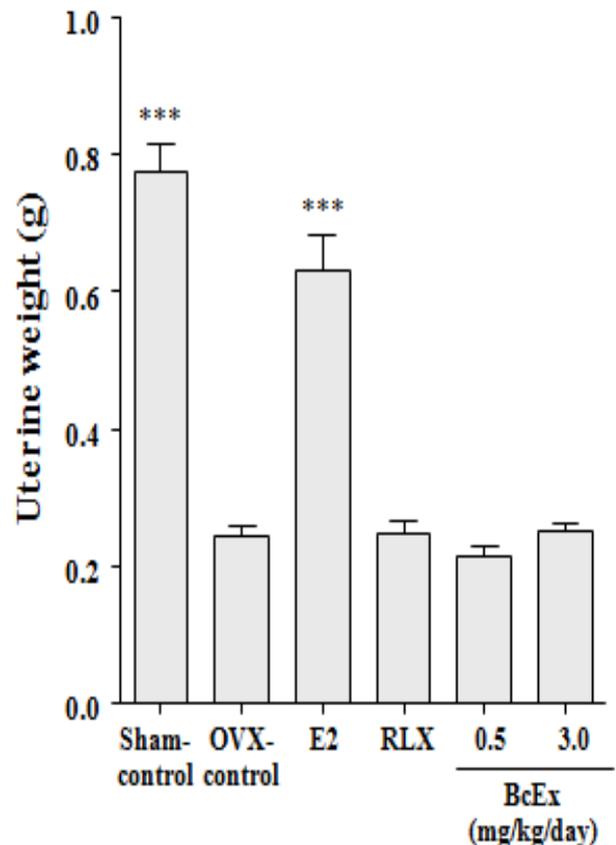
3. Results

3.1. Effects of BcEx, E2, and RLX on Body and Uterine Weights in OVX Rats

Body weight increased significantly in all OVX groups 2 weeks after surgery, compared with the sham-control group (Figure 1A). The lower concentration BcEx treatment (0.5 mg/kg/day) administered for 5 weeks had an increased effect on body weight gain to the level of the OVX-control group, but injections of E2, RLX, and higher concentrations of BcEx (3.0 mg/kg/day) significantly reduced body weight gain (Figure 1B). The uterine weight of the OVX subgroup decreased significantly in the BcEx and RLX treatment groups compared with the sham-control group, but the E2-treated rats did not differ (Figure 2).

3.2. Effects of BcEx, E2, and RLX on Serum Lipid Profiles in OVX rats

The lower concentrations of BcEx in OVX rats did not affect serum total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), or triglyceride (TG) levels compared with those in OVX-control rats (Table 1). However, rats that received higher concentrations of BcEx exhibited significantly lower TC, whereas TG and HDL-C levels remained unaffected. Additionally, rats injected with the higher concentration of BcEx exhibited lower LDL-C levels. RLX also exerted effects on serum TC, LDL-C, and TG levels similar to those of BcEx. In contrast, E2 significantly decreased TC and LDL-C levels, increased TG levels, but did not affect HDL-C levels.



*** $P < 0.001$ vs. the OVX-control group by one-way ANOVA followed by Dunnett's test ($n = 6-7$, respectively).

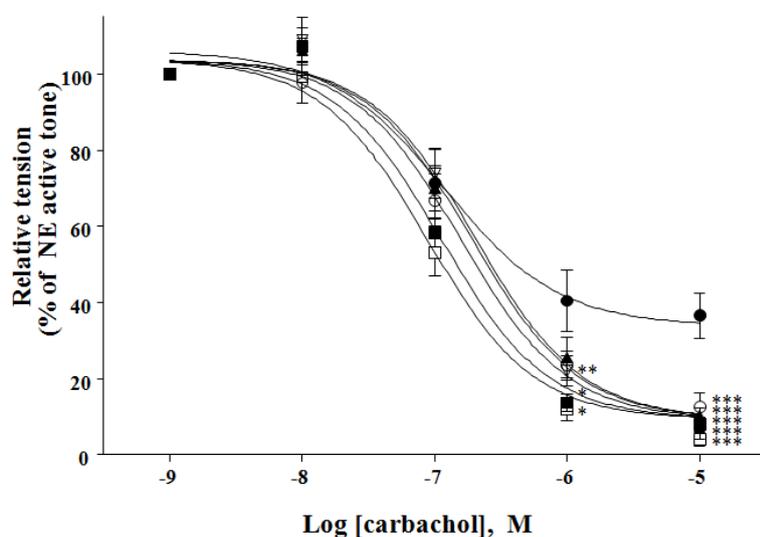
Figure 2. Effect of BcEx (0.5 or 3.0 mg/kg/day), 17 β -estradiol (E2, 0.5 mg/kg/day), and raloxifene (RLX, 2.5 mg/kg/day) on uterine weight in ovariectomized rats after 5 weeks of treatments

Table 1. Effects of subcutaneously injected BcEx (0.5 or 3.0 mg/kg/day), 17 β -estradiol (E2, 0.5 mg/kg/day), and raloxifene (RLX, 2.5 mg/kg/day) on serum lipid profiles in ovariectomized rats; serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels in sham and OVX rats after 5 weeks of treatments

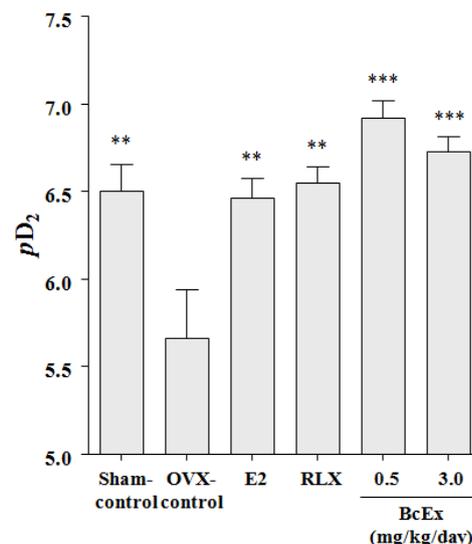
	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	TG (mg/dL)
Sham-control	88.14 \pm 8.41*	30.14 \pm 2.71	46.03 \pm 5.45	59.86 \pm 10.64
OVX-control	107.20 \pm 5.03	34.00 \pm 2.41	57.92 \pm 1.93	76.40 \pm 11.69
E2 (0.5 mg/kg/day)	81.60 \pm 3.66*	30.20 \pm 1.39	24.72 \pm 6.11***	133.40 \pm 23.70*
RLX (2.5 mg/kg/day)	78.60 \pm 3.28**	32.40 \pm 2.32	28.16 \pm 2.48**	90.20 \pm 5.27
BcEx (0.5 mg/kg/day)	107.00 \pm 3.59	32.40 \pm 1.78	59.88 \pm 3.89	73.60 \pm 10.98
BcEx (3.0 mg/kg/day)	82.00 \pm 6.57*	26.17 \pm 2.09	44.40 \pm 5.92	57.17 \pm 7.04

Values are expressed as mean \pm SEM (n = 6–7 per group). Statistical significance is indicated by *** P < 0.001, ** P < 0.01, and * P < 0.05 vs. the OVX-control group (one-way ANOVA followed by Dunnett's test).

A



B



(A) Each symbols indicate the relative tension observed in arteries from sham-operated (Sham-control, \circ) and ovariectomized rats treated with vehicle (OVX-control, \bullet), 17 β -estradiol (E2, ∇ , 0.5 mg/kg/day), raloxifene (RLX, \blacktriangle , 2.5 mg/kg/day), and BcEx (\square or \blacksquare , 0.5 or 3.0 mg/kg/day, respectively). (B) The pD_2 values were calculated as negative logarithm of the dilator concentration that induced 50% of the maximal relaxation. *** P < 0.001, ** P < 0.01, and * P < 0.05 vs. OVX-control group by one-way ANOVA followed by Dunnett's test (n = 5–7, respectively).

Figure 3. Vasomotor responses to carbachol in rat thoracic aorta pre-contracted with 300 nM norepinephrine

3.3. Effects of BcEx, E2, and RLX on Vasomotor Responses to Carbachol in NE-precontracted Isolated Aortic Rings

Carbachol relaxed NE-precontracted endothelium-intact rat aortic rings from OVX rats in a dose-dependent manner (Figure 3A). However, the relative magnitude of relaxation in response to carbachol was higher in arteries isolated from rats injected with BcEx, E2, or RLX. The pD_2 value of the relaxation response to carbachol was significantly lower in the OVX-control group compared with that in the other groups (Figure 3B), suggesting that BcEx, E2, and RLX improved the vasomotor responses to carbachol in OVX rat aorta.

4. Discussion

To our knowledge, this is the first study to compare the effects of BcEx, E2 and RLX on serum lipid profiles and vasomotor responses to those of carbachol in isolated

aortic rings contracted by NE. The cardiovascular protective effects of estrogen are mediated by inhibition of body weight gain, atrophy of the uterus and a decrease in serum cholesterol levels [20]. Additionally, estrogen protects against vascular injuries by increasing NO production in damaged arteries [21].

RLX is a second-generation SERM that improves the lipid profiles of menopausal women [22]. Although estrogen exerts cardiovascular protective effects by inhibiting body weight gain [20], the effects of RLX on body weight are a matter of debate [7]. In this study, RLX and E2 treatments attenuated body weight gain, but BcEx did not. Our findings conflict with a previous report that BcEx attenuated body weight gain in OVX rats [12]. The lower doses of BcEx used in our study (0.5 and 3.0 mg/kg/day), compared with the 135 mg/day used in previous studies, and the difference between the method of administration (subcutaneous injection vs. oral intake) could explain these differences. Estrogen exerts an uterotrophic effect in OVX female rats, but chronic estrogen replacement therapy increases the risk of cancer in reproductive tissues,

particularly the uterus [23]. However, Seidlova-Wuttke et al. [4] reported that RLX treatment did not increase uterine weight significantly in OVX rats. In this study, neither BcEx nor RLX influenced uterine weight, suggesting that BcEx may act on the OVX rat uterus in a SERM-like manner.

BcEx reduced TC, LDL-C and TG levels in OVX rats in a concentration-dependent manner, albeit to a lesser degree than did RLX. Postmenopausal changes in lipid profiles may contribute to an increased risk of cardiovascular disease [2]. As reported by previous studies, estrogen administration decreased serum TC and LDL-C levels but increased TG levels in OVX rats [24]. TGs are considered to play a key role in cardiovascular risk in postmenopausal women [25]; therefore, increases in TG levels are considered undesirable changes associated with estrogen therapy. In contrast, RLX reduces serum TC and LDL-C levels in humans [26] and TG levels in animal models [7]. Furthermore, clinical studies have reported that RLX reduces LDL-C levels but does not affect TG levels [26]. In this study, BcEx improved serum lipid profiles without increasing TG levels in OVX rats, suggesting that BcEx behaved in a RLX-like manner in terms of its effect on serum lipid profiles.

During the postmenopausal period, decreased NO availability in endothelial cells elicits morphological alterations, which is an important factor in the development of cardiovascular diseases [27]. RLX and E2 exert vasorelaxant effects by upregulating NO production in the endothelium [6,28]. In previous studies, we demonstrated that the vasorelaxant effects of BcEx are mediated by enhancement of the NO/cGMP system and inhibition of calcium influx into vascular smooth muscle cells [13]. Additionally, carbachol elicits endothelium-dependent, NO-mediated relaxation in isolated rat aortae [29]. In our study, the vasomotor response to carbachol in isolated aortas contracted by NE was reduced in OVX-control rats compared with sham-control rats. However, BcEx injection significantly increased the *pD2* value of carbachol-induced relaxation to the level of the sham-control, suggesting an improved vasomotor response. Wong et al. [14] showed that OVX did not affect the impaired endothelial function, but that relaxation was transformed to slight contraction in response to higher concentrations of acetylcholine. Additionally, Lamas et al. [30] suggested that RLX and E2 treatments restored the decreased acetylcholine-induced relaxant responses in OVX rats. Therefore, BcEx improved vasomotor responses in OVX rats in a manner similar to that by RLX and E2.

5. Conclusion

The findings of this study demonstrate that BcEx improved serum lipid profiles and increased the vasomotor responses to carbachol in aortic rings from OVX rats. Although the effects of BcEx on serum lipid profiles were similar to those of RLX rather than those of E2, the vasomotor responses to BcEx were consistent with those to E2 and RLX. Therefore, our results suggest that long-term administration of BcEx exerts cardioprotective effects by improving serum lipid profiles and vasomotor responses via a SERM-like mechanism.

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Conflicts of Interest

The authors declare no conflicts of interest.

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