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Original Article

A new formula CPC22 regulates bone loss, hot flashes, and dysregulated lipid metabolism in ovariectomized postmenopausal mice

Hee-Yun Kim[1], Hyunwoo Jee[2], Hosong Cho[3], Dongjun Park[3], Hyun-Ja Jeong[1*]

[1]*BioChip Research Center, Hoseo University, 20 Hoseo-ro, 79 Beon-gil, Baebang-eup, Asan 31499, Republic of Korea.*
[2]*Department of Science in Korean Medicine, Graduate School, Kyung Hee University, Seoul 02447, Korea*
[3]*LG Household &Healthcare Research Park, Seoul 07795, Republic of Korea*

ABSTRACT

Background and objective: A new formular CPC22 consists of *Cynanchum wilfordii* root, *Pueraria thomsonii* flower, and *Citrus unshiu* peel and has been developed to improve the postmenopausal symptoms. The research intended to evaluate whether CPC22 would regulate bone loss, hot flashes, and dysregulated lipid metabolism in ovariectomized (OVX) postmenopausal mice.

Method: The OVX mice were orally administered with CPC22 daily for 7 weeks.

Results: CPC22 regulated OVX-induced bon loss by enhancing serum osteoprotegerin, alkaline phosphatase, and osteocalcin levels and diminishing serum receptor-activator of the NF-κB ligand (RANKL), collagen type 1 cross-linked N-telopeptide, and tartrate-resistant acid phosphatase levels. As a result of CPC22 treatment, notable decreases in tail skin temperature and rectal temperature were observed, along with diminishment in hypothalamic RANKL and monoamine oxidase A levels and enhancement in hypothalamic serotonin (5-HT), norepinephrine, dopamine, 5-HT2A, and estrogen receptor-β levels. CPC22 enhanced levels of serum estrogen and diminished levels of serum follicle-stimulating hormone and luteinizing hormone. CPC22 regulated levels of serum lipid metabolites, including total cholesterol, triglycerides, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol. Furthermore, CPC22 diminished levels of serum blood urea nitrogen, creatine kinase, alanine transaminase, aspartate aminotransferase, and lactate dehydrogenase and restored vaginal dryness without affecting uterus atrophy index and vagina weights.

Conclusion: Therefore, these results indicated that CPC22 improves OVX-induced bone loss, hot flashes, and dysregulated lipid metabolism by compensating for estrogen deficiency without side effects, suggesting that CPC22 may be used for the prevention and treatment of post menopause.

Keywords CPC22, postmenopause, osteoporosis, hot flashes, lipid metabolites

*Correspondence: Hyun-Ja Jeong
E-mail: hjjeong@hoseo.edu
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INTRODUCTION

Women typically spend the last third of their lives in menopause after the end of their reproductive period. Approximately 70% of women experience symptoms due to estrogen

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deficiency during the menopause period.[1] The common symptoms are depression, insomnia, vasomotor symptoms, fatigue, night sweats, cognitive impairment, and increased risk for cardiovascular disorder, hyperlipidemia, and osteoporosis.[2-4] Among the various menopausal symptoms, many studies are being conducted on osteoporosis, hot flashes, and lipid metabolic diseases.

Healthy bones go through two opposing processes, including bone formation by osteoblasts and bone resorption by osteoclasts, and they are continually remodeled throughout their life.[5] However, imbalance of bone formation and bone resorption causes osteoporosis.[6] Osteoporosis is characterized by a high risk of fractures, reduction of bone mineral density (BMD), and breakdown of bone architecture.[7] During menopause, estrogen deficiency is the most important risk factor for induction of osteoporosis.[6]

Vasomotor symptoms (hot flashes and night sweats) trouble most women during the menopause period and reduce quality of life.[8] Women with hot flashes have a reduced thermoneutral zone and an increased core temperature by thermoregulatory dysfunction.[9] Estrogen is a neuromodulator of the central serotonin (5-hydroxytryp, 5-HT) system and affects serotonin synthesis and reuptake.[10] Serotonin increased by estrogen in brain plays an important role in thermoregulation.[11] Therefore, decrease in estrogen during menopause causes thermoregulatory dysfunction by blocking serotonin pathway.[12] Recently, RANKL has been reported as a major causative factor in menopausal dysthermoregulation.[13]

Dyslipidemia is caused by abnormal changes in the amount of lipids in the blood due to abnormal lipoprotein metabolism, resulting in hypercholesterolemia, hypertriglyceridemia, and low-density lipoproteinemia.[14] Such dyslipidemia is known to be the cause of cardiovascular disease during menopause.[15] Levels of blood lipid in postmenopausal women are closely associated with endogenous estrogen levels.[16] Estrogen is synthesized in the ovaries using low-density lipoprotein cholesterol (LDL), but since LDL in the blood of postmenopausal women cannot be used for estrogen synthesis, estrogen deficiency and dyslipidemia are caused.[17] In postmenopausal women and animal model, dysregulated lipid metabolism results in increased LDL levels and diminished high-density lipoprotein-cholesterol (HDL) levels.[16,18]

Although estrogen therapy has beneficial effects in managing postmenopausal symptoms, the debates about hormone therapy have continues because of the risk of ovarian and breast cancer and cardiovascular disease.[19,20] Therefore, research on effective and safe alternative therapy that can alleviate postmenopausal symptoms is needed. In Asia, people have used herbal medicine to relieve various diseases for a long time. Extract of *Cynanchum wilfordii* (CW), *Pueraria thomsonii* (PT), or *Citrus unshiu* (CU) has been used to alleviate osteoporosis, hot flashes, and/or anxiety.[21-23] A new formular CPC22 consists of *Cynanchum wilfordii* root, *Pueraria thomsonii* flower, and *Citrus unshiu* peel and has been developed in an optimal ratio to alleviate various postmenopausal symptoms through an *in vitro* assay. Herein, the aim of the present study is to demonstrate the regulatory effect of CPC22 against menopause in estrogen deficiency *in vivo* model induced by ovariectomy.

MATERIALS AND METHODS

Preparation of CPC22

CPC22 powder was manufactured by mixing water-extracted CW powder (DAEDONG

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KOREA GINSENG CO., LTD., Geumsan, Republic of Korea), 70% ethanol-extracted PT powder (HYUNDAI BIOLAND.CO., LTD, Ansan, Republic of Korea), and water-extracted CU powder (SK bioland CO., LTD.) in a ratio of 2:0.57:0.43. It was supplied by LG Household & Health Care Ltd. (Seoul, Republic of Korea). CPC22 was dissolved in distilled water. Concentrations of CPC22 (170 mg/kg, 345 mg/kg, and 690 mg/kg) were used according to previous studies.[21,24] β-estradiol (E_2, Sigma Chemical Co., St. Louis, MO, USA) was used as a positive control.

Animals and treatments

Ovariectomized (OVX) mice (weight 27~29 g, eight-week-old, an experimental model mimicking postmenopausal women) were purchased from Dae-Han Experimental animal center (Eumsung, Republic of Korea). Mice were used in this study after acclimatization for one week. All procedures for the Care and Use of Laboratory Animals were reviewed and approved by the Animal Ethics Committee of Kyung Hee University (KHSASP-21-285). The animals were maintained under standard condition (a 12 h light and 12 h dark cycle, 50~60% humidity, and 20~23°C temperature). The mice were randomly divided to six groups (n = 5 per group): Sham mice, OVX mice treated with distilled water, OVX mice treated with 170 mg/kg, 345 mg/kg, and 690 mg/kg CPC22, and OVX mice treated with 100 nM E_2. The OVX mice were orally administered with distilled water, CPC22, and E_2 daily for 7 weeks. Changes of tail skin temperature (TST, an indicator of hot flashes) and core body temperature (CBT, rectal temperature) were measured according to previous report.[21] Samples of blood, bone, hypothalamus, vagina, and uterus were obtained on the last day after CPC22 feeding for 7 weeks.

Analysis of menopause-related biomarkers in serum and hypothalamus

The ELISA kits for osteoprotegerin (OPG*)*, collagen type *1* cross-linked N-telopeptide (Ntx1*),* tartrate-resistant acid phosphatase (TRACP*)*, E_2, follicle-stimulating hormone (FSH), luteinizing hormone (LH), 5-HT, norepinephrine (NE), and dopamine were obtained from MyBioSource Inc. (San Diego, CA, USA). The ELISA kit for RANKL was purchased from R&D Systems, Inc. (Minneapolis, MN, USA). The ELISA assay kit for alkaline phosphatase (ALP) was obtained from Abcam (Cambridge, MA, USA). The ELISA kit for osteocalcin was purchased from *LSBio (Seattle*, WA, *USA).* The levels of total cholesterol, triglyceride, LDL, HDL, blood urea nitrogen (BUN), creatine kinase (CK), alanine transaminase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) in serum were detected using a DRI-CHEM NX500i (FUJIFILM Co., Tokyo, Japan), respectively. The levels of nitric oxide (NO) in serum were evaluated by Griess method.[25]

RNA isolation and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNAs were isolated from the tissues of uterus and hypothalamus using an easy-BLUE™ RNA extraction kit (iNtRON Biotech, Sungnam, Republic of Korea). RNA was reverse transcribed into complementary DNA using an AccuPower® RT PreMix (Bioneer Corporation, Daejeon, Republic of Korea). We conducted qRT-PCR with primers (Supplementary Table 1). The mRNA expression levels were analyzed using an ABI StepOne real-time PCR System (Applied Biosystems, Foster City, CA, USA) and normalized to housekeeping gene GAPDH. All data were analyzed using a ΔΔCT method.

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Microcomputed tomography (μCT)

The values of BMD, total porosity, trabecular number (Tb.N), trabecular bone volume (BV/TV), connectivity density (Conn.D), trabecular separation (Tb.Sp), and trabecular thickness (Tb.Th) in proximal tibia of OVX mice were analyzed by using a μCT according to previous report.[26]

Vagina histology

The vaginal tissues were fixed in 10% formaldehyde and embedded in paraffin. Vaginal tissue sections (4 μm) were stained with 1% methylene blue (Sigma Chemical Co.) for 45 min and epithelial layers of vagina were observed under microphotography. Images were taken at x 400 magnification.

Determination of uterus atrophy index

All mice were sacrificed under anesthesia. Each uterus was taken and weighed. For each mouse, uterus atrophy index was evaluated, which was defined as the weight of the uterus divided by the total body weight.

Statistics analysis

All values were represented as mean ± standard error of the mean (SEM). The statistically significant was evaluated by an independent *t*-test or an ANOVA followed by Tukey or Dunnett's post-hoc test using a software SPSS 25.0 version for Windows (SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant when the P value < 0.05.

RESULTS

Improvement effect of CPC22 in bone loss of OVX mice

OVX mice exhibits the low levels of serum OPG, ALP, and osteocalcin and the high levels of serum RANKL, Ntx1, and TRACP.[18] Initially, to determine the improvement effect of CPC22 on bone metabolism-related biomarkers in OVX mice, levels of serum biomarkers of bone formation (OPG, ALP, and osteocalcin) and bone resorption (RANKL, Ntx1, and TRACP) were analyzed. In the OVX group, the levels of serum OPG, ALP, and osteocalcin were obviously diminished compared with those observed in the Sham group (Figs. 1A-C, $P < 0.05$). Treatment with CPC22 or E_2 significantly enhanced the levels of serum OPG, ALP, and osteocalcin compared with those measured in the OVX group (Figs. 1A-C, $P < 0.05$). Compared with the OVX group, treatment with CPC22 or E_2 significantly diminished the levels of serum RANKL, Ntx1, and TRACP (Figs. 1D-F, $P < 0.05$). The μCT tibia images representing each group are shown in Figure 1G (upper). As a result of image analysis, treatment with CPC22 (690 mg/kg) or E_2 significantly enhanced the values of BMD and Tb.N, while diminished the values of total porosity compared with those observed in the OVX group (Figs. 1G-I, $P < 0.05$). However, there were no significances in the values for BV/TV, Conn.D, Tb.Sp, and Tb.Th between the OVX group and CPC22 (690 mg/kg) group (Supplementary Table 2). Treatment with E_2 significantly enhanced the values of BMD, Tb.N, and Tb.Th, while diminished the values of total porosity and Tb.Sp compared with those observed in the OVX group (Figs. 1G-I and Supplementary Table 2, $P < 0.05$). In addition, treatment with CPC22 did not exhibit significant influences on body weight (data not shown).

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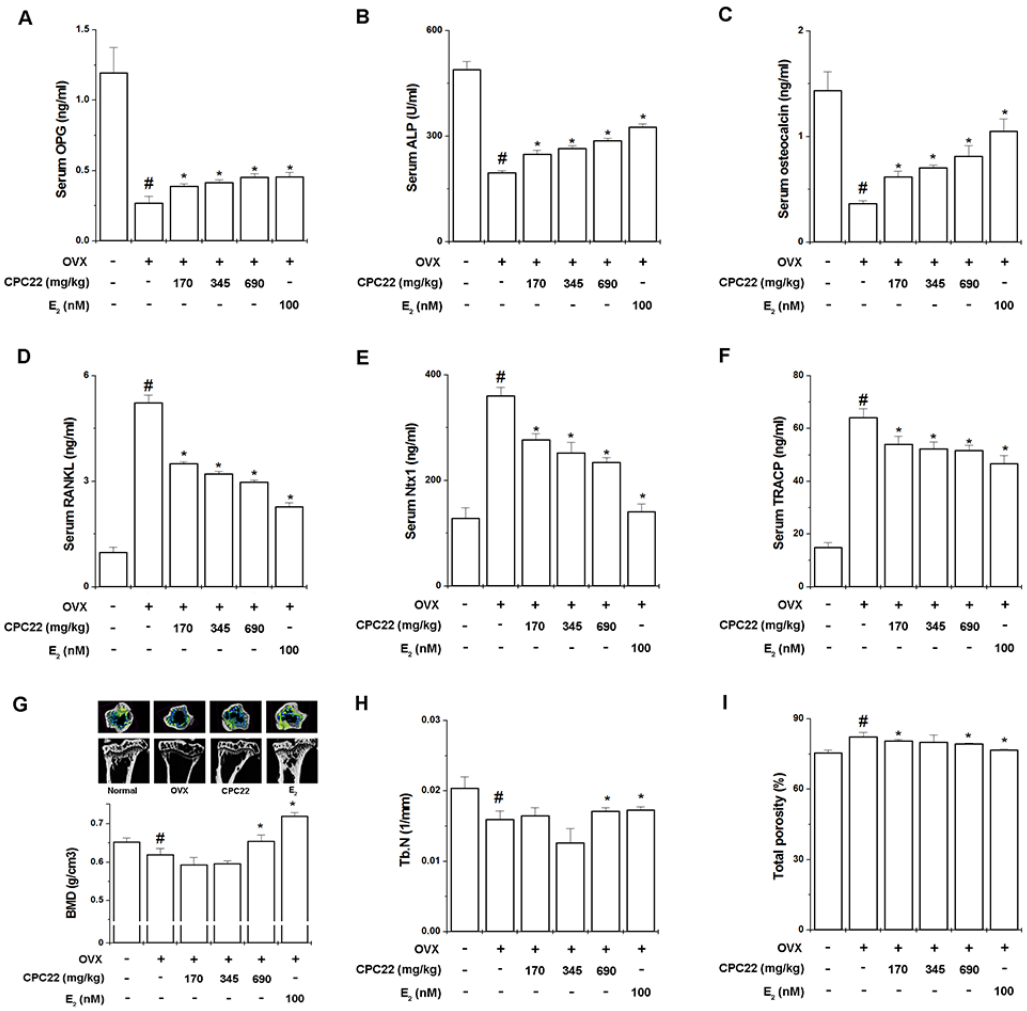

Figure 1. Improvement effect of CPC22 in bone loss of OVX mice. All values are expressed as mean ± SEM (n = 5). Serum (1A) OPG, (B) ALP, (C) osteocalcin, (D) RANKL, (E) Ntx1, and (F) TRACP. The various bone structural parameters in proximal tibia of OVX mice were analyzed by μCT. (G) cross-sectional μCT images of tibiae (upper) and BMD (lower), (H) Tb.N, and (I) Total porosity. # P < 0.05 vs. Sham group and * P < 0.05 vs. OVX group.

Improvement effect of CPC22 in hot flashes of OVX mice

Ovariectomy induces menopausal vasomotor diseases such as hot flashes.[27] To determine whether CPC22 would improve OVX-induced hot flashes, changes of TST and CBT and levels of hot flashes-related indicators were analyzed. As shown in Figures 2A-B, changes of TST and CBT in the OVX group were significantly higher than those observed in the Sham group for 7 weeks ($P < 0.05$). However, treatment with CPC22 or E_2 significantly diminished the changes of TST and CBT at 5~7 weeks compared with the OVX group (Figs. 2A-B, $P < 0.05$).

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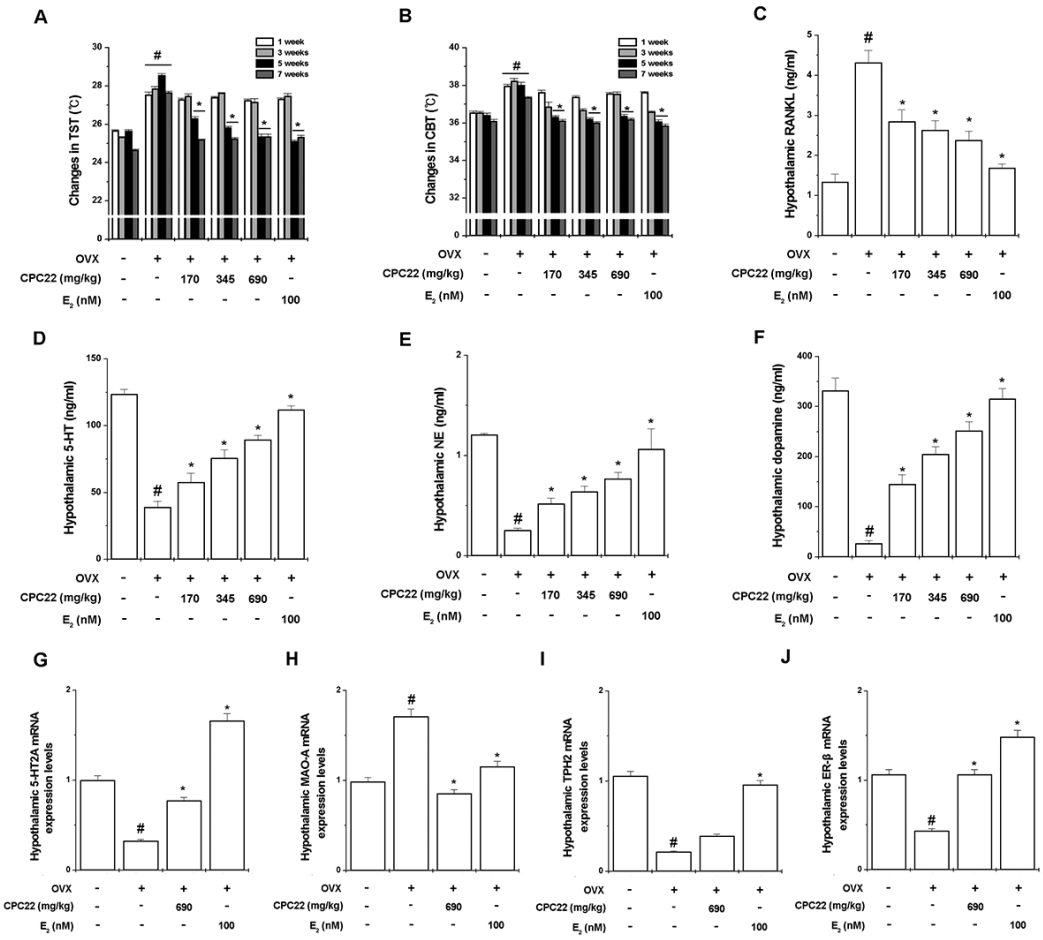

Figure 2. Improvement effect of CPC22 in hot flashes of OVX mice. All values are expressed as mean ± SEM (n = 5). Changes in (A) TST and (B) CBT of OVX mice for 7 weeks. Hypothalamic (C) RANKL, (D) 5-HT, (E) NE, and (F) dopamine levels. Hypothalamic (G) 5-HT2A, (H) MAO-A, (I) TPH2, and (J) ER-β mRNA expression levels. # P < 0.05 vs. Sham group and * P < 0.05 vs. OVX group.

Estrogen affects the levels of hypothalamic RANKL, 5-HT, NE, and dopamine, which regulate the hot flashes.[21,28] To investigate the regulatory mechanism of CPC22 in hot flashes, levels of hypothalamic RANKL, 5-HT, NE, and dopamine were analyzed by ELISA method. Ovariectomy resulted in increase of RANKL levels and decrease of 5-HT, NE, and dopamine levels in the hypothalamus compared with those observed in the Sham mice (Figs. 2C-F, $P < 0.05$). However, treatment with CPC22 or E_2 significantly diminished the levels of hypothalamic RANKL, while enhanced the levels of hypothalamic 5-HT, NE, and dopamine compared with those observed in the OVX group (Figs. 2C-F, $P < 0.05$). In addition, CPC22 (690 mg/kg) or E_2 significantly increased transcription levels of hypothalamic 5-HT receptor (5-HT2A) and estrogen receptor (ER)-β and reduced transcription levels of hypothalamic monoamine oxidase A (MAO-A) except for tryptophan hydroxylase2 (TPH2) (Figs. 2G-J, $P < 0.05$). Furthermore, treatment with CPC22 or E_2 significantly enhanced the transcription levels of uterine ER-β compared with those measured in the OVX group (Supplementary Fig. 1, $P < 0.05$).

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OVX mice resulted in lower levels of serum E_2 and NO and higher levels of FSH and LH compared with the Sham mice and these changes cause hot flashes.[21,29,30] The treatment with CPC22 or E_2 significantly enhanced the levels of serum E_2 and NO (Figs. 3A-B, $P < 0.05$). CPC22 at 690 mg/kg induced an approximately 3-fold increase in the E_2 level compared with the OVX group, but did not reach Sham control levels (Fig.3A).

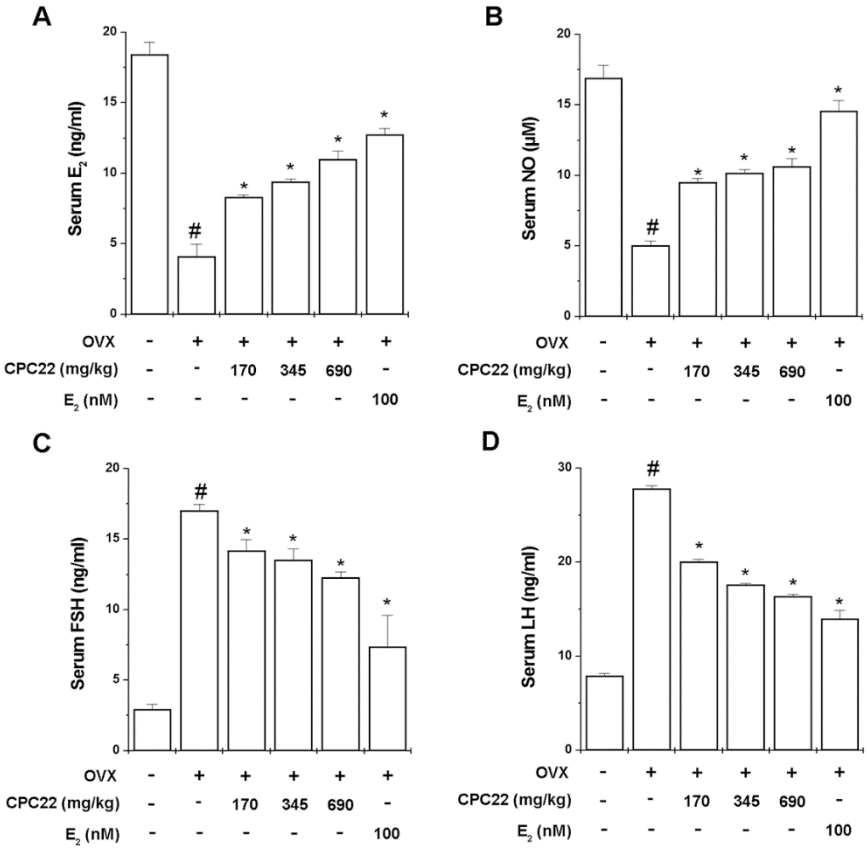

Figure 3. Improvement effect of CPC22 in serum E_2, NO, FSH, and LH levels of OVX mice. All values are expressed as mean ± SEM (n = 5). Serum (A) E_2, (B) NO, (C) FSH, and (D) LH. # $P < 0.05$ vs. Sham group and * $P < 0.05$ vs. OVX group.

Additionally, CPC22 treatment of OVX mice had significant regulatory effects in the levels of serum FSH and LH and there was a trend of dose-dependent manner (Figs. 3C-D, $P < 0.05$). CPC22 at 690 mg/kg resulted in an approximately 34% and 56% decrease in FSH and LH compared with the OVX group, respectively (Figs. 3C-D).

Improvement effect of CPC22 in dysregulated lipid metabolism of OVX mice

To investigate the improvement effect of CPC22 in levels of dysregulated lipid metabolite, we analyzed levels of serum total cholesterol, triglyceride, LDL, and HDL. In the OVX group, serum total cholesterol, triglyceride, and LDL levels were significantly higher than those observed in the Sham group, but serum HDL levels were significantly lower than those observed in the Sham group (Fig. 4, $P < 0.05$). Treatment with CPC22 or E_2 displayed markedly lower serum total cholesterol, triglyceride, and LDL levels and higher serum HDL levels than that of OVX

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group (Fig. 4, $P < 0.05$). CPC22 at 690 mg/kg diminished the levels of LDL by an approximately 28% and enhanced HDL by an approximately 62% (Figs. 4C-D).

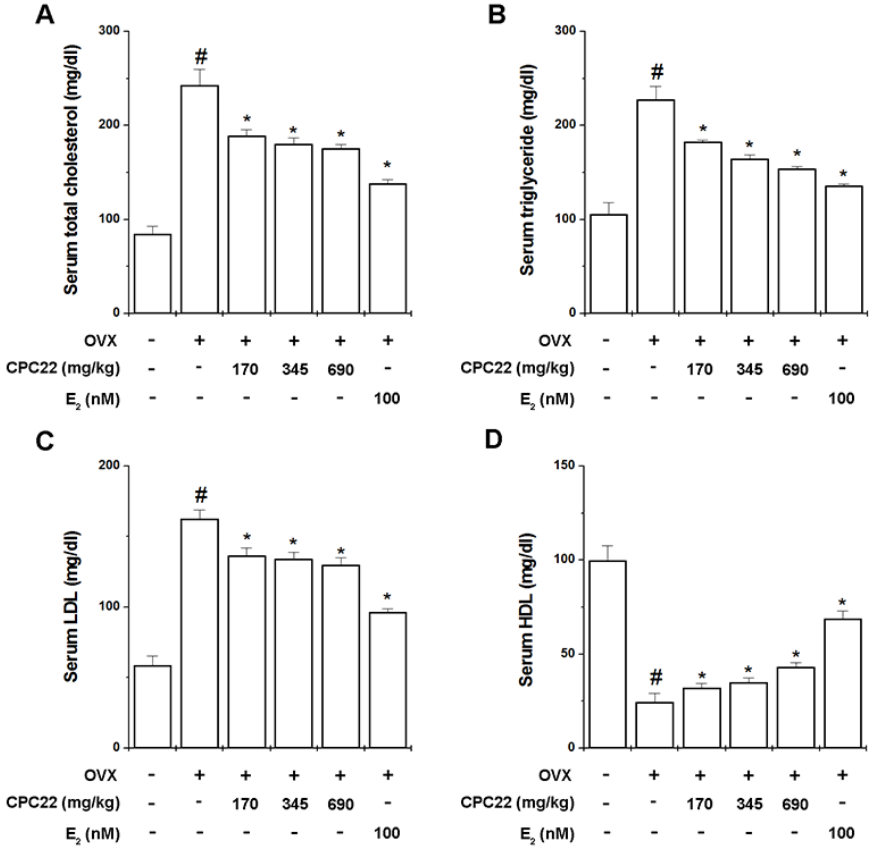

Figure 4. Improvement effect of CPC22 in serum lipid parameters of OVX mice. All values are expressed as mean ± SEM (n = 5). Serum (A) total cholesterol, (B) triglyceride, (C) LDL, and (D) HDL. # P < 0.05 vs. Sham group and * P < 0.05 vs. OVX group.

Improvement effect of CPC22 in serum biochemistry and vaginal histology of OVX mice

To investigate the side effects of CPC22, levels of serum biochemical parameters (BUN, CK, ALT, AST, and LDH) were analyzed using a DRI CHEM NX500 analyzer. As shown in Figures 5A-E, levels of serum BUN, CK, ALT, AST, and LDH in the OVX group were significantly higher than those in the Sham group, whereas treatment with CPC22 or E_2 significantly diminished the OVX-induced serum BUN, CK, ALT, AST, and LDH levels ($P < 0.05$).

In methylene blue-stained vagina tissues, treatment with CPC22 or E_2 showed the multilayered epithelium layers and restored the epithelial thickness damaged by OVX to Sham level (Fig. 5F). In addition, treatment with CPC22 did not affect uterus atrophy index values and vagina weights (Supplementary Fig. 2). However, treatment with E_2 markedly increased the uterus atrophy index and vagina weights compared with those observed in the OVX group (Supplementary Fig. 2).

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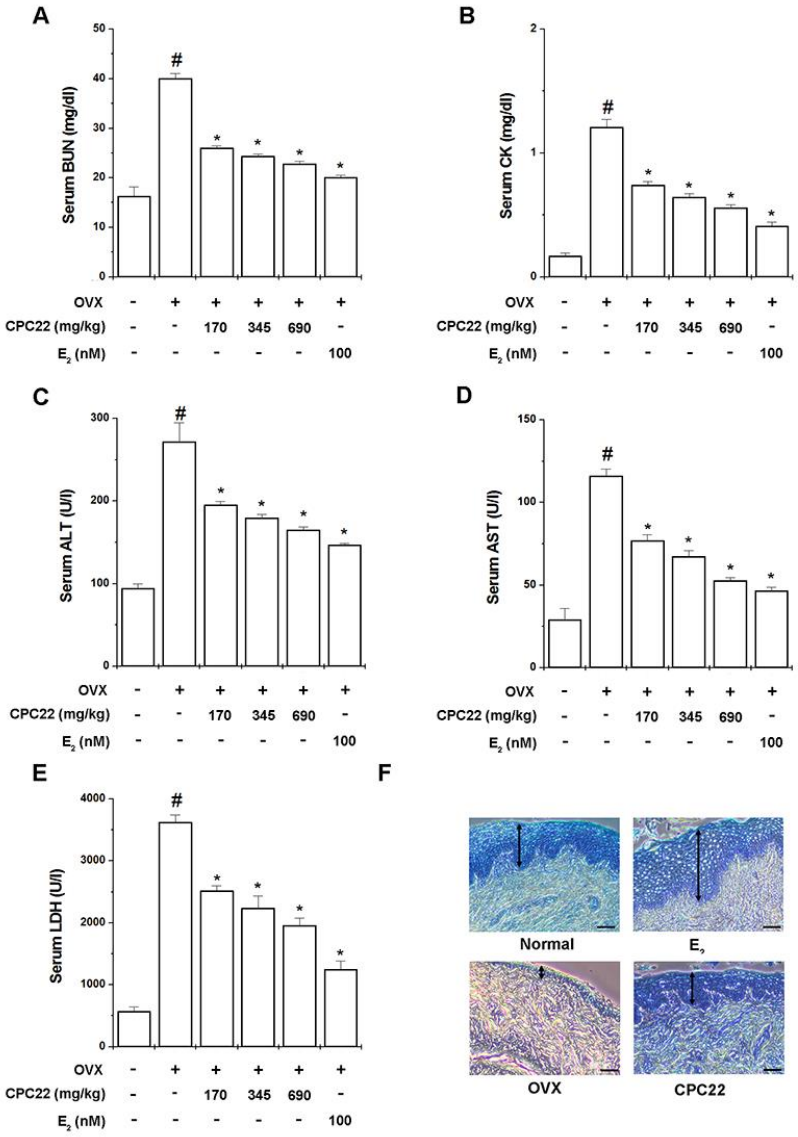

Figure 5. Improvement effect of CPC22 in serum biochemistry and vaginal histology of OVX mice. All values are expressed as mean ± SEM (n = 5). Serum (A) BUN, (B) CK, (C) ALT, (D) AST, and (E) LDH. (F) Vaginal sections stained with methylene blue. # $P < 0.05$ vs. Sham group and * $P < 0.05$ vs. OVX group.

DISCUSSION

The OVX animal model shows many clinical features of postmenopause caused by estrogen deficiency in women and is widely used in research on improvement of postmenopause. In the present study, we showed that CPC12 regulated the levels of osteoporosis-, hot flashes-, and dyslipidemia-related biomarkers in OVX animal model.

The incidence of postmenopausal osteoporosis is rapidly increasing with the aging of the population.[6] Thus, compounds that can increase osteoblast activity and decrease osteoclast activity are highly needed for the improvement of osteoporosis. Currently, calcium, vitamin D, bone resorption inhibitors, and osteogenesis-promoting agents are used to treat osteoporosis. OVX mice show the decrease of OPG, ALP, and osteocalcin levels,

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whereas increases of RANKL, Ntx1, and TRACP levels.[18] Estrogen replacement therapy improves the values of bone loss through increasing the OPG levels and decreasing the RANKL levels.[31] Pyrroloquinoline quinone alleviates the OVX-induced osteoporosis by reducing osteoclastic bone resorption and enhancing osteoblastic bone formation.[32] Lee et al. (2018) reported that anti-osteoporotic effect of CW is associated with inhibition of bone resorption and induction of bone formation.[22] PCE17 (a mixed extract of flowers of PT and peels of CU), which has an improving effect on the bone health, increased the levels of OPG, ALP, and osteocalcin, whereas decreased the levels of RANKL, Ntx1, and TRACP in the OVX mice.[18] However, there was no significant change in the levels of serum RANKL between the OVX group and CW (120 mg/kg) group (data not shown). PCE12 (115 mg/kg) did not significantly affect the levels of OVX-induced RANKL, OPG, ALP, and osteocalcin.[18] In the present study, we demonstrated that CPC22 at dosages of 170, 345, and 690 mg/kg significantly improved the OVX-induced bone loss and showed a significant effect on all biomarkers related to bone formation and bone resorption. Therefore, we suggest that CPC22 synergistically enhanced the effectiveness of CW or PCE17 on management of osteoporosis induced by OVX.

Hot flash is a form of flushing due to reduced estrogen levels and is the most common postmenopausal symptom.[8] It is regulated by hypothalamic neurotransmitters.[33] Estrogen modulates the expression of 5-HT2A receptors, MAO-A (5-HT catabolizing enzyme), and TPH2 (a rate-limiting enzyme in the synthesis of 5-HT) and alleviates hot flashes by enhancing the levels of 5-HT.[34] Reduction in hypothalamic NE and dopamine induced by estrogen deficiency caused hot flashes during menopause.[21,28,35] An increase in FSH and LH along with a decrease in estrogen and NO increases the severity of hot flashes.[29,30] A significant increase in RANKL is also considered a biomarker for hot flashes.[36] Currently, estrogen alone or in combination with progesterone is used to improve the hot flashes. However, there is increasing interest in safe treatments that are more effective than hormone therapy and reduce side effects for improving hot flashes.[37] Sicat and Broka (2004) reported that non-hormonal drugs such as 5-HT and/or NE reuptake inhibitors managed the vasomotor symptoms to overcome the side effects of hormone therapy.[38] A previous study showed that buspirone administration has effectively alleviated the hot flashes in OVX mice.[39] In our previous study, we reported that PCE17 alleviated the hot flashes by regulation the levels of neurotransmitters, E_2, FSH, LH, NO, and RANKL.[21] In the present study, we demonstrated that CPC12 lead to a significant reduction in TST and CBT through the regulation of E_2, FSH, LH, NO, RANKL, 5-HT, NE, and dopamine levels. Therefore, we suggest that CPC22 has a regulatory effect in OVX-induced hot flashes.

Menopause is accompanied with cognitive deterioration, anxiety, and depression.[40] The estrogen alleviates menopause-induced depression by activation of brain 5-HT, NE, and dopamine systems.[41] Decreased 5-HT, NE, and dopamine levels in brain are associated with depression.[42] Therefore, we predict that the levels of hypothalamic 5-HT, NE, and dopamine increased by CPC22 may also have a beneficial effect on postmenopause-induced depression.

Dyslipidemia caused by estrogen deficiency during menopause is known to promote atherosclerosis, and low levels of HDL and high levels of LDL are closely

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related to cardiovascular disease.[14] The loss of estrogen results in a surge in the levels of total cholesterol, triglycerides, and LDL, leading to an increased risk for atherosclerosis.[43] In the OVX mice, the levels of total cholesterol, triglycerides, and LDL are significantly increased, while the levels of HDL are significantly reduced.[18] Lin et al. (2015) reported that atorvastatin, which is a statin medication used to prevent cardiovascular disease, prevented the OVX-induced dyslipidemia.[44] In the present study, we demonstrated that CPC22 diminished the levels of total cholesterol, triglycerides, and LDL and enhanced the levels of HDL. Therefore, we suggest that CPC22 may have a beneficial effect on postmenopause-induced cardiovascular diseases through the regulation of dyslipidemia.

Reduction of estrogen by OVX significantly increases the levels of serum BUN, CK, ALT, AST, and LDH and causes vaginal dryness.[18,21] In the present study, we demonstrated that CPC22 regulated the levels of BUN, CK, ALT, AST, and LDH. However, estrogen-dependent activation of ER induced the hormone imbalance and the proliferation of endometrium, resulting in endometrial hyperplasia.[45] According to recent report, a combination formular containing CW had an estrogen-like effect but did not bind to the ER.[46] In addition, it has been reported that active compounds of PT have a lower binding affinity to ER than soy isoflavones.[47] Although CPC22 significantly increased the levels of E_2, there were significant differences in the levels of FSH and LH compared with the E_2 administration. In addition, CPC22 had no obvious effect on uterus atrophy index and recovered the vaginal dryness to normal levels. Therefore, we suggest that CPC22 improved the postmenopausal symptoms without affecting side effect.

As described above, CPC22 consists of 3 different herbs. Previous research has demonstrated that linoleic acid, which is a major active compound of CW, may prevent the OVX-induced osteoporosis.[48] Tectorigenin, tectoridin, and tectorigenin 7-O-xylosylglucoside, isolated from PT, improved the postmenopausal symptoms through the regulation of OVX-induced osteoporosis and hot flashes.[18,21] CU and its active compound hesperidin have been observed to alleviate the hot flashes, osteoporosis, and dyslipidemia induced by OVX.[18,21] Therefore, it can be seen that the above-mentioned compounds are active compounds of CPC22 in improving postmenopause. However, as we already described, CPC22 is a natural product made up of three types of herbs, it contains many types of compounds. Therefore, further studies will be needed to elucidate other active compounds that appear to create the postmenopausal improving effects of CPC22.

CONCLUSIONS

In conclusion, these results indicated that CPC22 improves OVX-induced bone loss, hot flashes, and dysregulated lipid metabolism by compensating for estrogen deficiency without affecting side effects, suggesting that CPC22 may be used for the prevention and treatment of postmenopause.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

The authors have no competing interests to declare.

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REFERENCES

1. Hoga L, Rodolpho J, Gonçalves B, Quirino B. Women's experience of menopause: a systematic review of qualitative evidence. JBI Database System Rev Implement Rep. 2015;13(8):250-337.
2. del Giorno C, Fonseca AM, Bagnoli VR, Assis JS, Soares JM, Jr., Baracat EC. Effects of Trifolium pratense on the climacteric and sexual symptoms in postmenopause women. Rev Assoc Med Bras (1992). 2010;56(5):558-562.
3. Kang SJ, Choi BR, Kim SH, Yi HY, Park HR, Song CH, Ku SK, Lee YJ. Anti-climacterium effects of pomegranate concentrated solutions in ovariectomized ddY mice. Exp Ther Med. 2017;13(4):1249-1266.
4. Wolff LP, Martins MR, Bedone AJ, Monteiro IM. [Endometrial evaluation in menopausal women after six months of isoflavones]. Rev Assoc Med Bras (1992). 2006;52(6):419-423.
5. Kanis JA, Cooper C, Rizzoli R, Reginster JY. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporos Int. 2019;30(1):3-44.
6. He B, Yin L, Zhang M, Lyu Q, Quan Z, Ou Y. Causal Effect of Blood Pressure on Bone Mineral Density and Fracture: A Mendelian Randomization Study. Front Endocrinol (Lausanne). 2021;12:716681.
7. Kling JM, Clarke BL, Sandhu NP. Osteoporosis prevention, screening, and treatment: a review. J Womens Health (Larchmt). 2014;23(7):563-572.
8. Berin E, Hammar M, Lindblom H, Lindh-Åstrand L, Rubér M, Spetz Holm AC. Resistance training for hot flushes in postmenopausal women: A randomised controlled trial. Maturitas. 2019;126:55-60.
9. Shen W, Stearns V. Treatment strategies for hot flushes. Expert Opin Pharmacother. 2009;10(7):1133-1144.
10. Freedman RR. Menopausal hot flashes: mechanisms, endocrinology, treatment. J Steroid Biochem Mol Biol. 2014;142:115-120.
11. Steininger TL, Gong H, McGinty D, Szymusiak R. Subregional organization of preoptic area/anterior hypothalamic projections to arousal-related monoaminergic cell groups. J Comp Neurol. 2001;429(4):638-653.
12. Wang W, Cui G, Jin B, Wang K, Chen X, Sun Y, Qin L, Bai W. Estradiol Valerate and Remifemin ameliorate ovariectomy-induced decrease in a serotonin dorsal raphe-preoptic hypothalamus pathway in rats. Ann Anat. 2016;208:31-39.
13. Kalkan R, Altarda M, Tosun O. RANKL is a new Epigenetic Biomarker for the Vasomotor Symptom During Menopause. Balkan J Med Genet. 2020;23(1):51-56.
14. Huang D, Hu H, Chang L, Liu S, Liang J, Song Y, Wang X, Zhang H, Wei C, Wu Y. Chinese medicine Bazi Bushen capsule improves lipid metabolism in ovariectomized female ApoE-/- mice. Ann Palliat Med. 2020;9(3):1073-1083.
15. Sima P, Vannucci L, Vetvicka V. Atherosclerosis as autoimmune disease. Ann Transl Med. 2018;6(7):116.
16. Marchand GB, Carreau AM, Weisnagel SJ, Bergeron J, Labrie F, Lemieux S, Tchernof A. Increased body fat mass explains the positive association between circulating estradiol and insulin resistance in postmenopausal women. Am J Physiol Endocrinol Metab. 2018;314(5):E448-456.
17. Thaung Zaw JJ, Howe PRC, Wong RHX. Postmenopausal health interventions: Time to move on from the Women's

www.kci.go.kr

Health Initiative? Ageing Res Rev. 2018;48:79-86.
18. Jeong HJ, Kim MH, Kim H, Kim HY, Nam SY, Han NR, Lee B, Cho H, Moon PD, Kim HM. PCE17 and its active compounds exert an anti-osteoporotic effect through the regulation of receptor activator of nuclear factor-κB ligand in ovariectomized mice. J Food Biochem. 2018;42(5):e12561.
19. Fernandez E, Gallus S, Bosetti C, Franceschi S, Negri E, La Vecchia C. Hormone replacement therapy and cancer risk: a systematic analysis from a network of case-control studies. Int J Cancer. 2003;105(3):408-412.
20. Mennenga SE, Gerson JE, Koebele SV, Kingston ML, Tsang CW, Engler-Chiurazzi EB, Baxter LC, Bimonte-Nelson HA. Understanding the cognitive impact of the contraceptive estrogen Ethinyl Estradiol: tonic and cyclic administration impairs memory, and performance correlates with basal forebrain cholinergic system integrity. Psychoneuroendocrinology. 2015;54:1-13.
21. Han NR, Nam SY, Hong S, Kim HY, Moon PD, Kim HJ, Cho H, Lee B, Kim HM, Jeong HJ. Improvement effects of a mixed extract of flowers of Pueraria thomsonii Benth. and peels of Citrus unshiu Markovich on postmenopausal symptoms of ovariectomized mice. Biomed Pharmacother. 2018;103:524-530.
22. Lee H, Kim MH, Choi YY, Hong J, Yang WM. Effects of Cynanchum wilfordii on osteoporosis with inhibition of bone resorption and induction of bone formation. Mol Med Rep. 2018;17(3):3758-3762.
23. Lim DW, Lee Y, Kim YT. Preventive effects of Citrus unshiu peel extracts on bone and lipid metabolism in OVX rats. Molecules. 2014;19(1):783-794.
24. Lee G, Choi CY, Jun W. Effects of Aqueous Extracts of Cynanchum wilfordii in Rat Models for Postmenopausal Hot Flush. Prev Nutr Food Sci. 2016;21(4):373-377.
25. Lim JH, Kim HY, Lee JS, Kim HM, Jeong HJ. Dp44mT regulates the levels of inflammatory mediators through blocking NF-κB nuclear translocation in LPS-stimulated RAW 264.7 macrophages. In Vitro Cell Dev Biol Anim. 2021;57(3):332-341.
26. Kim MH, Jeong HS, Moon PD. Effect of KH-BaRoKer-SeongJangTang based on traditional medicine theory on longitudinal bone growth. TANG. 2014;4(2):e14.
27. Stearns V, Ullmer L, López JF, Smith Y, Isaacs C, Hayes D. Hot flushes. Lancet. 2002;360(9348):1851-1861.
28. McEwen BS, Alves SE. Estrogen actions in the central nervous system. Endocr Rev. 1999;20(3):279-307.
29. Shuto H, Tominaga K, Yamauchi A, Ikeda M, Kusaba K, Mitsunaga D, Hirabara Y, Egawa T, Takano Y, Kataoka Y. The statins fluvastatin and pravastatin exert anti-flushing effects by improving vasomotor dysfunction through nitric oxide-mediated mechanisms in ovariectomized animals. Eur J Pharmacol. 2011;651(1-3):234-239.
30. Tataryn IV, Meldrum DR, Lu KH, Frumar AM, Judd HL. LH, FSH and skin temperaure during the menopausal hot flash. J Clin Endocrinol Metab. 1979;49(1):152-154.
31. Zhang Y, Hua F, Ding K, Chen H, Xu C, Ding W. Angiogenesis Changes in Ovariectomized Rats with Osteoporosis Treated with Estrogen Replacement Therapy. Biomed Res Int.

www.kci.go.kr

2019;2019:1283717.
32. Geng Q, Gao H, Yang R, Guo K, Miao D. Pyrroloquinoline Quinone Prevents Estrogen Deficiency-Induced Osteoporosis by Inhibiting Oxidative Stress and Osteocyte Senescence. Int J Biol Sci. 2019;15(1):58-68.
33. Dalal PK, Agarwal M. Postmenopausal syndrome. Indian J Psychiatry. 2015;57(Suppl 2):S222-232.
34. Hildebrandt T, Alfano L, Tricamo M, Pfaff DW. Conceptualizing the role of estrogens and serotonin in the development and maintenance of bulimia nervosa. Clin Psychol Rev. 2010;30(6):655-668.
35. Charoenphandhu N, Nuntapornsak A, Wongdee K, Krishnamra N, Charoenphandhu J. Upregulated mRNA levels of SERT, NET, MAOB, and BDNF in various brain regions of ovariectomized rats exposed to chronic aversive stimuli. Mol Cell Biochem. 2013;375(1-2):49-58.
36. Hanada R, Leibbrandt A, Hanada T, Kitaoka S, Furuyashiki T, Fujihara H, Trichereau J, Paolino M, Qadri F, Plehm R, et al. Central control of fever and female body temperature by RANKL/RANK. Nature. 2009;462(7272):505-509.
37. Krause MS, Nakajima ST. Hormonal and nonhormonal treatment of vasomotor symptoms. Obstet Gynecol Clin North Am. 2015;42(1):163-179.
38. Sicat BL, Brokaw DK. Nonhormonal alternatives for the treatment of hot flashes. Pharmacotherapy. 2004;24(1):79-93.
39. Shumilov M, Touitou E. Buspirone transdermal administration for menopausal syndromes, in vitro and in animal model studies. Int J Pharm. 2010;387(1-2):26-33.
40. Perich T, Ussher J, Meade T. Menopause and illness course in bipolar disorder: A systematic review. Bipolar Disord. 2017;19(6):434-443.
41. Garcia AN, Depena C, Bezner K, Yin W, Gore AC. The timing and duration of estradiol treatment in a rat model of the perimenopause: Influences on social behavior and the neuromolecular phenotype. Horm Behav. 2018;97:75-84.
42. Kim HY, Jeong HJ, Kim HM. Antidepressant-like effect of Ikwitang involves modulation of monoaminergic systems. Mol Med Rep. 2016;13(3):2815-2820.
43. Sun B, Yin YZ, Xiao J. An In Vivo Estrogen Deficiency Mouse Model for Screening Exogenous Estrogen Treatments of Cardiovascular Dysfunction After Menopause. J Vis Exp. 2019(150). doi: 10.3791/59536.
44. Lin S, Huang J, Fu Z, Liang Y, Wu H, Xu L, Sun Y, Lee WY, Wu T, Qin L, et al. The effects of atorvastatin on the prevention of osteoporosis and dyslipidemia in the high-fat-fed ovariectomized rats. Calcif Tissue Int. 2015;96(6):541-551.
45. Kim JH, Kim YJ. Effects of genistein in combination with conjugated estrogens on endometrial hyperplasia and metabolic dysfunction in ovariectomized mice. Endocr J. 2015;62(6):531-542.
46. Kang S, Jo H, Kim MR. Safety Assessment of Endocrine Disruption by Menopausal Health Functional Ingredients. Healthcare (Basel). 2021;9(10):1376.
47. Kamiya T, Takano A, Kido Y, Matsuzuka Y, Sameshima-Kamiya M, Tsubata M, Ikeguchi M, Takagaki K, Kinjo J. Evaluation of the Estrogenic Activity of Pueraria (Kudzu) Flower Extract and Its Major Isoflavones Using ER-Binding and Uterotrophic Bioassays. Pharmacology & Pharmacy. 2013;4(2):255-260.
48. Schlemmer CK, Coetzer H, Claassen N,

www.kci.go.kr

Kruger MC. Oestrogen and essential fatty acid supplementation corrects bone loss due to ovariectomy in the female Sprague Dawley rat. Prostaglandins Leukot Essent Fatty Acids. 1999;61(6):381-390.

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