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Effect of St. John's Wort (*Hypericum perforatum)* on obesity, lipid metabolism and uterine epithelial proliferation in ovariectomized rats

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BACKGROUND/OBJECTIVES: This study was conducted to assess the potential of St. John's Wort (*Hypericum perforatum*) to prevent obesity and abnormalities in lipid metabolism induced by ovariectomy in a rat model without stimulatory activity on uterus.
MATERIALS/METHODS: Ovariectomized (OVX) rats were treated for 6 weeks with 70% ethanol extracts of *Hypericum perforatum* [HPEs: whole plant (WHPE) and flower and leaves (FLHPE)], β-estradiol-3-benzoate at a dose of 50 μg/kg/day (E2) or vehicle (distilled water).
RESULTS: As expected, OVX increased body weight gain and adiposity and showed higher food efficacy ratio. OVX also increased the serum cholesterol as well as insulin resistance, while reducing uterus weight and uterine epithelial proliferation rate. HPEs (WHPE and FLHPE) showed estrogen-like effect on body weight gain, adipose tissue weight and food efficacy ratio in OVX rats. HPEs prevented hypercholesterolemia induced by OVX more effectively than E2. E2 increased uterus weight and epithelial proliferation rate in OVX rats, while HPEs maintained them at the level of the sham-operated animals.
CONCLUSIONS: Our finding demonstrates that HPEs can be considered as an effective agent to prevent OVX-induced obesity without stimulatory activity on uterus.

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Keywords: St John's Wort (*Hypericum perforatum*), insulin resistance, obesity, ovariectomy, uterine epithelial proliferation

INTRODUCTION

Metabolic syndrome is characterized by the co-occurrence of multiple metabolic disorders, including obesity, insulin resistance, hyperglycemia, hypertension and dyslipidemia [1,2]. The data, comparing pre- and postmenopausal women cross-sectionally, have indicated that postmenopausal women have greater intra-abdominal fat than premenopausal women [3]. In animal models, ovariectomy (OVX) induces a marked increase in body weight [4,5]. The increase in body weight caused by OVX is followed by concurrent alteration of lipid metabolism including the induction of fat accumulation and elevated levels of circulating lipoproteins [6].

The use of postmenopausal hormone replacement therapy has been associated with attenuation of body composition and fat distribution changes associated with menopause. However, discrepancies among the studies are caused by different protocols [7-9], and the metabolic benefits provided by hormone replacement therapy are often associated with increased risk of heart disease and breast and endometrial cancers. Therefore, research efforts on phytoestrogens are being directed toward determining whether phytoestrogens can provide protective effect on the systems affected by menopause without exerting the adverse effects on the breast and uterus encountered with hormonal therapy [1,10].

St. John's Wort (*Hypericum perforatum*) is an herbaceous perennial plant long known for its putative medical properties. St. John's Wort (*Hypericum perforatum*) extract (HPE) has been used for the treatment of neuralgia, fibrosis, depression and anxiety as an alternative to classic antidepressant [11-13]. HPE contains different groups of compounds such as hypericin, hyperforin and flavonoides. Hypericin and hyperforin are suggested to be responsible for its antidepressant effect [11]. However, there are few reports concerning the anti-obesity effect of HPE in OVX rats.

In the present study, we determined the potential of HPE to antagonize the effects of OVX on obesity, insulin resistance and lipid metabolism and to compare its action with that of estrogen replacement. We also determined if HPE affected uterine epithelial proliferation in order to demonstrate a lack of adverse effect on uterus.

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MATERIALS AND METHODS

Preparation of St. John's Wort (Hypericum perforatum: HP) extract (HPE)

500 g of powder HP was immersed in 5 L of 70% ethanol at 90℃ for 8~12 h, and this procedure was repeated in 2.5L of 70% ethanol at 90℃ for 8~12 h. The extract was concentrated under reduced pressure using a rotary evaporator (Daesin Machine Industry, Korea) at 60℃ and then freeze-dried. The yields obtained from whole plant of HP(WHPE) and flowers and leaves HP(FLHPE) were 25.3% and 16.3%, respectively.

Animals

Female sham-operated and ovariectomized Sprague Dawley rats (9 weeks old) were purchased from Central Laboratory Animal Inc. [SLC. Inc.(Japan)]. Animals were housed in a climate-controlled room (22 ± 2℃, 50 ± 10% relative humidity) under a 12 h light/dark cycle and provided diet and water *ad libitum*. The rats were acclimated to diet for 2 weeks, and then the ovariectomized rats were divided into four groups: ovariectomized control, estrogen-treated, WHPE treated and FLHPE treated groups. We provided the animals with β-estradiol-3-benzoate at a dose of 50 μg/kg/day, and HPEs at a dose of 500 mg/kg/day for 6 weeks. Animals in the all-group were provided a modified AIN-93G control diet (7% corn oil replacing soybean oil).

Body and uterus weight and abdominal adipose tissue

The weight of the animals was recorded every week. Abdominal adipose tissues were removed and weighed after sacrifice. The relative uterus weight (uterus weight/weight) was calculated by dividing the uterus weight by body weight.

Biochemical analysis

Fasting blood glucose levels were measured using a kit (Medisense 2, Korea) on the first and last day of the experiment. Serum insulin (Shibayagi, Japan), triglyceride and total cholesterol (Asan, Seoul) were determined using kits. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to the homeostasis of assessment, as follows [14,15].

$$\text{HOMA-IR} = \frac{\text{Fasting insulin}(mIU/L) * \text{fasting glucose}(mmol/L)}{22.5}$$

Histochemistry of uterus epithelium

Rat uterus was fixed by 4% paraformaldehyde for 1-2 days. H&E staining was performed and scored for the uterine epithelial proliferation as follows: single layered luminal epithelial cell (1 point); double or triple layered luminal epithelial cell (2 point); multi-layered luminal epithelial cell (3 point); and persistent proliferative stage (4 point).

Statistical analysis

All data are expressed as mean ± SE. Statistical analyses were performed using the SPSS program (Version SPSS 20, Chicago, IL, USA). Group comparisons were carried out using variance analysis followed by the Duncan's multiple range test. Statistical significance was considered at $P < 0.05$.

RESULTS

Effect of HPE on body weight, adipose tissue and food efficacy ratio

Two weeks after ovariectomy, the body weight of untreated OVX rats was significantly increased compared to that of intact (Sham-C) rats. We treated animals with HPEs or E2. Both extracts of HPEs [whole *hypericum perforatum* (WHPE) and flowers and leaves of *hypericum perforatum* (FLHPE)] and E2 significantly decreased body weight gains compared to untreated OVX rats (OVX-C)($P < 0.05$). Especially, there was no significant difference in final body weight between FLHPE-treated OVX rats and Sham-C rats, although body weight of rats in WHPE group was significantly higher than that of rats in Sham-C group at the beginning of treatment (two weeks after OVX) (Fig. 1). Untreated OVX rats showed a greater increase in adipose tissue weight than Sham-C rats at the end of experiment (Table 1). Both extracts of HPEs and E2 also decreased adipose tissue weight compared to untreated OVX rats and even Sham-C rats (Table 1). There were no significantly differences in the food intake among the groups throughout the experimental period,

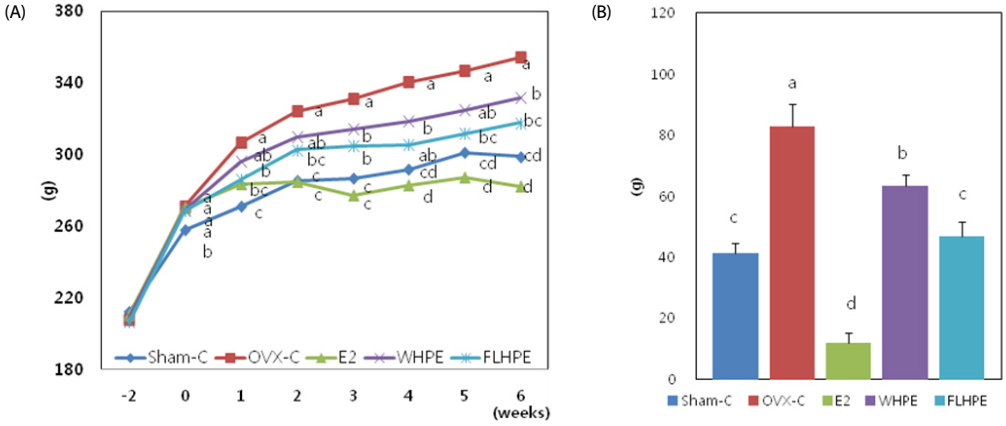

Fig. 1. Effect of *Hypericum perforatum* extracts on body weight (a) and body weight gain (b) during experimental period. Means with the same letter are not significantly different by Duncan' s multiple range test ($P < 0.05$). Sham-C: Sham operation + distilled water, OVX-C: Ovariectomy + distilled water, E2: Ovariectomy + estradiol, WHPE: Ovariectomy + Hypericum perforatum whole extract, FLHPE: Ovariectomy + Hypericum perforatum flower & leaf extract

Table 1. Effect of *Hypericum perforatum* extracts on the adipose tissue

	Sham-C	OVX-C	E2	WHPE	FLHPE
Visceral fat	20.76 ± 1.22^{b}	24.89 ± 1.78^{a}	10.92 ± 0.81^{c}	14.88 ± 1.24^{c}	13.37 ± 1.00^{c}
Mesenteric fat	3.91 ± 0.51^{b}	5.03 ± 0.35^{a}	2.27 ± 0.15^{d}	2.82 ± 0.28^{c}	2.59 ± 0.15^{cd}
Total fat	24.67 ± 1.70^{b}	29.92 ± 2.03^{a}	13.20 ± 0.74^{d}	17.70 ± 1.51^{c}	15.96 ± 1.14^{cd}

Means with the same letter are not significantly different by Duncan's multiple range test (P< 0.05). Sham-C: Sham operation + distilled water, OVX-C: Ovariectomy + distilled water, E2: Ovariectomy + estradiol, WHPE: Ovariectomy + Hypericum perforatum whole extract, FLHPE: Ovariectomy + Hypericum perforatum flower & leaf extract

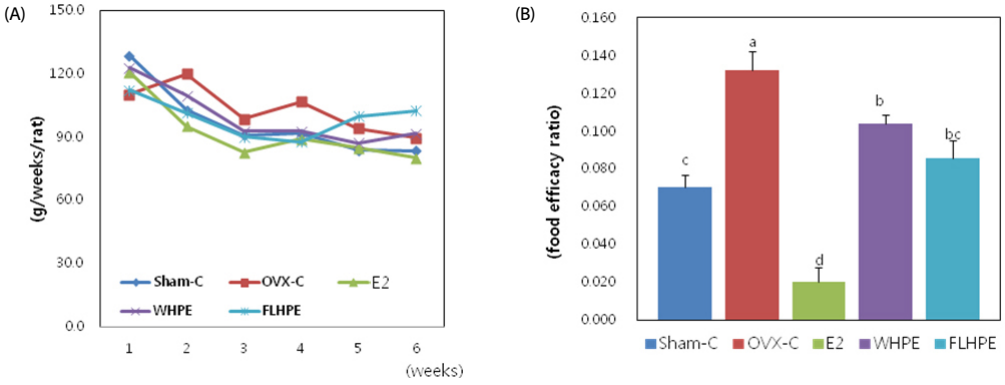

Fig. 2. Effect of *Hypericum perforatum* extracts on the food intake(a) and the food efficacy ratio(b). Means with the same letter are not significantly different by Duncan's multiple range test (P< 0.05). Sham-C: Sham operation + distilled water, OVX-C: Ovariectomy + distilled water, E2: Ovariectomy + estradiol, WHPE: Ovariectomy + Hypericum perforatum whole extract, FLHPE: Ovariectomy + Hypericum perforatum flower & leaf extract

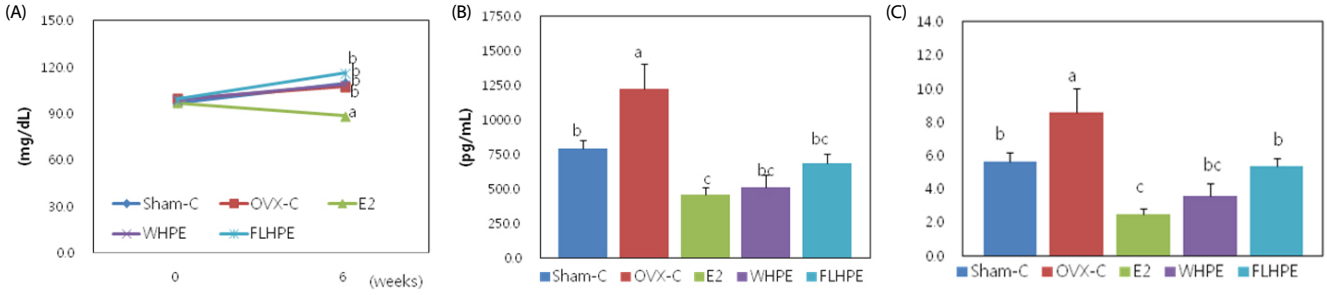

Fig. 3. Effect of *Hypericum perforatum* extracts on the blood glucose(a), fasting insulin(b) and HOMA-IR(c). Means with the same letter are not significantly different by Duncan's multiple range test (P< 0.05). Sham-C: Sham operation + distilled water, OVX-C: Ovariectomy + distilled water, E2: Ovariectomy + estradiol, WHPE: Ovariectomy + Hypericum perforatum whole extract, FLHPE: Ovariectomy + Hypericum perforatum flower & leaf extract

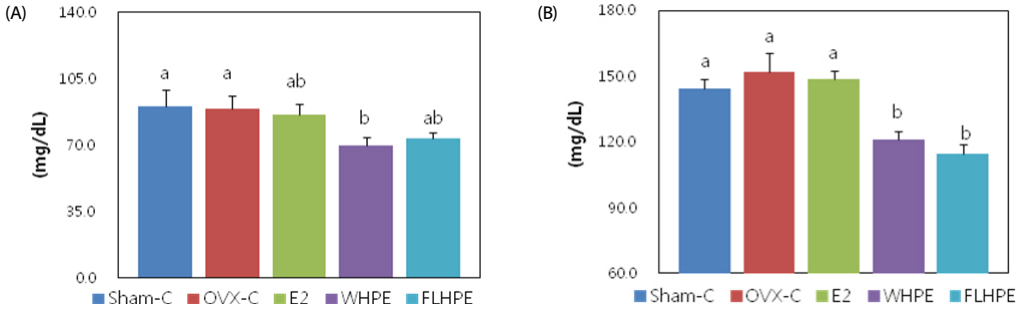

Fig. 4. Effect of *Hypericum perforatum* extracts on the serum triglyceride(a) and total cholesterol(b). Means with the same letter are not significantly different by Duncan's multiple range test (P< 0.05). Sham-C: Sham operation + distilled water, OVX-C: Ovariectomy + distilled water, E2: Ovariectomy + estradiol, WHPE: Ovariectomy + Hypericum perforatum whole extract, FLHPE: Ovariectomy + Hypericum perforatum flower & leaf extract

but the food efficacies in E2, WHPE and FLHPE groups were lower than that of untreated OVX group. Although E2 treatment showed lowest food efficacy, food efficacies between Sham-C and FLHPE groups were not significantly different (Fig. 2). In summary, HPEs (especially FLHPE) showed estrogen-like effect on body weight gain, adipose tissue weight and food efficacy ratio, although E2-treated OVX rats showed greatest effects among the groups.

Effect of HPE on insulin resistance and blood lipids

There was no significant difference in fasting blood glucose levels among the groups except for E2-treated group. The fasting glucose in E2-treated rats was lower than those of other groups (Fig. 3). OVX significantly increased fasting insulin compared to Sham-C animals. E2 and HPE treatment significantly decreased the fasting insulin level and thereby, HOMA -IR compared to untreated OVX rats. E2 and HPEs showed no significant changes in triglyceride level. However, total cholesterol tended to be increased by OVX and significantly lowered by both HPEs. E2 treatment was not effective on total serum cholesterol (Fig. 4).

Effect of HPE on uterus weight/ body weight and epithelial proliferation of uterus

As expected, relative uterus weight (uterus weight/ body weight) was significantly decreased by OVX compared to intact animals. E2 significantly increased relative uterus weight, increasing it to a level higher than that of sham-operated control (Fig. 5). However, the relative uterus weights of OVX rats treated with WHPE and FLHPE were not significantly different compared to untreated OVX rats. According to our analysis of uterine histology, luminal epithelium from sham-C and E2-treated OVX rats showed marked proliferation. Untreated OVX rats showed single-layered luminal epithelium and low proliferation grade, and generally multi-layered luminal epitheliums were observed in WHPE and FLHPE-treated OVX rats (Fig. 6a). Statistical analysis of uterus epithelial proliferation showed that OVX significantly decreased proliferation score and E2 significantly increased proliferation score up to the level of sham-C animals. However, HPEs significantly decreased proliferation scores and especially, FLHPE-treated OVX rats showed no significant difference in proliferation score compared to untreated OVX control (Fig. 6b).

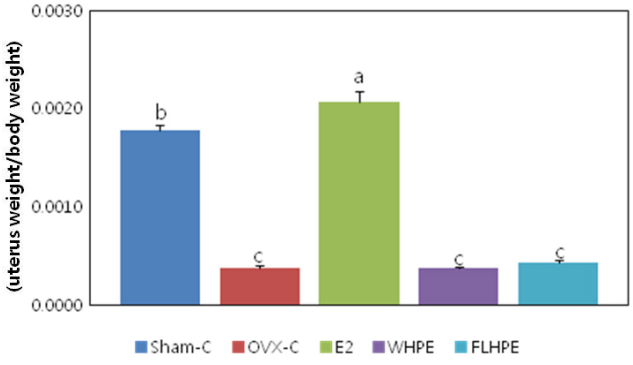

Fig. 5. Effect of *Hypericum perforatum* extracts on the uterus weight/ weight. Means with the same letter are not significantly different by Duncan' s multiple range test (P< 0.05). Sham-C: Sham operation + distilled water, OVX-C: Ovariectomy + distilled water, E2: Ovariectomy + estradiol, WHPE: Ovariectomy + Hypericum perforatum whole extract, FLHPE: Ovariectomy + Hypericum perforatum flower & leaf extract

DISCUSSION

In the present study, we observed that OVX increased body weight gain and adiposity. As mentioned in other studies [16-18], OVX-induced body weight gain was attenuated by exogenous E2. As such, it is seen that body weight gain in OVX animals is caused by estrogen deficiency. Overeating is not the cause for the adiposity in estrogen-depleted OVX rats, because food intake of untreated OVX rats was not significantly different from that of sham-C rats. In other study, it was also reported that ERα deficiency in female mice caused obesity without hyperphagia [16,17]. HPEs, like estrogen, reduced body weight gain, abdominal fats and food efficacy ratio in this study. Especially FLHPE reduced body weight gain and food efficacy ratio to the levels of sham-C rats. HPEs reduced food efficacy ratio without reduction in food intake. Therefore, reduction in food intake is not the mechanism for the reduced adiposity of HPEs, which is similar to estrogen.

Hyperinsulinemia has been suggested as a marker for metabolic abnormalities in individuals that do not have diabetes [19]. Estrogen not only participates in the regulation of body adiposity, but modulates insulin sensitivity [20,21]. Li *et al* . [22] reported that estrogen replacement could lower fasting plasma glucose and insulin in postmenopausal women. In our study, E2 reduced fasting blood glucose in rats, whereas OVX and HPEs did not affect fasting blood glucose levels. However, we observed that HPEs, similar to E2, attenuated hyperinsulinemia induced by OVX and thereby, attenuated HOMA-IR. The HOMA

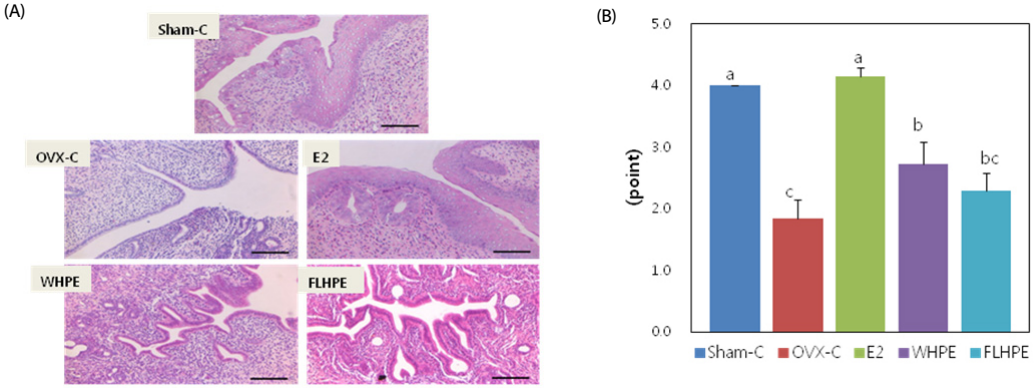

Fig. 6. Microscopic findings of uterus stained for Hematoxylin & Eosin(a) and proliferation score(b). Means with the same letter are not significantly different by Duncan's multiple range test (P< 0.05). Sham-C: Sham operation + distilled water, OVX-C: Ovariectomy + distilled water, E2: Ovariectomy + estradiol, WHPE: Ovariectomy + Hypericum perforatum whole extract, FLHPE: Ovariectomy + Hypericum perforatum flower & leaf extract Scale bar = 100 μm. Cell proliferation score measured by degree of luminal epithelium proliferation

model has been used to estimate β-cell function and insulin sensitivity from fasting insulin and glucose concentrations [14]. The results of present study showed minor increase in serum cholesterol levels by OVX, and E2 did not affect the triglyceride and total cholesterol levels in OVX rats. In other studies with OVX rats treated with E2 or soy extract, E2 did not correct a slight increase of serum cholesterol in OVX rats [23], and soy extract also showed a lack of hypocholesterolemic effect [10]. However, HPEs reduced triglyceride and total cholesterol in OVX rats. Therefore, we suggest that HPEs, like E2, can prevent metabolic abnormalities by a reduction in insulin resistance. Moreover, HPEs showed hypocholesterolemic and hypotriglyceridemic effect in OVX animals, while E2 did not.

We also demonstrated that the anti-obesity effects of HPEs were not accompanied by an uterotrophic effect, as shown by the relative uterus weight and histological analysis of epithelial proliferation. HPEs did not affect relative uterus weight, whereas E2 significantly increased relative uterus weight in OVX rats. The histological analysis also demonstrated that the uterine epithelial proliferation scores of HPEs-treated OVX rats were significantly decreased compared to that of untreated OVX rats.

The present study demonstrated that HPEs act as an estrogen agonist on body weight gain, body adiposity and insulin resistance, partially preventing OVX-induced obesity and metabolic abnormalities, without exerting stimulatory effects on uterine tissue. The implication that HPEs may be used to attenuate metabolic syndrome in postmenopausal women, without affecting uterine tissue, deserves further investigation.

REFERENCES

1. González-Castejón M, Rodriguez-Casado A. Dietary phytochemicals and their potential effects on obesity: a review. Pharmacol Res 2011;64:438-55.
2. Reaven GM. The metabolic syndrome: time to get off the merry-go-round? J Intern Med 2011;269:127-36.
3. Toth MJ, Tchernof A, Sites CK, Poehlman ET. Effect of menopausal status on body composition and abdominal fat distribution. Int J Obes Relat Metab Disord 2000;24:226-31.
4. Richard D. Effects of ovarian hormones on energy balance and brown adipose tissue thermogenesis. Am J Physiol 1986;250:R245-9.
5. Wade GN, Gray JM, Bartness TJ. Gonadal influences on adiposity. Int J Obes 1985;9 Suppl 1:83-92.
6. Deshaies Y, Dagnault A, Lalonde J, Richard D. Interaction of corticosterone and gonadal steroids on lipid deposition in the female rat. Am J Physiol 1997;273:E355-62.
7. Sørensen MB, Rosenfalck AM, Højgaard L, Ottesen B. Obesity and sarcopenia after menopause are reversed by sex hormone replacement therapy. Obes Res 2001;9:622-6.
8. Gambacciani M, Ciaponi M, Cappagli B, De Simone L, Orlandi R, Genazzani AR. Prospective evaluation of body weight and body fat distribution in early postmenopausal women with and without hormonal replacement therapy. Maturitas 2001;39:125-32.
9. Hänggi W, Lippuner K, Jaeger P, Birkhäuser MH, Horber FF. Differential impact of conventional oral or transdermal hormone replacement therapy or tibolone on body composition in postmenopausal women. Clin Endocrinol (Oxf) 1998;48:691-9.
10. Gallo D, Zannoni GF, Apollonio P, Martinelli E, Ferlini C, Passetti G, Riva A, Morazzoni P, Bombardelli E, Scambia G. Characterization of the pharmacologic profile of a standardized soy extract in the ovariectomized rat model of menopause: effects on bone, uterus, and lipid profile. Menopause 2005;12:589-600.
11. Mennini T, Gobbi M. The antidepressant mechanism of Hypericum perforatum. Life Sci 2004;75:1021-7.
12. Brenner R, Azbel V, Madhusoodanan S, Pawlowska M. Comparison of an extract of hypericum (LI 160) and sertraline in the treatment of depression: a double-blind, randomized pilot study. Clin Ther 2000;22:411-9.
13. Schrader E. Equivalence of St John's wort extract (Ze 117) and fluoxetine: a randomized, controlled study in mild-moderate depression. Int Clin Psychopharmacol 2000;15:61-8.
14. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
15. Matsuzawa Y, Funahashi T, Kihara S, Shimomura I. Adiponectin and metabolic syndrome. Arterioscler Thromb Vasc Biol 2004;24:29-33.
16. Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS. Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. Proc Natl Acad Sci U S A 2000;97:12729-34.
17. Ribas V, Nguyen MT, Henstridge DC, Nguyen AK, Beaven SW, Watt MJ, Hevener AL. Impaired oxidative metabolism and inflammation are associated with insulin resistance in ERalpha-deficient mice. Am J Physiol Endocrinol Metab 2010;298:E304-19.
18. Gao Q, Mezei G, Nie Y, Rao Y, Choi CS, Bechmann I, Leranth C, Toran-Allerand D, Priest CA, Roberts JL, Gao XB, Mobbs C, Shulman GI, Diano S, Horvath TL. Anorectic estrogen mimics leptin's effect on the rewiring of melanocortin cells and Stat3 signaling in obese animals. Nat Med 2007;13:89-94.
19. Després JP, Lamarche B, Mauriège P, Cantin B, Dagenais GR, Moorjani S, Lupien PJ. Hyperinsulinemia as an independent risk factor for ischemic heart disease. N Engl J Med 1996;334:952-7.
20. Lombard L, Augustyn MN, Ascott-Evans BH. The metabolic syndrome--pathogenesis, clinical features and management. Cardiovasc J S Afr 2002;13:181-6.
21. Saglam K, Polat Z, Yilmaz MI, Gulec M, Akinci SB. Effects of postmenopausal hormone replacement therapy on insulin resistance. Endocrine 2002;18:211-4.
22. Li C, Samsioe G, Borgfeldt C, Bendahl PO, Wilawan K, Aberg A. Low-dose hormone therapy and carbohydrate metabolism. Fertil Steril 2003;79:550-5.
23. Picard F, Deshaies Y, Lalonde J, Samson P, Labrie C, Bélanger A, Labrie F, Richard D. Effects of the estrogen antagonist EM-652.HCl on energy balance and lipid metabolism in ovariectomized rats. Int J Obes Relat Metab Disord 2000;24:830-40.