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Effects of *Urtica dioica* hydro-alcoholic extract on blood serum glucose and lipid profiles of female Wistar rats with long-term estrogen deficiency

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Abstract

In this study, the effects of *Urtica dioica* hydro-alcoholic extract were investigated on the blood glucose and lipid profiles of female ovariectomized and non-ovariectomized rats. In total, 32 adult female rats were divided into four groups (eight each) including control and ovariectomy groups as well as non-ovariectomy and ovariectomy groups treated with 200 mg kg^{-1} of *Urtica dioica* extract orally in the last five weeks of the study starting from the week 56^{th}. The duration of the study was 60 weeks. Glucose, serum lipid profiles and pancreatic pathological alterations were determined in these groups at the end of experiment. Serum glucose, triglyceride (TG), very-low-density lipoprotein (VLDL), and TG/high-density lipoprotein (HDL) ratio indicated a significant increase in the healthy female rats under treatment with *Urtica dioica* extract compared to others. The TG, cholesterol, HDL, low-density lipoprotein (LDL) and VLDL showed a significant increase in menopaused rats compared to others. The interaction of consuming *Urtica dioica* extract and ovariectomy caused significant decreases in glucose, TG, VLDL, HDL/LDL ratio and TG/HDL ratio. Consumption of *Urtica dioica* extract by non-menopaused rats damaged the beta cells in Langerhans islets. Results of the present study revealed that the consumption of *Urtica dioica* extract is not beneficial and has diabetogenic effects in female non-ovariectomized rats compared to ovariectomized ones.

آثار عصاره هیدروالکلی گزنه بر گلوکز و فراسنجه های چربی سرم خون موش های صحرایی ماده نژاد ویستار با کاهش طولانی مدت استروژن

چکیده

در این مطالعه آثار عصاره هیدروالکلی گزنه بر گلوکز و فراسنجه‌های چربی خون در موش‌های صحرایی ماده اواریکتومی شده و اواریکتومی نشده بررسی شد. در مجموع ۳۲ سر موش صحرایی ماده بالغ به چهار گروه ۸ تایی شامل گروه های شاهد و اواریکتومی و نیز گروه های اواریکتومی نشده و اواریکتومی شده دریافت کننده عصاره گزنه به میزان ۲۰۰ میلی‌گرم بر کیلوگرم به صورت خوراکی در ۵ هفته پایانی مطالعه از هفته ۵۶ تقسیم شدند. مدت زمان مطالعه ۶۰ هفته در نظر گرفته شد. در پایان مطالعه، قند، فراسنجه‌های چربی سرم و تغییرات پاتولوژیکی پانکراس در گروه‌های تحت مطالعه تعیین شد. گلوکز سرم، تری گلیسرید، لیپوپروتئین با چگالی بسیار پایین و نسبت تری گلیسرید به لیپوپروتئین با چگالی بالا افزایش معنی‌داری را در موش‌های صحرایی ماده سالم تحت درمان با عصاره گزنه نسبت به سایر موش‌های صحرایی نشان داد. در موش‌های صحرایی منوپوز شده تری‌گلیسرید، کلسترول، لیپوپروتئین با چگالی بالا، لیپوپروتئین با چگالی پایین و لیپوپروتئین با چگالی بسیار پایین افزایش معنی‌داری را در مقایسه با سایر موش های صحرایی نشان دادند. اثر تداخلی عصاره گزنه و اواریکتومی باعث کاهش های معنی‌داری در میزان گلوکز، تری گلیسرید، لیپوپروتئین با چگالی بسیار پایین، نسبت لیپوپروتئین با چگالی بالا به لیپوپروتئین با چگالی پایین و نسبت تری گلیسرید به لیپوپروتئین با چگالی بالا شد. مصرف عصاره گزنه توسط موش‌های صحرایی منوپوز نشده باعث آسیب سلول های بتا در جزایر لانگرهانس شد. نتایج مطالعه حاضر نشان داد که مصرف عصاره گزنه در موش‌های صحرایی ماده اواریکتومی نشده در مقایسه با موش‌های صحرایی اواریکتومی شده سودمند نمی‌باشد و دارای آثار دیابت زایی است.

واژه های کلیدی: برداشت تخمدان، فراسنجه‌های چربی، گزنه، گلوکز، موش صحرایی

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Introduction

With decreasing levels of estrogen and progesterone, menopause leads to symptoms such as hot flashes, night sweats, insomnia, malaise, fatigue, anxiety, irritability, depression, tachycardia, and joint pain.[1] Other menopause comorbidities include bone density reduction,[1,2] blood cholesterol increase and cardiovascular diseases.[1] Decreased estrogen in menopausal women results in body mass index, visceral fat and body weight increases due to decreased energy consumption.[3] Studies have shown that the over-consumption of calorie is the main factor causing insulin resistance, followed by diabetes.[4] In rats, increase in fasting insulin level five weeks after the ovaries removal can be a sign of insulin resistance in the whole body.[5] Ovariectomy in animals as models of menopause can be used as a factor to increase food absorption, body weight, insulin resistance, osteoporosis and skeletal muscle atrophy.[6] It has been proven that decreased estrogen stimulates visceral fat aggregation, decreases lipid consumption and increases insulin resistance as well as risks of cardiovascular diseases.[7,8] Moreover, by affecting the insulin signal, increase in the flow of non-esterified fatty acids decreases glucose absorption in muscles and increases triglyceride (TG) synthesis.[9]

Herbal medications are mostly used by women to treat and alleviate symptoms of menopause. In most countries, these medications are utilized as dietary supplements. Nevertheless, few studies have been conducted on their effectiveness for the abovementioned factors during menopause.[1] The effects of flavonoids on ovariectomized rats have only been studied in the past few years.[10] Flavonoids, the most important metabolites of vascular plants, have anti-inflammatory, anti-oxidant, anti-tumor, analgesic, anti-depressant, tranquilizing and other biologic activities.[11,12] Nettle (*Urtica dioica* L.) belonging to the Urticaceae family is an herbal medication known and used in the Iranian traditional medicine.[13] The consumable parts of *Urtica dioica* with medicinal use are young leaves, root and sap.[14] Nettle extensively grows in areas located within 2°34'N and 20°75'E, 2400 m above the sea level, with the mean annual precipitation of 1075 mm.[15] Extensive studies have been conducted on its anti-oxidant, anti-inflammatory, anti-diabetic, anti-viral,[16] anti-cancer,[17] anti-microbial, anti-fungal and anti-androgen activities,[18] introducing it as a supplementary medication for diabetes treatment and blood glucose decrease.[14] Nettle leaves have chlorophyll, carotene, xanthophyll and flavonoid compounds,[13] sitosterol, flavonoids, quercitin, retin, kaempferol, vitamin C, vitamin K, potassium, calcium and 5-hydroxytryptamine.[19] Quercitin (7,5,4,3,3-pentahydroxy-flavone) which is among the most important flavonoids in *Urtica dioica* leaves decreases histamine release from basophils and mast cells and has anti-inflammatory activities.[19,20] Studies on laboratory animals indicated the role of *Urtica dioica* hydro-ethanolic extract in low-density lipoprotein (LDL) cholesterols and the ratio of LDL to high-density lipoprotein (HDL) cholesterols reductions[21] and lipid profiles improvement.[22] No study has been conducted on the effect of *Urtica dioica* hydro-alcoholic extract on glucose and some lipid profiles in female ovariectomized and non-ovariectomized rats and most works have been focused on male mice and rats. Therefore, the present study was performed to examine the effects of *Urtica dioica* hydro-alcoholic extract on blood glucose and lipid profiles in female ovariectomized and non-ovariectomized rats.

Materials and Methods

Animals and experimental design. The present experimental study was performed on 32 female Wistar rats with the mean weight of 220-250 g for 60 weeks. The rats were obtained from the animal house of Pasteur Institute, Ahvaz, Iran and kept in polycarbonate cages at 20 ˚C, 60% humidity and 12:12 hr light-dark cycles in Pathology Research Center at Veterinary Hospital, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran. In order to accommodate the rats to the new conditions, all the tests were conducted after minimum two weeks of animals lodging. The rats were given access to standard food and water *ad libitum*.[23] All the experiments performed in this study were approved by Ethics Committee of Islamic Azad University, Shahrekord Branch, Shahrekord, Iran and all the ethical considerations related to the animal experiments were taken into account (IAUSHK, 4164).

***Urtica dioica* hydro-alcoholic extract preparation.** Dried *Urtica dioica* leaves and stems were purchased from credible centers and after being confirmed by a botanist at Medicinal Herbs Research Center, Shahrekord University of Medical Sciences, a herbarium specimen was recorded in this center (No. 412). Extraction was done via soaking. *Urtica dioica* leaves and stems were slightly powdered using an electric mill. The powder was then mixed with 70 ˚C ethanol such a way that the ethanol level would be some centimeters above the powder level. After 48 hr, the content was filtrated using a filter paper and glass funnel. The filtrate was transferred to a flask and then, its solvent was extracted using a rotary evaporator and a vacuum pump (set at 48-50 ˚C). The resulting thick liquid was poured into a glass container and dried in the oven at 37 ˚C.[24] Afterwards, the resulting powder was collected from the container. In order to prepare the 200 mg kg^{-1} dose, 1 g of the dried *Urtica dioica* extract was solved in 10 mL of physiological saline solution and gavage-fed to the female rats daily for five weeks based on their weight.[25]

Phenolic compounds determination. First, 0.10 mL of diluted *Urtica dioica* extract (0.01 g in 10 mL of 60.00%

ethanol) was added to 0.50 mL of 10.00% Folin–Ciocalteu solution (Sigma-Aldrich Co, St. Louis, USA). After 3-5 min, 0.40 mL of 7.50% sodium carbonate was added. After 30 min of incubation, the optical absorption of the specimens was read against the distilled-water blank at the wavelength of 765 nm and laboratory ambient temperature. Simultaneously, gallic acid was prepared by testing different dilutions, tested based on the abovementioned method and the standard curve was drawn. The absorption of the specimen was compared with the standard curve and the level of total phenol in *Urtica dioica* extract was measured (mg per 1 g of the dried extract). [26]

Flavonoid compounds determination. First, 0.50 mL of diluted *Urtica dioica* extract (0.01 g in 10 mL of 60.00% ethanol) was mixed with 0.50 mL of 2.00% aluminium chrolide (Merck, Darmstadt, Germany) and then, 3.00 mL of 5.00% sodium acetate (Merck) was added. After 40 min, the absorption of the specimens was read against distilled water at the wavelength of 415 nm. The absorption of the specimens was compared with the standard curve and the level of flavonoid in *Urtica dioica* extract was measured (mg per 1 g of the dried extract). [26]

Surgical procedures of ovariectomy in female rats. Prior to the surgery, the female rats were anesthetized by the intra-muscular injection of 100 and 10 mg kg^{-1} of the combination of ketamine and xylazine, respectively and then, undergone gonadectomy as a model of menopause following the method described by Sadeghi *et al*. [27] The rats were randomly divided into four groups, eight of each[28,29] including:

Group 1 (control) was included the rats kept in standard conditions for 60 weeks.

Group 2 (ovariectomy) was consisted of rats kept for 60 weeks following ovariectomy. In the last five weeks, they were gavage-fed 1.00 mL of the *Urtica dioica* solvent.

Group 3 was comprised non-ovariectomized rats kept for 60 weeks. In the last five weeks, they were gavage-fed 200 mg kg^{-1} of the *Urtica dioica* extract.

Group 4 was included ovariectomized rats kept for 60 weeks following the surgery. In the last five weeks of the research period, they were gavage-fed 200 mg kg^{-1} of the *Urtica dioica* extract.

Serum glucose and lipid profiles determination. At the end of week 60 and 12 hr after the last dose of *Urtica dioica* extract administration (day 35), following a night of fasting with access to water, the rats were anesthetized using chloroform. After opening the thoracic cavity, blood samples were collected from their heart. Blood was transferred to the tubes without anti-coagulant agents and sera were removed by centrifugation for 10 min at 3000 rpm. Serum glucose, TG, cholesterol, HDL and LDL were measured using an auto-analyzer device (Model BT-3000, Biotecnica, Rome, Italy) via commercial kits (Pars Azmoon, Tehran, Iran) and based on the recommended guidelines. In addition, the level of very-low-density lipoproteins (VLDL; using Friedewald equation),[30] risk of heart disease and atherogenic coefficient[31] were determined as follows:

$$VLDL = \frac{TG}{5}$$

$$Heart\ disease\ risk\ ration = \frac{Total\ cholesterol}{HDL\ cholesterol}$$

$$Atherogenic\ coefficient = \frac{Total\ cholesterol - HDL\ cholesterol}{HDL\ cholesterol}$$

Histological examination. To examine the alterations in the pancreatic tissues, immediately after blood collection, pancreas was removed from duodenal flexure and fixed in 10% formalin buffer. Paraffin blocks were prepared via common histological methods. Using microtome, 5 µm-wide strips were cut and stained by hematoxylin and eosin technique.[30]

Statistical analysis. Data were expressed as mean ± standard deviation. The data were analyzed using one-way ANOVA followed by LSD post–hoc test (version 20.0, IBM Corp., Chicago, USA).

Results

In the present study, serum glucose, TG, VLDL, and total cholesterol/HDL, HDL/LDL and TG/HDL ratios were significantly higher in the healthy female rats (no-ovariectomized rats) which had received *Urtica dioica* extract than those which had not received *Urtica dioica* extract ($p < 0.01$; Table 1). Levels of TG, cholesterol, HDL, LDL and VLDL were significantly increased in ovariectomized rats (group 2) compared to the non-ovariectomized rats (group 1; $p < 0.01$; Table 1). In group 4 (ovariectomized rats treated with *Urtica dioica* extract), the serum TG was significantly elevated ($p < 0.01$) compared to group 1 (Table 1). In group 3 (non-ovariectomized rats treated with *Urtica dioica* extract), glucose, TG, VLDL, and HDL/LDL and TG/HDL ratios significantly ($p < 0.01$) increased compared to group 1 (Table 1). Results of one-way ANOVA and post-hoc LSD tests showed that serum levels of glucose, TG, TG-HDL, and HDL-LDL were maximal in the non-ovariectomized rats treated with *Urtica dioica* extract ($p < 0.01$; Table 1).

In this study, the levels of phenol and flavonoid were 18.00 and 11.00 mg g^{-1} of the dried extract of *Urtica dioica*, respectively.

Pathological findings. Microscopic studies of pancreatic tissue in the control group indicated the normal structure of beta cells in the Langerhans islets (Fig. 1A). Consumption of 200 mg kg^{-1} of *Urtica dioica* extract in the female non-ovariectomized (healthy) rats severely damaged beta cells, decreased the number of Langerhans islets and caused cell death (Fig. 1B).

Table 1. Changes in glucose, and serum lipid profile in the studied female rats based on the results of one-way ANOVA and post-hoc LSD test. Data are presented as mean ± SD (n = 8).

Parameters	Saline solution		*Urtica dioica* extract (200 mg kg^{-1})	
	Intact (control)	Ovariectomized	Intact (control)	Ovariectomized
Glucose (mg dL^{-1})	164.33 ± 39.21^{b}	159.00 ± 27.42^{b}	490.80 ± 27.83^{a‡}	176.80 ± 21.55^{b}
TG (mg dL^{-1})	42.83 ± 8.15^{c}	104.50 ± 23.77^{ab}	122.00 ± 33.05^{a‡}	77.80 ± 25.83^{b}
TC (mg dL^{-1})	53.66 ± 13.92^{b}	82.66 ± 20.79^{a†}	53.600 ± 6.22^{b}	65.20 ± 5.77^{ab}
HDL-C (mg dL^{-1})	42.43 ± 18.81^{b}	87.30 ± 29.65^{a†}	43.56 ± 9.72^{b}	59.72 ± 15.28^{b}
LDL-C (mg dL^{-1})	19.83 ± 5.49^{bc}	35.65 ± 12.66^{a†}	14.72 ± 2.08^{c}	29.44 ± 13.26^{ab}
VLDL (mg dL^{-1})	8.56 ± 1.63^{c}	20.90 ± 4.75^{ab}	24.40 ± 6.61^{a†}	15.56 ± 5.16^{b†}
TC/HDL	1.36 ± 0.27^{a}	0.96 ± 0.08^{c†}	1.25 ± 0.16^{ab}	1.10 ± 0.08^{bc}
HDL/LDL	2.08 ± 0.44^{b}	2.48 ± 0.26^{ab}	3.02 ± 0.92^{a*}	2.15 ± 0.38^{b}
TG/HDL	1.18 ± 0.50^{b}	1.25 ± 0.33^{b†}	2.78 ± 0.47^{a‡}	1.34 ± 0.50^{b†}

^{abc} Values down the horizontal rows carrying different superscripts are significantly different from each other ($p < 0.05$). *$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ as compared to control group.

TG: Triacylglycerol; TC: Total cholesterol; HDL: High-density lipoprotein, LDL: Low-density lipoprotein, C: Cholesterol, HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; VLDL: Very-low-density lipoprotein.

No unnatural pathological alteration was observed in the pancreatic tissue of ovariectomized group. The pancreatic tissue of female ovariectomized rats under treatment with *Urtica dioica* showed signs of edema among acini and a relative increase in the number of cells in the Langerhans islets (Fig. 1C).

Discussion

Consumption of the hydro-alcoholic extract of *Urtica dioica* at the dose of 200 mg kg^{-1} of body weight for 35 consecutive days resulted in an increase in blood glucose in healthy female rats. Therefore, the *Urtica dioica* extract did not have a hypoglycemic effect, but hyperglycemic and polyuric effects, in contrast to the results of some previous studies.[15,32-35] in the male rats. In pathological examinations of the pancreatic tissue samples of the female non-ovariectomized rats under treatment with *Urtica dioica*, decreases in the number of cells as well as degeneration and necrosis in pancreatic islets were observed, which seems to contradict previous results using 200 mg kg^{-1} for female healthy rats with a diabetogenic activity. The interesting result, however, was that the hydro-alcoholic extract of *Urtica dioica* did not affect serum glucose in female ovariectomized rats, in contrast to the female non-ovariectomized ones under treatment. A reason for the increase in the blood glucose level of female non-ovariectomized rats under treatment with *Urtica dioica* extract, which contradicted previous studies, can be the animals' sex and estrogen presence.[17-19] Consumption of *Urtica dioica* extract caused no alteration in the structure of pancreas or blood glucose among female ovariectomized rats. The increase in the blood glucose of healthy female rats under treatment with *Urtica dioica* may be related to insulin resistance syndrome. This syndrome has four signs including increase in visceral fat, dyslipidemia, glucose tolerance disorder and insulin-mediated glucose uptake disorder in skeletal muscles.[36] According to research, 17β-estradiol in physiologic densities protects pancreatic beta cells from lipotoxicity, oxidative stress and programmed death.[37] It seems that the consumption of this extract blocks estrogen receptors

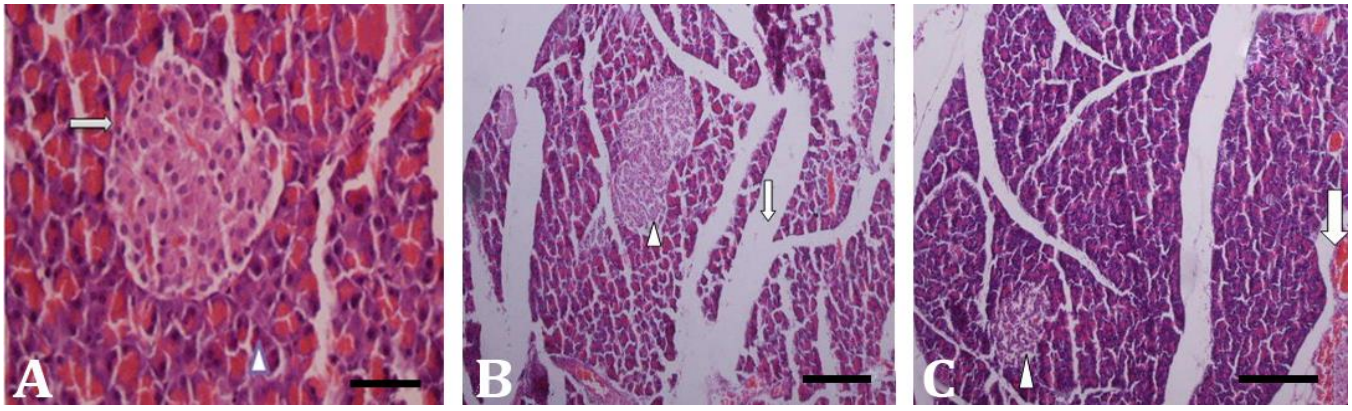

Fig. 1. A) Microscopic cross-section of pancreas in control healthy rats. Acini (arrowhead) and the normal structure of Langerhans islets (arrow) can be observed. **B)** Pancreatic tissue of non-ovariectomized rats under treatment with *Urtica dioica* extract, normal acini, edema among acini (arrow) and decrease in the size and number of Langerhans islets (arrowhead) are obvious; **C)** Pancreatic tissue of female ovariectomized rats under treatment with *Urtica dioica*, edema among acini (arrow) and relative increase in the number of cells in the Langerhans islets (arrowhead) can be seen (Hematoxylin and eosin staining; Bar = 20 µm).

and decreases the beta cells survival in healthy (non-ovariectomized) rats. Hernandez *et al*. have indicated that menopaused animals have lowered plasma total antioxidant status, reduced thiol groups and increased plasma lipoperoxides, all alleviated by estrogen therapy.[38] The role of angiotensin II in the insulin-sensitivity control in skeleton muscles, which decreases the insulin- and GLUT4-mediated glucose absorption, has been confirmed.[39] Moreover, it has been proved that angiotensin II stimulates the reactive oxygen species production in endothelium and vascular smooth muscle cells. This path plays a significant role in insulin sensitivity control.[40] On cell surface, angiotensin II and aldosterone increase oxidative stress, alter insulin signal and decrease glucose transfer, thereby inducing insulin resistance and decreasing glucose transfer.[41] It has been confirmed that by inhibiting the angiotensin-converting enzyme or blocking the angiotensin II-receptive gene, the inhibitors of renin–angiotensin–aldosterone system (RAAS) affect glucose metabolism and insulin- resistance and improve glucose hemostasis through insulin secretory response improvement in human and animal models.[41] Therefore, *Urtica dioica* extract may have increased the angiotensin II activity and decreased insulin sensitivity in target organs, thereby increasing blood glucose in female non-ovariectomized rats. This hypothesis requires further research. In the present study, serum levels of TG, cholesterol, HDL, LDL and VLDL were increased in ovariectomized, untreated rats with long-term decrease of estrogen compared to the control group. These findings were in line with those by Tawfik *et al*.[9] After menopause and with the decrease in estrogen production, lipids aggregate, lipid metabolism decreases and visceral fat aggregation, risk of cardiovascular diseases and insulin resistance increase.[42,43] In the present study, consumption of *Urtica dioica* extract resulted in significant increases of glucose, TG, VLDL, TG/HDL and HDL/LDL ratios in non-ovariectomized rats compared to the control group, contradicting the findings by Daher *et al*.[22] and Ahangarpour *et al.* [44] in male rats. By affecting the insulin signal, the increase in the flow of non-esterified fatty acids decreases the glucose absorption in muscles and increases the TG synthesis, followed by the induction of glucose production in the liver, a mechanism related to beta cell insufficiency.[45] In the present study, pancreatic beta cells in non-ovariectomized rats under treatment with *Urtica dioica* extract were accompanied by degenerative alterations and necrosis. According to Meguro *et al*., plant sterols and cholesterol have similar structures, while the former are highly hydrophobic. Consequently, *Urtica dioica* extract may decrease cholesterol storage.[46] In the present work, the cholesterol level of rats under treatment showed a significant decrease. Moreover, phytoestrogenic isoflavones and lignans of diphenolic compounds with a molecular weight similar to that of steroid estrogens and a structure similar to that of estradiol may bond to estrogen receptors and have stronger estrogen-like effects in ovariectomized rats.[47] In the present study, the interaction of consuming *Urtica dioica* extract and ovariectomy caused significant changes in serum levels of glucose, TG, VLDL, and HDL/LDL and TG/HDL ratios in the female rats. These results confirmed the positive role of plant extracts containing flavonoids and estrogen-like compounds in preventing the increase of lipid profiles in the ovariectomized rats. In this study, the glucose, TG, TG/HDL ratio, and VLDL showed a significant increase in healthy rats under treatment with *Urtica dioica* compared to the control group and the treated ovariectomized group, which is an appropriate predictor for coronary heart disease and atherosclerosis.[48] In addition, the increased level of TG in healthy rats and the rats which were under treatment with *Urtica dioica* extract is one of the main signs of insulin resistance, increased blood glucose and metabolic syndrome, which strongly correlate with coronary hearth diseases.[49]

Consumption of *Urtica dioica* extract in ovariectomized rats with a long-term decrease in estrogen may improve tissue sensitivity to insulin. Disordered glucose hemostasis, after the consumption of *Urtica dioica* extract among female healthy (non-ovariectomized) rats, was accompanied by serious disorders in lipid profiles, e.g. a significant increase in TG and TG/HDL and TG/LDL ratios. As a result, *Urtica dioica* extract must be consumed with care in premenopausal women.

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Conflict of interest

The authors declare that there is no duality of interest associated with this article.

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