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ORIGINAL ARTICLE

Distribution of Xanthine Oxidase in Selected Tissues of a Mouse Model of Menopause

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KEYWORDS

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ABSTRACT: The goal of this study was to investigate the common clinical chemistry and distribution of xanthine oxidase (XO) in selected tissues of a mouse model of menopause. Twenty four NMRI female mice were divided into three groups: normal control (NC), and ovariectomized (OVX) groups and an estrogen-treated ovariectomized (OVX+E) group which received subcutaneous injection of estradiol benzoate (2 mg/kg). After 8 weeks, blood samples were collected for determining plasma clinical chemistry. Tissue XO activity was measured spectrophotometrically based on monitoring uric acid (UA) formation. Plasma levels of total cholesterol (TC), total protein (TP), albumin (ALB), globulin (G), and calcium, and enteric XO activity increased in OVX group as compared with NC group. Hepatic XO activity in OVX group declined in comparison with NC group. E2, TP, and G levels and liver and brain XO activities increased in OVX+E group when compared with OVX group. However, TC, high density lipoprotein cholesterol, UA, and ALB levels decreased in OVX+E group compared with OVX group. The brain and heart XO activities increased in OVX+E group as compared to that of NC group. XO activity was not detected in womb, spleen and stomach of all studied groups. XO activity was not detected in muscles of NC group while OVX and OVX+E groups showed muscle XO activity. Induction of ovariectomy produced a hypoestrogenic state that coincident with an adverse alteration of plasma clinical chemistry in mice. XO activity also changed after ovariectomy and estrogen-replacement therapy with a tissue-specific manner.

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INTRODUCTION

Metabolic syndrome (MeS) is a pre-morbid condition characterized by abdominal obesity with visceral adiposity, impaired glucose tolerance due to insulin resistance with hyperinsulinemia, hypertriglyceridemia, decreased high density lipoprotein cholesterol (HDL-C), increased blood pressure, and possibly hyperuricemia. The hyperuricemic state is also accompanied by gout, MeS and alteration of cardiovascular system [1, 2]. The hyperuricemia is defined as serum uric acid (UA) $\geq$ 7 mg/dL for men and $\geq$ 6.0 mg/dL for women, and serum UA remains about 1 mg/dL lower in women than in men until menopause, when their levels rise to equal those of males [1]. Many factors can influence the concentrations of UA, e.g. diet, obesity, and MeS, however its upper limits are found mainly in postmenopausal women, African American, patients with renal disease and alcohol intake [2, 3]. In this context, we still do not know why and how serum UA is increased in (post) menopausal women.

Uric acid is considered as a final catabolic product of both endogenous and exogenous purines in higher animals [4]. Man and Dalmatian dog are the only mammals lacking the enzyme uricase and are thus incapable of digesting UA that makes them susceptible to hyperuricemia [4]. In this sense, xanthine oxidoreductase (XOR) catalyzes the oxidation of hypoxanthine to xanthine or xanthine to UA in the metabolic pathway of purine degradation [5]. This housekeeping enzyme originally exists in its xanthine dehydrogenase (XDH; EC 1.17.1.4) isoform, but is readily converted to xanthine oxidase (XO; EC 1.17.3.2) isoform by irreversible proteolysis or reversible sulfhydryl oxidation of its Cys residues [6]. XDH favors the cofactor NAD^+ as its primary electron acceptor, yet XO uses O_2 as its electron acceptor. With both isoforms, but particularly with the XO isoform, numerous reactive oxygen species (ROS) and reactive nitrogen species (RNS) are synthesized. Consequently, the synthesis of both an antioxidant (here UA) and numerous free radicals (ROS and RNS) makes XOR an important protective regulator of the cellular redox potential. In this report we will use the traditional name XO, intending this name to encompass all the various chemical reactions this enzyme can catalyze.

The distribution and characterization of XO have been investigated in various organisms from prokaryotes to eukaryotes. However, the concise role and tissue distribution of XO have been not studied in pathologic conditions like hyperuricemic state that occurred in menopause. Therefore, this study was undertaken to investigate the tissue distribution of XO activity in a mouse model of menopause.

MATERIALS AND METHODS

Animal subjects

The protocol of this study has been reviewed and approved by Ethical Committee of the School of Veterinary Medicine, Razi University (Kermanshah, Iran) that fulfills and follows declaration of Helsinki as revised in Tokyo 2004. All surgical and experimental procedures were performed according to the guidelines of the Animal Care and Use Review Committee (Razi University, Iran).

Female NMRI male mice (28±2 g) were prepared from our colony maintained in Laboratory Animal House. Our group-housed mice were maintained under 12-h light–dark cycle (lights on from 0600 to 1800 h) with food and water *ad libitum*. Age- and weight-matched female mice were allocated into three groups (n=8 for each) as follows: normal control (NC) group received subcutaneous (s.c.) injection of distilled water (final

volume 0.03 ml); ovariectomized (OVX) group treated similar to NC group; and ovariectomized and estrogen-treated (OVX+E) group received s.c. injection of estradiol benzoate (2 mg/kg at final volume 0.03 ml; Vetastrol®, Aburaihan Company, Iran).

Bilateral ovariectomy throughout dorsolateral approach was performed under anesthesia by intraperitoneal (i.p.) injection of ketamine/xylazine cocktail and ovariectomized mice were fed the commercial solid diet during a 7-d recovery period. The success of the ovariectomy was checked by analysing vaginal smear during 5 consecutive days after the surgery. Ovariectomized mice presented only the diestrus pattern while in control mice the 4 stages of the estrous cycle (proestrus, estrus, metestrus and diestrus) were clearly verified.

Clinical chemistry

On the last day of experiment (day 56), after 14-16 h fasting whole blood samples were taken from all mice by cardiac puncture under deep anesthesia with an i.p. injection of ketamine/xylazine cocktail and collected into tubes containing heparin as an anticoagulant. Plasma was separated by centrifugation at 1400 × *g* at 4 °C for 15 min, and stored at –20 °C until analysis. The concentrations of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and triglycerides (TGs) in the plasma were assayed using diagnostic kit (ELI TECH Diagnostic, French). Plasma HDL-C was determined by immunoinhibition method (ELI TECH Diagnostic, French). Very low density lipoprotein-cholesterol (VLDLC) was calculated by formula: VLDL-C = TGs/5 [7]. Atherogenic index (AI) was calculated according to the following equation: AI = LDL-C/HDL-C [8].

Plasma samples were thawed and the levels of albumin (ALB), total protein (TP), urea (U), uric acid (UA), creatinine (CRT), calcium (Ca), and phosphorus (P) were determined by biochemical kits (Pars Azmon, Tehran, Iran) using an autoanalyzer (Heitachi 896, Germany). Ca/P ratios also were calculated. The level of globulin (G) was obtained by subtracting albumin concentration from plasma TP, then calculating the albumin/globulin (A/B) ratio.

Plasma testosterone (T) level was measured using an enzyme linked immunosorbent assay (ELISA) kit (IBL International GmbH, Hamburg, Germany) with intra- and inter-assay coefficients of variation less than 6.8% and a sensitivity of 0.083 ng/mL. Plasma 17 beta-estradiol (E2) level was determined using an ELISA kit (IBL International GmbH, Hamburg, Germany). The sensitivity and intra- and inter-assay coefficients for E2 were 9.7 pg/ml, 6.8% and 9.4%, respectively. T/E2 ratios were then calculated.

Assay of xanthine oxidase activity

All reagents were purchased from Sigma-Aldrich, UK unless otherwise stated. After blood collection, selected tissues like stomach, gut, kidney, liver, heart, spleen, muscle, brain, womb, and lung were excised, labeled individually and snap frozen in liquid nitrogen as described previously. All tissues were stored at -80 °C and subsequently lyophilized for storage before analysis. Tissues were homogenized in 5-10 volume of potassium phosphate buffer (pH 7.4) containing 5 mM ethylenediamine tetraacetic acid disodium salt (EDTA-Na) and 1 mM phenylmethanesulfonyl fluoride (PMSF) using a Wise Tis homogenizer (HG-15D, Korea). The homogenate was centrifuged at 12,000 × *g* at 4°C for 15 min. The supernatant fraction was centrifuged at 12,000 × *g* at 4 °C for 15 min once again and the supernatant fraction was used to detect XO activity.

XO activity was spectrophotometrically assayed based on monitoring uric acid formation throughout XO reaction, xanthine + H_2O + O_2 ⇌ uric acid + H_2O_2. In brief, the test reaction is started by adding 0.1 ml of the supernatant in 3.9 ml of a phosphate buffer solution (pH 8.0, 50 mM, containing 1mM EDTA-Na) with 1 ml of

xanthine (500 μM, final concentration 100 μM) as the substrate. The mixture (total 5 ml) is incubated at 37 °C for 30 min. The reaction is stopped by the addition of 0.5 ml 0.58 M HCl. We considered a blank reaction containing all components of test reaction but stopped by the addition of 0.5 ml 0.58 M HCl at once. Then blank mixture (total 5.5 ml) was incubated at 37 °C for 30 min. The production of UA was measured by determining the UV absorbance (A) at 290 nm ($A = A_{Test\ tube} - A_{Blank\ tube}$). One unit of XO enzyme activity was calculate as the amount of enzyme required to convert 1 nmol of xanthine to UA per minute per mg protein at 37°C using a standard curve of UA. Each assay was performed in triplicate and protein concentration was determined by the method of Bradford (1976) using bovine serum albumin as the standard [9].

STATISTICAL ANALYSIS

Statistical analyses performed using SPSS version 16 (SPSS, Chicago, IL, USA). Comparison of means between groups was carried out using one-way analysis of variance (ANOVA) and *post hoc* Tukey's tests. Furthermore, Pearson's correlation coefficients were applied to investigate the relation between some variables. P-value < 0.05 was considered statistically significant. Data were expressed as mean ± SEM.

RESULTS

Final bodyweights of studied groups have been changed (P_{ANOVA} = 0.007). After an 8-week period of administration of estradiol benzoate in OVX+E group (25.2±0.925 g), their body mass gain decrease when compared to OVX (28.6±0.736; P = 0.016) and NC (28.8±0.627; P = 0.010) groups. However, induction of ovariectomy did not change the final bodyweight of OVX group in comparison to that of NC group (*P* = 0.976). There was no significant decrease in plasma E2 level in the OVX group (80±10.3 pg/ml; *P* = 0.085) when compared with the control value (167±42.0; Figure 1). Estradiol benzoate administration in OVX+E group (218±16.9 pg/ml) produced significant increment in E2 level when compared with OVX group (*P* = 0.008; Figure 1). Plasma T level reduced non-significantly in OVX group (14±8.4 ng/ml) compared with NC group (50±17.0 ng/ml; *P* = 0.085; Figure 1). Injection of estradiol benzoate in OVX+E group (7±1.7 ng/ml) induced an even greater reduction of T than observed in the OVX group (*P* = 0.923; Figure 1). However, plasma T was not significantly different in OVX and OVX+E groups (*P* = 0.923; Figure 1). The T/E2 ratio did not changed among all studied group (P_{ANOVA} = 0.128). However, the T/E2 ratio decreased by 48 and 91% in OVX (0.195±0.12) and OVX+E (0.037±0.01) groups, respectively as compared to that of NC group (0.379±0.12).

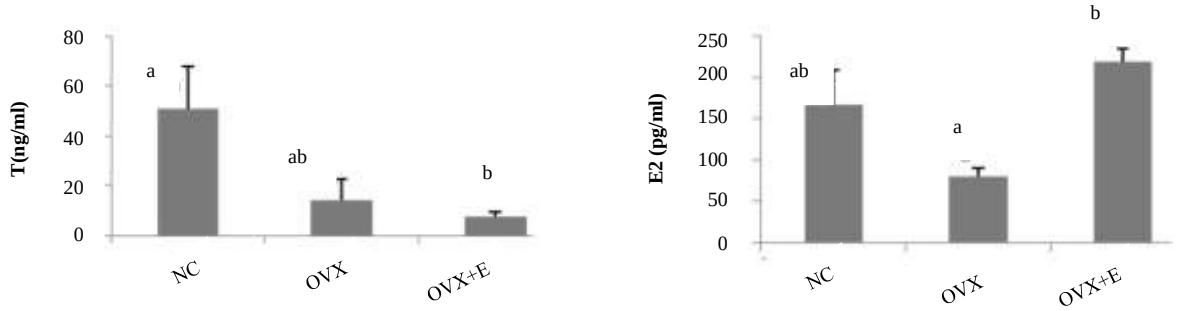

Figure 1. Steroid hormone profile of ovariectomized mice. Values are mean ± SEM (n = 8). T testosterone, E2 17 beta-estradiol, NC normal control group, OVX ovariectomized group, and OVX+E ovariectomized and estrogen-treated group. Columns with different letters are significantly different at $P < 0.05$

OVX mice demonstrated a significant increase in plasma TC compared with NC group ($P = 0.028$; Table 1). However, TGs ($P = 0.147$), HDL-C ($P = 0.095$), VLDL-C ($P = 0.147$), LDL-C ($P= 0.225$), and AI ($P = 0.707$) increased non-significantly in OVX group as compared with NC group (Table 1). Estradiol benzoate administration in OVX+E group exerted an ameliorative effect on TGs ($P = 0.842$), HDL-C ($P = 0.012$), VLDL-C ($P = 0.842$), LDL-C ($P= 0.372$) levels as compared with OVX group (Table 1). However, AI ($P = 0.974$) level decreased non-significantly in OVX+E group as compared with OVX group (Table 1).

Table 1. Lipid and lipoprotein profiles and their related indices in ovariectomized mice

	Group			
	NC	**OVX**	**OVX+E**	**P_{ANOVA}**
TGs (mg/dl)	50.5±4.41	64.4±5.32	60.3±5.49	0.158
TC (mg/dl)	112.2±5.66 ^{a}	136.5±5.78 ^{b}	104.5±7.16 ^{a}	0.005
HDL-C (mg/dl)	97.0±5.72 ^{ab}	116.4±6.15 ^{b}	97.0±5.72 ^{a}	0.014
VLDL-C (mg/dl)	10.1±0.88	12.8±1.06	12.0±1.09	0.158
LDL-C (mg/dl)	5.1±0.76	7.2±0.96	5.5±0.95	0.215
AI (LDL-C/HDL-C)	0.054±0.0089	0.065±0.0115	0.062±0.0066	0.717

Values are mean±SEM ($n = 8$). In rows, data with no common letters differ significantly ($P < 0.05$). TC total cholesterol, HDL-C high density lipoprotein cholesterol, LDL-C low density lipoprotein cholesterol, VLDL-C very low density lipoprotein cholesterol, TGs triglycerides, AI atherogenic index. NC normal control group, OVX ovariectomized group, and OVX+E ovariectomized and estrogen-treated group.

Ovariectomy induced significant increase in TP ($P = 0.001$), ALB ($P = 0.025$), G ($P = 0.004$) and Ca ($P = 0.022$) in OVX group when compared with NC group (Table 2). Estradiol benzoate administration in OVX+E group had an increasing effect on TP ($P = 0.001$), and G ($P = 0.000$) levels when compared with NC group (Table 2). The A/G ratio significantly decreased in OVX+E group as compared with NC group ($P = 0.008$; Table 2). Estradiol benzoate administration in OVX+E group produced significant reduction in UA ($P = 0.043$), and ALB ($P = 0.033$) levels when compared with OVX group (Table 2). The levels of FBS, U, CRT, *P*, and Ca/*P* ratio did not change in studied groups ($P_{ANOVA} > 0.05$; Table 2).

Table 2. Selected plasma metabolite chemistry of ovariectomized mice

	Group			
	NC	**OVX**	**OVX+E**	**P_{ANOVA}**
FBS (mg/dl)	117±19	154±13	146±5	0.193
U (mg/dl)	54.0±3.98	58.4±5.91	66.4±5.56	0.297
UA (mg/dl)	1.5±0.35 ab	1.6±0.25 a	0.5±0.09 b	0.035
CRT (mg/dl)	0.17±0.035	0.28±0.034	0.25±0.034	0.080
TP (g/dl)	4.6±0.15 a	5.6±0.14 b	5.6±0.16 b	0.000
ALB (g/dl)	2.0±0.10 a	2.3±0.04 b	2.0±0.06 a	0.014
G (g/dl)	2.6±0.12 a	3.3±0.12 b	3.6±0.16 b	0.000
A/G ratio	0.76±0.056 a	0.69±0.023 ab	0.56±0.035 b	0.010
Ca (mg/dl)	8.6±0.42 a	9.8±0.15 b	9.3±0.16 ab	0.028
***P* (mg/dl)**	7.7±0.84	6.8±0.90	4.9±0.29	0.057
Ca/ *P* ratio	1.95±0.794	1.60±0.235	1.94±0.148	0.856

Values are mean±SEM (n = 8). In rows, data with different letters were significantly different at $P < 0.05$. FBS fasting blood sugar, U urea, UA uric acid, CRT creatinine, TP total protein, ALB albumin, G globulin, A/G ratio albumin/globulin ratio, Ca calcium, *P* phosphorus, Ca/ *P* ratio calcium/phosphorus ratio. NC normal control group, OVX ovariectomized group, and OVX+E ovariectomized and estrogen-treated group.

According to the methodology of this study, XO activity has been not detected in womb, spleen, and stomach of all studied groups (Table 3). There was a significant decrease in liver XO activity in the OVX group when compared with the value of NC group ($P = 0.027$; Table 3). Estradiol benzoate administration in OVX+E group produced an increase of liver XO activity when compared with OVX group ($P = 0.019$; Table 3) and NC group ($P = 0.962$; Table 3). The XO activities of kidney, lung and muscle were comparable among studied groups ($P_{ANOVA} > 0.05$; Table 3). The XO activity has been not detected in muscles of NC group while OVX and OVX+E groups showed XO activities (Table 3). The brain XO activity increased in OVX ($P = 0.328$) and OVX+E ($P = 0.021$) groups as compared to that of NC group (Table 3). There was significant increase in gut XO activity in the OVX group when compared with the value of NC group ($P = 0.017$; Table 3). Estradiol benzoate administration in OVX+E group restored gut XO activity to its level in NC group ($P = 0.074$; Table 3). The heart XO activity increased in OVX ($P = 0.052$) and OVX+E ($P = 0.005$) groups as compared to that of NC group (Table 3). Finally, the XO activities were not different in all studied tissue except liver in OVX and OVX+E groups ($P > 0.05$; Table 3).

Table 3. Xanthine oxidase activity in the selected tissues of ovariectomized mice

	Group			
	NC	**OVX**	**OVX+E**	**P_{ANOVA}**
Liver	521±52.6 a	275±56.3 b	545±81.9 a	0.010
Kidney	146±33.4	61±14.9	79±46.1	0.081
Brain	55±9.1 a	167±44.9 ab	294±139.9 b	0.024
Gut	433±97.3 a	1302±271.1 b	1146±119.9 ab	0.017
Lung	144±46.2	354±101.3	159±38.4	0.285
Heart	35±13.8 a	112±7.7 ab	152±19.3 b	0.006
Muscle	ND	90±12.8	143±80.8	0.378

Values are mean±SEM (n = 8). In rows, data with different letters were significantly different at $P < 0.05$.
NC: normal control group; OVX: ovariectomized group; and OVX+E: ovariectomized and estrogen-treated group.

DISCUSSION

In the present work, our first objective was to evaluate the effects of bilateral ovariectomy upon biochemical parameters in mice. Our findings confirmed that OVX mice presented increased plasma TP, ALB, G, and Ca levels and considerable changes in the lipid profile independent to weight gain. In this study, similar to our previous study results have shown that ovariectomy put ovariectomized rodents in a hypoestrogenic status similar to postmenopausal women and led to elevation of plasma calcium levels [10]. As previously reported in menopausal women, plasma TP, ALB and G levels have been increased after ovariectomy which confirms previous findings of an increase in serum protein with age [11]. Induction of ovariectomy did not lead to weight gain in comparison to that of normal group. However, administration of estradiol benzoate in ovariectomized group for 8-week period decreased weight gain. In addition, induction of ovariectomy did not cause a significant loss in plasma E2 level when compared with the control value. This is probably due to extra-ovarian sources of 17 beta-estradiol. 17 beta-estradiol is also produced in adrenal cortex and mesenchymal cells of adipose tissue including that of the breast, osteoblasts and chondrocytes of bone, the vascular endothelium and aortic smooth muscle cells, and numerous sites in the brain [12]. However, plasma E2 level declined post-ovariectomy by 52% as compared to the normal group. Estradiol benzoate administration in the presence of ovariectomy led to an accrual change (23%) in plasma E2 level compared to the normal group. We considered a 7-d recovery period after ovariectomy as washout period of endogenous E2, however washout period may be omitted from estradiol-replacement therapy (ERT) in ovariectomized rodents since this period does not significantly influence the levels of 17 beta-estradiol [13].

A crucial finding in the current study is T level that tended to decrease following ovariectomy (- 257%) and ERT further decreased its level (- 614%) compared to the normal group ($P > 0.05$). To our knowledge, no published studies address this possibility. We postulate that this decline of testosterone after ovariectomy may be related to the aromatase enzyme activity that converts androgens to estrogens in peripheral tissues, particularly in adipose tissue [12]. T is made by stroma of menopausal ovaries in a gonadotropin responsive process in old women [14]. Similar to our results, women who have their ovaries surgically removed experience a sudden drop in blood T levels [15]. It the present study, injection of estradiol benzoate in ovariectomized mice led to an even greater reduction of T level as compared to that of control group. This crucial effect may be due to the inhibitory effect of E_2 on T biosynthesis of adrenal gland as a major organ of T production in ovariectomized mice [16].

Estrogen deficiency accounts for the loss of protection against coronary heart disease after natural menopause or following bilateral ovariectomy [17]. In the present study, plasma TC has been significantly increased in ovariectomized mice compared with NC group; however the levels of TGs, HDL-C, VLDL-C, LDL-C, and AI tended to increase ($P > 0.05$). An important atheroprotective impact of estrogen may be mediated by its beneficial influence on lipid metabolism. Postmenopausal women usually exhibit increased levels of LDL-C, lipoprotein (a), and TC and decreased HDL-C level [18]. In the present investigation, estradiol benzoate administration in ovariectomized mice only restored the levels of TGs, HDL-C, VLDL-C, LDL-C, and AI comparable to those of control group ($P > 0.05$). Altogether, induction of ovariectomy for an 8-week period may not completely translated menopausal condition of women, but could reflect hypoestrogenic state that usually occurred in perimenopausal period.

Our second objective was to evaluate the effects of ovariectomy upon the level of plasma UA and XO activity in selected tissues of mice. Our results suggest that plasma UA tended to increase ($P > 0.05$) following induction of ovariectomy while ERT restored its level comparable to control value. On the other hand, the data did not show a dramatic increase in plasma UA levels in ovariectomized mice. Increased UA levels were associated with increased risk for MeS in both premenopausal and postmenopausal women [19]. However our mouse model of menopause does not reliably translate hyperuricemia that occurred after menopause in women. Another study suggests that menopause explains partially age-associated increase of UA among women [19]. Behr et al. showed a decrease in plasma UA levels in ovariectomized rats [20]. Further translational investigations are requested to find a more compatible animal model that shows post-menopausal hyperuricemia. Inverse correlations have been detected between plasma T ($r = - 0.517$; $P = 0.034$) and E2 ($r = - 0.013$; $P = 0.960$) levels and plasma UA in the present study. It seems that enodogenous or exogenous steroids mediate their effects upon plasma UA via alteration of XO activity since UA and H_2O_2 are major products of XO. In this regards, Felty reported that the XO is one of the sources of ROS in estrogen-treated vascular endothelial cells that led to thickening of the vasculature [21]. On the other hand both estradiol stereoisomers, 17β- or 17α-estradiol prevent the hypoxia-induced increase in XDH/XO enzymatic activity [22]. We did not find any significant correlation between tissue XO activity and plasma UA level (data not shown). However, plasma E2 level has been only inversely correlated with XO activities of heart ($r = - 0.677$; $P = 0.011$) and gut ($r = - 0.589$; $P = 0.016$) among tissues that demonstrated XO activity. Although our mouse model of menopause did not show increased UA levels, we still hypothesize that postmenopausal hyperuricemia is resulted from unbalanced production and excretion of uric acid.

To the best of our knowledge, the present paper is the first work demonstrating tissue distribution of XO activity in the selected tissues of ovariectomized mouse model of menopause. Detection of XO activity in various tissues of different species depends on methodologies employed. In this study, XO activity has been not detected in womb, plasma, spleen and stomach of all studied groups. In contrast to our results, XO activities have been reported in the stomach, spleen, and womb of other species [23].

The XO activities of kidney, lung and muscle were similar among studied groups. One of the most striking features of the present study is pronounced XO activity in the muscles of OVX and OVX+E groups compared to normal group that showed no muscle XO activity. In this line, Powers et al. have shown that XO activity increases UA production and the superoxide radical which could elevate ROS and it could be the main mechanism for the reduction of muscle mass [24]. However our results did not show any reduced muscle mass in all studied group (data not shown). In another study, Bravard et al. reported that increased oxidative stress in muscle of streptozotocin-induced diabetic mice is associated with an increase of XO expression and activity and is mediated by an induction of H_2O_2 production by both mitochondria and XO [25]. They also concluded that XO might be a potential therapeutic target for improving oxidative capacities of muscle in diabetic states. In this investigation, FBS levels tended to increase after ovariectomy without resulting hyperglycemia, therefore we cannot explain the reason of increased muscular XO in OVX and OVX+E groups. Further molecular investigations are requested to find mechanism of ovariectomy-induced XO activity in muscles.

There was significant decrease in liver XO activity in the OVX group when compared with the value of NC

group. Liver is a well-known source of UA and XO activity [26]. Although this study did not show significant correlation between plasma UA and hepatic XO activity, the decreased hepatic XO activity is an interesting finding. Estradiol benzoate administration restored liver XO activity in ovariectomized mice to its level in control group. In addition, the XO activities in OVX and OVX+E groups were not different in all studied tissue except liver. We cannot explain the reason of this phenomenon at present study, but it may be due to a minor positive correlation that is between hepatic XO and plasma E2 levels ($r = 0.202$; $P = 0.394$). Search of the available literature revealed no investigations concerning the XO activity in tissues of menopause or animal models of menopause. In the present study, enteric XO has been increased following ovariectomy while ERT restored to its level in control values. In addition, a significant inverse correlation ($r = - 0.589$; $P = 0.016$) was between enteric XO and plasma E2 levels. The XO has long been considered an important host defense molecule in the liver and intestine [27]. The intestinal XO has been also suggested to participate in the mucosal processing of iron and production of urate and H2O2 that involved in infectious enteritis [27]. Further investigations are requested to understand and characterize the altered enteric XO activity in hypoestrogenism.

Heart and brain appeared to contain very low or even undetectable levels of XO activity that involving in ROS and RNS production particularly during tissue injuries [28]. The ERT in ovariectomized mice led to the more pronounced increased of XO activity in brain and heart tissues in the present investigation. In addition, induction of ovariectomy tended to increase XO activity of brain ($P > 0.05$) and heart ($P < 0.05$). The increased XO activity after induction of ovariectomy may explain increased AI in the present study. In this sense, XO inhibition improves endothelial function and plasma markers of oxidative stress in patients with, or at risk of, cardiovascular disease [29]. XO is induced and increases its activity in animal model of acute brain injury where local UA concentrations also increased significantly [30]. The incidence of stroke increases substantially after menopause and hormone therapy is also associated with an increased risk of stroke in menopaused women [31, 32]. In addition, there is a huge body of evidence to support the neuroprotective effect of UA administration after brain ischemia [33]. Therefore, neuroprotective or neurodestructive roles of XO as a main enzyme that produced UA in brains need to be elucidated in further experimental studies.

CONCLUSIONS

An 8-week period after ovariectomy in mice translates reliable hypostrogenic state in women that reflects some characters of menopause including hypercholesterolemia and increased susceptibility to cardiovascular diseases through increasing AI. The ERT also could restore alterations made by induction of ovariectomy in mice. Strict relationships between plasma UA levels and tissue XO activities were not found, therefore measuring the rate of UA excretion and/or altered activity of other enzymes that involving in purine metabolism and hyperuricemia (eg. hypoxanthine-guanine phosphoribosyl transferase) requested to be investigated in animal models of menopause. Since remarkable hyperuricemia did not occur in our mouse model of menopause during 8-week period, the etiology of postmenopausal hyperuricemia remained still mysterious.

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Conflict of interests

There is no conflict of interest.

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