

Effect of saponin extract of *Glycyrrhiza glabra* in activity of hepatic enzymes and some biochemical parameters in serum of adults ovariectomized female rats

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Abstract

This research was planned to verify the influence of saponin, that extracted from *Glycyrrhiza glabra* in activity of hepatic enzymes and some biochemical parameters of serum ovariectomized adult female rats. 15 adult female rats were randomly divided into three groups, which included sham-operated (sham), ovariectomized (ovx), and ovariectomized rats treated orally with (250 mg/kg/day) of saponin extract for (25) days. The results revealed, that ovx rats showed a significant elevation in alkaline phosphatase (ALP) activity, Calcium level, a significant reduction in activities of creatine kinase (CK), aspartate amino transferase (AST), γ -Glutamyl transferase (GGT), and level of albumin, creatinine without any significant change in alanine amino transferase (ALT) activity and magnesium level comparison with sham -operated rats. Treatment ovx rats with 250 mg/kg of saponin caused a significant reduction in ALT and CK activities, and elevation in albumin and creatinine levels, and AST, GGT activities. In conclusion, the present results revealed that, saponin extracted of *Glycyrrhiza glabra* have an effect in hepatic enzyme activity and some biochemical parameters in ovariectomized female rats with osteoporosis results from reduction in estrogen level.

Keywords: Saponin, *Glycyrrhiza glabra*, Ovariectomized rats, Enzymes

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تأثير الصابونين المستخلص من عرق السوس *Glycyrrhiza glabra* في فعالية بعض أنزيمات الكبد وبعض المتغيرات الكيموحيوية في مصل دم الجرذان البالغة المستأصلة المبايض

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الخلاصة

صممت الدراسة لمعرفة تأثير الصابونين المستخلص من عرق السوس في فعالية أنزيمات الكبد وبعض المتغيرات الكيموحيوية في مصل إناث الجرذان البالغة المستأصلة المبايض. خمسة عشر من إناث الجرذان قسمت عشوائياً إلى ثلاثة مجاميع والتي تضمنت مجموعة مستأصلة المبايض الكاذب، مجموعة مستأصلة المبايض ومجموعة مستأصلة المبايض المعاملة بمستخلص الصابونين 250 ملغم/كغم/يوم فمويًا ولمدة 25 يوم. أظهرت النتائج وجود ارتفاع معنوي في فعالية أنزيم الفوسفاتيز القلوي ومستوى الكالسيوم مع وجود انخفاض معنوي في فعالية كل من الكرياتينين كايينيز والاسبارتات ناقله الأمين وكاما كلوتاميل ترانسفيريز ومستويات الألبومين والكرياتينين وبدون أي تغير معنوي في فعالية الألبومين ناقله الأمين ومستوى المغنيسيوم في مصل الجرذان المستأصلة المبايض مقارنة مع مجموعة الجرذان المستأصلة المبايض الكاذب. غير أن معاملة الجرذان المستأصلة المبايض بمستخلص الصابونين تسبب في انخفاض معنوي في فعالية كل من أنزيم الفوسفاتيز القلوي والكرياتينين كايينيز مع ارتفاع معنوي في فعالية الاسبارتات ناقله الأمين وكاما كلوتاميل ترانسفيريز ومستوى كل من

الألبومين والكرياتينين. نتائج الدراسة اظهرت ان الصابونين المستخلص من عرق السوس يمتلك تأثير في فعالية أنزيمات الكبد وبعض المتغيرات الكيموحيوية في إناث الجرذان التي استأصلت مبايضها وأصيبت بهشاشة العظام نتيجة نقص الأستروجين.

Introduction

Osteoporosis following menopause which associated with reduction in hormones of ovarian, is the most common cause of bone loss (1). Osteoporosis is a common systemic skeletal disease among the elderly (2,3). This case generalized by reduction in mass of bone. It is a major health problem, that accelerated loss of bone, which lead the increasing the probability of bone fracture and its mortality risk. (4).

Aging decreased bone mass and menopause stimulate bone of loss. Ovariectomy cause a lack of estrogen and this deficiency produces osteoporosis in human and rats (5-8).

Estrogen replacement therapy is an active method that used to reduce the rate of bone loss in post menopausal women, but it has side effects. Because of the side effect of hormone replacement therapy (HRT) which is used to slow the development of osteoporosis, the present study was undertaken to attain the effect of saponin extracted from *Glycyrrhiza glabra* on estrogen deficiency, since we utilized the ovx rats as animal model for postmenopausal women.

Glycyrrhiza is an ancient herbal remedy in world. It was used in India as Ayurvedic medicine before 4000 years. The function of this plant in respiratory system is due to glycyrrhizin that act as an expectorant which decrease and prevent coughing. Glycyrrhizin utilize as an anti-inflammatory and also have anti-allergenic effect, especially in asthma (9,10). Glycyrrhizin, is a saponin have a sweet taste which found in *Glycyrrhiza* plants, it is a conjugate of two molecules glucuronic acids and glycyrrhetic acid (11).

The major component in the roots and stolons of *Glycyrrhiza* plants that imparts a sweet flavor-glycyrrhizin is an oleanane-type triterpene saponins (12). Glycyrrhizin also known as glycyrrhizic acid which is found together with other substances including other triterpenoids, polyphenols, polysaccharides, essential oils, pectins, flavonoids, amino acids, etc (13). It was established that it presents various types of pharmacological activity (antiviral, mineral corticoid) therefore it is used for numerous medical purposes such as treatment of disorders in lungs, liver, respiratory tract, stomach, kidney and hormones equilibrium (14-16).

Our study was aimed to investigate the estrogenic-like effect of saponin extracted from *Glycyrrhiza glabra* in hepatic enzyme activities and some biochemical markers of ovariectomized rats.

Materials and methods

Animal and experiment groups

Fifteen female rats (albino species), 4 months old, weighting 250-300 grams, were housed at conditions of temperature between 22-25°C, 12 hours light-dark cycle in animals house of veterinary college of University of Mosul. Standard diet and water were provided. The rats were divided into three groups, five rats in each group, group one contained five rats (ovaries were not removal, sham-operated =sham), group two contained five rats (ovaries were removal =ovx) and taken as a model of postmenopausal women, group three contained five rats (ovaries were removed and treated with 250 mg/kg bw /daily of saponin). The weights of all rats were measured for determine the actual dose to be given. Oral administration was used in this experiment.

Plant

Powdered *Glycyrrhiza glabra* was obtained from the local market in Mosul city. Extraction saponin from *Glycyrrhiza glabra*. 500 gm of *Glycyrrhiza glabra* was extracted with 70% ethanol for three times at 24 hours at room temp. The combined ethanol solutions were concentrated to small volume and extracted in succession with chloroform three times at 24 hours and n-Butanol three times at 24 hours. The n-Butanol layer was concentrated to dryness to give saponins extract (17).

Treatment

Group 1 (sham-operated rats) given standard diet with distilled water for 25 days. Group 2 (Ovx rats) given standard diet with distilled water for 25 days. Group 3 (Ovx rats) given standard diet and distilled water with 250 mg/kg/day of saponins for 25 days.

Blood collection

Blood samples were collected from the optical vein, at the ending of the experiment period (18), into sterile specimen tubes free of anticoagulant, left to clot, then centrifuged for 15 minutes and serum was separated and stored in -18°C for biochemical measurements.

Biochemical parameters

Biochemical analysis was included ALP activity that was detected spectrophotometrically by using Biomerieux kit which depend on the method of Belfeld and Goldbery (19), the activity of CK was determined by using kit which manufactured by Biolabo, France. AST and ALT activities were determined by colorimetric method developed by

Tonhazy, White, and Umbreit (20) using kit of Biolabo, France and GGT activity was measured by Biolabo, France kit which depended on Szasz, Rosalki and Tarlow method (21).

Kits manufactured by Biolabo, France were utilized to quantify calcium, magnesium and albumin levels. Colorimetric method of manufactured by Fortress diagnostics kit was used for determination of creatinine.

Statistical Analysis

The results were expressed as means \pm SE, one-way analysis of variance (ANOVA) test was used to detect the statistical significance by using sigma stat 16 within probability at $P < 0.001$.

Results

Results of table 1 appeared an elevation of serum ALP in ovariectomized rats comparison with sham rats, also elevated in treatment ovariectomized rats that treated with

250 mg/kg/day compared with sham-operated rats, a significant reduction of serum CK activity in ovariectomized comparison with sham group. decrease AST activity were significantly reduce when compared with sham group, and significantly elevated in treated ovariectomized rats compared with ovariectomized rats, but there was no differences in ALT activity within all groups as shown in table 1, also showed that serum GGT activity was decreased significantly in ovariectomized rats contrast with sham group but return to level of sham-operated rats after treatment with saponins.

Serum calcium level was increase significantly ($P < 0.001$) in untreated and treated ovariectomized rats compared with sham rats, but serum albumin level was decreased in both groups contrast with sham rats as shown in table 2. Serum creatinine level was significant decreased in untreated and treated ovx compared with sham rats as shown in table 2. While no significant differences in serum magnesium in all groups shown in table 2.

Table 1: Serum hepatic enzymes activities in sham-operated, ovx and treated ovx rats

Groups	Mean \pm SE (5 rats at each group)				
	ALP U/L	CK U/L	AST U/L	ALT U/L	GGT U/L
sham-operated rats	86.88 \pm 1.95 A	46.66 \pm 10.804 A	34.5 \pm 0.866 A	19.75 \pm 0.25 A	1.451 \pm 0.168 A
Ovx rats	211.25 \pm 7.589 B	39.00 \pm 8.28 B	23.75 \pm 0.479 B	20.75 \pm 0.47 A	1.107 \pm 0.02 B
Ovx + saponins	208 \pm 7.528 C	34.50 \pm 6.238 C	27.75 \pm 0.250 C	20.76 \pm .25 A	1.410 \pm 0.15 A

Letters different vertical mean a significant variance at $P < 0.001$.

Table 2: Serum biochemical parameters in sham-operated, ovx and treated ovx rats

Groups	Mean \pm SE (5 rats at each group)			
	Calcium mg/dl	Magnesium mg/dl	Albumin g/dl	Creatinine mg/dl
sham-operated rats	6.48 \pm 0.602 A	2.027 \pm 0.01 A	3.51 \pm 0.281 A	1.91 \pm 0.040 A
Ovx rats	6.75 \pm 0.250 B	2.01 \pm 0.03 A	2.50 \pm 0.28 B	1.58 \pm 0.042 B
Ovx + saponins	6.80 \pm 0.707 B	2.07 \pm 0.04 A	2.75 \pm 0.250 C	1.60 \pm 0.047 B

Letters different vertical mean a significant variance at $P < 0.001$.

Discussion

The elevation of both ALP activity and calcium level in ovx rats in this study was agree with other results of (22). The significance increase in ALP activity after ovariectomy of female rats in contrast to sham rats, perhaps due to an increase in bone resorption since high ALP activity that related to a high turnover rate of bone, which heighten bone destruction and lead to a slight rise in serum calcium (23). This elevation in ALP activity in our study agree with Armine *et al* (24) who showed that there was a raise in ALP activity in ovariectomized rats because of the resorption of bone by estrogen deficiency (24). Also, Li *et al* (25) were reported that ovariectomy increase bone resorption in rats

and ALP activity increased (25). Also, our results agree with other study, that they reported the ovariectomy cause altitude of ALP activity (26). On the other hand, administration ovx rats with 250 mg/kg of saponin extract for 25 days result in a reduction in serum ALP activity in comparison to ovx rats this perhaps due to the possible role of saponins to intensify bone formation and reduction bone resorption that done by ovariectomy. The decreasing in serum ALP activity in our study was agree with previous study reported that ALP increased in ovx and lowered after treatment with vanillic acid (27). Also, our study agrees with (28) since they showed that, there were a significant decrease in serum ALP activity after treatment ovx with soybean-Hop. The reduction in serum CK activity in ovx

compared with sham group in this study may be due to the less muscle damage occurred in ovx rats compared with sham group. In addition, the treatment of ovx rats with 250 mg/kg of saponin extracted from *Glycyrrhiza glabra* results in a significant decrease in serum CK activity compared with ovx rats, this may be due to estrogenic-like role of saponins which reduce CK activity in blood. In a study of (29) plasma CK level was significantly lower in the estrogen supplement and ovariectomized animals, which suggests that estrogens may have a protective effect on muscle tissue possibly due to their antioxidant and membrane stabilizing properties. Low serum creatine kinase values also have been reported in contraceptive steroid users (30) and this supports our hypothesis about the role of saponin as estrogen effect. In addition, study of (31) indicated, that increased testosterone level resulted in decreased CK level, this mean there are associations between sex steroid hormones and biochemical enzymes activities. Also, our results agree with result of (32) that showed, ovariectomy decreased creatine kinase activity. Some studies have shown, that serum CK level is significantly decrease after administration of steroid (33). As shown in table 1, the results indicated a significant decrease in serum AST activity of ovx rats compared with sham group and a significant increase in AST in treated ovx rats compared with ovx rats, this may be due to the effect of estrogen deficiency by ovariectomy and protective effect of *Glycyrrhiza glabra* in enzyme activity. On the other hand, there was no significant differences in ALT activity in all groups. Also, serum GGT activity was decreased significantly in ovx rats compared with sham group, but returns to its activity in treated ovx rats with 250 mg/kg of saponins.

The results in table 2 show a significant increase in calcium level in ovx rats compared with sham-operated rats. Our results is agreement with (34) they found, that ovariectomy result in an increase in serum calcium. This result indicates, that there is a decrease in calcium in bone because of the resorption of bone. Also our result is agree with (35) she reported that there was a significant increase in serum calcium after one month of ovariectomy, this may be due to the stimulation of parathyroid gland as a response of low calcium level in serum, since parathyroid hormone conjugated with receptors on osteoblasts this lead to production of messenger stimulate the osteoclasts to secrete hydrolysis enzymes which hydrolyze, bone matrix leading to release of calcium in to blood (36). A significant decrease in albumin levels in ovx rats compared with sham-operated rats, this may be due to estrogen deficiency. Our results is agree with another study of (37) they noted a reduction in the serum of albumin level for at least 30 days after ovariectomy and they reinstructed this reduction to estrogen deprivation. Albumin also acts a essential role as a major calcium-binding in serum (38). Consequently, it

should, have more direct effect on bone metabolism. The decreasing of albumin level results in an increase in free calcium (39). On other hand, treatment ovx with 250 mg/kg of saponin results in a significant increase of serum albumin compared with ovx rats, this already due to saponin role which act as estrogen. Hypoalbuminemia may cause hyperparathyroidism by increasing the level of phosphate in serum and lowering ionized calcium, hence loss of bone following ovariectomy (40). Our results agree with results of (41) they reported a significant decrease in serum albumin in ovx rats. Serum creatinine levels were significant decreased in ovx compared with sham-operated rats. On the other hand, given ovx rats with 250 mg/kg of saponin results in a significant increase in serum creatinine compared with ovx rats, this may be due to the estrogenic effect of *Glycyrrhiza glabra*.

Conclusion

The present results revealed that, saponin extracted of *Glycyrrhiza glabra* have an effect in hepatic enzyme activity and some biochemical parameters in ovariectomized female rats with osteoporosis results from reduction in estrogen level.

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Conflict of interest

Conflict of Interest the authors declare that there is no conflict of interest.

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