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Effect of germinated chickpea flour on bone healthofovariectomized ratsas model of menopause

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Abstract

Menopause affects a woman on all levels, from organs to bio-mentalpsycho-social functioning. Postmenopausal osteoporosisis a common disease related by the decrease of bone mass and bone structure changes, usually attributed to estrogen deficiency. The present study aimed to assess the effect of germinated chickpea flour on ovariectomized (ovx) rats (as model of menopause).The study was performed on thirty five adult female albino rats. They were divided into five equal groups. The first group was sham operated (7 rats) and the other four groups(7 rats each) were ovariectomized. The sham group (negative control) and one ovx group (positive control) were fed basal diet, whereas the remaining three ovx groups were fed basal diet containing 2.5, 5 and 7.5% of germinated chickpea flour. Supplementation rats diet with germinated chickpea flour led to the improvement of serum calcium, ionized calcium, phosphorus, magnesium, alkaline phosphatase activity, bone-specific alkaline phosphatase activity, osteocalcin, calcitonin, parathyroid hormone and lipid levels compared to positive control group. Also calcium, phosphorus, magnesium and BMD in femur and tibia in ovx groups supplemented with different concentration of germinated chickpea flour were significantly higher than in the positive control group. However, no significant differences were observed in estrogen level, femur and tibia weights and lengths between positive control and ovx groups supplemented with 2.5% of germinated chickpea flour. These results suggest that germinated chickpea flour is effective in improving lipid levels and bone health in ovariectomized rats.

Keywords: isoflavone, femur, tibia,osteocalcin.

Introduction

Estrogen deficiency due to menopause affects a woman on all levels, from subcellular structures, organs, regulatory systems to bio-mentalpsycho-social functioning **(Dijk*et al*., 2015)**.During menopause, estrogen deficiency affects bone metabolism **(Noh *et al*., 2019)**.Menopause increases bone fragility in women,by decreasingbone mass and bone mineral density (BMD).Estrogen deficiency is associated with an unbalanced between resorption and formation in favour of bone resorption, gradually leading to bone loss **(Farlay*et al*., 2019)**.Osteoporosis in menopausal women is mainly managed using estrogen replacement therapy (ERT), bisphosphonates, selective estrogen receptor modulators, and calcitonin. Unfortunately, the positive effects of these drugs are counteracted by serious side effects such as increased risk of cancer and thromboembolism. Therefore, it is necessary to search for new, less toxic, drugs that can prevent osteoporosis. The potential medical applications of phytochemicals are becoming more recognized in the scientific community and there have been many efforts to identify the effects of natural compounds on bone health in women with menopausal symptoms **(Noh *et al*., 2019)**.Isoflavone is a chemical compound isolated from di-phenolic secondary metabolites of phtoestrogens with a structure similar to that of human estrogen **(Hsiao and Hsieh, 2018).** The primary sources of isoflavone in the diet are the plants of the Leguminosae family such as chickpea **(Merlanti*et al*., 2018)**. Chickpeas contain a variety of important organic compounds, notably bioactive phenolic compounds such as isoflavones**(Mekky*et al*., 2015).**Chickpea is a good source of protein and carbohydrates, and protein quality is considered to be better than other pulses .It also contains significant amounts of all the essential amino acids except sulphurcontaining amino acids, which can be complemented by adding cereals to the daily diet **(Jukanti*et al*., 2012).** The isoflavones, especially daidzein and genistein, have been reported to have direct protective effects against several diseases such as osteoporosis, cancers, and metabolic syndromes **(Levis *et al*., 2011).**The aim of this study was to determine the effect of germinated chickpea flour on bone healthofovariectomized rats.

Materials and methods

Materials

Chickpea seeds(Cicerarietinum) werepurchased from the Agriculture Research Center, Giza, Egypt. Kits were purchased from (Alkan Medical Company, St. El Doky, Cairo, Egypt). All other chemicals and reagents were obtained from El-Gomhoreya Company, Cairo,Egypt.

Preparation ofchickpea flour

The chickpea seeds were sterilized by soaking in 95% ethanol for 1 min. The seeds were soaked in tap water for 12 h at room temperature. The soaked seeds were kept between thick layers of cotton cloth and allowed to germinate in dark at room temperature for 3 days. The germinated seeds were rinsed with tap water, mashed and dried under vacuum at 40^{o}C for 10 h.The dried germinated seeds were ground to pass through a 400mm sieve then packed in polyethylene bags and stored in a freezer until used.

Experimental design

Thirty five adult female albino rats at three months of age were purchased from the faculty of medicine, benha university, Egypt,after2 weeksconvalescenceperiod from conducting surgery a sham operated (7 rats) and bilateral ovariectomy (28 rats). Rats were housed in enviromentally controlled atmosphere and were fed standard diet according to AIN-93 guidelines **(Reeves *et al.*,1993)** in animal laboratory in the faculty of Home Economics foradaptation period (one week). After adaptation period rats were divided into fiveequal groups. The first group was sham operated and the other four groups were subjected bilateral ovariectomy. The sham group (negative control) and one ovariectomized (ovx) group (positive control) were fed a casein-based diet, whereas the remaining threeovx groups were fed a similar diet in which starch was replaced with 2.5, 5 and 7.5% of chickpea flour. Ethical guidelines for the care and treatment of animals were strictly followed in accordance with the rules of the Egyptian animal protection. At the end of experimental period (8 weeks), rats were anesthetized with diethyl ether after fasting for 12h and blood samples were collected, and serum was separated by centrifugation. Serum were frozen and kept at−20°C for later analysis. Left femur and tibiafrom each rat were removed and stored at - 4 C^{o} until determination of bone mineral density (BMD). After determination of bone mineral density(BMD),the left

femur and tibia of each rat were cleaned of soft tissue and stored at - 4 Cº until determination of bone mineral contents.

Methods

Protein, fat, moisture, and ash contents were determined in chickpea flour according to **AOAC (2010)**. The carbohydrate was calculated by difference.

Bone mineral density

Bone mineral density (BMD) of the left femur and tibia of each rat were measured by dual x-ray absorptiometry (DXA; model DCS-600A; Aloca, Tokyo, Japan).

Determination bone calcium, phosurous and magnesium

The left femurs and tibias weredried at 80°C for 18 hours to evalute bone weight, and then ashed at 600°C for 24 hours. Ashed samples were dissolved in 4 ml of 0.1 NHCl, and then diluted apppropriately with distilled water for atomization. Bone calcium, phosurous and magnesium were analyzed using flame atomic absorption spectrophotometry (Model 5100 PC, Perkin-Elmer, Norwalk, CT) acorrding to **Fraser *et al.* (1986)**.

Blood parameters

Serum calcium,ionized calcium,phosphorus and magnesium were carried out by colorimetric methods described by **Gindler and King (1972)**; **Boink*et al.* (1991);Maria*et al.* (1983) and Abdulsahib(2011)**respectively. Osteocalcin, estrogen, PTH, calcitonin, bone alkaline phosphatase (BALP), alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase (TRAP) activities were determined with kits according to **Lee *et al.* (2003); Owens and Ashby(2002);Julie *et al*. (2010)**;**Daumerie*et al*. (2013);Rosalki*et al.*(1993); Varley*et al.*(1980) and Smith *et al.*(2005)**respectively. Serum total cholesterol, triglyceride (TG) and high density lipoprotein (HDL-c) were determined by using methods of **Allain*et al.* (1974); Fossati and Prencipe (1982) and Lopez-virella (1977)** respectively. The determination of low density lipoprotein cholesterol (LDLc) and very low density lipoprotein cholesterol (VLDLc) were carried out according to the methods of **Lee and Nieman (1996)** as follows:

VLDLc = TG/5 and LDLc = Total cholesterol - (HDLc + VLDLc).

Statistical Analysis

Results were expressed as the mean ± SD. Data for multiple variable comparisons were analyzed by one-way analysis of variance

(ANOVA). For the comparison of significance between groups, Duncan's test was used as a post hoc test according to the statistical package program (**Armitage and Berry, 1987).**

Results and discussion

Chemical composition of germinatedchickpea flour were presented in Table (1). Data showed that protein(19.16%), carbohydrates(60.84%), Ca(101.75mg/100g), P (192.75mg/100g) and Mg (177.62mg/100g) were high in chickpea flour while, moisture (11.43%),fat (6.06%), ash (2.49%),fiber (8.4%) and Zn (6.09%) were low. These results are in the same trend of **Kahramana*etal*. (2018) and Rey *etal*. (2019)**who showed that the chemical composition of chickpea flour was 57, 6.8, 23, 3.09 and 9.15% for carbohydrates, fat, protein, ash and moisture respectively.In comparison with **(Jukantil*et al*., 2012)**who reported that chickpea contained Zn (4.1 mg/100g), Mg (138mg/100g), Ca (160mg/100g).

Effect of germinated chickpea flour on serum Ca, ionized calcium, P and Mg of experimental rats are shown in Table (2). The calcium, ionized calcium, phosphorus and magnesium in ovxpositive and ovx groups treatment with 2.5 and 5 % of germinated chickpea flour were lower than in sham group.These results are agreement with those reported by**Wahba and AL-Zahrany(2013)** who found that ovariectomy led to asignificant decrease in both calcium and phosphorus in the serum. Also, **Hassan *et al*. (2010)** reported that ovariectomy has been shown to alter phosphate homeostasis and lead to significant decrease of its level.Feeding ovariectomized rats with different concentration of germinated chickpea significantly ($p \leq 0.05$) improved the levels of Ca, ionized calcium, phosphorus and magnesium compared with ovx control group. This improvement may be due to genestien content and calcium, phosphorus and magnesium in germinated chickpea flour (table 1). **Breitaman*et al.* (2003)**suggested that the combination of isoflavones and supplemental Ca provides greater protection against ovariectomy-induced bone loss than either isoflavones or high Ca diet alone. On the other side there were no significant difference ($p>0.05$) in serum ionized calcium between sham and ovx group supplemented with 7.5% germinated chickpea flour. Also, supplementation rat diets with 5 and 7.5% germinated chickpea flour did not differ in their effect onionized calcium.The highest improvement of

calcium, ionized calcium, phosphorus and magnesium were observed in ovxgroup feeding with 7.5% germinated chickpea flour.

Table (3) showed the effect of germinated chickpea flour on serum bone parameter of experimental rats. Ovariectomized rats had significant increased (P≤ 0.05) levels of ALP, TRAP and PTH compared with sham and ovariectomizedtreatment groups while, BALP, calcitonin, osteocalcin and estrogen had opposite trend. These results are agreement with**Karmakar*et al*. (2012)**and **Park *et al*. (2011)** who reported that serum TRAP and ALP activity increased significantly in the ovx group compared to the sham group. Also, **Canpolat*et al*. (2010)** found that a significant increase in PTH level and a significant decrease in calcitonin level of ovx positive group.**Sontakke and Tare (2002)** showed that the decrease in the activity of BALP may be due to the disturbance in osteoblastic function and/or an imbalance between osteoclastic and osteoblastic activities.Feeding ovx rats on diet supplemented with germinated chickpea flour resulted in improvement the levels of ALP, TRAP, PTH, BALP, calcitonin, osteocalcin and estrogen compared with positive ovx group. These results had the same trend of **Youssef, (2016)** who found that treatment ovx rats with soybean as a source of isoflavones resulted in an increase in osteocalcin and ALP and BALP compared with ovx positive group.These improvements in results may be due to presence of high amount of isoflavones in germinated chickpea flour.The isoflavone contents(genistein, formononetin and biochanin A) in chickpeas dramatically increase during germination**(Gao*et al*., 2015)**. Also, biochanin A and formononetin can be demethylated to genistein and daidzein, respectively, by intestinal microflora and by the human liver **(Megías*et al*., 2016).** The actions of genistein on bone were shown to be reduction of osteoclasts and genistein stimulates bone formation and inhibits bone resorption (**Horiuchi and Onouchi, 2006)**. In the same table no significant difference was observed in ALP, PTH, calcitonin and estrogen between positive ovx rats and ovx rats fed diet supplementedwith 2.5% germinated chickpea flour.Feeding rats on diet supplemented with 5 and 7.5% did not significantly differ (p>0.05) in their effect on ALP, PTH and estrogen.Supplementation ovx rats diet with 7.5% of germinated chickpea was more effective (P≤ 0.05) in increasing BALP, calcitonin and osteocaclin than those supplemented with 2.5 and 5% of germinated chickpea flour.

Effect of germinated chickpea flour on lipids profile of experimental rats is shown in Table (4). Overiectomized rats had the highest (P≤0.05) values of TC, TG, LDL and VLDL compared to sham group while, HDL had opposite trend. These results are agreement with **Harini*et al*. (2015)**who showed that ovariectomy increased the cholesterol content in serum and the deficiency of estrogen is known to increase in cholesterol levels, both in humans and animals. No significant differences (P<0.05) were found in HDL between sham and ovx rats supplemented with different concentration of germinatedchickpea flour. The TC, TG, LDL and VLDL in ovxgroups treatment with germinated chickpea had lower than ovx positive control while, HDL had opposite trend. These results were agreement with **Legette*et al*. (2011)** who showed that rats receiving genestien had significantly lower total serum cholesterol concentration than ovx positive control. Feedinggerminated chickpea to ovx rats reversed the changes of ovariectomy in terms of serum lipid profile. The beneficial effects wereattributed to the presence of phytoestrogenic flavonoids, biochanin A, formononetin, which are found in higher concentration in germinated seeds of chickpea **(Harini*et al*., (2015)**.Similar results demonstrated that Phytoestrogens may affect other pathophysiologic vascular processes such as lipid profile (reduce levels of LDL cholesterol), angiogenesis, inflammation, tissue damage by reactive oxygen species, and these effects could delay the progression of atherosclerosis **(Sirotkin and Harrath, 2014)**.

Table (5) demonstrated the effect of germinated chickpea flour on bone parameters of experimental rats. The bone mineral content (BMC) and bone mineral density (BMD) of the left femur and tibia in the ovx positive control were lower (p≤0.05) than sham and ovx groups supplemented with germinated chickpea. These results agreement with **Fahmy*et al*. (2015)** who found that ovariectomy significantly reduced the femoral and tibial BMD and BMC.Supplementation ovx rats diet with germinated chickpea flour led to improvement weight, length, BMC and BMD in femur and tibia than ovx positive control. These results are agree with **Fu*et al*. (2014)** who showed that the phytoestrogen treatment group experienced a significantly higher femur BMD compared control positive group. Moreover,phytoestrogen used for the prevention of postmenopausal osteoporosis by stimulating osteoblasttic activity and inhibit osteoclast formation **(Rashid*et al*., 2010).** Ovx rats

supplemented with 7.5% of germinated chickpea flour was more effective (P≤ 0.05) in increasing calcium, phosphorus and magnesium levels in femur and tibia than those supplemented with 2.5 and 5% of germinated chickpea flour and ovx group. This may be due to high content of calcium, phosphorus and magnesium in rat diets supplemented with 7.5% of germinated chickpea flour. However ovx rats supplemented with 2.5 and 5% ofgerminated chickpea flour had the same effect on femur BMD and tibia length.There were no significant differences in femur length and tibia BMD between sham and ovx rats fed on diets supplemented with different concentration of germinated chickpea flour groups.**Hai-rong*et al.* (2013)** reported that chickpea contained strong phytoestrogens and could be used as an alternative therapy to relieve menopausal symptoms and prevent bone loss caused by estrogen deficiency during menopause.

Conclusion

Germinated chickpea flour supplementation for six weeks showed potentially beneficial effects on bone metabolism and on serum lipids in ovariectomized rats. So we recommend testing the ingestion of germinated chickpea flour for menopausal women for its potential effect to reduce the risks of postmenopausal osteoporosis.

Table (1): Chemical compositionof germinated chickpea flour

Parameter	**Germinated chickpea flour**
Moisture (g/100g)	11.43±0.24
Protein (g/100g)	19.16±0.20
Fat (g/100g)	6.06±0.14
Ash (g/100g)	2.49±0.1
Carbohydrate (g/100g)	60.84±0.12
Fiber (g/100g)	8.40±0.1
Zn (mg/100g)	6.09±0.1
P (mg/100g)	192.75±5.8
Mg (mg/100g)	177.62±1.3
Ca (mg/100g)	101.75±2.5

Each value in the table is the mean± standard deviation

Table (2): Effect of germinated chickpea flour on serum Ca, ionized Ca,P and Mg of experimental rats

Variables	Sham	Ovariectomized groups			
		OVX control	OVX+2.5% GCF	OVX+5% GCF	OVX+7.5% GCF
Ca (mg/dl)	10.79^{a}±0.3	6.31^{e}±0.5	7.10^{d}±0.53	8.03^{c}±0.31	9.23^{b}±0.31
Ionized Ca (mg/dl)	1.32^{a}±0.02	0.82^{d}±0.04	0.97^{c}±0.11	1.15^{b}±0.05	1.24^{ab}±0.03
P (mg/dl)	6.64^{b}±0.4	4.84^{d}±0.3	5.62^{c}±0.37	6.57^{b}±0.47	7.88^{a}±0.33
Mg(mg/dl)	2.39^{a}±0.14	1.58^{d}±0.02	1.74^{cd}±0.05	1.87^{c}±0.05	2.07^{b}±0.11

Values are expressed as means ± SD; means in the same raw with different letter are significantly different (P < 0.05. ovx: ovariectomized; GCF: germinated chickpea flour

Table (3): Effect of germinated chickpea flour on serum bone parameters of experimental rats

Variables	Sham	Ovariectomized groups			
		OVX control	OVX+2.5% GCF	OVX+5% GCF	OVX+7.5% GCF
ALP (u/L)	189.27^{c}±14.6	431.40^{a}±27.4	403.03^{a}±20.6	333.06^{b}±23.2	303.93^{b}±9.1
BALP (U/L)	0.61^{a}±0.03	0.14^{e}±0.01	0.26^{d}±0.01	0.33^{c}±0.02	0.46^{b}±0.01
TRAP(ng/ml)	0.1^{e} ±0.01	0.39^{a}±0.01	0.31^{b}±0.01	0.23^{c}±0.02	0.16^{d}±0.01
PTH(pg/ml)	0.71^{c}±0.04	1.42^{a}±0.19	1.24^{a}±0.14	1.003^{b}±0.07	0.90^{bc}±0.04
Calcitonin(pg/ml)	0.54^{a}±0.03	0.12^{d}±0.01	0.14^{d}±0.01	0.36^{c}±0.01	0.49^{b}±0.01
Osteocalcin(Pg/ml)	0.51^{a}±0.03	0.11^{d}±0	0.16^{c}±0.01	0.43^{b}±0.01	0.5^{a}±0.01
Estrogen (mg/dl)	1.01^{a}±0.02	0.42^{c}±0.02	0.66^{bc}±0.02	0.76^{ab}±0.02	0.83^{ab}±0.36

Values are expressed as means ± SD; means in the same raw with different letter are significantly different (P < 0.05. ovx: ovariectomized; GCF: germinated chickpea flour. ALP: alkaline phosphatase; BALP: bone specific alkaline phosphatase; TRAP: tartrate-resistant acid phosphatase.

Table (4): Effect of germinated chickpea flour on lipids profile of experimental rats

Variables	Sham	Ovariectomized groups			
		OVX control	OVX+2.5% GCF	OVX+5% GCF	OVX+7.5% GCF
TC (mg/dl)	109.31^{e}±6.7	233.05^{a}±8.4	171.30^{b}±7.5	149.16^{c}±6.7	121.73^{d}±1.8
TG (mg/dl)	82.99^{e}±6.02	205.90^{a}±7.7	153.23^{b}±5.5	126.75^{c}±6.1	104.16^{d}±6.4
LDL (mg/dl)	40.91^{e}±4.9	148.95^{a}±8.9	90.98^{b}±7.7	72.93^{c}±4.7	50.31^{d}±1.1
HDL(mg/dl)	51.80^{a}±1.3	45.01^{b}±1.8	49.67^{a}±1.8	50.88^{a}±0.9	50.45^{a}±1.5
VLDL(mg/dl)	16.59^{e}±1.2	39.08^{a}±3.9	30.65^{b}±1.1	25.35^{c}±1.2	20.83^{d}±1.3

Values are expressed as means ± SD; means in the same raw with different letter are significantly different (P < 0.05). ovx: ovariectomized; GCF: germinated chickpea flour, TC: Total cholesterol, TG: Triglyceride**,**LDL: Low density lipoproteins, HDL: High density lipoproteins, VLDL: very low density lipoproteins.

Table (5): Effect of germinated chickpea flour on bone parameters of experimental rats

Variables	Sham	Ovariectomized groups			
		OVX control	OVX+2.5% GCF	OVX+5% GCF	OVX+7.5% GCF
Femur					
Weight(g)	0.74^{a}±0.05	0.49^{c}±0.01	0.64^{b}±0.07	0.65^{b}±0.01	0.76^{a}±0.01
Length(cm)	3.23^{a}±0.1	2.27^{b}±0.06	3.07^{a}±0.06	3.2^{a}±0.1	3.2^{a}±0.2
Ca (mg/dl)	12.33^{a}±0.05	6.74^{e}±0.1	7.83^{d}±0.04	8.47^{c}±0.08	9.79^{b}±0.08
P (mg/dl)	10.79^{a}±0.1	5.67^{e}±0.05	6.95^{d}±0.04	7.5^{c}±0.06	8.19^{b}±0.05
Mg(mg/dl)	2.54^{a}±0.1	1.34^{d}±0.1	1.55^{c}±0.05	1.84^{b}±0.1	2.42^{a}±0.04
BMD(mg/ cm^3)	0.11^{a}±0	0.08^{c}±0.002	0.1^{b}±0.002	0.1^{b}±0.003	0.11^{a}±0.001
Tibia					
Weight(g)	0.5^{a}±0.04	0.34^{b}±0.03	0.36^{b}±0.04	0.5^{a}±0.04	0.54^{a}±0.07
Length(cm)	3.47^{ab}±0.1	3.23^{b}±0.1	3.3^{b}±0.1	3.47^{ab}±0.06	3.73^{a}±0.2
Ca(mg/dl)	10.81^{a}±0.1	6.61^{a}±0.1	7.42^{d}±0.08	8.19^{c}±0.04	9.25^{b}±0.08
P(mg/dl)	8.88^{a}±0.07	6.22^{d}±0.1	6.89^{c}±0.03	7.13^{b}±0.05	8.86^{a}±0.07
Mg(mg/dl)	2.39^{a}±0.07	1.25^{e}±0.1	1.56^{d}±0.1	1.83^{c}±0.06	2.17^{b}±0.08
BMD(mg/cm^3)	0.087^{a}±0.004	0.068^{b}±0.001	0.08^{a}±0.004	0.084^{a}±0.004	0.092^{a}±0.01

Values are expressed as means ± SD; means in the same raw with different letter are significantly different ($P < 0.05$. ovx: ovariectomized; GCF:germinatedchickpea flour.; BMD: bone mineral density.

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تأثير حمص الشام المنبت على صحة عظام الفئران المستأصلة المبايض كنموذج لانقطاع الطمث

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الملخص العربي

يؤثر انقطاع الطمث على المرأة على جميع المستويات ، من الأعضاء إلى الأداء النفسي والذهني والاجتماعي.هذا وتعتبرهشاشةالعظام التي تحدث بعد انقطاع الطمث المرض الأكثر شيوعاوالذي يرتبط بانخفاض في كتلة العظام وتغيرات فيالهيكل العظميوعادة ما يكون ناجم عن نقص هرمون الاستروجين. هدفتهذهالدراسةإلىتقييمتأثيردقيق حمص الشام المنبت علىإناثالفئرانمستأصلة المبيض(محاكاة لفترة انقطاعالطمث). تم اجراء الدراسة علي٣٥ من اناثالفئران الالبينوالبالغة .تمتقسيمهمإلىخمسمجموعاتالمجموعة الاولي (٧ فئران) أجرى لها عملية جراحية وهمية تسمى sham(المجموعة الضابطة السالبة) وكانتالمجاميع الأربعة الأخرىمستأصلةالمبايض (٧فئرانلكل منهما). تغذت مجموعة sham السالبة والمجموعة الضابطة الموجبة مستأصلة المبايض علي الوجبة القياسية،في حين غذيت الثلاثة مجموعات المستأصلة المبايض المتبقية علي نفس الوجبة معاس تبدال الوجبة بحمص الشام المنبت بنسبة 2.5و ٥ و 7.5٪ على التوالي .أدى التدعيم بدقيق حمص الشام المنبت إلىتحسين الكالسيوم،الكالسيوم المتأين,الفوسفور, الماغنسيوم ,نشاط انزيم الفوسفاتيز القاعدي ، نشاط انزيم الفوسفاتيز القاعدي الخاص بالعظم ،أوستيوكالسين, كالسيتونين,هرمون الباراثرمون ومستويات دهون في السيرم مقارنةبالمجموعةالضابطة الموجبة. وجد أيضاارتفاع فى مستوى الكالسيوم, الفوسفور,الماغنسيوم وكثافة المعادن في العظم لعظمة الفخذ والساق في المجموعات المستأصلة المبايض المدعمة بتركيزات مختلفة من حمص الشام المنبت مقارنة بالمجموعة الضابطة الموجبة.بينما لم يلاحظ وجود فروق معنوية فى مستوى هرمون الاستروجين ووزن وطول عظام الفخذ والساق بين المجموعة الضابطة الموجبة والفئران مستأصلة المبايض المدعمة بنسبة 2.5% من دقيق حمص الشام المنبت. وتشيرهذهالنتائجإلىأندقيق حمصالشام المنبت فعال في تحسين مستويات الدهون وصحة عظام الفئران مستأصلةالمبيض.

الكلمات الكشافة:ايزوفلافون, عظمة الفخذ, عظمة الساق و اوستيوكالسين.