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LEPIDIUM SATIVUM SEEDS CAN CONTROL OSTEOPOROSIS AND HYPELIPIDEMIA IN OVERIECTOMIZED RATS

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ABSTRACT

Osteoporosis and elevation of both blood cholesterol and low-density lipoprotein are an inevitable results of menopause in women as well as in overiectomized rats, both problems can be complicated by multiple health hazards. **Objective:** This study aimed to evaluate the effect of Lepidium sativum seeds on both problems in overiectomized rats. **Material and method**: Phenolic profile of LS was analyzed by HPLC. Thirty Sprague-dawely adult female rats will be divided into two groups G1 (N 10) control group (NOVXC) (sham incision), G2 (N 20) (COVX) overiectomized rats. Both groups will receive basic diet for 4weeks at which blood sample were collected. G2 will be divided into two groups one,treatment group, which will receive 20% of the basic diet as Lepidium sativum (OVX LS) (N10) for 8 weeks,the other will continue on basic diet (COVX). At the end of the experiment period, animals were fasted over night and sacrificed. Blood samples were collected for analysis of cholesterol, serum low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides TG, Malondialdhyde (MDA) Zinc, phosphrous, calcium and bone formation marker(PINP).Dual x ray Absorpiometry (Dexa) will be done for all overiectomized rats before and after treatment. **Results**: Dexa showed that group of overiectomized treated rats OVXLS have significant improvement of bone mineral density with elevation of Mg, zinc and PINP versus COVX. Lipid profile showed significant lowering of cholesterol and LDL as well as decrease of lipid peroxidation marker MAD in treatment group ($P \leq 0.05$). **Conclusion**: LS seeds improved bone density and controlled

elevated lipids in blood of overiectomized rats, treatment may has valuable action in osteoporosis and dyslipidemia due to menopause.

KEYWORDS: Lepidium Sativum, overiectomy, rats, osteoporosis, hyperlipidemia.

INTRODUCTION

Bone fragility as well as hyperlipidemia coincides with aging and decreased estrogen level in menopausal women.[1,2] Similar condition occurs in female rats after overiectomy. Osteoporosis is a systemic skeletal disease characterized by low bone density and deterioration of bone tissue with consequent increase in bone fragility. Women are more affected than men with 34% compared to 17% for men respectively.[2,3] Decreased level of estrogen promotes osteoclast (bone resorption cells) activity on expense of osteoblasts proliferation (the bone forming cells) leading to loss of bone mass.[3,4] Optimum Estrogen level prevents accumulation of lipids in central part of the body and in blood vessels wall, which leads to atherosclerosis and cardiovascular disease when this level decreases. Females at bearing age are less liable for such problems but incidence increase by arrival of menopause. The menopausal status not only increases plasma LDL-cholesterol and triglyceride levels, but also increases the HDL2/HDL3 ratio which is associated with elevation of plasma triglyceride levels. These changes may increase the risk for cardiovascular diseases (CHD) due to enlargement of the lipid pool.[1] Phytoesrogens of plant origin, found to have estrogenic activities in postmenopausal women which control bone loss[5,6] as well as dyslipidemia.[1,4,7]

Most of whole cereals and seeds are rich sources of fiber and polysaccharides which have low glycemic index and less liability to elevated lipids in blood eg. (Beans, Soya bean, wheat germ oats millet) and others which have good benefit for health and bone.[8]

Lepidium sativum (L. sativum) is commonly known as chandrasura (Tuffa). This is a small, annual, 15-45 cm hight plant cultivated as salad supplement. The seeds are reddish in colour, oblong, somewhat angular and curved slightly on one side with rugous surface. Near the point of attachment there is a white scar, from which a small channel extends to 1/3 the length of the seeds. Seeds are odorless and taste is pungent and mucilaginous.[9]

LS is recommended in the treatment of hypertension, diabetes[10] and as anticarcinogenic[11], and antibacterial[12] agent. LS help in healing bone fracture[13,14] and control of loss of bone

mass in menopausal women.[15,16] The effective control of these diseases are due to α linolinic acid content of LS.[17,18] Another active component are isothiocyanates which are formed with glucosinolates in LS and known to have a hepato-protective role against hepatotoxicants and fatty liver.[11,19-20]

The aim of this study was to investigate the effect of LS on osteoporosis and hyperlipedemia occurring as a result of overiectomy in rats.

MATERIALS AND METHODS

Phenolic acids profile analysis

The ethanolic extraction of powdered LS was done with the modification reported by Rajeswari et al.[21] HPLC analysis for LS was carried out using Agilent Technologies 1100 series liquid chromatography equipped with an auto sampler and a diode-array detector. The analytical column was an Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 ml/min for a total run time of 70 min and the gradient program was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. The injection volume was 50 µl and peaks were monitored simultaneously at 280 and 320 nm for the benzoic acid and Cinnamic acid derivatives, respectively. Samples were filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards.[22]

Animal experiment

Thirty female Sprague Dawley rats (200-250 g body weight) were obtained from the animal house of the National Research Center. Animals were treated according to the known ethical approval for ethical approval committee for animals. Twenty animals (COVX) were surgically bilaterally ovariectomized according to Khajuriam et al[23] while the skin of other ten animals has been surgically incised without ovariectomy (NOVXC) (Sham operation). All animals were acclimatized at room temperature and given control diet for four weeks till complete healing of the surgical wound and osteoporosis model is clear. Blood sample will be collected from all rats at this step.The operated (COVX) group will be divided into treatment group (N10) which will be fed on diet with 20 % of the starch was replaced by LS (OVXLS) for 8 successive weeks and second COVX group will be feed on basic diet. At the

end of the experiment period, animals were fasted over night and sacrificed. Blood samples were collected for analysis of serum cholesterol, low-density lipoprotein (LDL),high density lipoprotein (HDL), triglycerides TG, Malondialdhyde (MDA),Zinc, phosphorus,calcium and bone formation marker (PINP) before and after treatment. the cascade of the operated rats will be frozen in specific position and X rayed by Dual energy X ray absorpiometry (Dexa) (DXA scanner LUNAR Madison, WI prodigy/DPX, model 7681 USA) for calculation of mineral bone density (MBD) for all overiectomized rats before and after treatment. All blood and plasma analysis were done using Bio-diagnostic kits and Eliza for PINP. Data were computed and statistically analyzed.

Table (1): Composition of control diet and treatment group.[24]

Ingredients	Control diet	Treatment group
Casein	10	10
Cellulose	10	10
Corn oil	10	10
Slat mixture	4	4
Vitamin mixture	1	1
L-Cystine	0.018	0.018
Choline chloride	0.025	0.025
Corn starch	64.957	44.957
LS	0	20

RESULTS AND DISCUSSION

Results of HPLC profile showed that LS was rich in may phenolic compounds table (2). p-coumaric acid was highly detected in the extract of LS and was the most prominent, other compounds found are (rutin, Protocatechuic, p-hydroxybenzoic, Cateachin and Ferulic)respectively others phenolic acids were less detected such as Rosmarinic acid, Caffeic,Vanillic, Sinapic, Gallic and Syrngic acids Table (2).

Table 2: HPLC Profile of compounds in the LS extract.

Compound	Ethanolic extract (ug/g)اد
Gallic	5.39
Protocatechuic	21.70
p-hydroxybenzoic	17.02
Gentisic	ND
Cateachin	14.01
Chlorogenic	ND
Caffeic	3.97
Syringic	1.92
Vanillic	3.29
Ferulic	15.60
Sinapic	0.82
p-coumaric	1926.11
Rutin	32.72
Apigenin-7-glucoside	ND
Rosmarinic	13.31
Cinnamic	0.45
Qurecetin	ND
Apigenin	ND
Kaempferol	ND
Chrysin	ND

P-coumaric(CA) was the highest and most abundant phenolic in LS extract tested by HPLC (1926 µg/g) Table (2).Previous study showed that treatment with P-coumaric acid increased the bone mass/body mass ratio and bone mineral mass/body mass ratio in the long bones in overiectomized rats in comparison with the ovariectomized control rats. while ferulic acid which is represented by only (15.6 µg/g) improved some bone histomorphometric parameters, impaired by estrogen deficiency due to overiectomy However, did not increase the ratio of bone mineral mass to bone mass.[25]

In another study the treatment of rats with P-coumaric acid showed reduction of the expression of osteoclastogenic factors (RANKL and TRAP)responsible for bone breaking down It also down regulated the pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-17), and inflammatory enzymes (iNOS and COX-2) in arthritic rats.

Radiological (CT and DEXA scan) and histological assessments authenticated that CA inhibited bone destruction and cartilage degradation in association with enhanced bone mineral density.[26]

Treatment with CA significantly increased the tibial length of male rat, through the elevation of serum levels of growth hormone and insulin-like growth factor 1, both were significantly increased in the p-Coumaric acid-treated group.[27]

Another study aiming to evaluate the effects of ferulic acid on bone loss in ovariectomized female rats treated with either ferulic acid and/or 17a-ethynylestradiol daily for 8 weeks. These results indicate that ferulic acid promotes bone remodeling, leading to a predominantly osteoblastic phase compared to bone resorption by osteoclasts. Elevation of BMD in both treatments with estrogen or ferulic acid, but ferulic acid has different mechanism than estrogen in this elevation.[28]

Treatment of overiectomized rats with Caffeic acid (pearly presented in our extract by(4 µg/g) decreased bone mass when compaired to overictomized control in same experiment.[25]

Rutin, was represented in current study HPLC By (32.72 µg/g).Rutin is known as potent anti osteoporotic flavonoids as they stimulate estrogen receptor and bone formation.[29]

Protocatchuic and gallic acid were reported to have free radical scavenging and antioxidant activities by increasing the scavenging of hydrogen peroxide (H2O2) and diphenylpicrylhydrazyl (DPPH). They restore glutathione (GSH) related enzymes and lower the oxidized low-density lipoprotein levels (LDL). As Osteoblasts and adipocytes differentiate from bone marrow mesenchymal stem cells.[30] There is an inverse relationship between differentiation of mesenchymal stem cells to osteoblasts and adipocytes. Gallic acid, hydroxyl Cinnamic and Protocatchuic predominate osteoblasts differentiation.[30-32]

Protocatchuic acid also has been shown to exert an insulin-like activity in oxidized LDL-induced insulin resistance in adipocytes via increased PPARγ activation.[33] And as Bone loss is induced in the diabetic state[34], the protocatchuic acid enhance health of bones through decreased blood glucose levels in streptozotocin induced diabetic rats via restored carbohydrate metabolic enzyme activity, increased plasma insulin level, and normalized the activity of pancreatic islets.[35,36] These findings suggest that protocatchuic acid provides antihyperglycemic effects in addition to its reported antioxidant and anti-inflammatory effects which improve loss of bone mass. Gallic acid and p-coumaric acid exhibited marked antidiabetic action that could be mediated via modulation of TNF-α and adipocytokines

secretions as well as upregulation of PPARγmRNA expression which consequently improve bone density.[37]

Rosmarinic acid was represented in the current extract by (13.31 µg/g). In one study it is mentioned that it exerts an antiosteoporotic effect in the RANKL-induced mouse model of bone loss by promotion of osteoblastic differentiation and inhibition of osteoclastic differentiation.[38]

Results of animal experiment showed that overiectomy caused significant deterioration of bone density and lipid profile. (Table 3) This is due to loss of estrogen hormone which is the hormone responsible for activation of osteoblasts function in expense of the bone resorption cell (the osteoclasts) where this loss may cause apoptosis of osteoclasts progenitors, mediated by estrogen receptors.[39] Also hyperlipidemia is an inevitable event as eostrogen presence in normal concentration is responsible for keeping lipids away from dangerous sites as blood vessels and central abdominal adiposity which precipitates cardiovascular diseases and atherosclerosis.[1, 40-41]

Table (3).

	COVX	OVX LS	NOVXC
Calcium (mg/dl)	8.575±0.1	8.58±1.1	9.0±0.3
Phosphorus (mg/dl)	4.5± 0.78	4.38±0.69	5.5±0.14
Magnesium (mg/dl)	1.2± 0.12	1.73±0.05*	1.78±0.05**
Zinc(µg/dL)	82.9± 3.9	89.75±8.46*	110±6.05**
MDA(mMol/L)	8.6± 0.17	3.8±1*	1.95±0.11**
TAC (nMol/L)	3.5± 0.4	4.6±0.12 *	5.02 ± 0.11**
BMD	0.16±0	0.128±0.008*	---
PINP (µ /l)	25.4± 5.4	32±5.7	30.00±1

Chemical analysis of plasma of treatment group (OVX LS) versus ovariectomized (*P< 0.05) and control COVX versus NOVXC rats. (**P< 0.05).

In overiectomized rats treated with LS results showed significant improvement of BMD in Dexa results Table (2) (0.16±0.006 versus 0.128±0.008), P< 0.05**)** as well as elevation of bone formation marker PINP, Zinc and Mg in treatment group versus COVX group. Previous studies' observations and statistics showed that Lepidium Sativum has a significant role in accelerating bone fracture healing which supports the rationality of its traditional use for this purpose and several studies showed similar results.[15,16] Recent clinical study demonstrated statistically significant improvement in T-score and bone mineral density of DEXA done for patients of Osteopenia after the intake of Tuffa (L.S.). Provided data from other clinical trials

showed that LS was effective in improving bone health, decreases incidence of future fracture as well as arthritis and body inflammations in patients.[15] The consumption of LS improved osteoporotic periodontitis in menopausal women and have synergistic effect with alendronate drug to improve bone mass in those women.[16]

Table (4):

	NOVXC	COVX	OVX LS
S.cholesterol	67.6±2.07	142.9±2.5**	77.5±1.8*
LDL	50± 1.8	142.7± 2**	82.5±4.3*
HDL	22.8± 1	20.1± 15	24.5±0.9
TG	76 ±4	171.6±10 **	141± 12.5*

Lipid profile differences in serum of rats: (*P< 0.05 in COVX versus treated (OVX LS) group, ** P< 0.05 in COVX versus NOVXC.

Lipid profile in the current study showed improvement in overiectomized rats treated with LS (Table 4), were total cholesterol and LDL after treatment reached almost the level of control NOVX, after marked significant deterioration after overiectomy. Other studies mentioned the same observation. One study indicated that the administration of Lepidium sativum showed better lipid profile as well as decreases in the sugar level in hypercholesterolemia rats.[42] The hypolipidemic effect of Lepidium sativium might be attributed to inhibition of absorption and enhanced excretion of lipids.[43] Lipid peroxidation was decreased in this study and proved by decreased level of MAD in treated rats (table 3), which pointed at elevated antioxidant effect as well as elevation of MG and Zinc whom are the co-factors for antioxidant enzymes. The rich content in LS of glycoside, alkaloids, tannin (Phenolic compound), flavonoids, and amino acids like glutamine, cysteine, and glycine, may be the cause of lipid level control. The tannin and flavonoids may have antioxidant activity whenever glutamate, cysteine, glycine are intermediates for synthesis of the endogenous antioxidant glutathione these result agree with.[17] The primary fatty acids found in LS oil were oleic (30.6 wt %) and linolenic acids (29.3 wt %).[44] LS seeds oil contained high concentrations of tocopherols and the primary phytosterols in L S were sitosterol, campesterol, with avenasterol. Diets rich in alpha linolenic acid have been associated with a reduced risk of fatal ischemic heart disease, a reduction in heart attacks and mortality from chronic vascular disease.[45] Feeding alpha linolenic acid has also been shown to decrease platelet aggregation, total cholesterol, LDL cholesterol and triglycerides in humans and rats these founding concordance with our study. Another study demonstrated that histopathological protective effects of LS on hepatic fatty changes induced by administration of high cholesterol diet in rats.[46]

CONCLUSION

This study concluded that LS can control osteoporosis and elevated lipid profile in ovariectomized rats and can be used in menopausal women and more studies are needed**.**

Ethics approval and consent to participate: The agreement of animal ethical committee In NRC was taken.

Data availability: all relevant data are within the paper and its supporting information files.

Consent for publication: All authors agreed for publication of this article which is original and not published before.

Competing interests: No conflict of interest (not declared).

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Authors' contributions: Moetazza M. Alshafei is the corresponding author who wrote, revised the data, statistical analysis and editing. Also participated in designing experiment and concept of the study, with **Emtenan M.** Hanafi. **Seham S. Kassem, Emtenan M. Hanafi** are the biochemistry team who analyzed the compound and the biochemistry and Eliza test. **Manal M.Ramadan** has done the phenolic analysis of The LS.

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