The Possible Protective Role of Sesame on Experimentally Induced Osteoporosis

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Abstract: objective of this work will be based on two folds: one of them is to study the different effect of calcium and vitamin D deficiency on growing and mature bone of female albino rat. The other one is to asses a possible protective role of a separate sesame component on osteoporotic bone. The two folds will be based on; biochemical,histological, radiological and Statistical studies.
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1. Introduction

Osteoporosis is defined as a condition characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture **(Taes *et al.,* 2010).**

Osteoprosis must be differs from osteopenia is a term to define bone density that is not normal but also not as low as osteoporosis. By definition from the W.H.O, osteopenia is defined by bone densitometry as a T score -1 to-2.5. **(Kaats *et al*., 2010).**

Osteoporosis is a major public health problem in the elderly population around the world, particularly in women. The risk of developing osteoporosis after menopause is determined largely by the peak bone mass obtained at young adult age and by the rate of bone loss after menopause. Postmenopausal bone loss is caused primarily by the sharp decrease in estrogen levels that is inherent to menopause **(Marcu *et al.,* 2011).**

With the increasing life expectancy, osteoporosis is becoming a major worldwide health problem. The magnitude of the disease may become larger in developing countries**. (Larijani *et al.,* 2003).**

Osteoporosis is a chronic, systemic, metabolic disease of the skeleton, characterised by reduced bone mass, architectural defects and lowered mechanical resistance to injuries, what eventually leads to higher risks of fractures (**Lasota & Danowska-Klonowska, 2004).**

Nutritional factors have a significant influence on the cause of osteoporosis. Calcium supplementation may be particularly effective in populations with a low calcium diet. Supplementations of 500 mg/d may produce about 4% gain in skeletal calcium in adolescents. Supplementations of 800 mg/d may prevent bone loss in postmenopausal women. The results of clinical trials also suggested that such supplementation may prevent hip and vertebral fractures in the elderly. The largest effect of calcium supplementation occurs in the first year of treatment, whereas sustained effects are not proven **(Ackerman and Misra,2011).**

Calcium, phosphate, and vitamin D are essential for normal bone structure and function also several other micronutrient have essential roles in bone. Calcium (Ca) is the most abundant inorganic element in human body along with sodium **(Spiechowicz** *et* ***al.,* 2003).**

Osteoporosis may be classified as primary type 1, primary type 2, or secondary. The form of osteoporosis most common in women after menopause is referred to as primary type 1 or postmenopausal osteoporosis. Primary type 2 osteoporosis or senile osteoporosis occurs after age 75 and is seen in both females and males at a ratio of 2:1. Finally, secondary osteoporosis may arise at any age and affects men and women equally. This form of osteoporosis results from chronic predisposing medical problems or disease, or prolonged use of some medications**. (Alldredge *et al.,* 2009).**

Postmenopausal osteoporosis is a major health problem for women society. It is estimated that 1 in 3 women and 1 in 12 men over the age of 50 worldwide have osteoporosis **(Lasota&Danowska-Klonowska., 2004).**

Nutrition has an important and complex role in maintenance of good bone. Identified risk factors include low dietary calcium and/or phosphorus, magnesium, zinc, boron, iron, fluoride, copper, vitamins A, K, E,D and C. Excess sodium is also a risk factor. High blood acidity may be diet-related, and is a known antagonist of bone **(Ilich & Kerstetter, 2000)**. Some have identified low protein intake as associated with lower peak bone mass during adolescence and lower bone mineral density in elderly populations **(WHO Scientific Group,2003).** Conversely, some have identified low protein intake as a positive factor, protein is among the causes of dietary acidity. Imbalance of omega 6 to omega 3 polyunsaturated fats is yet another identified risk factor (**Alldredge *et al.*,2009).**

Free radicals may increase bone resorption through activation of oxidative stress responsive nuclear factor NF-kB which has been associated with osteoclastogenesis **(Basu *et al.*,2001).**

Sesame (*Sesamum indicum*, Pedaliaceae) is one of the most important oil seed crops worldwide, that mainly cultivated in developing countries, where it has been used in many food products **(Nzikou *et al.*, 2009),** Sesame seeds are a powerhouse of organic minerals, especially calcium and zinc, and is an alkaline food that supports bone and general health. Whole sesame seeds contain about 88 mg of calcium per tablespoon of seeds. Just a quarter cup of natural sesame seeds provides more calcium than a whole cup of milk. Plus, they are alkaline whereas milk is acidic. (**Vivian Goldschmidt,2011).**

There is a little bit of controversy about sesame seeds and calcium, because there is a substantial difference between the calcium content of hulled versus unhulled sesame seeds. When the hulls remain on the seeds, one tablespoon of sesame seeds will contains about 88 milligrams of calcium. When the hulls are removed, this same tablespoon will contain about 37 milligrams (about 60% less) (**Sirato-Yasumoto *et al.*,2001.)**

Sesame oil is a source of vitamin E. Vitamin E is an anti-oxidant and has been correlated with lowering cholesterol levels. As with most plant based condiments, sesame oil contains magnesium, copper, calcium, iron, zinc, and vitamin B6. (**Hirata *et al.*, 1996).**

One of the main benefits of the use of sesame oil is the inclusion of polyunsaturated fatty acids including Omega-3, Omega-6 and Omega-9,. It is also claimed sesame oil can help prevent osteoporosis by raising the estrogen level of the body through fatty-acid intake (**Dee Braun, 2009**). it is unique in that it keeps at room temperature. This is because it contains two naturally-occurring preservatives, sesamol and sesamin. (Normally, only oils predominately composed of the omega-9 monounsaturated oil, like olive oil, keep at room temperature(**Pugalendi, 2003).**

Mammalian lignans; enterolactone (EL) and enterodiol (ED) are of interest because of their chemical structural similarity to estrogen and their ability to bind to estrogen receptors. Sesame seed contains high concentrations of the plant lignans sesamin (SES), sesamolin (SMN), and sesaminol that might consider as mammalian lignans precursor (**Karen *et al.*,2005).**

2. Material and Methods

Casein, starch, cellulose, choline chloride, Dl-methionine, vitamins mixture and minerals mixture were obtained from Morgan Co. Cairo, Egypt. Chemical kits used in this study (ca,p) were purchased from El-Gomhoria Company for chemicals and Drugs, El-Ameria, Cairo, Egypt.

Sesame and sesame hulls were purchased, Corn oil were purchased from local market of Shibin El-Kom, Minufiya, Egypt.

Adult and baby female albino rats, Sprague Dawley strain, were obtained from Research Institute of Ophthalmology, Giza, Egypt.

Sesame and sesame without hulls were ground to pass through a Gomesh sieve then kept at -4°C until use, sesame hulls were washed and dried at 50°C in an electric draught oven.The dried hulls were ground to pass through a Gomesh sieve then kept at -4°C until use.

2.1. Experimental design

Adult and baby female albino rats of Sprague dawly strain, with an average weight 140±5g and 80±3g respectively were used in this study. Each rat was housed individually in stainless steel wire cage under controlled condition. Diets were introduced to the rats in a special non-scattering feeding cup to avoid loss of food and contamination. Tap water was provided to rats by mean of glass tubes projecting through wire cages from inverted bottles supported to one side of the cage.

The standard diet, were formulated according to **Ain (1993)**. Salt mixture and vitamins mixture were prepared according to **Hegested *et al.*, (1941) and Campbell, (1961),** as shown in (Table, D and E), Fifty four baby and adult female albino rats were fed standard diet for one week as an adaptation period. Rats were divided into two main Experiments.

***First experiment(prevention),** Baby rats were randomly divided into four groups, 6 rats per group.

Group I (Control Group):

Rats fed standard diet**.**

Group II (Rat fed on sesame seed instead of calcium):

Rats fed on diet free calcium plus (dry sesame seeds/kg standard diet), which allowance a amount of the daily recommended dietary of calcium.

Group III (Rat fed on sesame seed without hulls instead of calcium):

Rats fed on diet free calcium plus (dry sesame seeds without hulls/kg standard diet), which allowance a amount of the daily recommended dietary of calcium

Group IV (Rat fed on sesame hulls instead of calcium):

Rats fed on diet free calcium plus (dry sesame hulls/kg standard diet), which allowance a amount of the daily recommended dietary of calcium. Each group was fed the experimental diet for 45 days

***Second experiment,** Adult rats were randomly divided into two groups.

Group1: (Negative Group)

Six rats fed standard diet**.**

Group2: (Osteoporotic group)

Twenty four Rats fed on standard diet free calcium and vitamin D for 4 weeks, then rats were randomly divided into four subgroups,6 rats per group.

Subgroup I (Positive Group):

Rats fed on standard diet free calcium only.

Subgroup II (Osteoporotic with sesame seeds)

Rats fed on standard diet free calcium plus (dry sesame seeds/kg standard diet), which allowance a amount of the daily recommended dietary of calcium.

Subgroup III (Osteoporotic with sesame seeds without hulls)

Rats fed on standard diet free calcium plus (dry sesame seeds without hulls/kg standard diet), which allowance a amount of the daily recommended dietary of calcium.

Subgroup IV (Osteoporotic with sesame hulls)

Rats fed on standard diet free calcium plus (dry sesame hulls/kg standard diet), which allowance amount of the daily recommended dietary of calcium. Each group was fed the experimental diet for 45 days.

At the beginning of experiment, A 5 ml blood sample were taken to determine hemoglobin, haematocrit serum iron, serum ferritin, red blood cell, and total iron - binding capacity. As the data obtained basis, the rats were divided into five groups, 6 rats per group. The first (control group) fed basal diet, the second group fed thyme diet (21g dry thyme leaves/kg basal diet)), the third group fed thyme diet + double amount of the recommended dietary allowance of Ca (10 g/kg diet) from $CaCO_3$, the fourth group fed thyme diet + double amount of the recommended dietary allowance of P (8 g/kg diet) from sodium phosphate mono hydrogen ($Na\ H\ PO_4$) and the fifth group fed thyme diet + double amount of the recommended dietary allowance of Ca and P from calcium phosphate ($Ca\ PO_4$) as described by (14). Feed intake was recorded daily. Faces were collected of each animal daily. Body weight was recorded at the beginning and at the end of experimental period.

At the end of experimental period (6 weeks), the rats fasted overnight and were anaesthetized. Blood sample were collected and aliquots were analyzed to measure the hematological parameters. The remaining blood was centrifuged to obtain serum for determination serum iron, serum ferritin and total iron binding capacity.

2.2. Statistical Analysis:

The experimental data were subjected to an analysis of variance (ANOVA) for a completely randomized design using a statistical analysis system **(SAS, 2000).** Duncan's multiple range tests were used to determine the differences among means at the level of 95%. Differences between treatments of ($p \leq 0.05$) were considered significant.

2.3. Histopathological studies:

The histopathological studies were carried out on sections of decalcified right femur (fixed in formalin). The bones were dehydrated in an ethanol series and embedded in paraffin and cut into longitudinal sections of 5 µm thickness. The sections were stained with hematoxylin phloxine saffron.

2.4.Radiologyoligical studies:

Femur and scapula were removed and dissected free of soft tissue, contact radiographs were taken using a Faxitron model 805 radiographic inspection system, at 22 kv voltage and with a four minute exposure time X-Omat TLfilm was used and processed routinely.

3. Result and Discussion

A-Biochemical parameters

1. Body weight

Table (1) showed Mean values ±SD of food intake, body weight gain (%) and food efficiency ratio (FER) for normal rats fed on sesame seed instead of calcium, sesame seed without hulls instead of calcium and sesame hulls instead of calcium.

1. Feed intake (Fl):

Data in table (1) indicated that, the mean value of feed intake in the control group was 321.33± 10.11 g/28day, while the mean value of rats group that is fed on basal diet containing sesame seed instead of calcium was 304.66± 9.96g/ 28day.

Data also showed that, the mean values of feed intake of normal group fed on basal diet and sesame seed without hulls instead of calcium and sesame hulls instead of calcium were 293.33 ± 8.43 and 272 ± 6.65g/28day.

2. Food efficiency ratio (FER):

Mean values ±SD of feed efficiency ratio (FER) for normal rats fed on sesame seed instead of calcium, sesame seed without hulls instead of calcium and sesame hulls instead of calcium are presented in table (1).

The mean value ± SD of PER for the control group was 0.21 ±0.02, while it was 0.25±0.02 for rats fed on sesame seed instead of calcium. Results in this table showed a significant decrease in FER of group fed on sesame hulls instead of calcium as compared to control group whereas there is no significant differences between control group, sesame seed instead of calcium and sesame seed without hulls instead of calcium and these results were agree with those obtained by ***Gouvcia et al., (2004).***

Although, there were significant between control group and the other groups in case of feed intake, the feed efficiency ratio showed no significant differences compared with the control except the group fed on sesame hulls instead of calcium.

3. Body weight gain (BWG %):

The result of BWG in rats fed on basal diet, and diets containing sesame seed instead of calcium, sesame seed without hulls instead of calcium and sesame hulls instead of calcium under the current investigation were shown and summarized in table (1). BWG of the group fed on diet containing sesame seed instead of calcium showed significant increase as compared to the other groups.

Rats fed diet with sesame seed without hulls instead of calcium and sesame hulls instead of calcium led to significant decrease in BWG when compared with the others.

Table (1): Mean values ±SD of food intake, body weight gain (%) and food efficiency ratio (FER) for normal rats in 1st expermental.

Diet	Feed intake (g)	Body weight gain (g)	FER
Control group	321.33± 10.11 [a]	69.83 ± 5.03 [b]	± 0.02 0.21 [a]
Rat fed on sesame seed instead of calcium	304.66 ± 9.96 [b]	78.54 ± 1.54 [a]	0.25 ± 0.02 [a]
Rat fed on sesame seed without hulls instead of calcium	293.33 ± 8.43 [b]	60.83 ± 1.83 [c]	0.21 ± 0.01 [a]
Rat fed on sesame hulls instead of calcium	272 ± 6.65 [c]	24.16 ± 1.60 [d]	0.09 ± 0.007 [b]
L.S.D	11.48	3.41	0.02

Each value represent the mean of 6 rats ± SD. Values significantly different compared to normal: $P \leq 0.05$. Same letter means non-significant.

The effect of feeding sesame seeds, sesame seeds without hulls and sesame hulls on feed intake, feed efficiency ratio and body weight gain are shown in Table (2). Concerning the feed intake, it were 321.33 ± 10.11 g / 28 days for negative control and 375.83 ± 6.49g /28 days for positive control. However, the feed intake for osteoporotic groups fed on sesame seeds, sesame seeds without hulls and sesame hulls decreased compared to positive control and increased when compared with negative control. The values were 368.33 ± 6.77, 355.83 ± 11.54 and 342.50 ± 8.11g / 28days for groups fed on sesame seeds, sesame seeds without hulls and sesame hulls respectively.

The body weight gain was 69.83 ± 5.03 for control (-), while the highest value was in group control (+), the value was 97.83± 2.92. the other values were 51.33 ± 1.21, 52.83 ± 4.87 and 32.33 ±2.73 for sesame seeds, sesame seeds without hulls and sesame hulls groups respectively. However, such decrease is statistically significant.

Results of feed efficiency ratio was 0.21 ± 0.02 for negative control group and decreased gradually for groups control (+),sesame seeds, sesame seeds without hulls and sesame hulls respectively. The values were 0.26 ± 0.01, 0.14 ± 0.005, 0.15 ± 0.01 and 0.09 ± 0.009 respectively. There is no significant between negative and positive controls. Also, there is no significant between osteoporotic with sesame seeds and osteoporotic with sesame seeds without hulls.

From the obtained results, the feed intake significantly decreased when osteoporotic rats fed on sesame seeds, sesame seeds without hulls and sesame hulls,The highest effect in sesame hulls group, The mean value ± SD of PER for the control group was 0.21 ±0.02, while it was 0.25±0.02 for rats fed on sesame seed instead of calcium. Results in this table showed a significant decrease in FER of group fed on sesame hulls instead of calcium as compared to control group whereas there is no significant differences between control group, sesame seed instead of calcium and sesame seed without hulls instead of calcium and these results were agree with those obtained by **Gouvcia *et al.*,** (**2004**).

Rats fed diet with sesame seed without hulls instead of calcium and sesame hulls instead of calcium led to significant decrease in BWG when compared with the others and the obtained results were in agreement with those reported by **Nakai *et al*.** (**2003**).

From the obtained results in second experimental, the feed intake significantly decreased when osteoporotic rats fed on sesame seeds, sesame seeds without hulls and sesame hulls. The highest effect in sesame hulls group. These results are in agreement with those reported by **Hirata *et al.* (1996)** who reported that, sesame hulls was able to reduced feed intake for containing dietary fiber. Results of body weight gain decreased gradually and this decrease is statistically significant, These results are in agreement with those reported by **Hu *et al.* (2007)** who reported that; sesame hulls can able reduced the cardiovascular disease risk, diabetes and weight loss.

Table (2): Mean values ±SD of feed intake, body weight gain and food efficiency ratio (FER) for Osteoporotic rats.

Diet	Feed intake (g)	Body weight gain (g)	FER
(Negative control	321.33± 10.11 [d]	69.83 ± 5.03 [b]	0.21 ± 0.02 [a]
Positive group	375.83 ± 6.49 [a]	97.83± 2.92 [a]	0.26 ± 0.01 [a]
Osteoporotic with sesame seeds)	368.33 ± 6.77 [a]	51.33 ± 1.21 [c]	0.14± 0.005 [b]
(Osteoporotic with sesame seeds without hulls)	355.83 ± 11.54 [b]	52.83 ± 4.87 [c]	0.15 ± 0.01 [b]
Osteoporotic with sesame hulls	342.50 ± 8.11 [c]	97.83 ± 2.92 [a]	0.09± 0.009 [c]
L.S.D	13.21	3.74	0.01

Each value represents the mean of 6 rats ± SD. Values significantly different compared to normal: $P\leq$ 0.05.

B - Blood

Effect of **feeding** sesame seed instead of calcium, sesame seed without hulls instead of calcium and sesame hulls instead of calcium on calcium, phosphorous, calcium ions and calcium /phosphorous.

Table (3) illustrated the effect feeding sesame seed instead of calcium, sesame seed without hulls instead of calcium and sesame hulls instead of calcium on calcium, phosphorous, calcium ions and calcium /phosphorous of normal rats.

Data in this table showed that, calcium level (mg/dl) was increased in the group fed on sesame seed instead of calcium and decreased in groups fed on sesame seed without hulls instead of calcium and sesame hulls instead of calcium when compared with control group. There is no significant between control group sesame seed and sesame seed without hulls groups while, there was significant differences between control group and sesame hulls, For phosphorous, there were significant differences between rats fed on basal diet and all tested groups except group fed on basal diet containing sesame seed without hulls.

The statistical analysis showed non significant between all treated groups with sesame seed, sesame seed without hulls and sesame hulls when compared with control group, on the other hand, it could be observed a significant difference between control group, sesame seed and sesame seed without hulls groups. There is no significant in group fed on sesame hulls and control group in case of calcium ions.

Table (3): Effect of feeding sesame seed instead of calcium, sesame seed without hulls instead of calcium and sesame hulls instead of calcium on calcium, phosphorous, calcium ions and calcium /phosphorous.

Diet	ca	P	Ca/p	Ca ion
Control group	9.38±0.19 [a]	5.11 ± 0.11 [b]	158.1 ± 4.05 [a]	4.87± 0.18 [b]
(Rat fed on sesame seed instead of calcium	9.67 ± 0.57 [a]	5.42± 0.07 [a]	178.53 ± 1.99 [a]	5.02±0.04 [a]
Rat fed on sesame seed without hulls instead of calcium	9.36± 0.27 [a]	5.12± 0.12 [b]	170.93 ±4.33 [a]	5.09±0.07 [a]
Rat fed on sesame hulls instead of calcium	8.39± 0.33 [b]	4.58 ± 0.15 [c]	164.61 ± 1.04 [a]	4.69±0.09 [b]
L.S.D	0.4	0.14	2.93	0.13

Means in the same column with different letters are significantly different ($p \leq 0.05$)

The data presented in table (4) revealed the Effect of feeding sesame seed instead of calcium, sesame seed without hulls instead of calcium and sesame hulls instead of calcium on calcium, phosphorous, calcium ions and calcium /phosphorous of Osteoporotic rats. The statistical analysis of calcium levels showed a significant differences between treatments groups and positive control, while there were significant differences between negative control and all tested groups except Osteoporotic rats fed on sesame seeds. Significant differences were found concerning phosphorous of both controls and Osteoporotic rats fed on sesame seeds while, there is no significant differences between negative control and Osteoporotic rats fed on sesame seeds without hulls and sesame hulls. For calcium ions, there is no significant between both controls and all tested groups except rats fed on sesame seeds. In case of ca/p, there were significant differences between negative control group and the treatment groups while, there is no significant between positive control and group fed on sesame hulls, In conclusion, treatment with sesame seeds under study improve the minerals content of Osteoporotic rat's serum, The statistical analysis showed non significant between all treated groups with sesame seed, sesame seed without hulls and sesame hulls when compared with control group, on the other hand, it could be observed a significant difference between control group, sesame seed and sesame seed without hulls groups. There is no significant in group fed on sesame hulls and control group in case of calcium ions.

Our results were in accordance with that of **Hirata *et al.*(1996**) who found that Sesame seeds are a very good source of magnesium and calcium. Moreover the results of present work were in agreement with **Bedigian, 2000 and Bedigian, 2003.**

Revealed the Effect of feeding sesame seed instead of calcium, sesame seed without hulls instead of calcium and sesame hulls instead of calcium on calcium, phosphorous, calcium ions and calcium /phosphorous of Osteoporotic rats. The statistical analysis of calcium levels showed a significant differences between treatments groups and positive control, while there were significant differences between negative control and all tested groups except Osteoporotic rats fed on sesame seeds. Significant differences were found concerning phosphorous of both controls and Osteoporotic rats fed on sesame seeds while, there is no significant differences between negative control and Osteoporotic rats fed on sesame seeds without hulls and sesame hulls. For calcium ions, there is no significant between both controls and all tested groups except rats fed on sesame seeds. In case of ca/p, there were significant differences between negative control group and the treatment groups while, there is no significant between positive control and group fed on sesame hulls.

In conclusion, treatment with sesame seeds under study improve the minerals content of Osteoporotic rat's serum.

These results are in the same line of the work of **Bedigian, 2000 and Bedigian, 2003** who stated that Sesame seeds are a very good source of magnesium and calcium and improve the minerals status of Osteoporotic patients.

Table (4): Effect of feeding sesame seed instead of calcium, sesame seed without hulls instead of calcium and sesame hulls instead of calcium in 2nd expermental on calcium, phosphorous, calcium ions and calcium /phosphorous.

Diet	ca	P	Ca/p	Ca ion
(Negative Group)	9.10± 0.08 [b]	5.71 [b] ± 0.33	± 1.99 178.53 [a]	4.81± 0.18 [b]
Osteoporotic group)	7.33± 0.13 [d]	3.91± 0.3 [c]	153. 36 ± 3.13 [d]	4.47±0.16 [b]
Osteoporotic with sesame seeds)	9.33± 0.17 [b]	6.25± 0.16 [a]	164.61 ±1.04 [c]	5.35±0.19 [a]
(Osteoporotic with sesame seeds without hulls)	10.65 ± 0.42 [a]	5.48 ± 0.27 [b]	170.93 ± 4.33 [b]	4.92±0.07 [b]
Osteoporotic with sesame hulls	8.36± 0.14 [c]	5.33±0.21 [b]	158.1 ± 4.05 [d]	4.72±0.15 [b]
L.S.D	0.62	0.31	3.73	0.15

Means in the same column with different letters are significantly different ($p \leq 0.05$)

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