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Effect of Calcium, Vitamin D and Chia Seeds on Rats Suffering from Osteoporosis

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Abstract

Osteoporosis is a major health disease that affects people all over the world. It happens when there is a considerable of bone loss and it's linked to a higher risk of fracture. The goal of this study was to see how calcium, vitamin D, and chia seeds affected rats with osteoporosis. In this study, 48 female albino rats (Sprague Dawley strain) weighing an average of 200g±10g were divided into eight groups. Throughout the experiment, the negative control group was fed a basic diet. The experimental groups, which included 42 rats, received oral prednisone acetate (4.5ml/kg body weight / day twice a week) rats fed on a 5 percent chia, 1 percent calcium, and 400mg vitamin D diet treated rats with osteoporosis at groups 3,4,5,6,7, and 8, received oral prednisone acetate (4.5ml/kg body weight / day twice a week) rats fed on a 5 percent chia, 1 percent. The results showed that prednisone acetate treatment resulted in a significant ($P \leq 0.05$) increase in serum total cholesterol, triglyceride, low density lipoprotein-cholesterol (LDL-c), very low-density lipoprotein-cholesterol (VLDL-c), glucose, and liver enzymes (AST, ALT, and ALP), as well as a decrease in high density lipoprotein–cholesterol (HDL-c) concentration. These measures were markedly enhanced for osteoporotic rats treated with 5% chia, 400mg vitamin D, and 1% calcium. Calcium, phosphorus, BMD, and BMC levels enhanced in osteoporotic rats treated with 400 mg vitamin D and 5% chia. As a result, determining risk and treatment recommendations requires a thorough understanding of the systems that regulate bone health.

***Keywords**: Minerals, Vitamins, Plant seeds, Rats, Bone health.*

Introduction

Low bone mass and structural destruction of bone tissue produce osteoporosis, a degenerative bone condition that causes bone fragility [(1)]. Women undergo an enhanced period of bone loss during and immediately after menopause, with 20-30% loss of chancellor's bone and 5-10% loss of cortical bone [(2)]. Two body structure problems, osteoporosis and obesity, are on the rise in the United States and around the world [(3)]. Osteoporosis is a serious consequence in people who require long-term glucocorticoid medication [(4)]. Osteoporosis is a global health issue. It happens when there is a lot of bone loss and it's linked to a higher risk of fracture. It affects three times as many women as it does males, partially because of women's lower peak bone mass and partly because of hormonal changes that occur after menopause. Approximately 75 million people in Europe, Japan, and the United States suffer from osteoporosis, which leads to over 2.3 million fractures per year in Europe and the United States alone. In 1995, it was estimated that direct medical expenses for osteoporotic fractures in the United States would total USS billion [(5)]. Adults in Egypt have a 14 percent male prevalence and a 12.6 percent female prevalence of osteoporosis. The prevalence of osteoporosis increases with age, peaking at 21.9 percent in the 40-50 age range. 16.7% of male adolescents and 0.9 percent of female adolescents had relative osteoporosis [(6)]. According to [(7)], calcium and vitamin D are pharmacologically active, healthful, and cost-effective for the prevention and treatment of osteoporosis, and can thus be safely supplied to most men and women indefinitely, starting as early as feasible. When taken with antiresorptive medicines, they have a synergistic effect on bone mineral density (BMD), bone strength, and fracture prevention. According to current recommendations, most therapy will benefit by adding 1000-1500mg of calcium and 400-800IU of vitamin D per day. Today, an estimated 44 million people in the United States, or 55% of those aged 50 and more, have osteoporosis or reduced bone mass. By 2010, the number of women and men in the same age range was predicted to have climbed to over 52 million. In the United States, the cost of osteoporosis-related fractures is expected to exceed $18 billion per year [(8)]. In glucocorticoid-induced bone loss, increased osteoplastic activity, decreased intestinal calcium absorption, increased renal calcium excretion, disturbances in vitamin D and gonadal hormone metabolism, and increased death of osteoblasts and osteocytes are all involved [(9)]. According to [(10)], "healthy older women randomized to calcium supplementation had an elevated risk of myocardial infarction.". In terms of bone health, this effect could outweigh the benefits of calcium supplementation. Inadequate calcium and vitamin D intake leads to decreased calcium absorption, secondary hyperparathyroidism, and subsequent bone loss. Because bone loss is a good predictor of fracture, calcium supplementation with vitamin D has been one of the most well-known strategies in primary and secondary prevention of osteoporosis. Calcium supplementation has been found to be effective in reducing or preventing bone loss in numerous studies [11].

In recent years, chia (*Salvia hispanica*, L.) has been explored as a source of dietary fiber. In studies, eating chia seeds reduced systolic blood pressure, postprandial blood glucose, and inflammation while raising &-linoleic acid seeds and plasma eicosapentaenoic acid levels. Chia seeds are a one-of-a-kind seed that is gaining popularity as a novel cuisine and attracting scientific interest. It is a good source of omega 3/omega 6 fatty acids, soluble dietary fiber and includes a considerable quantity of protein and phytochemicals. As a result, it has nutritional qualities that help prevent obesity, hypertension, cardiovascular disease (CVDs), cancer, and diabetes, among other noncommunicable diseases [12]. Chia is a twice-yearly cultivated plant in the mint family (Labiate), spermatophyte super division, and plant kingdom. The seed's oil content ranges from 25% to 40%, with 60% of it being (omega) 3 alpha linolenic acid and 20% being (omega) 6 linoleic acid. Both essential fatty acids are required for public health and cannot be manufactured artificially [13].

The objective of this study was to see how calcium, vitamin D, and chia seeds affected rats with osteoporosis.

Materials and methods

Materials

Plant materials:

Chia seeds were purchased from Harraz market in Cairo governorate, Egypt.

Chia seeds(S. hispanica L.)were harvested from Ixtlahuacan ,Colima, Mexico, in Noember2010 and provided by Ingo. Roberto Nahum A Maya Zamora. The seeds were manually separated from extraneous matter (dust, vain seeds, and straw from threshed seeds) and kept in a sealed container with a relative humidity less than 40%, until use. Then ,samples(mix of chia seeds from several plants)were ground using liquid nitrogen to obtain a powder and were stored at 4^{o}c until use.

Rats

Forty-eight -old female albino rats, weighting 200±10g were purchased from Medical Insects Research Institute, Dokki, Cairo, Egypt.

Casein, vitamins, minerals, cellulose, choline chloride, vitamin D3 and calcium: Prednisone acetate as a source of Glucocorticoid (GC) was purchased from a pharmacy in Cairo, Egypt, and utilized at a dose of 4.5mg/kg body weight.

Chemical and kits

Al-Gomhoria Company for Trading Drug, Chemical, and Medical Instruments, Cairo, Egypt, provided the chemical kits used in this study (TC, TG, HDL-c, ALT, AST, ALP, glucose, calcium, and phosphorus).

Methods

Experimental design:

The experimental was carried out and approved in the faculty of Home Economics, Menoufia University, Shebin EL-Kom, Egypt. Rats were kept in wire cages at a room temperature of 25°C and were kept in typical, healthy conditions.

Forty-eight female albino rats (Sprague Dawley Strain) weighing an average of (200g±10g) were housed in well-aerated cages in hygienic conditions and fed a basal diet for one week to adapt. The basal fed was prepared according to instructions [(14)]. After this period, the rats were divided into two main groups, as a following.

The first main group (6 rats) fed on basal diet, as a control negative group.

The second main group (42 rats) was given oral prednisone acetate (4.5ml/kg body weight/day, twice a week) for the duration of the study in order to create osteoporotic models [(15)].

The second main group was divided into seven subgroups (6 rats each) as follows:

Group (1): Rats were fed on basal diet (control positive group).

Group (2): Rats were fed on basal diet and treated daily with 1% calcium in drinking water, ad libitum (Ca).

Group (3): Rats were fed on basal diet and supplemented with (400mg vitamin D3/Kg diet).

Subgroup (4): Rats were fed on balanced diet fortified with (5% chia seeds).

Group (5): Rats were fed a balanced diet that included 5% chia seeds and were given 1% calcium in their drinking water on a daily.

Group (6) Rats were fed on balanced diet containing 5%chia seeds and supplemented with 400mg vitamin D3 /Kg.

Group (7): Rats were fed on balanced diet containing 5% chia seeds and supplemented with400mg vitamin D3/kg also this group was treated daily with 1% calcium in drinking water.

Animals were starved for 12 hours at the end of the trial (4 weeks) before being scarified. Blood samples were taken from the portal vein into dry, clean centrifuge tubes for serum separation, and the serum was separated by centrifugation for 10 minutes at 3000 rpm [(16)]. Until chemical analysis, serum samples were kept frozen at -20 °C.

Lipid profile

Total cholesterol was determined according to [(17)], Triglycerides (T.G) according to [(18)]. High Density Lipoprotein (H.D.L- c) according to [(19)], Low Density Lipoprotein (L.D.L-

c) and Very low lipoprotein (V.L.D-c) were calculated according to the following equation: LDL- cholesterol=Total cholesterol – (HDL-c + TG/5). VLDL-c=TG/5 [20].

Liver functions

Alanine amino transferase (ALT) activities were measured in serum using the modified kinetic method of [21]. Aspartate amino transferase (AST)activities were measured in serum using the modified kinetic method of [22]. While ALP was carried out according to the method of [23].

Serum glucose

Enzymatic determination of Serum glucose was carried out calorimetrically according to the method of [24].

Bone analyses:

Calcium:

According this procedure, the amount of calcium in the right femur was determined using an atomic absorption spectrophotometer [25].

Phosphorus:

The amount of phosphorus in the right femur was determined by calorimetrically according to the method described by [26].

Bone mineral density(BMD) and bone mineral concentration (BMC)

DEXA Unite (Lunar OX IQ 4716) was used to determine the density of bone in the right femur as well as the BMC according to the procedure [27].

Statistical analysis

The date was statistically analyzed using a Computerized Costat Program by One Way ANOVA. The results are presented as mean+ SD. Differences between treatments at ($P \leq 0.05$) were considered significant [28].

Results and Discussion

Table (1) shows the mean total cholesterol (TC) values of rats fed varied diets. The control (+) group's mean value of (TC) was significantly higher ($P \leq 0.05$) than the control (-) group's, which were 95.00 and 51.67 mg/dl, respectively. The positive control group's mean serum cholesterol level rises by 83.859 % more than the negative control groups.

The osteoporotic groups fed on (basal diet and treated with 1% Ca in drinking water "G3"), (basal diet supplemented with 400 mg Vit. D3 "G4"), (basal diet containing 5% chia "G5"), (basal diet containing 5% chia and treated with 1% Ca in drinking water "G6"), (basal diet containing 5% chia and supplemented with Vit. D3 "G7"), (basal diet containing 5% chia and supplemented with Vit. D3, in addition to treat this group daily with calcium in drinking water "G8") induced significant decrease ($P \leq 0.05$) in mean values of (TC), as compared to control (+). Non-significant differences in mean total cholesterol values were identified across all treated groups, according to the results in this table. When compared to the negative control group, all treated rats groups demonstrated a significant rise (P≤0.05) in serum cholesterol levels.

In the same table, the mean value of triglyceride (TG) of rats fed various diets was shown (1). The positive control group (+ve) had a substantially higher (P0.05) mean value of (TG) than the negative control group (-ve), which were 131.00 and 54.67 mg/dl, respectively. The positive control group's mean serum TG increased by about 139.619 % more than the negative control groups. In comparison to the positive control group, treating osteoporotic groups with Ca, Vitamin D, chia, (Ca and chia), (Vit. D and chia), and (Vit. D, Ca and chia) resulted in a significant decrease (P≤0.05) in serum TG. Data in this table revealed that, osteoporotic groups which were fed on (basal diet and treated with1% Ca in drinking water "G3"), (basal diet supplemented with 400 mg Vit. D3 "G4"), (basal diet containing 5% chia "G5"), (basal diet containing 5% chia and treated with 1% Ca in drinking water "G6"), (basal diet containing 5% chia and supplemented with Vit. D3 "G7"), or fed on (basal diet containing 5% chia, Vit. D3 and treated daily with calcium in drinking water "G8") decreased the mean values of serum (TG) by about 36.893%, 41.221%, 43.511%, 47.839%, 45.801% and 46.053% than that of the positive control group. Osteoporotic group of rats which treated with (1% Ca and 5% chia) recorded the best results in (TG).

Table 1 shows the mean value of high-density lipoprotein-cholesterol (HDL-c) in rats fed varied diets (1). The control (+) group (G2) had a substantially lower (P≤0.05) mean value of (HDL- c) than the control (-) group (G1), with 16.67 mg/dl and 31.33 mg/dl, respectively. The mean blood HDL- C level in the positive control group was 46.792 percent lower than in the negative control group. Data in this table revealed that, osteoporotic groups which were fed on (basal diet and treated with1% Ca in drinking water "G3"), (basal diet supplemented with 400 mg Vit. D3 "G4"), (basal diet containing 5% chia "G5"), (basal diet containing 5% chia and treated with 1% Ca in drinking water "G6"), (basal diet containing 5% chia "G7" and supplemented with Vit. D3 which), and (basal diet containing 400 mg Vit. D3, 5% chia and treated daily with calcium in drinking water "G8") induced significant increase (P≤0.05) in mean values of (HDL-c), as compared to control (+) G2. The values were 25.33, 24.33, 21.33, 24.00 and 23.67 and 26.33 mg/dl, respectively. There were no significant differences in HDL-c between all treated groups, according to the data in the same table.

Table (1) also shows the mean value of low-density lipoprotein (LDL) in rats fed varied diets. The control (+) group's mean value of (LDL) was substantially greater (P≤0.05) than the control (-) group, which were 52.13 9.40 mg/dl, respectively. Data in this Table revealed that, treating osteoporotic groups with 1% Ca in drinking water, 400 mg Vit. D3, 5% chia, (5% chia and treated with 1% Ca in drinking water), (Vit. D3 and 5% chia) and (Vit. D3, 5% chia and treated daily with calcium in drinking water) induced significant decrease ($P \leq 0.05$) in mean values of (LDL), as compared to control (+). Mathematically, osteoporotic rats treated with 400 mg Vitamin D (G4) had the best results in (LDL-c), followed by the group treated with (400 mg vitamin D3, 1% calcium, and 5% chia) combined (G8).

The mean value of very low-density lipoprotein (VLDL-c) of rats fed on various diets was shown in table (1). The mean value of the positive control group (+ve) increased significantly ($P \leq 0.05$), as compared to the negative control group (-ve) being 26.2 mg/dl and 10.93 mg/dl, respectively. The mean value of serum VLDL-c increased by about 139.707% in the positive control group than that of the negative control group. Treating osteoporotic groups with Ca, vit. D, chia, (Ca and chia), (Vit. D and chia) and (vit. D, Ca and chia) induced significant decrease ($P \leq 0.05$) in serum VLDL-c, as compared to the positive control group. Data in this table revealed that, osteoporotic groups which were fed on (basal diet and treated with1% Ca in drinking water "G3"), (basal diet supplemented with 400 mg Vit. D3 "G4"), (basal diet containing 5% chia "G5"), (basal diet containing 5% chia and treated with 1% Ca in drinking water "G6"), (basal diet containing 5% chia and supplemented with vit. D3 "G7"), or fed on (basal diet containing 5% chia, Vit. D3 & treated daily with calcium in drinking water "G8") decreased the mean values of serum (VLDL-c) by about 36.908%, 41.221%, 43.511%, 47.824%, 45.801% and 46.068% than that of the positive control group. Osteoporotic group of rats which treated with (1% Ca and 5% chia) recorded the best results in (VLDL-c).

The results in this table show that prednisone acetate injections to induce osteoporosis (control +ve) resulted in significant increases ($P \leq 0.05$) in serum cholesterol, triglycerides, LDL-c, and VLDL-c, whereas HDL-c increased, when compared to non-injected rats (control-ve). The use of calcium, Vitamin D, chia, (Ca and chia), (Vit. D and chia), and (Vit. D, Ca and chia) in osteoporotic groups improved the mean lipid profile values (cholesterol, triglycerides, LDL-c, HDL-c and VLDL-c). Calcium (Ca) intakes may increase cardiovascular disease risk by affecting body composition (body weight and fat) and serum lipid profile, according to [(29)]. [(30)] discovered a clear relationship between BMI, serum triglyceride, TC, and LDL-c levels and glucocorticoid GC dosage response. According to [(31) & (32)], the mechanism of calcium's effect on cholesterol and its fractions in blood serum is incompletely defined. Calcium is thought to bind with bile acids and cholesterol in the gastrointestinal tract. This results in the formation of insoluble salts, which are excreted because they are not absorbed into the bloodstream from the gastrointestinal system.

Taking calcium + Vitamin D during a weight-loss intervention increased the favorable effect of body weight loss on the lipid and lipoprotein profile in overweight or obese women who had a poor calcium intake daily [(33)].

Chia seeds are an excellent source of omega-3 fatty acids (about 65 % of the oil content). Many physiological activities in the human body have been linked to omega-3 fatty acids. Chia seeds have the potential to be a good source of antioxidants with increased concentrations of healthy unsaturated fatty acids, gluten free protein, vitamin, minerals, and phenolic compounds, omega-3fatty acids are also a great source of dietary fiber, which is excellent for the digestive system and regulating diabetes mellitus and obesity. Chia seeds have anti-diabetic, anti-lipidemic, and antioxidant properties [(34)].

Table (1): Effect of calcium, vitamin D and chia seeds on lipid profile of rats suffering from osteoporosis

Group \ Parameters		TC	TG	HDL	LDL	VLDL
		mg/dl				
G1	Control (-ve)	51.67^{a}±1.155	54.67^{a}±5.687	31.33^{a}±4.163	9.40^{a}±3.995	10.93^{a}±1.137
G2	Control (+ve)	95.00^{b}±10.536	131.00^{b}±6.083	16.67^{b}±1.155	52.13^{b}±10.441	26.20^{b}±3.512
G3	1% Ca	70.33^{bc}±7.506	82.67^{bc}±1.528	25.33^{b}±3.055	28.47^{b}±7.855	16.53^{bc}±0.306
G4	400 mg Vit. D	65.00^{bc}±5.292	77.00^{bc}±2.000	24.33^{b}±1.155	25.27^{b}±6.804	15.40^{bc}±0.400
G5	5% chia	72.67^{bc}±5.033	74.00^{bc}±3.000	21.33^{b}±1.528	36.33^{b}±6.004	14.80^{bc}±0.600
G6	1% Ca and 5% chia	73.67^{bc}±4.041	68.33^{bc}±5.860	24.00^{b}±1.000	36.00^{b}±5.103	13.67^{bc}±1.172
G7	400 mg Vit. D and 5% chia	67.00^{c}±8.185	71.00^{c}±5.568	23.67^{b}±2.517	29.13^{b}±11.321	14.20^{c}±1.114
G8	400 mg Vit. D, 1% Ca and 5% chia	66.00^{d}±2.646	70.67^{d}±5.508	26.33^{c}±3.215	25.53^{c}±4.649	14.13^{d}±1.102
LSD (P≤0.05)		9.48	8.68	4.64	11.81	1.74

Values are given as mean ± SD. Mean values in each column with same letters are not significantly different. LSD: Least significant differences (P≤0.05)

Table (2) shows the mean blood glucose levels of rats fed various diets. The control (+) group's mean serum glucose level was considerably higher (P0.05) than the control (-) group, at 139.33 mg/dl and 82.67 mg/dl, respectively. The positive control group's mean serum glucose level increased by 68.537 percent more than the negative control groups. The results in this table revealed that osteoporotic groups which treated with1% Ca in drinking water, 400 mg Vit. D3 5% chia, (5% chia and treated with 1% Ca in drinking water), (400 mg Vit. D3 and 5% chia), (400 mg Vit. D3, 5% chia and treated daily with calcium in drinking water) induced significant decrease (P≤0.05) in mean values of glucose, as compared to control (+). Osteoporotic rats which treated with 5% chia (G5) recorded the best results in serum glucose, followed by the group which treated with (400 mg vitamin D, 1% calcium and 5% chia (G8). From the results in this table, it could be observed that, injected rats with prednisone acetate to induce osteoporosis (control +ve) led to significant increase (P≤0.05) in glucose as compared to non-injected rats (control-ve). All treated groups with tested diets towered the mean values of glucose. Chia seed intake enhanced glucose and insulin tolerance in both preventative and treatment groups

[36]. Vitamin D, alone or in combined with calcium, has been shown in a few trials to reduce the occurrence of oxidative stress in diabetics [35 and 36].

Complications in the etiology of diabetes mellitus, according to [37] are caused by oxidative stress and can be prevented or limited by oral vitamin D and calcium supplements. Oral vitamin D and calcium supplementation may be a beneficial treatment for diabetic patients to reduce/prevent diabetic pathological consequences.

Table (2): Effect of calcium, vitamin D and chia seeds on glucose of rats suffering from osteoporosis

Groups \ Parameters		Glucose mg/dl
G1	Control (-ve)	82.67[a]±2.517
G2	Control (+ve)	139.33[b]±5.859
G3	1% Ca	125.00[b]±6.000
G4	400 mg Vit. D	108.00[b]±2.646
G5	5% chia	101.67[c]±5.508
G6	1% Ca and 5% chia	117.33[c]±6.658
G7	400 mg Vit. D and 5% chia	118.67[c]±3.512
G8	400 mg Vit. D, 1% Ca and 5% chia	105.00[d]±2.000
LSD (P≤0.05)		8.090

Values are given as mean ± SD. Mean values in each column with same letters are not significantly different. LSD: Least significant differences (P≤0.05)

Table (3) shows the mean value of Alkaline Phosphatase (ALP) in rats fed various diets). The control (+) group's mean value of (ALP) was considerably greater (P≤0.05) than the control (-) group's, which were 136.33 and 86.33 U/L, respectively. The obtained results in this table revealed that osteoporotic groups which were (fed on basal diet and treated with1% Ca in drinking water "G3"), or (basal diet containing 5% chia "G5"), or (basal diet containing 5% chia and treated with 1% Ca in drinking water "G6"), or (supplemented basal diet with Vit. D3 which containing 5% chia "G7") or fed on (supplemented basal diet with Vit. D3 which containing 5% chia & treated daily with calcium in drinking water "G8") induced significant decrease(P≤0.05) in mean values of (ALP), as compared to control (+) G2. Osteoporotic rats which treated with 400 mg Vit. D3, 1% Ca and 5% chia together (G8) recorded the best results in (ALP), followed by the treated group with 1% Ca and 5% chia (G6) and the treated group with 400 mg Vit. D and 5% chia (G7), respectively.

In the same table, the mean value of Aspartate Aminotransferase (AST) of rats fed various diets was shown. The control (+) group (G2) had a considerably higher ($P \leq 0.05$) mean value of (AST) than the control (-) group (G1), which were 139.00 and 116.33 U/L, respectively. When compared to the positive control group, all treated osteoporotic groups with tested diets in this investigation caused non-significant changes in blood AST mean values. Osteoporotic rats which treated with 400 mg Vit. D3, 1% Ca and 5% chia together (G8) recorded the best results in (AST) mathematically.
The mean value of (ALT) of the control (+) group was significantly higher ($P \leq 0.05$), as compared to the control (-) group being 55.00 and 29.00 U/L, respectively. Data in this table revealed that, osteoporotic groups which were (fed on basal diet and treated with1% Ca in drinking water "G3"), or (basal diet supplemented with 400 mg Vit. D3 "G4") or (basal diet containing 5% chia "G5"), or (basal diet containing 5% chia and treated with 1% Ca in drinking water "G6"), or (supplemented basal diet with Vit. D3 which containing 5% chia "G7") or fed on (supplemented basal diet with Vit. D3 which containing 5% chia and treated daily with calcium in drinking water "G8") induced non-significant differences($P \leq 0.05$) in mean values of (ALT), as compared to control (+).
Mathematically, the best results in (ALT) were observed by osteoporotic rats treated with 400 mg Vit. D3, 1% Ca, and 5% chia combined (G8), followed by the treated group with 400 mg Vit. D and 5% chia (G7). From the results in this table, it could be observed that, injected rats with prednisone acetate to induce osteoporosis (control +ve) led to significant increased ($P \leq 0.05$) in AST, ALP and ALT, as compared to non-injected rats (control-ve). All treated groups with 1% Ca, 400mg Vit. D3, 5% chia, 1% Ca and 5% chia, 400mg Vit. D3 and 5% chia & 400mg Vit. D3, 1% Ca and 5% chia improved the mean values of ALT, AST and ALP. In this respect [(38)] reported that, calcium plays an essential role in nearly all cellular processes. As such, cellular and systemic calcium concentrations are tightly regulated.
For the above parameters, there was a 5% improvement liver profile permanents' in the case of tested seeds, followed by a 10% improvement. So, according to this study, CCl_4-induced liver damage in rats can be reduced by giving 5% chia seeds [(39)].
The mean value of calcium of rats fed on various diets was shown in table (4). The mean value of calcium of the control (+) group was non-significantly lower ($P \leq 0.05$), as compared to the control (-) group being 10.33 and 10.00 mg, respectively. Data in this table revealed that, Ca, Vit D3 and chia in all osteoporotic groups induced non-significant differences in calcium content, as compared to the control groups. Osteoporotic rats which treated with 400 ml vitamin D, 1% calcium and 5% chia (G8) recorded the best results in calcium, followed by the treated group with 400 ml vitamin D & 5% chia (G7).
The mean value of phosphorus of rats fed on various diets was shown in the same table (4). The mean value of phosphorus of the control (+) group was significantly lower

(P≤0.05), as compared to the control (-) group being 4.83 and 5.67 mg, respectively. All tested diets which contain Ca, VitD3 and chia seeds induced non-significant changes in bone phosphorus, as compared to the negative control group. This treatment improved the levels of phosphorus in the bone. Osteoporotic rats which treated with 400 mg vitamin D and 5% chia (G7) recorded the best results in phosphorus mathematically. From the results in this table, it could be observed that, injected rats with prednisone acetate to induce osteoporosis (control +ve) led to significant decreased (P≤0.05) in as compared to non-injected rats (control-ve) while Ca content did not change. All treated groups with 1% Ca, 400 mg Vit. D3, 5% chia, 1% Ca and 5% chia, 400mg Vit. D3 and 5% chia and 400mg Vit. D3 ,1%Ca and 5% chia improved the mean values of Ca and P. In this respect **[40]** it was concluded that dietary Ca, AP, vitamin D and strain of broiler chickens influenced the metabolism of Ca, P and that, as a consequence, the tolerance to high dietary Ca. A lean strain of chickens tolerated high dietary calcium better than its fat counterparts.

Table (3): Effect of calcium, vitamin D and chia seeds on Liver Profile of rats suffering from osteoporosis

Groups	Parameters	ALP (U/L)	AST (U/L)	ALT (U/L)
G1	Control (-ve)	$86.33^{c}\pm4.163$	$116.33^{a}\pm0.578$	$29.00^{a}\pm2.646$
G2	Control (+ve)	$136.33^{a}\pm3.512$	$139.00^{a}\pm8.185$	$55.00^{b}\pm5.000$
G3	1% Ca	$124.67^{b}\pm3.215$	$135.00^{ab}\pm7.810$	$39.67^{b}\pm5.033$
G4	400 mg Vit. D	$137.67^{b}\pm2.082$	$126.00^{ab}\pm6.083$	$37.67^{b}\pm2.516$
G5	5% chia	$125.00^{b}\pm2.000$	$126.33^{ab}\pm5.132$	$37.67^{b}\pm4.041$
G6	1% Ca and 5% chia	$109.33^{c}\pm7.638$	$126.33^{ab}\pm6.110$	$36.33^{b}\pm0.577$
G7	400 mg Vit. D and 5% chia	$121.00^{d}\pm2.000$	$126.33^{ab}\pm4.041$	$34.33^{b}\pm6.658$
G8	400 mg Vit. D, 1% Ca and 5% chia	$102.00^{e}\pm3.000$	$124.00^{b}\pm5.292$	$33.33^{b}\pm3.512$
LSD		6.57	9.94	7.40

Values are given as mean ± SD. Mean values in each column with same letters are not significantly different. LSD: Least significant differences (P≤0.05)

Chia seeds (*salvia hispanica, L.*) is good source of calcium; however, it is not previously reported its bioavailability associated with an inflammatory condition. This study aimed to evaluate the effect of chia on calcium bioavailability, inflammation, and oxidative stress in Wister rats fed a high –fat diet or standard diet or standard diet for 35 days **[41]**. Chia consumption resulted in lower calcium balance and calcium absorption and retention rates. In addition, the urinary calcium concentration was lower in groups that were fed chia. The bone resistance of animals fed chia was lower than that in rats fed the standard diet receiving calcium carbonate Animals that were fed chia showed lower total, very low-density lipoprotein, and low-density lipoprotein cholesterol levels than animals fed calcium carbonate. Chia intake presented low calcium bioavailability regardless of the type of diet consumed and was able to improve inflammation and the lipid profile in young waster rat. Besides this, the consumption of this seed decreased the activity of antioxidants enzymes [42].

Table (4): Effect of calcium, vitamin D and chia seeds on bone analysis of rats suffering from osteoporosis

Groups	Parameters	Calcium	Phosphorus
		Mg	
G1	Control (-ve)	$10.33^{a}\pm0.153$	$5.67^{a}\pm0.208$
G2	Control (+ve)	$10.00^{a}\pm0.100$	$4.83^{ab}\pm0.252$
G3	1% Ca	$10.17^{a}\pm0.153$	$5.47^{ab}\pm0.379$
G4	400 mg Vit. D	$10.13^{a}\pm0.252$	$5.20^{ab}\pm0.265$
G5	5% chia	$10.07^{a}\pm0.252$	$5.40^{ab}\pm0.300$
G6	1% Ca and 5% chia	$10.13^{a}\pm0.115$	$5.40^{ab}\pm0.100$
G7	400 mg Vit. D and 5% chia	$10.23^{a}\pm0.306$	$5.50^{ab}\pm0.361$
G8	400 mg Vit. D, 1% Ca and 5% chia	$10.37^{a}\pm0.513$	$5.27^{b}\pm0.208$
	LSD	0.49	0.43

Values are given as mean ± SD. Mean values in each column with same letters are not significantly different. LSD: Least significant differences (P≤0.05)

From the results in table (5), it could be observed that, injected rats with prednisone acetate to induce osteoporosis (control +ve) led to significant decrease (P<0.05) in BMD and BMC, as compared to non-injected rats (control-ve). All treated groups with 1% Ca, 400mg Vit. D3, 5%chia, 1% Ca and 5% Chia, 400 mg Vit. D3 and 5% chia and 400 mg Vit. D3, 1% Ca and 5%chia improved the mean values of BMD and BMC. In this respect, [43] reported that, calcium and vitamin D are considered to be pharmacologically active, safe and cost effective for prevention and treatment of osteoporosis hence can be administered safely to most men/women indefinitely starting as early as possible, they exert a synergistic effect with antiresorptive agents on bone mineral density BMD, bone strength and in term of fracture prevention it is appropriate to add 1000-1500mg/day calcium and 400-800IUvitamin D to most therapies under current guidelines.

Currently for Indian population calcium supplementation is recommended in all regardless of BMD [44] reported that calcium supplementation (alone or in combination with vitamin D) is effective in preventing osteoporotic fracture in elderly women and men. In a randomized placebo-controlled trial among healthy community-dwelling older men and women.

Chia seeds (*salvia hispanica*) provide an unusually high content of α-linoleic acid with several potential health benefits, but few studies have examined the long-term intake of n-3fatty acid-rich plant foods such as chia **(45)** Omega-3,however enhanced, calcium absorption by modifying the lipid composition of the intestinal cell membrane and intestinal calcium loss **(46)**.

Table (5): Effect of calcium, vitamin D and chia seeds on BMD and BMC of rats suffering from osteoporosis

Groups \ Parameters		BMD	BMC
		g/cm^2	
G1	**Control (-ve)**	0.1815^a± 0.01025	0.0745^a± 0.01396
G2	**Control (+ve)**	0.0765^e± 0.00331	0.0200^f± 0.0072
G3	**1% Ca**	0.1225^d± 0.0124	0.0483^{cd}± 0.0013
G4	**400 mg Vit. D**	0.1241 ^{d}± 0.0102	0.0340^e± 0.0052
G5	**5% chia**	0.1377^{cd}± 0.0106	0.0410^{de}± 0.0080
G6	**1% Ca and 5% chia**	0.1539^{bc}± 0.0100	0.0581^{bc}± 0.0058
G7	**400 mg Vit. D and 5% chia**	0.1381^{cd}±0.0055	0.0445^{de}± 0.0013
G8	**400 mg Vit. D, 1% Ca and 5% chia**	0.1639^b±0.0074	0.0687^{ab}± 0.0067

BMD: Bone Mineral Density. BMC: Bone Mineral Concentration.
Values are given as mean ± SD for 3 rats in each group. Mean values in each column with same letters are not significantly different. LSD: Least significant differences (P<0.05).

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تأثير الكالسيوم وفيتامين د وبذور الشيا على الفئران المصابة بهشاشة العظام

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الملخص العربي

هشاشة العظام مرض صحي رئيسي يصيب الناس في جميع يحدث ذلك عندما يكون هناك قدر كبير من فقدان العظام ويكون مرتبطًا بخطر أكبر للإصابة بالكسور. الهدف من هذه الدراسة هو معرفة كيفية تأثير الكالسيوم وفيتامين د وبذور الشيا على الفئران المصابة بهشاشة العظام. في هذه الدراسة، تم تقسيم ٤٨ أنثى من الفئران البيضاء سلالة سبراج دوللي بمتوسط وزن (٢٠٠± 10 جم) إلى ثماني مجموعات ومقسمة إلى ٨ مجموعات. حيث تغذت المجموعة الضابطة السالبة النظام الغذائي الأساسي طوال الفترة التجريبية. بينما تناولت المجموعات المعاملة التي ضمت ٤٢ فأرًا ، أسيتات بريدنيزون عن طريق الفم (٤,٥ مل / كجم من وزن الجسم / يوم مرتين في الأسبوع) وتم تغذيتها على نظام غذائي بنسبة ٥ في المائة من الشيا ، ١ ٪من الكالسيوم ، ٤٠٠ ملجم من فيتامين د عولجت من هشاشة العظام في المجموعات ٣، ٤، ٥، ٦، ٧، ٨.، تناولت أسيتات بريدنيزون عن طريق الفم (٤,٥ مل / كجم من وزن الجسم / يوم مرتين في الأسبوع) تم تغذيتها على ٥ ٪ من بذور الشيا ١ ٪. أظهرت النتائج أن معاملة أسيتات بريدنيزون أدت إلى زيادة معنوية (P≤0.05) في الكوليسترول الكلي في الدم، والدهون الثلاثية ، والبروتين الدهني منخفض الكثافة (LDL-c) ، والبروتين الدهني منخفض الكثافة (VLDL-c) ، ومستوى الجلوكوز ، أنزيمات الكبد (ALP ، ALT، AST) ، بالإضافة إلى انخفاض تركيز البروتين الدهني عالي الكثافة والكوليسترول (HDL-c). تم تحسين هذه الإجراءات بشكل ملحوظ بالنسبة للفئران التي تعاني من هشاشة العظام والتي عولجت بـ 5٪ شيا، ٤٠٠ مجم فيتامين د ، ١٪ كالسيوم. الخلاصة، حدث تحسن فى مستويات الكالسيوم، الفوسفور ، كثافة العظام ، BMC في الفئران التي تعاني من هشاشة العظام والتي عولجت بـ ٤٠٠ مجم فيتامين د ، ٥٪ شيا. نتيجة لذلك، يتطلب تحديد توصيات المخاطر والعلاج فهماً شاملاً للأنظمة التي تنظم صحة العظام.

الكلمات المفتاحية: الأملاح المعدنية، الفيتامينات، بذورالنباتات، الفئران، صحة العظام