ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 4, Issue 2, pp: (113-127), Month: April - June 2016, Available at: www.researchpublish.com

Protective Effect of Soybean and Mushroom against Glucocorticoid-Induced Osteoporosis in Female Rats

Bushra S. ALSahafi[1], Maryam A. Al-Ghamdi [1,2,3,4] Etimad A. Huwait[1,2,3,4] , Taha A. Kumosani[1,2,6], Reem AL-Wafi[7] ,Turkyah J. Al-Bogami[1], Moselhy SS [1,2,5,8]

[1]Biochemistry Department, Faculty of science, King Abdulaziz University
[2]Experimental biochemistry unit, King Fahad Medical Research center (KFMRC), King Abdulaziz University P.O. Box 21424, Jeddah, Saudi Arabia.
[3]Biocell Bank unit, King Fahad Medical Research center (KFMRC), King Abdulaziz University
[4]Vitamin D Pharmacogenomics Research Group, King Abdulaziz University
[5]Bioactive Natural Products Research Group King Abdulaziz University
[6]Production of bio-products for industrial applications Research group King Abdulaziz University
[7]Center of Nanotechnology and Physics Department, Faculty of science, King Abdulaziz University
[8]Department of Biochemistry, Faculty of Science, Ain Shams University

Abstract: **The present study was conducted to evaluate the possible protective effect of soybean and mushrooms on the bone turnover in glucocorticoid-induced osteoporosis female rats. Healthy female divided into six groups: Group I (n=10): Rats were fed on normal diet as negative control. Groups (II to VI)(n=60): Animals was injected with 2 mg dexamethsone sodium phosphate (glucocorticoid) to induce osteoporosis for 3 weeks daily. Group II (n=15): Rats were injected with 2 mg dexamethsone sodium phosphate as positive control. Group III (n=15): Rats were injected estrogen (25 mg/kg.b.w/day). Group IV (n=10): Rats were fed on diet containing 20% mushroom. Group V (n=10): Rats were fed on diet containing 20% soybean. Group VI (n=10): Rats were fed on normal diet containing 10 % soybean and 10 % mushroom. Data obtained revealed that group II exhibited significantly decrease in body and femur bone weight, calcium and phosphorus concentrations in serum and femur bone , serum osteocalcin , alkaline phosphatase, vitamin D and significantly increases in parathyroid hormone when compared with group I. Treatment with mushroom or combined with soybean revealed improvement in body and femur weight, calcium , phosphorus and magnesium concentrations in serum and femur bone , serum osteocalcin , alkaline phosphatase, vitamin D and significantly decrease in parathyroid hormone when compared with group II. Histopathological examination showed cracks-wide Haversian canals-thin trabeculae. This study demonstrates that mushroom alone or combined with soybean possesses positive effect to protect against osteoporosis.**

Keywords: **Osteoporosis, mushroom, soybean, glucocorticoid, rats.**

1. INTRODUCTION

Vitamin D has long been recognized for its role in bone health. The major biological function of vitamin D in humans is to maintain serum calcium (Ca) and phosphorus(P) levels within the normal range. It is well known that vitamin D is essential for optimal skeletal growth and density [1]. The overall nutritional vitamin D status of the individual depends on endogenous (sun rays) and exogenous (dietary, supplements) sources, with the former being the major source [2].

The role of vitamin D in bone health has far been recognized through the numerous intervention and observational studies. Before going into details of the studies that elucidate the impact of vitamin D sufficiency on bone health we will describe the biology of the bone tissue. Bone is dynamic and continuously being turned over throughout life. Throughout one's lifetime, old bone is removed (resorption or remodeling) and new bone is added to the skeleton [3].

Osteoporosis is the result of vitamin D deficiency characterized by low bone mass and microarchitectural deterioration of bone tissue, with increase in bone fragility and susceptibility to fracture [4]. Certain risk factors are linked to the development of osteoporosis some of them cannot be change like Gender, age, body size and ethnicity. The other factors can be change like sex hormones, Ca and vitamin D intake, medication use and lifestyle [5].Osteoporosis are greater in woman than men, because women have less bone tissue and lose bone faster than men as of the changes that happen with menopause [6]. In spite of the Middle East being one of the sunniest regions worldwide, several studies have been conducted that the prevalence of hypovitaminosis D there was among the highest in the world [7].

Osteoporosis treatment includes a focus on pharmacologic treatment and proper nutrition. Several medications are available for the prevention or treatment of osteoporosis, including: bisphosphonates, estrogen , parathyroid hormone (PTH), calcitonin(CT) and hormone therapy. Adequate nutrition contains a multiplicity of vitamins, minerals, and other important nutrients that help keep our bodies healthy. All of these nutrients are needed in balanced proportion. particularly, Ca and vitamin D are needed for strong bones, and plays a major role in the prevention and treatment of osteoporosis [8].

Therefore bone disease was caused by a deficiency of vitamin D sources present in the diet. Mushrooms and soybean products been researched for their medicinal benefits. Mushrooms that are exposed to UV-B radiation contain enhanced vitamin D_2 than unmodified (non-irradiated) mushrooms [9].

Soybean products are common dietary sources of protein and Ca and containing high concentration of isoflavones with potential health enhancing properties, such as decreasing the symptoms of postmenopausal women [10-13] and cardiovascular disease and antimutagenic effects [14]. It have many health benefits to prevent of breast, prostate cancer and osteoporosis [15-16].

Glucocorticoids (GCs) are classes of steroid hormones .They are widely used to treat a number of medical disorders, like inflammatory arthritis, immunosuppressive or other health problem may weaken bones. This, in turn, can lead to osteoporosis [17]. The administration and long-term use of oral GCs is associated with a significant increase in fracture risk at the hip and spine [18]. Glucocorticoid-induced osteoporosis has distinct characteristics, including the speed of bone loss early after beginning of therapy, the accompanying increase in fracture risk during this time and the combination of suppressed bone formation and increased bone resorption during the early phase of therapy[19]..The objective of this study is to investigate the effect of soybean combined with mushrooms on the bone turnover in glucocorticoid-induced osteoporosis female rats.

2. MATERIALS AND METHODS

Experimental Animals:

Anesthetic method and animal handling were approved in accordance with the ethical guidelines of Medical Ethics Committee of the King Abdulaziz University and ensure that animals did not suffer throughout the experiment. .Healthy Female *albino* rats (n=70 rats) weighting about (160-200g) were obtained from the animal experimental unit of King Fahd Center for Medical Research (KFCMR), King Abdulaziz University. The animal were housed in large polypropylene cages with utmost five animal per cage and maintained under standard laboratory condition (temperature 25° ±2° C), relative humidity (50-55%) and a 12 h light/dark cycle . All animals were allowed to one week to acclimatize in animal housing conditions before being used for the study. All animals fed standard nutritionally balanced diet and drinking water *ad libitum*. Standard nutritionally balanced diet was obtained from (KFMRC), the diet consists of the following ingredients; protein 20.0%, fat 4.0 %, fiber 5.0 %, vitamin mix 1.0%, mineral mix 3.50%, choline chloride 0.25%, the remained formula up to 100% cornstarch, and its energy equals 2850 kcal/kg. The diet manufactured by Grain Silos and Flour Mills Organization, KSA**.** After the adaption period, rats were divided into six groups (n=70) according to administrated herbal food or chemical drug as shown in Table 1.

At the end of experiment, A total of 5 ml blood was withdrawn from rats by heparinized capillary tube from the retro orbital plexu of each rat under anesthesia with diethyl ether. The blood samples were allowed to clot for 15-30 minutes, and then, separated by a cold centrifuge (4ºC) at 3000 rpm for 15 minutes. Serum was aliquot into 0.5 ml labeled eppendorf tubes and were promptly frozen at -20ºC until use it for analysis. The samples have not been subjected to more than 3 freeze/thaw cycles. The body weight was measured before and the end of the experiment. Then animals was sacrificed and right and left femurs were harvested. Each right femur was carefully cleaned and the weight was recorded, and then stored in 10% formalin for histopathological studies. Each left femur was carefully cleaned, which stored at -80° C until biochemical analysis.

Chemicals and Kits:

Dexamethasone sodium phosphate injection was purchased from Amriya for Pharmaceutical Industries, Alexandria-Egypt. Estrogen tablets (Ethinyloestradiol) were purchased from Kahira Pharmaceutical and Chemical Industries Company, Cario-Egypt. Reagent kits needed for chemistry analysis were purchased from Human Gesellschaft fur Biochemica and DiagnosticambH, Germany. These included Ca ,P and Mg were purchased from Spinreact, Spain. Alkaline phosphatase activity colorimetric assay kit was purchased from BioVision Incorporated Milpitas Boulevard, Milpitas, USA. Osteocalcin ELISA kit and 1,25$(OH)_2$vitamin D_3 ELISA kit were purchased from Cloud-Clone Corp, as smebled by USCN Life Science Inc.,USA. Parathyroid hormone ELISA kit was purchased from Cusabio, China. 25(OH)vitamin D ADVIA Centaur assay kit was purchased from Siemens Healthcare Diagnostics Inc, USA.

Statistical Analysis:

Data were expressed as mean ± standard deviation (SD). Statistical analyses were performed with one-factor analysis of variance (ANOVA) and paired t-test using MegaStat. The statistical significance difference was considered when (P<0.05, P<0.01 and P<0.001).

3. RESULTS

Results in figures (1, 2) showed the body weight and femoral weight in different groups. It was found that, there was a significant decrease in body weight and femoral weight in rats induced osteoporosis as compared with negative control. Treatment with mushroom or soybean or combined increased body weight but not returned to normal. The mushroom showed better effects than soybean alone or combined. A significant improvement in femoral weight by combined treatment higher than negative control and osteoporosis treated with esterogen.

Data in figures (3, 4) showed serum Ca, P and Mg levels in different groups. It was found that, a significant reduction in serum Ca and P in osteoprotic rats compared with control (P<0.05). Rats treated with mushroom or soybean or combined tend to correct the level of serum Ca more than control while the level of P not return to normal. Administration of mushroom, soybean or their combined on osteoporotic rats showed significant elevation in serum Ca more than positive control (P<0.001) while mushroom alone showed significant elevation in serum P more than control positive (P<0.01). The effect of mushroom is better than soybean and combined in Ca and P improvement compared with estrogen. Also, it was found that no significant changes in serum Mg in osteoproteic rats compared with negative control. It was found that, bone Ca, P and Mg levels in different groups. It was found that, a significant reduction in bone Ca and P in osteoprotic rats compared with control (P<0.001) and (P<0.01) respectively. Rats treated with mushroom or soybean or combined tend to correct the level of bone Ca but not returned to normal while the level of P showed to be higher than negative control. Administration of mushroom, soybean or their combined to osteoporotic rats showed significant elevation in bone Ca, P and more than positive control. The action of mushroom and combined effect is better than estrogen in elevation of P level. Also, it was found that no significant changes in bone Mg level in osteoproteic rats compared with negative control groups**.**

Data in figures (5,6) showed levels of 25(OH) vitamin D, 1,25$(OH)_2$ vitamin D_3 and PTH in different groups. It revealed that, a significant reduction in serum 25(OH) vitamin D and 1,25$(OH)_2$ vitamin D in osteoprotic rats compared with negative control (P<0.001). Rats treated with mushroom or combined tend to elevate the levels of these vitamin D forms more than negative control. Administration of mushroom, soybean or their combined to osteoporotic rats showed significant elevation in serum 25(OH) vitamin D and 1,25$(OH)_2$ vitamin D_3 compared with positive control (P < 0.001) except soybean in serum 25(OH) vitamin D. The action of mushroom effect is better than combined effects. On the other

hand the effect of mushroom or combined is better than esterogen (P<0.001) in elevation of 25(OH) vitamin D and 1,25$(OH)_2$ vitamin D_3 .

It was found that, a significant elevation in serum PTH in osteoprotic rats compared with negative control (P<0.001). Rats treated with mushroom or soybean or combined tend to lower the level of PTH compared with negative control. Administration of mushroom, soybean or their combined to osteoporotic rats showed significant reduction in serum PTH compared with positive control (P < 0.001). The action of mushroom effect is better than soybean and combined effects. On the other hand the effect of mushroom or combined is better than estrogen (P<0.01) in reduction of PTH.

Data in figures (7,8) showed serum OC level and activity of ALP in different groups. It revealed that, a significant reduction in serum OC level and the activity of ALP in osteoprotic rats compared with control (P<0.001) (P<0.05) respectively. Rats treated with mushroom or combined tend to elevate the levels of OC to be normalized but not returned to normal while mushroom or soybean tend to elevate the levels of ALP more than negative. Administration of mushroom and combined to osteoporotic rats showed significant elevation in serum OC compared with positive control (p < 0.001) and (p < 0.001) respectively The effect of mushroom or combined is better than esterogen (p<0.05) in elevation of OC. In addition, administration of mushroom and soybean to osteoporotic rats showed significant elevation in the activity of ALP compared with positive control (p < 0.001) and (p < 0.001) respectively. The effect of mushroom showed elevation in the activity of ALP compare with estrogen.

TABLE.1: The examined rats were divvied in to six group (n= 70) according to administrator food or drug.

Animal Group	Number	Administrated (Food or Drug)
Group I (Normal)	10 rates	Rats were fed on normal diet and served as a negative control group**.**
Group (II- VI)	60 rates	Rats were injected with (2mg/kg.b.wt/day) dexamethsone sodium phosphate ,intraperitonial for 3 weeks.* [1]
Group II (Osteoporotic untreated)	15 rates	Rats were fed on normal diet and served as a positive control group.
Group III (osteoporotic treated with estrogen)	15 rates	Rats were injected with estrogen (25mg/kg.b.wt /day) in10 mL of Tween80 . [2]
Group IV (osteoporotic treated with mushroom)	10 rates	Rats were fed on diet containing 20% Mushroom powder.[3]
Group V (osteoporotic treated with soybean)	10 rates	Rats were fed on diet containing 20% soybean powder.[1]
Group VI (osteoporotic treated with mushroom and soybean)	10 rates	Rats fed on diet containing 10% soybean and 10% Mushroom powder .

[1]dose of dexamethsone sodium phosphate and soybean according to (Soltan 2013).

[2]dose of estrogen according to (Wronski *et al.* 1988).

[3]dose of mushroom according to (Handayani *et al*. 2014)

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 4, Issue 2, pp: (113-127), Month: April - June 2016, Available at: www.researchpublish.com

TABLE.2: Initial and final body weight (g), bone weight (g) and relation between bone weight / body weight (final) in all studied groups (Mean ± SD).

Experimental groups / variables	Control (-ve control)	Osteoporotic (+ve control)	Osteoporotic+ Estrogen	Osteoporotic+ Mushroom	Osteoporotic+ Soybean	Osteoporotic+ (Mushroom&Soybean)
Body weight (initial) (g)	162.14±11.39	188±8.06	173.28±10.59	185.11±10.67	188.88±18.48	173.28±10.59
Body weight (final) (g)	196.43±11.71	126.8±15.15	143±17.53	164.88±10.70	152±18.99	143±17.53
Bone weight(g)	0.560±0 .03	0.491±0.03	0.573±.07	0.617±.09	0.617±.10	0.716±0.15
Relation between Bone weight / Body weight (final)	0.003	0.004	0.004	0.004	0.004	0.005

TABLE.3: Levels of calcium, phosphorus and magnesium in serum of different groups (Mean ± SD)

Experimental groups / Parameters	Control (-ve control)	Osteoporotic (+ve control)	Osteoporotic+ Estrogen	Osteoporotic+ Mushroom	Osteoporotic+ Soybean	Osteoporotic+ (Mushroom& Soybean)
Calcium (mg/dl)	4.21±1.26	2.48±1.72	5.51±1.49	6.71±1.01	5.24±0.83	6.497±1.18
P-Value	-- -- --	.0215[a*] -- --	.0549[a] .0002[b***] --	.0003[a] *** .000[b]*** --	.1109[a] .0004[b]*** --	.0014[a**] .000[b]*** .1941[c]
phosphorus(mg/dl)	9.99±2.65	7.65±0.49	9.88±1.96	10.75±1.49	7.64±1.33	7.51±1.39
P-Value	-- -- --	.0234[a]* -- --	.9040[a] .0303[b]* --	.3746[a] .0021[b]** --	.0075[a]** .9876[b] --	.0093[a]** .8892[b] .0231[c]*
Magnesium(mg/dl)	±1.05	3.01±0.36	3.76±0.38	3.28±0.29	3.62±0.88	3.17±0.35
P-Value	--	.123[a] -- --	1.000[a] 0.105[b] --	.720[a] .764[b] --	.999[a] .170[b] --	.583[a] .928[b] .0117[c*]

[a:] Comparison between control and osteoporotic groups; [b:] Comparison between osteoporotic and osteoporotic treated groups;

[c:] Comparison between osteoporotic treated with estrogen and osteoporotic treated with mushroom & soybean.

(*P< 0.05, **P < 0.01 and ***P< 0.001).

Levels of calcium, phosphorus and magnesium in serum of different groups (Mean ± SD)

[a:] Comparison between control and osteoporotic groups; [b:] Comparison between osteoporotic and osteoporotic treated groups;

[c:] Comparison between osteoporotic treated with estrogen and osteoporotic treated with mushroom & soybean.

(*P< 0.05, **P < 0.01 and ***P< 0.001).

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 4, Issue 2, pp: (113-127), Month: April - June 2016, Available at: www.researchpublish.com

TABLE.4: Levels of calcium, phosphorus and magnesium in bone of different groups (Mean ± SD)

Experimental Groups / Parameters	Control (- ve control)	Osteoporotic (+ ve control)	Osteoporotic+ Estrogen	Osteoporotic+ Mushroom	Osteoporotic+ Soybean	Osteoporotic+ (Mushroom& Soybean)
Calcium (mg/g)	1076.09±138.25	764.27±72.24	1032.80±95.46	947.87±77.50	962.42±140.46	882.33±112.67
P-Value	-- -- --	.000[a]*** -- --	.4752[a] .0002[b]*** --	.0291[a] * .0056[b] ** --	.0468[a] * .0026[b] ** --	.0025[a] ** .0804[b] .0195[c]*
phosphorus(mg/g)	572.69±122.96	364.82±73.85	547.03±185.15	666.59±105.24	617.25±93.50	647.85±132.07
P-Value	-- -- --	.0058[a] ** -- --	.6949[a] .0144[b] * --	.1333[a] .0001[b] *** --	.4614[a] .0005[b] *** --	.2543[a] .0003[b] * .2636[c]
Magnesium(mg/g)	30.1±3.44	24.34±2.58	30.45±10.33	37.95±3.48	36.76±5.30	43.63±9.50
P-Value	-- -- --	.1362[a] -- --	.8994[a] .1095[b] --	.0208[a] * .0005[b] *** --	0433[a] *. .0012[b] ** --	.0004[a] *** .000[b] *** .0297[c] *

[a:] Comparison between control and osteoporotic groups; [b:] Comparison between osteoporotic and osteoporotic treated groups; [c:] Comparison between osteoporotic treated with estrogen and osteoporotic treated with mushroom & soybean.

(*P< 0.05, **P < 0.01 and ***P< 0.001).

TABLE.5: Levels of 25(OH) vitamin D, 1,25(OH)$_2$ vitamin D$_3$ and parathyroid hormone in different groups (Mean ± SD).

Experimental groups / Parameters	Control (- ve control)	Osteoporotic (+ ve control)	Osteoporotic+ Estrogen	Osteoporotic+ Mushroom	Osteoporotic+ Soybean	Osteoporotic+ (Mushroom& Soybean)
25(OH)vitamin D (pg/ mL)	24570.9±2669.9	10930.5±823.3	12642.0±1556.0	55958.2±2375.8	10406.0±1332.9	34029.6±2975.0
P-Value	-- -- --	.000[a]*** -- --	.000[a] *** .1720[b] --	.000[a] *** .000[b] *** --	.000[a] *** .6509[b] --	.000[a] *** .000[b] *** .000[c] ***
1,25(OH)$_2$ vitamin D$_3$ (pg/ mL)	14152.0±2812.4	6302.3±1360.3	7301.7±2459.8	15892.8±1978.3	12587.5±2560.1	15689.0±2920.7
P-Value	-- -- --	.000[a] *** -- --	.000[a] *** .4926[b] --	.1943[a] .000[b] *** --	. 000[a]*** .8329[b] --	.2506[a] .000[b] *** .0001[c] ***
Parathyroid hormone (pg/ mL)	181.087±147.39	957.139±214.02	574.401±230.75	285.721±256.58	474.305±306.43	308.417±179.77
P-Value	-- -- --	.000[a] *** -- --	.0082[a] ** .0022[b] ** --	.4655[a] .000[b] ***	.0716[a] * .0002[b] *** --	.7363[a] .000[b] *** .0059[c]**

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 4, Issue 2, pp: (113-127), Month: April - June 2016, Available at: www.researchpublish.com

[a:] Comparison between control and osteoporotic groups; [b:] Comparison between osteoporotic and osteoporotic treated groups; [c:] Comparison between osteoporotic treated with estrogen and osteoporotic treated with mushroom & soybean.

([*]P< 0.05, [**]P < 0.01 and [***]P< 0.001).

TABLE.6: Levels of osteocalcin and activity of alkaline phosphatase in different groups (Mean ± SD)

Experimental groups / Parameters	Control (- ve control)	Osteoporotic (+ ve control)	Osteoporotic+ Estrogen	Osteoporotic+ Mushroom	Osteoporotic+ Soybean	Osteoporotic+ (Mushroom& Soybean)
Osteocalcin (pg/ mL)	1789.37±429.99	510.84 ± 65.20	602.19±118.31	1073.47±345.95	494.87±106.55	989.56±363.92
P-Value	-- -- --	.000[a***] -- --	.000 [a ***] .5555 [b] --	.000 [a ***] .0008 [b ***] --	.000 [a ***] .9132 [b] --	.000 [a ***] .0035[b**] .0201 [c *]
Alkaline phosphatase (U/ mL)	111.57±46.63	66.2 ±19.01	142.71±65.99	159.38±75.63	137.9±44.86	99.28±14.31
P-Value	-- -- --	.0.0467 [a *] -- --	.2680 [a] .0038 [b **] --	.0749 [a] .0005 [b ***] --	.3092 [a] .0034 [b **] --	.6600 [a] .2845 [b] .1146 [c]

[a:] Comparison between control and osteoporotic groups; [b:] Comparison between osteoporotic and osteoporotic treated groups; [c:] Comparison between osteoporotic treated with estrogen and osteoporotic treated with mushroom & soybean.

([*]P< 0.05, [**]P < 0.01 and [***]P< 0.001).

Histopathological Examination of Femoral Bone:

In the present study histological examination of negative control revealed that both cortical and trabecular bone of female rat femur showed normal structure described in literature. cortical bone showed regular Haversian system that consists of central Haversian canal, Volkmann canals and osteocyte lamellae within their tiny lacunae .The matrix of bone was homogeneously stained acidophilic (pink). The outer periosteal and the inner endosteal layers showed smooth appearance as in figure(1 A-B). Boney trabecular of epiphysis are of normal thickness separated by marrow spaces as in figure (1C).

Dexamethasone sodium phosphate administration in this study was found to induce histological changes in both cortical and trabecular bone of female rat femur much similar to those described for osteoporosis in animal models. There was widening of Haversian canals. Disorganization of concentric lamellae. Bone matrix showed unstained rarified regions . There is roughness and increase fibrous tissue in both the inner and outer surfaces as in figure(2 A-B). Tubercular bone showed marked thinning of individual trabeculae, and widening of marrow spaces as in figure (2C).Estrogen as a replacement therapy given to rats previously treated with dexamethoxone sodium phosphate showed protection against induced osteoporosis in both cortical and trabecular bone. However , Haversian system still showed disorganized pattern (black arrows) and bony matrix of cortical bone showed poor ossified regions(stars). The inner surface showed irregular outlines with aggregation of bone marrow cells as in figure (3 A). Trabecular bone showed normal thickness but also showed bluish regions indicating lack of complete ossification as in figure (3 B).

Administration of mushroom to animals receiving dexamethasone sodium phosphate result in protection against osteoporosis induced histological changes seen in non-treated groups . Both cortical and trabecular bones showed normal structure which is nearly similar to those seen in negative control animals as in figure (4 A). There was no osteoprotic changes in Haversian system or thickness of bony trabecular as in figure (4B).Soybean in the present study was found to prevent osteoporotic changes induced by dexamethasone sodium phosphate. The outer surface is regular and smooth. Haversian canals looked of normal size. Bone matrix is homogenously stained pink(acidophlic). The cortical bone of femur showed signs of new bone formation as dark lines near the inner surface of the bone as in figure (5A). Trabecular bone showed branching trabeculae with normal thickness and separated by marrow spaces of normal width which are

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 4, Issue 2, pp: (113-127), Month: April - June 2016, Available at: www.researchpublish.com

similar to those seen in control negative group as in figure (5A).The combination of both mushroom and soybean provided protections against osteoporotic changes induced by dexmethazone sodium phosphate. Similar results to what was observed in the groups treated with mushroom and soybean alone were observed . Haversian canals , osteocytes lamellae looked similar to control. In this group features of new bone formation in the form of layers marked by basophilic calcified lines were observed .Bone matrix was homogenous as in figure (6 A).Trabecular bones also showed normal trabecular thickness and marrow spaces as in figure (6 B).

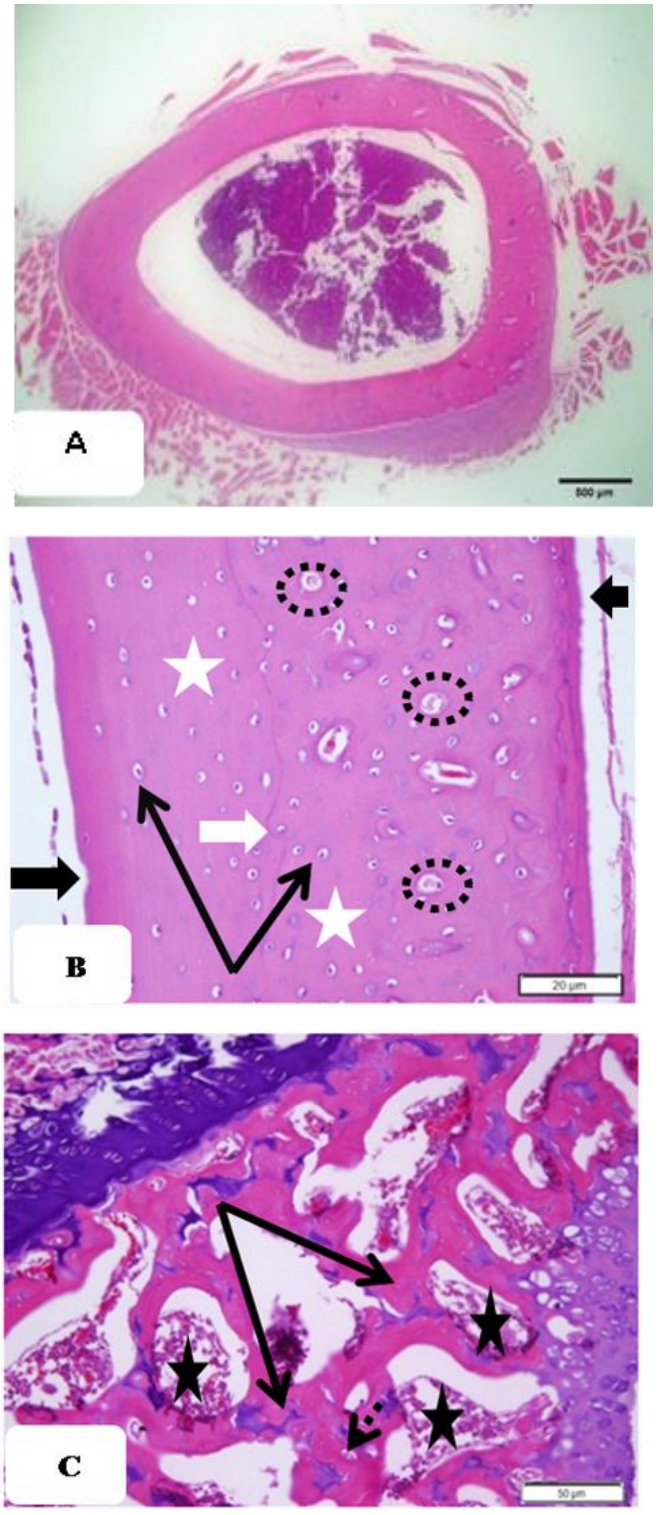

Figure 1. Sections from the middle part of right femur of negative control showing : A. Low power of a cross section of femur . **B**. Magnified power to show the normal regular Haversian canals (Rounded dotted circles),Volkman

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 4, Issue 2, pp: (113-127), Month: April - June 2016, Available at: www.researchpublish.com

canals (white arrow) . Osteocyte nuclei could be seen as dark basophilic spots within the tiny bony lacunae around Haversian canals (thin black arrows). Bone matrix is homogenously stained pink or acidophilic (white stars). Notice the smooth outer and inner surfaces of bone (thick black arrows). **C**. Boney trabeculae with normal thickness (thin black arrow). Notice marrow spaces with normal width (black stars).

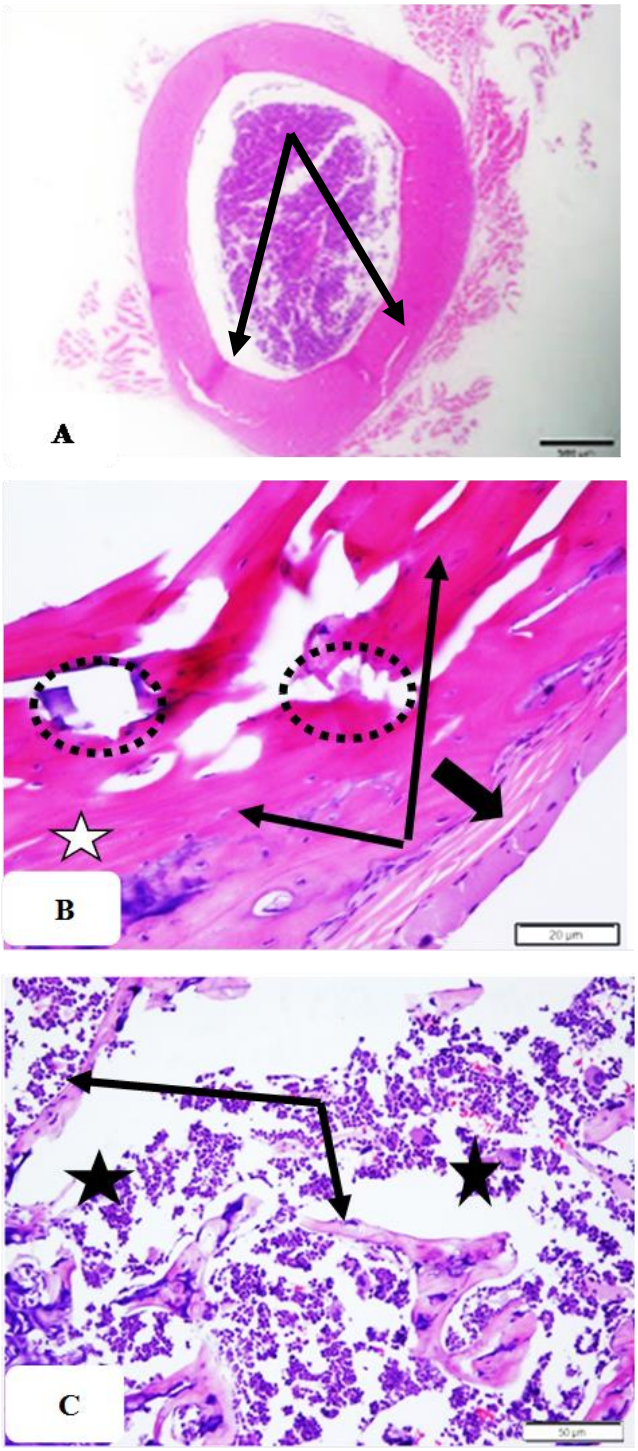

Figure.2. Sections from middle shaft of rat femur of osteoporotic group untreated showing: A. low power with fissures or cracks in cortical bones(thin black arrows). **B**. Magnified power to show marked cracking and fissuring of cortical bones with disorganization of both Haversian canals (dotted circles) and concentric osteocyte lamellae (thin black arrows) . Bone matrix (white star) showed regions of weak staining (rare factions) . There is irregularity and

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 4, Issue 2, pp: (113-127), Month: April - June 2016, Available at: www.researchpublish.com

fibrosis of the inner bone limning(thick black arrow) . **C**. Marked thinning of bone trabecular (thin black arrows) and widening of marrow spaces(black stars).

Figure.3.sections in right femur of estroporotic rat treated with estrogen showing : A. Cortical bone with narrow Haversian canals(dotted circles) . Disorganized less regular bone lamellae(thin black arrows).Bone matrix still showed large poorly stained regions(white stars) . The inner surface is irregular and showed adherence of cells (thick black arrows). **B**. Trabecular bone are of normal thickness(thin black arrows) with narrow marrow spaces (black stars).The matrix of trabecular showed rarefied regions (white stars).

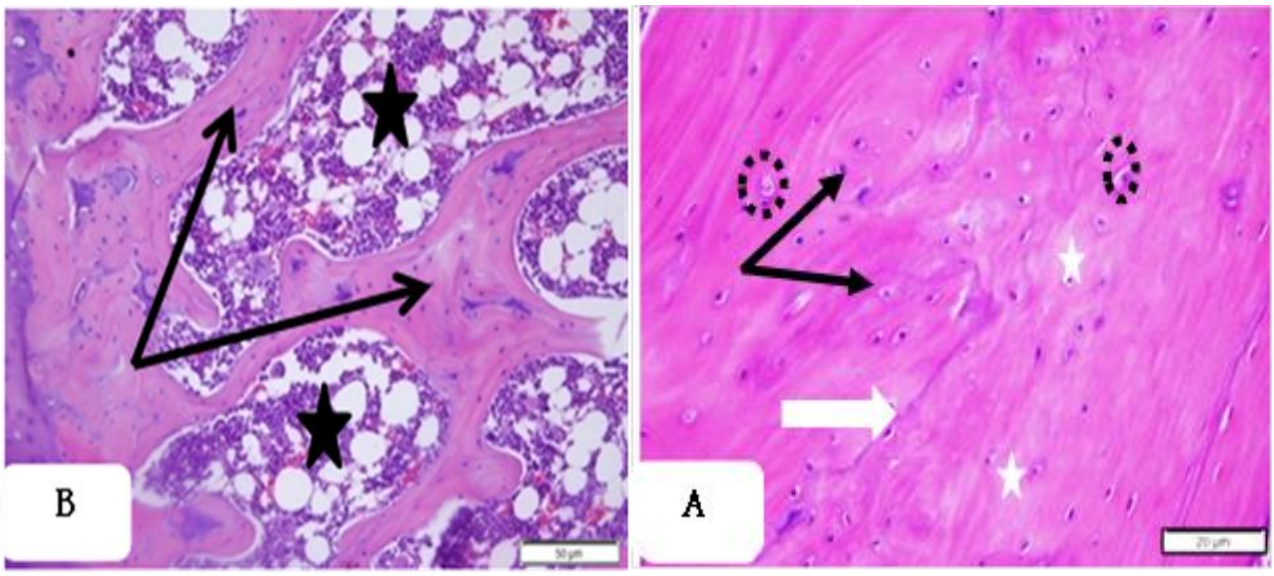

Figure.4.sections from right femure of osteoporotic rats treated with mushroom showing : A. Cortical bone with normal Haversin canals width(dotted circles). Osteocyte lamellae are of normal appearance (thin black arrows). Bone matrix still showed unstained rarfied regions(white stars) . Blue lines marked new bone formation (white arrow) . **B**. Trabcecular bone showed nearly normal thickness(black arrows). Bone marrow spaces return to normal width (black stars).

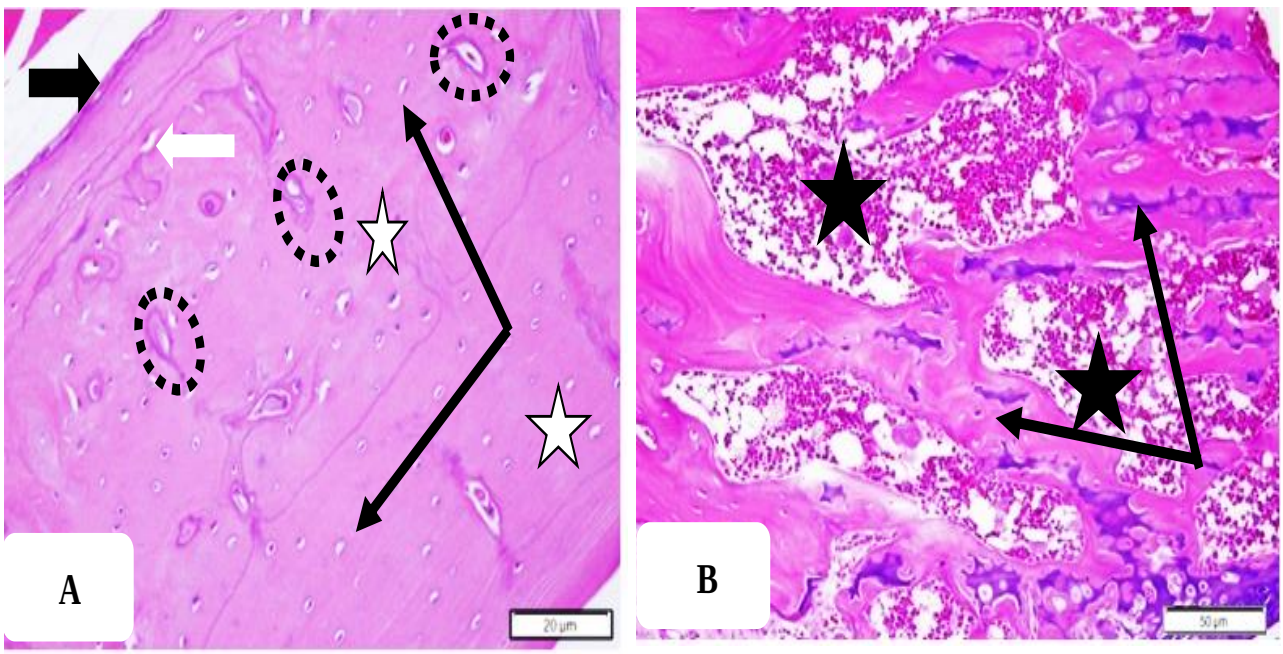

Figure.5.sections from right femure of osteoporotic rats treated with soybean showing: A. Cortical bone with normal size Haversian canals. and surrounded by bluish calcium deposition indicating new bone formation (dotted circles). Osteocyte lacunae showed regular arrangement (thin black arrows). The matrix is homogenously pink (

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 4, Issue 2, pp: (113-127), Month: April - June 2016, Available at: www.researchpublish.com

acidophilic) indicating good ossification (star).The outer surface is smooth (thick black arrow). Signs of new bone formation (Blue regions and lines due to deposition of calcium was seen) are observed (white arrows). **B**. Trabecular bone with normal thickness trabeculae(black arrows) and marrow spaces(black stars).

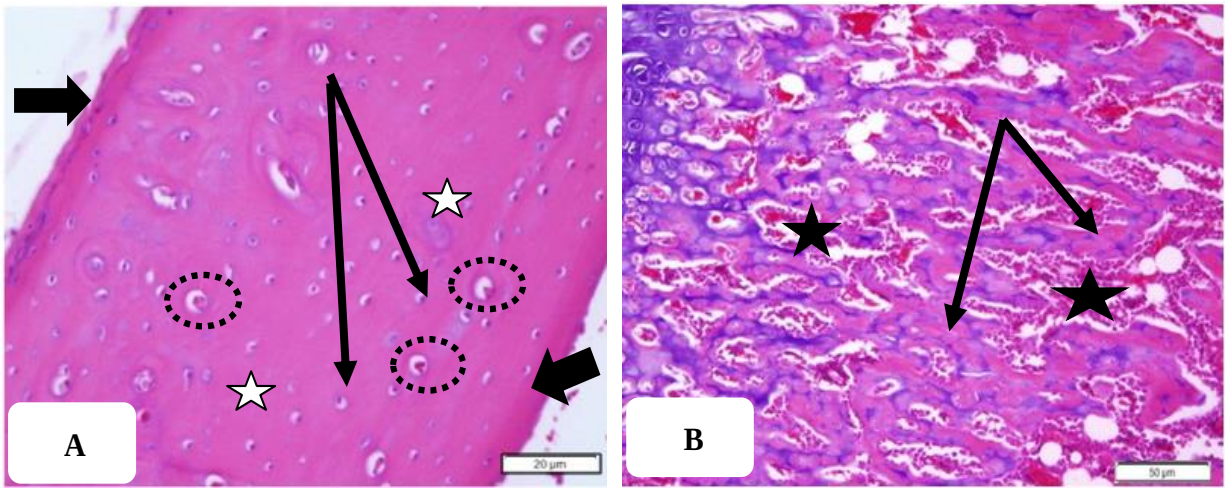

Fig 6. sections from rat femur of osteoporotic rat treated with muahroom and soybean showing : A. Cortical bone with narrow Haversian canals (dotted circles) osteocyte lamellae are of normal appearance (thin black arrows). Bone matrix was homogenous (white stars). The outer surface is smooth (thick black arrow). **B**. Trabecular bone with normal thickness trabeculae(black arrows) and marrow spaces (H&E stain)

4. DISCUSSION

Osteoporosis is one of the most common skeletal disease in humans. It is characterized by low bone mass and microarchitectural deterioration of the bone tissue, leading to decreased bone strength and increased risk of low-energy fractures, or so-called fragility fractures [23] .Osteoporosis is a major complication in patient who requires chronic GC treatment [24]. The GC therapy induces osteoporosis through the decrease in Ca intestinal absorption, the increase in renal Ca excretion and the stimulated osteoclast activity as well as suppression of bone formation [25]Moreover, GCs have been shown to decrease the number of osteoblasts by apoptosis [26.]

The present study was undertaken to evaluate the impact of soybean and mushroom on calcification in rats treated with GCs to induce experimental osteoporosis. This study was carred out on six groups of rats: control (negative control), osteoporotic untreated (positive control), osteoporotic treated with estrogen, osteoporotic treated with mushroom, osteoporotic treated with soybean and osteoporotic treated with mushroom and soybean. In current study body weight and femur bone weight of rats treated with GCs showed a significant decrease compared with negative control. The obtained results were in agreement with who reported that osteoporotic rats showed a significant decrease in body weight and femur bone weight compared with control group. Moreover, It was reported that rats treated with methylprednisolone showed a significant decrease in femur weight and length compared with negative control. Methylprednisolone administration significantly decreased the body weigh compared with negative control. The decrease in body weight and femur bone weight may be due to increased lipolytic effect of GCs [27-29] and decreased absorption of Ca. Treatment with mushroom or soybean or combined improvement the body and bone weight. This is may be due to antagonistic effect of these nutrients and enhancement of Ca absorption.

In the present experiment, we observed that osteoporotic rats showed a significant decrease ($P<0.05$) in serum Ca and P in osteoprotic rats compared with negative control. The obtained results were in a greement with results who reported significant decrease in serum Ca and P in GC treated group compared with control group. In addition, It was found that rats injected with methylprednisolone showed significantly decreased serum P concentration compared with control group. reported that methylprednisolone administration significantly decreased serum Ca level compared with vehicle control group. The decreased Ca may be due to side effect of GC therapy. In addition, GC enhances urinary excretion of Ca and P; and reduces its intestinal absorption. Also, we found that no significant changes in serum Mg in osteoproteic rats compared with negative control. These results were in agreement with result that reported the methylprednisolone had no effect on Mg absorption in adolescent rats [30]. Treatment of osteoporotic rats with mushroom, soybean or their combined

showed elevate the levels of serum Ca more than positive control (P<0.001) while mushroom alone showed elevate the levels of serum P more than positive control (P<0.01). The effect of mushroom is better than soybean and combined in Ca and P improvement compared with estrogen. This is may be due to their action to antagonize steroid signal or their ability to increase absorption of macrominerals from small intestine because mushroom have higher content vitamin D present in irradiated mushroom and isoflavones in soybean increases Ca and p absorbtion. These results are in line with the results, treatment with soy total extract and genistein restored the decreased serum Ca concentrations induced by ovariectomised rats to normal levels[30].

In the current study, osteoporotic rats showed a significant reduction in femur bone Ca and P levels compared with negative control (P<0.001)(P<0.01) respectively. This results are in accordance with the findings of Soltan (32) who reported that, osteoporotic rats showed a significant reduction in Ca and P femur bone levels compared with control group . The decreased in Ca may be due to the effect of GC in urinary excretion of Ca and P; and reduces its gastrointestinal absorption, thus its decreased bone uptake of Ca and P for calcification. Also, we found that no significant changes in bone Mg level in osteoproteic rats compared with negative control groups.

Treatment of osteoporotic rats with mushroom, soybean or their combined showed a significant elevation in bone Ca and P more than positive control. The action of mushroom and comnined effect is better than estrogen in elevation of P level. Their action may be due to enhancement of absorption from small intestine and increased rate of calcification to increase bone mass density because higher bioavailability of vitamin D_2 in mushroom and isoflavones in soybean. These results are in line with the results obtained, that treatment with soy total extract and genistein restored the increased urinary Ca and P concentrations induced by ovariectomied rats to normal levels and increased femur bone Ca content [30].

It was found that, a significant reduction in serum OC level and the activity of ALP in osteoprotic rats compared with control (P<0.001) and (P<0.05) respectively. It was revealed that rats treated with GC showed significant decrease serum the activity of ALP compared with vehicle group, this is may be due to inhibitory effect or suppression its rate of synthesis. Treatment of osteoporotic rats with mushroom or soybean showed significant elevation in serum ALP compared with positive control (p < 0.001) , (p < 0. 01) respectively. This is may be due to antagonized action of these nutrients to inhibit the signals mediated by steroids because isoflavone present in soybean promotes osteoblast proliferation and differentiation which could be help in increased ALP activity[34].In addition, vitamin D_2 present in irradiated mushroom increases osteoblast differentiation and ALP activity [35]. On the other hand the effect of mushroom is better than esterogen in elevation of ALP, this is may be due to higher bioavailability of vitamin D_2 in irradiated mushroom . It was reported that rats treated with GCs showed a significant decrease serum OC level compared with control. OC is another specific marker of bone formation that is a bone-specific protein released into the blood by osteoblasts during new matrix formation and is considered a sensitive ,specific marker of osteoblastic activity and bone formation[34]. Treatment of osteoporotic rats with mushroom and combined showed significant elevation in serum OC compared with positive control (p < 0.001) and (p < 0.001) respectively . Also, the effect of mushroom or combined is better than esterogen (p<0.05) in elevation of OC. This is may be due to higher vitamin D content in irradiated mushroom which increases osteoblast differentiation and synthesis of OC. Moreover, isoflavone present in soybean promotes osteoblast proliferation and differentiation.

In the present study, osteoporotic rats showed a significant reduction in serum 25(OH) vitamin D and 1,25$(OH)_2$ vitamin D_3 compared with control (P<0.001) respectively. It was stated that treatment with prednisolone resulted in a significant decrease in serum 1,25$(OH)_2$ vitamin D_3 level as compared to its corresponding value in the control. The decrease in 1,25$(OH)_2$ vitamin D3 may be because GCs have an inhibitory effect on renal cell 1-α-hydroxylase which catalyzes the conversion of 25(OH) vitamin D_3 to 1,25$(OH)_2$ vitamin D_3 leading to the reduction of serum level of 1,25$(OH)_2$ D_3 (Gyetko *et al.* 1993) or may be due to decreased rate of vitamin D absorption. Treatment of osteoporotic rats with mushroom, soybean and combined showed a significant elevation in serum 1,25$(OH)_2$vitamin D_3 compared with positive control (p < 0.001). These results may be due to isoflavones in soybean increases activity of 1-α-hydroxylase enzyme. The effect of combined is better than esterogen (P<0.001) in elevation of 1,25$(OH)_2$ vitamin D ,this is may be due to their synergestic action of both than individual. Results indicated that GCs therapy is associated with severe 25(OH) vitamin D deficiency, this is may be due to decreased of bioavailabity of cholesterol for vitamin D synthesis . Treatment of osteoporotic rats with mushroom and combined showed a significant elevation in serum 25(OH) vitamin D compared with positive control (p < 0.001) and estrogen (p < 0.001). This is may be due to higher activiation of 25-hydroxylase in liver induced by mushroom.

Moreover, the study revealed that, there were a significant elevation in serum PTH in osteoprotic rats compared with control (P<0.001). This finding was in agreement with who stated that administration with prednisolone caused significant increase in serum PTH level as compared to the corresponding value in the control. The increase in PTH may be in response to hypocalcemia induced by GCs. In addition, GCs act directly at parathyroid gland to affect PTH secretary dynamics via their effect in inducing the secretary behavior of parathyroid gland. Treatment of osteoporotic rats with mushroom, soybean and combined showed significant reduction in serum PTH compared with positive control (p < 0.001). The action of mushroom effect is better than soybean or combined effects. On the other hand the effect of mushroom or combined is better than esterogen (P<0.01) in reduction of PTH level . due to feed back inhibithion of hypercalceumia induced by mushroom and soybean. Becouse mushroom have higher content Ca. Also, vitamin D_2 present in irradiated mushroom and isoflavones in soybean increases Ca absorption.

In the present histological study of both cortical and trabecular bone of femoral rat female were found to confirm biochemical parameter used as bone marker for both osteoporotic changes and protection. It was concluded that, Supplementation of mushroom alone or combined with soybean exert positive effect on bone mass density. This is a target for improving the osteoporosis with less side effects of hormone replacing therapy. A further study should be carried out to elucidate the mechanism of these nutritional compounds.

ACKNOWLEDGMENT

The authors would like to thanks Prof. Suad Shakir , Anatomy Department Faculty of Medicine for her histopathological examination and King Abdulaziz City for Science and Technology for its financial support under grand number (399-35-AT).

REFERENCES

[1] Mccann, T., Mason, W., Meikle, M. and Mcdonald, F. (1997) A collagen peptide motif activates tyrosine kinase-dependent calcium signalling pathways in human osteoblast-like cells. Matrix biology,16: 273-283.

[2] Rossini, M., Bianchi, G., Di Munno, O., Giannini, S., Minisola, S., Sinigaglia, L. and Adami, S. (2006) Determinants of adherence to osteoporosis treatment in clinical practice. Osteoporosis International.17: 914-921.

[3] Rosalki, S. B., Foo, A. Y., Burlina, A., Prellwitz, W., Stieber, P., Neumeier, D. and Bodenmüller, H. (1993) Multicenter evaluation of Iso-ALP test kit for measurement of bone alkaline phosphatase activity in serum and plasma, Clinical chemistry, 39:648-652.

[4] Romberg R.W., Werness P.G., Riggs B.L. and Mann KG (1986) Inhibition of hydroxyapatite crystal growth by bonespecific and other calcium-binding proteins. Biochemistry 25:1176-1180.

[5] Robey, P. and Boskey , A. (2008) The composition of bone, in Primer on the metabolic bone diseases and disorders of mineral metabolism, Edited by: Rosen,C.J., Washington: American Society for Bone and Mineral Research.

[6] Rizzoli, R., Boonen, S., Brandi, M., Burlet, N., Delmas, P. and Reginster, J.-Y. (2008) The role of calcium and vitamin D in the management of osteoporosis. Bone, 42**:** 246-249.

[7] Rissanen, J. (2013) Markers of Bone Turnover In Preclinical Development of Drugs for Skeletal Diseases. Ph.D.Thesis, University of Turku, Turku.

[8] Rinker, D. and Chalmers ,W. (1998) Specialty mushroom industry in Canada . Mushroom orld,vol.9:7-8.

[9] Ren, Z., Yang, L., Xue, F., Meng, Q., Wang, K., Wu, X. and Fu, Q. (2013) Yeast-Incorporated Gallium Attenuates Glucocorticoid-Induced Bone Loss in Rats by Inhibition of Bone Resorption. Biological trace element research,vol.152: 396-402.

[10] Reginster, J.-Y. and Burlet, N. (2006) Osteoporosis: a still increasing prevalence. Bone, 38:4-9.

[11] Reddy, S. V.(2004) Regulatory mechanisms operative in osteoclasts. Critical Reviews in Eukaryotic Gene Expression, 14:255-270.

[12] Recker, R. R., Kimmel, D. B., Parfitt, M. A., Davies, M. K., Keshawarz, N. and Hinders, S. (1988) Static and tetracycline-based bone histomorphometric data from 34 normal postmenopausal females. Journal of Bone and Mineral Research, 3: 133-144.

[13] Prentice, A. (2004) Diet, nutrition and the prevention of osteoporosis. Public health nutrition, 7: 227-243.

[14] Poulsen, R. C., Moughan, P. J. and Kruger, M. C. (2007) Long-chain polyunsaturated fatty acids and the regulation of bone metabolism. Experimental Biology and Medicine, 232: 1275-1288.

[15] Post, T. M., Cremers, S. C., Kerbusch, T. and Danhof, M. (2010) Bone Physiology, Disease and Treatment. Clinical pharmacokinetics.49**:** 89-118.

[16] Phillips, K. M., Ruggio, D. M., Horst, R. L., Minor, B., Simon, R. R., Feeney, M. J. and Haytowitz, D. B. (2011) Vitamin D and sterol composition of 10 types of mushrooms from retail suppliers in the United States. Journal of agricultural and food chemistry, vol.59: 7841-7853.

[17] Pevsner-Fischer, M., Levin, S. and Zipori, D.(2011) The origins of mesenchymal stromal cell heterogeneity. Stem Cell Reviews and Reports, vol.7: 560-568.

[18] Pentti, K., Honkanen, R., Tuppurainen, M. T., Sandini, L., Kröger, H. and Saarikoski, S. (2006) Hormone replacement therapy and mortality in 52-to 70-year-old women: the Kuopio Osteoporosis Risk Factor and Prevention Study.European journal of endocrinology,vol. 154: 101-107.

[19] Peckett, A. J., Wright, D. C. and Riddell, M. C. (2011) The effects of glucocorticoids on adipose tissue lipid metabolism. Metabolism,vol. 60: 1500-1510.

[20] Parfitt, A. (1994) Osteonal and hemi-osteonal remodeling: The spatial and temporal framework for signal traffic in adult human bone. Journal of cellular biochemistry, vol.55: 273-286.

[21] Palacios, C. (2006) The role of nutrients in bone health, from A to Z. Critical reviews in food science and nutrition,vol. 46: 621-628.

[22] Olfati, J. A., Peyvast, Gh. A. and Mami, Y. (2009) Identification and chemical properties of popular wild edible mushrooms from northern Iran. Journal of Horticulture and Forestry ,vol. 1: 048-051.

[23] Okubo, H., Sasaki, S., Horiguchi, H., Oguma, E., Miyamoto, K., Hosoi, Y. and Kayama, F. (2006). Dietary patterns associated with bone mineral density in premenopausal Japanese farmwomen. The American journal of clinical nutrition, vol.83: 1185-1192.

[24] Ogbuewu, I. P., Uchegbu, M. C., Emenalom, O. O., Okoli, I. C. and Iloeje, M. U. (2010) Overview of the chemistry of soy isoflavones, potential threats and potential therapeutic benefits. Ejeafche,vol. 9: 682-695.

[25] Nuttelman, C.R., Benoit, D.S., Tripodi, M.C. and Anseth, K.S. (2006) The effect of ethylene glycol methacrylate phosphate in PEG hydrogels on mineralization and viability of encapsulated hMSCs. Biomaterials, vol.27:1377-1386.

[26] Nurmi-Lawton, J. A., Baxter-Jones, A. D., Mirwald, R. L., Bishop, J. A., Taylor, P., Cooper, C. and New, S. A. (2004) Evidence of Sustained Skeletal Benefits From Impact-Loading Exercise in Young Females: A 3-Year Longitudinal Study. Journal of bone and mineral research, vol.19:314-322.

[27] Nowicka, W., Machoy, Z., Gutowska, I., Noceń, I., Piotrowska, S. and Chlubek, D. (2006) Contents of Calcium, Magnesium, and Phosphorus in Antlers and Cranial Bones of the European Red Deer (CervusElaphus) from Different Regions in Western Poland,Polish Journal Environ. Stud, 15:297-301.

[28] NLM; National Library of Medicine ~ the National Institutes of Health, October 2007.

[29] Nizamutdinova, I. T., Kim, Y. M., Chung, J. I., Shin, S. C., Jeong, Y.-K., Seo, H. G., Lee, J. H., Chang, K. C. and Kim, H. J.(2009) Anthocyanins from black soybean seed coats preferentially inhibit TNF-α-mediated induction of VCAM-1 over ICAM-1 through the regulation of GATAs and IRF-1. Journal of agricultural and food chemistry, 57: 7324-7330.

[30] NIH; Osteoporosis and Related Bone Diseases . National Resource Center, June 2015.

[31] Nagata, C., Takatsuka, N., Kawakami, N. and Shimizu, H. (2001) Soy product intake and hot flashes in Japanese women: results from a community-based prospective study. American Journal of Epidemiology,vol. 153: 790-793.

[32] Muthyala, R. S., Ju, Y. H., Sheng, S., Williams, L. D., Doerge, D. R., Katzenellenbogen, B. S. and Katzenellenbogen, J. A. (2004) Equol, a natural estrogenic metabolite from soy isoflavones: convenient preparation and resolution of R-and S-equols and their differing binding and biological activity through estrogen receptors alpha and beta. Bioorganic and medicinal chemistry, 12: 1559-1567.

[33] Mundy , G. R., Chen, D. and Oyajobi , B. O . (2003) Bone remodeling, In Primer on the metabolic bone diseases and disorders of mineral metabolism,Edited by:Favus, M. J., Washington: American Society for Bone and Mineral Research.

[34] Mccue, P. and Shetty, K. (2004) Health benefits of soy isoflavonoids and strategies for enhancement: a review. Critical reviews in food science and nutrition, 44: 361-367.