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Anti-osteoporotic influence of biscuits fortified with single and combined mixtures of sesame, peanut, and sprouted sunflower seeds on DEX-induced osteoporosis rats

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Abstract

The current study looked at the effects of fortified biscuits with Sesame seeds (S), Peanut seeds (P) and sprouted whole Sunflower seeds (SF) on Dexamethasone (DEX) -induced osteoporosis in female rats. The results reported that biscuits fortified with S, P and SF at levels 30% (S1, P1 and SF1biscuits) or 20% P+20% S (PS) or 20% P+20%S+ 10% SF (PSSF) carried out development crude protein, ash, crude fiber, energy, and crude fat contents compared with biscuits without fortification. It might be demonstrated significant increased ($p \leq 0.05$) at PSSF sample which recorded the highest values of Ca, P, K, Zn, Mn and Cu (42.78, 65.00, 296.78, 1.90, 0.61and 0.42mg/100g, respectively). Also, the highest value of Fe was observed in S1 sample (1.68mg/100g), the highest Mg value recorded in SF1sample (5.83 mg/100g). Sixty-four non-pregnant female albino rats were divided into two groups, the first main group: (8 rats) fed on basal diet, as a control negative (normal group), the second main group (56 rats) were injected intramuscularly with DEX at dose (7 mg/kg b.wt.) once weekly up to four weeks to cause osteoporosis then divided into 7 groups of eight rats each , as follows: group (2) fed on basal diet, as a positive control (DEX-osteoporosis group), group (3) fed on basal diet + control biscuits , group (4) fed on basal diet + S1biscuits, group (5) fed on basal diet + P1biscuits, group (6) fed on basal diet + SF1biscuits, group (7) fed on basal diet +PS biscuits and group (8) fed on basal diet + PSSF biscuits. When compared to the positive control group, all osteoporosis groups treated with fortified biscuits had significant decreases in liver enzymes, kidney function, but increased bone mineral density, and bone mineral content assay. The best result for SF1 and PSSF groups compared with inducted group (control DEX-osteoporosis group). After two months, x-rays and histopathology of the positive control group revealed bone loss in various parts such as the fibula, tibia, and femur, as well as bone demineralization and osteoporosis. In comparison to the positive control group, these findings revealed that biscuits fortified with 20% Sesame seeds, 20%Peanut seeds and 10% sprout whole Sunflower seeds powder at levels used improved the nutritional and biochemical parameters of rats suffering from osteoporosis.

Keywords: Oil seeds; nutritive value; Fortified biscuits; Osteoporosis; DEXA; x-ray and histopathology

1. Introduction

Osteoporosis (OP) is a major public health concern, with a high economic burden in developed and emerging societies. OP is not only a major cause of fractures, but it also ranks high among diseases causing disability, dependence and bedridden. These may cause life-threatening complications in elderly people **[1,2]**. The WHO **[3]** defines osteoporosis as 'a disease characterized by low bone mass and micro-architectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk in osteoporosis the bone mineral density (BMD) is reduced, bone micro architecture is disrupted. It is a bone condition defined by low bone mass, increased fragility, decreased bone quality and an increased fracture risk **[3, 4].** Currently, the use of medicinal plants has emerged as one of the most common and preferred modalities of complementary and alternative medicine. Evidence provided that certain vegetables and fruits are essential for maintaining bone mass and preventing osteoporosis **[5].** Plants, seaweeds, microalgae, and food by-products are the most important sources of functional compounds such as: dietary fiber, phenolic compounds, flavonoids, oils, plant sterols, proteins, prebiotics, probiotics, anthocyanins, carotenoids, and many others **[6,7, 8, 9, 10, 11].** Sesame seeds (*Sesamum indicum*) annual plant herb or shrub family Pedaliaceae is one of the most important oil seed crops worldwide, especially developing countries, where it has been used in many food products **[12, 13].** Sesame seeds are a powerhouse of organic minerals, especially calcium and zinc, and are an alkaline food that supports bone and general health **[14, 15].**

Sesame lignans are clinically important nutraceuticals that exhibit several pharmacological properties **[16,17].** Among sesame lignans: sesamin and sesamolin are the primary ones that have gained the attentiveness of pharmacologists and clinicians due to their health-promoting properties against lifestyle-related diseases **[16,18,19]**. Sesamin and sesamolin owned various pharmacological proprieties such as antioxidative, anti-cancerogenic, anti-inflammatory, anti-proliferative, anti-hypertensive, and anti-melanogenesis **[20].** Peanut (*Arachis hypogaea*), family: *Fabaceae* of bean/legume, commonly known as groundnut, is very important food crop of tropical and subtropical areas **[21],** it is particularly important in Asia, where its production is

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about 64% of the world, it is the fourth important oilseed crop of the world in production after soybean, cottonseed, and rapeseed **[22].** Peanuts are rich in protein, a good source of Co-enzyme Q10 and contain all the 20 amino acids with highest amount of arginine, oil, and fibers, and rich in calories **[22,23].** It remains an essential requirement. The nutritionists may indicate a recommended quantity and quality of fat **[24].** sprouts are known to be richer in terms of phytochemical substances, proteins, vitamins, minerals, polyphenol, and resveratrol **[25].** Resveratrol compound has many pharmacologic effects such as anti-apoptosis, neuroprotection, protection of heart and blood vessel system, antitumor, immunological regulation, and anti-inflammatory effects **[26].**

Sunflower seed (*Heliantus annus*) family *Asteraceae*, sunflower seeds contain around 20% protein, high levels of potassium (710 mg/ 100 g) and magnesium (390 mg/100 g) and are especially rich in polyunsaturated fatty acids in comparison with other oilseeds **[27,28]**. Sunflower is the world's fourth most important source of edible oil, after palm oil, soybean, and rapeseed/canola **[29].** Sunflower oil is a widely consumed product with a high nutritional value and significant health benefits. It contains vitamin A, D, and enough vitamin E. Besides anti-oxides, fats in sunflower seeds are also heart-healthy fats that can increase high density lipoprotein cholesterol and provide cardiovascular protection **[30].** Scaffolds with sunflower seed extract significant enhancement cell growth bone, enzymatic resistance and collagen content **[31].** peptide–calcium complexes from sunflower seeds and peanuts made enhancing bone mineral density **[32].** Sunflowers contain phenolic compounds in kernel and hull which improved nutritional value **[33].** Sunflower sprouts have higher antioxidant activity than sunflower seeds, mainly due to the increase of total phenolic, melatonin, and total isoflavone contents during sprouting **[34].** sprouts are known to be richer in terms of phytochemical substances, proteins, vitamins, minerals, polyphenol, and resveratrol **[35].**

Modifications of basic recipe and incorporation of new ingredients such as fat and fibers, replacers, cereals other than wheat, *etc*. have led to biscuit formulations with improved functionality and nutritive value **[36, 37, 38].** Biscuits are one of the most widely consumed snacks in the world and are fast becoming a popular snack due to their good eating quality and long shelf life. Most bakery products including biscuits are made from refined flour **[39].** So, the aim of this research was to investigate anti-osteoporotic influence of biscuits fortified with single and combined mixtures of sesame, peanut, and sprouted sunflower seeds on DEX-induced osteoporosis rats.

2. MATERIALS AND METHODS

2.1. MATERIALS

The experiment was carried out Food Technology Research Institute, Agriculture Research Center, Giza, Egypt with ethical approval ARC-APRI-58-23. Wheat flours was obtained from Al Dohha Company, Egypt. While, sesame seeds (Shandawel 3 varity), peanut seeds (Giza 6 verity) and sunflower seeds (Sakha 53 verity) were obtained from the Field Crops Research Institute, Agriculture Research Center, Giza, Egypt. Fresh egg, corn oil, baking powder, Vanillin, and powder sugar were obtained from the local market. While Commercial kits were obtained from Bio diagnostic Co. Dokky, Egypt. Dex globe (Dexamethasone) was obtained from Memphis Company for Pharmaceutical and Chemical industries, Cairo, Egypt.

2.2. Experimental procedures:

2.2.1. Preparations of the raw materials:

2.2.1.1. Sesame seeds roasting and powder preparation:

Whole sesame seeds prepared according to the method described by **Elleuch *et al*. [40]**. Only seeds that were not damaged were sieved for removing stone and dust. Sieving, humidification, washing with water, spinning was conducted. Whole sesame seeds were arranged in single layer in aluminium foil dishes and then placed in electric forced air oven (Model R-5550, Sharp, and Osaka, Japan) at 120 ˚C for one hour. After roasting, the seeds were allowed to cool to ambient temperature, ground in an electric grinder by using a mixer (MIEN TA super blender) (Model BL-721) then the flour was sieved to pass through a 280 µm sieve. The resultant sieved powder was kept in an airtight container and stored at-18 °C until using.

2.2.1.2. Peanut seeds roasting and powder preparation:

Kernel of peanut seeds prepared according to the method described by **Aljuhaimi and Özcan [41]**. About 1Kg of up damaged seeds kernels, were distributed uniformly as a thin layer on the trays and roasted in an oven at 120°C for 15 min (Model R-5550, Sharp, Osaka, Japan). The heated samples were left to cool at room temperature, and then hulls were removed and seeds were ground into powder using a grinder before analysis.

2.2.1.3. Sunflower seeds sprouting roasting and powder preparation:

Sunflower seeds germinated according to the method described by **Aishwarya and Anisha [42]** with some modification. Whole seeds (up to insects and diseases), then sunflower seeds were washed under running water to remove any foreign particles and dust, then soaking for 3-4 hours, then draining (in colander) to removed water, and left for germination in the air for 48 hr, then put germinated seeds into drying in oven (Model R-5550, Sharp, Osaka, Japan) at 115°C for 6-8 hours, then grinding , sieved a 280 µm and finally were storage at airtight containers (polyethene bags) and stored at -18 °C until using.

2.2.2. Preparation of biscuits:

The biscuits were prepared in the lab of Pastries and Bread Research Dept., Food Technology Research Institute. Agriculture Research Center. Ingredients used to make biscuits were given in Table (1) Biscuits were made according to the method described by **Wade [43]** with some modifications as follow : sugar –corn oil was creamed and were added to egg-vanilla mixture and well beaten at low speed for 15 min., dry ingredients (wheat flour or its blends and baking powder) were stirred together and added to the mixture gradually followed by beaten continuously until the blend became smooth, and the resulted dough was left to rest for 15 min. The dough was rolled in a cookie sheet using a guide roll. The dough was cut in circles (5 cm diameter and 0.3 cm thick). Transferred to greased plate, then, the baking process was carried out in an electrically heater oven at170°C for 12-15 min. After baking, biscuits were allowed to cool at room temperature for 1hr.

Table (1): The formula used for preparing sweet biscuits.

Ingredients	Amount (g)
Wheat flour (72%)	100
Egg (Fresh)	24
Sugar (Powder)	30
Corn oil	22
Baking powder	2.5
Vanilla essence	1

2.2.2.1. Blends of different types of biscuits:

Pretest experiment has been carried out to determine the best mix ratio of suggested raw materials which were chosen for this study as shown in the following Table (2) and Fig (1).

Table (2). The blends used for preparing sweet biscuits.

Treatments.	Blends composition	Con.
Control	100% Wheat flour	Con.
Single mixes	70% Wheat flour +30% sesame seeds powder (S)	S1
	70% Wheat flour +30% Peanut seeds powder (P)	P1
	70% Wheat flour +30% sprouted sunflower seeds powder (SF)	SF1
combined Mixes	60% Wheat flour +20% peanut powder (P) +20% sesame seeds powder (S)	PS
Multi mixes	50% wheat flour +20% peanut seeds powder (P) + 20% sesame seeds powder (S) +10% Sprout sunflower seeds powder (SF).	PSSF

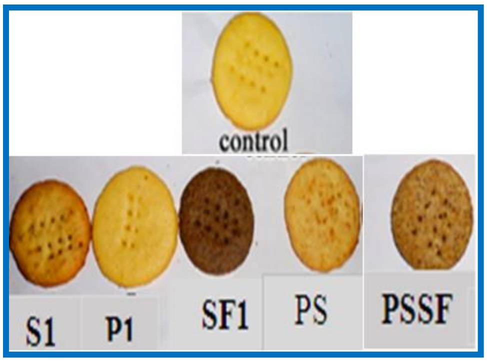


Fig (1): The best mix ratio of suggested raw materials to make biscuits.

2.3. Analytical methods

2.3.1. Chemical Composition

Moisture, crude protein, ash, crude fat, crude fiber contents were determined according to the method described in **AOAC [44].** Available carbohydrates content of the sample was calculated by the difference as mentioned by **Fraser and Holmes, [45].**

Available carbohydrates (% on dry basis) =100 - (%Ash+% crude Fat+% crude Protein+ % crude Fiber).

The approximate energy of biscuits was calculated according to the **FAO/WHO [46]** as follows:

Total energy (K. Cal/100g) = 4(%Available carbohydrates +% crude protein) +9 (%crude fat).

2.3.2. Minerals determination

Calcium (Ca), magnesium (Mg), Potassium (K), Iron (Fe), Zinc (Zn), Phosphor (p), Manganese (Mn), Copper (Cu), and Sodium (Na) were determined according to the method described by **AOAC [44].** The Perkin Elmer (Model 3300, USA) Atomic Absorption Spectrophotometer was used to determine this mineral, in the laboratories of Agricultural Research Center, Giza, Egypt.

2.4. Biological experiment

Sixty-four non-pregnant female albino rats of Sprague Dawley weighing 150 ± 10 g were obtained from Agricultural Research Center, Giza, Egypt. The animal groups were kept in an atmosphere of filtered, pathogen-free air, water, and a temperature of 20-25^{o}C for 8 weeks, with a 12-hour light/dark cycle and a light cycle (8-20 h) and a relative humidity of 50%. For one week, all rats were fed a basal diet. The basal diet was designed to contain 14% casein, 10% sucrose, 4% corn oil, 5% fiber (cellulose), 3.5 percent mineral mixture, 1% vitamin mixture, 0.25 percent choline chloride, 0.3 percent D-L methionine, and 61.95 percent corn starch by **Reeves *et al*. [47].** All the experimental procedures were carried out Institutional Animal Care and Use Committee Protocol-Agricultural Research Center Egypt (IACUC) protocol number ARC-APRI 5823, then starting the experiment to female rat's acclimatization. All the experimental procedures were carried out in accordance with international guidelines for the care and use of laboratory animals. The experiment was conducted at the Agricultural Research Center, Giza, Egypt.

2.4.1. Experimental design:

After one week of adaptation, the rats were divided into two groups, **the first main group:**(8rats) fed on basal diet, as a control normal group **the second main group** (56 rats) were injected intramuscularly with dexamethason (DEX) at dose (7 mg/kg b.wt.) once weekly up to four weeks to cause osteoporosis, according to **Thakur *et al*. [48]** then divided in to 7 groups of eight rats for each , as follows: **group (2)** fed on basal diet, as a DEX-osteoporosis group, **group (3)** DEX-induced osteoporosis+ 100% wheat flour biscuit , **Group (4)** DEX-induced osteoporosis+ S1 biscuits (70% WF + 30% S), **group (5)** DEX-induced osteoporosis +P1 biscuits (70% WF+ 30% P), **group (6)** DEX-induced osteoporosis +SF1 biscuits (70% WF+ 30% SF) , **group (7)** DEX-induced osteoporosis +PS biscuits (60%WF+20%P+20%S) and **group (8)** DEX-induced osteoporosis +PSSF biscuits (50% WF+20% P+20% S+10%SF).

2.4.2. Radiological examination:

Take ventrodorsally view 40 kv, lateral view 38kv on femur and tibia. At the end of the experiment, animals from each group were slaughter, and the blood was collected in a clean dry centrifuge tube, left at room temperature until the clot was formed, completely retracted, and then centrifuged to separate serum by centrifugation at 3000 rpm, for 15 minutes at room temperature, followed by storage in a plastic vial(well stoppered) until analysis, as the method described by **Gribnau *et al*. [49]**.The experiment was conducted at Food Technology Res. Inst., Agricultural Research Center, Giza, Egypt.

2.5. Biochemical Analysis

2.5.1. Biological Determination

Biological evaluation of the effect of different tested diets was carried out by determination of body weight gain (BWG %) according to **Chapman *et al.* [50]**, and organs weight / body weight (%).

BWG (%) = [(Final weight- Initial weight)/ (Initial weight)] X 100.

Organ weight /body weight (%) = (Organ weight /Final weight) X 100.

Notce : Organ weight (femer bone and kideny weight)

2.5.2. Biomarker Analysis:

Blood samples were withdrawn from orbital plexus venous by using fine capillary glass tubes, placed in centrifuge tubes without anticoagulant and allowed to clot. After the serum prepared by centrifugation (3000 rpm for 15 min), serum samples were analyzed by Bio diagnostic kits:

Serum uric acid was determined according to **Barham and Trinder [51]** using spectrophotometer (model DU 4700) adjusted at 510 nm. Serum urea was determined according to the method described by **Fawcett and Soctt [52]** using spectrophotometer (model DU 4700) adjusted at 550 nm Serum creatinine was determined according to **Tietz [53]** using spectrophotometer (model DU 4700) adjusted at 510 nm. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined calorimetrically using spectrophotometer (model DU 4700) at 505 nm according to the method of **Reitman and Frankel [54]**. Alkaline phosphatase (ALP) activity was determined calorimetrically using spectrophotometer (model DU 4700) at 510 nm according to the method of **Belfield and Goldberg [55].**

2.5.3. Bone mineral density and bone mineral content assay

The right femur of each animal was dissected and carefully cleaned for measuring bone mineral density (BMD) and bone mineral content (BMC) by dual energy x-ray absorptiometry (DEXA) using XR 46, version 3.9.6/2.3.1 instrument equipped with dedicated software for small animal measurements in bone mineral density unit, Medical Service Unit, National research Center, Doki, Egypt., to measure the bone mineral content (BMC, g) and bone area (BA, cm2). The BMD was calculated as BMC/BA by **Bagi *et al*. [56].**

2.5.4. Histopathology Investigation :

Autopsy samples were taken from the bone and joint of rats in different groups and fixed in 10% formal saline for twenty-four hours then decalcified by formic acid. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl, and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degrees in hot air oven for twenty-four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slide microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin & eosin stain for examination through the light electric microscope according to the method of **Banchroft *et al*. [57].**

2.5.5. Scanning X-ray:

Radiographic views were undertaken for each rat including lateral, ventrodorsally by using Fischer x-ray apparatus (40 Kilovolt, 100 mA and 100cm FFD) by **Farrow [58].** Finaly, all eight groups analyzed for all previously tests

2.6. Statistical Analysis:

The statistical analysis was carried out by using SPSS, PC statistical software (version 25.0, SPSS Inc., Chicago. USA). The results were expressed as mean ± SD. Data was analyzed by one way analysis of variance (ANOVA). The Differences between means were tested for significance using Least Significant Difference (LSD) test at $P \leq 0.05$ **Snedecor and Cochran [59].**

3. RESULTS AND DISCUSSION

3.1. Proximate chemical composition of biscuits.

To ensure the consumer's health, it is essential to have a thorough understanding of the chemical composition of food **[60]**. The chemical alterations of chosen sweet biscuits as a result of the addition of various raw materials is presented in Table (3). The results demonstrated significant ($p \leq 0.05$) increases in crude protein, crude fat, and crude fiber The crude protein content ranged from 13.08% in the PSSF sample to 9.74% in the SF1 sample, compared to 8.80% in the control sample. In addition, the crude fat content varied from 34.06% in the PSSF sample to 22.64% in the SF1 sample, compared to the control sample18.32%.

On the other hand, it was determined the ash content to get information relating to minerals in the present investigation, there was significant (*p≤0.05*) difference in the ash content of fortified samples, with values ranging from 1.94 (P1 Sample) to 2.47 (S1 sample) percent compared to 1.53 percent for the control. In comparison to the control sample, this contained 0.26%. Crude fiber, the various samples' crude fiber contents ranged from 3.27% for the S1 sample to 5.01% for the PSSF sample. In terms of available carbohydrates, the results ranged from 45.39% in the PSSF sample to 61.91% in the SF1 sample, compared to 71.00% in the control. The total energy levels ranged from 490.26Kcal/100g in the SF1 sample to 540.40 Kcal/100g in the PSSF sample, compared to 485.06 Kcal/100g in the control. Additionally the highest dry matter recorded in SF1 sample 98.92% , the lowest in PSSF sample96.09% compared with control 96.70% ; variation in values is owing to the various nutritional values of the raw materials employed; lipid, protein, and glucose not only give energy, but also supply important nutrients for regular physiological activities such as fat storage and muscle development **[61]**.These results are in agreement with **Obeta *et al*., [62]** who reported that 20% benniseed flour substitution of wheat led to improved macronutrient. Due to the high nutrient contents of peanuts, they have been used to combat osteoporosis in most developing countries. These results are in agreement with the results given by **Mohammady Assous *et al.* [63].** While, our results are comparable to those of **Suleman *et al*. [38]; Al-Alwani *et al*. [39]** who discovered that cookies made from fatted or defatted peanut flour enhanced macronutrients than cookies without fortified. While, our results agreed with **Kamali *et al*. [64]** who found that wheat flour substituted with sunflower seeds meal powder at level 15% and 20% and rose flower waste extract at levels 1 and 2% led to high nutrition values biscuits compared with control. **Al-Kuraieef [65]** found that wheat flour substitution of up to 10% of sunflower powder could improve macro nutrient value of biscuits.

Table (3): Major chemical constituent of wheat flour, sesame seeds powder, peanut seeds powder, sprout whole seeds sunflower powder and fortified biscuits g/100g (on dry matter).

	Moisture	Dry matter*	Crude Protein	Crude fat	Ash	Crude fiber	Available carbohydrates	Total energy (Kcal/100g)
Raw material Wheat flour (F) sesame (S), peanut (P) and sunflower (SF)								
WF	11.03[a] ±0.35	88.97[d] ±0.17	12.60[d] ±0.23	2.34[d] ±0.07	0.43[c] ±0.02	0.43[c] ±0.02	84.15[a] ±0.31	407.26[d] ±0.15
S	3.78[c] ±0.22	96.22[b] ±0.22	18.63[b] ±0.21	51.56[b] ±0.42	4.65[a] ±0.01	23.41[a] ±0.10	1.75[d] ±0.13	94.56[c] ±0.21
P	2.88[d] ±0.07	97.12[a] ±0.07	27.19[a] ±0.09	54.67a ±0.03	2.47c ±0.01	10.93[b] ±0.09	4.76[c] ±0.09	619.81[a] ±0.15
SF	5.45[b] ±0.21	94.55[c] ±0.21	14.11[c] ±0.40	36.89c ±0.11	3.04b ±0.04	23.60[a] ±0.27	22.35[b] ±0.27	477.89[c] ±1.47
Fortified biscuits								
Control	3.30 [b] ±0.05	96.70 [c] ±0.05	8.80[d] ±0.35	18.34[e] ±0.54	1.53[d] ±0.07	0.26[d] ±0.05	71.00[a] ±0.06	485.06[d] ±3.07
S1	3.47[b] ±0.09	96.53 [c] ±0.09	11.24[b] ±0.16	27.62[c] ±0.30	2.48[a] ±0.08	3.27[bc] ±0.92	55.46[c] ±0.39	515.30[c] ±4.82
P1	2.93[c] ±0.01	97.08[b] ±0.01	12.56[a] ±0.50	27.88[c] ±0.02	1.94[c] ±0.19	1.98[c] ±0.25	55.65[c] ±0.57	523.70[bc] ±0.17
SF1	1.08[d] ± 0.07	98.92[a] ±0.07	9.74[c] ±0.78	22.64[d] ±1.87	2.16[bc] ±0.25	3.55[ab] ±0.56	61.91[b] ±2.34	490.26[d] ± 10.44
PS	2.9[8c] ±0.19	97.01[b] ±0.19	12.71[a] ±0.14	31.00[b] ±0.23	2.24[ab] ±0.10	3.21 [bc] ±1.44	50.85[d] ±1.70	533.11[ab] ±4.34
PSSF	3.91[a] ±0.08	96.09[d] ±0.08	13.08 [a] ±0.20	34.06[a] ±1.16	2.47 [a] ±0.16	5.01[a] ±1.13	45.39[e] ±0.07	540.40[a] ±10.94

Data represents as mean ±SD. Each value within the same row followed by the same letter is not considered significantly different; at $P \leq 0.05$.

*Dry matter=100- Moisture

Control = 100%WF; **S1** =70%WF +30% S; **P1** =70%W F +30% P; **SF1=**70%WF +30% SF; **PS** = 60% WF + 20% P + 20% S; **PSSF** =50% WF +20% P + 20% S+10% SF.

3.2. Minerals content.

The outcomes in the Table (4) demonstrated increased significant (*p≤0.05*) for PSSF sample (50% WF +20% p+ 20% S+ 10% SF) which recorded the highest values of Ca, P, Mg, Mn, K, Zn and Cu (42.78, 65.00, 4.74, 0.61, 296.78, 1.90 and 0.42 mg/100g), respectively. Also, the highest value of Fe was observed in S1 (70% WF +30%S) sample (1.68 mg/100g), the highest Mg value showed in SF1 (70%WF+30%SF) sample, but all samples had not significant differences (*P>0.05*) in Na value in comparison with control. The control sample had the lowest value for Ca, P, Mg, Mn, Fe, K, Na, Zn and Cu (24.32, 47.70, 3.09, 0.42, 0.98, 137.08, 50.58, 1.41and 0.07 mg/100g, respectively). Our results demonstrated increased potassium level could be an added advantage to the product as potassium is important to preserved osmotic pressure and acid-base balance. Sodium is an important mineral that assists to regulate thirst and control body water. Zinc is essential for reproduction, regulate glucose level, and stress-controlled **[66].** These results agreed with **Obeta *et al*. [62]; Agrahar-Murugkar [67]; Ogundele and Doosuun [68]** who studied that wheat flour substituted with sesame, cumin, and moringa) or defatted sesame seeds were a good source of bio-accessible minerals like Ca and Zn and may assisting to enhancement healthy human. Substituted wheat flour at levels 5%, 10%, 15% and 20% defatting, cooking, roasting, germination, and fermentation sesame seeds to produce cookies recorded increased micronutrients values **[69]**. Meanwhile, these results nearly with using sunflower, peanut, and sesame seeds together in bar production led to increasing micronutrients **[70,10,32]** who reported that amino acids chain combining with calcium from sunflower seeds and peanuts carried out enhancing bone health. It must be intake of minerals in suitable amounts is importance for life in children, adolescent and adult and balanced elements between Ca and Mg **[27,36,71]** led to enhanced bone health. Our studies are agreement with **Ray and Singh [71]** who found that wheat flour supplemented with sunflower seeds powder enhanced calcium, magnesium and zinc values in cookies compared to cookies without treated.

Table (4): Minerals of Wheat flour, Sesame seeds powder, peanut seeds powder, sprout whole seeds Sunflower powder and fortified Biscuits (mg/100g).

Sample	**Constituents(mg/100g)**								
	Calcium	Phosphor	Magnesium	Manganese	Iron	Potassium	Sodium	Zinc	Cupper
	Raw material: Wheat flour (WF), sesame (S), peanut (P) and sunflower (SF)								
WF	33.65^{d}	19.53^{c}	3.84^{c}	0.65^{b}	1.08^{c}	192.35^{c}	40.76^{c}	2.13^{c}	0.13^{d}
	±0.65	±1.08	±0.399	±0.0s	±0.08	±4.19	±2.65	±0.13	±0.03
S	114.01^{a}	54.72^{b}	30.25^{a}	1.75^{a}	2.37^{a}	441.33^{c}	90.69^{a}	4.50^{ab}	1.50^{b}
	±6.02	±0.42	±1.3 3	±0.45	±0.56	±9.51	±9.66	±0.50	±0.02
P	95.53^{b}	48.31^{b}	19.94^{b}	1.56^{a}	1.46^{bc}	531.18^{b}	89.19^{a}	3.50^{b}	1.71^{a}
	±0.95	±5.95	±0.66	±0.24	±0.14	±2.55	±3.27	±0.50	±0.04
SF	69.60^{c}	65.16^{a}	30.73^{a}	1.40^{a}	1.67^{b}	554.45^{a}	65.53^{b}	5.00^{a}	0.42^{c}
	±6.18	±5.62	±1.30	±0.11	±0.05	±5.95	±12.53	±1.00	±0.03
	Fortified biscuits								
Con.	24.32^{d}	47.70^{c}	3.09^{b}	0.42^{c}	0.98^{c}	137.08^{d}	50.58^{ab}	1.41^{e}	0.07^{e}
	±2.47	±6.54	±0.75	±0.06	±0.02	±7.81	±2.31	±0.02	±0.01
S1	40.02^{ab}	51.36^{bc}	4.87^{ab}	0.68^{a}	1.68^{a}	193.88^{c}	60.65^{a}	1.75^{c}	0.28^{c}
	±3. 51	±3.78	±0.67	±0.10	±0.34	±10.08	±3.60	±0.04	±0.02
P1	35.54^{bc}	46.08^{c}	5.66^{a}	0.51^{bc}	1.14^{bc}	195.43^{c}	52.33^{ab}	1.57^{d}	0.33^{b}
	±2.32	±1.25	±1.11	±0.03	±0.08	±7.13	±9.53	±0.03	±0.02
SF1	31.82^{c}	49.98^{bc}	5.83^{a}	0.46^{bc}	1.17^{bc}	197.53^{c}	43.79^{b}	1.86^{ab}	0.12^{d}
	±1.22	±2.32	±2.83	±0.0 3	±0.14	±12.48	±3.79	±0.02	±0.03
PS	41.84^{ab}	61.55^{ab}	4.54^{ab}	0.52^{b}	1.16^{bc}	265.60^{b}	57.21^{a}	1.81^{b}	0.41^{a}
	±0.88	±12.95	±0.14	±0.04	±0.11	±10.86	±6.64	±0.03	±0.02
PSSF	42.78^{a}	65.00^{a}	4.74^{ab}	0.61^{a}	1.29^{b}	296.78^{a}	59.08^{a}	1.90^{a}	0.42^{a}
	±7.99	±5.58	±0.18	±0.05	±0.10	±8.15	±10.02	±0.03	±0.02

*Data represents as mean ±SD. Each value within the same row followed by the same letter is not considered significantly different; at $P \leq 0.05$.

Control = 100% wheat flour; **S1** =70% wheat flour +30% sesame seeds powder; **P1** =70% wheat flour +30% pea nut seeds powder; **PS** = 60% wheat flour + 20% peanut + 20% sesame seeds powder; **PSSF** =50% wheat flour +20% peanut + 20% sesame seeds powder + 10% whole sprout sunflower seeds powder.

Table (5): Mean body weight gain (%) of experimental rats which treated with different ratios of fortified biscuits by Sesame seeds, Peanut seeds and sprout whole seeds Sunflower powder.

Parameters	**Groups**							
	Normal group (-Ve)	**DEX-osteoporosis with**						
		Control osteoporosis group (+ve)	**control biscuits**	**S1**	**P1**	**SF1**	**PS**	**PSSF**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
IBW	180.2^{a}	181$^{a.}$	166.2^{c}	189^{a}	178^{a}	168^{c}	171.2^{b}	172.8^{b}
	±3.7	±4.69	±12.43	±13.22	±10.38	±8.08	±5.44	±17.58
FBW	226.2^{bc}	200.4^{d}	216.8$^{c\backslash}$	211.6^{c}	215^{ab}	214.2^{c}	231^{a}	206.6^{d}
	±15.5	±10.52	±13.88	±27.65	±18.88	±8.7	±12.3	±15.2
%BWG/W	25.6^{b}	10.71^{d}	31.06^{a}	11.76^{d}	20.69^{c}	27.36^{b}	35.14^{a}	20.68^{c}
	±9.61	±5.07	±12.92	±9.9	±12.59	±9.43	±10.59	±16.7

Data were presented as means ± SDM (n=8). a, b, c and d: Means with different letter among treatments in the same row are significantly different ($P \leq 0.05$) **IBW**= Initial body weight; **FBW**= Final body weight; **BWG**= Body Weight gain. **W**: Week.

Control biscuit = 100% wheat flour. **S1**=70% wheat flour +30%sesame seeds powder**.**

P1=70% wheat flour+30%peanut seeds powder.

PS = 60% wheat flour+20% peanut seeds powder + 20% sesame seeds powder.

PSSF=50% wheat flour+20% peanut seeds powder + 20% sesame seeds powder+ 10% sprout whole sunflower seeds powder.

3.3.2. Organs weight / body weight (%)

Osteoporosis described with bone deterioration which led to imbalance between osteoclast and osteoblasts **[11]**. In the femur bone of experimental animals treated with "glucocorticoids" that several studies have revealed marked degradation in femur bone **[7,11].** Table (6) compares the related percentages for organ weight of femur bone and kidney in rats suffering from osteoporosis and feeding on different ratios of fortified biscuits containing sesame seeds, peanut seeds and sprout sunflower seeds as powder. % Femur bone weight/body weight of the DEX-osteoporosis group (+ve) showed a decreased significantly$P \leq 0.05$ than the normal group (-ve) (2.34±0.4 and 3.04±0.26 %, respectively). According to **Li *et al* [7]** " Glucocorticoids" have damaging results on bone structure turnover and reliability. Cortisone affected firstly on osteoblasts, reducing replication and weaking differentiation and maturation, leading to reduced osteoblasts, increased osteoclast which led to weak bone structure, and significant fragility fracture risk **[7,11]**

There are increased significant differences $P \leq 0.05$ in % femur bone weight/body weight among the con. DEX-osteoporosis group (+ve) and the treated groups with different ratios of fortified biscuits containing sesame seeds powder, peanut seeds powder and sprout Sunflower seeds powder. The best result PSSF group (8) recorded for all treated group by (50%WF + 20% P +20%S +10% SF) which increased significant 2.94±0.4 in comparison with Con. DEX-osteoporosis group (+ve) 2.34±0.4(%) and Con. biscuits 2.43± 0.2(%), while decreased significant in comparison with DEX-osteoporosis group 2.34 ± 0.4 (%), followed that {PS group **(7)** (60% WF + 20% S+20% P) and P1 group (5) (70% WF+ 30% P) }(2.94±0.4, 2.84±0.22 and 2.83±0.24, respectively). Looking in the same Table (6) can be observed that % kidney weight/body weight of the (DEX-osteoporosis group) control group (2) had increased significant $P \leq 0.05$ than the (normal group) control group (1) (0.8±0.13and 0.63±0.13, respectively). But decreased significant differences $P<0.05$ in % kidney weight/ body weight among the (DEX-osteoporosis group) control group (2) and the treated groups with different ratios from fortified biscuits containing sesame seeds, peanut seeds and sprout whole Sunflower seeds powders and increased significant differences in treated groups in comparison with normal control group. The best group (6) which fed on SF1 biscuits (70% WF+30%SSF) 0.65 ± 0.06 in comparison with Con. biscuits 0.68 ± 0.12 and Con. (DEX-osteoporosis group) 0.8 ± 0.13, but normal group 0.63±0.13**.** Our results agreed with **El Sayed and El Hawary [79]** who found that reduced kidney weight of rats 0.80% which fed on 5% sunflower seeds to protect from obesity compared to control positive 1.14 % but, differences in control negative 1.26 %. Also, our results agreed with **Li *et al*. [80]** who studied that decreased "kidney index" rats group which fed on70% high fat diet and 30% white sesame seeds for three weeks were 0.577 compared to control (DEX-osteoporosis group) 0.599 and normal control 0.666.

Table (6): Organ weight/body weight (%) of experimental rats which treated with different ratios of fortified biscuits by Sesame seeds, Peanut seeds and sprout whole seeds Sunflower.

Organ weight / body weight (%)	Groups							
	Control			Fortified biscuits				
	Normal group (-ve)	DEX-osteoporosis group (+v)	Control biscuits	S1	P1	SF1	PS	PSSF
	1	2	3	4	5	6	7	9
Femur bone	3.04^{a} ±0.26	2.34^{f} ±0.4	2.43^{e} ±0.2	2.44^{e} ±0.28	2.83^{c} ±0.24	2.54^{d} ±0.21	2.84^{c} ±0.22	2.94^{b} ±0.4
Kidney	0.63^{d} ±0.13	0.8^{a} ±0.13	0.68^{c} ±0.12	0.73^{b} ±0.19	0.71^{b} ±0.19	0.65^{c} ±0.06	0.71^{b} ±.15	0.76^{b} ±0.06

Data were presented as means ± SDM (n=8). a, b, c and d: Means with different letter among treatments in the same row are significantly different (P ≤ 0.05).
Control biscuits = 100% wheat flour. **S1**=70% wheat flour+30% sesame seeds powder**.**
P1=70% wheat flour+30%peanut seeds powder.
PS = 60% wheat flour+20% peanut seeds powder + 20% sesame seeds powder.
PSSF=50% wheat flour+20% peanut seeds powder + 20% sesame seeds powder+10%whole sunflower seeds powder.

3.3.3. Bioassay Results

3.3.3.1. liver enzyme activities

The activity enzymes of alanine amino transferase, aspartate amino transferase " ALT and AST and " levels are indication for hepatotoxicity **[81].** Increased activity of liver enzymes may be due to the deterioration of the integrity function of the liver cells membranes and the infiltration of these enzymes outside the cells **[82].** "Alkaline phosphatase"(ALP) level is a biochemical indicator for osteoid formation and mineralization **[9,11].** Results in Table (7), showed that liver enzymes values for osteoporotic all groups animals which are feeding on standard diet and injected with DEX (control DEX-osteoporosis group) group (2) showed increased significant ($P \leq 0.05$) elevation of serum (AST, ALT and ALP) levels until the end of the experiment (110.63±8.8, 70.10±0.52 and 157.5±5.74, respectively) compared to group (1) control normal (65.57±0.56, 32.88±0.92 and 84.13±0.99, respectively). About one fifth of hospitalized patients with liver damage, high "ALT" level which consumption antibacterial and glucocorticoids as medicine therapy disease **[83,84].** Additionally uncovered that prebirth glucocorticoid over presentation in rodents expands glucocorticoid over presentation in rodents expands "hepatic lipid aggregation" with steatosis. Research proved that elevated levels of alkaline and acid phosphatases are contributing factors in the pathogenesis of several bone diseases **[85].** Increased ALP level can associate to higher bone turnover rate characterized by an increase in bone formation and resorption, but bone resorption is predominating, silicon can enhance the resistance of vascular "endothelial cells "to oxidative stress and well impact bone health **[86].**

Table (7): Liver enzymes activities of experimental rats which treated with different ratios of fortified biscuits by Sesame seeds, Peanut seeds and sprout whole seeds Sunflower.

Parameters	Groups							
	Control normal group (-ve)	Control DEX-osteoporosis group (+ve)	Control biscuits	Fortified biscuits				
				S1	P1	SF1	PS	PSSF
	1	2	3	4	5	6	7	8
AST (U/I)	65.57^{d} ±0.56	110.63^{a} ±8.8	92.43^{b} ±0.11	90.27^{b} ±0.14	82.81^{c} ±0.53	64.86^{d} ±0.23	71.29^{c} ±1.08	65.71^{d} ±0.45
ALT (U/I)	32.88^{d} ±0.91	70.10^{a} ±0.52	44.55^{b} ±0.44	$32.16^{d\backslash}$ ±0.16	39.48^{c} ±.45	32.25^{d} ±0.44	31.0^{d} ±8.18	31.1^{d} ±2.15
ALP (U/I)	84.13^{d} ±0.98	157.5^{a} ±5.74	95.55^{b} ±0.51	83.35^{d} ±0.24	94.52^{b} ±0.30	82.94^{d} ±0.18	88.18^{c} ±0.96	80.5^{d} ±1.73

Data were presented as means ± SDM (n=8). a, b, c and d: Means with different letter among treatments at the same row are significantly different ($P \leq 0.05$). **AST**: Aspartate amino transferase. **ALT**: Alanine amino transfease **ALP**: Alkaline phosphatase.
Control biscuits = 100% wheat flour. **S1**=70% wheat flour+30%sesame seeds powder**.**
P1=70% wheat flour+30%peanut seeds powder.
PS = 60% wheat flour+20% peanut seeds powder + 20% sesame seeds powder.
PSSF=50% wheat flour+20% peanut seeds powder + 20% sesame seeds powder+ 10% sprout whole sunflower seeds powder.

Our data showed that decrease significantly ($P≤0.05$) Liver enzymes such as "ALT, AST and ALP" levels in group (6) (70%WF+30% SF), group (4) (70%WF+30% S), group (7) (60%WF+20%S+20%P), group (5) (70%WF+30%P), group (8) (50%WF+2 0%S+20%P+10%SF) and control biscuits, respectively)when compared to the control (DEX-osteoporosis group) group(2). In the final, it could be observed that the best result for "AST, ALT and ALP" levels were for SF1 group (70%WF+30 %SF) followed, PSSF group (50%WF+20%P+20%S+10%SF) groups (64.86±0.23, 32.25±0.44 and 82.94±0.18) and (65.7±0.45, 31.1±2.15 and 80.5±1.7) compared to inducted group control (DEX-osteoporosis group) 110.63 ±8.8, 70.10±0.52 and 157.5±5.74 respectively.

Consumption biscuits fortified with pumpkin and sunflower seeds, improved significantly ($P≤0.05$) the level of glucose in the serum blood of rats, increased significantly "HDL-C" value and improved liver functions by decreasing "ALT", "AST", in the rats compared to the induced group **[87]. Li *et al*. [80]** studied rats feeding with different doses of black and white sesame seeds and kernel might be reduced the sever lipid on the liver. **Hu *et al.* [88]** studied that sesamol mitigate metabolic terribles joined obesity, and reduced lipid accumulation in skeletal muscle in obese mice. **Atefi *et al*. [89]** found that sesame oil had a safety effect on liver in albino rats tissues and enhanced rates alanine aminotransferase, aspartate aminotransferase, and fatty liver grade. *In vitro* and *in vivo* **Yuan *et al*. [9,10[** found that rats feeding at high dose from both Sunflower and peanut protein combined calcium groups complexes carried out decreased significant "ALP" level in serum compared to calcium carbonate control group and sunflower oil enhanced from liver and bone health. **Li *et al*. [90]** reported that resveratrol in peanut oil promoted the conversion of saturated triglycerides into unsaturated triglycerides, increased the linolenic acid content, and did not facilitate the formation of trans fatty acids.

3.3.3.2. Kidney Function

The present study in Table (8) results of kidney functions for all Osteoporotic animals' group which fed on standard diet (control DEX-osteoporosis group) showed increased significant ($P≤0.05$) of serum urea, uric acid and creatinine levels recorded the end of the experiment (53.18±2.1, 2.70±0.16 and 0.81±0.07), respectively compared to normal control group (25.66±1.3, 1.86±0.03 and0.56±.07), respectively. The kidney has main role in glucocorticoid metabolism and the major source of cortisone production in humans. Reduced Type2"11β-hydroxysteroid dehydrogenase" (11b-HSD2) activity contributes to the pathophysiology of salt and water retention and, possibly, high pressure blood in "renal failure" **[91].** Glucocorticoids (GCs) have important effects on "renal function", including increases in glomerular filtration and the keeping of nitrogen waste, developing" gut dysbiosis" and the excessive production of bacterial metabolites. These metabolites act as toxins **[92].** Our experimental rats which treated with different ratios of fortified biscuits by Sesame seeds, Peanut seeds and sprout whole Sunflower seeds powder showed decreased significant ($P≤0.05$) on serum urea, uric acid and creatinine when compared with the control (DEX-osteoporosis group). In the final, it could be observed that the best result for SF1 group (6) (70%WF+30%SF) and PSSF group (8) (50%WF+20%S+20%P+10%SF) (24.5±0.43, 1.65±0.08 and 0.58±0.05) and (26.56±2.95, 1.58±0.05 and0.56±0.03) respectively in comparison with inducted group 2 (control DEX-osteoporosis group)**.** PSSF and SF1 groups are no significant differences in urea, and creatinine test in comparison with control normal group.

Ghaffar [19] found that female rats fed on 2.5% and 5% sesame seeds carried out reduced creatinine, urea and uric acid amount in comparison with control positive induced osteoporotic**. El-Kholie *et al*. [87]** studied that decreased urea, uric acid and creatinine levels when feeding rats on biscuits supplemented with 5% Sunflower seeds in comparison with control induced diabetic. **Ajobiewe *et al.* [93]** found that sunflower seed extract had protection role as antibacterial activity, therapy diarrhea.

Table (8): Kidney functions (mg/dl) of experimental rats which treated with different ratios of fortified biscuits by Sesame seeds, Peanut seeds and sprout whole seeds Sunflower.

Parameters	Groups							
				Fortified biscuits				
	Con. (normal group) (-ve)	Con. DEX-osteoporosis group (+ve)	Control biscuits	S1	P1	SF1	PS	PSSF
	1	2	3	4	5	6	7	8
Urea(mg/dl)	25.66^{d} ±1.3	53.18^{a} ±2.1	30.63^{b} ±0.80	35.23^{b} ±6.9	33.83^{b} ±1.56	24.5^{d} ±0.43	27.96^{c} ±40.55	26.56^{d} ±20.95
Uric Acid (mg/dl)	1.86^{b} ±0.03	2.70^{a} ±0.16	1.88^{b} ±0.22	1.87^{b} ±0.16	2.17^{a} ±0.18	1.65^{c} ±0.08	1.91^{ab} ±0.54	1.58^{c} ±0.05
Creatinine (mg/dl)	0.56^{c} ±0.07	0.81^{a} ±0.07	0.62^{b} ±0.03	0.60^{b} ±0.09	0.62^{b} ±0.06	0.58^{c} ±0.05	0.58^{c} ±0.04	0.56^{c} ±0.03

Data were presented as means ± SDM (n=8). a, b, c and d: Means with different letter among treatments in the same row are significantly different (P ≤ 0.05).
Control biscuits = 100% wheat flour. **S1**=70% wheat flour+30%sesame seeds powder**.**
P1=70% wheat flour+30% peanut seeds powder.
PS = 60% wheat flour+20% peanut seeds powder + 20% sesame seeds powder.
PSSF=50% wheat flour+20% peanut seeds powder + 20% sesame seeds powder+ 10% sprout whole sunflower seeds powder.

3.4. Bone mineral density and bone mineral content

In Egypt BMD test (T score) gives indication for detection about osteoporotic disease; this parameter gives the quantity value of mineral in bone, which one of indicated on strength bone with low costin comparison with other tests for osteoporosis **[7,9,19].** Table (9) and Fig. (2) the results present in experimental rats treated with different ratios of fortified biscuits containing sesame seeds powder (S1), peanut seeds powder (P1), and whole sunflower seeds powder (SF1), the measurements both “Bone Mineral Density” and “Bone Mineral Content” are recorded in grams per square centimeter (g/cm^2) for BMD and in Grams (g) for BMC. Two parameters were decreased significantly in osteoporotic rats positive group (2) control (DEX-osteoporosis group) fed on “basal diet” (0.12±0.01 and 0.03±0.004, respectively) compared to control normal group (0.45±0.51 and 0.10±0.08, respectively) as shown in Table (9). Research had low amount calcium absorption carried out decreased BMD amount and weaking bone strength and deficiency of calcium long period led to osteoporosis disease **[19].**

The present studies showed that all groups female rats which treated with different ratios of fortified biscuits by sesame seeds, peanut seeds and sprout whole sunflower seeds powder had increased significant in "BMD" and " BMC" in comparison with induced control (DEX-osteoporosis group) group2. In the final, it can be noticed that the best result for SF1 group (70% WF+ 30% SF) and PSSF group (50% WF + 20% S+ 20% P+10% SF) groups (0.48±0.59 and 0.11±0.14) and (0.44±0.31 and 0.10±.0.57) respectively of the "proximal tibia" and "distal femur" in rats, which are rich in trabecular bone. **Tachibana *et al*. [94]** showed that Sesame included sesemol promoted osteoblastic differentiation of rats, and improved rat bone structure. **Zdzieblik *et al.* [95]** found that supplementation with specific bioactive collagen peptides demonstrates to be effective in loss in BMD. **Bao *et al*. [32]; Yuan *et al*. [10]** found that the calcium peptides from both sunflower and peanut seeds can effectively increase bone mass and structure. **Ghaffar [19]** reported that bone minerals density and bone minerals content for rats fed on 5% sesame seeds were 0.127 g/cm^2 and 0.036 g Compared to control induced osteoarthritic 0.072 g/cm^2 and 0.025 g respectively. Magnesium and potassium intake led to increased bone minerals density related which the potential protective role avoids osteoporosis. All of these elements are available in sesame, peanut and sun flower seeds to avoid osteoporotic disease.

Table (9): Femur minerals density (FMD) and bone minerals content (BMC) assay of experimental rats which treated with different ratios of fortified biscuits by Sesame seeds, Peanut seeds and sprout whole seeds Sunflower.

Parameters	Groups							
				Fortified biscuits				
	Con. normal group (-ve)	Con. DEX-osteoporosis group (+ve)	Control biscuits	S1	P1	SF1	PS	PSSF
	1	2	3	4	5	6	7	8
BMD (g/cm^2)	0.45^{a} ±0.51	0.12^{d} ±0.01	0.16^{c} ±0.03	0.19^{b} ±0.08	0.14^{c} ±0.07	0.48^{a} ±0.59	0.20^{b} ±0.09	0.44^{a} ±0.31
BMC (g)	0.10^{a} ±0.08	0.03^{c} ±0.004	0.03^{c} ±0.009	0.03^{c} ±0.02	0.03^{c} ±0.01	0.11^{a} ±0.14	0.07^{b} ±0.05	0.10^{a} ±0.57

Data were presented as means ± SDM (n=8). a, b, c and d: Means with different letters among treatments in the same row are significantly different (P ≤ 0.05). **BMD**: Bone mineral density, **BMC**: Bone mineral concentration.
Control biscuits = 100% wheat flour. **S1**=70% wheat flour+30% sesame seeds powder**.**
P1=70% wheat flour+30% peanut seeds powder.**;** **PS** = 60% wheat flour+20% peanut seeds powder + 20% sesame seeds powder.; **PSSF**=50% wheat flour+20% peanut seeds powder + 20% sesame seeds powder+ 10% sprout whole sunflower seeds powder.

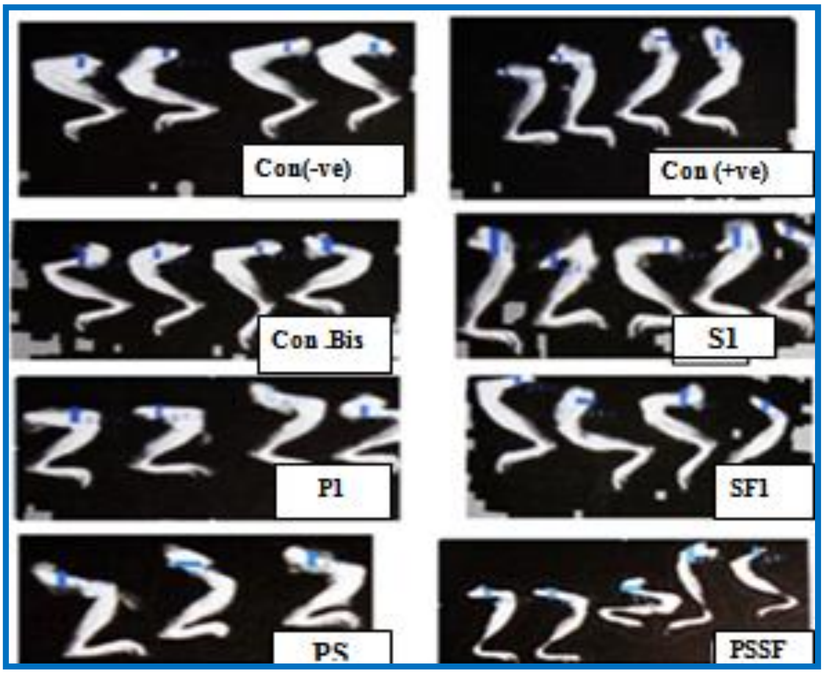


Fig 2. Bone minerals density and bone mineral content assay.

3.5. X-ray and Histopathological examination

Femur bone was examined by X-ray, Radiological examination of bone of rats from normal control (–ve) group (1) revealed no radiographic changes Fig (3) included Fig (1-8) Looking for Fig.1. Meanwhile, bone of rats control (DEX-osteoporosis group) group (2) showed demineralization in distal extremity (Fig. 2). From group control biscuits showed femoral cortical corrugation and thinning (Fig.3). On the other hand, group 4 (70%WF +30%S) described normal radiographic findings (Fig. 4). Furthermore, group 5 (70% WF+30%P) revealed of thinning femoral (Fig.5). Likewise, group (70%WF+30%SF) showed no radiographic changes (Fig.6). Meanwhile, group 6 (60%WF+20%S+20% P) described cortical thinning of femur and tabia (Fig.7). On the other hand, group 8 (50% WF + 20% S+20% P+10% SF) demonstrated normal radiographic finding (Fig.8). These finding revealed the Sesame seeds are a powerhouse for calcium and zinc, and are an alkaline food that supports bone and general health so it fortify the osteoporotic bone in agreement with **Wolf and Beh [14]** especially with 30% concentrate on Peanuts are rich in protein, a good source of Co-enzyme Q10 and contain all the 20 amino acids with highest amount of arginine, oil, and fibers, and rich in calories so it don't fortify the osteoporotic bone in agreement with **Morya *et al*. [22]and De-Oliveira *et al*.[23].** While sunflower seeds contain Vitamin A, D and peptide–calcium complexes enhancing bone mineral density so it fortifies the osteoporotic bone in agreement with **Yuan *et al*. [9,10]; Bao *et al*. [32]; Puraikalan and Scott [30].**

3.6. Histopathological examination Femur bone

Osteoporosis is the most common bone disorder and a major growing health problem in worldwide. Several risk factors are involved in the occurrence and progression of osteoporosis including aging, sedentary lifestyle, and estrogen deficiency due to menopause, ovariectomy, and hormonal therapying **[19,6,83].** Assessment bone health, especially in the femur, is important for decision making in osteoarthritic patients. The most common form of arthritis is known osteoarthritis (OA) which affects the hips **[96]**

Microscopical examination of bone of rats from (control –ve) group revealed no histopathological changes with normal bone cortex (Fig. 4).

Meanwhile, bone of rat's control (DEX-osteoporosis group) group 2 showed thin cortical bone with presence of cracks and fissures, dilated bone marrow cavity and thin bone trabeculae (Fig.4)."Trabecular bone" constitutes a major portion of "distal femur" and it is a highly sensitive to glucocorticoids **[7].**

Administration glucocorticoids (GC) for three weeks revealed bone loss of different parts such as "fibula", "tibia", and "femur" in addition to bone demineralization and thinning of femoral cortex in rats, disappeared of cortex of "tibia" in others and "fibula" and these agree with the observations of femur **[7,11].**

Otherwise, some sections from group control biscuits showed fissure in the bone cortex (Fig.5). On the other coronary, examined sections from group (4) (70%WF+30%S) described normal thick bone cortex (Fig.5). Furthermore, some examined sections from group (5) (70%WF+ 30%P) revealed few cracks in the cortex. Likewise, femur sections of rats from group (6) (70%WF+30%SF) manifested no histopathological alterations. Meanwhile, sections from group (7) (60%WF+20%S+20%P) described few cracks in t0he corte. On the other side, examined femur sections from group (8) (50%WF+20%S+20%P+10%SF) demonstrated apparent histologically normal bone architecture. Researchers have shown that the phenolic compounds in various plant species can modulate the osteoblastic cells functions, including their maturation and proliferative capacity, by increasing the activity of alkaline phosphatase and calcium deposition in the extracellular matrix **[9,10,11].**

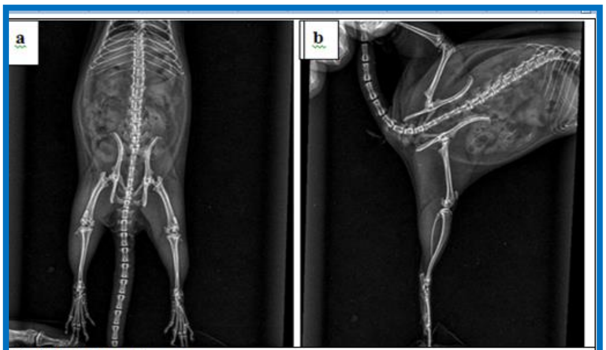


Fig.(1) Control negative group.
a. Ventra dorsal view showing normal radiographic finding.
b. Lateral vie w showing normal radiographic finding.

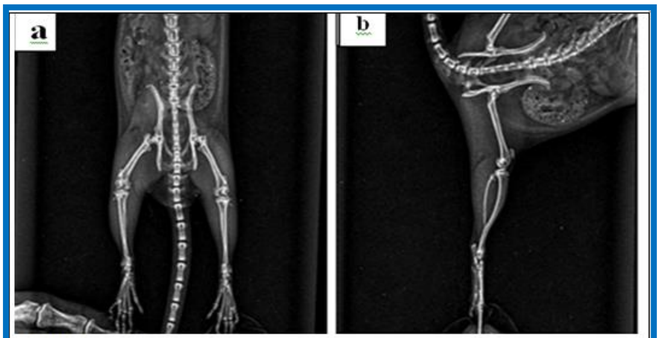


Fig.(2) Control positive group.
a. Ventra dorsal view showing deformity in distal extremity of femur and thin tibia cortex.
b. Lateral view showing demineralization in distal extremity of femur.

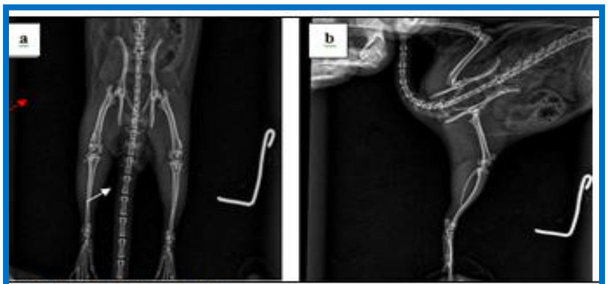


Fig.(3) Biscuits (Control)l group.
a. Ventrodorsal view showing thinning cortex of thin (white arrow and femoral cortical corrugation (red arrow)
b. Lateral view showing cortical corrugation..

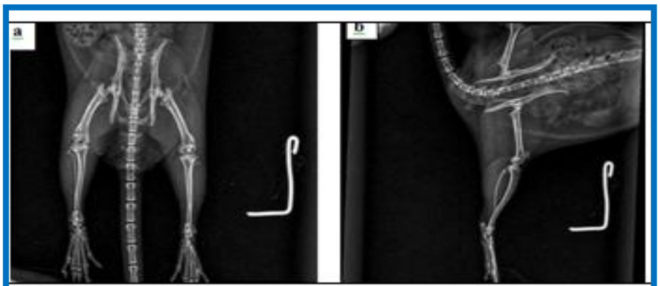


Fig.(4) Biscuits (70%WF+30%S) group.
a. Ventral Dorsal view showing demineralization of femur and thin tibia .
b. Lateral view showing demineralization of femur and tibia.

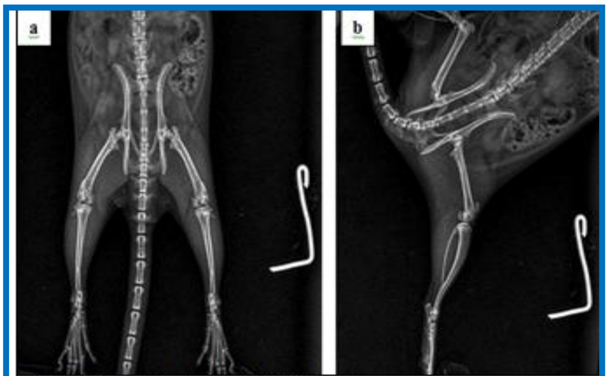


Fig.(5) Biscuits (70%WF+30%P)l group.
a. View showing thinning femoral cortex.
b. Lateral view showing thinning of femoral cortex.

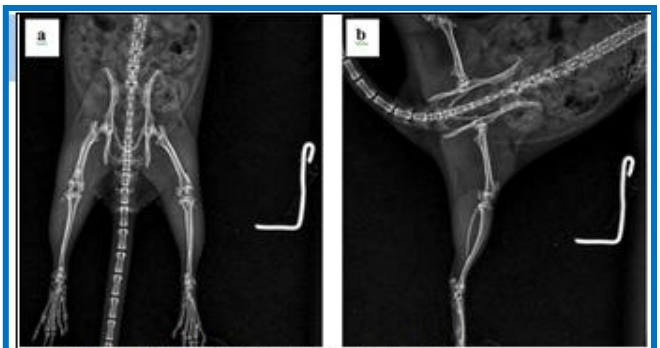


Fig. (6) Biscuits (70% WF+30% Whole sun flower powder) group.
a. Ventra dorsal view showing normal radiographic finding.
b. Lateral vie w showing normal radiographic finding.

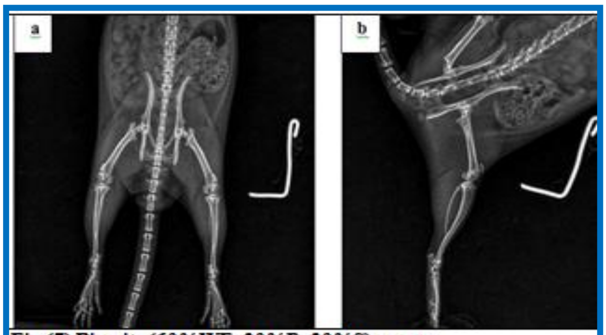


Fig.(7) Biscuits (60%WF+20%P+20%S) group.
a. View showing thinning femoral cortex.
b. Lateral view showing thinning of femoral cortex.

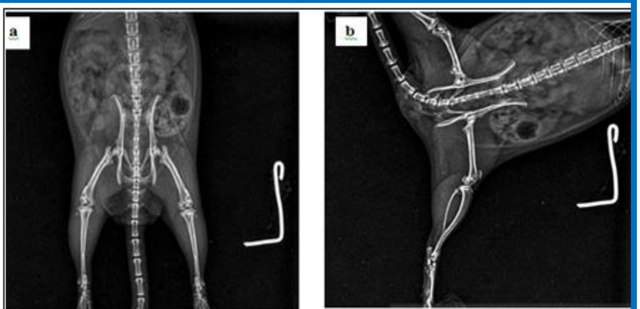
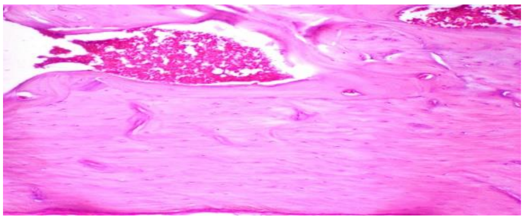
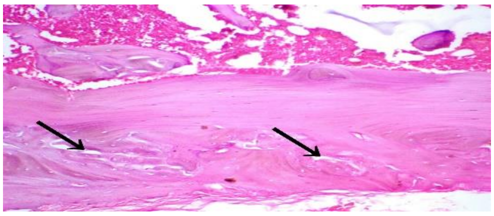


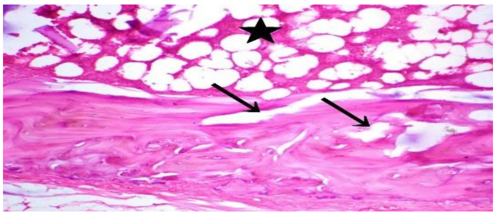
Fig. (8) Biscuits (50% WF20% peanut+20%sesame +10% Whole sun flower powder) group.
a. Ventra dorsal view showing normal radiographic finding.
b. Lateral vie w showing normal radiographic finding.

Fig 3. X-ray exanimation on female rats fed biscuits fortification with sesame and /or peanut and /or sprout whole sun flower seeds powder, from Fig (1) to Fig. (8).



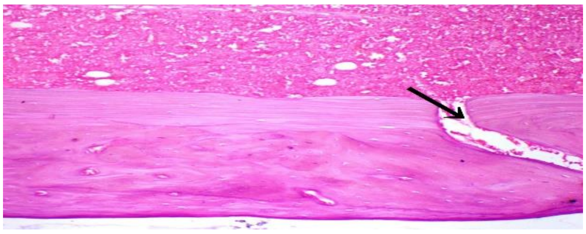
G (1) Control normal (-ve)



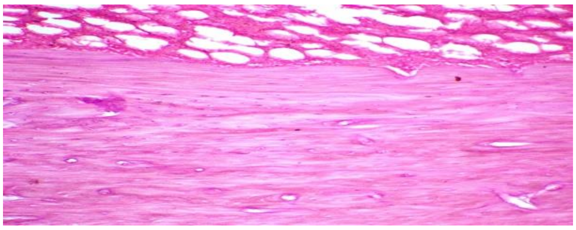


G (2) Control DEX-osteoporosis (+ve)

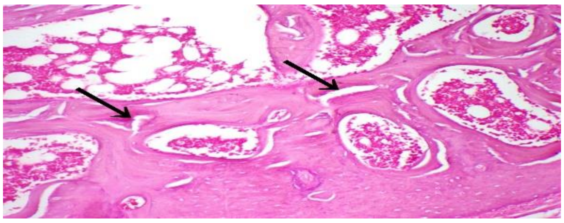
Fig. 4. Photomicrograph of femur sections in control (normal group) G1 and control (DEX-osteoporosis group) G2 groups (H&E), control (–ve) group revealed no histopathological changes with normal bone cortex, bone of rats control (DEX-osteoporosis group) G2 showed thin cortical bone with presence of cracks and fissures, dilated bone marrow cavity and thin bone trabeculae.



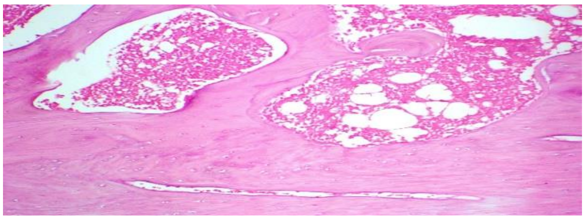
G (3) Control biscuits



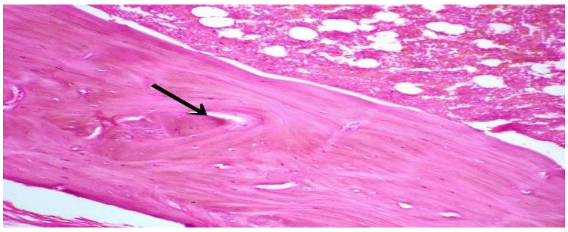
G (4) (70% WF + 30% S) group



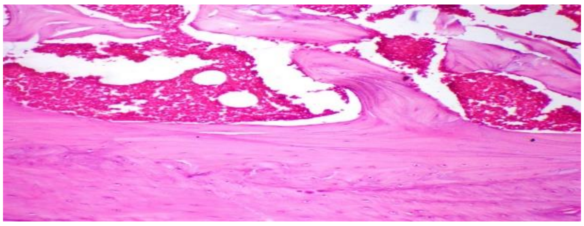
G (5) (70%WF+30% P)



G (6) (70% WF+ 30% SF)



G (7) (60% WF + 20% S+20% P)



G (8) (50% WF + 20% S+20% P+10% SF)

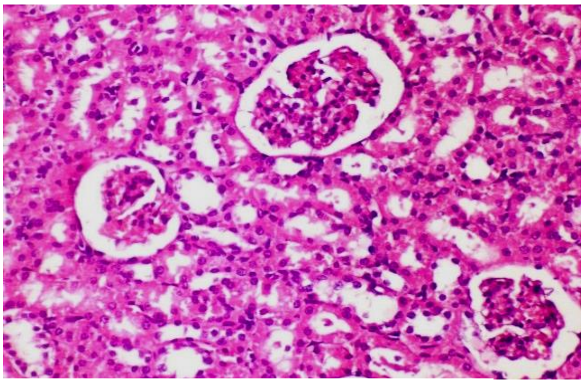
Fig. 5. Photomicrograph of Femur bone in experimental rats which treated with different ratios of fortified biscuits by Sesame seeds, Peanut seeds and sprout whole seeds Sunflower groups (H&E).

Femur bone of animals injected by DEX and fed on "spirulina algae" showed diminished in the thickness and length of the" bone trabeculae" with wide areas of bone marrow spaces in between as recorded in (Fig.5). In contrast, no histopathological alteration in femur bone was observed in the animals affected by DEX and fed on (6% dried SP algae) in (Fig.5) group (8) (50%WF+20%S+20%P+10%SF). Specific marine plants have recently attracted attention for their ability to improve bone metabolism, since they are rich in minerals which led to body health **[6].** Sunflowers contain phenolic compounds in kernel and hull which improved nutritional value **[23]. Hsu *et al*. [97]** studied that the daily supplywith0.25 or 0.5 mL/kg/day for 4 months daily sesame oil carried out increased bone mineral density, and "trabecular areas" in female.

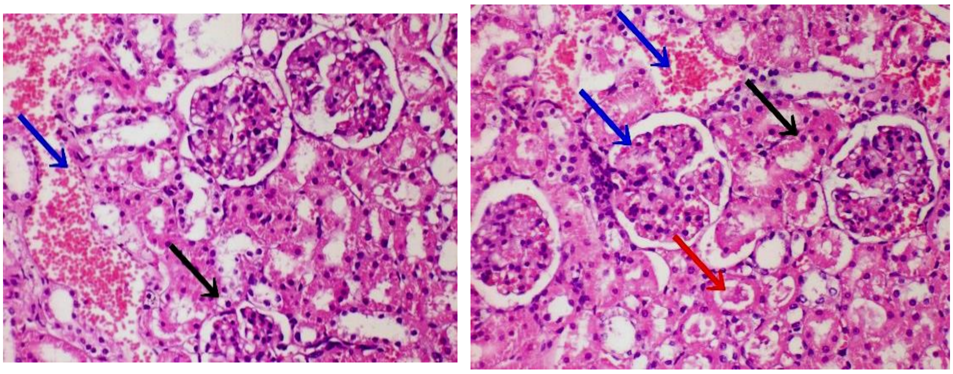
3.7. Histopathological results of kidneys:

Histological examination of kidneys rats from group control (–ve) revealed the normal histological architecture of renal parenchyma (Fig.6) Con (normal group). On contrary, kidneys of rats from group control (DEX-osteoporosis group) exhibited histopathological damage characterized by vacuolar degeneration of epithelial lining renal tubules and congestion of renal blood vessel and glomerular tufts as well as necrobiosis of epithelial lining renal tubules and proteinaceous cast in the lumen of renal tubules (Fig.6). Age-related loss of "renal function" is common in patients with osteoporosis**,** in general, kidney Patients have low bone content resulted osteoporotic, which carried out increased risk of fracture **[98]**.

Meanwhile, kidneys of rats from control biscuits group described slight vacuolar degeneration of epithelial lining some renal tubules and congestion of renal blood vessel (Fig.7). Furthermore, some examined sections from (70% WF +30%S) group (4) showed no histopathological lesions (Fig. 7). Moreover, some sections from (70%WF+30%P) group (5) showed slight vacuolar degeneration of epithelial lining some renal tubules, necrobiosis of epithelial lining some renal tubules and congestion of renal blood vessel in the same figure. On the other hand, renal sections of rats from (70%WF+30%SF) group (6) manifested no histopathological alterations (Fig.7). However, kidneys of rats from (60%WF+ 20% S+20%P) group (7) revealed mild changes as slight vacuolar degeneration of epithelial lining some renal tubules in the same figure. Furthermore, apparent normal renal parenchyma was demonstrated in renal sections of rats from (5**0%** WF+20%S+20%P+10%SF) group (8). Sesame lignans are clinically important nutraceuticals that exhibit several pharmacological properties **[19, 81]** sesame oil had a safety effect on kidney in female albino rats tissues.

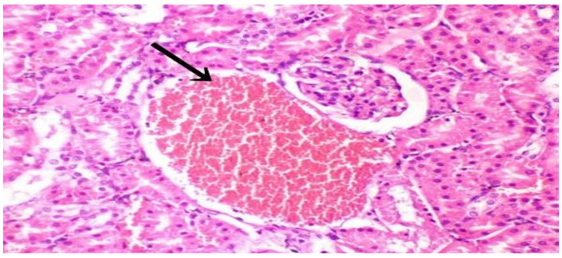
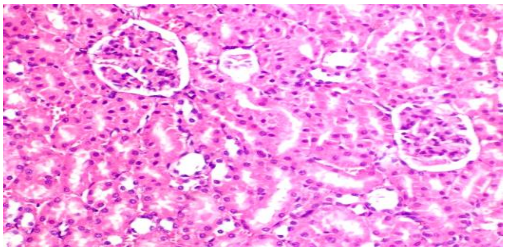


Control normal group (–ve) (group 1)

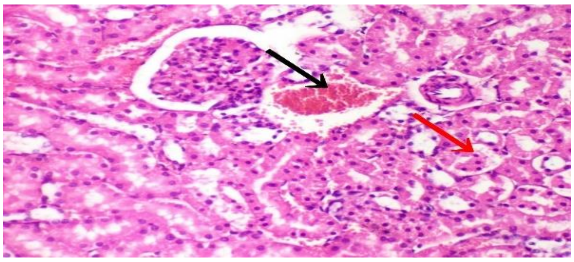
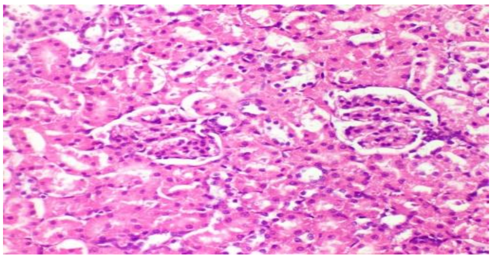


Control DEX-osteoporosis group (+ve) (group 2)

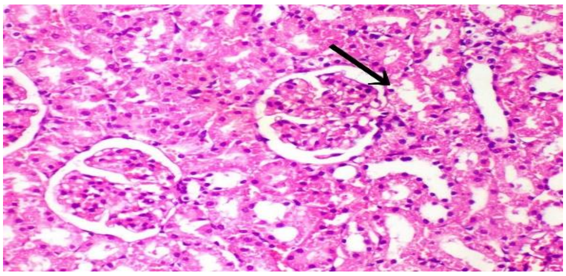
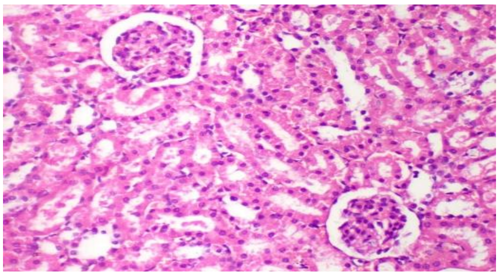
Fig. 6. Photomicrograph of kidney sections in control (normal group) and control (DEX-osteoporosis group) groups (H&E).

Control biscuits group (3) **(70%WF+30%S) group (4)**

(70% WF+ 30% P) group (5) **(70% WF+ 30% SF) group (6)**

(60% WF + 20% S+20% P) group (7) **(50% WF + 20% S+20% P+10% SF) group (8)**

Fig. 7. Photomicrograph of Kidney in experimental rats which treated with different ratios of fortified biscuits by Sesame seeds, Peanut seeds and sprouted Sunflower seeds groups (H&E).

4. Conclusion

Biscuits fortified with 20% peanut, 20% sesame and 10% sprout sun flower recorded the highest macro and micronutrients values, PER and BV and unsaturated fatty acids which prevent from osteoporosis. The best results of biological analysis female rats' osteoporosis, which fed on both biscuits fortified with 30% sprouted sun flower and biscuits fortified 20% peanut, 20% sesame and 10% sprout sun flower. Therefore, it can be recommended to incorporate the studied nutritional sources into bakery products to obtain healthy baked goods with high nutritional and biological values, especially with regard to resistance to osteoporosis due to their high content of required nutrients. Also, it can be used as food aid in institutional nutrition programs for students at different educational levels. It is also easy for mothers to prepare at home to serve to their families as healthy snacks.

5. Conflicts of interest

"There are no conflicts to declare".

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