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Research Article
Innovative Food Supplement of Functional Seeds Mixture Improved Bone Mineral Density in Menopausal Egyptian Women

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Abstract

Background and Objective: Postmenopausal women frequently have osteoporosis due to deficient estrogen secretion. Phytoesteroids are natural alternatives, of plant origin, can play the role of estrogen substitute in menopause. This study was planned as a trial to formulate a novel safe seeds mixture that supplement important functional nutrients to minimize bone mineral deterioration due to menopause in Egyptian women. **Methodology:** Mixed dietary supplement was formulated from seeds mixture, grounded to be given to patients on yoghurt. Methanolic and water extract were prepared and analyzed using HPLC, to stand on the present bioactive compounds. Analysis for total isoflavons, polyphenols and antioxidant power were done. Twenty nine postmenopausal women, proved to have bone fragility, supplemented by vitamin D and calcium for at least 2 months, were enrolled in the study. Patients were given 40 g of the seeds mixture twice daily for 3 months. The patients were examined by DEXA (dual energy X-ray densitometry) before and after supplementation. Serum minerals, oxidative marker (MDA) and liver and kidney functions were performed. Serum human procollagen 1 N terminal peptide (P1NT) and osteocalcin bone formation markers were estimated. All data were expressed as Mean±Standard Deviation, differences between groups were detected using t-test. The $p \leq 0.05$ considered statistically significant. **Results:** Analysis of seed mixture showed high antioxidant capacity, high total isoflavons and polyphenols which are beneficial for bone health. Patient showed significant elevation of T score ($p \leq 0.01$), P1NT, osteocalcin, zinc, magnesium and decreased MDA after supplementation. **Conclusion:** It is concluded that the seed mixture showed valuable effect in improving bone mass density of patients. It has no side effect on organ function. It can be considered as a novel supplement for bone health for post menopausal women having bone fragility.

Key words: Functional seeds mixture, dual energy X-ray absorptiometry, osteoporosis, menopausal women, palm pollen, HPLC

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bone compromise and fragility coincide with aging and decreased estrogen level in menopausal women[1]. Previous studies showed proliferation of osteoblasts (bone formation cells) on expense of osteoclasts (bone resorption cells) in bone tissue culture treated with estrogen[2]. Phytoestrogens are the natural compounds, of plant origin, found to have estrogenic activities in postmenopausal women who have low estradiol levels[3,4]. Flaxseed meal is the richest source of lignins including enterodiol, enterolactone, matairesinol and secoisolariciresinol which were previously reported to exert positive effect on bone of postmenopausal women[5,6].

Free radicals generated in the bone environment enhance osteoclasts formation and bone resorption[7]. Isoflavons such as genistein and daidzein, present in flaxseed, possess antioxidant and anti inflammatory function, with positive effect on aging and osteoporosis[5,7]. Also, vitamin E, K and magnesium, available in flaxseed, are essential for bone health. Omega 3 and 6 in flax seed are present in unique ratio that possesses antiprostaglandin effect decreasing bone loss[8]. Sesame seeds are the richest source of sesamin which has direct stimulatory effect on osteoblasts and the key enzymes necessary for bone formation and mineralization[9,10]. In the same time sesame seeds are excellent source of copper and manganese, calcium, iron, phosphorus, zinc, molybdenum, vitamins E and selenium. The later elements act as natural antioxidants and anti-inflammatory that down regulate osteoclasts activity[11]. Also, procyanidins, present in sesame, play a role in the protection of collagen and elastin in both connective tissues and bone[12]. Sesame seeds are rich in phytosterol which enhance bone formation and immune system[13].

Garbanzo or chickpeas is a good source of calcium for menopausal women who may have low intake of calcium and less intestinal absorption due to decreased estrogen[14]. Chickpeas controls elevation of glycemic index and decrease insulin resistance which stimulate osteoblasts function[15]. It contains antioxidant like vitamin C, E and beta-carotene. Also flavonoids, quercetin, kaempferol, myricetin and phenolic acids as ferulic, chlorogenic, caffeic and vanillic acids are present in sufficient amount[16]. Not only estrogen but also androgens have a role in bone formation and osteoblasts function. Palm Pollen Grain (PPG) stimulate testosterone secretion which repair the compromised bone through its' anabolic effect[17]. Androgens in females are essential pro-hormones for other steroids synthesis. The PPG had been prescribed for fertility promotion in women in ancient Egypt as it has gonadal stimulating potency[18,19].

This study was planned to investigate the possibility to prepare a new food supplement containing phyto-esterol, composed of the mentioned seeds mixture. This mixture was proposed to have synergistic positive effect on bone, to improve the low bone mass density in menopausal women having osteopenia or osteoporosis, taking into consideration to be acceptable in taste and has no hazardous effects on health.

MATERIALS AND METHODS

Formulation of seed mixture: Four compounds, flaxseed (*Linum usitatissimum*), sesame indicum, garbanzo (*Cicer canaries*) and palm pollen grain were purchased fresh from the experimental station of medicinal plants, Ministry of Agriculture, Cairo, Egypt, finely ground and mixed together in the ratio 20:10:10:1 from flaxseeds, sesame, chickpeas and palm pollen grain respectively. The seeds powder was freshly prepared every 2 weeks. Fourty grams of the seed mixture were given mixed with cup of yoghurt twice daily for 3 months, patients were adviced to keep the seed powder in refrigerator.

Organoleptic (sensory) characteristics of supplement were evaluated according to Hoojjat and Zabik[20], where the formula was subjected to sensory analysis by 20 panelists.

Biochemical analysis of the seed mixture

Analysis of total phenolic content: The total phenolic content was determined according to the Folin-Ciocalteu procedure[21]. The total phenolic content was determined by means of a calibration curve prepared with gallic acid and expressed as microgram of Gallic Acid Equivalent (GAE) per gram of sample.

Analysis of total flavonoids content: The total flavonoids content was determined according to Thaipong *et al.*[22]. The total flavonoids content was expressed as microgram of Catechin Equivalent (CE) per gram of sample.

Determination of radical DPPH scavenging activity: Free radical scavenging capacity was determined using the stable 1, 1-diphenyl-2-picryl-hydrazyl (DPPH). Percent inhibition of the DPPH free radical was calculated by means of a calibration curve prepared with trolox and expressed as microgram of Trolox Equivalent (TE) per unit (weight) of sample[22].

Phenolic acids profile: The HPLC analysis was carried out using agilent technologies 1100 series liquid chromatography equipped with an auto sampler and a diode-array detector. The analytical column was an Eclipse XDB-C18 (150×4.6 μm, 5 μm) with a C18 guard column (Phenomenex, Torrance,

CA). Peaks were identified by congruent retention times and UV spectra and compared with those of the standards[23].

Patients and treatment trial: Patients visiting complementary medicine clinic present in Excellence Center of National Research Center, with complain of back pain or arthritis and were seeking medical advice, gave the agreement to be enrolled in the treatment trial and acceptance to give blood samples and do some clinical investigation. Patients' history, clinical examination and 24 h dietary recall, to estimate calcium and vitamin D dietary intake, were done. Only 29 postmenopausal women completed the study and were eligible for recommended criteria. They were not under any medications known to affect bone metabolism or having any history of major health problem or fracture. Patient's age ranged between 45-65 years old with body weight <100 kg. They reported previous history of DEXA examination with bone compromise and were supplemented with vitamin D and calcium for more than 3 months. Their menstrual period stopped at least 2 years ago. Patients were send before and after supplementation of the compound, to do reassessment of their DEXA by (Lunar DPX DXA system manufactured by GE Health care)) on three sides (Right and left femur and lumbar vertebrae L1-L4). Only patients who had osteopenia (T score of -1 to -2.5) or osteoporosis (T score $\geq$-2.5 according to WHO) on any of the three sides were considered eligible to take the seeds mixture. Also, blood sample were collected before and after supplementation. Serum calcium, phosphorus, zinc, magnesium and MDA were done using colorimetric methods. The P1NT and osteocalcin were estimated by ELISA kit (Glory Science Co., LTD USA). Liver and kidney functions were performed to monitor and confirm the biosafety of the supplement. Questionnaire for any side-effects were recorded.

Statistical analysis: All data were expressed as Mean±Standard Deviation (SD). Differences between groups were detected using paired sample t-test using Microsoft excel version 2007[24]. The $p \leq 0.05$ was considered statistically significant.

RESULTS

Patient's data analysis showed that their average age was 52±4.5 years and body weight 75.4±16.4 kg. The physical activity was minimal in all cases and they were kept on conservative treatment of vitamin D and calcium alongside the treatment trial (1000 mg calcium, vitamin D 600 IU day^{-1}). Their dietary intake calculation from 24 h dietary recall showed that calcium and vitamin D intake per day were 552±225.6 mg, 204±83.5 IU, respectively which is considered low dietary intake.

The panel test for the seed formula showed that the seed mixture has good taste. Scores were odour 7.3±2, colour 7.4±1.4, appearance 7±1.4, flavor 7.4±1.9 and consistency 6.9±1.5. Also the dietary supplement proved its biosafety as there were no adverse symptoms detected from patient questionnaire and the liver or kidney functions were traced as normal after treatment (Table 1).

Chemical analysis of the methanolic extract (80%), showed that the seeds mixture is rich in phenolic compounds 1014.596±47.8 (expressed as gallic acid equivalent per gram (GAE g^{-1})), flavonoids 326.669±10.2 (expressed as catachin equivalent per gram (CE g^{-1})) and have high antioxidant power 1109.87±31.5 (expressed as trolox equivalent per gram (TE g^{-1})).

Screening the methanolic and water extracts, by HPLC, for the presence of bioactive compounds showed that the seed mixture is rich in flavonoids such as qurecetin, rutin, chyrsin and kaempferol and the phenolic acids such as vanillic, ferulic, sinapic, protochatchuic, gallic, gentisic and syrngic acids (Table 2).

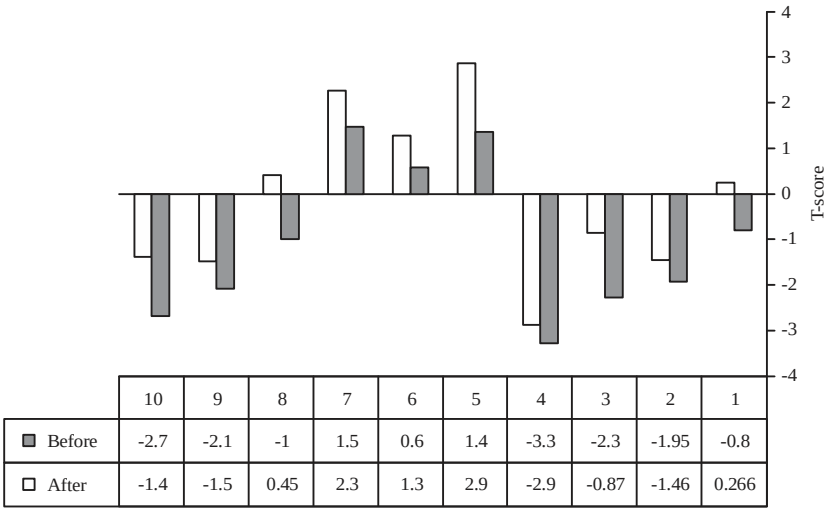
Bone examination using DEXA showed significant improvement in T score of left femur (-0.61±2 to 0.25±2.1) ($p \leq 0.01$), right femur (-0.80±2.25 to -0.07±2.1) ($p \leq 0.0001$) and lumbar spine L1-L4 (-2.68±1.04 to -1.9±1.2) ($p \leq 0.001$), respectively, after supplementation, part of individual data presented in (Fig. 1-3).

Serum analysis of the participants after supplementation showed significant increase $p \leq 0.05$ in zinc, P1NT and osteocalcin and decrease of MDA, while there were no changes in both liver and kidney function (Table 1).

Table 1: Serum parameters tested in patients before and after treatment

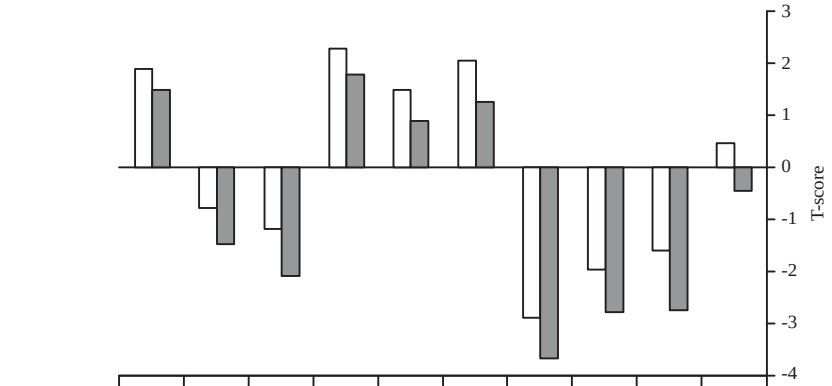
Parameters	Before	After
Calcium (mg dL^{-1})	12.6±2.1	12.60±5.1
Phosphorus (mg dL^{-1})	9.1±2.1	8.98±1.5
Mg (mmol L^{-1})	1.8±0 .43	2.44±0.49
Zinc (µmol L^{-1})	112.9±44.9	172.60±57.7*
AST (µ L^{-1})	19.2±10.1	20.50±8.2
ALT(µ L^{-1})	29.3±21	26.00±19
Creatinine (mg dL^{-1})	0.8±0.26	0.77±0.3
Urea (mg dL^{-1})	31.0±6.0	31.50±6
P1NT (µ L^{-1})	21.8±1.5	28.60±5.8*
Osteocalcin (ng mL^{-1})	13.1±6.5	15.90±2.7*
MDA (nmol mL^{-1})	8.6±0.2	3.10±1.8*

*Significant $p \leq 0.05$, values were mentioned as Means±Standard Deviation, AST: Aspartate aminotransaminase, ALT: alanine aminotransaminase, MDA: Malondialdehyde, P1NT: Procollage 1 N terminal peptide



	10	9	8	7	6	5	4	3	2	1
Before	-2.7	-2.1	-1	1.5	0.6	1.4	-3.3	-2.3	-1.95	-0.8
After	-1.4	-1.5	0.45	2.3	1.3	2.9	-2.9	-0.87	-1.46	0.266

Fig. 1: T-score of left femur before and after treatment in some patients



	10	9	8	7	6	5	4	3	2	1
Before	1.5	-1.5	-2.1	1.8	0.9	1.275	-3.7	-2.8	-2.77	-0.33
After	1.9	0.8	-1.2	2.3	1.5	2.75	-2.9	-1.99	-1.62	0.14

Fig. 2: T-score of right femur before and after treatment in some patients

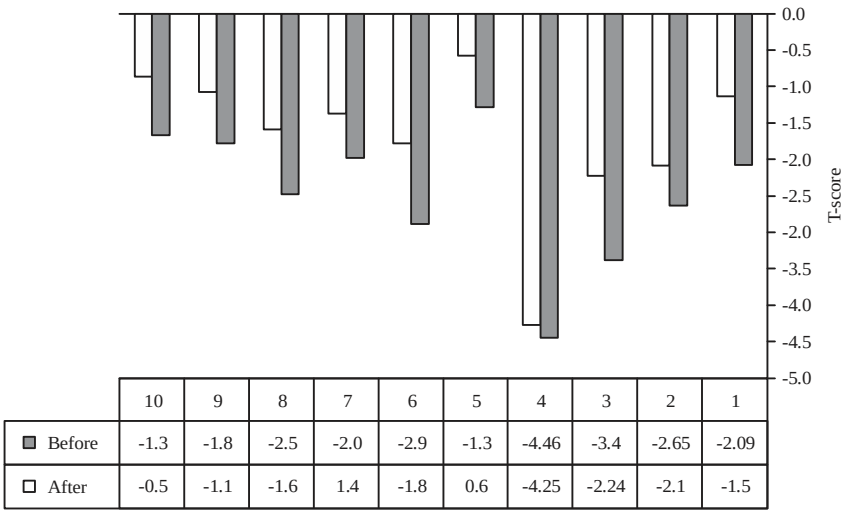
Table 2: HPLC profile of compounds found in the seed mixture

Compounds (μg g^{-1})	Methanol	Water
Rutin	76.280	ND
Qurecetin	118.040	ND
Chyrsin	2.215	ND
Kaempferol	ND	2.738
Vanillic acid	7.591	2.513
Ferulic acid	14.394	ND
Sinapic acid	23.694	1.134
Coumaric acid	ND	ND
Rosmarinic acid	ND	ND
Protochatchuic acid	ND	49.915
Cinnamic acid	ND	9.068
Gallic acid	ND	24.293
Gentisic acid	ND	19.325
Syrngic acid	ND	5.856

ND: Not detected

DISCUSSION

Estrogen is the maestro of bone strength in women, where it distribute subcutaneous fat away from bone, so that bone mesenchymal cells differentiate to osteoblasts rather than adipocytes[25,26] leading to increased bone formation and mineralization[3]. Also, it stimulates insulin secretion which consequently increases Vitamin D3 (1, 25(OH) 2D3), the necessary element for bone mineralization[25,26]. That is why decreased estrogen secretion in menopause is one of the important causes of osteoporosis[2]. Vitamin D and calcium intake in the studied group was considered low[27], that's why supplementation was continued.



	10	9	8	7	6	5	4	3	2	1
Before	-1.3	-1.8	-2.5	-2.0	-2.9	-1.3	-4.46	-3.4	-2.65	-2.09
After	-0.5	-1.1	-1.6	1.4	-1.8	0.6	-4.25	-2.24	-2.1	-1.5

Fig. 3: T-score of lumber spines before and after treatment in some patients

Because therapeutic estrogen may have hazardous side-effects for other organs as uterus and breast[28,29]. Phytoestrogens, in estrogen compromised menopausal women acts as estrogen substitute and it binds to estrogen receptors and mediates stimulation of osteoblasts, while isoflavons of plant origin inhibits osteoclastic differentiation and bone resorption whicn add to bone strength[30,31].

The current study showed significant improvement $p \leq 0.01$ of T score of DEXA results for both femurs (left and right) and vertebrae (L1-L4) at the end of supplementation period (Fig. 1-3). Also, significant elevation $p \leq 0.01$ of both P1NT and osteocalcin bone formation markers were observed (Table 1). The current results were considered as good indicators of bone health improvement and increased mineralization[32].

The positive effect of this supplement on bone formation can be explained in the light of its biochemical composition. The supplement is rich in phenolic compounds (1014.596±47.8 μg GAE g^{-1}), isoflavons (326.669±10.2 μg CE g^{-1}) and have high antioxidant capacity (1109.87±31.5 μg TE g^{-1}). As osteoporosis is enhanced by accumulation of free oxygen radical in the body[7], administration of phytonutients rich in polyphenols and isoflavons, were mentioned to improve bone formation as they are powerful antioxidant elements[31]. This explains the positive effect of the current seed mixture that added to bone health of examined patients.

The identified chemical components of the methanolic and water extracts of the seed mixture showed the presence of flavonoids such as qurecetin, rutin, chyrsin and kaempferol and the phenolic acids such as vanillic, ferulic, sinapic, protochatchuic, gallic, gentisic and syrngic acids. Chyrsin was traced in high concentration in the seed mixture; it decreases testosterone hormone metabolism enhancing its anabolic effect and help in bone building[33]. Also, quercetin, kaempferol and rutin were known as potent antiosteoporotic flavonoids as they stimulate estrogen receptor and bone formation[34-36].

Garbanzo was previously mentioned as rich source of antioxidants presented as flavonoids, quercetin, kaempferol and myricetin. Beside the phenolic acids such as ferulic, chlorogenic, caffeic and vanillic acids[37] which some of them were traced in HPLC of this study. Ferulic acid was previously mentioned as antiosteoporotic compound in ovarictomized rats[38].

Adding palm pollen powder for such formula showed synergestic action added to bone health. The PPG was previously reported to enhance testosterone secretion in rats[17]. Testosterone is the anabolic androgen responsible for bone and muscle strength[18,19]. The PPG is rich in chyrsin which is detected in this mixture and play a role as antioxidant, anti-inflammatory and anti-diabetic which add to bone health[33,39].

Also, lignans present in flaxseed and sesamin present in sesame have the ability to trigger osteoblasts differentiation and bone formation[3,5-7]. Genistein and daidzein, present in flaxseed, previously showed cessation of bone loss in ovariectomized rats[40]. Where they may cause apoptosis of osteoclasts progenitors, mediated by estrogen receptors[41]. Dose of 20-50 g day^{-1} of flaxseed meal is considered of value in improving bone fragility in menopause women other studies used similar doses[42]. Sesame is also rich in the phytosterol β-sitosterol which positively affects bone formation[11]. In the same time, testosterone secretion has been enhanced by palm pollen which possibly

undergoes aromatization and leads to increased estrogen in post menopausal women[17,43].

Because unbalanced nutrition is one of the causes of osteoporosis and may be aggravated with aging due to increased demand to certain elements such as calcium, magnesium, zinc, manganese, selenium, molybdenum, copper and others which are the building rocks of bone[44]. The seed mixture was reported as rich source of these elements where, flaxseed contains vitamin E, ALA and LA (omega 3 and 6) in good amount that were known as good antioxidants and inhibitors of prostaglandin secretion[45]. Also, sesame contains tocopherol as natural antioxidants and anti-inflammatory[11].

By aging, insulin resistance increased causing increased bone fragility[9,10]. Sesame was mentioned as good solution as it contains procyanidine that inhibits collagen and elastin breakdown in bone and body and control high blood sugar level[46].

The current formulated seed mixture, flaxseed, sesame seeds and garbanzo, were mentioned as very good source "Together rather than individual" of minerals[5,11,47].

Participant women administered calcium and vitamin D at least 3 months before the study with non significant improvement of bone fragility. Twenty four hours dietary recall showed low daily intake of calcium and vitamin D far from the daily dietary allowance for postmenopausal women[27].

The present study showed elevated plasma level of magnesium and zinc, non significant changes of calcium and phosphorus and lower level of MDA of participant women after supplementation (Table 1).

Zinc is an important element for bone health, it is essential element for several enzymes necessary for bone mineralization[48]. Calcium and phosphorus may be directed to bone mineralization as the T score increased in women after supplementation. The antioxidant power of seed mixture decreased oxygen radical indicated by decreased of MDA.

The supplementation of only minerals is not sufficient and should be combined with other elements as Phytoestrogens, antioxidant, UFA and others to improve bone health[49,50]. Synergistic effect of all these factors together improved bone strength rather than calcium and vitamin D alone. These entire compounds were supplemented in the current mixture beside vitamin D.

The biosafety of the current supplement as there were no adverse symptoms recorded by patient questionnaire and analysis of plasma enzymes for liver and kidney function were within normal level at the end of the study period.

It is worthy to mention that follow up studies are difficult, as we started with bigger number of patients but some does not show up later, some does not continue on the dose acuratly, whom were excluded from the study. Bigger scale of patients is needed, other causes of bone fragility may be tested and other forms of the mixture will be procceded in the coming studies.

CONCLUSION

The current study proved that combination between functional seeds was for the synergistic action of flavonoids, phenolic compounds, minerals, trace elements, antioxidants, anti-inflammatory and anti-aging compounds. Beside the supplemented vitamin D and calcium were effective to maximize the beneficial effects for bone health and mineralization.

The chemical medication and hormonal therapy of osteoporosis may have many side effects on other remote organs in patients. While safe plant remedies as this dietary supplement can improve bone mineralization, act as antioxidant and good source of minerals, with no side effect on remote organs and with good taste.

SIGNIFICANCE STATEMENTS

This study discovers the possible synergestic effect of multiple functional seeds rich in phytoestrogen and micronutrients such as flaxseeds, sesame seeds, garbenso and palm pollen combination, that can be beneficial, together rather than individually, for osteoporosis and bone compromise affecting menopausal women. This study will help to uncover the critical area of post menopausal bone loss that many researcher were unable to explore. Thus anew concept on combining multiple functional seeds or others can be explored, also treatment of such problems from Mother Nature will be encouraged. The previously mentioned functional seeds were not gathered in one supplemented formula before, which gives its novelty. This formula can be used by a wide society section suffering from bone compromise.

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REFERENCES

1. Rachner, T.D., S. Khosla and L.C. Hofbauer, 2011. Osteoporosis: Now and the future. Lancet, 377: 1276-1287.
2. Tremollieres, F. and C. Ribot, 2010. Bone mineral density and prediction of non-osteoporotic disease. Maturitas, 65: 348-351.
3. Sacco, S.M., J.M.Y. Jiang, S. Reza-Lopez, D.W.L. Ma, L.U. Thompson and W.E. Ward, 2009. Flaxseed combined with low-dose estrogen therapy preserves bone tissue in ovariectomized rats. Menopause, 16: 545-554.
4. Hall III, C., M.C. Tulbek and Y. Xu, 2006. Flaxseed. Adv. Food Nutr. Res., 51: 1-97.
5. Alshafe, M.M., S.S. Kassem, M.M. Abdelkader and E.M. Hanafi, 2015. Flaxseed as functional food. Res. J. Pharm. Biol. Chem. Sci., 6: 1944-1951.
6. Boulbaroud, S., A. Mesfioui, A. Arfaoui, A. Ouichou and A. El Hessni, 2008. Preventive effects of flaxseed and sesame oil on bone loss in ovariectomized rats. Pak. J. Biol. Sci., 11: 1696-1701.
7. Lean, J.M., J.T. Davies, K. Fuller, C.J. Jagger and B. Kirstein *et al*., 2003. A crucial role for thiol antioxidants in estrogen-deficiency bone loss. J. Clin. Invest., 112: 915-923.
8. USDA., 2011. USDA national nutrient database for standard reference, release 24. Nutrient Data Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, USA. http://www.ars.usda.gov/Services/docs.htm?docid=8964.
9. Wanachewin, O., K. Boonmaleerat, P. Pothacharoen, V. Reutrakul and P. Kongtawelert, 2012. Sesamin stimulates osteoblast differentiation through p38 and ERK1/2 MAPK signaling pathways. BMC Complementary Alter. Med., Vol. 12. 10.1186/1472-6882-12-71.
10. Wan, Y., H. Li, G. Fu, X. Chen, F. Chen and M. Xie, 2015. The relationship of antioxidant components and antioxidant activity of sesame seed oil. J. Sci. Food Agric., 95: 2571-2578.
11. Chakraborthy, G.S., G. Sharma and K.N. Kaushik, 2008. Sesamum indicum: A review. J. Herb. Med. Toxicol., 2: 15-19.
12. Takahashi, T., A. Kamimura, A. Shirai and Y. Yokoo, 2000. Several selective protein kinase C inhibitors including procyanidins promote hair growth. Skin Pharmacol. Physiol., 13: 133-142.
13. Pathak, N., A.K. Rai, R. Kumari and K.V. Bhat, 2014. Value addition in sesame: A perspective on bioactive components for enhancing utility and profitability. Plant Rev., 8: 147-155.
14. O'Loughlin, P.D. and H.A. Morris, 1998. Oestrogen deficiency impairs intestinal calcium absorption in the rat. J. Physiol., 511: 313-322.
15. Pittaway, J.K., I.K. Robertson and M.J. Ball, 2008. Chickpeas may influence fatty acid and fiber intake in an ad libitum diet, leading to small improvements in serum lipid profile and glycemic control. J. Am. Dietetic Assoc., 108: 1009-1013.
16. USDHHS. and USDA., 2005. Dietary Guidelines for Americans 2005. U.S. Government Printing Office, Washington DC, USA., ISBN: 0-1-16-072398-1.
17. Iftikhar, S., A. Bashir, M.S. Anwar, S.M. Mastoi and M. Shahzad, 2011. Effect of Date Palm Pollen (DPP) on serum testosterone levels in prepubertal albino rats. Pak. J. Med. Health Sci., 5: 639-644.
18. Tan, R.S., 2005. Testosterone replacement therapy for female androgen insufficiency syndrome. Int. J. Pharm. Compound., 9: 259-264.
19. Bahmanpour, S., T. Talaei, Z. Vojdani, M.R. Panjehshahin, A. Poostpasand, S. Zareei and M. Ghaeminia, 2015. Effect of Phoenix dactylifera pollen on sperm parameters and reproductive system of adult male rats. Iran. J. Med. Sci., 31: 208-212.
20. Hoojjat, P. and M.E. Zabik, 1984. Sugar-snap cookies prepared with wheat-navy bean-sesame seed flour blends. Cereal Chem., 61: 41-44.
21. Zilic, S., A. Serpen, G. Akıllıoglu, M. Jankovic and V. Gokmen, 2012. Distributions of phenolic compounds, yellow pigments and oxidative enzymes in wheat grains and their relation to antioxidant capacity of bran and debranned flour. J. Cereal Sci., 56: 652-658.
22. Thaipong, K., U. Boonprakob, K. Crosby, L. Cisneros-Zevallos and D.H. Byrne, 2006. Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Comp. Anal., 19: 669-675.
23. Arnao, M.B., A. Cano and M. Acosta, 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem., 73: 239-244.
24. Box, J.F., 1987. Guinness, gosset, fisher and small samples. Stat. Sci., 2: 45-52.
25. Michel, B.A., D.A. Bloch and J.F. Fries, 1989. Weight-bearing exercise, overexercise and lumbar bone density over age 50 years. Arch. Internal Med., 149: 2325-2329.
26. Albala, C., M. Yanez, E. Devoto, C. Sostin, L. Zeballos and J.L. Santos, 1996. Obesity as a protective factor for postmenopausal osteoporosis. Int. J. Obesity Related Metab. Disorders, 20: 1027-1032.
27. Geer, F.R. and N.F. Krebs, 2006. Optimizing bone health and calcium intakes of infants, children adolescents. Pediatrics, 117: 578-585.
28. Davey, D.A., 2012. Update: Estrogen and estrogen plus progestin therapy in the care of women at and after the menopause. Women's Health, 8: 169-189.
29. Iannitti, T., S. Rosini, D. Lodi, B. Frediani, V. Rottigni and B. Palmieri, 2012. Bisphosphonates: Focus on inflammation and bone loss. Am. J. Therapeutics, 19: 228-246.
30. Geller, S.E. and L. Studee, 2006. Soy and red clover for mid-life and aging. Climacteric, 9: 245-263.

31. Kawakita, S., F. Marotta, Y. Naito, U. Gumaste, S. Jain, J. Tsuchiya and E. Minelli, 2009. Effect of an isofl avones-containing red clover preparation and alkaline supplementation on bone metabolism in ovariectomized rats. Clin. Interventions Aging, 4: 91-100.
32. Biver, E., F. Chopin, G. Coiffier, T.F. Brentano, B. Bouvard, P. Garnero and B. Cortet, 2012. Bone turnover markers for osteoporotic status assessment? A systematic review of their diagnosis value at baseline in osteoporosis. Joint Bone Spine, 79: 20-25.
33. Kasala, E.R., L.N. Bodduluru, R.M. Madana, K.V. Athira, R. Gogoi and C.C. Barua, 2015. Chemopreventive and therapeutic potential of chrysin in cancer: Mechanistic perspectives. Toxicol. Lett., 233: 214-225.
34. Liang, W., Z. Luo, S. Ge, M. Li and J. Du *et al*., 2011. Oral administration of quercetin inhibits bone loss in rat model of diabetic osteopenia. Eur. J. Pharmacol., 670: 317-324.
35. Horcajada-Molteni, M.N., V. Crespy, V. Coxam, M.J. Davicco, C. Remesy and J.P. Barlet, 2000. Rutin inhibits ovariectomy-induced osteopenia in rats. J. Bone Miner. Res., 15: 2251-2258.
36. Choi, E.M., 2011. Kaempferol protects MC3T3-E1 cells through antioxidant effect and regulation of mitochondrial function. Food Chem. Toxicol., 49: 1800-1805.
37. Sreerama, Y.N., V.B. Sashikala and V.M. Pratape, 2010. Variability in the distribution of phenolic compounds in milled fractions of chickpea and horse gram: Evaluation of their antioxidant properties. J. Agric. Food Chem., 58: 8322-8330.
38. Sassa, S., T. Kikuchi, H. Shinoda, S. Suzuki, H. Kudo and S. Sakamoto, 2002. Preventive effect of ferulic acid on bone loss in ovariectomized rats. *In Vivo* (Athens Greece), 17: 277-280.
39. Gambelunghe, C., R. Rossi, M. Sommavilla, C. Ferranti and R. Rossi *et al*., 2003. Effects of chrysin on urinary testosterone levels in human males. J. Med. Food, 6: 387-390.
40. Bitto, A., F. Polito, B. Burnett, R. Levy and V. Di Stefano *et al*., 2009. Protective effect of genistein aglycone on the development of osteonecrosis of the femoral head and secondary osteoporosis induced by methylprednisolone in rats. J. Endocrinol., 201: 321-328.
41. Filipovic, B., B. Sosic-Jurjevic, V. Ajdzanovic, D. Brkic, M. Manojlovic-Stojanoski, V. Milosevic and M. Sekulic, 2010. Daidzein administration positively affects thyroid C cells and bone structure in orchidectomized middle-aged rats. Osteoporosis Int., 21: 1609-1616.
42. Dodin, S., A. Lemay, H. Jacques, F. Legare, J.C. Forest and B. Macsse, 2005. The effects of flaxseed dietary supplement on lipid profile, bone mineral density and symptoms in menopausal women: A randomized, double-blind, wheat germ placebo-controlled clinical trial. J. Clin. Endocrinol. Metab., 90: 1390-1397.
43. Dhawan, K., S. Kumar and A. Sharma, 2002. Beneficial effects of chrysin and benzoflavone on virility in 2-year-old male rats. J. Med. Food, 5: 43-48.
44. Mutlu, M., M. Argun, E. Kilic, R. Saraymen and S. Yazar, 2007. Magnesium, zinc and copper status in osteoporotic, osteopenic and normal post-menopausal women. J. Int. Med. Res., 35: 692-695.
45. Arjmandi, B.H., 2001. The role of phytoestrogens in the prevention and treatment of osteoporosis in ovarian hormone deficiency. J. Am. Coll. Nutr., 20: 398S-402S.
46. Chang, L.W., W.J. Yen, S.C. Huang and P.D. Duh, 2002. Antioxidant activity of sesame coat. Food Chem., 78: 347-354.
47. George Mateljan Foundation, 2015. Garbanzo beans (chickpeas). World's Healthiest Foods Rating System. http://www.whfoods.com/genpage.php?tname=foodspice&dbid=58.
48. Yamaguchi, M., 2010. Role of nutritional zinc in the prevention of osteoporosis. Mol. Cell. Biochem., 338: 241-254.
49. Price, C.T., J.R. Langford and F.A. Liporace, 2012. Essential nutrients for bone health and a review of their availability in the average North American diet. Open Orthopaedics J., 6: 143-149.
50. Lappe, J., I. Kunz, I. Bendik, K. Prudence, P. Weber, R. Recker and R.P. Heaney, 2013. Effect of a combination of genistein, polyunsaturated fatty acids and vitamins D3 and K1 on bone mineral density in postmenopausal women: A randomized, placebo-controlled, double-blind pilot study. Eur. J. Nutr., 52: 203-215.