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The Possible Protective Role of Powder Cuttlefish Bone, Crabshell and Eggshell on Osteoporotic Rats

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ABSTRACT

Bone mass declines alongside maturing, particularly for ladies after menopause as a result of diminishing estrogen emission together with low calcium consumption. This examination was directed to examine the impact of powdered cuttlebone, crab shell and eggshell, on female rats with induced osteoporosis. Fifty adult female albino rats, of (200 ± 10 g) were haphazardly divided into five groups, the first group, negative control group (-ve) fed on basal diet only, the other four groups (10 rats each) were fed on basal diet containing 100 mg prednisone acetate as source of glucocorticoid/ kg diet to induce osteoporosis for two weeks. One group of them was selected as a positive control group, the other three groups were fed on prednisone acetate diets containing powder of cuttlebone, crabshell and eggshell, at the level of 2.5% individually. Blood samples and femur bones were gathered for measure both serum and bone markers of osteoporosis. The results indicated that, supplementation with powder of cuttlebone, crabshell and eggshell significantly ($P<0.05$) increase of Ca and P in serum of osteoporotic rats. There were a significant increment in serum free thyroxin (T4) and a significant decrease in parathyroid hormone (PTH). Femur bone mineral content (BMC) and bone mineral density (BMD) were likewise increased as compared to positive control group. In addition, liver functions were significantly ($P<0.05$) improved compared to the positive control group, while serum lipid profiles were significantly decreased. These results recommend that sustaining postmenopausal rats with osteoporosis on diets supplemented with powder of cuttlebone, crab shell and eggshell instigated an enemy of osteoporotic impact. This impact may be due to upgrade of bone arrangement and direction of some metabolic hormones which control calcium. Therefore, it could be recommended using powder of cuttlebone, crabshell and eggshell for postmenopausal women suffering from osteoporosis.

Keywords: Osteoporosis, Postmenopausal, Cuttlebone, Sepia officinalis, Crabshell, Eggshell powder, Calcium Carbonate.

INTRODUCTION

The danger of osteoporosis, particularly in ladies, increments as a feature of the maturing procedure and the estimation of their bone thickness is imperative (Delmas and Fraser, 1999). Osteoporosis is regularly called a quiet ailment of maturing in light of the fact that bone misfortune happens without symptoms until microarchitectural weakening and bone break happens (Deyhim *et al*., 2006). Essential type small osteoporosis or decrepit osteoporosis happens after age 75. Auxiliary osteoporosis may emerge at any age and influences people similarly (Brian *et al*., 2009). Osteoporosis has transformed into an essential prosperity hazard disease starting late, besetting more than of 2000 million individuals around the world (Rachner *et al*., 2011).

Menopause is regularly connected with genuine open issues in moderately aged ladies (Ye *et al*., 2015). A diminishment in estrogen levels is ordinarily accepted to cause mental and state of mind changes, and also physiological changes that outcome in side effects (Streicher *et al*., 2017). Accordingly, hormone substitution treatment (HRT) has been used to upgrade menopausal symptoms (Nelson *et al*., 2002). Be that as it may, long haul HRT expands the danger of a few genuine illnesses, for example, bosom and endometrial malignancy, thromboembolic occasions and vaginal dying (Morrow *et al*., 2009 and Curtis *et al*., 2016).

Nutrition has a critical and complex role in support of good bone (Sophocleous *et al*., 2003).Specialists are searching for characteristic materials thinking about them unrivaled and more attractive (Dimitriou *et al*., 2011 and Sarkar and Lee, 2015). Up to this time a few crude materials, for example, eggshell and animal bone have been utilized to get ready hydroxyapatite (Kim *et al*., 2014 and Kattimani *et al.,* 2014). Sea-going living beings, for example, Coral, Nacre and Cuttlefish have been utilized as a part of request to improve bone recovery as of late (Clarke *et al*., 2011 and Silva *et al.,* 2014).

Cuttlebone (CB), otherwise called cuttlefish spine, is an inner shell of marine creatures known as cuttlefish (Sepia), having a place with the phylum mollusca, class cephalopod, arrange Sepiidae (Zhao *et al*., 2011 and North *et al*., 2017). Cuttlefish bone is for the most part made out of calcium carbonate. A few test considers have been directed on normal cuttlefish bone as a calcium hotspot for bone substitutes (Rocha *et al*., 2006, Kannan *et al*., 2007 , Dermience *et al*., 2015 and Hongmin *et al*., 2015). Cuttlefish bone will in general be utilized and masterminded by various fillers as a marine typical opposing to Osteoporosis (Hemmatti *et al*., 2018).

Crustacean's shells such as crab contain natural ingredients, and its principal components are chitin (20-30%), protein (30-40 %), calcium carbonate salts (30-50%) and antioxidant compounds such as selenium and carotenoids (astaxanthin, astatine, and can-thaxanthi) (Akhuemokhan *et al*., 2013 and Rezakhani *et al*., 2014). Distinctive marine sources polysaccharides have been utilized for treatment of bone illnesses like osteoporosis (Iwata *et al*., 2005) and joint pain (Porporatto *et al*., 2009).

Eggshell components are inorganic salts (91.87%), the main ones of which are calcium carbonate (98.4%), magnesium carbonate (0.8%), tricalcium phosphate (0.8%) (Dri *et al.,* 2011). Chicken eggshell powder (CESP) is a source of Ca, which is available at home and an excellent replacement material for important crustacean shells that can be used as Ca supplementation (Brun *et al*., 2013). CESP has superb antirachitic impacts in rats and humans. It diminishes agony and osteoresorption in postmenopausal ladies and ladies with decrepit osteoporosis. It additionally expands bone mineral thickness in such patients making it sensible in the killing action and treatment of osteoporosis when taken as an oral supplement for a year (Schaafsma *et al*., 2000 and Rovenský *et al*., 2003). The present examination was intended to research the possible protective role of cuttlebone, crabshell and eggshell powdered, on adult female rats with induced osteoporosis.

MATERIALS AND METHODS

Materials

Cuttlefish bone: was collected and removed from cuttlefish *(Sepia esculenta*) and its treatment.

Crabshell: was collected from household waste for this purpose and its treatment.

Eggshell: Chicken egg shell was collected from household waste for this purpose and the processing operation was carried out through a series of steps. It's collected from local shops in Port Said, Egypt.

Rats: Fifty adult female albino of Sprague Dawley strain rats (3 months old) in a body weight range of (200 ± 10 g) were obtained from Helwan Farm for Experimental Animals, Cairo, Egypt.

Diet: Casein, vitamins, cellulose, minerals, methionine and choline were obtained from Morgan Company for Chemicals, Cairo, Egypt.

Chemicals: Kits for biochemical examination were bought from Biodiagnostic Company for Pharmaceutical and chemicals, Dokki, Egypt. Prednisone Acetate as source of glucocorticoid (GC) was obtained from Morgan Chemical Factory, Cairo, Egypt.

Methods

Preparation of Cuttlefish bone Powder: Squares cuttlefish bone was removed from cuttlefish *(Sepia esculenta*, from the Mediterranean Sea), delicately washed with refined water, and dried in free air to lose its smell. After drying, the clean cuttlebone was powdered and totally blended to 60-100 mesh size (Kim *et al*., 2012).

Preparation of Crabshell Powder: Crab shells (*Brachyura*) were arranged appropriately: altogether washed, the inward layer was expelled, dried in oven dried in the oven at 70 °C for 24 h or longer until the point when they were totally dried, crushed in an espresso processor and smashed in an artistic mortar and pestle (Shahidi and Synowiecki 1991).

Preparation of Eggshell Powder: Eggshells was washed twice and prepared utilizing hot air oven at 60°C for 2h then was ground to powder by using household Mill (Braun, Germany) (Fred *et al*., 2006).

Determination of Minerals Content: Minerals content, magnesium (Mg), calcium (Ca), potassium (K) and phosphor (p) were digestion as described by Kirleis *et al.,* (1984) and determined by using the atomic absorption spectrophotometer (Perkin - Elmer 3300, USA).

Determination of Vitamin (D3) Content: Vitamin D3 contents were determined in Cuttlebone, Crabshell and Eggshell powdered, using the method by (VDLUFA, 2007).

Experimental Animal Design:

Fifty adult female albino of Sprague-Dawley strain rats, (3 months old) in a body weight range of (200 ± 10 g) were placed in well aerated cages under hygienic condition and fed for one week on basal diet for adaptation, then were divided into five groups as follows:

The first group (10 rats) was kept as negative control group (-ve) and fed on basal diet only Reeves *et al*., (1993). The other four groups (10 rats each) were fed on basal diet containing 100 mg Prednisone Acetate as source of glucocorticoid/ kg diet to induce osteoporosis for two weeks (Liao *et al*., 2003). One group of them was served as a positive control group, the other three groups were fed on prednisone acetate diets containing powder Cuttlebone, Crabshell and Eggshell at the level of 2.5%, respectively.

At the finish of the experiment (8weeks) the rats were fasted for 12 hour, and after that sacrificed under ether anesthesia. Blood samples were gathered from medial canthus of the eyes of rats by means of fine capillary glass tubes in a centrifuge tube without any anticoagulant and centrifuged for 20 minutes at 3000r.p.m. to obtain serum which was stored at -20°C until used for subsequent analysis.

Chemical analysis :

Serum levels of calcium and phosphorus were determined according to Gindler and King, (1972) and El-Merzabani *et al*., (1977), respectively. Additionally, serum parathyroid hormone (PTH) was estimated through enzyme linked immunosorbent assay (ELISA), according to Norazlina *et al*., (2010). Free thyroxine (T4) concentrations were measured using radioimmunoassay (RIA) method as described by Wang *et al*., (2009). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured according to Reitman and Frankel, (1957). Serum was analyzed for the following biochemical parameter: total cholesterol (TC) by the method of Richmond, (1973), HDL-cholesterol by Albers *et al*., (1983), triglyceride (TG) by Jacobs and Vander mark, (1960). Calculation of LDL-c and VLDL-c by the equation of Fridewald *et al*., (1972).

Measurement of bone minerals content (BMC) and bone minerals density (BMD): The BMD and BMC of the total skeleton, femur and tibia were estimated in anesthetized rats using dual energy X-ray absorptiometry (Gao *et al*., 2013).

Statistical analysis:

Results were communicated as mean±standard Error (SE). Data were analyzed statistically by SPSS program, one way ANOVA followed by post hoc multiple were used to make a comparison among different groups (Snedecor and Cochron, 1989).

RESULTS

Results illustrated in Table (1) revealed minerals composition of decalcified cuttlefish bone, crabshell and eggshell. Regarding the minerals content, cuttlefish bone contains a high level of Ca, and trace amounts of other micro elements such as K, Mg and P (26.16 mg, 14.24mg, 7.50 mg and 2.42mg), respectively. Crabshell contains a high level of Ca, and trace amounts of other micro elements such as K, Mg and P (34.72mg, 1.35mg, 0.083mg and 0.103mg), respectively. Eggshell contains a high level of Ca, and trace amounts of other micro elements such as K, Mg and P (37.73 mg, 0.054mg, 4.07mg and 0.087mg), respectively.

Table 1. Minerals composition of decalcified cuttlefish bone, crab shell and eggshell. Values (g/100 g dry matter)

Minerals	Ca (mg/g)	K (mg/g)	Mg (mg/g)	P (mg/g)
Cuttlefish bone	26.16	14.24	7.50	2.42
Crabshell	34.72	1.35	0.083	0.103
Eggshell	37.73	0.054	4.07	0.087

Results illustrated in Table (2) revealed vitamin (D) content of cuttlefish bone, crabshell and eggshell powdered. Powder of cuttlefish bone, crab shell and eggshell contains a high level of vitamin (D) in the following concentrations (3.3mg, 3.7mg and 4.3mg), respectively.

Table 2. Vitamin (D) content of cuttlefish bone, crabshell and eggshell powdered. (g/100 g dry matter)

Vitamin (D)	Values (g/100 g dry matter)
Cuttlefish bone	3.3 mg
Crabshell	3.7 mg
Eggshell	4.3 mg

Results showed in Table (3) revealed the effect of cuttlefish bone, crab shell and eggshell on serum contents of calcium and phosphorus on osteoporotic rats. Serum calcium and phosphorus of the positive control group were significantly (P<0.05) diminished when contrasted with the negative control rats. Feeding rats with powder of cuttlefish bone, crabshell and eggshell, caused a significant increase (P<0.05) in serum Ca and P, when contrasted with the positive control group. Likewise, no significant changes in serum levels of Ca and P was observed between the groups nourished on cuttlefish bone, crabshell and eggshell powdered. It was noticed that the group nourished on crabshell had the highest significant increase in serum Ca and P when contrasted with the other tried groups. Moreover, serum P level at powder of cuttlefish bone, crabshell and eggshell had no significant difference when stood out from the negative control group.

Table 3. Effect of cuttlefish bone, crabshell and eggshell, on serum calcium and phosphorus contents in osteoporotic rats

Parameters / Groups	Ca	P
	mg/g	
G(1):Control (-ve)	15.25±0. 35^{a}	7.00±0.20^{ab}
G(2):Control (+ve)	7.65±0.75^{c}	3.30±0.30^{c}
G(3): Cuttlefish bone (2.5%)	11.25±0.35^{b}	5.45±0.65^{b}
G(4): Crabshell (2.5%)	12.95±0.45^{b}	7.65±0.95^{a}
G(5): Eggshell (2.5%)	12.55±0.55^{b}	5.30±0.10^{b}

Values are expressed as means ± SE.
Values at the same column with different letters are significantly different at P<0.05.

Table (4) showed the impact of cuttlefish bone, crabshell and eggshell on serum levels of thyroxin and parathyroid hormone on osteoporosis in rats. The positive control had a significant (P<0.05) diminish in serum level of T4 and an increase in PTH as compared to the negative control group. Supplementation with powder of cuttlefish bone, crabshell or eggshell significantly increased (P<0.05) the lowered levels of serum T4 and significantly decreased (P<0.05) the elevated levels of serum PTH respectively when contrasted with positive control rats. In addition, there was no significant difference in serum levels of T4 and PTH among the three treated groups. In addition, Serum T4 level at eggshell powdered aggregate had no significant difference when contrasted with the negative control group.

Table 4. Effect of cuttlefish bone, crabshell and eggshell on serum thyroxin and parathyroid hormone in osteoporotic rats

Parameters / Groups	Thyroxin (T4)	Parathyroid hormone (PTH)
	(ng/mL)	(pg/mL)
G(1):Control (-ve)	7.45±0.45^{a}	0.95±0.50^{c}
G(2):Control (+ve)	3.00±0.20^{c}	3.00±0.10^{a}
G(3): Cuttlefish bone (2.5%)	5.30±0.60^{b}	1.80±0.30^{b}
G(4): Crab shell (2.5%)	5.65±0.35^{b}	2.10±0.20^{b}
G(5): Eggshell (2.5%)	6.10±0.30^{ab}	1.90±0.10^{b}

Values are expressed as means ± SE.
Values at the same column with different letters are significantly different at P<0.05.

Impact of diet supplemented with cuttlefish bone, crab shell and eggshell on Bone Minerals Content (BMC) and Bone Minerals Density (BMD) of osteoporotic rats was shown in Table (5) and figures (1, 2, 3, 4 and 5). The mean bone minerals (content and density) of the positive control group was significantly (P<0.05) diminished, contrasted with the negative control rats. The supplementation with powder of cuttlefish bone, crab shell and eggshell significantly (P<0.05) increased the mean value of BMC and BMD, compared to the positive control group. There was no significant change in BMC and BMD among the three treated groups, (G3,G4 and G5) respectively.

Table 5. Effect of cuttlefish bone, crabshell and eggshell on bone minerals (content and density) in femur bone of osteoporotic rats

Parameters / Groups	Bone minerals content (BMC)	Bone minerals density (BMD)
	(g/cm^2)	
G(1):Control (-ve)	0.717±0.117^{a}	0.168±0. 015^{a}
G(2):Control (+ve)	0.084±0. 029^{c}	0.018±0. 007^{c}
G(3): Cuttlefish bone (2.5%)	0.441±0. 051^{b}	0.091±0.006^{b}
G(4): Crabshell (2.5%)	0.342±0. 070^{b}	0.098 ±0.011^{b}
G(5): Eggshell (2.5%)	0.388±0. 065^{b}	0.094 ±0.015^{b}

Values are expressed as means ± SE.
Values at the same column with different letters are significantly different at P<0.05.

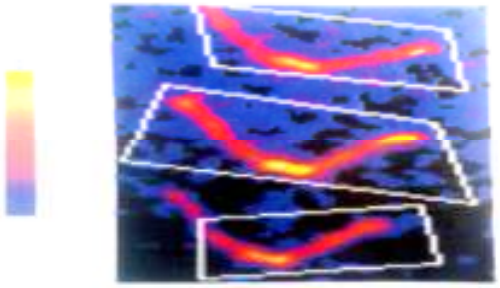

Fig. 1. Dexa scan for negative control (G1)

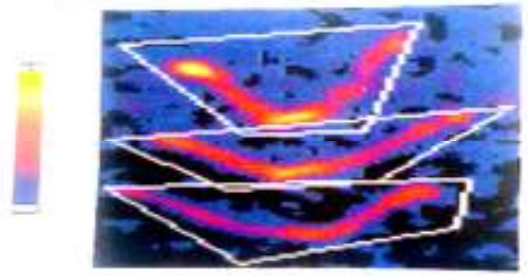

Fig .2. Dexa scan for positive control

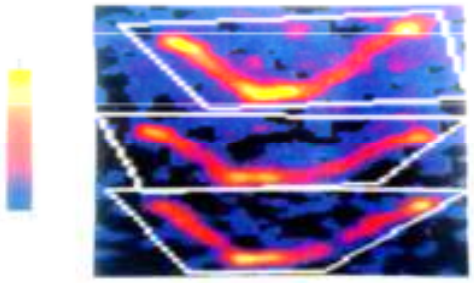

Fig .3. Dexa scan for cuttlefish bone(G3)

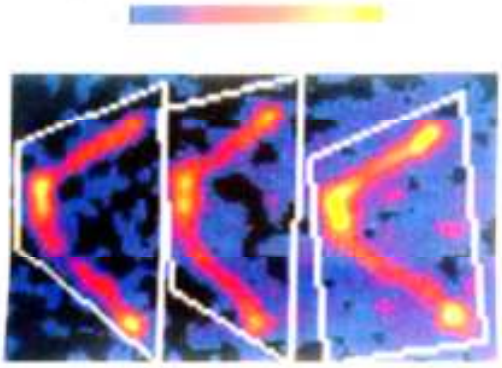

Fig .4. Dexa scan for crabshell(G4)

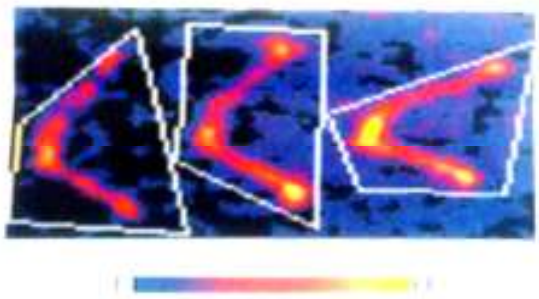

Fig .5. Dexa scan for eggshell (G5)

Results illustrated in Table (6) revealed the effect of cuttlefish bone, crabshell and eggshell on serum liver functions of osteoporotic rats. The activities of serum (ALT) and (AST) were significantly increased (P<0.05) in the positive control group, contrasted and the comparing estimation of ordinary control bunch because of nourishing prednisone acetate diet. Supplementation with powder of cuttlefish bone, crabshell and eggshell at the tested level significantly decreased (P<0.05) the elevated levels of both serum ALT and AST contrasted with the positive control group. In addition treating rats on experimental diet supplemented with powder of cuttlefish bone, crabshell and eggshell basically (P<0.05) institutionalized serum levels of AST and ALT contrasted with positive control rats. While, there was no significant change in serum ALT or AST among the three treated groups (G3,G4 and G5). The particular best delayed consequences of liver functions were recorded at the group fed on basal diet supplemented with 2.5% of eggshell powdered.

Table 6. Effect of cuttlefish bone, crabshell and eggshell on serum liver functions in osteoporotic rats

Parameters	AST	ALT
Groups	(µ/L)	
G(1):Control (-ve)	101.30±6.70^{c}	25.65±0.35^{c}
G(2):Control (+ve)	145.00±3.00^{a}	45.55±2.45^{a}
G(3): Cuttlefish bone (2.5%)	125.15±3.85^{b}	37.55±0.45^{b}
G(4): Crab shell (2.5%)	119.35±1.65^{b}	34.90±1.10^{b}
G(5): Eggshell (2.5%)	117.95±2.05^{b}	35.80±1.20^{b}

Values are expressed as means ± SE.
Values at the same column with different letters are significantly different at P<0.05.

Results illustrated in Table (7) uncovered the impact of cuttlefish bone, crabshell and eggshell on lipid profile of osteoporotic rats. The positive control group obtained significant increment in serum levels of (TC), (TG), (VLDL-c), and (LDL-c) and a significant diminish in serum (HDL-c), when contrasted with the healthy control group due to reinforcing prednisone acetate diet. Supplementation with powder of cuttlefish bone, crabshell and eggshell at the tried level altogether significantly decreased (P<0.05) the lifted levels of TC, TG, VLDL-c, and LDL-c showed up distinctively in connection to the positive control group, While had a significant increment (p<0.05) in serum level of serum HDL-c, contrasted with a similar group.

Additionally, there was no significant modifications in the levels of TC, HDL-c, and LDL-c, among the three treated groups, however there was significant changes in our levels of TG and VLDL-c, between powder of cuttlefish bone and eggshell. Besides, there are no significant changes in our levels of TG and VLDL-c, between powder of cuttlefish bone and crabshell, likewise there were no significant changes in the levels of TG and VLDL-c, between powder of crabshell and eggshell. Clearly, basal diet supplemented with powder of eggshell at the level of 2.5% gave the most beneficial effect in enhancing lipid profile on postmenopausal osteoporosis in rats.

Table 7. Effect of cuttlefish bone, crabshell and eggshell on lipid profile in osteoporotic rats

Parameters	TC	TG	HDL	VLDL-C	LDL-C
Groups			(mg/dl)		
G(1): Control (-ve)	68.00±3.00^{c}	79.00±2.00^{d}	46.15±0.85^{a}	15.80±0.40^{d}	6.05±2.55^{c}
G(2):Control (+ve)	88.50±1.50^{a}	118.00±2.00^{a}	34.10±1.90^{c}	23.60±0.40^{a}	30.80±3.80^{a}
G(3): Cuttlefish bone (2.5%)	77.50±1.50^{b}	104.50±0.50^{b}	42.50±1.50^{ab}	20.90±0.10^{b}	14.10±0.10^{b}
G(4): Crabshell (2.5%)	79.00±1.00^{b}	98.50±1.50^{bc}	44.00±1.00^{ab}	19.70±0.30^{bc}	15.30±0.30^{b}
G(5): Eggshell (2.5%)	73.40±3.40^{bc}	93.50±3.50^{c}	40.00±2.00^{b}	18.70±0.70^{c}	14.70±0.70^{b}

Values are expressed as means ± SE.
Values at the same column with different letters are significantly different at P<0.05.

DISCUSSION

Postmenopausal osteoporosis is a noteworthy medical issue for ladies society. It is evaluated that 1 out of 3 ladies and 1 out of 12 men beyond 50 years old worldwide have osteoporosis (Lasota and Danowska-Klonowska, 2004 and McNamara, 2010). With the lessening in estrogen levels, bone thickness is diminished by 5% for each a year, and by up to half of the premenopausal level (Cole *et al*., 2008).

These days, side effects, drug interaction and different issues of substance drugs have caused increment being used of herbal drugs or home grown medications in treatment of disease. Besides, one of the critical contemplations is the expense. We think of it as imperative to consider the potential medical advantages of cuttlebone, crab shell and eggshell powdered as a conceivable new compelling and safe remedial choice in the counteractive action of postmenopausal osteoporosis and adds to the field of ecological security, which is an expanding request in the public arena.

Bee, (2011) mentioned that eggshells contain calcium and follow measures of other smaller scale components, i.e. magnesium, boron, copper, iron, manganese, molybdenum, sulphur, silicon and zinc. Eggshell calcium is probably the best natural source of calcium and it is about 90% absorbable. It is a vastly improved wellspring of calcium than limestone or coral sources. The piece of an eggshell is fundamentally the same as that of our bones and teeth. These results are agreed with present study.

Cuttlebone (CB) similarity with bone mineral creation was shown by quantitative examination of sodium, magnesium, pottasium and calcium ions in CB and human elbow bone (Cadman *et al*., 2012 and Dogan and Okumus, 2014). Various explores concentrated on assessment of CB affect on bone recuperation, alone or in blend with joins together and advancement factors (Liu *et al*., 2013 and Venkatesan *et al*., 2014).

Results of the present study showed that treating rats with powder of cuttlefish bone, crabshell and eggshell caused a significant increase in serum calcium and phosphorus and this could be expected to an expanded osteoblastic movement, therefore upgrading bone development. These results are concurred with Jang *et al*., (2007) and Srikanta *et al*., (2011) who announced that the seaweed powder based, water-soluble calcium supplement was relied upon to be more helpful to bone minerals density as far as weight bearing on the skeleton than the insoluble calcium supplement.

Calcium, vitamin D and parathyroid hormone are basic controllers of bone redesigning (Lu *et al*., 2013). Serum calcium and phosphorus are ordinarily utilized as biochemical markers of bone development and building. The abatement in serum levels of calcium and phosphorus in rats experiencing osteoporosis actuated as detailed in this examination was like the past reports (Tamir *et al*., 2001 and Coxam, 2005). The diminished serum calcium levels were additionally answered to be because of estrogen inadequacy in rats experiencing osteoporosis initiated (Choi and Seo, 2013).

Kim *et al*., (2012) reported that natural cuttlefish bone is composed of calcium carbonate and contains little measures of overwhelming metals and other natural parts. These outcomes were in accordance with Hemmatti *et al*., (2018) who announced that cuttlebone is a natural compound with a high level of CaCO3, it tends to be utilized and figured by various fillers as a marine natural anti-Osteoporosis.

Dogan and Okumus (2014) found that the capability of utilizing little bits of pulverized CB for xenogeneic unions advancing the recuperating of bone deformities in vivo. Also, Kloping *et al*., (2016) and Mansouri *et al*., (2018) reported that CB showed promissive effects on bone healing. Palaveniene *et al*., (2017) reported that the components of the CB material may supplant those in the mineral period of bone when utilized as a bone substitute material.

Hirasawa *et al*., (2001) demonstrated that eggshell calcium is a standout amongst the best wellsprings of Ca – eggshell Ca could have more noteworthy impacts to $CaCO_3$ on bone digestion. It propose that egg-shell Ca might be a powerful supplement in Ca digestion for individuals treated with vitamin D_3. Daengprok *et al*., (2003) announced that chicken eggshell contains around 1.0% lattice proteins not withstanding a noteworthy type of calcium carbonate (95%). Brun *et al*., (2013) and Swiatkiewicz *et al*., (2015) reported that calcium from crushed eggshell powder was absorbed easier than commercial $CaCO_3$ in the rat small intestine.

Crabshell fingernail skin is another competitor material for upgrading the recuperating and redesigning of bone. The nearness of beginning period collagen fiber groups with sprinkled mineral precious stones inside the main week recommends that crabshell shows osteogenic properties (Otto *et al*., 2012). Crabshell has been presented as one of the conventional solutions for the treatment (Rezakhani *et al*., 2014). Selenium is a basic piece of the human eating regimen and is found in crab shell (Brozmanová *et al*., 2010). Likewise, selenium has cell reinforcement and mitigating impacts (Akhuemokhan *et al*., 2013 and Makalani *et al*., 2017).

Chitosan is a fiber got from the shells of shellfish (Kato *et al*., 2003). Has centered on the bone-prompting action of chitosan and its utilization as a bone join material (Mukherjee *et al*., 2003). In light of an investigation of ovariectomized rats, Li *et al*., (1999) revealed that oral organization of low-atomic weight chitosan counteracts bone demineralization. Additionally, Klokkevold *et al*., (1996) suggested that it may improve the separation of osteoprogenitor cells and advance new bone development.

Exploratory and clinical examinations in postmenopausal women performed demonstrated various positive properties of eggshell powder. In vitro eggshell powder fortifies chondrocyte partition and tendon improvement and expands versatility and bone thickness and decreases torment and osteoresorption (Daengprok *et al*., 2003). Clinical and trial thinks about demonstrated that eggshell powder effect sly affects bone and ligament and that it is appropriate in the avoidance and treatment of osteoporosis (Rovenský *et al*., 2003).

Makai, (2002) and Tavangar, (2011) reported conceivable employments of eggshell (ES) as a calcium

supplement. In addition, different components in ES, for example, strontium and fluorine are known to effects bone digestion. It has been demonstrated that the bioavailability of calcium from ES is equivalent or genuinely superior to anything that of business calcium supplements. In light of this, we inspected techniques for enhancing the bioavailability of ES as another option to calcium supplementation, which may anticipate and weaken osteoporosis.

With respect to metabolic hormones, PTH, protein hormone discharged by the parathyroid organ, is a noteworthy controller of bone digestion and calcium homeostasis (Papavasiliou *et al*., 2003 and Lu *et al*., 2013). The present outcomes uncovered that rats experiencing osteoporosis brought about an exceptionally huge increment in PTH levels contrasted with the control gathering. The acquired outcomes are in accordance with Taguchi *et al*., (2006) and Zhu *et al*., (2012).

Narayana *et al*., (2012) announced that estrogen lack instigates bone resorption by discharging calcium into the extracellular space, which thus smothers PTH emission, calcitriol combination, and intestinal retention of calcium in cancellous bone prompting general bone misfortune and decimation of neighborhood design and decreased bone quality bringing about osteoporosis (Sachdeva *et al*., 2005 and Justesen *et al*., 2006).

Also, the present results denoted that supplementation with powder of (cuttlefish bone, crabshell and eggshell) significantly elevated serum T4 and decreased PTH. These findings were partially in accordance with those announced by Norazlina *et al*., (2010) and Dumic- Cule *et al*., (2014).

Gomez-Basauri (1998) and Ceylan and Scheideler (1999) considered eggshell powder with vitamin D3 could enhance bone mineral thickness without essentially expanding blood calcium levels. Any sort of eggshells (chicken, goose and duck) can be utilized, however it is best to utilize shells from flying creatures that get adjusted minerals in the eating routine. Sakai *et al*., (2017) detailed that eggshell calcium was more convincing in extending bone mass than calcium carbonate in postmenopausal Vietnamese women.

In the present study bone mineral (content and density) significantly ($P<0.05$) increased in rats fed basal diet and supplemented with powder of cuttlefish bone, crab shell and eggshell. Chang, (2003) showed that ESP was a legitimate calcium source practically identical or better than $CaCO_3$. Calcium is generally utilized as a marker for bone development as it assumes a crucial part in bone mineralization (Choi and Seo, 2013). These discoveries were like the past report by Al Mijan *et al*., (2014) nano powdered eggshell (NPES) lessened the bone misfortune prompted by ovariectomy in rats.

Chen *et al*., (2013), discovered that high – calcium in addition to vitamin D3 in diet plays an essential job in bone mineralization as it builds BMD thus can avoid osteoporosis. Satisfactory admission of calcium and vitamin D is essential for bone wellbeing (Gambacciani and Ciaponi, 2000). On the contrary, Agata *et al.,* (2013) proposed that low calcium consumption amid period of quick bone misfortune caused by estrogen inadequacy in ovariectomized rats may be one conceivable reason for bone loss. Kim *et al*., (2013) suggested the conceivable utilization of ES-CPP in avoiding or constricting the seriousness of postmenopausal osteoporosis.

Eggshell powder (ESP) is a characteristic wellspring of calcium and other nutritious. Eggshell powder contains a little measure of strontium that should anabolically affect bone AL Mijan *et al*., (2014). Schaafsma *et al*., (2002) indicated that solid late post-menopausal ladies with a sufficient Ca admission at benchmark may build BMD of the hip inside a year following supplementation with the chicken eggshell powder-enhanced supplement.

The investigation of Rovenský *et al*., (2003) and Ruff *et al*., (2009) announced that chicken eggshell powder (ESP) has been proposed as an alluring wellspring of calcium for human wellbeing to build bone mineral thickness in an elderly populace with osteoporosis. The study of Masuda, (2005) proposed that calcium-fortified foods containing eggshell could be used as a source of nutraceuticals.

A vitro study, carried out by Neunzehn *et al*., (2015) exhibited the high capability of the blend of eggshell particles and hyaluronan as essential segments for bone recovery and tissue designing. These results were in accordance with Krithiga and Sastry, (2011) and Bee, (2011) who reported that the utilization of chicken eggshell powder may be useful, it could expand bone thickness and diminish torment in patients with osteoporosis. Sakai *et al*., (2017) proposes the value of eggshell for the treatment of osteoporosis in elderly ladies.

Results of the present study showed that supplementation with powder of cuttlefish bone, crab shell and eggshell at the tested level significantly decreased the elevated levels of both serum ALT and AST compared to the positive control group. These results are agreed with Soliman, (2011) who showed that organization of CB diminished lipid peroxidation, enhanced cell reinforcement status and consequently keep the harming to the liver and spillage of its chemicals (AST and ALT). Rajalakshmy and Pharm, (2013) reported that a general decline in the exercises of serum AST and ALT of rats treated with sepia IE all through the analysis, when contrasted with control positive gathering. Also, oral organization of the marine mollusk (Gelonia eros) for 30 d diminished the activities of AST and ALT in rats (Yeh *et al*., 2012). Additionally, Ramasamy *et al*., (2014) found that rats treated with chitosan against CCl_4 poisonous quality indicated significantly decreased levels of ALT and AST exercises, total cholesterol, triglyceride and free unsaturated fat in plasma and tissue. These results were according to Makalani *et al.*, (2017) who reported that crab shell expel affected an imperative decline in serum levels of ALT and AST.

Eggshell membrane (ESM) may be useful for maintaining human health, especially with regards to liver cirrhosis. (Jiaa *et al*., 2013). These outcomes were in accordance with Jia *et al*., (2014) who found that dietary ESM displayed potential impacts on hepatic damage and fibrosis induced by CCl4 in rats.

Results of the present study showed that supplementation with powder of cuttlefish bone, crab shell and eggshell at the tested level significantly decreased the

elevated levels of lipid profile of osteoporotic rats. These outcomes were in accordance with Lee *et al*., (2000) who announced that the crab shell diminished triglyceride add up to cholesterol, LDL-c, atherogenic list were diminished and focus in plasma and liver, and expanded convergence of HDL-c. In this manner, the crab shell powder is accepted to show capacity of chitin or chitosan that consequences for lipid digestion and cholesterol reabsorption.

Chitin and its subordinates like chitosan and chit oligosaccharides demonstrated against tumor, hostile to microbial and hypoallergenic, antioxidant (Lin and Chou, 2004), calming and cholesterol-bringing down properties (Shields *et al*., 2003 and Kumar *et al*., 2004). Kim and Yoon, (2008) proposed that supplementation with chitosan oligosaccharide organization was compelling in the avoidance and change of the lipid level, lowering plasma cholesterol and chemical exercises in serum of hypercholesterolemic rats.

Jiaa *et al*.,(2013) and Jia *et al*., (2014) who indicating that ESM is a sheltered and common side-effect of egg preparing which has intense defensive biochemical capacities against liver damage and fibrosis. ESM may have a cholesterol cutting down effect that can cover liver harm, and at last liver fibrosis.

CONCLUSION

This study showed that powder of cuttlefish bone, crab shell and eggshell had an extraordinary potential part to be an elective source of calcium supplement particularly in rats experiencing osteoporosis on postmenopausal inferable from its absorbability and its capacity to counteract maternal bone loss by advancing bone arrangement and decreasing bone resorption. As powder of cuttlebone, crab shell and eggshell have been approved to be a sheltered and characteristic nourishment side-effects, the present examination not just gives data about the functional and nutritional accessibility of cuttlebone, crab shell and eggshell powdered, but additionally adds to the field of natural security, which is an expanding request in society. It is imperative to show and offer to general society the safeness and adequacy of this natural food products and also its remedial advantages on treating osteoporosis.

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الدورالوقائي المحتمل لمسحوق عظام السيبيا وقشرالكابوريا وقشرالبيض على الفئران المصابة بهشاشة العظام

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تتناقص كتلة العظام مع التقدم في السن ، خاصة بالنسبة للنساء بعد انقطاع الطمث بسبب انخفاض إفراز الإستروجين مع تناول كميات قليلة من الكالسيوم. أجريت هذه الدراسة لدراسة تأثير مسحوق عظم السيبيا ، قشور الكابوريا ، قشر البيض على الفئران التي تعاني من هشاشة العظام بعد سن اليأس. تم تقسيم عدد ٥٠ من اناث الفئران البالغة من سلالة الألبينو(٢٠٠ ± ١٠ جم) الي خمس مجموعات، المجموعة الاولي وهي المجموعة الضابطة السالبة وتم تغذيتها علي النظام الغذائي الأساسي فقط . وتم تغذية المجموعات الأربعة الأخرى (١٠ فئران لكل منها) على النظام الغذائي الأساسي المحتوي على ١٠٠ ملجم بريدنيزون أسيتات كمصدر للكورتيزون/ كجم من النظام الغذائي لمدة أسبوعين لاحداث هشاشة العظام. تم اختيارمجموعة واحدة منهم كمجموعة ضابطة موجبة ، وكانت المجموعات الثلاثة الأخرى تتغذى على وجبات بريدنيزون أسيتات تحتوي على مسحوق عظم السيبيا ، وقشرالكابوريا وقشرالبيض عند مستوى (٥٪.٢) على التوالي، تم جمع عينات الدم وعظام الفخذ لتقدير نسبة الهشاشة فى الدم والعظم. أشارات النتائج الي أن الوجبات الغذائية المدعمة بعظم السيبا ،قشور الكابوريا ، قشر البيض أدت الي حدوث ارتفاع معنوي ($P<0.05$) في مستوى الكالسيوم والفسفور. كانت هناك زيادات معنوية في هرمون الثيروكسين في الدم (T4) وانخفاض في هرمون الباراثيرويد (PTH). كما تم زيادة محتوى عظم الفخذ (BMC) والكثافة المعدنية للعظام (BMD). علاوة علي ذلك ، قد تحسنت وظائف الكبد بدرجة ذات دلالة احصائية ($P<0.05$) مقارنة بالمجموعة الضابطة الموجبة. وانخفضت مستويات الدهون في الدم.هذه النتائج تشير إلى أن تغذية الفئران التى تعانى من هشاشة العظام بعد سن اليأس على الوجبات الغذائية المدعمة بمسحوق عظم السيبيا ، وقشر الكابوريا ، وقشر البيض تحدث تاثير مضاد لهشاشة العظام . قد يكون هذا التأثير بسبب تعزيز تكوين العظام ، وتنظيم بعض الهرمونات التي تتحكم في الكالسيوم. لذلك توصى الدراسة الحالية باستخدام مسحوق عظم السيبيا ، قشر الكابوريا ، قشر البيض للوقاية من هشاشة العظام في النساء بعد سن اليأس.

الكلمات المفتاحية : هشاشة العظام ، بعد سن اليأس ، عظم السيبا ، السيبا ، قشر الكابوريا ، مسحوق قشر البيض ، كربونات الكالسيوم .