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Evaluation of Sibia, Sardine and Salmon fish as Anti-osteoporotic Effect in Female Rats

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Abstract

Fatty fish and fish bone have been used successfully in the traditional management of Osteoporosis. The present study was carried out to investigate the role of sibia, sardine and salmon fish as anti-osteoporotic effect in female rats.Fifty adult female albino rats were divided into five groups as follows:The first group (10 rats) were kept as negative control group and were fed only on a basal diet. The other four groups (10 rats each) were fed on a basal diet containing 100 mg Prednisone Acetate as a source of glucocorticoid/ kg diet for two weeks to induce osteoporosis, four rats were selected to insure induction of osteoporosis by DEXA scan then they were divided as follow:The second group was served as a positive control group. The third group was fed on a basal diet supplemented with salmon powder at the level of (5%).The fourth group was fed on a basal diet supplemented with sardine powder at the level of (5%).The fifth group was fed on a basal diet supplemented with sibia powder at the level of (5%).Results revealed that three type of fish especially sibia fish caused a significant decrease in serum urea, creatinine, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT)and parathyroid hormone. In addition, a significant increase in calcium andphosphorus, vitamin D,bone mineral density and bone mineral content.In conclusion, thethree types of fish especially sibia fish protect rats against osteoporosis through its vitamin D, calcium and phosphorus content. So, it may be beneficial to the human beings.

Keywords: Sibia, Sardine, Salmon, Prednisone Acetateas, osteoporotic.

Introduction

Osteoporosis (OP) is defined as a condition depicted by low bone mass and microarchitectural crumbling of bone tissue, with a subsequent augmentation resulting increment in bone delicacy and defenselessness to split ***(Mansour et al., 2015).*** Osteoporosis is regularly called a quiet ailment of maturing in light of the fact that bone misfortune happens without symptoms until micro architectural weakening and bone break happens **(*Deyhim et al., 2006).***Osteoporosis has turned into a noteworthy wellbeing risk infection as of late, besetting more than 2000 million individuals around the world **(*Rachner et al., 2011).***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).***

Based on different studies in Egypt, it has been calculated that 53.9% of postmenopausal women have osteopenia and 28.4% have OP. On the other hand, 26% of men have osteopenia and 21.9% have OP***. (Taha., 2011). Salem et al., (2000)*** reported that 16.7% of 1190 Egyptian menopausal females had lumbar OP. OP awareness has increased in the last 20 years with the introduction of several effective pharmaceutical agents for treating those at high risk ***(Barrett-Connor et al., 2012).***

Nutrition has an important and complex role in maintenance of good bone ***(Sophocleous et al., 2003).*** Adequate calcium (Ca) intake is considered as one of the important nutritional factors to

establish peak bone mass ***(Weaver et al., 2016).*** Vitamin D which is necessary for optimal dietary Ca absorption should be sufficient as well ***(Kanchiku et al., 2017).*** Researchers are looking for natural materials as anti-osteoporotic effect and more desirable ***(Sarkar and Lee., 2015).***

The findings indicated that frequency of fish food consumption was independently and significantly associated with OP. The prevalence of OP was less frequent in Chinese men preferring fish food habits ***(Li et al., 2017).*** Fish bones may be a useful and well absorbed calcium source. Due to the high mineral content of the bone fraction, salmon bones can be well suitable as a natural calcium and phosphorus source ***(Malde et al., 2010).Jung et al., (2006)*** proved the beneficial effects of fish-meal in preventing Ca deficiency due to increased Ca bioavailability by Fish-bone peptides intake.

Some studies have indicated that a diet rich in n-3 polyunsaturated fatty acids (n-3PUFAs) is associated with higher bone mineral density (BMD) and decreased bone loss ***(Cooper et al., 2011).*** Fish, which constitutes a major food group in diets throughout the world, contains high levels of n-3PUFAs, high-quality protein, and vitamins and essential minerals, and thus may be beneficial to bone health ***(Xia et al., 2012).***

Cuttlebone (CB), otherwise called cuttlefish spine, is an inner shell of marine creatures known as cuttlefish (Sebia), having a place with the phylum Mollusca, class Cephalopod, arrange Sepiidae. CB is recorded in the Chinese Pharmacopeia; in conventional Chinese solution ***(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 ***(Kannan et al., 2007). Hemmatti et al., (2018)*** recommend that common cuttlefish bone may likewise be utilized as a bone substitute without handling its calcium carbonate to

hydroxyapatite. Since cuttlebone is a characteristic compound with a high level of $CaCO_3$, it can be utilized and planned by various fillers as a marine common hostile to Osteoporosis sedate.

The aim of this study was to evaluate the effect of sibia, sardine and salmon fish supplementation as anti-osteoporotic in female rats.

Materials and methods

Materials:

Sibia, Sardine and Salmon fish: Egyptian sibia, sardine and salmon fish were obtained from Local market.

Rats: Fifty adult female albino rats of Sprague Dawley strainweighing (170 ± 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 analysis were purchased from Biodiagnostic Company for Pharmaceutical and Chemicals, Dokki, Egypt.

Prednisone Acetateasas source of glucocorticoid was obtained from Pharmacy, Cairo, Egypt.

Methods:

This experiment was carried out at the Animal House of Agriculture Research Center, Gzia, Egypt.

Chemical composition: Protein, calcium, phosphorus, magnesium, zinc and vitamin D content in sibia, sardine and salmon were determined according to the official methods ***(A.O.A.C., 2005).***

Sibia, Sardine and Salmon fish: were dried in oven for 15 minutes at 40°C, grinded up in a grinder and crushed in a ceramic mortar.

Preparation of Sibia, Sardine and Salmon bone Powder: blocks fish bone was removed from sibia, sardine and salmon,each one gently washed with distilled water, and dried in free air in order to lose its smell. After drying, the clean fish bone was powdered.

Experimental Animal Design:

Fifty adult female albino rats were housed in well aerated cages under hygienic condition and were fed on a basal diet for one week for adaptation. After this week the rats were divided into five groups as follows:

The first groups (10 rats) were kept as negative control group and were fed only on a basal diet. The other four groups (10 rats each) were fed on a basal diet containing 100 mg Prednisone Acetate as a source of glucocorticoid/ kg diet for two weeks to induce osteoporosis ***(Liao et al., 2003),*** four rats were selected to insure the induction of osteoporosis by DEXA scan then the osteoporotic group were divided as follow:

The second group was served as a positive control group and fed on a basal diet.

The third group was fed on a basal diet supplemented with sibia powder at the level of (5%).

The fourth group was fed on a basal diet supplemented with sardine powder at the level of (5%).

The fifth group was fed on a basal diet supplemented with salmon powder at the level of (5%).

All rats were observed each day. Their feed intake were determined daily and body weights were obtained every week. Feed Efficiency Ratio and body weight gain were calculated according to the method of ***(Chapman et al., 1959).*** At the end of the experiment (8 weeks) the rats were fasted for 12 hour, and then were sacrificed under ether anesthesia. Blood samples were collected from medial canthus of the eyes of rats by means of fine capillary glass tubes in a centrifuge tube without any anticoagulant and were centrifuged for 20 minutes at 3000 r.p.m. to obtain the serum which was stored at -20°C until used for subsequent analysis.

Biochemical analysis:

Serum 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 immune sorbent assay (ELISA), according to ***(Norazlina et al., 2010).***Serum vitamin D was determined by enzyme linked immunosorbent assay (ELISA) according to the method described by ***(Zerwekh,2008).***Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured according to ***(Reitman and Frankel.,1957).***Serum urea according to***(Kaplan, 1984),***creatinine according to ***(Murray, 1984),*** and uric acid was measured according to ***(Patton and Crouch, 1977).***Measurement of bone mineral density (BMD) and bone mineral content (BMC): The BMD and BMC of femur bone were estimated in anesthetized rats using dual energy X-ray absorptiometry (DEXA) according to ***(Gao et al.,2013).***

Statistical analysis:

The obtained results were analyzed according to SPSS program. ANOVA test was used to compare results among groups and $P<0.05$ was consider being significant ***(SPSS, 1986).***

Results and discussion

Body weight gain and food efficiency ratio (BWG% and FER):

Table 1 indicated that significant differences ($P \leq 0.05$) of BWG% between the negative (-) control group and tested groups. Rats in groups 2, 3 and 4 had significantly ($P \leq 0.05$) BWG% differedcompared to those of the negative (-) control. While the mean value of BWG% in the group 5 recorded non-significant change as compared to the negative (-) control group. BWG% in groups 2, 3, 4 and 5 were: 16.52 ± 1.50; 21.80 ± 1.22; 23.15 ± 1.48 and 30.61 ± 1.12, respectively vs. 41.05 ± 1.43 for the negative (-) control.Also BWG% in groups 3,4 and 5 showed significant variations ($P \leq 0.05$) as compared to the control “group 2” (21.80 ± 1.22; 23.15 ± 1.48 and 30.61 ± 1.12, respectively vs. 16.52 ± 1.50.Data in this table recorded non-significant changes in BWG% between all treated groups.

The tabulated data revealed that osteoporocess significantly ($P \leq 0.05$) altered the FER in rats. Rats in groups1, 3, 4 and 5 had significantly ($P \leq 0.05$) differed to those of the positive (+) control group. The mean values of FER for groups 1, 3, 4 and 5 were: 0.16 ± 0.137; 0.13 ± 0.004; 0.14 ± 0.007and 0.15 ± 0.009, respectively vs. 0.05 ± 0.003 for the positive (+) control. All treated groups showed non-significant changes in FER as compared to the negative control group.

These results showed significant decrease ($P \leq 0.05$) in body weight gain and FER of control positive (suffering from osteoporosis) compared to control negative (healthy rat).These results are in agreement with the findings of ***Oliveira et al., (2010)*** and ***Soltan, (2013) ,*** who reported that glucocorticoids induced a significant lower weight gain (-9.7%) compared to control group. Meanwhile, rat's administration diet supplemented with 20% sardine fish recorded significant increase in final body weight and body weight gain

compared to positive control group. These results are accordance with the results obtained by ***Gouda,(2012),*** who reported that control positive (osteoporotic rats) showed significant decrease in body weight gain. The decrease in body weight gainmay be due to high dose from cortisone and long-term.

These results are agreed with***EL- Sayed, (2017)*** showed that Rats which were fed on basal diet containing fish as source of protein recorded higher feed intake and BWG value than other treated groups.Feed intake and BWG was increase in the negative control group than that of positive control group. On the other hand treated rat with fish illustrated significant decrease inBWG% when compared them with negative control group.

Daidj and Lamri-Senhadji, (2021) observed that the sardine by-product oil and sardine fillet oil groups showed similar significant decreases in body weight, weight gain, and food intake in the obese control group. Moreover, obese rats consuming fillet and by-product sardine oils had negative FER values compared with control obese and normal rats; FER ratios indicate the animal's ability to transform energy consumed into body mass ***(De Sibio et al., 2013).*** Fatty fish such as sardines help the body burn fat in addition to generating a feeling of satiety. This satietogenic effect may explain the decrease in food intake in obese groups consuming sardine oil and explains the negative FER values.

Calcium and phosphorus:

Table 2indicated that rats in groups1, 3, 4 and 5 had significant ($P \leq 0.05$) differences of serumcalciumas compared to positive (+) control group. Themean values of calcium for groups 1, 3, 4 and 5 were: 11.71 ± 0.12; 12.18 ± 0.37; 11.74 ± 0.13and 13.08 ± 0.27, respectively vs. 8.16 ± 0.11 for the positive (+) control also group 5 recorded significant ($P \leq 0.05$) increase in serum calcium, as

compared to the negative (-) control (13.08 ± 0.27vs. 11.71 ± 0.12respectively) and no significant differences ($P \leq 0.05$) were recorded of serumcalciumbetween groups 1, 3 and 4.

Also the data revealed that rats in groups 1, 3, 4 and 5had significantly ($P \leq 0.05$) levels of serumphosphorus that differed to those of the positive (+) control group. The mean values of phosphorus for groups 1,3,4 and 5 were: 13.40 ± 0.21; 12.18 ± 0.37; 12.72 ± 0.13and 12.91 ± 0.17, respectively vs. 9.38 ± 0.15 for the positive (+) control, also groups 3showed significant decrease ($P \leq 0.05$) as compared to the negative (-) control (12.18 ± 0.37; 12.72 ± 0.13 vs. 13.40 ± 0.21 respectively) and no significant differences ($P \leq 0.05$) were recorded of serumphosphorusbetween groups 1 ,4 and 5.

De Nijs ., (2008) stated that bone loss is one of the most important side effects of glucocorticoid use, even in low doses. The main effect of glucocorticoids on bone is inhibition of osteoblast function, leading to a decrease in bone formation. Also nongenomic effects (mediated by glucocorticoid interactions with biological membranes, either through binding to membrane receptors or by physicochemical interactions) may have a role in the pathogenesis of glucocorticoid-induced osteoporosis (GIOP). in addition secondary hyperparathyroidism induced by negative balance of calcium due to inhibition of absorption and increase of excretion is an important systemic mechanism of GIOP ***(Kaneko and Kawai .,2011).***

The anti-inflammatory effects of *n*-3 FAs are well-known. Recently, a promising association between higher *n*-3 FA intake and improved bone turnover markers and bone mineral density (BMD) in humans has been reported ***(Coetzee et al., 2007).***One of the mechanisms by which dietary n-3 fatty acids reduce bone loss in ovariectormized mice is by inhibition of osteoclast generation and activation ***(Matsushita et al.,2008).***

McManus et al., (2010) stated that “seafood is a rich source of both calcium and Vitamin D, important bone-building micronutrients.High intake of sea fish is independently associated with greater bone mass and lower osteoporosis risk in women, especially those consuming more than 250 grams per week of seafood. An abundance of nutrients can be found in mackerel, which include calcium, potassium, selenium and magnesium These nutrients help maintain proper function of the body and are important for the heart, bones and teeth, nerves and muscles, and proper metabolic function. Mackerel is a good source for vitamins like niacin (vitamin B3), choline, folate, vitamin E, vitamin D, vitamin A, vitamin K, vitaminC and vitamin B12.

Calcium sources for these supplements include calcium carbonate ores, animal skeletons, seashells and crustaceans. Therefore, other resources including marine resources, have gained attention due to their high safety and biological activity in recent years ***(Martiniakova et al., 2022).*** Similar results have been obtained in the study of ***Brennan et al. (2017)*** using calcium-rich marine multimineral complex which significantly preserved trabecular bone microarchitecture and slowed the onset of bone loss in comparison with calcium carbonate.

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 report ***(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).***

The previous results were in line with***Soltan, (2013)***who showed that all treatment showed significant increase ($p \leq 0.05$) in serum calcium and phosphorus. These results may be due to sardine fish have higher calcium content and easy absorbed by body. ***Larsen et al. (2000)*** reported calcium from fish would be easily absorbed by body and the intake of small fish with bones could increase calcium bioavailability. ***EL- Sayed, (2017)*** observed that fish meat caused a significant increase ($P \leq 0.05$) in serum Ca and P, when contrasted with the positive control group.

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. ***Negm, (2018)*** showed that feeding rats with powder of cuttlefish bone caused a significant increase ($P \leq 0.05$) in serum Ca and P, when contrasted with the positive control group. The author in this study recommended using powder of cuttlebone for postmenopausal women suffering from osteoporosis.

Parathyroid hormone and vitamin D:

Table 3 revealed that osteoporocess significantly ($P \leq 0.05$) altered the levels of parathyroid hormone and Vitamin D in female rats.Parathyroid hormone increasedsignificantly in rats of group 2 as compared to negative (-) control group. Rats in groups 1, 3, 4 and 5 had levels of parathyroid hormone that differed significantly ($P \leq 0.05$) from those of the positive (+) control. Themean values of parathyroid hormone for groups 1, 3, 4 and 5 were: 18.94 ± 1.01; 24.21 ± 1.43; 23.17 ± 1.95 and 21.50 ± 1.85, respectively vs. 26.50 ± 1.55 for the positive (+) control.

On the other hand Vitamin D decreasedsignificantly in rats of group 2 as compared to negative (-) control group. Rats in groups 1, 3, 4 and 5 had levels of Vitamin D that differed significantly ($P \leq$

0.05) from those of the positive (+) control. Mean values of Vitamin Dfor groups 1, 3, 4 and 5 were: 25.15 ± 0.12; 17.35 ± 0.25; 18.94 ± 0.36 and 20.15 ± 0.56, respectively vs. 15.15 ± 0.24 for the positive (+) control. All treated groups showed significant increase in vitamin D as compared to the positive (+) control group.

With respect to metabolic hormones, PTH, protein hormone discharged by the parathyroid organ, is a noteworthy controller of bone digestion and calcium homeostasis ***(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 ***Negm, (2018).***

Fish and seafood are also sources of n-3 fatty acids, and AA in particular may exert protective effects on bone ***(De Caterina et al., 2000 and Delerive et al., 2002).*** Current recommendations by the National Osteoporosis Foundation do not explicitly recommend fish consumption to increase the intake of long-chain PUFAs, but rather recommend the consumption of certain fish types to increase the intake of calcium and vitamin D ***(NOF, 2010).***

Soltan, (2013) indicated that injection of rats with cortisone caused osteoporosis. Osteoporosis caused decreases in body weight gain, calcium (Ca) and phosphorous (P) concentration in serum and femur bone, Boon mineral density (BMD), Boon mineral concentration (BMC) and increases in weight of liver and Parathyroid hormone (PTH) compared to negative control group (healthy rat). However, supplementation diet of osteoporotic rat with 20% dried sardine fish lead to increases in body weight gain, serum Ca, P in serum and femur, BMD, BMC and decreases in weight of liver and PTH.

The previous results were agreement with ***Davey andMorris, (2005)*** showed that salmon calcitonin (sCT) treatment produces an immediate decrease in ionized calcium in sham and ovx rats. The sCT induced hypocalcemia was more pronounced in the ovx rats and was accompanied by an increase in PTH secretion.Of interest was the observation that PTH levels were elevated in ovariectomized rats compared with those in sham rats for the same ionized calcium range.

Abaza et al. (2005) reported serum PTH was significantly lower in the osteopenic group than osteoporotic group. Increased PTH recreation contributes to an increase in bone resorption and osteoporosis ***(MuCkane, et a., 1977)*****,** over production of PTH lead to an increase in bone resorption compared with bone formation and contributes to general skeleta demineralization. ***Soltan, (2013)*** showed that rats feeding on sardine fish revealed significantly increased in Vitamin D and significantly decreased in PTH. These results are in agreement with those obtained by ***Micheal et al. (2011)*** who reported that supplementation diet with calcium showed a decrease in PTH. These results are in agreement with the findings of ***Farina et al. (2011)*** who reported that women with high intake of dark fish (Salmon) have protective effect of bone loss because the increase the intake of calcium and vitamin D, increasing the calcium intake preventing bone loss, possibly due to the effect of calcium in suppressing PTH secretion. ***Negm, (2018)*** denoted that supplementation with powder of cuttlefish bone significantly decreased PTH.

Liver enzymes (ASTand ALT):

Table 4 revealed that osteoporocess significantly ($P \leq 0.05$) altered the levels of AST in female rats. Rats in groups 1, 3, 4, 5 had levels of serum AST that differed significantly ($P \leq 0.05$) from those of the positive (+) control.Themean values of AST for groups 1, 3, 4 and 5 were: 102.00 ± 0.61; 110.21 ± 0.88; 112.34 ± 0.98 and 105.54

± 0.57, respectively vs. 189.87 ± 0.82 for the positive (+) control. But no differences in the mean values of AST were found between groups. (groups1, 3, 4 and 5). The data in this table showed that the mean values for ALT were not significantly ($P \leq 0.05$) altered between the groups (3, 4 and 5),also the data recorded that groups 3, 4 and 5 differed significantly($P \leq 0.05$) from negative (-) control (207.33 ± 0.62; 217.87 ± 0.85 and 208.00 ± 0.78, respectively vs. 129.56 ± 0.21).The mean values of ALT for groups 3, 4 and 5 decreased significantly ($P \leq 0.05$) as compared to the positive (+) control (207.33 ± 0.62; 217.87 ± 0.85 and 208.00 ± 0.78, respectively vs. 243.11 ± 0.93).

The current results denoted the effect of osteoporocess on liver enzymes, such effect was lowred by three type of fish especialy sibia fish is significantly ($P \leq 0.05$) lowered the increments in liver enzymes levels.

The previous results were in line with ***Xu et al.,(2012)*** who reported that drug-induced liver injury is mostly caused by use of antibacterial and glucocorticoids. ***Drake et al.,(2010)*** also revealed that prenatal glucocorticoids overexposure in rats increases hepatic lipid accumulation with steatosis. ***Saka et al. (2011*)** showed that elevation of transaminase activities, in particular aspartate aminotransferases (AST), is linked to liver damage. Moreover, experimental studies have shown beneficial effects of diets rich of PUFAs on transaminase (asparate aminotrans-ferase and AST) activities ***(Ketsa and Marchenko, 2014).***

In this regard, ***Jackson et al,.(2008)*** also found that serum ALT,AST, sorbitol dehydrogenase and glutamate dehydrognase increased after 2,3 and 4 days of dexamethasone ,a potent synthetic member of the glucocorticoids class of steroid drugs. ***Chiofalo et al., (2012)*** found that the alkaline phosphatase responsible for maintenance of hematic levels of calcium and phosphorus through

the bone turnover. A rise in serum alkaline phosphatase (SAP) activity, urinary hydroxyproline and urinary calcium to creatinine ratio have been linked with collagen degradation, bone resorption and osteoporosis ***(Stepan, 1999)*** .The beneficial effects of n-3 fatty acids appear to be associated with down regulation of PGE2 formation with a net enhancement of bone formation ***(Watkins et al.,2001).***

Soltan, (2013), who reported that liver function (AST, ALT and ALP) of osteoporotic rats revealed significantly increase compared to negative control group. The levels of AST, ALT and ALP decreased gradually with increasing sardine fish. These results are agreed with***EL- Sayed, (2017)*** found that supplementation with fish meat which were added to the diet of osteoporotic rats led to decrease the mean value of AST, ALT and ALP significantly were noticed in comparison to control positive group.

Daidj, and Lamri-Senhadji (2021) suggest that the oils from both sardine by-products and fillets do not have toxic effects in rats, since hepatic function indicated by transaminase activity was not negatively altered (a reduction was observed).

Additionally,***Soliman, (2011)*** who showed that organization of sepia 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 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).Negm, (2018)*** found that supplementation with powder of cuttlefish bone significantly decreased the levels of both serum ALT and AST contrasted with the positive control group.

Kidney Functions:

Table 5 revealed that osteoporocess significantly ($P \leq 0.05$) altered the Kidney functions in rats. Rats in groups 2, 3, 4 and 5 had significantly ($P \leq 0.05$) levels of serum Urea differedcompared to those of the negative (-) control. Urea in groups 2, 3, 4 and 5 were: 71.66 ± 0.60; 69.04 ± 0.20; 69.16 ± 0.74 and 67.66 ± 0.10, respectively vs. 45.68 ± 0.38 for the negative (-) control.But this groups (2, 3, 4 and 5) showed no significant differences ($P \leq 0.05$) between each other.

Rats in groups 1, 3, 4 and 5 had levels of serum creatinine that differed significantly ($P \leq 0.05$) from those of the positive (+) control. Themean values of Creatinine for groups 1, 3, 4 and 5 were: 0.41 ± 0.05; 0.48 ± 0.02; 0.44 ± 0.05 and 0.40 ± 0.04, respectively vs. 0.63 ± 0.03 for the positive (+) control. But no differences were found between all treated groups and the negative (-) control group.

The means value of serumuric acid followed the same pattern of creatinine. Levels of Uric Acid were (1.13 ± 0.12; 1.26 ± 0.12 ;1.26 ± 0.13 and 1.10 ± 0.33 respectively vs. 1.70 ± 0.11) for the positive (+) control.

Patients with chronic kidney failure are advised to consume a diet with modest protein restriction in order to limit the development of toxic nitrogenous metabolites, uremic symptoms and other metabolic complications ***(Group,2009).*** However, information is lacking in regard to whether different dietary proteins may have dissimilar impact on kidney function, and it is of interest that intake of fish has been associated with reduced risk of developing kidney disease ***(Gopinath, et al., 2011).***

The previous results were in line with ***EL-Sayed, (2017)*** who the finest results of the mean values for serum uric acid, urea nitrogen and creatinine for the group which fed on diet containing fish as source of protein.In a randomized trial in 40 patients with osteoporosis, subjects taking a supplement rich in n-3 PUFA showed better calcium absorption and increased markers of bone formation, while those taking a placebo showed no improvement ***(van et al.,1995).***

Drotningsvik et al., (2019) showed that dietary consumption of salmon led to lower urine concentrations of several established renal function markers in Zucker fa/fa rats, indicating a better kidney function, whereas salmon intake did not affect renal function markers in Long-Evans rats. We also found that urinary concentrations of creatine, as well as plasma concentrations of creatine were higher in rats fed salmon, and may thus be useful as biomarkers of salmon intake in rats. Concentrations of kidney function markers were lower after salmon intake only in rats.

Bone mineral density (BMD) and bone mineral content (BMC):

Table 6revealed that osteoporocess significantly ($P \leq 0.05$) altered the bone mineral density (BMD) and bone mineral content (BMC) in female rats.

Rats in groups 1, 3, 4 and 5 had bone mineral density (BMD) that differed significantly ($P \leq 0.05$) from those of the positive (+) control. Mean values of bone mineral density (BMD) for groups 1, 3, 4 and 5 were: 0.181 ± 0.010; 0.122 ± 0.021; 0.138 ± 0.005 and 0.163 ± 0.007, respectively vs. 0.076 ± 0.011 for the positive (+) control.

Also rats in groups 1, 4 and 5 had bone mineral content (BMC) that differed significantly ($P \leq 0.05$) from those of the positive (+) control. Mean values of bone mineral content (BMC) for groups 1, 4 and 5 were: 0.232 ± 0.004; 0.211 ± 0.008 and 0.228 ± 0.006,

respectively vs. 0.201 ± 0.012 for the positive (+) control. But no significant differences ($P \leq 0.05$) were found between group 2 and 3 for bone mineral content (BMC).

The Glucocorticoid has harmful effects on bonedensity ***(Sedo Sarkis et al., 2012).*** Glucocorticoid induced osteoporosis and leads to a suppression of bone formation by decrease thenumber and function osteoblast and induced bone loss ***(DiMunnoand Delle Seide, 2006).*** There are many reported to decrease BMD andBMC in osteoporosis disease ***(Wu et al., 2001 and Matsumoto et al., 2002).***These results are in agreement with the findings of ***Soltan, (2013)*** showed that the mean BMD and BMC of osteoporotic rats fed diet supplemented with 20 %sardine fish .The increases in BMD and BMC due to increases calcium and phosphorus in diet lead to increases ostoblast cell which lead to increases in bone formation. The previous results were agreement with ***Farina et al., (2011)*** observed that Fish consumption may protect against bone loss. Also, ***Luo et al., (2015)*** showed that salmon calcitonin treatment was effective in delaying the process of the disc degeneration in OVX rats. ***Negm, (2018)*** showed that bone mineral (content and density) significantly ($P \leq 0.05$) increased in rats fed basal diet and supplemented with powder of cuttlefish bone.

Table (1): Effect of Sibia, Sardine and Salmon fish on body weight gain and food efficiency ratio (BWG% and FER) in Female Rats withosteoporocess.

Parameters / Groups	BWG%	FER
G1: Control (-ve)	41.05 ^{a} ±1.43	0.16 ^{ab} ±0.137
G2: Control (+ve)	16.52 ^{c} ±1.50	0.05 ^{c} ±0.003
G3: 5% Salmon	21.80 ^{bc} ±1.22	0.13 ^{ab} ±0.004
G4: 5% Sardine	23.15 ^{bc} ±1.48	0.14 ^{ab} ±0.007
G5: 5% Sibia	30.61 ^{ab} ±1.12	0.15 ^{b} ±0.009

Values are expressed as means ± S

Values at the same column with different letters are significantly different at P≤0.05.

Table(2): Effect of Sibia, Sardine and Salmon fish on levels of serum calcium and phosphorusin Female Rats with osteoporocess.

Parameters / Groups	Ca	P
G1: Control (-ve)	11.71b ±0.12	13.40a ±0.21
G2: Control (+ve)	8.16c ±0.11	9.38c ±0.15
G3: 5% Salmon	12.18b ±0.37	12.18b ±.37
G4: 5% Sardine	11.74b ±0.13	12.72ab ±-.13
G5: 5% Sibia	13.08a ±0.27	12.91a ±0.17

Values are expressed as means ± SE.

Values at the same column with different letters are significantly different at P≤0.05.

Table (3): Effect of Sibia, Sardine and Salmon fish on the levels of parathyroid hormone and Serum vitamin D in Female Rats withosteoporocess.

Parameters / Groups	PTH pg/ml	Vitamin D ng/ml
G1: Control (-ve)	18.94^{c} ±1.01	25.15^{a} ±0.12
G2: Control (+ve)	26.50^{a} ±1.55	15.15^{d} ±0.24
G3: 5% Salmon	24.21^{ab} ±1.43	17.35^{c} ±0.25
G4: 5% Sardine	23.17^{b} ±1.95	18.94^{bc} ±0.36
G5: 5% Sibia	21.50^{bc} ±1.85	20.15^{b} ±0.56

Values are expressed as means ± SE.

Values at the same column with different letters are significantly different at P≤0.05.

Table (4): Effect of Sibia, Sardine and Salmon fish on Liver Enzymes in Female Rats withosteoporocess.

Parameters / Groups	AST	ALT
G1: Control (-ve)	102.00^{b} ±0.61	129.56^{c} ±0.21
G2: Control (+ve)	189.87^{a} ±0.82	243.11^{a} ±0.93
G3: 5% Salmon	110.21^{b} ±0.88	207.33^{b} ±0.62
G4: 5% Sardine	112.34^{b} ±0.98	217.87^{b} ±0.85
G5: 5% Sibia	105.54^{b} ±0.57	208.00^{b} ±0.78

Values are expressed as means ± SE.

Values at the same column with different letters are significantly different at P≤0.05.

Table(5): Effect of Sibia, Sardine and Salmon fish on Kidney functionsin Female Rats withosteoporocess.

Parameters / Groups	Urea	Creatinine	Uric Acid
G1: Control (-ve)	45.68^{b} ±0.38	0.41^{b} ±0.05	1.13^{b} ±0.12
G2: Control (+ve)	71.66^{a} ±0.60	0.63^{a} ±0.03	1.70^{a} ±0.11
G3: 5% Salmon	69.04^{a} ±0.20	0.48^{b} ±0.02	1.26^{b} ±0.12
G4: 5% Sardine	69.16^{a} ±0.74	0.44^{b} ±0.05	1.26^{b} ±0.13
G5: 5% Sibia	67.66^{a} ±0.10	0.40^{b} ±0.04	1.10^{b} ±0.33

Values are expressed as means ± SE.

Values at the same column with different letters are significantly different at P≤0.05.

Table (6): Effect of Sibia, Sardine and Salmon fish on bone mineral density (BMD) and bone mineral content (BMC) in Female Rats withosteoporocess.

Parameters / Groups	BMD	BMC
G1: Control (-ve)	0.181^{a} ±0.010	0.232^{a} ±0.004
G2: Control (+ve)	0.076^{f} ±0.011	0.201^{c} ±0.012
G3: 5% Salmon	0.122^{d} ±0.021	0.209^{c} ±0.009
G4: 5% Sardine	0.138^{c} ±0.005	0.211^{b} ±0.008
G5: 5% Sibia	0.163^{b} ±0.007	0.228^{ab} ±0.006

Values are expressed as means ± SE.

Values at the same column with different letters are significantly different at P≤0.05.

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تقييم تأثير سمك السيبيا والسردين والسلمون كمضادات لهشاشة العظام في إناث الفئران

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الملخص العربى

تم استخدام الأسماك الدهنية وعظام السمك بنجاح في العلاج التقليدي لهشاشة العظام. الهدف من الدراسة هو معرفة دور أسماك السيبيا والسردين والسلمون في التأثير المضاد لهشاشة العظام في إناث الفئران ، تمت التجربة على خمسين من اناث الفئران البالغة وتم تقسيمهم خمس مجموعات على النحو التالي: المجموعة الأولى:سلبية (10 فئران) وهى المجموعة الضابطة وتم تغذيتها فقط على النظام الغذائي الأساسي. وتم تغذية المجموعات الأربع الأخرى (10 فئران لكل منها) على نظام غذائي أساسي يحتوي على 100 مجم بريدنيزون أسيتات كمصدر للجلوكوكورتيكويد / كجم غذاء لمدة أسبوعين للحث على هشاشة العظام ، تم اختيار أربعة فئران لضمان حدوث هشاشة العظام عن طريق مسح(DEXA) وتم تقسيمهم على النحو التالي: المجموعة الثانية كانت بمثابة مجموعة ضابطة إيجابية. المجموعة الثالثة تم تغذيتها على علف مدعم بمسحوق السلمون بنسبة (5٪) ، المجموعة الرابعة تغذت على علف مدعم بمسحوق السردين بنسبة (5٪) ، المجموعة الخامسة تم تغذيتها على علف مدعم بمسحوق السيبيابنسبة (5٪) وأظهرت النتائج أن الثلاثة أنواع من الأسماك خاصة أسماك السيبيا أدت إلى انخفاض معنوي في مصلاليوريا والكرياتينين وحمض اليوريكوAST و ALT وهرمون الغدة الجار درقية. بالإضافة إلى زيادة كبيرة فى الكالسيوم والفوسفور وفيتامين د وكثافة المعادن في العظام والمحتوى المعدني للعظام. وفي الختام ، هناك ثلاثة أنواع من الأسماك خاصة أسماك السيبيا تحمي الفئران من هشاشة العظام من خلال محتواها من فيتامين د والكالسيوم والفوسفور. لذلك ، يتوقع أن يكون لها آثر وقائي على الإنسان.

الكلمات المفتاحية : سيبيا ، سردين ، سلمون ، بريدنيزون اسيتات ، هشاشة العظام.