INTRODUCTION:

Osteoporosis a systemic skeletal disease characterized by low bone mass density and microarchitectural deterioration of bone tissue, is a global public health problem currently affecting more than 75 million people worldwide **(Schuiling et al.,2011)**.Eighty percent of people who suffer from osteoporosis are women **(Chang et al.,2009)**. The type of osteoporosis which is associated with ovarian hormone deficiency following menopause (postmenopausal osteoporosis) is so far the most common cause of age-related bone loss ,but any estrogen deficiency state also increases the risk of osteoporosis **(Arjmandi et al., 1996).**

There are several risk factors progression to osteoporosis. Among the more important of these are lack of exercise, mineral and vitamin deficiencies, excessive protein intake, excessive consumption of coffee, cola drinks, smoking, salt intake, certain medications (steroids such as prednisone, thyroid medications ,antidepressants and hyperparathyroidism **(Kado .,2002).**

There are two main types of cells involved in the process of bone formation, osteoblasts, which promote the formation of new bone structure by increasing calcium content, and osteoclasts, which promote the resorption (demineralization of old bone) by releasing calcium into the blood circulation. The hormone calcitonin promotes the transfer of calcium into the bones, while parathyroid hormone (PTH) promotes the release of calcium from the bones **Wright ., (2006).**

Glucocorticoids are effective anti inflammatory agents, but prolonged use results in many adverse effects and induce rapid bone loss and increase the risk for osteoporotic fractures. Glucocorticoids inhibit the function of mature osteoblasts and suppress the synthesis of insulin-like growth factor-I, an agent that enhances bone formation. Glucocorticoids alter the growth hormone/insulin-like growth factor axis in cartilage and, as a consequence, suppress linear growth **Klein.,(2004).**

Phytoestrogens perform their anti osteoporotic effect by stimulating osteoblastic activity through an estrogen receptor mediated action , or by increasing the production of insulin like

growth factor-1 (IG-F) which is known to enhance osteoblastic activity **Choi et al.,(2001).**

Garlic oil has numerous uses in the world today, its uses include the flavouring like salads and sauces**(Chadha., 2001).** The regular consumption of garlic oil can reduce blood pressure, prevent heart disease including atherosclerosis, high cholesterol and cancer**(Andreatta et al., 2005**). Garlic oil is an effective antibiotic, anti-viral, anti-fungal agent, which could be used to prevent nausea, diarrhea, ease coughs, even treatment in conditions such as malaria and cholera probably an immune system enhancement, some studies have found lower rates of certain types of cancer in people **Turner., (2004).**

The n−3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are found in fish and fish oils **(Vanek and Connor.,2007).** In a study of premenopausal women, those with the highest bone mineral density (BMD) scores consumed more fish and shellfish, fruit, mushrooms, and dark-yellow vegetables than those women with greater intake of meat, fats, and oils **Okubo,(2006).**

The aim of the present study is to evaluate the effect of garlic oil and dried fish meat on glucocorticoid-induced osteoporosis in rats.

MATERIALS AND METHODS:

Materials:

- Casein, vitamins, minerals, cellulose and choline chloride were purchased from El-Gomhoreya Company, Cairo, Egypt.
- Forty two female albino rats (Sprague Dawley Strain) were obtained from Helwan farm.
- Mackerel fish and garlic were purchased from local market , Cairo, Egypt.
- Prednisone acetate was purchased from pharmacies, Cairo, Egypt.

Methods:

Extraction of garlic oil:

The extraction of garlic oil was conducted with a soxhlet extractor using n-hexane (boiling point of 40°C - 60°C) for six hours. The oils were obtained after the solvent was removed under reduced temperature and pressure and refluxing at 70°C so as to remove any excess solvent used for the oil extracted. The extracted garlic oil was

stored in refrigerator freezer at 2° C for chemical analyses and tested biologically as the method described by **Warra et al.,(2011).**

Preparation of dried fish meat:

Raw fish was firstly eviscerated to separate the head, fins, tail, viscera and backbone, and then the body cavity of fish was washed with tap water to remove any traces of blood. Mackerel fish was cutting into similar parts and dried in oven at 50 C° for 48 hr., then grind by electric blender and stored in freezer till analysis **Aba and Ifannyi., (2013).**

The chemical analysis of fish Compositions:

Moisture, ash, total protein, crude fat were determined according to **(A.O.A.C. 1990).**

Fatty acids composition of fish and garlic oil:

Fatty acids composition of mackerel fish and garlic oil were determined according to **(Gunstone et al., 1994 and Yeshajahu, 1994).**

The biological assay:

Forty two female albino rats (200 - 210g) were kept in individual stainless steel cages under hygienic conditions and fed one week on basal diet for adaptation in adlibitum in the animal house of faculty of Home Economics, Helwan University. The basal diet consisted of 10% protein from casein, 10% corn oil, 5% cellulose, 4% salt mixture, 1% vitamin mixture, 0.2% choline chloride and the remainder is corn starch according to (**Lane-Peter and Pearson., 1971).** Vitamin composition of diets prepared according to **(A.O.A.C., 1975)** and the salt mixture was prepared according to **Hegested et al., (1941).**

After a period of adaptation on basal diet (one week), the rats (n=42) were divided into two main groups, the first main group (n=6 rats) was fed on basal diet as a control negative group. The second main group was received oral glucocorticoid twice weekly (prednisone acetate 4.5 mg/kg body weight) to induce osteoporosis (**Liao et al ., 2003),** as the same time it was divided into six subgroups (n=6 rats for each), subgroup (1) was fed on basal diet as a control positive group, subgroup (2) was fed on basal diet and treated daily orally with 100mg garlic oil/kg body weight **(Mukherjee et al., 2006)**, subgroup(3) was fed on diet containing half amount of protein from casein and the other from fish, subgroup(4) was fed on diet

containing half amount of protein from casein and the other from fish and treated with garlic oil(100mg/kg bw/daily/oral), subgroup(5) was fed on diet containing fish as source of protein, and subgroup(6) was fed on diet containing fish as source of protein and treated with garlic oil(100mg/kg bw/daily/oral).

During the experimental period (28 days), the diets consumed and body weights were recorded twice weekly.

At the end of the experiment, the animals were fasted overnight, then the rats were anaesthetized and sacrificed, and blood samples were collected from the aorta. The blood samples were centrifuged and serum was separated to estimate some biochemical parameters, i.e. uric acid (***Fossati et al., 1980***), urea nitrogen **(*Patton and Crouch.,1977*),** Creatinine **(*Bartels and Bohmer.,1971*),** aspartate amino transferase (AST) and alanine amino transferase (ALT) (***Ritman and Frankel., 1957*),** serum alkaline phosphates (ALP) (***Belfield and Goldberg., 1971)***, serum calcium (***Biggs and Moorehead., 1974)*** and serum phosphorus (***Henry., 1974).***

Liver, kidney and femur bones were separated from each rat and weighted to calculate organs to body weight %. The femur bones in each group were examined histopathologicaly, according to ***(Sheehan and Hrapchak., 1980)***.

The data obtained was analyzed statistically for mean value± standard deviation by using one way ANOVA test ***(Steel and Torri.,1980)***.

Results and Discussion:

- ***Analytical Results:***

  Chemical composition of fish: Mackerel fish was analyzed and illustrated in table (1). The percent of moisture was 69.812. On dry matter the % contents of mackerel fish were 69% protein, 27% lipid and 3.97% ash.

Table (1): Chemical composition of Mackerel fish:

Component / Type of fish	Moisture	% on dry matter		
		Protein	Lipid	Ash
Mackerel	69.812	69.00	27.00	3.97

Table (2): Fatty acids composition of fish:

Fatty acids	Carbon number	Mackerel Fish g/100g
Lauric	C12:0	0.81
Myristic	C14:0	7.72
Palmitic	C16:0	10.04
Palmitolic	C16:1	7.15
Stearic	C18:0	3.27
Oleic	C18:1	8.51
Linoleic ω-6	C18:2	1.65
Linolenic ω -3	C18:3	0.82
Arachidic	C20:0	1.76
Eicosenoic	C20:1	1.54
Eicosadienoic ω -6	C20:2	0.72
Arachedonic ω -6	C20:4	0.91
Eicosapentaenoic ω -3	C20:5	25.57
Behenic	C22:0	3.40
Erucic	C22:1	1.46
Docosapentaeenoic ω -3	C22:5	2.60
Docosahexaenoic ω -3	C22:6	18.30
Other	-------	3.77
Saturated (SFA)		**27.00**
Monounsaturated (MUFA)		**18.66**
Polyunsaturated (PUFA)		**50.57**
PUFA/SFA ratio		**1.87**

Fatty acid composition of mackerel fish:

Table (2) shows the fatty acid composition of mackerel fish. It could be observed from the results that saturated fatty acid SFA was 27%, the palmitic acid C16:0 was the major SFA presented in this type from fish. Monounsaturated fatty acid MUFA was 18.66%, oleic acid C18:1 achieved the highest amount in MUFA, followed by palmitolic acid C16:1. The percent of polyunsaturated fatty acid PUFA in mackerel fish was 50.57%, the Eicosapentaenoic ω-3 (C20:5) was the predominant PUFA presented in mackerel, followed by Docosahexaenoic ω-3 (C22:6). The data in this table revealed that, mackerel fish lipid contain more unsaturated fatty acids than saturated ones.

Table (3):Fatty acids composition of Garlic oil

Fatty acids	*Garlic oil* g/ 100g
C10:0 (Capric acid)	0.54
C12:0 (Lauric acid)	0.55
C14:0 (Myristic acid)	0.03
C16:0 (Palmitic acid)	24.22
C18:0 (Stearic acid)	0.04
C18:1 (Oleic acid)	3.25
C18:2, n-6 (Linoleic acid)	65.21
C18:3, n-3 (Linolenic acid)	5.90
Total SFA	25.38
Total UFA	74.36

Table (3) shows the fatty acid composition of garlic oil. It could be observed that, total saturated fatty acid SFA was 25.38%, the palmitic acid C16:0 was the major SFA presented in garlic oil. Oleic acid C18:1 recorded 3.25%, The percent of polyunsaturated fatty acid PUFA in garlic oil was 74.36% , the linoleic acid (C18:2, n-6) was the predominant PUFA presented in garlic oil, followed by Linolenic acid, n-3 (C18:3). The data in this table revealed that, garlic oil contain more unsaturated fatty acids than saturated ones.

- ***Biochemical Results*:**

Effect of garlic oil and fish meat on feed intake, body weight gain % and some organs weight / body weight % of osteoporotic rats

Feed intake (g/day for each rat) was increase in the negative control group than that of positive control group (15.432 vs. 11.678 g, respectively).

Rats which were fed on basal diet containing fish as source of protein recorded higher feed intake value than other treated groups. The lowest value of feed intake in treated groups was noticed in rats fed on basal diet and treated with garlic oil(100mg/kg bw/daily/oral). All tested groups had high values of food intake as compared to the positive control group.

There was a significant decrease in BWG% for control positive group, as compared to the negative control group. BWG% of all treated groups recorded significant decrease $P< 0.05$, as compared

to control positive group, On the other hand all treatments illustrated significant decrease when compared them with negative control group.

Concerning organs weight/body weight %, statistical analysis showed a significant increase in liver and kidney weight/body weight % for control positive group compared with control negative group at $P< 0.05$, while bone weight/body weight % for control positive group compared with control negative group cleared a significant decrease. The best results for all organs weight/body weight % among the treatment groups as compared to control positive group was for group fed on diet containing fish as source of protein and treated with garlic oil(100mg/kg bw/daily/oral) **Table (4).**

Table (4): ***Effect of garlic oil and fish meat on feed intake, body weight gain % and some organs weight / body weight % of osteoporotic rats.***

Parameter / Groups	Feed Intake (g/day/rat)	Body weight gain%	Liver weight / body weight%	Kidney weight / body weight%	Bone weight / body weight%
Control (-ve) fed on basal diet.	15.432	16.460 [a] ± 1.514	2.824 [e] ± 0.129	0.634 [d] ± 0.026	1.150 [a] ± 0.058
Control (+ve) fed on basal diet	11.678	-29.904 [d] ± 2.672	5.437 [a] ± 0.346	1.158 [a] ± 0.126	0.700 [c] ± 0.045
Fed on basal diet and treated with garlic oil(100mg/kg bw/daily/oral).	11.992	-15.947 [c] ± 0.411	3.624 [d] ± 0.165	0.775 [c] ± 0.019	0.787 [bc] ± 0.126
Fed on diet containing half amount of protein from casein and the other from fish.	12.102	-13.790 [c] ± 2.773	3.459 [d] ± 0.321	0.772 [c] ± 0.094	0.808 [bc] ± 0.121
Fed on diet containing half amount of protein from casein and the other from fish and treated with garlic oil(100mg/kg bw/daily/oral).	12.243	-11.920 [b] ± 6.954	4.687 [b] ± 0.390	0.797 [c] ± 0.051	0.835 [bc] ± 0.127
Fed on diet containing fish as source of protein.	13.121	-8.356 [b] [c] ± 1.439	4.207 [c] ± 0.035	0.967 [b] ± 0.030	0.893 [b] ± 0.144

Fed on diet containing fish as source of protein and treated with garlic oil(100mg/kg bw/daily/oral).	12.871	-11.133 [b] [c] ± 1.133	3.399 [d] ± 0.373	0.761 [c] ± 0.025	0.947 [b] ± 0.076

LSD: Least significant differences (P<0.05)
Mean values in each column with same letters are not significantly different

The previous results were agreement with **Liu et al.,(2011)** who found that glucocorticoids (GCs) treatment decreased appetite and body weight, induced apparent glucolipid metabolic disturbances and hyperinsulinemia, while down-regulated mRNA expression levels of the orexigenic neuropeptides, neuropeptide Y (NPY), agouti-related protein (AGRP) , and anorexigenic neuropeptide known as cocaine and amphetamine-regulated transcript (CART) , in the hypothalamus in the rats. Loss of leptin an adipocyte- derived hormone or leptin receptor expression in both humans and animals models led to striking elevation in feed intake**. (Halaas et al.,1995&Montague et al.,1997)**. **Mukherjee et al.,(2004)** observed that the significant increase in weight of the heart ($p < 0.05$), liver ($p < 0.01$)and kidneys ($p < 0.01$) of ovariectomized rats could be significantly reduced by supplementation with oil extract of garlic on the other hand ovariectomized group of rats showed significantly decreased weight of femur bones level, but treatment of ovariectomized rats with oil extract of garlic could significantly elevate the weight of all these bones tested.

Effect of garlic oil and fish meat on some kidney functions of osteoporotic rats

Uric acid, urea nitrogen and creatinine values (mg/dl) for control positive group showed highly significant increase in concerning to control negative group. The mean values ± SD were (2.465 ± 0.071 , 58.987 ± 3.421 and 1.264 ± 0.059 vs. 1.610 ± 0.036, 31.969 ± 1.406 and 0.508 ± 0.027), respectively.

All treated groups showed a significant reduction in uric acid, urea nitrogen and creatinine mean values (mg/dl) at $p<0.05$ in comparison to control positive group, but the finest results of the mean values for serum uric acid, urea nitrogen and creatinine was for group which fed on diet containing fish as source of protein and treated with garlic oil(100mg/kg bw/daily/oral) **Table (5).**

The oil extract of garlic suppressed the urinary loss of both calcium and phosphate ions, suggesting that it may have some positive effect in restoring calcium and phosphate balance. **(Mukherjee, 2004)**

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 etal.,1995)**

Table (5): ***Effect of garlic oil and fish meat on some kidney functions of osteoporotic rats.***

Parameters / Groups	Uric Acid	Urea Nitrogen	Creatinine
	mg/dl		
Control (-ve) fed on basal diet.	1.610 [e] ± 0.036	31.969 [f] ± 1.406	0.508 [f] ± 0.027
Control (+ve) fed on basal diet	2.465 [a] ± 0.071	58.987 [a] ± 3.421	1.264 [a] ± 0.059
Fed on basal diet and treated with garlic oil(100mg/kg bw/daily/oral).	2.153 [b] ± 0.063	52.173 [b] ± 2.356	1.028 [b] ± 0.086
Fed on diet containing half amount of protein from casein and the other from fish.	2.086 [b] ± 0.056	48.441 [c] ± 2.783	0.911 [c] ± 0.025
Fed on diet containing half amount of protein from casein and the other from fish and treated with garlic oil(100mg/kg bw/daily/oral).	1.938 [c] ± 0.117	41.594 [d] ± 2.051	0.771 [d] ± 0.025
Fed on diet containing fish as source of protein.	1.855 [d] ± 0.060	39.754 [d] ± 1.705	0.796 [d] ± 0.009
Fed on diet containing fish as source of protein and treated with garlic oil(100mg/kg bw/daily/oral).	1.780 [d] ± 0.036	34.866 [e] ± 1.657	0.659 [e] ± 0.038

LSD: Least significant differences (P<0.05)
Mean values in each column with same letters are not significantly different

Effect of garlic oil and fish meat on some liver functions of osteoporotic rats

In respect of AST , ALT and ALP enzymes , it could be noticed that the rats in control negative group had lower mean values than that of osteoporotic rats as follows (76.836 ± 2.771 , 30.004 ± 1.689 and 84.531 ± 3.012 vs. 152.197 ± 4.819, 65.559 ± 4.049 and 178.281 ± 5.751 , respectively).

Generally, all treated groups illustrated significant decrease concerning the mean values of AST, ALT and ALP comparing with control positive group, especially when garlic oil and /or fish meat were added to the diet of osteoporotic rats a significant decrease of AST, ALT and ALP values were noticed in comparison to control positive group. On the other hand, high level of fish meat powder with garlic oil induced significant decrease than other treated groups. So, the best results were for groups of rats fed on diet containing fish as source of protein and treated with garlic oil(100mg/kg bw/daily/oral), followed by group that received diet containing fish as source of protein **Table (6).**

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. In th**is** 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., (2011)** 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).** But treatment with oil extract of garlic significantly reduced all these changes in serum parameters **Mukherjee, (2004).**

Allicin has been found to be the compound most responsible for the spiciness of the raw garlic and being a powerful antibiotic and antifungal compound, it believe to be the agent responsible for the

speed recovery from strep throat or other mild ailments when garlic is used. **Block.,(1992).**

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)**

Table (6): ***Effect of garlic oil and fish meat on some liver functions of osteoporotic rats.***

Parameters / Groups	AST	ALT	ALP
	U/L		
Control (-ve) fed on basal diet.	76.836 [g] ± 2.771	30.004 [f] ± 1.689	84.531 [f] ± 3.012
Control (+ve) fed on basal diet	152.197 [a] ± 4.819	65.559 [a] ± 4.049	178.281 [a] ± 5.751
Fed on basal diet and treated with garlic oil(100mg/kg bw/daily/oral).	143.051 [b] ± 1.737	56.077 [b] ± 4.009	156.407 [b] ± 3.301
Fed on diet containing half amount of protein from casein and the other from fish.	137.143 [c] ± 1.884	52.373 [c] ± 3.941	153.880 [b] ± 2.933
Fed on diet containing half amount of protein from casein and the other from fish and treated with garlic oil(100mg/kg bw/daily/oral).	125.986 [d] ± 3.660	43.843 [d] ± 3.149	143.801 [c] ± 3.799
Fed on diet containing fish as source of protein.	121.810 [e] ± 2.959	44.561 [d] ± 1.576	138.640 [d] ± 2.658
Fed on diet containing fish as source of protein and treated with garlic oil(100mg/kg bw/daily/oral). .	112.721 [f] ± 2.849	37.639 [e] ± 1.699	116.876 [e] ± 3.302

LSD: Least significant differences (P<0.05)
Mean values in each column with same letters are not significantly different

Effect of garlic oil and fish meat on serum calcium and phosphorus of osteoporotic rats.

Concerning serum calcium and phosphorus , statistical analysis showed a significant increase in both of serum calcium and phosphorus for control negative group compared with control positive group at P< 0.05. Also all treated groups cleared significant increase in both of serum calcium and phosphorus compared with the control positive group. The best result for serum calcium and phosphorus comparing to control positive group was for group fed on diet containing fish as source of protein and treated with garlic oil(100mg/kg bw/daily/oral) **Table (7).**

Table (7): ***Effect of garlic oil and fish meat on serum calcium and phosphorus of osteoporotic rats.***

Parameters / Groups	Calcium	Phosphorus
	mmol/l	
Control (-ve) fed on basal diet.	3.486 [a] ± 0.121	2.572 [a] ± 0.049
Control (+ve) fed on basal diet	2.220 [f] ± 0.062	1.568 [f] ± 0.085
Fed on basal diet and treated with garlic oil(100mg/kg bw/daily/oral).	2.432 [e] ± 0.035	1.749 [e] ± 0.052
Fed on diet containing half amount of protein from casein and the other from fish.	2.554 [d] ± 0.059	1.922 [d] ± 0.025
Fed on diet containing half amount of protein from casein and the other from fish and treated with garlic oil(100mg/kg bw/daily/oral).	2.770 [c] ± 0.033	2.132 [c] ± 0.104
Fed on diet containing fish as source of protein.	2.841 [c] ± 0.056	2.085 [c] ± 0.035
Fed on diet containing fish as source of protein and treated with garlic oil(100mg/kg bw/daily/oral).	3.104 [b] ± 0.119	2.302 [b] ± 0.005

LSD: Least significant differences (P<0.05)
Mean values in each column with same letters are not significantly different

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).**On the other hand, it was previously stated that hydrocortisone was capable of partially blocking the intestinal absorption of phosphate **(Ferraro et al.,1976).**

Chang et al.,(2009) explain that nuclear factor kappa B(NF-KB)is identified as a critical factor responsible for inhibiting bone formation in a model of osteoporosis. The suppression of NF-KB in differentiated osteoblasts prevents osteoporotic bone loss by maintaining osteoblast function.

High rate of bone turnover was well corrected by supplementation with oil extract of garlic, suggesting that garlic oil may have protective action against ovarian hormone insufficiency-related bone resorption. **Mukherjee,(2004)** As estrogen, the female gonadal hormone, is the most potent inhibitor of bone resorption, the most widely recommended method to reduce the rate of postmenopausal bone loss is estrogen replacement therapy **(Gambacciani and Ciaponi, 2000).** Long term use of estrogen replacement therapy may have serious side-effects **(Barnes, 2001).**

Oil extract of garlic also has some significant role in reducing enhanced osteoclastic activity and bone resorption. **(Mukherjee, 2004)** Garlic (Allium sativum Linn.), a common ingredient of food with many medicinal effects, was found to be a substantial contributor of phytoestrogens **(Horn-Ross et al., 2000).** It is considered as a dietary source of lignans, a type of phytoestrogen**.** Oil extract of garlic by its phytoestrogenic efficacy could influence the various physiological phenomena; namely, serum estradiol, in vivo intestinal transference of calcium and the activities of relevant enzymes, alkaline phosphatase and calcium ATPase, and the relation of this to bone turnover in an ovariectomized rat model of osteoporosis **Hernandez et al., (2004).**

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, vitamin C and vitamin B12.

Histopathological results of bones :

Specimens of femur bone were fixed with 10% neutral formalin and decalcified with 5% nitric acid solution for 1 day. The routine tissue processing for light microscopy was performed, and tissues were embedded in parafin. Cross-sections of 5-µm intervals were taken and stained with hematoxylin and eosin for measurement of femur bone thickness and Masson Trichrom for collagen fiber evaluation. Diaphyseal femur bone thickness was measured with ocular micrometer. Ten random areas were selected and average thickness was calculated for each femur (**Comelekoglu et al., 2007**).

Microscopically, bone of rat from group received basal diet (control –ve) revealed no histopathological changes **Photo.(1).** Examined bone of rat from group received basal diet (+ve control group) revealed thin trabecular bones and enlarged medullary cavity **Photo.(2).** Bone of rat from group fed **on** (diet containing garlic oil) revealed thin cortex **Photo (3).** Meanwhile, bone of rat from group fed on diet containing half amount of protein from casein and the other from fish revealed thin bone trabecullae **Photo (4).** No histopathological changes were noticed in bone of rat from group

received diet containing half amount of protein from casein and the other from fish + garlic oil **Photo (٥).** Examined section from group received diet containing fish as source of protein revealed no histopathological changes **Photo (٦).** Some sections from group received diet containing fish as source of protein + garlic oil revealed thick cortex **Photo (٧),**whereas other sections showed no histopathological changes **Photo (٨).**

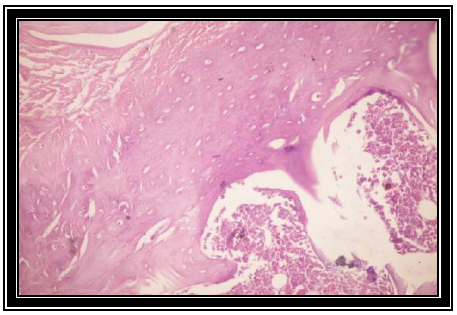

Photo. (1): Bone of rat from group received basal diet (control –ve) showing no histopathological changes (H and Ex100) .

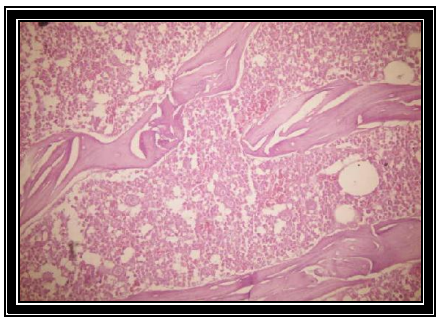

Photo. (2): Bone of rat from group received basal diet (+ve control group) showing thin trabecular bones and enlarged medullary cavity (H and Ex100).

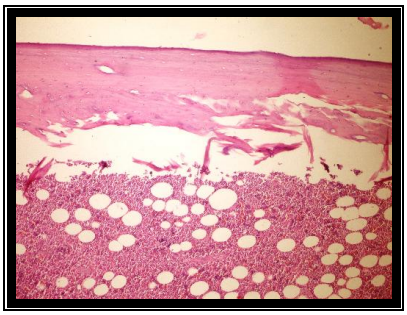

Photo (3) : Bone of rat from group received (diet containing garlic oil) showing thin cortex (H and E x 400)

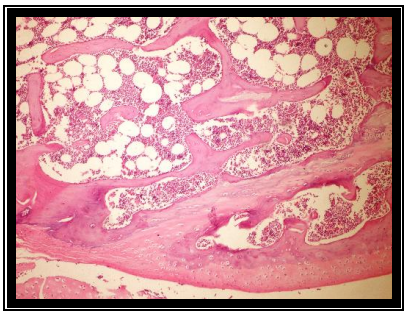

Photo (٤) : Bone of rat from group received diet containing half amount of protein from casein and the other from fish showing thin bone trabeculae
(H and E x 400)

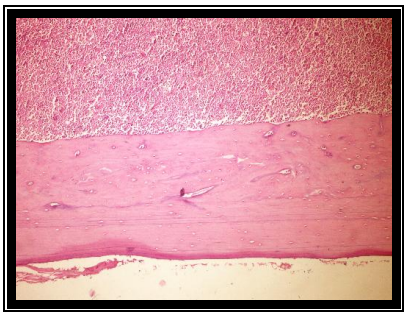

Photo (٥): Bone of rat from group received diet containing half amount of protein from casein and the other from fish + garlic oil. showing no histopathological changes
(H and E x 400)

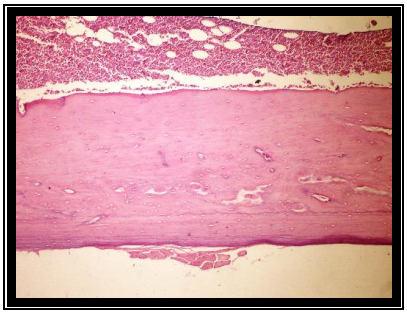

Photo (6): Bone of rat from group received diet containing fish as source of protein showing no histopathological changes (H and E x 400)

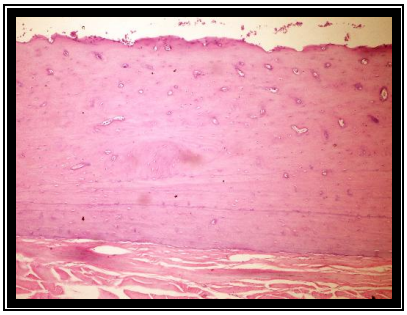

Photo (7): Bone of rat from group received diet containing fish as source of protein + garlic oil showing thick cortex (H and E x 400)

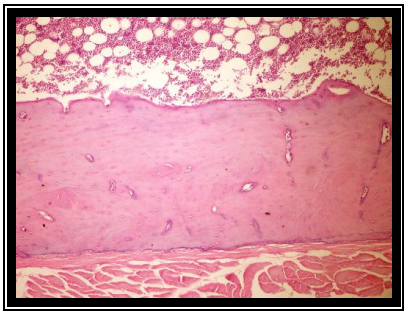

Photo (٨): Bone of rat from group received diet containing fish as source of protein + garlic oil showing no histopathological changes (H and E x 400)

CONCLUSION

According to the current findings, it could be concluded that dietary supplementation of bakery products with fish powder and garlic oil is recommended in order to prevent or reduce glucocorticoids-induced osteoporosis.

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