THE ROLE OF GENISTEIN AND FISH OIL IN MOLECULAR AND BIOCHEMICAL DISORDERS OF OSTEOCLASTOGENESIS AS A RISK FACTOR FOR OSTEOPOROSIS INDUCED BY ANASTROZOLE IN LABORATORY RATS (*Rattus norvegicus*)

Yahya Abdulhadi Owaid Jihad Abdul ameer Ahmed

Department of Pathology and poultry diseases , College of Veterinary Medicine, University of Basrah, Iraq.

(Received 24 July 2018 ,Accepted 24September 2018)

Key words: Anastrazole, Geneistein, Osteoporosis.

Corresponding author: yalbaji123456@gmail.com

ABSTRACT

In order to determine the toxic effects of anastrazol and its relationship to the incidence of osteoporosis, this study was conducted (56 females laboratory rats) aged (3-4 month) divided into (7 groups) each group composed 8 female rats as follows: The first group was treated with a anastrazol (0.02 mg/1 kg body weight) for a 60 days. The second group was treated with anstrazol (0.02 mg/1 kg body weight) and fish oil (3750 mg/1 kg of body weight) for 60 days. The third group was treated with anstrazol (0.02 mg/1 kg body weight), and phytoestrogen (genstien) (20 mg/1 kg of body weight) for 60 days. The fourth group was treated with anstrazol (0.02 mg/1 kg body weight) , fish oil (3750 mg/1 kg of body weight) and phytoestrogen (genstien) (20 mg/1 kg of body weight) for a 60 Day. The fifth group is treated with a drug phytoestrogen (genstien) (20 mg/1 kg of body weight) for a 60-day. The sixth group were treated with fish oil (3750 mg/1 kg of body weight) for a 60-day. The seventh group is a group control are not treated by any drugs for a 60 days.

The biochemical results showed a significant decline in value of estrogen in the GI (43.925±2.895) compared with GII, GIII and GIV while significant improvement observed in the estrogen level of GII, GIII and GIV respectively (70.308±2.140), (67.42±3.812) and (74.15± 3.603) compared with the first group (43.925±2.895). Also the GIV showed the optimal improvement in estrogen level (74.15±3.603), as well, the biochemical results showed significant increase in the level of the alkaline

phosphatase in the GI which showed (190.02±3.98) compared with GII, GIII and GIV while showed significant improvement of alkaline phosphatase values in the GII, GIII and GIV respectively (180.57±1. 89), (181.37±5. 03), (176. 8±2, 41).In Real-Time PCR RANK in the first group showed up-regulation (12.157±1.59) compared with the second, third and fourth groups (5.50±90.601), (6.903±0.130) and (3.018±0.027) respectively. The results also showed an up-regulation RANKL in Group I (8.458±0.766) compared with the second, third and fourth groups respectively (6.071±0.53), (4.970±0.228) and (3.398±0.114). While the results showed an up-regulation OPG in the second, third and fourth respectively (1.924±0.08), (1.014±0.064) and (5.567±1.419) compared in the first group (0.009±0.0076).We concluded that the derivatives like genestein and fish oil lead to ameliorate the toxic effect of anastrazole, also these derivatives may minimized the risk of osteoporosis caused by estrogen deficiency.

INTRODUCTION

Osteoporosis is a disease characterized by low bone mass and deterioration of the bone architecture leading to increase fragility and fractures, these fractures can result from minimal trauma such as fall from standing height or less, and can occur at any area but most commonly in hips, spine and wrist, primary osteoporosis related to aging and menopause while secondary type results from specific diseases or drugs (1).

The relationship of estrogen depletion and bone loss has been well established and it is estimated that estrogen deficiency is responsible for 75% of bone loss that occurs in postmenopausal women, immediately after the menopause, bone mass decreases by 3 to 5% per year and increased bone turnover is associated with increased bone loss and the risk of fractures (2).

The bone stromal cells include the osteoblasts that formed from pluripotent mesenchymal stem cells and are responsible for the mineralization of bone, and osteoclasts that formed from hematopoietic stem cells, differentiate into multinucleated giant cells and occur in the bone absorption zone, the interaction between these cells is controlled by the bone metabolism mediators RANK, RANKL and OPG (3).

The tumor necrosis factor family molecule RANKL (RANKL, TRANCE, ODF) and its receptor RANK are key regulators of bone remodelling and regulate T cell/ dendritic cell communications, and lymph node formation, moreover, RANKL and RANK are expressed in mammary gland epithelial cells and control the development of a lactating mammary gland during pregnancy and the propagation of mammalian species, importantly, RANKL and RANK are essential for the development and activation of osteoclasts and bone loss (4).While, OPG has an antiosteoclastogenic property due to its ability to act as a decoy by binding to RANK-ligand and blocking the RANK-ligand/RANK interaction (5).

In addition, Aromatase (cytochrome P-450) catalyzes the rate-limiting step (conversion of steroidal C-19 androgens to C-18 estrogens) in estrogen biosynthesis, aromatization is the final step in steroid biosynthesis and, therefore, aromatase is an attractive target for selective inhibition; aromatase is expressed primarily in the ovary (6).

Aromatase inhibitors (AIs) such as anastrozole is currently part of the standard endocrine therapy in postmenopausal women with hormone-receptor positive early breast cancer, the AIs inhibit the conversion of androgen to estrogen in peripheral fat tissues and tumor cells, which leads to a marked reduction in plasma estrogen (7).

MATERIALS AND METHODS

Fifty-six laboratory Females rats (*Rattusnorvegicus*) approximately 3-4 months of age and weight ranged 200±10 gram (which bought from consultant office of veterinary medicine college at Basra university) were randomly allocated to seven groups. These 7 groups were: Group A,B,C,D,E,F and G. At first, the animals were left two weeks before the beginning of experiment under controlled condition for adaptation at animal's house, where they fed on special feed pellets and given ad-libitum water as well as controlled temperature (25±2 °C). All ratswere treatedfor two months by daily oral gavage as the following:

1- Group (I) : Treated orally with anastrazole 1 ml containing (0.02mg/ 1kg BW) for 60 days according to (8).
2- Group (II): Treated orally with combination of anastrazole and fish oil in (0.02mg/ 1kg BW) ,(3750mg/ 1kg BW) respectively for 60 days daily.

3- Group (III): Treated orally with combination of anastrazole and genestein in (0.02mg/ 1kg BW) and (20mg/ 1kg BW) respectively for 60 days daily.
4- Group (IV): Treated orally with combination of anastrazole and genestein and Fish oil in (0.02mg/ 1kg BW), (20mg/ 1kg BW) and (3750mg/ 1kg BW) respectively for 60 days daily.
5- Group (V): Treated orally with Genestein 1 ml containing (20mg/ 1kg BW) for 60 days daily according to (9)
6- Group (VI): Treated orally with fish oil 1 ml containing (3750mg/ 1kg BW) for 60 days daily according to (9).
7- Group (VII): Treated orally with normal saline daily for 60 days and served as control group.

At the end of experiment, all of experimental groups was estimatedthe estrogen and alkaline phosphatase concentrations,in whichthe estrogen concentration considered to be the most reliable tool to evaluate the activity of ovarian follicles,the kit was used (E2(Estradiol) ELISA Kit. USA,product Catalogue No.:EU0390, Range:0.703-45ng/ml) and the procedure was done according to (10).The serum alkaline phosphatase concentrationwas assessed according to(11).

The RT-PCR done by using the following materials:

1. Total RNA Extraction Kit AccuZolTM (Bioneer, Korea).
2. DNase I enzyme kit (Promega, USA).
3. M-MLV Reverse Transcriptase kit (Bioneer, Korea).
4. NEXpro™ qPCR Master Mix (Genes Laboratories,Korea).

The primers for target genes and housekeeping gene were designed by pervious study (12) and provided by Macrogencompany, Korea as following:

1. RANK primers F 3- GGGAAAACGCTGACAGCTAATC -5, R5-GGTCCCCTGAGGACTCCTTATT-3.
2. RANKL primers F 3- CCGTGCAAAGGGAATTACAAC-5, R5-GAGCCACGAACCTTACATCA-3.
3. OPG primers F 3 – CACAGCTCGCAAGAGCAAACT – 5, R 5-ATATCGCGTTGCACACTGCTT-3.
4. CYC-A primers F 3- GAGCTGTTTGCAGACAAAGTTTC 5- R 5 – CCCTGGCACATGAATCCTGG-3.

The total RNA were extracted from frozen blood by using (TRIzol® reagent kit) and done according to company instructions, while the total RNA were extracted from frozen blood by using (TRIzol® reagent kit) and done according to company instructions. The extracted total RNA was assessed and measurement by Nanodrop spectrophotometer (THERMO. USA), There are two quality controls were performed on extracted RNA. The genomic RNA extraction was done by using the following: 10 µl of total RNA (100ng/µl), 1µl of DNase I enzyme, 4µl of 10X buffer and 5 µl of DEPC water. After that, the mixture was incubated at 37C° for 30 minutes. Then, 1µl stop reaction was added and incubated at 65C° for 10 minutes for inactivation of DNase enzyme action.

The DNase-I treated RNA samples were also used in cDNA synthesis step for Pten and GAPDH gene by using M-MLV Reverse Transcriptase kit and done according to company instructions as following: 8 µl of total RNA 100ng/µl , 1µl of random hexamer primer, 1ul of DEPC water in which the total concentration was 10 µl. Then RNA and primer was denatured for 10 min at 65 °C, after that immediately cool on ice as the following: 10 µl of the step 1 RT master mix, 1ul of M-MLV RTase (200u), 4 µl of 5X M-MLV RTase reaction buffer, 2 µl of 100 mM DTT, 2µl of DNTPs, 1µl of RNase inhibitor, in which the total amount was 20 µl.

Then the tubes were placed in vortex and briefly spinning down. The RNA converted into cDNA in thermocycler under the following thermocycler conditions: 42 °C of cDNA synthesis (RT step) for 1 hour, then 95 °C of Heat inactivation for 5 min.

The quantitative Real-Time PCR used in quantificationoftarget genes expression analysis that normalized by housekeeping gene in treatment and control group by using Real-Time PCR technique and this method was carried out according to method described by (9) and include the following steps: the qPCR master mix composed 2.5µL of cDNA templet (100ng), 1 µL of forward primer (10 pmol), 1 µL of reverse primer (10pmol), 10 µl of qPCR Master Mix, then 5.5 µl of DEPC water in which the total volume was 20 µl.After that, these qPCR master mix component that mentioned above placed in qPCR plate strip tubes and mixed by Exispin vortex centrifuge for 3 minutes, then placed in Miniopticon Real-Time PCR system.The qPCR plate was loaded and the following thermocycler protocol in table (1).

The data results of qRT-PCR for target and housekeeping gene were analyzed by the relative quantification gene expression levels (fold change) (The ΔCT Method Using a reference gene) that described by (12).

Table (1): The thermocycler protocol.

as following equation:

qPCR step	Temp.	Time	Repeat cycle
Initial Denaturation	95 °C	5min	1
Denaturation	95 °C	20 sec	45
Annealing\Extention	60 °C	30 sec	

$$\text{Ratio (reference/target)} = 2^{CT(reference) - CT(target)}$$

The other statistical analysis was performed using Chi-Square test and the ORs (odds ratios) and 95% CIs (95% confidence intervals) were calculated and $p \leq 0.05$ were considered significant (13).

RESULTS

I. Biochemical results:

There are a significant ($P \leq 0.05$) difference in the level of estrogen and alkaline phosphatase among groups. In the estrogen level there are a significant ($P \leq 0.05$) decrease of all treated groups when comparing to control group, which showed GI (43.925±2.895), GII (70.308±2.140), GIII (67.42 ± 3.812), GIV (74.15 ±3.603) and control group GVII (77.92 ±2.001), GI showed the highestsignificant ($P \leq 0.05$) decrease when compared to GII, GIII, GIV and other control groups (table.2).

The ALP level showed significant ($P \leq 0.05$) increased in GI, GII, GIII and GIV groups when comparing to control group, which showed GI (190.02 +3.98), GII (180.57 +1.89), GIII (181.37 + 5.03), GIV (176.8+2.41) and control group GVII

(153.12+2.47). The GI showed the highest significant (P≤0.05) increased when compared to GII, GIII, GIV and control groups (table.2).

Table (2): Biochemical evaluation after 60 days of the experiment.

Group	Estrogen (pg/ml) Mean ± SE	ALP (U/L) Mean ± SE
G.I (Anas.)	43.925±2.895 e	190.02 ±3.98 a
G.II (Anas. +Gn)	70.308±2.140 c	180.57 ±1.89 b
G.III (Anas.+Fo.)	67.42 ± 3.812 d	181.37 ± 5.03 b
G.IV (Anas.+Gn+Fo)	74.15 ±3.603 b	176.8±2.41 c
G.V(Gn.)	77.75 ±3.736 a	153.06±3.78 d
G. VI (Fo.)	77.85 ±1.947 a	154.71±1.91 d
G. VII (control)	77.92 ±2.001 a	153.12±2.47 d
P-value	0.0004	0.0002
LSD	3.628	3.980

- **Different letters vertically means significant differences between groups.**

II. Molecular results:

The molecular results of RANK, RANKL and OPG gene expressions were details as the following:

Group I : (Which received Anastrazole 1 ml (0.02mg/ 1kg BW) alone), it showed that RANK mRNA levels up-regulation significantly (P ≤ 0.05) 12.157 fold change in 60 days exposure (12.157 ± 1.59) in comparison with the control group (Group VII) (1.842± 0.0278) as in table (3) and Fig. (1).In addition, it showed that RANKL

mRNA levels up-regulation significantly (P ≤ 0.05) 8.456 fold change in 60 days exposure (8.458± 0.766) in comparison with the control group (Group VII) (2.072± 0.207) as in table (3) and Fig. (2). Also, it showed that OPG mRNA levels down-regulation significantly (P ≤ 0.05) was 0.076 fold change in 60 days of exposure (0.076± 0.009) in comparison with the control group (Group VII) (7.146± 0.0562) as in table (3) and Fig. (3).

Group II : (Which received anastrazole and fish oil in (0.02mg/ 1kg BW) ,(3750mg/ 1kg BW)), it showed that RANK mRNA levels up-regulation significantly (P≤0.05) 5.509 fold change in 60 days exposure (5.509 ±0.601) in comparison with the control group (Group VII) (1.842± 0.0278) as in table (3) and Fig. (1). In addition, it showed that RANKL mRNA levels up-regulation significantly (P ≤ 0.05) was 6.071 fold change in 60 days exposure (6.071± 0.53) in comparison with the control group (Group VII) (2.072± 0.207) as in table (2) and Fig. (2). Also, it showed that OPG mRNA levels down-regulation significantly (P ≤ 0.05) was 1.014 fold change in 60 days exposure (1.924± 0.08) in comparison with the control group (Group VII) (7.146± 0.0562) as in table (3) and Fig. (3).

Table (3): The RANK, RANKL and OPG gene mRNA expression.

Group	RANK	RANKL	OPG
GI (Anas.)	12.157 ± 1.59 a	8.458± 0.766 a	0.076± 0.009 d
GII (Anas.+Gn.)	5.509 ±0.601 c	6.071± 0.53 b	1.924± 0.08 c
GIII (Anas.+Fo.)	6.903 ± 0.130 b	4.970± 0.228 c	1.014± 0.064c
GIV (Anas.+Gn+Fo)	3.018± 0.027 d	3.398± 0.114 d	5.567± 1.419 b
GV (Gn.)	1.646 ± 0.046 e	2.003± 0.186 e	7.154± 0.836 a
GVI (Fo.)	1.735± 0.0394 e	2.026± 0.079 e	7.135± 0.698 a
GVII (control)	1.842± 0.0278 e	2.072± 0.207 e	7.146± 0.0562 a
P value	0.0004	0.0004	0.0002
LSD	3.980	3.980	3.628

- Different letters vertically refers to presence significant differences between group

Group III : (Which received Anastrazole and Genestein in 0.02mg/ 1kg BWand 20mg/ 1kg BW, it showed that RANK mRNA levels up-regulation significantly ($P \leq 0.05$) was 6.903 fold change in 60 days exposure (6.903 ± 0.130) in comparison with the control group (Group VII) (1.842± 0.0278) as in table (3) and Fig. (1). In addition, it showed that RANKL mRNA levels up-regulation significantly ($P \leq 0.05$) was 4.970 fold change in 60 days exposure (4.970± 0.228) in comparison with the control group (Group VII) (2.072± 0.207) as in table (3) and Fig. (2). Also, it showed that OPG mRNA levels down-regulation significantly ($P < 0.05$) was 1.924 fold change in 60 days exposure (1.014± 0.064) in comparison with the control group (Group VII) (7.146± 0.0562) as in table (3) and Fig. (3).

Group IV : (Which received Anastrazole and Genestein and fish oil), it showed that RANK mRNA levels up-regulation significantly ($P < 0.05$) was 3.018 fold change in 60 days exposure (3.018± 0.027) in comparison with the control group (Group VII) was (1.842± 0.0278) as in table (3) and Fig. (1). While there were non- significant ($P \leq 0.05$) difference in RANK mRNA in groups GV, GVI and GVII which showed 1.646, 1.842 and 1.842 fold changes respectively in the same period exposure at (1.646 ± 0.046), (1.735± 0.0394) and (1.842± 0.0278) respectively as in table (3) and Fig. (1). In addition, it showed that RANKL mRNA levels up-regulation significantly ($P \leq 0.05$) was 3.398 fold change in 60 days of exposure (3.398± 0.114) in comparison with the control group (Group VII) (2.072± 0.207) as in table (3) and Fig. (2). While, there was non- significant ($P \leq 0.05$) difference in level of RANKL mRNA in groups GV, GVI and GVII which showed 2.003, 2.072 and 2.072 fold changes respectively in the same period exposure at (2.003± 0.186), (2.026± 0.079) and (2.072± 0.207) respectively as in table (3) and Fig. (2).Also, it showed that OPG mRNA levels down-regulation significantly ($P \leq 0.05$) was 5.567 fold change in 60 days of exposure (5.567± 1.419) in comparison with the control group (Group VII) was (7.146± 0.0562) as in table (3) and Fig. (3). While, there was non- significant ($P \leq 0.05$) difference in level of OPG mRNA in groups GV, GVI and GVII which showed 7.154, 7.146 and 7.146 fold changes respectively in the same period of exposure (7.154± 0.836), (7.135± 0.698) and (7.146± 0.0562) respectively as in table (3) and Fig. (3).

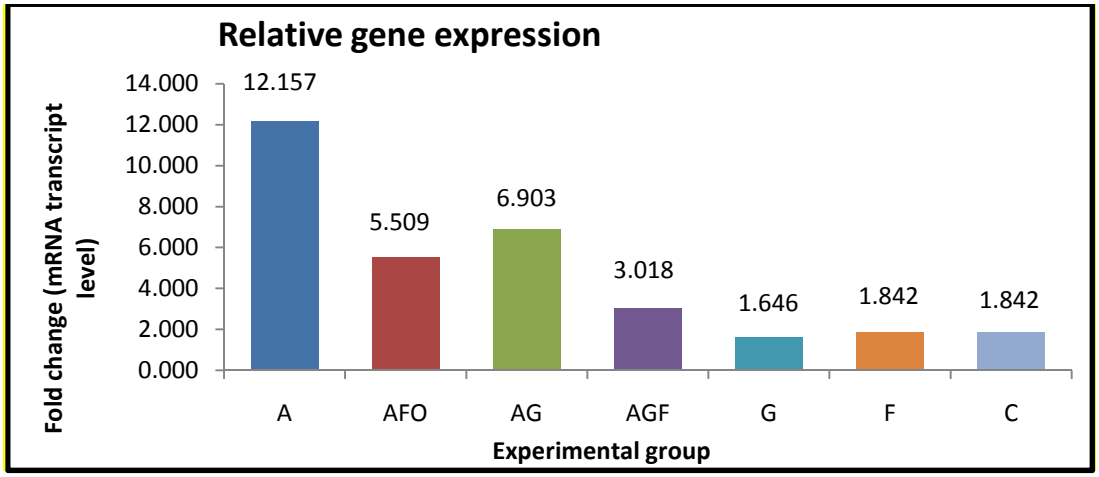

Figure (1): Relative mRNA levels of RANK in the marrow of rats in all groups were determined by quantitative RT-PCR. Two-month of exposure in all groups showed significant up-regulation expression of RANK (GI,GII,GIII and GIV). While the (GV,GVI,GVII) showed significant (P≤0.05) decreased in the expression of RANK.

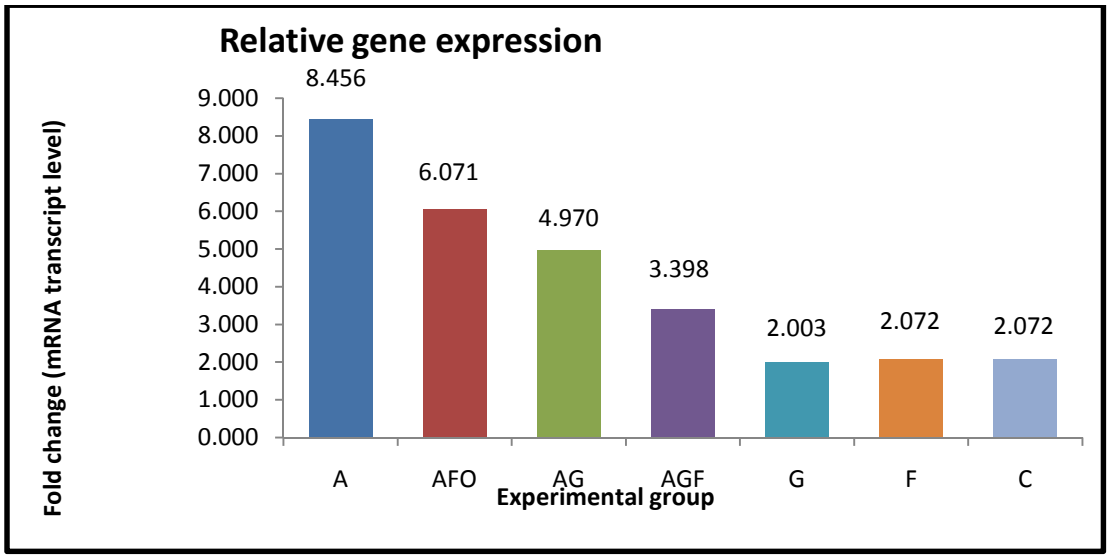

Figure (2): Relative mRNA levels of RANKL in the marrow of rats in all groups were determined by quantitative RT-PCR. Two-month of exposure in all groups showed significant upregulation expression of RANK (GI,GII,GIII and GIV). while the (GV,GVI,GVII) caused significant (P≤0.05) decreased in the expression of RANKL.

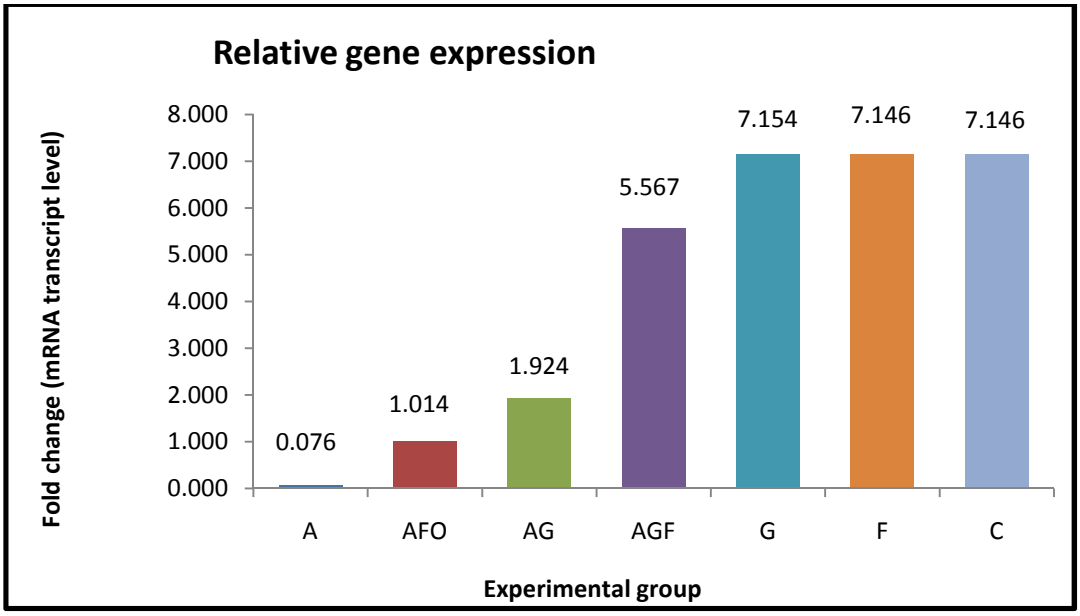

Figure (3): Relative mRNA levels of OPG in the marrow of rats in all groups were determined by quantitative RT-PCR. Two-month of exposure in all groups showed significant down-regulation expression of OPG (GI,GII,GIII and GIV). while the (GV,GVI,GVII) caused significant (P≤0.05) increase in the expression of OPG.

DISCUSSION

The current results showed that the estrogen level was significantly (P< 0.05) decreased in GI when comparing to other groups, these may due to anastrazole can inhibit the action of aromatase in which the aromatase is the final enzymatic step in catalyzing the biosynthesis of estrogens, as a result the current study showed the decrease estrogen level in group I, theses idea may agree with the report of (14) which documented that all AIs are similar in that action in which inhibit estrogen synthesis by blocking aromatase activity, thereby reducing endogenously synthesized estrogen. In addition, the current results showed significant (P< 0.05) improvement of estrogen level in group II, group III and group VI, these may due to beneficial action of both genistein and fish oil in decreasing the harmful effect of anastrazole in estrogen biosynthesis and release in which the genistein regarded a phytoestrogen that leading to synergic effect to ameliorate the effect of anastazole in estrogen synthesis also the fish oil showed improved estrogen level by increases converting of estrogen precaurser to final active form of estrogen, thses results as similar to the results that mentionted by (15) who reported that increased 2-hydroxylation of estradiol occurs in

MCF-7 cells grown with omega-3-fatty acids, which results in more estradiol converted in to estrogens.

The other biochemical results showed significant ($P < 0.05$) increase in ALP level in group I when comparing to other groups, these may due to release of this enzyme from activated osteoclast in status of estrogen deficiency and osteoporosis induced by anastrazole, these idea agree with (16) which documented that the estrogen deficiency status initiated and increased the rate of bone resorption by increasing osteoclastic activity and the serum alkaline phosphatase levels elevated significantly ($P < 0.05$) both in osteoporotic female as well as elderly against normal healthy individuals, Because osteoporotic bone was more porous due to increased number of osteoclast cells . In addition the results of this study showed significant ($P<0.05$) improvement of ALP level in group II, group III and group VI, these occur due to beneficial action of genistein and fish oil in decreasing the harmful effect of anastrazole due to the ability of these supplements to not only suppress osteoclastogenesis/osteoclast numbers but also to preserve the bone marrow osteogenesis/bone surface osteoblast numbers which agreed with(17). In addition our biochemical result showed that significant ($P<0.05$)decreased in ALP levels in G II, GIII and G IV are may due to the action of genistein and /or fish oil in increasing the estrogen level that leading to suppress the action of osteoclast cells.

The current molecular study showed significant ($P<0.05$) up-regulation in RANK and RANKL mRNA levels in GI comparing to other groups that's may due to the harmful effect of anastrazole leading to decreased of estrogen level that causing activation of osteoclast and that mean as a risk factor for osteoporosis, these facts agreed with (18) who mentioned that estrogen is a well-known regulator of bone metabolism and that's also involved in immune function. Seventy-five years ago, Fuller-Albright noted that estrogen deficiency after menopause is associated with a decline in bone mineral density (BMD) and osteoporosis. Also theresultshowed significant ($P<0.05$) downregulation in RANK and RANKL mRNA levels in G II and G IV when comparing to GI that's may due to the beneficial effect of geneistein leading to improvement of estrogen level that causing activation of osteoblast and its mean as a bone support, these fact agreed with (19) which reported that geneistein has been shown to anabolically modulate bone cells and benefit bone by stimulating protein synthesis, alkaline phosphatase release, differentiation of osteoblasts. In addition the

current molecular study showed significant (P<0.05) down-regulation in OPG mRNA levels in GI comparing to other groups that's may due to harmful effect of anastrazole leading to decreased of estrogen level that causing inactivation of osteoblast cells and its mean as a risk factor for osteoporosis, these facts agreed with (20) which demonstrated that chemotherapy may decreases trabecular bone volume, which is associated with increased adipogenesis, enhanced osteoclastogenesis, and decreased osteogenesis potential within the bone marrow, and thus a lower osteoblast number but a higher osteoclast density on the bone surface as well as a higher adipocyte density in the bone marrow.

Also theresultshowed significant (P<0.05) upregulation in OPG mRNA levels in GII, GIII and GIV comparing to GI that's may due to the beneficial effect of genestein and/ or fish oil the leading to improvement of estrogen level that causing activation of osteoblast and its mean as a bone support, these may inconsistence with (21) that mentioned the Genistein has also been shown to suppress the activation of protein phosphatases and nuclear factor-kappa B (NF-kappa B) and Akt signaling pathways, which are known to maintain a homeostatic balance between cell survival and apoptosis, to inhibit osteoclast formation, induce their apoptosis and to suppress bone resorption. In other hand, the n-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) abundant in fatty fish such as salmon, menhaden and tuna or in their oils are known to have significant anti-inflammatory properties and positive effects on bone metabolism, possibly via suppressing pro-inflammatory mediators like prostaglandin E2 (PGE2), IL-1, IL-6 and TNF-a, which are known to promote osteoclastogenesis and increase bone loss (22). The current molecular results also agreed with our biochemical results in which the anastrazole significantly lead to increases in bone resorption and decrease in bone formation, in addition that the genestein and or fish oil leaded to significant improvement in bone formation and decreases in bone resorption.

دور عقار الجنستين وزيت السمك في الاختلالات الجزيئية والكيمياء الحياتية في عملية الهدم العظمي كعامل خطورة في حدوث هشاشة العظام المستحدثة بواسطة عقار الانسترازول في الجرذان المختبرية

يحيى عبد الهادي عويد جهاد عبد الامير احمد

فرع الامراض وامراض الدواجن، كلية الطب البيطري، جامعة البصرة ، البصرة ، العراق.

الخلاصة

لغرض تحديد التأثيرات السمية لعقار الانسترازول وعلاقته في حدوث هشاشة العظام، أجريت هذه الدراسة على(٥٦ جرذ مختبري انثى) ، تتراوح أعمارهم بين ٣- ٤ اشهر مقسمة الى سبعة مجاميع بواقع ٨ جرذان مختبرية لكل مجموعة وعملت على النحو التالي:-

مجموعة الأولى تمت معالجتها ب الانسترازول (٠,٠٢ملغ/كلغ وزن الجسم) لمدة ٦٠ يوم.

مجموعة الثانية تمت معالجتها ب الانسترازول(٠,٠٢ملغ/كلغ وزن الجسم) و زيت السمك ٣٧٥٠ملغ/كلغم وزن الجسم) لمدة ٦٠ يوم.مجموعة الثالثة تمت معالجتها ب الانسترازول (٠,٠٢ملغ/كلغ وزن الجسم) و الجنستين ٢٠ملغ/كلغم وزن الجسم) لمدة ٦٠ يوم.

مجموعة الرابعة تمت معالجتها ب الانسترازول (٠,٠٢ملغ/كلغ وزن الجسم) و زيت السمك ٣٧٥٠ملغ/كلغم وزن الجسم) و الجنستين ٢٠ملغ/كلغم وزن الجسم) لمدة ٦٠ يوم.مجموعة الخامسة تمت معالجتهابالجنستين ٢٠ملغ/كلغم وزن الجسم) لمدة ٦٠ يوم.مجموعة السادسة تمت معالجتها بزيت السمك (٣٧٥٠ملغ/كلغم وزن الجسم) لمدة ٦٠ يوم.مجموعة السابعة هي مجموعة السيطرة لم تعامل ب أي علاج.أظهرت النتائج الكيمياء الحياتية نقص معنوي في مستوى الاستروجين في المجموعة الأولى (٢.٨٩٥±٤٣.٩٢٥)مقارنتا مع بقية المجاميع بالتتابع مج٢ (٢,١٤٠±٧٠,٣٠٨)، مج٣(٣,٨١٢±٦٧,٤٢)، مج٤(٣,٦٠٣±٧٤,١٥) ومج السيطرة (٢.٠٠١±٧٧,٩٢). وكما أظهرت النتائج زيادة معنوية في مستوى انزيم الفوسفوتيز القاعدي في المجموعة الأولى (٣,٩٨±١٩٠,٠٢) مقارنتا مع بقية المجاميع بالتتابع مج٢ (١٨٠,٥٧ ±١,٨٩)، مج٣ (٥,٠٣±١٨١,٣٧)، مج٤ (٢,٤١ ±١٧٦,٨) و مجموعة السيطرة (٢,٤٧±١٥٣,١٢). أظهرت نتائج الكوثرهالانية :- جين RANK في المجموعة الأولى أظهرت زيادة معنوية فيه (١,٥٩+_١٢,١٥٧) مقارنتا مع المجاميع الثانية والثالثة والرابعة(٠,٦٠١+_٥,٥٠٩)،(٠,١٣٠+_٦,٩٠٣)و(٠,٠٢٧+_٣,٠١٨) بالتتابع . كذلك أظهرت النتائج وجود زياده معنويه في جين RANKL في المجموعة الأولى (٠,٧٦٦+_٨,٤٥٨) مقارنتا مع المجاميع الثانية والثالثة والرابعة بالتتابع (٠,٥٣+_٦,٠٧١)، (٠,٢٢٨+_٤,٩٧٠) و(٠,١١٤+_٣,٣٩٨) . في حين أظهرت النتائج وجود زياده معنويه في جين OPG في المجموعة الثانية والثالثة والرابعة بالتتابع (٠,٠٨+_١,٩٢٤)، (٠,٠٦٤+_١,٠١٤) و (١,٤١٩+_٥,٥٦٧) مقارنتا في المجموعة الأولى (٠,٠٠٩+_٠,٠٧٦) .استنتجنا من خلال الدراسة الحاليه ان عقار الجنستين و

زيت السمك أدى الى معادلة التاثير السمي لعقار الانسترازول و من هنا نستنتج ان الجنستين و زيت السمك يقلل خطورة هشاشة العظام في لحيوانات .

REFERENCES

1. **Bowles, S. K. (2011).** Drug-induced osteoporosis. *PSAP-VII Women's and Men's Health*. Boyce, B. F., & Xing, L. (2007). The Rankl/Rank/Opg Pathway. *Current osteoporosis reports*, *5*(3), 98-104.
2. **Fitzpatrick, L. A. (2006).** Estrogen therapy for postmenopausal osteoporosis. *ArquivosBrasileiros de Endocrinologia&Metabologia*, *50*(4), 705-719.
3. **Sundermeyer, M., Schlüter, R., & Ney, H. (2012).** LSTM neural networks for language modeling. In *Thirteenth annual conference of the international speech communication association*.
4. **Jones, D. H., Kong, Y. Y., &Penninger, J. M. (2002).** Role of RANKL and RANK in bone loss and arthritis. *Annals of the Rheumatic Diseases*, *61*(suppl 2), ii32-ii39.
5. **Krassas, G. E., &Papadopoulou, P. (2001).** Oestrogen action on bone cells. *Journal of Musculoskeletal and Neuronal Interactions*, *2*(2), 143-152.
6. **Velasco, R., Zharkikh, A., Troggio, M., Cartwright, D. A., Cestaro, A., Pruss, D., ...&Malacarne, G. (2007).** A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. *PloS one*, *2*(12), e1326.
7. **Reddel, H. K., Bateman, E. D., Becker, A., Boulet, L. P., Cruz, A. A., Drazen, J. M., ... &Lemanske, R. F. (2015).** A summary of the new GINA strategy: a roadmap to asthma control. *European Respiratory Journal*, *46*(3), 622-639.
8. **Altintas, D.; Kokcu, A., Kandemir, B.; Tosun, M. and Cetinkaya, M.B. (2010).**Comparison of the effects of raloxifene and anastrozole on experimental endometriosis. Eur J ObstetGynecolReprod Biol. 150(1):84-87.
9. **Nadhanan, R. R., Skinner, J., Chung, R., Su, Y. W., Howe, P. R., & Xian, C. J. (2013).** Supplementation with fish oil and genistein, individually or in combination, protects bone against the adverse effects of methotrexate chemotherapy in rats. *PloS one*, *8*(8). 71592.
10. **Frank, A.; Rohrbach, B.; Bailey, C.; West, A. and Oliver, C. (2003).** Steroid hormone concentration profiles in healthy intact and neutered dogs before and after cosyntropin administration. Domestic Animal Endocrinology 24. 43–57.

11. **Charles, P.; Poser, J.; Mosekilde, L. and Jensen, F. (1985).** Estimation of bone turnover evaluated by 47Ca-Kinetics efficiency of serum bone Gamma-Carboxyglutamic acid-containing protein, serum alkaline phosphatase, and urinary hydroxyproline excretion. J. Clin. Invest. 2254-2259.
12. **Kenneth, J.; Livak, and Thomas, D. (2001).** Analysis of Relative Gene Expression Data Using RealTime Quantitative PCR and the 22DDCT Method. Methods 25, 402–408.
13. **Al.Rawi, K.M. (2000).** Introduction to statistics.2nd Ed. college of Agriculture. University of Mosil.
14. **Shi, S., Zheng, S., Li, X. F., & Liu, Z. D. (2017).** The Effect of Estradiol on the Growth Plate Chondrocytes of Limb and Spine from Postnatal Mice in vitro: The Role of Estrogen-Receptor and Estradiol Concentration. *International journal of biological sciences*, *13*(1), 100.
15. **Miller, W. R., Bartlett, J., Brodie, A. M., Brueggemeier, R. W., Di Salle, E., Lønning, P. E., ... & Goss, P. E. (2008).** Aromatase inhibitors: are there differences between steroidal and nonsteroidal aromatase inhibitors and do they matter?. *The oncologist*, *13*(8), 829-837.
16. **Poulsen, R. C., Moughan, P. J., & Kruger, M. C. (2007).** Long-chain polyunsaturated fatty acids and the regulation of bone metabolism. Experimental Biology and Medicine, 232(10), 1275-1288.
17. **Bharathi, R., & Baby, D. (2017).** Osteoporosis risk assessment among rural women using osteoporosis self-assessment tool for Asians (OSTA). *IJAR*, *3*(4), 106-109.
18. **Cauley, J. A. (2015).** Estrogen and bone health in men and women. *Steroids*, *99*, 11-15.
19. **Klionsky, D. J., Abdalla, F. C., Abeliovich, H., Abraham, R. T., Acevedo-Arozena, A., Adeli, K., ...&Ahn, H. J. (2012).** Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy*, *8*(4), 445-544.
20. **Georgiou, K. R., Scherer, M. A., Fan, C. M., Cool, J. C., King, T. J., Foster, B. K., & Xian, C. J. (2012).** Methotrexate chemotherapy reduces osteogenesis but increases adipogenic potential in the bone marrow. Journal of cellular physiology, 227(3), 909-918.
21. **Ullmann, U., Bendik, I., &Fluhmann, B. (2005).** BonisteinTM (synthetic genistein), a food component in development for a bone health nutraceutical. Journal of Physiology and Pharmacology. Supplement, 56(1), 79-95.
22. **James, M. J., Gibson, R. A., & Cleland, L. G. (2000).** Dietary polyunsaturated fatty acids and inflammatory mediator production. The American journal of clinical nutrition, 71(1), 343-348.