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# Study Some Biochemical Parameters in Serum and Bone of Adults Female Rats Treated with Normal Omega-3 Fatty Acids

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**Abstract**

Bone diseases are increasingly reported as a challenging dilemma in a health care setting, due to limited therapeutic options, slow response of bone diseases to therapy and long-term needs to induce effects. Omega-3 Fatty acids (ω3FA) have recently been introduced in the treatment of some bone diseases mainly as adjuvant therapy. Herein we explored the potential therapeutic effects of ω3FA on bone formation and resorption in adult female rats if any. To do so, serum samples were collected from rats treated with ω3FA versus the control group and the serum was analysed for determination of the levels of "vitamin D, IGF-1, Alkaline phosphatase activity, Calcium, Phosphorus and Albumin" alongside femur bones were collected for bone ash study of rats treated with ω3FA versus a control group. Results showed a significant increase in vitamin D, Calcium, Phosphorus, and albumin and a significant reduction in alkaline phosphatase activity, Insulin-like growth factor-1 (IGF-1) in a group which gave 20 mg/kg of ω3FA compared to the control group, while treatment animals with 40 mg/kg of ω3FA caused a significant reduction in vitamin D and Phosphorus also a significant increasing in IGF-1, Alkaline phosphatase activity, and Albumin. Also, there was a significant increase in calcium and phosphorus content in bone ash of the group which took 20 mg/kg ω3FA while these contents were reduced in bone ash of the group taking 40 mg/kg ω3FA.

**Keywords:** ω3FA, vitamin D, IGF-1, Bone ash.

## Introduction

Omega-3 Fatty acids (ω3FA) refer to the location of the unsaturation bond relative to the omega end of this fatty acid. So the PUFAs ω3FA include fatty acids which are formed from other fatty acids or diets, and then converted in the body(Hahn et al.,2022; Ramirez et al., 2019). There are four major ω3FA, "α- linolinic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docasahexaenoic acid (DHA)". In the body, ALA has converted into EPA and then DPA and then to DHA. EPA and DHA are considered the main two ω3FA which have bioactivity of "polyunsaturated fatty acids". Polyunsaturated fatty acids have a critical function in the fluidity and integrity of the structure of the membrane, and also have a role in the expression of genes and substrate in the synthesis of mediators like eicosanoids (Husson et al., 2016; Chen et al., 2021). ω3FA have a good abundance in nature and they are a member of some supplements like GRAS(Generally recognized as safe) which are generally identified as safe. ω3FA like EPA and DHA has an inflammatory effect (Mc Claskey et al., 2007; Hathaway et al., 2020). The ability of EPA and DHA for reducing inflammations came from their ability to decrease the arachidonic acid(ARA) in phospholipids that are found in the membrane which is responsible for producing ARA-Lipid mediators and inflammation process(Calder P, 2015; Calder P, 2017). ω3FA also can reduce triacylglycerides and heart disease, in addition, ω3FA showed positive effects on patients with hypertriglyceridemia, and reduce blood pressure, also inflammation process(Marijana and

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Carla, 2021; Skulas et al., 2019). ω3FA has a good role in regulating bone metabolism, and its positive effects on bone mineral density (Lavado et al., 2018; Saini and Keum, 2018). ALA, EPA and DHA have an important role in osteoblastogenesis control and inhibited the resorption of bone (Kelly et al., 2013). The major origin of ω3FA is fish which protect the liver, kidney and cardiovascular system (Marco et al., 2021; Miles and Calder, 2017). ω3FA was used in clinical parenteral nutrition(Hylde et al., 2020). Therefore, the present study was applied to investigate the effects of different normal doses of ω3FA in some parameters of bone.

**Methodology**

1. Animals and Experimental Design: Fifteen female rats (3.5-4 months) weighing (200-230) gm were kept at conditions of temperature between 22-25 Cº, 12h light -12h dark in the animal's house in the college of veterinary medicine of Mosul university. Water and a standard diet were given. All rats were divided as follows, 5 rats for each group:

Group 1: Considered control group, and given D.W

Group 2: Rats were orally given 20 mg /Kg ω3FA

Group 3: Rats were orally given 40 mg /Kg ω3FA

2. Blood Collection: After the experimental period (40 days), the blood samples have withdrawn from the optical vein and left at room temperature to set and clot, and serum is collected and stored at -20 Cº for biochemical estimations(Elizabeth et al., 2021; Chawla, 2020).

3. Biochemical tests: Rat IGF-1 (Insulin-Like Growth Factor) and Vitamin D in serum determined by using ELISA kits from Elabscience company(Oster et al., 1995). Spectrophotometric estimation of serum alkaline phosphatase activity was done using the Biomerieux kit depending on Belfeld and Goldberg method (Wang et al., 2009). Biolabo, France kits were used to determine serum calcium and phosphorus spectrophotometrically. Albumin was estimated using a spectrophotometric kit (Al-Hakeim et al., 2022).

4. Bone ashing: After removing the soft tissues, the right femur bone was weighed, dried and incineration in muffle apparatus at 600 Cº for 12 hours. The ash was weighed and dissolved in 6NHCL, then converted into (100 ml) volumetric flask and completed the volume with 6NHCL. Calcium in bone ash is estimated according to the titration method(Yang et al., 2008). Inorganic phosphate in bone ash was determined spectrophotometrically by Plummer's method(Al-Hashemi et al., 2013).

**Data Analysis**

Statistical Analysis: All results expressed as means± S.D. Statistical significance was evaluated by one-way analysis of variance (ANOVA) using the SPSS test selector which was used to compare all groups within probability($p \leq 0.05$)(Umakantha N, 2016).

**Results**

Serum vitamin D was significantly elevated in group 2 treated with 20 mg/kg of ω3FA in comparison to the control group as shown in table (1), while there was a significant ($p \leq 0.05$)

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reduction in vitamin D in the group 3 that received 40 mg/kg compared with a control group and group 2. Table (1) also shows a significant ($p \leq 0.05$) reduction in IGF-1 levels in groups 2 and 3 in comparison with the control group, but group 3 which was treated with 40 mg/kg increase in IGF-1 levels significantly compared with group 2 which treated with 20 mg/kg but not returned to the level of the control group. The results in table(2) revealed a significant ($p \leq 0.05$) reduction in alkaline phosphatase activity in group 2 given 20 mg/kg compared with the control group. Although treatment of rats in group 3 with 40 mg/kg ω3FA caused a significant ($p \leq 0.05$) elevation in the activity of alkaline phosphatase compared with the control group and group 2( given 20 mg/kg). Serum calcium was increased significantly ($p \leq 0.05$) by increasing the dose of ω3FA, so there was a positive relationship between serum calcium and doses of ω3FA as shown in table 2. Serum phosphorus was significantly ($p \leq 0.05$) elevated in group 2, given 20mg/kg compared with the control group, while there was a significant ($p \leq 0.05$) decrease in phosphorus in group 3 given 40 mg/kg ω3FA in comparison with the control group and group 2(given 20 mg/kg). Results in Table 2 indicated the covariant increase in serum albumin in group 2 and group 3 that were treated with 20 mg/kg and 40 mg/kg of ω3FA respectively, this increase in serum albumin with increasing the doses of ω3FA consort with increasing of serum calcium levels. The results in table 3 showed a significant ($p \leq 0.05$) elevation in calcium percentage of bone ash in group 2 which was given 20 mg/kg compared with the control group. But a significant ($p \leq 0.05$) decrease in calcium percentage was noticed in bone ash of group 3 given 40 mg/kg compared with group 2 and the control group. Also, table 3 shows a significant increase in phosphorus percentage in bone ash of group 2 given 20 mg/kg and group 3 given 40 mg/kg ω3FA in comparison with the control group.

**Table 1.** Effect of different doses of ω3FA in serum Vitamin D, IGF-1

| Treatments | Vitamin D ng/ml | IGF-1 ng/ml |
|---|---|---|
| Control | 167.238±77.648 $^{b}$ | 188.093±16.086 $^{a}$ |
| 20 mg/kg | 242.980±41.245 $^{a}$ | 107.662±52.274$^{ab}$ |
| 40 mg/kg | 124.949±41.146 $^{ab}$ | 158.204±45.126 $^{b}$ |
| The values represent the mean± SD for 5 animals/group.<br>Different letters in the vertical mean a significant variance. | | |

**Table 2.** Effect of different doses of ω3FA in serum ALP activity, Ca, P and Albumin

| Treatments | ALP (U/L) | Ca (mg/dl) | P (mg/dl) | Albumin (mg/dl) |
|---|---|---|---|---|
| Control | 69.862±6.029$^{b}$ | 8.472±0.723$^{ab}$ | 7.692±0.493 $^{b}$ | 3.637±0.472$^{ab}$ |
| 20 mg/kg | 45.155±7.962$^{ab}$ | 10.415±0.240$^{b}$ | 8.712±0.850 $^{a}$ | 5.667±0.441 $^{b}$ |
| 40 mg/kg | 144.44±26.083$^{a}$ | 12.365±0.459$^{a}$ | 6.837±0.803$^{ab}$ | 6.902±0.442 $^{a}$ |
| The values represent the mean± SD for 5 animals/group.<br>Different letters in the vertical mean a significant variance. | | | | |

**Table 3.** Mineral contents in bone ash of right femur from studies groups

| Treatments | % Calcium | % Phosphorus |
|---|---|---|
| Control | 29.0±0.400 $^{ab}$ | 12.26±0.087 $^{ab}$ |
| 20 mg/kg | 34.5±0.425 $^{b}$ | 15.28±0.038 $^{b}$ |

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| | | |
|---|---|---|
| 40 mg/kg | 42.2±0.435 [a] | 16.09±0.190 [a] |
| The values represent the mean± SD for 5 animals/group. Different letters in the vertical mean a significant variance. | | |

## Discussions

The treatment of the rats (group 2) with 20 mg/kg ω3FA causes a considerable decline in serum alkaline phosphatase in comparison to the control group, this result revealed the improvement role of a small dose of ω3FA on osteogenesis. Our result is in line with Habib et al., 2020, were noticed a significant reduction in alkaline phosphatase and gamma-glutamyl-transferase after supplementation the diabetic patients with ω3FA. On other hand, 40 mg/kg of ω3FA that was given to group 3 caused a high elevation ($p \geq 0.05$) in alkaline phosphatase activity in comparison with the control group and group 2. This upregulation in the activity of alkaline phosphatase in group 3 may be responsible for the uplifted rate of bone turnover, which indicated an increase in both formation and resorption, but the resorption process exceeds the bone formation, which leads to loss of bone since Rachel et al., 1992 referred to the elevation of serum alkaline phosphatase and acid phosphatase in rats with ovariectomy lead to a high bone turnover rate, because of the high osteoblastic and osteoclastic activity, that leading to in net bone loss. There is a bone turnover that which bone resorption increased, so bone formation also increased, but resorption more than formation (El-Wakf et al., 2015; Hassan et al., 2013). These findings implied the importance of the dose, which means the improvement in bone after ω3FA supplementation is dose-dependent. A significant elevation in vitamin D was noticed in the group supplemented with 20 mg/kg ω3FA, while group 3 which was supplemented with 40mg/kg ω3FA showed a decrease in the level of vitamin D compared with the control group and group 2. These results indicated that a dose of 20 mg/kg is better than a dose of 40 mg/kg. There is a good relation between ω3FA and vitamin D (Jaafarnejad et al., 2013). As known the active form 1,25(OH) D is produced by the 1α hydroxylase enzyme in the kidney. ω3FA-PUFAs enhance the level of "1,25-dihydroxy vitamin D" level in dialysis patients (An et al., 2012). The decrease in serum levels of vitamin D in group 3 which was treated with 40 mg/kg, may result from the inhibition of the 1α-hydroxylase enzyme because the high of level of the enzyme. This result shows, that too many ω3FA doses have a negative effect on vitamin D3 levels and activation of 1α-hydroxylase. The results showed a significant decrease in IGF-1 in group 2 which treated ω3FA at a dose of 20 mg/kg, this result agrees with a study by (Lindsay et al., 2013), Who proposed the concentrations of glucose, Insulin and IGF-1 decreased but the concentration of IGF binding protein-3 (IGF BP-3) increased when low-fat diet with or without ω3FA was given. In addition, growth hormone GH and IGF-1 levels were decreased in old ageing. However study in Singapore reported a relation between IGF BP-3 and too much intake of ω3FA(Mayer., 1993). Some studies have shown no relationship between IGF-1, ω3FA and a low-fat diet(Aronson et al., 2011).

The elevation in serum calcium may result from increases in the calcium absorption through the intestine (Heaney et al., 2005), they noticed an increase in the absorption of calcium in humans when supplemented with ω3FA fatty acid. Our data revealed a significant increase in

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serum calcium levels after being treated with ω3FA, this result agrees with(Sun et al., 2004), they were reported an association between fish oil and calcium in osteoporotic rats, these results boost the hypothesized mechanism of excretion and calcium absorption can be adjusted after fish oil intake. ω3FA, have a favourable effect on the health of bone by a melioration of the balance of calcium and bone turnover. An increase in calcium content in the bone of the femur rats was noticed in our study. The ω3FA influence the structure of bone by its effect on osteoclastic and osteoblastic differentiation and their activity (Lau et al., 2013; Lavado et al., 2018).

Our present study revealed a significant elevation in phosphorus level of group 2 that was treated with 20 mg/kg in comparison to the control group, but serum phosphorus in group 3 which was given 40 mg/kg ω3FA was decreased compared with the control group and group 2. Table 2 showed a significant elevation in bone phosphorus in group 2 that was treated with 20 mg/kg in comparison with the control group, also there was a significant elevation in bone phosphorus in group 3 that was treated with 40 mg/kg ω3FA. This elevation in serum and bone phosphorus in group 2, refers to the improvement in bone mineral content, this result agrees with (Lau et al., 2013; Hamdoon et al., 2000; Silverman et al., 2015) who denoted, that fish oil which contains too much of DHA and EPA levels make rats with high bone mineral density and stronger bone than rats that given soybean oil, which contain a good source of ALA. The low level of serum phosphorus in group 3 that was treated with 40 mg/kg ω3FA may result from renal excretion. Our result showed a significant increase in albumin with increasing the dose of ω3FA, this elevation consort with the increase of serum calcium levels.

Some chronically used drugs were reported to be associated with side effects on kidney (Merkhan et al., 2022), thyroid glands (Merkhan, 2013a; Faisal et al., 2020), liver (Abdulqader et al., 2022), lipid profile (Merkhan, 2013b; Abdulrazzaq et al., 2020; Abdullah et al., 2021, Almukhtar et al., 2021), immune system (Faisal et al., 2019; Merkhan et al., 2020a; Almukhtar et al., 2022), or multiple sclerosis (Abdullah et al., 2012). In these cases, ω3FA could find an application as an adjuvant therapy to protect these vital organs either alone or combined with vitamins (Sulaiman et al., 2021; Merkhan et al., 2020b), minerals (Althanoon et al., 2021; Younis et al., 2022), or herbal remedies (Hamed et al., 2022). Nonetheless, these steps need approval via conducting in vivo experimental animal study and once approved, large scale application in human is wisable.

The limitation of the present study is small sample size, using ω3FA instead of ω3and/or ω6FA or a mixed. Another drawback of the study, is that only two doses were tested while its wisable to use a range of doses in such study. The duration of therapy might needs to be extended further to achieve better outcome.

## Conclusion

We concluded from our study, The importance of the selection of the appropriate dose of ω3FA to keep healthy bones and fit level of minerals.

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**Conflicts of interest**
The authors acknowledge that there were no difficulties with this manuscript.
**Ethical approv**al for this study was obtained from the laboratory animals house in the college of veterinary medicine of Mosul university with the number of approval UM.VET.2022.03 and date 11/1/2022

**References**

[1] Al-Hakeim, H. K., Al-Jassas, H. K., Morris, G., & Maes, M. (2021). Increased angiotensin-converting enzyme 2, sRAGE and immune activation, but lowered calcium and magnesium in COVID-19: association with chest CT abnormalities and lowered peripheral oxygen saturation. *medRxiv*.

[2] M Al-Hashemi, H., M Al-Khashab, E., & A Hamdoon, A. (2013). Effect of high doses of omega-3 fatty acids on the metabolism of bones in adult females rats. Rafidain Journal of Science, 24(5), 17-26.

[3] An, W. S., Lee, S. M., Son, Y. K., Kim, S. E., Kim, K. H., Han, J. Y., ... & Park, Y. (2012). Omega-3 fatty acid supplementation increases 1, 25-dihydroxyvitamin D and fetuin-A levels in dialysis patients. Nutrition Research, 32(7), 495-502.

[4] Galet, C., Gollapudi, K., Stepanian, S., Byrd, J. B., Henning, S. M., Grogan, T., ... & Aronson, W. J. (2014). Effect of a Low-Fat Fish Oil Diet on Proinflammatory Eicosanoids and Cell-Cycle Progression Score in Men Undergoing Radical ProstatectomyLow-Fat Fish Oil Diet, Serum Eicosanoids, and Prostate Cancer. Cancer prevention research, 7(1), 97-104.

[5] Calder, P. C. (2015). Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1851(4), 469-484.

[6] Calder, P. C. (2017). Omega-3 fatty acids and inflammatory processes: from molecules to man. Biochemical Society Transactions, 45(5), 1105-1115.

[7] Chawla, R. (2014). Practical clinical biochemistry: methods and interpretations. JP Medical Ltd.

[8] Chen, J., & Vitetta, L. (2021). Modulation of Gut Microbiota for the Prevention and Treatment of COVID-19. Journal of Clinical Medicine, 10(13), 2903.

[9] Duchow, E. G., Duchow, M. W., Plum, L. A., & DeLuca, H. F. (2021). Vitamin D binding protein greatly improves bioactivity but is not essential for orally administered vitamin D. Physiological Reports, 9(23), e15138.

[10] El-Wakf, A. M., Hassan, H. A., Mahmoud, A. Z., & Habza, M. N. (2015). Fenugreek potent activity against nitrate-induced diabetes in young and adult male rats. Cytotechnology, 67(3), 437-447.

[11] Habib, T. B., Akhter, Q. S., Imam, H., Yeasmin, N., Rahman, F., Nahar, S., ... & Akhter, T. (2020). Useful Effects of Omega-3 Fatty Acids on Serum Alkaline phosphatase And Gamma-glutamyl-transferase Levels in Middle Aged Patients with Diabetes mellitus. Bioresearch Communications-(BRC), 6(1), 806-809.

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Article Received: 10 April 2022; Revised: 15 June 2022; Accepted: 25 June 2022; Publication: 31 August 2022

[12] Hahn, J., Cook, N. R., Alexander, E. K., Friedman, S., Walter, J., Bubes, V., ... & Costenbader, K. H. (2022). Vitamin D and marine omega 3 fatty acid supplementation and incident autoimmune disease: VITAL randomized controlled trial. bmj, 376.

[13] Hamdoon, A. A., Al-khashab, E. M., & Al-hashemi, H. M. (2020). Effect of saponin extract of Glycyrrihiza glabra in activity of hepatic enzymes and some biochemical parameters in serum of adults ovariectomized female rats. Iraqi Journal of Veterinary Sciences, 34(2), 411-415.

[14] Hassan, H. A., El Wakf, A. M., & El Gharib, N. E. (2013). Role of phytoestrogenic oils in alleviating osteoporosis associated with ovariectomy in rats. Cytotechnology, 65(4), 609-619.

[15] Hathaway III, D., Pandav, K., Patel, M., Riva-Moscoso, A., Singh, B. M., Patel, A., ... & Abreu, R. (2020). Omega 3 fatty acids and COVID-19: a comprehensive review. Infection & chemotherapy, 52(4), 478.

[16] Heaney, R. P., Carey, R., & Harkness, L. (2005). Roles of vitamin D, n-3 polyunsaturated fatty acid, and soy isoflavones in bone health. Journal of the American Dietetic Association, 105(11), 1700-1702.

[17] Spector, P. E., Fox, S., Penney, L. M., Bruursema, K., Goh, A., & Kessler, S. (2006). The dimensionality of counterproductivity: Are all counterproductive behaviors created equal?. Journal of vocational behavior, 68(3), 446-460.

[18] Zirpoli, H., Chang, C. L., Carpentier, Y. A., Michael-Titus, A. T., Ten, V. S., & Deckelbaum, R. J. (2020). Novel approaches for omega-3 fatty acid therapeutics: chronic versus acute administration to protect heart, brain, and spinal cord. Annual review of nutrition, 40, 161.

[19] Jaafarnejad, F., Hosseini, S. F., Mazlom, S. R., & Hami, M. (2013). Comparison of the effect of fish oil and vitamin E on the duration of Cyclic mastalgia. Evidence Based Care, 3(1), 69-76.

[20] Kelly, O. J., Gilman, J. C., Kim, Y., & Ilich, J. Z. (2013). Long-chain polyunsaturated fatty acids may mutually benefit both obesity and osteoporosis. Nutrition research, 33(7), 521-533.

[21] Lau, B. Y., Cohen, D. J., Ward, W. E., & Ma, D. W. (2013). Investigating the role of polyunsaturated fatty acids in bone development using animal models. Molecules, 18(11), 14203-14227.

[22] Lavado-García, J., Roncero-Martin, R., Moran, J. M., Pedrera-Canal, M., Aliaga, I., Leal-Hernandez, O., ... & Canal-Macias, M. L. (2018). Long-chain omega-3 polyunsaturated fatty acid dietary intake is positively associated with bone mineral density in normal and osteopenic Spanish women. PloS one, 13(1), e0190539.

[23] Young, L. R., Kurzer, M. S., Thomas, W., Redmon, J. B., & Raatz, S. K. (2013). Low-fat diet with omega-3 fatty acids increases plasma insulin-like growth factor concentration in healthy postmenopausal women. Nutrition Research, 33(7), 565-571.

[24] Sartorio, M. U. A., Pendezza, E., Coppola, S., Paparo, L., D'Auria, E., Zuccotti, G. V., & Berni Canani, R. (2021). Potential role of omega-3 polyunsaturated fatty acids in pediatric food allergy. Nutrients, 14(1), 152.

[25] Tadic, M., Sala, C., Grassi, G., Mancia, G., Taddei, S., Rottbauer, W., & Cuspidi, C. (2021). Omega-3 fatty acids and coronary artery disease: more questions than answers. Journal of Clinical Medicine, 10(11), 2495.

[26] Mayer, E. J., Newman, B., Quesenberry Jr, C. P., & Selby, J. V. (1993). Usual dietary fat intake and insulin concentrations in healthy women twins. Diabetes care, 16(11), 1459-1469.

[27] McClaskey, E. M., & Michalets, E. L. (2007). Subdural hematoma after a fall in an elderly patient taking high-dose omega-3 fatty acids with warfarin and aspirin: case report and review

of the literature. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 27(1), 152-160.

[28] Miles, E. A., & Calder, P. C. (2017). Can early omega-3 fatty acid exposure reduce risk of childhood allergic disease?. Nutrients, 9(7), 784.

[29] Oster, M. H. (1995). Fielder PJ, Levin N, and Cronin MJ. Adaptation of the growth hormone and insulin-like growth factor-I axis to chronic and severe calorie or protein malnutrition. J Clin Invest, 95, 2258-2265.

[30] Wahnon, R., Cogan, U., & Mokady, S. (1992). Dietary fish oil modulates the alkaline phosphatase activity and not the fluidity of rat intestinal microvillus membrane. The Journal of nutrition, 122(5), 1077-1084.

[31] Ramirez, J. L., Gasper, W. J., Khetani, S. A., Zahner, G. J., Hills, N. K., Mitchell, P. T., Grenon, S. M. (2019). Fish oil increases specialized pro-resolving lipid mediators in PAD (the omega-pad II trial). Journal of Surgical Research, 238, 164-174.

[32] Saini, R. K., & Keum, Y. S. (2018). Omega-3 and omega-6 polyunsaturated fatty acids: Dietary sources, metabolism, and significance—A review. Life sciences, 203, 255-267.

[33] Silverman, S., Curtis, J., Saag, K., Flahive, J., Adachi, J., Anderson, F., ... & Compston, J. (2015). International management of bone health in glucocorticoid-exposed individuals in the observational GLOW study. Osteoporosis international, 26(1), 419-420.

[34] Skulas-Ray, A. C., Wilson, P. W., Harris, W. S., Brinton, E. A., Kris-Etherton, P. M., Richter, C. K., Welty, F. K. (2019). Omega-3 fatty acids for the management of hypertriglyceridemia: a science advisory from the American Heart Association. Circulation, 140(12), e673-e691.

[35] Sun, L., Tamaki, H., Ishimaru, T., Teruya, T., Ohta, Y., Katsuyama, N., & Chinen, I. (2004). Inhibition of osteoporosis due to restricted food intake by the fish oils DHA and EPA and perilla oil in the rat. Bioscience, biotechnology, and biochemistry, 68(12), 2613-2615.

[36] Levene, H. (1960). Contributions to probability and statistics. Essays in honor of Harold Hotelling, 278, 292.

[37] Wang, J. H., Wang, K., Bartling, B., & Liu, C. C. (2009). The detection of alkaline phosphatase using an electrochemical biosensor in a single-step approach. Sensors, 9(11), 8709-8721.

[38] Yang, L. C., Wu, J. B., Ho, G. H., Yang, S. C., Huang, Y. P., & Lin, W. C. (2008). Effects of poly-γ-glutamic acid on calcium absorption in rats. Bioscience, biotechnology, and biochemistry, 72(12), 3084-3090.

[39] Almukhtar HM, Faisal IM, Merkhan MM. Short-term treatment with Atorvastatin selectively decreases Lymphocyte count. Research Journal of Pharmacy and Technology. 2022 Feb 1;15(2):689-94.

[40] Almukhtar HM, Faisal IM, Merkhan MM. Acute effect of atorvastatin in comparison with rosuvastatin on glucose homeostasis in hypercholesteremic patients. Pharmacology. 2021;25:25-34.

[41] Merkhan MM, Abdullah E, Althanoon Z. Effect of Esomeprazole on serum creatinine and urea in patients with Peptic Ulcer. Research Journal of Pharmacy and Technology. 2022;15(1):160-4.

[42] Abdullah E, Dhiaa S, Saleh K, Merkhan M. Effect of esomeprazole on lipid profile in patients with peptic ulcer. Pharmacia. 2021 Aug 17;68:613.

[43] Merkhan MM, Faisal IM, Alsaleem DZ, Shindala OM, Almukhtar HM, Thanoon IA. Immunodepressant and oxidant potential of standard leukaemia drug regimen. International Journal of Research in Pharmaceutical Sciences. 2020a;11(4):1-4.

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*http://www.veterinaria.org*
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[44] Abdullah KS, Majdal HM, Mohamad M. Oxidative Stress in Patients with Multiple Sclerosis on Interferon Therapy. Tikrit Medical Journal. 2012 May 1;18(2).

[45] Abdulqader SW, Faisal IM, Saeed MG, Merkhan MM. Fluvoxamine Suppressed Oxidative Stress associated with Tissue Erosion. Research Journal of Pharmacy and Technology. 2022 Feb 1;15(2):819-24.

[46] Faisal IM, Almukhtar HM, Merkhan MM, Alobaidi RW. Comparative anti-inflammatory effect of risperidone versus olanzapine in schizophrenic patients. Indian Journal of Public Health Research & Development. 2019 Aug 1;10(8):964-.

[47] M Merkhan M. The effects of glibenclamide on thyroid function tests in type 2 diabetic patients. Iraqi Journal of Pharmacy. 2013 Dec 28;13(2):55-61.

[48] Sulaiman EA, Dhiaa S, Merkhan MM. Overview of vitamin D role in polycystic ovarian syndrome. MMSL, 91(1), 37-43. doi: 10.31482/mmsl.2021.027.

[49] Althanoon ZA, Merkhan MM. Effects of zinc supplementation on metabolic status in patients with metabolic syndrome. Acta Poloniae Pharmaceutica. 2021 Jul 1;78(4):521-6.

[50] Younis HY, Thanoon IA, Fadhil NN, Merkhan MM. Effect of Zinc as an Add-On to Metformin Therapy on Glycemic control, Serum Insulin, and C-peptide Levels and Insulin Resistance in Type 2 Diabetes Mellitus Patient. Research Journal of Pharmacy and Technology. 2022 Mar 24;15(3):1184-8.

[51] Hamed ZS, Abed RR, Almashhadany MS, Merkhan MM. Effects of Hypericum perforatum on serum lipid vascular systems in mice. Iraqi Journal of Veterinary Sciences. 2022 Mar 22;36(2):525-30.

[52] Faisal IM, Merkhan MM, Almukhtar HM. Effect of chronic Allopurinol therapy on Thyroid function in patients with urate stones. Journal of Advanced Pharmacy Education & Research| Oct-Dec. 2020;10(4):5.

[53] Abdulrazzaq G, Khalaf MM, Merkhan MM. Allopurinol therapy impairs lipid metabolism in patients with renal stone. Pharmacology. 2020;1:1.

[54] Merkhan MM, Abdullah KS. The role of vitamin C and E in improving hearing loss in patients with type 2 diabetes. Annals of the College of Medicine, Mosul. 2020b Jan 29;41(2):184-9.

[55] Merkhan MM. Effect of metformin, glibenclamide and insulin on lipid profile in type 2 diabetic patients. Tikret Journal of Pharmaceutical Sciences. 2013b;9(2).