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Effect of Atorvastatin Drug on The Liver Enzymes of Pregnant Mice and The Deformities of Their Fetus

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ABSTRACT

Atorvastatin is used by many people around the world as a cholesterol-lowering medication in blood . And this study aimed to identify the physiological changes in the liver enzye of experimental mice . Where 30 mice were used, and they were divided into five groups, group A is the control consisting of 6 mice, Group B included 6 pregnant mice that were fed a high-fat diet, Group C included 6 pregnant mice dosed with concentration 20mg/kg of atorvastatin, Group D included 6 pregnant mice dosed at a concentration of 40mg /kg , Group E included 6 pregnant mice dosed at a concentration of 80mg /kg ,Starting from the seventh day of pregnancy, the mice were dissected on the 18th day, and the concentrations of liver enzymes Alkaline phosphatase(ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), were measured. Where the results of the study showed an increase in liver enzymes in the blood serum. The fetus showed phenotypic changes from swelling of the body and face, shortness of the limbs, lack of phalanges, fusion of the phalanges, enlargement of the hind limb, long and hooked tail, short mandible, small size of the fetus, curvature of the trunk, and the appearance of fetus with one foot, And the most deformities were in the group that was dosed at a concentration of 80 mg. From the foregoing, we conclude that atorvastatin has harmful effects on the physiological parameters of liver enzymes and fetus in pregnant mice.

Introduction

Statins are divided into two groups of natural and synthetic products based on the origin. They are classified as natural (such as Lovastatin),semi-synthetics (such as simvastatin and pravastatin), and synthetic compounds (such as fluvastatin, atorvastatin, cerivastatin, rosuvastatin, and pentavstatin) (Tajabadi *et al*., 2015; Martín *et al*., 2011). Cholesterol-lowering statins include Atorvastatin, Fluvastatin, Lovastatin, Pitavastatin, Pravastatin, Rosuvastatin, and Simvastatin confirmed that among these statins, atorvastatin and rosuvastatin are the most effective as they can reduce the level of lipoprotein LDL-C by 30% even in the case of low doses.(Tobert, 2003; Endo, 2010). Atorvastatin is available in the form of atorvastatin calcium tablets in doses (10, 20, 40 and 80 mg). The drug can be taken with or without food at any time of the day. Doses of atorvastatin can be based on its ability to lower LDL-C intensity(Stone et al.,2014). The American College of Cardiology/American Heart Association Guidelines recommend either low-dose atorvastatin 10-20 mg or high-dose atorvastatin 40-80 mg (Stone .,2014) .Atorvastatin is rapidly absorbed after oral administration and reaches its peak concentration in blood plasma within 1-2 hours, as it is highly bound to plasma proteins by 98%, especially albumin (Pfizer, 2015). The liver and intestines produce lipoprotein compounds, which transport cholesterol through the blood. These are large, complex compounds consisting of a mixture of cholesterol, an ester salt, phospholipids, triglycerides, and various proteins(Harvard .,2023; Better .,2023). Statins lower cholesterol levels through selective and competitive inhibition of HMG-CoA reductase, the enzyme that slows the conversion of HMG-CoA into mevalonic acid, a precursor of sterols including cholesterol.Inhibiting this enzyme initially leads to a decrease in cholesterol in the liver ,however, compensatory mechanisms induce greater expression of enzyme receptorsHMG-CoA reductase and LDL-C(Raghow,2017) . Statins act indirectly by increasing LDL-C receptor uptake and thus reducing plasma LDL-C, leading to a marked decrease in plasma triglycerides (Stender et al., 2005).Statins are still the first-line treatment as they have been shown to reduce the risk of major vascular disease by lowering LDL-C, yet many patients do not reach target LDL-C levels.Ezetimibe, Fibrates, and Nicotinic acid are type II drugs that should be used in combination with statins if lipid targets cannot be reached (Zodda et al., 2018).Several studies in rats indicate that the use of HMG-CoA inhibitors is teratogenic at toxic doses (Henck et al.,1998).

MATERIALS AND METHODS

Preparation of Animals and Rifam

30 Swiss albino mice of the Mus musculus strain were used, their ages ranged between (10-12) weeks and weights (25 ±2) grams, as they were obtained from the animal house of the University of Kirkuk, and all the mice were in good health. The animals were housed under suitable conditions in terms of temperature, lighting cycle, and ventilation. Cholesterol was obtained from the store of the Faculty of Science, and atorvastatin was used at a concentration of 10 mg ⁄ kg to obtain all concentrations, and all concentrations were obtained according to the following steps:

First concentration * first volume = second concentration * second volume

Group **A** is the control group

Group **B**, group that was fed a high-fat diet

Group **C** was treated with atorvastatin at a concentration of 20mg/kg

Group **D** was treated with atorvastatin at a concentration of 40mg/kg
Group **E** was treated with atorvastatin at a concentration of 80mg/kg
Where they were dosed by stomach tube.

Physiolgical analysis

After the end of the dose period, the rats were partially anesthetized with chloroform to ensure the survival of the heart, and blood was drawn directly by puncturing the heart with medical syringes, and it was placed in tubes without anticoagulant and placed in a centrifuge for 15 minutes at a speed of 3000 rpm / min to obtain serum. To conduct serological tests to estimate the following serum titres AST, ALT, ALP.

Statistical Analysis

The statistical results were analyzed by ANOVA analysis of variance, and the arithmetic means were compared based on the Duncans

Results

The results showed as shown in Figure (1,2,3) an increase in the activity of liver enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) at the level of ($p<0.05$) in the serum of mice treated with atorvastatin at concentrations of 20, 40, 80 mg⁄kg when compared with the control group.The results showed a significant increase in the level of ALT in group B that was fed a high-fat diet, which amounted to (41.7 ± 3.33)) compared with the healthy control group, and a significant increase in the group treated with atorvastatin C, D, and E, which amounted, respectively (29.51 ± 1.44 IU/L , 31.59 ± 3.84 IU/L,). , 63.33 ± 2.61) compared to the control group, which amounted to (27.67 ± 1.74) (IU/L). As well as a significant increase in the level of AST in group B, which was fed a high-fat diet (127.11 ± 5.83) compared with a healthy control group (68.01 ± 3.95), and an increase in the concentration of AST in treated groups C, D, and E, which reached (191.7 ± 4.82 IU/L). 91.05 ± 6.21, 152.67 ± 4.31), compared to the control group, which was (68.01 ± 3.95 IU/L).As for the level of ALP, it recorded a rise in group B, which was fed a high-fat diet (234.75 ± 6.57 IU / L) compared with the healthy control group 47.13 ± 1.82 IU/L, C, E, D, which amounted to (IU / L 178.09 ± 3.89, 214.5 ± 5.73, 237.7 ± 8.93), compared to the control group, which was (47.13 ± 1.82 IU/L).

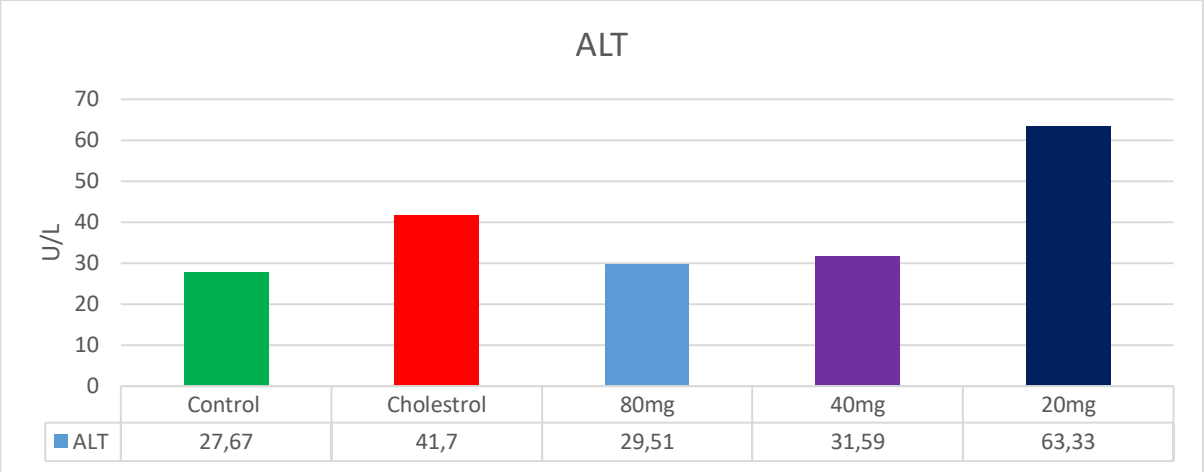

Figure (1) shows the effect of atorvastatin with the three concentrations on the concentration of the enzyme alanine aminotransferase (ALT (IU/L) in the serum of pregnant female rats.

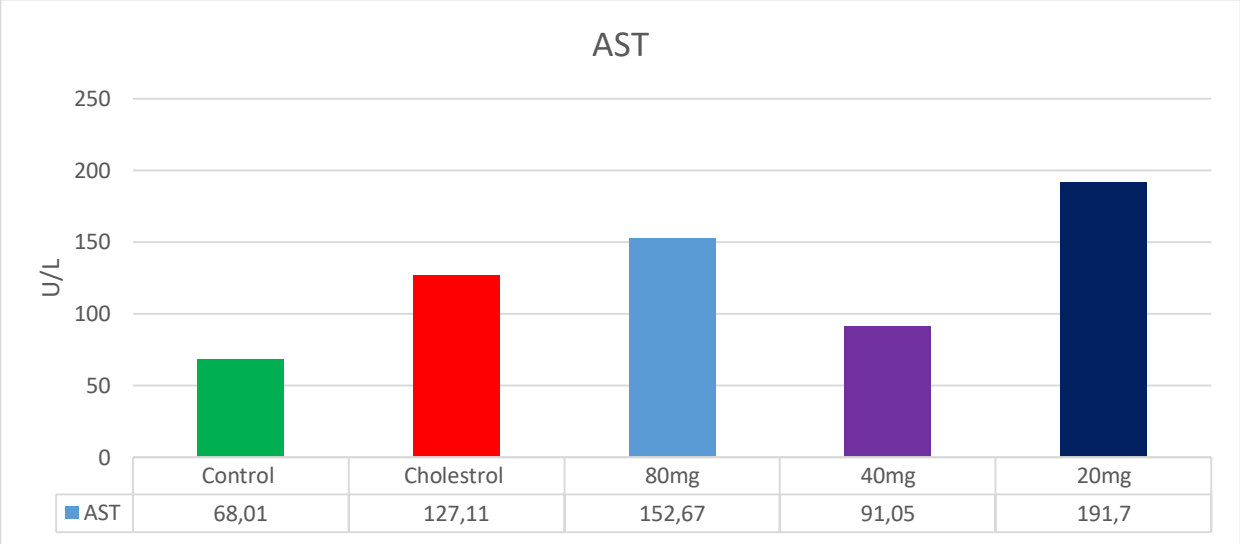

Figure (2) shows the effect of atorvastatin with the three concentrations on the concentration of the enzyme aspartate aminotransferase (AST (IU/L) in the serum of pregnant female rats.

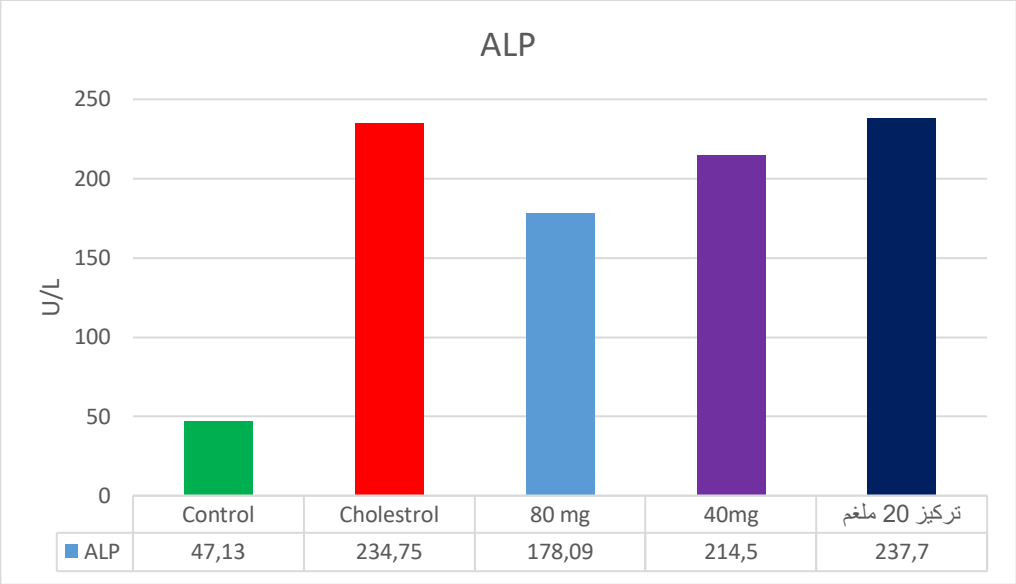

Figure (3) shows the effect of atorvastatin at three concentrations on the alkaline phosphate enzyme ALP in the serum of pregnant rats.

Fetal abnormalities

The results showed the emergence of many phenotypic deformities in all experimental groups at concentrations (20, 40, 80 mg⁄kg) as follows: The current study showed swelling in some areas of the fetus's body, including, curvature of the tail, blurring of facial features, prominence and congestion of the brain, prosencephalon, exophthalmia, congestion near the ear and eyes, curvature of the face due to cranial flexure, enlargement of the ear pinna, prolapse of the auditory disc Dctormity of ear placoid, as for the deformities occurring in the limbs, the limbs Abnormality, the results showed the occurrence of deformities in the front and back limbs as a result of the drug treatment, as it showed the shortness of the front limbs Micromelia, and the appearance of embryos with a raised hand to the top and bleeding in the foot, while the deformities that appeared at a concentration of 40mg⁄kg showed embryos with an oval shape, scaphocephaly, the meeting of the hind limbs with the forelimbs, the enlargement of the lower limbs and its contact with a thick tail, graininess of the skin, hemorrhage in the foot, and incomplete growth of the phalanges, as for for the embryos that were treated with a concentration of 80mg⁄kg, the embryos appeared deformed, decreased in weight, and the embryos appeared with one foot and a dark red color due to the occurrence of bleeding under the skin.

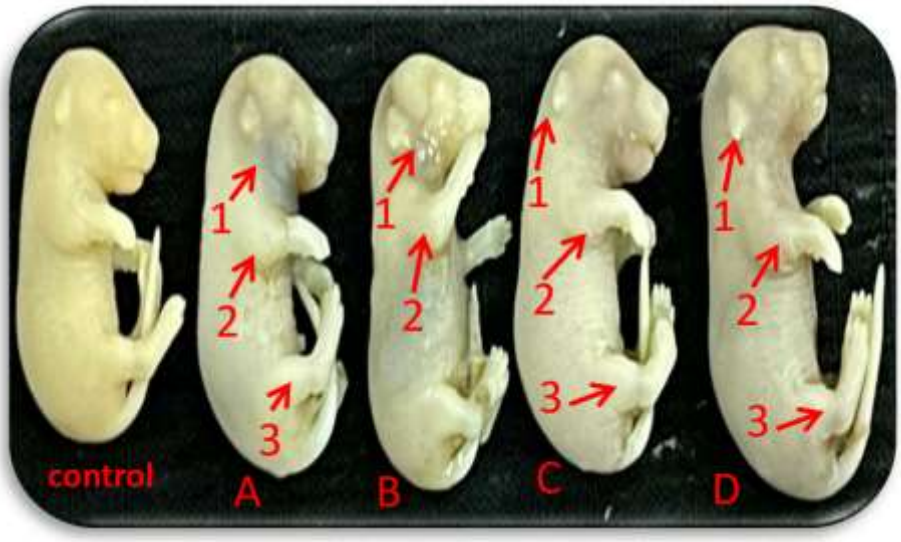

Figure (4) is aside view of a laboratory mouse embryo with Atorvastatin concentration 20mg⁄kg A-(1) Swelling of the neck area,(2) Micromelia,(3) Thickness of hind limbs. B-(1)Granularity in face ,(2)front end length. C-(1) Swelling of the eye socket,(2) Micromelia,(3)Lower extremity shortness. D- (1)Auditory Disc Flow,(2) Micromelia,(3) Lower extremity shortness.

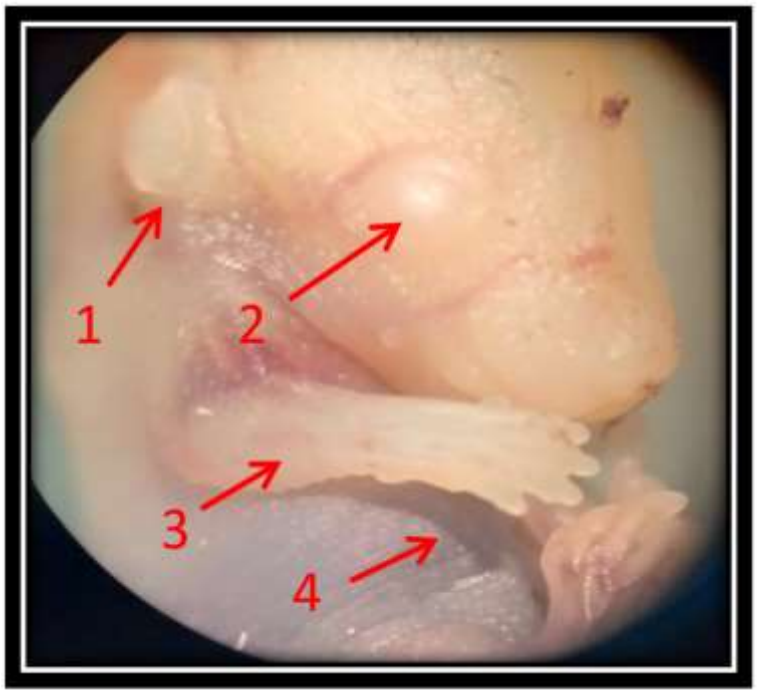

Figure (5) Image in a dissecting microscope ×2 side view of mouse embryo treated Atorvastatin concentration 20mg⁄kg :-1) Auditory Disc Flow,2) Exophthalmia , with 3) Micromelia , 4)Swelling of the abdominal area

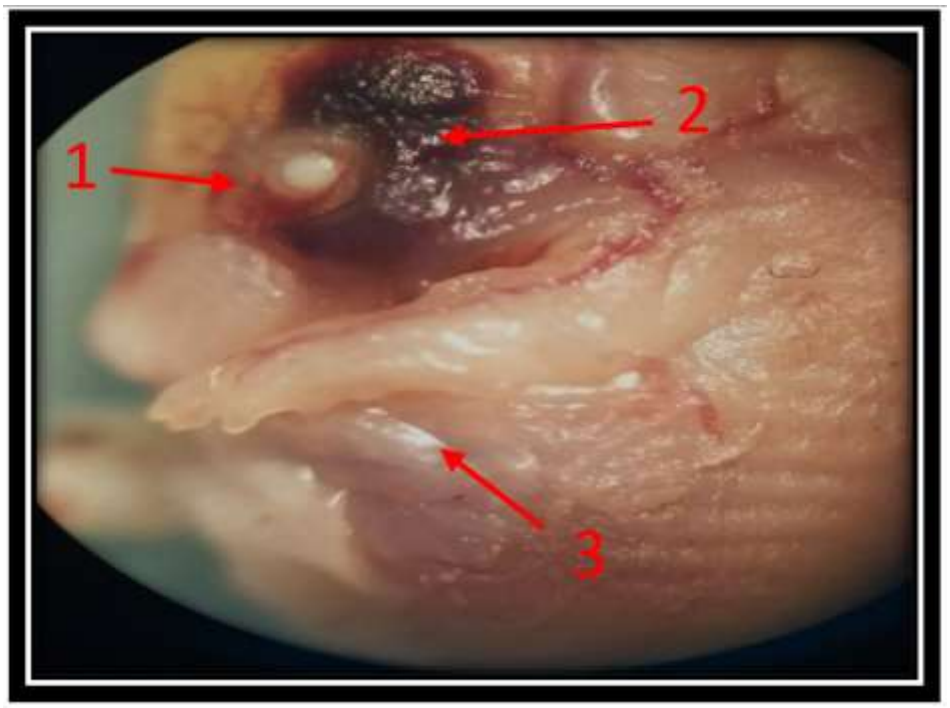

Figure (6) Image in a dissecting microscope ×2 side view of mouse embryo treated with Atorvastatin concentration 40mg⁄kg :-1) Exophthalmia 2)Hemorrhage and swelling of eye socket , 3) Micromelia

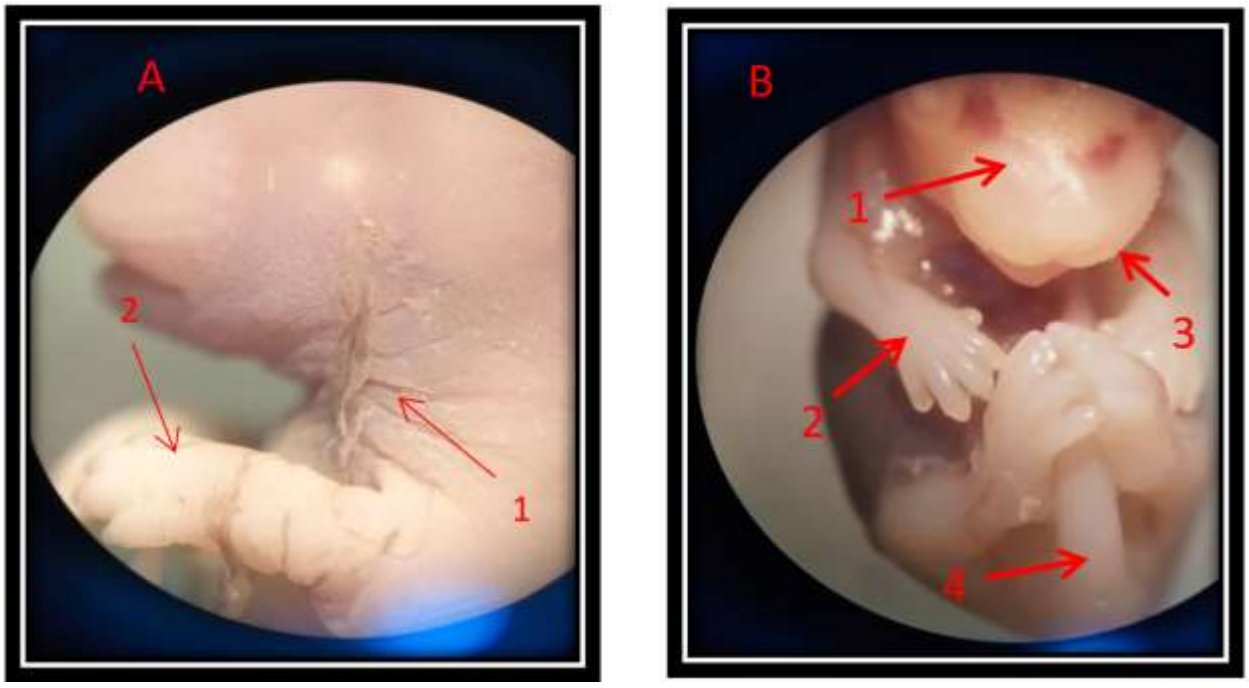

Figure (7): Image in a dissecting microscope ×2 view of mouse embryo treated with Atorvastatin concentration 40mg⁄kg :- A)1-Furrows in the neck area,2-the en largement of the phalanges B) 1-bleeding in nose, 2-Swelling of the snout area 3-Adhesion of the lower extremity phalanges, 4-Torision Tail

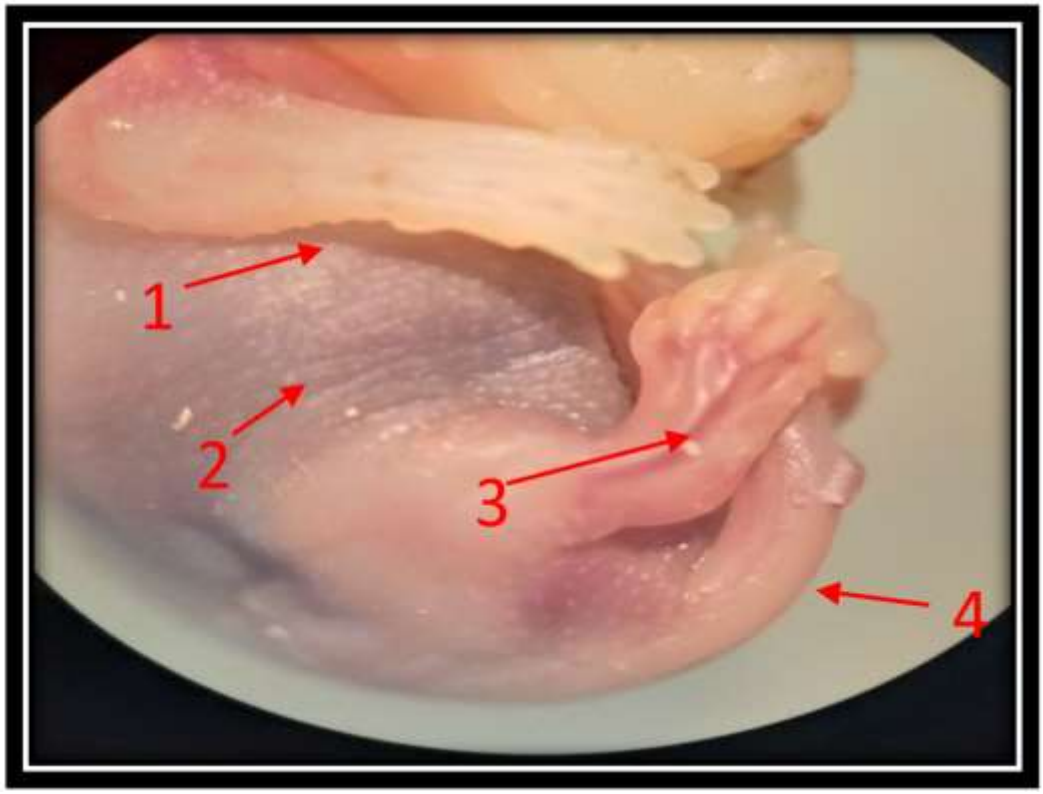

Figure (8) Image in a dissecting microscope ×2 side view of mouse embryo treated with Atorvastatin concentration 40mg⁄kg :-1) Micromelia ,2) Sowllen abdomn, 3)bleeding in the foot twisted tail towerd the abdomen 4)Twisted thic tail

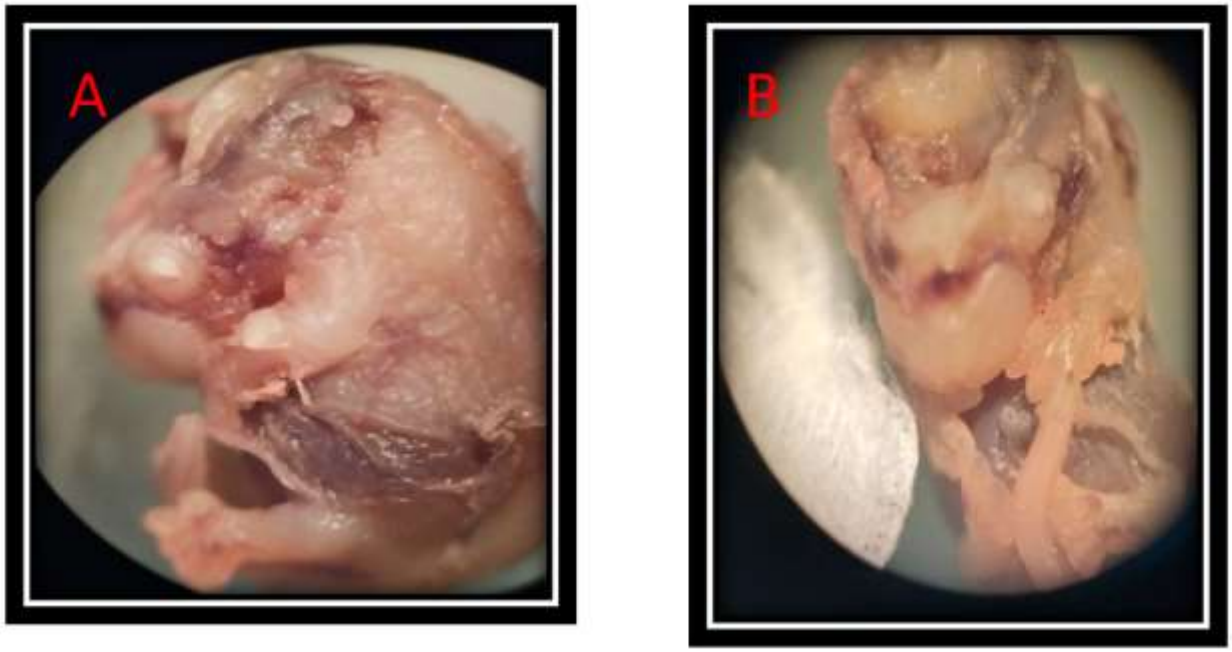

Figure (9) Image A and B in a dissecting microscope ×2 view of mouse embryo treated with Atorvastatin concentration 80mg ⁄kg view Deformed embryos

Discussion

The results of the current study showed that hyperlipidemia in mice led to an increase in the concentrations of liver enzymes ALT, AST, and ALP in serum compared with the control group,

and this was consistent with Liu et al (2021) and Alghamdi and his group (2018) who indicated in their study an increase in the level of ALT AST in mice fed cholesterol. These two enzymes are essential in the formation of amino groups that are necessary for entry into the urea cycle, and the concentrations of these enzymes are an important indicator of amino acid metabolism as well as an important indicator of liver function (Umarani et al, 2015), these enzymes are vital indicators of liver function in treated mice compared with the control group Its height is a result of damage to the histological structure of the liver, which causes the release of enzymes into the blood circulation, and this indicates the development of hepatotoxicity (Al- Jameil et al., 2014, Hoan et al., 2018). The researcher Ma et al. (2011) Malgrozta, et al. (2008) indicated an increase in the concentration of AST enzyme with the progression of drug administration, and in our study, mice were vaccinated for three weeks.The reason for the high concentration of ALT enzyme is attributed to changes in the hepatic cell membrane and leakage of the enzyme instead of direct injury, and this in turn leads to an increase in its concentration in the serum of mice (Mills, 2006), and the increase in ALT concentration in low doses in the group that was treated with a concentration of 20 mg of atorvastatin was consistent with (Rallidis et al. 2004), where they noted in their study a higher concentration of ALT in patients using statins compared to patients who did not use statins (Kashani, et al., 2006). The increase in the concentration of alkaline phosphatase enzyme ALP in the group fed a high-fat diet was consistent with (Al- Jameil et al., 2014, Hoan Im et al.,2018), and as the results of our study were consistent with the study of (Sowmya et al. 2016) which showed an increase in the concentration of alkaline phosphate enzyme and the reason for this is that statins caused necrosis of hepatic cells, and in our study the highest concentration was reached ALP in the group that received atorvastatin at a concentration of 20 mg compared to the control group, and this is not consistent with the results of the study(Sowmya 2016, Pasternal 2002), as we showed an increase in the concentration of the alkaline phosphate enzyme ALP in high doses. The reason for this may be that these studies were on sick people, but our study was on laboratory animals (mice). Elevated activity of the liver enzymes AST, ALT, and ALP is a biomarker of liver function. This is due to damage to the histological structure of the liver, thus causing the release of enzymes into the circulation after cellular damage that indicates the development of hepatotoxicity (Dias et al., 2009).High levels of AST, ALT, and ALP enzymes in the cytoplasm of hepatocytes as a result of liver injury lead to increased permeability of cell membranes(Albrahim and Alonazi.,2020) In this study, the effect of atorvastatin on liver enzymes in pregnant female mice was known.The current study showed the emergence of many fetal abnormalities in all groups treated with atorvastatin. Therefore, the use of statins should be avoided because it leads to a defect in the function of the developing fetus, which leads to miscarriage, premature birth, or mental and physical disabilities (Buhimschi 2009). The deformities that appeared in the limbs and the spine in the current study agreed with studies on rats and rabbits treated with statins, and they showed the occurrence of structural deformities (the spine and the limbs) (Dostal et al 1994). As the current study showed, teratogenic fetus and limb deficiency in groups that were given high doses of atorvastatin at a concentration of (80mg⁄kg) and these results are consistent with the studies of (Edison and Muenke, 2004).

Conclusions

Atorvastatin caused an increase in the concentration of liver enzymes, where the results showed a high rise in ALP and a slight increase in AST, ALT, that the use of the drug during pregnancy is unsafe because it causes many deformities in fetus, such as protrusion of the brain,

short limbs, exophthalmos, torsion tail, trunk curvature and others.

References

1. Albrahim T, Alonazi MA. 2020 .Role of Beetroot (Beta vulgaris) Juice on Chronic Nanotoxicity of Silver Nanoparticle-Induced Hepatotoxicity in Male Rats. Int J Nanomedicine.: 3471–3482.
2. Alghamdi ES., (2018). Protective Effect of Quinoa (Chenopodium Quinoa Willd.) Seeds Aginast Hypercholesterolemia in Male Rats. 9(6). Pages: 11-21.
3. Al-Jameil N., Farah A Khan, Sadia Arjumand, Mohammad F Khan and Hajera Tabassum. (2014). "Associated liver enzymes with hyperlipidemic profile in type 2 diabetes patients". Int J Clin Exp
4. Beth, D.; Robert, G. and Trapp. (2004). Basic and clinical biostatistics, 4th ed. Lung Medical book, Mc Graw-Hill Medical publishing Division.New York,PP: 83-154.
5. Better Health.2023, Cholesterol - healthy eating tips. Retrieved on the 30th of April,
6. Buhimschi CS, Weiner CP. Medications in pregnancy and lactation, part 1: teratology. Obstet Gynecol. 2009;113(1):166-188. doi:10.1097/AOG.0b013e31818d6788
7. Dias, R. J.; Satish B B.; Kailas K M. and Fransis J D. (2009). ASSESSMENT OF HEPATIC FUNCTION: A REVIEW. IJPRD. 1(10): 1-18.
8. Dostal LA,Schardein JL. Developmental toxicity of the HMG-CoA reductase inhibitor, atorvastatin, in rats and rabbits. Teratology. 1994;50(6):387-394.
9. Edison, R. J., and Muenke, M. (2004). Central nervous system and limb anomalies in case reports of first-trimester statin exposure N. Engl. J. Med. 350 (15), 1579–1582
10. Endo, A. (2010). A historical perspective on the discovery of statins. Proceedings of the Japan Academy, Series B, 86(5), 484-493.

a. Focus on old and new drugs. Pharmacy, 6(1), 10.

11. Harvard T.H CHAN. 2023 , Cholesterol. Retrieved on the 30th of April.
12. Henck JW, Craft WR, Black A, Colgin A, Ander test (100 mg/kg) group had a curvy internal- son JA. Pre- and postnatal toxicity of the HMG-Co reductase inhibitor atorvastatin in rats. Toxi-col sci 1998; 41:88- 99.
13. Hoan Im K., Choi J, Baek A. and Lee TS. (2018). "Hyperlipidemic Inhibitory Effects of Phellinus pini in Rats Fed with a High Fat and Cholesterol Diet". Mycobiology; 46(2): 159-167.
14. Kashani A, Phillips CO, Foody JM, et al. Risks associated with statin therapy: a systematic overview of randomized clinical trials. Circulation. 2006 .2797-2788:(25)114
15. Liu, Y.; Cheng, Z.; Ding, L.; Fang, F.; Cheng, K. A.; Fang, Q. and Shi, G. P. (2010). " Atorvastatin-induced acute elevation of hepatic enzymes and the absence of cross-toxicity of pravastatin" Int. J Clin Pharmacol Ther, 48(12): 798-802
16. Ma, R.C.; Liu, K.; Lam, P.M. and Cheung, L. (2011). Sonographic measurement of mesenteric fat predicts presence of fatty liver among subjects with polycystic ovary syndrome. The Journal of Clinical Endocrinology & Metabolism, 96,(3):799-807
17. Malgrozata, M. B.;George, O. and Martin, D. W. (2008). "An association between nonalcoholic fatty liver disease and polycystic ovarian syndrome" J.Gastrol & Haptol 24 (2):243-247.

18. Martín, J., Buchberger, W., Alonso, E., Himmelsbach, M., and Aparicio, I. (2011). Comparison of different extraction methods for the determination of statin drugs in wastewater and river water by HPLC/Q-TOF-MS. Talanta, 85(1), 607-615.
19. Mills, P. R. and Clarke, A. T. (2006). "Atorvastatin associated liver disease". Dig Liver Dis.; 38:772-777.]
20. Pasternal, R. C.; Smith, S. C; Bairez-Merz, C.; Grundy, S. M.; Cleeman, J. I. and Lenfant, C. S. L. (2002) "Circulation, Vol. 106,Patients With Hypercholesterolemia and Without Active Arterial Disease The American Journal of Cardiology. 91..
21. Pfizer. (2015)." Lipitor (atorvastatin calcium) tablets prescribing information" New York,NY.
22. Rallidis LS, Drakoulis CK, Parasi AS. Pravastatin in patients with nonalcoholic steatohepatitis: results of a pilot study. Atherosclerosis. 2004;174:193-196.
23. 23. Raghow, R. (2017). Statins redux: A re-assessment of how statins lower plasm cholesterol. World J. Diabetes, 8, 230–234.
24. Sowmya,P.;Muthiah, N.S.; Susshmitha,R. & Arvinth. A.(2016). Study of liver function abnormalities during statin therapy in a tertiary care center Available online www.jocpr.com Journal of Chemical & Pharmaceutical Research8(7):515-519 Res. Art. ISSN: 0975-7384.
25. Stender, S., Schuster, H., Barter, P., Watkins, C., Kallend, D., and MERCURY I Study Group. (2005). Comparison of rosuvastatin with atorvastatin, simvastatin and pravastatin in achieving cholesterol goals and improving plasma lipids in hypercholesterolaemic patients with or without the metabolic syndrome in the MERCURY I trial. Diabetes, Obesity and Metabolism, 7(4), 430-438.
26. Stone NJ. Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, Goldberg AC, Gordon D, Levy D, Lloyd-Jones DM. et al.(2014). ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation:129 (25 Suppl 2): S1-S45.
27. Tajabadi, F., Ghambarian, M., and Yamini, Y. (2015). Evaluation of three-phase hollow fiber microextraction based on two immiscible solvents coupled to GC and HPLC for determination of statin drugs in biological fluids. Analytical Methods, 7(7), 2959-2967.
28. Tobert, J. A. (2003). Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors. Nature reviews Drug discovery, 2(7), 517.
29. Umarani V., Sudhaker Muvvala A., Ramesh B.V., Lakshmi N., Sravanthi (2015). Rutin potentially attenuates fluoride induced oxidative stress mediated – cardiotoxicity, blood toxicity and dyslipidemia in rats. Toxicology mechanisms and methods 25 (2) 143-149
30. Zodda, D., Giammona, R., and Schifilliti, S. (2018). Treatment strategy for dyslipidemia in cardiovascular disease preven