International Journal of Medical Toxicology & Legal Medicine Volume 27, No. 4, 2024
e-ISSN: 0974-4614
p-ISSN: 0972-0448

Effect of Hypervitaminosis D on Histological Structure of Liverin Albino Rats

Aliaa Hanoon Al-Kaabi[1]*, Ali Fayadh Bargooth[2]

[1,2]Department of Biology, College of Education for Pure Sciences, University of Wasit, Wasit, Iraq
Email: aliaah@uowasit.edu.iq
*Corresponding Author

Received: 17.08.2024 Revised: 20.09.2024 Accepted: 11.10.2024

ABSTRACT
Vitamin D is essential for overall health, wellbeing, andincreased mortality and a variety of adverse consequences have been caused by hyper vitamin D insufficiency. This study was designed to determine the effects of toxic doses of vitamin D on the structures of liver and calcification in rats due to hypervitaminosis D. Forty adult Wistar rat males were divided to four groups at random and administered both oral gavage of vitamin D excepted control group water. For a period of sixty days, the 10 rats in the control group received distilled deionized water every day. Three of the experimental groups received varying doses of vitamin DI.U. per day for 60 days: a low dose (the LD, 10 rats) (3000 IU/rat/day), intermediate dose (ID, 10rats/8000 IU/rat/day, and high dose (HD, 10 rats, 20000 IU/rat/day). The LD group's organ histopathology exhibited fewer changes but the ID and HD groups' liver showed more deeply restricted degenerative deviations. In conclusion, the histological structure of liver calcification were affected with increased vitamin D dosage' with appeared of calcification.

Keywords: Vitamin D, Wistar rat, Immunohistochemistry, Fat-soluble vitamin, Iraq

INTRODUCTION

A nutritious diet is essential for every system of the body to function properly. An excess or deficiency of any one of these nutrients may cause negative effects on health and increase an individual's susceptibility for different diseases (Mahassni and Al-Sheikh, 2013).Over two billion people worldwide are impacted with major micronutrient deficiencies, becoming the main cause of chronic diseases and greater rates of morbidity and mortality. These nutritional deficiencies are especially prevalent in low-income but also developed nations (Tulchinsky, 2010). Vitamin D as a fat-soluble vitamin may be obtained through eating well, exercising, and exposure to sunlight. Vitamin D is necessary for the absorption of calcium and phosphate, bone health, and general health,manyindividuals around the world, of all ages and genders have a vitamin D deficiency, which makes it one of the most common deficiencies in nutrition (Mazahery and Hurst, 2015). In addition to raising the risk of fractures, osteoporosis, osteomalacia, and muscle weakness in adults, a vitamin D deficiency may lead to rickets in children. (Wanget al., 2012; Christakos et al., 2013)Furthermore, it has also been related to inflammatory, autoimmune, cancer, infectious, and cardiovascular diseases. Obesity, hypertension, and even depression and decline in cognition (Calvo et al.,2005; Urashima et al., 2010; DiRosa et al., 2011) Although it is highly unusual, using excess vitamin D supplements may cause vitamin D overdose, which is referred to as hypervitaminosis or intoxication. Vitamin supplements are employed in numerous countries for humans as well as animals (Hathcock et al., 2007; Holick, 2007). Hypervitaminosis d leads to the blood's calcium concentration increase, affected to the, liver, , in addition to soft tissues and organs, become calcified quicker than the bones (Morrow, 2001; Peterson and Fluegeman, 2013). Calcification Phosphate and deposits of calcium in an organic matrix are the reason for this. Various types of soft tissues are susceptible to calcification. The accumulation of calcium and other salts in the blood and tissues because of a systemic imbalance in their metabolism results in metastatic calcifications. They often damage the liver, (Skinner et al.,2003; Fathi and Sakr, 2014). Vitamin D is essential for overall health, wellbeing, and increased mortality and a variety of adverse consequences have been caused by hyper vitamin D insufficiency. This study was designed to determine the effects of toxic doses of vitamin D on the structures of liver and calcification in rats due to hypervitaminosis D.

MATERIALS AND METHODS

The study was performed by using 40 males of albino rats, were used with average weight (200g-250g) aged between(3-4) months

1. Group A Control group : normal diet and distilled water were given during thirty day period

2. Group B low dose of vitamin D3 / orally dose whith 3000IU/day for 60 day
3. Group C medium dose of vitamin D3/orally dosewhith8000 IU /day For 60 day
4. Group D high dose of vitamin d / orally dose whith20000 IU / day for 60 day

The scarification of animalwas performed and the targeted organ was collected (liver) and itwas preserved in 10%of neutral buffered formalin for histological study. The organs were prepared for histological technique to stain with Hematoxylin and Eosin (H and E) stain and Alizarin stain which detection abnormal calcium accumulation (Bancroft, and Laytonred, 2013)

RESULT

Histological examination of liver of negative control group revealed normal rat liver showedcentral vein, liver cords of hepatocytes radiating from the central vein and separated by blood sinusoid. Hepatocytes appearednormal rounded nuclei with prominent nucleoli;the liver was composed of a thin capsule and an indistinct parenchyma of the lobule and the peripheral portal area. The portal area is composed of branching of portal vein, hepatic artery and one or two bile ducts (Figure 1).

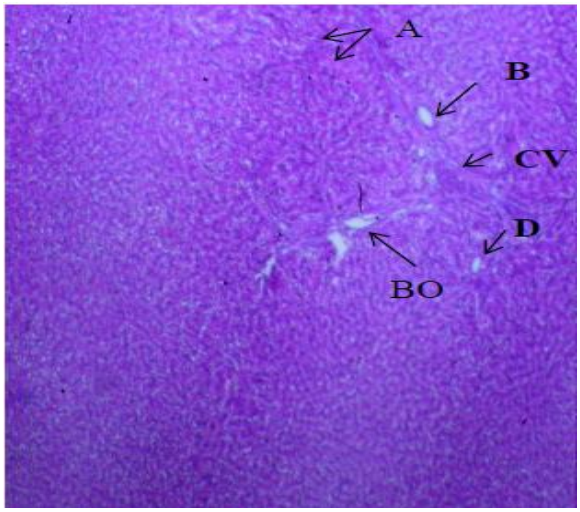

Figure 1: Normal liver (A):Hepatocyte (B):Blood vessels (CV):Connective tissue (D):Central vein (BO):Portal area (H and E stain 40x)

In current study showed that the histological properties of the liverwere appeared few changed when given low doses of vitamin D, hepatocytes were arranged in well-defined plates or cords radiating from the few dilation of central vein and portal triad was clearly visible (Figure 2).

An increase in the dose of vitamin D had led to the appearance of an increased focal thickening of the capsule and to the appearance of a higher number of underlying cells with dark inactive nuclei. Dark-stained cytoplasm and small dark nuclei, indicating apoptosis, appeared in clusters of hepatocytes in the intermediate dose (ID) liver (Figure 3). Darker apoptotic cells were seen in the high dose (HD) liver compared with the low dose(LD) and intermediate dose (ID) groups. The portal vein was dilation and congestion of portal vessels were observed in ID and HD groups as was the number of infiltrating inflammatory cells around bile ducts and necrotic hepatocytes were appeared (Figure 4).

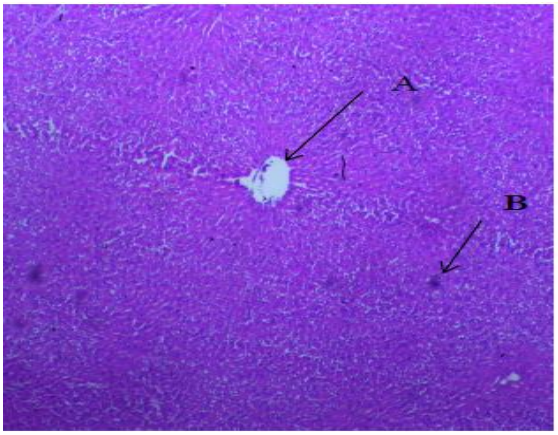

Figure 2: Low dose; (A): Dilation of central vein; (B): Small spot of calcification (H and E 40x)

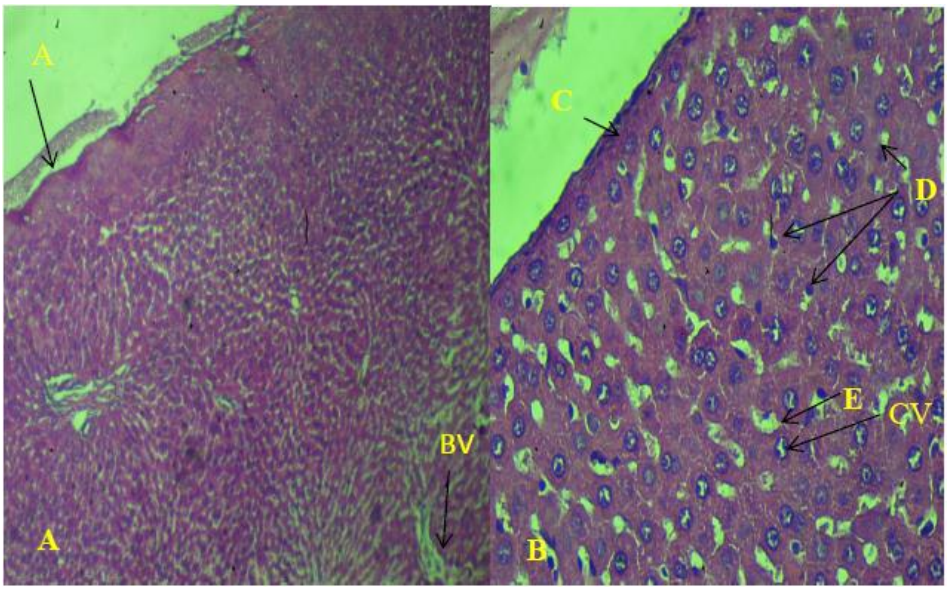

Figure 3A: Intermediate dose; (A):Thickening of capsule; (BV): Thickening of capsule(H and E40x);(3B):(C):Apoptosis; (D: Congestion of blood vessels; (E):Karyolysis; (CV):Karyohexis(H and E 400x)

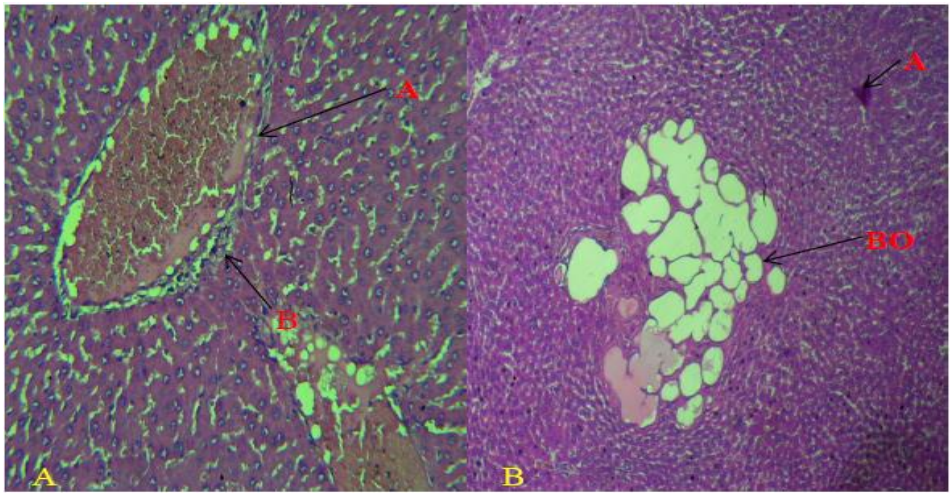

Figure 4A: High dose; (A) Sever congestion of central vein;(4B): (A): Spots of calcification (BO):Fatty change (H and E40x). Infiltration of lymphocyte (inflammatory cells) (H and E100x)

Fatty change was also observed(Figure 5).

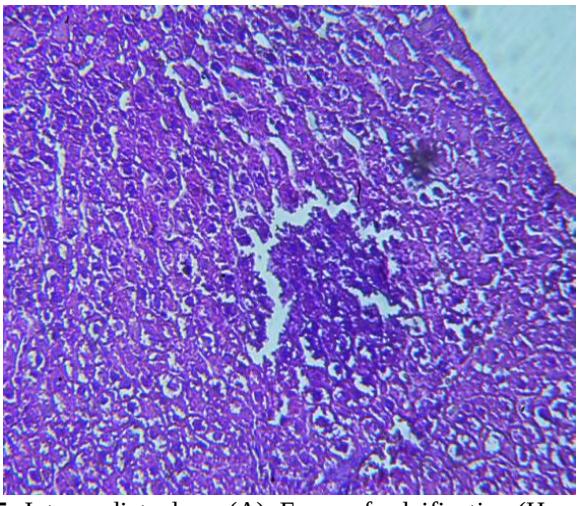

Figure 5: Intermediate dose; (A): Focus of calcification (H and E40x)

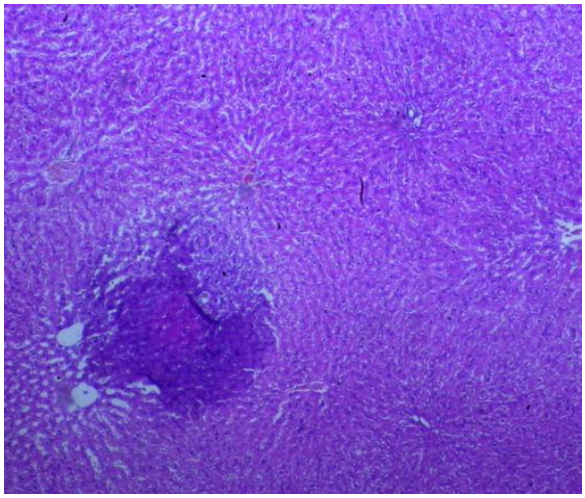

Figure 6: High dose; (CV): Focus of calcification(H and E100x)

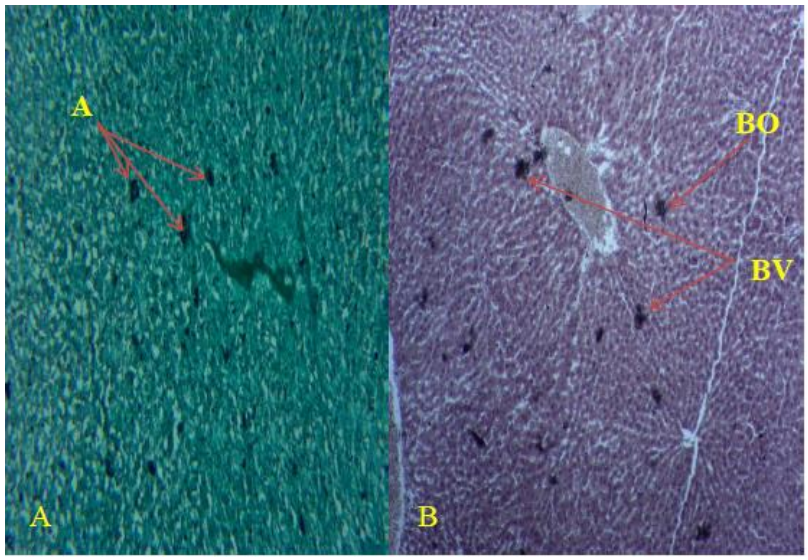

Figure 7A: Intermediate dose; (A):Area of calcification;(7B):High dose; (BV, BO):Area of calcification(alizarin100x)

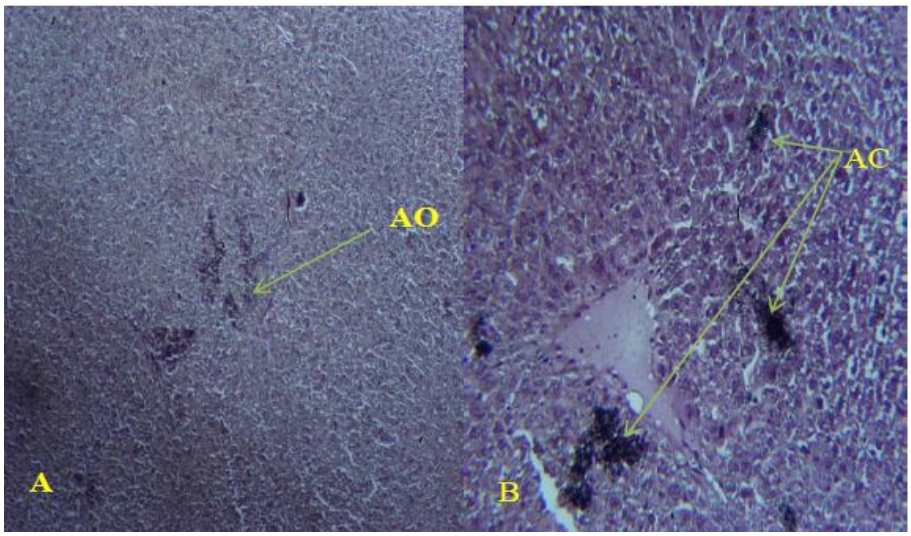

Figure 8A: Intermediate dose; (AO):Area calcification; (8B): Intermediate dose;(AC):Area calcification (DAB and Mayer hematoxylin, 100X)

In alizarin stain the liver showed the calcified area which is darker in colour, necropsied tissue surrounded by fibrous connective tissue with low dose (LD) and intermediate dose(ID)groups(Figures 8-10). The Immunohistochemical technique of liver sections was shown a slight calcification in low dose, mild calcification in intermediate dose and high calcification with high dose of vitamin d as dark to brown.

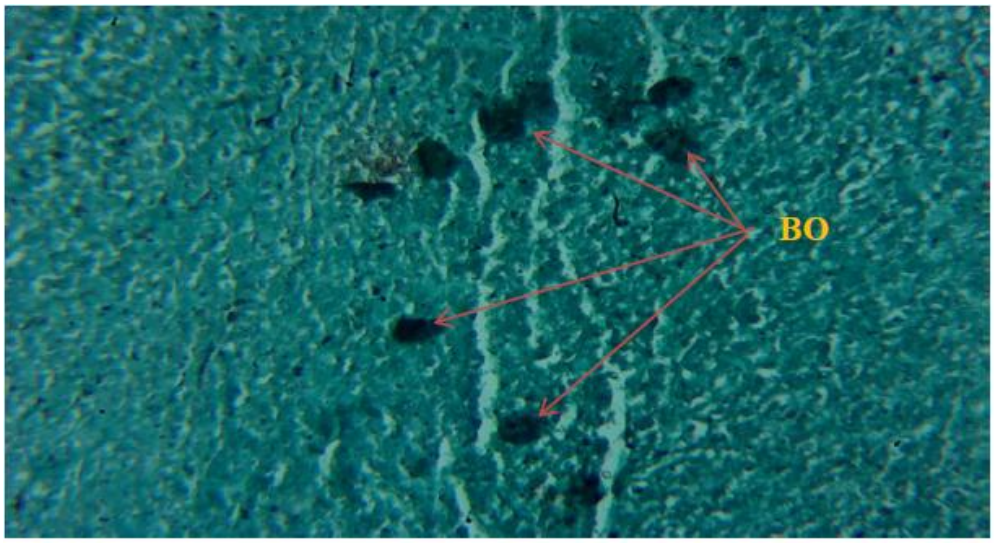

Figure 9: High dose;(BO):Area calcification (DAB and Mayer hematoxylin, 40X)

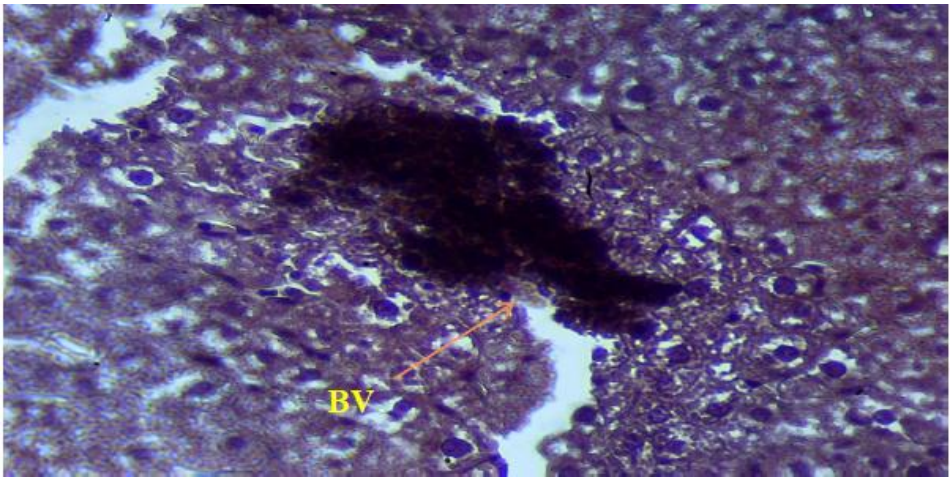

Figure 10: Intermediate dose; (BV):Area calcification (DAB and Mayer hematoxylin, 400X)

DISCUSSION

The present study appeared rats were take the low dose (LD) showed slight changes in the liver tissue, with a few cells exhibiting small dark nuclei and dark cytoplasm,other cells exhibited normal prominent nucleoli, this result agree with (Ali et al.,2018) and (Holick, 2017)that suggest the safety of low-dose vitamin D for normal hepatic structure and function,this support the beneficial effects of physiological vitamin D levels to the liverand the role of vitamin D in maintaining hepatocyte function and preventing liver inflammation, while our results confirmedthe ID and HD in ratsvery distinct histological changes were reported, which are characteristic of the hepatotoxic effects of excess vitamin D. These observations are consistent with experimental findings in rodents by (Wang et al, 2019) who reported that high doses of vitamin D enhanced hepatic apoptosis and fibrosis. The appearance of dark cytoplasm with small dark nuclei is typical features of apoptotic hepatocytes. This might suggest that high doses of vitamin D could activate pro-apoptotic pathway in hepatocytes. This is consistent with the higher number of apoptotic bodies found in the livers of rats given higher doses of ergocalciferol.Hepatocyte fatty/vacuolative degeneration was one of the histological alterations seen in the liver this agreement with (chavhanet al., 2011) who reported that high doses of vitamin D enhanced hepatic apoptosis and fibrosis. The appearance of dark cytoplasm with small dark nuclei are typical features of apoptotic hepatocytes this might suggest that high doses of vitamin D could activate pro-apoptotic pathway in hepatocytes this is consistent with the higher number of apoptotic bodies found in the livers of rats given higher doses of ergocalciferol These observations are consistent with experimental findings in rodents by(Ali et al.,2018).

The dilation of the portal vein and congestion of portal vessels were observed in ID and an HD group which implies vascular changes resulted from excessive vitamin D intake. Similar results were reported by Trépo et al. (2018) where hypervitaminosis D was associated with endothelial dysfunction and alteration of hepatic blood flow. Furthermore, the increased infiltration of inflammatory cells around the bile duct at high doses suggested the possibility that high vitamin D levels might promote hepatic inflammation and liver damage associated with high vitamin D levels could be a consequence of over activation of immune responses These observations are consistent with experimental findings in rodents byBarchetta et al. (2019). The Oxidative stress is enhanced by high levels of vitamin D, which associated with cell death as reported by other studies (Gharbanet al., 2019; Targher et al., 2020; Hussen et al., 2024). Vitamin D plays a complex role in liver health. Physiological doses of vitamin D are needed to maintain liver function (shown by the normal hepatic architecture in the LD group whilesupraphysiological doses induce apoptosis and inflammation, potentially leading to hepatic damage. This biphasic effect is in line with the hormesis theory, which suggests that substances beneficial at low doses become toxic at higher concentrations, vitamin D supplementation at low levels can aid liver health, but caution should be exercised at higher levels, with clinicians being mindful of the hepatotoxic effects of excessive vitamin D to prevent exacerbation of liver disease in patients who are already affected Holick (2017) acknowledges, ‘vitamin D is a powerful hormone’, and as such achieving optimal levels is important but only once adverse effects from high-dose supplementation have been carefully monitored.

The fact that the number of apoptotic and inflammatory cells in the liver increased with higher doses of vitamin D suggests that high-dose supplementation should be discouraged, especially in those at risk of liver disease. Public health recommendations should highlight the toxicity of high-dose vitamin D supplementation without clinical monitoring, as Trépo and colleagues (2018).The pathways of hyper Vit D vital in designing targeted therapies to combat the adverse effects of high dose of VitD. Hydroxylated products have been shown to have increased biological activity These observations are consistent with experimental findings in rodents by (Tieu et al. 2012; Slominski et al. 2012). The calcified area showing black in colour Some section of liver showed necrosed and calcified area surrounded by fibrous connective tissue and fatty changes were also observed. These observations reported are similar to those mentioned by (Makawana et al.,2022). Suggested mechanism leading to ectopic soft tissue calcium deposition: in met astatic calcification, high serum calcium and phosphate levels result in crystal formation in tissues. Calciphylaxis is a term suggested to include development of ectopic calcifications when both factors are present [i.e., high calcium and/or phosphate levels and tissue injury this consistent with (Pounder,1985)described a calcified hepatocytes were associated with centrilobular necrosis and chronic passive congestion of the liver latter showing granular calcium deposits. The mechanism of calcification is thought to be a function of hyperphosphatemia, but direct roles for vitamin D and high levels of parathyroid hormone have also been postulated our result agreement whith(Slatopolskyet al., 1978)

CONCLUSION

The histological structure of liver calcification were affected with increased vitamin D dosage' with appeared of calcification.

REFERENCES

1. Skinner, L. J., Conlon, B. J., Hegarty, S., and O'Dwyer, T. P. (2003). Ectopic ossification in the parotid gland. Revue de laryngologie-otologie-rhinologie, 124(4), 243-245.
2. Ali, S. S., Mahassni, S. H., and Alnefaie, R. M. (2018). The effects of hypervitaminosis D in rats on histology and weights of some immune system organs and organs prone to calcification. Int J Pharmaceutical and Phytopharmalogical Res, 8, 59-71.
3. Bancroft, J. D., and Layton, C. (2012). The hematoxylins and eosin. Bancroft’s theory and practice of histological techniques, 7, 173-186.
4. Barchetta, I., Del Ben, M., Angelico, F., Di Martino, M., Fraioli, A., La Torre, G., and Cavallo, M. G. (2016). No effects of oral vitamin D supplementation on non-alcoholic fatty liver disease in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. BMC medicine, 14, 1-10.
5. Calvo, M. S., Whiting, S. J., and Barton, C. N. (2005). Vitamin D intake: a global perspective of current status. The Journal of nutrition, 135(2), 310-316.
6. Chavhan, S. G., Brar, R. S., Banga, H. S., Sandhu, H. S., Sodhi, S., Gadhave, P. D., and Kammon, A. M. (2011). Clinicopathological studies on vitamin D3 toxicity and therapeutic evaluation of Aloe vera in rats. Toxicology international, 18(1), 35.
7. Christakos, S., Hewison, M., Gardner, D. G., Wagner, C. L., Sergeev, I. N., Rutten, E., and Bikle, D. D. (2013). Vitamin D: beyond bone. Annals of the New York Academy of Sciences, 1287(1), 45-58.
8. Di Rosa, M., Malaguarnera, M., Nicoletti, F., and Malaguarnera, L. (2011). Vitamin D3: a helpful immuno-modulator. Immunology, 134(2), 123-139.
9. Fathi, I., and Sakr, M. (2014). Review of tumoral calcinosis: a rare clinico-pathological entity. World Journal of Clinical Cases: WJCC, 2(9), 409.

10. Gharban, H. A., Al-Shaeli, S. J., Al-Fattli, H. H., andAltaee, M. N. (2019). Molecular and histopathological confirmation of clinically diagnosed lumpy skin disease in cattle, Baghdad Province of Iraq. *Veterinary world*, *12*(11), 1826.
11. Hathcock, J. N., Shao, A., Vieth, R., and Heaney, R. (2007). Risk assessment for vitamin D. The American journal of clinical nutrition, 85(1), 6-18.
12. Holick, M. F. (2007). Vitamin D deficiency The New England journal of medicine 357: 266–281.
13. Holick, M. F. (2017). The vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention. Reviews in Endocrine and Metabolic Disorders, 18, 153-165.
14. Hussen, T. J., Al-Shaeli, S. J. J., Al-Mahna, B. H. R., andGharban, H. A. J. (2024). Biochemical and histological effects of long-term administration of estrogen on female mice. *Adv. Anim. Vet. Sci*, *12*(8), 1563-1572.
15. Mahassni, S. H., and Al-Shaikh, N. A. (2013). Effects of vitamin A overdose on the immune system in rats. International Journal of Pharma Medicine and Biological Sciences, 2(4), 80-91.
16. Makawana, P., Mehra, M., Dadhich, H., Burdak, S., Pankaj, D. K., and Rathi, R. (2022). Occurrence and pathology of hepatic abscess in liver of sheep.
17. Mazahery, H., and Von Hurst, P. R. (2015). Factors affecting 25-hydroxyvitamin D concentration in response to vitamin D supplementation. Nutrients, 7(7), 5111-5142.
18. Morrow, C. (2001). Cholecalciferol poisoning. Vet Med, 96(12), 905-911.
19. Peterson, M. E., and Fluegeman, K. (2013). Cholecalciferol. Topics in companion animal medicine, 28(1), 24-27.
20. Pounder, D. J. (1985). Hepatocellular calcification. Pathology, 17(1), 115-118.
21. Slatopolsky, E., Rutherford, W. E., Hruska, K., Martin, K., and Klahr, S. (1978). How important is phosphate in the pathogenesis of renal osteodystrophy?. Archives of internal medicine, 138(Suppl_5), 848-852.
22. Slominski, A. T., Zmijewski, M. A., Skobowiat, C., Zbytek, B., Slominski, R. M., Steketee, J. D., and Steketee, J. D. (2012). Melatoninergic system in the skin (pp. 27-36). Springer Berlin Heidelberg.
23. Tieu, K. S., Tieu, R. S., Martinez-Agosto, J. A., and Sehl, M. E. (2012). Stem cell niche dynamics: from homeostasis to carcinogenesis. Stem cells international, 2012(1), 367567.
24. Trépo, E., Ouziel, R., Pradat, P., Momozawa, Y., Quertinmont, E., Gervy, C., and Moreno, C. (2013). Marked 25-hydroxyvitamin D deficiency is associated with poor prognosis in patients with alcoholic liver disease. Journal of hepatology, 59(2), 344-350.
25. Tulchinsky, T. H. (2010). Micronutrient deficiency conditions: global health issues. Public health reviews, 32, 243-255.
26. Urashima, M., Segawa, T., Okazaki, M., Kurihara, M., Wada, Y., and Ida, H. (2010). Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. The American journal of clinical nutrition, 91(5), 1255-1260.
27. Wang, Y., Zhu, J., and DeLuca, H. F. (2012). Where is the vitamin D receptor?. Archives of biochemistry and biophysics, 523(1), 123-133.