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Effect of hypervitaminosis A on the histological structure of liver in albino rat

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ABSTRACT

The importance of vitamin A as it is important for normal vision, the immune system, reproduction, growth and development. The aim of the experiment is to study the histological effects in liver from the hypervitaminosis A .Forty adult male Wistar rats were divided into four groups randomly that were given vitamin A orally For sixty days, 10 rats in the control group received distilled deionized water every day. Three experimental groups received varying doses of vitamin A. IU daily for 60 days: low dose (LD, 10 mice) (8000 IU/mouse/day), intermediate dose (ID, 10 mice, 12000 IU/mouse/day), and high dose (HD, 10 mice, 15000 IU/mouse/day). The LD groups appeared less of histological changes in liver, but the livers in the ID and HD groups showed deeply histological changes with classification and fibrosis . In conclusion, the histopathology of the liver was affected by increasing vitamin A dose with the appeared of calcification and fibrosis

Keywords: Vitamin A , Calcification ,liver, Alizarin Red, Rats , Hypervitaminosis A.

INTRODUCTION

Vitamin A(VA) is fat soluble compounds. There are two types of VA , the first type is retinol, retinal and retinoic acid (derivative of animal source) and the second type is carotenoid (derivative of plant source (Harrison, 2012 ; Polcz and Barbul, 2019). In 1928, Green and Mellandy reported that VitA could enhance the anti-inflammatory response of organisms and called VitA the "anti-inflammation vitamin (Huang et al., 2018).

Vitamin A is a naturally present in many foods. Vitamin A is important for normal vision, the immune system, reproduction, and growth and development. Vitamin A also helps your heart, lungs, and other organs work properly(AL-Khatawi et al.,2019).Carotenoids are pigments that give yellow, orange, and red fruits and vegetables their color. Human body is able to convert some carotenoids into vitamin A. There are two different sources for vitamin A (Ross and Harrison 2007) . VA also has been reported to influence the gut microbiota composition and diversity(Amimo et al., 2022).

Preformed vitamin A is found in fish, organ meats (such as liver), dairy products, and eggs.Provitamin A carotenoids are turned into vitamin A by your body. They are found in fruits, vegetables, and other plant-based products. The most common provitamin A carotenoid in foods and dietary supplements is beta-carotene (Ross and Harrison 2007).Vitamin A toxicity or Hypervitaminosis A is resulted from excessive consumption of the active (preformed) vitamin A, while hypervitaminosis with provitamin A is largely impossible. Hypervitaminosis A may be acute or chronic. Acute hypervitaminosis A is rare and has many symptoms such as; vomiting, diarrhea and headaches (Safi and Filbrun, 2014; Daher et al.,2017). Chronic vitamin A toxicity leads to hepatotoxicity, nephrotoxicity, hypercalcemia, hyperglycemia, hyperostosis, hypercholesterolemia and increased cerebrospinal fluid pressure (Al-Gharbawi et al.,2021).

(Ibrahim, 2015; ; Daher et al.,2017).bones and teeth are use almost all the calcium in your body (about 99%). Soft tissues and organs calcify more rapidly than bones (Morrow, 2001; Peterson and Fluegeman, 2013). Phosphate calcification and calcium deposits in the organic matrix are the cause. Different types of soft tissues are susceptible to calcification, the accumulation of calcium and other salts in the blood and tissues due to systemic imbalances in their metabolism, leading to metastatic calcifications. They often damage the liver, (Skinner et al., 2003; Fathi and Saqr, 2014). Some calcification is normal as calcium remaining in the blood dissolves and travels to different parts of the body through the bloodstream. This is the body's response to injury or inflammation. Calcium deposits, or calcification, occur when calcium builds up in the body. (Habeeb,2023). This calcium buildup can harden tissues, organs, or blood vessels, which can cause normal body processes to not function properly (Bargooth,2022). But some conditions can cause calcium deposits to appear in places

where they shouldn't. This includes areas such as the brain, kidneys, and blood vessels.This can cause problems with how your organs and blood vessels work (Izawa et al., 2012). The aim of the experiment is to study the histological effects in liver from the hypervitaminosis A in adult male rats.

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,in the animal house in Collage Education of Pure Sciences / Wasit university.
1-Group A Control group, normal diet and distilled water were given during sixty day period.
2-Group B low dose of vitamin A / orally dose whith 8000 IU/day for sixty day.
3-Group C medium dose of vitamin A/orally dose whith 12000 IU/day For sixty day.
4-Group D high dose of vitamin d / orally dose whith15000 IU/ for sixty day.
Histological study was performed using three stains, hematoxylin and eosin(H.E) to show the general histological structure, Massontrichom to appeared fibrosis and Alizarin red stain to detect the deposite of calcium salts in the tissues **(Bancroft and Laytonred,2013).**Immunohistochemical technique was also performed using antibodies that to detect calcifications in the tissues body.

RESULT

Histological examination of the control group showed that the liver consist of several hepatic lobules separated from each other by very fine connective tissue Each hepatic lobule contains a central vein with a thin wall surrounded by radiating hepatic cords composed from hepatocytes with sinus capillaries(fig1).
With Low dose LD the results showed less histological changes in the structures of liver(fig2)
The histological results appeared thickened of the portal vein wall with dilation, fibrosis, degeneration of hepatocytes, Necrosis of hepatocytes, accumulating of inflammatory cells connective tissue, congestion with dilation of central vein and hyper atrophy of epithelium with hemorrhage when taken with intermediate dose ID(fig3,4).
On the other hand there were increase in the histological effected in liver tissue with increase of fibrosis when given animal high doses HD of vitamin A (fig5,6,7).With the alizarin stain and immunohistochemical technique, the less of calcification appeared in low dose LD (fig 8,9) mild calcification in intermediate dose ID(fig10,11) and high calcification with high dose of vitamin A as dark to brown in color(fig12,14).

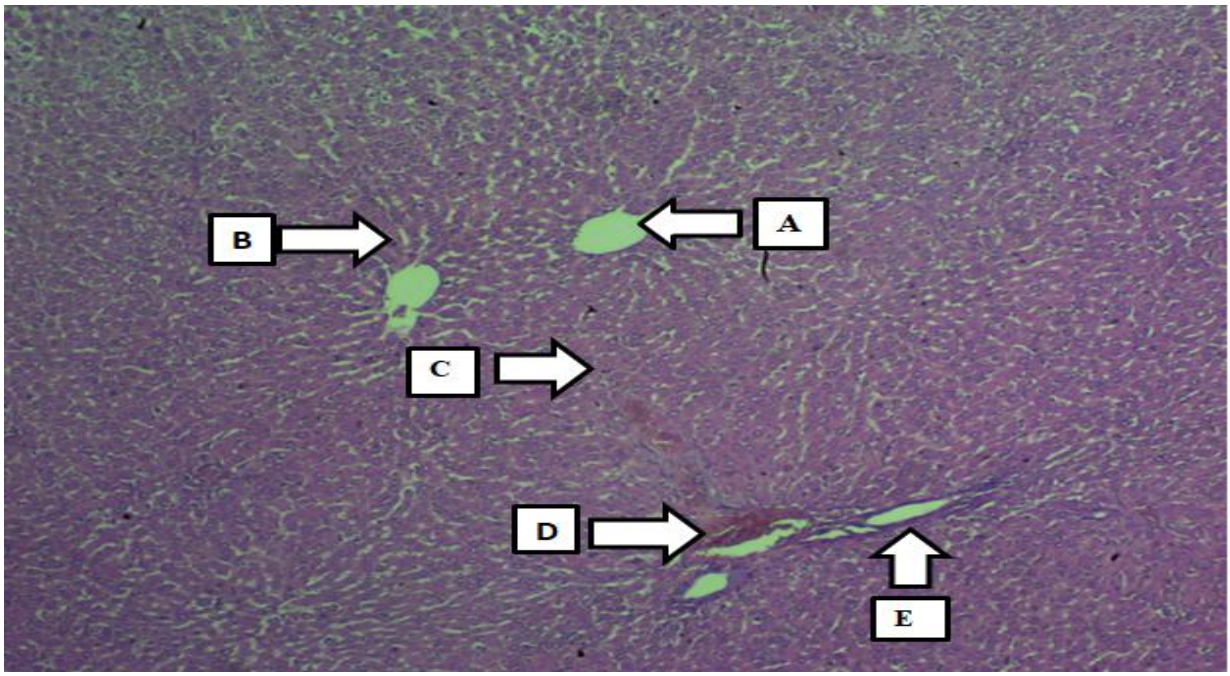

fig 1: Light micrograph of liver of control group rats appeared,central vein (A),sinusois (B),hypato cyte (C),hypatic artery(D),hypatic vein(E)),(H&E 100X).

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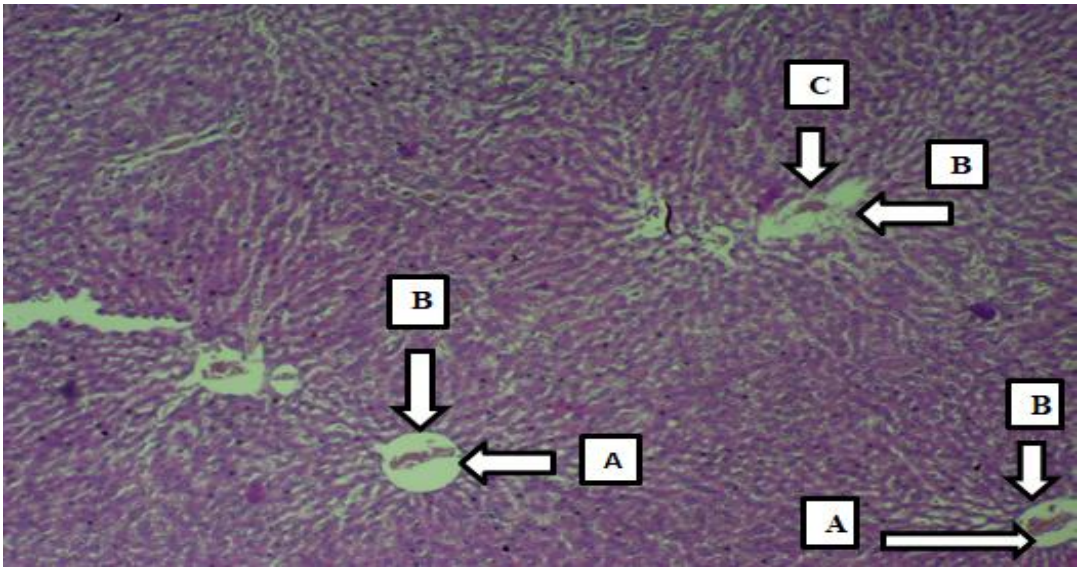

Fig 2: Light micrograph of liver of Low dose LD group rats of VA appeared, dilation of portal vein(A),congestion of blood vessels(B),inflammatory cells(C),(H&E 100X).

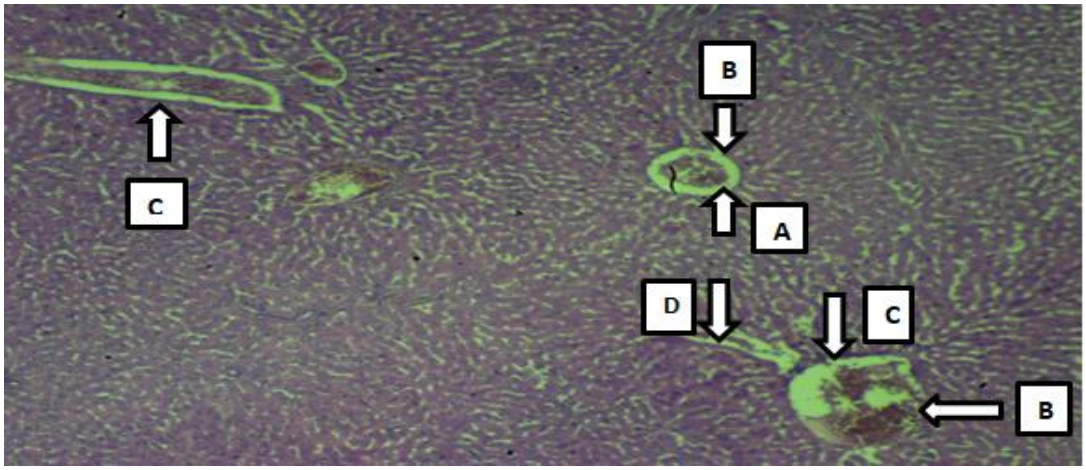

Fig 3: Light micrograph of liver of Intermediat dose ID group of VA appeared , dilationof portal vein (A),congestion of blood vessles(B),thiclcening of wall of blood vessles(C),accumulation in flammatory cells(D),(H&E 40x).

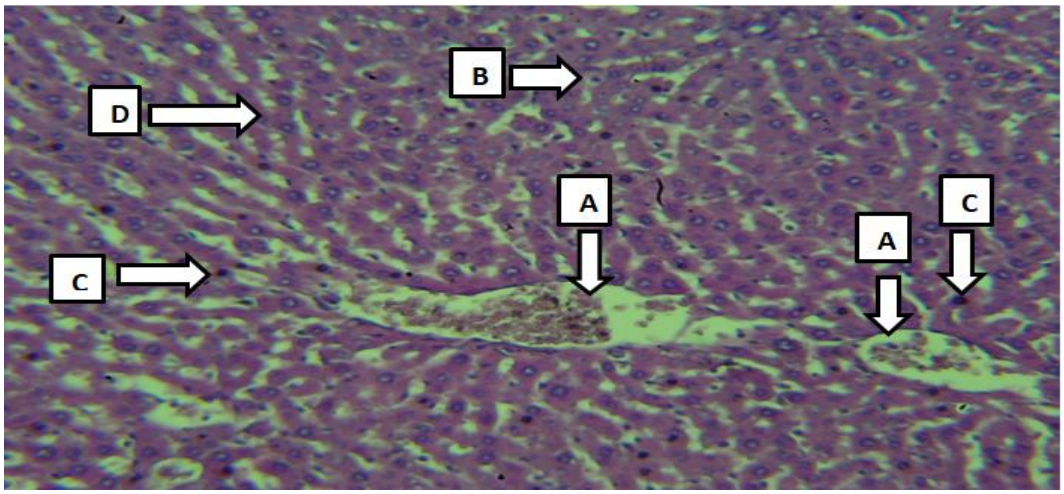

Fig 4: Light micrograph of liver of Intermediat dose ID group rats of VA appeared, congestion of blood vessels(A),degeneration of hypatocytes(B),pyknotic of hypatocytes(C),karyolysis of hypatocytes(D).(H&E 400X).

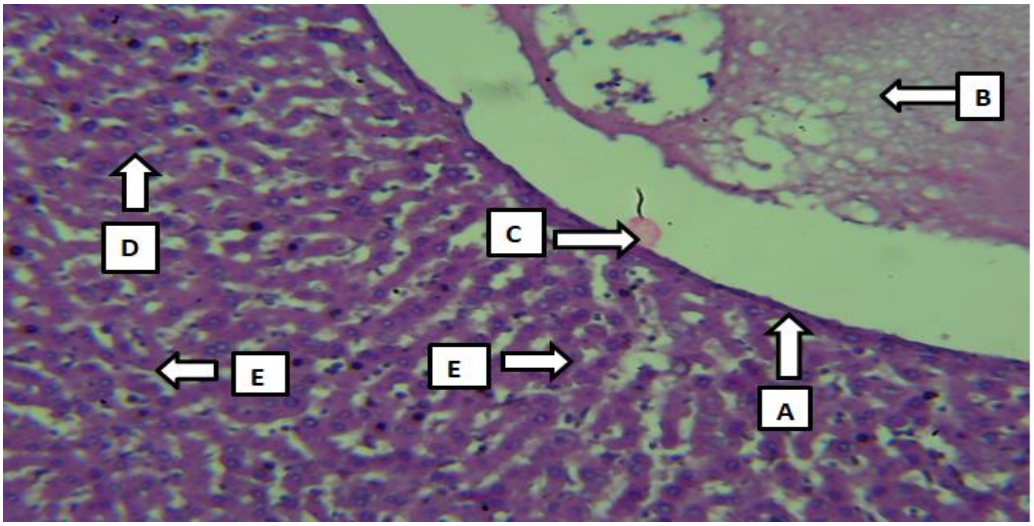

Fig 5: Light micrograph of liver of High dose group HD rats of VA appeared , incresed dilation of portel vein (A),increased congestion of blood vessels(B),in creased of thickening of portal vein(C), degeneration of hepatocyte(D),necrosis of hepatocytes(E).(H&E 40x).

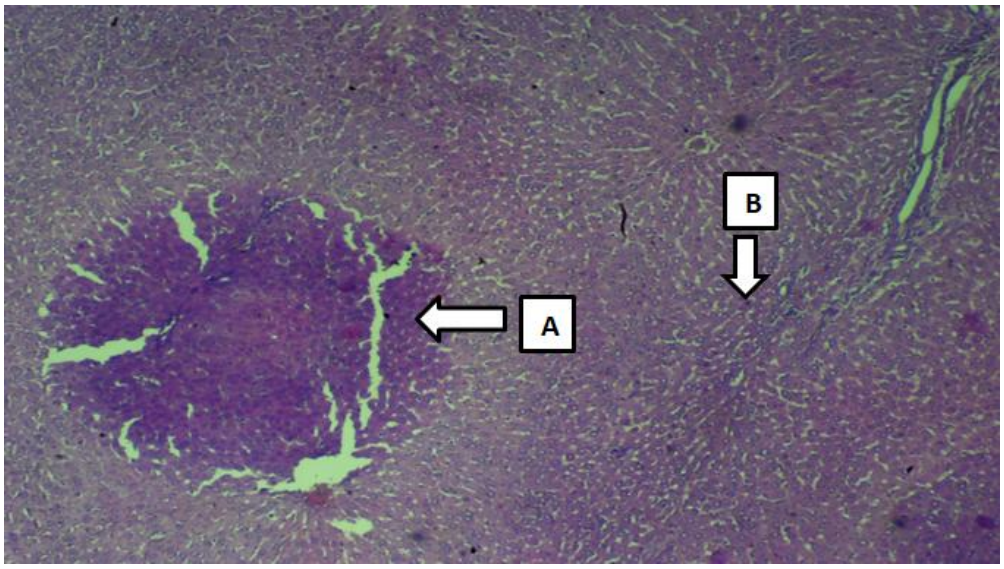

Fig 6: Light micrograph of liver of High dose rats of VA appeared , fibrosis in stracture of liver(A),accumulation of inflammatory cells(B),(H&E 40X).

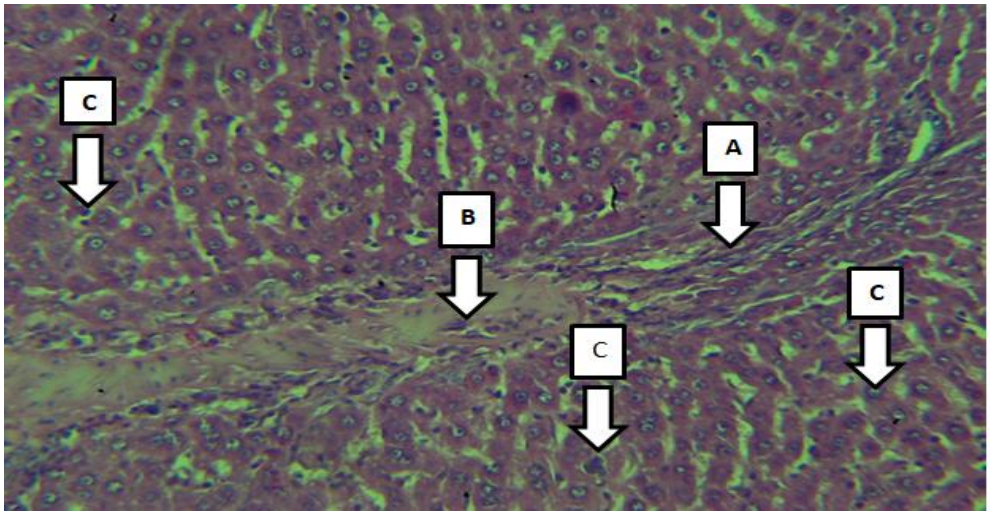

Fig 7: Light micrograph of liver of High dose HD group rats of VA appeared ,high inflammatory cells(A),high congestion of blood(B),different stages of necrosis(C).(H&E 100X).

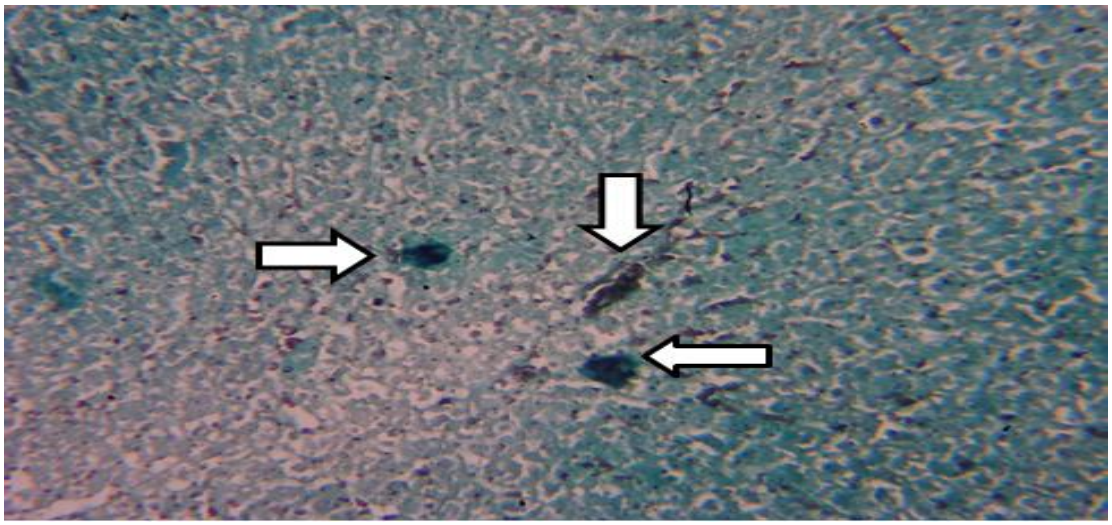

Fig 8: Light micrograph of liver of low dose LD group rats of VA appeared ,less positive reaction of calcium ⇨ (alizarin stain 40x).

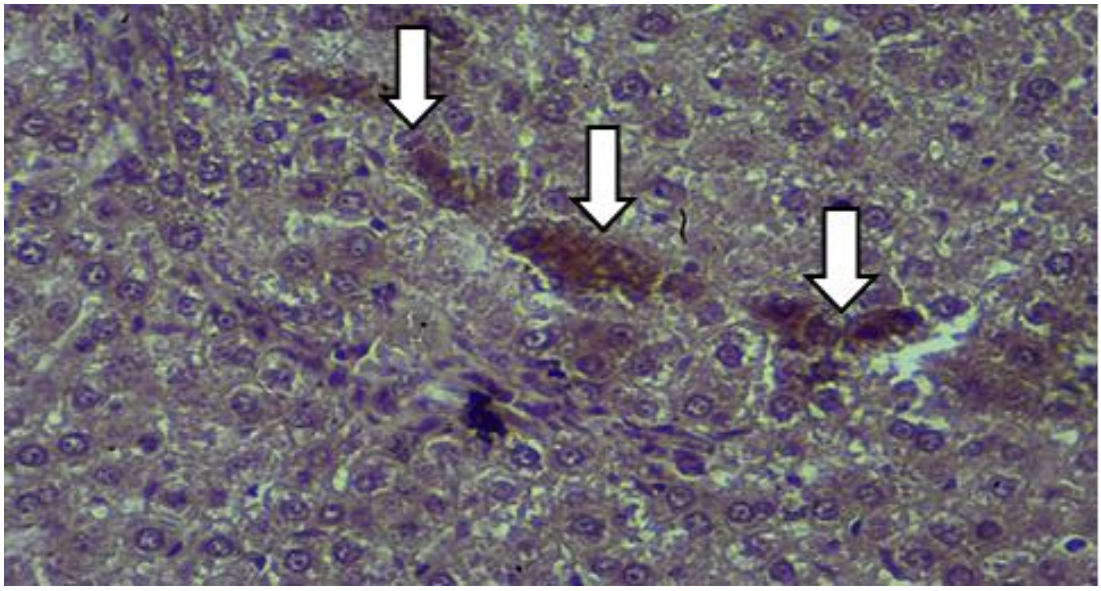

Fig 9: Light micrograph of liver of low dose LD group rats of VA appeared, less intensity of positive reaction of positive reaction of calcium ⇨ (immunohisto chemical technique DAB & mayer H400X).

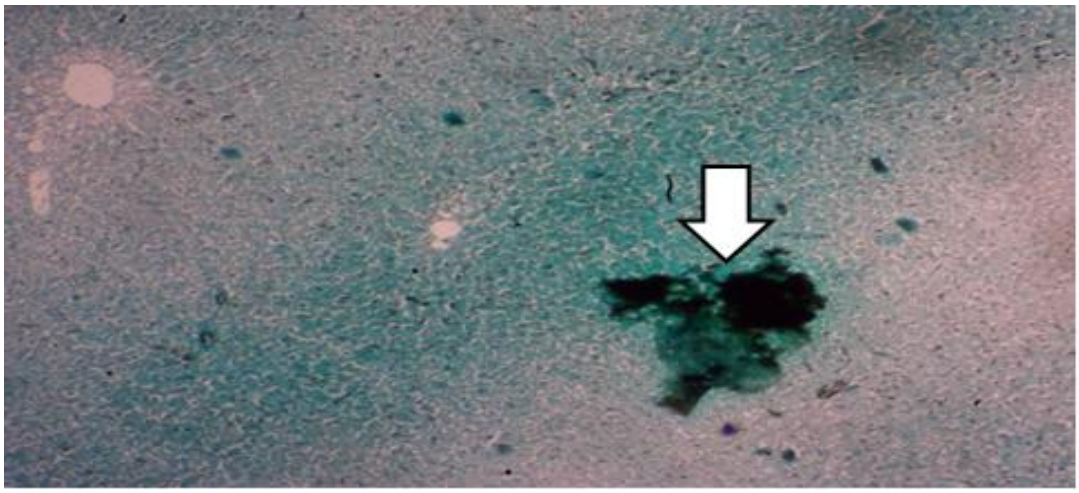

Fig 10: Light micrograph of liver of intermedieat dose ID group rats appeared ,moderate postive reaction of calcium ⇨ (alizarin stain 40x).

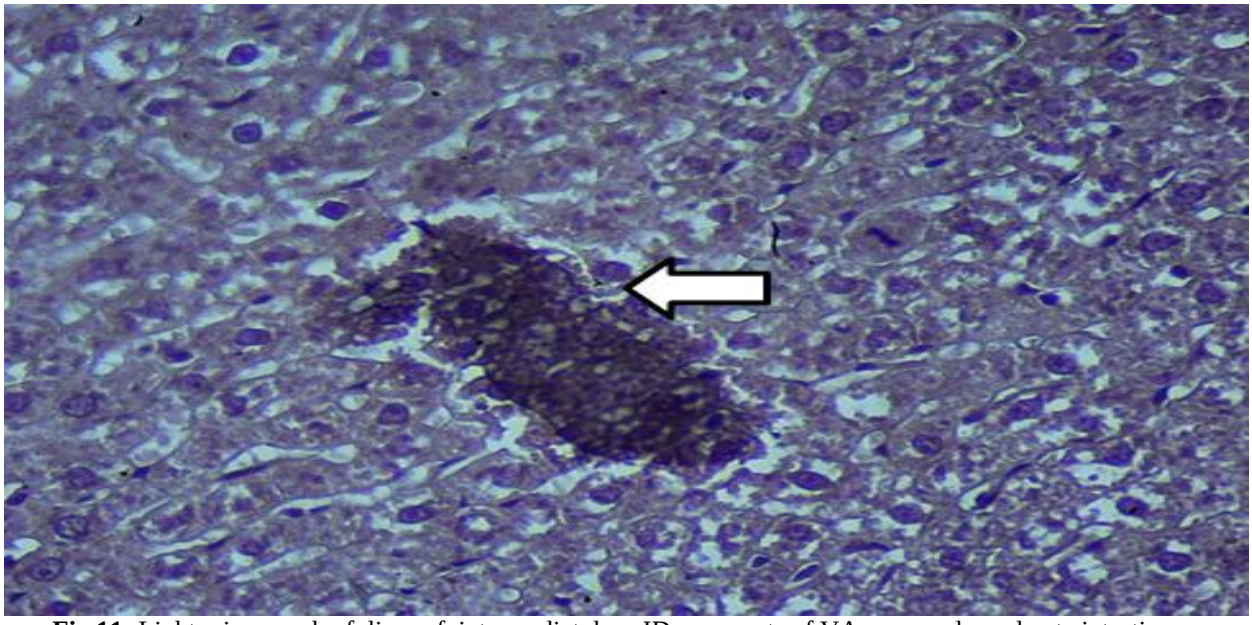

Fig 11: Light micrograph of liver of intermediat dose ID group rats of VA appeared, moderate intesting positive reaction of calcium ⇨ (immunohisto chemical technique DAB & mayer H400X)

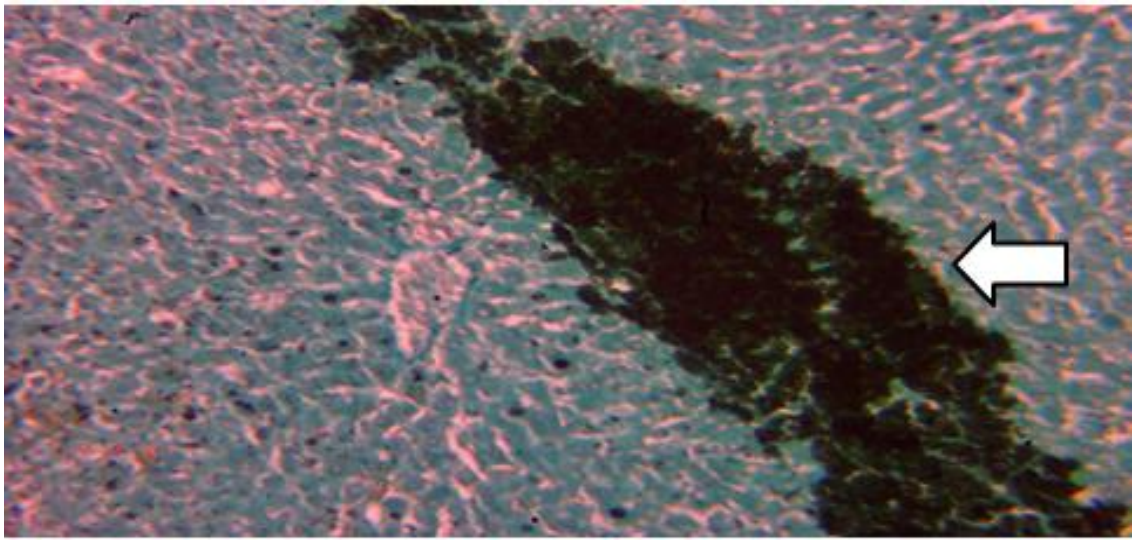

Fig 12: Light micrograph of liver of high dose HD group rats of VA appeared ,high positive reaction with calcium ⇨ (alizarin stain 40x).

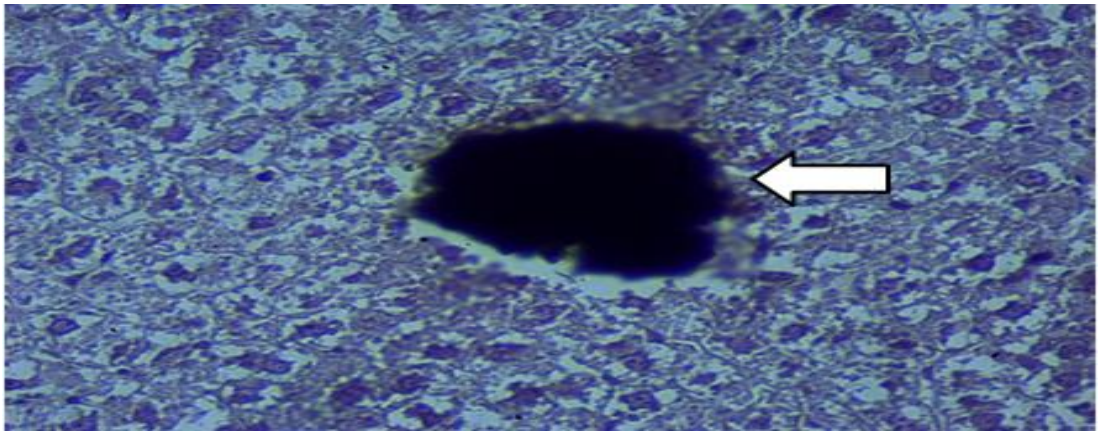

Fig 13: Light micrograph of liver of high dose HD group rats of VA appeared, high intesting positive reaction of calcium ⇨ (immunohisto chemical technique DAB & mayer H400X)

DISCUSSION

The present study showed that hypervitaminosis A caused histological changes in liver tissue and loss of its normal structures, represented by dilatation of the central vein with its congestion, hyperplasia of the endothelium, hemorrhage in different stages of necrosis , degeneration of lymphocytes and infiltration of lymphocytes and macrophages into the portal area . These observations are consistent with the experimental results in rats reported by many researchers on liver toxicity and histological changes caused by hypervitaminosis as the observations were reported by **(Liu *et al*., 1982)** who believed that vitamin A caused necrosis, infiltration of lymphocytes and macrophages into the portal area. **(Ibrahim and Okdah, 2015).** who showed that hypervitaminosis A had been caused vacuolization in hepatocytes, which might be attributed to mitochondrial swelling and proliferation of the endoplasmic reticulum leading to liver fibrosis, and that high-dose supplementation should be discouraged, especially in those at risk of liver disease.The histological results showed that the portal area of liver tissue appeared as bridging fibrosis, collagen fibers increased significantly and appeared as thick bundles of collagen fibers surrounding the sinusoids, central vein and portal vein. (Figure) . Excessive accumulation of vitamin A leads to the activation of stellate cells, which stimulate myofibroblast-like cells surrounding newly formed collagen fibers. The fact that showed by **(Nollevaux *et al*., 2006)** that Activation of hepatic stellate cells leads to the expression of the smooth muscle actin (SMA) gene, which then increases the secretion of extracellular matrix (ECM) components and matrix-degrading enzymes Thus, vitamin A-induced fibrosis results from retinol toxicity and the release of inflammatory cytokines and fibrogenic material by injured hepatocytes. On the other hand (**Guerra *et al*., 2016)** remained the cause of hypervitaminosis A-induced liver fibrosis remains unknown because there are limited studies on the direct effect of high amounts of vitamin A on the fibrotic reaction **.**Our present study showed light brown calcification in the low-dose LD group, darker in the medium-dose MD group, and highly dark brown in the high-dose HD group. The researcher **(Pounder, 1985)** demonstrated that increased vitamin A intake leads to calcium absorption in bones and teeth, which leads to calcium deposition in many organs of the body, including the liver, blood vessels, heart, and kidneys. The proposed mechanism leading to abnormal soft tissue by calcium deposition in it lead to abnormal calcification, where high levels of serum calcium and phosphate lead to the formation of crystals in the tissue. **(Slatopolsky *et al*., 1978)** described calcified hepatocytes as being associated with central lobular necrosis and chronic passive congestion of the liver, the latter showing granular calcium deposits. The mechanism of calcification is believed to be a function of hyperphosphatemia.In general, some authors have described liver tissue calcification in different vertebrate species, such as rats **(Makawana *et al*. 2022),** and the appearance of calcium deposition in the present study in the group with a high dose of vitamin A was similar to that reported in the liver tissue of rats in the study **(Tayeh, 2024).** This increase in liver tissue calcification may also be related to age, as well as the type of their diet, which is often accompanied by a physiological disturbance in the level of calcium in the blood. This is what previous authors announced that the differences in calcifications may be attributed to the age of the rat, as well as the type of its diet **(Genchev *et al.* 2008**).

CONCLUSION

The histological structure of liver calcification was affected by increased vitamin A intake with the appearance of fibrosis.

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