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Physiological and Histological Alterations in Liver of Domestic Male Rabbits Exposed to High Doses of Vitamins D and C

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التغيرات الفسيولوجية والنسيجية في كبد ذكور الأرانب المنزلية المعرضة لجرعات عالية من فيتاميني د، ج

الملخص:

تعتمد وظائف الكبد والصحة العامة على فيتامين د. وللجرعات العالية من فيتامين D3 آثار سلبية عديدة. هدفت هذه الدراسة إلى دراسة التغيرات الفسيولوجية والنسيجية المحتملة التي قد تُسببها الجرعات الكبيرة من فيتامين د3، ج على كبد ذكور الأرانب. تلقت ذكور الأرانب حقنًا من فيتامين د3 بجرعات شهرية مقدارها 200,000، 400,000، 600,000، و800,000 وحدة دولية على مدى ثلاثة أشهر. ظهرت عدة مؤشرات فسيولوجية في الكبد بعد العلاج، بما في ذلك زيادة ملحوظة في نشاط إنزيمي AST و ALT في بلازما الدم في المجموعة الثانية، التي تلقت أعلى جرعات من فيتامين D3. في المقابل، لم يُلاحظ فرق يُذكر في نشاط إنزيمي ASTو ALT بين المجموعات G3 و G4 و G5 والمجموعة الضابطة. كما أظهرت النتائج أن نشاط ALP في المجموعتين G2 و G3 كان أعلى بكثير منه في المجموعة الضابطة، إلا أن المجموعتين G4 و G5 لم تختلفا بشكل ملحوظ عن المجموعة الضابطة. بالإضافة إلى ذلك، وبالمقارنة مع المجموعة الضابطة، لوحظ ارتفاع كبير في مستوى T. bilirubin في جميع المجموعات التي عولجت بفيتامين D3 علاوة على ذلك، كشف بحثنا عن إصابة أنسجة الكبد بتلف شديد، والذي تفاقم مع زيادة جرعة فيتامين D3 وشمل هذا التلف تكلسًا، وتليفًا في المسالك البابية، واحتقانًا في الوريد البابي بخلايا الدم الحمراء، والتهابًا وتوسعًا في الوريد المركزي.

الكلمات المفتاحية: *فيتامين د3، AST، ALT، ALP، الكبد، التوسع، الاحتقان، التكلس.*

Abstract:

Liver function and general health depend on vitamin D. High vitamin D3 dosages have a number of negative effects. The purpose of this study was to look into the possible physiological and histological changes that large dosages of vitamin D3 and C could have on liver of male rabbits. Male rabbits received injections of vitamin D3 at monthly doses of 200,000, 400,000, 600,000, and 800,000 IU over three months. Several physiological indicators in the liver following treatment, including a marked increase in AST and ALT activity in blood plasma in group 2, which received the highest vitamin D3 dosages. In contrast, there was no discernible difference in the AST and ALT enzyme activity between the G3, G4, and G5 groups and the control group. Also, the results showed that ALP activity in G2 and G3 was much higher than in the control group, but that G4 and G5 did not differ significantly from the control group. Additionally, when compared to the control group, a substantial rise in T. bilirubin was observed in all vitamin D3-treated groups. Moreover, our research revealed that the liver tissue exhibited severe damage to the liver, which worsened as the dosage of vitamin D3 increased. This damage included calcification, fibrosis in the portal tracts, congestion of the portal vein by red blood cells, inflammatory and dilatation of the central vein.

Keywords: *Vitamin D3, AST, ALT, ALP, Liver, dilatation, congestion, Calcification.*

Introduction:

Vitamin D, a fat-soluble vitamin, plays a vital role for maintaining good health (1). It helps reduce the risk of obesity, insulin resistance, prediabetes, cardiovascular disease, metabolic syndrome, cardiovascular diseases and cancer (2-3). It is also necessary for maintaining calcium homeostasis and helps control the immune system (4-5).

Furthermore, many researches have search for investigate the association between liver disease and a deficiency in vitamin D. Vitamin D3 has been found to improve hepatic steatosis (6) and protect mice from hepatic ischemia-reperfusion injury (7). Vitamin D3 administration in animal models of acute liver injury also decreases apoptotic gene expression (8).Previous studies suggest a link between vitamin D deficiency and liver disease, particularly nonalcoholic fatty liver disease (NAFLD), with high vitamin D intake improving liver function markers in adolescents (9-10).

Many studies examine the deficiency of vitamin D, In contrast, there has been a rarity of research regarding overdose of vitamin D3 when compared to the extensive studies on its deficiency. Prolonged administration of high doses of vitamin D can be detrimental and result in several complications.Hence, the present study was carried to investigate the potential physiological and histological alterations of high doses of vitamin D3 and C on the liver of male rabbits.

2. Materials and methods:

2.1 Drugs used.

Cholecalciferol (vitamin D3) 200.000 IU/ml injections (Bouchara-recordari.France) and Vitamin C 1000 mg (Superdyn, Europe,Italy) were purchased from local pharmacy.

2.2 Experimental animals.

40 Male rabbits were used in this experiment; every 8 rabbits were housed in a cage within a room equipped with standard conditions, including a ventilation system and a 12-h light/12-h dark cycle with controlled temperature (22 ± 3 °C) for 12 weeks. Their ages ranged between 4 and 6 months, and their weights were ranged between 800 and 1200 grams. The rabbits were provided with water and food, including dried clover and specialized feed. The experiment started in September 2023 and ended in December 2023. They were divided into five groups (8 animals each) and treated as follows: **Group I:** control group each animal was injected corn oil (100 mg) for 3 months. **Group II:** each animal was injected with vitamin D3 (200,000 IU/mL) once a week (four doses per month), for 3 months. **Group III:** each animal was injected with vitamin D3 (200,000 IU/mL) every 10 days (three doses per month) for 3 months. **Group IV:** each animal was injected with vitamin D3 (200,000 IU/mL) twice a month (every 15 days) for 3 months. **Group VI:** This group was injected with vitamin D3 (200,000 IU/mL) once a month for 3 months. All groups given vitamin C (1000 mg) orally in parallel with the doses of vitamin D3.

2.3 Measurement of biochemical parameters:

The blood serum was collected by centrifugation of whole blood at 1500 rpm for 15 minutes. This serum is used for the estimation of different biochemical measurements including (level of vitamin D3, ALT, AST and ALP enzymes, Tot.Bil (Serum total bilirubin concentration), Dir.Bil (Serum direct bilirubin concentration) and In .Dir.Bil (Serum Indirect bilirubin concentration). The biochemical tests were estimated on auto analyser by using diagnostic reagent kits supplied by Bayer Diagnostics India.

2.4 Histopathological study:

At the end of experiment the animals were scarified and the liver dissected and removed for histopathological study. Samples of liver tissues were fixed with 10% formalin after that the samples were washed in distilled water, dehydrated in graded series of ethanol and embedded in paraffin wax .The sections were cut at 4-5um thickness on a microtome. Then the slices were staining with haematoxylin and eosin (H&E), the pathologist used a light microscope (Olympus BH-2, Tokyo, Japan) to see the evaluating of the histopathological changes caused by high dose of cholecalciferol in liver tissues of treated rabbit.

2.5 Statistical analysis:

The result was presented in the form of mean ± SD. One-way analysis of variance (ANOVA) was used to examine the data for comparisons between various variables. According to the statistical package software (SPSS version 25), Duncan's test was employed as a post hoc test for the comparison of significance across groups.Statistical significance was defined as a value of less than 0.05.

3. Results:

3.1 Clinical signs

The effects of high doses of vitamin D3 in liver of the rabbits revealed some changes in the behaviour of visible individuals. For instance, as the dose and the duration increased, there was a decrease in feed intake and an increase in drinking water. Difficulty moving was observed in the last month of the exposure. Also some animal was died.

3.2 measurements **the activity of** liver enzymes:

3.2.1 Change in the level of AST enzyme:

The comparisons of the activity of AST enzyme in treated groups and the control group indicated that, there was a significant increase in the level of AST enzyme in the animals of group-2 (47.5±2.72). The increase rate was approximately 12%, While, there was no significant difference (P>0.05) between the means of the control group and the rest of treated groups as shown in figure 1.

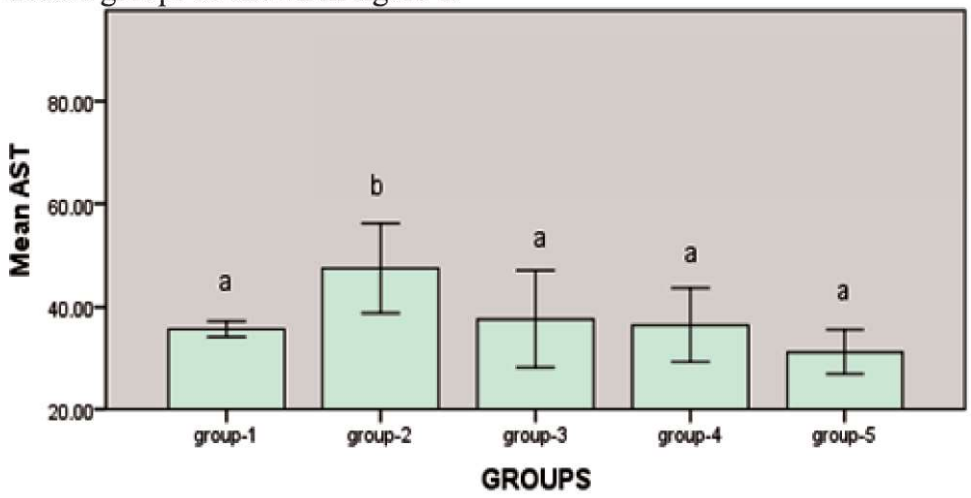

Figure 1: The activity of AST enzyme in control group (group 1) and treated groups (2,3,4 and 5) with different doses of vitamin D3 (800,000IU/ml, 600,000IU/ml, 400,000IU/ml and 200,000IU/ml per month) respectively for 3 months . Bars followed by the same letter are not significantly different.

3.2.2 Change in the level of ALT enzyme

The activity of ALT in blood plasma was significant increase ($P<0.01$) in the animals of group-2 (84.2500±4.66146) as compared to the control group. The increase rate was about 46%. However, there was no significant difference ($P>0.05$) between the means of the control group and the rest of the study groups as presented in figure 2.

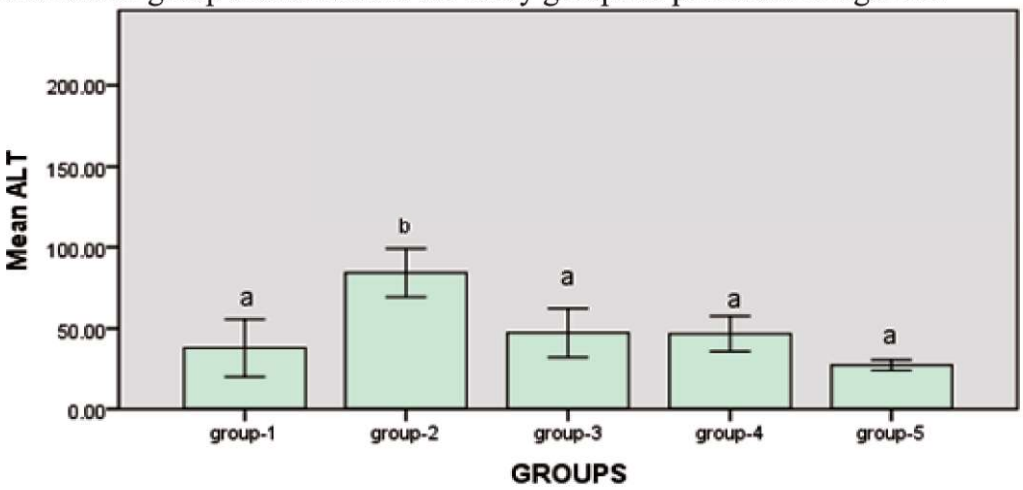

Figure 2: The activity of ALT enzyme in control group (group 1) and treated groups (2,3,4 and 5) with different doses of vitamin D3 (800,000IU/ml, 600,000IU/ml, 400,000IU/ml and 200,000IU/ml per month) respectively for 3 months . Bars followed by the same letter are not significantly different

3.2.3 Change in the level of ALP enzyme

Regarding to the level of ALP enzyme It was noted that there was a significant increase in the level of ALP enzyme in the animals of group-2 (188.75 ± 5.57) when compared to the control group, the increase rate was around 114%. Also, there was a significant increase in the level of ALP in group-3 animals (113.0±7.31) when compared to the control group, where the percentage of increase was almost 39%. On the other hand, there is no significant difference between the means of the control group and the rest of the study groups according to the indicator ALP as revealed in figure 3.

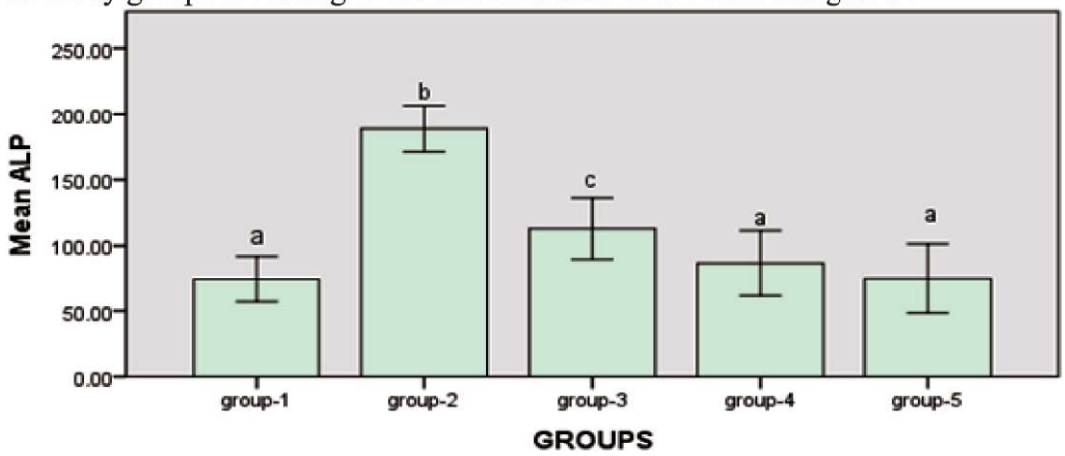

Figure 3: The activity of ALT enzyme in control group (group 1) and treated groups (2,3,4 and 5) with different doses of vitamin D3 (800,000IU/ml, 600,000IU/ml, 400,000IU/ml and 200,000IU/ml per month) respectively for 3 months . Bars followed by the same letter are not significantly different.

3.2.4 Change in the level of **T.BILIRUBIN**

The result of the activity of T.BILIRUBIN remarked that, there was a significant increase in the level of T.BILIRUBIN in the animals of group-2 animals (1.74±0.04) as compared to control group, where the percentage of increase was roughly 1.4%. As well, there was a significant increase in the level of T.BILIRUBIN in group-3 (1.6 ±0.13) when compared to the control group, the increase rate was around 1.3%. Moreover, there was a significant increase in the level of T.BILIRUBIN in group-4 animals (1.08±0.06) when compared to the control group, where the percentage of increase was about 0.73%. Additionally, there was a significant increase in the level of T.BILIRUBIN in group-5 animals (0.93±0.011) when compared to the control group, where the percentage of increase was 0.58% approximately as presented in figure 4 .

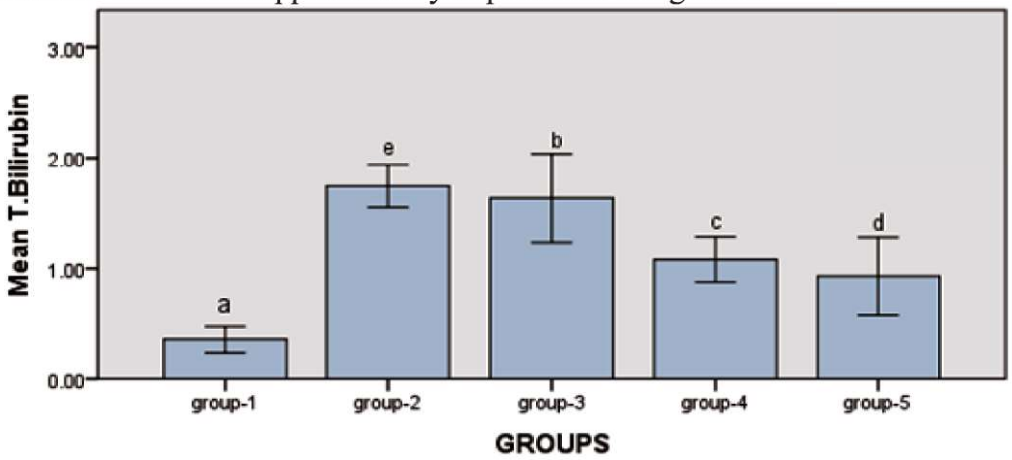

Figure 4: The activity of T.BILIRUBIN in control group (group 1) and treated groups (2,3,4 and 5) with different doses of vitamin D3 (800,000IU/ml, 600,000IU/ml, 400,000IU/ml and 200,000IU/ml per month) respectively for 3 months . Bars followed by the same letter are not significantly different.

3.3 Histological alteration of liver tissue.

3.3.1 Histological structure of liver tissue in the control group: When liver sections from the control group were examined under a light microscope and stained with H&E, the central vein, the hepatocytes surrounding it, and the lumen of the sinusoidal capillaries all displayed typical histological characteristics as illustrated in figure 5.

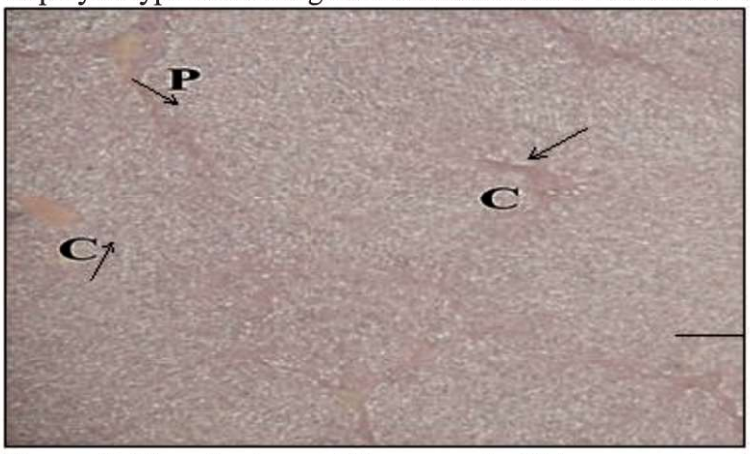

Figure 5. Hepatic tissue of the control rabbits group showing normal hepatic architecture, hepatocytes, central vein (C) , and portal tracts (P). Sections stained with H&E, (X10).

3.3.2 Histology of supplemented rabbit liver

Histopathological analysis of liver sections from group 2, which received the highest doses of vitamin D3 in this study (800,000 IU/ml per month) for three months, revealed that the liver tissue had changed to include severe dilatation of the central vein, severe congestion by red blood cells, moderate fibrosis in the portal tracts, and severe dilatation and congestion by red blood cells in the portal vein as presented in figure 6 A and B.

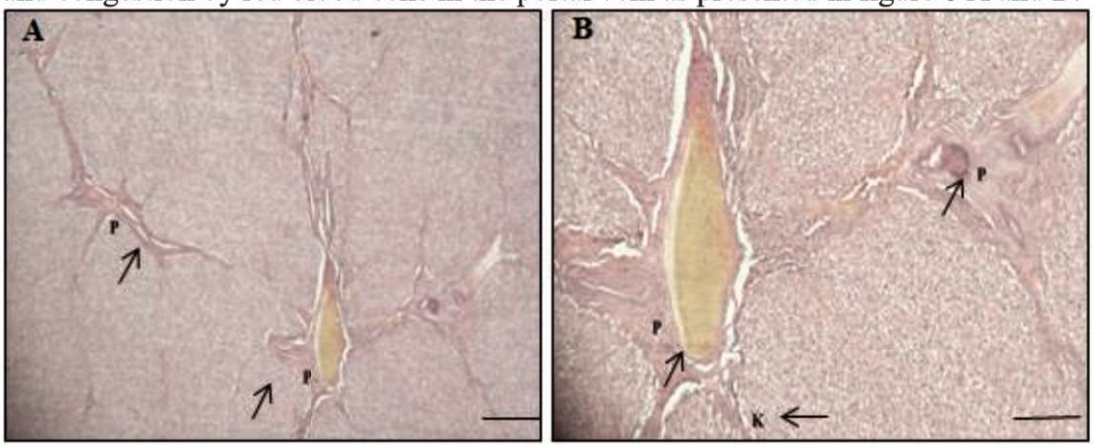

Figure 6. Hepatic tissue of vitamin D3-treated rabbits received (800,000IU/ml) for 3 months showing (A and B) portal tracts (P) with sever fibrosis, dilatation and congestion. 20X (B) liver parenchyma show some kupffer cells (K) within sinusoids. HE stained section, 20X.

Microscopic analysis of liver sections in group 3 revealed the following conditions: mild fibrosis in the portal tracts, moderate dilatation in the central vein, moderate congestion by red blood cells, and mild dilatation and congestion in the portal vein by red blood cells, as illustrated in figure 7 A and B.

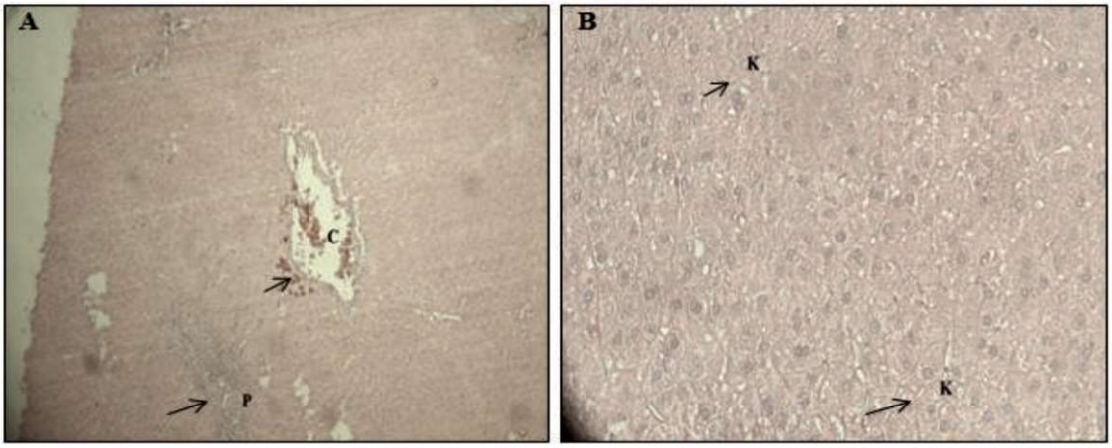

Figure 7 A and B. Hepatic tissue of vitamin D3-treated rabbits received (600,000IU/ml) for 3 months showing (A) portal tracts (P) with moderate fibrosis and portal vein (P) display moderate dilatation and congestion.20 X (B) liver parenchyma show some kupffer cells (K) within sinusoids. HE stained section, 40X.

The liver sections in group 4 showed slight dilatation and congestion of the central vein by red blood cells, while the liver parenchyma displays mild inflammatory and kupffer cells within sinusoids as demonstrated in figure 8 A and B.

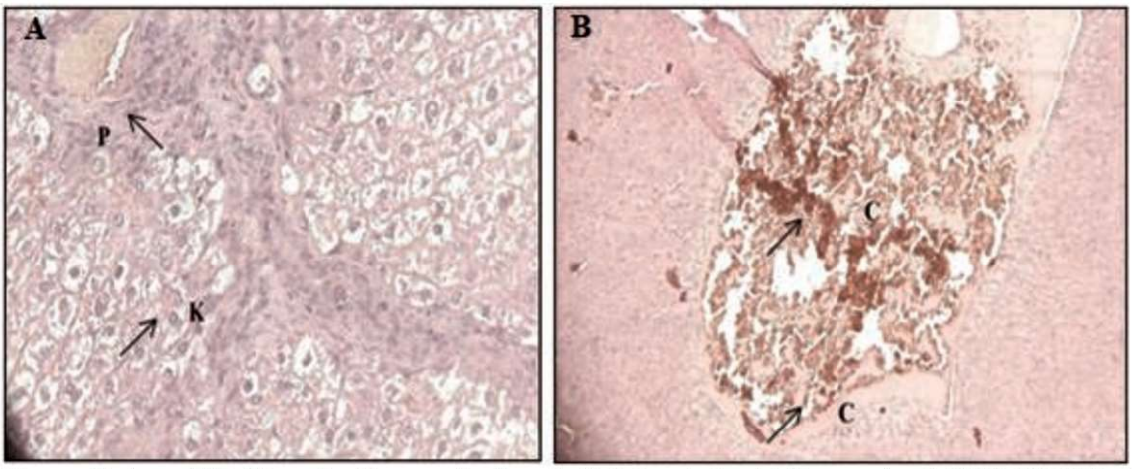

Figure 8 A and B. Hepatic tissue of vitamin D3-treated rabbits received (400,000IU/ml) for 3 months showing (A) kupffer cells(K) within sinusoids, portal tracts (P) show mild fibrosis. 40 X (B) Liver tissue shows Central vein (C) with moderate dilatation and congestion by red blood cells. HE stained section, 40X .

Group 5's liver sections displayed very slight dilatation in the central vein, and mild inflammatory and kupffer cells are seen in the liver parenchyma. Portal tracts and sinusoids exhibit moderate fibrosis as shown in figure 9 A and B.

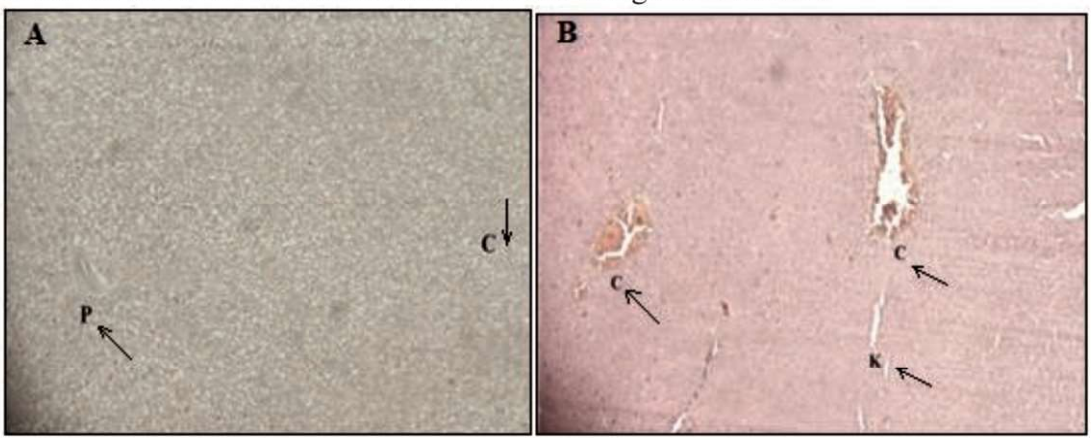

Figure 9 A and B. Hepatic tissue of vitamin D3-treated rabbits received (200,000IU/ml) for 3 months shows (A) portal tracts (P) with mild fibrosis and Central vein (C). 40 X (B) Liver tissue shows Central vein (C) with slight dilatation and congestion and kupffer cells (K) within sinusoids. HE stained section, 40X.

4- Discussion:

This study shows that the administration of high doses of vitamin D3 over 3 months on male rabbit results in an increase in the activity of AST and ALT in blood plasma significantly ($P \leq 0.05$) in group 2 which, had the highest doses of vitamin D3 over the three months. Whereas, the AST and ALT enzyme activity in the G3, G4 and G5 groups did not differ significantly from that in the control group. Moreover, the findings

revealed that (ALP) activity in G2 and G3 was significantly higher (P≤0.05) than in control group, but there was no significant difference (P≤0.05) in G4 and G5 when compared to control group. While elevated levels of these enzymes in the blood are indicative of cellular damage (11), decreased levels indicate stable and healthy liver function (12). Generally speaking, the liver enzymes (AST, ALT and ALP) are used as biomarkers for assessing the health of the liver; they produce more free radicals and experience more oxidative stress as a result of their metabolism, which compromises healthy liver function. The liver enzyme levels (AST, ALT, and ALP) significantly increased in group 2 with the highest doses of vitamin D3 in our study; this increase indicates that vitamin D3 may be toxic to liver. Therefore, the high levels of vitamin D3 used in the study were considered toxic, leading to hypervitaminosis D. The very high monthly doses used in this study were higher than the daily level that considered toxic to rats by the other researchers (13-14).

A statistically significant increase was found in T.bilirubin in all groups treated with vitamin D when compared to control group. This result was in contrast with

Mutlu 2023 who suggests that low level of serum vitamin D may associate with hyperbilirubinaemia in full-term neonates (15).

Furthermore, our study found that liver tissue of the rabbit that treated with vitamin D3 showed significant liver tissue damage and this damage increasing with increasing the doses of vitamin D3 including; dilatation of the central vein, congestion by red blood cells, fibrosis in the portal tracts, congestion by red blood cells in the portal vein, inflammatory and Kupffer cell hyperplasia and calcification. This finding could be due to hypercalcemia-induced vascular dysfunction; this result was along with outcomes stated by Ali et al (16).

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