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Functional and physiological role of vitamin D3 in blood, lipid, aortic, and liver tissues induced by Triton x-100

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I. Conclusion

This study was conducted in the Central Laboratories Research Building of the College of Agriculture and the Animal House of the College of Veterinary Medicine, Tikrit University. The study took place from25/2/2024 until 15/4/2025. Its aim was to evaluate the health benefits of Vitamin D3 in male albino rats after injection of 0.25 ml of Triton X-100 into the peritoneal layer. The abdominal wall was inoculated, and the animal was given an oral dose of vitamin D 0.5 ml/ml once daily for 21 days, continuing the treatment until the end of the experiment. After the experiment, the animals were dissected for biological analyses, including blood counts, liver enzymes, lipid profile, and histological analysis of the liver and coronary arteries. The blood test results for the group treated with vitamin D3, compared to the infected group and the healthy group, showed a significant difference, indicating an improvement in the animals' health. The platelet count results were as follows: (PLT 10^3/L 892.0±31.4 b) Recording a highly significant increase with a significant difference (P<0.05) However, the results for cell size in the same group reached (PCV%44.12±7.02 a،) Significantly increased (P<0.05). White blood cell counts and red blood cell count showed improvement as follows (PLT 10^3/L892.0± 31.4 b) (PCV%44.12± 7.02 a) (WBC 10^3/uL13.321± 3.11 ab) (RBC 10^6/uL8.370± 2.33 bc) Compared to the infected group, these results indicate the effectiveness of vitamin D3; the increased platelet count, cell volume, and white and red blood cell counts reflect the poor health of the animals, as follows (PLT 10^3/L 926.1± 33.4 a)(PCV% 43.41± 6.33 a)(WBC 10^3/uL 14.724± 3.84 a)(10.201±2.257a RBC 10^6/uL) These results demonstrate the therapeutic effect of vitamin D3. As for the effect of the treatments on liver enzymes and kidney function, the results for the affected group were as follows(1.5±0.417 b Creatinine) (90.57±12.4 a Urea mg/dL) (65.34±9.40 an ALT U/L) (170±24.8 an AST U/L) This value indicates that the mean AST enzyme level is 162, with a standard deviation of 19.3. The letters "a" and "b" indicate statistically significant differences between this group and the infection groups. The mean ALT enzyme level is 60.08, with a standard deviation of 8.14. "a" and "b" also indicate statistically significant differences with the infection group at a significance level of p<0.05.

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II. Introduction:

Vitamin D3, a fat-soluble vitamin, is essential for many physiological processes, in addition to its well-known role in calcium metabolism and bone health. Understanding the sources of vitamin D3 is crucial for maintaining optimal levels in the body (Pahwa R, 2023). Vitamin D3 can be obtained from dietary sources and sun exposure. Dietary sources of vitamin D3 include fatty fish such as salmon, mackerel, and tuna, as well as fortified dairy products such as milk, yogurt, and cheese. These food sources provide vitamin D3 (cholecalciferol), which is the most readily available form of vitamin D3 for the body. (Ross AC, 2011). Sun exposure is another important source of vitamin D3. When skin is exposed to sunlight, specifically ultraviolet B (UVB) rays, a precursor molecule in the skin is converted into cholecalciferol. This inactive form of vitamin D3 is then converted into its active form, calcitriol. This occurs through a series of processes in the liver and kidneys. Vitamin D3 synthesis in the skin is considered the primary source for individuals with adequate sun exposure (Wacker M, 2013). However, several factors can affect the efficiency of vitamin D synthesis from sunlight. Geographic location plays a role, as people living at higher latitudes with less direct sunlight exposure may experience reduced vitamin D3 synthesis. Seasonal changes also affect vitamin production. Reduced sun exposure during the winter months can lead to lower vitamin D3 levels. Additionally, skin pigmentation affects the amount of ultraviolet radiation absorbed. Darker skin requires longer sun exposure to produce the same amount of vitamin D as lighter skin. Finally, sunscreen with a high sun protection factor (SPF) can block ultraviolet (UV) radiation and inhibit vitamin D3 synthesis. Given these factors, individuals should aim to obtain vitamin D3 from dietary sources and moderate sun exposure. Dietary supplements may be necessary for those with limited sun exposure or who are at higher risk of vitamin D3 deficiency due to certain conditions or lifestyle factors. Consulting a healthcare professional can help identify appropriate sources and strategies for maintaining adequate vitamin D3 levels based on individual needs (Mendes MM, 2018).

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III. Materials and working methods:

Preparation of Triton X-100

The substance was diluted using physiological saline, which consisted of 100 ml of the prepared dilution solution. Ten milliliters of this solution were withdrawn, and ten milliliters of Triton X-100 were added to dilute it by a ratio of 10/90 ml. After the Triton X-100 was added to the physiological saline, it was not dissolved directly It was directly in the form of a suspended solution until it was dissolved at a temperature of (37) degrees Celsius to ensure that it was completely dissolved. After that, the animals were injected into the peritoneal layer of the abdominal wall at a rate of 0.25/ml.

Vitamin K2 preparation:

0.103 mcg of vitamin D3 was ground and dissolved in distilled water at a rate of 10 ml per weight used, to be administered orally to animals at a rate of 0.5 ml of the dose throughout the experimental period.

Preparing animals:

This study used adult male laboratory rats of the white albino breed, weighing (140-150) g, obtained from the Veterinary Medicine College Research Center at Tikrit University. They were placed in cages measuring 20×40×20cm made of stainless-steel alloy After the animals were prepared and conditioned in a suitable environment, the procedure was carried out over a period of 21 days, including two days at the beginning of the trial period in order to acclimate the animals to the environmental conditions. After that, they were injected with the triton x-100 substance after it had been prepared in advance and injected into the peritoneal layer (abdominal wall). With 0.25ml/m of diluted triton x-100, after the animals were infected, they were left for two days in order to confirm the infection and prove the apparent symptoms that ensure the infection. The symptoms of infection appeared on the animals, including lethargy and the occurrence of two deaths. After confirming the infection, the animals were divided into three groups, as follows.

1 -Control group: Given drinking water (distilled water) and food daily for (21) days.

2- Triton-treated group: Treated with 0.25 ml in the peritoneal layer of the abdominal wall. This is the infected control group, as they were infected and received no treatment other than food and water.

3- The group treated with vitamin K2 (therapeutic effect): They were treated with vitamin D3 at a dose of 0.5 ml/ml once a day for (21) days, with the treatment with vitamin D3 continuing until the end of the experiment.

Blood samples:

After the end of the 27-day experiment, the animals were fasted for 12 hours and then anesthetized with chloroform. The animals were dissected to obtain the liver and coronary artery, and blood samples were drawn by cardiac puncture in the amount of (3-5 ml). Using 5ml syringes in two sets of blood collection tubes, one containing the anticoagulant (EDTA) Ethyl diamine tetra acetic acid for normal blood tests and the other without it, which were placed in a centrifuge at a speed of 3000 rpm For 10 minutes to obtain the plasma, the plasma

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(blood serum) was then placed in special Eppendorf tubes which were kept frozen at -18°C until the necessary biochemical tests were carried out (Titez, et al.2005).

Complete Blood Cell (CBC) Blood Count Standards:

Twenty-seven days after the treatments, blood was withdrawn from the animals after anesthetizing them with 97% chloroform following a thoracotomy. Blood was then withdrawn via cardiac puncture, and after the blood withdrawal was complete, sections of both the liver and coronary artery were taken. The blood was placed in plastic test tubes containing the anticoagulant Ethyl Diaminic Tetraacetic Acid (EDTA). And others that do not contain an anticoagulant, for the purpose of measuring certain blood parameters, according to (Turgeon, 2012), which included the following:

PLT- Platelets

PCV -Packed Cell Volume

WBC - White Blood Cells

RBC - Red Blood Cells

Creatinine

Urea

Estimation of cholesterol concentration in serum:

Basic Principle

Cholesterol levels in serum are estimated using the enzymatic method, which relies on the conversion of cholesterol to a dye (quinonimine) in the presence of three enzymes: cholesterol esterase, cholesterol oxidase, and peroxidase, as shown in the following equations:

Cholesterol es **Cholesterol Oxidase** Cholesterol+freefatty acids

Cholesterol $+O_2$ **Cholesterol Oxidase** Choles ten 4-one3$+H_2O_2$

$2H_2O_2$+phenol+PAP **POD** uinoneimine $+4H_2O$

Measurement of high-density lipoprotein (HDL-C) cholesterol levels in serum

Measurement of low-density lipoprotein (LDL-C) cholesterol levels in serum

Measurement of triglyceride levels in serum

Histological Sections Prepare:

The tissue sections were prepared based on the method mentioned in (Al-Hajj, 2010) in the Life Sciences Laboratory - College of Science / Tikrit University, as follows:

Fixation:

The targeted organs were immediately fixed after dissecting the rats of the studied groups with a 10% formalin fixative (90 ml water + 10 ml of 40% formaldehyde) for 24 hours.

Washing:

The samples were washed with running water to remove any remaining fixative for half an hour.

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Dehydration:

The samples were passed through increasing concentrations of ethyl alcohol (30-50-70-80-95-100) % for the purpose of gradually removing water from them. The duration of each pass was (30) minutes, and the absolute alcohol step (100%) was repeated twice to complete the final removal of water.

Clearing:

The samples were placed in xylene for (30) minutes in two stages in order to make the tissue more transparent.

Infiltration:

The samples were placed in a mixture of xylene and molten paraffin wax at 58ºC in a ratio of (1:1), for (15) minutes, after which they were passed through molten wax for half an hour in three passes to ensure that the wax penetrated evenly.

IV. Results and discussion:

Effect of Treatments on Liver Enzymes and Kidney Function

The results of the current study, shown in Table (1), indicate a significant increase ($P \leq 0.05$) in red blood cell count (RBC 106/µL, ± 2.18 bc), white blood cell count (WBC 14724 ± 3.84 aµL, PCV 43.41 ± 6.33 aµL), and platelet count (PLT 926.1 aµL, ± 103/µL) in the group treated with the induced substance from Triton X-100. This is consistent with the findings of Moulin (2018), who observed an increase in white blood cell count due to Triton X-100 being a non-ionic surfactant that disrupts protein or organelle liquefaction, increases cell membrane permeability, and promotes liver fat accumulation Cholesterol absorption from the intestines is inhibited through emulsification, and the action of the lipoprotein lipase enzyme is inhibited, while the activity of the cholesterol acyltransferase enzyme, which is stimulated in insulin deficiency, is increased by Triton X-100. Compared to the healthy control group, whose blood cell counts were 8.538 RBC 106/uL, WBC 103/uL 8.476, PCV% 45.42, PLT 103/L 726.0.

Table (1) Effect of different treatments on some blood types

n	PLT 10^3/L	PCV%	WBC 10^3/uL	RBC 10^6/uL
Proper control	726.0 ± 29.7 e	45.42 ± 7.41 a	8.476 ± 1.64 d	8.538 ± 2.18 bc
Infected control	926.1 ± 33.4 a	43.41 ± 6.33 a	14.724 ± 3.84 a	10.201 ± 2.57 a
The group treated with vitamin D3	892.0 ± 31.4 b	44.12 ± 7.02 a	13.321 ± 3.11 ab	8.370 ± 2.33 bc

Effect of Vitamin D3 Treatment on Blood Counts

The study indicates that taking VD3 at a concentration of 0.5 mg/ml led to a reduction in the number of white blood cells, which amounted to (8,370, 13,321, 44,12, 892). The result of the study was consistent with what was stated in (AJG.2013), that VD3 in the T3 group reduced the levels of RBC 106/uL. This indicates that it has anti-inflammatory, anti-fibrotic, and immune-enhancing effects and its ability to produce cytokines that activate red blood cells and reduce the effect of free radicals. It did not affect the reduction of WBC levels 103/uL, but it

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improved PCV levels compared to the infected group, as there was no significant difference between it and the healthy group. As for PLT, the number of platelets, the vitamin led to a significant reduction in its levels, which is attributed to the fact that vitamin VD3 has anti-inflammatory effects. As for the number of PLT, the results showed through Table (1-4) that there were significant differences between the experimental treatments, as the average (PLT 103/L926.1) increased in the infected treatment compared to (PLT 103/L726.0) in the control treatment. These results are consistent with (Minnee et al.2025), which showed that triton will lead to an increase in the level of red blood cells as a result of the inflammation caused by them. The functional activity was shown, as stated in (Feketea,2021), that the main function of vitamin D3 is to activate platelets in hemostasis and the immune response. It shows its ability to attract white blood cells and release pro-inflammatory and anti-inflammatory factors

.

Effect of VD3 treatment on lipid profile

The results in the current study shown in Table (2) show a significant increase $P \leq 0.05$ in the concentration of total cholesterol, as its concentration in the group infected with triton x-100 reached (128.35) mg/100 ml and in the healthy group it reached (95.58) mg/100 ml. The group treated with VD3 showed a decrease in the concentration of total cholesterol compared to the healthy control group. The study showed that the vitamin has an effect on reducing total cholesterol levels.

Table (2) Effect of treatments on liver enzymes and kidney function

n	Creatinine mg/dL	Urea mg/dL	ALT U/L	AST U/L
Proper control	0.8 ± 0.111 d	47.32 ± 6.42 d	42.57 ± 7.12 d	92 ± 18.6 e
Infected control	1.5 ± 0.417 b	90.57 ± 12.4 a	65.34 ± 9.40 a	170 ± 24.8 a
The group treated with vitamin D3	1.2 ± 0.216 c	70.05 ± 8.83 c	55.20 ± 8.85 b	161 ± 22.7 ab

Similar letters mean there is no semantic difference between them.

A significant increase ($p \leq 0.05$) in cholesterol (cholesterol) levels was observed in the Triton X100 treatment, along with a decrease in high-density lipoprotein (HDL) levels and an increase in low-density lipoprotein (LDL), triglycerides (TG), and the TG/HDL ratio. These values reached 128.35, 26.24, 67.06, 125.00, and 4.76 mg/dl, respectively. These results are consistent with those of Abdelgadir et al. (2020), who found an increase in blood lipids after injection with Triton X100, a non-ionic surfactant that promotes cholesterol synthesis. In the liver, fat absorption in the intestines occurs through emulsification. It inhibits the action of the lipoprotein lipase enzyme, preventing lipoprotein absorption. This leads to hyperlipidemia (high blood lipid levels) and cardiovascular disease. Alternatively, it may be due to increased activity of the cholesterol acyltransferase enzyme, which is responsible for cholesterol absorption in the intestines and is stimulated by insulin deficiency, as seen with Triton X100.

Meanwhile, cholesterol (Chol) levels decreased, high-density lipoprotein (HDL) levels increased, and low-density lipoprotein (LDL) and triglyceride (TG) levels decreased. The TG/HDL ratio in the blood decreased to (112.00, 32.00, 58.34, 98.11, and 3.06 mg/dL, respectively). The aforementioned indicators were consistently higher compared to the infected control group. This is attributed to the functional role of VD3, as demonstrated in other studies. VD3, through its receptors (VDRs), affects the liver. These receptors are found in liver cells,

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and their presence may contribute to reducing inflammation in chronic liver diseases, lowering cholesterol and triglyceride levels that contribute to plaque buildup, and reducing blood flow to the rest of the body. Studies have indicated that VD3 receptors, by enhancing muscle expression of glucose transporter and modulating free fatty acids, increase insulin sensitivity. This, in turn, reduces the activity of lipolytic enzymes such as lipoprotein lipase and adrenaline, helping to regulate blood sugar levels. Its effects extend to numerous metabolic processes, energy regulation, and overall metabolism. Insulin plays a pivotal role in regulating blood sugar levels and supports many processes that maintain the efficiency of bodily functions. Furthermore, VD3 exhibits anti-fibrotic and anti-inflammatory effects in the liver. In addition, the vitamin can reduce the concentration of the programmed cell death process, a series of cellular processes that occur without the release of harmful substances into the surrounding area, such as cytokeratin, which is an indicator of liver damage (Barchetta et al., 2016). Previous studies indicate that vitamin D deficiency in patients with non-alcoholic fatty liver disease (NAFLD) and metabolic syndrome increases the risk of cardiovascular disease (Oxentenko, 2025). Furthermore, it has been found that vitamin D3 intake has a positive effect on improving blood lipid levels, as it is an antioxidant capable of inhibiting the action of an enzyme Hydroxy methyl glutamyl reductase Vitamin D3, found in the liver and involved in cholesterol formation, also has the ability to remove free radicals from the body and reduce LDL-C oxidation, leading to lower cholesterol and blood lipids and helping to reduce the risk of cardiovascular disease (Hafez et al., 2019). Other studies have linked vitamin D3 deficiency to elevated LDL cholesterol and triglycerides. Studies indicate that vitamin D3 supplementation increases HDL cholesterol levels and may have the opposite effect of lowering triglycerides and total cholesterol (Duggan, 2015).

Tissue analysis results

The following images of the healthy control group and the infected group show the results of the interaction between the two groups.

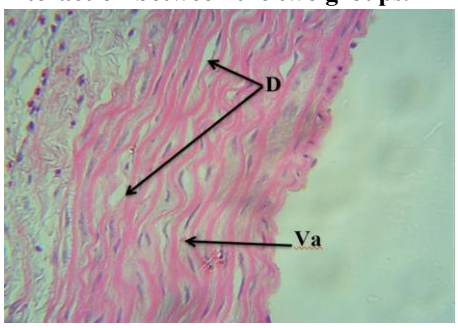

Image (2) A section of the control group showing the tunica albuginea (Te), tunica media (Tm), and tunica externa (Ta) in normal condition and smooth muscle fibers in order. H & E 400X

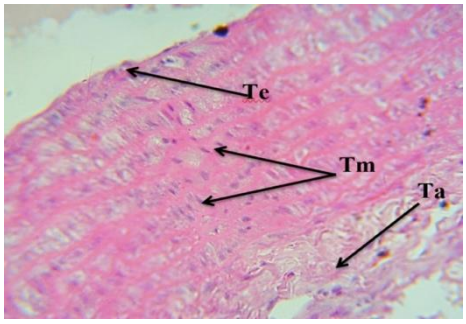

Image (1) A section of the aorta of the control group showing the tunica albuginea (Te), tunica media (Tm), and tunica externa (Ta) in normal condition and smooth muscle fibers arranged H&E400X

The analytical study of coronary artery tissue, interpreting images of both the healthy control and affected groups, revealed that the aortic wall of the healthy group (T1) exhibits the tunica albuginea (Ta), lined with squamous endothelial cells. The innermost layer of blood vessels consists of a single layer of endothelial cells. The capillaries, in turn, are the primary function of this layer, which facilitates the exchange of gases and nutrients. Arteries and veins generally consist of three layers: an inner layer adjacent to the lumen called the tunica albuginea, a middle layer called the tunica media, and an outer layer called the tunica externa. The tunica albuginea is composed of a flat, smooth layer of cells called endothelial cells، The tunica albuginea lines the

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lumen. The endothelial cells are supported by subendothelial connective tissue composed of a small number of fibroblasts, smooth muscle, elastic fibers, and a small amount of collagen. The tunica media is the thickest layer, consisting mainly of smooth muscle and elastic fibers with fewer fibroblasts and collagen. The tunica externa is somewhat integrated with the surrounding tissues and is composed of fibroblasts, collagen, elastic fibers, and nerve fibers. Larger vessels also include the vascular endothelium, which consists of small blood vessels and capillaries that supply the tunica externa and tunica media (Abhimanyu, 2021).

A healthy endothelial layer is essential for normal blood flow through veins or arteries. Biochemical interactions between the endothelium and blood maintain an environment that prevents the formation of clots (thrombus). Endothelial rupture and exposure of the underlying connective tissue lead to a localized release of chemical mediators and cellular responses that result in clot formation and cessation of bleeding (Lyon, 2017). The middle layer, composed of elastic laminae and smooth muscle cells, forms the tissue layer that constitutes the walls of blood vessels. In contrast, the middle layer of arteries contains more smooth muscle than that of veins. This allows arteries to contract and dilate to regulate the blood volume needed by the tissues they supply. The innermost layer, the outermost layer, is composed of elastic and colloidal fibers and represents the intact structure of the blood vessels. As for picture (2) of the infected group, there appeared to be shedding of the endothelial cells in the endothelial layer with the breakdown of the elastic platelets in the middle layer (va) and vacuole degeneration in the middle layer (D) and the outer layer in it, the production of white blood cells due to the effect caused by the substance induced by triton x-100, as this study explains that the infected group had the effect of the breakdown of elastic platelets in the middle layer as shown in picture above (2).

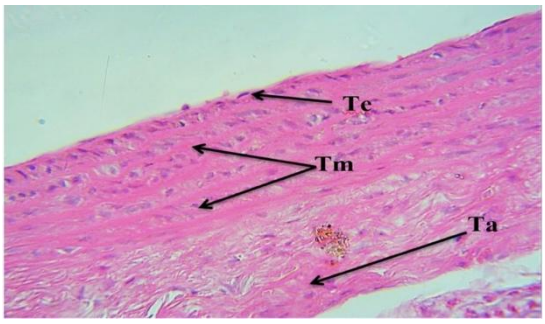

Image (3) A section that impressed the group treated with triton and vitamin D3 showing the tunica endothelium (Ta), tunica media (Tm), and tunica externa (Ta) in a near-normal state. H & E 400X

The wide-ranging effect of vitamin D3 on various physiological processes, and the association of its deficiency with numerous disorders and cardiovascular diseases, point to its potential therapeutic role. Histological analysis, as shown in Figure 3 above, demonstrated that vitamin D3 treatment resulted in improved levels of function. Images of the affected group treated with the vitamin show that the tunica albuginea is lined with squamous endothelial cells resting on the basement membrane, the middle layer is lamellar and elastic, and there are wavy colloidal fiber bundles in the tunica adventitia and fatty tissue at the arterial periphery, indicating the therapeutic efficacy of vitamin D3. In the regeneration and repair of tissues damaged by the induced substance, improved levels were observed in tissue images compared to the affected group, which showed endothelial cell shedding in the endothelial layer with the breakdown of elastic lamellae in the middle layer. This is in contrast to other studies that have shown that vitamin D inhibits the peripheral renin-angiotensin system and improves vascular endothelial function in hypertensive patients with vitamin D3 deficiency. It

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reduces the expression of angiotensin-1 receptors in endothelial cells, thus improving vascular endothelial function and preventing excessive production of reactive oxygen species. (Carrara D, et al. 2016)

Molecular studies have indicated a beneficial role for vitamin D in cardiovascular diseases, through the regulation of coagulation, atherosclerosis, endothelial function, the renin-angiotensin-aldosterone system, vascular calcification, and myocardial hypertrophy. Vitamin D and its derivatives have been shown to inhibit coagulation by suppressing coagulation factors such as vascular smooth muscle-derived tissue factor, protease receptor-activated 2 (PAR-2), plasminogen activator inhibitor 1, and thrombospondin. By increasing the anticoagulant factor thrombomodulin, the anti-inflammatory effect of vitamin D3 in vascular endothelial cells has been shown to prevent the initiation or development of atherosclerosis. Furthermore, some studies have indicated that vitamin D3 may protect against atherosclerosis by inhibiting the transformation of macrophages into foam cells and by stimulating vascular relaxation (Pilz et al., 2016). The anti-inflammatory effect of vitamin D3 in vascular endothelial cells has been shown to prevent the initiation or development of atherosclerosis by inhibiting the transformation of macrophages into foam cells and by stimulating vascular relaxation (Sarhan et al., 2023).

As for the liver tissue sections taken from the three groups on which the experiment was conducted, they are shown in the following images.

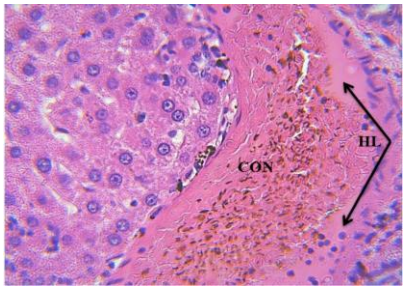

Image (2) Liver section of the Triton treatment group showing central venous congestion (CON) and hemolysis (HL). H & E 400X

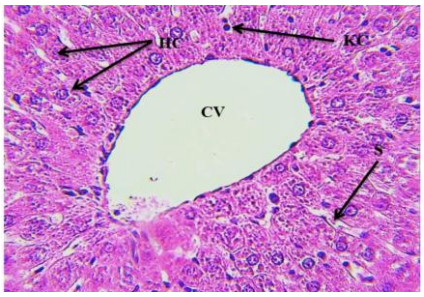

Image (1) Liver section of the control group showing the central vein (CV), hepatocytes (HC), and sinusoids (S). Kover cells (KC) can also be observed. H & E 400X

The liver tissue in the first histological section of the control group contained a large central vein devoid of blood and surrounded by rows and clusters of dark acid-stained, polygonal hepatocytes with spherical nuclei and blood channels to the sinusoids containing some Cowper cells. The second histological section of the affected group showed a central vein in the middle of the liver lobe congested with blood and surrounded externally by some white blood cells, in addition to the presence of polygonal hepatocyte groups with dark-stained spherical nuclei. This result indicates poor health and tissue damage caused by the stimulating substance given to the laboratory animal used in the experiment.

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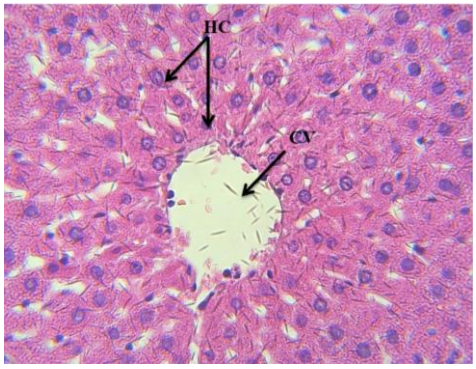

Figure (3) shows a section of the liver from the group treated with tritons and vitamin D3, indicating a near-normal central vein (CV) and hepatocytes (HC). H & E 400X

The histological section of the group treated with vitamin D3 shows that the liver lobe contained a central vein devoid of blood, continuous at its periphery with blood sinuses containing red blood cells and Koverf cells, and within it were rows of radial hepatocytes arranged in a uniform pattern, demonstrating the extent of therapeutic and preventative efficacy and improvement in health status. And the extent of the protective and therapeutic effect of vitamin D3 towards the substance that induces heart disease and hardening of the blood vessels.

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