Wasit Journal for Pure Science

Journal Homepage: https://wjps.uowasit.edu.iq/index.php/wjps/index
e-ISSN: 2790-5241 p-ISSN: 2790-5233

Effect of Hypervitaminosis D on the Histological Structure of Heart in Albino Rat

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DOI: https://doi.org/10.31185/wjps.617
Received 26 November 2024; Accepted 08 December 2025; Available online 30 March 2025

ABSTRACT: Vitamin D is essential for overall health, wellbeing, and Increased mortality and a variety of adverse consequences have been caused by hypervitain D insufficiency. This study was designed to determine the effects of toxic doses of vitamin D on the structures of heart 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 d I.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 few changes but the ID and HD groups showed more deeply restricted degenerative deviations in heart with calcification. the histological structures of heart were affected with increased vitamin D dosage' and appeared calcification.

Keywords: hypervitaminosis D, histological structure of heart, IHC, albino rat, Calcification

1. 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 to different diseases (1). 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. (**2**) Vitamin D is a fat-soluble vitamin that 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, many individuals around the world, of all ages and genders, have a vitamin D deficiency, which makes it one of the most common deficiencies in nutrition (3). In addition to raising the risk of fractures, osteoporosis, and osteomalacia, and causing muscle weakness in adults, a vitamin D deficiency may lead to rickets in children. (4,5,22) Furthermore, it has also been related to inflammatory, autoimmune, cancer, infectious, & cardiovascular diseases. Obesity, hypertension, and even depression and decline in cognition (6, 7, 8) 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 (9, 10,11) Hypervitaminosis D on Phosphate and deposits of calcium in an organic mataffectingheheart this. Various types of soft tiorgans, whichre 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, (13, 14,23) Vitamin D is essential for overall health, well-being, and Increased mortality and a variety of adverse consequences have been caused by hypervitamin D insufficiency. This study was designed to determine the effects of toxic doses of vitamin D on the structures of the heart and calcification in rats due to hypervitaminosis D.

2. MATERIAL AND METHODS

The study was performed using 40 albino rats, which were used with an average weight (200g-250g) and aged between (3-4) months.
1-Group A Control group: normal diet and distilled water were given during a thirty-day period.
2 – Group B received a low dose of vitamin D3/oral dose of 3000 IU/day for 60 days.
3. Group C received a medium dose of vitamin D3/oral dose of 8000 IU/day for 60 days.
4-Group D high dose of vitamin D/oral dose with 20,000 IU/day for 60 days
The total body weight of the animals for all groups, as mentioned in the study design, was recorded before and after during the experiment period for 30 days. The scarifications of the animals were performed, and the targeted organ was collected (heart), and tissue was dissected and cleaned, then preserved in 10% neutral buffered formalin for histological study. The organs were collected from all groups and prepared for histological technique to stain with hematoxylin and eosin (H&E) stain and alizarin (15). Immunohistochemistry is a primary antibody for calcium-binding protein (S100B).

3. RESULTS AND DISCUSSION

Histological examination of the heart of the control group revealed a normal heart of a rat; the heart wall consists of three chief layers: the inside stratum endocardium, the intermediate myocardium, and the outside stratum epicardium. The epicardium stratum involves a thin layer of mesothelium as well as a connective tissue layer. The epicardium stratum is relatively thin in contrast with the myocardium stratum, which consists of heart muscles that compose the majority of the heart wall. It consists of striated cardiac muscle fibers (cardiomyocytes), which are characterized by branched muscle fibers. The myocyte comprises a single centrally located nucleus enclosed by a sarcolemma. Endocardium stratum had simple squamous epithelium (heart endothelium), which lined the heart's cavity. This endothelial layer is continuous with the sub endothelial lining connective tissue with the great blood vessels (Figures 1, 2).

In current study showed that the histological properties of the heart, were appeared few changed when given low doses of vitamin D (LD) (Figure 1,2).while an increase in the dose of vitamin D had led to the appearance highly thickened of heart wall distorted cardiac muscle fibres,irregular distribution of nuclei of myocytes of cardiac fibers ,deeply stained nuclei (pyknotic) and Hypertrophy of cardiac muscle (Figure 3 AB).Mild congestion of coronary artery was observed with mild degeneration of cardiac muscle fibers (figure4B). Apoptotic changes as well as nuclear pyknosis and karyolysis was observed of intermediate dose (ID) (Figure 4A). while high dose, (HD) was showed localized damaged of areas of sub-endothelial and cardiac fibers were inflammatory cells, mainly macrophages, variety of histological abnormalities of the heart structure (Figure 4 B). the calcification was seen in epicardium, myocardium, endocardium, cardiac valves, coronary arteries and certain valves, as well as deposition of white plaques on the luminal surface, degenerative changes and necrotic changes in the cardiac muscle were appeared via alizarin red dye with intermediate and high dose (Figure 5 B,6). Among the histological alterations were calcification, degeneration, fibrous tissue replacement of heart muscle cells, and infiltration of cells..The Immunohistochemical technique of heart sections was shown a slight calcification in low dose (Fig 7) ,mild calcification in intermediate dose(Fig 8) and high calcification with high dose of vitamin d as dark to brown .(Fig ,9)

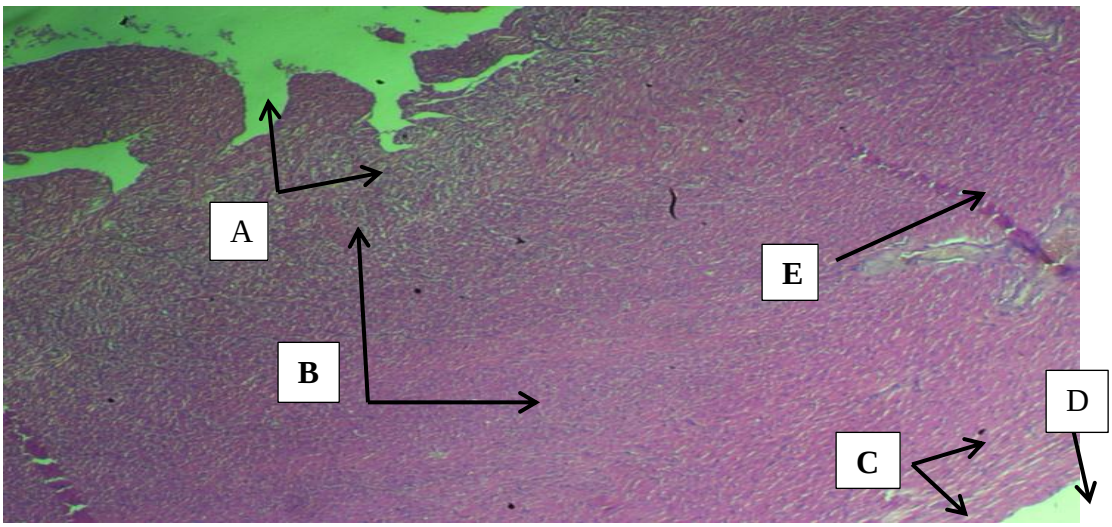
4. DISCUSSION

The present study appeared rats were taking the low dose (LD) showed slight changes in the heart tissue that the histological properties of the heart, were appeared few changed when given low doses of vitamin D (LD). These structural changes in the LD group are consistent with previous studies that document the cardio-protective effect of vitamin D levels. (**16)** note that 'Vitamin D improves myocardial contractility and overall cardiac function by modulating calcium homeostasis.' while an increase in the dose of vitamin D had led to the appearance of Hypertrophy of cardiac muscle heart. mild congestion of coronary and mild degeneration of cardiac muscle fibers were observed. Our result in agreement with (**17**). who reported when the dose of vitamin d increased in mice it led to hypertrophy of the heart muscle, the capillaries between the fibers were dilated and crowded. present study appeared hypertrophy with more degeneration of cardiac muscles fibers and more congestion in blood vessels. Many reports on the non-benefits of high-dose appearedto this fact as (**18**) who reported that vitamin D toxicity caused hypertrophy and congestion and potential damage of heart. This suggests that there is a tipping point above which vitamin D supplementation would have effects on the heart had black degenerated fibers, which are the signs of apoptotic changes was observed in intermediate dose (ID) while high dose (HD) showed localized areas of sub-endothelial damage of cardiac fibers, inflammatory cells, mainly macrophages. variety of histologic abnormalities of the heart and aorta, in this agreement with (**17**)who remained the hypercalcemia resulting from an excessive vitamin D intake could lead to alterations in cardiac function, such as increased cardiac contraction and increased cardiac oxygen demand, degenerative and necrotic changes in the cardiac muscle There was a

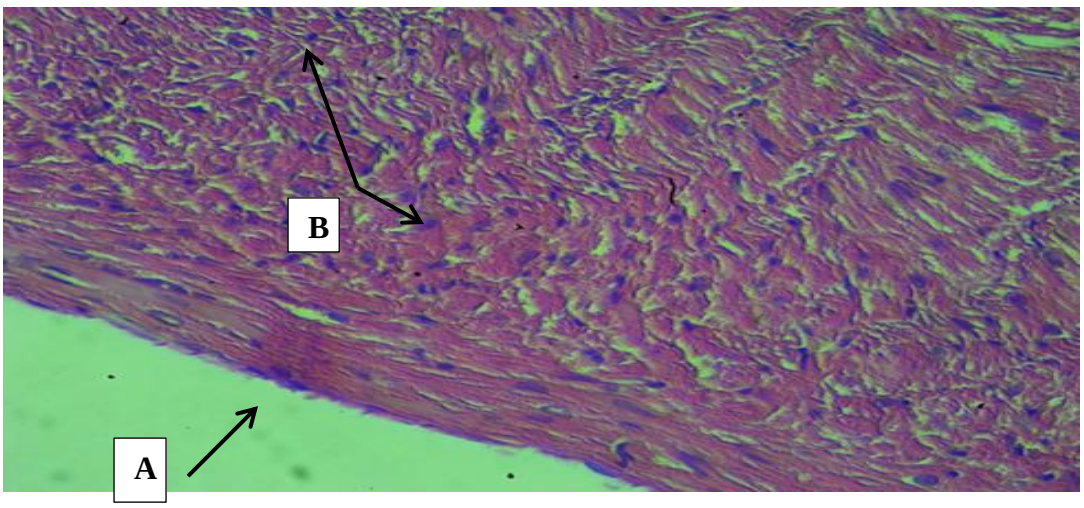
positive linear correlation between calcium and coronary artery wall thickness this result in agreement with (**19**) who proposed that vitamin D, in excess, may lead to pro-apoptotic pathway activation, particularly through the cardiovascular system. well established that perturbations in intracellular Ca2+ store levels or Ca2+ overload can trigger apoptosis or necrosis (**20**) remained the increased signs of apoptosis that we observe and supports the notion that although low doses of vitamin D are beneficial, high doses may predispose cardiac tissues to cell death and dysfunction. Within alizarin staing, the present study showed calcification in epicardium, myocardium and endocardium, cardiac valves and coronary arteries This calcification is where it appears red in the alizarin stain. Among the histological alterations were calcification, degeneration, fibrous tissue replacement of heart muscle cells, and infiltration of inflammatory cells, there was significant inflammatory infiltration and widespread fibrous tissue proliferation in the epicardium and myocardium this result paralled with (**21**) who noted to calcification in heart and explained that calcification caused by deposition of calcium ions in the layers of the heart. With Alizarin Red stain revealed that the first evidence of present calcification emergence as very small black point which found in the myocardium of heart wall. The immunohistochemical examination appeared the beginning of a simple precipitate of calcium salts in heart wall. This finding is similar to mineralization pattern which noted in early age of mice when he mentioned that small granular deposits of calcium in myocardium.With Alizarin Red stain the calcification appeared as small brown spots in valves at this age. Immunohistochemical results of valve showed calcification in the form of spread small points.

5. CONCLUSION

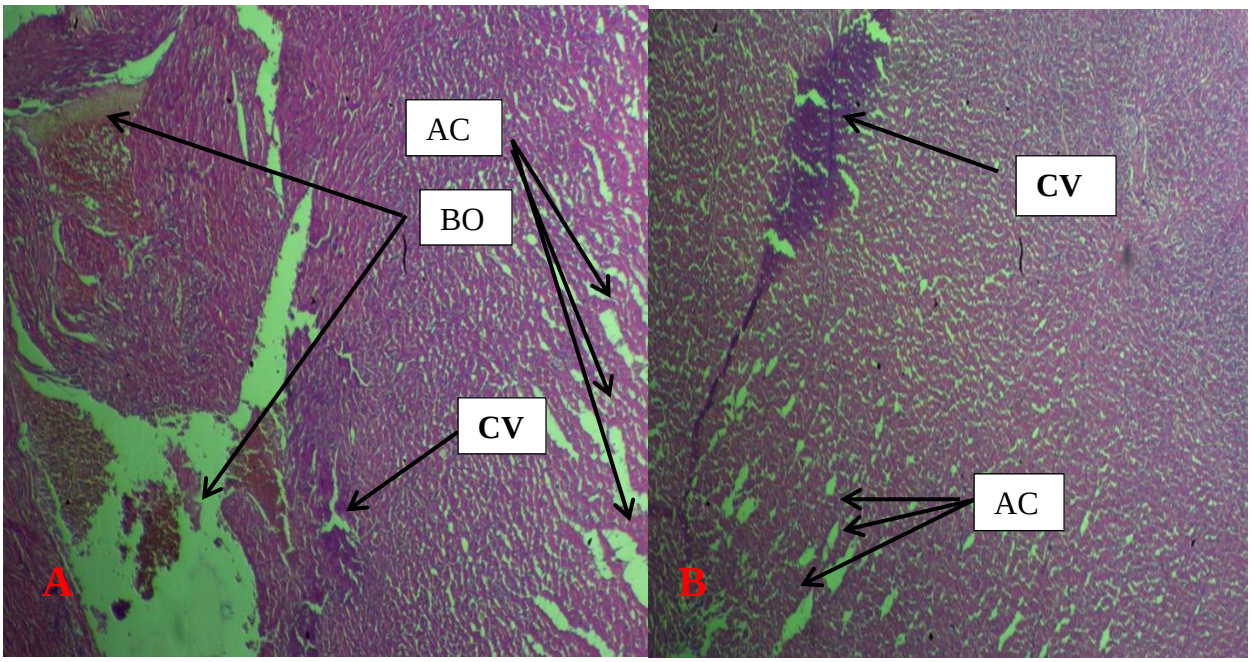
The histological structure of heart was affected with increased vitamin D dosage' and appeared of calcification.



(Fig 1):-showed endocardium(A), myocardium, (B), epicardium(C), mesothelium (D) blood vessels (E) (H&E40X)

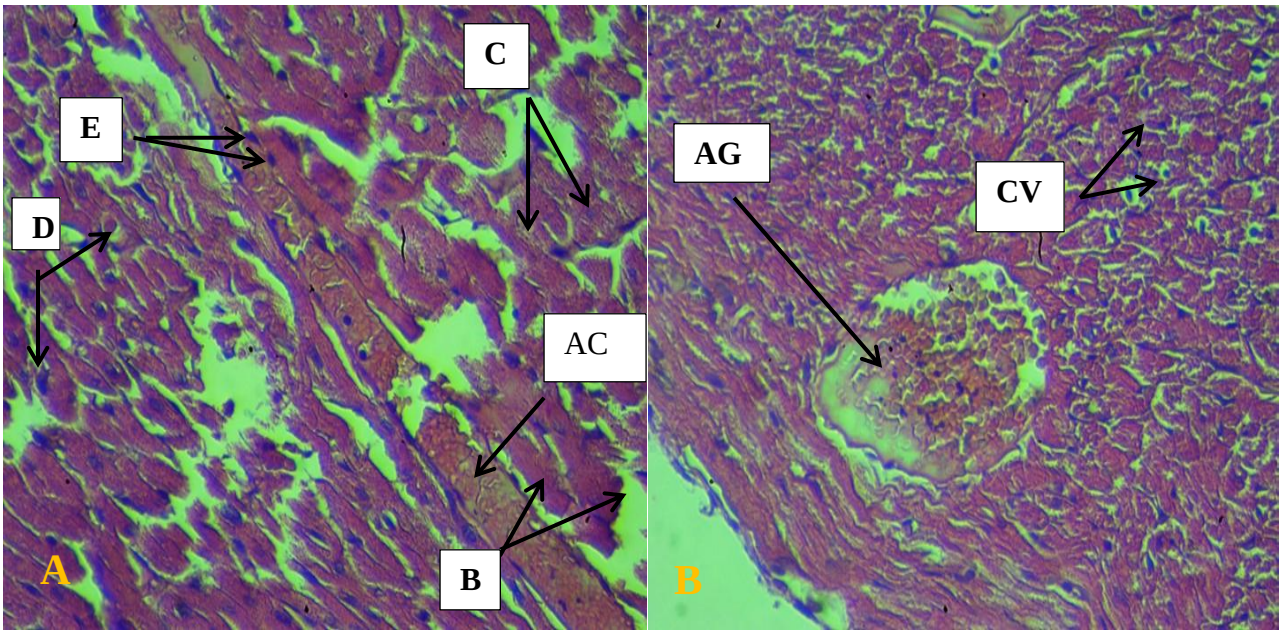


(Fig 2): showed mesothelium(A), Cardiumyocyte (B) (H&E 200 X)



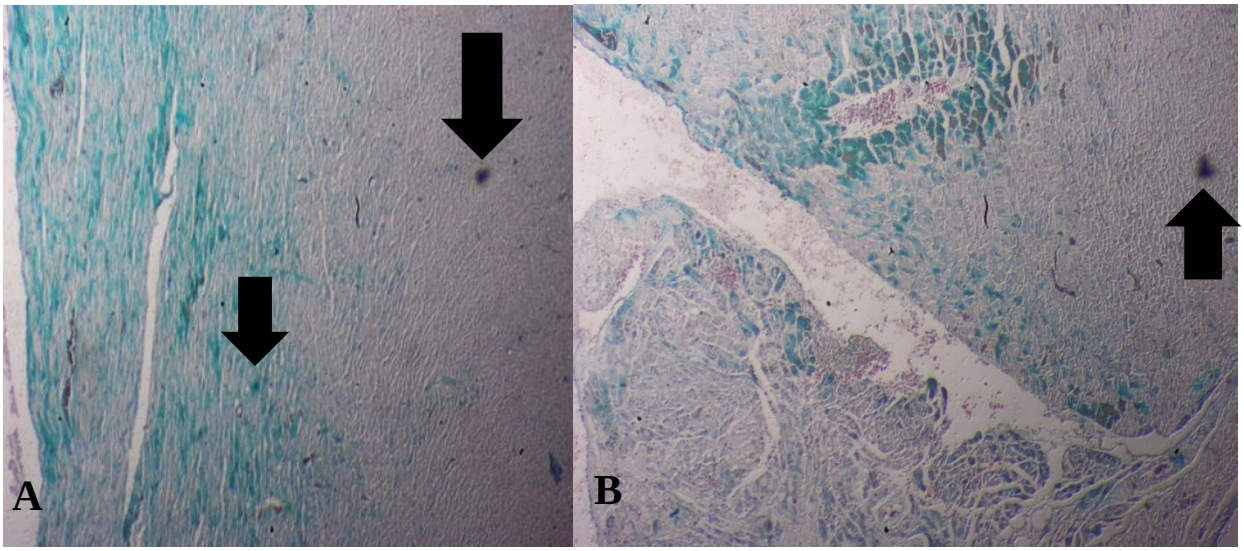
(Fig 3 A, B) (A): high dose of vit d appeared thickened of heart wall, distroid cardiac muscles (AC), congestion of coronary artery (BO), deposit of calcium (CV) (H&E40X)

(Fig B): an intermediate dose Vit D. appeared distroied of some parts of cardiac muscles (AC), deposit of calcium (CV)



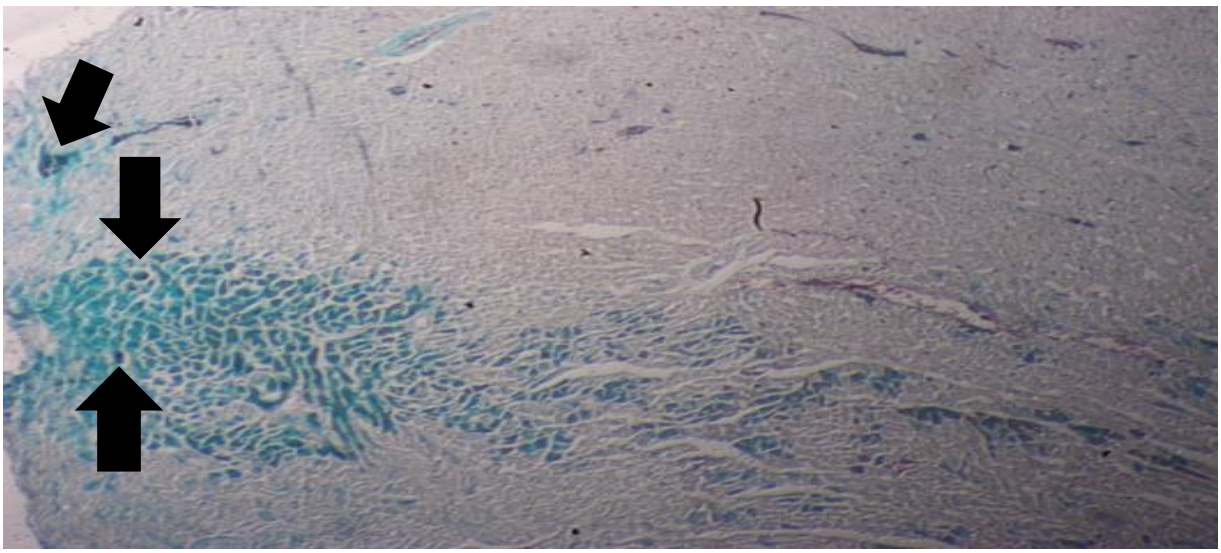
(Fig4 A, B) A: high dose of vit d appeard congestion of blood vessel (AC), degeneration of muscle fiber (B), karyolysis of muscle fiber(C), pykontic(D), inflammntary cells (E)(H&E400X)

(Fig B): intermediate dose of Vit d. appeared congestion of coronary artery (AG), hypertrophy of muscle fiber (CV), damaged epicaridium (BO) (H&E100 X)

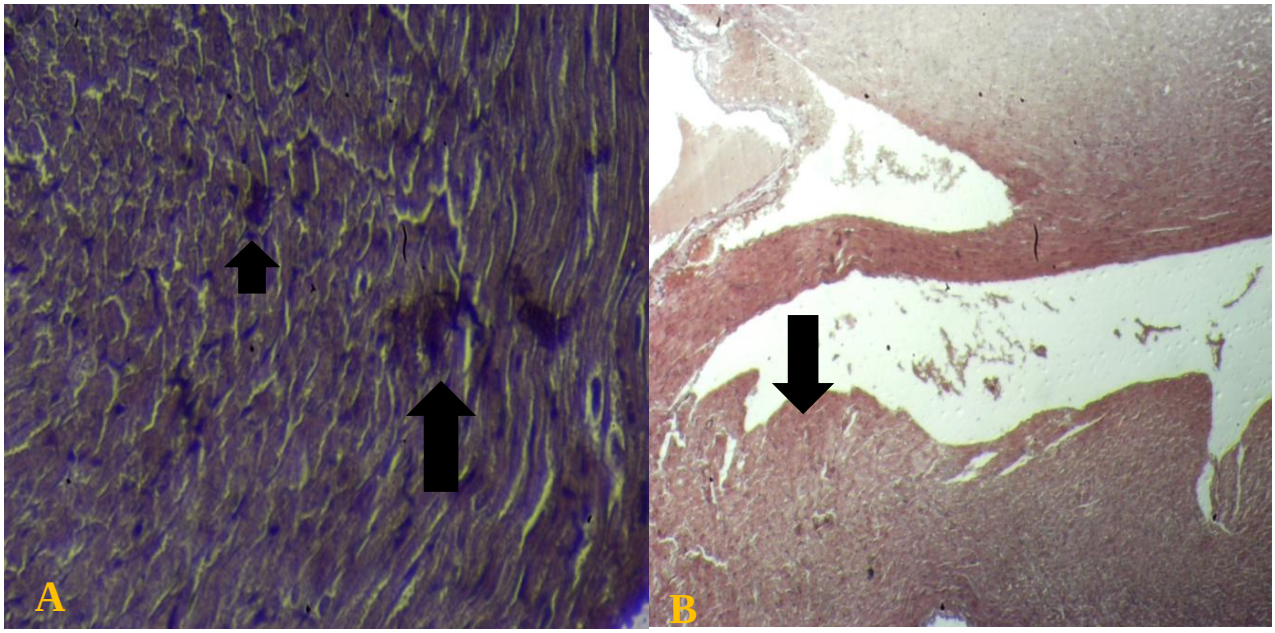


(Figure **5** A, B) A: low dose Vit d showing little amount of calcium scattering (black arrow) (40X alizarin)

(Fig B): high dose of Vit D area of calcification (black arrow) (40X alizarin)

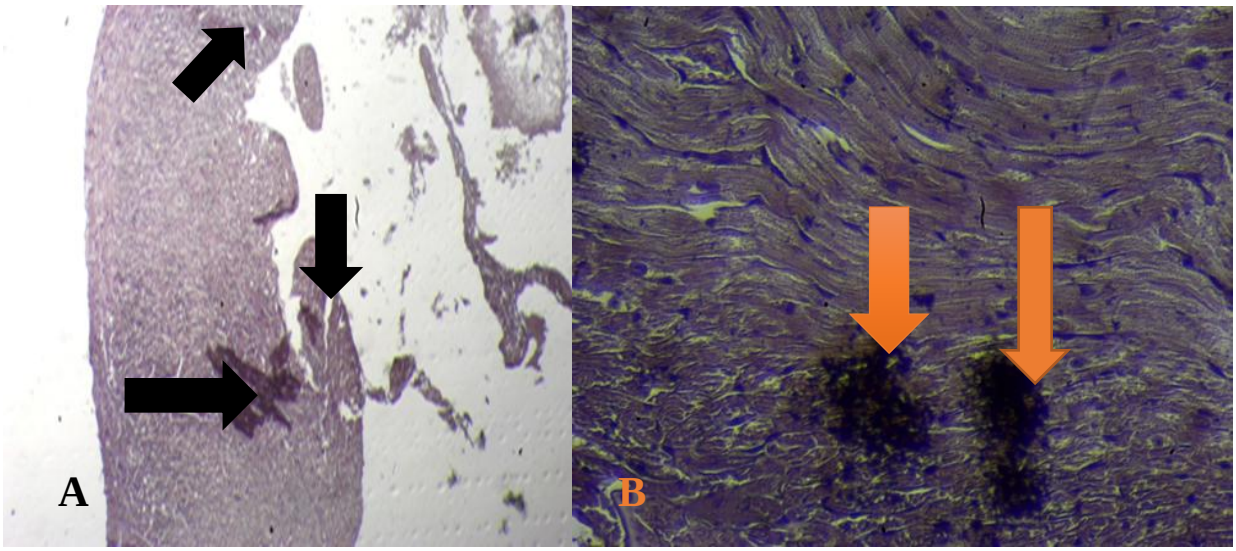


(Figure 6) intermediate dose showing large amount of calcium scattering (black arrow) (40X alizarin)



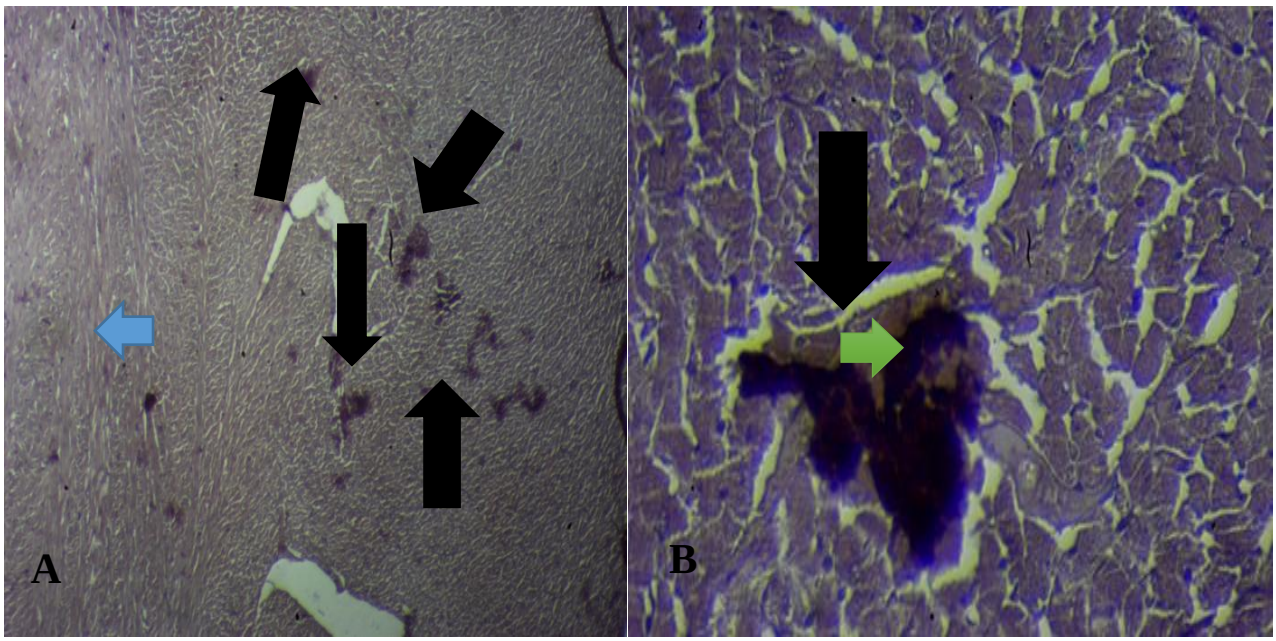
(Figure 7 A, B): low dose (black arrows) slight calcification (Immunohistochemistry 400X)

(Figure B): low dose (black arrow) thickness of heart muscle (Immunohistochemistry 40X)



(Figure 8 A, B) A: High dose areas calcification (black arrows) (40X immunohistochemistry)

(Figure B): intermediate dose areas of calcification (red arrows) (40X immunohistochemistry)



(Figure 9 A, B) A: High dose focus calcification (black arrows) (40X Immunohistochemistry)

(Figure B): High dose area calcification (black arrow) (400X Immunohisochemistry)

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