American Journal of Biology and Natural Sciences
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Effect of Hypervitaminosis a on the Histological Structure of Heart in Male Albino Rat

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Received: 2024, 22, Dec
Accepted: 2025, 23, Jan
Published: 2025, 24, Feb

Annotation: Vitamin A (VA) is a fat-soluble compound. There are two types of VA, the first type is retinol and retinoic acid (derived from animal sources) and the second type is carotenoid derived from plant sources (1,2). In 1928, Green and Milandi reported that vitamin A could enhance the anti-inflammatory response of organisms and called vitamin A the "anti-inflammatory vitamin" (3). VA is naturally present in many foods. VA is important for normal vision, the immune system, reproduction, growth, and development. VA also helps the heart, lungs, and other organs function properly. Carotenoids are pigments that give yellow, orange, and red fruits and vegetables their color. The human body is able to convert some carotenoids to VA (4). VA has also been reported to influence the composition and diversity of gut microbes (5).

Preformed VA is found in fish, organ meats (such as liver), dairy products, and eggs. Carotenoids are converted to VA by your body. The most common provitamin A carotenoid in foods and supplements is beta-carotene **(4).** Vitamin A toxicity or hypervitaminosis A results from excessive

consumption of active (preformed) vitamin A, while hypervitaminosis A with provitamin A is largely uncommon.

Hypervitaminosis A can be acute or chronic. Acute hypervitaminosis A is rare and has many symptoms, including vomiting, diarrhea, and headache **(6,7).** Chronic vitamin A toxicity can lead to liver toxicity, kidney toxicity, hypercalcemia, hyperglycemia, hyperosteomalacia, hypercholesterolemia, and increased cerebrospinal fluid pressure **(8,7).**

Your bones and teeth use almost all of the calcium in your body (about 99%). Soft tissues and organs calcify faster than your bones **(9,10).** Phosphate calcification and calcium deposits in the matrix are the cause. Different types of soft tissues are susceptible to calcification, a buildup of calcium and other salts in the blood due to systemic metabolic imbalances, leading to metastatic calcifications. They often damage the heart **(11,12).**

Some calcification is normal as calcium left 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. This calcium buildup can harden tissues, organs, or blood vessels, which can cause normal body processes to not work properly. But some conditions can cause calcium deposits to appear in places where they shouldn't. This includes areas like the brain, kidneys, liver, and blood vessels. This can cause problems with how your organs and blood vessels function **(13).** The aim of the experiment was to study the tissue changes and calcifications caused by excessive VA in the heart of adult male rats.

MATERIAL AND METHODS:

The study was conducted in the animal house laboratory of the College of Education for Pure Sciences / University of Wasit, for a period of 60 days, with the provision of appropriate conditions of food and temperature, on 40 male white mice with an average weight of (200-250 g) and ages ranging between (3-4) months.

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 with 8000 IU/day for sixty day.
3. Group C medium dose of vitamin A/orally dose with 12000 IU/day For sixty day.
4. Group D high dose of vitamin d / orally dose with15000 IU/ for sixty day.

The body weight of the animals of all groups was measured before and after the experimental period for 30 days. Then the animals were dissected, the target organ (heart) was collected, cleaned, and then preserved in 10% neutral buffered formalin. For histological study, the histological changes were detected by collecting the organs from all groups and preparing them for histological technique using hematoxylin and Eosin (H&E) stain to show the general histological structure and alizarin red to detect calcium salt deposits (calcifications) and Masson Trichom to appeared fibrosis (1**4**).

RESULTS AND DISCUSSION

Histological examination of normal rat hearts has shown that the walls of the four chambers of the heart consist of three main layers or membranes (endocardium, middle myocardium, and outer pericardium). The myocardium is the thickest and contains muscle fibers. The heart is externally covered by simple squamous epithelium (mesothelium) supported by a thin layer of connective tissue that forms the pericardium. The pericardium consists of a single layer of squamous endothelial cells resting on a thin layer of loose connective tissue that contains elastic and collagen fibers as well as some smooth muscle cells. **(Fig 1,2,3).**

The present study showed that the low dose LD group of VA showed less histological changes in the heart structures and that the histological characteristics of the heart did not change significantly when given low doses of vitamin A (LD) represent by Little degeneration and

destructures of the myocardial tissue **(Fig 4).**

Histological examination of animals with Intermediate ID dose of VA showed tissue abnormalities such as destructures, fragmentation and degeneration of myocardial fibers, large gaps and necrosis with loss of normal aortic structure, fragmentation and degeneration of its wall and extensive separation of elastic fibers of the media with little calcium deposition with fibrosis **(Fig 5,6).**

On the other hand, there were clear histological changes in the heart tissues of the high dose HD of VA, as they suffered from cardiac hypertrophy, myocardial necrosis, an increase in the number of mononuclear cells and infiltration of inflammatory cells, and increased degeneration with increased fibrosis of the thick muscle wall of the heart **(Fig 7, 8, 9,10).**

Using alizarin stain and immunohistochemistry, there were less calcifications and calcium deposition in the low dose LD animals **(Fig 11,12,13).** and mild calcifications in the Intermmediat dose ID animals **(Fig 14,15,16).**

In the high-dose animals, there was an increase in the accumulation of calcium deposits in the affected tissues, which led to increased calcifications in the pericardium, myocardium, endocardium, heart valves and coronary arteries in a dark brown color **(Fig 17,18,19).**

DISCUSSION

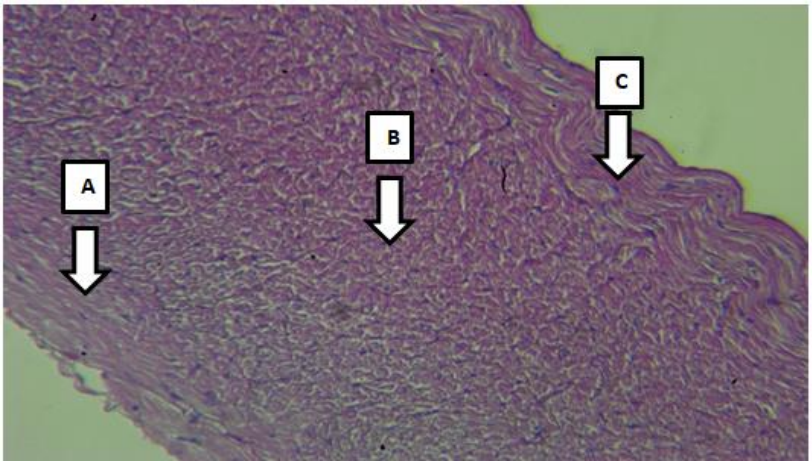
The present study showed that hypervitaminosis A causes histological changes in heart tissue that affect its normal structure, represented by a slight degeneration and deterioration of myocardial tissue in animals exposed to a low dose(LD). Our results were consistent **w**ith**[15],** who showed that the interlocking discs and myocardial fibers still function well and that the heart maintains its normal structure if not exposed to high doses of nutritional supplements and vitamins necessary to ensure healthy heart function. While histological examination of the heart of animals with a Intermediate dose(ID) showed cardiac hypertrophy, myocardial necrosis, and infiltration of inflammatory cells, with degeneration and fibrosis of the thick myocardial wall. These results are consistent with the research published by **[16]**, which confirmed the occurrence of tissue abnormalities such as destructure, fragmentation, dissolution, and fibrosis in the heart muscle when exposed to high doses(HD) of vitamin A. Large gaps and necrosis with loss of normal aortic structure, fragmentation and disintegration of its wall, and extensive separation of elastic fibers of the media with calcium deposition. These results are consistent with **[15]** who reported that hypervitaminosis A in high-dose(HD) animals can cause cardiotoxicity leading to cardiac hypertrophy, myocardial necrosis, increased inflammatory cell counts, and increased degeneration with increased fibrosis of the thick muscle wall. Our results are consistent with those of **[17]** who described a case of left ventricular fibrosis and calcification after severe myocardial infarction leading to progressive heart failure due to acute vitamin A toxicity. Severe myocardial degeneration and fibrosis with increased numbers of inflammatory mononuclear cells. Our results were consistent with **[18]** who observed fibrotic hypertrophy in heart tissue after exposure to hypervitaminosis A for eight weeks. The accumulation of calcium deposits in the affected tissues led to a state of calcification, and increased calcification of the pericardium, myocardium, endocardium, heart valves, and coronary arteries, and this result is consistent with **[19].**

who confirmed that oxidative stress and cellular damage caused by retinoids and steroids is an inflammatory response and progressive fibrosis. With the increase in calcifications, this is consistent with what **[20],** confirmed about degenerative and fibrotic changes in rodents due to hypervitaminosis A.our current study showed light brown calcification in the low dose (LD)group, darker in the Intermediate dose (ID) group, and very dark brown in the high dose (HD) group. The researcher **[21]**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 mechanism of calcification is believed to be a function of hyperphosphatemia. In general, according to some authors' descriptions of cardiac calcification in different vertebrate species, such as mice **[22]**, the occurrence of calcium

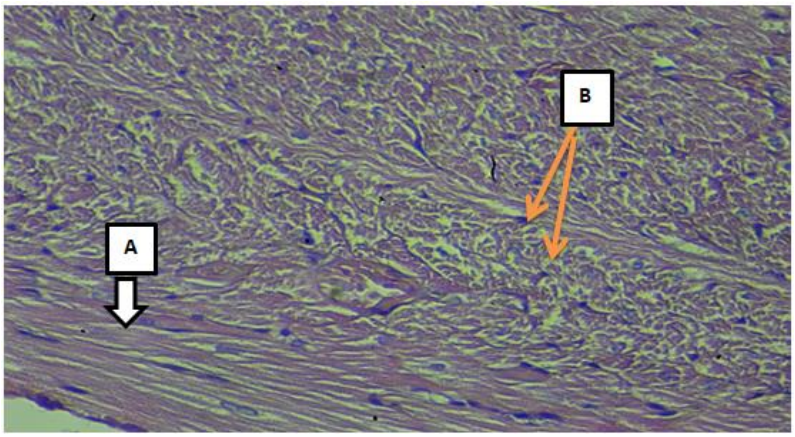
deposition in our study in the high-dose(HD) vitamin A group was similar to that of **[23].** This increase in cardiac calcification may also be related to age, and the type of their diet, which is often accompanied by a physiological disturbance in the level of calcium in the blood. This is what **[24]** reported that the differences in calcifications may be attributed to the age of the mouse, as well as the type of diet.

Conclusion:

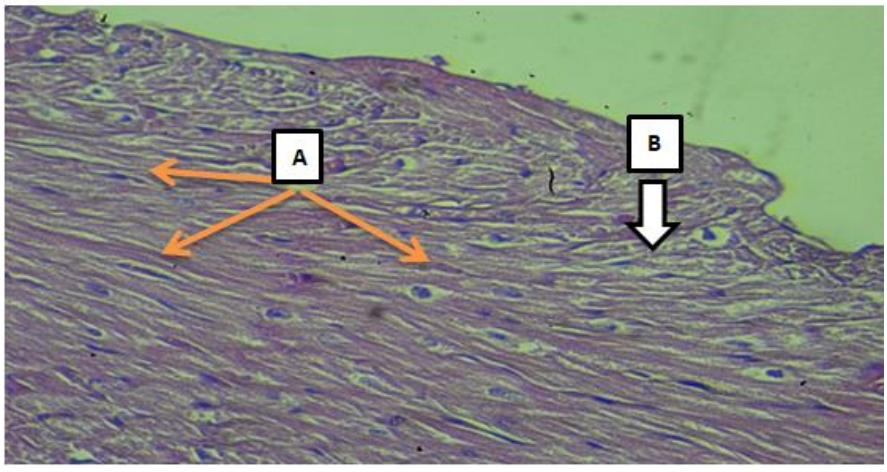
The tissue structure of the heart was affected by severe calcifications due to increased intake of vitamin A doses with the appearance of fibrosis.



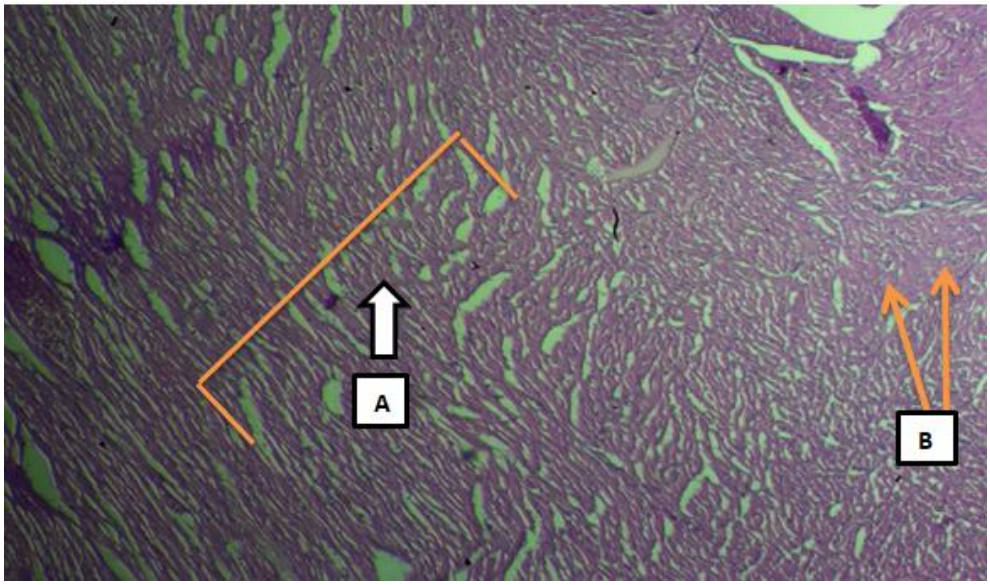
(Fig1): control group appeared,endocardium(A),Myocardium(B),epicardium(C),(H&E 100X).



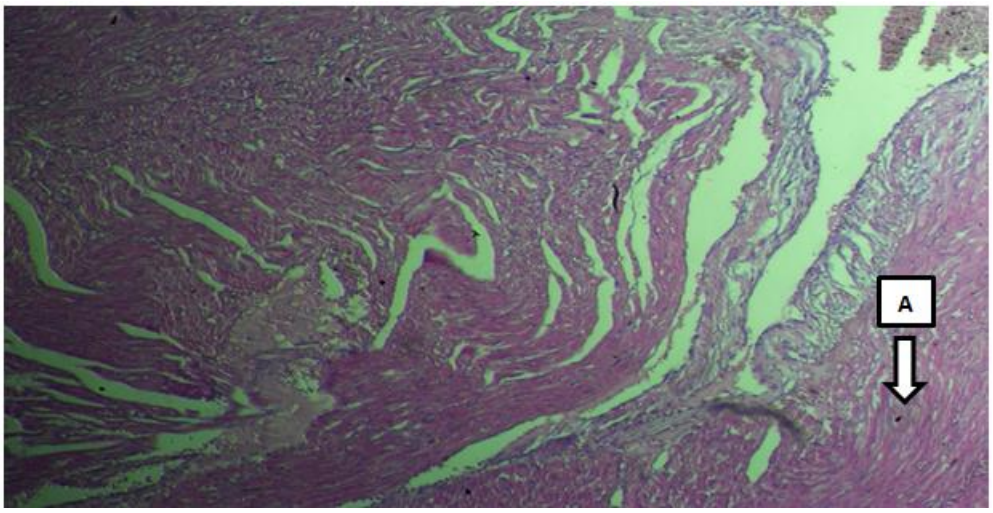
(Fig2): control group appeared, epithelial squamous cells (A) ,myocardial cells(B) ,(H&E 100X).



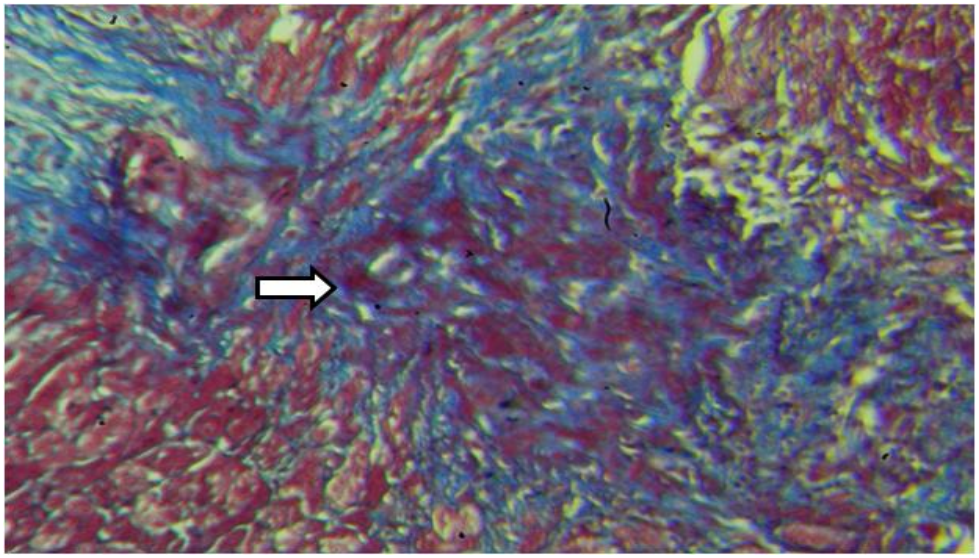
(Fig3): control group appeared,myocardial cell (A) ,epicardium (B). (H&E 100X).



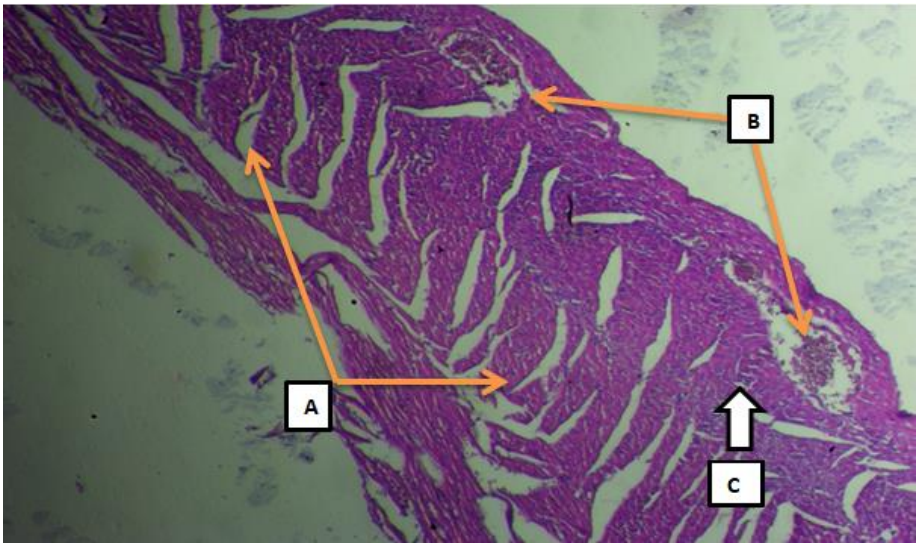
(Fig4):low dose LD group appeared,destructures(A),degeneration(B). (H&E 100X).



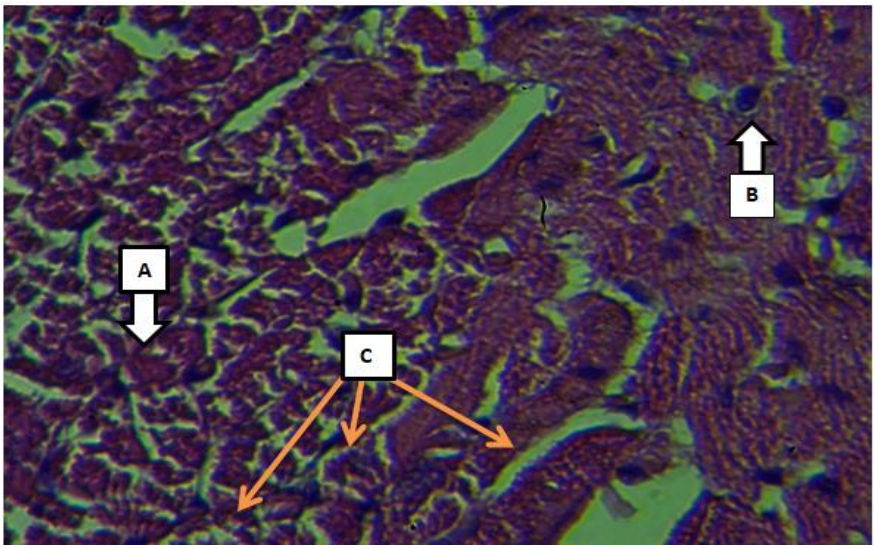
(Fig5):intermediate dose ID group appeared,increased of destructures (A), increased of degeneration (B). (H&E 100X).



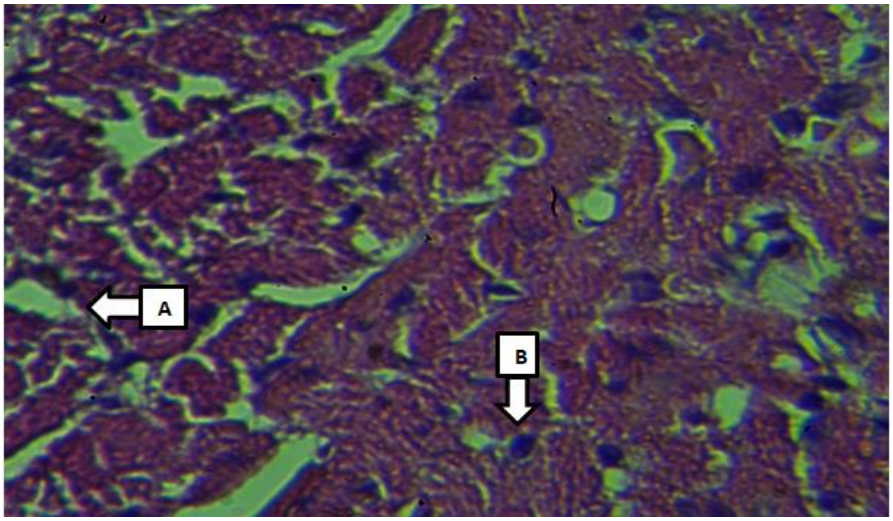
(Fig6):intermediate dose ID group appeared, increase fibrosis(white arrow),(Masson Trichom100X).



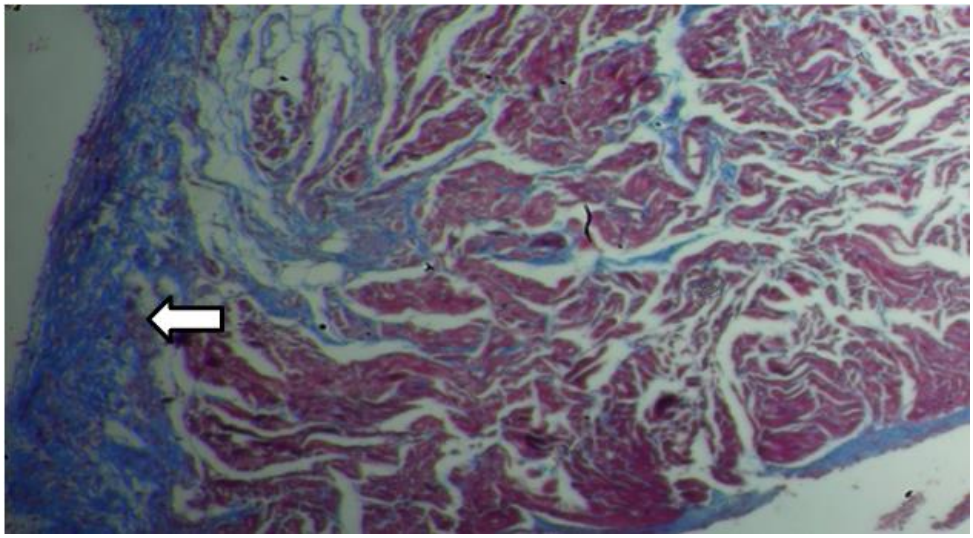
(Fig7): high dose HD group appeared,increaeed destructures (A),hemoragted of coronary artery(B),fibrosis(C), (H&E 100X).



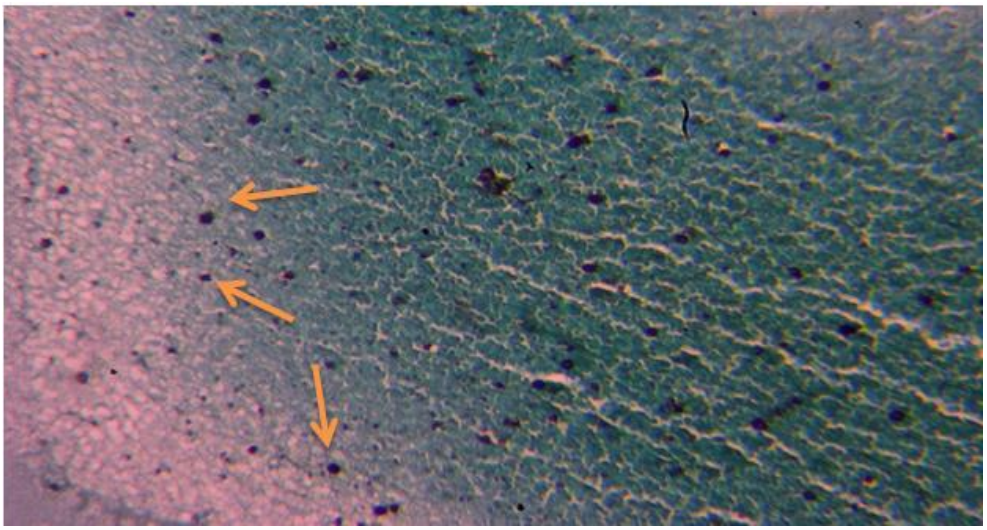
(Fig8): high dose HD group appeared,degeneration of myocyte(A), necrosis of myocyte(B)fragmentation(C). (H&E 100X).



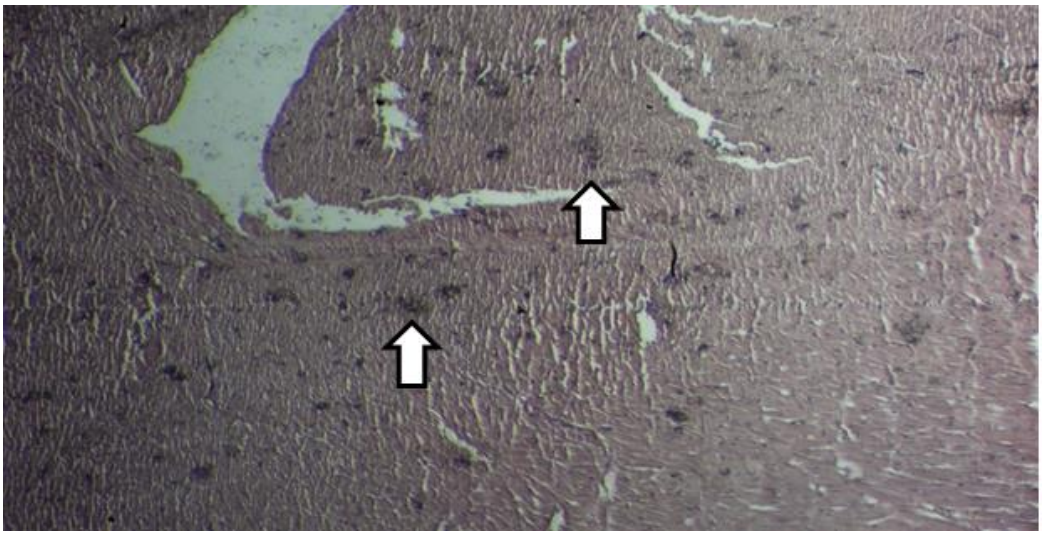
(Fig9): high dose HD group appeared,fragmentation(A),necrosis(B),). (H&E 100X).



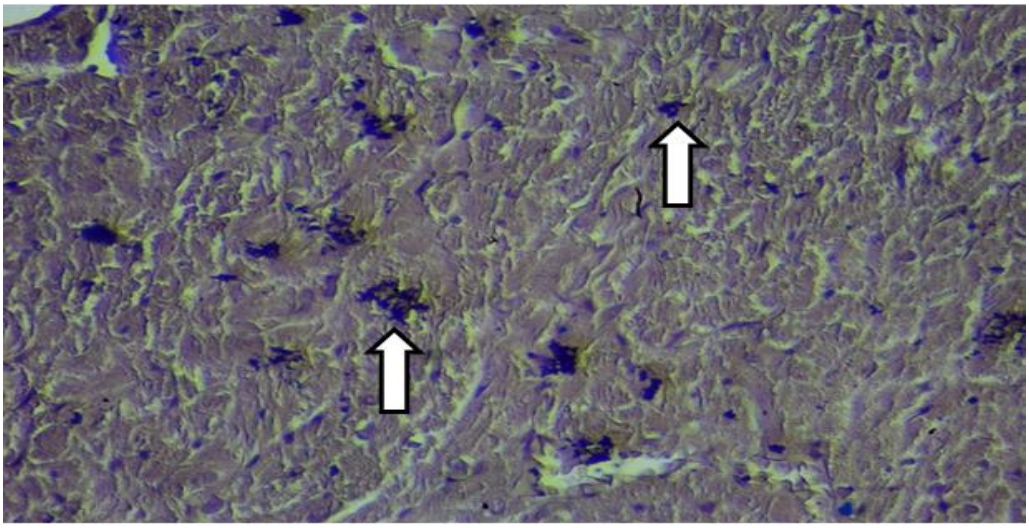
(Fig10): high dose HD appeared , very high fibrosis **(white arrow)** ,(Masson Trichom 100X).



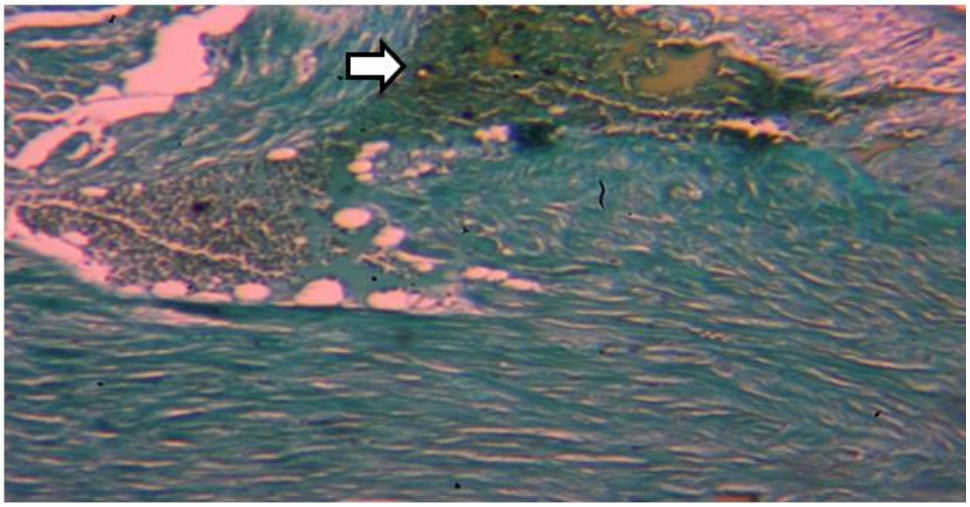
(Fig11): Low dose LD group appeared ,calcification → ,(alizarin stain 40x).



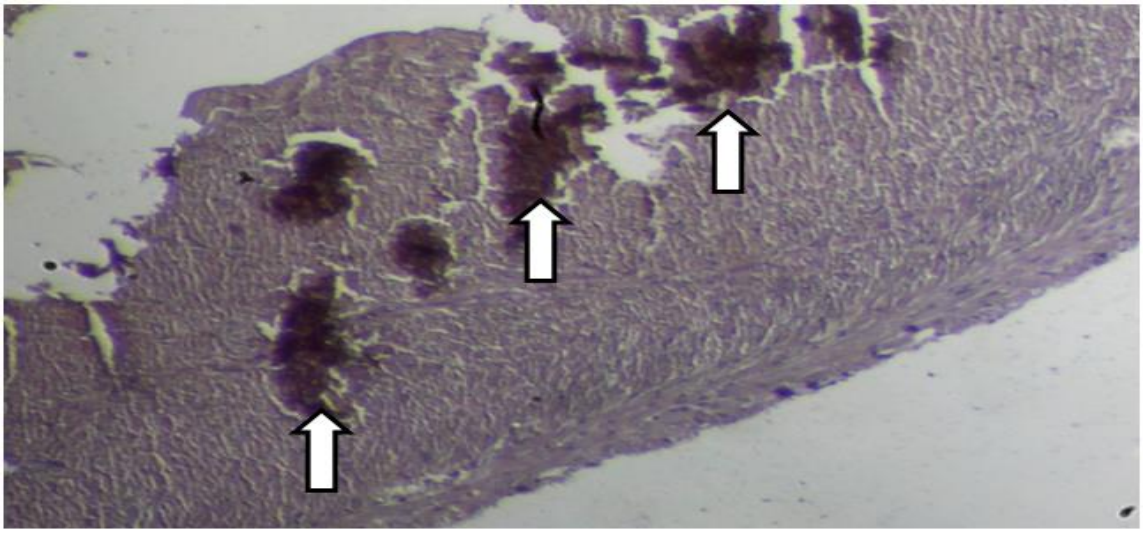
(Fig12): low dose LD group appeared ,less calcium immunhisto chemistry (white arrow), (alizarin stain 10x).



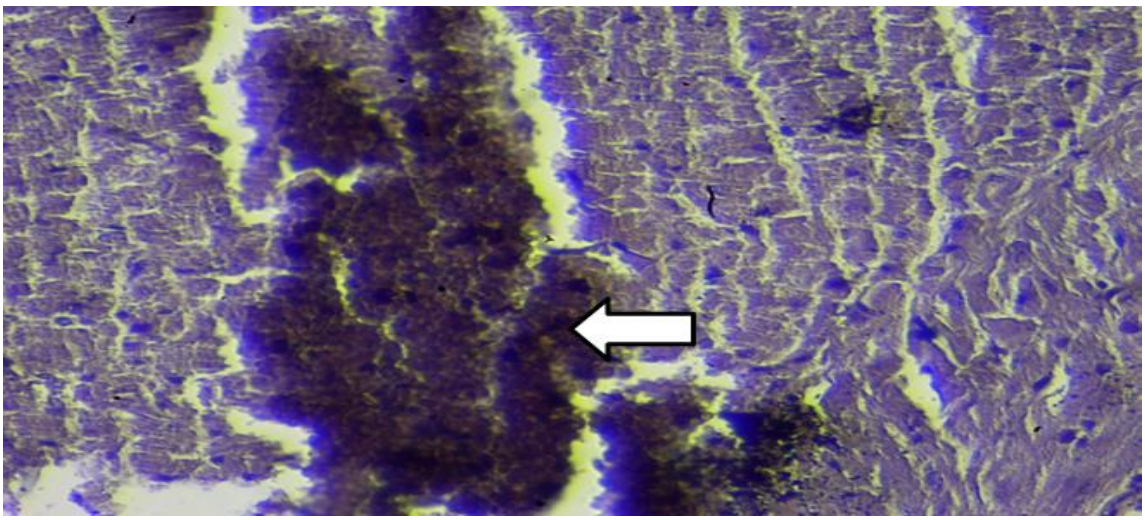
(Fig13): Low dose LD group appeared,less calcification(white arrow),(immunohistochemistry technique DAB & mayer hematoxy line).



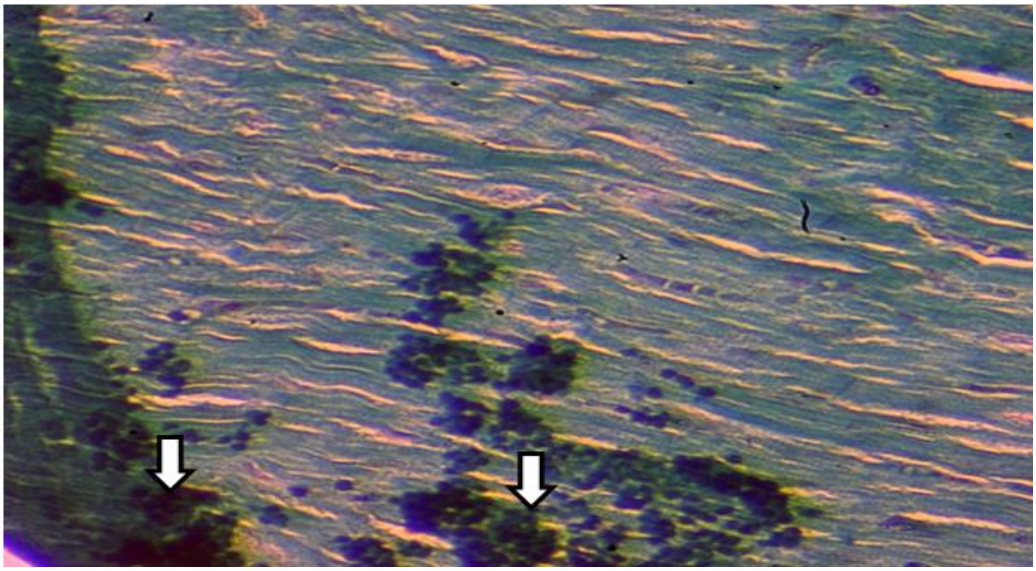
(Fig14): Intermediate dose ID appeared,calcification, (white arrow),(alizarin stain 40x).



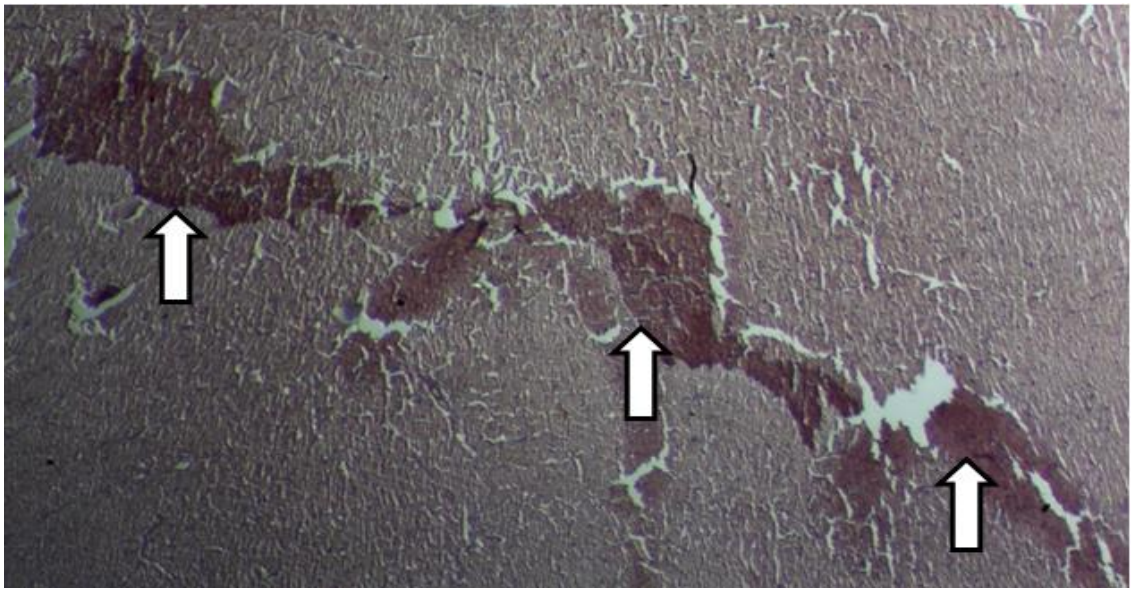
(Fig15):intermediate dose ID group appeared,increased calcification(whitearrow),(immunohistochemistry technique DAB & mayer hematoxy line).



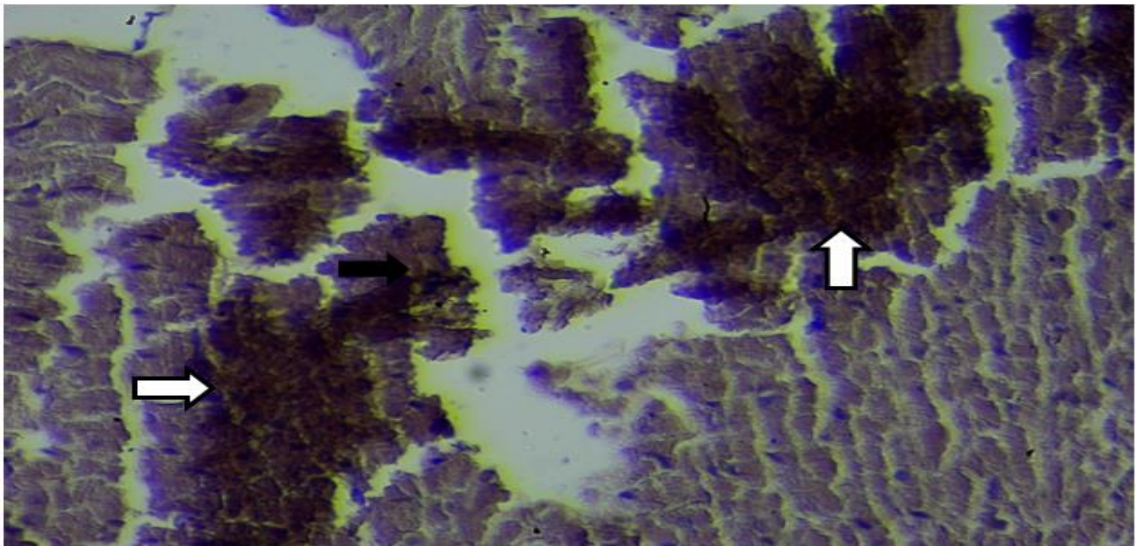
(Fig16) :intermedieate dose ID group appeared ,increased calcification (white arrow),(immuhistochemistry technique DAB & mayer hematoxy line 40x).



(Fig17): high dose HD group appeared, calcification (**(white arrow)**, (alizarin stain 40x).



(Fig18);high dose HD group appeared,very high calcification(white arrow),(immuhistochemistry technique DAB & Mayer Hematoxy line 10x).



(Fig19); high dose HD group appeared,very high calcification (whitearrow) ,(immunohistochemistry technique DAB & Mayer Hematoxy line 400x) .

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