ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Evaluation of Red Beet Root Activity Physiologically and Histologically in Males Rats

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Abstract

This research design to study the effect of cadmium chloride (Cdcl2) and red beet root juice on heart and kidney by measured the physiological parameters in the experimental male's rats for 30days. Twenty males rats were assigned to 4 groups each group contain 5 rats. First group was the control group (G1), the second group was cadmium chloride group (G2), third group was cadmium chloride &red beet juice (G3), than last group was red beet juice (G4). The Cd-exposure group (G2) obtained drinking a water daily containing cadmium chloride (CdCl2) in concentration of 2.0 mg Cd/L, while the G3 group treatment with red beet juice 2ml with 2ml of Cdcl2, while the last group G4 treatment with 2ml of red beet juice only. At the end of the experimental period, blood samples were collected to determine the changes of serum Total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), Total protein (TP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST). The results of the present study indicated that a significantly increased in serum levels of TC, TG, LDL_C, VLDL_C, ALT, AST, & significant decreased in the levels of HDL_C & TP in G2 group, while there are no significant changes of all parameters in G3&G4 group comparing with control group at Significant level ($p \leq 0.05$). Results registors also that are significantly decreased in some parameters contain T.C ,Triglycerides ,LDL_C,VLDL_C, ALT &AST concentration in G3 & G4 group ,while the results showed a significantly increased in parameters HDL_C & Total proteins comparing with G2 group that trated with cadmium chloride at Significant level ($p \leq 0.05$). In the end of this study we find that exposure to low Cd concentration can effected on the lipid profile & lipoprotein &liver enzymes by increased this parameters, while red beet root juice treatmenting decreased that parameters.

Keywords: *Cadmium chloride (Cdcl2), Total cholesterol(TC), Triglycerides(TG), High density lipoprotein cholesterol (HDL_C), Low-density lipoprotein cholesterol (LDL-C), Very low density lipoprotein cholesterol (VLDL-C), Total protein(TP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST).*

Introduction

Cadmium (Cd), it's one of the most important environmental and occupational toxic metals, its widely dispersed in the environment& can be found throughout the environment at low levels but can accumulate significantly through human activities such as mining and iron smelting [1].

In the environment, Cd is dangerous because humans consume both plants and animals that absorb Cd efficiently and concentrate it within their tissues [2, 3]. Depending on the dose of exposure, Cd can damage various organs include lung, liver, kidney, bones, testes rather than placenta [4]. Cd is implicated in the pathogenesis of several diseases, include cardiovascular disease (CVD) as a metabolic disorder, CVD may ensue from metabolic disorders such as diabetes & dyslipidemia, Cd exposure of animals and humans is known to impair myocardial function, possibly leading to heart failure [5]. Recent studies demonstrated that Cd stimulated free radical production, that's result oxidative deterioration of macromolecules [6].

The association between Cd exposure & occurrence of lipid abnormalities is well understood; however, few data have so far been published on the influence of chronic exposure to cadmium on the metabolism of lipids [7]. Alterations in lipid metabolism increase the cardiovascular risk, thus the

morbidity & mortality of diabetic patients [8]. More recently epidemiological studies linked increased cardiovascular disease mortality to low level exposure to Cd [9, 10]. Data from National Health & Nutrition Examination Survey (NHANES) led to the conclusion that increased Cd levels are associated with stroke & heart failure, peripheral arterial disease [11, 12], myocardial infarction and cerebrovascular diseases [13, 14].

Chronic toxic by cadmium lead to renal toxicity, Osteoporosis, cardiovascular disease &neural disorder [15].Cadmium may be accumulation in kidney without apparel its toxicity by formation a complex called Metallothionine, this complex decreased oxidative stress by lowering free radicals produced by Cadmium toxic, the risk of Cadmium contain its role of inhibition of enzymatic process & phophorelation in mitochondria, in addition to increased of lipid peroxidation [16].

Proteins and antioxidant enzymes becomes inactive if cadmium presence, that lead to obstruction antioxidant roles [17].In human & animal body, reactive oxygen species (ROS) can be neutralized by antioxidant defense systems including antioxidant enzymes & antioxidant compounds [18]. A number of possible mechanisms have been proposed with antioxidant nutrients through lowering oxidative stress, focused on natural antioxidants in fruits and vegetables [19]. Red beetroot (*Beta vulgaris* L.) growning in many countries worldwide & regularly consumed as part of the normal diet, and useful in manufacturing as a food colouring [20, 21].

It's a good source of natural antioxidants such as betalains, flavonoids, polyphenols, vitamins, & folic acid, total phenol content in red beet is the highest among 23 vegetables that have been studied, Betalains comprise betacyanins &betazanthins [22]. In red beet ROS induced a synthesis of betacyanin which could act as ROS scavengers, limiting damage caused by bacterial infection & wounding [23].

Betanin, the main betacyanin, showed strong antioxidant effects in lipid peroxidation of membranes and inhibition of LDL oxidation, in which the oxidation rate was better than that by catechin [24]. Recent interest about beetroot discovery that sources of dietary nitrate may have important implications for managing cardiovascular health [25]. There are many studies show that beetroot juice supplement significantly reduce systolic & diastolic blood pressure [26].Beetroot used as a popular folk remedy for liver & kidney diseases, for stimulation of immune & hematopoietic systems, as a special diet in the treatment of cancer [24].

Materials &Methods

Red beet root juice was prepared in a household juice extractor daily, and then put it in a glass container away of sunlight. The animals (20 male rats) breeding in the animal house, Tikrit university, Collage of Veterinary Medicine. The animals age proximatly 9weeks and there weighing are 160±15 g. the rats were kept in their own cages, maintained in a room with 22±2 °C, 12 h light/dark cycle, and were fed standard rat chow and water ad libitum.

The experimental contain ingestion control group water without any addition substance, while the group deal with cadmium chloride ingestion the Cdcl2 in adosge mainly 2ml every day with water, the group that deal with red beet root juice ingestion 2 ml of juice daily& the last group ingestion 2ml of Cdcl2 (in morning) with 2ml of beet root juice (in the evening) daily for 30 days.

Blood samples collected from experimental animals after the experimental period finishing, samples were collected form all four groups, each group contain (5) animals, the first group G1 was the control group, while the second group G2 was the cadmium chloride Cdcl2, the third group G3 was cadmium chloride with beetroot juice and the last group G4 was beetroot juice group.

The experimental began in January 2018 to February 2018, biochemical tests measured including; Total cholesterol(TC), Triglycerides(TG), High density lipoprotein_C(HDL_C),Total protein(TP), Alanine aminotransferase (ALT) & Aspartate aminotransferase (AST).All parameters masured by using commercial Kits from BIOLABO FRANCE, SA companies sequentially.

Statistical analysis used SPSS system in level ($p \leq 0.05$).The tissues for histological examination were fixed in 10% formalin since 24 hours, dehydration by ethyl alcohol in increasing concentrations(70%, 80%, 95%, 100% and100%), clearing with xylene and then embedded with paraffin. When analyzed,

all paraffin-embedded tissue was sectioned at 5 µm, and stained with Hematoxylin and eosin. These specimens were examined under a light microscope at 40X magnification power. Corresponding digital images were captured for later analysis [20].

Physiological Results

The Figure (1) showed that the Total cholesterol concentration(the column on the left) significantly increased in G2group (124±4 mg/100ml while there is no any significantly changes in cholesterol concentration in G3 group (86±3 mg/100ml) &G4 group (87±2 mg/100ml) compared with the G1 group (96±3 mg/100ml) at Significant level ($p \leq 0.05$) .while we comparing the cholesterol concentration in G3 group (86±3 mg/100ml) which the cholesterol level in G2 group (124±4 mg/100ml) found that there is a significantly decreased in cholesterol levels while there is no significantly changes in G4 group(87±2 100ml) when we comparing with G2 group(124±4 mg/100ml).

The middle column refer to the Triglycerides results that showed significantly increased at G2 group (168±4mg/100ml),while there is no any significants changes in triglycerides levels at G3 group (94± 24mg/100ml) & G4 group (92 ± **3** mg/100ml) comparing with control group (G1) (87±4mg/100ml).while the results show that triglycerides levels significantly decreased in G3 group (94±**2**mg/100ml) and G4 group (92±**3** mg/100ml) comparing with G2 group (168±44mg/100ml). Column on the right pointing to LDL_C results, showed that the Low deseity lipoprotein significantly increased in G2 group (87± 4mg/100ml),while G3 group (25±3mg/100ml) and G4 group (28±2mg/100ml) register significantly decreased comparing with the control group(G1) (33±5 mg/100ml).on another hand the data that obtained after masured LDL_C levels pointed that there is significantly decreased in LDL_C levels in G3 group (25± 3mg/100ml) and G4 group (28±2mg/100ml) comparing with G2 group (87± 4mg/100ml).

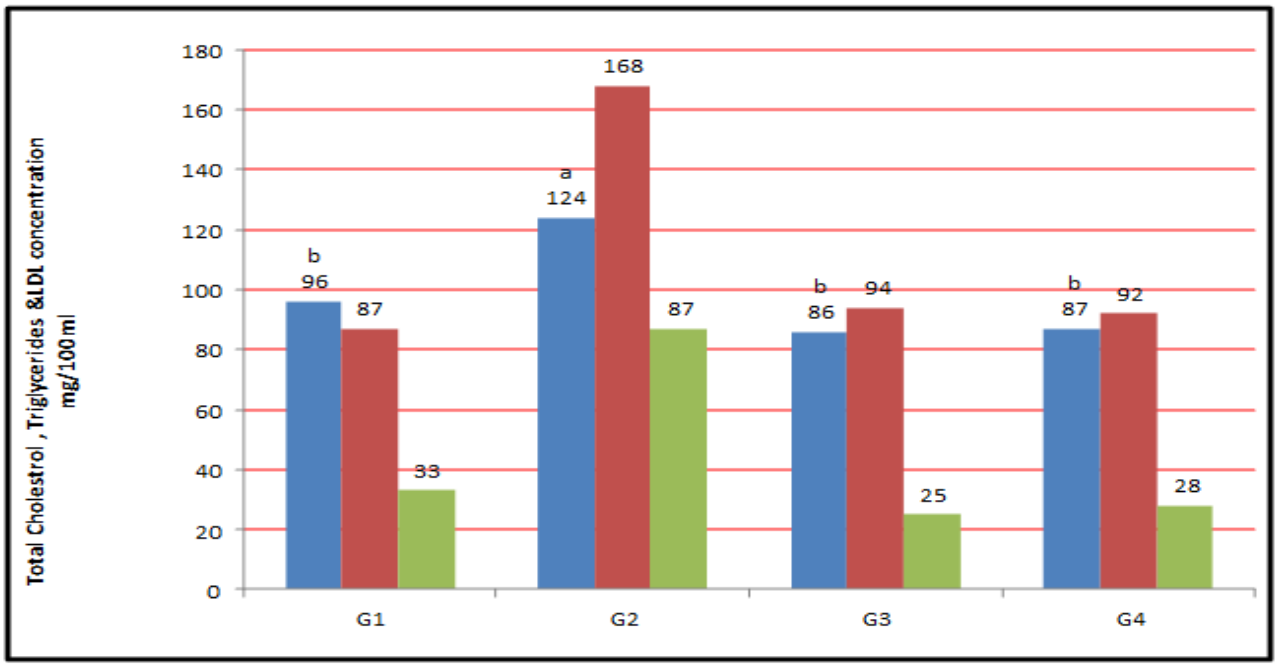

Figure 1: Mean level of Total cholesterol, Triglycerides &LDL concentration in Labrotary male rats compared to controls group.*left column (blue) T.C, middle column (red) T.G, right column (green) is LDL

The Figure (2) appeared that a very low density lipoprotein concentration (column on the left) significantly increased in G2 group (34±1 mg/100ml), while G3 group (19±1 mg/100ml) and G4 group (18±1 mg/100ml) do not find any significant changes comparing with G1 group (17±1 mg/100ml). On another hand the measured samples pointed that G3 group (19±1 mg/100ml) and G4 group (18±1mg/100ml) registered significantly decreased in VLDL_C levels comparing with the G2 group (34±1 mg/100ml). while column on the right refers to HDL_C result that appeared significant decreased registered in G2 group (37±3 mg/100ml),while there is no significant changes in G3 group (61±3 mg/100ml) and G4 group (59±3 mg/100ml) comparing with control group (63±2 mg/100ml) .if looking at the results in G3 group (61±3 mg/100ml) and G4 group (59±3 mg/100ml) a significant increased appeared comparing with G2 group (37±3 mg/100ml).

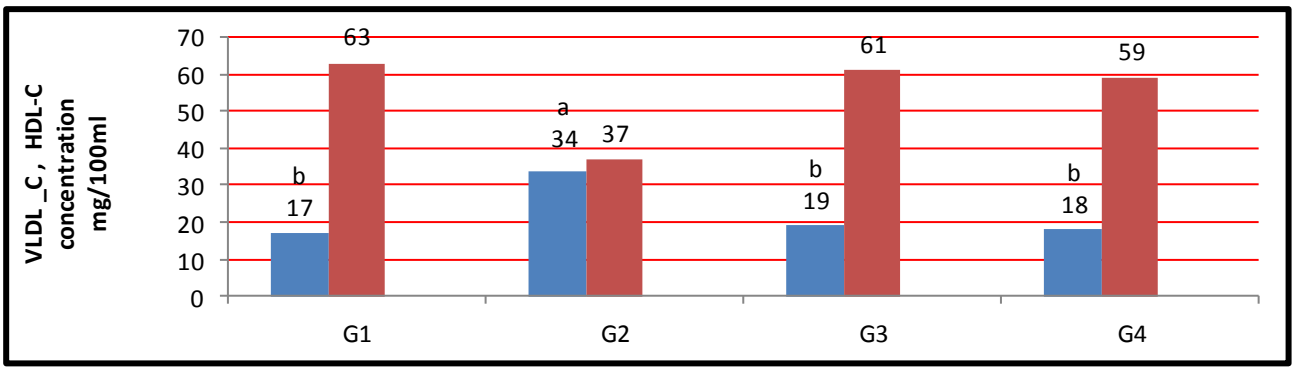

Figure 2: Mean level of very Low density lipoprotein, High density lipoprotein concentration in Labrotary male rats compared to controls group.*left column (blue) VLDL_C, right column (red) HDL

In Figure (3) the results showed that there is significant decreased in TP levels in G2 group (4.1± 0.6 gm/dl) while there is no significant changes in G3 group (5.6± 0.5 gm/dl) and G4 group (6.1± 0.3 gm/dl) comparing with the control group (5.8 ± 0.4 gm/dl). The data that registered showed significant increased in TP concentration in G3 group (5.6± 0.5 gm/dl) and G4 group (6.1± 0.3 gm/dl) comparing with the G2 group (4.1± 0.6 gm/dl).

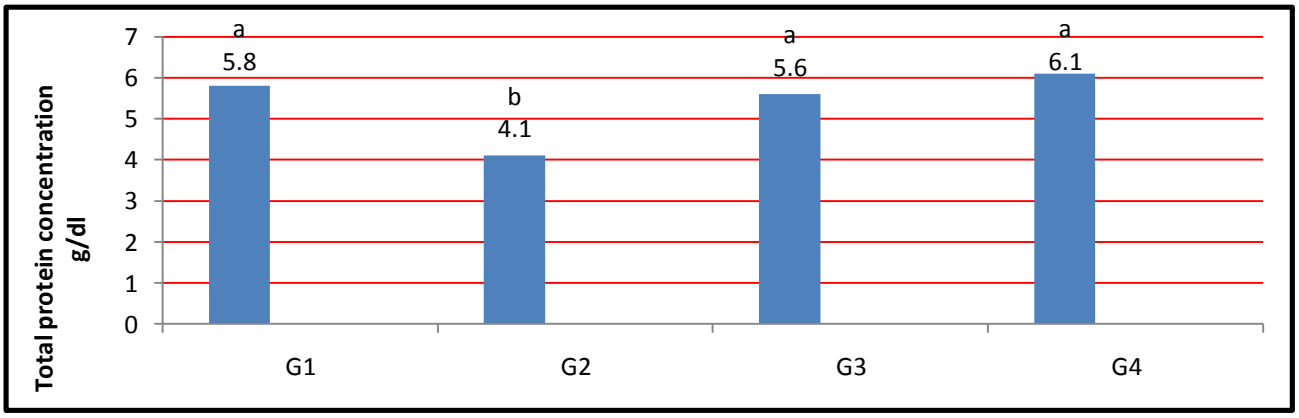

Figure 3: Mean level of Total protein concentration in Laboratory male rats compared to controls group

The Figure (4) show the results that conjugated with ALT(column on the left) concentration in serum, A significantly increased registered in G2 group (58±2mlU/ml),while G3 group (37±1mlU/ml) and G4 group(32±2mlU/ml) don't showed any significant changes comparing with control group (33±2 ml U/ml). When comparing the results of ALT levels in G3 group (37±1mlU/ml) and G4 group (32±2mlU/ml) with G2 group (58±2mlU/ml) noted that there is significantly decreased appeared.

While the results of AST(column on the right) parameters refers to finding significantly increased in G2 group (62±1 ml U/ml), while there is significantly decreased registered in G3 group (39 ±2 ml U/ml) and G4 group (39±3 ml U/ml) comparing with control group (G1) (38±2 ml U/ml). On another hand the results showed significantly decreased in G3 group (39 ±2 ml U/ml) and G4 group (39±3 ml U/ml) comparing with G2 group (62±1 ml U/ml).

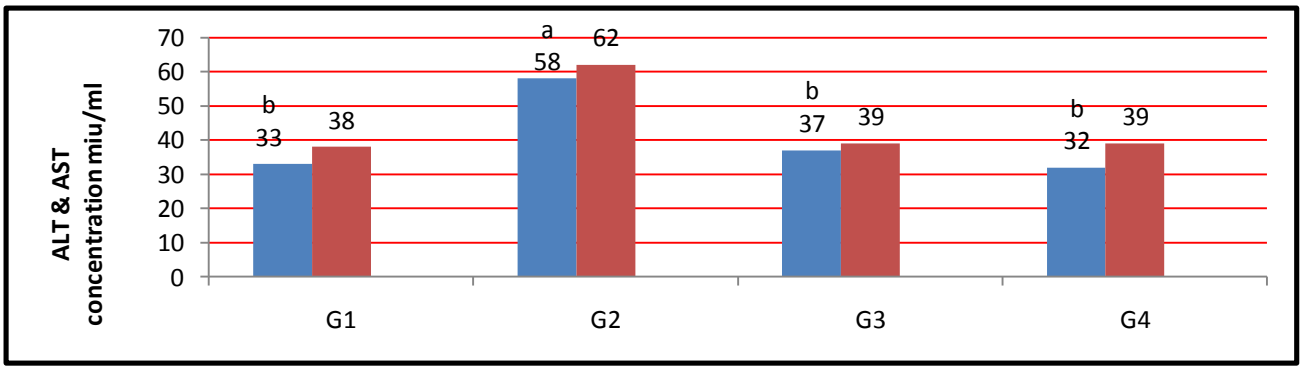

Figure 4: Mean level of ALT& AST concentration in Labrotary male rats compared to controls group *left column (blue) ALT, right column (red) AST

Histological Results

Histological examination of liver tissue showed congestion, fibrosis around the central vein, and necrosis within the liver tissue in G2 compared with the control group Fig (6, 7), while G3 showed that the tissue returned to the semi-normal state, with some legions, a cellular degeneration Fig (8), whereas G4 showed normal liver tissue Compare with control group Fig (9).

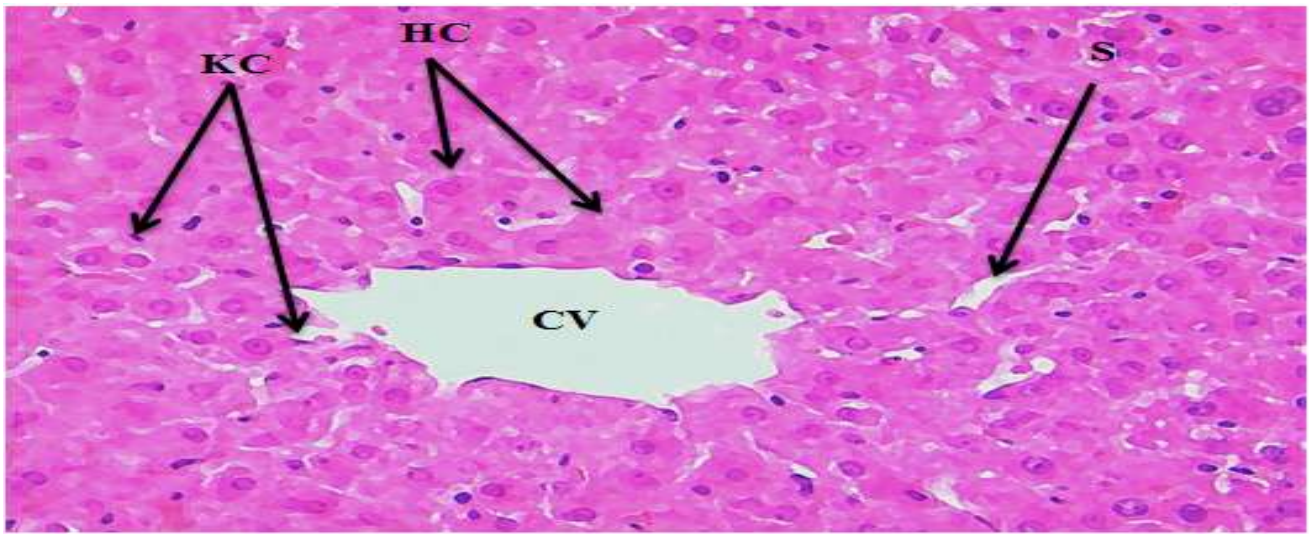

Fig 5: liver section G1 control group shows the central vein (CV) and normal hepatocytes (HC) as hepatic cords and K offer cells (KC) and possibility shows of sinusoid (S) H & E 400X

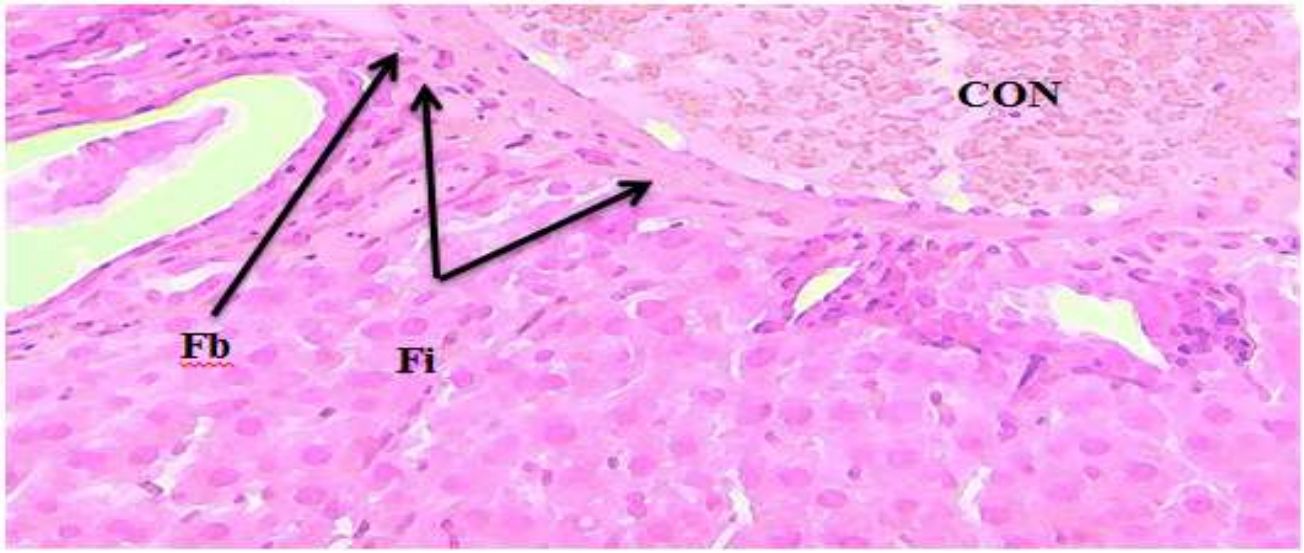

Fig 6: Liver section G2 shows congestion in the central vein (CON), the appearance of the fibrosis around the central vein (Fi) and fibroblast (Fb) H & E 400X

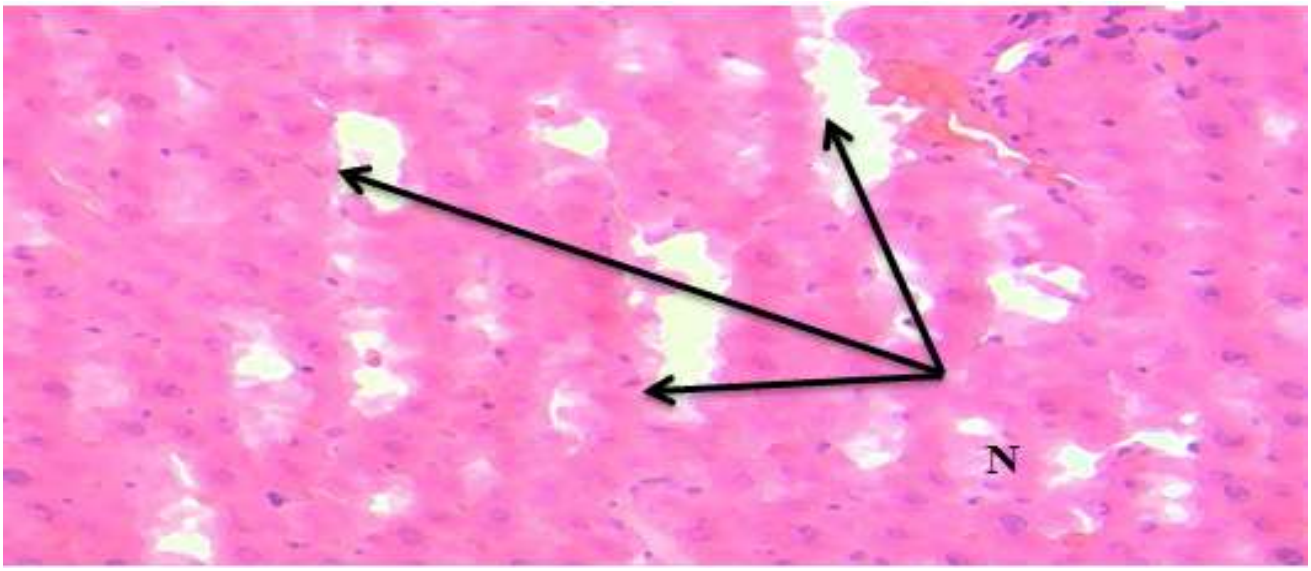

Fig 7: liver section G2 shows necrosis within the liver tissue (N) H & E 400X

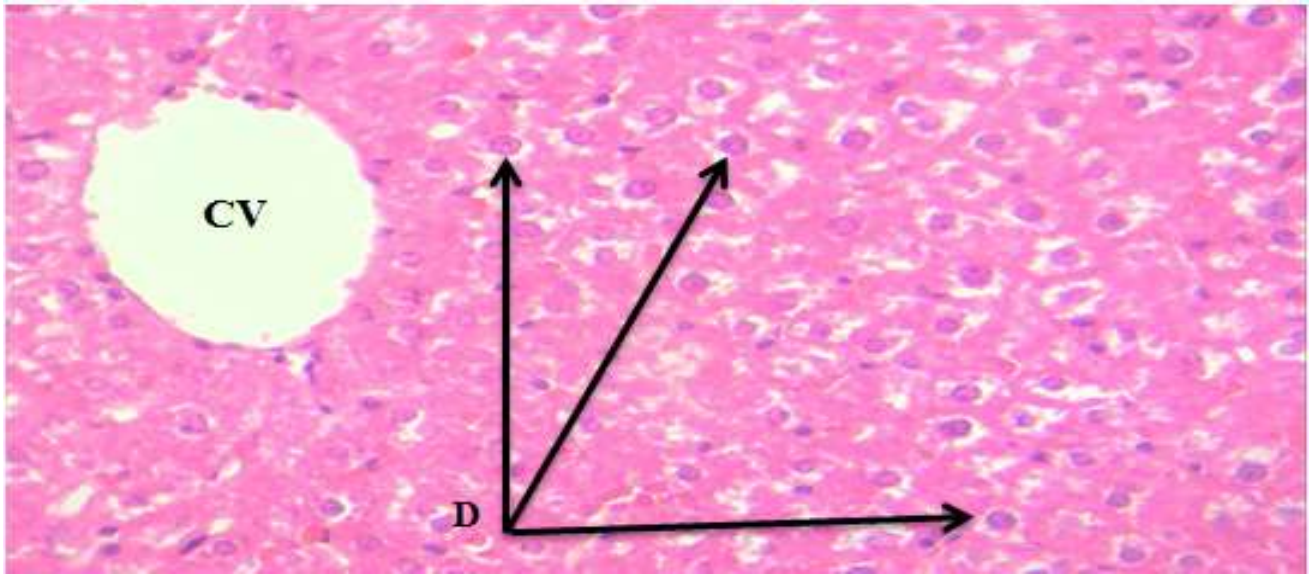

Fig 8: liver section G3 shows the central vein (CV) as normal and degeneration of some hepatocytes (D) within liver tissue H & E 400X

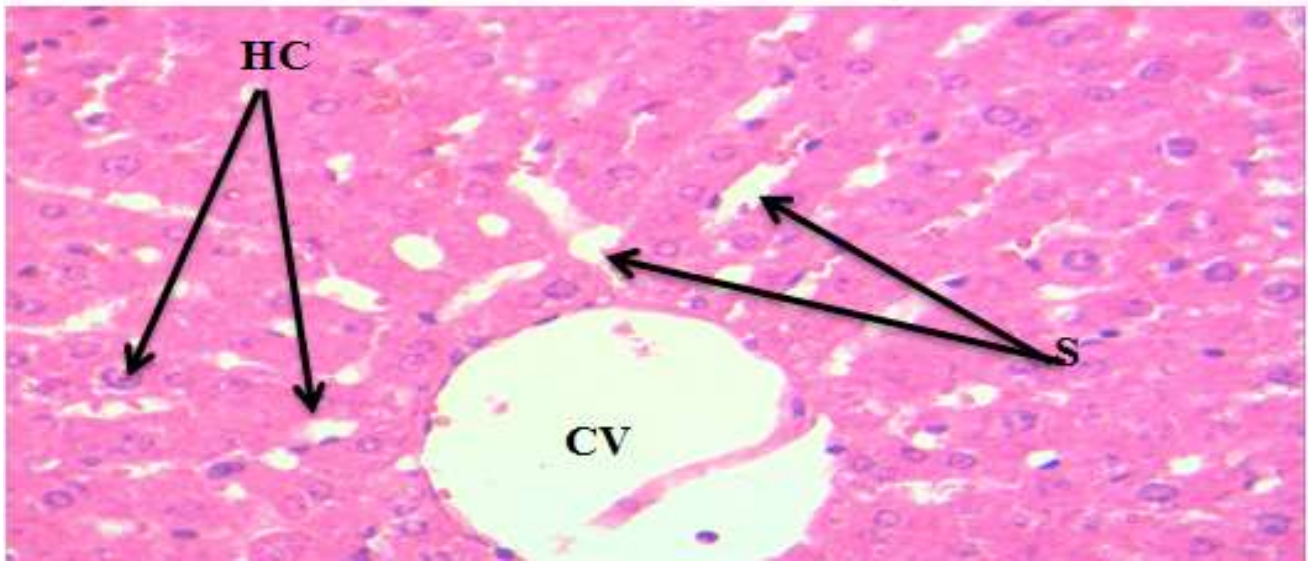

Fig 9: liver section G4 shows the central vein (CV), Hepatocytes (HC) and sinusoid (S) H & E 400X

Histological examination of Heart tissue showed dissociative of some cardiac muscles and necrosis within the heart tissue in G2 compared with the control group Fig (7), while G3 showed that the tissue returned to the semi-normal state with little disintegration between myofibers Fig (12), either G4 the tissue appearance normal state Compare with control group Fig (13).

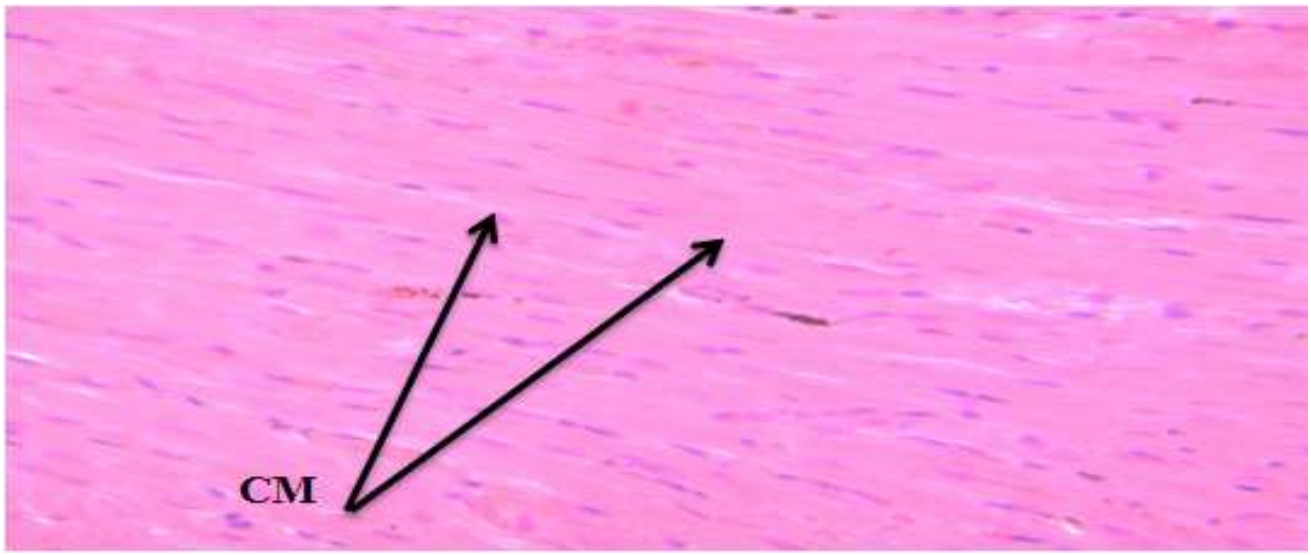

Fig 10: Heart section G1 control group shows Cardiac muscle (CM)H & E 400X

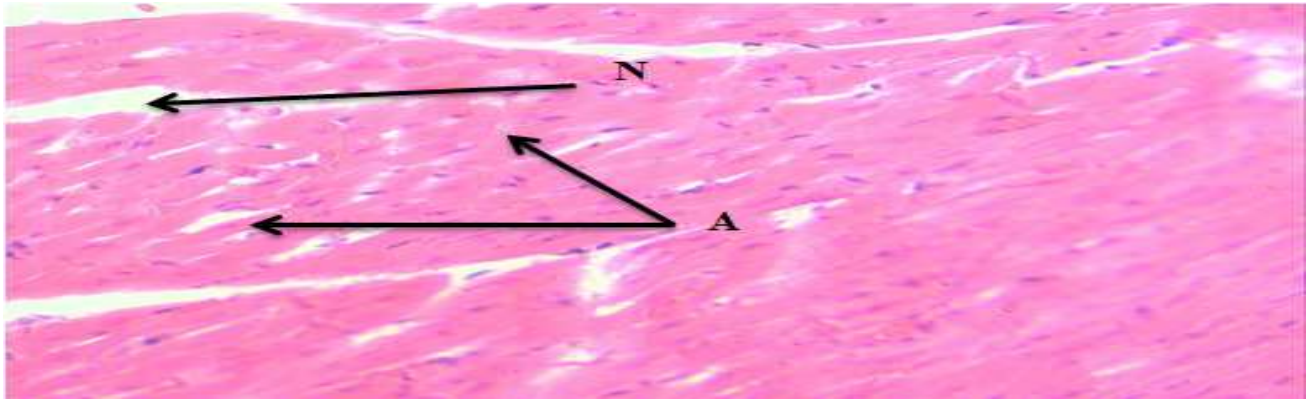

Fig 11: Heart section G2 shows the dissociative of some cardiac muscles (A) with necrosis (N) within the heart tissue H & E 400X

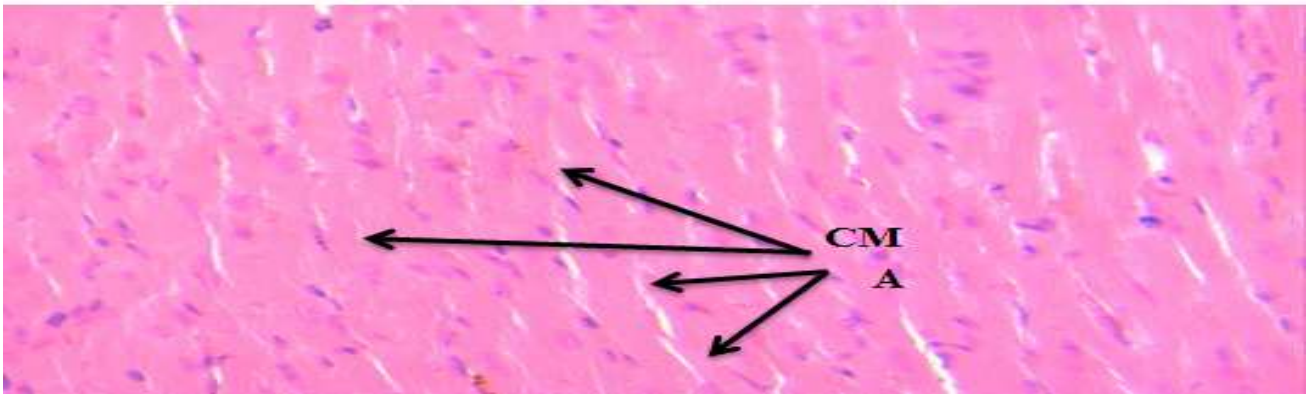

Fig 12: Heart section G3 shows the cardiac muscle (CM) and dissociative some cardiac muscles (A) H & E 400X

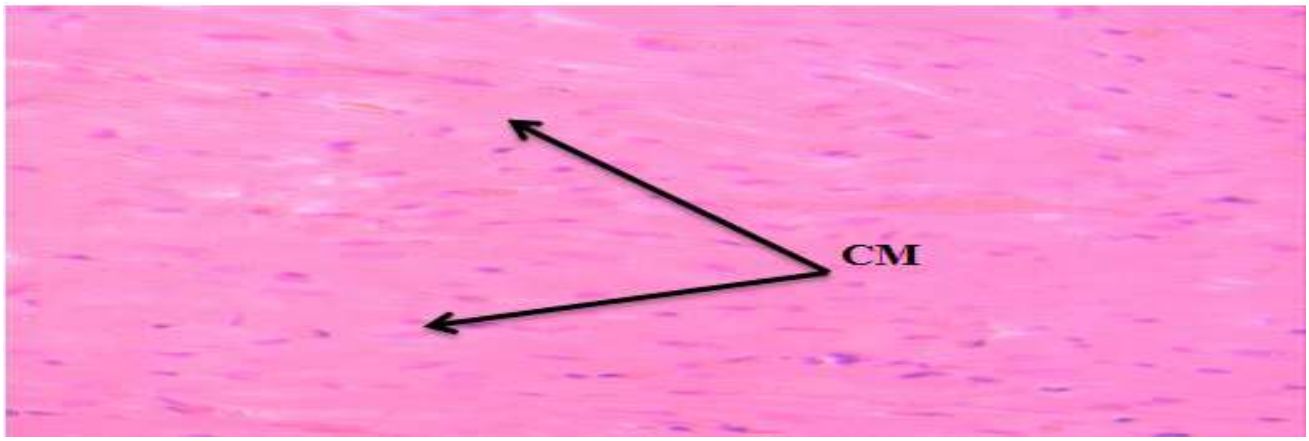

Fig 13: Heart section G4 shows the cardiac muscles (CM) as normal, H & E 400X

Histological examination of coronary artery shows the irregularity of smooth muscles within the wall of the artery and Vaculation in some layers of muscles and adventitia layer in G2 compared with the control group Fig (11), while G3 showed that the tissue returned to the semi-normal state with Vaculation in some layers of muscles Fig (12), either G4 the tissue appearance normal state Compare with control group Fig (13).

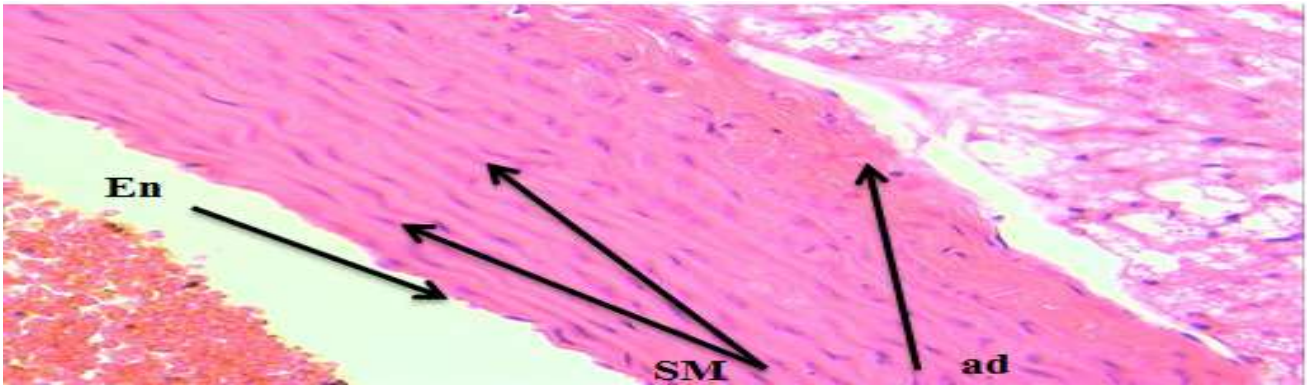

Fig 14: A section in the coronary artery G1 the control group shows the Endothelium the artery (En), smooth muscles (SM) and adventitia layer H & E 400X

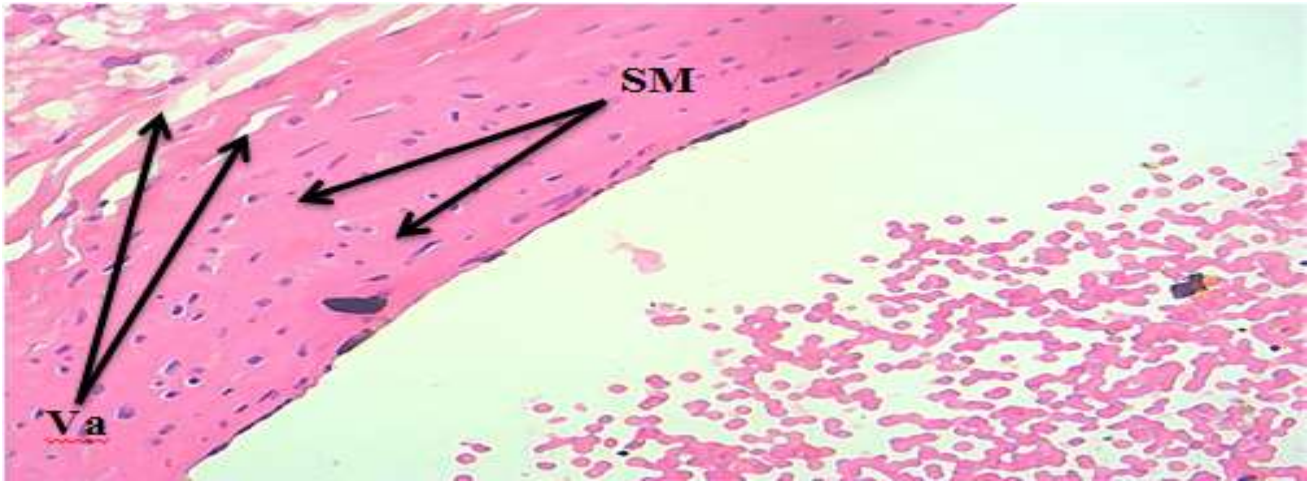

Fig 15: A Section in the coronary artery G2 shows the irregularity of smooth muscles (SM) within the wall of the artery and Vaculation (Va) in some layers of muscle and adventitia layer (ad) H & E 400X

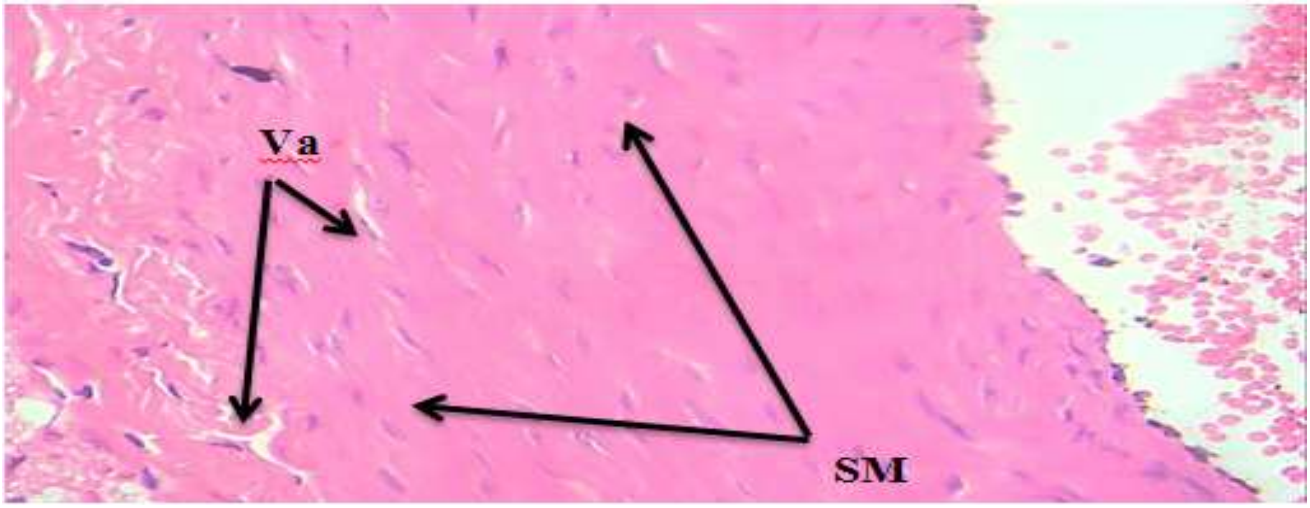

Fig 16: A section in the coronary artery G3 shows the smooth muscles (SM) within the wall of the artery with Vaculation (Va) in some muscle layers H & E 400X

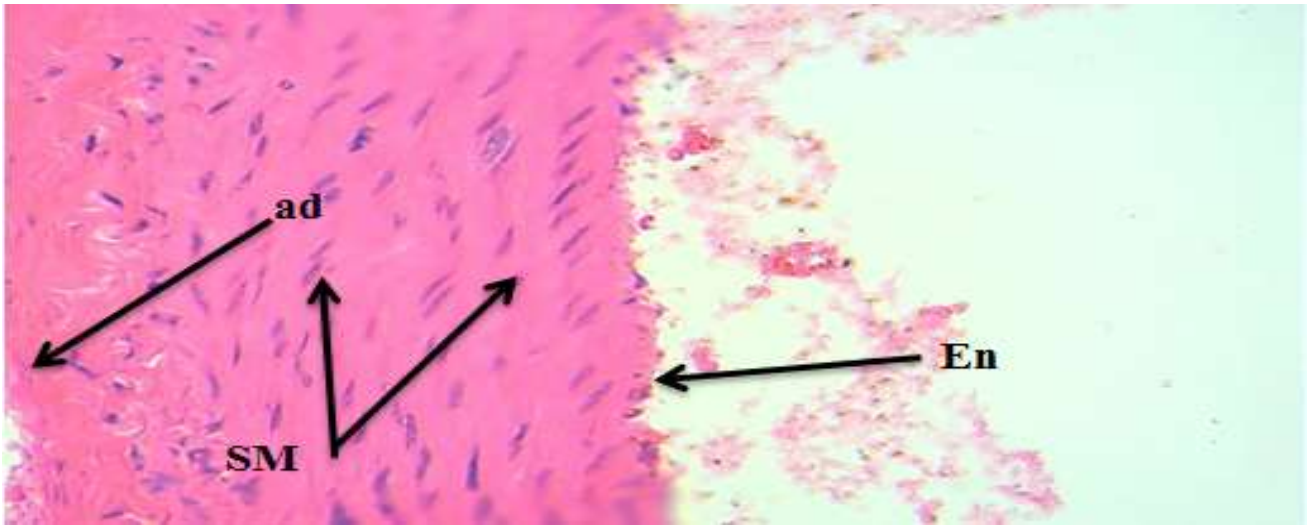

Fig 17: A section in the coronary artery G4 shows the endothelial layer of the artery (En) and smooth muscles (SM) within the wall and adventitia layer (ad) H & E 400X

Discussion

Results that obtained in this study showed a significantly increased in some parameters that masured including, Total cholesterol, Triglycerides, LDL_C, VLDL_ C,ALT & AST concentration, while the results registers significantly decreased in HDL_C & Total proteins concentration in Group that treatment with cadmium chloride (Cdcl2) comparing to control group at Significant level ($p \leq 0.05$). This result agree with research results that reported by [27] they finding that exposure to Cdcl2 leads to depletion of glutathione & protein-bound sulfhydryl groups that resulting in enhanced production of ROS such as, hydrogen peroxide, superoxide ion & hydroxyl radicals.

Cd-increased ROS to produced lipid peroxidation& results in DNA damage [28]. Oxidative stress is caused by an imbalance between production ROS & neutralization by antioxidant defense system [29]. Normally in metabolic processes the cells continuously produce ROS & cells can protect themselves against ROS by defense mechanism of antioxidants & antioxidant enzymes [30].This results agreement with results that obtained by [31] which suggested that oxidative stress result from Cd increased the production of ROS that lead to consumption Glutathione & increased MDA. While the results do not registers any significant changes in G3 & G4 group comparing with the control group.

In this study the results that obtained also showed a significantly decreased in some parameters like T.C, Triglycerides, LDL_C, VLDL_C, ALT & AST concentration in G3 & G4 group, while the results registered a significantly increased in parameters like HDL_C & Total proteins comparing with G2 group that trated with cadmium chloride Significant level ($p \leq 0.05$).

This results agree with the results that obtained by [32] which find that a Juice of red beet is a rich source of bioactive components such as: Betalaines, a water soluble antioxidants, free radical scavengers, anti-inflammatory & chemo-preventive activity *in vitro* and *in vivo*. Betalain found in beetroot in about 300-600 mg·kg-1 & it wsa the most effective inhibitor of lipid peroxidation its inhibited LDL oxidation catalyzed by H2O2 activated metmyoglobin [33]. This research results similar to results that obtained by [35] which reported that saponins contain in some medicinal plants reduced the triglycerides & cholesterol levels in rats.

Also a juice contain ß carotene, α tocopherol &polyphenols, these compounds contain a highly effective antioxidant properties, on another hand this juice source of vitamin C (antioxidant) that helps your immune system & protect cells from damaging by free radicals [38]. Red beet juice reduced lipid peroxidation in blood & the major organs including liver, heart, & kidney, the juice showed a positive anti-oxidative effect in experimental mice [39].When ew have oxidative stress the oxidative modifications of LDL happening; which are associated with an incidence of atherosclerosis [40, 41].

Resulted that betanin can bind LDLs in vivo plasma & increase their resistance to copper-induced oxidation also ß-carotene & α-tocopherol are effective chain-breaking antioxidants in tissues &LDLs & appeared to be protective nutrients against the development of Cardio heart diseases [33, 34].

Obtained data reported that flavonoids considered as active principles in some medicinal plants & natural products with benefit effect on human health; saponins has been also suggested that reduce heart diseases, red beet juice treatment decreased the levels of T.C, triglycerides &also increased levels of HDLC. Lipids lowering potential of beet root may be come from flavonoids & saponins (the main constituents of beet root extract), this results findings accordance with the earlier studies demonstrating about the effect of flavonoids on cholesterol metabolism [35, 36, 37].

Reported about endothelial dysfunction which that considered as a risk factor for several cardiovascular disorders and has been implicated in the pathogenesis of hypertension and atherosclerosis therefore, beetroot juice isa natural NO donor, its description as a nutritional way to preserve or restore endothelial function.

Beet root was a source of nitrate & increasing *in vivo* nitric oxide (NO) which preventing & managementing pathologies associated with diminished NO bioavailability & notably hypertension [32]. Cd hepatotoxicity is closely related to inflammation, since after acute exposure, the damaged liver is often infiltrated by polymorphonuclear neutrophils (PMN), which, in addition to Kupffer cells, contribute to the hepatotoxicity by enhancing inflammatory mediators and promoting necrosis [42].

Previous studies demonstrate that activated Kupffer cells release a number of inflammatory mediators that subsequently enhance the expression of adhesion molecules that initiate a cascade of cellular and humoral responses leading to inflammation and secondary liver damage during Cd-induced hepatotoxicity [43]. When Kupffer cells are selectively destroyed, inhibited or suppressed, the hepatotoxicity of Cd is dramatically reduced. Although the mechanism by which Kupffer cells contribute to Cd hepatotoxicity

is not fully understood, it is known that activated Kupffer cells, release a variety of cytotoxic mediators that can directly damage hepatocytes. These mediators include reactive oxygen species (ROS). The initiation and progression of Cd-induced hepatocellular injury involves multiple cell types. EC are the first liver cells that come into contact with free Cd and are clearly involved in the early stages of Cd-induced liver injury. In the current study, the endothelial cells of the F344 were damaged more extensively than the EC of the SD rats. Once damaged, these cells obstruct the hepatic microcirculation, which leads to localized ischemia. This occlusion of hepatic inflow could lead to hypoxic conditions that promote hepatocellular necrosis.

The liver responds to these conditions by synthesizing repair proteins. In fact, hsp72 has been shown to be produced early after total hepatic inflow occlusion [44]. The earlier synthesis of MT and hsp72 may contribute to the decreased sensitivity of the SD rat to Cd-induced hepatotoxicity, but at least with regard to MT, this does not appear to be a primary factor. The hepatocytes and EC respond to hepatocellular injury by releasing chemokines and cytokines that result in inflammation orchestrated in part by KC. Cadmium could potentially replace and interact with the homeostasis of several essential metals, such as zinc, iron and calcium.[45] It has been reported that cadmium could have cardiotoxic effects, which might explain the association between cadmium and HF. Chronic exposure to cadmium causes degenerative changes to myocardial cells in rats, and cardiac depressant effects following low dose cadmium exposure have been reported in mice [46, 47].

Cadmium may affect the tissue structure and integrity of the heart muscle by oxidative stress and increased reactive oxygen species production or by DNA methylation [48, 49]. Cadmium also affects the cardiac conduction system by interfering with calcium-mediated physiological and biochemical processes [50].Blockade of L-type calcium channels and altered outward potassium current in ventricular myocytes were also observed in vivo [51, 52].

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