

# Can raisins ameliorate the hypercholesterolaemia-induced cardiac affection?

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*Raisins were investigated for their protective role on cardiac muscle both biochemically and histopathologically in high cholesterol diet (HCD)-fed rats. Wister male rats were randomly divided into four groups (n = 10): control, raisin-fed, HCD-fed and HCD-raisin fed group. Animals were anaesthetized after 13 weeks. Hearts were dissected and processed for histopathological examination. Raisins administration with HCD significantly decreased the animals' blood glucose, insulin, cholesterol, triglycerides, and low density lipoprotein levels; while increased their high density lipoprotein levels compared with rats fed HCD alone. They also decreased cardiomyocytes' degeneration, cellular infiltration, haemorrhages and blood vessels affection. Raisins reduced fibrosis by decreasing the immuno-expression of alpha smooth muscle actin marker, whereas they significantly increased the immuno-expression of endothelial nitric oxide synthase. Raisins showed a cardioprotective effect and were able to alleviate the biochemical and the histopathological changes induced by the HCD. Consumption of raisins or their pharmaceutical product should be recommended specially for those eating a high-fat diet. (Folia Morphol 2015; 74, 1: 106–117)*

**Key words:** hypercholesterolaemia, raisin, cardiomyocytes, immunohistochemistry, actin, eNOS, cardiac muscle protection

## INTRODUCTION

Coronary heart disease (CHD) is a common cause of death. The main significant clinical risk factors for CHD are elevation of total serum cholesterol, low-density lipoprotein (LDL) and drop in high-density lipoprotein (HDL) level. Prevention of CHD has been given a high priority in healthcare through dietary adjustment [4].

Community awareness of dietary sources that might efficiently decrease plasma cholesterol level has increased due to established relationship between elevated plasma LDL concentrations and increased risk of heart diseases [20]. Hyperlipidaemia has a direct effect on the myocardium and the development of

atherosclerosis [19, 38]. High cholesterol diet is responsible for the accumulation of intracellular lipids in cardiomyocytes and several alterations in the structural and functional properties of the myocardium [46].

Desmin, a component of the muscle intermediate filaments, is concentrated at the intercalated discs [1]. It plays a vital role in the maturation, maintenance and recovery of skeletal and cardiac muscle fibres (CMFs) through the formation of an interlinking scaffold around myofibrils connected to the sarcolemma and nuclear membrane [26]. Although the normal adult cardiac myocytes don't show alpha smooth muscle actin (ASMA) expression, Bassiouny et al. [7], Borgers et al. [8] and Ausma et al. [3] reported that

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cardiac myocytes express ASMA in certain cardiac affections. Nitric oxide (NO) plays an important role in controlling vascular tone, mean arterial pressure and heart rate [15]. Cardiac myocytes express endothelial nitric oxide synthase (eNOS) where its activity is regulated by the contractile state of the heart and intracellular calcium activity [22, 44]. In addition, both the endothelial cells and NO have been involved in modulating the cardiovascular function [18, 53].

Various studies promoted the utilisation of fruits due to the presence of dietary fibres and antioxidant substances for the reduction of cardiovascular disease risk development [50, 52]. Sun-dried raisins are very nutritious; they contain dietary fibre, potassium and health-promoting phytochemicals. They also retain the minerals and antioxidants of the grape, including its resveratrol and polyphenols, which are potent antioxidants and may protect cell constituents against oxidative damage. Sun-drying enhances the antioxidant content of raisins [61]. Raisins chelate metals, modulate enzymatic activity, inhibit cellular proliferation and alter signal transduction pathways [35].

The health benefits of grapes have been studied and publicised extensively, whereas dried grapes (raisins) have received comparatively less attention. The present study aims to investigate the possible ameliorating effects of raisins on the cardiac muscle of adult rats fed high-cholesterol diet (HCD).

## MATERIALS AND METHODS

### Animals

Forty male albino Wister rats were purchased from the animal house of King Fahd Medical Research Centre (KFMRC), King Abdulaziz University (KAU), Jeddah, Saudi Arabia. All rats used in the experiment weighed 225–285 g and were 12–16 weeks old. Rats were housed at 22–24°C and 55% relative humidity, with 12:12 (light:dark) photoperiod. Rats were fed a commercial diet and water *ad libitum*. All procedures were conducted in accordance with guidelines and protocols reviewed and approved by the ethical committee for animal care and use in King Fahd Medical Research Centre (KFMRC), KAU, Jeddah, Saudi Arabia, which are in accordance with the guidelines of the Canadian Council on Animal Care. Animals were acclimatised for 1 week prior to experimental procedures. Animals were split randomly into four equal groups (n = 10). Group I served as control and received only saline through a nasogastric tube; group II

represented the positive control and received 0.5 g of raisin homogenate through a nasogastric tube daily for 13 weeks, according to Spiller et al. [51], after adjusting the human dose for a rat according to Paget and Barnes [40]; group III was the induction group where animals were fed a HCD, consisting of 95% rat chow supplemented with 4% cholesterol and 1% cholic acid, according to Thiruchenduran et al. [54], for 13 weeks; and group IV represented the treated group, where the rats were fed a HCD along with raisin homogenate at the same dose. Food and water consumption, and body weight were measured at the start of the experiment, after 6 weeks, and by the end of the experiment. Weight gain and food efficacy were calculated.

### Preparation of raisins

Raisins, imported from Yemen, were purchased from nut stores in Jeddah. They were verified by a senior botanist from Biology Department, Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. The chemical composition of 100 g of raisins was performed in the Analytical Chemistry Unit as shown in Table 1. Raisins were homogenised with

**Table 1.** Chemical composition of raisins used in the study

Element	Amount
Moist [g/100 g]	4.92
Protein [g/100 g]	2.40
Carbohydrates [g/100 g]	75.08
Fibres [g/100 g]	5.90
Mono-unsaturated fatty acids [g/100 g]	1.58
Poly-unsaturated fatty acids [g/100 g]	–
Cholesterol [g/100 g]	0.092
Mineral elements [g/100 g]	2.72
Iron [g/100g]	0.002
Potassium [g/100 g]	0.73
Calcium [g/100 g]	0.04
Magnesium [g/100 g]	0.012
Phosphorus [g/100 g]	0.071
Vitamin E [g/100 g]	0.09
Vitamin B1 [mg/100 g]	1.099
Vitamin B2 [mg/100 g]	1.79
Vitamin B3 [mg/100g]	4.91
Vitamin C [g/100 g]	0.003
Phenol compounds [g/100 g]	6.33
Flavones [g/100 g]	0.019
Anthocyanin [g/100 g]	0.002

small amount of water in a sterilized blender, packed into small blocks of 1 g each and stored in the refrigerator until the time of use.

#### Biochemical assays

Blood was collected from the retro-orbital sinus of overnight fasting rats using capillary glass pipettes. Samples were collected at the start (1<sup>st</sup> week), mid (6<sup>th</sup> week) and by the end of the experiment (13<sup>th</sup> week) for biochemical assessment. Blood glucose level, insulin and lipid profile (triglycerides, cholesterol, LDL and HDL levels) were recorded. Blood glucose was measured by Accu-Chek Active monitoring device (Roche Diagnostics, Mannheim, Germany) according to Brăslasu et al. [9]. Serum insulin concentration was determined by using rat-specific Insulin-Ak ELISA (DPC, Los Angeles, CA, USA) according to Kekow et al. [33]. The assay kits for the lipid profile were obtained from Randox Laboratories Ltd., Ardmore, Co. Antrim, UK and assessed according to Onyeike et al. [39].

#### Histological investigations

After 13 weeks, rats were euthanized and dissected. All procedures were conducted in accordance with protocols reviewed and approved by the ethical committee for animal care and use in King Fahd Medical Research Centre (KFMRC), KAU, Jeddah, Saudi Arabia, which are in accordance with the guidelines of the Canadian Council on Animal Care. Hearts were harvested, weighed and processed for obtaining paraffin blocks. Paraffin sections 5  $\mu\text{m}$  thick were stained using haematoxylin and eosin (H&E), periodic acid-Schiff (PAS) and Masson trichrome (MT), according to Bancroft and Gamble [6]. The Verhoeff-Van Gieson (EVG) stain was used to identify the elastic fibres (black stained) in the wall of the blood vessels, as well as the surrounding collagen fibres (red stained) [16]. A digital camera connected to a computer and attached to Olympus Microscope BX-51 was used for photographing. A total of 30 fields were used to measure the cardiac muscle diameter and cross sectional area using the Pro Plus image analyser computer system (Media Cybernetics, Rockville, MD, USA).

For immunohistochemical staining, hearts were fixed in neutral buffered formalin, embedded in paraffin and 4  $\mu\text{m}$  tissue sections were obtained according to Bruneval et al. [12]. Paraffin sections were deparaffinised, hydrated and 10% hydrogen peroxide was used to block the peroxidase endogenous activity. Sections were then incubated in primary

antibodies for 120 min, which included monoclonal anti-ASMA (Dako Cytomation, Heverlee, Belgium) at a dilution of 1/1000, anti-desmin (Dako, Trappes, France) at dilution of 1/100, and anti-eNOS (Abcam, Cambridge, MA) at a dilution of 1/50. After washing with phosphate buffer saline, the secondary antibody (biotinylated goat antirabbit) was applied. Slides were incubated with labelled avidin–biotin peroxidase which binds to the biotin of the secondary antibody. Antibody binding sites were visualised after adding (diaminobenzidine) chromogen, which is converted into a brown precipitate by peroxidase. Slides stained with the secondary antibody were only used as negative controls. The nuclei were counterstained with haematoxylin. Semiquantitative analysis of antibody immunoreactivity was assessed by using Pro Plus image analysis software version 6.0. The labelling intensity (mean intensity) and extension of the reaction (area percentage) of ASMA, desmin, and eNOS were examined in a 30 field (3 slides/animal) using a 400 magnification as described by Leslie et al. [34].

#### Statistical analysis

Data were analysed using Statistical Package for the Social Sciences (SPSS) version 16. For non-parametric data, Kruskal-Wallis analysis of variance (ANOVA), followed by a *post-hoc* test (based on the Dunn procedure) was used to analyse each pair of groups, thereby avoiding a multiple-comparison effect. For parametric data, groups were compared using ANOVA (f-test), followed by a Bonferroni *post hoc* test. A p value less than 0.05 was considered to be significant.

## RESULTS

#### Biochemical findings

Both control and raisins groups rats did not have any significant changes in weight gain, heart weight, and food or water intake. However, weight gain of HCD-fed rats was significantly higher compared with control group. HCD-raisins fed rats had significantly less weight gain. HCD-fed rats consumed significantly less food and water compared with control group, while HCD-raisins fed rats had a significantly higher food and water intake. Relative heart weight (heart weight/body weight) in HCD-fed rats was significantly higher than control and HCD-raisins fed groups, as illustrated in Table 2.

**Table 2.** Effect of high cholesterol diet (HCD) and raisins on body weight and food and water intakes

	Control	Raisins	HCD	HCD + raisins
Starting weight [g]	240.6 ± 14.4	255 ± 12.6	231 ± 18.9	256.6 ± 12.1
Ending weight [g]	376 ± 14.9	376 ± 24.2	462 ± 32.5 <sup>a, c</sup>	361.8 ± 38.0 <sup>b, c</sup>
Water intake [mL/d]	286.5 ± 13.5	297 ± 22	247 ± 21.3 <sup>a</sup>	257.1 ± 5.8 <sup>b</sup>
Food intake [g]	2046 ± 106	2026 ± 94	1831 ± 142 <sup>a</sup>	2814 ± 92 <sup>b, c</sup>
Weight gain [g]	136 ± 14.2	121 ± 12.5	231 ± 19.3 <sup>a</sup>	105 ± 24.4 <sup>b</sup>
Food efficacy*	0.066	0.059	0.126	0.037
Relative weight of heart**	0.34 ± 0.03	0.38 ± 0.05	0.39 ± 0.01 <sup>a</sup>	0.35 ± 0.01 <sup>b</sup>

Results are presented as mean ± standard deviation; Significance was considered at  $p < 0.05$ ; \*Weight gain/food intake; \*\*Weight of the heart/body weight; <sup>a</sup>Significant change in HCD compared with the control; <sup>b</sup>Significant change in HCD + raisins compared with the HCD group; <sup>c</sup>Significant change in at the end compared with that at the control start of the experiment.

**Table 3.** Effect of high cholesterol diet (HCD) and raisins on blood glucose, insulin levels and lipid profile

Groups	Time of measurement	Control	Raisins	HCD	HCD + raisins
Blood glucose level [mg/100 mL]	At the start	117 ± 16.4	111 ± 12	123 ± 3.7	105.2 ± 14.4
	At the end	100.2 ± 23.3	109 ± 10	139.9 ± 13.2 <sup>a, c</sup>	113.1 ± 9.8 <sup>b</sup>
Insulin level [ng/mL]	At the start	0.68 ± 0.25	0.65 ± 0.27	0.66 ± 0.29	0.61 ± 0.32
	At the end	0.85 ± 0.24	0.71 ± 0.25	1.77 ± 0.35 <sup>a, c</sup>	0.82 ± 0.15 <sup>b</sup>
Cholesterol level [mg/100 mL]	At the start	61.38 ± 12	59.5 ± 10	57 ± 8.5	60.9 ± 14
	At the end	50.63 ± 8.8	55.2 ± 7	108.8 ± 2.9 <sup>a, c</sup>	54.3 ± 8.4 <sup>b</sup>
Triglycerides level [mg/100 mL]	At the start	40.41 ± 16	42.3 ± 11	46.64 ± 7.5	42.17 ± 12
	At the end	47.4 ± 12	44.3 ± 10	81.12 ± 6 <sup>a, c</sup>	37.17 ± 11 <sup>b</sup>
HDL level [mg/100 mL]	At the start	26.6 ± 2.2	25.2 ± 2	24.92 ± 1.9	26.7 ± 3
	At the end	24.45 ± 2.5	23.2 ± 3.2	18.4 ± 2.1 <sup>a, c</sup>	25.9 ± 1.3 <sup>b</sup>
LDL level [mg/100 mL]	At the start	36.88 ± 2.2	35.8 ± 2	35.34 ± 1.9	36.23 ± 1.6
	At the end	35.43 ± 3	36.2 ± 2.5	86.59 ± 1.2 <sup>a, c</sup>	34 ± 3.8 <sup>b</sup>

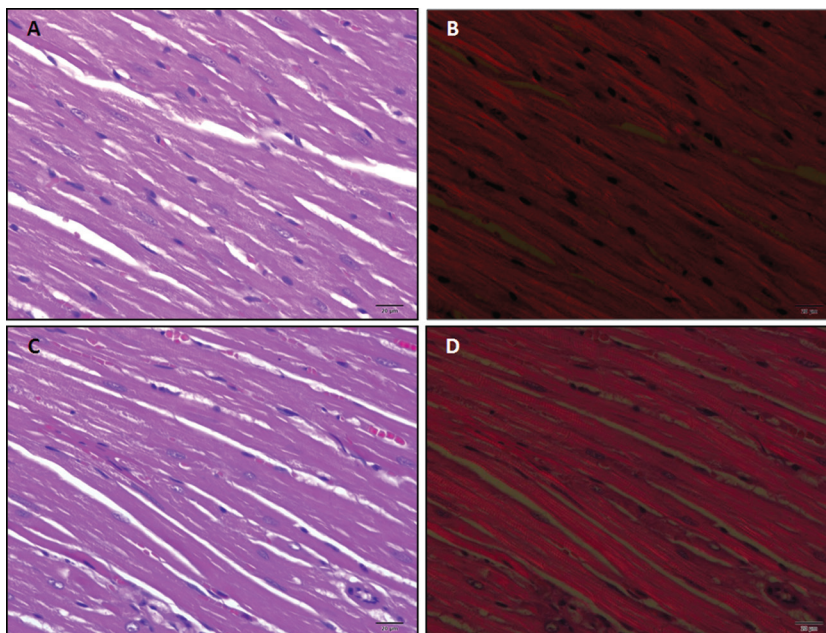
Significance was considered at  $p < 0.05$ ; Results are presented as mean ± standard deviation; <sup>a</sup>Significant change in HCD compared with the control; <sup>b</sup>Significant change in HCD + raisins compared with the HCD group; <sup>c</sup>Significant change in at the end compared with that at the control start of the experiment; HDL — high density lipoprotein; LDL — low density lipoprotein

Blood glucose and insulin levels of HCD-fed rats increased significantly after 13 weeks compared with their starting levels (week 1) and compared with the control group (week 13). HCD-raisins fed rats had significantly lower blood glucose and insulin levels by the end of the experiment compared with those who received HCD alone (week 13), as seen in Table 3. HCD-fed rats had significant elevation in cholesterol, triglycerides, and LDL levels and a significant decrease in the HDL levels in serum at the end of the experiment compared with their starting levels (week 1) and to the control group (week 13). Elevated serum lipids were significantly decreased at the end of the experiment in HCD-raisins fed rats compared with those that were fed HCD alone. A significant decrease in all parameters was noticed except HDL, which increased in HCD-raisins fed rats compared with HCD-fed rats, as seen in Table 3.

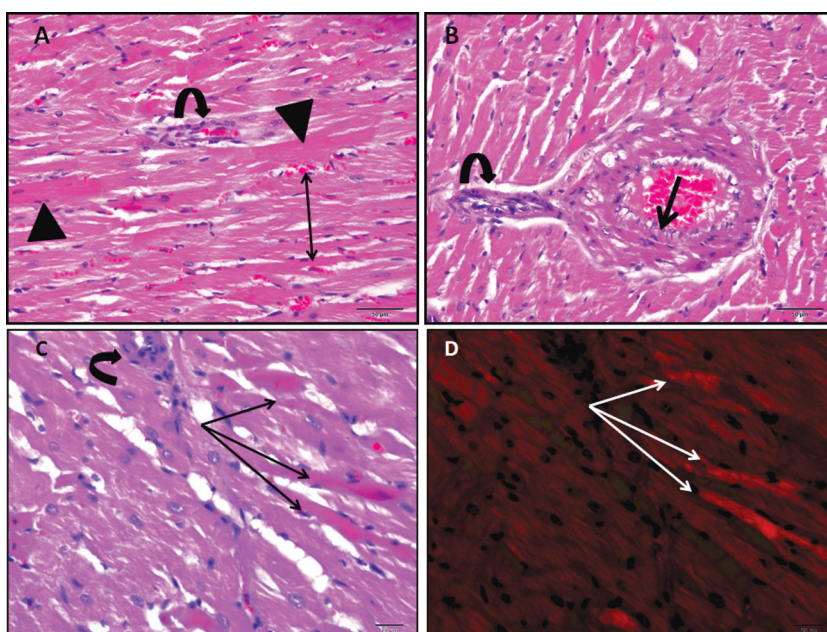
### Histological findings

Left ventricles of control (normal) rats were formed of cylindrical branching and anastomosing CMFs with acidophilic sarcoplasm and oval central nuclei. Left ventricle (LV) CMFs in both control and raisins-fed groups neither had birefringent colours nor histological changes were observed under the polarised light microscope (Fig. 1). However, HCD-fed rats had some degenerated LV CMFs with orange-red colour under polarised light, indicating a content of the highly birefringent type I collagen fibres. Extravasated red blood cells between the CMFs and cellular infiltrate around the blood vessels were also observed (Fig. 2). Left ventricle of HCD-raisins fed rats showed less pathologic changes and fewer degenerated CMFs compared with HCD-fed rats. HCD-raisins fed rats had a significant decrease in CMFs diameters and cross sectional areas compared with HCD-fed group (Fig. 3).





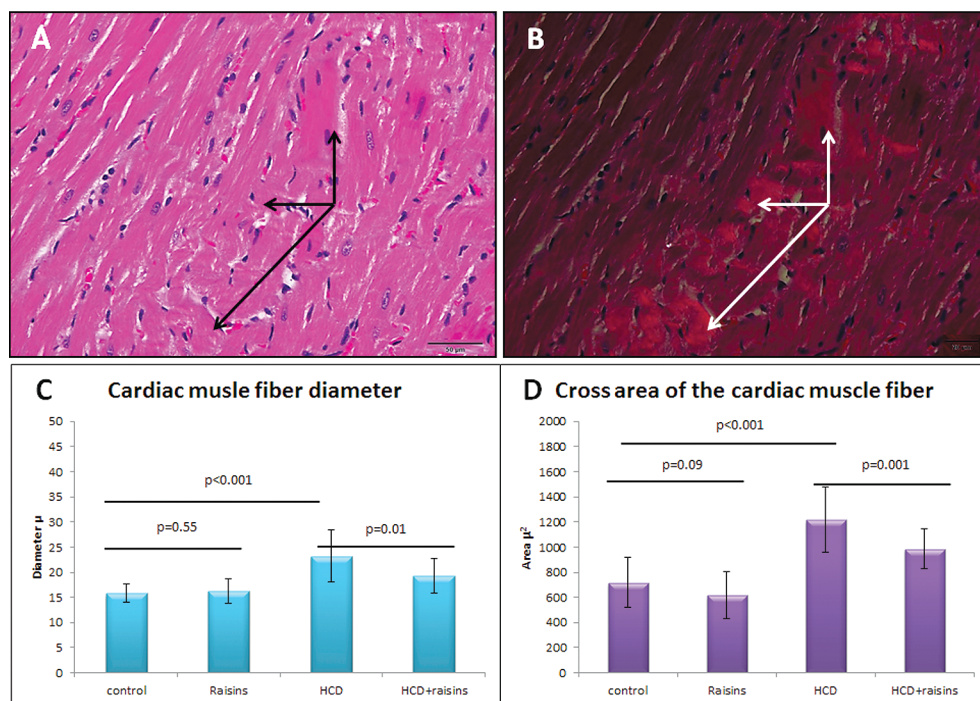
**Figure 1.** Section of left ventricle of control rat visualised in bright (A) and dark (B) fields. The later shows no birefringent colours. Section of left ventricle of raisins-fed rat visualised in bright (C) and dark (D) fields. There is no change from the control (H&E and polarized light  $\times 400$ ).



**Figure 2.** Sections in left ventricle of high cholesterol diet (HCD)-fed rats (A, B, C) showing extravasated red blood cells (two-headed arrow) between cardiac muscle fibres (CMFs), some CMFs with deep acidophilic cytoplasm and dark nuclei (arrow head) and cellular infiltrate (curved arrow) around the blood vessel. Some CMF appear degenerated (thin black arrow) in bright field and possess orange-red birefringent (white arrows) under polarised light (D) (A, B: H&E  $\times 400$ ; C, D:  $\times 600$ ).

Using EVG stain, some LVs of HCD-fed rats showed a remarkable disrupt in blood vessels and were missing their internal elastic lamina. Moreover, there was an increase in the amount of collagen fibres between the CMFs and around the blood vessels. These chan-

ges were less frequent in the LVs of HCD-raisins fed rats (Fig. 4). Statistical analysis showed a significant increase ( $p < 0.001$ ) in the percentage of collagen fibres area in LVs of HCD-fed rats compared with control and raisins-fed rats (Fig. 4).



**Figure 3.** Section in left ventricle of rats fed high cholesterol diet plus raisins (A) shows few degenerated cardiac muscle fibres (CMFs) (thin arrow) which appear moderately birefringent (white arrows) under polarised light (B) while most of the other CMFs appear intact (H&E  $\times$  600, polarised light  $\times$  600). Histograms show the changes in CMF diameter (C) and cross sectional area (D) in the studied groups.

### Immunohistochemical findings

Immunohistochemical expression of ASMA was used to assess the fibrotic changes in cardiac myocytes. Strong ASMA expression was observed in the blood vessel wall and some fibroblasts between the CMFs in LVs of both control and raisins-fed rats. However, HCD-fed rats had a moderate expression in many CMFs and significant increase in ASMA expression in the LVs compared with control group. HCD-raisins fed rats showed a weak ASMA expression in some CMFs (Fig. 5).

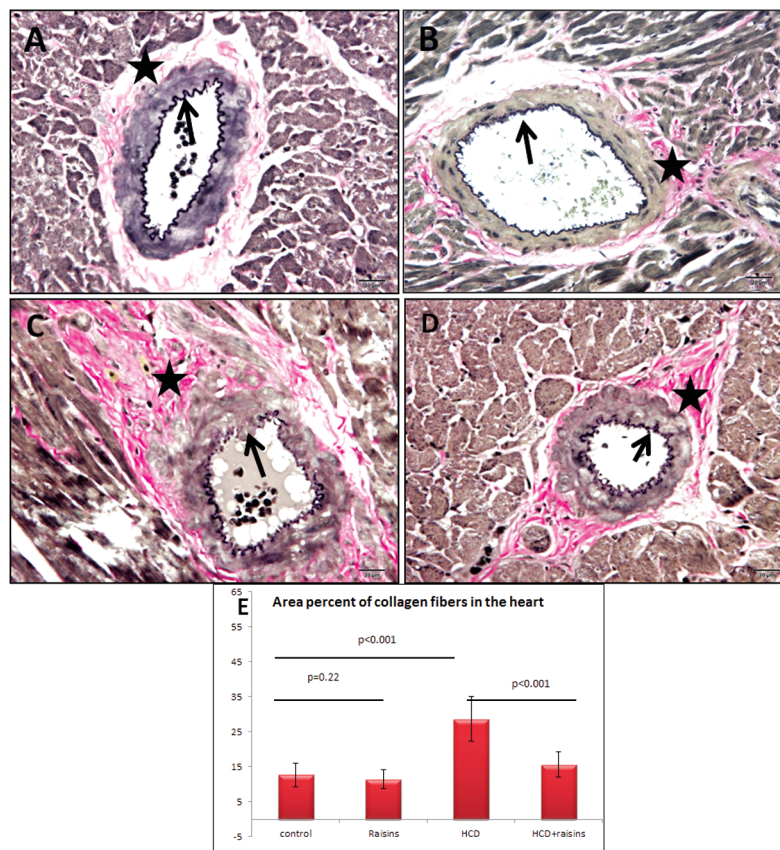
A strong desmin expression was observed in the CMFs of control and raisins-fed rats. HCD-fed rats showed a moderate expression in some fibres and weak expression in others (Fig. 6). A moderate eNOS expression was observed in the CMFs and endothelial lining the blood vessels in control and raisins-fed rats; however HCD-fed rats showed a very weak expression. Left ventricle of HCD-raisins fed rats had weak eNOS expression in CMFs and moderate expression in the endothelial cell lining the blood vessels (Fig. 7). There was a significant decrease in the expression of both desmin and eNOS in the LVs of HCD-fed rats compared with the control and HCD-raisins fed rats (Figs. 6, 7).

### DISCUSSION

Atherosclerotic cardiovascular disease is the leading cause of morbidity and mortality worldwide. Dyslipidaemia is one of the main risk factors leading to atherosclerosis [17]. The present study was carried out to investigate the possible hypocholesterolaemic effect of raisins on the cardiac muscle in HCD-fed rats. Results showed that HCD diet induced a significant increase in the weight of rats, which is in agreement with Kang et al. [32] and Thiruchenduran et al. [54]. Supplementation of a HCD with raisins significantly reduced this weight gain. A previous study on children reported that raisins improved satiety, reduced appetite and were related to less food intake [41]. The increase in relative heart weight of HCD-fed compared with control rats could be attributed to the addition of fibrous tissue around the blood vessels and the fibrotic changes in the cardiac myocytes that was evident by the expression of ASMA. Puskas et al. [46] explained the increase in heart weight as a response to a cholesterol diet and the accumulation of intracellular lipids in cardiomyocytes. The latter was not observed in this study.

HCD-raisins diet significantly decreased the elevated blood glucose and insulin levels that were induced





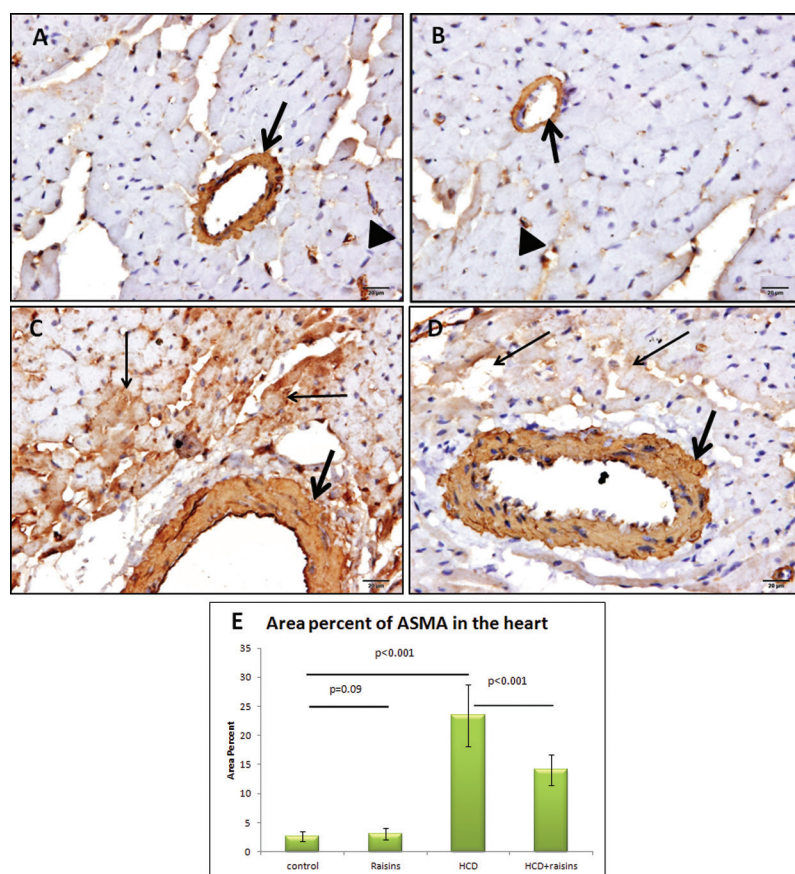
**Figure 4.** Sections in left ventricle of control rat (A), raisins-fed rat (B) and high cholesterol diet (HCD)-fed rat (C). The latter shows interrupted internal elastic lamina (IEL) and increase in collagen fibres (star) around the blood vessel, while that of rat fed HCD plus raisins (D) shows continuous IEL and increased collagen fibres (star) (EVG  $\times$  600). Histogram (E) shows the changes in collagen fibres area per cent in the studied groups.

in HCD-fed rats, which may be due to the hypoglycaemic effect of raisins on HCD-fed rats described by Yugarani et al. [58] and the presence of flavonoids in raisins that preserve the insulin-secreting capacity and viability of pancreatic beta cells [25]. HCD-fed rats were reported to have higher serum lipids levels [29, 53], which was in accordance with our findings where HCD-raisins fed rats had a significant decrease in the levels of serum lipids. Puglisi et al. [45] found that using one cup of raisins per day for 2 weeks in humans significantly decreased total cholesterol, triglycerides and LDL. This hypolipidaemic effect of raisins might be due to polyphenols content interference with cholesterol absorption [60] and decrease of hepatic cholesterol concentrations as observed in guinea pigs [61] after diet supplementation with lyophilised grape powder. Similarly, the hypocholesterolaemic effects of raisins were proven by Spiller et al. [51]. In addition, Bruce et al. [11] and Gardner et al. [24] reported that daily consumption of raisins

improved lipid profile and increased plasma antioxidant capacity.

Histopathological examination of rat hearts revealed that HCD induced degeneration of CMFs, increased collagen fibres deposition and some degenerative changes in the blood vessel walls. Previous studies reported alterations in the structural and functional properties of the myocardium in response to HCD [2, 28, 46]. Wittenstein et al. [57] showed that hyperlipidaemia induces oxidative stress, which plays a key role in atherogenesis.

The significant increase in ASMA immunorepression, observed in the LV of HCD-fed rats in this study, indicates myofibroblast hyperactivity with subsequent fibrosis. A significant increase in the collagen fibres percentage area recorded in this group, suggests that the collagen fibres were derived from ASMA expressing cells. Previous studies indicated similar responses that in myocardial stress and heart diseases, the cardiomyocytes are lost due to necrosis and that



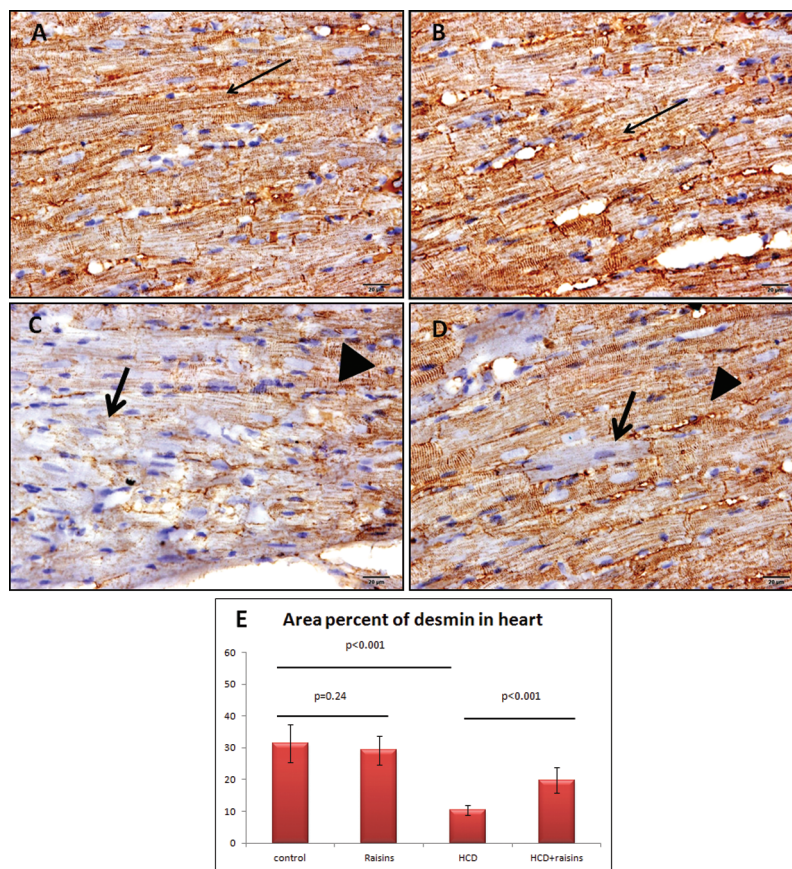
**Figure 5.** Sections in left ventricle of control rat (A) and raisins-fed rats (B) showing strong alpha smooth muscle actin (ASMA) expression in the wall of the blood vessels (thick arrow) and few fibroblast (arrow head) between the cardiac muscle fibres (CMF), while those of high cholesterol diet (HCD)-fed rats (C) and rats fed HCD plus raisins (D) showing weak to moderate expression in many CMFs (thin arrow) in addition to the blood vessels (ASMA immunohistochemistry  $\times 600$ ). Histogram (E) shows the changes in ASMA area per cent in the left ventricle of the studied groups.

myofibroblasts are activated to initiate a reparative fibrosis with a subsequent increase in ASMA-positive cells [14, 37, 56]. ASMA, a protein that is present in foetal heart muscle cells, but absent in adult cardiomyocytes, is re-expressed in cardiomyocytes during heart hypertrophy induced by cardiac overload [27, 55], which could explain the ASMA expression observed in some LV myocytes. The significant decrease in desmin expression in LV CMFs of HCD-fed rats indicates an affection of the structural integrity of these fibres, as was reported by Paulin et al. [42]. Mice lacking desmin developed numerous muscle architectural and ultrastructural defects, especially in extensively-used muscles such as the heart Milner et al. [36] and Capetanaki et al. [13].

Nitric oxide, an important vasodilator, is produced by coronary endothelial cells and has a cardioprotective effect. It minimizes the deleterious effects of superoxide and other reactive oxygen species (ROS) by serving

as an oxidant scavenger [48]. In addition, it inhibits contractile tone and the proliferation of underlying vascular smooth muscle cells and promotes diastolic relaxation [5]. In this study, moderate eNOS immunoreactivity was observed in ventricular myocytes of the control group while HCD administration significantly decreased it. Results resemble those of Jones et al. [30] who stated that a cholesterol-enriched diet markedly reduces cardiac NO levels with subsequent deterioration of cardiac performance. Stapleton et al. [43] reported that elevated cholesterol triggers the release of the inflammatory mediator, C-reactive protein, which may exacerbate vascular dysfunction by inhibiting eNOS and stimulating production of ROS. Elevated cholesterol may also initiate the expression and stimulation of adhesion molecules, chemokine production, and thrombus formation within endothelial cells.

Structural changes could be explained in the light of some previous studies. Hypercholesterolaemic diet



**Figure 6.** Sections in left ventricle of control rat (A) and raisins-fed rat (B) showing strong desmin expression (thin arrow) in cardiac muscle fibres (CMFs) while those of rat fed high cholesterol diet (HCD) (C) and rat fed HCD plus raisins (D) showing decreased expression (thick arrow) in some CMFs (desmin immunohistochemistry  $\times 600$ ). Histogram (E) shows changes of desmin area per cent in the studied groups.

induces excessive production of ROS and lipid peroxidation with a subsequent impaired protein stability and membrane destruction [10, 31], diminishes effectiveness of the antioxidant defence system and decreases catalase and superoxide dismutase activities in hypercholesterolaemic rats [21]. Restoration of antioxidant defence mechanism might lead to CMFs improvement in hypercholesterolaemic rats fed raisins. The existence of antioxidant properties in raisins was supported by many previous studies. Flavonoids, hydrocinnamic acids and resveratrol are among the specific antioxidants that have been identified in raisins [47, 59]. Flavonoids have the capacity to scavenge ROS when consumed by animals and humans [49]. Such compounds can increase the radical scavenging capacity of plasma [23]. It is expected that same mechanism enabled raisins to scavenge ROS that were produced due to the HCD, hence ameliorated its negative effects on cardiac muscles.

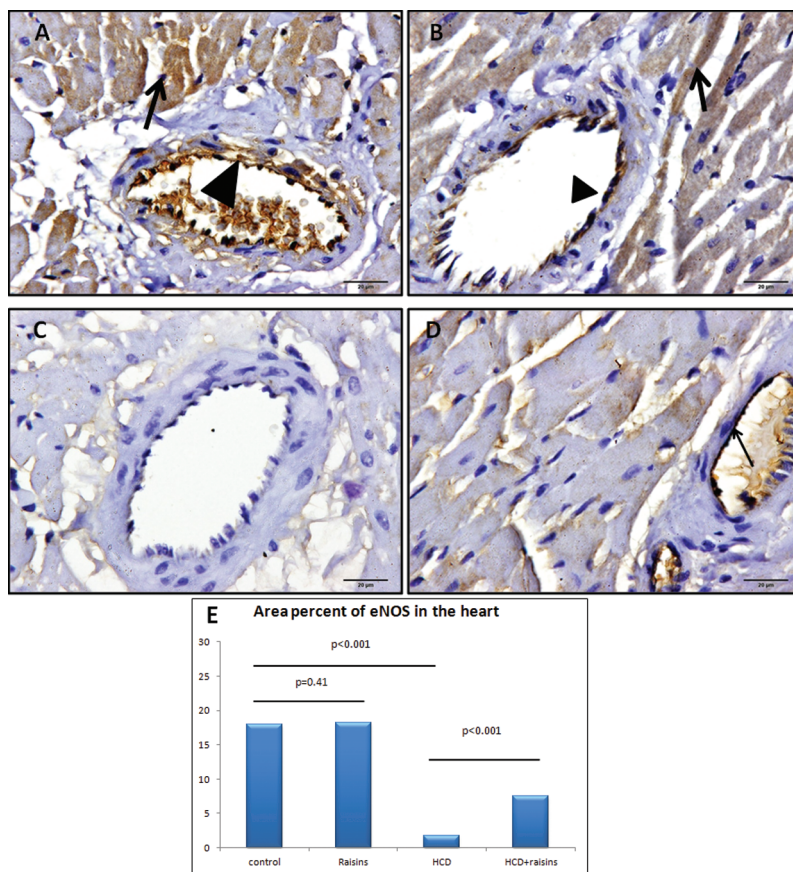
## CONCLUSIONS

In conclusion, HCD induced hyperglycaemia, hyperinsulinaemia, elevated serum cholesterol, triglyceride, LDL and decreased HDL levels. It also resulted in degeneration and fibrosis of CMFs and cardiac blood vessel walls, along with an increase in ASMA expression and a decrease in desmin and eNOS expressions in CMFs. Raisins consumption could alleviate all these changes. Results suggest that habitual consumption of raisins or its pharmaceutical preparations can protect against the hypercholesterolaemia-induced cardiac affection.

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**Figure 7.** Sections in left ventricle of control rat (A) and raisins-fed rat (B) show moderate endothelial nitric oxide synthase (eNOS) expression in cardiac muscle fibres (CMFs) (thick arrow) and endothelial lining (arrow head) of the blood vessels while those of high cholesterol diet (HCD)-fed rat (C) and rat fed HCD plus raisins (D) show very weak and weak expression respectively (eNOS immunohistochemistry  $\times 600$ ). Histogram (E) shows changes of eNOS area per cent in the studied groups.

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