Global Journal of Pharmacology 11 (1): 01-08, 2017
ISSN 1992-0075

DOI: 10.5829/idosi.gjp.2017.01.08

Ameliorative Role of Red Grape Juice on Hypercholesteremia - Induced changes in Aorta and Coronaries in Rats

[1]Abeer Khaled Al Ansary, [2]Hassan El-Hendy, [3]Mohamed Nabil Alama, [4]Soad Ali and [5,6]Gamal Karrouf

[1]Department of Nutritional and Food Science,
Educational Collage for Home Economic, Umm al-Qura University, Makka, Saudi Arabia
[2]Nutrition and Food Science Home Economics Department, Faculty of Agriculture. Alex. University
[3]Consultant Adult Interventional Cardiologist, Facp, Facc, Fscai, Fesc, Faha,
King Abdulaziz Hospital, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[4]Department of Anatomy (Cytology and Histology), Faculty of Medicine, King Abdulaziz University,
Jeddah 21589, Saudi Arabia and Assuit University, Faculty of Medicine,Histology Department.Egypt
[5]Faculty of Science, Medical Physics Department, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[6]Surgery, Anesthesiology and Radiology Department,
Faculty of Veterinary Medicine, Mansoura University, Mansoura, 35516 Dakahlia, Egypt

Abstract: A lot of researches reported the role of free radicals in comprising structural integrity and function of cardiovascular elements.Red grape juice was known to be rich in antioxidants.Thus the main objective of the present study was focused on evaluating its efficacy for protection against hypocholesteremia induced changes in rat aorta and coronary vessels.A total number of 24 apparently healthy albino male rats (200 to 250 gm) were allocated into 4 groups (N=6) GI: (Control negative) received standard rat pellet; GII: fed standard diet + cholesterol; GIII: fed a high fat meal plus 50% grape juice (1:1 D.W.) as an alternative to drinking water; GIV: red grape juice was given as 100% for 8 hours then replaced by water. The experiment lasted for 3 months. Samples of blood were collected before and after the experiment for the analysis of Lipid Profile and blood pressure were measured before animal sacrifice. The heart and thoracic aorta were dissected for microscopic examination. The results showed that red grape juice 100% provided protection against fat deposition in aortic wall., followed by the group that administrated 50% juice. Similar protection was observed in coronaries. Lipid profile proved marked amelioration of increased serum levels of hyperlipidemic elements. Also blood pressure was controlled in treated animals. Conclusion, red grapes juices exerted effective reduction of lipid profile in rat model of hypercholesteremia which seemed to have impact on the protection of the aorta and coronaries against high cholesterol induced atherosclerosis.

Key words: Grape · Antioxidant · Cardiovascular Disease · Atherosclerosis · Aorta

INTRODUCTION

Cardiovascular diseases (CVD) were ranked as the first causes of death in the world and is expected to increase in the coming years. It has been registered about 17.5 million deaths due to heart disease in 2005, representing 30% of the proportion of deaths [1]. CVD occupies according to figures from the Ministry of Health Saudi Arabia ranked as the second in pathological cases where the rate among Saudis in 2004 (19.9%) [2]. The primary pathophysiological basis for most CVD is atherosclerosis and dysfunction of endothelial elements which has been considered an early feature of atherosclerosis progression and a guide for poor

Corresponding Author: Gamal Karrouf, Faculty of Science, Medical Physics Department, King Abdulaziz University, 21589 Jeddah, Saudi Arabia and Surgery, Anesthesiology and Radiology Department, Faculty of Veterinary Medicine, Mansoura University, Mansoura, 35516 Dakahlia, Egypt.
E-mail: drgamalkarrouf1966@gmail.com, gkarrouf@kau.edu.sa.

prognosis in many CAD[3,4].Endothelial function improvement in daily life has been ruledin recent years [5] Polyphenols in grape were reported to have protective effects on cardiovascular systemand endothelial function improvement in many experimental studies [6,7].

Grape was known for its high nutritional value and was mentioned in AL-Quran AL-Kariem many times [8].The juice of black, red and purple colored grape was rich in antioxidant compared with white or green ones [9].Several human and in vitro researches have declared that grape products especially grape juice might help in reducing risk of CVD by decreasing the susceptibility of low density lipoprotein (LDL) to oxidation,based on reports that 2 weeks of concord grape juice(CGJ) supplementation (10 mL/kg body weight/day) increased LDL lag time and decreased the LDL oxidation rate [10]. Shanmuganayagam *et al.*[11]noticed that the daily intake of CGJ reduces the pool and clotting of blood platelets, high blood pressure, high total cholesterol in serum of rabbits model with a high level of cholesterol, leading to prevention of atherosclerosis. Based on what has been reached that the grape is a source of many of antioxidants and fiber that are important to protect the heart and blood vessels, so the present research aimed to demonstrate the role of red grape juice in reducing the risk of feeding high fat diet with excess cholesterol on weight body, blood cholesterol level, blood pressure and histological integrity of aorta and coronary vessels in rat model of hypercholesterolemia.

MATERIALS AND METHODS

Animals: The present study was conducted at King Fahd Medical Research Center (KFMRC),King Abdulaziz University, Jeddah, Saudi Arabia.A total number of 24 apparently healthy albino male rats,weighted from 200 to 250 grams were used.The animals were housed in groups (n=6). Rats had free access to commercial rat pellets and tap water for one week before experiment. The study was performed according to animal care ethics recommended by the University Committee.

Grape: Red grape of high quality (chilly source) was purchased from well-known market. Grapes were weekly crushed in three stages to extract the largest amount of juice then well filtered and packed in transparent plastic boxes and freeze at - 25°C until use.

Cholesterol Powder: The Powder was purchased from Bayouni Trading, Company, Jeddah Saudi Arabia. Cholesterol was added to rat pellets in 5% w/ w standard diet + 20% sheep fat

Experimental Design and Animal Groups: The rats had randomly assigned into 4 groups each group had 6 rats. The rats were fed a standard diet for an adjustment period for a week. Group I: received standard rat pellet; Group II fed standards rat pellets + cholesterol paste (5% w/ w standard diet) + 20% sheep fat; Group III: fed a high fat meal plus 50% grape juice (1:1 D.W.) as an alternative to drinking water; Group IV: red grape juice was given as 100% for 8 hours then replaced by water. The experiment lasted for 3 months.

Assessment of the Nutritional Status and Health of the Rats: The amount of food intake was estimated per day during the trial period, water and juice put in the bottle for drinking were measured every day. All rats were weighted before the start of the experiment using a sensitive electronic balance and then their weights were recorded weekly. At the end of experiment animals were anesthetized by ether. Blood samples from all groups were collected via retro-orbital venous plexus. Centrifugation was carried at 4000 rpm (10 minutes) and the separated sera were used for estimation of total cholesterol (CHOL), high density lipoproteins (HDL) low-density lipoproteins (LDL), triglycerides (TRIG). The analysis were performed according to David [12].

Histological Study: Following blood collection, animals were euthanized by cervical dislocation, abdomen and chest were opened, the heart was perfused with normal saline followed by 10% neutral buffered formalin for 10 minutes. heart and aorta were removed and re-fixed in the same fixative for 24 hours. trimming was done for selecting the left ventricles and samples from all length of the aorta. They were routinely processed by standard paraffin embedding technique, sectioned at 5micron and stained with Hematoxylin and Eosin (H&E) for general structure and orcein for elastic fibers and examined microscopically according to Bancroft and Gamble [13].

Statistical Analysis: The recorded quantitative data were analyzed using SPSS version 20. The data was presented as mean and standard deviation (Mean $\pm$ SD). Analysis was made using unpaired student “t” test between groups. The one-way ANOVA was used between untreated and treated subgroups. The level of significance was considered at $P < 0.05$.

RESULTS

During the experiment, no gross or behavioral changes were observed in all groups. The highest amount of drinking water and grape juice consumption (ml) during the period of the experiment (13 weeks) was observed in GIV of a 50% grape juice concentration Overall average for the consumption was during the probationary period is 3380 ± 196ml/ week.

The Average Food Intake (gm): Fluctuation in the amount of food intake per week between different groups was observed. When calculating the overall average food intake turned out to be the highest amount of food intake are shown in the negative control group was 2046 ± 106 gm/week, respectively then the control positive group and a grape juice 50%, where the average of general food intake was 1831 ± 142, 1806 ± 123 gm/week, respectively while the least amount of food intake for group III that take grape juice concentration of 100% and it was 1370 ± 123 gm/week. The results of the statistical analysis of the existence of significant differences (P less than 0.01) between the control group negative and positive groups and all experimental groups on the other hand. The efficiency of the use of food were 0.028, 0.045, 0.035, 0.036 for each of the control positive and negative groups rats and that takes grape juice concentration of 50%, 100% of the meal, respectively.

Body Weight: The body weight and weight gain of the rats showed increase in the different groups while the highest increase was in the weight at 13 weeks for the control group positive, then a grape juice 50%, the control negative and group grape juice 100%. Highest weight was acquired at the end of the experiment for positive control group (81.8 ± 15.8 g), while the lowest was acquired weight of rats which take the grape juice 100%.

Biochemical Analysis: The results of the blood cholesterol level did not indicate any significant differences between the control and rats that consume grape juice concentration of 50%, 100%. The average values of blood triglycerides do not showed significant differences between the levels of triglycerides between different groups of rats at the beginning of the experiment, while the emergence of significant differences (Probability less than 0.05) between the control negative group and control positive group and also a group rats that take grape juice concentrate 50% were reported. On the other hand at the end of the experiment continued high level of triglycerides in the positive control group compared to negative. Experimental groups did not observe significant differences with the negative control group. The level of HDL in the blood of rats in controls or experimental groups showed lack of significant differences but a non-significant decline were noticed in the level of high density lipoproteins in the positive control group and also for a grape juice 100% at the beginning of the experiment and during the experiment and also at the end of the experiment (13 weeks). Estimating the level of LDL in the blood indicated lack of significant differences between all groups in the beginning of the experiment. The highest value for a grape juice100%, then the control positive group, then a grape juice 50% and finally the control negative group. At the end of the experiment continued rises of LDL(Probability of less than 0.01) in the positive control group and all experimental groups compared to the control negative group (Table1).

Histological Findings:

Aorta: Histology of Rat Aorta in Control Group: showed that aorta of control rat has normal ordered layers described in literature. the inner intima layer showed smooth intact endothelial layer. Media was mainly made of elastic fibers stained by Verhoff stain (b) with few smooth muscles and collagen fibers. Those two layers merge together to make one intima-media layer. Adventitia or the most outer layer consisted from loose collagen fibers and connective tissue cells (Fig. 1.).

Table 1: Showing the comparison between the effect of grape and raisins on lipid profile and kidney functions of high cholesterol fed animals

Parameter	Cholesterol (mmol/L)	Triglycerides (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	Creatinine (mmol/L)	BUN (mmol/L)
Control	56.9±11.4	31.5±1.5	22.5±1.5	0.68±0.05	36.1±9.02	4.5±1.2
Hypercholesterolemia	81.8±1.8	27.1±2.3	20.1±1.6	0.72±0.07	51.7±10.8	6.7±1.26
Hypercholesterolemia+G	49.8±19.7	31.0±2.4	30.2±2.8	0.54±0.05	43.68±5.75	5.97±0.23

Significance was considered at P <0.0

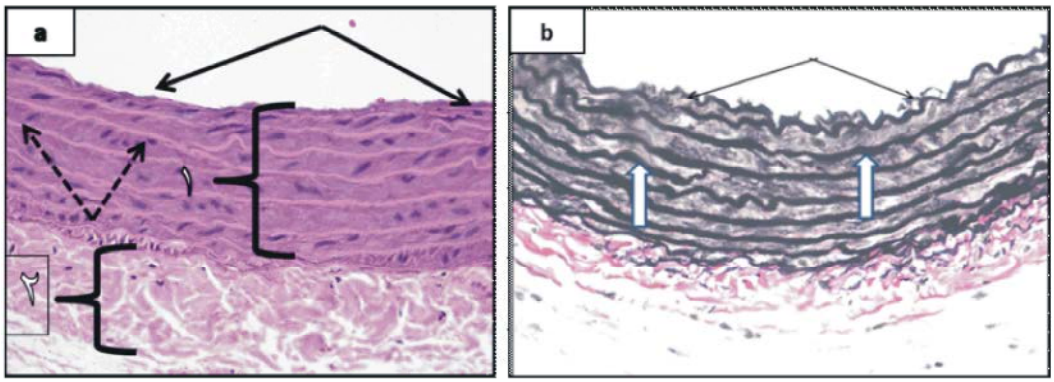

Fig. 1: Control rat aorta: a.H&E stain showing normal intima –media and adventitia thickness (brackets). Smooth intact endothelial lining (thin black arrows) and normal elastic fibers (dotted arrows). b. Elastic fibers stained by orcein (white arrows) and internal elastic lamina (thin arrows) stained by orcein

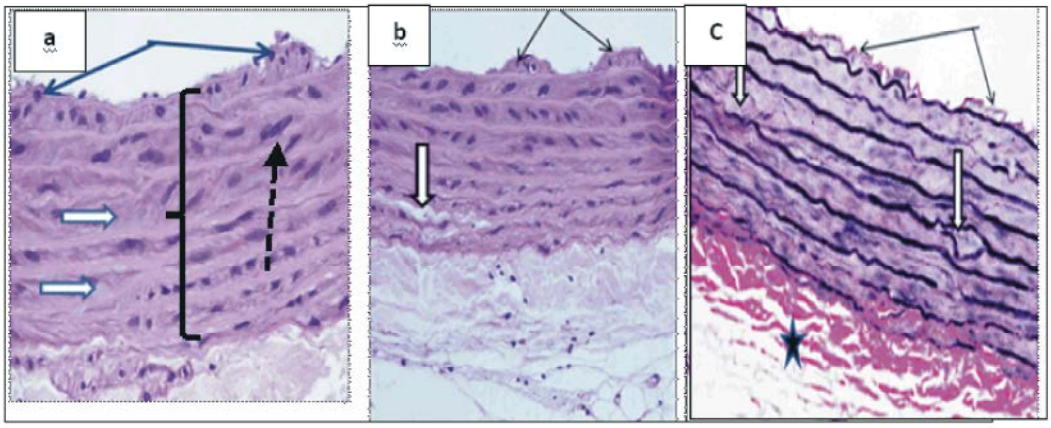

Fig. 2: High cholesterol diet aorta showing:a. Sub endothelial intima thickness (thin black arrows) and increase intima media thickness(brackets), deposition of fat among media elements (white arrows) and smooth muscle proliferation(dotted arrows).b. shows raised intima regions(thin black arrows) and degenerated media regions (white arrow).c. elastic fibers spacing (white arrows). intima thickening (thin black arrows) and increased collagen in adventitia (black star).

Histology of Rat Aorta in Hypercholesteremic Group: Histological examination of the wall of aorta in cholesterol group showed more pronounced arterial wall thickening with the presence of undyed areas that are between the components of the middle layer due to the accumulation of lipid. An increase in e nuclear size was observed in smooth muscle fibers. (Fig. 2a,b,c).

Histology of Rat Aorta of hypercholesteremic Group + Red Grape Juice: Aorta of animals receiving grape juice (50%) showed a relative improvement in histological changes caused by high cholesterol where fatty deposits in the arterial wall, as well as focal intema thickening were declined. Marked protection was observed in the aorta of animals receiving 100% grape juice. But it must be noted that there are individual differences between rats in the degree of response to treatment and some of them showed average improvement with treatment (Fig.3)

Coronaries: Figure 4: Rat coronary arteries: a, GI: control group showing normal wall thickness(white arrow) nearby cardiac muscles looked normal. b, GII: (Cholesterol group) showed marked congestion and red corpuscle lysis and increased wall thickness with deposition of fatty droplets (vacuolation) among media layer components and in contact with endothelial lining. c,d, GIII:Coronaries of rats of 100% grape juice group showed nearly normal appearance compared to those of cholesterol group. Cardiac muscles looked also normal.

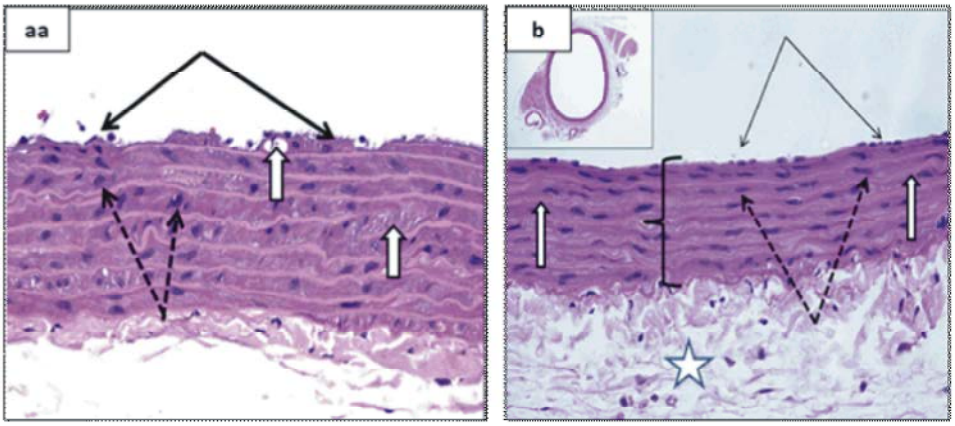

Fig. 3: Showing. a. aorta from rat receiving high cholesterol diet + 50% red grape juice,there is still focal intima thickening (thin black arrows). Cholesterol deposition among media elements(white arrows) and smooth muscle proliferation.b.aorta from rat receiving high cholesterol diet +100% red grape juice showing marked preservation of normal structure (smooth endothelium(thin black arrows) elastic fibers(white arrows) and smooth muscles(dotted arrows (H&E x400).

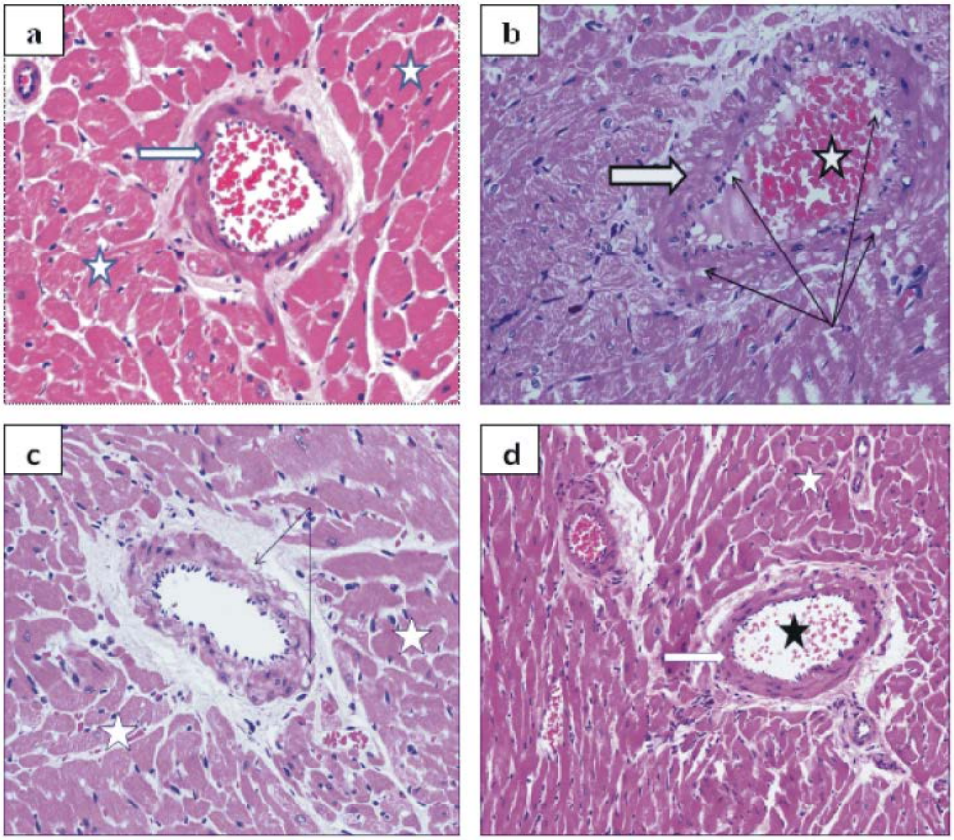

Fig. 4: Showed sections in cardiac muscles surrounding coronary artery branches. compared to control (a).Those from high cholesterol diet group (b) showed marked thickening of arterial wall with deposition of fatty droplets near endothelial lining and among media layer elements.Nearby muscles looked somewhat rarified and smaller in size most probably due ischemic. Coronaries from rats receiving 50% red grape juice (C) showed moderate protection while those from100% grape juice group (d) showed nearly normal appearance compared to those of cholesterol group. Cardiac muscles also looked also normal

DISCUSSION

The results of the current study showed that frequent intake of diet rich in cholesterol and animal fat result in increased risk of both biochemical alteration of lipid profile and damaging vascular tissue especially those of cardiovascular system such as aorta and coronaries [14]. Natural products were recently tried as adjuvant therapies for many diseases including hypercholesteremia [15]. Red grape was known for its high content of antioxidants such as flavonoids , in the present study , concentrated red grape juice was found to control hyperlipidemia induced in rat by feeding on high cholesterol plus high animal fat, changes observed in vascular wall of both aorta and coronaries were much reduced by 50% red juice administration and nearly completely prevented in rats allowed free access to 100% red grape juice for 8 hours. The present results are consistent with what was reported by Castilla *et al*. [16, 17, 18, 19] who found that intake of grapes powder led to a 41% decline in arterial sclerosis due to lowering of oxidative stress.

Liu *et al.*[20] found that red grape extract contains high levels of phenolics and flavonoids compared to other types of grapes and pointed to their effectiveness as an antioxidants to get rid of free radicals. The results of Xu *et al.*[17] on the purple grape juice indicated the ability of its flavonoid compounds to prevent atherosclerosis. The ingestion of red grape juice as a dietary supplement reduces the likelihood of the risk of CVD as the it enhanced flow-mediated vasodilatation and reduces LDL susceptibility to oxidation in CAD patients. Improved endothelium-dependent vasodilatation and prevent the oxidation of LDL [15, 19].The absorptive efficiency of grape polyphenols has detected by Goldberg *et al.*[21].

The recorded decreased body weight, which was seen as a result of grape juice consumption of 100% compared to the control group, may be due to the positive effect of polyphenols in grape juice available to absorb nutrients and energy metabolism. Also the present study agreed with the findings of Lim [22] that Proanthocyanidins as one of the many phenols available in grape led to a significant decrease in cholesterol and triglycerides in the blood serum of rats that dealt with meals high in cholesterol content has added 100 mg Proanthocyanidins grape seed / kg body weight compared to mice that dealt with meals high in cholesterol content. While my results disagreed with the findings of Kang *et al.*[23]who declared that no change in the level of each of cholesterol or triglycerides in the blood plasma of rats fed a high meals fat content with eating extract containing red grape juice (400 or 800 mg / kg / day) for 8 weeks compared to the negative or positive control groups.

The histological study revealed that eating a diet with a high proportion of cholesterol leads to an increase in the thickness of the inner layer of the wall of the aorta with an increase in smooth muscle cells activity [22] who declared that numerous phenols such as grapes rich product lead to a reduction ratio of triglycerides and LDL in the blood and therefore less cholesterol accumulation of in the aorta. The present study agreed with Zern *et al.*[24] that The ingestion of Red grape juice as a dietary supplement 14 days has been decreased cholesterol level, alter levels of lipoprotein, and lowered the percentage of atheromas . While the result of Kang *et al.*[22] showed no change in the level of LDL in the blood plasma of rat fed a diet high in fat content with eating extract containing red grape juice (400 or800 mg / kg / day) for a period of 8 weeks, compared to the negative or the positive control. While the level of HDL significantly increased in the group that dealt with meals high in fat content with eating extract containing red grape juice.

Histological examination of the wall of the aorta of the rat in the cholesterol group showed more pronounced arterial wall thickening with the presence of undyed areas between the components of the intema layer due to the accumulation of simple cholesterol and showed exponentially overgrown the nuclei of some smooth muscle. In some samples, congestion of vessel in the outer layer was observed with a gathering of some inflammatory cells as described by Galkina and Ley [25].

We Notes that drinking grape juice (100%) has led to protection of the aorta from the changes caused by the high experimental cholesterol and the wall appeared in the normal way with the regularity of endothelial cells as well as fiber elastic and the disappearance of lipid droplets accumulated in the smooth muscle cells. However, it should be noted that there are individual variations between rats in the degree of response to treatment, and some of them showed an average improvement with treatment. This is because the effect of grape juice, especially in high concentrations in the protection of the occurrence of changes caused by eating diets high in cholesterol in the wall of the aorta in the rat to the role of polyphenols in grapes as they change chemical structures of medium density fat thus preventing the progression of atherosclerosis[18,19,22].

Arts and Hollman [25] noticed that feeding diet rich in phenols like in grapes down incidence of heart disease and flavonoids are considered powerful antioxidants

making her health benefits through the mechanics contrast to oxidation And grapes has great role in getting rid of these free radicals and inhibiting an adverse effect on the cells lining the blood vessels and this is what was observed in the histological study as polyphenols, which are found in abundance in red grape extract has great role in the disposal of the metals that have a role in the oxidation of fat that destroy the membrane process the cells lining the arteries next to their role in the activation of antioxidant enzymes.

Many phenols extracted from grapes reduce the oxidation of low-density lipoprotein, as well as clotting platelets. Also, these compounds have protective properties of the heart and blood vessels and these effects which are resistant to atherosclerosis and heart rhythm disorders and it also work to relax the blood vessels. So it may be useful eating grapes and grapes extracts and its products in the prevention of chronic cardiovascular diseases [27].

Conclusion, red grapes juices exerted effective reductionof lipid profile in rat model of hypercholesteremia which seemed to have impact on the protection of the aorta and coronaries against high cholesterol induced atherosclerosis.

REFERENCES

1. WHO, 2007. Cardiovascular Diseases Fact Sheet No.317.
2. Saudi Ministry of Health, 2005. Deaths from cardiovascular diseases.-10th ed. Part VI.
3. Cai, H. and D.G. Harrison, 2000. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res., 87: 840-844.
4. Nemati, H.M. and B. Astaneh, 2010. Optimal management of familial hypercholesterolemia: treatment and management strategiesVasc Health Risk Manag, 6: 1079-1088.
5. Hooper, L., P.A. Kroon and E.B. Rimm, 2008. Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. Am. J. Clin. Nutr., 88: 38-50.
6. Zern, T.L. and M.L. Fernandez, 2005. Cardioprotective effects of dietary polyphenols. J. Nutr., 135: 2291-2294.
7. Leifert, W.R. and M.Y. Abeywardena, 2008. Cardioprotective actions of grape polyphenols. Nutr. Res. Nov., 28(11): 729-37.
8. Badwilan, Salah Ben Salem, 2005. Therapeutics grapes. Third Edition. House civilization Lynch and distribution. Ariyad. almofh Saudi Arabia.
9. Carper, J., 2009. Stop Aging Now: The ultimate plan for staying young and reversing the aging process. A New York Times Bestseller, Harper Collins Publishers, pages, 174: 177-78.
10. Sano, A., R. Uchida and M. Saito , 2007. Beneficial effects of grape seed extract on malondialdehyde-modified LDL. J. Nutr. Sci. Vitaminol., (Tokyo), 53: 174-182.
11. Shanmuganayagam, D., T.F. Warner, C.G. Krueger, J.D. Reed and J.D. Folts, 2007. Concord grape juice attenuates platelet aggregation, serum cholesterol and development of atheroma in hypercholesterolemic rabbits. Atherosclerosis, 1: 135-42.
12. David, M.D., 1994. Tietz Textbook of Clinical Chemistry JAMA., 272(8): 644-645.
13. Bancroft, J.D. and M. Gamble, 2008. Theory and practice of histological techniques. 6 th.Churchil Living StoneElsevier. Elsevier Limited
14. Gollücke, A,P. and D.A. Ribeiro, 2012. Use of grape polyphenols for promoting human health: a review of patents. Recent Pat Food Nutr Agric. 1; 4(1): 26-30.
15. Puglisi, M., G. Mutungi, P. Brun, M. McGrane, C. Labonte and J. Volek, 2009. Raisins and walking alter appetite hormones and plasma lipids by modifications in lipoprotein metabolism and up-regulation of the low-density lipoprotein receptor. Metabolism Clinical and Experimental, 58: 120-128.
16. Castilla, P., R. Echarri, A. Dávalos, F. Cerrato, H. Ortega, J.L. Teruel, M.F. Lucas, D. Gómez-Coronado, J. Ortuño and M.A. Lasunción, 2006. Concentrated red grape juice exerts antioxidant, hypolipidemic, and antiinflammatory effects in both hemodialysis patients and healthy subjects. Am. J. Clin Nutr., 1: 252-62.
17. Xu, Y., J.E. Simon, C. Welch, J.D. Wightman, M.G. Ferruzzi, L. Ho, G.M. Pasinetti and Q. Wu, 2011. Survey of polyphenol constituents in grapes and grape-derived products. J Agric Food Chem., 12; 59(19): 10586-93.
18. Georgiev, V., A. Ananga and V. Tsolova, 2014. Recent advances and uses of grape flavonoids as nutraceuticals. Nutrients. 21; 6(1): 391-415.
19. Giovinazzo, G. and F. Grieco, 2015. Functional Properties of Grape and Wine Polyphenols. Plant Foods Hum Nutr. Dec., 70(4): 454-62.
20. Li, S.H., H.B. Tian, H.J. Zhao, L.H. Chen and L.Q. Cui, 2013. The acute effects of grape polyphenols supplementation on endothelial function in adults: meta-analyses of controlled trials. PLoS One. 24;8.

21. Goldberg, D.M., J. Yan and G.J. Soleas, 2003. Absorption of three wine-related polyphenols in three different matrices by healthy subjects. ClinBiochem, 36: 79-87.
22. Lim, T.K., 2013. Edible Medicinal and Non-Medicinal Plants: Vol. 6, Fruits Book • January 2013 .DOI: 10.1007/978-94-007-5628-1.
23. Kang, J., W. Lee, C. Lee, W. Yoon, N. Kim, S. Park and H. Lee, 2011. Improvement of high-fat diet-induced obesity by a mixture of red grape extract, soy isoflavone and L-carnitine: Implications in cardiovascular and non-alcoholic fatty liver diseases. Food and Chemical Toxicology, 49: 2453-2458.
24. Zern, T.L., K.L. West and M.L. Fernandez, 2003. Grape polyphenols decrease plasma triglycerides and cholesterol accumulation in the aorta of ovariectomized guinea pigs. J Nutr., 133: 2268-72.
25. Galkina, E. and K. Ley, 2009. Immune and Inflammatory Mechanisms of Atherosclerosis. Annu Rev. Immunol., 27: 165-197.
26. Arts, I. and P. Hollman, 2005. Polyphenols and disease risk in epidemiologic studies. Am. J. Clin Nutr., 78: 559S-5569.
27. Ren-You Gan, Xiang-Rong Xu, Feng-Lin Song1, Lei Kuang and Hua-Bin Li., 2010. Antioxidant activity and total phenolic content of medicinal plants associated with prevention and treatment of cardiovascular and cerebrovascular diseases. Journal of Medicinal Plants Research, 4(22): 2438-2444.