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Original Article

Cardioprotective effect of resveratrol on atherogenic diet-fed rats

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Abstract: Atherogenic or high fat diets were known to induce cardiovascular diseases, and several active compounds were tested to protect/prevent the risk of cardiovascular diseases. We aimed to investigate the cardio protective effect of resveratrol against atherogenic diet fed rats. Male Wistar rats were administered atherogenic diet for 30 days and further continued for 15 days with or with resveratrol in the diet. The serum lipid profile, antioxidant enzyme activity, lipid peroxidation, lipid metabolic proteins and cardiac tissue markers were examined. The histopathology of myocardium and aorta were also examined. The abnormal serum lipid profile found in atherogenic rats was reversed by the administration of resveratrol. Similarly, the enzymatic (catalase, superoxide dismutase, glutathione-peroxidase), non-enzymatic (reduced-glutathione, Vitamin C, E) antioxidants were improved by the resveratrol fed against atherogenic diet. Interestingly, resveratrol activated the lipid metabolic proteins (SIRT1, eNOS and AMPKa), suggesting its protective effect on lipid metabolism. Further analysis on tissue damage revealed that resveratrol had significantly protected the tissue damage and maintains the morphology of cardiac tissue. Altogether, our results suggest that resveratrol played a significant role in the prevention of cardiovascular system against the high fat diet. Emphasising the anti-atherogenic property of resveratrol, we propose resveratrol as a potential compound to be consumed for the healthy life-style.

Keywords: Antioxidants, atherosclerosis, resveratrol, lipid metabolism

Introduction

Coronary heart disease (CHD) is a major and preventable cause of morbidity and death in the United States. Studies have shown that despite an increased prevalence of smoking and consumption of diets containing significant amounts of saturated fats, the incidence of cardiovascular disease is actually lower in the French population than in the American population [1]. The prophylactic and therapeutic effect of many plant foods and extracts in reducing cardiovascular disease has been reviewed [2].

Resveratrol, trans-3, 5, 4'-trihydroxy stilbene is a naturally occurring phytoalexin present in many different types of nutrients, which we consume on daily basis. Resveratrol is a polyphenol found in the skin of grapes, berries and peanuts that can activate AMPK and sirtuins [3]. Resveratrol possess antioxidant properties [3], increases nitric oxide synthase production [4] and improves mitochondrial function by activating AMPK and sirtuins [3-5]. Resveratrol has anti-cancer and anti-inflammatory effects and beneficial cardiovascular effects [6]. It is well known that resveratrol has beneficial effects on the cardiovascular system. It plays the most important role in the epidemiological phenomenon called "French paradox" meaning that existence of cardiovascular risk factors with low incidence/mortality rates which may attribute to moderate consumption of red wine [7, 8].

Resveratrol protects the cardiovascular system by a number of mechanisms, including resveratrol-mediated inhibition of low-density lipoprotein oxidation, inhibition of platelet aggregation, synthesis of proatherogenic eicosanoids, inhibition of cell proliferation, and increased vasorelaxation. Recent studies have also shown that resveratrol suppresses the induction of procoagulant tissue factor, one of the key components thought to be responsible for high mortality from cardiovascular disease [9-11].

The present study was designed to evaluate the protective effect of resveratrol against atherogenic diet particularly focusing on its possible role of cardio protection in rat model.

Materials and methods

Experimental animals and grouping

The Male Wistar rats (n = 24) were housed in room temperature with regular 12 h day/night cycle, the animals were accessed to food and water *ad libitum*. The animals were used in accordance with institutional guidelines and approved protocols by the Animal Ethical Committee. The animals were initially divided into three groups. A control group (C, n = 6) was given access to water and normal standard diet, *ad libitum*. To study influence of resveratrol in standard diet-fed conditions, a (Res, n = 6) group received standard diet and 6 mg/l resveratrol (trans-3, 40, 5-trihydroxystilbene) in its drinking water, approximately 1 mg/kg body weight/day. In order to study the effects of resveratrol in atherogenic diet-fed condition, a group (AD, n = 12) received water and atherogenic diet formulated based on Diniz [12]. After 30 days of the experimental period, the AD group was randomly divided into two subgroups (n = 6/group): (AD) group remained receiving atherogenic diet and water, and (AD-Res) group given atherogenic diet and 6 mg/l resveratrol in its drinking water. The experiment with AD and AD-Res group continues further for 15 days. Rats in the C and Res groups remained with the same treatment during whole experimental period of 45 days.

Sample preparation

After sacrificing the animals the blood samples, serum was separated and a haemolysate was prepared according to the modified procedure of Quist [13]. A lipid profile analysis was performed on the serum samples, while antioxidant levels were analyzed in haemolysate samples. All the samples were stored at -80°C until analysis. Prior to biochemical analysis, cardiac tissue (100 mg tissue/ml buffer) was homogenized in 50 mM phosphate buffer (pH 7.2); the homogenate was then centrifuged at 1200 g for 15 min and the supernatant was used for biochemical analysis. The protein concentration in each fraction was determined by the method of Bradford [14], using crystalline bovine serum albumin as a standard.

Evaluation of serum lipid profile

The lipid profile includes total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL), very low-density lipoprotein cholesterol (VLDL) and high-density lipoprotein cholesterol (HDL). The serum levels lipid profile was determined by using standard assay kits (Diasys, Holzheim, Germany). The units were expressed as mg/dl.

Determination of lipid peroxidation

The lipid peroxidation was evaluated in both tissue homogenate and haemolysate samples. The mean concentration of malondialdehyde (MDA) was determined as a measure of lipid peroxidation, and MDA was assayed in the form of thiobarbituric acid-reacting substances (TBARS) by the method of Ohkawa [15].

Enzymatic antioxidant activities

The activity of enzymes in antioxidant system was evaluated in both tissue homogenate and haemolysate samples by following the previously reported methods. Catalase (CAT) activity was determined by the method of Sinha [16]. The activity of CAT was expressed as units/mg protein (μmol of H_2O_2 consumed/min/mg protein). Superoxide dismutase (SOD) activity was determined by the method of Marklund and Marklund [17]. The enzyme activity was expressed as units/mg protein. Glutathione peroxidase (Gpx) was determined essentially as described by Rotruck [18]. The activity of Gpx was expressed in terms of μg of GSH consumed/min/mg protein. The enzyme activity was expressed as lmol of CDNB formed/min/mg protein.

Non-enzymatic antioxidant levels

The levels of non-enzymatic antioxidants in cardiac tissue homogenate samples were determined by following the previously reported methods. Reduced glutathione (GSH) content was estimated by the method of Moron [19]. Ascorbate (vitamin C) was measured by the method of Omaye [20]. α-Tocopherol (vitamin E) was estimated by the method of Desai [21]. For

Table 1. Administration of Resveratrol improves serum lipid profile

Lipid Profile	Control	Res	AD	AD + Res
TC	50 ± 3.76	40 ± 5.07	425 ± 7.34[a,*]	125 ± 5.38[b,*]
TG	80 ± 3.4	75 ± 3.5	180 ± 5.6[a,*]	115 ± 3.8[b,*]
LDL	20 ± 2.2	17 ± 2.8	205 ± 2.1[a,*]	60 ± 2.6[b,*]
HDL	70 ± 2.3	76 ± 2.5	38 ± 3.1[a,*]	50 ± 3.2[b,*]
VLDL	20 ± 1.5	16 ± 1.2	50 ± 1.9[a,*]	23 ± 1.3[b,*]
Cardiac ratio	5 ± 0.2	5 ± 0.1	17 ± 1.3[a,*]	6 ± 0.2[b,*]

TC: Total cholesterol; TG: Triglycerides; LDL: low-density lipoprotein, VLDL: Very low-density lipoprotein, HDL: High-density lipoprotein. Values are expressed as mean ± SD of six animals. Statistical analyses *represents significance at $P < 0.01$. [a]Control vs. AD values; [b]AD vs. AD + Res values.

Table 2. Resveratrol prevent lipid peroxidation (LPO)

LPO	Control	Res	AD	AD + Res
Tissue	0.8	0.6	1.5[a,*]	1.0[b,*]
Haemolysate	1.7	1.5	3.2[a,*]	2.0[b,*]

Values are expressed as mean ± SD of six animals. Statistical analyses *represents significance at $P < 0.01$. [a]Control vs. AD values; [b]AD vs. AD + Res values.

all these experiments, the results were expressed as μg/mg protein.

Western blot analysis

Cells were washed with Hanks buffer and scraped in 50-100 mL of lysis buffer (with protease inhibitors), centrifuged, and the supernatant was collected. Protein content was determined by BCA protein assay. Total cell extracts containing 16-20 mg of protein were prepared in SDS sample buffer and subjected to SDS-PAGE and western blot analysis. Proteins were transferred to nitrocellulose membranes prior to immuno-detection. The antibodies for SIRT1, P-AMPKα (Thr172) and endothelial nitric oxide synthase (eNOS) were purchased from Cell Signaling (Beverly, MA), and GAPDH (Cambridge, MA) were used to detect the protein level in the heart tissues.

Assessment for cardiac tissue damage markers

The activity of cardiac tissue damage makers such as lactate dehydrogenase (LDH), alkaline phosphatase (ALP), aspartate transaminases (AST) and alanine transaminases (ALT) were determined by following the methodology of King [22].

Histopathological examination

Conventional techniques of paraffin wax sectioning and haematoxylin-eosin (HE) staining were used for this study. Slices of fresh thoracic aorta were cut and fixed in buffered neutral formalin fixative for 24 h. Following fixation, the tissue slices were washed and processed through an ascending series of alcohol (30%, 50%, 70%, 90% and 100%), cleared in methyl salicylate and infiltrated with wax at 57°C. The aorta, thus cleared was embedded in paraffin. Sections of thickness 4-6 μm were cut, stained by aqueous haematoxylin and alcoholic eosin and the sections were examined by bright-field microscopy (Carl Zeiss Axioskop 2 plus; Jena, Gera, Germany).

Statistical analysis

The values are expressed as mean ± standard deviation (SD) for six animals in each group. Differences between groups were assessed by a one-way analysis of variance (ANOVA) and students T-test using SPSS software package for Windows (Version 11.5; SPSS Inc., Chicago, IL, USA). Post hoc testing was performed for intergroup comparisons using the least significance difference (LSD) test.

Results

Resveratrol improves serum lipid profile

A significant ($P < 0.001$) increased levels of serum TC, TG, bad cholesterols (LDL, VLDL) and the cardiac risk ratio values were measured in AD fed rats than those fed with a normal diet or Res fed rats. However, AD + Res fed rats had significantly ($P < 0.001$) decreased these levels and showed a significant ($P < 0.001$) increase in the level of HDL cholesterol. Rats fed with resveratrol alone showed improved lipid profile compared to that of normal diet fed (control) rats (**Table 1**).

Resveratrol precludes lipid peroxidation both in cardiac tissue and haemolysate

The lipid peroxidation (LPO) is determined by measuring the mean concentration of MDA. The cardiac tissue and haemolysate samples of AD rats showed significantly ($P < 0.001$) high-

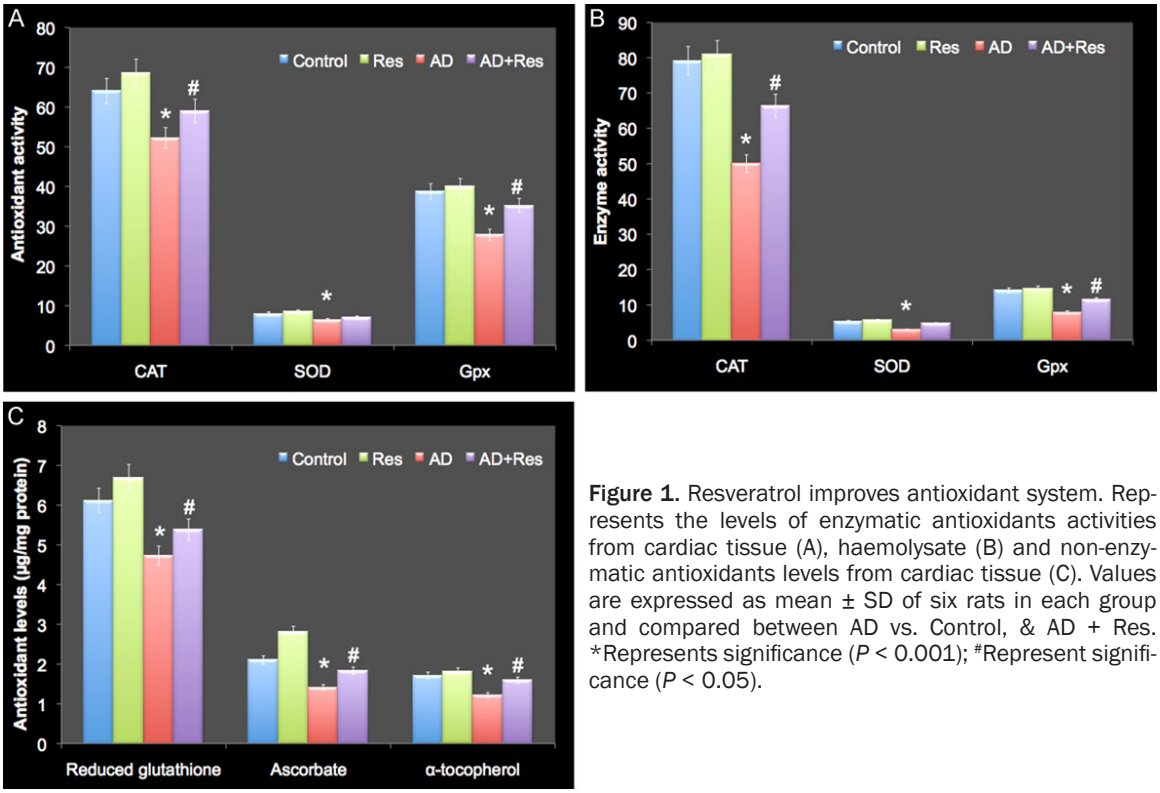

Figure 1. Resveratrol improves antioxidant system. Represents the levels of enzymatic antioxidants activities from cardiac tissue (A), haemolysate (B) and non-enzymatic antioxidants levels from cardiac tissue (C). Values are expressed as mean ± SD of six rats in each group and compared between AD vs. Control, & AD + Res. *Represents significance ($P < 0.001$); #Represent significance ($P < 0.05$).

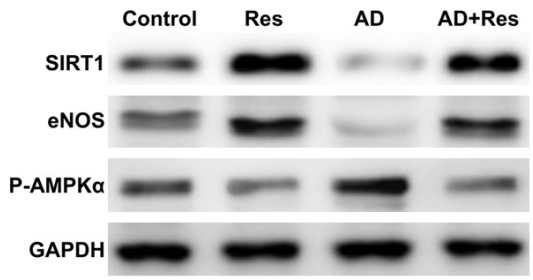

Figure 2. Represent the western blot analysis of lipid metabolic proteins in rat heart tissues of experimental groups. Resveratrol regulates the key proteins SIRT1, eNOS, P-AMPKα involved in lipid metabolic pathway.

er than that in control or Res fed rats (**Table 2**). The mean MDA concentrations in cardiac tissue of AD + Res fed rats were significantly ($P < 0.05$) lower than that in AD rats. Similarly, pattern of MDA concentration was found in haemolysate sample. However, the Res fed rats showed much improved protection of LPO than that of control rats (**Table 2**).

Resveratrol improves enzymatic antioxidant activity and non-enzymatic antioxidants levels

The mean activity of CAT, SOD and GPx found significantly ($P < 0.01$) lower in cardiac tissue and haemolysate samples of AD fed rats while compared to that of control or Res fed rats. However, no significant differences in mean activities of antioxidant enzymes found between control and AD + Res fed rats (**Figure 1A**). Similar pattern of enzyme activities was found in haemolysate sample. However, the Res fed rats showed much improved antioxidant activity than that of control rats (**Figure 1B**). The mean level of GSH, ascorbate and α-tocopherol in cardiac tissue of AD rats were found significantly ($P < 0.001$; $P < 0.05$) lower than that of control or Res fed rats (**Figure 1C**). The mean concentration of ascorbate in cardiac tissue of AD + Res fed rats was significantly higher ($P < 0.05$) than that in AD rats; however, no significant differences were observed in mean levels of GSH and α-tocopherol in cardiac tissue samples of AD + Res and AD rats (**Figure 1C**).

Resveratrol regulates lipid metabolic pathway

The western blot analysis of key proteins involved in lipid metabolism showed that resveratrol significantly activated the protein levels of SIRT1, eNOS and decreased the phosphorylated AMPKa in heart tissue (**Figure 2**). However, the AD fed rats showed the reverse effect.

Table 3. Resveratrol prevents cardiac tissue damage

Cardiac markers	Control	Res	AD	AD + Res
LDH	33.98 ± _4.7	30.2 ± _6.7[a,*]	57.2 ± _6.7[a,*]	31.55 ± 4.5[b,*]
ALP	0.10 ± _0.01	0.07 ± _0.018[a,*]	0.17 ± _0.018[a,*]	0.12 ± 0.10[b,*]
ALT	0.08 ± _0.007	0.07 ± _0.010[a,*]	0.11 ± _0.010[a,*]	0.08 ± 0.09[b,*]
AST	0.22 ± _0.02	0.21 ± _0.04[a,*]	0.35 ± _0.04[a,*]	0.25 ± 0.03[b,*]

LDH: lactate dehydrogenase; ALP: alkaline phosphatase; ALT: alanine transaminase; AST: aspartate transaminase. Values are expressed as mean ± SD of six animals. Statistical analyses *represents significance at $P < 0.01$. [a]Control vs. AD values; [b]AD vs. AD + Res values.

These results might be a key evidence to speculate the mechanism of action of resveratrol against lipid deposition in heart tissue.

Resveratrol improves the activity of cardiac marker enzymes

The marker enzymes lactate dehydrogenate (LDH), alkaline phosphatase (ALP), alanine amino transferase (AST) and aspartate amino transferase (ALT) were found significantly ($P < 0.01$) elevated in AD rats than that of control/Res fed rats (**Table 3**). There was a significant ($P < 0.001$) decrease in the activity of cardiac markers in AD + Res fed rats when compared to AD rats. On administration of resveratrol, the changes in the activity of cardiac markers were reverted to near normal (**Table 3**).

Resveratrol prevents structural integrity of heart muscles

We had examined the histopathological studies on myocardial tissue and aorta. The control and Res fed rats shows healthy morphology of myocardial tissue. The AD fed rats show extensive myocardial damage like edema, leukocyte infiltration and necrosis. However, these damages were found significantly decreased in AD + Res fed rats (**Figure 3A**). The intima layer of the aorta was found thickened (blocks of fat deposition) in the AD fed rats while compared with control or Res fed rats. However, the AD + Res fed rat showed the intima of the aorta was found slightly thickened than that of rats fed with control rats (**Figure 3B**).

Discussion

The results of our present study represent the possible implications of resveratrol as a therapeutic agent for cardiovascular disease with an emphasis on resveratrol's effect on cholesterol metabolism. The cholesterol diet influences the cholesterol deposit in the aorta and other tissues in the form of cholesterol esters [23]. Our study demonstrated that the experimental animals fed with atherogenic diet (AD) exhibited augmented lipid levels in the cardiac tissue. The deposited cholesterol esters in the tissue need hydrolysis to release free cholesterol. One of the hydrolysis factors is HDL, since HDL-cholesterol level was found to be decreased in atherogenic diet fed rats [24], the insufficient HDL level may lead to free cholesterol in plasma, enhancing the pathogenesis. Lipoproteins are the vehicle for transporting plasma lipids to the blood. Resveratrol significantly alleviates the serum lipid profile, considering the importance of HDL to the reverse transport of cholesterol. Our results showed that resveratrol enhanced HDL in Res-fed rats. The most obvious effect of resveratrol on lipid profile was its action on in vivo ox-LDL. Resveratrol reduced the ox-LDL in both dietary conditions. Ox-LDL promotes atherosclerosis both by providing lipids signals that initially activate macrophages, and by stimulating foam cell formation [25].

Oxidative stress is one of the causative factors that link hyper cholesterolemia with atherogenesis. There was enhanced lipid peroxidation or MDA level observed in the group II animals due to atherogenic diet that induces free radical production. Lipid peroxidation is a chain event that enhances MDA production [26]. Resveratrol also induced a strong decrease in alcohol-induced lipid peroxidation of heart; this could partly explain the cardiovascular beneficial effects of red wine consumption [27]. The lipophilic property of resveratrol enables it to associate with the lipid moiety of lipoproteins and prevent the oxidation of their unsaturated fatty acids [28]. Resveratrol increases the expression of antioxidant enzymes like superoxide dismutase, catalase, and glutathione peroxidase, thereby reducing the formation of free radicals and preventing endothelial injury [28]. Consistently, our results in present study; resvera-

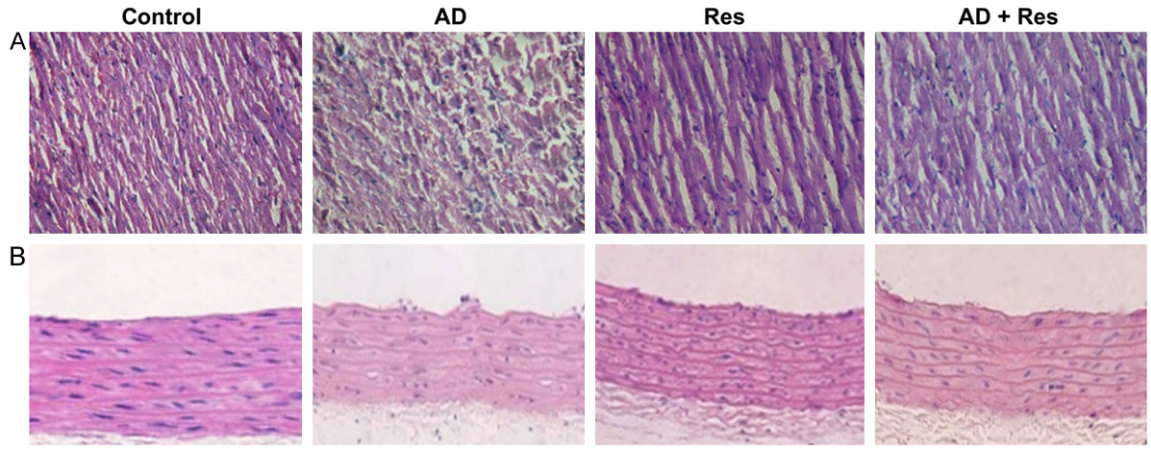

Figure 3. Represent the histopathological studies on rat myocardial tissue (A) and aorta (B) of experimental groups. The resveratrol had reduced the damage induced by atherogenic diet. Scale bar 200× and 100× respectively.

trol administration had improved the enzymatic and non-enzymatic antioxidant system against the atherogenic diet as well as in normal condition. These effects were similar in both heart tissue and haemolysate which is consistent with the previous study, treatment of apoE knockout mice with resveratrol for 7 days results in the upregulation of superoxide dismutase, glutathione peroxidase, and catalase in heart tissue [29]. Some researchers believe that the expression of these enzymes is the actual mechanism by which resveratrol prevents oxidative injury rather than the direct scavenging activity of reactive oxygen species.

Lipid metabolism in macrophages is an important process in the context of hypercholesterolemia. Uptake of excessive amounts of native and modified lipoproteins leads to their conversion into foam cells, which accumulate to create fatty streaks, a central feature of the early phase of atherosclerotic lesion development. Networks of proteins associated with macrophage lipid metabolism have been found in recent years to be affected by resveratrol [30]. In the present study, resveratrol activated the SIRT1, eNOS and regulated the phosphorylation of AMPK against the atherogenic diet. Resveratrol was discovered to be a strong activator of SIRT1, which regulates lipid metabolism by de-acetylation of modified lysine residues on histones and various transcriptional regulators [31]. SIRT1 has several effects associated with protection from the development of atherosclerosis. SIRT1 is an important signaling molecule in endothelium, which improves its function. SIRT1 binds directly to eNOS and has been shown to target eNOS for deacetylation, thereby stimulating nitric oxide production and promoting vascular relaxation [32]. Endothelial-derived nitric oxide controls vascular tone and has atheroprotective effects. AMPK is a sensor of cellular energy status and a key controller in the regulation of whole-body energy homeostasis [33]. It plays an integral role in lipid metabolism by switching on the oxidative process for fatty acids and by inhibiting the synthesis of lipids [34]. It also aids in endothelial relaxation and dilation.

Next, we examined the tissue damage induced by high cholesterol diet. The cardiac tissue markers were measured. The rats exposed to atherogenic diet had increased the activity of cardiac markers such as LDH, ALP, AST and ALT. This tissue damage causes the leakage of these markers in the plasma [35]. Resveratrol administration prevents this damage. Similarly, our histopathological examination of myocardium and aorta showed the abnormal morphology in atherogenic diet fed rats. However these changes have been prevented in resveratrol fed rats. These results were supported by the earlier reports with similar studies but with difference in treatments like fluvastatin and methanol extract of sorbus cortex [35, 36]. Moreover, resveratrol has potent antiatherosclerotic effects in in-vitro and in-vivo that indicate potential clinical utility in preventing the onset and/or progression of atherosclerotic cardiovascular disease. Despite a good deal of preclinical evidence, data on cardiovascular effects in humans are quite limited [36].

In conclusion, the results of the present study have demonstrated that administration of resveratrol had significantly prevented cardiac abnormalities induced by atherogenic diet. Our data revealed that resveratrol posses the cardio protective effect by improving the serum lipid profile, antioxidant system, improving lipid metabolism and cardiac tissue damages either in myocardium or aorta. Our findings support a role for regular consumption of dietary resveratrol by consumption of resveratrol rich fruits or vegetables to avoid the risk of coronary artery disease.

Disclosure of conflict of interest

None.

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References

[1] Ferrieres J. The French Paradox: lessons for other countries. Heart 2004; 90: 107-111.
[2] Walker AF. Of hearts and herbs. Biologist 1996; 43: 177-180.
[3] Catalgol B, Batirel S, Taga Y and Ozer NK. Resveratrol: French paradox revisited. Front Pharmacol 2012; 3: 141.
[4] Haigis MC and Sinclair DA. Mammalian sirtuins: biological insight and disease relevance. Annu Rev Pathol 2010; 5: 253-295.
[5] Canto C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P and Auwerx J. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature 2009; 458: 1056-1060.
[6] Mukherjee S, Dudley JI and Das DK. Dose-dependency of resveratrol in providing health benefits. Dose Response 2010; 8: 478-500.
[7] Opie LH and Leceur S. The red wine hypothesis: from concept to protective signalling molecules. Eur Heart J 2007; 28: 1683-1693.
[8] Liu Z, Zhang LP, Ma HJ, Wang C, Li M and Wang QS. Resveratrol reduces intracellular free calcium concentration in rat ventricular myocytes. Acta Physiol Sin 2005; 57: 599-604.
[9] Pendurthi US and Rao LV. Resveratrol suppresses agonist-induced monocyte adhesion to cultured human endothelial cells. Thromb Res 2002; 106: 243-248.
[10] Wilson T, Knight TJ, Beitz DC, Lewis DS and Engen RL. Resveratrol promotes atherosclerosis in hypercholesterolemic rabbits. Life Sci 1996; 59: 15-21.
[11] Pace-Asciak CR, Rounova O, Hahn SE, Diamandis EP and Goldberg DM. Wines and grape juices as modulators of platelet aggregation in healthy human subjects. Clin Chim Acta 1996; 246: 163-182.
[12] Diniz YS, Cicogna AC, Padovani CR, Santana LS, Faine LA and Novelli EL. Diets rich in saturated and polyunsaturated fatty acids: metabolic shifting and cardiac health. Nutrition 2004; 20: 230-234.
[13] Quist EE. Regulation of erythrocyte membrane shape by Ca2+. Biochem Biophys Res Commun 1980; 92: 631-637.
[14] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72: 248-254.
[15] Ohkawa H, Ohishi N and Yagi K. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-358.
[16] Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972; 47: 389-394.
[17] Marklund S and Marklund G. Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974; 47: 469-474.
[18] Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG and Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science 1973; 179: 588-590.
[19] Moron MS, Depierre JW and Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta 1979; 582: 67-78.
[20] Omaye ST, Turnbull JD and Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. Methods Enzymol 1979; 62: 3-11.
[21] Desai ID. Vitamin E analysis methods for animal tissues. Methods Enzymol 1984; 105: 138-147.
[22] King J. The dehydrogenases or oxidoreductase-lactate dehydrogenase. Practical clinical enzymology. In: Van D, editor. London: Nostrand Company Ltd; 1965. pp. 83-93.
[23] Hodis HN, Crawford DW and Sevanian A. Cholesterol feeding increases plasma andaortic tissue cholesterol oxide levels in parallel: further evidence for the role of cholesterol oxidation in atherosclerosis. Atherosclerosis 1991; 89: 117-126.
[24] Brown MS, Ho YK and Goldstein JL. The cholesterol ester cycle in macrophage foam cells. Continual hydrolysis and re-esterification of cy-

toplasmic cholesteryl esters. J Biol Chem 1980; 225: 9344-9352.
[25] Schaffer JE. Lipotoxicity: when tissues overeat. Cur Opin Lipidol 2003; 14: 281-287.
[26] Lee MK, Park YB, Moon SS, Bok SH, Kim DJ, Ha TY, Jeong TS, Jeong KS and Choi MS. Hypocholesterolemic and antioxidant properties of 3-(4-hydroxyl) propanoic acid derivatives in high-cholesterol fed rats. Chem Biol Interact 2007; 170: 9-19.
[27] Bradamante S, Barenghi L and Villa A. Cardiovascular protective effect of resveratrol. Cardiovasc Drug Rev 2004; 22: 169-188.
[28] Spanier G, Xu H, Xia N, Tobias S, Deng S, Wojnowski L, Forstermann U and Li H. Resveratrol reduces endothelial oxidative stress by modulating the gene expression of superoxide dismutase 1 (SOD1), glutathione peroxidase 1 (GPx1) and NADPH oxidase subunit (Nox4). J Physiol Pharmacol 2009; 60: 111-116.
[29] Xia N, Daiber A, Habermeier A, Closs EI, Thum T, Spanier G, Lu Q, Oelze M, Torzewski M, Lackner KJ, Munzel T, Forstermann U and Li H. Resveratrol reverses endothelial nitric-oxide synthase uncoupling in apolipoprotein E knockout mice. J Pharmacol Exp Ther 2010; 335: 149-154.
[30] Voloshyna I, Hussaini SM and Reiss AB. Resveratrol in cholesterol metabolism and atherosclerosis. J Med Food 2012; 15: 763-773.
[31] Bordone L and Guarente L. Calorie restriction, SIRT1 and metabolism: understanding longevity. Nat Rev Mol Cell Biol 2005; 6: 298-305.
[32] Stein S and Matter CM. Protective roles of SIRT1 in atherosclerosis. Cell Cycle 2011; 10: 640-647.
[33] Steinberg GR and Kemp BE. AMPK in health and disease. Physiol Rev 2009; 89: 1025-1078.
[34] Misra P. AMP activated protein kinase: a next generation target for total metabolic control. Expert Opin Ther Targets 2008; 12: 91-100.
[35] Mitani H, Egashira K and Kimura M. HMG-CoA reductase inhibitor, fluvastatin, has cholesterol-lowering independent “direct” effects on atherosclerotic vessels in high cholesterol diet-fed rabbits. Pharmacol Res 2003; 48: 417-427.
[36] Turan B, Tuncay E and Vassort G. Resveratrol and diabetic cardiac function: focus on recent in vitro and in vivo studies. J Bioenerg Biomembr 2012; 44: 281-296.