

Full Length Research Paper

Reduces cholesterol induced atherosclerotic lesions in aorta artery in hypercholesterolemic rabbits

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Atherosclerosis is the leading cause of death in developed countries with many proved risk factors. Vinegar has been proved to have some medical uses and some potential protective effects on atherosclerosis. In this study, effects of vinegar on various risk factors of atherosclerosis and development of atherosclerosis in hypercholesterolemic rabbit have investigated. Rabbits were assigned to four groups and each group received one of experimental diets: normal diet, high cholesterol diet (1% cholesterol), 1% cholesterol supplemented with 5 ml vinegar and 1% cholesterol supplemented with 10 ml vinegar for 8 weeks. Blood samples were collected before and after 8 weeks of experimental diets for measurement of serum C-reactive protein (CRP), nitrite, nitrate, apolipoprotein A (ApoA₁) and apolipoprotein B (ApoB₁₀₀), total cholesterol (TC), fibrinogen and factor VII. At the end of study, fatty streak formation in aorta was determined in all groups. Using vinegar (5 and 10 ml) with hypercholesterolemic diet caused significant reduction in CRP, fibrinogen, factor VII, ApoB₁₀₀, ApoB/ApoA ratio, TC levels, atherosclerotic lesions in aorta and increased nitrite and nitrate in comparison with hypercholesterolemic diet. Vinegar did not significantly change ApoA₁ level compared to high cholesterol diet. These results suggest that vinegar reduced atherosclerosis processes in high cholesterol diet fed animals. More studies are needed to find the exact mechanisms of action.

Key words: Vinegar, atherosclerosis, risk factor, fatty streak, C-reactive protein.

INTRODUCTION

The principal cause of death in the world and the most dangerous diseases in industrial countries is atherosclerosis (Henneken and Gaziano, 1993). Atherosclerosis is associated with hypercholesterolaemia, oxidative modification of low density lipoproteins (LDL), endothelial dysfunction, and platelet hyperactivity in developed form (Woodward et al., 2004). Although lipid profile and LDL are important in diagnosis of

atherosclerosis, some recent studies indicate that apolipoprotein measurements especially ApoB and ApoB/ApoA ratio are useful predictors of risk of cardiovascular diseases (Chan and Watts, 2006).

It has been shown that disorders of both coagulation and fibrinolysis play a role in development of cardiovascular diseases such as coronary artery disease, essential hypertension, ischemic stroke and deep vein thrombosis. In a number of trials, it has been identified that high plasma fibrinogen and factor VII activity are independent predictors of cardiovascular mortality (Noto et al., 2002; Kelleher, 1992; Meade et al., 1993). In all stages of atherosclerosis, inflammation is critical (Libby

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and Ridker, 2002) and C-Reactive protein (C-RP) has been shown to predict both cardiovascular events and prognosis post-event (Liuzzo et al., 1994; Morrow et al., 1998) C-RP may actively promote atherogenesis (Torzewski et al., 2000; Cermak et al., 2003; Pasceri et al., 2000) causing lesion formation through mechanisms such as endothelial dysfunction and leukocyte activation (Pasceri et al., 2001) and changes in plaque structure resulting in stability reduction and enhancing rupture.

NO is a strong endogenous vasodilator and endothelial damage can decrease its availability (Cuevas and Germain, 2004). Increased ROS release in states of oxidative stress convert NO to peroxynitrite and neutralizes its beneficial effects. Release of NO and superoxide simultaneously results in the generation of the cytotoxic oxidant peroxynitrite (Aldini et al., 2003).

A considerable amount of epidemiological and clinical evidence has demonstrated a significant reduction of morbidity and mortality among fruit and vegetable consumers (Gey et al., 1993; Hertog et al., 1995). Such diets because of their polyphenolic compounds, and particularly flavonoids, which are known to possess antioxidant effect, have positive influence (Morel et al., 1993).

Vinegar is one of the components of grape. A number of studies have demonstrated that grape juice can decrease cholesterol (Shanmuganayagam et al., 2000), improve endothelial function (Stein et al., 1999), and enhancing the resistance to oxidative modification of LDL (Stein et al., 1999).

Vinegar is not only used commonly as a condiment but also traditionally as a folk medicine. Acetic acid is the main component of vinegar. Some other constituents include, anthocyanins (e.g. cyanidin-3-glucoside) flavonols (e.g. quercetin, kaempferol), flavanols (catechin, epicatechin) (Shahidi et al., 2008), vitamins, mineral salts, amino acids and nonvolatile organic acids (eg. tartaric, citric, malic, lactic) (Johnston and Gaas, 2006). Vinegar has shown such effects as enhancement of glycogen repletion (Fushimi et al., 2001), prevention of hypertension (Kondo et al., 2001), stimulation of Ca^{+2} absorption (Kishi et al., 1997) and reduction of serum total cholesterol and triacylglycerol in animal studies (Fushimi et al., 2006). This study was performed to determine the effects of vinegar on markers of inflammation, coagulation, endothelial function, oxidative factors, lipid profile and progression of atherosclerosis.

MATERIALS AND METHODS

Preparation of the plant

The genus and species were verified by a botanist from the Research Center of Isfahan Province Natural Resources. The grapes were collected from Aminabad region of Isfahan and vinegar was produced with traditional methods. Two factors require special attention when making vinegar with traditional method: oxygen supply and temperature. Oxygen is spread throughout the mixture

by stirring it daily and by letting air reach the fluid through a cheesecloth filter, which is used in place of a regular lid. The temperature of fermenting cider should be kept between 60 and 80 degrees Fahrenheit. Steps for making vinegar including: Clean ripe grapes; change all of the fruit sugar to alcohol (this is called "yeast fermentation"), change all of the alcohol to acetic acid (this is called "acetic acid fermentation)," clarify the acetic acid to prevent further fermentation and decomposition (Victoria, 2009). In order to standardize the vinegar, some factors such as density, vitamin C, anthocyanin, flavonoids and acetic acid were measured.

Animals and experimental design

Thirty two male New Zealand rabbits weighing between 2030 ± 237 g were purchased from Razi Institute of Iran. The animals were acclimatized under room temperature and were housed in cages under a 12 h light/dark cycle according to approved standards for laboratory animal care for two weeks and had free access to water and a standard powdered purified diet (PARS Co, Iran) which consisted of 15% protein, 40 to 50% carbohydrates, 2% vegetable fat, 15 to 25% fiber, 2.5% calcium carbonate and bisphosphate, mineral mixture 3.9% and 0.5% vitamin mixture. At the end of this period, the rabbits were randomly divided into four groups. The animals were fasted for 12 to 15 h and venous blood samples were taken to determine baseline values.

After this, each group received one of the four experimental diets: normal diet, hypercholesterolaemic diet (1% cholesterol), 1% cholesterol with 5 ml vinegar, 1% cholesterol with 10 ml vinegar every day. Cholesterol-rich diet was prepared by adding 1 g /L (Merck, Germany) in 4 ml olive oil to 0.1 kg of commercial rabbit chow (Boger, 1997; Singer et al., 1997). Vinegar (5 or 10 ml) was given orally to animals by oral gavage once daily (Decorde et al., 2008). The duration of experiment was 60 days and the animals had unlimited access to food and water. The study was reviewed and approved by the ethics committee of Isfahan University of Medical Sciences. At the end of experiment, the blood samples were taken.

Measurement biochemical factors in rabbit

Blood samples were centrifuged at 3500 rpm for 20 min to obtain serum and plasma. The plasma was used for fibrinogen, and factor VII measurements and the serum for other biomarkers.

CRP (Kamiya Biomedical Co, Cat #KT-097, USA) was measured using enzyme-linked immuno-sorbent assay kit according to manufacturer's instructions. Fibrinogen was measured using coagulation kit (Mahsayaran Co, Iran). The serum level of nitrite and nitrate were measured using a colorimetric assay kit (R and D Systems, Cat #KGE001, USA) that involves the Griess reaction. Factor VII was measured using clotting time, in the presence of the STA-Neoplastine reagent of a system in which all the factors are present, constant and in excess except factor VII which is derived from the sample being tested (Diagnostic Stago, French). Apolipoprotein A₁ (ApoA), apolipoprotein B₁₀₀ (ApoB) and total cholesterol (TC) were determined using standard enzymatic kits (Pars Azmoon Co, Iran) and an automated chemistry analyzer (Hitachi model 902, Japan).

Assessment of the severity of atherosclerotic lesions

At the end of the study, the animals were sacrificed. Following chest incision, the animals' aortas were excised for assessment of fatty streaks formation. After slicing and staining with Hematoxylin-Eosin, atherosclerotic thickness was assessed on an arbitrary scale 1-4 (Chekanov, 2003):

Table 1. Measured factors in vinegar used in experiment.

pH	Density (g/ml)	Vitamin C mg/dl	Acetic acid (%)	Flavonoids g/ 100 ml	Anthocyanin mg / 100 g	Factors
3.58 ± 0.01	1.042 ± 0.002	8.02 ± 0.02	15.81 ± 0.04	1.071 ± 0.06	3.25 ± 1.02	Vinegar

Trace: Minimal thickness of subintimal with little injury to aorta artery.

Grade 1: Atherosclerotic thickness less than half as thick as the media with some form of endothelial dysfunction, macrophages and isolated foam cell inside the endothelium.

Grade 2: Atherosclerotic thickness half as thick as the media with accumulation of intracellular lipid, macrophage and smooth muscle cells.

Grade 3: Atherosclerotic thickness as thick as the with an abundance of macrophages, smooth muscle cells and connective tissue.

Grade 4: Atherosclerotic thickness more than as thick as the media with a large extracellular intimal lipid core that appears as a large nucleus from the endothelial surface (Chekanov, 2003).

Measurement of physiochemical factors after vinegar treatment

The pH was determined using pH meter (Fisher Scientific, Germany), density by densitometer (Erma Tokyo, Japanes), vitamin C assayed by spectrophotometric (ShimadzoUV-3100, Japanese) method at 520 nm and determined photometrically with 2,4 dinitrophenyl hydrazine to form the red bis-hydrazone which is reduced to a colorless form (McCormick and Greene, 1994). Total flavonoid content was measured using aluminum chloride colorimetric assay. The absorbance was measured against prepared reagent blank at 510 nm (Kumar et al., 2008). Total anthocyanin was assayed by spectrophotometric method at 535 nm (Francis, 1982) and the total acidity of the vinegar was measured using acid-base titration (Scarf and Malerich, 2008).

Statistical analysis

Results are given as Mean ± SD. Data were analyzed statistically using One-Way-ANOVA test followed by LSD post test. The differences between the baseline values and the two months values of all measured parameters calculated and used in statistical analysis. In all instances, p value less than 0.05 was considered significant. For histological data, SPSS software was used to compare mean values between the groups. One-Way ANOVA and Tukey tests were used for histological data.

RESULTS

Determination of some physiochemical factors in vinegar

Analyzing vinegar factors showed that the amount of vitamin C was 8.02 ± 0.02 (mg/dl), acetic acid percent was 15.81 ± 0.04%, total anthocyanin in 100 g of vinegar was 3.25 ± 1.02 (mg/100 g) and total flavonoids in 100 ml of vinegar was 1.071 ± 0.06 (g/100 ml equivalent

naringenin). The density and pH were 1.042 ± 0.002 (g/ml) and 3.58 ± 0.01, respectively (Table 1).

Endothelial marker

Analyzing vinegar factors showed that the amount of vitamin C was 8.02 ± 0.02 (mg/dl), acetic acid percent was 15.81 ± 0.04%, total anthocyanin in 100 g of vinegar was 3.25 ± 1.02 (mg/100 g) and total flavonoids in 100 ml of vinegar was 1.071 ± 0.06 (g /100 ml equivalent naringenin). The density and pH were 1.042 ± 0.002 (g/ml) and 3.58 ± 0.01, respectively.

Determination of some physiochemical factors in rabbit

The serum level of nitrite and nitrate in normal diet control were significantly decreased compared to hypercholesterolaemic diet (p < 0.05). Nitrite and nitrate concentrations were increased in low-and high-dose vinegar with cholesterolemic diet compared to rabbits fed high cholesterolaemic diet (p < 0.05) (Table 2).

Inflammatory factors

High cholesterol control induced a significantly increase in fibrinogen, CRP and factor VII levels compared to normal diet (p < 0.05). Following concurrent use of 5 and 10 ml vinegar with hypercholesterolaemic diet, fibrinogen, CRP and factor VII levels were significantly decreased in comparison with high-cholesterol group (p < 0.05) (Table 3).

Lipid profile

In the high-cholesterol group, ApoB₁₀₀ and ApoB/ApoA ratio were increased significantly and ApoA₁ decreased compared to normal-diet group (p < 0.05). Both doses of vinegar with cholesterolaemic diet induced a significant decrease ApoB₁₀₀ and ApoB/ApoA ratio compared with hypercholesterolaemic diet (p < 0.05). No significant difference was found between doses of vinegar compared with high cholesterol diet in ApoA₁ (p > 0.05). Both doses of vinegar induced a significant decrease in TC compared to high cholesterol group (p < 0.05) (Table 4). No significant difference was found between 5 and

Table 2. Comparison of endothelial markers values before (baseline) and end of 2 months experimental diet.

Biochemical factors ($\mu\text{mol/l}$)		Groups			
		Cholesterolemic diet	5 ml Vinegar with 1% chol	10 ml Vinegar with 1% chol	Normal diet
Nitrite	Baseline	21.3 \pm 7.20	19.5 \pm 8.96	22.30 \pm 10.12	31.9 \pm 16.10
	End of 2 months	35.61 \pm 10.64	64.63 \pm 24.19*	79.03 \pm 19.92*	27.17 \pm 11.01*
Nitrate	Baseline	15.49 \pm 2.92	11.73 \pm 4.86	17.03 \pm 2.12	8.11 \pm 0.84
	End of 2 months	22.54 \pm 5.88	26.74 \pm 3.56*	30.41 \pm 1.61*	8.2 \pm 1.63*

Mean nitrite and nitrate ($\mu\text{mol/l}$) \pm SD, in each group (n = 8 for each experimental group). *p < 0.05, Pairwise comparison of delta (differences baseline and end of 2 months) between cholesterolemic diet group and each of other 3 groups (5 and 10 ml vinegar with 1% cholesterol and normal diet).

Table 3. Comparison of inflammatory factors values before (baseline) and end of 2 months experimental diet.

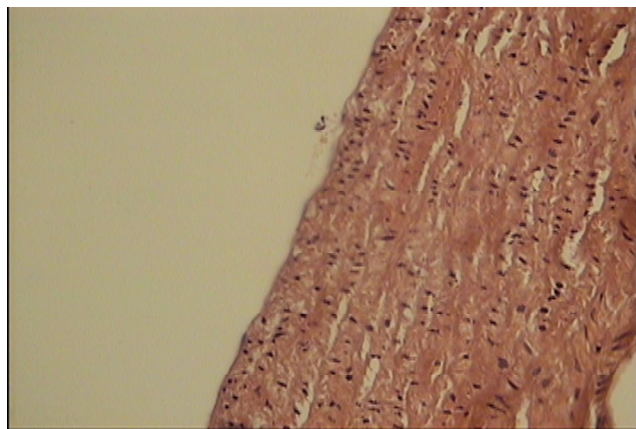
Biochemical factors		Groups			
		Cholesterolemic diet	5 ml Vinegar with 1% chol	10 ml Vinegar with 1% chol	Normal diet
CRP($\mu\text{g/ml}$)	Baseline	2.53 \pm 0.35	2.17 \pm 0.26	2.28 \pm 0.32	2.45 \pm 0.12
	End of 2 months	3.9 \pm 0.21	3.22 \pm 0.25*	3.15 \pm 0.18*	2.63 \pm 0.21*
Fibrinogen (mg/dl)	Baseline	205.17 \pm 20.81	234.56 \pm 51.48	209.17 \pm 20.26	240.75 \pm 16.8
	End of 2 months	293.67 \pm 35.10	239.33 \pm 37.89*	213.83 \pm 47.32*	244.75 \pm 13.96*
VII (% activity)	Baseline	150.17 \pm 29.54	219 \pm 58.03	216.5 \pm 39.88	230 \pm 2.06
	End of 2 months	338.83 \pm 78.62	279.67 \pm 63.03*	273.33 \pm 44.20*	231.5 \pm 31.46*

Mean CRP (C-Reactive protein), fibrinogen and VII factor, $\mu\text{g/ml}$, mg/dl and % activity \pm SD respectively, in each group (n = 8 for each experimental group). *p < 0.05, pairwise comparison of delta (differences baseline and end of 2 months) between cholesterolemic diet group and each of other 3 groups (5 and 10 ml vinegar with 1% cholesterol and normal diet).

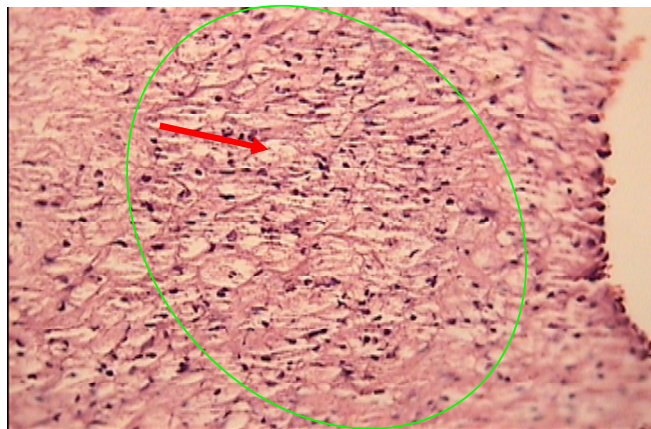
Table 4. Comparison of lipid profile values before (baseline) and end of 2 months.

Biochemical factors (mg/dl)		Groups			
		Cholesterolemic diet	5 ml vinegar with 1% chol	10 ml vinegar with 1% chol	Normal diet
TC	Baseline	61.8 \pm 12.1	101.5 \pm 22.36	108.5 \pm 46.04	97.8 \pm 23.7
	End of 2 months	1413.67 \pm 80.10	801.67 \pm 437.33*	821.33 \pm 345.77*	116.75 \pm 19.14*
ApoA1	Baseline	20.8 \pm 2.5	26.83 \pm 9.45	26.5 \pm 7.42	19 \pm 2.4
	End of 2 months	28.67 \pm 6.77	37.33 \pm 4.76	35.38 \pm 1.17	42.75 \pm 2.87*
ApoB100	Baseline	5.17 \pm 2.5	6.67 \pm 4.3	6.67 \pm 2.5	4.5 \pm 1.9
	End of 2 months	26.67 \pm 10.19	12.83 \pm 8.7*	12.17 \pm 9.04*	4.25 \pm 1.7*
ApoB/ApoA	Baseline	0.26 \pm 0.14	0.27 \pm 0.20	0.21 \pm 0.09	0.18 \pm 0.11
	End of 2 months	1.26 \pm 0.59	0.36 \pm 0.26*	0.38 \pm 0.23*	0.12 \pm 0.04*

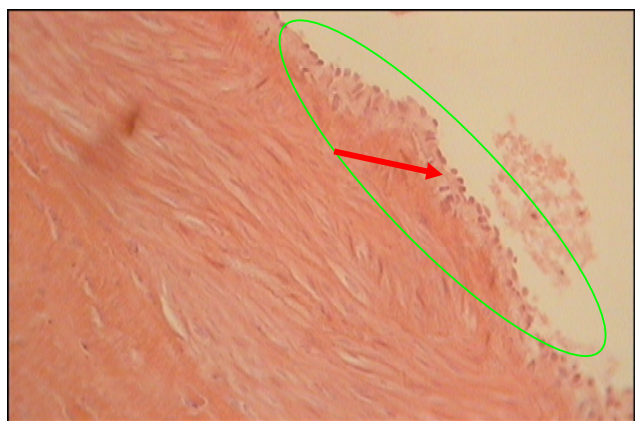
Mean TC (Total cholesterol), ApoA1 (Apolipoprotein A1), ApoB100 (Apolipoprotein B100), (mg/dl) \pm SD and ApoB/ApoA \pm SD in each group (n = 8 for each experimental group). *p < 0.05, pairwise comparison of delta (differences baseline and end of 2 months) between cholesterolemic diet group and each of other 3 groups (5 and 10 ml vinegar with 1% cholesterol and normal diet).



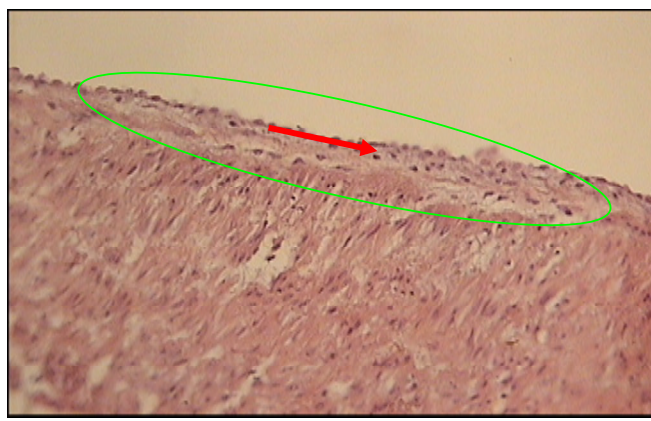
a: Normal diet (X40)



b: Hypercholesterolemic diet (X40)



c: 5 ml vinegar +1% hypercholesterolemic diet (X40)



d: 10 ml vinegar +1% hypercholesterolemic diet (X40)

Figure 1. Aorta artery intima cross-section in the studied groups.

10 ml vinegar groups with regard to CRP, ApoA₁, ApoB100, nitrite, nitrate, fibrinogen, factor VII and TC concentrations.

Fatty streak formation

Histological sections of aorta artery stained from the 4 groups are shown in Figure 1 and the results of atherosclerotic thickness grading in these groups were summarized in Figure 2. Normal diet had completely normal arteries without any lesion in intima or media (Figure 1a). In high cholesterol group atheroma plaque were formed with macrophages filled with fat created foamy cells. Plaque thickness was also increased to more than half of media thickness, equal to degree 3 of Chekanov scale (Figure 1b). In the vinegar groups some endothelial dysfunction along a few foam cell and macrophages were seen in the intimal surface of the aorta artery and plaque degree were 1 (Figures 1c and d). Atherosclerotic thickness grade in the vinegar groups

decreased significantly compared to high cholesterol group ($p < 0.05$) (Figure 2).

DISCUSSION

Concomitant consumption of cholesterol enriched diet with vinegar modifies the atherogenic effects of cholesterol and significantly prevents the increase of CRP, fibrinogen, factor VII, ApoB, ApoB/ApoA ratio, and TC. It also increases serum nitrite and nitrate concentrations. Histological results indicated that vinegar significantly reduced atherosclerotic lesion of aorta artery.

In our study, nitrite and nitrate increased in high cholesterol control compared to normal diet. It has been suggested that enhanced NO synthesis might be a defense mechanism to compensate for continuous inactivation of NO and protection against damaging factors (Ferlito et al., 1999).

Both 5 and 10 ml vinegar with cholesterolaemic diet caused significant increase in nitrite and nitrate compared

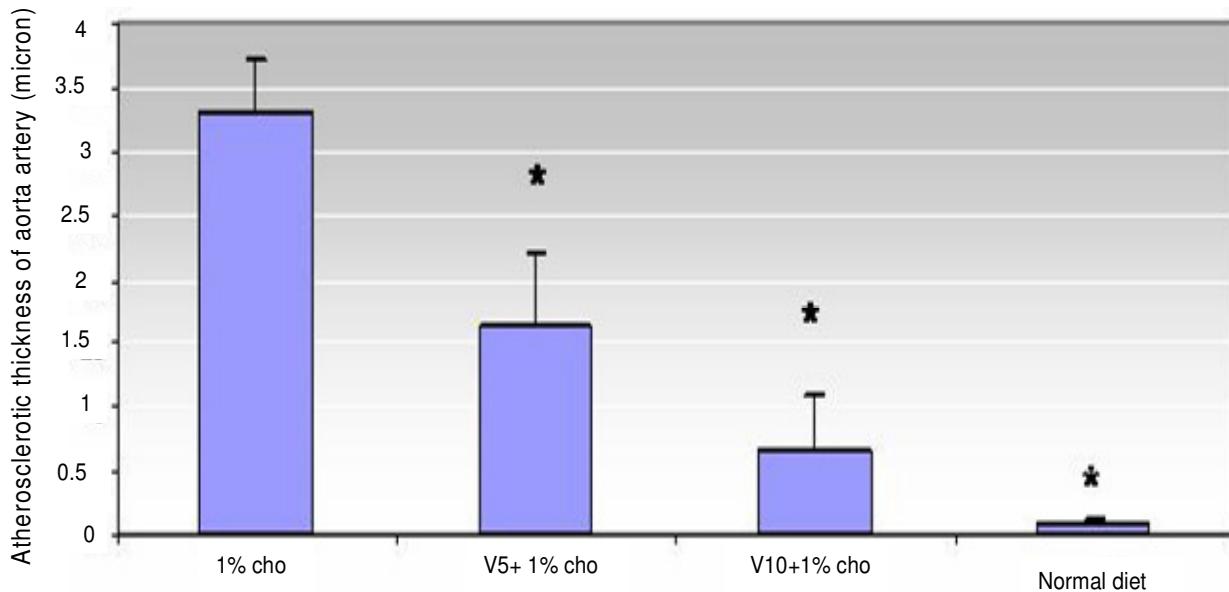


Figure 2. Mean of atherosclerotic thickness grade in the studied groups (aorta artery). * $p < 0.05$, Comparison between groups (V5 + 1% cho, V10 + 1% cho and normal diet) with respect to hypercholesterolemic group, V5 + 1% cho: 5 ml vinegar + 1% hypercholesterolemic diet, V10 + 1% cho: 10 ml vinegar + 1% hypercholesterolemic diet.

with high cholesterolaemic diet without vinegar. Some of the polyphenols through improving of NO synthase activity can cause endothelium dependent relaxation. The critical phase of NO synthase activity in endothelium cells is the increase of intracellular calcium concentration leading to the endothelium- dependent vasorelaxation (Andriambelason et al., 1999). By the scavengers of oxygen-free radicals, biological activity of NO can be effectively increased (Bouloumie et al., 1997). Increase in $[Ca^{2+}]_i$ can be due to either an influx of extracellular Ca^{2+} or a release of Ca^{2+} from intracellular stores (Luckhoff et al., 1998). Polyphenolic components can increase the NO release by stimulating the NO activity and preservation or stabilization of NO and protection of NO from destruction by superoxides (Schuldt et al., 2000).

In other study, 35% (v w⁻¹) DRW (dealcoholised red wine), 0.3% (w w⁻¹) quercetin and 0.3% (w w⁻¹) catechin increased the endothelium dependent NO without changing the release of the O₂ in rat's aorta. This component causes vasorelaxation (Benito et al., 2002).

According to the results of Leikert et al. (2002), red wine polyphenols increase the eNOS expression and endothelial NO release (Leikert et al., 2002). Released nitric oxide from endothelial cells through eNOS is a vascular protective molecule (Li and Fostermann, 2000).

Following concurrent use of 5 and 10 ml vinegar with cholesterolaemic diet, fibrinogen and factor VII levels were significantly decreased. Studies show that red wine consumption caused significant decreases in fibrinogen and factor VII (48) (Mukamal et al., 2001; Yarnel et al., 2000). Earlier experiments showed that coffee improved

fibrinolytic potential and t-PA activity (Samarrae and Truswell, 1997). In another study by Grenett et al. (2004), ethanol acts through MAPK cascades include the mitogen-activated protein kinase, c-jun NH₂-terminal kinase (jnk) and the extracellular signal regulated kinase (erk1/2) that activate transcription factors. This cascade activity regulates the expression of fibrinolytic proteins (t-PA, u-PA, PAI). Ethanol cause down regulation PAI-1 and up regulation t-PA, u-PA (Grenett et al., 2004). Investigation of Ogston et al. (1985) on the effect of apple cider vinegar procyanidine on t-PA, u-PA activity showed that it inhibits the t-PA, u-PA activity but its phelordizine and chlorogenic acids have no inhibitory effect (Ogston et al., 1985).

Using both doses of vinegar with cholesterolaemic diet induced a significant decrease in serum CRP level. Several studies indicated that flavonoids can inhibit some active enzymes during the inflammatory processes (Kwon et al., 2005). Prostaglandins and nitric oxide biosynthesis is involved in inflammation, and isoforms of inducible nitric oxide synthase (iNOS) and of cyclooxygenase (COX-2) are responsible for the production of a great amount of these mediators. Studies have indicated that nitric oxide production and the expression of iNOS inhibited by flavonoid quercetin (Martinez-Flores et al., 2005). Flavonoids can modulate the molecular events cascade in several critical steps that results in iNOS or COX-2 overexpression. Activation of a transcription essential for the expression of proinflammatory genes, the nuclear factor kappa B (NF-kappa B) converge the iNOS and COX-2 induction path

ways (García-Mediavilla et al., 2005).

CRP can be induced by IL-6 mechanism that involves NF-kappaB activation (Ahmad et al., 2002). Effects of flavonoids on CRP expression could be mediated by the modulation of the NF-kappaB-dependent pathway. It is indicated that 8 weeks use of apple cider vinegar by patients with diabetes 2 had no effect on CRP but could decrease the IL6, significantly (Golzarand et al., 2008). It is suggested that there is a reverse reaction between the use of vegetable and apple flavonoids (such as quercetin) and CRP also rich flavonoid food may decrease CR (Chung et al., 2008).

In this study, both doses of vinegar induced a significant decrease and ApoB/ApoA ratio. The effect of taxifolin (a plant flavonoid) on modulation of hepatic lipoprotein synthesis and secretion studied by Theriault et al (2000) and they concluded that ApoA secretion was found to increase by $36 \pm 10\%$. In contrast, an average reduction of $61 \pm 8\%$ in labeled ApoB was apparent with taxifolin (Theriault et al., 2000). Decrease of ApoB₁₀₀ suggests an effect on lipoprotein production, such as a delay in fat reabsorption and or a decrease in the secretion of the particles from enterocytes. Polyphenols have been shown to play a role in the controlling of key intracellular enzymes contributed in the synthesis and secretion of ApoB-containing lipoproteins (Wilcox et al., 2003). In this study dietary consumption of 5 and 10 ml vinegar with cholesterol diet significantly decreased serum TC. The results of Fushimi et al. (2006), indicated that dietary acetic acid reduces serum cholesterol concentration in rats fed a cholesterol-rich diet through inhibition of metabolic pathways of cholesterologenesis and lipogenesis in the liver, together with a concomitant enhancement of fatty acid oxidation and a stimulation of faecal bile acid excretion (Fushimi et al., 2006).

Histological results indicated that vinegar intake reduced atherosclerotic lesion in aorta, significantly compared to hypercholesterolaemic group. Since inflammation has an important role in atherosclerosis development, significant reduction in inflammatory lesion may be due to anti-inflammatory effect of vinegar.

Conclusion

In conclusion, vinegar attenuated several risk factors for atherosclerosis progression by lowering levels of ApoB, ApoB/ApoA ratio, fibrinogen, factor VII, CRP, TC, MDA, ox-LDL and increasing levels of nitrate, nitrite. Future studies must focus on determining similar effects of vinegar on human.

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REFERENCES

- Ahmad N, Chen LC, Gordon MA, Laskin J D, Laskin DL (2002). Regulation of cyclooxygenase-2 by nitric oxide in activated hepatic macrophages during acute endotoxemia. *J. Leuk. Biol.*, 1: 1005-1011.
- Aldini G, Carini M, Piccoli A, Rossoni G, Facino RM (2003). Procyanidins from grape seeds protect endothelial cells from peroxynitrite damage and enhance endothelium-dependent relaxation in human artery: new evidences for cardio-protection. *Life Sci.*, 73: 2883-2898.
- Andriambelosen E, Stoclet JC, Andratsitohaina R (1999). Mechanism of endothelial nitric oxide dependent vasorelaxation induced by wine polyphenols in rat thoracic aorta. *J. Cardiovasc. Pharmacol.*, 33: 248-254.
- Benito S, Lopez D, Sáiz MP, Buxaderas S, Sánchez J, Puig-Parellada P, Mitjavila MT (2002). A flavonoid-rich diet increases nitric oxide production in rat aorta. *Br. J. Pharmacol.*, 135: 910-916.
- Boger RH, Bode-Boger SM, Brandes RP, Phivthongam L, Bohme M, Nafe R (1997). Dietary L-arginine reduces the progression of atherosclerosis in cholesterol fed rabbits: comparison with lovastatine. *Circ.*, 96: 82-90.
- Bouloumie A, Bauersachs J, Linz W, Schölkens BA, Wiemer G, Fleming I, Busse R (1997). Endothelial dysfunction coincides with an enhanced nitric oxide synthase expression and superoxide anion production. *Hypertension*, 30: 934-941.
- Cermak J, Key NS, Bach RR, Balla J, Jacob H S, Vercellotti G M (1993). C-reactive protein induces human peripheral blood monocytes to synthesize tissue factor. *Blood*, 82: 513-520.
- Chan DC, Watts GF (2006). Apolipoproteins as markers and managers of coronary risk. *QJM*, 99: 277-287.
- Chekanov VS (2003). Low frequency electrical impulses reduce atherosclerosis in cholesterol fed rabbits. *Med. Sci. Monit.*, 9: 302-309.
- Chung OK, Chang SJ, Claycombe KJ, Song WO (2008). Serum C-reactive protein concentrations are inversely associated with dietary flavonoid intake in U.S. adults. *J. Nutr.*, 138: 753-760.
- Cuevas AM, Germain AM (2004). Diet and endothelial function. *Biol. Res.*, 37: 225-230.
- Decorde K, Teissedre PL, Auqe C, Cristo JP, Rouanet JM (2008). Phenolics from purple grape, apple, purple grape juice and apple juice prevent early atherosclerosis induced by an atherogenic diet in hamsters. *Mol. Nutr., Food Res.*, 2: 400-407.
- Ferlito S, Gallina M, Catassi S, Bisicchia A, Di Salvo MM (1999). Nitrite plasma levels in normolipemic and hypercholesterolemic patients with peripheral occlusive arteriopathy. *Panminerva Med.*, 41: 307-309.
- Francis FJ (1982). In anthocyanins as food colors. New York: Academic press, pp. 181-207.
- Fushimi T, Tayama K, Fukaya M, Kitakoshi K, Nakai N, Tsukamoto Y, Sato Y (2001). Acetic acid feeding enhances glycogen repletion in liver and skeletal muscle of rats. *J. Nutr.*, 131: 1973-1977.
- Fushimi T, Suruga K, Oshima Y, Fukiharuru M, Tsukamoto Y, Goda T (2006). Dietary acetic acid reduces serum cholesterol and triacylglycerols in rats fed a cholesterol-rich diet. *Br. J. Nutr.*, 95: 916-924.
- García-Mediavilla V, Crespo I, Collado PS, Esteller A, Sánchez-Campos S, Tuñón MJ, González-Gallego J (2007). The anti-inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein, and down-regulation of the nuclear factor kappaB pathway in Chang Liver cells. *Eur. J. Pharmacol.*, 28: 557(2-3).
- Gey KF, Stahelin HB, Eichholzer M (1993). Poor plasma status of carotene and vitamin C is associated with higher mortality from ischemic heart disease and stroke. Basel prospective study. *Clin. Invest.*, 71: 3-6.
- Golzarand M, Ebrahimi-Mamaghani M, Arefhosseini SR (2008). Effect of processed Berberis vulgaris in apple vinegar on blood pressure and inflammatory markers in type 2 diabetic patients. *Iran. J. Diab. Lipid Disorders*, 7: 3.
- Grenett HE, Wolkowicz PE, Lucchesi PA, Booyse FM (2004). Mapkinases mediate expression of fibrinolytic proteins by ethanol and

- polyphenols in human endothelial cells(ECS). *Biol. Res.*, 37(2): 343.
- Henneken CH, Gaziano JM (1993). Antioxidants and heart disease: epidemiology and clinical evidence. *Clin. Cardiol.*, 16: 110-115.
- Hertog MG, Kromhout D, Aravanis C (1995). Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch. Int. Med.*, 155: 381-386.
- Johnston CS, Gaas CA (2006). Vinegar: Medicinal uses and antiglycemic effect. *Med. Gen Med.*, 8(2): 61.
- Kelleher CC (1992). Plasma fibrinogen and factor VII as risk factors for cardiovascular disease. *Eur. J. Epidemiol.*, S1: 79-82.
- Kishi M, Fukaya M, Tsukamoto Y, Nagasava T, Takehana K, Nishizawa N (1997). Enhancing effect of dietary vinegar on the intestinal absorption of calcium in ovariectomized rats. *Biosci. Biotechnol. Biochem.*, 63: 905-910.
- Kondo S, Tayama K, Tsukamoto Y, Ikeda K, Yamori Y (2001). Antihypertensive effects of acetic acid and vinegar on spontaneously hypertensive rats. *Biosci. Biotechnol. Biochem.*, 65: 2690-2694.
- Kumar S, Kumar D, Rakash O (2008). Evaluation of antioxidant potential, phenolic and flavonoid contents of hibiscus tiliaceus flowers. *EJAFche*, 7: 2863-2871.
- Kwon KH, Murakami A, Tanaka T, Ohigashi H (2005). Dietary rutin, but not its aglycon quercetin, ameliorates dextran sulfate sodium-induced experimental colitis in mice: Attenuation of proinflammatory gene expression. *Biochem. Pharmacol.*, 69: 395-406.
- Leikert JF, Rathel TR, Wohlfart P, Cheynier V, Vollmar AM, Dirsch VM (2002). Red wine polyphenols enhance endothelial nitric oxide synthase expression and subsequent nitric oxide release from endothelial cells. *Circulation*, 106: 1614-1617.
- Li H, Fostermann U (2000). Nitric oxide in the pathogenesis of vascular disease. *J. Pathol.*, 190: 244-256.
- Libby P, Ridker PM (2002). Inflammation and atherosclerosis. *Circulation*, 105: 1135-1143.
- Liuzzo G, Biasucci LM, Gallimore JR (1994). The prognostic value of C-reactive protein and serum amyloid A protein in severe unstable angina. *New Engl. J. Med.*, 331: 417-424.
- Luckhoff A, Pohl U, Mulsch A, Busse R (1998). Differential role of extra and intracellular calcium in the release of EDRF and prostacyclin from cultured endothelial cells. *Br. J. Pharmacol.*, 95: 189-196.
- Martinez-flores S, Gutierrez-fernandez B, Sanchez-camps S, Gonzalez-gallego (2005). Quercetin prevents nitric oxide production and nuclear factor kappa B activation in interleukin 1- β activated rat hepatocytes. *J. Nutr.*, 135: 1359-1365.
- Mccormick DB, Greene HL (1994). Vitamins. In: Tietz Text Book Of Clinical Chemistry, edited by Carl A, Britis Edward R, Ashwood. Tietz Text Book Of Clinical Chemistry. Philadelphia, WB Saunders, 2: 1313-1314.
- Meade TW, Ruddock V, Stirling Y, Chakrabarti R, Miller GJ (1993). Fibrinolytic activity, clotting factors, and long-term incidence of ischaemic heart disease in the Northwick Park Heart Study. *Lancet.*, 342: 1076-1079.
- Morel I, Lescoat G, Cogrel P (1993). Antioxidant and iron chelating activities of flavonoids of the catechin, quercetin and diosmetin on ironloaded rat hepatocyte cultures. *Biochem. Pharmacol.*, 1: 13-19.
- Morrow DA, Rifai N, Antman EM (1998). C-reactive protein is a potent predictor of mortality independently of and in combination with troponin T in acute coronary syndromes: a TIMI 11A substudy. *J. Am. Coll. Cardiol.*, 31: 1460-1465.
- Mukamal KJ, Jadhav PP, DAgostino RB, Massaro JM, Mittleman MA, Lipinska I, Sutherland PA, Mathney T, Levy D, Wilson PW, Ellison RC, Silbershatz H, Muller JE (2001). Alcohol consumption and haemostatic factors: analysis of the Framingham offspring cohort. *Circulation.*, 104: 1367-1373.
- Noto D, Barbagallo CM, Cefalu' AB (2002). Factor VII activity is an independent predictor of cardiovascular mortality in elderly women of a Sicilian population: results of an 11-year follow-up. *Thromb. Haemost.*, 87: 206-210.
- Ogston D, Lea AG, Langhorne P, Wilson SB (1985). The influence of the polyphenols of cider on plasmin and plasminogen activators. *Br. J. Haematol.*, 60: 705-713.
- Pasceri V, Cheng JS, Willerson JT, Yeh ET, Chang J (2001). Modulation of C-reactive protein-mediated monocyte chemoattractant protein-1 induction in human endothelial cells by anti-atherosclerosis drugs. *Circulation*, 103: 2531-2534.
- Pasceri V, Willerson JT, Yeh ETH (2000). Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation*, 102: 2165-2168.
- Samarrae WA, Truswell AS (1997). Short-term effect of coffee on blood fibrinolytic activity in health adults. *Atherosclerosis*, 26: 255-260.
- Scharf W, Malerich CH (accessed Feb. 2008). Determination of acetic acid content of vinegar. http://www.baruch.cuny.edu/wsas/departments/natural_science/chemistry/chm_1000/vinegar.doc.
- Schuldt EZ, Ckless K, Simas ME, Farias MR, Riberio-Do-Valle RM (2000). Butanolic fraction from *Cuphea carthagenensis* Jacq McBride relaxes rat thoracic aorta through endothelium-dependent and endothelium independent. *J. Cardiovasc. Pharmacol.*, 35: 234-239.
- Shahidi F, McDonald J, Chandrasekara A, Zhong Y (2008). Phytochemicals of foods, beverages and fruit vinegars: chemistry and health effects. *Asia Pac. J. Clin. Nutr.*, 17(Suppl 1): 380-382.
- Shanmuganayagam D, Warner T, Folts J (2000). Effect of grape juice flavonoids on experimental atherosclerosis. *FASEB J.*, 14: A455-A455.
- Singer AH, Tsao PS, Wang BY, Bloch DA, Cooke JP (1995). Discordant effects of dietary L-arginine on vascular structure and reactivity in hypercholesterlemic rabbits. *J. Cardiovasc. Pharmacol.*, 25: 710-716.
- Stein JH, Keevil JG, Wiebe DA, Aeschlimann S, Folts JD (1999). Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation*, 100: 1050-1055.
- Theriault A, Wang Q, Van Iderstine SC, Chen B, Franke AA, Adeli K (2000). Modulation of hepatic lipoprotein synthesis and secretion by taxifolin, a plant flavonoid. *J. Lipid Res.*, 41: 1969-1979.
- Torzewski M, Rist C, Mortensen RF (2000). C-reactive protein in the arterial intima: role of C-reactive protein receptor-dependent monocyte recruitment in atherogenesis. *Arterioscler Thromb. Vasc. Biol.*, 20: 2094-2099.
- Victoria A (2009). Vinegar. Available from: <http://www.Madehow.com/volume7/vinegar.html>, access.
- Wilcox JN, Subramanian RR, Sundell CL, Tracey WR, Pollock JS, Harrison DG, Marsde PA (1997). Expression of multiple isoforms of nitric oxide synthase in normal and atherosclerotic vessels. *Arterioscler Thromb. Vasc. Biol.*, 17: 2479-2488.
- Wilcox LJ, Borradaile NM, de Dreu LE, Huff MW (2003). Secretion of hepatocyte apoB is inhibited by the flavonoids, naringenin and hesperetin, via reduced activity and expression of ACAT2 and MTP. *J. Lipid Res.*, 42: 725-734.
- Woodward D, Shanmuganayagam D, Folts J (2004). Grape extracts and risk factors for cardiovascular disease. *US Cardiol.*, 18: 45-55.
- Yarnell JW, Sweetnam PM, Rumley A, Lowe GD (2000). Lifestyle and hemostatic risk factors for ischemic heart disease: The caerphilly study. *Arterioscler Thromb. Vasc. Biol.*, 20: 271-279.