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Full Length Research Paper

Effects of unripe grape juice (verjuice) on plasma lipid profile, blood pressure, malondialdehyde and total antioxidant capacity in normal, hyperlipidemic and hyperlipidemic with hypertensive human volunteers

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There is a widespread belief in Iranian society that verjuice consumption has useful effects on blood lipid profile and hypertension. This study was designed to examine this hypothesis. This study subjects included three groups: (A) healthy individuals volunteers of age 20 to 30 years; (B) hyperlipidemic patients of age 30 to 60 years old; and (C) hyperlipidemic plus hypertensive patients of age 30 to 60 years. All subjects were asked to consume 200 ml of verjuice twice per day for one month. At the beginning of this study and then every two weeks, blood pressure and heart rate were measured. Blood sample were also collected and all parameters consisted of plasma lipid profile, total antioxidant capacity (TAC), and malondialdehyde (MDA) were measured. There was no significant difference between the levels of lipid profile components, heart rate, and blood pressure before and after consumption of the verjuice in healthy volunteers. However, in all the three groups, the concentration of TAC and high density lipoprotein cholesterol (HDL-C) to low density lipoprotein cholesterol (LDL-C) ratio was increased and MDA concentration was reduced respectively, in all subjects after verjuice consumption. In hyperlipidemic and hyperlipidemic plus hypertensive peoples, administration of verjuice, after four weeks (no two weeks) resulted in significant reduction of blood pressure along with the reduction of LDL-C, triglyceride (TG), and total cholesterol (TC) concentrations. In conclusion, although blood pressure and lipid profile disorders did not completely return to normal levels by verjuice consumption, it seems that it is used as a flavoring, not devoid of benefit may be due to its antioxidant effects.

Key words: Verjuice, blood pressure, blood lipid profile, malondialdehyde (MDA), total antioxidant capacity (TAC).

INTRODUCTION

Hypertension (HTN) is a progressive cardiovascular (CV) syndrome arising from complex and interrelated etiologies (Giles et al., 2011). It is the most common form of CV disease, affecting millions of people throughout the world and about 20% of the adult population in many countries. HTN is interconnected with coronary artery disease, stroke, congestive heart failure, and renal dysfunction. HTN, as one of the major risk factors for CV-related mortality accounts for 20 to 50% of all deaths (Yang et al., 2004). HTN is defined as a constantly elevated blood pressure further than 140 over 90 mmHg, a systolic pressure above 140 with a diastolic pressure above 90 mmHg. Chronic HTN is a "silent" condition; it

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has no symptoms and is the leading cause of many life threatening or disabling diseases. Many people have HTN without knowing it. Therefore, it is important to have blood pressure checked regularly. Blood pressure measurements are classified in stages, according to severity. Normal blood pressure: less than 120/80 mmHg, pre-HTN: 120 to 129/80 to 89 mmHg, stage 1 HTN: 140 to 159/90 to 99 mmHg and stage 2 HTN: at/or greater than 160 to 179/100 to 109 mmHg (Giles et al., 2011). Although, many factors including age, sex, race, heredity, self sensitivity, obesity, inactive life style, etc., are involved in HTN (secondary HTN); nevertheless, the cause of most HTN is not known (Primary or essential HTN) (Reaven et al., 1996). Adequate treatment of HTN can diminish health risks, recover quality and quantity of life, and reduce overall costs of health care. Treatment to lower blood pressure may include dropping salt intake, reducing fat intake, losing weight, getting regular exercise, quitting smoking, reducing alcohol consumption, managing stress, and taking antihypertensive medications (Sacks et al., 2001).

For many years, it has been a widespread belief in our society that certain foods and herbal drugs have useful effects for lipid and blood pressure lowering and several studies also established the efficacy of some of them (Aminian et al., 2006; Caron and White, 2001). Unripe grape juice (verjuice or verjus) is one of these agents. Verjuice is an unfermented green grape juice obtained by directly pressing green grapes. It has a unique flavor and sour taste. Verjuice is used as an alternative to vinegar and lemon juice (Aminian et al., 2003). Verjuice is produced and consumed locally, particularly in the Mediterranean, Southeastern regions of Turkey, and many different regions of Iran. It has been used to augment the flavor of traditional meals, salads, and appetizers, and as an ingredient in the production of various drinks and several sausages such as mustard sausage (Hildebrandt and Matchuk, 2002; Hayoglui et al., 2009). The use of verjuice as a food ingredient and as a medicine has a long history, from 370 to 460 B.C until today (Pour Nikfardjam, 2008). Verjuice, which is known as "Abe ghureh" in Iran, is still widely used for cooking in our country. According to Iranian folk medicine, verjuice has antilipidemic and antihypertensive effects. The interest of Western countries has been attracted to this product in recent years. Although, some information are currently available on the chemical composition and polyphenolic composition of this food stuff; however, few studies have been conducted regarding the medical use of verjuice, which analyze the effect of its ingestion on health. Aminian et al. (2006) have investigated the impact of verjuice on plasma lipid level in rabbit. Their results show that verjuice has no preventive or therapeutic effect in hypercholesterolemia (Pour Nikfardjam, 2008). To the best of our knowledge, very limited information is available regarding the effect of verjuice on blood pressure, plasma lipid profile, and oxidative stress indices in humans. Thus, this work aimed to investigate the effects of 4 weeks Iranian verjuice ingestion on blood pressure, plasma lipid profile, total antioxidant capacity (TAC) and malondialdehyde (MDA) in normal, hyperlipidemic, and hypertensive human volunteers.

SUBJECTS AND METHODS

This study was carried out in the Department of Physiology, Zanjan University of Medical Sciences and Department of Biology, Zanjan Payam Noor University, Zanjan, Iran. The study of verjuice effects on HTN, lipid profile TAC, and MDA was conducted on three groups of people. Group 1: normal volunteers, thirteen male of healthy and nonsmoking volunteers (Students of Payam Noor University) aged between 20 and 30 years, who were under no medication and without any history of disease. Group 2: this group included 11 male patients of age 30 to 60 years old, who had abnormal blood lipids and normal blood pressure, but they were unaware of their blood lipid disorders. Group 3: this group included 7 male patients of age 30 to 60 years old, who had high blood pressure and abnormal blood lipids as well. They were all aware of their condition, previously. Adequate and accurate description was given to all the people about their condition and the research. Written consent was obtained from all the participants.

Verjuice preparation and consumption

Unripe green grapes were bought from the market and its juice was extracted by means of pressing grapes using the juice making machine and the juice obtained was sieved through a nonmetal filter. The juice was transferred into bottles and was kept in refrigerator for consumption. All subjects were asked to consume 200 ml of verjuice twice per day (night, before sleeping and morning, after waking up) for one month. All persons sustained their habitual diets throughout the study. At three time intervals, the start of the study and after two and four weeks of the verjuice consumption, blood pressure and heart rate was measured. Also, blood samples (15 ml) were collected in the morning from each individual after 12 h of fasting and plasma was immediately separated by centrifugation at 5000 rpm for 15 min at room temperature for determination of biochemical parameters.

Blood pressure measurement and heart rate

Baseline, blood pressure was measured by the auscultatory method with a random zero mercury sphygmomanometer and standard cuff (12 × 34 cm). The blood pressure measurement was taken in the seated position, quietly in a chair with feet on the floor and an arm support at the heart level. After recording the systolic and diastolic pressures, mean arterial pressures (MAP) was calculated by the following formula: MAP = diastolic pressure + (systolic pressure - diastolic pressure) / 3 (Bern and Levy, 2010). Heart rate (HR) was also measured by finding the pulse of the radial artery via pressuring the thumb on the ventral aspect of the wrist (Sharma et al., 2011).

Biochemical measurements

Blood samples were drawn after fasting for 12 h before verjuice administration in order to plasma lipid profile, MDA, and TAC measurements. All persons continued their habitual diets during the study.

Table 1. Plasma lipid levels (mg/dl), MAP (mmHg), and HR in human healthy volunteers during one month verjuice consumption.

Variable	Start (Mean ± SD)	2 weeks (Mean ± SD)	4 weeks (Mean ± SD)	p-value
LDL-C	170.9 ± 7.2	168.4 ± 7.3	165.5 ± 7.4	0.04
HDL-C	48.2 ± 4.9	49.6 ± 4.9	50.7 ± 5	0.45
HDL-C/LDL-C	0.28 ± 0.03	0.29 ± 0.03	0.30 ± 0.03	0.13
TG	180.6 ± 16	178.2 ± 15.2	175.3 ± 14.7	0.65
TC	254.7 ± 3.8	252.3 ± 4.1	249.5 ± 4.5	0.04
MAP	94.7 ± 2.2	94.6 ± 1.6	94.1 ± 1.6	0.56
RH	74.8 ± 1.9	74.2 ± 1.9	73.8 ± 1.8	0.41

Measurements were performed at the start (before verjuice consumption) and every 2 weeks (after start of verjuice consumption). TC, Total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; MAP, mean arterial pressure; HR, heart rate. Significant level ($p < 0.05$).

Plasma lipid profile including total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglyceride (TG) were determined by enzymatic methods using automatic analyzer (Abbott, Alcyon 300, USA). Plasma TAC concentration was determined in 600 nm at 37°C against air by spectrophotometer, according to the guidelines coming with the relevant kit (TAC kit, Randox Co, Germany) and its concentration was calculated by the related formula (kit direction) and was expressed as mmol/L. The amount of malondialdehyde (MDA) was determined by thiobarbituric acid (TBA) assay. All reagents that were used in this assay were obtained from Merck (Darmstadt, Germany). Briefly, 0.50 ml of plasma was added to 3 ml of 1% phosphoric acid, 1 ml of 0.60% TBA, and 0.15 ml of 0.20% butylated hydroxytoluene in 95% methanol. The samples were heated in a boiling water bath for 45 min, cooled, and 4 ml of 1-butanol was added. The butanol phase was separated by centrifugation at 3000 rpm for 10 min, and absorbance was measured at 532 nm. The concentration of MDA was calculated and was expressed as µM (Alipour et al., 2006).

Statistical analyses

Data are expressed as the mean ± SD. All statistical analyses were performed using Kruskal–Wallis test, followed by the Mann–Whitney U test (Statistical Package for Social Sciences (SPSS) software version 16). In all statistical evaluations, $p < 0.05$ was considered as the criterion for statistical significance.

RESULTS

Based on our results, the plasma levels of LDL-C, HDL-C, TG, and TC after one month verjuice consumption (200 ml twice per day) were not significantly lesser than the equivalent levels before the verjuice consumption in healthy volunteers. Also, no significant difference was observed between the blood pressure and heart rate before and after verjuice consumption (Table 1). However, in these subjects, the levels of TAC for two and four weeks after starting the trial (verjuice consumption), were significantly higher than the corresponding levels before the verjuice consumption ($p = 0.005$). In contrast to TAC and MDA concentration, an index of lipid peroxidation was significantly reduced ($p = 0.005$) in response to 4 weeks verjuice consumption (Figure 1).

Table 2 shows the plasma LDL-C, HDL-C, TG, and TC concentrations in hyperlipidemic subjects (group II) before and two and four weeks after verjuice consumption. Means comparison showed significant differences only in TC and LDL fractions. Significant reduction was observed in plasma levels of LDL-C ($p = 0.019$) and TC ($p = 0.016$) in hyperlipidemic subjects receiving verjuice for four weeks. Positive changes but non-significant statistically was found in other parameters including HDL-C, TG, and HDL-C/LDL-C. MDA values were high in hyperlipidemic subjects when compared with the normal subjects (Figure 2). Mean amounts of MDA was reduced noticeably ($p = 0.005$) after 2 and 4 weeks of verjuice consumption in hyperlipidemic subjects than before verjuice consumption (Figure 2). Unlike MDA, a signify-cant increase was found in plasma levels of TAC after 4 weeks of verjuice consumption. Reduction of MDA and elevation of TAC levels were depended on the duration of the verjuice consumption. That is, MDA concentration was lower after 4 weeks of verjuice consumption than 2 weeks. The same results, but unlike the MDA, was seen for TAC levels (Figure 2).

In group 3 (hyperlipidemic along with hypertension subjects), in addition to the significant decrease of LDL-C and TC, that were also seen in the previous trial (group 2; HDL-C concentration and the proportion of HDL-C to LDL-C were significantly increased following the consumption of verjuice as compared to starting stage ($p = 0.002$ to 0.007)) (Table 3). A significant reduction and a significant elevation were found in plasma levels of MDA and TAC, respectively, in subjects receiving verjuice for 2 and 4 weeks (Figure 3).

Interestingly, 2 and 4 weeks of verjuice consumption resulted in the significant reduction of mean arterial pressure in hypertensive-hyperlipidemic subjects in comparison to the starting stage (before verjuice consumption) ($p = 0.003$, $p = 0.005$, respectively). There was no statistically significant difference between the heart rate at the start of the study and following 2 and 4 weeks of verjuice consumption.

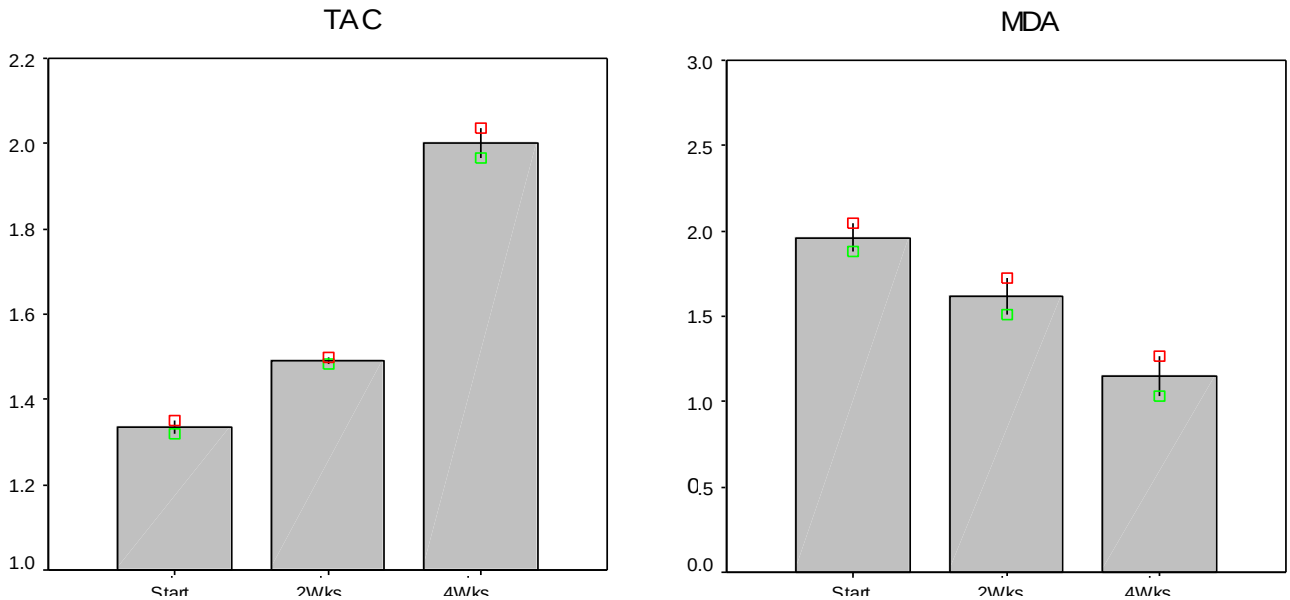

Figure 1. TAC (left) and MDA (right) concentrations before and after 2 and 4 weeks of verjuice consumption in healthy volunteers. The concentration of TAC increased during verjuice consumption as compared to before consumption ($p < 0.005$). The reduction of MDA concentration was significant ($p < 0.005$) after 4 weeks of verjuice consumption. Data are mean ± SD. Abbreviations: TAC, total antioxidant capacity; MDA, malondialdehyde; wks, weeks.

Table 2. Comparison of plasma lipid levels (mg/dl), MAP (mmHg) and HR in hyperlipidemic subjects before and after verjuice consumption (every 2 weeks).

Variable	Start (Mean ± SD)	2 weeks (Mean ± SD)	4 weeks (Mean ± SD)	p-value
LDL-C	170.9 ± 7.2	168.4 ± 7.3	165.5 ± 7.4	0.04
HDL-C	48.2 ± 4.9	49.6 ± 4.9	50.7 ± 5	0.45
HDL-C/LDL-C	0.28 ± 0.03	0.29 ± 0.03	0.30 ± 0.03	0.13
TG	180.6 ± 16	178.2 ± 15.2	175.3 ± 14.7	0.65
TC	254.7 ± 3.8	252.3 ± 4.1	249.5 ± 4.5	0.04
MAP	94.7 ± 2.2	94.6 ± 1.6	94.1 ± 1.6	0.56
RH	74.8 ± 1.9	74.2 ± 1.9	73.8 ± 1.8	0.41

Measurements were performed at the start (before verjuice consumption) and every 2 weeks (after start of verjuice consumption). TC, Total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; MAP, mean arterial pressure; HR, heart rate; wks, weeks. Significant level ($p < 0.05$)

Table 3. Comparison of plasma lipid levels (mg/dl), MAP (mmHg) and HR in hyperlipidemic plus subjects before and after verjuice consumption (every 2 weeks).

Variable	Start (Mean ± SD)	2 weeks (Mean ± SD)	4 weeks (Mean ± SD)	p-value
LDL-C	210 ± 13.5	201.6 ± 10.1	194.7 ± 7.4	0.05
HDL-C	51.6 ± 2.6	57.6 ± 1.9	59.9 ± 2.2	0.001
HDL-C/LDL-C	0.25 ± 0.02	0.28 ± 0.02	0.31 ± 0.01	0.001
TG	212.9 ± 14	208.7 ± 13.9	203.6 ± 12.9	0.35
TC	268.4 ± 44.4	246.3 ± 13.4	240.2 ± 13.5	0.001
MAP	111.1 ± 2.1	107.1 ± 1.8	105.9 ± 2	0.002
RH	84.1 ± 6.1	83.3 ± 5.9	81.6 ± 5.1	0.64

Measurements were performed at the start (before verjuice consumption) and every 2 weeks (after start of verjuice consumption). TC, Total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; MAP, mean arterial pressure; HR, heart rate. Significant level ($p < 0.05$)

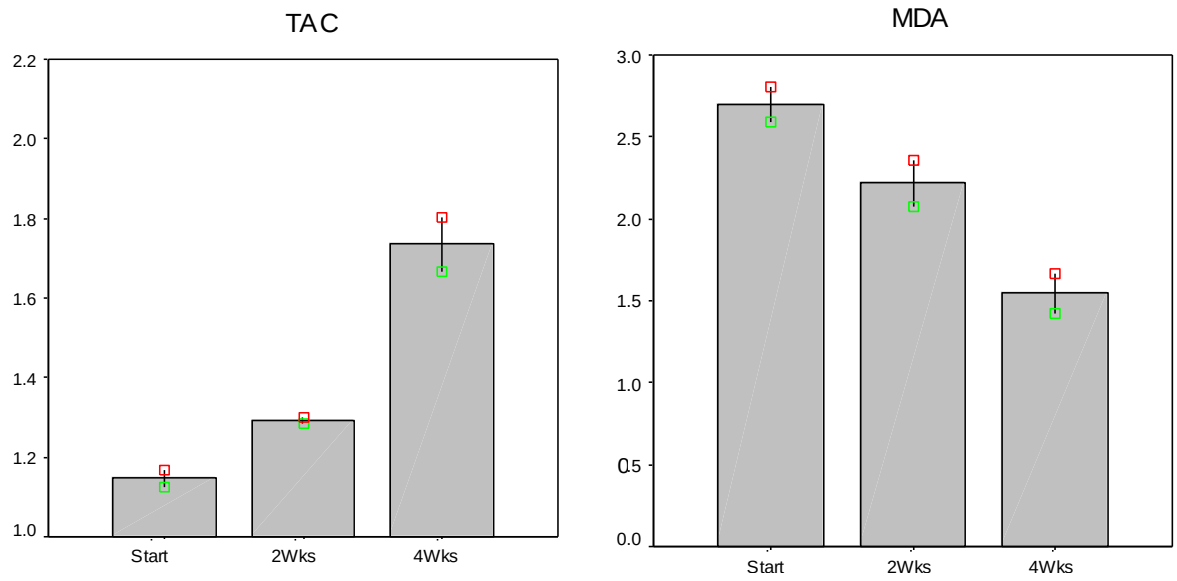

Figure 2. TAC (left) and MDA (right) concentrations before and after 2 and 4 weeks of verjuice consumption in hyperlipidemic subjects. Concentration of TAC was augmented in response to 4 weeks of verjuice consumption compared to start stage (p = 0.005). A significant reduction in MDA concentration was seen after 2 and 4 weeks of verjuice consumption compared with the start stage (p = 0.005). Data are mean ± SD. TAC, total antioxidant capacity; MDA, malondialdehyde; wks, weeks.

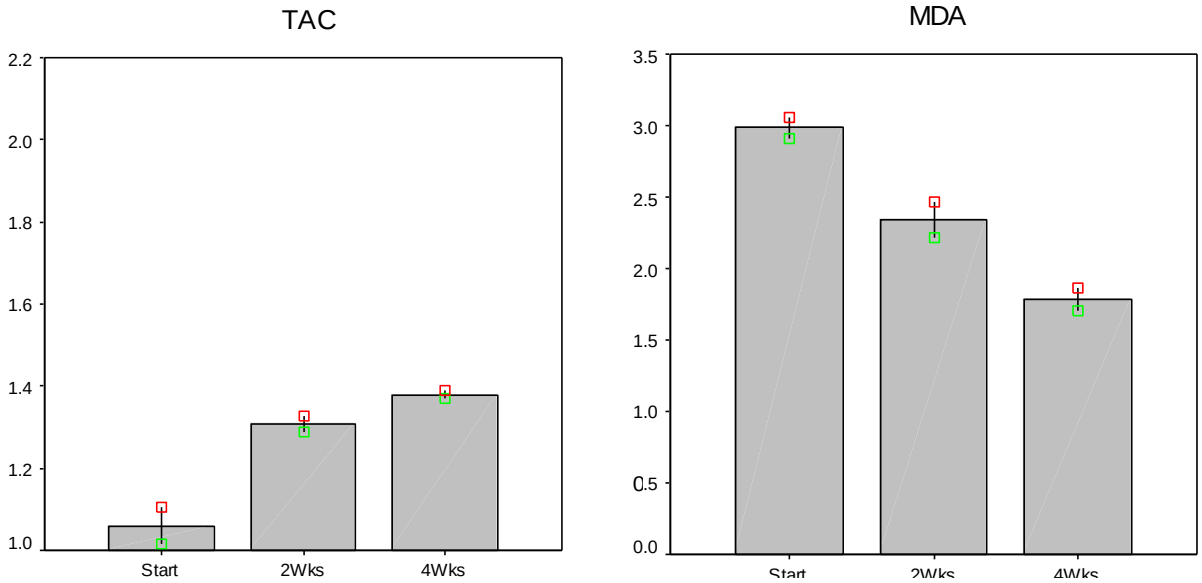

Figure 3. TAC (left) and MDA (right) concentrations before and after 2 and 4 weeks of verjuice consumption in hyperlipidemic along with hypertension subjects. Concentration of TAC was augmented in response to 2 and 4 weeks of verjuice consumption compared to start stage (p = 0.005). A significant reduction in MDA concentration was seen after 2 and 4 weeks of verjuice consumption compared with the start stage (p = 0.005). Data are mean ± SD. TAC, total antioxidant capacity; MDA, malondialdehyde; wks, weeks.

DISCUSSION

The major findings of this study were that consumption of verjuice for one month ameliorates mean arterial pressure that was with the reduction of MDA as an indirect measure of oxidative stress and elevation of TAC. In addition, positive changes were observed in plasma lipid profile including decrease of LDL-C and TC and increase

of HDL-C and HDL-C to LDL-C ratio.

The consumption of verjuice as a food flavoring and as a medicine has a long history. It has been used as food ingredient and as a medicine for treatment of ulcers and as a digestive agent after the ingestion of fatty foodstuff such as brain and especially a particular type of food known as "kalah-pacheh" in Iran (Pour Nikfardjam, 2008). Despite the widespread uses of verjuice, limited scientific information regarding the chemical composition and particularly about its effects on physiological and pathological conditions such as hypertension and lipid profile are available. Aminian et al. (2006) studied the effect of verjuice on plasma lipid levels in rabbits after egg yolk ingestion. In contrast to our findings with regard to the effect of verjuice as a lipid lowering agent, their results show that verjuice has no preventive or therapeutic effect in hypercholesterolemia. In human studies, it has been reported that verjuice has no lipid-lowering effect. Based on their work, in hyperlipidemic subjects, who had received 80 ml verjuice daily at lunch time for the 4 months, TC and TG decreased significantly at the end of the study, but authors suggested that this result may be the result from the effect of diet and not verjuice (Aminian et al., 2003).

HDL-C is known to be a significant and independent predictor of coronary heart disease (CHD) risk (Eccleston et al., 2002). A significant increase was found in plasma levels of HDL-C in subjects receiving verjuice for 4 weeks. However, Aminian et al. (2006) showed that verjuice consumption had no incremental effect on serum HDL-C levels in healthy individuals and hyperlipidemic subjects. To the best of our knowledge, regardless limited studies of Aminian et al. (2006), no animal or human study regarding the effect of verjuice consumption on plasma lipid profile have been published. On the other hand, few studies show the effect of grape juice on hyperlipidemia. In support of our results, it has been reported that red grape juice consumption is associated with parallel reduction in the LDL-C and elevation in HDL-C concentrations (Castilla et al., 2006). However, Coimbra et al. (2005) reported no effect on HDL-C levels after consumption of purple grape juice in healthy persons for 14 days. In addition, red grape juice consumption is coupled with significantly lower cholesterol/HDL as atherogenic index, which is usually used as the best lipid parameter for determining human heart disease risk (Vinson et al., 2001).

In vitro studies showed that grape juice has significant antioxidant activity and can inhibit the oxidation of LDL, and consequently, nonalcoholic red grape extract may have a similar beneficial effect to red wine (Day et al., 1997). According to a few published studies, general composition and polyphenolic content of verjuice show various differences in many regions and countries. Polyphenolic content of Iranian verjuice reported about 780 to 1330 mg/L (Pour Nikfardjam, 2008). Discrepancies between our results and other studies (related to lipid profile) may be connected to differences in the composition and polyphenols content of verjuice, time of consumption, and amount of consumption. Grape juice and verjuice are very similar in structure and both have a flavonoid component, which may have an antioxidant effect on plasma lipoproteins and other molecules. This study was designed to assess whether verjuice consumption could in particular contribute to the MDA decrease and TAC increase as indicators of oxidative stress conditions and changes in lipid profile. In this study, we observed a significant increase in the MDA level of hyperlipidemic and hypertensive patients before treatment with verjuice which suggests the presence of increased oxidant stress. The total antioxidant status in these patients decreased significantly in comparison to the after treatment with verjuice. Therefore, it seems that a negative correlation between the total antioxidant status and lipid peroxidation in the plasma of healthy, hyperlipidemic, and hyperlipidemic-hypertensive patients.

MDA reflects both autoxidation and oxygen mediated peroxidation of polyunsaturated fatty acids in particular. It reflects the oxidative status of the biological system. MDA causes damage to LDL which in turn forms foam cells finally. Due to increased production of reactive oxygen species (ROS) and increased oxidative stress, lipid peroxidation products are found to be elevated in hyperlipidemic and hypertensive patients (Meera, 2011). In healthy conditions, a balance exists between free-radical generation and antioxidant defense system which prevents occurrence of disease. This study implies that hyperlipidemia and hypertension shift the balance in favor of free-radical generation which leads to oxidative tissue damage (Das et al., 2000), whereas, it seems the verjuice consumption increases the total antioxidant capacity and eliminates the harmful effects of free radicals. Thus, we can conclude that verjuice has antioxidant properties, and its beneficial effects may be related to this effect.

To the best of our knowledge, any information is currently available about the effects of verjuice consumption on plasma levels of MDA and TAC in normal subjects, hyperlipidemia, and hypertension in both animals and humans.

Furthermore, this is the first study that investigates the effect of verjuice consumption on blood pressure in human. Hypertension is linked with a number of alterations in the vascular system, including augmentation of vascular tone, amplified shear stress, and activation of the sympathetic nervous and renin-angiotensin-aldosterone systems, because these functional and structural changes promote endothelial damage. Hyperlipidemia is prevalent in hypertension, but the cause of this association is unknown.

Impaired endothelial function is associated with increased total-C/HDL-C values, perhaps as the result of increased vascular oxidative stress and inflammation (Sugiura et al., 2011). Oxidative stress may contribute to

the production and/or preservation of hypertension via the number of possible mechanisms, including quenching of the vasodilator nitric oxide (NO) by ROS such as superoxide, production of vasoconstrictor lipid peroxidation products, such as F2-isoprostanes and MDA, diminution of tetrahydrobiopterin (BH4), an important NO synthase (NOS) cofactor, as well as structural and functional alterations within the vasculature. These vascular changes may be mediated in different ways (Ward and Croft, 2006). In this study, plasma TAC level was significantly reduced and plasma MDA level was significantly raised in hyperlipidemic and hyperlipidemic with hypertension. These changes suggest an association between increased oxidative stress and the mentioned disorders. Reverse relations in MDA and TAC levels that were observed following consumption of verjuice may be attributed to the antioxidative properties of verjuice which resulted in the reduction of oxidative stress and thus improvement of blood pressure.

In conclusion, this study indicates that the consumption of verjuice resulted in noticeable raise in TAC levels and marked reduction in MDA levels in hyperlipidemic and hyperlipidemic with hypertensive patients, which are associated with the reduction of mean arterial pressure. Although, blood pressure and lipid profile disorders did not completely return to normal levels by verjuice consumption; it seems that its drinking as a flavoring, is not devoid of benefits, may be due to its antioxidant properties.

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REFERENCES

Alipour M, Mohammadi M, Zarghami N, Ahmadiasl N (2006). Influence of chronic exercise on red cell antioxidant defense, plasma MDA and TAC in hypercholesterolemic rabbits (2006). J. Sport Sci. Med. 5:682-691.

Aminian A, Aminian B, Nekooian AA, Hoseinali F (2006). Effects of unripe grape juice (verjuice) on plasma lipid level in rabbits rendered hypercholesterolemic by feeding egg yolk. Acta Medica Iranica. 44(4):232-234.

Aminian B, Massoompour SM, Sadeghalvaad A (2003). Unripe grapes juice (vejuice) as a lipid lowering agent: fact or fiction. Arch Iranian Med. 6:32-34.

Bern RM, Levy MN, Koeppen BM, Stanton BA (2010). Physiology. 5th Ed. Mosby, Missouri, pp. 357-365.

Caron MF, White CM (2001). Evaluation of the antihyperlipidemic properties of dietary supplements. Pharmacotherapy 21(4):481-487.

Castilla P, Echarri R, Davalos A, Cerrato F, Ortega H, et al (2006). Concentrated red grape juice exerts antioxidant, hypolipidemic, and antiinflammatory effects in both hemodialysis patients and healthy subjects. Am. J. Clin. Nutr. 84:252-262.

Coimbra SR, Lage SH, Brandizzi L, Yoshida V, da Luz PL (2005). The action of red wine and purple grape juice on vascular reactivity is independent of plasma lipids in hypercholesterolemic patients. Braz. J. Med. Biol. Res. 38:1339-1347.

Das S, Vasisht S, Nibhriti Das S, Srivastava LM (2000). Correlation between total antioxidant status and lipid peroxidation in hypercholesterolemia. Current Sci. 78:486-487.

Day AP, Kemp HJ, Bolton C, Hartog M, Stansbie D (1997). Effect of concentrated red grape juice consumption on serum antioxidant capacity and low-density lipoprotein oxidation. Ann. Nutr. Metab. 41:353–357.

Eccleston C, Baoru Y, Tahvonen R, Kallio H, Rimbach GH, Minihane AM (2002). Effects of an antioxidant-rich juice (sea buckthorn) on risk factors for coronary heart disease in humans. J. Nutr. Biochem. 13:346-354.

Giles TD, Materson BJ, Cohn JN, kostis JB (2011). Definition and Classification of Hypertension: An Update. J. Clin Hypertension. 11(11):61-614.

Hayoglui I, Kola O, Kaya C, Ozer S, Turkoglui H (2009). Chemical and sensory properties of verjuice, a traditional Turkish non-fermented beverage from Kabarcik and yediveren grapes. J. Food Process. Preserv. 33:252–263.

Hildebrandt A, Matchuk J (3003). Publishing Co, Northbrook, IL. http:// www.foodproductiondesign.com (accessed March 6, 2003).

Meera KS (2011). Oxidative imbalance in smokers with and without hypertension. Biomed. Res. *22*(3):267-272.

Pour Nikfardjam MS (2008). General and polyphenolic composition of unripe grape juice (verjus/ verjuice) from various producers. Mitteilungen klosterneuburg. 58:28-31.

Reaven GM, Lithell H, Landsberg L (1996). Hypertension and associated metabolic abnormalities-The role of insulin resistance and the sympathoadrenal system. N Engl J Med. 334 (6): 374-381.

Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, Obarzanek E, Conlin PR, Miller III ER, Simons-Morton DG, Karanja N, Lin PH (2001). Effects on blood pressure of reduced dietary sodium and the dietary approaches to stop hypertension (dash) diet. N Engl. J. Med. 344:1-9.

Sharma SK, Ghimire A, Radhakrishnan J, Thapa L, Shrestha NR, Paudel, Gurung K, Maskey R, Budathoki A, Baral N, Brodie D (2011). Prevalence of Hypertension, Obesity, Diabetes, and Metabolic Syndrome in Nepal. Int. J. Hypertension. pp.1-9.

Sugiura T, Dohi Y, Yamashita S, Yamamoto K, Wakamatsu Y, Tanaka S, Kimura G (2011). Impact of lipid profile and high blood pressure on endothelial damage. J Clin Lipidol. 5(6):460-666.

Vinson JA, Teufel K, Wu N (2001). Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in a hamster model. Atherosclerosis 156:67-72.

Ward NC, Croft KC (2006). Hypertension and oxidative stress. Clin. Exp. Pharmacol. Physiol. 33:872–887.

Yang Y, Lu FH, Wu JS, Wu CH, Chang CJ (2004). The protective Effect of Habitual Tea Consumption on Hypertension. Arch Int. Med. 164(14):1534-1540.