

EFFECT OF RED GRAPE POLYPHENOLS EXTRACT ON SERUM LIPID PROFILES AND HOMOCYSTEINE CONCENTRATIONS IN HYPERCHOLESTEROLEMIC RATS

(Received: 27.6.2007)

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ABSTRACT

The aim of this study was to investigate the effect of two levels of red grape polyphenols extract on serum lipid profiles and total homocysteine (tHcy) concentration in hypercholesterolemic rats. Twenty four male albino rats were classified into four groups (6 rats each one). The first group received standard diet (negative control), the second group received hypercholesterolemic diet (positive control). The last two groups received hypercholesterolemic diets and orally administered with 0.5 mg and 1 mg/kg body weight of the redgrape polyphenols extract, respectively. Results showed that treatment with red grape polyphenols extract at a level of 0.5 mg/kg body weight significantly reduced serum concentration of triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) by 29.5%, 57.2%, 68.3% and 18.2 %, respectively compared to the positive control. By comparing to the negative control, the reduction in serum concentration of TG, TC and LDL-C was 27.4%, 21.2% and 40.0%, respectively. Serum HDL-C concentration was elevated compared to negative control, but not significantly. Increasing the treatment dose to 1 mg/kg body weight did not alter the previous results compared to treatment with 0.5 mg/kg body weight. Treatment with both levels of the polyphenols extract slightly reduced serum tHcy concentration compared to both controls. There was no significant difference between the two levels of the extract on serum tHcy concentration. These results suggest that the lower concentration of red grape polyphenols extract was effective in prevention and treatment of cardiovascular disease *via* hypolipidemic action rather than lowering blood tHcy concentration.

Key words: *atherogenesis, cardiovascular, cholesterol, disease, homocysteine, polyphenols, red grape and triglycerides.*

1. INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality all over the world. CVD including coronary heart disease, strokes and peripheral vascular disease, is the clinical expression of atherosclerosis. There are two major nutritional causes for CVD; first is hypercholesterolemia which has been reported in several studies as a major factor of CVD (Frohlich and Lear, 2002; Kruth, 2001). The second cause is homocysteine. Several studies reported that a high total homocysteine (tHcy) concentration in blood is associated with an increase risk of CVD, and considered as independent risk factor of CVD (Refsum *et al.*, 1998; Hankey and Eikelboom, 1999). Epidemiological studies have shown that consumption of foods and beverages rich in phenolic compounds can reduce the risk of CVD.

Total extracts from traditional natural plants, rich in phenolic compounds, have been shown to inhibit the development of CVD in animal models (Chen *et al.*, 2003; Kim *et al.*, 2003). Although polyphenols and related compounds are widely distributed and abundant in plant foods, especially fruits and vegetables, and some beverages such as fruit juices and tea, polyphenols in grape and its products have received much attention in treatment and prevention of CVD (Folts, 2002; Teissedre *et al.*, 1996; Vinson *et al.*, 2001). Grape seed polyphenolic flavonoids were examined for their anti-atherosclerotic effect in cholesterol-fed rabbits (Yamakoshi *et al.*, 1999). Feeding proanthocyanidin rich extract, 0.1 and 1% in the diet (w/w) to rabbits significantly reduced severe atherosclerosis in the aorta. Immunohistochemical analysis revealed a decrease in the number of

oxidized low density lipoprotein (LDL) positive macrophage-derived foam cells in atherosclerotic lesions in the aorta of rabbits fed proanthocyanidin rich extract. Grapes are comprised of a wide variety of polyphenols including resveratrol (Sitolbene), flavonoids and its derivatives, flavons, flavonoids and anthocyanins. These polyphenols were thought to have beneficial effect on CVD *via* lowering blood cholesterol. This was examined by individual phenolic compound such as the lowering effect of flavonoid intake on risk of CVD (Hertog *et al.*, 1995) or by the consumption of grape products, especially wine. For years, red wine was thought to have beneficial effect on CVD (Renaud and Delorgeril, 1992). In addition, concentrated red grape juice was found to have a hypolipidemic effect in human subjects (Castilla *et al.*, 2006). On the other hand, the effect of grape polyphenols on homocysteine was examined in very limited studies. Resveratrol, a phenolic antioxidant synthesized in grapes and present in grape products such as wine, was found to prevent homocysteine accumulation in human subjects (Schroeksnadel *et al.*, 2005). Other polyphenols in other foods were found to have different effects. Chlorogenic acid, a major polyphenol in coffee and black tea, was found to raise tHcy in human subjects (Olthof *et al.*, 2001). In contrary, moderate to high consumption of black tea was not found to effect tHcy concentrations in human subjects (Hodgson *et al.*, 2003). The effect of red grape polyphenols (total extract) on blood lipid and tHcy concentration in hypercholesterolemic models has not been investigated yet. Therefore, the aim of this study was to investigate the effect of two different levels of red grape polyphenol extract on serum lipid profile and tHcy concentrations in hypercholesterolemic rats.

2. MATERIALS AND METHODS

Dark red grapes (*Vitis vinifera* L.) were purchased from the local market in Riyadh-Saudi Arabia. Briefly, red grapes were washed with water at 60°C for 2 hr. and then soaked and homogenized with petrolcum either (80-100 °C) , 500 g/L and stored at room temperature overnight for defatting. The mixture was filtered and the petroleum ether fraction was discarded. The defatted grapes were homogenized again in 80 % aqueous ethanol (20 g / L) and stored overnight at 5°C then filtered. The ethanolic extract was evaporated using rotary evaporator at a temperature below 35°C under vacuum (Falenzuella *et al.*, 2004). The residue aqueous

extract was lyophilized to obtain the phenolic compounds, which triturated in saline solution (0.9% NaCl) at a concentration of 100 mg/dl of saline to be orally administrated to hypercholesterolemic rats. Twenty four male albino rats (weight 50±5g) were classified to four groups, six rats in each group. The first group received standard diet and used as a negative control, the second group received standard diet with cholesterol (1.5% of diet weight) and used as a positive control. Groups 3 and 4 received the standard diet with cholesterol and each animal was orally administered once a day with 0.5 mg and 1mg/kg body weight of polyphenols extract, respectively. All animals were maintained under conventional conditions of temperature 25°C, relative humidity 52% and light (12 hr of illumination/day). The experiment was conducted in the animal house at the College of Medicine, King Saud University, Riyadh – Saudi Arabia. Rats were given free access of water and the standard diets that were prepared according to the American Institute of Nutrition (Reeves *et al.*, 1993). Rats were allowed to acclimate to the new environmental condition and received standard diet for one week before the experiment period which extended to 30 days. After the experiment period, the rats were fasted overnight and blood samples were collected *via* cardiac puncture. Blood was allowed to clot at room temperature, and then was centrifuged at 12,000×g at 5°C for 15 min. for serum separation. Serum samples were analyzed in duplicate for triglycerides (T.G.), total cholesterol (T.C.) and high-density lipoprotein cholesterol (HDL-C) according to the methods of Fossati and Prencipe 1982, Allain *et al.*, 1974 and Lopes-Virella *et al.*, 1977 respectively, using reagents from Sigma Chemical Co., St.Louis, MO. (Kits No. 339, 352 and 352-3.). Low-density lipoprotein cholesterol (LDL-C) was calculated from the following equation: $(T.C - (T.G/5 + HDL-C))$ (Van Horn *et al.*, 1988). Serum tHcy concentration was analyzed by a fluorescence polarization immunoassay on an Abbott IMX analyzer (Abbott Laboratories, Abbott Park, IL.) as described by (Leino, 1999). Food intake (FI) and weight gain (WG) were recorded daily and reported as averages. Food efficiency ratio (FER) was calculated as WG / FI . Atherogenic index (AI) was calculated as $LDL-C / HDL-C$. All data are reported as means and standard deviations. Comparisons between the four groups were performed by one-way analysis of variance (ANOVA) procedure with Duncan's test. Statistical analysis was performed by SPSS

version 10. Significance of difference was set at p-value of ≤ 0.05 .

3. RESULTS

Data of food intake (FI), weight gain (WG) and food efficiency ratio (FER) are presented in Table 1. Adding cholesterol to diet, comparison between groups 1 and 2, and treatment with two levels of the red grape polyphenols extract, as well as comparison between groups (3 and 4) vs. 2, did

percentage of 27.4%, 21.2% and 40.5%, respectively (p-value = 0.03, 0.03 and 0.01, respectively). Serum HDL-C concentration was increased in group 3 (compared to the negative control), but not significantly. However, serum TC concentration was reduced due to the reduction in the LDL component. These results indicated that the treatment with red grape polyphenol extract caused a reduction in serum concentration of TG and TC, especially in the

Table (1): Food intake (FI), Weight gain (WG) and Food efficiency ratio (FER) in rats administered two levels of grape polyphenol extracts.

	Group 1	Group 2	Group 3	Group 4
FI (g/day)	17.9±2.1	18.3±1.4	18.8±0.8	16.7±2.0
WG (g/day)	4.5±2.2	4.6±1.3	6.1±0.9	5.8±1.2
FER	0.25±0.06	0.25±0.02	0.32±0.01	0.31±0.04

Table (2): Triglycerides (TG), Total cholesterol (TC), Low-density lipoprotein cholesterol (LDL-C), High-density lipoprotein cholesterol (HDL-C), Atherogenic index (AI) and Total homocysteine (tHcy) concentrations in serum rats administered two levels of grape polyphenol extract.

	Group 1	Group 2	Group 3	Group 4
TG (mg/dL)	44.6±1.8 ^a	45.9±0.9 ^a	32.4±1.7 ^b	32.3±0.6 ^b
TC (mg/dL)	69.2±3.9 ^b	127.3±1.6 ^a	54.5±2.3 ^c	50.8±1.5 ^c
LDL-C (mg/dL)	52.1±1.7 ^b	97.9±1.9 ^a	31.0±3.1 ^c	30.4±1.4 ^c
HDL-C (mg/dL)	15.8±1.0 ^b	20.9±0.9 ^a	17.1±1.0 ^b	16.9±1.0 ^b
AI	3.3±0.2 ^b	4.7±0.3 ^a	1.9±0.2 ^c	1.7±0.3 ^c
tHcy (µmol/L)	8.4±1.8 ^a	8.6±1.7 ^a	6.0±1.4 ^a	6.1±1.8 ^a

Different letters in a given row denote significant difference, $p \leq 0.05$.

not significantly alter any of the previous characteristics. Results of lipid profile and tHcy are shown in Table 2. As expected, adding cholesterol to the diet significantly increased serum concentration of TC, LDL-C and HDL-C (group 1 vs. group 2). This elevation was not significant in TG and tHcy. The treatment with 0.5 mg / kg red grape polyphenol extract compared to the positive control caused reduction of 29.5%, 57.2%, 68.3% and 18.2% in serum concentration of TG, TC, LDL-C and HDL-C, respectively (P-value = 0.01, 0.01, 0.0 and 0.03, respectively). Almost similar results were observed when the rats were treated with 1mg/kg red grape polyphenol extracts. Increasing the dose of the extract from 0.5 g to 1mg/kg lowered the concentration of the previous lipid components, but not significantly compared to group 3. As a result, AI was reduced in the same previous trend. The treatment with 0.5 mg polyphenol extract (groups 3) compared to the negative control (group 1) significantly reduced serum concentration of TG, TC and LDL-C with a

LDL particles, and as a result, lowered AI. Increasing the dose of the extract did not lead to further significant reduction. The treatment with both levels of the extract (0.5 and 1 mg / kg) reduced serum tHcy concentration, but not significantly. Also, no significant difference was observed between the two levels of the extract.

4. DISCUSSION

Results of this study showed that administration of red grape polyphenol extract at both levels increased WG and FER. This is partially in agreement with the results of Sembries *et al.*, (2006), who showed that intake of grape juice extraction was greater compared to control (water intake) in rats. However, the increases of WG and FER in this study were not significant compared to the control. Other studies on animal models including treatment with grape powder polyphenols in apolipoprotein E deficient mice (Führman *et al.*, 2005) and the treatment with proanthocyanidin rich extract from grape seeds in hypercholesterolemic rabbits (Yamakoshi *et al.*,

1999) showed no significant effect on weight gain, which is in consistence with the results of this study.

The major finding of this study is the hypolipidemic effect of red grape polyphenol extract in normal and hypercholesterolemic rats. This is in agreement with the previous studies conducted in animal models (Zern *et al.*, 2003; Shanmuganayagam *et al.*, 2007; Vinson *et al.*, 2001) and also in human (Nikitina *et al.*, 2006; Castilla *et al.*, 2006; Zern *et al.*, 2005). The unique finding of this study is the hypolipidemic effect of grape polyphenol extract, whereas the previous mentioned studies found hypolipidemic effect of either grape juice, grape powder or single phenolic compounds of grape polyphenols. Few studies that used grape juice, especially those conducted in human (O'Byrne *et al.*, 2002; Stein *et al.*, 1999), gave hypertriglyceridemic effect. This effect was attributed to the large amount of carbohydrates consumed *via* the juice and not to the grape polyphenols (Hertog *et al.*, 1997), which is illustrated in this study. Mechanisms underlying the hypolipidemic effect of grape polyphenols are not clear and have yet to be elucidated. However, several mechanisms have been suggested. These mechanisms include increase in LDL receptor expression and activity (Pal *et al.*, 2003), reduction in oxidative stress in the cells (Fuhrman *et al.*, 2005), decrease in secreted VLDL particles, which lower plasma TG and TC (Zern *et al.*, 2003), altering hepatic cholesterol metabolism *via* decreasing the activity of biosynthesis enzymes (Zern *et al.*, 2003) and a reduction in compounds that play a major role in the inflammation process (Zern *et al.*, 2005).

This study was not designed to investigate the mechanism of the hypolipidemic effect of grape polyphenols. However, it is important to notice that some of the previous mechanisms were related to certain phenolic compounds found in grape. It is known that variety of polyphenols are present in grape including flavonols, anthocyanins, procyanidins, quercetin, myricetin, kaempferol, resveratrol and phenolic acids (Zern *et al.*, 2003; Davalos *et al.*, 2006). These phenolic compounds may differ in their presence and concentration by different varieties and different climates in which grapes were grown (Montealegre *et al.*, 2006). From a nutritional point of view, the consumption of grape and/or its products lower blood cholesterol and triglycerides, which are a primary goal in attenuating major risk factor of CVD. From the biochemical and pharmacological point of view, further studies

need to be done to clarify which phenolic compound(s) and what mechanism are responsible for the hypolipidemic effect.

The other major finding of this study was that the treatment with red grape polyphenol extract at both levels did not significantly alter tHcy concentration. Comparison with other studies is not possible since according to our knowledge there is no study investigated this effect. Few previous studies reported that the consumption of tea and coffee increased plasma tHcy concentration due to phenolic compounds present in tea and coffee (Olthof *et al.*, 2001). However, a comparison is not applicable since phenolic compounds in grapes are different. Schroecksadel *et al.*, (2005) reported that resveratrol, may prevent homocysteine accumulation in human subjects. This is in consistence with the results of this study. However, the reduction in serum tHcy concentration found in this study was not significant. It is possible that single phenolic compounds or more of grape polyphenols may reduce tHcy when given in a high dose and can be used as a supplement. Further investigations are needed to clarify this point. Our results suggest that beneficial effects of red grape polyphenols in CVD are mainly due to their hypolipidemic effects rather than lowering tHcy concentration.

In conclusion, this study showed that red grape polyphenol extract at two levels effectively lowered serum TC and TG concentration and lowered tHcy concentration but not significantly. There was no significant reduction by increasing the dose level of the extract. These results suggest that red grape polyphenol extract is useful in the prevention and treatment of CVD by hypolipidemic effect rather than by lowering serum tHcy concentration.

5. REFERENCES

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تأثير مستخلص المركبات الفينولية العديدة من العنب الأحمر على تركيزات الليبيدات والهيوموستين في بلازما الجردان المحدث لها ارتفاع كولسترول الدم

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ملخص

استهدفت هذه الدراسة معرفة تأثير مستويين من مستخلص المركبات الفينولية العديدة من العنب الأحمر على تركيز الليبيدات والهيوموستين في الدم للجرذان المحدث لها ارتفاع كولسترول الدم. استخدمت التجربة ٢٤ جرذ تم تقسيمهم لأربعة مجموعات (٦ جرذان في كل مجموعة). غذيت جرذان المجموعة الأولى على عليقة مرجعية، بينما غذيت المجموعة الثانية على عليقة مرجعية مضاف لها كولسترول. أما الجرذان في المجموعتين الثالثة والرابعة غذيت على علائق مرجعية مضاف لها الكولسترول وتم إعطاء الجرذان بالفم (orally) مستخلص المركبات الفينولية العديدة من العنب بتركيز ٠,٥ ملجم و ١ ملجم/كجم وزن الجسم للمجموعتين الثالثة والرابعة على التوالي. أظهرت النتائج أن المعاملة بتركيز ٠,٥ ملجم/كجم وزن الجسم مقارنة بالمجموعة المرجعية (الثانية) أدت إلى خفض معنوي لتركيزات الجلسريدات الثلاثية (٢٩,٥%)، الكولسترول الكلي (٥٧,٢%)، كولسترول البروتينات الدهنية منخفضة الكثافة (LDL-C) (٦٨,٣%) وكولسترول البروتينات الدهنية مرتفعة الكثافة (HDL-C) (١٨,٢%). بالمقارنة مع المجموعة المرجعية (الأولى) كانت نسبة الانخفاض لتركيزات الجلسريدات الثلاثية (٢٧,٤%)، الكولسترول الكلي (٢١,٢%) و (LDL-C) (٤٠%) بينما ازداد تركيز (HDL-C) بدرجة غير معنوية. لم يؤد زيادة تركيز الجرعة من ٠,٥ ملجم إلى ١ ملجم/كجم وزن جسم لاختلاف النتائج السابقة، كذلك لم تكن هناك فروق معنوية بين المستويين من المعاملة (٠,٥ ملجم و ١ ملجم) في التأثير على تركيز الليبيدات السابقة في الدم. أدت المعاملة بكلا المستويين من المستخلص (٠,٥ ملجم و ١ ملجم) إلى خفض غير معنوي لتركيز الهيوموستين (المرتبط بتصلب الشرايين و أمراض القلب) الكلي في السيرم مقارنة بالمجموعتين المرجعية (الأولى والثانية) ولم يكن هناك فرق معنوي بين المستويين. استنتجت هذه الدراسة أن مستخلص المركبات الفينولية العديدة من العنب الأحمر ذو تأثير إيجابي في العلاج والوقاية من أمراض القلب الوعائية وأن هذه التأثيرات أساساً تعود لفعالها المخفض لليبيدات الدم أكثر من خفضها لتركيز الهيوموستين الكلي في الدم.

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