Short Communication

Citrus Juice Modulates Antioxidant Enzymes and Lipid Profiles in Orchidectomized Rats

Farzad Deyhim,1,2 Erica Lopez,1 Julia Gonzalez,1 Michelle Garcia,3 and Bhimanagouda S. Patil2

Departments of 1Human Sciences and 3Animal and Wildlife Sciences, Texas A&M University–Kingsville, Kingsville; and 2Vegetable and Fruit Improvement Center, Department of Horticultural Sciences, Texas A&M University, College Station, Texas

ABSTRACT Oxidative stress and hypogonadism are two factors linked to the increased incidence of cardiovascular disease in males. Eating fruits and vegetables is known to reduce the incidences of oxidative stress. The objective of this research was to delineate whether drinking daily squeezed orange juice (OJ) or grapefruit juice (GJ) modulates oxidative stress and antioxidant enzymes while impacting cardiovascular risk factors in hypogonadal male rats. In the present study, 36 1-year-old male rats were equally divided among the following four treatments: sham (control), orchidectomized (ORX), ORX/OJ, and ORX/GJ. After 60 days of drinking OJ or GJ, antioxidant capacity, cholesterol, and triglycerides in serum and superoxide dismutase (SOD), catalase (CAT), cholesterol, and triglycerides in liver were evaluated. Serum antioxidant capacity and SOD and CAT activities decreased \( P < .05 \), while serum cholesterol and liver triglycerides increased \( P < .05 \) in the ORX group compared with the sham group. In contrast to the ORX group, drinking OJ was ineffective while drinking GJ decreased \( P < .05 \) cholesterol concentration in liver and in serum. Nevertheless, OJ and GJ decreased \( P < .05 \) triglyceride concentration in liver and increased \( P < .05 \) serum antioxidant capacity and SOD and CAT activities compared with the ORX group. In conclusion, drinking OJ or GJ prevented oxidative stress by enhancing total antioxidant capacity and elevating liver antioxidant enzymes while modulating cardiovascular risk factors.

KEY WORDS: • antioxidant capacity • catalase • cholesterol • citrus juice • superoxide dismutase • rats

INTRODUCTION

Cardiovascular disease (CVD), the number one killer in the United States, is a growing epidemic of great magnitude with approximately 960,000 individuals dying from heart disease and stroke each year.1 According to the Centers for Disease Control and Prevention’s annual report, 356,598 men died of heart disease in 2001.2 As of 2005, approximately 5.8 million men have suffered a CVD-related illnesses in the United States.2 Age and male sex hormones are two independent risk factors for CVD.3 While the incidence of CVD is considerably lower among young men, it increases exponentially in older men. The primary reason is related to a decline in serum levels of testosterone with aging, which contributes to the atherosclerotic process.3 Low levels of serum testosterone are associated with several cardiovascular risk factors, including hypercholesterolemia, dyslipidemia, and insulin resistance.4–6 Testosterone replacement therapy in hypogonadal men delays CVD, because testosterone administration lowers total cholesterol concentration in the serum.4 However, testosterone administration in hypogonadal men is not without risk. The incidence of prostate cancer has been seen to increase with testosterone administration.7 Certainly any remedy that improves cardiovascular status in elderly men without having an adverse effect on health is worth investigating.

Accumulated findings suggest that eating fruit and vegetables is beneficial against CVD.8–11 Several national studies, including the Framingham Heart Study and Nurses’ Health Study, have concluded that there are protective effects of fruit and vegetables against the risk of developing CVD.12–14 In a recent Baltimore Longitudinal Study of Aging study, participants ate more than five servings of fruits and vegetables while consuming less than 12% of calories from saturated fats. As a result, there was a 76% decline in CVD-related illnesses relative to those who consumed fewer than five servings of fruits and veg-
etables, while eating greater than 12% of calories from saturated fat. The protective effects of fruits and vegetables against CVD may be either due to having serum lipid profile values within a normal range or having hypolipidemic and hypocholesterolemic effects as suggested previously. In another study, eating large amounts of fruits and vegetables significantly decreased serum levels of total cholesterol and low-density lipoprotein cholesterol. Overall benefits of eating fruits and vegetables against CVD may be related to their nutrient compositions and bioactive compounds with antioxidant activity. In other studies, orange juice (OJ) and grapefruit juice (GJ) were shown to be rich sources of antioxidants and polyphenols, and they collectively reduced oxidative stress and blood lipid profiles, making them a valuable choice for disease prevention in particular among the elderly. The potential benefits of drinking OJ and GJ may be mediated through their bioactive compounds. OJ and GJ are certainly an alternative to drug and hormone therapy, and their nutrient contents and bioactive compounds potentially may improve CVD status and antioxidants in orchidectomized (ORX) rats. Our recent study provides evidence that both flavonoids and limonoids have antioxidant activity.

Despite the known antioxidant properties of OJ and GJ, recent evidence suggests that OJ and GJ have hypolipidemic effects, though it is unclear whether the hypolipidemic effects are due to a decrease in production of lipids by the liver. The objective of this study was to evaluate whether drinking OJ or GJ enhances serum antioxidant capacity and liver antioxidant enzymatic activity while having a hypolipidemic and hypocholesterolemic effect in ORX rats.

**MATERIALS AND METHODS**

*Animals and diets*

In this experiment, 36 1-year-old male Sprague-Dawley rats upon arrival at our institution were housed in an environmentally controlled animal laboratory and acclimated with a standard laboratory diet for 3 days before surgery. The animals were weighed and divided into four groups. One group was sham-operated (control; n = 9), and the complete procedure was performed in three groups (ORX), which were then equally divided (nine rats per group) among the three treatment groups (ORX, ORX + OJ, and ORX + GJ). All animals consumed a semipurified, powdered casein-based diet (AIN-93M, Teklad, Madison, WI) for the duration of the study. The rats were pair-fed to the mean food intake of the sham group. Drinking water, daily squeezed OJ, and daily squeezed GJ were provided for ad libitum consumption. To ensure that caloric intake from drinking was similar among treatment groups, table sugar (42 calories/100 mL) was added to the drinking water. The pH of the OJ and GJ was adjusted to 7.45 ± 0.25 using 2% sodium bicarbonate (20 g/L), and the amount of sodium bicarbonate was added to the drinking water for the sham group and the ORX group.

**Blood and liver parameters**

Sixty days after drinking OJ, GJ, or salinated water, animals were anesthetized using ketamine/xylazine (100 and 5 mg/kg of body weight, respectively) and bled from the abdominal aorta. Blood samples were collected in tubes and centrifuged (4°C) at 1,500 g for 15 minutes. Serum was separated, and an aliquot of serum was refrigerated for total antioxidant status, defined as the capacity of red blood cells to withstand free radical-induced hemolysis, which was measured as described previously. Total cholesterol and triglycerides were evaluated using commercially available kits (Thermo Electron, Louisville, CO). The liver was separated and cleaned from surrounding tissues and analyzed for liver concentrations of cholesterol and triglycerides as detailed previously. Superoxide dismutase (SOD) (Calbiochem, San Diego, CA) and catalase (CAT) (Calbiochem) activities were also evaluated in liver. Results were expressed as unit activity per milligram of liver protein.

**Statistical analyses**

Data were analyzed by analysis of variance using the General Linear Models procedure of SAS (SAS Institute, Cary, NC) to determine the effects of citrus juice supplementation on serum and liver lipid profiles, serum total antioxidant status, and activities of SOD and CAT as the primary outcome variables. When a significant F statistic (P = .05) from analysis of variance was calculated, the least square means procedure was performed for separating means that were significantly (P = .05) different.

**RESULTS**

In the present study, total serum antioxidant status was significantly (P < .05) lowered in the ORX group compared with the sham group (Table 1). In contrast to the ORX group, rats that drank either OJ or GJ maintained their serum total antioxidant status to the level of the sham group (Table 1).

Compared with the sham group, the ORX group showed a significant (P < .05) decrease in SOD and CAT activities (Table 1). However, SOD and CAT activities were significantly higher (P < .05) in rats drinking citrus juice compared with the ORX group drinking water.

The concentration of triglycerides in the serum numerically increased, while the level of triglycerides in liver was significantly elevated (P < .05) in the ORX group in comparison to the sham group. In contrast, rats that drank either OJ or GJ exhibited no increase in serum and liver triglycerides compared with the sham group.

Compared with the sham group, concentrations of cholesterol in serum of the ORX group and the ORX + OJ group increased (P < .05), while the hepatic cholesterol concentration was not affected by either of the two treatment groups. In contrast to drinking OJ, drinking GJ atten-
A recent animal study has reported that in mice, naringin reduced lipid peroxidation status in tissues by enhancing tissue antioxidant status. This report concurs with our present findings that OJ and GJ containing bioflavonoids enhance serum antioxidant capacity.

As expected, similar to previous findings, castration decreases SOD and CAT activities from decreasing, suggesting natural products from dietary components prevent free radical accumulation. In one human study the erythrocyte SOD activity of phenol-depleted subjects significantly decreased, while after 6 days of phenol-rich intervention, the activity of SOD was significantly increased by 41%. In the current study, the results suggested that the nutritional antioxidant status is hormone dependent. This conclusion follows from the significant reduction in antioxidant capacity of the ORX group. Previous results also suggest similar findings: that testosterone depletion induces oxidative stress, increases reactive oxygen species production, and attenuates antioxidant detoxification. The current study also suggested that OJ and GJ maintained serum total antioxidant status to the level of the sham group despite being the animals’ ORX status. In a previous study, rats that ate a diet rich in cholesterol while drinking either red GJ or naringin exhibited a higher antioxidant capacity than the control group. In a similar study, an increase in the plasma antioxidant activity was observed in rats receiving either fresh OJ or GJ. The authors attributed the high antioxidant capacity largely to the bioactive compounds. A recent animal study has reported that in mice, naringin reduced lipid peroxidation status in tissues by enhancing tissue antioxidant status. This report concurs with our present finding that OJ and GJ containing bioflavonoids enhance serum antioxidant capacity.

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In the current study, the results suggested that the numerical increase in plasma triglycerides of the ORX group may be due to increased triglyceride synthesis or triglyceride accumulation by the liver. These results also imply that in ORX rats, drinking either OJ or GJ inhibits the increase in hepatic triglycerides. In several animal studies, when normolipidemic rats and diet-induced hyperlipidemic rats were given flavonones, the triglyceride level in serum or plasma was reduced. In a similar study, when rats were fed a diet containing 10% hesperidin, they showed a significant reduction in plasma triglyceride level. In another study in ovariectomized mice, a diet containing 0.5% hesperidin decreased serum and hepatic triglyceride concentrations compared with the control diet. It is likely that bioactive compounds from OJ and GJ mediated the low triglyceride concentration in liver.

It appears that drinking OJ does not have hypocholesterolemic effects, while GJ has a significant hypocholesterolemic effect in ORX rats. The hypocholesterolemic property of GJ may be related to certain specific bioactive compounds.

**DISCUSSION**

The present study suggests that serum antioxidant status is hormone dependent. This conclusion follows from the significant reduction in antioxidant capacity of the ORX group. Previous results also suggest similar findings: that testosterone depletion induces oxidative stress, increases reactive oxygen species production, and attenuates antioxidant detoxification. The current study also suggested that OJ and GJ maintained serum total antioxidant status to the level of the sham group despite being the animals’ ORX status. In a previous study, rats that ate a diet rich in cholesterol while drinking either red GJ or naringin exhibited a higher antioxidant capacity than the control group. In a similar study, an increase in the plasma antioxidant activity was observed in rats receiving either fresh OJ or GJ. The authors attributed the high antioxidant capacity largely to the bioactive compounds. A recent animal study has reported that in mice, naringin reduced lipid peroxidation status in tissues by enhancing tissue antioxidant status. This report concurs with our present finding that OJ and GJ containing bioflavonoids enhance serum antioxidant capacity.

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<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>ORX</th>
<th>ORX + OJ</th>
<th>ORX + GJ</th>
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</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antioxidant capacity</td>
<td>62 ± 1a</td>
<td>58 ± 1b</td>
<td>61 ± 1a</td>
<td>62 ± 1a</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>98 ± 3a</td>
<td>102 ± 3a</td>
<td>97 ± 3a</td>
<td>82 ± 3b</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>98 ± 3b</td>
<td>120 ± 3b</td>
<td>123 ± 3a</td>
<td>106 ± 3b</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAT (U/mg of liver protein)</td>
<td>38.5 ± 5.3a</td>
<td>19.6 ± 4.7b</td>
<td>40.8 ± 4.0a</td>
<td>44.8 ± 5.3a</td>
</tr>
<tr>
<td>SOD (U/mg of liver protein)</td>
<td>33.7 ± 1.4a</td>
<td>19.1 ± 1.7c</td>
<td>24.0 ± 1.3b</td>
<td>37.2 ± 2.59a</td>
</tr>
<tr>
<td>Triglycerides (mg/g of liver)</td>
<td>44.99 ± 1.79b</td>
<td>62.37 ± 1.42a</td>
<td>46.41 ± 1.41b</td>
<td>47.94 ± 1.62b</td>
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<tr>
<td>Cholesterol (mg/g of liver)</td>
<td>18.46 ± 0.79a</td>
<td>18.41 ± 0.60a</td>
<td>17.01 ± 0.55b</td>
<td>13.8 ± 0.67b</td>
</tr>
</tbody>
</table>

Antioxidant capacity was defined as the percentage of red blood cells not hemolysed after 60 minutes. Unlike superscripts within a row are significantly different (P < .05).
In conclusion, frequent drinking of OJ and GJ can be used as a nonpharmacologic cardiovascular protective agent that enhances total antioxidant status and antioxidant enzymes while it reduces oxidative stress in hypogonadal rats. Furthermore, hypolipidemic and hypocholesterolemic effects of daily drinking of GJ can significantly protect against atherosclerosis.

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