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Antihypertensive, vasodilator and antioxidant effects of a vinifera grape skin extract

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Abstract

Cumulative evidence suggests that moderate wine consumption exerts a cardioprotective effect. We investigated the occurrence of an antihypertensive effect of an alcohol-free hydroalcoholic grape skin extract (GSE) obtained from skins of a vinifera grape (*Vitis labrusca*) in experimental rodent hypertension models. The vasodilator effect of GSE (polyphenols concentration 55.5 mg g^{-1}) was also assessed in the isolated mesenteric vascular bed of Wistar rats and the antioxidant effect was studied on lipid peroxidation of hepatic microsomes. Oral administration of GSE significantly reduced systolic, mean and diastolic arterial pressure in Wistar rats with desoxycorticosterone acetate-salt and N^G-nitro-L-arginine methyl ester (L-NAME) induced experimental hypertension. In the rat isolated mesenteric vascular bed pre-contracted with norepinephrine, bolus injections of GSE induced endothelium-dependent vasodilatation that was substantially inhibited by L-NAME, but not by indometacin, tetraethylammonium or glibenclamide. Lipid peroxidation of hepatic microsomes estimated as malondialdehyde production was concentration-dependently inhibited by GSE. In conclusion, the antihypertensive effect of GSE might be owing to a combination of vasodilator and antioxidant actions of GSE. These findings also suggest that the beneficial effect of moderate red wine consumption could be owing to an antihypertensive action induced by compounds occurring in the skin of vinifera grapes.

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Introduction

Epidemiological studies have shown that red wine consumption may have a protective effect against coronary heart disease (St Leger et al 1979). This aspect is observed mainly in some regions of France where a high intake of saturated fats is not accompanied by increased risk of cardiovascular disease, a condition termed the "French paradox" (Renaud & de Lorgeril 1992). This paradox has been attributed in part to the moderate consumption of red wine. The mechanism of the cardioprotective effect of wine is not completely established, but experimental evidence suggests that the beneficial effects of wine are probably owing to coronary vasodilatation (Flesch et al 1998), inhibition of platelet aggregation (Demrow et al 1995), inhibition of oxidation of low-density lipoprotein (Frankel et al 1993) and reduction of endothelin synthesis (Corder et al 2001). Biochemical studies have shown that wine is rich in polyphenols (Scalbert & Williamson 2000), compounds that have several pharmacological properties, including vasodilator (Andriambeloson et al 1997), hypotensive (Diebolt et al 2001) and antioxidant effects (Frankel et al 1993). As hypertension is also a very important risk factor for coronary heart disease, the present study addressed the possibility that an antihypertensive effect of grape skin compounds could also participate in the cardioprotective effect of wine. We tested the hypothesis that an alcohol-free hydroalcoholic grape skin extract (GSE) obtained from skins of *Vitis labrusca*, a vinifera grape largely used in Brazil to produce red wine, might possess antihypertensive action. Antioxidant and vasodilator effects of the extract were also assessed in the lipid peroxidation of microsomal protein and in the rodent isolated mesenteric vascular bed, respectively.

Materials and Methods

Preparation of GSE

V. labrusca (Isabel varietal) red grapes were obtained from selected vineyards located it the State of Rio Grande do Sul, Brazil. The grapes were washed in tap water and the skins were separated from the pulp. Approximately 100 g of grape skin was boiled in 400 mL of distilled water for 5 min and then minced. Ethanol (400 mL) was added to the decoction, which was then shaken for 4 h and kept in dark bottles inside a refrigerator (4°C) for 20 days. The hydro-alcoholic extract of *V. labrusca* skins was filtered through Whatman no. 1 filter paper, and the ethanol was evaporated under low pressure at 55°C. The extract was then lyophilized and frozen at −20°C until use. Typically, 100 g of wet skins yields about 8.9 g of lyophilized extract.

Drugs

Norepinephrine (noradrenaline) bitartrate, acetylcholine chloride, N^G-nitro-L-arginine methyl ester (L-NAME), tetraethylammonium (TEA), indometacin, deoxycholic acid, deoxycorticosterone acetate and chemical reagents were purchased from Sigma (St Louis, MO, USA). Nitroglycerin and glibenclamide were gifts from Cristalia Produtos Quimicos Farmaceuticos Ltd, and Hoechst do Brazil, respectively. All drug solutions were freshly prepared before each experiment. Norepinephrine, acetylcholine, nitroglycerin, L-NAME, TEA and deoxycholic acid were dissolved in water or physiological salt solution (PSS), deoxycorticosterone was dissolved in corn oil, indometacin was dissolved in ethanol, and glibenclamide was dissolved in 50% ethanol/50% dimethylsulfoxide. The extract was dissolved in PSS, drinking water or in saline.

Animals

All experiments were reviewed and approved by the Ethics Committee of Animal Experiments of State University of Rio de Janeiro. The experiments were performed with adult male Wistar rats (250–350 g) obtained from the Animal Care Facility of the Department of Pharmacology, State University of Rio de Janeiro. The animals were housed in plastic cages (three rats per cage). Bodyweight, heart rate, systolic, mean and diastolic arterial pressure were measured three times a week. Cardiovascular parameters (systolic, mean and diastolic arterial pressure and heart rate) were measured by the tail cuff method using a Letica LE 5000 device. For arterial pressure measurements, animals were trained for at least 2 weeks to stay still under a piece of cloth until the arterial pressure was steadily recorded with minimal restraint and stress. The first measurement of cardiovascular parameters was discarded and the mean of two or three subsequent measurements was recorded each day.

L-NAME hypertension

The animals were treated orally with 50 mg kg^{-1} L-Name (L-NAME group, n = 10), or with 50 mg kg^{-1} L-NAME plus 100 mg kg^{-1} GSE (L-NAME+GSE group, n = 10) dissolved in drinking water, daily for 28 days. Bodyweight and cardiovascular parameters were measured three times a week.

Desoxycorticosterone acetate (DOCA)-salt hypertension

Twelve rats were uninephrectomized under ether anaesthesia. After recovery from surgery, the rats were trained for measurements of systolic, mean and diastolic arterial pressure. After the period of adaptation, the animals were treated subcutaneously with deoxycorticosterone acetate (12.5 mg kg^{-1} per week) and were given a 0.9% NaCl/0.2% KCl drinking solution *ad libitum* for 30 days. Sixteen days after the beginning of DOCA-salt treatment, when the animals became hypertensive, a group of rats was treated orally with 100 mg kg^{-1} GSE, (DOCA-salt+GSE group, n = 6) daily from Day 16 until Day 28. Another group of rats was continuously treated with DOCA and 0.9% NaCl/0.2% KCl drinking solution (n = 6) until the end of the experiments.

Isolated mesenteric vascular bed

The rat superior mesenteric vascular bed was isolated according to McGregor (1965). Briefly, male Wistar rats (250–350 g) were killed with inhaled CO_2. Then the mesenteric vascular bed was cannulated and perfused with PSS at flow rate of 4 mL min^{-1} generated by a peristaltic pump (Lifecare Model 4; Abbott/Shaw). The PSS had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, EDTA 0.02, and glucose 11. The PSS (37°C) was bubbled with 95% O_2/5% CO_2. Perfusion pressure was measured with a transducer connected to a preamplifier and chart recorded. Drugs were either dissolved in PSS and perfused at the desired concentration or were administered as bolus injections directly into the perfusion stream (volume < 100 μL). Perfusion pressure was elevated (80–110 mmHg) with norepinephrine (1–0.1 μM) added to the perfusion fluid. The vasodilator effect of drugs was expressed as percentage decrease in relation to the pressor effect of norepinephrine. When the pressor effect of norepinephrine reached a plateau, the dose response to bolus injections of GSE was obtained. Acetylcholine (10 pmol) and nitroglycerin (1 μmol) were administered as bolus injections to assess endothelial function. The vasodilator effect of GSE was studied in vessels pre-treated with deoxycholic acid (2.5 mM) dissolved in PSS for 4 min to chemically remove the endothelium, L-NAME (300 μM), TEA (1 mM), glibenclamide (1 μM) or indometacin (0.1 μM). As the GSE induced a long-lasting inhibitory effect on the norepinephrine constrictor effect, only one dose–response curve to GSE was obtained in each vascular bed preparation.

Lipid peroxidation of hepatic microsomes and estimation of polyphenols

Hepatic microsomes were prepared from livers of Wistar rats by tissue homogenization in 5 vols of ice-cold 0.25 mM sucrose, 50 mM Tris-HCl, pH 7.4, using a Teflon Potter-

Elvehjem homogenizer. The homogenate was centrifuged for 20 min at 10000 *g*, and the resulting supernatant was centrifuged for 60 min at 100000 *g*. The microsomal pellet was suspended in 20% glycerol, 50 mM Tris-HCl, pH 7.4, and stored at −80°C. Microsomal protein was determined by the method described by Petersen (1977), with bovine serum albumin as standard. For induction of lipid peroxidation, 1 mg of microsomal protein was treated with 50 μM ferrous iron (stock solution: 100 μM $Fe(NH_4)_2$ in 0.05 M HCl) and 500 μM H_2O_2 in 500 μL (final volume) of 50 mM Tris-HCl, pH 7.4, in the absence or presence of different concentrations of GSE. After incubation for 40 min at 37°C, the samples were treated with 500 μL of 20% trichloroacetic acid (w/v) and 10 μL of 2% butylated hydroxytoluene (w/v) in ethanol. After centrifugation at 3000 *g* for 5 min, supernatants were reacted with an equal volume of 0.67% thiobarbituric acid (TBA) (w/v) and incubated for 1 h in a boiling water bath. The malondialdehyde (MDA)/TBA complexes were detected on a Shimadzu high-performance liquid chromatography system using the method described by Young & Trimble (1991) with minor modifications.

The concentration of polyphenols in lyophilized GSE, measured by analysing for total phenol by the Folin-Ciocalteau procedure (Singleton & Rossi 1965), was 55.5 mg g^{-1}.

Statistical analysis

All results are presented as mean ± s.e.m. for the number of rats. One-way analysis of variance and the Student's unpaired *t*-test were used for statistical analysis. Values of $P < 0.05$ were considered statistically significant.

Results

Antihypertensive effect of GSE in L-NAME hypertensive rats

The baseline systolic, mean and diastolic arterial pressures in L-NAME and L-NAME+GSE groups were not significantly different (L-NAME group: 157±3, 96±2 and 76±4 mmHg; L-NAME+GSE group: 160±2, 100±3 and 72±4 mmHg, respectively). Four weeks of L-NAME administration increased systolic, mean and diastolic arterial pressure to 198±2, 154±3 and 131±3 mmHg in the L-NAME group, and to 170±3, 119±3 and 103±3 mmHg in the L-NAME+GSE group, respectively ($P < 0.05$ between L-NAME and L-NAME+GSE groups). Figure 1 shows the time course of changes in arterial pressure in both groups. Heart rate decreased in both groups in the first week of treatment. At the end of treatment, the heart rate of the L-NAME group (256±4 beats min^{-1}) was significantly lower then that of the L-NAME+GSE group (281±5 beats min^{-1}). The bodyweight of the L-NAME group was not different compared with the L-NAME+GSE group at the beginning of L-NAME treatment, but, at the end of the study, the bodyweight of the L-NAME+GSE group (342±8 g) was significantly higher than that of the L-NAME group (306±8 g).

Antihypertensive effect of GSE in DOCA-salt hypertensive rats

The baseline systolic, mean and diastolic arterial pressure in DOCA-salt and DOCA-salt+GSE groups were not significantly different (DOCA-salt group: 193±3, 113±8 and 73±3 mmHg; DOCA-salt+GSE group: 194±5, 120±3 and 83±2 mmHg, respectively). Administration of DOCA in both groups induced a continuous increase in arterial pressure. By Day 16 of DOCA-salt treatment, the systolic, mean and diastolic arterial pressures were 223±9, 165±10 and 137±11 mmHg in the DOCA-salt group, and 212±4, 156±6 and 130±8 mmHg in the DOCA-salt+GSE group, respectively. The oral administration of GSE to the DOCA-salt+GSE group from Day 16 to Day 28 reduced the increases in arterial pressure. By Day 28, the systolic, mean and diastolic arterial pressures in the DOCA-salt+GSE group were significantly lower than the arterial pressures observed in the DOCA-salt group (DOCA-salt group: 246±5, 192±9 and 164±12 mmHg; DOCA-salt+GSE group: 221±3, 146±3 and 107±4 mmHg, respectively). Figure 2 shows the time course changes in systolic, mean and diastolic arterial pressure in both groups.

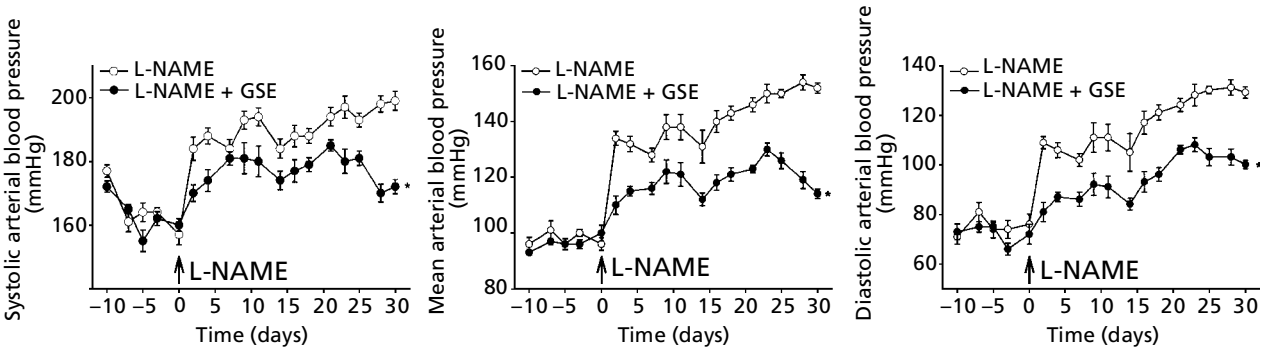

Figure 1 Effects of grape skin extract (GSE) on systolic, mean and diastolic arterial pressure in rats treated with N^G-nitro-L-arginine methyl ester (L-NAME). From Day 0 (arrow) the animals were treated daily with oral L-NAME, (50 mg kg^{-1} day^{-1}) or with oral L-NAME (50 mg kg^{-1} day^{-1}) plus GSE (100 mg kg^{-1} day^{-1}) in drinking water. *$P < 0.05$ (analysis of variance).

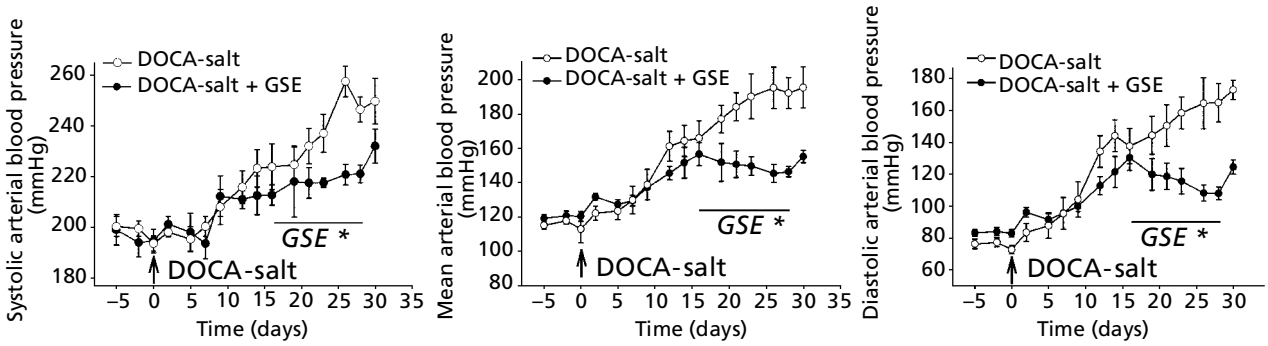

Figure 2 Effects of grape skin extract (GSE) on systolic, mean and diastolic arterial pressure in uninephrectomized rats treated with deoxycorticosterone acetate (DOCA) and salt solution. From Day 0 (arrow) rats were treated subcutaneously with DOCA (12.5 mg kg^{-1} per week) and 0.9% NaCl/0.2% KCl solution for drinking. From Day 16, six rats were treated with DOCA-salt plus oral GSE (100 mg kg^{-1} day^{-1}) until Day 28. The other six rats were treated only with DOCA-salt from Day 0 (arrow) until Day 30. $*P < 0.05$ (analysis of variance).

Vasodilator effect of GSE in the isolated vascular mesenteric bed

The basal perfusion pressure was 29 ± 3 mmHg. The increase in perfusion pressure induced by norepinephrine in control, deoxycholic acid-, L-NAME-, TEA-, indometacin- and glibenclamide-treated groups were 85 ± 12, 83 ± 10, 119 ± 9, 113 ± 6, 80 ± 6 and 88 ± 11 mmHg, respectively. In vessels pre-contracted with norepinephrine, bolus injections of GSE induced a dose-dependent vasodilator response. The vasodilator effect of GSE was not altered by TEA, glibenclamide or indometacin, but was significantly reduced by L-NAME (Figure 3). The percentage vasodilator effects of acetylcholine and GSE (30, 60 and 100 μg) before deoxycholic acid were 40 ± 3, 31 ± 10, 61 ± 9 and 76 ± 5, respectively. The percentage vasodilator effects of acetylcholine and GSE were significantly reduced after endothelium removal (0 ± 0, 0 ± 0, 1 ± 1 and 3 ± 2, respectively). However, the vasodilator effect of nitroglycerin obtained before deoxycholic acid ($65 \pm 8\%$) was not significantly changed by endothelium removal ($63 \pm 7\%$).

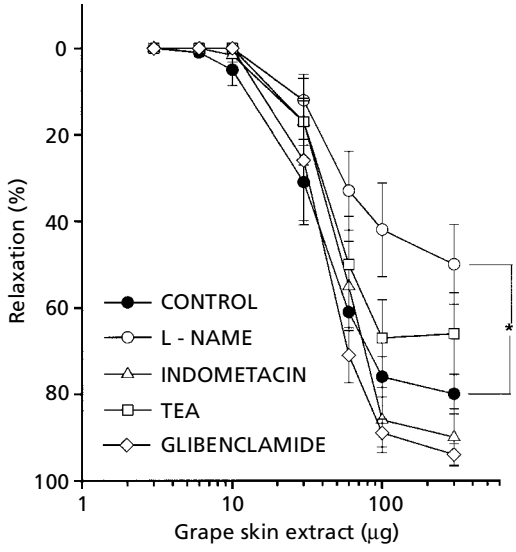

Figure 3 Dose–response curves for grape skin extract in norepinephrine pre-contracted rat vascular mesenteric bed in the absence (control) or presence of N^G-nitro-L-arginine methyl ester (L-NAME), indometacin, tetraethylammonium (TEA) or glibenclamide. $*P < 0.05$ (analysis of variance).

Antioxidant effect and polyphenol concentration

As shown in Figure 4, GSE induced a concentration-dependent inhibition of iron-induced lipid peroxidation in rat liver microsomes as measured by the decrease in MDA formation.

Discussion

The aim of the present study was to investigate the antihypertensive effect of an alcohol-free hydroalcoholic extract prepared from skins of *V. labrusca*, a vinifera red grape largely used in Brazil for wine production. Approximately 220 million litres of wine obtained from *V. labrusca* are consumed in Brazil each year. The present study shows that oral administration of 100 mg kg^{-1} day^{-1} *V. labrusca* GSE, which corresponds to 5.5 mg kg^{-1} day^{-1} of polyphenols (approximately the amount occurring in about 2.75 mL kg^{-1} day^{-1} of red wine; López et al 2001), induces a substantial antihypertensive effect in DOCA-salt hypertensive rats, and prevents L-NAME-induced hypertension in rats. Similarly, the ethanol-free hydroalcoholic extract obtained from Cabernet sauvignon grape skin has been shown to produce an antihypertensive effect (Soares de Moura 2001). We have also observed that hydroalcoholic extract obtained from the pulp of *V. labrusca* grapes did not reduce the hypertension induced by L-NAME in Wistar rats (R. Soares de Moura, unpublished results). It is possible that the antihypertensive effect of the

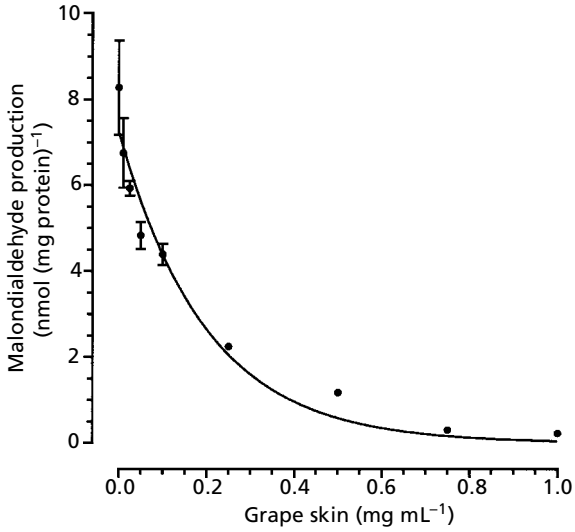

Figure 4 Effect of grape skin extract on the iron-induced lipid peroxidation of rat liver microsomes.

compounds of the hydroalcoholic extract of vinifera grape skins, which may also occur in wine, could potentially play an important role in the cardioprotective effect of moderate red wine consumption (St Leger et al 1979).

The mechanism of the antihypertensive effect of GSE is not known. Given that our extract was obtained from a vinifera grape, the extract may share some effects similar to those encountered in wine and other grape products. Wine and grape products have been shown to induce many important cardiovascular effects. The effects of wine on vascular smooth muscle are controversial. Red wine has been shown to induce either vasodilatation (Fitzpatrick et al 1993; Flesch et al 1998) or no vascular effect (Rending et al 2001) in isolated vessels. These controversial findings may be owing to different wine varieties or even diverse vessels studied. A direct endothelium-dependent vasodilator effect of wine produced in oak barrels, but not in steel tanks, has been demonstrated in isolated vessels (Flesch et al 1998). Products obtained from red wine, such as polyphenol compounds, induce vasodilatation (Andriambeloson et al 1997, 1999) and reduce arterial pressure in normotensive rats (Diebolt et al 2001).

The present results demonstrate that GSE also has a substantial direct vasodilator effect in the isolated mesenteric vascular bed of the rat. This inhibitory effect on the vascular smooth muscle is not inhibited by indometacin, TEA or glibenclamide, suggesting that the release of vasodilator prostanoids, activation of Ca^{+2}- or ATP-dependent potassium channels do not play an important role in the vasodilator effect of GSE. The vasodilator effect of GSE is endothelium-dependent because in the isolated mesenteric vascular bed pre-treated with deoxycholic acid, the vasodilatation induced by GSE and acetylcholine, but not nitroglycerin, was significantly reduced. Nitric oxide, an important modulator of vascular function (Vanhoutte & Mombouli 1996), participates in the vasodilator effect of wine (Fitzpatrick et al 1993; Flesch et al 1998) and wine-derived polyphenols (Andriambeloson et al 1999; Stoclet et al 1999). Importantly, nitric oxide is also released after red wine ingestion in humans (Matsuo et al 2001). Therefore, nitric oxide probably plays a very important role in the vasodilator effect of GSE, given that L-NAME, an inhibitor of nitric oxide synthase, significantly reduced the vasodilator effect of GSE in the isolated mesenteric vascular bed of rats. We speculate that the antihypertensive effect of GSE may also be owing to its vasodilator effect.

The antihypertensive effect of GSE probably does not involve modulation of the renin–angiotensin system since the antihypertensive effect was observed in DOCA-salt hypertensive rats, a low-renin model of experimental hypertension. An anti-adrenergic action of GSE also appears to be unlikely because the vasodilator effect of the extract was significantly reduced in vessels without endothelium and contracted with norepinephrine. We could speculate that the antioxidant effect of the extract demonstrated in our study could play an important role in the antihypertensive effect, since GSE reduced the MDA formation in-vitro. Previous experimental results have shown that oxidative stress increases in hypertension induced by inhibition of NO synthase (Usui et al 1999) or by salt supplementation in uninephrectomized rats treated with DOCA (Wu et al 2001). However, our in-vitro results are apparently not in agreement with the findings of Young et al (2000) who demonstrated that oral ingestion of GSE in humans did not change the MDA plasma levels. This difference may be owing to the distinct grape skin preparation or dose used. Recently, it has been demonstrated that polyphenols from red wine decrease endothelin-1 synthesis in cultured bovine aortic endothelial cells (Corder et al 2001). Considering that endothelin-1 plays a very important role in DOCA-salt (de Carvalho et al 1990) and in L-NAME hypertension (Naruse et al 2000), we also speculate that the reduction of arterial pressure induced by GSE might involve the reduction of endothelin-1 synthesis.

Increases in arterial pressure induced by L-NAME produced a decrease in heart rate, probably owing to baroreceptor activation. However, in rats treated with L-NAME plus GSE, the bradycardia was smaller probably due to lesser increases in arterial pressure.

Rats treated with L-NAME showed a reduction in bodyweight as the pressure increased. However, in animals treated with L-NAME plus GSE, the decrease in bodyweight was significantly smaller. The mechanism of weight reduction induced by chronic L-NAME treatment is not known. As NO seems to modulate food intake in rodents (Morley & Flood 1991), cerebral reduction on NO formation by L-NAME could participate in this effect. At the moment, we cannot propose a mechanism for the GSE-dependent inhibition of L-NAME-induced weight loss.

The quality and chemical composition of wine varies according to many factors, such as grape species, cultivar, vineyard terroir, time of fermentation and ageing. The percentage of wine polyphenols, compounds that induce vasodilatation in isolated vessels, varies among wines (Viñas et al 2000), and in-vitro studies have shown that only red wine produced in oak barrels induces vasodilatation in isolated rat aorta (Flesch et al 1998). However,

wine ageing in oak barrels does not seem to be important for the vasodilator effect of vinifera grapes, as previously suggested (Flesch et al 1998), since we demonstrated that GSE, which contains 55.5 mg g^{-1} polyphenols, a concentration similar to that obtained in the lyophilized residue of the red wine Cabernet sauvignon (95.2 mg g^{-1}; L. M. S. Rubenich, unpublished observation) has substantial vasodilator, antihypertensive and antioxidant effects even though the extract was not submitted to the ageing process. Nevertheless, augmentation of some pharmacological properties of compounds present in grape skin during the process of wine ageing in oak barrels might occur.

In conclusion, the present results provide experimental evidence that an ethanol-free extract obtained from vinifera grape skin exhibits significant antihypertensive, vasodilator and antioxidant effects. The combination of these actions present in the vinifera grape skin may help explain why moderate red wine consumption has a protective effect against cardiovascular diseases. Although wine consumption might protect against coronary heart disease, as suggested by many studies, the harmful effects of ethanol, such as pancreatitis, liver dysfunction and addiction, could limit the medical indication of chronic wine ingestion. Therefore, an alcohol-free product obtained from vinifera grapes with similar pharmacological actions as wine might be a potential medicine for prevention and treatment of coronary heart diseases and hypertension.

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