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# Antidiabetic Activity of Red Wine Polyphenolic Extract, Ethanol, or Both in Streptozotocin-Treated Rats

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A polyphenol extract from a Corbières (France) red wine (P, 200 mg/kg), ethanol (E, 1 mL/kg), or a combination of both (PE) was administered by daily gavage for 6 weeks to healthy control or streptozotocin (60 mg/kg i.v.)-induced diabetic rats (180−200 g). Treatment groups included C or D (untreated control or diabetic) and CP, CE, or CPE (treated control) or DP, DE, or DPE (treated diabetic). P treatment induced a reduction in body growth, food intake, and glycemia in both CP and DP groups. In DP, hyperglycemia was reduced when measured 1 h after daily treatment but not at sacrifice (no treatment on that day). The hyperglycemic response to the oral glucose tolerance test (OGTT) and plasma insulin at sacrifice were impaired similarly in DP and D groups. In contrast, in DE or DPE, body growth was partially restored while hyperglycemia was reduced both during treatment and at sacrifice. In addition, hyperglycemia response to OGTT was reduced and plasma insulin was higher in DE or DPE than in D animals, indicating a long-term correction of diabetes in ethanol-treated animals. Morphometric studies showed that ethanol partially reversed the enlarging effect of diabetes on the mesenteric arterial system while the polyphenolic treatment enhanced it in the absence of ethanol. In summary, our study shows that (i) a polyphenol extract from red wine ("used at a pharmacological" dose) reduces glycemia and decreases food intake and body growth in diabetic and nondiabetic animals and (ii) ethanol ("nutritional" dose) administered alone or in combination with polyphenols is able to correct the diabetic state. Some of the effects of polyphenols were masked by the effects of ethanol, notably in diabetic animals. Further studies will determine the effect of "nutritional" doses of polyphenols as well as their mechanism of action.

**KEYWORDS: Red wine; polyphenols; alcohol; diabetes; streptozotocin rats; glycemia; insulin; levels; antidiabetic activity; microangiopathy**

## INTRODUCTION

Considerable evidence has accumulated indicating that moderate prolonged red wine consumption is beneficial for health (*1*−*3*). This could be explained by the intake of significant amounts of natural antioxidant phenolics present in red wine (*4*). Indeed, epidemiological studies show that intake of these compounds is correlated with reduced incidence of coronary heart disease (CHD) (*5*, *6*). Furthermore, red wine and its antioxidant phenolic components increase serum antioxidant capacity in vivo (*7*, *8*), inhibit low-density lipoprotein (LDL) oxidation in vitro (*6*, *9*−*11*) and ex vivo (*8*, *12*), inhibit platelet aggregation in vitro (*6*), and demonstrate scavenging capacity against free radicals in vitro (*6*). Taken together, these observations indicate that red wine phenolic compounds provide protection against oxidation although the mechanism of this effect is not totally understood (*7*).

Recently, an aqueous solution of green tea polyphenols (GTP) was found to inhibit lipid peroxidation and scavenge hydroxyl and superoxide radicals in vitro (*13*). Administration of GTP (500 mg/kg body weight) to normal rats increased glucose tolerance significantly ($P < 0.005$) at 60 min. GTP was also found to significantly reduce serum glucose levels in alloxan diabetic rats at a dose level of 100 mg/kg. Continued daily administration (15 days) of the extract at 50 and 100 mg/kg produced 29 and 44% reduction in the elevated serum glucose level produced by alloxan administration. Elevated hepatic and renal enzymes produced by alloxan were found to be reduced

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($P < 0.001$) by GTP. The serum LP levels were increased by alloxan and were reduced significantly ($P < 0.001$) by the administration of 100 mg/kg of GTP. The GTP-treated group showed an increased antioxidant potential as seen from improvements in superoxide dismutase and glutathione levels. These results indicate that alterations in the glucose utilizing system and oxidation status in rats increased by alloxan were partially reversed by the administration of GTP.

In another study (*14*), streptozotocin (STZ)-induced diabetic rats exhibited alterations in cardiac function, ventricular remodeling, and changes in cell signaling, including protein kinase C (PKC) isoforms. The effect of low-dose ethanol consumption on cardiac function, geometry, and PKC isoforms in STZ-induced diabetic rats was studied. Consumption of 4% ethanol by diabetic animals prevented the decrease in left ventricular contraction and the increase in left ventricular internal dimension observed in untreated diabetic rats. Up-regulation of PKC cytosolic and membrane protein content (PKC-$\alpha$, -$\delta$, and -$\epsilon$) occurring in the diabetic animals was also prevented by ethanol consumption, whereas ethanol had no effect on PKC isoform pattern in nondiabetic animals. This work suggested that STZ-induced cardiac remodeling and dysfunction are associated with increases in PKC activity, particularly PKC-$\alpha$, -$\delta$, and -$\epsilon$, and that consumption of 4% ethanol can prevent those changes.

In the Zutphen elderly study (*15*), after adjustment for age, education, and smoking status, men with cardiovascular diseases (CVD)/diabetes and low to moderate alcohol intake had a significantly lower risk for poor cognitive functions (mini-mental state examination < 25) than abstainers (odds ratios of 0.3 for less than one drink and 0.2 for 1−2 drinks per day). Alcohol intake was not associated with cognitive decline. This study concluded that alcohol may result in an acute beneficial effect on cognitive function among those with CVD/diabetes.

More recently, in a prospective study with 8663 men (aged 30−79 years old), fasting plasma glucose measurements and alcohol intake (all types: beers, wines, and spirits) were classified into five groups (nondrinkers and four quartiles) according to the alcohol intake levels (*16*). Type 2 diabetes was diagnosed according to the 1997 American Diabetes Association criteria (fasting glycaemia > 1.26 g/L). There were 149 incident cases of type 2 diabetes during the 6 years of follow-up. A U-shaped association between alcohol intake and diabetes was observed, with the lowest incidence of diabetes observed for 61.9−122.7 g alcohol/week (corresponding to the moderate consumption of 1−2 glasses/per day). Nondrinkers and heavy drinkers had a higher risk of developing diabetes (2.3- and 2-fold, respectively) when compared with moderate drinkers (1 or 2 glasses of alcoholic beverages/day). These changes persisted after adjustment for age, fasting plasma glucose, smoking, body mass index, blood pressure, serum triglyceride concentration, cardiorespiratory fitness, HDL cholesterol, waist circumference, and parental diabetes. It was calculated that if nondrinkers become moderate drinkers, there would be a 10% reduction in diabetes in their population and if heavy drinkers become moderate drinkers a 24% incident reduction in diabetes would occur. The authors concluded that a light or moderate consumption of alcoholic beverages is associated with an improvement of insulin sensitivity. However, this study provided no insights into whether the beneficial effects of consuming moderate amounts of alcoholic beverages are due to alcohol and/or to the presence of phenolic compounds, which are present in high concentrations in red wine.

Our previous study (*17*) indicated that white wine, when enriched in polyphenols, was able to induce ethanol-independent in vivo effects in STZ-induced diabetes characterized by a major oxidative stress. In the present study, we investigated the in vivo effect of red wine polyphenols extract (RWPE), ethanol, or both on the same model of insulin deficient diabetes (*18*). General physical and biochemical parameters were also determined. Because polyphenols have been described as vasoactive agents (*19*, *20*), we also studied the influence of RWPE, ethanol, or both on the morphological changes of mesenteric arteries previously described in the same model of diabetes. The aim of our study was to know whether RWPE and ethanol are able to correct diabetes and whether they possess additive effects on glycemic control.

## MATERIAL AND METHODS

**Preparation and Characterization of Red Wine Polyphenolic Extract.** The RWPE dry powder from red French wine (Corbières A.O.C.) was prepared according to the following procedure: briefly, phenolic compounds were adsorbed on a preparative column (20% Polyclar AT, 10% silica G gel, and 70% silica 60 gel; this adsorbant mix was treated by 12 N HCl during 30 min and rinsed by twice-distilled water until neutrality); then, the wine was added to the column; and an elution with twice-distilled water was realized to flush out sugars, acids, and minerals. In a second time, phenolics were eluted with methanol; the alcoholic eluent was gently evaporated; the concentrated residue was lyophilized and finely sprayed by atomization to obtain the RWPE dry powder.

One liter of red wine produced 2.9 g of extract, which contained 471 mg/g of total phenolic compounds expressed as gallic acid. Total phenol content was determined using the Folin−Ciocalteu method (*21*). Samples were calibrated against gallic acid, and the results were expressed as gallic acid equivalents (GAE). Phenolic levels in the extract were obtained according to high-performance liquid chromatography (HPLC) analysis procedure as described in the following section. In particular, the extract contained 8.6 mg/g catechin, 8.7 mg/g epicatechin, dimers (B1, 6.9 mg/g; B2, 8.0 mg/g; B3, 20.7 mg/g; and B4, 0.7 mg/g), anthocyanins (malvidin-3-glucoside, 11.7 mg/g; peonidin-3-glucoside, 0.66 mg/g; and cyanidin-3-glucoside, 0.06 mg/g), and phenolic acids (gallic acid, 5.0 mg/g; caffeic acid, 2.5 mg/g; and caftaric acid, 12.5 mg/g).

**Standards and HPLC Analysis.** (+)-Catechin and (−)-epicatechin were obtained from Aldrich (St. Quentin Fallavier, France). Procyanidin dimers B1, B2, B3, and B4 were obtained from grape seeds as previously reported (*22*). HPLC analysis with UV detection at 280, 313, 365, and 520 nm was used. A Hewlett-Packard model 1090 with three low-pressure pumps and a diode array detector coupled to a Hewlett-Packard Chem Station was used for solvent delivery and detection. A Hewlett-Packard column packed with Nucleosil 100 C18 (250 mm × 4 mm, 5 $\mu$m particle size) was used for the stationary phase. The flow was 0.7 mL/min. The solvents used for separation (*22*) were as follows: solvent A, 50 mmol/L dihydrogen ammonium phosphate adjusted to pH 2.6 with orthophosphoric acid; solvent B, 20% A with 80% acetonitrile; solvent C, 200 mmol/L orthophosphoric acid adjusted with ammonia to pH 1.5. Elution was performed with a gradient previously described (*23*).

**Animals and Treatment Groups.** Seventy-two Wistar rats (180−200 g) from Iffa Credo (Labresle, France) were used for this study. Half of the animals were injected with STZ (60 mg/kg) i.v. Treatments were administered for 6 weeks under a single daily 10 mL/kg gavage performed between 8 and 9 a.m. to healthy control or diabetic rats. Untreated animals received water only. Treatment groups (nine animals per group) included untreated control or diabetic (C and D), treated control with 200 mg/kg RWPE (CP), 1 mL/kg ethanol (CE), or both (CPE), and diabetic treated with the same doses of RWPE (DP), ethanol (DE), or both (DPE) animals. The weight was recorded three times a week while food and water intakes were recorded daily. Glycemia was checked every week from a cut to the tail using a Glucometer apparatus. The oral glucose tolerance test (OGTT) was performed on overnight fasted animals (no treatment on that day). Briefly, the glucose solution (5 g/kg) was administered by gavage under a volume of 10 mL/kg

b.w. and glycemia followed for 5 h using the Glucometer apparatus. At the end of the 6 week treatment period, unfasted and untreated animals were killed by decapitation and blood was collected on a heparinized tube. Plasma was stored at −80 °C until further use.

**Measurement of Plasma Antioxidant Capacity.** At the end of treatment, blood samples were collected on heparinized tubes and centrifuged for 20 min (2000 $min^{-1}$), and the plasma was stored at −80 °C until processed.

The plasma antioxidant capacity was determined by the total antioxidant status method of Randox. A Kit Randox catalog no. NX2332 (Randox Laboratories LTD, Crumlin, U.K.) was used. This assay is based on the 2,2′-azino-di-(3-ethylbenzothiazoline sulfonate) (ATBS) incubated with a peroxidase (POD) (methmyoglobin) and hydrogen peroxyde ($H_2O_2$) to produce the radical cation $ATBS^{\bullet+}$. This has a relatively stable blue-green color, which is measured at 600 nm.

Antioxidants in the added sample cause suppression of this color production to a degree proportional to their concentration. This analytical procedure has been applied to physiological antioxidant compounds and radical scavenging drugs, and an antioxidant ranking based on their reactivity relative to a 1.0 mmol/L Trolox standard has been established. The Trolox equivalent antioxidant capacity of plasma from an adult reference population has been measured, and the method was optimized and validated (*24*). We used this automated method to investigate the total plasma antioxidant capacity of plasma rats.

**Biochemical Parameters.** Assessment of glycemia, total cholesterol, triglycerides, HDL cholesterol (high-density lipoprotein), ALP (alkaline phosphatase), AST (aspartate aminotransferase), ALT (alanine aminotransferase), and urea were performed on final blood sampling with an HITACHI 704 apparatus.

Triglycerides analysis was performed with a Kit no. 1488872 Roche/Hitachi, Roche Diagnostics, Gmbh-D-68298 Mannheim. This enzymatic triglycerides assay required a liporotein lipase from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of POD to form a red dyestuff (Triender end point reaction).

*Glycemia.* Analysis was performed with a Kit no. 1448668 Roche/Hitachi, Roche Diagnostics. Sample and addition of R1 (buffer/enzymes/4-aminophenazone/phenol) and start a reaction where glucose is oxidized by glucose oxidase (GOD) to gluconolactone in the presence of atmospheric oxygen, $H_2O_2$ is also created. This resultant hydrogen peroxide oxidizes 4-(*p*-benzoquinone-monoimino)phenazone in the presence of POD. The color intensity of the red dye is directly proportional to the glucose concentration and can be measured photometrically.

*Total Cholesterol.* Analysis was performed with a Kit no. 1489232 Roche/Hitachi, Roche Diagnostics. Cholesterol is determined enzymatically using cholesterol esterase and cholesterol oxidase. Sample and addition of R1 (cholesterol reagent) conducts start the reactions. Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol is converted by oxygen with the aid of cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide created forms a red dyestuff 4-(*p*-benzoquinone-monoimino)phenazone by reacting with 4-aminophenazone and phenol under the catalytic action of POD. The color intensity is directly proportional to the concentration of cholesterol and can be determined photometrically.

*HDL Cholesterol.* Analysis was performed with a Kit no. 1930672 Roche, Roche Diagnostics. The principle is based on an homogeneous enzymatic colorimetric test. Sample and addition of R1 (sulfated *a*-cyclodextrin/buffer) are realized. In the presence of slightly alkaline buffer and magnesium sulfate, sulfated a-cyclodextrin and dextran sulfate selectively form water soluble complexes with LDL, VLDL, and chylomicrons, which are resistant to PEG-modified enzymes (PEG = poly(ethylene glycol)). Addition of R2 (PEG-modified enzymes/4-aminophenazone/buffer) starts the reaction where the cholesterol concentration of HDL cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approximately 40%). Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to $D^4$-cholestenone and hydrogen peroxide. In the presence of POD, the hydrogen peroxide generated reacts with 4-aminophenazone and HSDA (HDSA = N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline) to form a purple-blue dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.

*Urea.* Analysis was performed with a Kit no. 1489364 Roche/Hitachi, Roche Diagnostics. The principle is based on a kinetic UV assay with addition to the sample of R1 (buffer/NADH) and addition of R2 (buffer/enzymes/substrate) to start the reaction where urea is hydrolyzed by urease to form $CO_2$ and ammonia. The ammonia formed then reacts with a-ketoglutarate and NADH in the presence of GLDH (glutamate dehydrogenase) to yield glutamate and $NAD^+$. The decrease in absorbance due to consumption of NADH is measured kinetically.

*AST.* Analysis was performed with a Kit no. 851124 Roche/Hitachi, Roche Diagnostics. The principle is based on a UV test according to a standardized method in which the addition to the sample of R1 (buffer/enzyme/coenzyme) and R2 (a-ketoglutarate) starts the reaction where a-ketoglutarate and L-aspartate can give L-glutamate and oxaloacetate. AST is the enzyme catalyzing this equilibrium reaction. The oxaloacetate increase is measured in a subsequent indicator reaction, which is catalyzed by malate dehydrogenase. Oxaloacetate and NADH in the presence of $H^+$ give L-malate and $NAD^+$. In this second reaction, NADH is oxidized to NAD. The rate of decrease in NADH (measured photometrically) is directly proportional to the rate of formation of oxaloacetate and thus to the AST activity.

*ALT.* Analysis was performed with a Kit no. 851132 Roche/Hitachi, Roche Diagnostics. The principle is based on a UV test according to a standardized method in which the addition to the sample of R1 (buffer/enzyme/coenzyme) and R2 (a-ketoglutarate) starts the reaction where a-ketoglutarate and L-alanine can give L-glutamate and pyruvate. ALT is the enzyme catalyzing this equilibrium reaction. The pyruvate increase is measured in a subsequent indicator reaction, which is catalyzed by lactate dehydrogenase. Pyruvate and NADH in the presence of $H^+$ gives L-lactate and $NAD^+$. In this second reaction, NADH is oxidized to NAD. The rate of decrease in NADH (measured photometrically) is directly proportional to the rate of formation of pyruvate and thus to the ALT activity.

*ALP.* Analysis was performed with a Kit no. 816388 Roche, Roche Diagnostics. The principle is based on a colorimetric assay in accordance with a standardized method with addition of R1 (buffer: diethanolamine and magnesium chloride) to the sample and the addition of R2 (buffer: diethanolamine and magnesium chloride/substrate: *p*-nitrophenyl phosphate) to start the reaction. In the presence of magnesium and zinc ions, *p*-nitrophenyl phosphate is hydrolyzed by phosphatases (ALP) to form phosphate and *p*-nitrophenol. The *p*-nitrophenol released is proportional to the ALP activity and can be measured photometrically.

**Histomorphometric Study of Arterial Mesenteric System.** Histomorphometric analysis of mesenteric arterial system was performed as previously described (*25*). Briefly, the mesenteric vascular tree was collected by dissecting the superior mesenteric artery and its branches until their penetration into jejunum, was immerged for 6 h in Bouin solution, and was kept in 70% ethanol until processed. Tissue samples were cut cross-way to the general direction of vessels, at the distal portion of jejunal arteries resulting from the division of the cranial mesenteric artery, and embedded in paraplast (Histomed Standard, Labo Moderne, France); 3 mm thick transverse sections, obtained at ≈5 mm from the distal end of the arteries, were stained with PAS for histomorphometric analysis in order to visualize elastic laminae.

Morphometric measurements were performed using the axioHOME system (Carl Zeiss, Oberkochen, Germany). Briefly, the system consists of an IBM-PC compatible computer using the 2.04 version of the Zeiss-Alcatel TITN Answare software (Meylan, France) and a light microscope in which a built-in high-resolution image is superimposed on the optical image of the specimen (*25*). Five to thirteen arteries (vessels showing several elastic laminae) per animal were quantified, for a total of 69−90 vessels per group. For each artery examined, the parameters were measured as follows: diameter (measured for each vessel at its minimal value, D), total vessel (T), media + lumen (ML), and lumen

**Table 1.** Food Intake, Water Intake, and Body Growth of the Various Treatment Groups[a]

| treatment group | water intake (mL/day/cage) ($n = 87$) | food intake (g/day/cage) ($n = 87$) | body growth (g) ($n = 9$) |
|---|---|---|---|
| C | 78.74 ± 0.96 | 71.83 ± 1.42 | 110.89 ± 10.61 |
| CP | 39.43 ± 1.46 | 63.40 ± 1.26 | 83.33 ± 9.62 |
| CE | 65.98 ± 0.95 | 67.86 ± 1.48 | 111.33 ± 7.06 |
| CPE | 74.89 ± 1.19 | 64.75 ± 1.49 | 100.0 ± 6.58 |
| D | 554.65 ± 6.86 | 112.07 ± 3.19 | 40.22 ± 6.07 |
| DP | 354.60 ± 4.70 | 99.95 ± 1.89 | 26.56 ± 5.89 |
| DE | 297.82 ± 8.69 | 87.03 ± 1.85 | 52.22 ± 8.32 |
| DPE | 318.45 ± 35.94 | 91.74 ± 2.17 | 68.89 ± 12.10 |

[a] For water and food intakes, values are given as means ± SEM of 87 values/group recorded on three cages per group (three animals per cage) during the 29 day study period. For body growth, values are means ± SEM of nine animals/group.

(L) areas. Adventitia (A) and media (M) areas were calculated as (T − ML) and (ML − L), respectively, assuming that the intima area was negligible in the conditions of quantification.

**Expression of Results and Statistical Analysis.** Data were expressed as means ± standard error of the mean (SEM). Body growth and blood glucose (BG) changes were calculated as means of individual differences between recordings at the end minus recordings at the initiation of treatment of weight and Glucometer BG determinations, respectively. For morphometric data, areas were transformed into their logarithmic values so that normal distribution and homogeneity of variance between groups were obtained for each measured or calculated parameter.

Statistical analyses were realized using Statgraphics plus 4.0 software. The effects of three factors, namely, pathology (A), ethanol (B), and RWPE (C) treatment on the distribution of the variables indicated above, were determined by a three way analysis of variance (ANOVA) ($p$: significance level) in which we observed the effect of each factor A, B, and C and the interactions between them (AB, AC, BC, and ABC). For a better understanding of the results obtained in each pathological group, another multifactorial variance analysis with two factors only (ethanol, A, and RWPE, B) in a fixed pathological condition was performed, in which the effect of the two factors ethanol and RWPE and the interaction between them (AB) were analyzed. In some cases, one factor analysis followed by the Newmann−Keuls test was used to compare the different groups two by two.

## RESULTS

**General Feature of the Animals. Table 1** indicates the general feature of the animals; **Figure 1** illustrates the evolution of glycemia during the treatment period. No mortality was observed in any treatment group.

As expected, three factor analysis (**Table 2**) indicated that diabetes induced a significant increase in food and water intake consumption ($p < 10^{-4}$) and a decrease in body growth ($p < 10^{-4}$) in diabetic animals, along with a major increase in glycemia levels ($p < 10^{-4}$; **Tables 3** and **4**; **Figure 1**).

Three factor analysis indicated that ethanol induced a significant increase in body growth ($p = 0.004$) and a decrease in food ($p < 10^{-4}$) and water ($p < 10^{-4}$) intakes. Two factor analysis showed that those changes were manifested in diabetic animals only ($p = 3 \times 10^{-3}$), suggesting that ethanol treatment induced the correction of diabetes. Indeed, in nondiabetic animals, ethanol induced no change in food and an increase in water intake ($p < 10^{-4}$). Correction of diabetes by ethanol was confirmed by the level of glycemia taken either during the study (**Figure 1** and **Tables 1** and **2**; $p = 0.003$) or at sacrifice (**Tables 3** and **4**; $p = 0.04$). Three factor analysis indicated that RWPE induced a significant decrease in food ($p < 10^{-4}$) and water ($p < 10^{-4}$) intakes.

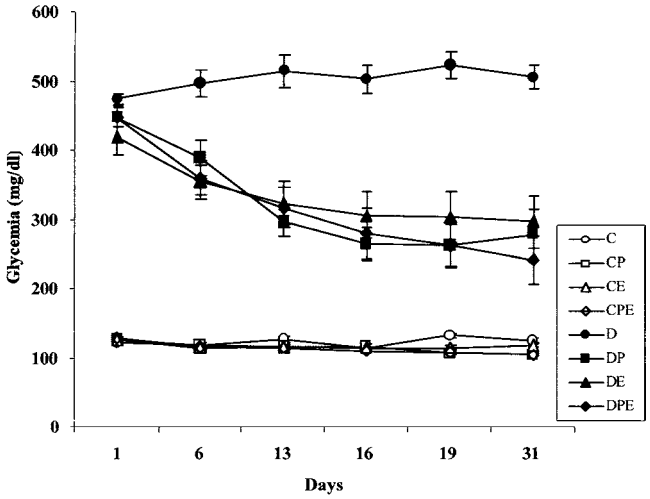


**Figure 1.** Evolution of BG in untreated (C or D) or treated control or diabetic rats by polyphenolic extract (CP or DP), ethanol (CE or DE), or both (CPE and DPE, respectively). Treatments were administered for 31 days and BG recorded at the days indicated using a Glucometer apparatus. Treatment induced no significant change in control animals. BG was significantly ($p \leq 0.05$) lower in DP, DP, and DPE than in D animals from day 6 to day 31.

In nondiabetic animals, two factor analysis showed that both effects were manifested in association with a decrease in body growth ($p = 0.031$) and a slight but significant decrease in BG (**Tables 1** and **2**; $p = 0.0002$). A significant interaction was found between ethanol and RWPE ($p < 10^{-4}$) on water intake. One factor analysis of water intake decrease showed that the effect of RWPE was manifested in the absence ($p < 10^{-4}$), but not in the presence, of ethanol.

In diabetic animals, two factor analysis indicated that RWPE alone had no effect on food intake and lowered water intake ($p < 10^{-4}$), although a significant interaction was observed between ethanol and RWPE for both parameters ($p = 0.004$ and $p < 10^{-4}$, respectively). One factor analysis showed that RWPE lowered food or water intake in the absence ($p = 0.0013$ and $p < 10^{-4}$, respectively) but not in the presence of ethanol. Those effects were associated with a decrease in glycemia recordings during treatment (**Figure 1** and **Tables 1** and **2**; $p < 10^{-4}$) but not on day of sacrifice (**Table 4**), i.e., in the absence of treatment on that day.

**Biochemical Analyses (Tables 3 and 4).** Three factor ANOVA indicated that diabetes was associated with lower plasma insulin levels ($p = 0.035$). Two factor analysis indicated that ethanol treatment was associated with higher plasma insulin levels in diabetic animals ($p = 0.038$), confirming the antidiabetic effect of ethanol previously noted.

Three factor ANOVA indicated that polyphenols induced an increase in AST ($p = 0.0412$) or ALT ($p = 0.0005$) levels, while an interaction was noted in the latter case between polyphenols and pathology ($p = 0.0141$). The two factor analysis confirmed that the ALT increase was manifested in diabetic animals only ($p = 0.02$).

**OGTT.** OGTT (**Figure 2**) was performed in all treatment groups on the third week of the experiment. In control groups, no significant difference was observed in glycemia from all time points after glucose administration or in corresponding AUC (mean values comprised between 632 and 697 mg $dL^{-1}$ h). In

**Table 2.** Three and Two Factor ANOVA Analysis of Food Intake, Water Intake, Body Growth, and BG Changes[a]

Three Factors

| | food | water | body growth | BG change |
|---|---|---|---|---|
| | | Pathology (A) | | |
| 0 | 66.96 ± 0.96 | 64.75 ± 6.71 | 101.39 ± 4.28 | –12.42 ± 10.58 |
| 1 | 97.69 ± 0.96* | 381.38 ± 6.71* | 46.97 ± 4.28* | –117.72 ± 10.58 |
| *p* | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| | | Ethanol (B) | | |
| 0 | 86.81 ± 0.96 | 256.85 ± 6.71 | 65.25 ± 4.28 | –39.89 ± 10.58 |
| 1 | 77.84 ± 0.96* | 189.28 ± 6.71* | 83.11 ± 4.28* | –90.25 ± 10.58 |
| *p* | 0.0000 | 0.0000 | 0.0044 | 0.0013 |
| | | Polyphenol (C) | | |
| 0 | 84.69 ± 0.96 | 249.30 ± 6.71 | 78.67 ± 4.28 | –24.64 ± 10.58 |
| 1 | 79.96 ± 0.96* | 196.84 ± 6.71* | 69.69 ± 4.28 | –105.5 ± 10.58 |
| *p* | 0.0005 | 0.0000 | NS | 0.0000 |
| interaction: *p* | AB: 0.0000 | AB: 0.0000 | | AB: 0.0042 |
| | BC: 0.0001 | AC: 0.0001 | NS | AC: 0.0001 |
| | ABC: 0.0354 | BC: 0.0000 | | BC: 0.0421 |
| | | ABC: 0.0000 | | |

Two Factors
Pathology = 0

| | food | water | body growth | BG change |
|---|---|---|---|---|
| | | Ethanol (A) | | |
| 0 | 67.61 ± 1.00 | 59.08 ± 0.82 | 97.11 ± 6.11 | –9.44 ± 3.29 |
| 1 | 66.31 ± 1.00 | 70.43 ± 0.82* | 105.67 ± 6.11 | –15.39 ± 3.29 |
| *p* | NS | 0.0000 | NS | NS |
| | | Polyphenol (B) | | |
| 0 | 69.84 ± 1.00 | 72.36 ± 0.82 | 111.11 ± 6.11 | –2.78 ± 3.29 |
| 1 | 64.08 ± 1.00* | 57.15 ± 0.82* | 91.67 ± 6.11* | –22.06 ± 3.29 |
| *p* | 0.0001 | 0.0000 | 0.0313 | 0.0002 |
| interaction | | AB | | |
| *p* | NS | 0.0000 | NS | NS |

Two Factors
Pathology = 1

| | food | water | body growth | BG change |
|---|---|---|---|---|
| | | Ethanol (A) | | |
| 0 | 106.01 ± 1.65 | 454.63 ± 13.40 | 33.39 ± 5.99 | –70.33 ± 20.90 |
| 1 | 89.38 ± 1.65* | 308.13 ± 13.40* | 60.55 ± 5.99* | –165.11 ± 20.90 |
| *p* | 0.0000 | 0.0000 | 0.003 | 0.0030 |
| | | Polyphenol (B) | | |
| 0 | 99.55 ± 1.65 | 426.24 ± 13.40 | 46.22 ± 5.99 | –46.5 ± 20.90 |
| 1 | 95.84 ± 1.65 | 336.52 ± 13.40* | 47.72 ± 5.99 | –188.94 ± 20.90 |
| *p* | NS | 0.0000 | NS | 0.0000 |
| interaction | AB | AB | | |
| *p* | 0.0004 | 0.0000 | NS | NS |

[a] * Significant difference ($p < 0.05$). $p$ = level of significance. Values are given as means ± SEM of 29 values per cage for food and water intakes and nine values per group for body growth.

**Table 3.** Plasma Parameters for Control or Diabetic Groups at the End of the Treatment Period[a]

| treatment groups | glucose (g/L) | cholesterol (g/L) | AST (UI/L) | ALT (UI/L) | insulin (ng/mL) | TAS (mmol/L) |
|---|---|---|---|---|---|---|
| C | 1.05 ± 0.07 | 0.92 ± 0.03 | 91.0 ± 7.3 | 24.6 ± 1.5 | 3.37 ± 0.24 | 1.29 ± 0.02 |
| CP | 1.19 ± 0.09 | 0.93 ± 0.01 | 105.1 ± 7.2 | 28.4 ± 1.0 | 2.54 ± 0.37 | 1.25 ± 0.03 |
| CE | 1.22 ± 0.06 | 0.92 ± 0.02 | 92.7 ± 5.8 | 27.9 ± 1.8 | 2.38 ± 0.41 | 1.22 ± 0.01 |
| CPE | 1.21 ± 0.07 | 0.92 ± 0.03 | 113.1 ± 8.6 | 28.0 ± 1.5 | 2.89 ± 0.23 | 1.31 ± 0.01 |
| D | 4.70 ± 0.17 | 1.09 ± 0.03 | 100.9 ± 4.3 | 33.6 ± 1.5 | 1.52 ± 0.24 | 1.28 ± 0.03 |
| DP | 4.85 ± 0.07 | 1.10 ± 0.03 | 96.4 ± 10.4 | 51.4 ± 4.1 | 1.17 ± 0.23 | 1.23 ± 0.03 |
| DE | 3.63 ± 0.51 | 1.00 ± 0.04 | 91.3 ± 7.1 | 33.2 ± 2.4 | 2.19 ± 0.62 | 1.30 ± 0.02 |
| DPE | 3.53 ± 0.54 | 0.99 ± 0.03 | 127.8 ± 27.2 | 45.4 ± 7.7 | 2.5 ± 0.60 | 1.25 ± 0.03 |

[a] Values are given as means ± SEM of nine animals per group.

diabetic animals, a significant difference was observed between D (AUC = 1731.3 ± 107.3 mg $dL^{-1}$ h) or DP (AUC = 1742.9 ± 155.7 mg $dL^{-1}$ h) on one hand and DE (AUC = 1178.0 ± 111.7 mg $dL^{-1}$ h) or DPE (AUC = 1352.0 ± 176.8 mg $dL^{-1}$ h) groups on the other hand ($p < 10^{-3}$).

**Histomorphometric Analysis of Mesenteric Arterial System (Tables 5 and 6).** Three factor analysis confirmed that diabetes had a significant enlarging effect on mesenteric vessels, inducing a significant increase of all parameters ($p < 10^{-4}$). Three factor analysis indicated that ethanol induced a reduction

**Table 4.** Three and Two Factor ANOVA Analysis of Biochemical Parameters[a]

| | glucose | cholesterol | triglycerides | AST | ALT | insulin | antioxidant capacity |
|---|---|---|---|---|---|---|---|
| **Three Factors** | | | | | | | |
| | | | | Pathology (A) | | | |
| 0 | 1.21 ± 0.14 | 0.91 ± 0.02 | 1.26 ± 0.06 | 109.64 ± 8.76 | 27.61 ± 1.72 | 2.79 ± 0.31 | 1.29 ± 0.02 |
| 1 | 4.18 ± 0.14* | 1.05 ± 0.02* | 1.06 ± 0.06* | 104.11 ± 8.76 | 40.92 ± 1.72* | 1.84 ± 0.31* | 1.26 ± 0.02 |
| *p* | 0.0000 | 0.0000 | 0.0189 | NS | 0.0000 | 0.0348 | NS |
| | | | | Ethanol (B) | | | |
| 0 | 2.95 ± 0.14 | 1.01 ± 0.02 | 1.20 ± 0.06 | 98.36 ± 8.76 | 34.5 ± 1.72 | 2.15 ± 0.31 | 1.26 ± 0.02 |
| 1 | 2.44 ± 0.14* | 0.95 ± 0.02* | 1.13 ± 0.06 | 115.39 ± 8.76 | 34.03 ± 1.72 | 2.49 ± 0.31 | 1.29 ± 0.02 |
| *p* | 0.0146 | 0.0129 | NS | NS | NS | NS | NS |
| | | | | Polyphenol (C) | | | |
| 0 | 2.65 ± 0.14 | 0.98 ± 0.02 | 1.21 ± 0.06 | 93.97 ± 8.76 | 29.81 ± 1.72 | 2.36 ± 0.31 | 1.27 ± 0.02 |
| 1 | 2.74 ± 0.14 | 0.98 ± 0.02 | 1.12 ± 0.06 | 119.78 ± 8.76* | 38.72 ± 1.72* | 2.27 ± 0.31 | 1.28 ± 0.02 |
| *p* | NS | NS | NS | 0.0412 | 0.0005 | NS | NS |
| interaction | AB | | AB | | AC | | AC |
| *p* | 0.0011 | NS | 0.0019 | NS | 0.0141 | NS | 0.0401 |
| **Two Factors Pathology = 0** | | | | | | | |
| | glucose | cholesterol | triglycerides | AST | ALT | insulin | antioxidant capacity |
| | | | | Ethanol (A) | | | |
| 0 | 1.12 ± 0.08 | 0.92 ± 0.02 | 1.43 ± 0.10 | 98.06 ± 13.87 | 26.5 ± 1.16 | 2.96 ± 0.53 | 1.27 ± 0.04 |
| 1 | 1.30 ± 0.08 | 0.90 ± 0.02 | 1.09 ± 0.10* | 121.22 ± 13.87 | 28.72 ± 1.16 | 2.63 ± 0.53 | 1.31 ± 0.04 |
| *p* | NS | NS | 0.0264 | NS | NS | NS | NS |
| | | | | Polyphenol (B) | | | |
| 0 | 1.13 ± 0.08 | 0.92 ± 0.02 | 1.37 ± 0.10 | 91.83 ± 13.87 | 26.22 ± 1.16 | 2.87 ± 0.53 | 1.25 ± 0.04 |
| 1 | 1.29 ± 0.08 | 0.91 ± 0.02 | 1.16 ± 0.10 | 127.44 ± 13.87 | 29.00 ± 1.16 | 2.72 ± 0.53 | 1.33 ± 0.04 |
| *p* | NS | NS | NS | NS | NS | NS | NS |
| interaction | | | | | | | AB |
| *p* | NS | NS | NS | NS | NS | NS | 0.0397 |
| **Two Factors Pathology = 1** | | | | | | | |
| | glucose | cholesterol | triglycerides | AST | ALT | insulin | antioxidant capacity |
| | | | | Ethanol (A) | | | |
| 0 | 4.78 ± 0.27 | 1.09 ± 0.02 | 0.96 ± 0.06 | 98.67 ± 10.69 | 42.5 ± 3.24 | 1.34 ± 0.33 | 1.25 ± 0.02 |
| 1 | 3.58 ± 0.27* | 1.00 ± 0.02* | 1.16 ± 0.06* | 109.56 ± 10.69 | 39.33 ± 3.24 | 2.34 ± 0.33* | 1.27 ± 0.02 |
| *p* | 0.0041 | 0.0123 | 0.0190 | NS | NS | 0.0385 | NS |
| | | | | Polyphenol (B) | | | |
| 0 | 4.17 ± 0.27 | 1.04 ± 0.02 | 1.05 ± 0.06 | 96.11 ± 10.69 | 33.39 ± 3.24 | 1.85 ± 0.33 | 1.29 ± 0.02 |
| 1 | 4.19 ± 0.27 | 1.05 ± 0.02 | 1.08 ± 0.06 | 112.11 ± 10.69 | 48.44 ± 3.24* | 1.83 ± 0.33 | 1.24 ± 0.02 |
| *p* | NS | NS | NS | NS | 0.0024 | NS | NS |
| interaction | | | | | | | |
| *p* | NS | NS | NS | NS | NS | NS | NS |

[a] * Significant difference ($p < 0.05$). $p$ = level of significance. Values are given as means ± SEM of nine animals per group.

**Table 5.** Histomorphometric Parameters for Control and Diabetic Groups[a]

| | diameter | total surface [ln($\mu$m$^2$)] | lumen [ln($\mu$m$^2$)] | media [ln($\mu$m$^2$)] | adventice [ln($\mu$m$^2$)] |
|---|---|---|---|---|---|
| control | 171.0 ± 2.9 | 10.21 ± 0.03 | 8.69 ± 0.04 | 9.36 ± 0.03 | 9.16 ± 0.03 |
| CP | 166.5 ± 2.7 | 10.18 ± 0.03 | 8.60 ± 0.06 | 9.29 ± 0.03 | 9.18 ± 0.03 |
| CE | 169.4 ± 2.9 | 10.22 ± 0.03 | 8.73 ± 0.04 | 9.33 ± 0.03 | 9.18 ± 0.03 |
| CPE | 166.4 ± 2.4 | 10.18 ± 0.03 | 8.59 ± 0.05 | 9.30 ± 0.03 | 9.16 ± 0.03 |
| D | 193.3 ± 3.5 | 10.49 ± 0.04 | 9.16 ± 0.06 | 9.50 ± 0.04 | 9.43 ± 0.04 |
| DP | 212.4 ± 4.7 | 10.69 ± 0.05 | 9.49 ± 0.06 | 9.68 ± 0.05 | 9.55 ± 0.05 |
| DE | 191.5 ± 2.9 | 10.50 ± 0.04 | 9.13 ± 0.06 | 9.56 ± 0.04 | 9.40 ± 0.03 |
| DPE | 186.7 ± 4.2 | 10.43 ± 0.04 | 9.12 ± 0.06 | 9.47 ± 0.04 | 9.33 ± 0.05 |

[a] Values are given as means ± SEM of 69–90 values per group.

in diameter, total surface, adventice, and lumen ($p = 0.015$). A significant interaction between ethanol and pathology was noted for diameter, total surface, and lumen ($p < 0.02$). Two factor analysis indicated that the effect of ethanol was manifested in diabetic animals only ($p < 0.003$), suggesting that it was related to the correction of diabetes induced by ethanol.

Three factor analysis indicated that RWPE had no effect on the various parameters. However, a significant interaction was noted between all three factors for diameter, total surface, and media ($0.005 < p < 0.0137$) and between RWPE and ethanol for all parameters ($0.005 < p < 0.0282$). In nondiabetic animals, two factor analysis showed that RWPE induced an enlargement of lumen ($p = 0.0188$). In diabetic animals, two factor analysis showed a significant interaction between ethanol and RWPE for all parameters ($0.0012 < p < 0.0157$). One factor analysis showed that RWPE induced an increase in all morphometric parameters in diabetic animals in the absence ($0.0001 < p < 0.048$) but not in the presence of ethanol.

## DISCUSSION

Various epidemiological studies indicate that moderate red wine consumption may be beneficial for health, in particular in CVD. As diabetes constitutes a major risk for CVD, the effect of wine constituents on diabetes deserves to be established. Among the components of wine, ethanol itself may play a major

**Table 6.** Three and Two Factor ANOVA of Histomorphometric Parameters[a]

Three Factors

| | diameter | total surface [ln($\mu$m)] | media [ln($\mu$m)] | adventice [ln($\mu$m)] | lumen [ln($\mu$m)] |
|---|---|---|---|---|---|
| | | Pathology (A) | | | |
| 0 | 168.33 ± 1.62 | 10.20 ± 0.02 | 9.32 ± 0.02 | 9.17 ± 0.02 | 8.65 ± 0.03 |
| 1 | 196.01 ± 1.68* | 10.53 ± 0.02* | 9.55 ± 0.02* | 9.43 ± 0.02* | 9.22 ± 0.03* |
| *p* | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| | | Ethanol (B) | | | |
| 0 | 185.83 ± 1.68 | 10.40 ± 0.02 | 9.46 ± 0.02 | 9.33 ± 0.02 | 8.98 ± 0.03 |
| 1 | 178.51 ± 1.62* | 10.33 ± 0.02* | 9.41 ± 0.02 | 9.27 ± 0.02* | 8.89 ± 0.03* |
| *p* | 0.0017 | 0.0152 | NS | 0.0159 | 0.0164 |
| | | Polyphenol (C) | | | |
| 0 | 181.32 ± 1.64 | 10.36 ± 0.02 | 9.44 ± 0.02 | 9.29 ± 0.02 | 8.93 ± 0.03 |
| 1 | 183.01 ± 1.66 | 10.37 ± 0.02 | 9.43 ± 0.02 | 9.31 ± 0.02 | 8.95 ± 0.03 |
| *p* | NS | NS | NS | NS | NS |
| interaction: *p* | AB: 0.0056 | AB: 0.0133 | BC: 0.0282 | AB: 0.0144 | AB: 0.0042 |
| | AC: 0.0197 | AC: 0.0484 | ABC: 0.0050 | BC: 0.0194 | AC: 0.0005 |
| | BC: 0.0163 | BC: 0.0048 | | | BC: 0.0134 |
| | ABC: 0.0067 | ABC: 0.0137 | | | |

Two Factors
Pathology = 0

| | diameter | total surface [ln($\mu$m)] | media [ln($\mu$m)] | adventice [ln($\mu$m)] | lumen [ln($\mu$m)] |
|---|---|---|---|---|---|
| | | Ethanol (A) | | | |
| 0 | 168.75 ± 1.96 | 10.20 ± 0.02 | 9.32 ± 0.02 | 9.17 ± 0.02 | 8.64 ± 0.03 |
| 1 | 167.90 ± 1.92 | 10.20 ± 0.02 | 9.32 ± 0.02 | 9.17 ± 0.02 | 8.66 ± 0.03 |
| *p* | NS | NS | NS | NS | NS |
| | | Polyphenol (B) | | | |
| 0 | 170.20 ± 1.96 | 10.22 ± 0.02 | 9.34 ± 0.02 | 9.17 ± 0.02 | 8.71 ± 0.03 |
| 1 | 166.45 ± 1.92 | 10.18 ± 0.02 | 9.30 ± 0.02 | 9.17 ± 0.02 | 8.59 ± 0.03* |
| *p* | NS | NS | NS | NS | 0.0188 |
| interaction *p* | NS | NS | NS | NS | NS |

Two Factors
Pathology = 1

| | diameter | total surface [ln($\mu$m)] | media [ln($\mu$m)] | adventice [ln($\mu$m)] | lumen [ln($\mu$m)] |
|---|---|---|---|---|---|
| | | Ethanol (A) | | | |
| 0 | 202.90 ± 2.78 | 10.59 ± 0.03 | 9.59 ± 0.03 | 9.49 ± 0.03 | 9.33 ± 0.05 |
| 1 | 189.11 ± 2.65* | 10.47 ± 0.03* | 9.51 ± 0.03 | 9.37 ± 0.03* | 9.12 ± 0.04* |
| *p* | 0.0004 | 0.0027 | NS | 0.0024 | 0.0010 |
| | | Polyphenol (B) | | | |
| 0 | 192.44 ± 2.66 | 10.50 ± 0.03 | 9.53 ± 0.03 | 9.42 ± 0.03 | 9.14 ± 0.04 |
| 1 | 199.58 ± 2.78 | 10.56 ± 0.03 | 9.57 ± 0.03 | 9.44 ± 0.03 | 9.30 ± 0.04* |
| *p* | NS | NS | NS | NS | 0.0111 |
| interaction | AB | AB | AB | AB | AB |
| *p* | 0.0021 | 0.0012 | 0.0017 | 0.0157 | 0.0070 |

[a] * Significant difference ($p < 0.05$). $p$ = level of significance. Values are given as means ± SEM of 309–333 values per factor.

role. Effects of ethanol on carbohydrate metabolism have been thoroughly studied. Acute ingestion of ethanol may induce hypoglycemia related to insulin secretion and inhibition of gluconeogenesis. The health effect of chronic ethanol consumption on diabetes is U-shaped. While high ethanol intake (>50 g/day) will cause insulin resistance and is deleterious for diabetes, light to moderate (10−30 g/day) alcohol consumption is associated with enhanced insulin-mediated glucose uptake, lower plasma glucose and insulin concentrations in response to oral glucose, and a higher HDL cholesterol concentration (*26*). Epidemiological studies also suggest that regular moderate alcohol consumption has a favorable impact on diabetes risk, particularly in women; this may reflect a direct insulin sensitizing effect on muscle and, in women, a reduced risk for obesity (*27*). Among the other components of wine potentially beneficial to health, red wine polyphenols are thought to provide protection against oxidation (*7*), although other pharmacological properties, such as vasodilating properties (*19*), may play a significant role in vivo. Few studies have explored the possible role of polyphenols in preventing or correcting diabetes although some results obtained with GTPs have shown that polyphenols can indeed display some antidiabetic activity on alloxan-induced diabetes (*13*).

In the present study, we showed that a red wine polyphenol extract, ethanol, or their association have some antidiabetic activity on a model of diabetes linked to insulin deficiency, the STZ-induced diabetic rat, characterized by a major hyperglycemia (without insulin dependence), along with hyperphagia, polydipsia, and arrest of body growth. Hypercholesterolemia and hypoinsulinemia are two other characteristics of this model.

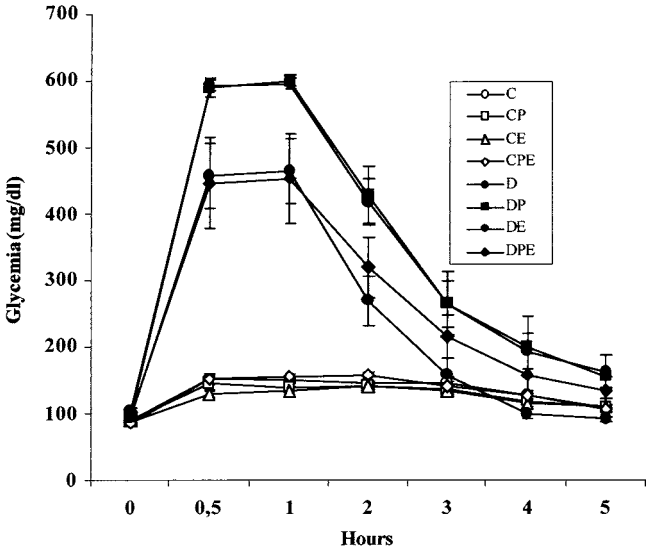


**Figure 2.** Evolution of BG recorded after an OGTT performed on day 26 of treatment in untreated (C or D) or treated control or diabetic rats by polyphenolic extract (CP or DP), ethanol (CE or DE), or both (CPE and DPE, respectively). Rats were fasted for 12 h and untreated before glucose (5 g/kg) oral administration. Treatment induced no significant change in control animals. BG was significant ($p \leq 0.05$) in DP or DPE than in D animals a time 0.5, 1, and 2 h.

The administration of a RWPE at a "pharmacological" dose (200 mg/kg) to diabetic animals is associated with a progressive decrease of BG along with a decrease in water and food intakes. Interestingly, glycemia was not different from those of untreated diabetic animals when measured during OGTT or at sacrifice, i.e., when glycemia was determined without previous daily treatment. As glycemia in the STZ-induced diabetic rat is greatly influenced by food intake (as illustrated by glycemia measured at time 0 of OGTT after an 18 h fast), the antihyperglycemic effect of the polyphenolic extract in diabetic animals can be explained by the reduction of food intake and/or the absorption of nutriments. Plasma insulin or cholesterol levels were not significantly different in DP as compared to D either, indicating that the decrease in glycemia noted during the experimental period was short-lived and not linked to the correction of the diabetic state.

The administration of RWPE to nondiabetic animals lowered food and water intakes as well as body growth. In addition, a decrease in BG was noted when RWPE was administered before glycemia determination. Those data reinforce the hypothesis that RWPE may influence the regulation of food and water intakes as well the absorption of nutriments.

As opposed to the administration of the polyphenolic extract, the administration of ethanol, at a dose corresponding to 500 mL of red wine per day in an adult human, was associated with a progressive decrease of glycemia in diabetic rats during treatment and at sacrifice (**Figure 1** and **Tables 1**−**4**), along with an OGGT curve lower in DE than in D animals. In addition, plasma insulin was higher and cholesterol lower in DE than in D. Food and water intakes were also lower and body growth higher in DE than in D, indicating that correction of glycemia was associated to an improved metabolic state in those animals. All those data indicate that the administration of ethanol was able to act not only on hyperglycemia per se but also to correct the diabetic state itself. As suggested by higher insulin levels in DE, the administration of ethanol at a moderate dose was able to induce the protection and/or correction of pancreatic insulin secreting capacities. As previously stated, the consumption of moderate dose of ethanol may have insulin sensitizing properties. Our data obtained on the same model with the insulin sensitizer vanadyl sulfate (*28*) or clinical data obtained with thiazolidine diones (*29*) indeed indicate that insulin sensitizing drugs may have some preventive effect on the impairment of insulin secretion associated with the diabetic state.

Data similar to those obtained with ethanol are observed in diabetic animals treated with the association of ethanol and polyphenols, with a progressive reduction of glycemia during the experimental period associated with the same correction of glycemia at sacrifice, along with higher insulin and reduced cholesterol levels. In this case, the proper effects of RWPE on food and water intakes are masked by the correction of the diabetic state induced by ethanol. Interestingly, in nondiabetic animals, the effect of RWPE on water intake cannot be shown in the presence of ethanol. As ethanol itself is diuretic and induces an increase in water intake, it is tempting to speculate that the effect of polyphenols is masked by the effect of ethanol (*30*).

The vascular consequences of insulin deficiency are particularly marked in small size arteries (microangiopathy). As previously shown in the mesenteric arterial system, vessels from diabetic animals were enlarged, with vasodilatation and an increase in media and adventice area (*17*, *25*). The administration of a moderate dose of ethanol was able to reduce the vascular size in diabetic, but not in nondiabetic, animals, suggesting that its effects were linked to the antidiabetic effects of ethanol. The administration of RWPE induced a further increase of vessel areas in diabetic animals, an effect that was not present in ethanol-treated animals, while some enlargement of lumen was noted in nondiabetic animals. Similar results were obtained in our recent study in which nondiabetic or diabetic animals were treated with polyphenol-enriched white wine or its desalcoolized version (*17*). Those data indicate that polyphenols are able to amplify some mechanisms associated with microangiopathy, an effect that is not present when diabetes is corrected by ethanol. In vitro vasodilatation properties of polyphenolic extracts have been shown to be endothelium-dependent and linked to NO production (*20*). As vasodilatation of small vessels in diabetes was shown to be related to an increased production of NO, it is tempting to speculate that polyphenols may amplify the vascular effects of the mediator.

Finally, the increase in plasma ALT levels induced by polyphenols in diabetic animals while ethanol itself induced no significant change may indicate some degree of toxicity, especially when polyphenols are administered at high dose. The high potential of polyphenols in the prevention or treatment of various chronic diseases (*31*) may therefore necessitate thorough safety studies.

In summary, our study showed (i) that ethanol (at a dose equivalent to 500 mL of red wine per day in an adult human) was able to correct the insulin deficient diabetic state induced by STZ and (ii) that a red wine polyphenolic extract (at a pharmacological dose) was able to reduce glycemia, an effect also manifested in nondiabetic animals and related to the reduction in food intake and food absorption. Some of the effects of polyphenols were masked by the effects of ethanol, notably in diabetic animals. Further studies will determine the effect of more "nutritional" doses of polyphenols as well as their mechanisms of action.

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