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Original Article

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Amelioration of myoglobinuric renal damage in rats by chronic exposure to flavonol-rich red wine

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Abstract

Background. Myoglobinuric acute renal failure causes increased oxidative stress. Since ethanol upregulates renal antioxidant enzymes and wine polyphenols behave as antioxidants, we tested the hypothesis that red wine components would ameliorate the renal damage caused by rhabdomyolysis.

Methods. Adult rats received water (control), alcohol-free red wine, ethanol 12.5% (v/v) or red wine for 10 weeks. Rhabdomyolysis was induced by glycerol injection (50%, 10 ml/kg, i.m.), and urine and blood samples were collected 6 h later to measure renal function parameters, creatine kinase (CK) activity, free F_2-isoprostanes and total antioxidant capacity. Kidneys were then harvested for morphological studies and determinations of lipid peroxidation, protein carbonylation, (Na + K)-ATPase and antioxidant enzyme activities.

Results. In the control group, myoglobinuria was associated with a 68% decrease in creatinine clearance and increases in plasma creatinine and blood urea nitrogen of 3.2 and 1.8 times above baseline, respectively. Controls also showed increases in plasma free F_2-isoprostanes levels and CK activity, together with enhanced renal expression of the antioxidant enzymes catalase, glutathione peroxidase and superoxide dismutase, as well as increased production of malondialdehyde and carbonyls. Rhabdomolysis reduced renal (Na + K)-ATPase activity and this reduction was associated with a 5-fold increase in fractional sodium excretion as well as morphological damage to the kidney. These changes were significantly attenuated by pretreatment with chronic red wine exposure prior to glycerol injection. A less marked degree of functional and biochemical protection was also observed in response to the administration of alcohol-free red wine and ethanol.

Conclusions. The present data suggest that red wine protects against functional, biochemical and morphological damage caused by rhabdomyolysis in the rat, and this protection may be due to the synergistic effects of ethanol and non-alcoholic red wine components.

Keywords: antioxidants; ethanol; free radicals; polyphenols; rhabdomyolysis; wine

Introduction

During rhabdomyolysis, potentially toxic myocyte contents are released into the systemic circulation, and the renal consequences of this disturbance have been attributed to both intense vasoconstriction and renal tubular necrosis. Myoglobinuria plays a key role in the pathophysiology of acute renal failure both in clinical settings that are characterized by muscle tissue injury [1] and in a widely used animal model of glycerol-induced rhabdomyolysis. The intratubular degradation of myoglobin results in the generation of reactive oxygen species (ROS), which are implicated in the pathogenesis of renal damage. Although the kidney possesses an antioxidant defence system that scavenges ROS, the capacity of this system can be overwhelmed during myoglobinuria.

Naturally occurring antioxidants act to reinforce the endogenous antioxidant systems that depurate ROS and this contributes to their beneficial health effects [2]. Although the mechanism of action of antioxidants has not yet been fully established, they may attenuate the effects of oxidative challenges to the kidney. Polyphenols, which are particularly abundant in Chilean red wine [3] may strengthen the antioxidant mechanisms, and this action would support the hypothesis that moderate red wine consumption is protective

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against renal disease [4]. In further support, the upregulation of antioxidant enzymes has been attributed to chronic exposure to moderate amounts of ethanol [5,6].

In previous work, red wine polyphenol resveratrol caused an attenuation of oxidative damage and decreased vulnerability of the kidney to ischaemia-reperfusion damage [7,8]. Nevertheless, it is not well established whether this protection is mechanistically linked to the reported effects of the various wine components since these effects have not been separately studied.

The aim of the present study was to test the hypothesis that chronic treatment with red wine results in the attenuation of myoglobinuric renal damage in the rat. We examined the long-term effects of red wine, ethanol and alcohol-free red wine on rhabdomyolysis while measuring functional, biochemical and morphological characteristics of the kidney.

Materials and methods

Experimental design and animals

The protocol was approved by the Comité de Bioética, Facultad de Medicina, Universidad de Chile. The management of the rats was carried out according to internationally accepted ethics rules. Adult male Wistar rats weighing 200 ± 12 g (Departamento de Nutrición, Facultad de Medicina Universidad de Chile) were divided into four groups of 20 rats. The rats were given, for 10 weeks, a balanced diet, and either tap water (control group), ethanol 12.5% (v/v) (ethanol group), red wine (Syrah, 2001 harvest, Ventisquero Vineyards, Chile) (red wine group) or dealcoholized red wine (alcohol-free red wine group). Red wine dealcoholization was carried out according to Serafini *et al.* [9], and achieved an extraction of 97% of ethanol, since the alcohol content of the alcohol-free solution was 0.38% (v/v). Red wine had an ethanol concentration of 12.5% (v/v) with a flavonol concentration (myricetin plus quercetin) of 37.5 mg/l. Flavonol was 35.0 mg/l in the alcohol-free red wine. As has been previously characterized, the four groups showed similar weight gain and fluid volume intake. In addition, the quantity of ethanol ingested by the ethanol and red wine groups were similar and their blood ethanol levels were comparable [6,10]. Arterial pressure was monitored in a subset of rats from each group ($n = 8$). Carotid catheters were connected to a pressure transducer coupled to a multichannel recorder. Mean arterial pressure (MAP) was derived electronically. Electrocardioghraphic recording was performed while the rats were under light ether anaesthesia at basal conditions and 6 h after glycerol injection.

Rhabdomyolysis-induced acute renal failure

The rats were deprived of fluid intake for 12 h, and then were injected with 50% glycerol in normal saline (10 ml/kg, intramuscularly) under light ether anaesthesia [11]. The control rats were injected with an equal volume of normal saline. The animals were then housed in metabolic cages to collect urine samples while allowing free access to water, ethanol, red wine or alcohol-free red wine. Six hours after glycerol injection, blood samples were obtained from the carotid artery in sodium pentobarbital (40 mg/kg, intraperitoneally) anaesthetized rats. The blood samples served to measure the antioxidant capacity of plasma, ferric reducing ability of plasma (FRAP) [12], plasma levels of urea nitrogen, uric acid and activity of creatine kinase (CK). The concentrations of free F_2-isoprostanes [13] and of sodium and creatinine were assessed in both blood and urine samples.

Biochemical studies in the kidneys

The anaesthetized animals were perfused with Earle's balanced salt solution of pH 7.40 (Sigma Chemical Co., St Louis, MO) and the kidneys were quickly removed, homogenized and the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were determined using previously published methods [6].

Lipid peroxidation and protein carbonylation

Assays for products of lipid peroxidation in renal tissue were performed spectrophotometrically at 532 nm using the thiobarbituric acid reaction at pH 3.5, followed by solvent extraction with a mixture of *n*-butanol/pyridine (15/1, v/v). The level of lipid peroxides was expressed as nanomoles of malondialdehyde (MDA) per milligram protein. The assessment of protein oxidation was performed by a spectrophotometric method based on the reaction of 2,4-dinitrophenylhydrazine with protein carbonyls, and the results were expressed as nanomoles of carbonyl per milligram protein.

Activity of (Na + K)-ATPase

The activity of this membrane-bound enzyme in renal cortex was determined by a method based on the measurement of ATP hydrolysis, and enzyme activity was expressed as micromoles of inorganic phosphate per milligram protein per hour.

Kidney morphological studies

Electron and light microscopy studies of kidneys were performed on 2 mm vertical slices from kidneys of the control and red wine groups at basal conditions and at 6 h following glycerol injection. For glomerular studies, thin sections were examined and photographed in an electron microscope (EM-109; Zeiss, Güttingen, Germany).

Statistical analysis

The results are expressed as means ± SEM. The sources of variation for multiple comparisons were assessed by one-way analysis of variance (ANOVA), followed by the Sheffé test. Differences were considered statistically significant at $P < 0.05$.

Results

Rhabdomyolysis, haemodynamics, and renal function

The activity of serum CK, a measure of muscle damage or myopathy, was similar at baseline in all groups, and

increased dramatically and equally after glycerol injection (Table 1). The systemic haemodynamics effects of rhabdomyolysis, assessed by MAP and heart rate (HR), are shown in Table 2. All groups were similar at baseline, and glycerol-injection comparably increased MAP by 25–30% and decreased HR by 15–20%. Fluid intakes (ml/day per kg body weight) for the control, ethanol, red wine and alcohol-free red wine groups ($n = 12$ for each group) were 110.5 ± 6.3, 103.8 ± 12.9, 98.6 ± 13.8 and 105.1 ± 10.6, respectively. In turn, urine flow rates (ml/day per kg body weight) for the control, ethanol, red wine and alcohol-free red wine groups ($n = 12$ for each group) were 55.6 ± 5.1, 62.7 ± 8.2, 57.3 ± 7.5 and 60.8 ± 6.8 respectively. Fluid intakes and diuresis were not different between the groups. After glycerol injection, plasma levels of uric acid increased by 10%, and were not different between the groups (data not shown). Plasma levels of creatinine and blood urea nitrogen (BUN) are shown in Figure 1. As expected, at 6 h following glycerol injection, serum creatinine and BUN were significantly increased, and reached levels that were 3.2 and 1.8 times greater than basal values, respectively ($P < 0.05$). Red wine administration diminished these increases to 1.5 and 1.2 times, respectively, indicating a renoprotective effect. This effect was also observed in the ethanol and alcohol-free red wine groups, although to a lesser extent. This renoprotection was confirmed by the creatinine clearance data, which showed a 68% decrease from control levels following glycerol-injection, whereas this diminution was 60, 47 and 21% in ethanol, alcohol-free red wine and red wine groups, respectively (Table 3). Thus, alcohol-free red wine causes renoprotection against rhabdomyolysis, and this effect was augmented in the red wine group ($P < 0.05$). Rhabdomyolysis elevated the fractional excretion of sodium in the control and ethanol groups by 4.9 and 2.7 times, respectively ($P < 0.05$), but these values were not changed in the red wine and alcohol-free red wine groups (Table 3). In the control, ethanol and alcohol-free red wine groups, this effect was paralleled by a diminution in activity of (Na + K)-ATPase to 75, 82 and 88% of basal values, respectively ($P < 0.05$). In contrast, kidneys from the red wine group showed no change in (Na + K)-ATPase activity following glycerol injection.

Table 1. Effects of glycerol-induced rhabdomyolysis on serum CK activity (U/ml)

Group	Basal	Glycerol
Control	0.18 ± 0.07	36.68 ± 3.80^{a}
Ethanol	0.25 ± 0.10	34.62 ± 4.90^{a}
Red wine	0.22 ± 0.08	30.71 ± 3.10^{a}
Alcohol-free red wine	0.20 ± 0.09	33.92 ± 3.75^{a}

Values are means ± SE ($n = 20$).
[a]$P < 0.05$ *vs* basal.

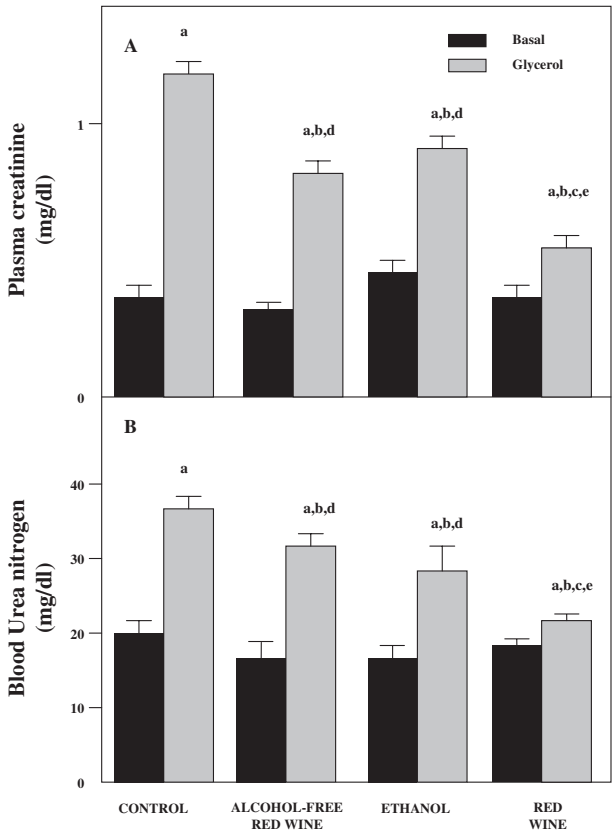

Fig. 1. Effects of rhabdomyolysis on serum creatinine (**A**) and BUN (**B**) in control, ethanol, red wine and alcohol-free red wine groups. Values are means ± SE ($n = 20$). Statistically significant differences, at $P < 0.05$, are indicated by superscript letters: [a]*vs* basal, [b]*vs* control-glycerol, [c]*vs* ethanol-glycerol, [d]*vs* red wine-glycerol and [e]*vs* alcohol-free red wine-glycerol.

Table 2. Effects of glycerol-induced rhabdomyolysis on MAP (mmHg) and HR (b.p.m.)

Group	MAP (mmHg)		HR (b.p.m.)	
	Basal	Glycerol	Basal	Glycerol
Control	102 ± 4	132 ± 3^{a}	218 ± 15	185 ± 12^{a}
Ethanol	105 ± 3	136 ± 5^{a}	215 ± 13	176 ± 14^{a}
Red wine	103 ± 3	130 ± 4^{a}	220 ± 12	176 ± 13^{a}
Alcohol-free red wine	105 ± 4	133 ± 5^{a}	225 ± 15	191 ± 17^{a}

Values are means ± SE ($n = 8$).
[a]$P < 0.05$ *vs* basal.

Table 3. Effect s of glycerol-induced rhabdomyolysis on creatinine clearance (ml/min/100 g BW), fractional excretion of sodium (FE_{Na}) and renal (Na + K)-ATPase activity

Parameter	Group	Basal	Glycerol
Creatinine clearance	Control	0.60 ± 0.02	0.19 ± 0.09^{a}
(ml/min/100 g BW)	Alcohol-free red wine	0.63 ± 0.08	$0.33 \pm 0.05^{a,b,d,e}$
	Ethanol	0.56 ± 0.05	$0.22 \pm 0.04^{a,c,e}$
	Red wine	0.62 ± 0.09	$0.49 \pm 0.03^{a,b,c,d}$
FE_{Na} (%)	Control	0.43 ± 0.03	2.10 ± 0.11^{a}
	Alcohol-free red wine	0.51 ± 0.12	$0.53 \pm 0.10^{b,d}$
	Ethanol	0.35 ± 0.05	$0.94 \pm 0.08^{a,b,c,e}$
	Red wine	0.37 ± 0.09	$0.51 \pm 0.13^{b,c,d}$
(Na ± K)-ATPase	Control	13.8 ± 0.5	10.3 ± 0.4^{a}
(μmol/Pi/mg protein/h)	Alcohol-free red wine	12.8 ± 0.3	$11.3 \pm 0.3^{a,b,d,e}$
	Ethanol	17.5 ± 0.7	$14.4 \pm 0.7^{a,b,c,e}$
	Red wine	13.2 ± 0.3	$12.6 \pm 0.4^{b,c,d}$

Values are means ± SE ($n = 1$–12). Pi, inorganic phosphate; BW, body weight. Statistically significant differences, at $P < 0.05$, are indicated by superscript letters: [a]*vs* basal, [b]*vs* control-glycerol, [c]*vs* alcohol-free red wine-glycerol, [d]*vs* ethanol-glycerol and [e]*vs* red wine-glycerol.

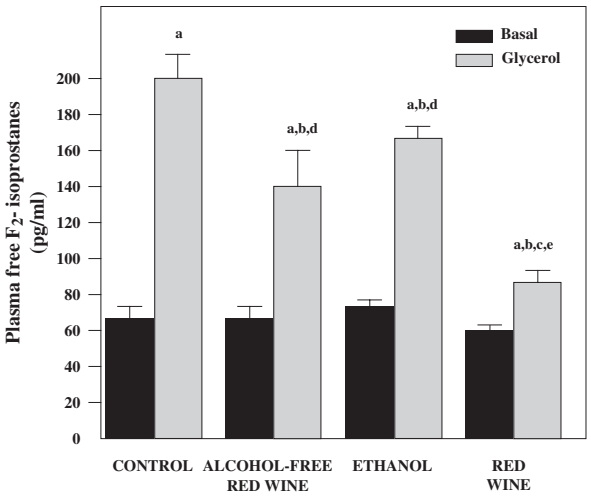

Fig. 2. Effects of rhabdomyolysis on plasma levels of free F_2-isoprostanes in control, ethanol, red wine and alcohol-free red wine groups. Values are means ± SE ($n = 20$). Statistically significant differences, at $P < 0.05$, are indicated by superscript letters: [a]*vs* basal, [b]*vs* control-glycerol, [c]*vs* ethanol-glycerol, [d]*vs* red wine-glycerol and [e]*vs* alcohol-free red wine-glycerol.

Antioxidant capacity of plasma and free F_2-isoprostane levels in plasma and urine

The basal values of FRAP (μM) in the control, ethanol, red wine and alcohol-free red wine groups ($n = 20$ for each group) were 249.6 ± 10.3, 255.8 ± 8.4, 356.7 ± 11.8 and 305.6 ± 9.6, respectively. These values were significantly higher in the latter two groups and were the highest in the red wine group ($P < 0.05$). Urinary levels of free F_2-isoprostanes (ng/24 h) at basal conditions in the control, alcohol-free red wine, ethanol and red wine groups ($n = 12$ for each group) were 7.5 ± 0.3, 6.2 ± 0.2, 7.7 ± 0.4 and 5.7 ± 0.2, respectively, and were significantly higher in the alcohol-free red wine and red wine groups than in controls ($P < 0.05$). Plasma levels of free F_2-isoprostanes at basal conditions were not different between the groups, and glycerol injection increased these levels by 3, 2.3, 1.5 and 2 times ($P < 0.05$) in the control, ethanol, red wine and alcohol-free red wine groups, respectively (Figure 2).

Renal antioxidant enzymes

Figure 3 shows the effects of rhabdomyolysis on the activity of CAT, GSH-Px and SOD in renal tissues. Basal values of CAT and GSH-Px were higher in the ethanol and red wine groups than in controls ($P < 0.05$), whereas the activity of SOD was similar in all groups. Glycerol injection caused decreased antioxidant enzyme activity in the four groups, and in the red wine group this decrease produced levels that were not different from basal values in the control group.

Lipid peroxidation and protein carbonylation

The MDA production and carbonyl content (nmol/mg protein) of the kidneys from the four groups are shown in Figure 4. Under basal conditions, MDA production in the red wine and alcohol-free red wine groups was significantly lower than in the control group ($P < 0.05$), whereas the carbonyl contents were similar across all groups. Following glycerol injection, MDA production and carbonyl content increased in the control, ethanol and alcohol-free red wine groups, and these increased by 1.6 times in controls but to a lesser degree in the other two groups. In turn, rhabdomyolysis in the red wine group did not change the protein carbonylation of kidneys but elevated its MDA production by 1.6 times. However, it should be noted that even rhabdomyolysis did not significantly alter MDA and carbonyl content values in the red wine group from values in the control group during basal conditions.

Morphological studies

The ultrastructural characteristics of glomeruli from control and red wine groups at basal conditions

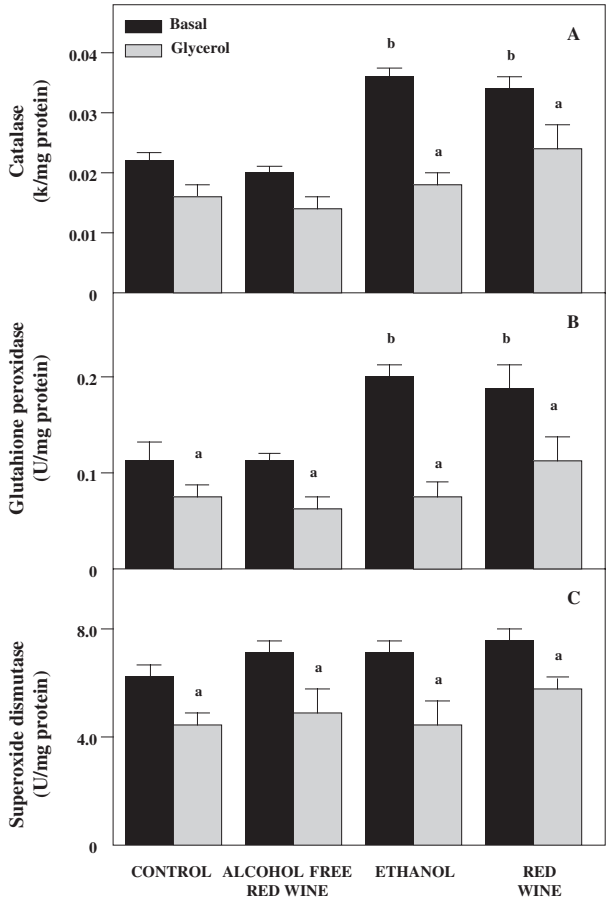

Fig. 3. Effects of rhabdomyolysis on the activity of the antioxidant enzymes CAT (**A**), GSH-Px (**B**) and SOD (**C**) in renal cortex in control, ethanol, red wine and alcohol-free red wine groups. Values are means ± SE ($n = 20$). Statistically significant differences, at $P < 0.05$, are indicated by superscript letters: [a]*vs* basal and [b]*vs* control-glycerol. k, CAT first-order kinetic constant for breakdown of hydrogen peroxide (M^{-1} s^{-1}).

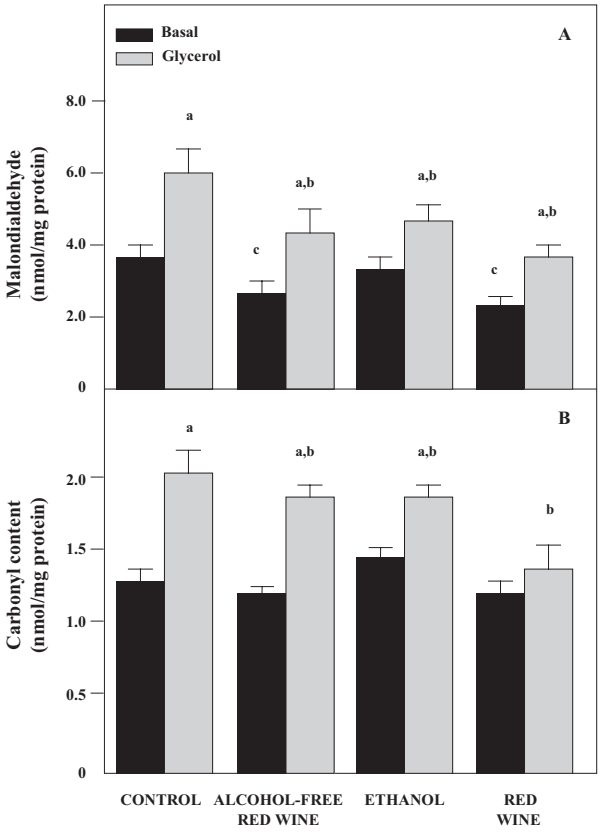

Fig. 4. Effects of rhabdomyolysis on lipid peroxidation (MDA production) (**A**) and protein oxidation (**B**) in renal cortex in control, ethanol, red wine and alcohol-free red wine groups. Values are means ± SE ($n = 20$). Statistically significant differences, at $P < 0.05$, are indicated by superscript letters: [a]*vs* basal, [b]*vs* control-glycerol and [c]*vs* control-basal.

(Figure 5) and at 6 h following glycerol injection (Figure 6) showed that red wine partly prevented morphological impairments caused by myoglobinuria. The renal glomeruli in specimens obtained from the red wine group under basal conditions show a normal filtration barrier but had more stained microfilaments of the cytoskeleton of foot processes (Figure 5A and B). Following glycerol injection, the glomeruli from the control group presented abundant accumulation of electron dense material in the endothelium, glomerular basement membrane (GBM) and foot processes of the podocytes causing the GBM to protrude into the lumen (Figure 6A and B). In contrast, this effect was appreciably less severe in glomeruli from the red wine group, which had a normal filtration barrier without accumulation of electron dense material. This graph also showed the presence of neutrophil cells in the lumen of capillary glomerular vessels (Figure 6C) and microfilaments of the cytoskeleton of foot processes that stained as strongly as those from the control group under basal conditions (Figure 6D). The red wine group also showed an amelioration of the morphological impairment caused by myoglobinuria as determined by light microscopy studies (Figure 7A and B). Many of the proximal convoluted tubules from the control group showed tubular necrosis (vacuolar and hydropic cell degeneration) and tubulorhexis, and the distal convoluted tubules showed a lumen containing exfoliated epithelial cells (Figure 7A). In contrast, proximal convoluted tubules from the red wine group had neither epithelial necrosis nor tubulorhexis (Figure 7B).

Discussion

Rhabdomyolysis is a cause of acute renal failure [1]. In the present study, we found that chronic treatment with flavonol-rich red wine ameliorated the initial renal damage caused by rhabdomyolysis. Oxidative stress has been implicated in the mechanism of renal injury. For example, other groups observed striking increases in the urinary excretion of F_2-isoprostanes accompanied by increased plasma levels of MDA [14], which indicated increased lipid peroxidation. These findings are in agreement with the present data from the control group, showing increased plasma levels of free

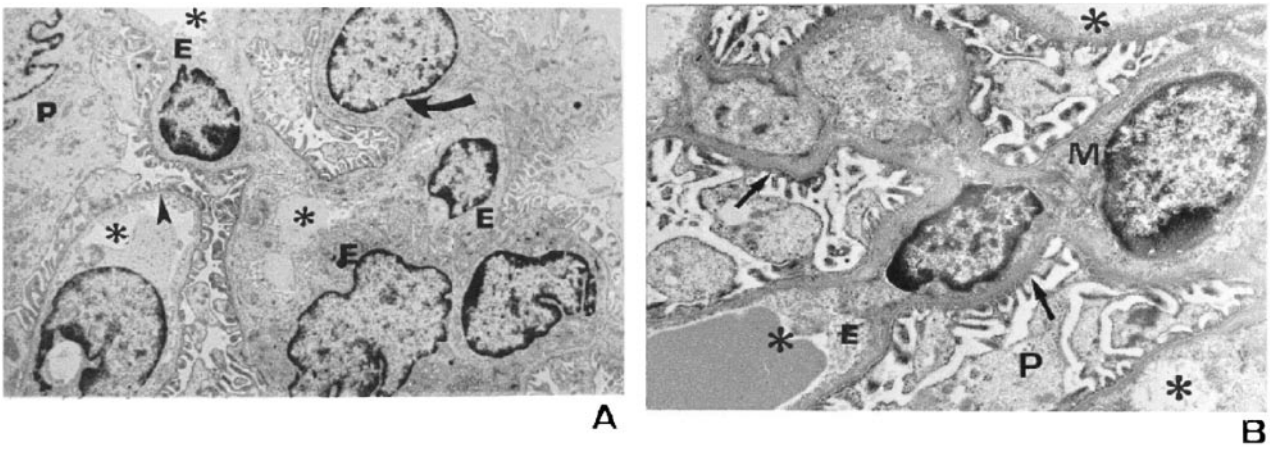

Fig. 5. Electron micrographs of kidneys from rats in the the control (**A**) and red wine (**B**) groups showing the ultrastructural characteristics of the glomerulus of a nephron at basal conditions. Endothelial cells (E) and lumen of the capillaries (*), mesangium (M), and podocytes (P) showed heavier cytoskeleton staining in the foot processes of the wine group (arrows). Note the normal filtration barrier in both groups (arrow heads) (original A, ×30 000 and B, ×38 500).

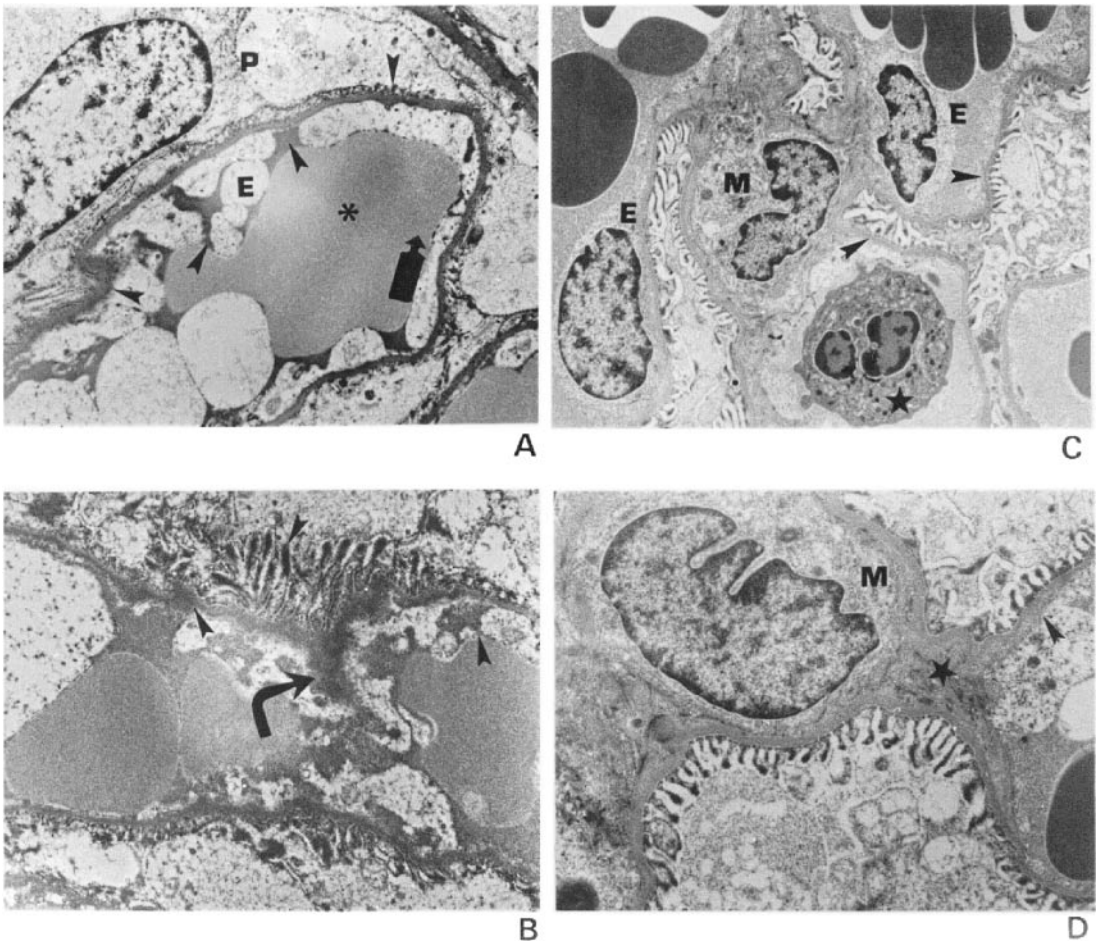

Fig. 6. Electron micrographs of the kidneys of rats from the control (**A** and **B**) and red wine (**C** and **D**) groups showing the ultrastructural characteristics of the glomerulus of a nephron, after 6 h of glycerol injection. (**A**) The swelling of the endothelium (E), electron-dense deposits within the endothelial cells, the foot processes and the GBM (arrow heads) and glomerular capillary lumen (thick arrow) with dark plasma (*) (original × 25 000) are shown. (**B**) The electron-dense deposits causing the GBM to protrude into the capillary lumen (arrow). The same deposits were present in the cytoskeleton of the foot processes, the endothelial cells, and the GBM (arrow heads). The foot processes were all intact and detachments from the GBM were not seen (original × 35 000). (**C**) The normal endothelium (E), mesangium (M) and filtration barrier (arrow heads). Presence of a neutrophil in the capillary lumen (star) (original × 35 000). (**D**) A normal mesangium (M) and filtration barrier (arrow heads) and mesangial matrix (star) are shown. Electron-dense deposits were not seen (original × 38 500).

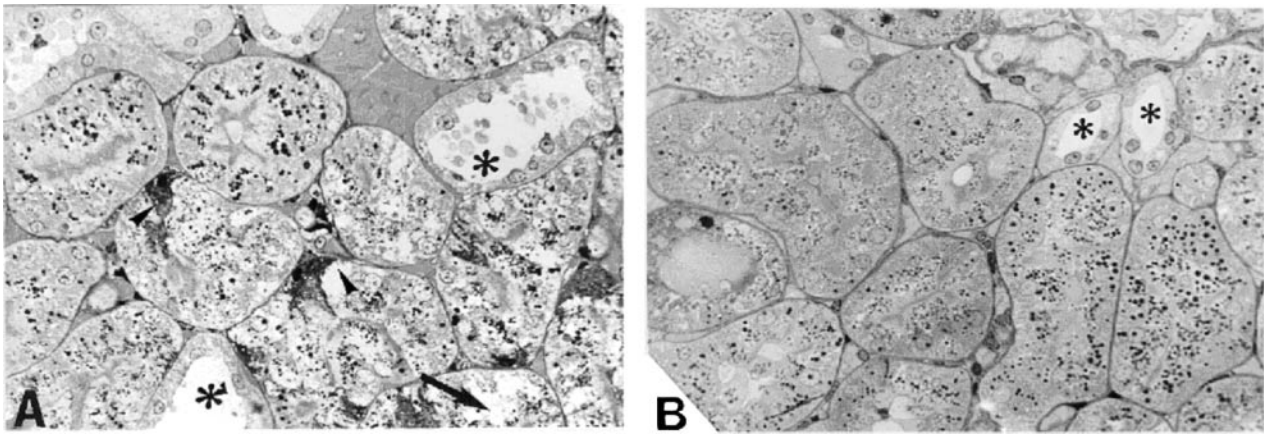

Fig. 7. Micrographs of the kidneys of rats from the control (**A**) and red wine (**B**) groups showing the histological characteristics of the tubular epithelium at 6 h after glycerol injection. (**A**) The proximal convoluted tubules displaying tubular necrosis with vacuolar and hydropic cell degeneration (arrow) and tubulorhexis (arrow heads) are shown. The distal convoluted tubules showed exfoliated cells (*) (original × 400). In contrast, kidneys from the red wine group (**B**) showed normal distal convoluted tubules (*) and proximal convoluted tubules without tubular necrosis (original × 400).

F_2-isoprostanes and significantly increased MDA production by the kidney at 6 h following glycerol injection. The release of ferrous iron from myoglobin may result in generation of ROS through the Fenton reaction to produce lipid peroxidation.

The oxidative stress that followed rhabdomyolysis was accompanied by significant impairments in renal function, as demonstrated by the creatinine clearance data. The decrease in glomerular filtration rate following glycerol injection was lowest in the red wine group (Table 3), which strongly supported a renoprotective effect in this group, at least in the short-term (6 h). The fact that alcohol-free red wine caused an intermediate diminution in creatinine clearance following glycerol injection suggests that there was a synergistic action of polyphenols and ethanol. Although ethanol by itself did not counteract the functional impairment of the kidney caused by glycerol, it reinforced the major renoprotective effects of polyphenols. The beneficial effect of ethanol probably arises from its ability to upregulate the activity of antioxidant enzymes (Figure 3) and to enhance the enteric absorption of polyphenols [15]. This effect is also associated with a significant decrease in morphological derangements in the red wine group. Alternatively, the drop in renal (Na + K)-ATPase activity induced by rhabdomyolysis may partly explain the augmented fractional sodium excretion (Table 3). The activity of this enzyme activity is partly modulated by the microenvironment made up of the physical and chemical properties of the membrane. Consequently, it is likely that the optimal interaction between membrane phospholipid and (Na + K)-ATPase may be impaired by lipid peroxidation, thereby diminishing the efficiency of sodium transport by tubular cells [16]. However, these functional derangements were markedly ameliorated by chronic red wine treatment given prior to glycerol injection. Therefore, these results support the view and show for the first time that long-term red wine exposure attenuates both the augmentation of lipid peroxidation and the impairment of renal function caused by rhabdomyolysis.

Importantly, the concentration of total flavonols in the red wine used in the present study (37.5 mg/l) was higher than in wines from other geographical regions [3]. It is therefore likely that these compounds significantly contributed to the elevation in FRAP levels observed in the red wine group. In fact, both the red wine and alcohol-free red wine group, showed increased antioxidant capacity in plasma, since their FRAP values were 43 and 23% higher than in controls, respectively.

This increased antioxidant capacity in plasma should contribute to enhancement of the renal antioxidant defence system *in vivo*, since the kidney is a highly perfused organ. In addition, previous studies using the same experimental model reported that red wine administration attenuated the ethanol-induced up-regulation of kidney microsomal cytochrome P450 2E1 activity [17], an effective generator of superoxide anion, to thereby diminish the occurrence of oxidative stress.

The renoprotective effects of red wine polyphenols have been also attributed to the release of endothelial nitric oxide (NO), a pivotal vasoprotective molecule, since increases in NO synthase expression have been observed [18]. These data are in agreement with the increased bioavailability of NO in the kidneys of rats receiving a dose of resveratrol designed to cause plasma levels that are similar to those found in moderate wine consumers, which was given prior to ischaemia-reperfusion induced oxidative stress [8]. However, oxidative stress should cause NO consumption via its reaction with the superoxide anion to form peroxynitrite, a highly peroxidant molecule. Therefore, it is very likely that the antioxidant effects of red wine contribute to the increased bioavailability of NO.

In agreement with the present data, other studies have shown that bioflavonoids from the seeds of grapes exert morphological and functional protection against

renal damage by experimental myoglobinuric acute renal failure [19]. Also, a renoprotective effect by the wine bioflavonoid proanthocyanidin-BP1 was reported in a study examining the same glycerol-induced rhabdomyolysis animal model used in the present experiments [20].

It is unlikely that extrarenal effects of the treatments and of rhabdomyolysis contributed to the observed renal protection since systemic parameters other than FRAP, such as haemodynamics and uricaemia, were not different between the groups at basal conditions or after glycerol injection.

In summary, the findings from the present study support the hypothesis that chronic red wine exposure reduces the vulnerability of the kidney towards the development of myoglobinuric acute renal failure at 6 h following glycerol injection. This protection may be due to synergistic effects of both non-alcoholic red wine components and ethanol, although the effects of polyphenols seem to dominate in this mechanism. However, the relative contributions of polyphenols and ethanol remain to be fully elucidated. These findings strongly suggest that attenuation of oxidative stress significantly contributes to the amelioration of the biochemical, functional and morphological impairments in the kidney caused by rhabdomyolysis.

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