

# Oxidative Stress Modulation by *Rosmarinus officinalis* in CCl<sub>4</sub>-induced Liver Cirrhosis

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***Rosmarinus officinalis* (Lamiaceae) possesses antioxidant activity and hepatoprotective effects, and so may provide a possible therapeutic alternative for chronic liver disease. The effect produced by a methanolic extract of *Rosmarinus officinalis* on CCl<sub>4</sub>-induced liver cirrhosis in rats was investigated using both prevention and reversion models. Over the course of the development of cirrhosis, the increased enzymatic activities of gamma-glutamyl transpeptidase and alanine aminotransferase, and the rise in bilirubin levels caused by CCl<sub>4</sub> administration, were prevented by *Rosmarinus officinalis* co-administration. When the cirrhosis by oxidative stress was evaluated as an increase on liver lipoperoxidation, total lipid peroxides, nitric oxide in serum, and loss of erythrocyte plasma membrane stability, *R. officinalis* was shown to prevent such alterations. On cirrhotic animals treated with CCl<sub>4</sub>, histological studies showed massive necrosis, periportal inflammation and fibrosis which were modified by *R. officinalis*. These benefits on experimental cirrhosis suggest a potential therapeutic use for *R. officinalis* as an alternative for liver cirrhosis. Copyright © 2009 John Wiley & Sons, Ltd.**

*Keywords:* *Rosmarinus officinalis*; antioxidative hepatoprotection; cirrhosis; carbon tetrachloride; lipoperoxidation.

## INTRODUCTION

Free radicals (FRs) induce an oxidation state that can lead to cellular membrane injury with the consequent alteration on metabolic processes. FRs are involved in various degenerative human diseases and have been implicated in atherosclerosis, lung and kidney damage, diabetes mellitus, aging and liver diseases (Halliwell and Gutteridge, 1999; Elejalde, 2001). Liver tissue alterations can progress to hepatic cirrhosis (Crawford, 2000), the third most important cause of death in men aged 35 to 74 years (INEGI, 2005), which is only diagnosed at autopsy in 30% to 40% of cases. In liver cirrhosis, the physiological capability to neutralize FRs is impaired, and natural products from the plant kingdom are being investigated as a source of antioxidants for the treatment of such disorders (Etter, 2005; Núñez-Sellés, 2005).

*Rosmarinus officinalis* (L.) (Labiatae) has been suggested as being of special interest as a hepatoprotector (Amin and Hamza, 2005). Its antioxidant activity is well documented (Wu *et al.*, 1982) and the activity has been ascribed to both the diterpene content, mainly carnosic acid and carnosol (Wijeratne and Cuppett, 2007), as well as to the essential oil constituents (Bozin *et al.*, 2007). Several methods have now been developed to obtain suitable extracts of *Rosmarinus officinalis* with high antioxidant activity (Cuvelier *et al.*, 1996). The hepatoprotective effects have been shown in studies on experimental acute liver damage (Sotelo *et al.*, 2002), and on liver experimental cirrhosis (Yahuaca *et al.*,

2005). Carnosic acid also provides protection from the liver carcinogen aflatoxin A (Costa *et al.*, 2007). We have therefore conducted a further investigation on the antioxidant properties of the total organic extract using a chronic liver damage model induced by carbon tetrachloride (CCl<sub>4</sub>), a well-known hepatotoxin (IPCS, 1999). CCl<sub>4</sub> is rapidly transformed to the trichloromethyl (CCl<sub>3</sub><sup>•</sup>) radical by cytochrome P450-2E1 (CYP2E1) in liver microsomes (Brent and Rumack, 1993). This FR and its highly reactive derivative, the trichloromethylperoxyl radical (Cl<sub>3</sub>COO<sup>•</sup>), is thought to interact with membrane lipids leading to their peroxidation (Muriel, 1997), which produces malondialdehyde (MDA) as a final product along with other metabolites (Brent and Rumack, 1993). Membrane disintegration, loss of membrane-associated enzymes (Yahuaca *et al.*, 1985; Muriel, 1998) and necrosis are some of the consequences of CCl<sub>4</sub>-induced lipid peroxidation. Based on these precedents, it can be inferred that blocking of FR propagation and lipid peroxidation can protect the liver from some of the deleterious effects of CCl<sub>4</sub>. This work evaluates a *Rosmarinus officinalis* polar (methanolic) extract for the ability to normalize biochemical, oxidative and histological parameters in chronic CCl<sub>4</sub>-induced liver injury.

## MATERIALS AND METHODS

**Chemicals.** Carbon tetrachloride, thiobarbituric acid (TBA), bovine serum albumin, anthrone and activated charcoal were obtained from Sigma Chemical Co. (St Louis, MO, USA); solvents for spectrophotometry and high performance liquid chromatography (HPLC) were from Mallinckrodt Baker (Phillipsburg, NJ, USA). The

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*R. officinalis* standard extract (Herbalox), and carnosol were a kindly gift from Dr Don Berdahl of Kalsec Industries, Inc., (Kalamazoo, MI, USA).

**Preparation of methanol extract from *Rosmarinus officinalis* ground leaves.** The leaves of *Rosmarinus officinalis* (Lamiaceae) were collected from cultivated plants in Zacatecas, México, and were authenticated by Jesús Balleza Cadengo at the Botanical Department of the Unidad Académica de Agronomía, Universidad Autónoma de Zacatecas, México. Leaves were shade-dried and the plant extract was obtained by a modification to the method reported by Wu *et al.* (1982). Briefly, 1 kg of dried leaves were powdered and extracted with 6 L methanol at 60 °C for 2 h. After extraction, the mixture was filtered and the filtrate treated three times with 200 g activated charcoal then filtered to yield a light-brown filtrate. The methanolic solution was concentrated to a final volume of 600 mL on a vacuum rotatory evaporator (Yamato) and filtered. 1 L of distilled water was added to the filtrate to form a precipitate that was filtered and air-dried to yield the *Rosmarinus officinalis* extract used for animal administration. This extract was suspended in distilled water to 200 mg/mL. The extract was compared to Herbalox by HPLC and by spectrophotometric scan analysis from 700 to 200 nm.

**HPLC analysis.** A sample of *Rosmarinus officinalis* extract dissolved in methanol (4 µg/mL) was analyzed by reverse phase HPLC analysis (Agilent Model 1100) using a 5-µm Zorbax Ultrasphere column C8 (4.6 mm × 150 mm), with gradient separation and ultraviolet detection (Agilent Model G1365B, MWD DE40502601) as described by Cuvelier *et al.* (1996). Briefly, the mobile phase was solvent A (acetonitrile/1% acetic acid in water; 15/85) with solvent B (methanol) according to a step gradient from 0% to 100% over 100 min, with a flow rate of 1 mL/min. The absorbance was monitored at 284 nm. Standard Herbalox and carnosol, one of the main active compounds of *Rosmarinus officinalis*, were used in order to quantify its content.

**Treatment of animals and induction of liver cirrhosis.** Male Sprague Dawley rats (90–100 g) were maintained under standard conditions (temperature of 24 °C, light/darkness cycles of 12 h) with free access to food (Harlan Teklad Global Diets) and water. All procedures were in accordance with the *Guide for the care and use of laboratory animals* of the Mexican Council for Animal Care (Mexican Official Norm, NOM-062-ZOO-1999) as approved by the Animal Care Committee (UAZ). Acute liver injury was induced by a weekly dose of CCl<sub>4</sub> (1 g · kg<sup>-1</sup> body weight), dissolved in mineral oil (Ehrinpreis *et al.*, 1980), given intraperitoneally.

The extract was suspended in water at 200 mg/mL and intragastrically administered through an esophageal cannula to assure a dose of 200 mg/kg of body weight, which corresponds to 6.04 mg/kg/day of carnosol (Sotelo *et al.*, 2002). Daily treatment with *Rosmarinus officinalis* was given using two schemes: the prevention model which involved simultaneous administration with CCl<sub>4</sub> for 12 weeks, and the reversion model, where the treatment started after cirrhosis was established, and lasted for 12 weeks. Control groups given either no treatment, vehicle (oil) or only

*R. officinalis*, were included, and compared with cirrhotic group (only CCl<sub>4</sub> administered). Twenty-four hours after the last administration period, animals (n = 10, each group) were anesthetized in an ether chamber. The liver was removed after perfusion with sodium phosphate buffer 0.2 M, pH 7.4, at 4 °C. Blood was collected by cardiac puncture and serum obtained by blood centrifugation at 1500 × g for 10 min, at 4 °C using an Avanti Beckman centrifuge.

**Measurement of enzymatic activity.** Alanine aminotransferase (ALT) activity in serum was measured as specified by commercial source (Diagnostica Merck-México, S.A., ALT Cat. 3364) and gamma-glutamyl transpeptidase (γ-GTP) activity determined as described by Szasz (1976). Briefly, a reaction mixture of 3 mL containing Tris-HCl buffer 200 mM pH 8.2, MgCl<sub>2</sub> 20 µmol, glycyl-glycine 4 µmol, γ-glutamyl p-nitroanilide 2 µmol and 100 µL of serum was allowed to react for 30 min at 37 °C; the reaction was stopped by adding cold acetic acid (1.5 M, cooled). P-nitroaniline production was measured by spectrophotometric analysis at 410 nm and activity reported in µmol · L<sup>-1</sup> · min<sup>-1</sup>.

**Determination of hepatic glycogen content and bilirubin.** The glycogen content in liver tissue was determined by the anthrone method (Fong *et al.*, 1953). Briefly, 3 mL KOH 30% (w/v in distilled water) was added to 1 g of liver tissue and heated at 100 °C for 30 min. After dilution with distilled water to 1:50, 20 µL of this mixture was transferred to 2 mL anthrone reagent (2 mg/mL in sulfuric acid) and the resultant mixture boiled for 10 min. The samples were cooled to room temperature and the absorbance determined at 620 nm. The bilirubin concentration was determined using a kit as specified by the manufacturer (Diagnostica Merck-México, S.A., Bilirubin Cat. 3328).

**Hepatic lipid peroxidation determination.** Malondialdehyde in the liver homogenate was determined by the reaction with thiobarbituric acid (TBA) and used as a lipid peroxidation index (Uchiyama and Mihara, 1978). Briefly, 1 g of liver was homogenized in 10 mL KCl 1.15% (w/v) and the homogenate filtered through 4-folded gauze. 0.5 mL of liver homogenate was mixed with 3 mL of H<sub>3</sub>PO<sub>4</sub> 1% (v/v) and 1 mL of TBA 0.6% (w/v), and boiled for 45 min. Samples were cooled to room temperature and 3 mL of 1-butanol added. After shaking, the butanolic phase was obtained by centrifugation at 4000 × g for 10 min and absorbance at 535 nm determined.

**Total lipid peroxides and nitric oxide.** Total lipid peroxides were measured according to Yagi (1998). Measurements were based on serum thiobarbituric acid reactive substances (TBARS) and are reported in mmol/mL. The concentration of nitric oxide (NO) in serum was measured by the method of Green *et al.* (1982); the analytical determination is based on a diazotization reaction with NaNO<sub>2</sub> in the presence of ammonium sulfamate and N-naftylethylenediamine; a colored compound is produced which can be measured spectrophotometrically at 515 nm. Erythrocyte plasma membrane stability was evaluated as a measure for oxidative

stress mediated events, as described by Mounnissamy *et al.* (2007). Briefly, a blood aliquot was washed once with cold Alsever solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, NaCl 0.42%) and twice with NaCl 0.85%, pH 7.2. A 10% erythrocyte suspension was prepared in NaCl 0.85%. The assay mixture was made using 0.5 mL of the erythrocyte suspension, 2 mL distilled water and 1 mL sodium phosphate buffer ( $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  0.15 M, pH 7.4), and incubated at 50°C for 30 min. After incubation the suspension was centrifuged and the supernatant used to measure the absorbance at 560 nm, for haemoglobin. The percentage protection from oxidative stress was calculated by comparing the absorbance of the test sample with that of an erythrocyte suspension in twice-distilled water (100% haemolysis, 0% protection) and a sample of isotonic solution (0% haemolysis, 100% protection).

**Histological studies.** Liver tissue was processed for histological observation by staining with haematoxylin-eosin and Masson's trichrome stain. A microscope (Carl Zeiss Axiostar plus model 440950 CP ACHROMAT) coupled with digital camera (Olympus, model C7070 Wide Zoom) was used.

**Statistical analysis.** All values are the mean  $\pm$  SEM obtained from ten different animals. For statistical analysis, ANOVA with the Bonferroni test was used to compare the groups. In all cases, a difference was considered significant when  $p$  value was  $<0.05$ .

## RESULTS AND DISCUSSION

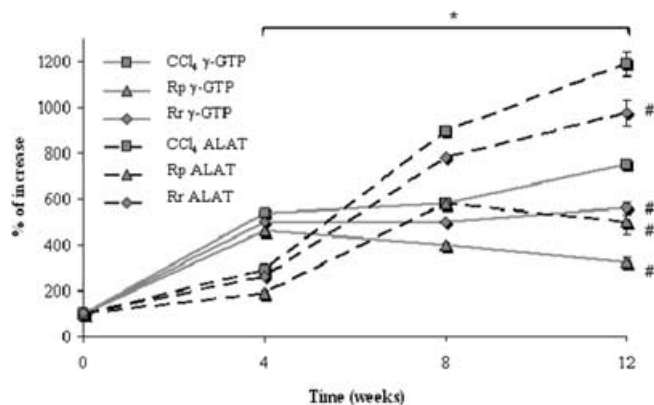
The *Rosmarinus officinalis* extract (yield around 12%) was a light, very aromatic, yellow powder.

### HPLC analysis

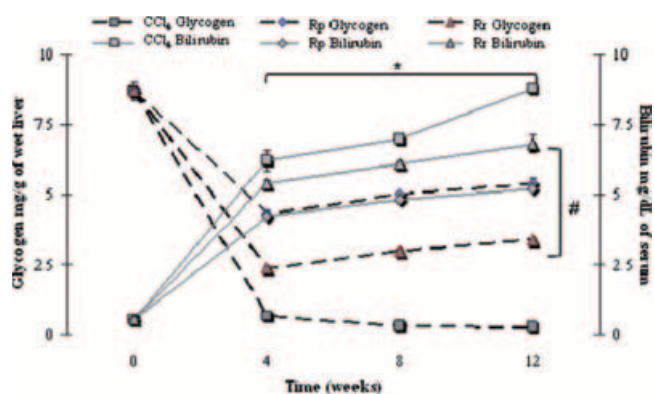
HPLC analysis of *Rosmarinus officinalis* extracts revealed the presence of chromatographic peaks consistent with the pattern showed by the standard Herbalox, showing in both cases a main peak at a retention time of 67.8 min. Carnosol, one of the main active principles, was clearly identified according to its retention time, confirming the presence of this compound in the extract, as previously described (Sotelo *et al.*, 2002). Quantitative HPLC analysis showed that carnosol content in the *Rosmarinus officinalis* extract was 3.02% (w/w).

### Effect of *Rosmarinus officinalis* extract on $\text{CCl}_4$ -induced biochemical parameters

To confirm that  $\text{CCl}_4$ -induced liver damage had occurred, serum enzymatic activities (ALT and  $\gamma$ -GTP), metabolic (liver glycogen content and serum bilirubins) and structural indicators were measured.  $\text{CCl}_4$  induced an increase in enzymatic activity over time, reaching a 12-fold increment for ALT activity and a 7-fold increment for  $\gamma$ -GTP (Fig. 1) at 12 weeks. *Rosmarinus officinalis* reduced those alterations by about 60% each



**Figure 1.** Effect of *Rosmarinus officinalis* extract on serum enzymatic activities of  $\gamma$ -glutamyl transpeptidase (solid lines) and alanine aminotransferase (broken lines) in rats with  $\text{CCl}_4$ -induced hepatic damage. Each point represents the mean value in percentage over the control  $\pm$  SEM for ten animals. Sera were assayed in duplicate. Rp = treatment with *R. officinalis* in the prevention model; Rr = treatment in the reversion model. (\*) = significantly different from control group; (#) = significantly different from the  $\text{CCl}_4$ -treated group;  $p$  value  $< 0.05$ .



**Figure 2.** Effect of *Rosmarinus officinalis* on  $\text{CCl}_4$ -induced modifications of hepatic glycogen (broken lines) and serum bilirubin (solid lines) over the time. Each point represents the mean value  $\pm$  SEM of ten animals. Samples were assayed in duplicate. Rp = treatment with *R. officinalis* in the prevention model; Rr = treatment in the reversion model. (\*) = significantly different from control group; (#) = significantly different from  $\text{CCl}_4$ -treated group;  $p$  value  $< 0.05$ .

case. The effect of *R. officinalis* in the reversion model (established cirrhosis) was partial, decreasing the enzymatic activity by 18% for ALT and 25% for  $\gamma$ -GTP respectively.

$\text{CCl}_4$  treatment decreased liver glycogen content by 96% with respect to the control, starting at the fourth week and continuing until 12 weeks after initial exposure ( $p < 0.0001$ ). *Rosmarinus officinalis* extract avoided (by about 59%) the  $\text{CCl}_4$ -induced decrease in hepatic glycogen (Fig. 2); it also significantly increased the glycogen content at the end of the study ( $p < 0.001$ ). *R. officinalis* treatment resulted in a partial reversion of cirrhosis over the time, compared to the  $\text{CCl}_4$ -treated group (by about 39%). Bilirubin levels in the  $\text{CCl}_4$  group increased 16-fold (Fig. 2), an effect observed from weeks 4 to 12. *Rosmarinus officinalis* extract partially prevented the  $\text{CCl}_4$ -induced increase in bilirubin levels, reducing it by about 50%. In the reversion model, *R. officinalis* extract reduced this alteration in cirrhotic rats by 23%.

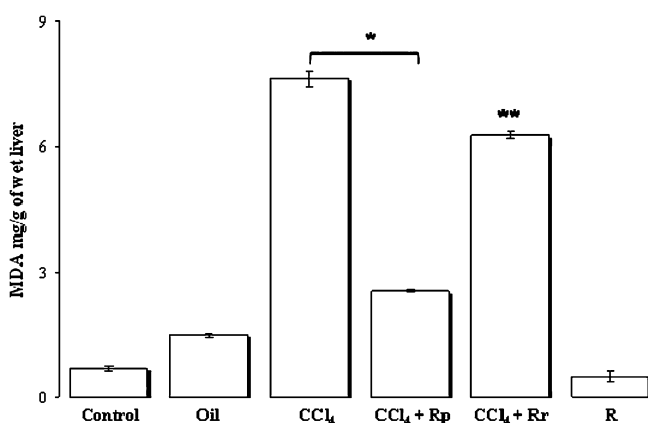


### Effect of *Rosmarinus officinalis* on CCl<sub>4</sub>-induced oxidative stress

CCl<sub>4</sub> increased the MDA content by up to 412% in liver homogenates from rats administered CCl<sub>4</sub> for 12 weeks, compared to the oil (vehicle control) treated animals (basal values) (Fig. 3). The oil group showed MDA basal values of  $1.49 \pm 0.04$   $\mu\text{g/g}$  wet tissue, while the untreated (control) group was  $0.68 \pm 0.05$   $\mu\text{g/g}$  wet tissue; values were stable during the period of study. When non-cirrhotic animals were administered with *Rosmarinus officinalis* extract alone, MDA levels were lower than for the control group, suggesting a FR scavenging effect (Fig. 3), and the extract was able to almost fully inhibit the MDA increase in CCl<sub>4</sub>-treated animals (Fig. 3). In the established cirrhosis model, *Rosmarinus officinalis* treatment produced a significant reversion of MDA levels (17.5%) at 12 weeks.

Serum total lipid peroxides increased in CCl<sub>4</sub> liver damaged animals (Fig. 4) to about 9.6-fold over the control value. *R. officinalis* treatment reduced this rise to only 2.5-fold. In the reversion model *Rosmarinus officinalis* partially decreased total lipid peroxides levels (by 33%) compared to the group with established cirrhosis. Administration of the vehicles to untreated animals did not modify total lipid peroxides basal levels in serum, but when animals were administered with *Rosmarinus officinalis* alone, the levels were again lower than for the control group, suggesting a FR scavenging effect (Fig. 4).

Table 1 shows nitric oxide values measured in serum. This indicator again displays the same trends as those previously examined in cirrhotic animals: *R. officinalis* prevented (66%) and reverted (28%) CCl<sub>4</sub>-induced increase nitric oxide levels, suggesting that *R. officinalis*



**Figure 3.** Effect of *Rosmarinus officinalis* on CCl<sub>4</sub>-induced lipid peroxidation on liver homogenates. Each bar represents the mean  $\pm$  SEM of ten animals. CCl<sub>4</sub> + Rp = therapy by *R. officinalis* in the damage prevention model; CCl<sub>4</sub> + Rr = therapy by *R. officinalis* in the cirrhosis reversion model; R = *R. officinalis* alone. (\*) = significantly different from control group; (\*\*) = significantly different from CCl<sub>4</sub>-treated group; p value < 0.05.

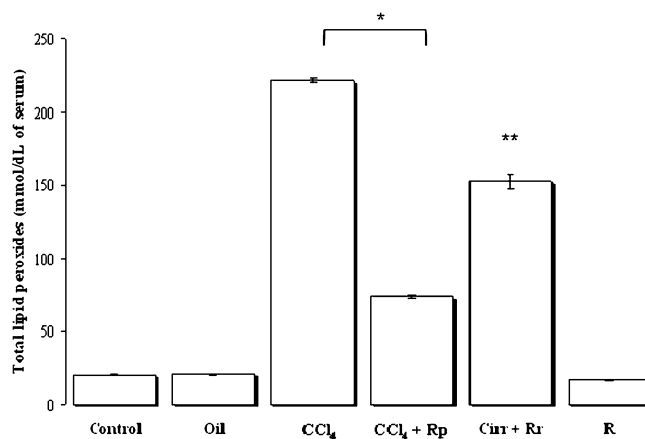
mediates its hepatoprotective effects through an anti-oxidant mechanism.

Erythrocyte plasma membrane stability was grossly impaired by CCl<sub>4</sub> administration, producing a labile membrane which was easier to lyse, and the instability was measured as an increase in hemoglobin release. Because the erythrocyte plasma membrane stability protection was very low on the treated animals compared to control (untreated) group (Table 1) membrane instability values are in accordance with other oxidative and damage indicators measured (metabolic, histological and enzymatic). *R. officinalis* treatment increased the erythrocyte plasma membrane stability in the treated animals and this effect was maintained over the experimental course from 4 to 12 weeks (p < 0.01).

### Effect of *Rosmarinus officinalis* on CCl<sub>4</sub>-induced histological alterations

Histological liver images from control animals show a normal and conserved architecture, with veins well defined (Fig. 5A). Livers from animals administered only with *Rosmarinus officinalis* were normal, and similar to the control group. The CCl<sub>4</sub>-treated group showed, at 4 weeks, reactive hepatitis and mild inflammation, progressing to reactive hepatitis with patch necrosis zones and mild collagen deposit (not shown). After 12 weeks of treatment, CCl<sub>4</sub> had induced massive necrosis and complete liver tissue destruction with lost of cellular structure, severe inflammation and extensive fibrosis (Fig. 5B).

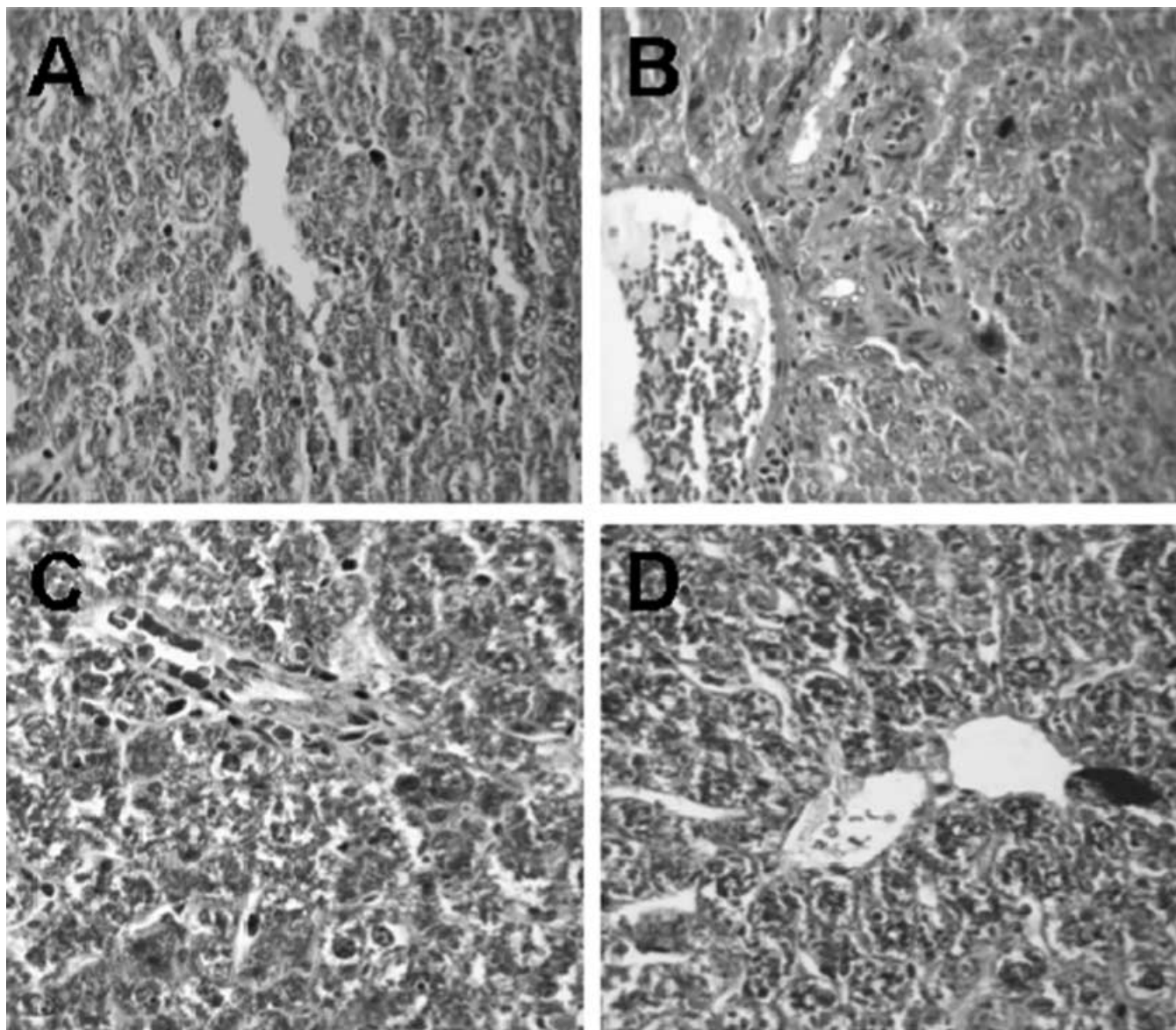
*R. officinalis* treatment prevented some of these alterations, and at 4 weeks only periportal inflammation and



**Figure 4.** Effect of *Rosmarinus officinalis* on CCl<sub>4</sub>-induced serum total lipid peroxides. Each bar represents the mean value  $\pm$  SEM of ten animals. CCl<sub>4</sub> + Rp = therapy by *R. officinalis* in the damage prevention model; Cirr + Rr = therapy by *R. officinalis* in the cirrhosis reversion model; R = *R. officinalis* alone. (\*) = significantly different from control group; (\*\*) = significantly different from CCl<sub>4</sub>-treated group; p value < 0.05.

**Table 1.** Effect of *Rosmarinus officinalis* on CCl<sub>4</sub>-induced serum nitric oxide and on erythrocyte plasma membrane stability

Parameter measured	Control	CCl <sub>4</sub>	CCl <sub>4</sub> + <i>R. officinalis</i> prevention model	Cirrhosis + <i>R. officinalis</i> reversion model	<i>R. officinalis</i>
Nitric oxide ( $\mu\text{mol/mL}$ )	$18.92 \pm 0.67$	$328.76 \pm 1.36$	$155.31 \pm 7.4$	$241.12 \pm 2.58$	$18.21 \pm 2.44$
Erythrocyte plasma membrane stability protection value (%)	$96.67 \pm 0.83$	$2.43 \pm 0.09$	$65.73 \pm 0.63$	$39.8 \pm 0.21$	$96.5 \pm 0.70$



**Figure 5.** Liver photomicrographs from cirrhotic CCl<sub>4</sub>-induced and control rats treated with *Rosmarinus officinalis*. Control animals (A), animals administered with CCl<sub>4</sub> alone (B) or with *Rosmarinus officinalis* in the prevention model (C) and cirrhotic *Rosmarinus officinalis*-treated animals in the reversion model (D). All tissue slices were stained with Masson's trichrome stain (final magnification 180X).

some regenerative nodules were observed (not shown). At week 12, livers of animals treated with *R. officinalis* showed mild fibrosis and some necrosis zones with regenerative nodules (Fig. 5C), showing an amelioration of the extensive damage found in the CCl<sub>4</sub>-treated group.

*R. officinalis* also promoted a partial reversion of established cirrhosis. In the reversion model, *R. officinalis* treatment for 12 weeks modified the course of damage as evidenced by bridge necrosis, fibrosis and notorious regenerative nodules (Fig. 6D). These effects on tissue damage and oxidative indicators suggest a hepatoprotective action of *R. officinalis* extract on CCl<sub>4</sub>-induced liver cirrhosis.

## CONCLUSIONS

CCl<sub>4</sub>-induced hepatotoxicity in the rat is an established model for studying liver damage (Pérez-Tamayo, 1983).

The metabolic role of the liver makes it a preferred target for oxidative attack and antioxidant action has been shown to confer hepatoprotective effects. Natural products have long been an important source of therapeutic agents for this purpose (Asha *et al.*, 2007; Jain *et al.*, 2008), and *R. officinalis* is well known for its antioxidant properties (Amin and Hamza, 2005; Rusu *et al.*, 2005). Our studies using an experimental cirrhosis model have confirmed these properties and provided evidence for the traditional use of *R. officinalis* as a hepatoprotective agent.

Our results show that daily administration of *R. officinalis* can limit the extent of histological changes and partially normalize altered biochemical parameters in experimental cirrhosis. *R. officinalis* prevented oxidative damage induced by CCl<sub>4</sub>, demonstrated by a decrement in lipoperoxidation and total lipid peroxide and NO levels, and an increase in erythrocyte plasma membrane stability. These results suggest that *R. officinalis* therapy, acting as an antioxidant and/or a free radical scavenger, can preserve cellular integrity and counter-

act the severe damage induced by  $\text{CCl}_4$ . The results of this and previously published acute model studies (Sotelo *et al.*, 2002) suggest a concomitant activation of physiological mechanisms in addition to antioxidant or free radical scavenging activity, as previously reported by Mantle *et al.* (2000).

The liver excretes the breakdown product of hemoglobin, namely bilirubin, so serum bilirubin levels have been used to evaluate chemically induced hepatic injury (Gressner *et al.*, 2007). In our study *R. officinalis* both prevented and reversed  $\text{CCl}_4$ -induced damage as evidenced by a reduction in bilirubin levels, suggesting an improvement in biotransformation. The increment in serum enzymatic activities is related to hepatic parenchymal damage since ALT is released from mitochondrial and cytosolic localization and  $\gamma$ -GTP from membranal sites, and cellular rupture allows the enzyme to escape into the blood (Gressner *et al.*, 2007). *R. officinalis* treatment generated a recovery of both indicator levels suggesting a hepatoprotective effect and preservation of plasma membranes by antioxidative action.

A decrease in liver glycogen is one of the functional and metabolic changes in liver damage, produced generally in response to stimulation of adenyl cyclase with a consequent rise in AMPc levels (Muriel, 1998). The present study showed a decrease in liver glycogen which

may be due to excessive adrenergic activity, as suggested by several authors (Müller *et al.*, 1999), although there are reports concerning other properties of *R. officinalis* that may contribute to the preventive and regenerative effect found in our studies (Fahim *et al.*, 1999; Zeng *et al.*, 2001; Sotelo *et al.*, 2002; Gutiérrez *et al.*, 2003; Yahuaca-Mendoza *et al.*, 2005).

The antioxidant effects of *R. officinalis* shown here are in agreement with previous reports (Aruoma *et al.*, 1992; Sotelo *et al.*, 2002; Yahuaca-Mendoza *et al.*, 2005) and blocking lipid peroxidation by may be due to either scavenging  $\text{Cl}_3\text{COO}^\bullet$  and  $^\bullet\text{OH}$  radicals, converting them into a less toxic substances, or acting as antioxidants (Aruoma *et al.*, 1992; Haraguchi *et al.*, 1995). In summary, our results suggest *R. officinalis* can prevent  $\text{CCl}_4$ -induced chronic liver damage, improve hepatocyte integrity through the scavenging activity of free radicals and, consequently, avoid the propagation of lipid peroxides.

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