Physiol. Res. 53: 515-521, 2004

Regeneration of Coenzyme Q_9 Redox State and Inhibition of Oxidative Stress by Rooibos Tea *(Aspalathus linearis)* Administration in Carbon Tetrachloride Liver Damage

J. KUCHARSKÁ[1], O. ULIČNÁ[1], A. GVOZDJÁKOVÁ[1], Z. SUMBALOVÁ[1], O. VANČOVÁ[1], P. BOŽEK[2], M. NAKANO[3], M. GREKSÁK[4]

[1]Pharmacobiochemical Laboratory, School of Medicine, Comenius University, Bratislava, Slovak Republic, [2]Department of Biochemistry and Hematology, State Hospital, Bratislava, Slovak Republic, [3]Institute of Medical Science of Aging, Aichi Medical University, Nagakute, Japan, [4]Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, Ivanka pri Dunaji, Slovak Republic

Received August 27, 2003
Accepted October 24, 2003

Summary

The effect of rooibos tea (*Aspalathus linearis*) on liver antioxidant status and oxidative stress was investigated in rat model of carbon tetrachloride-induced liver damage. Synthetic antioxidant N-acetyl-L-cysteine (NAC) was used for comparison. Administration of carbon tetrachloride (CCl_4) for 10 weeks decreased liver concentrations of reduced and oxidized forms of coenzyme Q_9 (CoQ_9H_2 and CoQ_9), reduced α-tocopherol content and simultaneously increased the formation of malondialdehyde (MDA) as indicator of lipid peroxidation. Rooibos tea and NAC administered to CCl_4-damaged rats restored liver concentrations of CoQ_9H_2 and α-tocopherol and inhibited the formation of MDA, all to the values comparable with healthy animals. Rooibos tea did not counteract the decrease in CoQ_9, whereas NAC was able to do it. Improved regeneration of coenzyme Q_9 redox state and inhibition of oxidative stress in CCl_4-damaged livers may explain the beneficial effect of antioxidant therapy. Therefore, the consumption of rooibos tea as a rich source of natural antioxidants could be recommended as a market available, safe and effective hepatoprotector in patients with liver diseases.

Key words

CCl_4-liver damage • Oxidative stress • Rooibos tea • Coenzyme Q_9 • α-tocopherol

Introduction

Increasing evidence indicates the role of oxidative stress in liver injury, cirrhosis development and carcinogenesis (Yamamoto *et al.* 1998, Yamamoto and Yamashita 1999, Stal and Olsson 2000). Chronic administration of carbon tetrachloride (CCl_4) is widely used as an animal model of liver damage caused by formation of trichloromethyl and trichloromethylperoxyl radicals, initiating lipoperoxidation and resulting in

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

fibrosis and cell necrosis (Recknagel 1973, Beyer 1990, Kishi *et al.* 1997, Kadiiska *et al.* 2000). Of course, some other hepatotoxic agents have also been used (Ferenčíková *et al.* 2003). It has been shown that oxidative stress can modulate fibroblast and hepatic stellate cells proliferation (Murrel *et al.* 1990, Lee *et al.* 1995) and collagen synthesis (Parola *et al.* 1993). Participation of defense antioxidant systems in liver protection under the conditions of oxidative stress has been confirmed, however, the exact pathogenetic and protective mechanisms have not been fully explained. Coenzyme Q (ubiquinone) besides its bioenergetic function in mitochondrial respiratory chain is a powerful lipid-soluble antioxidant synthesized in the liver (Littarru *et al.* 1994, Ernster and Dallner 1995, Rauchová *et al.* 1995). Dominant form of coenzyme Q in rats is coenzyme Q_9 and about 70 % of the total coenzyme Q in the liver is kept in reduced form (ubiquinol) by the activities of enzymatic systems (Takahashi *et al.* 1993, Kishi *et al.* 1997). The reduced form of coenzyme Q exerts its antioxidant function either directly on superoxide radicals or indirectly on lipid radicals, both alone and in cooperation with α-tocopherol (Kagan *et al.* 2000). Changes in endogenous coenzyme Q concentrations have been found in patients and experimental animals under conditions of oxidative stress (Kucharská *et al.* 1998, 2000, Gvozdjáková and Kucharská, 2000, Štefek *et al.* 2000). Protective effects of vitamin E and coenzyme Q administration found in experimental models of CCl_4-induced cell necrosis support the role of free radicals in liver damage (Parola *et al.* 1992, Naziroglu *et al.* 1999, Canturk *et al.* 1999).

Research of natural hepatoprotective compounds has become attractive in recent years. Beneficial effects of rooibos tea (*Aspalathus linearis),* indigenous to South Africa, based on its antioxidant activities, have been reported (Lamošová *et al.* 1997, Simon *et al.* 2000, Standley *et al.* 2001). In our previous paper (Uličná *et al.* 2003) we described a beneficial effect of rooibos tea on CCl_4-induced liver damage in rats in which blood biochemical parameters and histological examination of the liver tissue confirmed its hepatoprotective effect.

In this study, we tested whether rooibos tea as a rich source of natural antioxidant compounds could affect liver antioxidant capacity, the redox state of coenzyme Q and oxidative stress in a rat model of carbon tetrachloride liver damage. The effect of rooibos tea administration has been compared with a known synthetic antioxidant N-acetyl-L-cysteine (NAC).

Methods

Male Wistar rats weighing 210-280 g were used in our experiment. Animals were divided into four groups, each of 10 rats.

Control group (C): Animals were injected intraperitoneally with olive oil (1 mg/kg) twice a week for 10 weeks and received daily 5 mg/kg of water orally by gastric tube.

Carbon tetrachloride (CCl_4) group: Animals were injected intraperitoneally with 50 % CCl_4 in olive oil (1 ml/kg) twice a week for 10 weeks and received water orally as control group.

CCl_4 + rooibos tea (CCl_4 + RT) group: Instead of tap water the animals drank rooibos tea (*Aspalathus linearis*) freshly prepared by boiling of 2.5 g of dry tea in 1 l of water for 10 min, starting 7 days before CCl_4 administration and they also were given 5 ml/kg of rooibos tea once a day by gastric tube.

CCl_4 + N-acetyl-L-cysteine (CCl_4 + NAC) group: Animals received NAC 150 mg/kg in solution orally by gastric tube, starting 7 days before CCl_4 administration.

The rats had free access to standard Larsen pellet food and tap water. All experiments were carried out according to guidelines for the care and use of experimental animals and approved by the State Veterinary Administration of the Slovak Republic.

The rats were anesthetized with thiopental 48 h after the last treatment with CCl_4 and samples of the liver tissue were taken for biochemical analyses. Concentrations of oxidized and reduced forms of coenzyme Q_9 (CoQ_9 and CoQ_9H_2) and α-tocopherol were determined by high-performance liquid chromatography (HPLC - LKB, Sweden) according to Lang *et al.* (1986) with some modifications as follows. The liver tissue (50-100 mg) was homogenized using of Ultra-Turrax in water with an addition of t-butylhydroxytoluene and sodium dodecyl sulphate and extracted twice by mixture of hexane-ethanol (5/2, v/v, Merck). Collected organic layers were evaporated under nitrogen and the residue taken up in ethanol was injected on the column SGX C18 (7 μm, Tessek, Czech Republic). The mobile phase consisted of methanol-acetonitrile-ethanol (6/2/2, v/v/v, Merck). Concentrations of compounds were detected spectrophotometrically at 275 nm using external standards (Sigma). Reduced coenzyme Q_9 standard was prepared by reduction of CoQ_9 with sodium dithionite. Malondialdehyde (MDA) in liver tissue was determined

by HPLC (Pilz *et al.* 2000), cholesterol in liver tissue was determined according to Abell *et al.* (1952) and triacylglycerols according to Jover (1963).

The results were evaluated using Student´s t-test for unpaired data and linear regression analysis with Pearson´s correlation coefficient. Values of $p<0.05$ were considered statistically significant.

Table 1. Liver concentrations of α-tocopherol, oxidized and reduced coenzyme Q_9 (CoQ_9 and CoQ_9H_2), redox state of CoQ_9 and malondialdehyde (MDA) concentration in CCl_4-damaged rats and the effects of rooibos tea (RT) and N-acetyl-L-cysteine (NAC).

Liver	Control n=10	CCl_4 n=10	CCl_4+RT n=10	CCl_4+NAC n=10
α-tocopherol	32. 5±1.57	18.5±0.81	26.9±4.13	33.1±4.62
(nmol/g ww)		p<0.0001	p<0.05	p<0.002
CoQ_9	71.0±5.16	58.2±4.03	60.7±4.85	78.8±4.09
(nmol/g ww)		p<0.05	NS	p<0.005
CoQ_9H_2	132.8±12.5	40.8±6.34	155.0±16.1	131.1±19.7
(nmol/g ww)		p<0.0001	p<0.0001	p<0.0002
CoQ_9H_2/total CoO_9	65.2	41.2	71.9	62.5
(%)				
MDA	99.8±7.79	152.2±12.1	80.8±6.41	104.3±8.81
(nmol/g ww)		p<0.001	p<0.001	p<0.05

Data are means ± S.E.M., CCl_4 is compared to controls, CCl_4 + RT and CCl_4 + NAC are compared to CCl_4.

Table 2. Liver concentrations of total cholesterol (Chol) and triacylglycerols (TAG) in CCl_4-damaged rats and treated with rooibos tea (RT) and N-acetyl-L-cysteine (NAC).

Liver	Control n=10	CCl_4 n=10	CCl_4+RT n=10	CCl_4+NAC n=10
Chol	5.19±0.21	10.4±0.21	10.7±0.53	8.97±0.48
(nmol/g)		p<0.001	NS	p<0.01
TAG	7.56±0.74	39.4±2.59	32.9±1.98	25.3±1.86
(nmol/g)		p<0.001	p<0.05	p<0.0001

Data are means ± S.E.M., CCl_4 CCl_4 is compared to controls, CCl_4 + RT and CCl_4 + NAC are compared to CCl_4.

Results

The administration of carbon tetrachloride to experimental rats for 10 weeks developed significant changes in liver concentrations of lipophilic antioxidants and malondialdehyde (MDA) formation as a marker of lipid peroxidation (Table 1). Concentrations of reduced coenzyme Q_9 (CoQ_9H_2) and α-tocopherol were reduced highly significantly (by 69 % and 43 %, respectively), whereas oxidized coenzyme Q_9 (CoQ_9) decreased by 18 % ($p<0.05$). Treatment with rooibos tea (RT) increased antioxidant status in CCl_4-damaged livers. Concentrations of α-tocopherol and CoQ_9H_2 increased to the values comparable with healthy animals. Similar effects on α-tocopherol and CoQ_9H_2 concentrations were found when N-acetyl-L-cystein (NAC) was administered. Rooibos tea did not counteract the decrease of oxidized CoQ_9, whereas NAC was able to do so. Malondialdehyde formation in the liver was suppressed significantly both with RT and NAC administration. Redox state of CoQ_9 expressed in percentage of reduced CoQ_9 from total CoQ_9 was restored with RT and NAC treatment in CCl_4-treated rats. CCl_4 treatment of rats caused a severe increase of hepatic total cholesterol and triacylglycerols (Table 2). Rooibos tea did not affect cholesterol concentration, but decreased triacylglycerols concentration. N-acetyl-L-

cysteine lowered both cholesterol and triacylglycerols. Standardized concentration was used for expressing the ratio of the measured concentration of lipophilic antioxidant to cholesterol and triacylglycerol concentrations. In CCl_4-treated rats, these ratios were decreased (Table 3). Similarly to the absolute antioxidant concentrations, lipid-standardized concentrations of α-tocopherol and reduced CoQ_9 also increased with RT and NAC treatment. Rooibos tea did not counteract the decrease of oxidized CoQ_9, whereas NAC did. Concentrations of CoQ_9H_2 correlated inversely with MDA significantly in control (r = –0.70, p = 0.05) and non-significantly in RT-treated rats (r = –0.54, NS). Non-significant positive correlations were found in CCl_4-damaged livers (r = 0.58, p = 0.08, NS) and with NAC treatment (r = 0.55, p = 0.162) (Fig. 1).

Table 3. Cholesterol- and triacylglycerol-standardized liver concentrations of lipophilic antioxidants in CCl_4-damaged rats and the effects of rooibos tea (RT) and N-acetyl-L-cysteine (NAC).

Liver	Control n=10	CCl_4 n=10	CCl_4+RT n=10	CCl_4+NAC n=10
α-tocopherol/Chol	6.38±0.44	1.77±0.07	2.61±0.39	3.89±0.72
		p<0.0001	p<0.05	p<0.005
α-tocopherol/TAG	5.08±0.71	0.48±0.03	0.81±0.11	1.28±0.15
		p<0.0001	p<0.02	p<0.0001
CoQ_9/Chol	13.90±1.24	6.12±0.61	6.00±0.55	9.76±1.03
		p<0.0001	NS	p<0.01
CoQ_9/TAG	8.20±0.65	1.75±0.25	1.90±0.17	3.54±0.48
		p<0.0001	NS	p<0.005
CoQ_9H_2/Chol	25.5±2.73	3.96±0.65	15.0±1.91	15.3±2.51
		p<0.0001	p<0.0001	p<0.0002
CoQ_9H_2/TAG	20.4±3.20	1.08±0.19	4.80±0.60	5.26±0.83
		p<0.0001	p<0.0001	p<0.0001

Data are means ± S.E.M., CCl_4 CCl_4 is compared to controls, CCl_4 + RT and CCl_4 + NAC are compared to CCl_4.

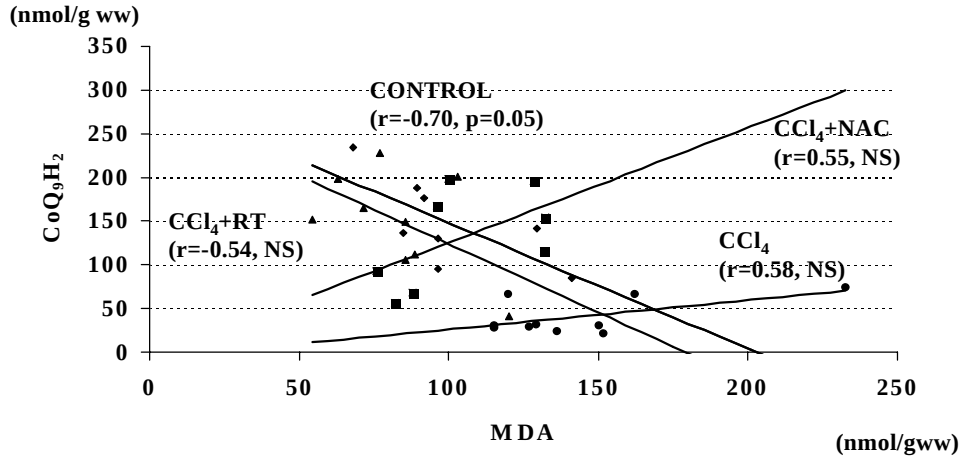

Fig. 1. Corelations between liver concentrations of reduced coenzyme Q_9 (CoQ_9H_2) and malondialdehyde (MDA) in CCl_4-damaged rats and treated with rooibos tea (RT) and N-acetyl-L-cysteine (NAC). ♦Control; ● CCl_4; ▲ CCl_4+RT; ■ CCl_4+NAC

Discussion

Chronic administration of carbon tetrachloride provides a suitable animal model for free radical damage in liver. CCl_4 is metabolically activated by cytochrome P450 into a trichloromethyl radical which is in the presence of oxygen converted into a peroxyl radical (Recknagel *et al.* 1989). As reported previously, under

our experimental conditions, CCl_4 treatment caused a severe increase of hepatic triacylglycerols, total cholesterol and plasma activities of aminotransferases as well as a decrease of albumin concentration as markers of liver function. Malondialdehyde formation, as marker of lipid peroxidation, increased significantly. In these livers, pathological changes developed, i.e. steatosis and fibrosis, which were confirmed histomorphologically and histometrically (Uličná *et al.* 2003).

The role of coenzyme Q in liver diseases has been investigated in few clinical and experimental studies. Bianchi *et al.* (1994) and Eaton *et al.* (2000) found a reduction in plasma coenzyme Q_{10} levels in patients with liver cirrhosis and in chronic alcohol abusers. Yamamoto and Yamashita (1999) and Kontusch *et al.* (1999) observed a lower percentage of plasma ubiquinol-10 (reduced form of coenzyme Q_{10}) in patients with liver diseases which may reflect its diminished reduction by the liver.

Kadiiska *et al.* (2000) measured several plasma antioxidants in an animal model after a single dose of CCl_4. Concentrations of total coenzyme Q (oxidized and reduced CoQ_9 + CoQ_{10}) and α-tocopherol decreased and consumption of total coenzyme Q was more sensitive to CCl_4 exposure than that of α-tocopherol. Ratio of oxidized to reduced glutathione decreased, suggesting an early oxidative stress. Kishi *et al.* (1997) published decreased concentrations of reduced coenzyme Q_9 plus Q_{10}, α-tocopherol, ascorbic acid and reduced glutathione in rat livers after CCl_4 injection. Stal and Olson (2000) exposed rats to the combination of diethylnitrosamine as initiator, followed by repeated injections of carbon tetrachloride to induce liver cirrhosis. Fourty-eight hours after CCl_4 administration, the content of hepatic α-tocopherol was increased and after 23 weeks of CCl_4 administration once a week, both reduced and oxidized forms of CoQ_9 were enhanced. The authors explain these results as an adaptation of the liver to toxic chemicals. In our experimental model of liver damage induced by CCl_4 administration for 10 weeks, liver concentrations of coenzyme Q_9 and α-tocopherol were significantly reduced. Redox state of coenzyme Q expressed as percentage of reduced CoQ_9 from total CoQ_9 decreased from 65 % in control rats to 41 % in CCl_4-treated rats and simultaneously increased MDA formation by 52 %. These findings support the presence of oxidative stress in CCl_4-damaged livers and its consequences on antioxidant status. We found higher utilization of CoQ_9H_2 than of α-tocopherol. This is in agreement with theories of Kishi *et al.* (1997) and Thomas and Stocker (2000) that CoQ_9H_2 is the first lipohilic antioxidant utilized under conditions of oxidative stress.

The administration of rooibos tea and N-acetyl-cysteine prevented the decline in liver concentrations of CoQ_9H_2 in CCl_4-treated rats (concentrations increased by 280 % with RT and 221 % with NAC, respectively). Concentration of α-tocopherol was increased with RT by 45 % and with NAC by 79 % to the values comparable with healthy animals. Both RT and NAC prevented the increased formation of malondialdehyde in CCl_4-damaged livers. In spite of little differences in liver antioxidant state of the rats treated with rooibos tea or NAC, we found a negative correlation between CoQ_9H_2 and MDA in RT-treated rats only. Although this correlation was not significant, it was similar as in control rats. It seems that sufficient antioxidant protection is important for the prevention of liver damage but treatment with natural antioxidants may be more advantageous to the liver protection. Improved regeneration of coenzyme Q redox state may explain the beneficial effect of antioxidant therapy. Therefore, the consumption of rooibos tea as a rich source of natural antioxidants could be advised as a market available, safe and effective hepatoprotector in patients with liver diseases.

Acknowledgements

The study was supported by Slovak Grant Agency for Science VEGA No. 1/7547/20 and 1/0546/03 and Science and Technology Assistance Agency (Slovak Republic) No. APVT-51-016502. Technical assistance of E. Benko, Ľ. Butašová, A. Štetková, P. Zaušková is gratefully appreciated.

References

ABELL LL, LEVEL BD, BRODIE BB: A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J Biol Chem* **152**: 357-362, 1952.

BEYER RE: The participation of coenzyme Q in free radical production and antioxidation. *Free Radic Biol Med* **8**: 545-565, 1990.

BIANCHI GP, FIORELLA PL, BARGOSSI AM, GROSSI G, MARCHESINI G: Reduced ubiquinone plasma levels in patients with liver cirrhosis and in chronic alcoholics. *Liver* **14**: 138-140, 1994.

CANTURK Z, CANTURK NZ, OZBILIM G, YENISEY C: Experimental cirrhosis of the liver and cardioprotective effect of alpha tocopherol. *East Afr Med J* **76**: 223-227, 1999.

EATON S, RECORD ChO, BARLETT K: A role for coenzyme Q in alcoholic liver disease? In*: Coenzyme Q: Molecular Mechanisms in Health and Disease*. KAGAN VE, QUINN DJ (eds), CRC Press, Boca Raton, 2000, pp 307-315.

ERNSTER L, DALLNER G: Biochemical, physiological and medical aspects of ubiquinone function. *Biochim Biophys Acta* **1271**: 195-204, 1995.

FERENČÍKOVÁ R, ČERVINKOVÁ Z, DRAHOTA Z: Hepatotoxic effect of D-galactosamine and protective role of lipid emulsion. *Physiol. Res* **52**: 73-78, 2003.

GVOZDJÁKOVÁ A, KUCHARSKÁ J: Implication of coenzyme Q depletion in heart transplantation. In: *Coenzyme Q: Molecular Mechanisms in Health and Disease.* KAGAN VE, QUINN DJ (eds), CRC Press, Boca Raton, 2000, pp 293-304.

JOVER A: Technique for the determination of serum glycerides. *J Lipid Res* **4**: 228-230, 1963.

KADIISKA MB, GLADEN BC, BAIRD DD, DIKALOVA AE, SOHAL RS, HATCH GE, JONES DP, MASON RP, BARRETT JC: Biomarkers of oxidative stress study: are plasma antioxidants markers of CCl_4 poisoning? *Free Radic Biol Med* **28**: 838-845, 2000.

KAGAN VE, FABISIAK YP, TYURINA YY: Independent and concerted antioxidant functions of coenzyme Q. In: *Coenzyme Q: Molecular Mechanisms in Health and Disease*. KAGAN VE, QUINN DJ (eds), CRC Press, Boca Raton, 2000, pp 119-129.

KISHI T, TAKAHASHI T, OKAMOTO T: Cytosolic NADPH-UQ reductase-linked recycling of cellular ubiquinol: its protective effect against carbon tetrachloride hepatotoxicity in rat. *Mol Aspects Med* **18** (Suppl): 71-77, 1997.

KONTUSCH A, SCHIPPLING S, SPRANGER T, BEISIEGEL V: Plasma ubiquinol-10 as a marker for disease: is the assay worthwile? *BioFactors* **9**: 225-229, 1999.

KUCHARSKÁ J, GVOZDJÁKOVÁ A, MIZERA S, BRAUNOVÁ Z, SCHREINEROVÁ Z, SCHRAMEKOVÁ E, PECHÁŇ I, FABIÁN J: Participation of coenzyme Q_{10} in the rejection development of the transplanted heart: a clinical study. *Physiol Res* **47**: 399-404, 1998.

KUCHARSKÁ J, BRAUNOVÁ Z, ULIČNÁ O, ZLATOŠ L, GVOZDJÁKOVÁ A: Deficit of coenzyme Q in heart and liver mitochondria of rats with streptozotocin-induced diabetes. *Physiol Res* **49**: 411-418, 2000.

LAMOŠOVÁ D, JURÁNI M, GREKSÁK M, NAKANO M, VANEKOVÁ M: Effect of rooibos tea (Aspalathus linearis) on chick skeletal muscle cell growth in culture. *Comp Biochem Physiol C* **116**: 39-45, 1997.

LANG JK, GOHIL K, PACKER L.: Simultaneous determination of tocopherols, ubiquinols, and ubiquinones in blood, plasma, tissue homogenates, and subcellular fractions. *Anal Biochem* **157**: 106-116, 1986.

LEE KS, BUCK M, HOUGLUM K, CHOJKIER M: Activation of hepatic stellate cells by TGFβ and collagen type I is mediated by oxidative stress through c-myb expression. *J Clin Invest* **96**: 2461-2468, 1995.

LITTARRU GP, BATTINO M, TOMASETTI A, MORDENTE A, SANTINI S, ORADEI A, MANTO A, GHIRLANDA G: Metabolic implications of coenzyme Q_{10} in red blood cells and plasma lipoproteins. *Mol Aspects Med* **15** (Suppl): 67-72, 1994.

MURREL GAC, FRANCIS MJO, BROMLEY L: Modulation of fibroblast proliferation by oxygen free radicals. *Biochem J* **265**: 659-665, 1990.

NAZIROGLU M, CAY M, USTUNDAG B, AKSANAL M, YEKELER H: Protective effects of vitamin E on carbon tetrachloride-induced liver damage in rats. *Cell Biochem Funct* **17**: 253-259, 1999.

PAROLA M, LEONARDUZZI G, BIASI F, ALBANO E, BIOCCA ME, POLI G, DIANZANI MV: Vitamin E dietary supplementation protects against carbon tetrachloride-induced chronic liver damage and cirrhosis*. Hepatology* **16**: 1014-1021, 1992.

PAROLA M, PINZANI M, CASINI A, ALBANO E, POLI G, GENTILINI A, GENTILINI P, DIANZANI MU: Stimulation of lipid peroxidation or 4-hydroxynonenal treatment increases procollagen alpha 1 (I) gene expression in human liver fat-storing cells. *Biochem Biophys Res Commun* **194**: 1044-1050, 1993.

PILZ J, MEINKE I, GLEITER CH: Measurement of free and bound malondialdehyde in plasma by high performance liquid chromatography as the 2,4-dinitrophenylhydrazine derivative. *J Chromatogr B* **742**: 315-325, 2000.

RAUCHOVÁ H, DRAHOTA Z, LENAZ G: Function of coenzyme Q in the cell: some biochemical and physiological properties. *Physiol Res* **44**: 209-216, 1995.

RECKNAGEL RO, GLENDEK EA Jr, DOLAZK JA, WALLER RL: Mechanism of carbon tetrachloride toxicity. *Pharmacol Ther* **43**: 139-154, 1989.

SIMON M, HOROVSKÁ L, GREKSÁK M, DUŠINSKY R, NAKANO M: Antihemolytic effect of Rooibos tea (Aspalathus linearis) on red blood cells of Japanese quials. *Gen Physiol Biophys* **19**: 365-371, 2000.

STAL P, OLSON J: Ubiquinone, oxidative stress, and liver carcinogenesis. In*: Coenzyme Q: Molecular Mechanisms in Health and Disease*. KAGAN VE, QUINN DJ (eds), CRC Press, Boca Raton, 2000, pp 317-329.

STANDLEY Y, WINTERTON P, MARNEWICK JL, GELDERBLOM WC, JOUBERT E, BRITZ TJ: Influence of processing stages on antimitogenic and antioxidant potentials of rooibos tea. *J Agric Food Chem* **49**: 114-117, 2001.

ŠTEFEK M, SOTNIKOVÁ R, OKRUHLICOVÁ L, VOLKOVOVÁ K, KUCHARSKÁ J, GAJDOŠÍK A, GAJDOŠÍKOVÁ K, MIHALOVÁ D, HOZOVÁ R, TRIBULOVÁ N, GVOZDJAKOVÁ A: Effect of dietary supplementation with pyridoindole antioxidant stobadine on antioxidant state and ultrastructure of diabetic rat myocardium. *Acta Diabetol* **37**: 111-117, 2000.

TAKAHASHI T, OKAMOTO T, MORI K, SAYO H, KISHI T: Distribution of ubiquinone and ubiquinol homologues in rat tissues and subcellular fractions. *Lipids* **28**: 803-809, 1993.

THOMAS RS, STOCKER R: Mechanism of antioxidant action of ubiquinol-10 for low density lipoprotein. In: *Coenzyme Q: Molecular Mechanisms in Health and Disease*. VE KAGAN, DJ QUINN (eds), CRC Press, Boca Raton, 2000, pp 131-145.

ULIČNÁ O, GREKSÁK M, VANČOVÁ O, ZLATOŠ L, GALBAVÝ Š, BOŽEK P, NAKANO M: Hepatoprotective effect of rooibos tea (Aspalathus linearis) on CCl_4-induced liver damage in rats. *Physiol Res* **52**: 461-466, 2003.

YAMAMOTO Y, YAMASHITA S: Plasma ubiquinone to ubiquinol ratio in patients with hepatitis, cirrhosis, and hepatoma, and in patients treated with percutaneous transluminal coronary reperfusion*. BioFactors* **9**: 241-245, 1999.

YAMAMOTO Y, YAMASHITA S, FUJISAWA A, KOKURA S, YOSHIKAWA T: Oxidative stress in patients with hepatitis, cirrhosis and hepatoma, evaluated by plasma antioxidants. *Biochem Biophys Res Commun* **247**: 166-170, 1998.

Reprint requests

Dr. Jarmila Kucharská, PhD., Pharmacobiochemical Laboratory, School of Medicine, Comenius University, Hlboká 7, 811 05 Bratislava, Slovak Republic, e-mail: jarmila.kucharska@stonline.sk