Does rooibos tea (*Aspalathus linearis*) support regeneration of rat liver after intoxication by carbon tetrachloride?

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Abstract. This study evaluates the effect of rooibos tea (RT, *Aspalathus linearis*) on biochemical and histological parameters during rat liver regeneration after intoxication by carbon tetrachloride (CCl_4). From the 10^{th} week, when the administration of CCl_4 was terminated, the liver tissue began to regenerate. Seven days later in the regeneration phase, the animals treated by RT during whole period of the experiment, and those which drunk RT only during the regeneration period, exhibited a trend for decrease in the activity of alanine aminotransferase and significant decrease in the activity of aspartate aminotransferase and in total bilirubin content when compared with the water-drinking group. At the same time, the concentration of plasma albumin was elevated and that of tissue malondialdehyde decreased in the both groups drinking RT. After 42 days of regeneration, all biochemical parameters in all three groups reached the level of control healthy animals. In both groups treated with RT, the extent of fibrotic tissue was lower than in the group which received water. We conclude that RT can be recommended not only for the prevention but also as a co-adjuvant for the therapy of liver diseases.

Key words: Liver regeneration — Rooibos tea — Carbon tetrachloride intoxication — Fibrosis — Cirrhosis

Introduction

Numerous studies reported the unique ability of liver to regenerate quickly after injury or intoxication when conditions are favorable. Besides investigating regeneration processes of the liver after partial hepatectomy of the donors and a recovery of the liver transplantation recipients (Fausto et al. 2006; Pahlavan et al. 2006), it is of high importance also to study regeneration of the liver affected by various hepatopathies since their prevalence is increasing worldwide (Vitaglione et al. 2004). These hepatopathies are characterized by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma. As a matter of fact is that approximately 20% of the general population in USA is suffering from non-alcoholic fatty liver and/or non-alcoholic steatohepatitis (Comar and Sterling 2006).

It is believed that oxidative stress is involved in the initiation and progression of damage to liver in a variety of disorders. Liver damage caused by carbon tetrachloride (CCl_4) is a proved model of free-radical-mediated injury (Slater et al. 1985) and it is widely used for induction of liver fibrosis and cirrhosis in experimental animals (Jiang et al. 1992). Thus induced fibrosis/cirrhosis resembles human cirrhosis in many aspects of morphology and pathophysiology. For example in both, humans and animals, regeneration of hepatocytes occurs

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after necrosis, and fibrotic infiltration is almost irreversible in the advanced stage of cirrhosis (Perez Tamayo 1983).

The need for inhibiting oxidative stress has promoted investigation of the compounds with antioxidant and hepatoprotective properties, which could represent a sound therapeutic strategy for prevention and treatment of acute and chronic liver diseases (Dhiman and Chawla 2005; Medina and Moreno-Otero 2005). Therefore, beside artificial antioxidants (Rozga et al. 1991; Svátková et al. 1997; Harrison 2006) also natural antioxidants (Drahota et al. 1999; Malinska and Winiarska 2005) including those obtained from plant sources (Rice-Evans et al. 1996; McKay and Blumberg 2007) have been exploring with the aim to find new, efficient means for supporting spontaneous regeneration processes in the liver.

As described elsewhere, rooibos tea (RT, *Aspalathus linearis*) seems to be a rich and easily accessible source of a wide range of powerful antioxidant compounds (Hitomi et al. 2004; Joubert and Schulz 2006; McKay and Blumberg 2007) which have also shown a potent hepatoprotective efficiency (Uličná et al. 2003, 2006; Kucharská et al. 2004). In comparison with the other brands of tea of plant or fruit origin, RT does not contain any mono- and disaccharides or stimulating alkaloids (Rabe et al. 1994; Joubert and Schulz 2006). Therefore, RT can be used without restriction also by the patients suffering from cardiovascular diseases or with diabetes mellitus, unless specifically noted. Because, to our knowledge, the studies of the effect of RT on liver regeneration after intoxication have not been reported, the purpose of our study was to test whether RT in a commonly used concentration for human use would positively affect biochemical and histological parameters during liver-regeneration process in the experimental model of cirrhosis in rats.

Materials and Methods

Chemicals

All chemicals used were of analytical grade purity and were purchased mostly from Centralchem (Slovakia) except for malondialdehyde tetrabutylamonium salt, 2,4-dinitrophenylhydrazine, tetrahydroxypropane, which were obtained from Fluka. Cholesterol, glycerol, n-hexane, acetonitrile, amonium acetate, sodium hydroxide, and hydrochloric acid were obtained from Merck. Phosphomolybdenic acid, picrosirius red, hematoxylin and eosin were purchased from Sigma. Morbital for anesthesis was obtained from Biowet Pulawy (Poland).

Plant material

Commercial best quality (Super Grade) RT (*Aspalathus linearis*) imported from South Africa was kindly provided by Rooibos World Co. (Nagoya, Japan). The aqueous extract of the tea used as a substitute for drinking water (see experimental design) was prepared daily by simple boiling 2.5 g dry tea in 1000 ml of deionized water for 10 min with subsequent standing for 20 min and cooling down to room temperature. After decantation of insoluble residue the extract was filtered through Whatman No. 4 filter paper. The volume of fluids drunk during this period was noted eight times in each group. Mean volumes in the groups were for control group 33.7 ± 6.16 ml/24 h/animal; CCl_4+H_2O 29.68 ± 3.82 ml/24 h/animal; RT+CCl_4 31.71 ± 4.97 ml/24 h/animal (mean ± SD).

Animals

Male Wistar rats (290–360 g) were maintained at a 12 h light/dark cycle, temperature 22°C and moisture from 60 to 70%. The animals had free access to standard Larsen pellet food and to either tap water or RT. All experiments were carried out according to the guidelines for the care and use of experimental animals and approved by State Veterinary Administration of the Slovakia, No. of the approval: 2797/05-221/3b.

Experimental procedure

The experimental approach used for repetitive *in vivo* intoxication of rats with CCl_4, is described elsewhere (Uličná et al. 2003). Animals (total number 121) were randomly assigned to three groups. The first group (n = 6) served as a healthy control (C). The second group of animals with free access to drinking water (n = 75) was treated with CCl_4 (1 ml/kg) by intraperitoneal injection of 50% solution of CCl_4 in olive oil twice a week. The control rats were injected with a corresponding volume of olive oil. After 10 weeks, CCl_4 administration was terminated and from the total of 57 animals, which survived the toxic effects of CCl_4, six animals were taken for blood and tissue analysis to determine baseline at 0 day (0 d) for monitoring changes in the biochemical and histological parameters during liver-regeneration process. The regeneration phase lasted 42 days. The rest of this second group was randomly divided into two subgroups. The first subgroup (n = 26) had a free access to the tap water (CCl_4+H_2O) and the second subgroup (n = 25) had a free access to RT (CCl_4+RT) instead of water. On the 1st, 7th, 21st and 42nd days of the regeneration phase, six rats from each subgroup were taken for blood and tissue analysis at each time point. The third group of animals (n = 40), denoted as RT+CCl_4, started to drink RT instead of water preventively one week before the first administration of CCl_4. Similarly, ten weeks after starting CCl_4 administration, 6 animals from the total of 30 animals, which survived the intoxication, were taken for blood and tissue analysis at the baseline 0 d. The rest, 24 animals, continued drinking RT until the end of the regeneration phase. The analyses were made at the same time points as in the CCl_4+RT subgroup.

Survival analysis

The loss of animals which drunk water during treatment with CCl_4 (CCl_4+H_2O), amounted to 24% and that in the regeneration phase another 8%. The loss in the subgroup of animals which drunk RT only in the regeneration phase (CCl_4+RT), was 4%. In the third group of animals, which preventively drunk RT (RT+CCl_4), the loss of animals during the intoxication with CCl_4 amounted to 25%, however, in the regeneration period it was zero per cent.

Tissue analysis

The rats were anesthetized with Morbital (64 mg/kg). Blood from aorta abdominalis was collected in heparinized tubes. Liver was removed and a part of right lobe was immediately fixed in 4% buffered formaldehyde solution for one day and then used for histological examination. Fixed liver tissue samples were embedded in paraffin, cut to 4 μm slices and stained routinely with hematoxylin and eosin. Modified picrosirius-red-staining technique described by Dolber and Spach (1993) was used to determine fibrosis. The sections were analyzed using a Leica DM 200 microscope (Leica Germany) equipped with polarization filters. The images were obtained under polarized light using 40 times magnification. The fibrosis extent was measured as a percentage of picrosirius-red-positive area in the microscopic field.

Other parts of the liver tissue were minced and homogenized in a chloroform-methanol mixture (1 : 1) for cholesterol determination, in a chloroform-methanol mixture (2 : 1) for triacylglycerol determination, and in 0.9% NaCl solution for the determination of malondialdehyde (MDA).

Biochemical analysis

Plasma activities of alanine aminotransferase (ALT), aspartate aminotranferase (AST), and concentrations of glucose, albumin and total bilirubin (tBil) were determined by standard automated techniques using of Hitachi Analyzer Model 911 and adequate kits from Roche Company (Switzerland). MDA content in the liver tissue was determined by HPLC method (Pilz et al. 2000), concentrations of cholesterol and triacylglycerols according to Abell et al. (1952) and to Jover (1963), respectively.

Statistics

All data obtained are presented as median and interquartile range (IQR: from lower quartile Q_1 (25%) to upper quartile Q_3 (75%)) if not otherwise stated. Non-parametric analysis of variance (Kruskal-Wallis test) and the post hoc all-pairwise comparisons (Conover-Inman test) were performed. Kaplan-Meier survival analysis was performed.

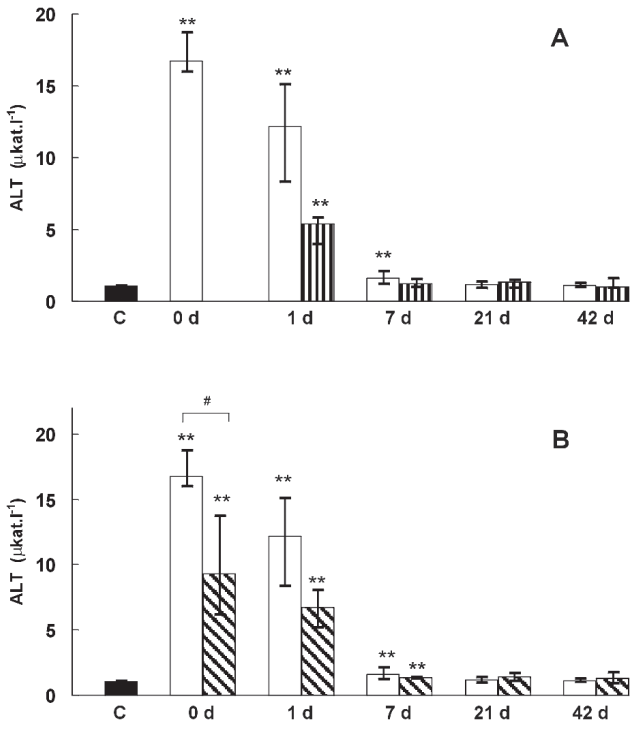
Results

The weight profiles among the all groups were not statistically different. Each group (except for control group) showed a significant decrease in the body weight during CCl_4 intoxication (10 weeks from starting intoxication) regardless of whether the animals drunk tea or water. From that point till the end of the experiment, the mean weights were gradually increasing in all groups.

Ten-week intoxication of rats with CCl_4 caused a significant increase in ALT (Fig. 1), AST (Fig. 2) activities, increase in the concentration of tBil in plasma (Table 1), as well as increase in the MDA, triacylglycerols and total cholesterol content in the liver tissue (Table 2). Together with observed decrease in plasma glucose and albumin (Table 1), all findings clearly demonstrated serious functional changes in the liver tissue due to CCl_4 intoxication. Morphological examinations of the tissue showed all signs of fibrosis leading to cirrhosis. The percentage of fibrotic area to the total field area was calculated. The extent of fibrosis/cirrhosis was estimated immediately after CCl_4 intoxication (0 day) and during the regeneration phase on the days 21 and 42 (Fig. 3). The group of animals which were preventively treated by RT one week before, during and after CCl_4 intoxication (RT+CCl_4) showed evidently lower extent of fibrosis/cirrhosis in comparison with non-treated group (CCl_4+H_2O) at the 0 day (Fig. 3). The RT+CCl_4 group had also significantly lower activities of ALT (Fig. 1), AST (Fig. 2) and tBil (Table 1), however, concentrations of glucose and albumin were not changed. In the liver tissue, concentration of MDA was significantly lower, while cholesterol and triacylglycerols were not changed at the 0 day (Table 2).

When the administration of CCl_4 was terminated (0 d), the injured liver tissue gradually began to recover. Seven days later, both treated groups – that treated by RT, before, during and after intoxication (RT+CCl_4) and that treated only during the regeneration phase (CCl_4+RT), exhibited significantly decreased activity of AST (Fig. 2) and trend for decrease in the activity of ALT (Fig. 1) when compared with the non-treated group CCl_4+H_2O. At the same time point the concentration of plasma albumin was elevated and tBil was decreased in the both groups drinking the tea (Table 1). Liver tissue MDA concentration was also decreased in both treated groups, in the RT+CCl_4 group significantly (Table 2).

On day 42 of the regeneration, all the biochemical parameters in all three experimental groups reached the corresponding levels found in the control healthy animals (C), regardless of whether the animals drunk RT or water. At that time, liver fibrosis in the group CCl_4+H_2O lasted, however, it was significantly less extended in comparison with the 0 d. Interestingly, the groups of



◄ **Figure 1.** The effects of rooibos tea (RT) on alanin aminotransferase (ALT) activity during liver regeneration after intoxication by carbon tetrachloride (CCl_4). **A**. Administration of RT during regeneration phase only. **B**. Administration of RT before and during CCl_4 intoxication as well as during regeneration phase. Data are presented as median (M) and interquartile range (lower quartile Q_1 – upper quartile Q_3). * $p < 0.05$, ** $p < 0.005$ – significantly different from the control group; # $p < 0.05$, ## $p < 0.005$ – significantly different from the non-treated group CCl_4+H_2O at given time. Each analyzed group contained 6 animals. ■ control group (C); □ CCl_4 + water (10 weeks), regeneration phase with water (42 days) (CCl_4+H_2O), ▥ CCl_4 + water (10 weeks), regeneration phase with rooibos tea (42 days) (CCl_4+RT); ▧ RT (1 week) before application of CCl_4+RT instead of water (10 weeks) and regeneration phase with RT (42 days) (RT+CCl_4).

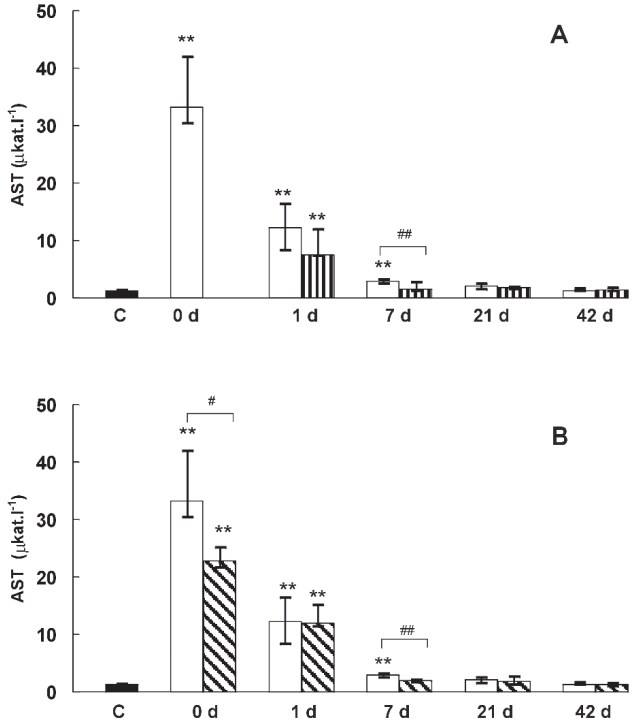


Figure 2. The effects of rooibos tea (RT) on aspartate aminotransferase (AST) activity during liver regeneration after intoxication by carbon tetrachloride (CCl_4). **A**. Administration of RT during regeneration phase only. **B**. Administration of RT before and during CCl_4 intoxication and also during regeneration phase. Labels and explanation as in Fig. 1.

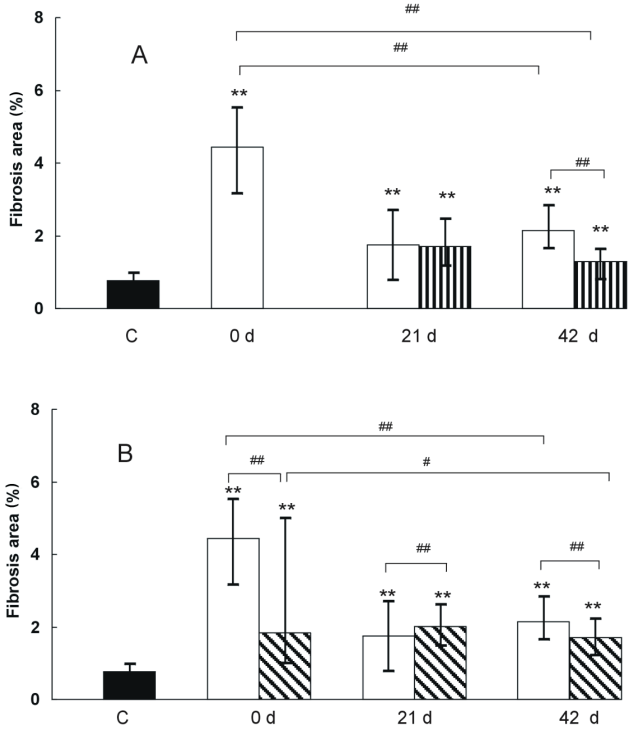


Figure 3. The effects of rooibos tea (RT) on extent of fibrosis area during liver regeneration after intoxication by carbon tetrachloride (CCl_4). **A**. Administration of RT during regeneration phase only. **B**. Administration of RT before and during CCl_4 intoxication and also during regeneration phase. Labels and explanation as in Fig. 1.

Table 1. The effects of rooibos tea (RT) on plasma glucose, albumin and total bilirubin of rats after carbon tetrachloride (CCl_4)-induced liver intoxication and during regeneration phase

	Glucose ($mmol \cdot l^{-1}$)	Albumin ($g \cdot l^{-1}$)	Total bilirubin ($\mu mol \cdot l^{-1}$)
Day	M (Q_1-Q_3)	M (Q_1-Q_3)	M (Q_1-Q_3)
Control	10.90 (10.58–11.54)	27.45 (26.53–28.15)	0.73 (0.44–1.21)
CCl_4+H_2O			
0	5.7 (5.5–5.94)**	20.25 (18.9–21.38)**	16.35 (9.3-21.18)**
1	7.97 (6.85–8.;91)**	22.05 (18.05–25.25)**	14.24 (3.93–7.98)**
7	7.87 (6.51–8.27)**	20.2 (19.13–21.2)**	5.87 (3.7–7.57)**
21	10.05 (9.69–10.81)	25.65 (24.8–26.28)*	1.8 (1.27–2.22)**
42	10.11 (10.05–10.62)	26.95 (26.4–27.2)	1.26 (0.55–1.59)
CCl_4+RT			
1	6.52 (5.63–6.55)**	18.6 (17.9–20.4)**	8.42 (7.58–11.47)**
7	9.79 (8.95–9.8)*	26.8 (26.7–27.3)	1.48 (1.3–2.76)** ##
21	10.49 (10.26–11.21)	27.85 (26.98–28.55) #	1.97 (1.79–2.07)**
42	10.22 (9.33–11.13)	26.35 (25.53–27.1)	0.84 (0.21–1.04)
RT+CCl_4			
0	5.19 (4.84–5.59)**	20.9 (20.45–21.35)**	9.83 (8.41–10.27)** #
1	6.44 (6.6–7.1)**	21.25 (18.8–22.95)**	10.54 (5.96–12.83)**
7	9.24 (9.1–10.69)*	27.7 (27.45–28.65)#	2.24 (2.05–2.45)** ##
21	9.44 (8.5–10.1)*	25.9 (23.25–26.98)*	1.36 (1.18–2.14)
42	10.71 (10.41–11.13)	28.75 (27.5–28.8)	0.91 (0.36–1.6)

Data are presented as median (M) and an interquartile range (lower quartile Q_1 – upper quartile Q_3); * $p < 0.05$, ** $p < 0.005$ – significantly different from the control group; # $p < 0.05$, ## $p < 0.005$ – significantly different from the non-treated group CCl_4+H_2O at given time. CCl_4+H_2O – CCl_4 + water (10 weeks), regeneration phase with water (42 days); CCl_4+RT – CCl_4 + water (10 weeks), regeneration phase with rooibos tea (42 days). RT+CCl_4 – RT (1 week) before application of CCl_4+RT instead of water (10 weeks) and regeneration phase with RT (42 days). The number of animals in each analyzed group was 6.

Table 2. The effects of rooibos tea (RT) on liver-tissue triacylglycerols, cholesterol and malondialdehyde of rats after carbon tetrachloride (CCl_4)-induced liver intoxication and during regeneration phase

	Triacylglycerols ($mmol \cdot kg^{-1}$)	Total cholesterol ($mmol \cdot kg^{-1}$)	Malondialdehyde ($\mu mol \cdot kg^{-1}$)
Day	M (Q_1-Q_3)	M (Q_1-Q_3)	M (Q_1-Q_3)
Control	13.47 (11.39–14.81)	5.60 (5.30–5.82)	91.11 (84.25–108.11)
CCl_4+H_2O			
0	44.29 (36.33–48.80)**	9.52 (8.36–10.07)**	125.24 (102.73–144.08)*
1	35.80 (33.58–40.12)**	8.37 (7.62–8.55)*	97.23 (91.66–103.90)
7	11.45 (9.71–12.61)	5.76 (5.56–5.81)	118.83 (105.14–122.39)*
21	5.02 (4.55–5.56)**	5.51 (4.87–6.04)	98.30 (81.16–116.34)
42	6.48 (5.99–7.04)**	5.56 (5.25–5.78)	97.17 (81.58–108.45)
CCl_4+RT			
1	21.55 (19.2–23.69)**	8.04 (6.25–8.20)*	107.43 (103.14–132.06)
7	16.98 (16.46–19.89)** ##	5.37 (4.84–5.61)	96.05 (90.60–112.87)
21	4.53 (4.27–5.62)**	5.92 (5.29–7.28)	133.50 (116.14–149.18)
42	5.54 (4.85–8.16)**	5.28 (5.0–5.46)	81.59 (75.24–106.26)
RT+CCl_4			
0	55.36 (28.61–60.78)**	11.65 (8.89–13.03)**	68.71 (65.72–82.50)##
1	36.06 (23.35–45.86)**	8.9 (8.59–12.50)**	67.08 (61.59–78.80)* #
7	26.40 (21.56-27.28)** ##	8.21 (7.94–8.86)** ##	90.10 (80.90–102.87)#
21	6.71 (5.84–8.46)**	5.27 (4.74–5.93)	84.40 (73.08–93.72)
42	6.07 (5.15–6.40)**	5.09 (4.73–5.35)* #	85.75 (75.13–100.65)

Legend: see Table 1.

animals, which drunk RT had the extent of fibrotic tissue significantly lower than the group which drunk water only. Nevertheless, the extent of cirrhosis/fibrosis in all three groups was significantly higher when compared with C group (Fig. 3).

Discussion

It is believed that liver cell membranes damage caused by reactive oxygen species is the predominant mechanism of hepatotoxicity of a various origin. Therefore, most of a wide scale of natural and also artificial compounds with an antioxidant and radicals scavenging activity have hepatoprotective properties. However, studies on the effect of antioxidants on liver regeneration processes are rather limited, moreover, they are often with equivocal or contradictory results due to various experimental designs (e.g. Vitaglione et al. 2004; Zhang et al. 2006). Assuming possible therapeutic significance of such compounds, it is rather surprising that investigation of at least natural antioxidants has not attracted more attention of the researchers. We suppose that it could be caused by the facts that liver regeneration is a complex process which starts immediately after injury, therefore, it is difficult to distinguish this naturally running process from hepatoprotective effect of an antioxidant (Nakata et al. 1985; Das et al. 2000; Ferenčíková et al. 2003; Medina and Moreno-Otero 2005).

In our work we did not focus on specifying which part from the observed regeneration could be attributed to RT. Our objective was to verify whether regular use of RT as a beverage could have a positive effect on the process of liver regeneration after intoxication. We stated the presence of positive effect when we found a significant, time-dependent declination of the monitored parameters from those in the non-treated group. Our results pointed to a positive effect of RT on the biochemical parameters, activity of ALT and AST as well as on the concentration of bilirubin, which are typically worsened in cirrhosis. We confirmed our previous findings that animals, which drink RT instead of water before and during liver intoxication by CCl_4, have lover extent of fibrosis/cirrhosis and lover level of MDA in the liver tissue after intoxication (Uličná et al. 2003).

In the present study we found an evidence of that drinking RT prevented the liver from large-scale fibrosis. The group which drunk RT before, during and after liver intoxication by CCl_4 (RT+CCl_4) and also the group which drunk the tea only from the beginning of liver regeneration period (CCl_4+RT) showed lower extent of liver cirrhosis when compared to the non-treated group CCl_4+H_2O at the end of the regeneration phase. Nevertheless, recovery was not completed yet and we still found statistically significant differences in the extent of cirrhosis/fibrosis in both treated groups when compared with healthy control animals (C). Hepatic fibrogenesis is an intricate process, which involves increasing oxidative stress, cytochrome P450 induction (Castillo et al. 1992), depletion of antioxidant defenses, lipid peroxidation, generation of aldehydic products, the effects of mitogenic and fibrogenic cytokines (Friedman et al. 1993). Oxidative stress could represent a common link between the different types of chronic liver injury and hepatic fibrosis (Baroni et al. 1998). Using experimental model with CCl_4, we induced oxidative stress which has been shown to stimulate fibroblasts (Murrel et al. 1990), stellate cells (fat-storing cells, Ito cells or hepatic lipocytes) proliferation (Lee et al. 1995) and synthesis of collagen (Parola et al. 1993). Hepatic stellate cells are a major source of extracellular matrix in normal and pathological conditions (Friedman et al. 1985, 1993), which is reflected in the extent of fibrosis (per cent of fibrosis area in Fig. 3).

A link between lipid peroxidation and fibrogenesis was demonstrated *in vivo* by evidence that antioxidant supplementation was also able to depress, to a significant extents, procollagen α1 (I) mRNA overexpression (Parola et al. 1992b) and collagen deposition in fibrotic livers (Mourelle et al. 1989; Parola et al. 1992a), two proven consequences of chronic CCl_4 administration (Parola et al. 1992a; Pierce et al. 1987). We assume that effects of the RT were in the reducing oxidative stress which subsequently depress the extent of fibrosis area and inhibiting formation of collagen fibers.

Oxidative stress arising from long-term application of CCl_4 could cause, first of all, damage to the membranes of hepatocytes and mitochondria, as evidenced by increased activities of ALT and AST enzymes in plasma, which are the most sensitive and early indicators of membrane-integrity perturbations. More pronounced increase in activity of AST at already elevated activity of ALT pointed to the presence not only the cytoplasmic but also the mitochondrial isoenzyme – a sign of necrosis. The rise in oxidation of both, cellular and subcellular membranes in hepatocytes was confirmed also by elevated concentration of MDA in the hepatic tissue. Monitoring of the biochemical parameters during the regeneration phase showed that the activities of ALT and AST were significantly lover in both treated groups RT+CCl_4 and CCl_4+RT already on the 1st and 7th day of the period, when compared to the non-treated group. Similarly, the concentration of tBil was significantly lover in the both groups treated with the tea when compared to the non-treated group on the 7th day of the regeneration phase. At the end of the experiment, on the 42nd day, all monitored biochemical parameters in all three experimental groups reached the control levels. These results indicate that antioxidant and scavenger activities of the compounds, which are present in RT, caused inhibition of oxidative stress during the chronic CCl_4 intoxication of the animals. Decrease in both, the activities of transaminases and the concentration of MDA after RT administration pointed to the preventive

effects of the tea on the membrane-bound processes such as oxidative phosphorylation, proteosynthesis, etc.

It was plausible that lipid peroxidation and necrosis were inhibited by antioxidants such as flavonoid silymarin and vitamin E (Mourelle et al. 1989; Parola et al. 1992a). Reported hepatoprotective effect of silymarin is mediated, first of all, by stabilization of cellular and mitochondrial membranes (Abrol et al. 2005). Proper functioning of the membranes is a prerequisite for undisturbed metabolic, detoxification and synthetic functions of the liver.

The present work is complementary to our previously reported findings on hepatoprotective effect of RT (Uličná et al. 2003). Our results show that administration of the tea, besides already observed hepatoprotection, also positively influenced the biochemical parameters during the liver-regeneration process, particularly in the early stage of regeneration. It is known that regeneration of the liver is an endergonic process which demands availability of energy generation and delivery of free energy in the form of ATP. The energy is needed for synthetic processes which secure reparation or replacement of damaged tissue. For successful protein biosynthesis, a rapid expression of genes in the nucleus and subsequent translation in the cytoplasma are required. All these processes increase energy demands, however, enhanced mitochondrial functions will also enhance basal electron leak and subsequent reactive oxygen species generation, which may retard the regeneration of liver tissue. Antioxidants contained in the RT might accelerate the regeneration process by scavenging reactive oxygen species and stabilizing the cellular and mitochondrial membranes, thus preserving their integrity and function.

As the effects were more pronounced in the animals which drunk the RT already before intoxication and continued drinking the tea during the time course of the experiment, it seems reasonable to conclude that regular drinking the RT can be recommended as a convenient alternative or supplement to commercially available antioxidants.

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