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Hepatoprotective Effect of Rooibos Tea (*Aspalathus linearis*) on CCl_4-Induced Liver Damage in Rats

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Summary

Hepatoprotective properties of rooibos tea (*Aspalathus linearis*) were investigated in a rat model of liver injury induced by carbon tetrachloride (CCl_4). Rooibos tea, like N-acetyl-L-cysteine which was used for the comparison, showed histological regression of steatosis and cirrhosis in the liver tissue with a significant inhibition of the increase of liver tissue concentrations of malondialdehyde, triacylglycerols and cholesterol. Simultaneously, rooibos tea significantly suppressed mainly the increase in plasma activities of aminotransferases (ALT, AST), alkaline phosphatase and billirubin concentrations, which are considered as markers of liver functional state. The antifibrotic effect in the experimental model of hepatic cirrhosis of rats suggests the use of rooibos tea as a plant hepatoprotector in the diet of patients with hepatopathies.

Key words

Rooibos tea • *Aspalathus linearis* • N-acetyl-L-cysteine • Hepatoprotective activity • CCl_4-induced cirrhosis.

Introduction

It is well known that a substantial increase in steatosis and fibrosis usually leads to potentially lethal cirrhosis of the liver in humans. The high global prevalence of these hepatopathies place them among the most serious diseases. Although the pathogenesis of liver fibrosis is not quite clear, there is no doubt that reactive oxygen species (ROS) play an important role in pathological changes in the liver, particularly in cases of alcoholic and toxic liver diseases (Poli and Parola 1997). Biological membranes are particularly prone to the ROS effect. The peroxidation of unsaturated fatty acids in biological membranes leads to a decrease of membrane fluidity and to a disruption of membrane integrity and function, which is implicated in serious pathological changes (Halliwell 1987). Several endogenous protective mechanisms have been evolved to limit ROS and the damage caused by them (Sies 1993). However, since this protection may not be complete, or when the formation of

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ROS is excessive, additional protective mechanisms of dietary antioxidants may be of a great importance. Therefore, many natural and artificial agents possessing antioxidative properties have been proposed to prevent and treat hepatopathies induced by oxidative stress (Lieber 1997, Červinková and Drahota 1998). There is increasing evidence for the hepatoprotective role of hydroxy- and polyhydroxy-organic compounds – particularly from vegetables, fruits and some herbs (Bass 1999). Especially in humans, different kinds of tea are amongst the most popular non-alcoholic beverages that contain a wide range of various natural antioxidants (Dreosti 1996, Cao *et al.* 1996, Kohlmeier *et al.* 1997).

Rooibos tea (*Aspalathus linearis*) (afr. rooi – red; bos – bush), indigenous to South Africa, has been shown to exhibit a remarkable antioxidant and superoxide anion scavenging activity (Yoshikawa *et al.* 1990, Nakano and Mizuno 1993) as it contains a wide range of phenolic compounds such as flavonoids, chalkones and hydroxylated fatty acids, whereas it contains no alkaloids (Rabe *et al.* 1994). Therefore, it may be anticipated that rooibos tea would have health promoting properties in humans.

The purpose of this study was to investigate the hepatoprotective effect of an aqueous extract of rooibos tea on cirrhosis development by CCl_4 intoxication in rats. For comparison we used N-acetyl-L-cysteine which has known therapeutic effects linked to the antioxidant and free radical scavenging action, and is commonly used as an antidote against drug-induced hepatopathies (Smilkstein *et al.* 1988).

Methods

Chemicals

All chemicals used were of analytical grade purity and were purchased mostly from Centralchem Bratislava, but N-acetyl-L-cysteine, malondialdehyde, cholesterol, 2,4-dinitrophenylhydrazine were obtained from Merck and tetraethoxypropane from Fluka.

Plant material

Commercial, best quality rooibos tea (*Aspalathus linearis*) was kindly provided by Rooibos World Co. (Nagoya, Japan). The aqueous extract of the tea was prepared daily by boiling 5 g dry tea in 2000 ml water for 10 min with subsequent standing for 20 min and cooling down to room temperature. After separation of undissolved residue, the solution was used for experiments.

Animals

Male Wistar rats (210-280 g) were maintained in a 12 h light/dark cycle at a constant temperature 25 ^{o}C with free access to standard Larsen pellet food and tap water. All experiments were carried out according to the guidelines for the care and use of experimental animals and approved by the State Veterinary Administration of the Slovak Republic.

Experimental procedure

Animals were assigned to one of four groups each of 10 rats. The first group was not treated with CCl_4 and served as a control (C). Three other groups were treated with i) tetrachloromethane (CCl_4), ii) tetrachloromethane and rooibos tea (CCl_4+RT), and iii) tetrachloromethane and N-acetyl-L-cysteine (CCl_4+NAC). Except of the control group, CCl_4 was applied intraperitoneally as a 50 % solution in olive oil (1 ml/kg) to all the other animals twice a week for 10 weeks. The rats of the control group were injected with olive oil (1 ml/kg, i.p.) at the same time. The rats in the third group (CCl_4+RT) had free access to rooibos tea solution instead of tap water, starting 7 days before CCl_4 administration. These animals were also given 5 ml/kg of rooibos tea once a day using a gavage technique. The average daily consumption of the tea was 32 ml per animal (28-34 ml). At the same time, animals of the control group and CCl_4-group received 5 ml/kg water in the same route. The last group of animals (CCl_4+NAC) received 150 mg/kg N-acetyl-L-cysteine in solution instead of rooibos tea by the same oral technique as above mentioned, starting 7 days before CCl_4 administration. Ten weeks after CCl_4 administration started, and 48 hours after the last treatment with CCl_4, the rats were anesthetized with thiopental and blood samples were taken into heparinized tubes from abdominal aorta.

The livers were removed and a part of the right lobe were sliced, fixed in 10 % buffered formaldehyde solution and used for histological examination. Liver tissue was processed by the paraffin slice technique and sections were stained with hematoxylin and eosin according to the commonly used van Gieson's method. The stained slices of liver tissue were subjected to the automated morphometric evaluation of steatosis and fibrosis. For this evaluation a method according to Babál *et al.* (1997) was used. TELEMET II (Tesla Piešťany, Slovakia) and special morphometry computer with CCD TV camera attached to microscope were used. This

equipment and its program were set in such a way that the tissue lipid infiltration characteristic for the normal physiological state was not registered. The results are expressed as the relative area showing steatosis or fibrosis. Other parts of the livers were minced in a beaker with a pair of scissors and used for analysis.

Samples of liver tissues for analysis were homogenized in a chloroform-methanol mixture (1:1) for cholesterol determination, in a chloroform-methanol mixture (2:1) for triacylglycerols determination and in physiological solution for the determination of malondialdehyde.

Biochemical analysis

Activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and concentrations of albumin, total proteins, cholesterol, triacylglycerols, glucose, urea, bilirubin and creatinine in plasma were determined by standard automated techniques using of Hitachi Analyzer Model 911 and the adequate kits from Roche Company (Switzerland). 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).

Statistics

The data are expressed as mean ± SEM and statistical analysis was performed using analysis of variance followed by Student's t-test with $P<0.05$ being considered as statistically significant.

Results

The long-term intraperitoneal administration of CCl_4 to experimental animals induced, as expected, pathological changes in the liver. Histomorphological and histometrical examination of the tissue showed steatosis with marked accumulation of lipid droplets and fibrosis which were both significant ($p<0.001$). These characteristics were not seen in animals to which rooibos tea (CCl_4+RT) or N-acetylcysteine (CCl_4+NAC) were administered (Fig. 1). While rooibos tea lowered the development of CCl_4-induced steatosis to 73 % and N-acetylcysteine to 20 % of that of CCl_4-treated animals, both of these antioxidants remarkably protected liver tissue from CCl_4-induced fibrosis. This finding correlated with markedly increased plasma aminotransferases and alkaline phosphatase activities, (used for assessing liver function) in rats treated with CCl_4 only (Fig. 2) and with significantly lower activities of these enzymes in animals additionally treated with rooibos tea or N-acetylcysteine. In the case of ALT and AST, N-acetyl-L-cysteine was more effective than rooibos tea, while in the case of alkaline phosphatase the effects of N-acetyl-L-cysteine and rooibos tea were similar. As seen from Figure 3, cirrhotic rats exhibited a decrease in plasma albumin and a remarkably high level of total billirubin. Both rooibos tea and N-acetyl-L-cysteine significantly inhibited the production of billirubin in CCl_4-treated rats (77 and 78 % of inhibition, respectively), while their effect on plasma albumin increase was rather weak, if any.

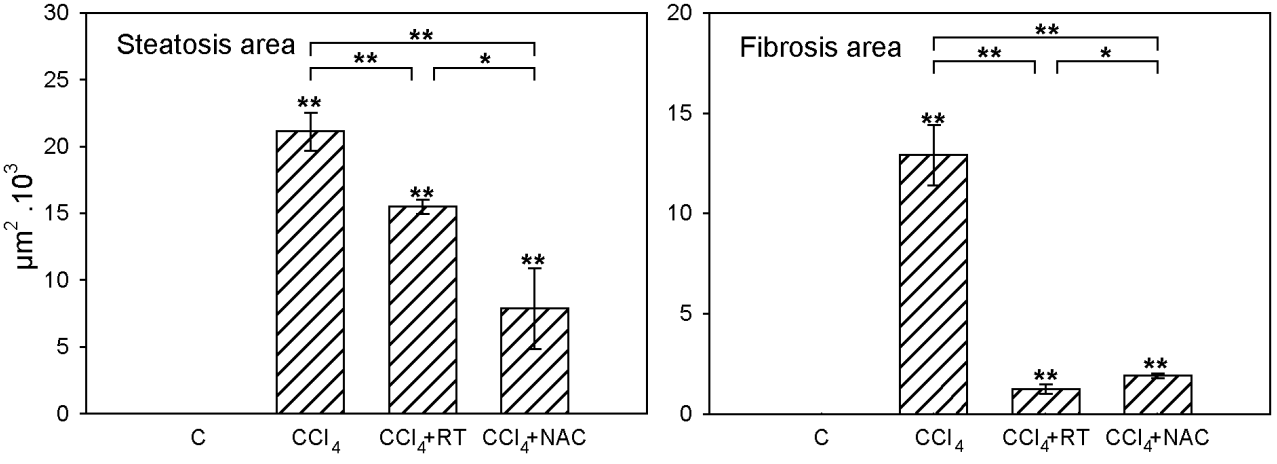

Fig. 1. *Effect of rooibos tea and N-acetyl-L-cysteine on CCl_4-induced liver steatosis and fibrosis in rats. Statistical significance *p<0.05, **p<0.001*

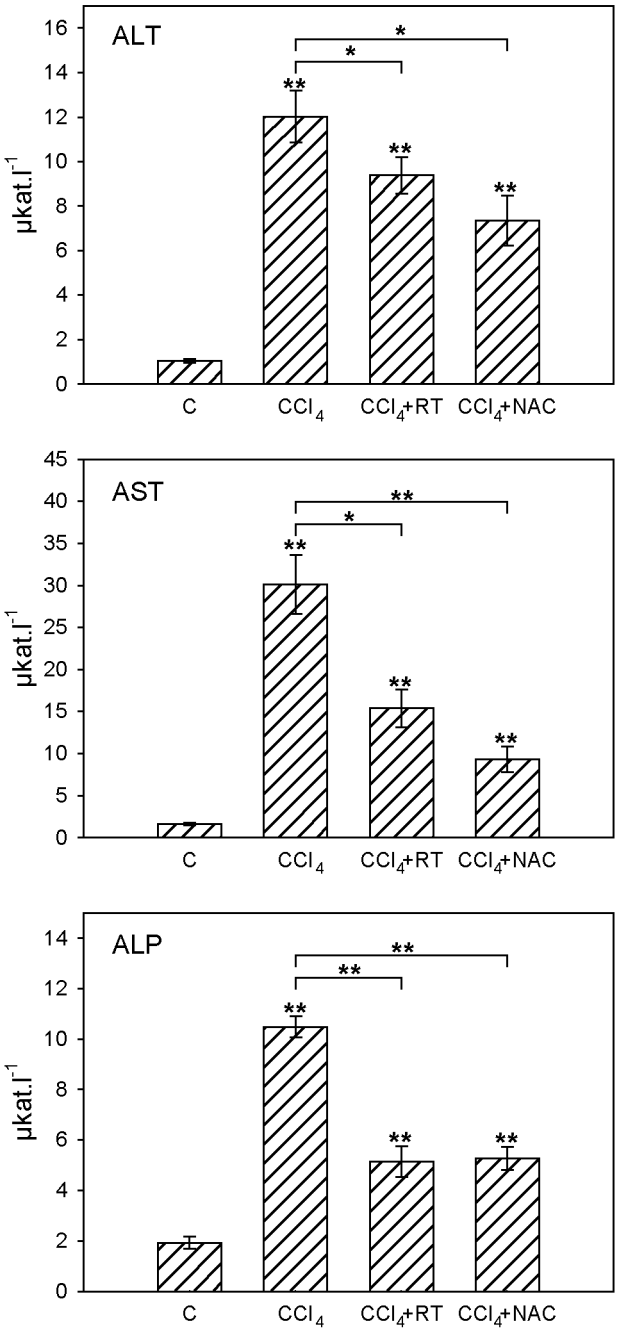

Fig. 2. *Effect of rooibos tea and N-acetyl-L-cysteine on plasma activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in CCl_4-induced cirrhosis in rats. Statistical significance *p<0.05, **p<0.001*

The other blood biochemical parameters examined in these experiments are shown in Table 1. CCl_4-treated rats exhibited a significant decrease in plasma concentrations of glucose, triacylglycerols, cholesterol and creatinine. It is evident that RT and NAC improved some of these parameters but not to the control values.

CCl_4 treatment of rats also caused a severe increase of hepatic triacylglycerols, total cholesterol and malondialdehyde (Fig. 4). *R*ooibos tea and N-acetylcysteine completely protected the formation of malondialdehyde in the liver tissue of CCl_4-treated rats, lowered triacylglycerol concentration by 17 % (CCl_4+RT) and 35 % (CCl_4+NAC). Rooibos tea did not affect total cholesterol concentration (CCl_4+RT), and N-acetylcysteine lowered its concentration by 14 % only (CCl_4+NAC).

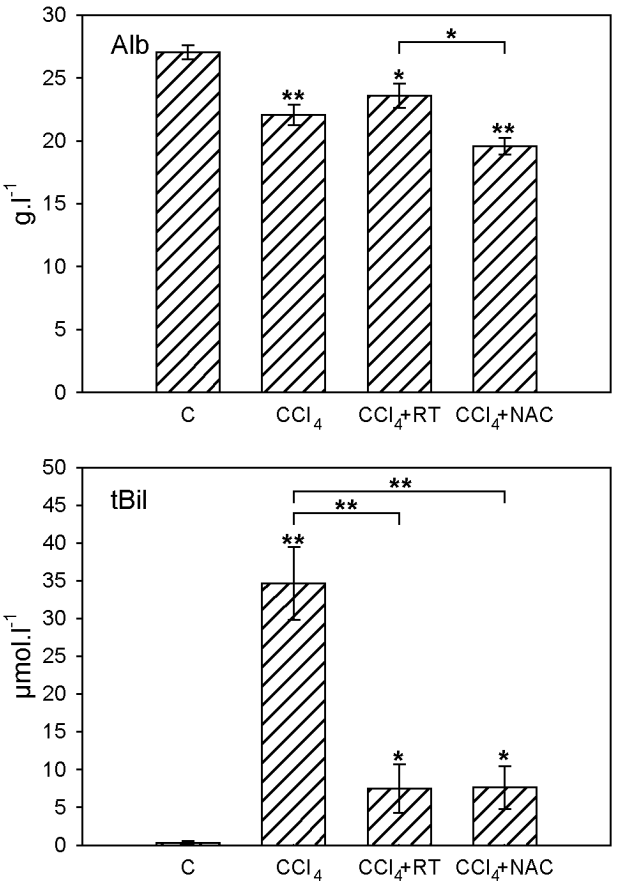

Fig. 3. *Effect of rooibos tea and N-acetyl-L-cysteine on plasma concentration of albumin and total billirubin in CCl_4-induced cirrhosis in rats. Statistical significance *p<0.05, **p<0.001.*

Table 1. The effect of rooibos tea and N-acetyl-L-cysteine on plasma biochemical parameters of rats with CCl_4-induced liver cirrhosis.

Parameter	C (n=10)	CCl_4 (n=10)	CCl_4+RT (n=10)	CCl_4+NAC (n=10)
Total protein (g/l)	63.16±1.00	61.23±1.32	61.00±1.46	57.94±0.72*
Glucose (mmol/l)	9.87±0.58	4.62±0.37*	5.04±0.41*	5.85±0.25* #
Triacylglycerols (mmol/l)	0.49±0.07	0.11±0.01*	0.18±0.02* #	0.16±0.02*
Cholesterol (mmol/l)	0.98±0.04	0.72±0.05*	0.57±0.02* #	0.71±0.06* +
Urea (mmol/l)	8.99±0.31	8.39±0.39	11.16±0.4* ##	9.57±0.57+
Creatinine (µmol/l)	66.18±2.95	51.81±1.49*	50.66±2.75*	43.65±1.92* #

*Data are mean ± SEM. * Significantly different from controls ($p<0.001$), # Significantly different from CCl_4 (# $p<0.05$, ## $p<0.001$), + Significantly different from CCl_4 +RT ($p< 0.05$).*

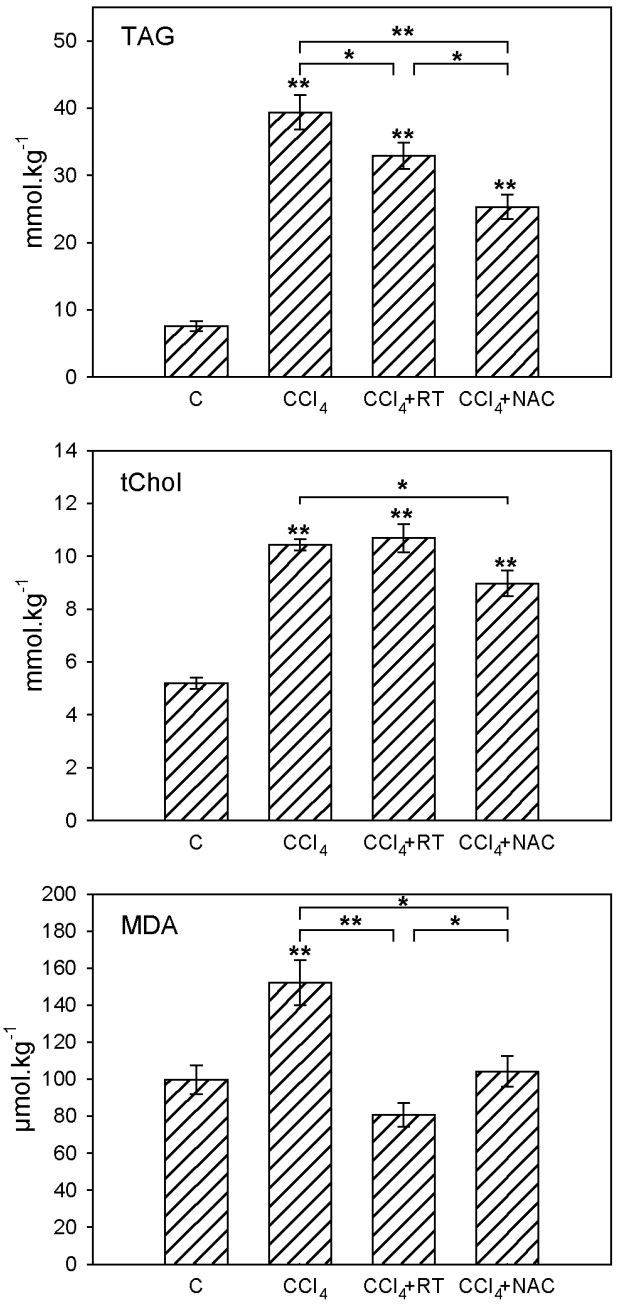

Fig. 4. *Effect or rooibos tea and N-acetyl-L-cysteine on concentration of triacylglycerols, total cholesterol and malondioaldehyde in liver tissue of rats with CCl_4-induced cirrhosis. Statistical significance *p<0.05, **p<0.001.*

Discussion

In recent years many studies have shown that various types of tea have a wide range of physiological, biochemical and pharmacological effects due to the properties of their constituents (Trevisanato and Kim 2000). In particular, they contain many types of polyhydroxy-compounds which can function as natural antioxidants in humans and animals. In contrast to teas derived from *Camelia sinensis*, rooibos tea does not contain caffeine or any other alkaloides which are often considered as the most physiologically effective constituents of teas (Puming *et al.* 2001). However, as mentioned above, rooibos tea does contain a variety of compounds with antioxidative and reactive oxygen species scavenging potency (Rabe *et al.* 1994).

To study the hepatoprotective effect of rooibos tea we used the well-described CCl_4-model of rat liver fibrosis (Wu and Norton 1996), in which the liver microsomal oxidizing systems connected with cytochrome *P*-450 produce reactive metabolites of CCl_4 such as trichloromethyl radical ($CCl_3^{\bullet}$) or trichloroperoxyl radical ($CCl_3O_3^{\bullet}$). These radicals cause lipid peroxidation which produces hepatocellular damage and enhanced production of fibrotic tissue. In line with this assumption our study revealed that simultaneous long-term *ad libitum* administration of rooibos tea (in a concentration commonly used as beverage by humans) to CCl_4-treated rats protected them from the liver damage. This hepatoprotective effect of rooibos tea was found to be similar to that observed in the group of rats long-term

treated with N-acetyl-L-cysteine. While the hepatoprotective properties of N-acetyl-L-cysteine have been reported in several studies (Smilkstein *et al.* 1988, Dobrzynska *et al.* 2000), the anticirrhotic effect of rooibos tea has not been previously shown. Since the model of CCl_4-induced hepatic fibrosis in the rat simulates many of the features of human liver fibrosis, we suggest that natural antioxidants and scavenging agents in rooibos tea might be the effective plant hepatoprotectors in the diets of patients with hepatopathies.

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