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Rooibos Tea (*Aspalathus linearis*) Partially Prevents Oxidative Stress in Streptozotocin-Induced Diabetic Rats

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Summary

The aim of this study was to investigate the effects of rooibos tea as a natural source of a wide scale of antioxidants on the prevention and treatment of oxidative stress in streptozotocin-induced diabetic rats. Expected significant changes of biochemical parameters characteristic for experimental diabetic state were found in plasma and tissues eight weeks after single dose streptozotocin application. Administration of aqueous and alkaline extracts of rooibos tea (or N-acetyl-L-cysteine for comparison) to diabetic rats did not affect markers of the diabetic status (glucose, glycated hemoglobin and fructosamine). Besides the parameters characterizing hepatotoxic effect of streptozotocin, rooibos tea significantly lowered advanced glycation end-products (AGEs) and malondialdehyde (MDA) in the plasma and in different tissues of diabetic rats, particularly MDA concentration in the lens. From these results we can conclude that antioxidant compounds in rooibos tea partially prevent oxidative stress and they are effective in both hydrophobic and hydrophilic biological systems. Therefore, rooibos tea as a commonly used beverage can be recommended as an excellent adjuvant support for the prevention and therapy of diabetic vascular complications, particularly for protecting ocular membrane systems against their peroxidation by reactive oxygen species.

Key words
Rooibos tea • *Aspalathus linearis* • Oxidative stress • Streptozotocin-induced diabetes

Introduction

Many current studies indicated that elevated oxidation of glucose leads to increased production of free radicals and other reactive oxygen species (ROS) as H_2O_2, organic peroxides and also singlet oxygen, which are believed together with glycation (Bucala and Cerami 1992) to be the main causes of a wide scale of diabetic complications such as nephropathy, diabetic cataract and retinopathy, neuropathy, cardiovascular diseases and

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others (West 2000, Bayraktutan 2002). In such a case of excessive production of ROS, endogenous protective mechanisms may not be sufficient to limit ROS and the damage they cause (Sies 1993). As additional mechanisms of dietary antioxidants may be of great importance, many artificial and natural agents possessing antioxidative and radical scavenging properties have been proposed to prevent and to treat oxidative damage induced by ROS developed pathological states (Martinez-Cayuela 1995, Furst 1996, Kucharská *et al.* 2004).

As mentioned above, along with a wide scale of artificially prepared antioxidants, numerous naturally occurring plants and fruits, containing compounds with antioxidative and radical scavenging properties, have been studied (Lampe 1999) for the purpose of preventing oxidative stress of different etiology. This is due to several advantages they possess, such as low or no toxicity, a wide scale of different antioxidants which they contain, covering dismutation and trapping of most or all types of reactive oxygen species, easy accessibility etc. Most popular among them are different kinds of herbal tea widely used as non-alcoholic beverages (Benzie and Szeto 1999, Trevisanato and Kim 2000).

Rooibos tea originates from leaves and fine stems of the indigenous South African plant *Aspalathus linearis.* Due to its rich content of different compounds with antioxidative properties (Bramati *et al.* 2002, 2003) recently gained much attention because of its potential use for clinical purposes (Hesseling and Joubert 1982, Inanami *et al.* 1995, Nakano *et al.* 1997a,b, Uličná *et al.* 2003, Marnewick *et al.* 2003). Therefore, it seems to be of interest to elucidate whether rooibos tea, in a commonly used concentration as beverage for humans, might have a positive effect on the oxidative stress coupled to the diabetic state.

The purpose of this study was to investigate the effect of aqueous and alkaline extracts of rooibos tea on streptozotocin-induced diabetes in rats. For comparison, we used N-acetyl-L-cysteine which has therapeutic effects linked to the antioxidative and free radical scavenging action (Straface *et al.* 2002), and is also commonly used as an antidote against drug-induced intoxication of the organism.

Methods

Chemicals

All chemicals used were of analytical grade purity and were purchased mostly from Centralchem Bratislava, with the excetion of N-acetyl-L-cysteine and malondialdehyde-tetrabutyl acetate were obtained from Merck and streptozotocin from Sigma.

Plant material

Commercial best quality (black – fermented) rooibos tea (*Aspalathus linearis*) was kindly provided by Rooibos World Co. (Nagoya, Japan). The aqueous extract of the tea (RT) was prepared daily by boiling 2.5 g dry tea in 1000 ml water for 10 min with subsequent standing for 20 min and cooling down to room temperature. After separation of insoluble residue, the solution was used for the experiments. The alkaline extract of rooibos tea (AERT) was prepared using of 1 % sodium carbonate or 1 % sodium hydroxide according Nakano *et al.* (1996, 1997b) procedure and provided by Tokyo Food Techno Co., Ltd. (Japan).

Animals

Male Wistar rats (290-340 g) were maintained under 12 h light/dark cycle at a constant temperature of 25 ^{o}C with free access to standard Larsen pellet food and tap water, unless otherwise indicated. 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 five groups of 10 rats each. The first group was not treated with streptozotocin and served as a control (C). Four other groups were treated with a) streptozotocin (STZ), b) streptozotocin and rooibos tea (STZ+RT), c) streptozotocin and alkaline extract of rooibos tea (STZ+AERT), d) streptozotocin and N-acetyl-L-cysteine (STZ+NAC). Streptozotocin was applied as the single dose administration (45 mg/kg in 0.5 mol.l^{-1} citrate buffer pH 4.5) into the tail vein seven days after starting of rooibos tea, its alkaline extract and N-acetyl-L-cysteine administration. Rats of the control group (C) received an injection of the above mentioned citrate buffer at the same time. The rats in the third group (STZ+RT) had free access to rooibos tea solution instead of tap water, starting seven days before STZ administration. These animals were also given 5 ml/kg of rooibos tea once a day using the gavage technique. The same technique was used for administration of an alkaline extract of rooibos tea (300 mg/kg of body weight) dissolved in water to the rats of the fourth group, which did not receive rooibos tea

instead of tap water (STZ+AERT). The last group of animals (STZ+NAC), drinking tap water, received 150 mg/kg N-acetyl-L-cysteine in solution instead of rooibos tea by the same oral technique as above mentioned, starting seven days before STZ administration. With respect to stress conditions while the gavage technique was applied to animals in STZ+AERT and STZ+NAC groups, C group with water and STZ+RT group with rooibos tea were treated by the same gavage technique. Consumption of liquid drunk by rats in each group was measured twice a week. The average volume of consumed liquid represented 35 ml/day in the case of control group and 145 ml/day for all streptozotocin-treated groups.

Eight weeks after streptozotocin administration to experimental animals and 24 h after the last administration of rooibos tea, its alkaline extract and N-acetyl-L-cysteine the rats were anesthetized with Morbital (64 mg/kg) obtained from Biowet Pulawy (Poland). Blood samples from aorta abdominalis were collected into heparinized tubes. Liver, kidney and lens were removed and a part of these tissues was immediately deeply frozen. An other part of these tissues was minced and homogenized in the physiological solution for the determination of malondialdehyde.

Biochemical analysis

Plasma activities of aminotransferases (ALT, AST), alkaline phosphatase (ALP) and concentration of glucose, creatinine, albumin, total proteins, total cholesterol, triacylglycerols, uric acid and urea were determined by a standard automated technique using Hitachi Analyzer Model 911 and adequate kits from Roche Company (Switzerland). Glycated hemoglobin in the blood was estimated according to Flückinger and Winterhalter (1976), fructosamine according to Johnson *et al.* (1982), advanced glycation end-products (AGEs) according to Münch *et al.* (1997) and advanced oxidation protein products (AOPPs) according to Witko-Sarsat *et al.* (1996). Malondialdehyde in the plasma, lens, liver and kidney tissue was determined by HPLC (Pilz *et al.* 2000).

Statistics

The data are expressed as means ± S.E.M. and statistical analysis was performed using analysis of variance followed by Student`s t-test with $P<0.05$ being considered as statistically significant.

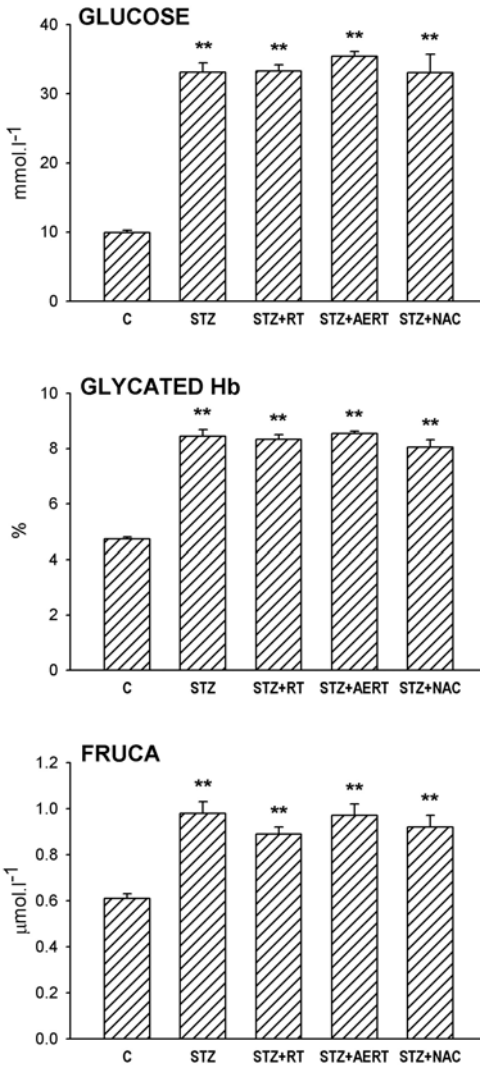

Fig. 1. Status of plasma glucose, glycated hemoglobin and fructosamine (FRUCA) after administration of aqueous (RT) and alkaline (AERT) rooibos tea extracts and N-acetyl-L-cysteine (NAC) in streptozotocin-induced (STZ) diabetic rats. C – control. Data are mean ± S.E.M. Statistical significance *$p<0.05$, **$p<0.001$

Results

As expected, a significant increase in blood glucose ($p<0.001$), glycated hemoglobin ($p<0.001$), fructosamine ($p<0.001$) (Fig. 1), enhanced glycation end-products ($p<0.001$), increased oxidation protein product levels ($p<0.001$) (Fig. 2), plasma triacylglycerols ($p<0.01$), total cholesterol ($p<0.001$), creatinine concentrations ($p<0.001$) together with higher activities of alanine aminotrasferase (ALT) ($p<0.001$), aspartate aminotransferase (AST) ($p<0.05$) and alkaline phosphatase (Table 1) were found in streptozotocin-induced diabetic rats (STZ).

Rooibos tea (STZ+RT), its alkaline extract (STZ+AERT) and N-acetyl-L-cysteine used for comparison (STZ+NAC), did not significantly affect glucose, glycated hemoglobin and fructosamine levels, while AGEs (expressed as arbitrary units) were significantly lowered and AOPPs were slightly decreased. Plasma concentration of triacylglycerols, total cholesterol, urea and activity of ALP and aminotransferases ALT, AST were only slightly lowered, however, a significant decrease occurred in the concentration of creatinine in animals taking rooibos tea, its alkaline extract and N-acetyl-L-cysteine when compared to diabetic rats (STZ). A marked but non-significant decrease in the concentration of uric acid, below the control (C) and diabetic rats (STZ), was observed in the case of animals given the above mentioned antioxidants (Table 1). Plasma albumin, total proteins did not show differences when antioxidants were administered.

At the end of the eight weeks lasting experiment, both plasma and tissue (liver, kidney, lens) malondialdehyde (MDA) levels were significantly higher in diabetic animals (STZ) than those in the control (C) group (Fig. 3). Administration of rooibos tea (STZ+RT), its alkaline extract and N-acetyl-L-cysteine significantly decreased MDA in the plasma and lens. MDA in the liver decreased only in the case of rooibos tea administration and in the kidney only after administration of N-acetyl-cysteine. It is interesting that all of these antioxidants reduced MDA levels more efficiently in case of the lens and plasma, while in all cases, excluding kidney tissue, rooibos tea or its alkaline extract were more effective than N-acetyl-L-cysteine. In all cases (STZ+RT, STZ+AERT and STZ+NAC), the level of malondialdehyde did not decrease to its concentrations in the control group.

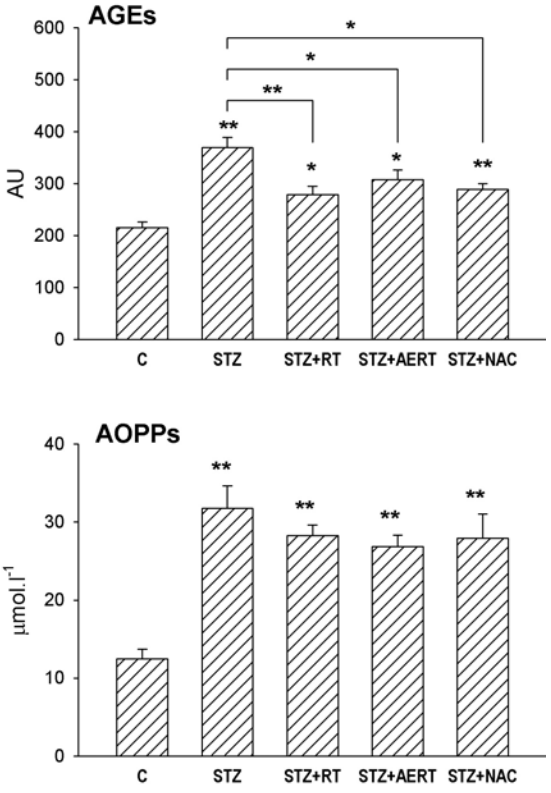

Fig. 2. Effect of aqueous (RT) and alkaline (AERT) extracts of rooibos tea and N-acetyl-L-cysteine (NAC) on the plasma level of advanced glycation end products (AGEs in arbitrary units) and advanced oxidation protein products (AOPPs) of streptozotocin-induced (STZ) diabetic rats. C – control. Data are mean ± S.E.M. Statistical significance * $p<0.05$, ** $p<0.001$

Table 1. Plasma parameter values of streptozotocin-induced diabetic rats (STZ) treated with aqueous (STZ+RT) and alkaline extracts (STZ+AERT) of rooibos tea and with N-acetyl-L-cysteine (STZ+NAC). C – control.

Parameter	C (n=10)	STZ (n=9)	STZ+RT (n=10)	STZ+AERT (n=9)	STZ+NAC (n=9)
Triacylglycerols (mmol/l)	0.66±0.09	1.29±0.19*	0.97±0.11*	1.04±0.06**	1.13±0.13*
Total cholesterol (mmol/l)	1.14±0.08	1.90±0.08**	1.59±0.05$^{* \#\#}$	1.75±0.09**	1.64±0.07$^{** \#}$
ALP (μkat/l)	2.47±0.21	11.76±1.52**	9.74±0.95**	10.25±1.50**	9.52±1.24**
ALT (μkat/l)	0.95±0.03	1.94±0.22**	1.67±0.142**	1.67±0.17**	1.58±0.22*
AST (μkat/l)	1.25±0.06	2.34±0.33**	1.79±0.20*	1.58±0.21	1.62±0.27
Albumine (g/l)	28.19±0.32	26.97±0.41*	26.86±0.42*	26.5±0.62*	26.39±0.32*
Total proteins (g/l)	59.96±1.11	54.64±0.68**	53.75±0.92**	55.13±0.83**	53.34±0.86**
Creatinine (μmol/l)	61.43±0.89	77.1±1.64**	71.24±1.49$^{** \#}$	72.28±1.39$^{** \#}$	70.81±1.44$^{** \#}$
Uric acid (μmol/l)	44.78±9.77	47.04±4.49	41.89±6.22	35.25±4.78	35.73±5.50
Urea (mmol/l)	7.72±0.36	9.98±0.490*	8.50±0.23	8.62±0.32	8.29±0.37

Data are mean ± S.E.M. Significantly different from control. (* $p<0.05$, ** $p<0.005$). Significantly different from STZ ($^{\#}$ $p<0.05$, $^{\#\#}$ $p<0.005$)

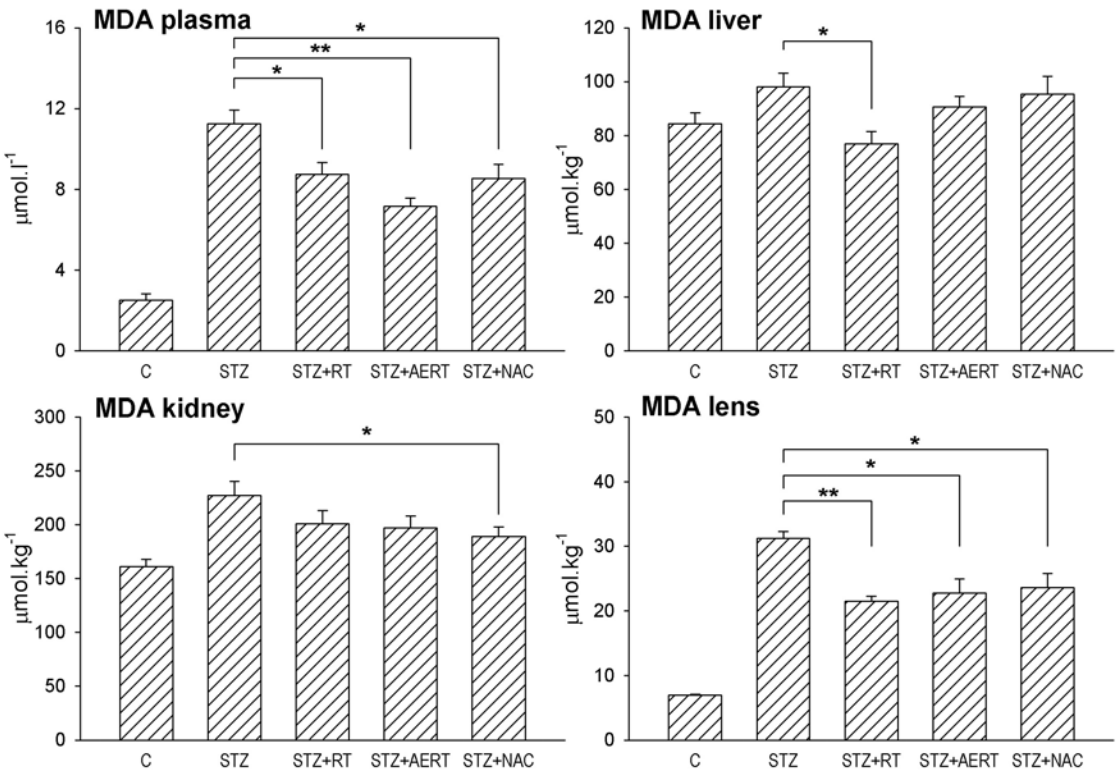

Fig. 3. Effect of aqueous (RT) and alkaline (AERT) extracts of rooibos tea and N-acetyl-L-cysteine (NAC) on plasma and tissue levels of malondialdehyde (MDA) of streptozotocin-induced (STZ) diabetic rats. C – control. Data are mean ± SEM. Statistical significance * $p<0.05$, ** $p<0.001$

Discussion

In both type 1 and type 2 diabetes mellitus the late diabetic pathological complications are mostly due to excessive elevated production of reactive oxygen species over the capacity of their removal by internal enzymatic and non-enzymatic mechanisms (Bonnefont-Rousselot, 2002). Therefore, additional numerous dietary artificial or natural antioxidants may be of great importance in such cases (Ruhe and McDonald 2001).

Various natural products have long been used in traditional medical systems for treating diabetes (Shapiro and Gong, 2002). Most of them contain a wide scale of antioxidants with a potent scavenging activity for reactive oxygen species. Therefore, it might be assumed that these products or isolated natural compounds could play a very important role in adjuvant therapy, at least in the case of non-insulin-dependent diabetes mellitus (type 2). From this point of view, rooibos tea manufactured from legume *Aspalathus linearis*, particularly its non-fermented form (green), containing a large amount of flavonoids and other kinds of antioxidants (Rabe *et al.* 1994) seems to be a useful candidate for the above mentioned purpose.

However, in these experiments, neither aqueous extract or alkaline extract of rooibos tea nor N-acetyl-L-cysteine administration affected the changes of blood parameters characteristic for diabetes which are also accepted as tools in diabetes diagnostics (glucose, glycated hemoglobin, fructosamine). This seems to be in accordance with many published data (Vural *et al.* 2001, Baydas *et al.* 2002) describing the effects of different types of mostly hydrophilic antioxidants on markers of artificially induced diabetes. These observations implicate that probably free radicals need not be the only causative reason for at least streptozotocin-induced diabetes (Szkudelski 2001). On the other hand, advanced glycation end-products (AGEs) and advanced oxidation protein products (AOPPs) levels were found to be lowered by both, rooibos tea and its alkaline extract administration and N-acetyl-L-cysteine, as well, when compared with diabetic rats. Both, AGEs and AOPPs are known to increase in the case of oxidative stress because of the reactive nature of elevated reducing sugars occurring in the case of both type 1 and 2 diabetes mellitus, preferentially in the case of type 2 (Kalousová *et al.* 2002) and in patients with renal insufficiency (Witko-Sarsat *et al.* 1996). As reactive oxygen species are involved in the formation of these metabolites exhibiting

several toxic effects, their decrease in the plasma of diabetic rats after administration of the used antioxidants refers to a kind of their preventive effect against microvascular and/or macrovascular diabetic complications.

Aqueous and alkaline extracts of rooibos tea slightly lowered plasma concentrations of triacylglycerols and total cholesterol, plasmatic urea, creatinine and aminotransferases (ALT, AST) when compared with non-treated diabetic rats. This might be due to hepatoprotective effects of these antioxidants (Uličná *et al.* 2003) against hepatotoxic effect of streptozotocin (Carnovale *et al.* 1991). Plasma albumin and total proteins did not change when antioxidants were administered to diabetic rats. The surprisingly marked decrease in plasma uric acid due to antioxidant administration was not significant due to the wide scatter of the values.

In the current study, both plasma and tissue malondialdehyde (MDA) levels were increased significantly in diabetic rats. Administration of aqueous and alkaline extracts of rooibos tea and N-acetyl-L-cysteine to diabetic rats caused a highly significant reduction in MDA concentrations, particularly in the blood plasma and in the lens, while lower MDA levels in the kidney and liver were not so remarkable. Nevertheless, the aqueous extract of rooibos tea was most effective in the case of liver MDA concentration, while the lowest plasma MDA concentration was observed after admnistration of alkaline extract of rooibos tea. A similar effect of melatonin on MDA levels in some tissues of diabetic rats was described by Baydas *et al.* (2002).

As it is generally known, diabetic retinopathy and cataracts are the most frequent causes of irreversible microvascular complications due to elevated lipid peroxidation with reactive oxygen species in ocular membrane systems (Altomare *et al.* 1995). From this point of view, our results suggest that a wide spectrum of antioxidants in roiboos tea (or at least some of them) are effective as antioxidants not only in hydrophilic but also in hydrophobic biological systems because they are able to protect membrane lipids against their peroxidation as in the above mentioned case.

From these results we can conclude that water extract of rooibos tea as a commonly used non-alcoholic beverage, lacking alkaloids and sugars, could be an excellent adjuvant support in the therapy of diabetic micro- and macrovascular complications, particularly in protecting against ocular pathological changes of diabetic patients. However, rooibos tea can be generally used as supportive therapy in the cases of every disease where free radicals are involved in a pathological process.

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Reprint requests

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