Antihemolytic Effect of Rooibos Tea (*Aspalathus linearis*) on Red Blood Cells of Japanese Quails

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Abstract. The antihemolytic activity of Rooibos and black tea on Japanese quail erythrocytes was studied. Peroxide and hypotonic hemolysis of the red blood cells of quails, either fed with Rooibos tea supplemented food or fed without tea, was performed. Long-term consumption of Rooibos tea did not change the erythrocyte fragility to either peroxide or hypotonia induced hemolysis. However, Rooibos and black teas decreased peroxide induced hemolysis of erythrocytes incubated with each of them, but not hemolysis induced by hypotonic NaCl solution. Stronger inhibition of hemolysis has been obtained when a boiled water extract of Rooibos tea was used for the inhibition. The degree of inhibition was comparable with the effect of ascorbic acid.

Key words: Antioxidants — Aspalathus linearis — Avian erythrocytes — Hemolysis — Japanese quails — Rooibos tea

Introduction

Free radicals, singlet oxygen and hydrogen peroxide, mostly of intracellular origin, are highly reactive oxygen species (ROS) which can readily oxidize and damage essential biological molecules. In particular, polyunsaturated fatty acids are prone to their effects. Therefore, biological membranes including cytoplasmic membranes may be a preferential target of an unfavourable ROS action.

Oxidation of erythrocytes has been used (Kondo et al. 1997) as a model system for oxidative damage of biomembranes. It has been shown that most of ROS attack erythrocyte membranes causing oxidation of the lipids and proteins, and they are also involved in some changes in hemoglobin structure resulting in hemolysis of red blood cells (Chiu et al. l982). Red blood cells of blood stream are protected from oxidative stress by a variety of enzymatic and non-enzymatic antioxidant systems. Free radicals mediated damage of erythrocytes may be inhibited *in vivo* and *in vitro* by some antioxidants, especially by vitamin C, vitamin E (Fischer et al. 1970) and

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by other blood constituents (Yamamoto et al. 1986; Niki et al. 1988; Frei et al. l989). Similar antioxidant effects of some food components, mostly hydroxy- and polyhydroxy- organic compounds from vegetables, fruits and some herbs, have been also observed (Stavric 1994). Especially in humans, different kinds of tea are the most popular 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) has been shown to exhibit a remarkable antioxidant and superoxide anion scavenging activity (Yoshikawa et al. 1990; Nakano and Mizuno 1993). It contains a wide range of phenolic compounds such as flavonols, flavones, chalcones and hydoxylated fatty acids (Komatsu et al. 1994; Rabe et al. 1994).

Therefore, in this study we attempted to investigate whether feeding Japanese quails on Rooibos tea or incubation of quail erythrocytes, which essentially differ from mammalian red blood cells, with the tea extracts could influence the resistance of their red blood cells to lysis.

Materials and Methods

The stability of red blood cell membrane to hemolysis was studied in two types of experiments.

In an *in vivo* experiment, Japanese quails (*Coturnix coturnix japonica*) of both sexes were separated after hatching into three groups. All birds were maintained on a standard commercial broiler mash diet *ad libitum* but the diet of the first group ($n = 15$) was enriched with Rooibos tea powder (3.1 g/1 kg); the second group ($n = 29$) received water extract of the tea (3.5 g/2 l water) instead of drinking water. The third group ($n = 23$) served as control. Birds were decapitated at the age of 180 days. Blood was collected into heparinized tubes and used for hydrogen peroxide or/and hypotonia induced hemolysis tests.

In the second experiment, 40 randomly chosen two to four months old Japanese quails of both sexes were bled and peroxide and hypotonia induced hemolysis of red blood cells and its inhibition *in vitro* was measured. The oxidative hemolysis was estimated according the method of Fisher et al. (1970). The red blood cell suspension contained 20 % red blood cells and 0.12 % hydrogen peroxide was used. After 2.5 hours of incubation at 37℃, the hemolysate was evaluated spectrophotometrically at 540 nm. Absorbance of samples was measured against blanks containing all the sample components excluding erythrocytes. Hypotonia-induced hemolysis was carried out according to the same scheme, using 0.45 % NaCl solution for erythrocyte hemolysis instead of peroxide solution. Hemolysis was inhibited with ascorbic acid (14 mg/l00 ml) or with aqueous extract of Rooibos or black tea (175 mg/100 ml).

The Rooibos tea of the best quality was kindly supplied by Rooibos World Co. (Nagoya, Japan). The aqueous extracts of the teas ($RT_{A,}$ BT_A) were prepared by pouring 2000 ml boiling water onto 3.5 g of dry tea. The extraction continued for 30 min while cooling down to room temperature. Where indicated, Rooibos

and black tea extracts (RT_B, BT_B) were prepared by boiling 3.5 g tea in 2000 ml water for 10 min with subsequent standing for 20 min and cooling down to room temperature.

All chemicals used were reagent grade purchased from commercial companies, except for ascorbic acid (Merck) and black tea which was purchased in a local store.

Results and Discussion

The observed antimutagenic properties (Sasaki et al. 1993), suppressive effect on oncogenic X-ray induced transformation of mouse cells (Komatsu et al. 1994) and the suppression of age related accumulation of lipid peroxides in rat brain (Inanami et al. 1995) of Rooibos tea are closely related to the scavenging of reactive oxygen species by its derived compounds. Therefore, it was of interest to test the antihemolytic properties of this tea on the stability of avian erythrocyte membranes treated with hydrogen peroxide and hypoosmotic conditions.

The results obtained by feeding tea-enriched food or aqueous extracts of the tea to quails are summarised in Fig. 1. The addition of powdered Rooibos tea (RT_P) to food or supplementation of drinking water with tea extract during four months had no significant influence on the stability of erythrocyte membranes. The strongest peroxide induced hemolysis was found in the group of birds which received the water tea extract. The differences among the tested groups of animals, however, were not statistically significant. Similarly, supplementation of food with powdered Rooibos tea or its extract had no influence on hypotonic hemolysis. At the same time, this long-term consumption of tea by the same experimental birds did not change the concentration of some blood constituents and biochemical blood

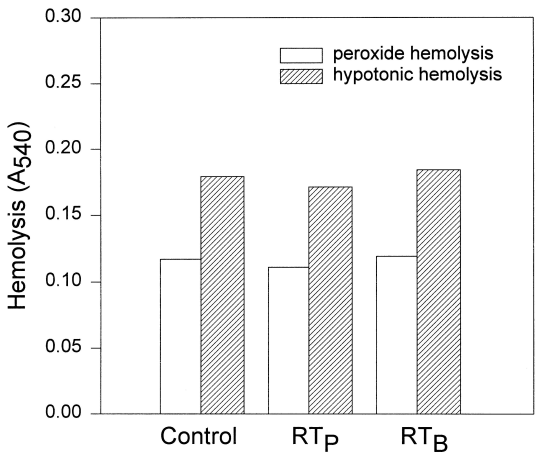

Figure 1. The effect of feeding tea enriched diet on hydrogen peroxide or hypotonia-induced hemolysis of Japanese quail erythrocytes expressed as absorbance at 540 nm. RT_P – powdered Rooibos tea; RT_B – boiled Rooibos tea.

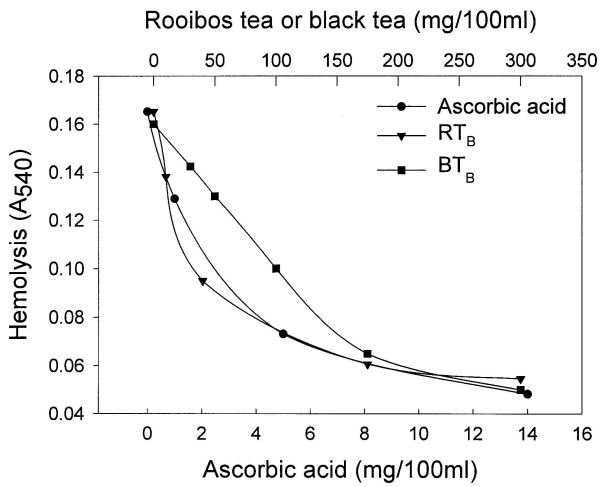

Figure 2. Concentration dependence of Japanese quail erythrocytes hemolysis induced by hydrogen peroxide for Rooibos tea, black tea and ascorbic acid. RT_B – boiled Rooibos tea; BT_B – boiled black tea.

parameters (not shown), indicating that there was no unfavourable effect of Rooibos tea on the physiological and biochemical homeostasis of the Japanese quails.

As shown in Fig. 2, the incubation of quail erythrocytes at 37 ℃ for 2.5 hours with different concentrations of Rooibos (RT_B) or black (BT_B) tea induced resistance of the erythrocytes to the peroxide-induced hemolysis comparable with the protection provided by ascorbic acid. Therefore, 175 mg/100 ml of both teas and 14 mg/100 ml of ascorbic acid were chosen as optimal concentrations.

The antihemolytic effect against peroxide-induced lysis of erythrocytes by Rooibos and black teas prepared by different ways, in comparison with ascorbic acid, is shown in Fig. 3. The weakest hemolysis was observed when the boiled extract of Rooibos tea (RT_B) was used for the inhibition of hemolysis. The differences in hemolysis between blood samples treated with teas or ascorbic acid and the control sample were statistically significant ($P = 0.05$). Weaker, but not significant inhibition of peroxide induced hemolysis was seen when hot water extract of Rooibos tea (RT_A) was used for inhibition compared to boiled tea (RT_B). This was not a surprising finding as prolonged tea extraction at high temperatures is needed to obtain maximum yield of substances showing antioxidant activity (Gadow et al. 1997). The "boiling effect" for the release of antioxidative substances from black tea was not as clear, as the hot water extract of black tea (BT_A) produced stronger inhibition than the boiled one (BT_B). This difference however, was also not significant. From this experiment it can be seen that the antihemolytic effect of Rooibos tea seems to be comparable to that of ascorbic acid.

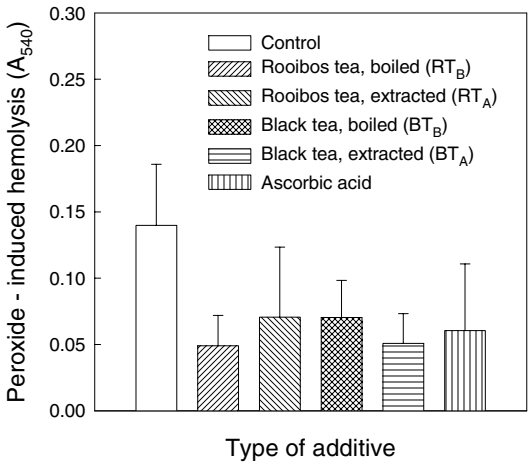

Figure 3. Inhibition of hydrogen peroxide-induced hemolysis of Japanese quail erythrocytes by Rooibos tea, black tea and ascorbic acid. RT_A – hot water extract of Rooibos tea; RT_B – boiled Rooibos tea; BT_A – hot water extract of black tea; BT_B – boiled black tea.

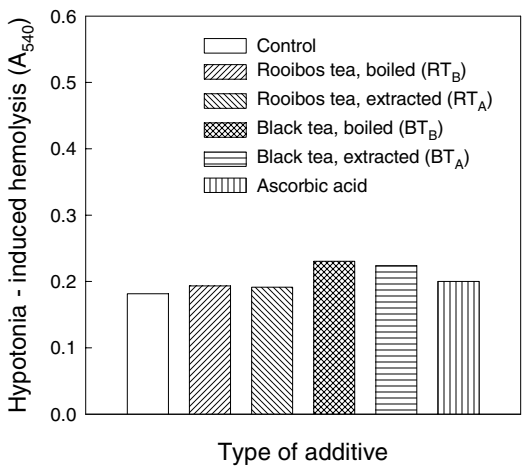

Figure 4. The effect of Rooibos tea, black tea and ascorbic acid on hypotonia-induced hemolysis of Japanese quail erythrocytes. RT_A – hot water extract of Rooibos tea; RT_B – boiled Rooibos tea; BT_A – hot water extracted of black tea; BT_B – boiled black tea.

The antihemolytic effect of ascorbic acid on human red blood cells has been already proven (Kondo et al. l997; Frei et al. l989), as being connected with its action as a free radical scavenger and its functioning in the redox interrelation-

ship with other antioxidants, particularly with hydrophobic vitamin E which is recognized as an essential antioxidant against hemolysis. In the experiments presented herein, all the antioxidants tested were effective in stabilising membranes of Japanese quail erythrocytes to peroxide-induced hemolysis. In contrast to peroxide induced-hemolysis, no inhibition of hypotonia-induced hemolysis with Rooibos tea, black tea and ascorbic acid was observed (Fig. 4). The osmotic fragility of quail erythrocytes seems to remain uninfluenced by the antioxidant properties of the tea extracts and ascorbic acid used in this study.

In conclusion, this study shows that the effects of hydrophilic compounds with potent antioxidative properties derived from Rooibos tea are comparable with that of ascorbic acid in protecting the bird erythrocytes against hydrogen-peroxide induced hemolysis.

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