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In vitro Antioxidant Effect of *Rosa canina* in Different Antioxidant Test Systems

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ABSTRACT

Rosa canina L. has been used since ancient times as a natural product and believed to be safe and almost without side effects. It can be administered easily for some diseases. The actual dose of *Rosa canina* being consumed is often variable, unpredictable or simply unknown. Therefore, in this study, five different (1%, 2%, 3%, 4%, 8%) concentrations of *Rosa canina* infusions were used to determine the optimum antioxidant dose of *Rosa canina* by using different *in vitro* test systems. Our results suggest that *Rosa canina* has the potential to be used as an antioxidant substance at 3% concentration. Therefore, it can be suitable to achieve this concentration at tissue level when used as a supplement to therapeutic regimens and for healthy living.

Keywords: *Rosa canina, Antioxidant, DNA Damage, In vitro.*

INTRODUCTION

Currently there is considerable interest in new natural antioxidants to replace the synthetic ones that are used in foods and therapeutic regimens. The use of the Rosaceae family in prescriptions dates back to Hippocrates but precisely what for is unclear. As a medicinal plant *Rosa canina* L. which is a member of the Rosaceae family, came into full bloom in the Second World War. When Britain was unable to import fresh citrus fruits the government organised the gathering of *Rosa canina* fruits, which were known to be a rich source of vitamin C. Today, the plant is valued as a rich source of vitamin C, organic acids, flavonoids, carotenoids, macro and microelements, and various lipids. (1–4). Recently it has been demonstrated that *Rosa canina* has anti-inflammatory (5–8), antioxidant (9–12), and anti-mutagenic activities (13). However, to the best of our knowledge, no detailed study concerning dose properties and the preventive effects on *in vitro* protein oxidation, carbohydrate damage and DNA damage of raw fruits of *Rosa canina* has been performed so far. On the other hand, the actual dose of active ingredients of *Rosa canina* being consumed is often variable, unpredictable or simply unknown. Dosage variation has greater effects especially on children due to their different capacity for detoxifying chemicals (14). Therefore, the objectives of the present study were to determine the proximate dose and *in vitro* antioxidant effect of wild *Rosa canina* in different antioxidant test systems.

MATERIALS AND METHOD

Rosa fruits were picked from Erzincan (Çayi1l1) province in September. The fruits were cleaned with deionized water, stored at −25 °C and then freeze dried. The seeds were removed. Following that, the dry material was cut into pieces. Infusion was made by pouring of boiling water on 8 grams of plant material. The mixture was left

to stand for 20 minutes and then filtered and diluted to the concentrations (1%, 2%,3%, 4%, 8%).

Linoleic acid was oxidized in a linoleic acid model system to measure the antioxidative activity following the method of Osawa and Namiki with slight modifications (15). Briefly, the sample (0.5 ml *Rosa canina* infusion) was dissolved in 5 ml of 50 mmol/L phosphate buffer (pH 7.0) and added into a mixture of 99.5% ethanol (5 ml) and linoleic acid (0.065 ml) in which the final volume was adjusted to 12.5 ml with distilled water. The mixed solution in a slightly sealed screw-cap conical tube was incubated at 40 °C in the dark. The degree of linoleic acid oxidation was measured by ferric thiocyanate method described by Mitsuda et al. (16) Aliquot (0.1 ml) of reaction mixture was mixed with 75% ethanol (4.7 ml), 30% ammonium thiocyanate (0.1 ml) and 0.02 mol/L ferrous chloride (0.1 ml) in 3.5% HCl. After 3 min, color change that represented linoleic acid oxidation was measured spectrophotometrically at 500 nm.

Protein carbonyl content was measured by the method of Lenz et al. (17). Briefly, 0.2 ml *Rosa canina* infusion, phosphate buffer, BSA, $FeCl_3$, H_2O_2 and ascorbic acid were mixed and the final volume was adjusted to 1.2 ml. Mixture was incubated at 37 °C for 1 hour. After that 1 ml DNPH, 1 ml TCA were added into the mixture. The mixture was centrifuged at 3000 g for 10 minutes. Following this, protein pellets were washed three times with 1 ml ethanol-ethyl acetate. The final protein was solved in 2ml guanidine hydrochloride and the absorbance was measured at 360 nm.

The carbohydrate damage assay was measured by deoxyribose method with a few modifications (18). Reaction mixtures contained, in a final volume of 3.5 ml, the following reagents at the final concentrations given: 0.2 ml *Rosa canina* infussion, phosphate buffer, pH 7.4 (20 mM KH_2PO_4-K_2HPO_4) deoxyribose; ascorbic acid (AA); hydrogen peroxide (H_2O_2); EDTA; $FeCI_3$; were premixed just before addition to the reaction mixture. Solutions of ascorbic acid were prepared in de-aerated water immediately before use. Reaction mixtures were incubated at 37 °C for 1 hour then 1 ml of 2.8 % cold trichloroacetic acid was added and reactivity was developed by adding 1 ml Thiobarbituric acid (TBA) (l%, w/v, in 0.05 M NaOH), followed by heating at 100 °C for 15 min. When the mixture was cool, the absorbance at 532 nm was measured against appropriate blank.

Bleomycin-lron Dependent DNA Damage was measured by method of Aruoma et al. (19) Briefly, except for bleomycin sulfate, all reactives were treated with Chelex to remove contaminating metal ions. Reaction mixtures contained, in a final volume of 3.5 ml, the following reagents at the final concentrations given: Calf thymus DNA (0.2 mg/ml), bleomycin sulfate (0.05 mg/ml), $FeCl_3$ (25 μM) KH_2PO_4−K_2HPO_4 buffer (20 mM, pH 7.4) $MgCl_2$ (5 mM), ascorbic acid (240 μM) and, *Rosa canina* were mixed. Reaction mixtures were incubated for 1 h at 37 °C. After incubation, 0.1 ml EDTA (0.1 M), 1 ml TBA (1% solution of TBA was prepared in the solution of 50 mM NaOH) and 1 ml TCA (2.8% solution of TCA) were added to the reaction mixture and the reaction mixture was incubated for 20 min. at 90 °C. The absorbance of mixture was measured at 532 nm against appropriate blank. Increased absorbance of the reaction mixture shows the increasing damage of DNA.

Statistics

Data are presented as mean ±S.D. of at least three independent experiments (n = i>3). One-way ANOVA (analysis of variance) followed by Scheffe's test were performed to determine statistical differences among groups with the aid of SPSS software version 11.0 (SPSS, Chicago, IL, USA). Statistical significance was defined as $p < 0.05$ for all tests.

RESULT AND DISCUSSION

There is continuing interest on the screening of medicinal plants with a view to determine new sources of natural antioxidants (20). In this study, the antioxidant activities of *Rosa canina* fruits' infusions were evaluated according to four *in vitro* tests systems; the lipid oxidation assay, protein oxidation test, DNA damage test and carbohydrate damage assay, which were used to assess the antioxidant properties of *Rosa canina* (Table 1). Together, all four tests provide a beter assessment of antioxidant properties than separately. To assess the inhibitory effects of *Rosa canina* on lipid peroxidation, linoleic acid was oxidized using a Fe^{2+}, accelerated lipid peroxidation. As observed in this research, the *Rosa canina* could retard lipid perxidation efficiently than that of control. The infussions of *Rosa canina*, exhibited lipid perxidation inhibitory activity. Among concentrations of *Rosa canina*, the concentration of 3 % g/100 ml, exhibited the highest activity. This activity increased up to 76.03% (Table 1). On the other hand, in previous study conducted by Rossnagel et al. was shown that rose hip extract inhibits lipid oxidation *in vitro* and reduces the chemotaxis and chemoluminescence of leucocytes (21). These findings support our research findings. So, it can be said that owing to high contents of antioxidants in *Rosa canina*, it's extract showed high antioxidant activity on lipid peroxidation at concentration of 3%.

Table 1: Antioxidant Effect of Rosa canina Concentrations in Different Antioxidant Test Systems
Concentrations of Rosa canina Infusions

Parameters	Control	1%	2%	3%	4%	8%
Lipid Oxidation (% inhibition)	0.00	66.05 ± 0.01a	70.19 ± 0.08b	76.03 ± 0.01c	70.16 ± 0.03b	65.07 ± 0.03d
Protein oxidation (% inhibition)	0.00	75.41 ± 0.50a	76.29 ± 0.02b	82.06 ± 0.03c	82.06 ± 0.02c	65.22 ± 0.02d
Carbohydrate Damage (A_{532})	0.402 ± 0.001a	0.361 ± 0.006b	0.323 ± 0.001c	0.313 ± 0.021c	0.318 ± 0.005c	0.383 ± 0.003ab
DNA Damage (A_{532})	0.417 ± 0.001a	0.084 ± 0.002b	0.046 ± 0.002c	0.016 ± 0.002d	0.080 ± 0.002b	0.278 ± 0.003e

Means ± SD, n=3.
Values in the same line with different lower-case letters are significantly different at $P < 0.05$.

Oxidation of proteins can lead to nitration of aromatic amino acid residues, oxidation of thiol groups, advanced oxidation protein products formation, and conversion of some amino acid residues to carbonyl derivatives. Alterations in protein conformations can lead to increased aggregation, fragmentation, distortion of secondary and tertiary structure, susceptibility to proteolysis, and diminution of normal function (22). Experiments on the inhibition of protein oxidation by *Rosa canina* showed that the antioxidant activity of *Rosa canina* increased with increasing concentration. The concentrations of 3% and 4% exhibited the highest activity (Table 1). According to the literature, there is no study on *in vitro* protein oxidation inhibitory effect of *Rosa canina*. Thus, our results may be the first study to provide data that the *Rosa canina* possess protein oxidation inhibitory effect.

The effects of various concentrations of *Rosa canina* (from 1 to 8 g/100 ml) on carbohydrate damage are shown in Table 1. The antioxidant activity of *Rosa canina* increased with increasing concentration (from 1 to 4 g/100 ml). But the same protection was not observed at 8% concentration (Table 1). This effect may be attiributed to polyphenol compounds of *Rosa canina* (23, 24). As a matter of fact, some studies have reported that the same polyphenol compounds could behave as both antioxidants and prooxidants, depending on concentration and free radical source (25–27). However, to the best of our knowledge, no detailed study concerning dose properties and the preventive effects on *in vitro* carbohydrate damage of raw fruits of *Rosa canina* has been performed hitherto. This may be the first study to provide data on this matter.

In the assay for DNA damage, the concentrations of 1%, 2%, 3%, 4%, 8% had protective effects on DNA damage. But, the antioxidative activity of the individual fractions differed significantly (Table 1). The concentrations of 3% demonstrated the strongest protective activity towards DNA damage. On the other hand, Vidushi et al. remarked that some of the plant extracts were able to stimulate oxidative damage to deoxyribose by possibly interacting with the iron ions in the reacting mixture (28). But, the stimulation of oxidative damage to deoxyribose wasn't seen at any concentration of *Rosa canina*. These results illustrate that *Rosa canina* has a strong protective effect on DNA damage. According to literature searched, we couldn't find any study on *in vitro* DNA damage inhibitory effect of *Rosa canina*. Therefore, depending on concentrations, these results may be the first to provide data on *Rosa canina*'s *in vitro* DNA damage inhibitory effect.

Finally, the results obtained from this study clearly indicate that the inhibition of protein oxidation, lipid peroxidation, carbohydrate damage and DNA damage at 3% concentration of raw fruits of *Rosa canina* provides promising opportunuties. Therefore, *Rosa canina* can be used as being easily accessible in pharmaceutical and food industries. On the other hand, further studies are needed to get proper information regarding the role of *Rosa canina* as an antioxidant or prooxidant and its involvement in the other dose depending processes and *in vitro* test systems.

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