In vivo Antioxidant Potentials of Rosa Damascena Petal Extract from Gilan, Iran, Comparable to α-tocopherol

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Abstract: Rosa damascena Mill, (Rosaceae) is a widely cultivated ornamental plant. Several therapeutic effects including calming, antiaxiety, laxative, and antispasmodic have been described for the flower of R. damascena. The petals of R. damascena are specially used as cardiotonic by the people of Gilan province. In this study antioxidant potential of R. damascena petals were determined by FRAP test and its ability to inhibit lipid peroxidation was determined by TBARS test in rat. In vivo examination was performed by oral administration of ethanol extract of R. damascena petals at doses of 50, 75, 100 and 200 mg/kg/day for 10 days which compared to vitamin E (10 mg/kg/day) and control groups. In vivo evaluation of antioxidant effects of R. damascena with these two methods showed that the extract of R. damascena has a high ability to inhibit lipid peroxidation and has a high antioxidant power with all doses comparing to control (p<0.001). The highest activity was observed with the dose of 200 mg/kg/day. This preliminary study indicates the interesting antioxidant stress activity of R. damascena, which is comparable to the known antioxidant compound, alpha-tocopherol. R. damascena can be considered as a medicinal source for the treatment and prevention of many free radicals related diseases.

Key words: Rosa damascena, antioxidant, lipid peroxidation, oxidative stress, alpha-tocopherol

INTRODUCTION

Oxidative stress results from an imbalance between the generation of oxygen derived radicals and the organism’s antioxidant potential playing an important role in many chronic diseases (Abdollahi et al., 2004). Antioxidants are generally believed to be protective against the oxidative stress and exert their activity by several mechanisms. These mechanisms include enzymatic degradation of free radicals and scavenging free radicals (Penckofar et al., 2002). Therefore, the intake of antioxidant vitamins and other natural products as preventive measures is suggested.

In recent years, many plants have been screened for their antioxidant potential (Ashtural Nakhai et al., 2006; Ghafar et al., 2006; Mehdipour et al., 2006; Ghazanfari et al., 2006). Several therapeutic effects including laxative, antispasmodic and cardiotonic have been proposed for the flowers of R. damascena (Penckofar et al., 2002). Flavonol glycosides were extracted from petals of R. damascena Mill. Among the 22 major compounds analyzed, kaempferol and quercetin glycosides were detected (Schlieber et al., 2005). The high flavonol content of approximately 16 g kg-1 on a dry weight basis revealed that distilled rose petals represent a promising source of phenolic compounds which might be used as a functional food ingredient, as a natural antioxidant, or as a color enhancer (Schlieber et al., 2005). Since previous studies have shown that R. damascena has a high antioxidant, hepatoprotective and antibacterial effect (Ozkan et al., 2004) the present in vivo study evaluates the lipid peroxidation level and antioxidant power of R. damascena in rats.

MATERIALS AND METHODS

Materials: Sodium acetate, 2, 4, 6-tripryridyl-s-triazine (TPTZ), 2-thiobarbituric acid (TBA), 1, 1, 3, 3-tetramethoxypropane, trichloracetic acid (TCA), sodium sulfate, vitamin E, FeCl, HCl, hydrochloric acid, distilled water, sulfuric acid and n-butyl alcohol from Merck Chemical Company (Germany) were used in this study.

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Extract preparation: Flowers of R. damascena were collected during May 2004 from the North of Iran (Gilan). Samples of the plant were deposited (No. 6555) in the herbarium of Pharmacognosy Department, Faculty of Pharmacy, TUMS. Two hundred gram of fresh petals were extracted by ethanol-water 80%. The final extract was collected, distilled and evaporated in low pressure and temperature. To purify this extract from fats, it has been extracted with petrol ether and chloroform to obtain a brown gummy extract which was soluble in water.

Animals: Thirty male Wistar rats of average weight 200±2 g were used in this experiment. They were housed in well ventilated cages and maintained under standardized environmental condition (22-28 °C, 60%-70% relative humidity, 12 h dark light cycle) with free access to stock laboratory diet and water.

Rats were divided into 6 groups, within 5 rats in each group. The groups were divided into control, vitamin E treated and R. damascena extract treated in 4 different doses groups.

Treatment: Animals from group 1 to 4 received doses of (50, 75, 100 and 200 mg/kg/day) of the extract of R. damascena by gavage for 10 days. Group 5 received vitamin E (10 mg/kg/day) by gavage. The 6th group of animals was assigned as control and received only vehicle.

Blood collection: About 4 mL of blood was collected through direct heart puncture from anesthetized (with sodium pentobarbital, 55 mg kg−1) rats and then blood was centrifuged at 2000 g for 10 min to separate serum. The serum was kept in −20°C for subsequent determination of lipid peroxidation and antioxidant status.

Lipid peroxidation assay in serum: It was determined using the thiobarbituric acid (TBA) test. To precipitate the proteins of serum, 2.5 mL of TCA 20% (w/v) was added into 0.5 mL of the sample, which then centrifuged at 1500 g for 10 min. Then 2.5 mL of sulfuric acid 0.05 M and 2 mL TBA 0.2% was added to the sediment, shaken and incubated for 30 min in a boiling water bath. Then 4 mL n-butanol was added and the solution was centrifuged and cooled, then absorption of the supernatant was recorded at 532 nm using a UV-VIS spectrophotometer (Shimadzu, UV-160A, JAPAN). The calibration curve was obtained using different concentrations of 1, 1, 3, 3-tetramethoxy-propan as a standard to determine the concentration of TBA-MDA adducts in samples (Satho, 1978).

Total antioxidant assay in serum: Antioxidant power of plasma was determined by measuring their ability to reduce Fe²⁺ to Fe³⁺ established as named FRAP test and described previously (Benzie and Strain, 1996). Briefly, in this test, the medium is exposed to Fe³⁺ and the antioxidants present in medium start to produce Fe²⁺ as an antioxidant activity. The reagent included 300 mmol L⁻¹ acetate buffer, pH 3.6 and 16 mL acetic acid per litre of buffer solution, 10 mmol L⁻¹ 2, 4, 6-tripryridyls-triazine (TPTZ) in 40 mmol L⁻¹ HCL, 20 mmol L⁻¹ FeCl₃ 6H₂O. Working FRAP reagent was prepared as required by mixing 25 mL of acetate buffer, 2.5 mL TPTZ solution and 2.5 mL FeCl₃ 6H₂O solution. Ten microliter of H₂O-diluted sample was added to 300 µL freshly prepared reagent and warmed at 37°C. The complex between Fe²⁺ and TPTZ gives a blue colour with absorbance at 593 nm.

Statistical analysis: The values are reported as mean±SE. Statistical analysis of data was carried out by computer using GraphPad software. One-way ANOVA and Tukey posthoc multicomparsion tests were used to analyze data. p-values lesser than 0.05 were considered significant.

RESULTS

The total antioxidant power of R. damascena is shown in Fig. 1. The total antioxidant power of R. damascena with all doses (50, 75, 100 and 200 mg/kg/day) was increased when compared to control (p<0.001).

Comparison of vitamin E and different dose groups of R. damascena showed that doses of 50 and 75 mg/kg/day of R. damascena has less antioxidant power than vitamin E (p<0.001). The 100 mg/kg/day of R. damascena showed almost the same total antioxidant power as vitamin E (p>0.05). The 200 mg/kg/day of R. damascena showed greater antioxidant effect than vitamin E (p<0.001).

![Graph showing FRAP assay results](image-url)

Fig. 1: Effect of different doses of R. damascena and vitamin E on rat blood antioxidant power; *means that the difference between control and treated groups is significant at p<0.001. *means that the difference between vitamin E and treated groups is significant at p<0.001.
The doses of 50 and 75 mg/kg/day showed the same total antioxidant activity and the difference was not significant (p>0.05) while 100 mg/kg/day of R. damascena showed greater antioxidant activity than dose of 50 mg/kg/day (p<0.001) but almost the same antioxidant power as dose of 75 mg/kg/day (p<0.05). The 200 mg/kg/day of R. damascena showed the most antioxidant power among others (p<0.001).

The lipid peroxidation formation in serum was effectively inhibited by extract of R. damascena (Fig. 2). All doses of R. damascena (50, 75, 100 and 200 mg/kg/day) and vitamin E (10 mg/kg/day) showed lesser lipid peroxidation than controls (p<0.001). Comparison of different doses of R. damascena with vitamin E (10 mg/kg/day) showed that doses of 50 and 75 mg/kg/day have less ability to inhibit lipid peroxidation than vitamin E (p<0.001). The 100 mg/kg/day showed the same effect as vitamin E and the difference was not significant (p>0.05) and dose of 200 mg/kg/day showed greater ability than vitamin E in inhibition of lipid peroxidation (p<0.001).

Comparison of different doses of R. damascena showed that doses of 50 and 75 mg/kg/day have the same power to inhibit lipid peroxidation and the difference was not significant (p<0.05) while the dose 100 mg/kg/day showed greater ability than doses of 50 and 75 mg/kg/day to inhibit lipid peroxidation (p<0.001). The 200 mg/kg/day dose group showed greater ability than other doses to inhibit lipid peroxidation (p<0.001).

**DISCUSSION**

Oxidative stress in biological systems, results from the overproduction of reactive oxygen species or decrease in antioxidant potential. These are capable of chemically altering all major biomolecules including lipids, proteins and nucleic acids by changing the structure and function. Humans and animals have developed mechanism to protect these biomolecules from damage of free radicals by endogenous antioxidants including enzymes like superoxide dismutase, glutathion peroxidase and catalase and non-enzymes like vitamins, uric acid, albumin and seroloplasmin (Abdollahi et al., 2004).

The toxicology data on rose essential oil showed that it is very safe. One study on the safety and low oral toxicity of rose oil reported that it has no cumulative effect and does not appear to have any adverse effects on the development of the embryo when taken internally (Kiror and Bainova, 1998). Rose is non-phototoxic and non-sensitizing, though undiluted it can be a mild skin irritant to some people. It has low oral toxicity in comparison to spearmint and citrus oils, both of which considered safe (Tisserand and Balacs, 1995).

Flavonoids such as quercetin and kaempferol (3, 5, 7, 4′-tetrahydroxy Flavone) were detected from petals of R. damascena (Schieber et al., 2005). Flavonoids, as a matter of fact, are antioxidants (Saia et al., 1995). A number of quercetin’s positive effects appear to be due to its antioxidant activity. Quercetin scavenges oxygen radicals (Miller, 1996; Saia et al., 1995), inhibit xanthine oxidase (Chang et al., 1993) and inhibit lipid peroxidation in vitro (Chen et al., 1990). As another indicator of its antioxidant effect, quercetin inhibit oxidation of LDL cholesterol In vitro, probably by inhibiting LDL oxidation itself, by protecting vitamin E in LDL from being oxidized or by regenerating oxidized vitamin E (DeWhalley et al., 1993). By itself and paired with ascorbic acid, quercetin reduced the incidence of oxidation damage to neurovascular structures in skin and inhibited damage to neurons caused by experimental glutathione depletion (Skaper et al., 1997). Kaempferol is a known antioxidant with effects such as inhibition of the state 3 oxidation rate of malate, NADH and succinate shown in intact mitochondria (Ravanel et al., 1982).

Previous studies have shown that R. damascena has a high antibacterial, hepatoprotective and antioxidant activity. In addition, administration of R. damascena extract at dose of 50 mg/kg/day significantly reduced the serum alkaline phosphate (ALP), glutamine pyruvate transaminase (GPT) and glutamine oxaloacetate transaminase (GOT) and lipid peroxides level in rats.

The present results strongly indicate that the extract of R. damascena has marked antioxidant activity in vivo. In vivo evaluation of antioxidant effects of R. damascena with FRAP and TBA methods have shown that the highest activity is observed with the dose of 200 mg/kg/day among tested doses. Surprisingly this
potential of *R. damascena* was comparable to vitamin E. Thus *R. damascena* may have a great potential to prevent disease associated with free radicals. It is favorable to do further studies on this plant to determine whether it has any effect on the diseases related to oxidative stress.

**ACKNOWLEDGMENTS**

This study was supported by a grant from Pharmaceutical Sciences research Center, TUMS.

**REFERENCES**


