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

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Abstract: *Rosa damascena* Mill, (Rosaceae) is a widely cultivated ornamental plant. Several therapeutic effects including calmative, antianxiety, 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 Guilan 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. damascen*a 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. damascene* 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 anti oxidative 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 (Penckofer *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 (Ashtaral Nakhai *et al*., 2006; Ghafari *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* (Penckofer *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 (Schiieber *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 (Schiieber *et al*., 2005). Since previous studies have shown that *R. damascena* has a high antioxidant, hepatoprotective and antibacterial effect (Özkan *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-tripyridyl-s-triazine (TPTZ), 2-thiobarbituric acid (TBA), 1, 1, 3, 3-tetramethoxypropane, trichloroacetic acid (TCA), sodium sulfate, vitamin E, $FeCl_3$ $6H_2O$, 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 (Guilan). 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, shaked 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 Fe^{3+} to Fe^{2+} established as named FRAP test and described previously (Benzie and Strain, 1996). Briefly, in this test, the medium is exposed to Fe^{3+} and the antioxidants present in medium start to produce Fe^{2+} 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-tripyridyl-s-triazine (TPTZ) in 40 mmol L^{-1} HCL, 20 mmol L^{-1} $FeCl_3$, $6H_2O$. Working FRAP reagent was prepared as required by mixing 25 mL of acetate buffer, 2.5 mL TPTZ solution and 2.5 mL $FeCl_3$, $6H_2O$ solution. Ten microliter of H_2O-diluted sample was added to 300 µL freshly prepared reagent and warmed at 37°C. The complex between Fe^{2+} 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 multicomparison 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 ($p<0.001$).

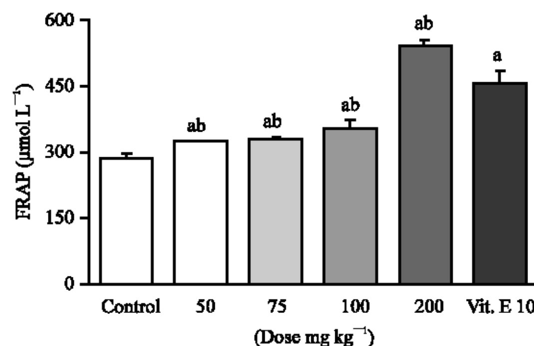

Fig. 1: Effect of different doses of *R. damascena* and vitamin E on rat blood antioxidant power, [a]means that the difference between control and treated groups is significant at $p<0.001$. [b]means that the difference between vitamin E and treated groups is significant at $p<0.001$

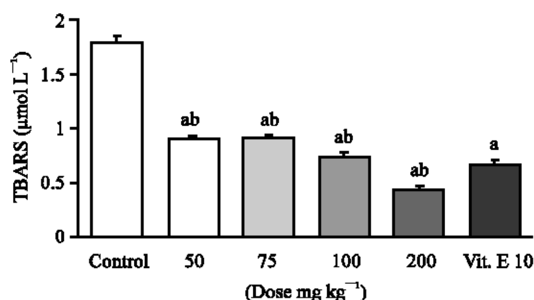

Fig. 2: Effect of different doses of *R. damascena* and vitamin E on rat blood lipid peroxidation, [a]means that the difference between control and treated group is significant at $p<0.001$. [b]means that the difference between vitamin E and treated group is significant at $p<0.001$

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 peroxides 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, gluthation 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 (Kirov and Bainova, 1998). Rose is non-phototoxic and non-sensitizing, though undiluted it can be a mild skin irritant to some people. It has a low oral toxicity in comparison to spearmint and citrus oils, both of which considered safe (Tisserand Balacs, 1995).

Flavonoids such as querecetin and kaemferol (3, 5, 7, 4' tetrahydroxy Flavone) were detected from petals of *R. damascena* (Schiieber *et al*., 2005). Flavonoids, as a matter of fact, are antioxidants (Saija *et al*., 1995). A number of quercetin's positive effects appear to be due to its antioxidant activity. Quercetin scavenges oxygen radicals (Miller, 1996; Saija *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. damascene* 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.

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