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Hypolipidemic activity of *Rhodiola rosea* L. root extract

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ABSTRACT : *Rhodiola rosea* L. ("golden root"), a perennial herbaceous plant belonging to the Crassulaceae family, has been used for centuries in Russian folk medicine. Recently *R. rosea* became popular in western countries, mainly for its properties of adaptogen and anabolic. The total phenolic content of *Rhodiola rosea* L. root hydro-alcoholic extract (269 mg/g) was evaluated by the Folin-Ciocalteau method. The radical scavenging activity of the extract was assayed by the DPPH method (IC_{50}=9.59 μg/ml). Moreover, the hypolipidemic activity of *R. rosea* (100 mg/kg, os) was evaluated by diet-induced hypercholesterolemia in rat; during all the experiment the food intake and growth rates were monitored. It was not observed a significative difference in food consumption in all groups throughout the experiment; however, the group administered with *R. rosea* extract did not show any increase in body weight versus hypercholesterolemic controls. The administration of *R. rosea* extract for 15 days provokes a reduction in plasma levels of cholesterol (52.63%), LDL (59.11%), triglycerides (20.04%), and an increase in HDL levels (83.30%). The hypolipidemic activity of *Rhodiola rosea* L. root extract could be related to the free radical scavenging properties of polyphenol compounds contained in high concentration in the extract.

KEYWORDS: Rhodiola rosea *L.; Hypolipidemic activity; DPPH test; Polyphenols.*

INTRODUCTION

The genus *Rhodiola* consists of approximately 100 species occurring mainly in Asia and Europe (1). *Rhodiola rosea* L. or rose root, also commonly known as "golden root" or "arctic root", is a perennial herbaceous plant belonging to the Crassulaceae family; it is widely distributed at high altitudes in arctic and mountainous regions throughout Europe and Asia. Its name derives from the characteristic flower scent of rose (2).

Several bibliographic data reported *R. rosea* root chemical composition (3-5); the main components are salidroside and its aglycon tyrosol (6, 7), and cinnamic glycosides such as rosin, rosavin and rosarin (4, 6). Rosin, rosavin and rosarin seem to be components of *R. rosea* alone. Other constituents from *R. rosea* are flavonoids (8), tannins (9), and essential oil (10, 2).

Medicinal preparations of *R. rosea* are used in Russian traditional medicine for the adaptogenic properties, and for the treatment of long-term illness and weakness due to infection (2). *R. rosea* has been classified as an adaptogen by Russian researchers; infact it increases resistance to a variety of chemical, biological, and physical stressors (11, 12). Several literature data report various biological activities relative to *R. rosea* root; its properties include antidepressant (13, 14), anticancer (15, 16), cardioprotective activity(17-19), and central nervous system enhancement (20, 12). In Russia *R. rosea* root has been used to increase the stress tolerance of astronauts; professional athletes utilize it for enhancing physical resistance, stimulating anabolic processes in skeletal muscles, and promoting subsequent recovery of the cardiovascular system (21, 22).

Recently *R. rosea* became popular in western countries mainly for its properties of adaptogen and anabolic; in fact it can be found in several formulations employed in body weight control. For this reason it seemed interesting to study the effects of a chronic treatment with *R. rosea* root extract on lipid metabolism. Moreover, the total phenolic content and the radical scavenging activity of the extract were evaluated.

MATERIALS AND METHODS

Plant Material

Rhodiola rosea L. is a perennial herbaceous plant with a thick, fleshy rhizome, bearing persistent. The leaves are orbicular-ovate to linear-oblong, usually dentate, broad-based and glabrous. The flowers are gathered in cymes somewhat corymbose. The petals are usually yellow, sometimes absent. The fruit is a follicle reddish (23) (Fig. 1).For the experiments *Rhodiola rosea* root dried hydro-alcoholic extract (CRUAL, Roma) was used.

Determination of total phenolic content

The total phenolic content of *R. rosea* extract was evaluated by the Folin-Ciocalteau method (24). 100 µl of the sample, approximately diluted, were added to 200 µl of Folin-Ciocalteau solution.

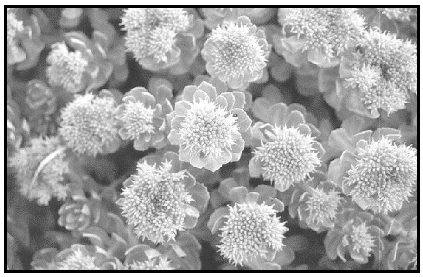


Fig. 1 *Rhodiola rosea* L.

After 3 min., 2 ml of water and 1 ml of sodium carbonate (15%) were added; then, the test tubes were shaken and allowed to incubate in darkness for 2 hours. The assay was carried out in triplicate. A blue coloration was developed, and the absorbance was read at 765 nm. Results were expressed as mg of gallic acid equivalent/g extract.

Radical scavenging activity on DPPH radical

Free radical scavenging activity of *R. rosea* extract was determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method (25). An aliquot (0.5 ml) of methanol solution containing different amounts of *R. rosea* extract (5, 10, 20, and 50 µg/ml) was added to 3 ml of daily prepared methanol DPPH (Sigma-Aldrich S.r.l., Italy) solution (0.1 mM); the maximum concentration of the extract employed was 100 µg/ml. The optical density change at 517 nm was measured 10 min. later by a spectrophotometer (Shimadzu UV-1601, Italy). The antioxidant assay was carried out in triplicate and the readings were averaged.

The scavenging activity was measured as the decrease in absorbance of the samples versus DPPH standard solution. Results were expressed as "percentage inhibition" of the DPPH ± Standard Deviation (S.D.) and "mean inhibiting concentration" (IC_{50}) of the DPPH. IC_{50} parameter is defined as the concentration, in µg/ml, of substrate required to inhibit DPPH radical formation by 50%, and it was calculated by using the Litchfield and Wilcoxon test (26).

Biological assay

Animals

Adult male Wistar rats (180-200 g) were maintained in standardized conditions (temperature 22 ± 2 °C; humidity 60 ± 4%; natural lighting), and water was provided *ad libitum*. During the experiment the food consumption was monitored every day, and weight-gain was evaluated once a week. Data, expressed as mean ± S.E., were analyzed by two-ways analysis of variance (ANOVA). Statistical significance of the difference of the means was evaluated by Duncan's test.

Hypolipidemic activity

The effects of *R. rosea* extract on lipid metabolism were evaluated by diet-induced hypercholesterolemia experimental model (27).

Animals were divided into four groups of ten rats each:

- Group I (normolipidemic controls) was kept with standard diet (S. Morini, Mil rat GLP) for 30 days;
- Group II (hyperlipidemic controls) was fed with hypercholesterolemic diet (Altromin-Rieper, Bolzano) for 30 days;
- Group III was fed with hypercholesterolemic diet for 30 days; from the 15th to the 30th day, each rat received by gavage *R. rosea* extract, suspended in water (1 ml/100 g b.w.), at the dose of 100 mg/kg;
- Group IV was maintained on a hypercholesterolemic diet for 30 days; from the 15th to the 30th day, each rat received by gavage Gemfibrozil (Lopid, Parke-Davis), suspended in water (1 ml/100 g b.w.), at the dose of 30 mg/kg, used as reference drug (28).

At the 30th day, the animals were fasted overnight. Blood was collected in heparinized tubes, from the carotid artery of rats under light diethyl-ether anaesthesia; the plasma was immediately centrifuged (2500 RPM; 4 °C), and kept at -20 °C until assay.

Total cholesterol (29), HDL (High Density Lipoproteins), LDL (Low Density Lipoproteins) (30-32), and triglycerides (33) were assayed with colorimetric and enzymatic methods (BioSystems S.A., Barcelona Spain).

Data are expressed as mean ± S.E. of ten determinations. The results were statistically analyzed by Student's *t*-test. P < 0.05, versus hyperlipidemic controls, was taken as significant.

RESULTS

Determination of total phenolic content

The total phenolic content of *R. rosea* extract, evaluated by the Folin-Ciocalteau method, was 269 mg/g extract.

Radical scavenging activity on DPPH radical

R. rosea extract showed, at the concentrations assayed, radical scavenging effects on DPPH radical. Actually, it inhibited DPPH formation by 50% at a concentration of 9.59 μg/ml (IC_{50}) (Table 1).

Table 1: Radical scavenging effects of Rhodiola rosea L. extract on DPPH radical. Values are expressed as mean ± S.D. of three determinations.

Dose (μg/ml)	DPPH percentage inhibition	IC_{50} (μg/ml)
50	95.98 ± 0.33	9.59
20	78.63 ± 0.45	
10	52.24 ± 0.37	
5	25.84 ± 0.43	

Hypolipidemic activity

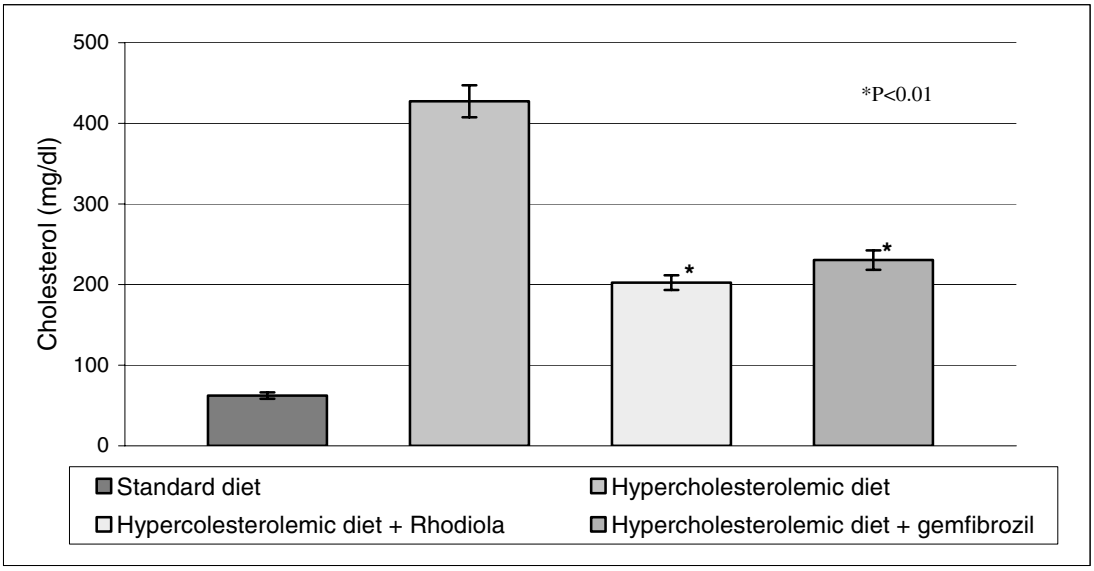
Throughout the experiment it was not observed a significative difference in food consumption in all groups (data not shown). At first and second week the body weight increased in all groups without any significant differences between them; instead, unlike the hypercholesterolemic controls (group II), at third and fourth week, the group treated with *R. rosea* extract did not show any increase in body weight (Table 2). The hypercholesterolemic diet administered for 30 days increased cholesterol, triglycerides, and LDL plasma levels. The administration of *R. rosea* extract for 15 days provoked a reduction in plasma levels of cholesterol of.52.63%, triglycerides of 20.04%, and LDL of 59.11%. Furthermore, HDL levels increased of 83.30%; in fact, in 100 mg of total cholesterol, HDL amount changed from 2.38 mg, in control hypercholesterolemic rats, to 11. 68 mg in hypercholesterolemic treated rats. Similar results were obtained in the group treated with gemfibrozil (Fig. 2 - 5).

Table 2 : Effect of Rhodiola rosea L. extract on body weight in rat.

Treatment	Body weight (g)				
	Weeks				
	0	1	2	3	4
Standard diet	200 ± 4.05	210 ± 6.09	229 ± 6.81	255 ± 4.40	280 ± 5.23
Hypercholesterolemic diet	208 ± 5.23	213 ± 5.46	240 ± 4.04	290 ± 4.56 [a]	340 ± 4.24 [a]
R. rosea	205 ± 3.90	218 ± 3.89	233 ± 5.23	232 ± 7.51 [b]	240 ± 7.90 [b]
Gemfibrozil	204 ± 4.56	215 ± 4.75	235 ± 4.39	267 ± 3.68 [c]	300 ± 5.53 [c]

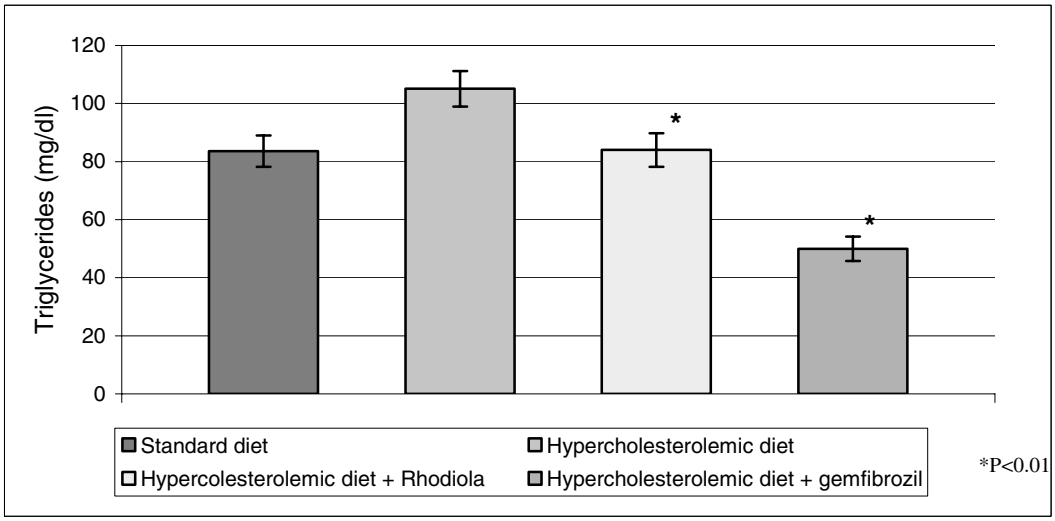
Values are expressed as mean ± S.E. of ten rats for each week of treatment. Means in 1st , 2nd and 3rd columns are non-significant using ANOVA test. Means with different superscripts (a, b, c) in 4th and 5th columns are significant at P< 0.01 using ANOVA test.

Fig. 2 : Cholesterol plasma levels in rat fed with a hypercholesterolemic diet, after 15 day-treatment with Rhodiola rosea L. extract.



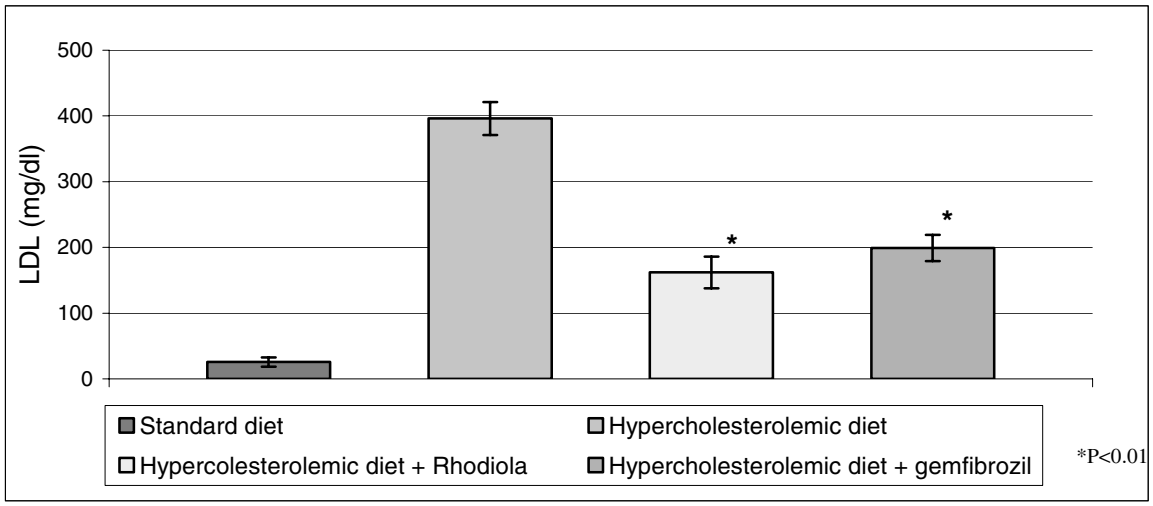
*Values are expressed as mean ± S.E. of ten rats; *p<0.01, compared with the hypercholesterolemic group; Student's t- test.*

Fig. 3: Triglycerides plasma levels in rat fed with a hypercholesterolemic diet, after 15 day-treatment with Rhodiola rosea L. extract.



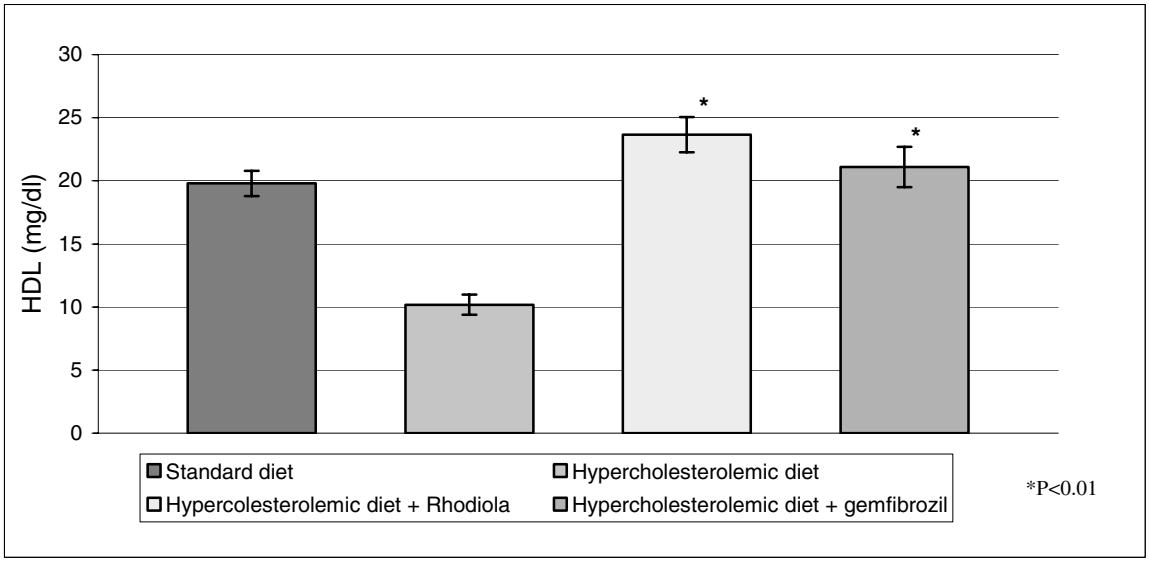
*Values are expressed as mean ± S.E. of ten rats; *p<0.01, compared with the hypercholesterolemic group; Student's t- test.*

Fig. 4 : LDL plasma levels in rat fed with a hypercholesterolemic diet, after 15 day-treatment with Rhodiola rosea L. extract.



*Values are expressed as mean ± S.E. of ten rats; *p<0.01, compared with the hypercholesterolemic group; Student's t- test.*

Fig. 5 : HDL plasma levels in rat fed with a hypercholesterolemic diet, after 15 day-treatment with Rhodiola rosea L. extract.



*Values are expressed as mean ± S.E. of ten rats; *p<0.01, compared with the hypercholesterolemic group; Student's t- test.*

DISCUSSION

It is evident from the results that *Rhodiola rosea* L. hydro-alcoholic extract, in our experimental conditions, influences lipid metabolism in rats fed with hypercholesterolemic diet. This activity could be related to the high content of polyphenols in the extract.

It was reported that some polyphenol compounds inhibit HMG-CoA reductase (Hydroxymethyl glutaryl coenzyme A reductase), the key enzyme of HMG-CoA transformation to mevalonate (34, 35). The decrease in cholesterol synthesis might provoke the up-regulation of LDL-receptors that, consequently, leads to a decrease of plasma LDL cholesterol. These

receptors bind and internalize IDL (Intermediate Density Lipoproteins) also and therefore the plasma triglyceride is reduced (36).

Therefore we can hypothesize that the significant decrease of cholesterol and LDL plasmatic levels could be related, in part, to this mechanism.

It is well known that *R. rosea* is able to stimulate lipoprotein lipase (LPL) activity; this enzyme is involved in fatty acids mobilization from adipose tissue. This effect has been linked to rosavin, cynnamic glycoside contained in this species only; in fact it was observed that other species belonging to *Rhodiola* genus did not manifest any activity on LPL (37).

Chronic administration of *R. rosea* extract did not provoke a loss of appetite, in fact it was not observed a reduction in food consumption. On the other hand it was not evident any increase in body weight in the group of rats treated with *R. rosea* extract *versus* hyperlipidemic controls. It can be hypothesized that *R. rosea* extract increases catabolism of lipids accumulated in adipose tissue producing a decrease in mean body weights.

Moreover, *R. rosea* extract showed antioxidant activity; the effects on lipid metabolism can be related to the free radical scavenging properties of polyphenol compounds contained in the extract. As it is known, several flavonoid compounds were isolated from *R. rosea* root: tricin, kaempferol, gossypetin derivatives, cinnamic glycosides and tyrosol (37). A number of flavonoids have been shown in *in vitro* studies to inhibit the oxidation of LDL (38-40), that has been recognized to play an important role in atherosclerosis (41, 42, 39). Circulating monocytes scavenge oxygen-modified LDL molecules with a very high affinity, up to ten times greater than "native LDL" (41); then, monocytes/macrophages penetrate into the subendothelial space, and this process leads to the formation of atherosclerotic plaques in the arterial wall. Flavonoids can reduce LDL lipid peroxidation by scavenging reactive oxygen/nitrogen species; they can also reduce macrophage oxidative stress by inhibition of cellular oxygenases or by activating cellular antioxidants (such as the glutathione system). Polyphenol compounds might inhibit free radical formation and the propagation of free radical reactions through the chelation of transition-metal ions, particularly those of iron and copper. Several studies have shown that certain flavonoids can protect LDL from being oxidized by these two mechanisms (43, 44).

In conclusion *R. rosea* extract lowers the increased levels of cholesterol, triglycerides and LDL, and increases HDL plasmatic levels; moreover, it possesses antioxidant activity. These preliminary results let us hypothesize that the extract could be effective in the treatment of atherosclerosis.

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