Medical Journal of the
Islamic Republic of Iran

Volume 18
Number 3
Fall 1383
November 2004

CHOLESTEROL LOWERING EFFECT OF *RHEUM RIBS* IN HYPERCHOLESTEROLEMIC RABBITS

M-AL-R. HADJZADEH,* H. PARSAEE,** AND A. SADEGHIAN

From the Departments of Physiology and Pharmacology,** Medical School, Mashhad University of Medical Sciences, Mashhad, Iran.*

ABSTRACT

The effects of ethanolic and aqueous extracts of *Rheum ribs L.*(RR) of the family polygonaceae was studied on serum cholesterol concentration in rabbits. To induce hypercholesterolemia, 0.5 g/kg/day of pure cholesterol powder was given orally to each rabbit for two weeks; then cholesterol was halved and each group of animals was treated with a different regimen for another two weeks. Both the ethanolic and the aqueous extracts significantly decreased serum cholesterol conc-entration. The ethanolic extract decreased serum cholesterol by the end of the first and the second week by 43.9% and 59.1% (both $p<0.001$) respectively; while the cholesterol reduction with aqueous extract was 25.01% ($p<0.05$) and 43.82% ($p<0.01$) at the end of the first and the second week of treatment respectively. The ethanolic extract was found to be more potent than the aqueous one. When comp- ared with nicotinic acid as a positive control, the hypocholesterolemic effect of ethanolic extract was significantly higher than that of nicotinic acid. Therefore, we may conclude that RR extracts have hypocholesterolemic effect and thus can be used in the treatment of hypercholesterolemia which is the major risk factor of cor-onary heart disease (CHD).

MJIRI, Vol.18, No.3, 277-280, 2004.

Keywords: *Rheum ribs*; Rhubarb; Hypercholesterolemia.

INTRODUCTION

Coronary heart disease (CHD) is one of the major causes of death in human communities both in industrial and underdeveloped countries. [1] Hypercholesterolemia is the main primary contributing factor to the development of atherosclerosis which is the leading cause of CHD.[2] Cholesterol lowering therapies reduce the risk of CHD and total morbidity and mortality in patients with CHD. [3,4] In all hypercholesterolemic subjects, in whom cholesterol lowering is recommended, non-drug therapy is a part of the treatment regimen.

**Corresponding author: E-mail: h-parsaee@mums.ac.ir

Both experimental and clinical studies have demonstrated that *rhubarb* species are potentially hypolipidemic plants. [5,6] *Rheum ribs L.* (RR) is a native plant of Iran which grows in mountainous areas of several provinces including Khorasan. It belongs to the polygonaceae family and is locally known as *"Rivas"*. [7] The thick leaf-stalk of RR is used as vegetable, jam or syrup by local people. [8] In traditional medicine leaf-stalk and powder of stalk and roots are used to relieve disorders such as: gastric illnesses, constipation, toothache, headache, kidney and bladder pain, uterine pain, liver disorders, icterus, cold headache due to bile retention, humidity and coldness on the brain, reducing thick and thin phlegm and GI tract hemorrhage. [9] It also is used for increasing appetite and bile secretion. [9,7]

Aqili Khorasani mentioned that Rivas improves digestion and appetite, renders renal secretion more healthy, acts as an excellent cathartic on the bowel and increases peristaltic action and is useful in disorders such as flatulence and irritation of the alimentary canal. [10] Moemen also described Rivas as: improving bile secretion and retention, increasing peristaltic action of the GI-tract and decreasing vapor and flatus. [11]

Various components have been isolated from RR. In a quantitative study from 400g of risoms and roots of RR, these compounds were found: chrysophanol (7mg), physcion (14mg), rhein (9mg), aleo-emodine (29mg), physcion-8-o-glucoside (12 mg), aleo-emodin-8-o-glycoside (35 mg), sennoside-A (4 mg) and rhaponticin (9 mg). [12]

Although hypolipidemic effects of other species of *rhubarb* such as *Rheum rhaponiticum*, [13] *Rheum palmatum*[14] and *Rheum undulatum* [5] have been reported, there was no report of RR; therefore, the hypolipidemic effects of this Iranian native plant was studied.

MATERIAL AND METHODS

A- Experimental animals and treatment

Male rabbits with about 2 kg weight were housed at 21-22^{oC} with a standard diet and tap water. To induce hypercholesterolemia, pure cholesterol powder (Merck, Germany) 0.5g/kg/day was given orally to each rabbit for two weeks; thes the dosage of cholesterol was halved and animals were randomly divided into groups of 6. Group A received distilled water as negative control; group B and group C received 4 g/kg/day of aqueous and ethanolic extract respectively and group D received 600 mg/kg/day of nicotinic acid as positive control for 2 weeks (the end of the study).

B- Preparation of Rheum ribs extract

Stalk and roots of RR were collected from heals of Zoshk (southwest of Mashhad) in July and identified by botanists in a herbarium of Ferdowsi university. Roots were cleaned and washed with water. It was then chapped into small pieces and dried while protected from light and wetness. The dried pieces were powdered and kept in a cupped container until extraction.

Ethanolic extract

Dried powder (120g) was mixed with a sufficient volume of ethanol (96%) and then extracted with a soxhelet apparatus. Extraction was continued for 12-18 hr; after removing the solvent in vacuum the extract was dried in a 50-60^{o}C oven. The dried extract weighed 38.3g, so the extract is 32.08% w/w. The extract was kept in a refrigerator until the experiment.

Aqueous extract

Dried powder (120g) was mixed with distilled water in a balloon; the balloon was shaken for 2 days at room temperature. The preparation was then filtered off through a Gauze mesh and the solvent was dried by evaporation under reduced pressure at 40^{o}C. The final product yielded an 8% w/w dried extract; it was stored in a refrigerator until the experiment.

C- Cholesterol assessment

Blood samples were collected from femoral veins of all rabbits before cholesterol treatment and at the end of each week throughout the experiment. Serum cholesterol was measured by enzymatic method with an autoanalyzer (Technicon /RA-1000/USA).

Statistical analysis

Data were expressed as mean ± SD. Cholesterol concentrations at day 0, 7 and 14 were compared by AVOVA and mean cholesterol concentrations at the end of the first and the second week of cholesterol treatment were compared with week 0 (before treatment) by paired t-test. The results after regimen treatment by the end of the first and the second week were also analyzed by ANOVA and t-test. The differences were considered to be significant when the P values were less than 0.05.

RESULTS

Serum cholesterol concentration was significantly increased in all animals as a result of cholesterol treatment at the end of the first and the second week (Table I). Group A (control) had remained hypercholesterolemic during the last two weeks of the experiment while the dose of cholesterol was halved. In group B, [illegible] tion was significantly decreased by the end of the first and the second week of extract treatment by 25.01% ($p<0.05$) and 43.82% ($p<0.01$) respectively. In group C, treatment with ethanolic extract also resulted in reduction of serum cholesterol significantly by the end of the first and second week by 43.9% and 59.17% (both $p<0.001$) respectively (Fig. 1). In group D, nicotinic acid treatment lead to reduction of serum cholesterol at the end of the first week by 4.19% (NS) and at the end of the second week by 37.1% ($p<0.01$).

DISCUSSION

In the present investigation we have shown that serum

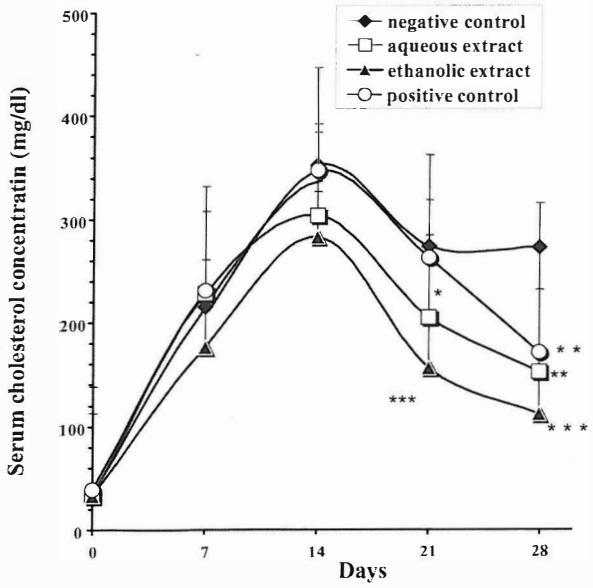

Fig 1. Effects of ethanolic and aqueous extracts of *Rheum ribs* (RR) on serum cholesterol concentration in hypercholesterolemic rabbit

Rabbits were treated with 0.5g/kg/day cholesterol for two weeks and from day 14 the dose of cholesterol was reduced to half and the animal group was treated with 10 mL distilled water as negative control, aqueous extract 4g/kg/day, ethanolic extract 4g/kg/day and nicotinic acid 0.3g/kg/day as positive control. Data are mean ± SD (n=6). Significantly different from negative control (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

cholesterol in hypercholesterolemic rabbits treated with aqueous and/or ethanolic extracts of *Rheum ribs* for 2 weeks, exhibited significant reduction (Table I). Although there are reports about the cholesterol lowering effects of other *rhubarb* species, our study so far is the first one which shows the cholesterol lowering effect of the extracts of *Rheum ribs* which is a native plant in Iran.

Cholesterol fed rabbits were clearly hypercholesterolemic at the end of the second week, and after the dose of cholesterol was halved, they also remained hypercholesterolemic throughout the experiment (Table I). The reduction of serum cholesterol by aqueous extract at the end of the first week after treatment was 25.01% (p=0.023) and after 2 weeks the reduction was 42.82% (p=0.01). Ethanolic extract of *Rheum ribs* also reduced serum cholesterol significantly by the end of the first and the second week of treatment by 43.9% (p=0.0001) and 59.7% (p=0.0001) respectively (Table I).

These results demonstrate that ethanolic extract is more effective in the reduction of cholesterol than the aqueous extract. Since ethanolic extract concentration is 32.08% w/w, and aqueous extract concentration is 8% w/w, and intraquinone derivatives of RR are more soluble in ethanolic extract, it seems that these parameters may be the effective cause for the more potent effect of ethanolic extract on cholesterol reduction.

In the present study, the effect of nicotinic acid on serum cholesterol reduction was insignificant at the end of the first week after treatment; but serum cholesterol was significantly reduced by nicotinic acid by the end of the second week after treatment. This finding shows that the cholesterol lowering effect of nicotinic acid develops after a period of several days after treatment, which is important to pay attention when this drug is used in hypercholesterolemic status.

When compared with nicotinic acid, the reduction in cholesterol concentration by aqueous extract was insignificant, indicating that the cholesterol lowering effects of aqueous extract are in the rank of nicotinic acid. On the

Table I. Serum cholesterol concentrations (mg/dL) in different groups of rabbits.

Days / Groups	0	7	14	21	28
A	34±7.61	215.5±49.09	353±62.33	273.83±33.87	272.66±41.17
B	33±6.23	228±44.18	304.17±55.41	* 205.33±50.59	** 153.17±42.02
C	33.33±8.38	176.66±45.77	283±43.28	*** 156.33±50.59	*** 111.33±45.57
D	38.83±10.46	231.5±45.07	347.33±39.73	262.67±44.46	** 171.5±43.03

All rabbits were supplemented with pure cholesterol powder 0.5g/kg/day for 2 weeks, then cholesterol dosage was halved and groups A, B, C and D were administered 10-15 mL of distilled water as negative control, aqueous extract (4g/kg/day), ethanolic extract (4g/kg/day) and nicotinic acid (600 mg/kg/day) as positive control respectively. The results are expressed as mean ±SD, n=6 (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

other hand, the reduction in serum cholesterol in rabbits treated with ethanolic extract was significantly greater than those treated wiht nicotinic acid. This finding shows that the ethanolic extract of RR can induce more reduction in cholesterol concentration of hypercholesterolemic rabbits and may be more effective in the treatment of hypercholesterolemia.

Li and Liu [15] have reported that *Rheum emodi* treatment in 5/6 nephrectomised rats significantly decreased plasma cholesterol and triglycerides. Li and Ye et al. [16] have also reported that the extract from *Rheum hotaoense* in normal and hyperlipidemic rabbits significantly reduced serum cholesterol, serum triglyceride/β-lipoprotein (LDL) and pre-β lipoprotein (VLDL). This result also supports the findings of Goel et al. [6] that daily ingestion of *rhubarb* stalk fiber in hypercholesterolemic men for 4 weeks, significantly reduced serum total cholesterol and LDL-cholesterol, while the HDL-cholesterol level remained unchanged. Their results clearly demonstrate the potential effects of rhubarb on cholesterol lowering in men. In another study, Goel et al. [13] have reported that in cholesterol-supplemented mice, *Rheum rhaponticum* stalk fiber significantly reduced plasma cholesterol, hepatic concentration of cholesterol and cholesteryl esters content. *Rhubarb* fiber feeding also significantly reduced the activity of acyl-coA and cholesterol acyl transferase; and also increased the fecal bile acid loss and the activity of cholesterol 7α-hydroxylase. They proposed that the hypocholesterolemic effect of *Rhubarb* fiber may be due to the increased excretion of bile acid and induction of 7α-hydroxylase activity in mice. These results are in agreement with our finding concerning the cholesterol lowering effect of RR which is in another rhubarb species.

Abe et al. [14] have suggested that the cholesterol lowering effect of *Rhubarb (Rheum palmatum)* may be due to the potent inhibitory effect of squalen epoxidase which is a rate-limiting enzyme of cholesterol biosynthesis. As mentioned by Mericli and Tuzlaci [12] the cholesterol lowering effects of RR may be due to rhaponticin which is present in this plant. Perhaps this is the first report depicting the hypocholesterolemic effect of RR extracts, suggesting its effectiveness in the treatment of a hypercholesterolemic status and thus its important role as a non-drug therapy in preventing CHD.

In conclusion, the present study provides substantial evidence for the cholesterol lowering effect of RR extracts. Therefore, it might be suggested that the leaf-stalk of the plant or the extracts of it may have beneficial effects in the hyperlipidemic status.

REFERENCES

1. Ernest JS, Margaret EB: Diet, lipoprotein and coronary heart disease. Endocrinology and Metabolism Clinics of North America September: 27 (3): 711-731, 1998.
2. Jeffrey MH: Lipoproteins and atherogenesis. Endocrinology and Metabolism Clinics of North America September: 27 (3): 569-584, 1998.
3. Scott MG: The role of cholesterol management in coronary disease risk reduction in elderly patients. Endocrinology and Metabolism Clinics of North America September: 27(3): 655-675, 1998.
4. Scott MG: Cholesterol management in the era of managed care. American Journal of Cardiology 85: 3A-9A, 2000.
5. Peigen XH, Liwei W: Ethnopharmacologic study of Chinese rhubarb. Journal of Ethnopharmacology 10: 275-293, 1984.
6. Goel V, Ooraikol B, Basu TK: Cholesterol lowering effects of rhubarb stack fiber in hypercholesterolemic men. Journal of American College of Nutrition Dec. 16: 600-604, 1997.
7. Zargari A: Medicinal Plants. University publication,Tehran (in Persian). 5th edition, vol. 4, pp. 233-241, 1992.
8. Miraldi E, Ferri S, Mostaghimi V: Botanical drugs and preparations in the traditional medicine of West Azarbaijan (Iran). Journal of Ethnopharmacology 75: 77-87, 2001.
9. Mir Heidar H: Encyclopedia of Medicinal Plants of Iran. Islamic Culture Press,Tehran (in Persian), vol. 1, pp. 92-95, 1993.
10. Aqili Khorasani MH: Ribas (in Persian) in: Makhzan-Al-Adviah, Aqili Khorasani, MH., (ed.), Enqelab-e Islami Publishing and Educational Organization, second ed., Tehran, Iran, p. 458, 1992.
11. Moemen Hosaini M: Rivand (Rivas in Persian) In: Tohfah Hakim, Moemen Hosaini M, (ed.), Mostafavy publication, Qhum, Iran, p: 124, 1969.
12. Mericli AH, Tuzlaci E: Constituents of *Rheum ribs*. Fitoteropia 67 (4): 375, 1990.
13. Goel V, Cheema SK, Agellon LB, Ooraikul B, Basu TK: Dietary rhubarb (*Rheum rhaponticum*) stalk fiber stimulates cholesterol 7-alpha-hydroxylase gene expression and bile acid excretion in cholesterol-fed C57BL/6J mice. British Journal of Nutrition, Jan. 81: 65-71, 1999.
14. Abe I, Seki T, Noguchi H, et al: Galloyl esters from rhubarb are potent inhibitors of squalene epoxidase, a key in cholesterol biosynthesis. Planta Med Dec 66: 753-6, 2000.
15. Li LS, Liu ZH: Clinical and experimental studies of rheum on preventing progression of chronic renal failure. Zhong Xi Yi Jie He Za Zhi 11:392-6, 387, 1991.
16. Li C, Ye Y: Effect of rhapontin of *Rheum hotaoense* on lipid and lipoprotein level serum. Acta Pharmaceutica Sinica 17: 699-702, 1981.