

Study on the Effect of *Paliurus spina-christi* on Cholesterol, Triglyceride and HDL Levels in Diabetic Male Rats Fed a High Cholesterol Diet

Mahmoud Mosaddegh^{*a}, Mohammad Javad Khoshnood^b, Mohammad Kamalinejad^a,
Elham Alizadeh^b

^aTraditional Medicine and Materia Medica Research Center, Shaheed Beheshti University of Medical Sciences, Tehran, Iran. ^bSchool of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.

Abstract

Paliurus spina-christi Mill (Rhamnaceae) has been used in folk medicine as a hypocholesterolemic agent. In this study, the effect of total fruit extract on lowering lipid content of serum was investigated. Male rats were introduced streptozocin, subsequently fed with a high cholesterol diet and concomitantly treated with various doses of the extract. After 8 days, serum levels of cholesterol, triglyceride and HDL were measured. It was found that the administration of 100 mg/kg/day of the extract had reduced the amount of total cholesterol by 45.5% and the administration of 50 mg/kg/day reduced the amount of triglyceride by 48.73%. No significant enhancement was found in the amount of HDL. It was concluded that of *Paliurus spina-christi* extract can inhibit the enhancement of cholesterol and triglyceride.

Keywords: Hypercholesterolemia; Triglyceride; Streptozocin; *Paliurus spina-christi*.

Introduction

Paliurus spina-christi Mill. (Rhamnaceae), also known as christ's thorn, is a perennial thorny shrub of wide distribution in dry and rocky places in the Mediterranean region and Asia (1). It grows mostly in the northern parts of Iran and is used as a diuretic and anti-hypertensive agent (2). Chemical investigation of fruits and leaves of *Paliurus spina-christi* Mill. have shown that rutin, quercetin and quercetin 3-O-rhamnoglucoside 7-O-rhamnoside are the main flavonoid components (3, 4). In addition, the fruits contain alkaloids (5, 6). Recently, a remarkable antibacterial activity of the ethanolic extracts from different plant parts has been reported (7).

Hyperlipidemia is well known to play a main role in the development of atherosclerosis

(8). Accumulation of lipids in the arterial wall plays a crucial role in the genesis of atherosclerosis by impairing the endothelial function. Endothelial dysfunction can initiate vasoregulation, platelet and monocyte adhesion, vascular smooth muscle cell growth and oxidization of LDL (9, 10). Atherosclerotic plaque contains foam cells that originate from macrophages and smooth muscle cells (11). Hence, it is obvious that lowering the lipid levels could reduce the risk of Cardiac Heart Disease (CHD) by regression of atherosclerosis. No literature has been found in respect of hypolipidemic properties of *Paliurus spina-christi* in the literature.

Experimental

Plant material

Fruits of *Paliurus spina-christi* were collected in July from Marzanabad

* Corresponding author:

Email: mmosaddegh@itmrc.org

(Mazandaran province). The plant was identified and a voucher specimen was deposited at TMRC, numbered as 123. The fruits were dried and stored.

Preparation of the extract

The fruits were weighed (500 g) and boiled in distilled water for 1 h. It was then maintained for 24 h and finally filtered. The filtrate was concentrated to a greasy material using a rotary evaporator. It was then dried under laboratory conditions.

Preparation of the standard and hypercholesterol diet

The standard diet was prepared by blending the standard animal diet. The hypercholesterol diet was prepared by mixing the standard powdered diet with 1.5% cholesterol and 0.2% cholic acid.

Condition and preparation of the animals

Fifty eight male rats weighing 280 to 320 g were divided into seven groups: three control and four test groups. The first group of rats were normal rats fed with the standard powdered diet and took normal saline (N.S.) as treatment. The second group were diabetic rats, fed with the standard powdered diet and N.S. as the treatment drug. The third group were diabetic rats fed hypercholesterol diet and N.S. as treatment. The other groups were the test groups, which were diabetic and fed with the hypercholesterol diet and 50, 100, 200 or 400 mg/kg/day of *Paliurus spina-christi* extract (Table1).

It should be mentioned that rats were made diabetic by administering a single dose of streptozocin (STZ) (60 mg/kg) at the beginning of the study. They were then kept for one week and at the beginning of the second week treated

Table 1. Characteristics of the different groups of rats used in this study.

Group	Number of rats	Rat	Diet	Drug administered
1	10	Naive	Standard	N.S.
2	8	STZ	Standard	N.S.
3	8	STZ	Cholesterol	N.S.
4	8	STZ	Cholesterol	50 mg/kg/day of the extract
5	8	STZ	Cholesterol	100 mg/kg/day of the extract
6	8	STZ	Cholesterol	200 mg/kg/day of the extract
7	8	STZ	Cholesterol	400 mg/kg/day of the extract

with the drug and a hypercholesterol diet concomitantly.

The animals were kept in well ventilated cages under standard conditions for 8 days. At the end, they were kept for overnight fasting. The samples were then collected by cardiac puncture. Livers of the animals were removed and investigated at the end of the study.

Serum cholesterol, triglyceride and HDL estimation

Serum cholesterol, triglyceride and HDL levels were measured using enzymatic methods.

Statistical analysis

Data are expressed as mean±SE. Differences between groups were considered to be significant at $P<0.05$, using the one-way analysis of variance (one-way ANOVA) followed by Dunnet's or scheffee multiple comparison tests.

Results and Discussion

In this study, serum values obtained from the parameters investigated groups 2 and 3 (control groups) were compared with those of the normal group (group1), while values of various parameters belonging to the test groups were compared with group 3.

Introducing only STZ (alone) to rats (group 2), had no significant effect on the amounts of cholesterol, triglyceride and HDL as well as the ratio of HDL/TG. However, introducing STZ to the rats along with feeding them with hypercholesterol diet (group 3) significantly increased cholesterol and triglyceride levels and decreased the ratio of HDL/TG compared with group 1; Nevertheless, in all cases it had no significant effect on the HDL level (Table 2).

When hypercholesterol diet was co-administered with different doses of *Paliurus spina-christi* fruit extract, the cholesterol and triglyceride increasing effect of diet was partially decreased. The reduction cholesterol level was significant in group 5 (dose of 100mg/kg/day) which showed a 45.5% reduction compared to group 3. The reduction observed in the triglyceride level was found to be significant in group 4 (dose of 50mg/kg/day), showing a 48.73% reduction, compared to group 3. Levels of HDL did not show any

Table 2. Effect of the treated regimen and *Paliurus spina-christi* fruit extract on various serum factors.

Group	Cholesterol*	Triglyceride*	HDL*	HDL/T.C.*
1	86.59 ± 2.33	64.19 ± 3.39	42.00 ± 2.46	0.49 ± 0.02
2	87.51 ± 3.87	88.21 ± 10.17	30.79 ± 3.34	0.39 ± 0.05
3	159.95 ± 29.03 ^a	126.92 ± 18.84 ^a	34.39 ± 3.91	0.26 ± 0.06 ^a
4	116.02 ± 6.73	65.06 ± 7.31 ^b	36.24 ± 2.83	0.33 ± 0.04
5	87.17 ± 7.76 ^b	112.09 ± 15.33	40.94 ± 8.07	0.46 ± 0.06 ^b
6	145.99 ± 27.36	110.18 ± 7.67	25.08 ± 4.82	0.18 ± 0.02
7	120.75 ± 12.55	101.78 ± 6.73	28.44 ± 2.11	0.24 ± 0.02

a) Significantly different from group 1

b) Significantly different from group 3

*Values are presented as mean±SE for 8 rats, except for group 1 which had with 10 rats.

significant changes, but the ratio of HDL/TG was significantly increased in group 5 compared to group 3. In the current study the amount of relative liver weight, weight differences of first and last day and food intake were also measured but no significant changes were observed and thus no data has been, were not mentioned.

Although high cholesterol diet hardly changes the plasma cholesterol concentration of rats (12), cholesterol feeding for one week induced a remarkable increase in plasma cholesterol level in STZ treated rats (Table 2) (13). Recent studies have shown an increased cholesterol absorption in STZ rats (14) thus, this method could be a fast and easy way for development of hypercholesterolemia.

Cholesterol levels in test groups were decreased compared to group 3 and the reduction was significant in the dose of 100 mg/kg/day. Chemical investigations on *Paliurus spina-christi* fruits indicated the presence of flavonoids (isoquercetin and rutin). Rhamnetin and isorhamnetin are metabolites of these flavonoids in rat plasma (15) and it has been shown that both quercetin and rhamnetin have cholesterol decreasing properties in rats fed a high cholesterol diet (16). This could be the reason for the cholesterol lowering efficacy of this plant.

As for cholesterol, the regimen administered to rats could effectively increase triglyceride level (Table 2). Administration of fruit extract can also decrease levels of triglyceride compared to group 3 (Table 2). This reduction is significant in group 4 (dose of 50mg/kg/day), suggesting that the triglyceride lowering effect observed with the extract is due to the presence of quercetin (17).

The ratio of HDL/TG has been significantly decreased in group 5, compared with group 3

(Table 2). The antioxidant effect of flavonoids, which could reduce the amount of oxidized LDL (18) (LDL is the main initial cause of atherosclerosis) is predicted to be the reason for observing this effect.

In conclusion, it seems that *Paliurus spina-christi* fruit could be a good candidate for clinical investigation towards the treatment of hyperlipidemia. This can explain the reason for its use in traditional medicine as a lipid-lowering plant. Further studies are needed to understand the exact mechanism of action of this plant.

References

- (1) Kustruk D and Males Z. Flavonoids of the leaves of christ's thorn. *Acta Pharm. Jugosl.* (1990) 40: 551-54
- (2) Zargari A. *Medicinal Plants*, Vol. 1, 4th ed, Tehran University Publishing Co., Tehran (1986) 506-608
- (3) Branter AH and Males Z. Quality assessment of *Paliurus spina-christi*. *J. Ethnopharmacol.* (1999) 66: 175-79
- (4) Branter A and Males Z. Investigation on the flavonoid glucosides of *Paliurus spina-christi*. *Planta Med.* (1990) 56, 582
- (5) Dalakishvili TM, Gusakova SD, Chachanidze NJ, Kuparadze KG and Kemertelidze EP. Lipids of *Paliurus spina-christi* seed. *Khimija Prirodnii Soedinenii* (1985) 5: 322-26
- (6) Velcheva, MP. Constituents of *Paliurus spina-christi*. *Fitoterapia* (1993) 64: 284-5
- (7) Branter AH, Males Z, Pepeljnjak S and Antolic A. Antimicrobial activity of *Paliurus spina-christi* Mill. (christ's thorn). *J. Ethnopharmacol.* (1996) 52: 119-22
- (8) Kaplan NM. The Deadly quarter; upper body weight, glucose intolerance hypertriglyceridemia and hypertension. *Arch. Intern. Med.* (1989) 149: 1514-15
- (9) Vogel RA. Coronary risk factors, endothelial function, and atherosclerosis, a review. *Clin. Cardiol.* (1997) 20: 426-32
- (10) Shaila HP, Udapa AL and Udapa SI. Preventive action of *Terminallia bellerica* in experimentally induced atherosclerosis. *Int. J. Cardiol.* (1995) 49: 101-106

- (11) Ross R. The pathogenesis of atherosclerosis: an update. *New Engl. J. Med.* (1986) 164: 553-8
- (12) Nakura H, Tanaka M, Tateishi T, Watanabe M, Kumai T and Kobayashi, S. The effect of streptozocine induced hypoinsulinemia on serum lipid levels in spontaneously hyperlipidemic rats. (1997) *Horm. Metab. Res.* 29: 437-57
- (13) Sakuma Y, Hagihara H, Nagayoshi A, Ohne K, Mutoh S, Ito Y, Nakahara K, Nosto Y, Okuhara M, Ohne K, Mutoh S, Ito Y and Nakahara K. Effect of FR 14523: an ACAT inhibitor on diet Induced hypercholesterolemia in rats. *Life Sci.* (1996) 60: 351-56
- (14) Holmojern PA and Brown AC. Serum cholesterol levels of non-diabetic and streptozocin-diabetic rats fed a high cholesterol diet. *Artery J.* (1993) 20: 337-45
- (15) Manach C, Morand C, Demigne C, Texier B, Regerat F and Remesy E. Bioavailability of rutin and quercetin in rats. *FEBS Letters* (1997) 409: 12-16
- (16) Igarashi K and Ohmuma M. Effects of isorhamnetin, rhamnetin and quercetin on concentration of cholesterol and lipoperoxidase in the serum and liver and on the blood and liver antioxidative enzyme activities of rats. (1995) *Biosci. Biotechnol. Biochem.* 59: 595-601
- (17) Lauridsen ST and Mortensen A. Probucol selectively Increases oxidation of atherogenic lipoproteins in cholesterol fed mice. *Atherosclerosis* (1999) 142: 169-78
- (18) Afanas've IB, Ostrachovitch EA, Abramova NE and Korkina LG. Different antioxidant activities of bioflavonoid rutin in normal and iron overloading rats. *Biochem. Pharmacol.* (1995) 80: 627-35

Archive of SID