Antihyperlipidemic Effect of Flavonoids from Pterocarpus marsupium

M. A. Farboodniay Jahromi, Anil B. Ray, and J. P. N. Chansouria

J. Nat. Prod., **1993**, 56 (7), 989-994• DOI:
10.1021/np50097a001 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 7, pp. 989-994, July 1993

ANTIHYPERLIPIDEMIC EFFECT OF FLAVONOIDS FROM *PTEROCARPUS MARSUPIUM*

M.A. FARBOODNIAY JAHROMI, ANIL B. RAY,*

Department of Medicinal Chemistry

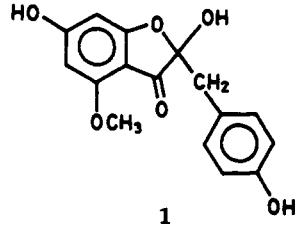
and J.P.N. CHANSOURIA

Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India

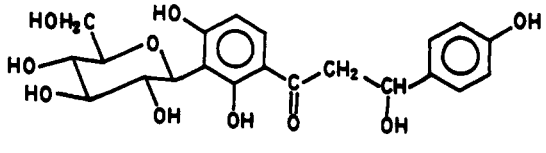
ABSTRACT.—Serum lipid levels in rats with hyperlipidemia induced by diet as well as by Triton were determined after oral administration of EtOAc extract of *Pterocarpus marsupium* heartwood and its flavonoid constituents, marsupsin [**1**], pterosupin [**2**], and liquiritigenin [**3**]. Administration of EtOAc extract for 14 consecutive days produced a significant reduction of serum triglyceride, total cholesterol, and LDL- and VLDL-cholesterol levels without any significant effect on the level of HDL-cholesterol. Liquiritigenin and pterosupin were able to effect a significant fall in serum cholesterol, LDL-cholesterol, and atherogenic index, pterosupin being additionally effective in lowering serum triglyceride.

The search for new drugs with the ability to reduce and/or regulate serum cholesterol and triglyceride concentrations has gained momentum over the years, resulting in a plethora of publications reporting significant activity of a variety of natural (1,2) and synthetic (3–5) agents. Molecular modification of naturally occurring lead compounds has also given rise to potent agents like pravastatin and simvastatin; the former prepared by replacement of the methyl group of the naturally occurring lovastatin by a hydroxyl group and the latter a methylated derivative of compactin (6).

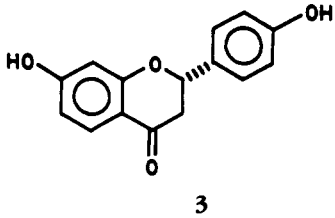
In continuation of our search for plant-derived antihypercholesterolemic and



1



2



3

hypolipidemic agents (7), we directed our attention to *Pterocarpus marsupium* Roxb. (Leguminosae), a plant essentially known for its hypoglycemic activity (8) but the hypolipidemic activity of which has also been reported in the Indian system of medicine (9). The reported hypocholesterolemic activity of the aqueous decoction of the heartwood of the plant (9) was substantiated by our work, and it was further observed to reduce triglyceride levels in the serum of experimental animals. In an attempt to locate the constituent(s) responsible for this activity, the aqueous decoction was exhaustively extracted with EtOAc, and the residue obtained from the organic solvent was found to be equally as potent as the aqueous decoction in lowering serum cholesterol and triglycerides (Table 1). This observation prompted us to explore further the possible hypolipidemic activity in pure constituents of the extract.

Pt. marsupium heartwood is known to be a rich source of flavonoids and related phenolic compounds, reported from our laboratory (10,11) and elsewhere (12,13). The detection of the compounds present in the EtOAc extract by tlc analysis and characterization of the isolated constituents by direct comparison with authentic samples was, therefore, easily achieved. Out of the seven compounds found to be present in the EtOAc-soluble fraction of the aqueous decoction, marsupsin [**1**], pterosupin [**2**], and liquiritigenin [**3**] were the major constituents. Hypolipidemic activities of these three compounds were investigated in two different experimental models.

MATERIALS AND METHODS

ANIMALS.—Male albino rats of Charles Foster strain (200–250 g), procured from the Central Animal House of the Institute, were used for this study. The animals were kept in polypropylene cages (3 in each cage) under ambient temperature of 25±2° and 55–65% relative humidity with a 12±1 h light-dark schedule. The animals had free access to H_2O and normal laboratory diet (Lipton India Ltd.).

PLANT MATERIAL.—The heartwood of *Pt. marsupium* used in this study was collected from Varanasi (10) and authenticated by Dr. R.L. Khosa, Division of Pharmacognosy, Department of Pharmaceutics, Banaras Hindu University. A specimen sample is being preserved in the department.

PREPARATION OF AQUEOUS DECOCTION.—Dried heartwood (100 g) of *Pt. marsupium* was pulverized, boiled with distilled H_2O (800 ml) until the volume was reduced to less than 100 ml, and filtered, and the volume was adjusted to 100 ml in order to obtain an extract 1 ml of which corresponds to 1 g of the drug.

PREPARATION OF EtOAc EXTRACT.—Aqueous decoction of *Pt. marsupium* (500 ml) was extracted with EtOAc in a liquid-liquid extractor. The EtOAc fraction was evaporated to dryness in a rotary vacuum evaporator to afford a brown hygroscopic powder (58.95 g).

DETECTION OF PHYTOCHEMICALS IN EtOAc EXTRACT.—Resolution of EtOAc extract over thin layer chromatoplates (SiO_2) with different developing solvents revealed the presence of seven compounds, six of which were identified as marsupsin [**1**], pterosupin [**2**], liquiritigenin [**3**], pterostilbene, isoliquiritigenin, and 3-(*p*-hydroxyphenyl)-lactic acid by co-chromatography with authentic samples previously isolated from this source (11). The seventh compound was characterized as pterocarpol, a sesquiterpene alcohol not previously reported from this species, by its isolation and spectral comparison with reported data (14).

ISOLATION OF MARSUPSIN, PTEROSUPIN, AND LIQUIRITIGENIN.—Marsupsin [**1**], pterosupin [**2**], and liquiritigenin [**3**] were isolated according to the procedure of Maurya *et al.* (10,11) and identified by direct comparison with authentic samples (mp, co-tlc, ^{1}H nmr).

EXPERIMENTAL HYPERLIPIDEMIC DIET.—Experimental diet consisted of a well pulverized mixture of cholesterol (2%), cholic acid (1%), sucrose (40%), peanut oil (10%), and normal laboratory diet (47%).

EXPERIMENTAL HYPERLIPIDEMIC AND HYPOCHOLESTEROLEMIC AGENTS.—A suspension of Triton-WR 1339 (Sigma, USA) in 0.15 M NaCl was used for inducing hyperlipidemia (15) in experimental rats. Aqueous suspension of cholestyramine (Laboratories Allard S.A., Paris) was used as a reference hypocholesterolemic agent (16).

EXPERIMENTAL PROCEDURE.—*Diet-induced hyperlipidemic model.*—The EtOAc extract suspended in gum acacia, and marsupsin, pterosupin, liquiritigenin, and cholestyramine suspended in 5% EtOH/saline were each administered at the doses indicated in the tables, once daily in the morning through gastric

intubation for 14 consecutive days to rats receiving hyperlipidemic diet. The control animals received the hyperlipidemic diet and the drug vehicle. Four hours after administration of last dose, rats were sacrificed by decapitation, and blood was collected through cardiac puncture. Serum was separated by centrifugation at 3000 rpm and stored at −20° pending biochemical analysis.

Triton-induced hyperlipidemic model.—Animals kept fasting for 24 h were administered ip a saline solution of Triton (15) at the dose of 400 mg/kg body wt. Pterosupin and liquiritigenin at a dose of 75 mg/kg body wt were administered orally through gastric intubation, the first dose being given immediately after Triton injection and second dose 20 h later. After 4 h of the second dose, the animals were sacrificed by decapitation, and serum from the blood samples was separated by centrifugation and stored at −20° until used for biochemical analysis.

STATISTICS.—The significance of differences between the control and drug treated groups was tested using Student's *t*-test.

BIOCHEMICAL ESTIMATIONS.—Serum samples obtained from control and drug-treated groups were analyzed for total cholesterol according to the method of Abell *et al.* (17), for triglycerides by the method of Gottfried and Rosenberg (18), and for serum total lipid according to the method of Frings and Dunn (19). Serum HDL-cholesterol was determined by use of a commercial kit (Span Diagnostic India, Ltd.). Serum LDL- and VLDL-cholesterol were calculated by the procedure of Friedwald *et al.* (20). The HDL-cholesterol/total cholesterol ratio and total cholesterol − HDL-cholesterol)/HDL-cholesterol were calculated. While the former ratio has been considered by some authors (21) to be an index of atherogenesis, others (27) have defined atherogenic index (A.I.) by the latter ratio, which has been used in this paper.

RESULTS

EFFECT OF EtOAc EXTRACT ON LIPID CONSTITUENTS IN SERUM.—Oral administration of EtOAc-soluble fraction of aqueous decoction of *Pt. marsupium* heartwood at a dose of 120 mg/kg body wt to hyperlipidemic rats for 14 days showed significant reduction of serum cholesterol, triglyceride, and LDL- and VLDL-cholesterol without any effect on HDL-cholesterol level (Table 1). Oral administration of aqueous decoction at a dose of 1 ml/kg body wt elicited nearly identical results (Table 1).

EFFECT OF MARSUPSIN, PTEROSUPIN, AND LIQUIRITIGENIN ON LIPID CONSTITUENTS IN SERUM.—Of the different pure principles tried on diet-induced hyperlipidemic rats, pterosupin [**2**] and liquiritigenin [**3**] were significantly effective in lowering serum cholesterol, LDL-cholesterol, and atherogenic index and raising the level of HDL-cholesterol and HDL-cholesterol/total cholesterol ratio. Pterosupin was additionally effective in reducing serum triglycerides. The results obtained revealed the activity of

TABLE 1. Effect of Oral Administration of Aqueous Decoction of *Pterocarpus marsupium* heartwood and of Its EtOAc-soluble Fraction on Serum Lipids and Lipoprotein Levels in Rats Fed on an Experimental Hyperlipidemic Diet.[a]

Treatment (14 days)	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Control	338.8±9.8	188.5±10.05	[b]	[b]	[b]
Aqueous decoction (1.0 ml/rat/day)	269.9±11.7[c]	155.2±7.8[d]	[b]	[b]	[b]
Control	323.3±6.8	198.1±10.8	28.5±3.1	255.1±6.3	39.6±2.2
EtOAc extract (120 mg/kg body wt)	282±12.4[e]	168.6±4.8[d]	28.2±3.9	220.3±12.6[d]	33.7±0.9[d]

[a]TC=Total cholesterol; TG=triglyceride; HDL-C=high-density lipoprotein cholesterol; LDL-C=low-density lipoprotein cholesterol; VLDL-C=very low-density lipoprotein cholesterol. Values are mean ± SEM for six rats.

[b]These parameters have not been studied with the administration of aqueous decoction.

[c]Significantly different from control value, $p<0.01$.

[d]Significantly different from control value, $p<0.05$.

[e]Significantly different from control value, $p<0.02$.

TABLE 2. Effect of Oral Administration of Major Flavonoid Constituents Present in Aqueous Decoction of *Pterocarpus marsupium* Heartwood on Serum Lipid and Lipoprotein Levels in Rats Fed on an Experimental Hyperlipidemic Diet.[a]

Treatment (14 days)	TC (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	*HDL-C*/TC	A.I.
Control	357.8±11.8	181.9±8.7	34.4±1.5	287.0±10.1	36.4±1.7	0.09±0.01	9.5±0.6
Cholestyramine (reference) (150 mg/kg body wt per day)	286.3±11.6[d]	171.9±4.6	39.9±1.4[b]	211.3±12.2[d]	34.4±0.9	0.13±0.02	6.1±0.3
Liquiritigenin [3] (40 mg/kg body wt)	323.2±6.0[b]	198.1±10.8[b]	38.01±0.6[c]	245.7±6.4[d]	39.6±2.2	0.11±0.02	7.5±0.2[d]
Marsupsin [1] (40 mg/kg body wt)	350.6±28.5	190.5±9.8	32.0±3.8	292.8±30.6	41.1±1.3	0.09±0.01	—
Pterosupin [2] (40 mg/kg body wt)	312.2±10.1[c]	146.2±9.1[c]	38.6±0.8[b]	244.2±11.1[c]	29.2±1.8[c]	0.12±0.01	7.05±0.3[d]

[a]Values are mean ± SEM for six rats.
[b]Significantly different from control level, $p<0.05$.
[c]Significantly different from control level, $p<0.01$.
[d]Significantly different from control level, $p<0.001$.

TABLE 3. Effect of Oral Administration of Liquiritigenin and Pterosupin on Serum Lipid Levels in Triton-induced Hyperlipidemic Rats.[a]

Treatment (2 days)	Total Cholesterol	Triglyceride	Total lipid
	mg%		
Control (Triton 400 mg/kg body wt)	498.9±27.3	614.3±28.6	1125.7 ±85.4
Triton + Liquiritigenin [**3**] (75 mg/kg body wt)	406.7±20.9[b]	572.9±23.1	837.3±71.5[b]
Triton + Pterosupin [**2**] (75 mg/kg body wt)	374.4±18.3[c]	402.9±34.3[d]	763.9±93.3[c]

[a]Values are mean ± SEM for six rats.
[b]Significantly different from control level, $p<0.05$.
[c]Significantly different from control level, $p<0.01$.
[d]Significantly different from control level, $p<0.001$.

pterosupin to be comparable to that of cholestyramine, a well-known hypocholesterolemic agent (Table 2).

Both total cholesterol and total lipid levels were significantly reduced by pterosupin and liquiritigenin in the Triton-induced hyperlipidemic group. Serum triglyceride level was also significantly reduced by administration of pterosupin but not with liquiritigenin (Table 3). The hypolipidemic activity of pterosupin was thus manifest in both the experimental models.

DISCUSSION

Pt. marsupium is primarily known for its hypoglycemic activity, and (−)-epicatechin, a constituent of the stem bark of this plant, is believed to be the principle responsible for this activity (22) though the validity of this claim has been questioned (23,24). The hypolipidemic activity of this plant is also on record, and following the lead of a preliminary communication (9) describing the cholesterol-lowering activity of the aqueous decoction of the heartwood of the plant, a systematic investigation was taken up. The investigation not only substantiated the reported efficacy of the aqueous decoction of the heartwood of the plant as a hypocholesterolemic agent (Table 1) but also succeeded in the isolation of the active constituents from this fraction. The observation that the active principles were extractable from the aqueous decoction by EtOAc permitted separation of the constituents present in this extract into three major and four minor constituents. The major constituents were identified as marsupsin; a benzofuranone flavonoid, pterosupin; a *C*-glucosyl-β-hydroxydihydrochalcone; and liquiritigenin, a well-known flavanone. Flavonoids are known for their diverse biological activities, including hypolipidemic activity. The flavonoids quercetin, epicatechin hesperidin, prunin, and hesperetin-5-*O*-glucoside have been shown to reduce cholesterol levels of experimental diet-induced hyperlipidemic rats (25–27). The isoflavones biochanin A, formononetin, and protensein are known to reduce serum cholesterol and triglyceride in rats treated with Triton-WR 1339 (28). In view of these reports, the major flavonoids of the EtOAc extract were tested for their possible hypolipidemic activity in two different experimental models, and pterosupin [**2**] and liquiritigenin [**3**] were proved to be effective agents in lowering total cholesterol and other lipid and lipoprotein levels. Pterosupin was particularly effective, and its hypolipidemic and hypocholesterolemic activity was comparable to that of cholestyramine.

The importance of pterosupin and liquiritigenin lies in their ability to reduce atherogenic index and elevate the ratio of HDL-cholesterol/total cholesterol. This ratio has been considered to be the major factor in predicting coronary heart disease in human beings, and an increase in this ratio is believed to have a beneficial effect since there is an inverse relationship between plasma HDL-cholesterol level and coronary heart disease (29).

Pt. marsupium thus possesses both hypoglycemic and hypolipidemic activity as is known in traditional use. Such dual property has also been reported in *Prunus davidiana* (Rosaceae) MeOH extract and its flavonoid constituent, prunin (27). The EtOAc-soluble fraction of the aqueous decoction of *Pt. marsupium* contains several other constituents in addition to the three major compounds studied, but the low yield of these compounds precluded determination of their probable hypolipidemic activity. Further work would be necessary to evaluate the activity of the minor constituents and to assess the mode of action of pterosupin and liquiritigenin.

ACKNOWLEDGMENTS

We thank Dr. S.C. Sinha, Scripps Clinic and Research Foundation, California, USA for providing spectra of pterocarpol and to Mr. S.M. Singh for his technical assistance in this work.

LITERATURE CITED

1. Y. Tsujita, M. Kuroda, and Y. Shimada, *Biochim. Biophys. Acta,* **877**, 50 (1986).
2. A.D. Patil, J.A. Chan, P.L. Flamberg, R.J. Mayer, and J.W. Westley, *J. Nat. Prod.,* **52**, 153 (1989).
3. P. Cozzi, U. Branzoli, P.P. Lovisolo, G. Orsini, G. Carganico, A. Pillar, and A. Chiari, *J. Med. Chem.,* **29**, 404 (1986).
4. I.H. Hall, O.T. Wong, A.R.K. Murthy, P.A. Day, and J. Calvin, *Pharm. Res. Commun.,* **19**, 839 (1987).
5. V.G. DeVries, J.D. Bloom, M.D. Dutia, A.S. Katocs Jr., and E.E. Largis, *J. Med. Chem.,* **32**, 2318 (1989).
6. D.R. Illingworth, *Drugs,* **41**, 151 (1991).
7. M.A.F. Jahromi, J.P.N. Chansouria, and A.B. Ray, *Phytother. Res.,* **6**, 180 (1992).
8. G.V. Satyavati, A.K. Gupta, and N. Tandon, "Medicinal Plants of India," Vol. 2, ICMR Publications, New Delhi, India, 1987, p. 533.
9. M.C. Pandey and P.V. Sharma, *Journal of Research on Medicine, Yoga, and Homeopathy,* **13**, 137 (1978).
10. R. Maurya, A.B. Ray, F.K. Duah, D.J. Slatkin, and P.L. Schiff Jr., *Heterocycles,* **19**, 2103 (1982).
11. R. Maurya, A.B. Ray, F.K. Duah, D.J. Slatkin, and P.L. Schiff Jr., *J. Nat. Prod.,* **47**, 179 (1984).
12. D. Adinarayan, S.K. Venkata, O. Seligman, and H. Wagner, *Z. Naturforsch.,* **13C**, 145 (1982).
13. J. Mathew and A.V. Subba Rao, *Phytochemistry,* **22**, 794 (1983).
14. M.R. Parthasarathy and T.R. Seshadri, *Tetrahedron,* **24**, 6231 (1968).
15. P.E. Schurr, J.R. Schultz, and T.M. Parkinson, *Lipids,* **7**, 68 (1971).
16. R.J. Jones and L.J. Dobrilovic, *J. Lab. Clin. Med.,* **75**, 953 (1970).
17. L.L. Abell, B.B. Levy, and F.E. Kendral, *J. Biol. Chem.,* **195**, 357 (1952).
18. S.P. Gottfried and B. Rosenberg, *Clin. Chem.,* **19**, 1077 (1977).
19. C.S. Frings and R.T. Dunn, *Am. J. Clin. Pathol.,* **53**, 89 (1970).
20. W.T. Friedwald, R.I. Levy, and D.S. Fredrickson, *Clin. Chem.,* **18**, 499 (1972).
21. G. Fenton, C.G. Newton, B.M. Wyman, P. Bagge, D.I. Dron, D. Riddell, and G.D. Jones, *J. Med. Chem.,* **32**, 265 (1989).
22. B.K. Chakravarthy, S. Gupta, S.S. Gambhir, and K.D. Gode, *Life Sci.,* **29**, 2043 (1981).
23. E.W. Sheehan, M.A. Zemaitis, D.J. Slatkin, and P.L. Schiff Jr., *J. Nat. Prod.,* **46**, 232 (1983).
24. H. Kolbe, U. Kiessel, B. Greulich, and J. Vender Bosch, *Lancet,* June 5, 1303 (1982).
25. P.W. Basarkar and N. Nath, *Indian J. Med. Res.,* **77**, 122 (1983).
26. P.W. Basarkar and N. Nath, *Indian J. Exp. Biol.,* **19**, 787 (1981).
27. J.S. Choi, T. Yokozawa, and H. Oura, *J. Nat. Prod.,* **54**, 218 (1991).
28. R.D. Sharma, *Atherosclerosis,* **33**, 371 (1979).
29. S.B. Schmidt, A.G. Wasserman, R.A. Muesing, S.A. Schlesselman, J.C. Larosa, and A.M. Ross, *Am. J. Cardiol.,* **55**, 1459 (1985).

Received 7 August 1992