Antihyperglycemic Activity of Phenolics from *Pterocarpus marsupium*

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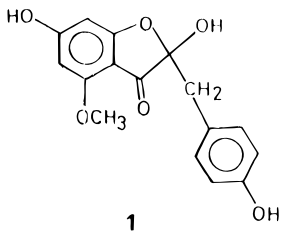
Glucose levels in rats with hyperglycemia induced by streptozotocin were determined after ip administration of marsupsin (**1**), pterosupin (**2**), and pterostilbene (**3**), three important phenolic constituents of the heartwood of *Pterocarpus marsupium*. Marsupsin and pterostilbene significantly lowered the blood glucose level of hyperglycemic rats, and the effect was comparable to that of 1,1-dimethylbiguanide (metformin).

In a previous paper,[1] we reported the antihyperlipidemic activity of marsupsin, pterosupin, and liquiritigenin obtained from *Pterocarpus marsupium* Roxb. (Leguminosae), a plant whose wood is primarily used in the treatment of diabetes in the Ayurvedic system of medicine.[2,3] Chakravarthy *et al*. isolated (−)-epicatechin from the bark of this plant, claimed it to be the antidiabetic principle of the plant, and suggested that the compound acts by regeneration of pancreatic β cells.[4−6] This claim was, however, questioned by Kolbe *et al*.[7] and Sheehan *et al*.,[8,9] who felt that further studies are necessary before (−)-epicatechin can be considered a viable antidiabetic agent for use in human clinical trials.[8] Moreover, it is the heartwood rather than the bark of *P. marsupium* that is commonly used in the treatment of diabetes by Ayurvedic physicians,[3] and we could not detect the presence of (−)-epicatechin in this part of the plant. It was, therefore, necessary to determine the active antihyperglycemic agent, if any, present in this part of the plant, particularly in its aqueous decoction, which is commonly used in the traditional system of medicine. Thus, the benzofuranone, marsupsin (**1**),[10] the dihydrochalcone, pterosupin (**2**),[11] and the stilbene, pterostilbene (**3**),[12] three of the major phenolic principles present in the EtOAc-soluble fraction of the aqueous decoction of the heartwood,[1] were tested for their putative antihyperglycemic activity against streptozotocin-induced hyperglycemic rats.

The plasma glucose level was significantly increased ($p < 0.001$) in streptozotocin (STZ)-treated rats as compared to control animals; the levels were 353 ± 12 ($n = 25$) and 55 ± 3 ($n = 5$), respectively.

The phenolic constituents marsupsin (**1**) and pterostilbene (**3**) significantly decreased the plasma glucose level of STZ-induced diabetic rats. Pterosupin (**2**) was found to be ineffective (Table 1). The antidiabetic activity of marsupsin was comparable to that of the reference compound, metformin.[13,14]

In nondiabetic animals, the basal plasma glucose level was not altered by **1** and **3**, but **2** significantly increased the basal glucose level. Furthermore, all three compounds significantly decreased the body weight in comparison to the vehicle-treated animals (Tables 2 and 3).



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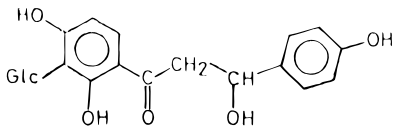

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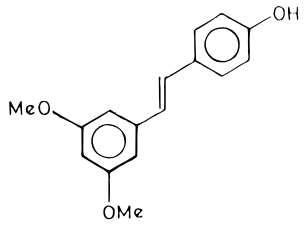

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It should be noted that the antidiabetic activity of the test compounds was evaluated in an animal model in which the β-cells are partially destroyed and the intrinsic insulin level is decreased. Hence, marsupsin and pterostilbene may have insulin-like effects on several tissues[15] as in the case of the oral hypoglycemic agents (metformin); i.e., they may suppress hepatic gluconeogenesis, stimulate glycolysis, inhibit glucose absorption from the intestine,[13] or act by other mechanisms.[16,17] However, further experiments are required to elucidate the exact mechanism of action of these test compounds.

In the control nondiabetic animals, all three compounds decreased the body weight, an effect that was found to be highly significant. Of these three compounds, however, only marsupsin and pterostilbene were found to be effective antidiabetic agents, which might be useful in non-insulin-dependent *diabetes mellitus*, which is usually accompanied by obesity. However, further work in different models would be required prior to their being considered for clinical application.

Experimental Section

Animals. Charles Foster strain albino rats of either sex (125−150 g), procured from the Central Animal

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Table 1. Effect of Major Phenolic Constituents of *Pterocarpus marsupium* on Plasma Glucose Levels in Diabetic Animals

	plasma glucose level in mg/100 mL ($n = 5$)		
treatment (3 days)	before treatment	after treatment	% activity[a]
normal	51 ± 4		
control (vehicle: 3% aqueous propylene glycol, 0.5 mL)	326 ± 5	325 ± 5	
metformin (reference)	358 ± 24	188 ± 36[b]	−48
marsupsin (**1**)	355 ± 36	238 ± 34[a]	−33
pterosupin (**2**)	351 ± 49	405 ± 32	+11
pterostilbene (**3**)	377 ± 37	219 ± 48[b]	−42

[a] "+" percent increase and "−" percent decrease in blood glucose level. a and b indicate statistical significance (before vs after treatment) at $p < 0.05$ and $p < 0.01$, respectively. Student's *t* test paired.

Table 2. Effect of Major Phenolic Constituents of *Pterocarpus marsupium* on Blood Glucose Levels in Nondiabetic Animals

treatment (3 days)	n	blood glucose level in mg/100 mL	*p* value[a] (Student's *t* test unpaired)
normal	5	51 ± 4	
control (vehicle: 3% aqueous propyleneglycol, 0.5 mL)	5	55 ± 3	
marsupsin (**1**) (20 mg/kg)	5	64 ± 4	n.s.
pterosupin (**2**)	5	74 ± 3	<0.001
pterostilbene (**3**)	5	65 ± 4	n.s.

[a] Control *vs* drug-treated group.

Table 3. Effect of Major Phenolic Constituents of *Pterocarpus marsupium* on Body Weight in Nondiabetic Animals ($n = 5$)

	body wt (g)		
treatment 3 days	before treatment	after treatment	% decrease
vehicle (3% aqueous propyleneglycol 0.5 mL)	128 ± 2	127 ± 4	
marsupsin (**1**)	124 ± 2	99 ± 2[a]	−20
pterosupin (**2**)	126 ± 2	96 ± 3[a]	−23
pterostilbene (**3**)	125 ± 2	99 ± 3[a]	−20

[a] Statistical significance at $p < 0.01$ vs vehicle treatment; Student's *t* test unpaired.

House of the Institute, were used for this study. The animals were housed in colony cages at an ambient temperature of 25 ± 2 °C and 55−65% relative humidity with a 12 h light−dark schedule. The animals had free access to H_2O and normal laboratory diet (Lipton India Ltd.).

Isolation of Marsupsin, Pterosupin, and Pterostilbene. Marsupsin, pterosupin, and pterostilbene were isolated from the heartwood of *P. marsupium* following the procedure of Maurya *et al.*[18,19] and identified by direct comparison with authentic samples (mp, Co-TLC, ^{1}H-NMR, MS). A specimen sample of the plant material is being preserved in the department.

Experimental Hyperglycemic and Hypoglycemic Agents. A solution of streptozotocin (STZ, Sigma) in citrate buffer at pH 4.5 was used for inducing hyperglycemia[20] in experimental nonfasted rats. Metformin (1,1-dimethylbiguanide, Franco Indian, India) was used as reference hypoglycemic agent.[13,14]

Preparation of Diabetic Rats. Nonfasted animals were administered ip a solution of STZ at the dose of 40 mg/kg body weight. The blood glucose level was measured 72 h after STZ injection. Experiments were conducted with rats having blood glucose levels above 275 mg/100 mL.

All the drugs other than pterostilbene were dissolved in aqueous propylene glycol (3%). Pure propylene glycol was used for dissolution of pterostilbene.

Diabetic animals were divided into five groups of five animals each. Marsupsin, pterosupin, and pterostilbene at a dose of 20 mg/kg body weight were administered ip for 3 days to three groups, and metformin, a widely used hypoglycemic agent, was administered ip to the fourth group at a dose of 30 mg/kg body weight for 3 days. The fifth group received an equivalent amount of vehicle (aqueous propylene glycol 3%, 0.5 mL) through the same route for the same period. Similarly, in another set of nondiabetic animals, pterostilbene, marsupsin, pterosupin, and vehicle were administered for 3 days to check the *per se* effect of these drugs on blood glucose level.

Glucose Estimation. Plasma glucose levels were estimated for all the groups 1 h after the administration of the last dose. Blood samples were collected from rat tail tip under mild ether anesthesia. The plasma was separated by centrifugation, and the glucose level was estimated by the GOD/POD method[21] with absorbance measured by a spectrophotometer (Du-70 Beckman) at 510 nm.

Statistics. The data are expressed as mean ± SEM. The significance of difference was tested by Student's *t* test.

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References and Notes

(1) Farboodniay Jahromi, M. A.; Ray, A. B.; Chansouria, J. P. N. *J. Nat. Prod.* **1993**, *56*, 989−994.
(2) Satyavati, G. V.; Gupta, A. K.; Tandon, N. *Medicinal Plants of India*; ICMR Publications: New Delhi, 1987; Vol. 2, p 533.
(3) Chatterjee, A.; Pakrashi, S. C. *The Treatise on Indian Medicinal Plants*: Publications & Information Directorate: New Delhi, 1992; Vol. 2, pp 115−117.
(4) Chakravarthy, B. K.; Gupta, S.; Gambhir, S. S.; Gode, K. D. *Life Sci.* **1981**, *29*, 2043−2047.
(5) Chakravarthy, B. K.; Gupta, S.; Gode, K. D. *Life Sci.* **1982**, *31*, 2693−2697.
(6) Chakravarthy, B. K.; Gupta, S.; Gode, K. D. *Lancet* **1982**, July 31, *2* (8292), 272−273.
(7) Kolbe, H.; Kiessel, U.; Greulich, B.; VanderBosch, J. *Lancet* **1982**, June 5, *1* (8284), 1303−1304.
(8) Sheehan, E. W.; Zemaitis, M. A.; Slatkin, D. J.; Schiff, P. L., Jr. *J. Nat. Prod.* **1983**, *46*, 232−234.
(9) Sheehan, E. W.; Stiff, D.; Duah, F.; Slatkin, D.; Schiff, P. L., Jr.; Zemaitis, M. *Life Sci.* **1983**, *33*, 593−597.
(10) Maurya, R.; Ray, A. B.; Duah, F. K., Slatkin, D. J.; Schiff, P. L., Jr. *Heterocycles* **1982**, *19*, 2103−2107.
(11) Adinarayana, D.; Syamasundan, K. V.; Seligmann, O.; Wagner, H. *Z. Naturforsch.* **1982**, *37C*, 145−147.
(12) Mathew, J.; Subba Rao, A. V., Subba Rao, N. V. *Curr. Sci.* **1977**, *46*, 337−338.
(13) Jackson, R. A.; Hawa, M. I.; Japan, J. B.; Sim, B. M.; Silvio, D.; Featherbe, L.; Kurtz, D. *Diabetes* **1987**, *36*, 632−640.
(14) Bailey, C. J. The anti-hyperglycaemic action of metformin. In *Diabetes and metformin. A Research and Clinical update*; Krans, E., Ed.; London: Royal Society of Medicine, 1984; pp 17−26 (ICSS No. 79).
(15) Hermann, L. S. *Diabetic Metab.* **1979**, *5*, 233−245.
(16) Lord, J. M.; Atkins, T. W.; Bailey, C. J. *Diabetologia* **1983**, *25*, 108−113.
(17) Puah, J. A.; Bailey, C. J. *Diabetologia* **1984**, *27*, 322A.
(18) Maurya, R.; Ray, A. B.; Duah, F. K.; Slatkin, D. J.; Schiff P. L., Jr. *J. Nat. Prod.* **1984**, *47*, 179−181.
(19) Maurya, R. Chemistry of Natural Products. Ph.D. Thesis, Banaras Hindu University, Varanasi, India, 1984.
(20) Ganda, O. P.; Rossini, A. A.; Like, A. A. *Diabetes* **1976**, *25*, 595−603.
(21) Trinder, P. *J. Clin. Pαthol.* **1969**, *22*, 158−161.

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