3. Sample preparation

To aliquots of 100 μL plasma, 10 μL internal standard (100 $\mu g \cdot mL^{-1}$, rutin in methanol) and 50 μL 0.5 $mol \cdot L^{-1}$ phosphoric acid were added. The mixture was vortex-mixed and extracted with 2 mL of ethyl acetate by shaking for 10 min. the organic phases were separated by centrifugation for 10 min (3 000 rpm), transferred to another tube and evaporated to dryness in a water bath at 40 °C under a flow of nitrogen. The residue was dissolved in 50 μL mobile phase. A 20 μL aliquot of the solution was injected into the HPLC system for analysis.

4. Pharmacokinetics study and data analysis

All animal studies were performed according to the Guidelines for the Care and Use of Laboratory Animals that was approved by the Committee of Ethics of Animal Experimentation of Shenyang Pharmaceutical University. Wistar rats (Male and female, 240–300 g) were provided by the Animal Center of Shenyang Pharmaceutical University (Shenyang, China). Animals were housed in a room with controlled temperature and humidity, and had free access to food and water. They were fasted overnight before the experiments. Two groups (6 rats/group) were randomly assigned to receive scutellarin solution via injection into the tail vein or intraportal injection at a single dose of 20 mg/kg, respectively. All rats were anesthetized with an intraperitoneal injection of urethane (250 $mg \cdot ml^{-1}$) at a dose of 1000 $mgkg^{-1}$ The jugular vein was cannulated and blood samples were collected at 0.05, 0.17, 0.33, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, and 4.0 h after the initiation of injection. The plasma samples were transferred into microcentrifuge tubes and stored at −20 °C until analysis.
The plasma concentration-time data were analyzed by noncompartmental analysis with 3p97 sofeware, a practical pharmacokinetic program (the Chinese Society of Mathematical Pharmacology).

References

Deng TF (2002) Application of breviscapine injection in clinic. Zhongguo Yao Ye 11, 72–73.

Ding GH (1999) Pharmacology research of breviscapus and its clinical investigation. Shizhen Guo Yi Guo Yao 10: 303–304.

He W, Zeng FD (2002) Effect of breviscapine on ischemic cerebrovascular diseases and its clinical investigation. Chin J Clin Pharmacol 18: 458–461.

Ilett KF, Tee LBG, Reeves PT, Minchin RF (1990) Metabolism of drugs and other xenobiotics in the gut lumen and wall. Pharmacol Ther 46: 67–93.

Liu H, Yang XL, Zhou LZH, Xu HB (2002) Study on effects of scutellarin on scavenging reactive oxygen. Zhong Yao Cai 25: 491–493.

Liu Y, Hu M (2002) Absorption and metabolism of flavonoids in the caco-2 cell culture model and a perfused rat intestinal model. Drug Metabol Dispos 30: 370–377.

Yan Cai, Nathan D Anavy, and H-H. Sherry Chow (2002) Contribution of presystemic hepatic extraction to the low oral bioavailability of green tea catechins in rats. Drug Metabol Dispos 30: 1246–1249.

Zhong DF, Yang BH, Chen XY, Li K, Xu JH (2003) Determination of scutellarin in rat plasma by high-performance liquid chromatography with ultraviolet detection. J Chromatogr B Analyt Technol Biomed Life Sci 796: 439–444.

Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, (Hamdard University), Hamdard Nagar, New Delhi, India

Effect of aqueous extract of *Pterocarpus marsupium* wood on alloxan-induced diabetic rats

H. M. Mukhtar, S. H. Ansari, M. Ali, Z. A. Bhat, T. Naved

Received August 31, 2004, accepted October 5, 2004

Dr. H. M. Mukhtar, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi – 11006
hayat18@rediffmail.com

Pharmazie 60: 478–479 (2005)

An aqueous extract of *Pterocarpus marsupium* wood was screened for hypoglycemic activity on alloxan-induced diabetic rats. During both acute and sub-acute tests, the water extract, at an oral dose of 250 mg/kg, showed statistically significant hypoglycemic activity.

Pterocarpus marsupium Roxb. (Leguminosae), commonly called as Bijasal, is a moderate sized to large deciduous tree, up to 30 m high, found commonly in hilly regions from Deccan Peninsula and entering to Gujarat, Madhya Pradesh, Uttar Pradesh, Bihar and Orissa (Kirtikar and Basu 1991; Anonymous 2001). In the traditional system of medicine it is used as an astringent, bitter, acrid, cooling, anti-inflammatory, haemostatic, antihelmintic, constipating, anodyne, alterant and rejuvenating agent, in fractures, bruises, leprosy, skin diseases, leucoderma, urethrorrhea, diabetes, rectalgia, rectitis, verminosis, diarrhoea, dysentery, gout, rheumatoid arthritis, cough, asthma, bronchitis and greyness of hair (Vaidyaratnam 1995; Nadkarni 1985; Chopra et al. 1956). An aqueous infusion of the wood is said to be useful in diabetes and water stored in vessels made of the wood is reported to have anti-diabetic qualities (Anonymous 1969a; Rastogi and Mehrotra 1990). Liquirtigenin, isoliquiritigenin, alkaloids, resins, essential oil, semidrying oil, tannins (Rastogi and Mehrotra 1991; Rastogi and Mehrotra 1993), terpenes, catechol, gallic acid and yellow colouring matter have been isolated from the plant (Anonymous 1969b).
The effect of an aqueous extract of the heartwood of *P. marsupium* on diabetic rats was evaluated in this study. The results indicated that *P. marsupium* wood possessed statistically significant hypoglycemic activity as was evident during acute and sub-acute treatments. It is generally accepted that the sulphonylureas, including gliclazide produce hypoglycaemia in normal animals by stimulating the pancreatic β-cells to release more insulin. These drugs, however, do not reduce blood glucose in alloxan diabetic animals. In contrast to the oral anti-diabetic agents, the exogenous administration of insulin is known to produce hypoglycaemia in both normal and alloxan-induced subjects. It is, therefore, conceivable that the hypoglycemic principle(s) in the aqueous extract of *P. marsupium* exert a direct effect in diabetic rats. In diabetic rats, aqueous extract cannot act indirectly by stimulating the release of insulin since alloxan treatment causes permanent destruction of β-cells (Pari and Maheshwari 1999). The antihy-

Table 1: Effect of acute treatment of *P. marsupium*, wood aqueous extract (250 mg/kg, p.o.), on blood glucose levels in alloxan induced diabetic rats[a]

Group	Treatment	Blood glucose (mg/dl)		
		Basal value	1 h	3 h
I	Normal control (Dist. water only)	75.00 ± 1.24	77.00 ± 1.87	74.80 ± 2.08
II	Diabetic control (Alloxan only)	353.80 ± 5.29	352.60 ± 4.67	352.00 ± 5.29
III	Standard (Alloxan + reference drug)	329.00 ± 4.85	315.60 ± 4.26^{NS}	308.20 ± 3.58 ^{NS}
IV	Test (Alloxan + extract)	348.80 ± 3.29	292.80 ± 2.27***	242.80 ± 2.30***

[a] Values are means ± S.E.; n = 5, $^{***}p < 0.001$, NS, not significant vs. group II.

Table 2: Effect of sub-acute treatment of *P. marsupium*, wood aqueous extract (250 mg/kg, p.o., once daily), on blood glucose levels in alloxan induced diabetic rats[a]

Group	Treatment	Blood glucose (mg/dl)				
		Basal value	Day 1	Day 3	Day 7	Day 10
I	Normal control (Dist. water only)	75.60 ± 1.24	93.80 ± 6.08	88.20 ± 5.45	91.80 ± 3.48	92.20 ± 1.77
II	Diabetic control (Alloxan only)	353.80 ± 5.29	353.40 ± 3.20	353.60 ± 3.65	354.40 ± 3.15	354.80 ± 4.83
III	Standard (Alloxan + reference drug)	329.00 ± 4.85	303.80 ± 3.62 ^{NS}	306.40 ± 4.77 ^{NS}	304.40 ± 4.09 ^{NS}	304.60 ± 5.40 ^{NS}
IV	Test (Alloxan + extract)	348.80 ± 3.29	290.20 ± 3.70***	243.60 ± 4.55***	226.60 ± 3.75***	189.60 ± 3.47***

[a] Values are means ± S.E.; n = 5, $^{***}p < 0.001$, NS, not significant vs. group II.

perglycemic effect in the alloxan-diabetic rats suggests that its main mechanism may not be due to potentiation of insulin release from pancreatic cells and thus the drug may be effective in insulin independent diabetes.

Experimental

Heartwood of *P. marsupium* was purchased from local market, Khari Baoli, Delhi and authenticated at Taxonomy Division, Faculty of Science. A voucher specimen (JH-PM-19) was deposited in the laboratory of Pharmacognosy. Shade dried, powdered heartwood (500 g) was extracted with water and filtered. The filtrate was dried by vacuum rotary evaporation, which yielded a solid residue of 17.5 g (yield 3.5%).

Wistar rats (180–200 g) of either sex were used. They were obtained from the Central Animal House, Jamia Hamdard and housed under standard environmental conditions at the animal house. The animals were fasted for 16 h prior to experiment, with water *ad libitum.* Hyperglycemia was induced by a single i.p. injection of 120 mg/kg of alloxan monohydrate in sterile saline (Joy and Kuttan 1999). Five days after alloxan injection, the diabetic rats (glucose level > 300 mg/dl) were separated and divided into three groups of five diabetic animals each. Group I was previously selected from normal animals and served as normal control and received distilled water and no alloxan. Group II served as diabetic control and received distilled water only. Group III received the reference anti-diabetic drug gliclazide at an oral dose of 25 mg/kg (Panacea Biotech Ltd., Batch No. 01030513). Group IV was treated orally with aqueous extract at an oral dose of 250 mg/kg; the dose was selected after preliminary behavioural and acute toxicity tests. Blood samples were collected from the tip of tail just prior to and 1 and 3 h after the extract/reference drug administration. In subacute treatment, the administration of extract/reference drug was continued for 10 days, once daily. Blood samples were collected from the tip of the tail just prior to and on days 1, 3, 7 and 10 of the extract/reference drug administration. The blood glucose levels were determined for all the samples by glucose-oxidase method (Varley et al. 1967). Statistical significance was determined by using, one-way analysis of variance (ANOVA) followed by Dunnet's t-test. $P < 0.05$ indicates significant differences between group means. The effect of *P. marsupium* aqueous extract on alloxan-induced diabetic rats (Table 1) has shown statistically significant effect ($P < 0.001$). In the untreated animals, blood glucose level did not change significantly. During sub-acute treatment, *P. marsupium* aqueous extract produced consistent reduction in the blood glucose levels in alloxan-induced diabetic rats (Table 2), as compared to diabetic control.

Acknowledgement: The authors whish to thank the UGC, New Delhi for providing financial support and Panacea Biotech Ltd. for providing complimentary sample of gliclazide.

References

Anonymous (2001) The Ayurvedic Pharmacopoeia of India, New Delhi, The Controller of Publications, India, p. 12.

Anonymous (1969a), The Wealth of India, Vol. VIII, Publications and Information Directorate, CSIR, New Delhi, p. 305.

Anonymous (1969b), The Wealth of India, Vol. VIII, Publications and Information Directorate, CSIR New Delhi, p. 304–305.

Chopra RN, Nayar SL, Chopra IC (1956) Glossary of Indian Medicinal Plants, PID, New Delhi, p. 206.

Joy KL, Kuttan R (1999) Antidiabetic activity of *Picrorrhiza kurroa* extract. J Ethnopharmacol 67: 143–148.

Kirtikar KR, Basu BD (1991) Indian Medicinal Plants, Vol. I, 2nd edition, Lalit Mohan Basu Prakashan, Allahabad, India, p. 828–829.

Nadkarni KM (1985) Indian Materia Medica, Vol. I, Popular Book Depot., Bombay, p. 1025.

Pari L, Uma Maheshwari J (1999) Hypoglycemic effect of *Musa sapientum* L. in alloxan-induced diabetic rats. J Ethnopharmacol 68: 231–325.

Rastogi RP, Mehrotra BM (1990) Compendium of Indian Medicinal Plants, Vol. 1, CDRI Lucknow and PID, New Delhi, p. 334.

Rastogi RP, Mehrotra BM (1991) Compendium of Indian Medicinal Plants, Vol. 2, CDRI Lucknow and PID, New Delhi, p. 570.

Rastogi RP, Mehrotra BM (1993) Compendium of Indian Medicinal Plants, Vol. 3, CDRI Lucknow and PID, New Delhi, p. 537.

Vaidyaratnam PSV (1995) Indian Medicinal Plants, Vol. 4, Orient Longman Ltd., Madras, India, p. 371.

Varley H, Gowenlok AH, Bel MC (1976) Practical Biochemistry, Heinmann, London, p. 389–391.