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Antihyperglycemic Activity of the Aqueous Stem Extract of *Coscinium fenestratum* in Non–insulin Dependent Diabetic Rats

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Abstract

The antihyperglycemic potential of the aqueous stem extract of *Coscinium fenestratum* Colebr. (Menispermaceae), a medicinal plant widely used in traditional Ayurveda and Siddha systems of medicine for the treatment of diabetes mellitus, was evaluated in the streptozotocin-nicotinamide–induced type 2 diabetic model. Graded doses of the aqueous stem extract were administered to normal and experimental diabetic rats for 12 days. In extract-treated diabetic rats, an insulin-independent action with significant reduction in blood glucose, serum triglyceride, and cholesterol levels was observed. In addition, other parameters like change in body weight, thiobarbituric acid reactive substance levels, glycosylated hemoglobin and liver glycogen levels, assessed in the extract-treated diabetic rats, were compared with diabetic control and normal animals. Significant results were observed in the above parameters thereby justifying the use of the plant in the indigenous system of medicine.

Keywords: Antihyperglycemic activity, aqueous extract, *Coscinium fenestratum*, Menispermaceae, streptozotocin-nicotinamide–induced diabetes.

Introduction

Diabetes mellitus is a syndrome of impaired carbohydrate, fat, and protein metabolism caused by either lack of insulin secretion or by decreased sensitivity of the tissues to insulin characterized by chronic hyperglycemia (Setter et al., 2000). The worldwide prevalence of diabetes has risen in the past two decades. Type 2 diabetes is more common, and its prevalence is expected to rise more rapidly in the future because of increasing obesity and reduced activity levels. Though several new pharmacological agents have been developed for the management of diabetes, the treatment of diabetes with herbal remedies has also been increasing among practitioners. Ancient Indian literature has prescribed various herbs for the cure of diabetes mellitus.

Many indigenous drugs have been used by the practitioners of the Ayurvedic system for the treatment of diabetes mellitus in India. Infusion and tincture preparations of the stems of *Coscinium fenestratum* are widely used for the treatment of diabetes in Ayurveda (Varier, 1994). In the Siddha system of medicine, the powdered stems in milk are given to diabetic patients (Chinnaiah, 2002). In Kanyakumari district, Tamilnadu, India, the rural folk use the decoction of the stems to cure diabetes (Kalavincela, 1998). The stem extract has been reported for its hypotensive activity (Singh et al., 1990). Preliminary screening of this plant showed good antidiabetic activity (Mahapatra, 1997). The current investigation is an attempt to study the antidiabetic activity of the aqueous stem extract of *Coscinium fenestratum* using the streptozotocin-nicotinamide–induced type 2 diabetes mellitus model.

Materials and Methods

Animals

Healthy adult male Wistar albino rats between 2 and 3 months of age weighing about 250–300 g were used for the study. The animals, housed in polypropylene cages maintained under standard conditions (12 h light/dark cycle; $25 \pm 3^{\circ}C$; 35–60% humidity), were fed standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. The Institutional Animal Ethical

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Committee of KMC, Manipal, India (IAEC/KMC/03/2003-04), approved the study.

Chemicals and instruments

Procurement of chemicals were as follows: STZ (Sigma Aldrich Chemie GmbH, Germany), trichloroacetic acid, glucose (NICE Chemical Pvt. Ltd., Cochin, India), nicotinamide (Qualigens Fine Chemicals Ltd, Division of Glaxo India Ltd., Mumbai, India), thiourea (S.D. fine-chem. Ltd., Mumbai, India), anthrone (SISCO Research Laboratories Pvt. Ltd., Mumbai, India).

Blood glucose levels were estimated by glucose oxidase peroxidase reactive strips (Accu-chek, Roche Diagnostics, USA). Serum insulin levels were estimated by radioimmuno assay kit issued by the Board of Radiation and Isotope Research, Bhaba Atomic Research Center (BARC), Mumbai, India. Serum lipid profiles and glycosylated hemoglobin levels were estimated by an auto analyzer (Hitachi 912, USA).

Plant material

The stems of the plant material of *C. fenestratum* were purchased from Jogappa Shanbag Ayurvedic Store (Udupi, Karnataka, India) during the month of August 2003. The plant was authenticated by Dr. Gopalakrishna Bhat, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. A voucher specimen (PP 526) has been deposited at the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, India.

Preliminary phytochemical screening

Preliminary phytochemical screening (Kokate, 1994; Harborne, 1998) of the aqueous extract revealed the presence of alkaloids, saponins, phenolic substances, and carbohydrates.

Preparation of stem extract

The aqueous extract was prepared by cold maceration of 125 g of the dried stem powder for 7 days in 500 ml of chloroform water [the chloroform water was prepared by shaking together water: chloroform (99:1) and then discarding the chloroform layer]. The extract was filtered, concentrated, dried *in vacuo*, and the residue (yield 34 g) was stored in the refrigerator at 2–8°C for use in subsequent experiments.

Acute toxicity studies

Healthy adult Wistar albino rats of either sex, starved overnight, were divided into four groups (n = 6) and orally fed with the aqueous extract of *C. fenestratum* in increasing dose levels of 100, 500, 1000, and 3000 mg/kg body weight (Ghosh, 1984). The rats were observed continuously for 2 h for behavioral, neurological, and autonomic profiles and thereafter at 24 and 72 h for any lethality (Turner, 1965).

Oral glucose tolerance test

The oral glucose tolerance test (Bonner-Weir, 1988) was performed in overnight fasted (18 h) normal animals. Rats divided into four groups (n = 6) were administered 2% gum acacia solution, aqueous extract (250 mg/kg), aqueous extract (500 mg/kg), and glibenclamide (0.25 mg/kg), respectively. Glucose (2 g/kg) was fed 30 min after the administration of extracts. Blood was withdrawn from the retro-orbital sinus under light ether anesthetized condition at 0, 30, 60, 90, and 120 min of extract administration, and fasting blood glucose levels were estimated.

Normoglycemic studies

Normoglycemic study was performed in normal rats without diabetic induction. Rats divided into four groups (n = 6) were administered 2% gum acacia solution, aqueous extract (250 mg/kg), aqueous extract (500 mg/kg), and glibenclamide (0.25 mg/kg), respectively. The blood glucose levels were estimated on day 0, day 5, and day 12.

Antihyperglycemic studies

Induction of experimental diabetes

Type 2 diabetes mellitus was induced (Masiello et al., 1998) in overnight fasted animals by a single intraperitoneal injection of 60 mg/kg streptozotocin (STZ), 15 min after the i.p. administration of 120 mg/kg nicotinamide. The elevated glucose level in the blood determined at 72 h and then on day 7 after injection confirmed hyperglycemia. The rats found with permanent non–insulin dependent diabetes mellitus (NIDDM) were used for the antidiabetic study.

Experimental design

The diabetic animals divided into four groups (n = 6) were administered 2% gum acacia solution, aqueous extract (250 mg/kg), aqueous extract (500 mg/kg), and glibenclamide (0.25 mg/kg), respectively, for 12 days. Fasting blood glucose levels were estimated on day 0, day 5, and day 12.

The effects of administration of *C. fenestratum* aqueous stem extract on diabetic rats were estimated on the 12th day after the animals were sacrificed by decapitation. The parameters evaluated were serum insulin level, serum lipid profile, liver glycogen

(Nicholas, 1956), glycosylated hemoglobin level, thiobarbituric acid reactive substances (TBARS) level (Ohkawa et al., 1979), and changes in initial and final body weight. The protein content in the homogenate was estimated by the method of Lowry et al. (1951).

Statistical analysis

Data were statistically evaluated by using one-way ANOVA, followed by *post hoc* Scheffe's test using SPSS computer software (ver. 7.5). The values were considered significant when $p < 0.05$.

Results

Acute toxicity studies revealed the nontoxic nature of the aqueous extract of *C. fenestratum*. There was no lethality or toxic reactions found at any doses selected through the end of the study. In the oral glucose tolerance test, the aqueous extract showed a significant reduction in blood glucose levels from 30 min onwards (Table 1). In normal animals, a significant reduction in the blood glucose level was observed as compared with the control (Table 2). Induction of diabetes in the experimental rats was confirmed by the presence of high blood glucose level. The effects of the aqueous stem extract on fasting blood glucose levels in diabetic animals are presented in Table 3. The difference between fasting blood glucose level in the diabetic rats and control rats were found to be statistically significant. The effects on serum insulin levels are presented in Table 4. Significant ($p < 0.05$) difference was observed in glycosylated hemoglobin levels (Table 4), serum lipid profiles (Table 5), changes in body weight (Table 6), liver glycogen levels, and thiobarbituric acid reactive substances levels (Table 7) of extract-treated diabetic animals when compared with the diabetic control and normal animals.

Discussion

The current study was undertaken with the objective of exploring the antidiabetic potential of *C. fenestratum*, which even at high concentrations did not produce any muscular weakness or cause any gross behavioral disturbances in the rats, thereby suggesting its nontoxic nature. The oral glucose tolerance test and normoglycemic study revealed the significant antihyperglycemic activity exerted by the aqueous extract. The high blood glucose level observed in the blood of streptozotocin-induced rats indicates the establishment of a diabetic state (Ladeji et al., 2003). Administration of graded doses of aqueous extract for a 12-day experimental period produced a statistically significant decrease in blood glucose concentration when compared with the diabetic control ($p < 0.05$). No significant change was noted in serum insulin levels of the diabetic animals treated with the aqueous extract, thereby suggesting that the extract probably exerts significant antihyperglycemic activity

Table 1. Effect of aqueous stem extract of *Coscinium fenestratum* on oral glucose tolerance test.

		Blood glucose concentration (mg/dl)				
Group	Treatment	0 min	30 min	60 min	90 min	120 min
I	Control (vehicle)	88.6 ± 9.8	110 ± 2.3	104 ± 11.8	101.5 ± 13.4	97.5 ± 6.2
II	Aq. extract (250 mg/kg)	82.4 ± 7.9	101 ± 11.2^a	97.3 ± 8.5	92.7 ± 8.4^a	89.7 ± 7.5^a
III	Aq. extract (500 mg/kg)	80.2 ± 3.2	98.9 ± 7.3^a	92.1 ± 8.9^a	88.1 ± 7.7^a	86.3 ± 9.4^a
IV	Glibenclamide (0.25 mg/kg)	82.1 ± 5.1	85.7 ± 6.1^a	78.9 ± 2.7^a	76.5 ± 5.2^a	68.6 ± 3.9^a

Each value represents mean ± SE, n = 6.
[a]Represents statistical significance vs. control ($p < 0.05$).

Table 2. Effect of aqueous stem extract of *Coscinium fenestratum* on normal animals.

		Blood glucose concentration (mg/dl)		
Group	Treatment	Day 0	Day 5	Day 12
I	Control (vehicle)	74.3 ± 1.7	74.8 ± 3.6	75.4 ± 4.8
II	Aq. extract (250 mg/kg)	78.7 ± 9.6	74.3 ± 6.1	70.1 ± 5.3^a
III	Aq. extract (500 mg/kg)	72.3 ± 5.4	68.8 ± 2.9^a	63.1 ± 2.6^a
IV	Glibenclamide (0.25 mg/kg)	74.7 ± 5.7	67.2 ± 5.9^a	59.8 ± 4.0^a

Each value represents mean ± SE, n = 6.
[a]Represents statistical significance vs. control ($p < 0.05$).

Table 3. Effect of aqueous stem extract of *Coscinium fenestratum* on diabetic animals.

Group	Treatment	Blood glucose concentration (mg/dl)		
		Day 0	Day 5	Day 12
I	Diabetic control	223.7 ± 18.6	230 ± 11.6	231.4 ± 14.3
II	Aq. extract (250 mg/kg)	171.3 ± 11.3	141.3 ± 18.5^{a}	118.3 ± 13.4^{a}
III	Aq. extract (500 mg/kg)	169.8 ± 15.4	126.8 ± 12.9^{a}	96.3 ± 18.7^{a}
IV	Glibenclamide (0.25 mg/kg)	187.7 ± 15.7	126.2 ± 13.9^{a}	103.8 ± 14.0^{a}

Each value represents mean ± SE, n = 6.
[a]Represents statistical significance vs. control ($p < 0.05$).

Table 4. Effect of aqueous stem extract of *Coscinium fenestratum* on serum insulin and glycosylated hemoglobin (whole blood) in diabetic rats.

Group	Treatment	Insulin (μU/ml)	Glycosylated hemoglobin (%)
I	Normal	125.7 ± 15.3	3.2 ± 0.5
II	Diabetic control	95.4 ± 9.8	7.1 ± 0.2
III	Aq. extract (250 mg/kg)	99.9 ± 8.4	$5.1 \pm 0.3^{a,b}$
IV	Aq. extract (500 mg/kg)	98.5 ± 7.9	$4.9 \pm 0.2^{a,b}$

Each value represents mean ± SE, n = 6.
[a]Represents statistical significance vs. control ($p < 0.05$).
[b]Represents statistical significance vs. normal ($p < 0.05$).

Table 5. Effect of aqueous stem extract of *Coscinium fenestratum* on serum lipid profile in diabetic rats.

Group	Treatment	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	HDL cholesterol (mg/dl)
I	Normal	92.4 ± 1.7	59.3 ± 2.6	53.0 ± 3.2
II	Diabetic control	183.0 ± 13.2	123.9 ± 15.2	35.2 ± 2.7
III	Aq. extract (250 mg/kg)	$132.8 \pm 11.3^{a,b}$	$73.5 \pm 1.3^{a,b}$	$39.2 \pm 6.3^{a,b}$
IV	Aq. extract (500 mg/kg)	$98.3 \pm 4.7^{a,b}$	$64.7 \pm 6.7^{a,b}$	$47.4 \pm 2.7^{a,b}$

Each value represents mean ± SE, n = 6. HDL, high-density lipoprotein.
[a]Represents statistical significance vs. control ($p < 0.05$).
[b]Represents statistical significance vs. normal ($p < 0.05$).

Table 6. Effect of aqueous stem extract of *Coscinium fenestratum* on changes in body weight in diabetic rats.

Group	Treatment	Initial (g)	Final (g)
I	Diabetic control	273.3 ± 14.2	201.9 ± 13.2
II	Aq. extract (250 mg/kg)	224.7 ± 17.4	215.4 ± 16.3^{a}
III	Aq. extract (500 mg/kg)	247.5 ± 15.6	233.9 ± 12.7^{a}

Each value represents mean ± SE, n = 6.
[a]Represents statistical significance vs. control ($p < 0.05$).

by an extra pancreatic mechanism, independent of insulin secretion. Similar findings on other medicinal plants have been reported in studies by Jouad et al. (2003), Hannan et al. (2003), and Sachdewa et al. (2003).

Lipoprotein abnormalities are very common in individuals with both non–insulin dependent and insulin dependent diabetes. Although lipoprotein alterations appear to be an intrinsic part of these disorders, such alterations are also induced by diabetes-associated complications such as obesity and renal diseases (Howard & James Howard, 1994). A report by The Expert Panel, National Cholesterol Education Program (1988) has focused attention on the necessity for managing lipid disorders. Recent studies show that triglycerides are also independent risk factors for coronary heart disease (Bainton et al., 1992; National Cholesterol

Table 7. Effect of aqueous stem extract of *Coscinium fenestratum* on liver glycogen and TBARS level in diabetic rats.

Group	Treatment	Liver glycogen (mg/kg)	TBARS (mM/mg)	
			Liver	Pancreas
I	Normal	3.9 ± 0.6	0.14 ± 0.01	0.08 ± 0.05
II	Diabetic control	1.2 ± 0.1	0.30 ± 0.03	0.18 ± 0.00
III	Aq. extract (250 mg/kg)	1.7 ± 0.2^{a}	26 ± 0.02^{a}	$0.13 \pm 0.01^{a,b}$
IV	Aq. extract (500 mg/kg)	$1.9 \pm 0.3^{a,b}$	$0.20 \pm 0.05^{a,b}$	$0.11 \pm 0.02^{a,b}$

Each value represents mean ± SE, n = 6. TBARS, Thiobarbituric acid reactive substances.
[a]Represents statistical significance vs. control ($p < 0.05$).
[b]Represents statistical significance vs. Normal ($p < 0.05$).

Education Program Expert Panel, 1994). In our study, the increase in serum triglycerides and cholesterol observed in diabetic rats is in agreement with the findings of Nikkila and Kekki (1973). Insulinopenia in STZ-diabetic rats suggests association with lipid overproduction in the basal (hyperglycemic) state (Burcelin et al., 1995). Oral administration of the aqueous extract resulted in a significant decrease in serum triglycerides and serum cholesterol. Preliminary screening of the aqueous extract of *C. fenestratum* revealed the presence of saponins, alkaloids, and phenolic substances. The underlying mechanism of lipid-lowering activity of *C. fenestratum* may be due to the inhibition of lipid absorption by saponins and phenolic substances present in the aqueous extract. In this context, similar findings have been observed in studies conducted on other medicinal plants by Dwivedi and Agarwal (1994), Vaidya (1994), Hostettman and Marston (1995), Ram et al. (1997), and Berrougui et al. (2003). In our investigation, weight loss associated with treated diabetic rats may be attributed directly to the lipid-lowering activity of the extract or indirectly to its influence on various lipid regulation systems.

Glycogen synthesis in the rat liver and skeletal muscles are impaired during diabetes (Huang et al., 2000). In diabetic animals treated with the extract, the significant increase in the liver glycogen may be due to the activation of the glycogen synthase system by the aqueous extract. Glycosylated hemoglobin is known to increase in patients with diabetes mellitus (Koeing et al., 1976), and the increase has been found to be directly proportional to the fasting blood glucose level (Jackson et al., 1979). The significant reduction in glycosylated hemoglobin of extract-treated diabetic rats indicates its efficiency in glycemic control. In earlier studies by the authors, an increase was observed in the TBARS level of STZ induced diabetic rats (Shirwaikar et al., 2004). In the current study, the elevated TBARS levels were significantly reduced on treatment with *C. fenestratum* extract.

Saponins have been reported to possess good antihyperglycemic activity in recent studies (Sauvaire et al., 1996; Vats et al., 2003). Hence, the significant antihyperglycemic activity exerted by the aqueous stem extract of *Coscinium fenestratum* in our study may be attributed to the presence of saponins, alkaloids, and phenolic substances. Studies are in progress to elucidate the molecular and cellular mechanism of the extract. Longer duration studies on chronic models may contribute toward the development of a potent antidiabetic drug.

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References

Bainton D, Miller NE, Botton CH, Yarnell JWG, Suretman PM, Baker IA, Lewis B, Elwood PC (1992): Plasma triglyceride and high density lipoprotein cholesterol as predictors of ischaemic heart disease in British men. *Br Heart J 68*: 60–66.

Berrougui H, Ettaib A, Herrera Gonzalez MD, Alvarez de Sotomayor M, Bennani-Kabchi N, Hmamouchi M (2003): Hypolipidemic and hypocholesterolemic effect of argan oil (*Argania spinosa* L.) in meriones shawi rats. *J Ethnopharmacol 89*: 15–18.

Bonner-Weir S (1988): Morphological evidence of pancreatic polarity of beta cells within islets of Langerhans. *Diabetes 37*: 616–621.

Burcelin R, Eddouks M, Maury J, Kande J, Assan R, Girard J (1995): Excessive glucose production, rather than insulin resistance, accounts for hyperglycaemia in recent-onset streptozotocin diabetic rats. *Diabetologia 38*: 283–290.

Chinnaiah A (2002): *1000 Siddha Medicine Notes* (Tamil), 1st ed. Sivakasi, Tamilnadu, India, Siva Publishers, p. 33.

Dwivedi S, Agarwal MP (1994): Antianginal and cardioprotective effects of *Terminalia arjuna*, an indigenous drug in coronary artery disease. *J Assoc Physicians India 42*: 287–289.

Ghosh MN (1984): Toxicity studies. In: *Fundamentals of Experimental Pharmacology*. Calcutta, Scientific Book Agency, 2, pp. 153–158.

Hannan JMA, Rokeya B, Faruque O, Nahar N, Mosihuzzaman M, Azad Khan AK, Ali L (2003): Effect of soluble dietary fibre fraction of *Trigonella foenum graecum* on glycemic, insulinemic, lipidemic and platelet aggregation status in type 2 diabetic model rats. *J Ethnopharmacol 88*: 73–77.

Harborne JB (1998): *Phytochemical Methods*. London, Chapman & Hall, pp. 60–66.

Hostettman K, Marston A (1995): *Saponins*. Cambridge, Cambridge University Press, pp. 232–286.

Howard BV, James Howard WM (1994): The pathophysiology and treatment of lipid disorders in diabetes mellitus. In: *Joslin's Diabetes Mellitus*, 13th ed. Philadelphia, Lea & Febiger, p. 372.

Huang X, Vaag A, Hanson M, Weng J, Goop L (2000): Impaired insulin stimulated expression of the glycogen synthase gene in skeletal muscle of type 2 diabetic patients in acquired rather than inherited. *Clin Endocrin Metabol 85*: 1584.

Jackson Rl, Hess RL, England JD (1979): Hb_1c values in children with over diabetes maintained in varying degree. *Diabetes Care 2*: 391–395.

Jouad H, Lemhadri A, Maghrani M, Ali Zeggwah N, Eddouks M (2003): Cholesterol lowering activity of the aqueous extract of Spergularia in normal and recent onset diabetic rats. *J Ethnopharmacol 87*: 43–49.

Kalavincela T (1998): Studies on rural medicine of Kanyakumari district with special reference to medicinal Plants. M. Phil. Thesis, Manonmaniam Sundranar University, Tirunelveli, Tamilnadu, India, p. 185.

Koenig RJ, Peterson CM, Jones RL, Saudek C, Lehrman M, Cerami A (1976): Correlation of glucose regulation and hemoglobin A_1c in diabetes mellitus. *New Engl J Med 295*: 417–420.

Kokate CK (1994): *Practical Pharmacognosy*. New Delhi, Vallabh Prakashan, pp. 107–113.

Ladeji O, Omekaran I, Solomon M (2003): Hypoglycemic properties of aqueous bark extract of *Ceiba pentandra* in STZ induced diabetic rats. *J Ethnopharmacol 84*: 139–142.

Lowry OH, Rosenburgh NJ, Farr AL, Randall RL (1951): Protein measurement with Folin-phenol reagent. *J Biol Chem 193*: 265–275.

Mahapatra B (1997): Hypoglycemic activity of *Coscinium fenestratum*. *J Res Ayu Siddha 18*: 89.

Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, Novelli M, Ribes G (1998): Development of a new model of type 2 diabetes in adult rats administered with streptozotocin and nicotinamide. *Diabetes 47*: 224.

National Cholesterol Education Program Expert Panel (1994): Second report of the expert panel on detection, evaluation and treatment of high blood cholesterol in adults. *Circulation 89*: 1329–1450.

Nicholas V (1956): The determination of glycogen in liver and muscle by use of anthrone reagent. *Ind J Biol Chem 220*: 583.

Nikkila EA, Kekki M (1973): Plasma transport kinetics in diabetes mellitus. *Metabolism 22*: 1.

Ohkawa H, Ohishi N, Yagi K (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem 95*: 351–358.

Ram A, Lauria P, Gupta R, Kumar P, Sharma VN (1997): Hypocholesterolemic effects of *Terminalia arjuna* tree bark. *J Ethnopharmacol 55*: 165–169.

Sachdewa A, Khemani LD (2003): Effect of *Hibiscus rosa sinensis* Linn. ethanol flower extract on blood glucose and lipid profile in STZ induced diabetes in rats. *J Ethnopharmacol 89*: 61–66.

Sauvaire Y, Baissae VH, Leconte O, Petit P, Ribes G (1996): Steroid saponins from fenugreek and some of their biological properties. *Adv Exp Med Biol 405*: 37–46.

Setter MS, White JR, Campbell RK (2000): Diabetes mellitus. In: *Herfindal's Textbook of Therapeutics Drug and Disease Management*. Philadelphia, Lippincott Williams & Willkins, pp. 377–405.

Shirwaikar A, Rajendran K, Dinesh Kumar C, Ramgopal Bodla (2004): Antidiabetic activity of aqueous leaf extract of *Annona squamosa* in streptozotocin-nicotinamide type 2 diabetic rats. *J Ethnopharmacol 91*: 171–175.

Singh GB, Singh S, Bani S, Malhotra S (1990): Hypotensive action of a *Coscinium fenestratum* stem extract. *J Ethnopharmacol 38*: 151–155.

The Expert Panel, National Cholesterol Education Program (1988): Report of the National Cholesterol Education Program Expert Panel on detection, evaluation and treatment of high blood cholesterol in adults. *Arch Intern Med 148*: 36–39.

Turner MA (1965): *Screening Methods in Pharmacology*, 2nd ed. New York, Academic Press, p. 26.

Vaidya AB (1994): *Terminalia arjuna* in cardiovascular therapy. *J Assoc Physicians India 42*: 281–282.

Varier PS (1994): *Coscinium fenestratum. Indian Medicinal Plants Compendium of 500 Species*, Hyderabad, Orient Longman Ltd, pp. 191–193.

Vats V, Yadav SP, Grover JK (2003): Effect of *T. foenum graecum* on glycogen content of tissues and the key enzymes of carbohydrate metabolism. *J Ethnopharmacol 85*: 237–242.