International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 4 (4): 292-296, 2008
ISSN 1811-7775

Effect of Hexane Extract of *Cassia fistula* Barks on Blood Glucose and Lipid Profile in Streptozotocin Diabetic Rats

A. Nirmala, J. Eliza, M. Rajalakshmi, Edel Priya and P. Daisy
Department of Biotechnology, Holy Cross College, Tiruchirapalli, Tamil Nadu, India

Abstract: Medicinal plants play a major role in the management of Diabetes mellitusespecially in developing countries. The present study investigated the possible protective effects of hexane extract of *Cassia fistula* bark on certain biochemical parameters in Streptozotocin (STZ) induced diabetes in rats. *Cassia fistula* (Caesalpinaceae) has been used in traditional medicine. The barks of *C. fistula* have already been scientifically proved to possess anti-oxidant properties. The hypocholesterolemic and hypoglycemic effects of the hexane extract of stem bark of *C. fistula*, in normal and streptozotocin induced diabetic rats, were investigated in the present study. Hexane extract of *C. fistula* bark at doses 0.15, 0.30, 0.45 g kg^{-1} body weight for 30 days suppressed the elevated blood glucose levels in diabetic rats. The extract at 0.45 g kg^{-1} was found to be comparable with glibenclamide, the reference drug. The lipid profile (total cholesterol, triglyceride, HDL-cholesterol, LDL and VLDL-cholesterol) after the extract treatment at 0.45 g kg^{-1} body weight showed remarkable improvement compared to the diabetic control animals. Antioxidant and polyphenol content present in the extracts might contribute to the antihyperglycemic and antilipidemic properties. Thus the results suggest that *Cassia fistula* barks would be effective in the treatment of diabetes and in prevention and management of coronary artery disease.

Key words: Antihyperglycemic effect, Bark, *Cassia fistula*, glucose level, lipid profile

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder affecting approximately 4% population worldwide and is expected to increase by 5.4% in 2025 (Kim *et al*., 2006). The worldwide prevalence of Diabetes mellitus is expected to increase by 42% from 51-72 million in the developed countries and by 170% from 84-228 million in the developing countries by the year 2025 (King *et al*., 1998).

With the increasing incidence of the disease, the field of herbal medicines and the demand to use natural products in the treatment of diabetes is also growing, as treatment with herbal medicines do not cause any harmful side effects. Therefore, it has become necessary to look for an economical as well as therapeutically effective treatment without side effects (Grover *et al*., 2003). This study describes the study of *Cassia fistula* Linn., (Caesalpinaceae, Common name: Golden shower, Indian Laburnum) native to India, the Amazon and Srilanka and found extensively diffused in various countries including Mauritius, South Africa, Mexico, China, West Indies, East Africa and Brazil as an ornamental tree. The plant is an important constituent in the traditional medicine of India and it possesses properties useful in the treatment of skin diseases, inflammatory diseases, rheumatism, ulcers, anorexia, jaundice and as laxatives (Kritikar and Basu, 1991). Different parts of this plant have been demonstrated to possess several medicinal values such as antitumor activity (Gupta *et al*., 2000), antioxidant activity (Luximon-Ramma *et al*., 2002) and hypoglycemic activity (Bhakta *et al*., 1997).

The leaf extract is indicated for its anti-tussive, wound healing and hepatoprotective properties (Bhakta *et al*., 1999). This fiber and mucilage content suggest that they can be used in the treatment of hypercholesterolemia (El-Saadany *et al*., 1991). Seeds of *C. fistula* are reported to have antibacterial (Perumal *et al*., 1998) antitumour (Gupta *et al*., 2000) and antfertility effect (Yadav and Jain, 1999) and four novel compounds have been isolated (Kuo *et al*., 2002). The antioxidant effect of the crude extracts of stem bark, leaves, flowers and fruit pulp of *C. fistula* have been assessed (Siddhuraju *et al*., 2002). Though the hypoglycemic efficacy of leaves and seeds of *C. fistula* have been studied (Esposito *et al*., 1991), a detailed antidiabetic screening of the *Cassia fistula* barks has not been reported. The present study reports the hypoglycemic and hypocholesterolaemic potential of hexane extract of *C. fistula* barks.

Corresponding Author: P. Daisy, Department of Biotechnology, Holy Cross College (Autonomous), Teppakulam, Tiruchirapalli-620002, Tamil Nadu, India Tel: 91-431-2700637 Fax: 91-431-2713312

MATERIALS AND METHODS

Plant material and extraction: During summer month of June and July, the freshly collected bark of this plant was chopped, shade dried and coarsely powdered. An authenticated voucher specimen (No. HC-15) of the plant has been preserved in our Department for future reference. The powder was defatted with petroleum ether (60-80°C) then extracted with hexane using soxhlet extractor. The hexane extracts were dried under reduced pressure using a rotary vacuum evaporator. The percentage yield was 7% w/w and the extracts were kept in refrigerator for further use.

Chemical used: Glibenclamide was obtained from Himedia Laboratory Limited, Mumbai, India and Streptozotocin was purchased from Sigma-Aldrich, St. Louis, USA. All other commercial reagents used were of analytical grade.

Animals: Wistar male albino rats weighing 150-220 g bred in the Animal Division of King's Institute, Chennai were used in this study. The animals were fed on a pellet diet (Sai Durga Feeds, Bangalore, India) and water ad libitum. The experimental protocol has been approved by the Institutional Animals Ethics Committee and by the regulatory body of the government (Reg. No. 585/05/A/CPCSEA).

Induction of experimental diabetes: A freshly prepared solution of streptozotocin (45 mg kg^{-1}) in 0.1 M citrate buffer pH 4.5 was injected intraperitoneally in a volume of 1 mL kg^{-1} (16). Forty eight hours after streptozotocin administration, rats with moderate Diabetes having glycosuria and hyperglycemia (i.e., with blood glucose of 200-300 mg dL^{-1}) were taken for the experiment. Same amount of citrate buffer (pH 4.5) was injected to normal rats through similar route as control.

Study design: In the experiment, a total of 36 rats (30 diabetic surviving rats, 6 normal rats) were used. The rats were divided into 6 groups of 6 rats each.

Group 1: Normal untreated rats

Group 2: Diabetic control rats given 1 mL of 0.3% CMC (carboxymethyl cellulose) solution using an intragastric tube

Group 3: Diabetic rats treated with hexane extract of *C. fistula* (0.15 g kg^{-1} body weight) in 1 mL of 0.3% CMC solution, daily using intragastric tube for 30 days

Group 4: Diabetic rats treated with (0.30 g kg^{-1} of *C. fistula* hexane extract body weight) in 1 mL of 0.3% CMC solution daily using intragastric tube for 30 days

Group 5 : Diabetic control rats given hexane extract of *C. fistula* (0.45 g kg^{-1} body weight) in 1 mL of 0.3% CMC solution daily using an intragastric tube for 30 days

Group 6: Diabetic rats given glibenclamide (600 μg kg^{-1} body weight) (Pari and Uma., 2000) in 1 mL of 0.3% CMC solution daily using an intragastric tube for 30 days

Blood preparation: At the end of 30 days the rats were anesthetized with diethlyl ether following a 12 h fast. Blood was drawn from retro-orbital plexus into plain EDTA tube. The blood was then centrifuged at 3000 rpm for 20 min using refrigerated centrifuge at 4°C. The plasma was used for determination of glucose and lipid profile.

Analytical procedure: Fastitng blood glucose was estimated by glucose oxidase-peroxidase method (Trinder, 1969). Plasma insulin level was assayed by Enzyme linked immunosorbent assay (ELISA) kit, using human insulin as standard. Glycosylated hemoglobin was estimated using the diagnostic kit from Biosystems, Spain. Serum was analyzed for total cholesterol (Allain *et al*., 1974), HDL, LDL and VLDL levels (Friedewald *et al*., 1972), triglycerides was measured by the method of Muller *et al*. (1977).

Statistical analysis: All values wee expressed as the mean obtained from a number of experiments (n). Data from Table 1-3 of normal, diabetic control, reference drug treated and *C. fistula* bark extract treated animals were compared by ANOVA followed by Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Table 1 shows the level of blood glucose, changes in body weight and urine sugar of normal and experimental rats. There was a significant elevation in blood glucose and urine sugar levels while the body weight decreased in streptozotocin diabetic rats when compared with normal rats. Administration of the hexane extract of *C. fistula* banks tends to bring the parameters significantly towards the normal indicating a better trend compared to the hypoglycemic drug (glibenclamide). The effect of the extract at a dose of 0.45 g kg^{-1} body weight

Table 1: Blood glucose levels, changes in body weight and urine sugar of normal and experimental animals

Groups	Body weight (g) Initial	Final	Fasting blood glucose (mg dL^{-1})	Urine sugar[A]
Normal	195±10.40	208±8.700	96.30±5.070[a]	Nil
Diabetic+control	203±13.60	150±11.65•••	237.00±14.76[b]	+++
Diabetic+*C. fistula* bark extract (0.15 g kg^{-1})	194±15.50	197±15.35***	218.43±18.70[b]	++
Diabetic+*C. fistula* bark extract (0.30 g kg^{-1})	197±16.30	206±10.23***	161.50±13.40[c]	+
Diabetic+*C. fistula* bark extract (0.45 g kg^{-1})	202±18.86	214±12.36***	111.40±10.70[ad]	Nil
Diabetic+Glibenclamide (600 μg kg^{-1})	196±11.20	207±13.63***	122.70±10.62[d]	Trace

Values are given as mean±SD for 6 rats in each group; Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT); Duncan procedure, range for the level 289, 3.03, 3.13, 3.20, 3.25; Diabetic control was compared with normal, •••$p<0.001$; Experimental groups were compared with diabetic control ***$p<0.001$; [A]: Indicates 0.25% sugar and (+++) indicates more than 1% sugar

Table 2: Changes in levels of plasma insulin and glycosylated hemoglobin of normal and experimental rats

Groups	Glycosylated hemoglobin m g^{-1} Hb	Plasma insulin (μU mL^{-1})
Normal	0.24<0.02[a]	16.12±1.02[a]
Diabetic control	0.83±0.06[b]	4.56±0.87[b]
Diabetic+*C. fistula* bark extract (0.45 g kg^{-1})	0.38±0.05[c]	13.80±0.55[c]
Diabetic+Glibenclamide (600 μg kg^{-1})	0.44±0.07[d]	12.23±0.67[d]

Values are given as mean±SD for 6 rats in each group; Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT)

was more highly significant than 0.15 and 0.30 g kg^{-1} body weight and therefore the dose was used for further biochemical studies.

In streptozocin induced diabetic rats oral administration of the hexane extract of *C. fistula* barks and glibenclamide, effectively lowered the high level of glycosylated hemoglobin and restored the low plasma insulin to normal levels (Table 2).

There was a significant increase in triglyceride level in control diabetic rats when compared to the normal animals. In diabetic rats supplemented with *C. fistula* bark extract and glibenclamide, the triglyceride level was significantly decreased (Table 3). Table 3 also shows the evaluations in total cholesterol, LDL and VLDL levels and a decrease in HDL level of streptozotocin diabetic rats when compared to the corresponding control rats. Administration of the extract and glibenclamide tends to bring back the levels to near normal.

Streptozotocin is well known for its selective pancreatic islet β-cell cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms (Papaccio *et al*., 2000). Intraperitoneal administration of streptozotocin (45 mg kg^{-1}) effectively induced diabetes in normal rats as reflected by glycosuria, hyperglycaemia and body weight loss when compared with normal rats.

In present study we have observed that the hexane extract of *Cassia fistula* bark can reverse these adverse effects caused by streptozotocin. The possible mechanism by which the extract brings about its antihyperglycemic action may be by potentiation of pancreatic secretion of insulin from β cells of islets or due to enhanced transport of blood glucose to peripheral tissue. This was clearly evidenced by the increased level o insulin in diabetic rats treated with extract.

The bark hexane extract administration to streptozotocin dosed animals reversed the weight loss. This ability to recover body weight loss seems to be due to its antihyperglycemic effect. The high glycosylated hemoglobin levels due to the oxidative stress of the erythrocytes (Andallu and Varadacharyulu, 2002), were also reversed by the treated of hexane extract of *C. fistula* barks.

These results indicating the antihyperglycemic efficacy of *C. fistula* barks are in accordance and similar to antidiabetic properties of other plant parts investigated (Esposito *et al*., 1991) further adding evidence to the traditional use of *C. fistula* in treating diabetes.

Cassia fistula barks, used extensively against a wide range of ailments, is known as a source of flavanoids, phenolic acids and xanthine glycosides (Gupta *et al*., 1989). It has been reported that the stembark of *C. fistula* is a potential source of lupeol, β-sitosterol and hexacosanol (Bahorun *et al*., 2005). These phenolic derivatives have also been reported to exhibit biological effects including antibacterial, antiviral, anti-inflammatory, antithrombotic, anticarcinogenic and vasodilatory actions. The bark extracts of *C. fistula* have been shown to have the highest antioxidant potential (Sen and Shukia, 1968). The antioxidant principles (sitosterols and lupeols) of *C. fistula* bark may also be responsible for hypoglycemic effects proved in this study.

Furthermore, the treatment of hexane extract of *C. fistula* barks showed to improve lipid profile by reducing the level of total cholesterol, triglyceride and LDL-cholesterol and at the same time increased the level of HDL-cholesterol. The improvement of lipid profile might be contributed by plant sterols (β sitosterol) found in barks. Plant sterol is well known for its cardioprotective properties by lowering the cholesterol level (Jones *et al*., 1997). This antihyperlipidaemic effect could represent a protective mechanism against the development of cardiovascular complications associated with diabetes (Stamler *et al*., 1993).

Table 3: Effect of *C. fistula* bark extract on the levels of triglycerides, total cholesterol and lipoprotein in normal and STZ- induced hyperglycemia rats

Groups	Triglycerides (mg dL^{-1})	Total cholesterol (mg dL^{-1})	HDL (mg dL^{-1})	LDL (mg dL^{-1})	VLDL (mg dL^{-1})
Normal	74.04±2.20^a	90.04±5.30^a	57.60±3.120^a	54.78±3.33^a	12.00±0.62^a
Diabetic control	103.73±6.50^b	216.41±6.1^b	17.64±1.52^b	93.07±5.32^b	21.31±3.40^b
Diabetic+*C. fistula* bark extract (0.45 g kg^{-1})	82.77±3.52^c	104.23±5.4^c	51.46±2.56^c	46.23±2.50^c	13.26±0.68^c
Diabetic+Glibenclamide (600 µg kg^{-1})	86.46±2.70^d	110.70±7.7^d	38.43±1.59^d	49.45±3.17^d	14.34±0.70^d

Values are given as mean±SD for six rats in each group; Values not sharing a common superscript letter differ significantly at $p< 0.05$ (DMRT); Duncan procedure, range for the level 2.95, 3.09, 3.20

CONCLUSION

The results showed that *C. fistula* bark extract displayed antihyperglycemic activities in experimental modes by reduced blood glucose level and improved lipid profile. Hence this work elicits the potential utilization of *C. fistula* barks in food system of diabetic patients or as prophylactics in nutritional or food supplement programs. The precise active substance(s), site(s) and mechanism (s) of its pharmacological effect are still to be determined.

ACKNOWLEDGMENTS

The financial support extended by the University Grants Commission (Project No. 32-505/(SR) is acknowledged. Special thanks to Ms. Poorani and Ms. Rajalakshmi for their help and technical assistance.

REFERENCES

Allain, C.C., L.S. Poom, C.S. Chan, W.S. Richmonal and P.C. Fu, 1974. Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.

Andallu, B. and N. Varadacharyulu, 2002. Control of hyperglycemia and retardation of cataract by mulberry (*Morus indica* L.) leaves in streptozotocin diabetic rats. Ind. J. Exp. Biol., 40: 791-795.

Bhakta, T., P.K. Mukherjee, K. Saha, M. Pal and B.P. Saha, 1997. Hypoglycemic activity of *Cassia fistula* Linn. (Leguminosae) leaf (methanol extract) in alloxan-induced diabetic rats. J. Ethnobot., 9: 35-38.

Bhakta, T., P.K. Mukherjee, M. Pal and B.P. Saha, 1998. Studies on antitussive activity of *Cassia fistula* leaf extract. Pharma Biol., 36: 140-143.

Bhakta, T., P.K. Mukherjee, K. Mukherjee, S. Banerjee, T.K. Mandar Maity, M. Pal and B.P. Saha, 1999. Evaluation of hepatoprotective activity of *Cassia fistula* leaf extract. J. Ethnopharmacol., 66: 277-282.

El-Saadany, S.S., R.A. El-Massry, S.M. Labib and M.Z. Sitohy, 1991. The biochemical role and hypocholesterolaemic potential of the legume *Cassia fistula* in hypocholesterolaemic rats. Die Nahrung, 35: 807-815.

Esposito Avella, M., A. Diaz, R. De Graia, I. De Tellor and M.P. Gupta, 1991. Evaluation of traditional medicine: Effects of *Cajanus cajan* L. and *Cassia fistula* L. on carbohydrate metabolism in mice. Rev. Med. Parama, 16: 39-45.

Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of LDL cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.

Grover, J.K., S.P. Yadav and V. Vats, 2003. Effect of feeding *Murraya koeingii* and *Brassica juncea* on kidney functions and glucose levels in streptozotocin diabetic mice. J. Ethnopharmacol., 85: 1-5.

Gupta, V., A. Agrawal and H.P. Tiwari, 1989. Isolation and characterization of two flavanoids and a xanthine glycoside from the stem bark of *Cassia fistula* Linn. Indian J. Chem., 28B: 282-284.

Gupta, M., U.K. Mazumder, N. Rath and D.K. Mukhopadhyay, 2000. Antitumour activity of methanolic extract of *Cassia fistula* L. seed against Ehrlich ascites carcinoma. J. Ethnopharmacol., 72: 151-156.

Kim, S.H., S.H. Hynn and S.Y. Choung, 2006. Antidiabetic effect of cinnamon extract on blood glucose in db/db mice. J. Ehthnopharmacol., 104: 119-123.

King, H., R.E. Aubert and W.H. Herman, 1998. Global burden of diabetes, 1995-2025: Prevalence, numerical estimates and projections. Diabetes Care, 22: 1414-1431.

Kritikar, K.R. and B.A. Basu, 1991. Indian Medicinal Plants. Vol. II, 2nd Edn., Periodical Experts Book Agency, New Delhi, India. pp: 856-860.

Kuo, H., P.H. Lee and Y.S. Wein, 2002. Four new compounds from the seeds of *Cassia fistula*. J. Nat. Prod., 65: 1165-1167.

Luximon-Ramma, A., T. Bahorun, M.A. Soobratte and O.I. Aruoma, 2002. Antioxidant activities of phenolic, proanthrocyanidin and flavanoid components in extracts of *Cassia fistula*. J. Agric. Food Chem., 50: 5042-5047.

Muller, P.H., R.M. Schmulling, H.M. Licbich and M. Eggstgein, 1977. A fully enzymatic triglyceride determination. J. Clin. Chem. Clin. Biochem., 15: 457-464.

Papaccio, G., F.A. Pisanti, M.V. Latronico, E. Ammendola and M. Galdieri, 2000. Multiple low dose and single high dose treatments with streptozotocin do not generate nitric oxide. J. Cell Biochem., 77: 82-91.

Pari, L.J. and Uma Maheswari, 2000. Antihyperglycaemic activity of *Musa sapientum* flowers: Effect on lipid peroxidation in Aloxan diabetic rats. Phytother. Res., 14: 136-138.

Perumal, R., S. Samy, S. Ignacimuthu and A. Sen, 1998. Screening of 34 medicinal plant's antibacterial properties. J. Ethnopharmacol., 62: 173-181.

Sen, A.B. and Y.N. Shukia, 1968. Chemical evaluation of *Cassia fistula*. J. India Chem. Soc., 45: 744-744.

Siddhuraju, P., P.S. Mohan and K. Becker, 2002. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.) : A preliminary assessment of crude extracts from stem, bark, leaves, flowers and fruit pulp. J. Agric. Food Chem., 79: 61-67.

Stamler, J., O. Vaccaro, J.D. Neaton and D. Wentworth, 1993. Diabetes, other risk, factors and 12 year cardiovascular mortality for men screened in Multiple Risk Factor Intervention Trial. Diabetes Care, 16: 433-444.

Trinder, P., 1969. Determination of glucose using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem., 6: 24-27.

Yadav, R. and G.C. Jain, 1999. Antifertility effect of aqueous extract of seeds of *Cassia fistula* in female rats. Adv. Contracept., 15: 293-301.