



Hypoglycemic activity of *Cassia auriculata* in neonatal streptozotocin-induced non-insulin dependent diabetes mellitus in rats

A. R. Juvekar*, G. V. Halade

Department of Pharmaceutical Sciences and Technology, University Institute of Chemical Technology, University of Mumbai, Nathalal Parikh Marg, Matunga, Mumbai-400019, India.

Abstract

Objective: The primary objective of the present research work was to investigate the effect of ethanol and aqueous extract of *Cassia auriculata* in streptozotocin induced neonatal model of Non-Insulin Dependent Diabetes Mellitus (NIDDM). **Methodology:** The ethanol extract of whole plant powder of *Cassia auriculata* was obtained by successive solvent extraction with petroleum ether (60-80°C) and ethanol (95%v/v). The aqueous extract was obtained by boiling plant material in distilled water and concentrated by evaporation. The rats were divided into 4 groups as diabetic treated and two groups maintained as control and diabetic control, each group contained six rats. The ethanol (400mg/kg) and aqueous extract (250mg/kg, 500mg/kg) of the *Cassia auriculata* administered orally to streptozotocin induced diabetic rats once a day and evaluated for hypoglycemic activity. The blood glucose, triglyceride, HDL cholesterol and total cholesterol were studied in streptozotocin induced neonatal diabetic rat model. **Results:** *Cassia auriculata* ethanol extract at dose of 400 mg/kg whereas aqueous extract at doses 250 and 500 mg/kg of their body weight were administered to diabetic rats for 28 days, lead to suppression in elevated glucose, cholesterol and triglycerides levels. The glibenclamide (0.45mg/kg) was used as reference standard. **Conclusion:** From the results obtained, it can be observed that ethanol and aqueous extract of *Cassia auriculata* have antidiabetic potential. The findings indicate that the *Cassia auriculata* possess the hypoglycemic as well as the antihyperlipidemic activity.

Key words: Blood glucose, *Cassia auriculata*, Streptozotocin, flavonoids.

1. Introduction

The plant *Cassia auriculata* Linn. (Cesalpiniaceae) is fast growing branched tall, evergreen shrub with reddish brown branches. It is a common plant in Asia, has been widely used

in traditional medicine as a potent adjunct in the treatment of rheumatism, conjunctivitis and diabetes. Seeds are used in ophthalmia and dysentery. [1, 2] Dried flowers and leaves of the

* Corresponding author
E-mail: arj@udct.org

Cassia auriculata are being used for medicinal treatment, [3] however fewer reports are available with respect to the pharmacological properties of the plant.

Hence the present investigation was undertaken to evaluate the effect of ethanol and aqueous extract of *Cassia auriculata* for antidiabetic activity in streptozotocin induced neonatal rat model. The effect of the extract was also compared with that of the standard drug glibenclamide, a well known antidiabetic drug.

Diabetes mellitus is one of the major threats to human health in 21st century. In the past two decades there has been an explosive increase in the number of people diagnosed with diabetes worldwide. The diabetes epidemic relates particularly to type II diabetes and is taking place both in the developed and developing nations. The global figure of people with diabetes set rise from the current estimate of 150 million to 220 million in 2010 and 300 million in 2025. [4]

Many traditional plant treatments for diabetes mellitus are used throughout the world. [5] Very few of the traditional folklore plant treatments for diabetes have received scientific scrutiny and that World Health Organization has recommended that this arena need priority. [6]

2. Materials and methods

2.1 Plant material

The entire plant powder ("Panchang") of *Cassia auriculata* Linn. L. (Cesalpiniaceae) was procured from the Anushka Herbals, Mumbai, India, which were collected during the month of March 2002 and authenticated at Quality Assurance department of Anushka Herbals, Mumbai, India. The certificate of analysis was received and preserved as authentication record for further correspondence. The powder was extracted using water and ethanol as solvents and physicochemically characterized.

2.2 Preparation of *Cassia auriculata* aqueous extract

Dried powder of *Cassia auriculata* were extracted with boiling distilled water for 2 h, evaporated to dryness and kept at 4°C. The yield of aqueous extract was 10.5%.

2.3 Preparation of *Cassia auriculata* ethanol extract

The dried plant powder of whole plant was defatted with petroleum ether (60-80°C) in Soxhlet extraction unit, subsequently it was extracted with ethanol and it was recovered in rota evaporator under reduced vacuum. The yield of ethanol extract was 2.7%.

2.4 Phytochemical investigation of the plant extracts

In order to detect the various constituents present in the aqueous and ethanol extract of *Cassia auriculata*, both extracts were subjected to the tests by Kokate [7] and Trease and Evans [8]. It was found that the major chemical constituents of the extract were polysaccharides and flavonoids.

2.5 Experimental Animals

Wistar albino rats of either sex (180-220g) randomly bred in well-controlled registered animal house facility of U.I.C.T., Mumbai, India. The animals were housed in standard environmental conditions of temperature (22±5°C) and humidity (55±5%) and 12 h light-dark cycles. They were fed on conventional laboratory pelleted diet and water *ad libitum*.

All the procedures were performed in accordance with the Institutional Ethical Committee constituted as per the directions of the committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) under Ministry of Animal Welfare Division, Government of India, New Delhi, India.

2.6 Induction of diabetes

The method of Portha *et al* was followed for the induction of diabetes. To induce type II diabetes, two day old Wistar neonates were injected intraperitoneally with 90mg/kg (n2STZ) streptozotocin (Sigma, USA) in 0.1 M citrate buffer (pH 4.3) solution. The control group received equivalent amount of citrate buffer. The pups were allowed to be with their respective mother and weaned at 4 weeks of age.

Eight weeks after the injection of STZ, the rats were checked for fasting blood glucose levels. [9,10] The animals showing fasting glucose more than 150mg/dl were considered as diabetic. The experimental animals were divided into six groups as I) Non-diabetic control, II) Diabetic control, III) Diabetic treated with ethanol extract of *Cassia auriculata* (400mg/kg), IV) Diabetic treated with aqueous extract of *Cassia auriculata* (250mg/kg), V) Diabetic treated with aqueous extract of *Cassia auriculata* (500mg/kg), VI) Diabetic treated with standard drug glibenclamide (0.45mg/kg body weight) [11].

2.7 Treatment Protocol

The blood samples were collected from 8 h. fasted animals under light ether anesthesia at the end of 8 weeks. The ethanol and aqueous extract of *Cassia auriculata* were suspended in 0.1% sodium alginate and orally administered to diabetic animals at concentration of 400 and 250, 500mg/kg respectively for 28 days. The serum was separated and analyzed for glucose, cholesterol, and triglycerides using diagnostic reagent kits (Emerck India Ltd., Mumbai, India).

2.8 Blood collection and serum separation

The blood was collected retro-orbitally from the inner canthus of the eye using micro hematocrit capillaries. The blood was collected in 1.5ml eppendorf tubes and centrifuged. The serum was separated from blood cells by centrifugation at

5000 rpm for 5 min. in temperature controlled centrifuge. (Superspin R-V/F_M, Plasto Crafts, Mumbai, India)

2.9 Estimation of Blood glucose

It was estimated by glucose oxidase-peroxidase (GOD-POD) method and expressed in milligrams per deciliter (mg/dl).

2.10 Estimation of serum lipid profile

Total cholesterol (TC), HDL cholesterol (HDLc) and triglycerides (TG) were estimated by enzymatic methods using commercially available kits.

2.11 Data and Statistical Analysis:

The data were expressed as the mean±SEM obtained from the number of experiments (n). One-way ANOVA with Dunnett's post test was performed using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. The Statistical significance was accepted at $p < 0.05$.

3. Results and discussion

In recent years, various plant extracts have been claimed to be useful for the treatment of diabetes mellitus [12]. Over 150 plant extracts and some of the active principle including flavonoids, are known to be used for the treatment of diabetes [13, 14].

Moreover tannin-containing drugs demonstrated anti-diabetic activity. [15-17] The intraperitoneal injection of streptozotocin (90 mg/kg) in Wistar rat pups (2 days old) produced mild hyperglycemia, impaired glucose tolerance and insulin resistance at the age of 8 weeks. Thus, this model slowly progresses to impaired glucose-stimulated insulin release and insulin resistance which are the marked features of type II diabetes [18].

Chronic treatment with aqueous extract of *Cassia auriculata* resulted in a 9% and 22% decrease in

Table 1.
Serum glucose and body weight changes in the normal and experimental animals.

Experimental Groups	Body Weight (g)		Fasting Blood Glucose (mg/dl)	
	Initial	Final	Initial	Final
Control	204 ± 5.14	213 ± 5.38	83.34 ± 3.58	97.28 ± 3.20*
Diabetic control	195 ± 5.43	205 ± 5.29	180.3 ± 3.51	173.0 ± 4.86
Diabetic + Ethanol <i>C. auriculata</i> (400 mg/kg)	201 ± 6.00	197 ± 7.81	167.0 ± 5.50	129.2 ± 3.55*
Diabetic + aqueous <i>C. auriculata</i> (250 mg/kg)	191 ± 3.54	207 ± 4.43	170.8 ± 4.70	155.42 ± 6.71**
Diabetic + aqueous <i>C. auriculata</i> (500 mg/kg)	206 ± 6.49	212 ± 6.52	169.2 ± 4.97	129.6 ± 5.60*
Diabetic + Glibenclamide (0.45 mg/kg)	209 ± 5.34	214 ± 4.99*	164.4 ± 3.03*	121.76 ± 7.19*

Values are given as mean ± SEM for six rats in each group. *P<0.01= significant vs diabetic control, **P<0.05 vs diabetic control

Table. 2
Changes in serum levels of cholesterol, triglyceride, and HDL cholesterol in control and experimental animals.

Experimental Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL Cholesterol (mg/dl)	Atherogenic Index (Total Cholesterol – HDL Cholesterol)
				HDL Cholesterol
Control	83.64 ± 3.44	50.40 ± 3.06	17.99 ± 1.57	3.88 ± 0.61*
Diabetic control	97.73 ± 3.99	82.23 ± 3.00	12.20 ± 1.25	7.56 ± 1.15
Diabetic + Ethanol <i>C. auriculata</i> (400 mg/kg)	93.90 ± 7.28	68.25 ± 3.28*	13.03 ± 1.99	7.34 ± 1.62
Diabetic + aqueous <i>C. auriculata</i> (250 mg/kg)	90.85 ± 3.37	72.31 ± 3.29*	17.81 ± 1.82	4.37 ± 0.60
Diabetic + aqueous <i>C. auriculata</i> (500 mg/kg)	84.91 ± 3.29*	64.38 ± 3.12**	21.35 ± 1.90*	3.17 ± 0.49**
Diabetic + Glibenclamide (0.45 mg/kg)	92.2 ± 3.32	60.53 ± 3.19*	16.64 ± 1.62	4.87 ± 0.72*

Values are given as mean ± SEM for six rats in each group. *p<0.01 = Significant vs diabetic control, **p<0.05 vs diabetic control.

blood glucose level at the dose of 250 and 500mg/kg in type II diabetic rats respectively, whereas ethanol extract lead to 23% decrease in blood sugar level at 400mg/kg.

The possible mechanism by which aqueous and ethanol extract brought about its hypoglycemic action may be due to enhanced transport of blood glucose to peripheral tissue or by increase

secretion of insulin from β -cell of islets. Bonner-Weir *et al.*, [18] have reported retarded growth of streptozotocin treated animals compared to control animals, however in the present investigation, the body weight, food and water consumption of diabetic and non-diabetic was not significantly different from each other as shown in Table no. 1.

It can be seen from the table no. 2 that chronic treatment with aqueous *Cassia auriculata* extract reduced cholesterol level by 13% at a dose of 500mg/kg, whereas in case of glibenclamide it was observed 6%. The triglyceride level declined to 17% and 21% at the dose of 400 and 500mg/kg of ethanol and aqueous extract respectively.

There was significant increase in HDL cholesterol in all doses of *Cassia auriculata* treatment eventually reduction in the atherogenic index indicates that the plant has cardio-protective potential along with antidiabetic effects.

The present investigation reveals that ethanol and aqueous extract of *Cassia auriculata* has shown significant pharmacological activity towards lowering of blood glucose, managing dyslipidemia and other cardiovascular risk associated with type II diabetes. From the phytochemical investigation it was found that the major chemical constituents of the extract were polysaccharides, flavonoids, anthracene derivatives and dimeric procyanidins [19, 20].

On the basis of above evidence it is possible that the presence of procyanidins could be responsible for the observed antidiabetic activity [21].

References

1. Kirtikar KR, Basu BD. (1988) In: Basu LM (Eds.) *Indian Medicinal Plants*, II edn. The Indian Press: Allahabad; 867.
2. Bopalal Vaidya. (1998) *Nighantu Aadarsha*, Part-I, Chaukhmba Bharati Academy, II Ed. Varanasi: India; 481.
3. Sawhney AN, Khan MR, Ndaalio G, Nkonya, MHH, Wavers H. (1978) *Pakistan J. Sci. Indigenous Res*, 21: 189-192.
4. Paul Zimmet, KG, Alberti MM. (2001) *Nature*, 414: 781-787.
5. Swanston Flatt SK, Day C, Bailey CJ, Flatt RR. (1990) *Diabetologia*, 33: 462-464.
6. WHO expert committee on diabetes mellitus second report. (1980) *Technical Report Series* 646, WHO: Geneva; 61.
7. Kokate CK. (1994) *Practical Pharmacognosy*, 4th Ed. Vallabh Prakashan: New Delhi, India; 107.
8. Trease GD, Evans WC. *Pharmacognosy*, 14th Ed. Harcourt Brace and Company: London, UK; 565.
9. Fantus IG, Chayoth R, O'Dea L, Marliss EB, Yale JF, Grose M. (1987) *Diabetes*, 36: 654-660.
10. Portha B, Picon L, and Rosselin G. (1979) *Diabetologia* 17: 371-377.
11. Goyal RK, Shinde UA, Mehta AA. (2001) *Indian J. Pharmacology*, 39: 864-870.
12. Shukla R, Sharma SB, Puri D, Prabhu KM, Murthy PS. (2000) *Indian J. Clinical Biochem.*, 15 (Suppl): 169-177.
13. Bailey CJ. (1989) *Diabetes care*, 12: 553-564.
14. Choi JS, Yokozava T, Oura H. (1991) *Planta Med*, 57: 208-211.
15. Erenmemisoglu A, Kelestimur F, Kokaer AH, Ustun H, Tekol Y, Ustul M. (1995) *J. Pharm Pharmacol*, 47: 72.
16. Iwu MM. (1983) *Fitoterapia*, 54: 243.
17. Iwu MM. (1980) *Planta Med*, 39: 247.
18. Bonner-WS, Trent DF, Honey RN, Weir GC. (1981) *Diabetes*, 30: 64-69.
19. Singh RB, Jindfal VK. (1990) *J. Econ Botany Phytochem*, 1: 25.
20. Rai KN, Dasundhi RA. (1990) *J. Bangladesh Acad Sci*, 14: 57.
21. Mario DA, Alessandra B, Errica B. (2004) *Cardiovas Res*, 63: 593-602.