

# Antihyperglycemic Activity of Various Fractions of *Cassia auriculata* Linn. in Alloxan Diabetic Rats

S. J. SURANA, S. B. GOKHALE, R. B. JADHAV, R. L. SAWANT AND JYOTI B. WADEKAR\*  
P. D. V. V. P. F's College of Pharmacy, Vilad Ghat, Post MIDC, Ahmednagar-414 111, India

Surana, *et al.*: Antihyperglycemic Activity of *Cassia auriculata* Linn. in Diabetic Rats

Present work describes the potent antidiabetic fraction from flowers of *Cassia auriculata* Linn. Hydromethanolic extract along with its ethyl acetate and n-butanol fractions were evaluated for antidiabetic activity in alloxan-induced diabetes in rats. The n-butanol fraction exhibited significant reduction ( $p < 0.001$ ) in blood glucose levels and was also found effective in restoring the blood lipids and proteins to normal level. The activity was found comparable with standard drug phenformin. The hydromethanolic extract and its fractions were subjected to preliminary qualitative chemical investigations which indicated the presence of phenolic compounds, carbohydrates, tannins, steroids and amino acids.

**Key words:** *Cassia auriculata*, antidiabetic activity, alloxan-induced diabetes, hydromethanolic extract

Diabetes Mellitus is a fast growing medical problem in affluent societies and critically attack on metabolic activity of patient. As per the WHO, Diabetes Mellitus

is a heterogeneous metabolic disorder characterized by common feature of chronic hyperglycaemia with disturbance in carbohydrate, fat and protein metabolism<sup>1</sup>. In India, this disorder is on alarming condition as compared to most of the developed countries. Despite advances in understanding of the disorder and the management, the mortality and

---

## \*For correspondence

Pushkar Apartment, Shivaji Chowk, Jangam Lane,  
Rahuri (Dist. Ahmednagar)-413 705, India.  
E-mail: jyotsfriend21@yahoo.com

morbidity due to this disease is increasing<sup>2</sup>. The focus has been shifted to treat the various ailments through plant-derived drugs due to their safety, efficacy, cultural acceptability and lesser side effects.

*Cassia auriculata* Linn., a member of genus *Cassia* belonging to family *Caesalpiniaceae* is commonly known as Tanner's *Cassia*<sup>3</sup>. It is a shrub found throughout southern, western and central India<sup>4</sup>. The various parts of the plant has been reported to possess a number of therapeutic activities to manage disease states like leprosy, asthma, gout, rheumatism<sup>5</sup> and diabetes<sup>6</sup>. It is also used as antipyretic, antiulcer and in the treatment of skin infections<sup>7</sup>. The flower has been reported to contain flavonoids, proanthocyanidins and  $\beta$ -sitosterol<sup>8-9</sup>.

In folk remedies, flowers of *Cassia auriculata* Linn. are proposed to have antidiabetic activity. From literature survey, it was evident that the aqueous extract of flowers has been reported for its antidiabetic activity in streptozotocin-diabetic rats at a dose of 0.45 g/kg body weight<sup>10</sup>. In the present investigation an attempt has been made to find out the potent fraction responsible for the said activity at a dose of 0.20 g/kg body weight. Thus, the present work describes effect of the hydromethanolic extract and its fractions on the blood glucose, total protein, cholesterol and triglyceride level in alloxan-diabetic rats.

The flowers of *Cassia auriculata* Linn. were collected from the premises of Shirpur situated in Dhule district of Maharashtra during March 2005. The crude drug was authenticated from the Botany Department, L. K., Dr. P. R. Ghogrey Science College, Dhule (MS). Herbarium specimen was deposited in Botany Department for future reference. The flowers were dried under shade and crushed into coarse powder.

Coarse powder was extracted by cold maceration method using methanol:water (1:1). The extract thus obtained was concentrated in rotary flash vacuum evaporator and further dried in vacuum oven. The hydromethanolic extract was subjected to fractionation with ethyl acetate and n-butanol. The fractions were dried in vacuum oven and utilized for animal studies.

In house bred Wistar male rats weighing between 180-220 g were utilized for study. Animals were housed in polypropylene cages and given standard pellet diet and water *ad libitum*. The ethical clearance was obtained from the Institutional Animal Ethical Committee (Approval no. 152/02/C/CPCSEA). Diabetes was induced in rats by intraperitoneal injection of alloxan monohydrate<sup>11</sup> (S. D. Fine Chemicals, Mumbai) in ice-cold citrate buffer, pH 4.5 at a dose of 0.12 g/kg. The diabetic state was confirmed 48 h after alloxan injection by weight loss and hyperglycemia<sup>12</sup>. Rats with blood sugar levels 200-350 mg/dl were selected for the study.

The rats were divided into six groups each carrying six animals. Group I served as diabetic control and received 0.3% w/v CMC, orally and alloxan. Group II served as positive control and received standard drug phenformin (0.04g/kg). Group III received hydromethanolic extract of flower (0.20g/kg). Group IV received n-butanol fraction of hydromethanolic extract (0.20g/kg). Group V received ethyl acetate fraction of hydromethanolic extract (0.20g/kg). Group VI served as normal untreated group. The treatment was continued for 8 d by administering the respective fractions/drug in 0.3% w/v CMC. On ninth day of the treatment, blood samples were collected by puncturing the retro-orbital plexus under mild ether anesthesia and kept aside for ½ h for clotting. Serum was separated by centrifuging the samples at 6000 rpm for

**TABLE 1: EFFECT OF EXTRACT AND FRACTIONS OF CASSIA AURICULATA FLOWERS ON BODY WEIGHT AND BLOOD SUGAR LEVEL**

Groups	Body weight (g)		Blood sugar level (mg/dl)		
	Before commencement of treatment	After end of the treatment	Normal	Alloxan treated	After extract/drug treatment
Normal control (Non-diabetic)	191.5±1.54	190±1.41	79.5±9.5	----	82.5±1.9
Diabetic control (Untreated)	188.8±0.92	175.6±1.92	83.2±5.6	291.2±1.6	----
Phenformin (0.04 g/kg)	183.6±1.67	182.6±1.25	90.5±8.6	372.0±2.1	141.8±1.5*
Hydromethanolic extract (0.20 g/kg)	216.5±5.02	210.6±4.1	86.5±0.9	293.2±1.6	201.8±2.6*
n-Butanol fraction (0.20 g/kg)	187.0±1.77	185.23±1.52	83.3±0.8	283.2±1.6	88.9±1.5*
Ethyl acetate fraction (0.20 g/kg)	206.0±1.31	201.2±1.29	88.6±0.9	289.6±1.9	127.2±1.5*

The observations are given as mean±SEM, n= 6, \*p<0.01, compared with diabetic control group

**TABLE 2: EFFECT OF EXTRACT AND FRACTIONS OF CASSIA AURICULATA FLOWERS ON BIOCHEMICAL PARAMETERS**

Groups	Biochemical parameters		
	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Total protein (g/dl)
Normal control (Non-diabetic)	67.2±1.1	95.8±1.7	7.1±0.1
Diabetic control (Untreated)	179.3±1.7	186.2±1.3	4.4±0.5
Phenformin (0.04 g/kg)	73.2±1.3*	112.9±1.0*	7.9±0.1*
Hydromethanolic extract (0.20 g/kg)	84.5±3.4*	125.7±1.3*	6.7±0.2*
n-Butanol fraction (0.20 g/kg)	74.6±1.2*	110.1±1.3*	7.4±0.1*
Ethyl acetate fraction (0.20 g/kg)	85.5±1.2*	121.6±2.5*	7.4±0.2*

The observations are given as mean±SEM, n= 6, \*P<0.01 compared with diabetic control group

20 mins and stored in the refrigerator until analyzed. The serum was analyzed for blood glucose level (GOD-PAP method)<sup>13</sup>, total protein (Biuret method)<sup>14</sup>, cholesterol (CHOD-PAP method)<sup>15</sup> and triglycerides (enzymatic method)<sup>16</sup> according to the kit manual. The quantitative measurements were made on six animals in each group and the values of biochemical estimations are expressed as mean±SE. The data obtained were subjected to one-way ANOVA followed by Bonferroni multiple comparison test<sup>17</sup>.

Alloxan-induced  $\beta$ -cell cytotoxicity is well established animal model for investigating antidiabetic activity. From the results, it is evident that hydromethanolic, n-butanol and ethyl acetate fraction shown significant ( $P<0.001$ ) reduction in blood glucose level. However n-butanol fraction was highly effective and results are comparable with that of reference drug, phenformin (Table 1). Alloxan treated rats shown substantial weight loss and also affect carbohydrate, lipid and protein metabolism. All three extracts were found to be effective in restoring the body weight of animals to the normal value. Elevated blood lipids especially cholesterol and triglycerides as well as reduction of protein level are other indicators of diabetic condition. Diabetic animals treated with hydromethanolic extract as well as ethyl acetate and n-butanol fraction shown significant ( $P<0.001$ ) effect on serum protein, cholesterol and triglyceride level (Table 2). In comparison to other extracts, n-butanol fraction was found to be more potent in normalizing the blood lipids and protein level. Proximate chemical analysis reveals the presence of steroids, flavonoids, tannins and carbohydrates in hydromethanolic extract

where as ethyl acetate and n-butanol fractions were found to be very rich in flavonoid content. Thus, flavonoids from *Cassia auriculata* Linn. may be responsible for its antidiabetic potency. This study, therefore, indicates n-butanol as a potent fraction responsible for antihyperglycemic effect of *Cassia auriculata* flowers in alloxan-induced diabetes.

## REFERENCES

1. Harsh M. Textbook of Pathology. 5<sup>th</sup> ed. New Delhi: Medical Publishers Ltd; 2005. p. 842.
2. Dhanbal SP. Evaluation of therapeutic activity and development of quality control profiles for some antidiabetic herbal drugs. Indian J Pharm Educ 2004;8:163-5.
3. Nadkarni KM. The Indian materia medica. Vol. I. Mumbai: Popular Prakashan; 2002. p. 284.
4. Anonymous. A dictionary of Indian raw materials and industrial products: The Wealth of India. Revised ed. Vol. III. New Delhi: Publication and information Directorate, CSIR; 2003. p. 334-7.
5. Kirtikar KR, Basu BD. Indian medicinal plants. 2<sup>nd</sup> ed. Vol. II. Dehradun: International Book Distributors; 2006. p. 868.
6. Joshi SG. Textbook of Medicinal Plants. New Delhi-Oxford: IBH Publishing Co.; 2000. p. 119.
7. Qadry JS. Shah and Qadry's Pharmacognosy. 12<sup>th</sup> Revised ed. Ahmedabad: B.S. Shah Prakashan; 2005. p. 243.
8. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants. Vol. II. New Delhi: National Institute of Science Communication; 1998. p. 147.
9. Asolkar LV, Kakkar KK, Chakre OJ. Second Supplement To, Glossary of Indian medicinal plants with active principles. Part I (A-K). New Delhi: National Institute of Science Communication; 1965-81. p. 177.
10. Pari L, Latha M. Effect of *Cassia auriculata* flowers on blood sugar levels, serum and tissue lipids in streptozotocin diabetic rats. Singapore Med J 2002;43:617-21.
11. Chattopadhyay S, Ramanathan M, Das J, Bhattacharya SK. Animal models in experimental diabetes mellitus. Indian J Expt Bio 1997;35:1141-5.
12. Siddique O, Sun Y, Lin JC, Chum YW. Facilitated transdermal transport of insulin. J Pharm Sci 1987;76:341-5.
13. Patrick N, Henri D, Vincent B, Etienne P. Measurement of postprandial incremental glucose and insulin changes in healthy dogs: Influence of food adaptation and length of time of blood sampling. J Nutr 1998;128:2659S-62S.
14. Burkhardt RT, Batsakis JG. An interlaboratory comparison of serum total protein analyses. Am J Clin Pathol 1978;70:508-10.
15. Pierre NM, Demacker A, Marja H, Helga TD, Henk B. Precipitation methods for high-density lipoprotein cholesterol measurement compared, and final evaluation under routine operating conditions of a method with a low sample-to-reagent ratio. Clin Chem 1997;43:663-8.
16. Quraishi R, Lakshmy R, Prabhakaran D, Mukhopadhyay A, Jaikhanil B. Use of filter paper stored dried blood for measurement of triglycerides. Lipids Health Dis 2006;5:20-2.
17. Moon KS, Hwang K, Mark JR, Diane MH, Dean TY, Ian Y, et al. Antihyperglycemic activity of zinc plus cyclo (his-pro) in genetically diabetic goto-kakizaki and aged rats. Exp Biol Med 2003;228: 1338-45.

Accepted 7 April 2008

Revised 18 September 2007

Received 2 February 2007

Indian J. Pharm. Sci., 2008, 70 (2): 227-229