Assessment of anti hyperglycemic fractions isolated from *Albizia procera* stem bark chloroform extract using STZ induced diabetic albino rats

Praveen kumar P*, Ramesh A[1] and Prasad K[2]

*[,2]*Shri Vishnu college of Pharmacy, Bhimavaram, India*
[1]Vishnu Institute of Pharmaceutical Education & Research,Vishnupur, Narsapur, Medak

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ABSTRACT

Objective: The present Study was to identify more effective hypoglycemic fractions from chloroform extract of *Albizia procera* stem bark. **Material and methods:** Isolated fractions of *Albizia procera stem bark chloroform extract* were given individually to different batches of rats both normal (80 mg/kg of b.wt animals) and STZ induced diabetic rats (160mg/kg b.wt animals) after an overnight fast. The blood glucose levels were measured at 0, 1, 2, 3, 5 and 6 hours after the treatment. Fractions were also treated to STZ induced diabetic rats by chronically (80mg/kg b.wt). **Results:** The fractions E of *Albizia procera stem bark chloroform extract was* shown maximum blood glucose lowering effect in both normal and STZ diabetic rats with acute and chronic treatment. The other fractions are also showing hypoglycemic and antihyperglycemic activity, but the effect is significantly less than that of fraction E. The antihyperglycemic activity of fractions of *Albizia procera stem bark chloroform extract* was compared with the treatment of glibenclamide. **Conclusion:** The present data confirm the anti diabetic activity of *Albizia procera* in Indian traditional medicine for Diabetes mellitus treatment. The anti hyperglycemic action attributed to the presence of valuable flavonoids, terpinoids in the fraction E.

Keywords: *Albizia procera, hyperglycemia,* Streptozocin (STZ)

INTRODUCTION

Diabetes Mellitus is a chronic metabolic disease resulting from insulin deficiency and insulin resistence.[1] It is a complex disorder in the way the body's ability to convert food into energy is impaired. Consequently, glucose builds up in the blood instead of moving into cells, called hyperglycemia, if left untreated, hyperglycemia can become severe and contribute to the development of microvascular and macrovascular complications of diabetes.[2] In the long term, persistent hyperglycemia, even if not severe, can lead to complications affecting your eyes, kidneys, nerves and heart. However, Diabetes care is complex and requires that many issues addressed, beyond glycemic control.[3]

Treatment of diabetes mellitus by insulin and oral hypoglycemic drugs fails to prevent diabetes related complications in many patients, indicating that additional alternative treatments could be helpful.[4] Herbal medicines have been used in medical practice for thousands of years and are recognized especially as a valuable and readily available healthcare resource. During the past decades, the contribution of herbal medicines and their preparations has attracted much interest in the pharmaceutical industry. However, most herbal medicines still need to be investigated scientifically, although the experience obtained from their traditional use over the years should not be ignored.[5, 14]

***Corresponding author.**

*P. Praveen kumar (Ph.D),
Asst. professor, Dept of Pharmacology,
Shri Vishnu College of Pharmacy, Vishnupur,
Bhimavaram. 534202
Tel: 9000561611

E-mail: praveenpharmaco@gmail.com

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Albizia procera is a medium-sized, fast growing, pioneer tree species belonging to the Mimosaceae. It also occurs in tropical semi-evergreen, moist deciduous and northern subtropical forests.[6] All parts of the plant are reported to show anticancerous activity. The plant is used for stomach & intestinal disease and during pregnancy; bark decoction is given for rheumatism and hemorrhages.[7] Arial parts of extracts reported α glucosidase inhibitory activity, which was useful to prevent postprandial elevated glucose level in type II diabetes mellitus.[8] The aim of present study is to evaluate the hypoglycemic and anti hyperglycemic activities of fractions isolated from chloroform extract of *Albizia procera* stem bark.

MATERIALS AND METHODS

Collection and processing of plant material

About 2 kg of the stem bark sheaths of *Albizia procera* were collected from the deciduous forest of Thirumala in Andhra Pradesh State, India, in the months of March and June 2013. Samples were authenticated by Dr. Madhave setti, Department of Botany, Sri Venkateswara University. The stem bark sheaths were cleaned and dried in hot air oven under 50°C for 2 days. These were ground to powder using the laboratory Hammer mill. Powdered samples were collected and stored in air- and water-proof containers protected from direct sunlight and heat until required for extraction.

Preparation of extract

Obtained powder (1Kg) was macerated with different solvents Petroleum ether, chloroform, ethanol and distilled water at room temperature for 7 days and obtained yield was 1.45g/kg, 2.9 g/kg and 315g/kg respectively.

Isolation process of fractions

Thin-layer chromatography method was carried out using silica gel aluminum plate 60F-254, 0.5mm (TLC plates, Merck). The solvent system used for TLC was Petroleum ether/ethyl acetate (7:3). The spots were visualized in UV light and 10% of H_2SO_4 in methanol. The chloroform extract was subjected to column chromatography (silica gel 60-100) for further purification. The column was equilibrated for one hour with petroleum ether at flow rate 5 ml/min. The sample was (1 g dissolve in chloroform) loaded on to the column, fourteen fractions were collected using Petroleum ether, Petroleum ether: Chloroform (9:1), Petroleum ether: Chloroform (7:3), Chloroform: Petroleum ether (9:1), Chloroform: methanol (9.5:0.5), Chloroform: methanol (9:1), Chloroform: Methanol (8:2). Above yielded product were pooled into six fractions based on TLC. The yield values of fraction A 72.5mg/g, fraction B 102.5 mg/g, fraction C 115 mg/g, fraction D 125mg/g, fraction E (107.5mg/g) and fraction F 227.5mg/g, respectively. All six fractions were checked for their hypoglycemic and anti hyperglycemic activity.

Phytochemical analysis

Phytochemical analysis of fractions was carried out by different methods.[9]

Experimental design

Ethics statement

All animal experiments were conducted with the approval of the Institutional Animal Care Committee and Committee for purpose conducting supervising experimental animals (IACC& 439/PO/01/a/CPCSEA) of Shri Vishnu college of Pharmacy, Bhimavaram, Andhra University, India.

Animals

Albino rats of Wistar strain weighing 150-200g were purchased from MKM, Hyderabad. The rats were kept in polypropylene cages (3 in each cage) at an ambient temperature of 25±2°C and relative humidity of 55–65%. A 12 h light and dark schedule was maintained in the air conditioned animal house. All the rats were fed with common diets for 1 week after arrival, and then divided into groups with free access to food and water.

Evaluation of hypoglycaemic effect of isolated fraction from Albizia procera stem bark of chloroform extract on normoglycemic rats[10]

Male Wistar normoglycemic rats (150–200gm) were used in the experiment. All experiments were carried out using six animals per group.

Groups	Treatment
I	Normal group (Tween 80)
II	Normoglycemic rats + Glibenclamide (10 mg/kg)
III	Normoglycemic rats + Fraction A (80 mg/kg)
IV	Normoglycemic rats + Fraction B (80 mg/kg)
V	Normoglycemic rats + Fraction C (80 mg/kg)
VI	Normoglycemic rats + Fraction D (80 mg/kg)
VII	Normoglycemic rats + Fraction E (80 mg/kg)
VIII	Normoglycemic rats + Fraction F (80 mg/kg)

Blood samples were collected from the tail vein at 0, 1, 2, 3, 4, 5 and 6 hr after oral administration. Blood glucose concentration was estimated by enzymatic glucose oxidase method using a commercial glucometer (Accue check active). The percentage variation of glycemia for each group was calculated in relation to initial (0 h) level, according to:

% glycemic change = G0 – Gt × 100/Go

Where G0 were initial glycemia values and Gx were the

STZ induced diabetic rats[10]

Diabetes was induced in male albino rats by intraperitoneal administration of STZ (a single dose 45 mg/kg b.wt) dissolved in freshly prepared 0.01 M citrate buffer (p^h 4.5). After 72 hrs rats with marked hyperglycemia (blood glucose ≥ 300 mg.dl) were selected and use for study.

Design of Anti hyperglycaemic effect of isolated fraction from Albizia procera stem bark of chloroform extract by acute treatment on STZ induced diabetic rats

Male Wistar normoglycemic rats (150–200 g) were used in the experiment. All experiments were carried out using six animals per group.

Group	I	Normal group (Tween 80)
Group	II	Diabetic rats
Group	III	Diabetic rats + Glibenclamide (20 mg/kg)
Group	IV	Diabetic rats + Fraction A (160 mg/kg)
Group	V	Diabetic rats + Fraction B (160 mg/kg)
Group	VI	Diabetic rats + Fraction C (160 mg/kg)
Group	VII	Diabetic rats + Fraction D (160 mg/kg)
Group	VIII	Diabetic rats + Fraction E (160 mg/kg)
Group	IX	Diabetic rats + Fraction F (160 mg/kg)

Blood samples were collected from the tail vein at 0, 1, 3, 4, 5 and 6 hr after treatment administration. Blood glucose concentration was estimated by enzymatic glucose oxidase method using a commercial glucometer (Accue check active) The percentage variation of glycemia for each group was calculated in relation to initial (0 h) level, according to:

% glycemic change = G0 – Gt × 100/Go

Where G0 were initial glycemia values and Gx were the final glycemia.

Design of Anti hyperglycaemic effect of isolated fraction from Albizia procera stem bark of chloroform extract by chronic treatment on STZ induced diabetic rats

Male Wistar normoglycemic rats (150–200 gm) were used in the experiment. All experiments were carried out using six animals per group.

Group	I	Normal group (Tween 80)
Group	II	Diabetic rats + Tween 80
Group	III	Diabetic rats + Glibenclamide (10 mg/kg)
Group	IV	Diabetic rats + Fraction A (80 mg/kg)
Group	V	Diabetic rats + Fraction B (80 mg/kg)
Group	VI	Diabetic rats + Fraction C (80 mg/kg)
Group	VII	Diabetic rats + Fraction D (80 mg/kg)
Group	VIII	Diabetic rats + Fraction E (80 mg/kg)
Group	IX	Diabetic rats + Fraction F (80 mg/kg)

Diabetic rats were treated with isolated fraction for 14 days, blood glucose concentrations were determined by Coralyzer 100. Percentage glycemic variation was calculated as a function of time (t) by using formula:

% glycemic change = G0 – Gt × 100/Go

Go and Gt represent glycemic value before (i.e., Zero time and glycemic value at 7days after administration of the extracts, respectively).

ANALYSIS

Results were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using Graph pad prism with one-way analysis of variance (ANOVA). A difference was defined as significant when $P<0.05$.

RESULTS

Effect of various fractions on the blood glucose level of normal rats.

Table 2, (Fig 1) shows the effect of isolated fractions on the fasting blood glucose level in normoglycemic rats. Among these, fraction D and E showed significant hypoglycemic activity 60.4%, 67.3% respectively. 53.1% and 51.2% of glucose reduction with fraction B and C, 44.01% and 36.7% of glucose reduction with fraction F and A with 80 mg/kg b.wt. after 6 hr treatment in normoglycemic rats.

Table 1. Phytochemical analysis

Tests	Fraction A	Fraction B	Fraction C	Fraction D	Fraction E	Fraction F
Alkaloids	–	–	–	–	–	–
Terpenoids	+	+	+	+	+	+
Saponins	–	+	–	+	–	–
Cardiac glycosides	–	+	–	–	+	–
Tanins	–	–	–	–	–	–
Carbohydrates	+	–	–	+	–	+
Proteins	–	–	–	–	–	–
Flavanoids	+	–	–	–	+	+

Table 2. Effect of different fractions isolated from *Albizia procera* stem bark chloroform extract on normoglycemic rats

Groups	Blood glucose levels at different time intervals (hrs)							% of glucose reduction
	0 hr	1 hr	2hr	3hr	4hr	5 hr	6hr	
I	123 ± 2.3	120.5 ± 1.9	119 ± 2.1	112 ± 1.5	110 ± 1.3	106 ± 1.4	109 ± 1.1	11.3
II	124.7 ± 2.1	72.5 ± 3.5	43.4 ± 1.4	48.3 ± 1.3	58.4 ± 2.1	64.2 ± 2.3	60.4 ± 3.2	51.5
III	131.6 ± 2.2	147.2 ± 2.5	103.3 ± 1.3	83.2 ± 1.4	85.4 ± 1.3	87.5 ± 1.8	92.2 ± 2.1	36.7
IV	116.3 ± 1.2	92.3 ± 1.6	86 ± 1.4	54.5 ± 1.3	69 ± 1.4	73.4 ± 2.3	81.3 ± 1.4	53.1
V	128.3 ± 2.4	102.3 ± 1.3	62.6 ± 1.4	70.3 ± 1.4	79.4 ± 2.3	72.6 ± 1.3	66.4 ± 1.9	51.2
VI	107.3 ± 1.3	91.3 ± 2.1	101.8 ± 2.5	86.3 ± 1.2	82.4 ± 1.2	42.4 ± 1.3	47.8 ± 1.3	60.4
VII	120.4 ± 2.3	109.5 ± 1.4	71.3 ± 1.2	60 ± 2	62.23 ± 1.2	39.3 ± 1.1	48.6 ± 1,2	67.3
VIII	90.2 ± 1.3	86 ± 2	70.4 ± 1.3	61.3 ± 1.4	64.2 ± 1.3	50.5 ± 1.4	64 ± 1.8	44.01

Values are given as mean ± SEM. Values were significant P < 0.05.

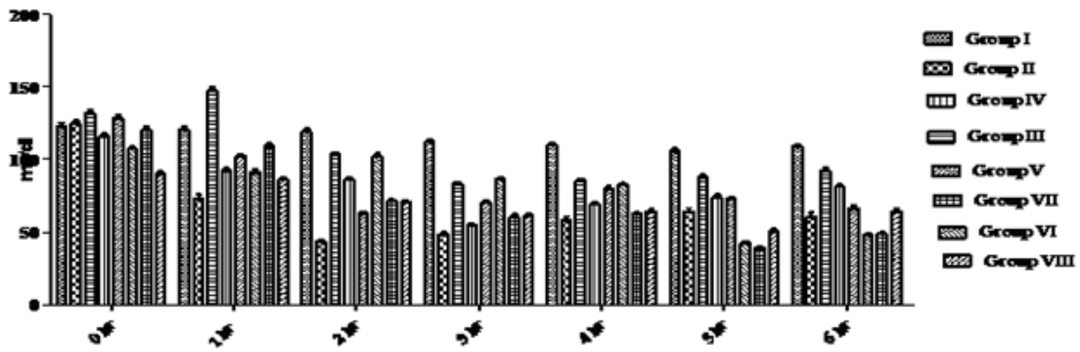

Figure 1. Effect of different fractions isolated from *Albizia procera* stem bark chloroform extract on normoglycemic rats.

Effect of acute treatment of different fractions isolated from Albizia procera stem bark chloroform extract on STZ induced diabetic rats.

Table 3, (Fig 2) shows, 41%, 49.3% and 42% of glucose reduction with fractions A, B, C (dose 160 mg/kg of body weight) at 3rd hr after oral administration in diabetic rats. Similarly, at 6th hr 76.4%, 81.02%, 51% of reduction of blood glucose levels in diabetic rats was observed with fraction D, E and F at 160 mg/kg b.wt. Treatment of glibenclamide at a dosage of 20 mg/kg.b.wt. Diabetic rats resulted in 91.3% of fall of blood glucose after 5 hrs.

Effect of chronic treatment of different fractions isolated from Albizia procera stem bark chloroform extract on STZ induced diabetic rats

The effects of isolated fractions on blood glucose in diabetic rats with chronic treatment are shown in Table 4, Fig 3. A significant decrease in blood glucose levels was

Table 3. Effect of acute treatment of different fractions isolated from *Albizia procera* stem bark chloroform extract on STZ induced diabetic rats

Groups	Blood glucose level (mg/dl)							% of reduced Glucose
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	
I	128 ± 3	120 ± 2	123 ± 1.5	120 ± 2.2	121 ± 2.4	115 ± 3.3	123 ± 4.2	10.1
II	358 ± 14	340 ± 13	342 ± 21	355 ± 16	341 ± 13	331 ± 12	353 ± 16	7.5
III	435 ± 13	321 ± 21.3	159 ± 13.4	53 ± 11.3	39 ± 4.1	46 ± 2.3	69 ± 3	91.03
IV	394 ± 12	342 ± 16	283 ± 12	232 ± 14	335 ± 11	361 ± 12	369 ± 10	41
V	384.6 ± 13	378.3 ± 17	352.4 ± 13	194 ± 9	342 ± 15	369 ± 14	398 ± 13	49.3
VI	392 ± 14	314.2 ± 12	252 ± 13	227.2 ± 13	289 ± 16	301 ± 12	329 ± 13	42
VII	492.7 ± 23	445 ± 21	386 ± 17	329 ± 15	291.5 ± 14	230 ± 12	121 ± 6	76.04
VIII	325.5 ± 14	281 ± 13	200 ± 12	170.5 ± 12	121.75 ± 5	104.7 ± 6	61.7 ± 4	81.03
IX	340.5 ± 18	370 ± 15	370.2 ± 13	369 ± 14	313 ± 18	261 ± 13	166.8 ± 9	51

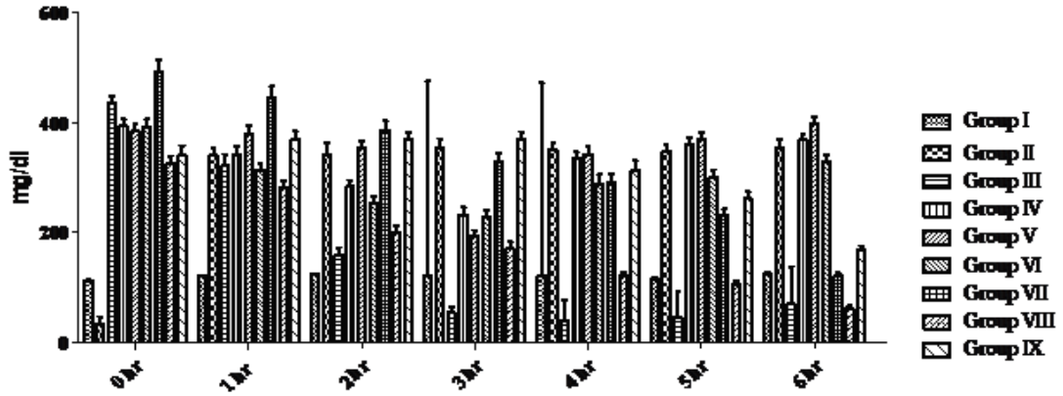

Figure 2. Effect of acute treatment of different fractions isolated from *Albizia procera* stem bark chloroform extract on STZ induced diabetic rats.

Table 3. Effect of acute treatment of different fractions isolated from *Albizia procera* stem bark chloroform extract on STZ induced diabetic rats

Groups	Blood glucose level (mg/dl)							% of reduced Glucose
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	
I	128 ± 3	120 ± 2	123 ± 1.5	120 ± 2.2	121 ± 2.4	115 ± 3.3	123 ± 4.2	10.1
II	358 ± 14	340 ± 13	342 ± 21	355 ± 16	341 ± 13	331 ± 12	353 ± 16	7.5
III	435 ± 13	321 ± 21.3	159 ± 13.4	53 ± 11.3	39 ± 4.1	46 ± 2.3	69 ± 3	91.03
IV	394 ± 12	342 ± 16	283 ± 12	232 ± 14	335 ± 11	361 ± 12	369 ± 10	41
V	384.6 ± 13	378.3 ± 17	352.4 ± 13	194 ± 9	342 ± 15	369 ± 14	398 ± 13	49.3
VI	392 ± 14	314.2 ± 12	252 ± 13	227.2 ± 13	289 ± 16	301 ± 12	329 ± 13	42
VII	492.7 ± 23	445 ± 21	386 ± 17	329 ± 15	291.5 ± 14	230 ± 12	121 ± 6	76.04
VIII	325.5 ± 14	281 ± 13	200 ± 12	170.5 ± 12	121.75 ± 5	104.7 ± 6	61.7 ± 4	81.03
IX	340.5 ± 18	370 ± 15	370.2 ± 13	369 ± 14	313 ± 18	261 ± 13	166.8 ± 9	51

observed in diabetic treated group from initial range 398.7± 19 mg/dl to the level of 119.6 ± 9 mg/dl with 70% glucose reduction after 14 days treatment of fraction E at dose 80 mg/kg b.wt. At the dose of 10 mg/kg b.wt. glibenclamide shows initial 512 ± 25 mg/dl to the level of 152.6 ± 13 mg/dl with 70.1% after 14 days treatment.

Table 4. Effect of chronic treatment of different fractions isolated from *Albizia procera* stem bark chloroform extract on STZ induced diabetic rats

Groups	Blood glucose levels (mg/dl)		% of glucose reduction
	Before treatment	After treatment	
I	123 ± 12	112.2 ± 15	8
II	395.14 ± 13	376.5 ± 15	4.7
III	512 ± 15	152.6 ± 13	70.1
IV	382.3 ± 13	224.8 ± 12	41.1
V	423.8 ± 12	201.3 ± 15	52.5
VI	398.4 ± 14	183.4 ± 13	53.9
VII	382.5 ± 13	139.3 ± 10	63.5
VIII	398.7 ± 19	119.6 ± 9	70
IX	387.4 ± 13	211.9 ± 14	45.3

Values are given as mean ± SEM. Values were significant $P < 0.05$.

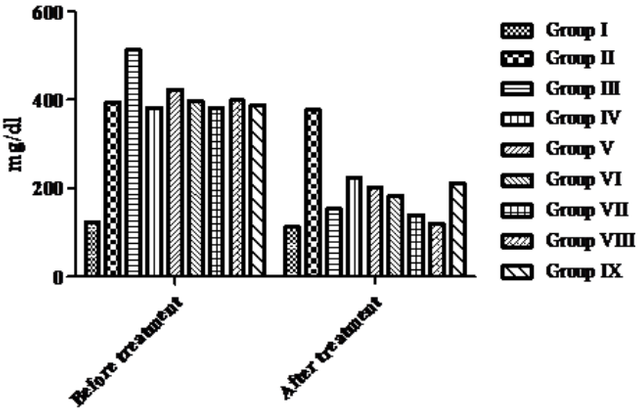

Figure 3. Effect of chronic treatment of different fractions isolated from *Albizia procera* stem bark chloroform extract on STZ induced diabetic rats.

DISCUSSION

In this study STZ was selected for induction of diabetes in rats rather than alloxan. STZ is well known for its selectively pancreatic beta- cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals[11] and it is less toxic than alloxan and allows a consistent maintenance of diabetes mellitus. The present results on the normal rats indicate that the fraction E induces a significant and pronounced hypoglycemic effect (Table 2, Fig 1). The light elevation of glycemia observed in rats treated with fraction A, due to the carbohydrates contained in these fractions. This study also reported fraction E produced maximum anti hyperglycemic activity in acute treatment (81.02%) and chronic treatment (70%) in diabetic rats (Table 3 and 4, Fig 2 and 3). However fraction E, like glibenclamide produced significant reduction in blood glucose in both normal and STZ treated diabetic rats. It indicates the hypoglycemic effect of fraction E would appear to be most probably exerted via mechanism similar to that of glibenclamide. However, the possibility exists that the fraction's mimic or improve insulin's action at the cellular levels.

The data obtained in the present study do not allow any definite conclusion to be drawn on the mechanisms of action of fractions in the experimental paradigms used. However, phytochemical screening of isolated fractions from *Albizia procera* stem bark revealed that the presence of traces of cardiac glycosides, terpinoids and flavonoids. A number of investigators reported that flavonoids, terpinoids posses hypoglycemic properties in various experimental models.[12, 13] The fraction E which has shown the maximum anti hyperglycemic action was considered as the active fraction from above isolated fractions due to presence of valuable phytochemicals.

The results of present study clearly indicate that fraction E isolated from *Albizia procera* stem bark chloroform extract posses the anti hyperglycemic principles. However, chemical and pharmacological investigations are necessary to identify the latter and confirm its mechanism of action and anti diabetic potentials.

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