

Research Article

Antidiabetic Activity of the Plant *Abutilon indicum* in Streptozotocin-Induced Experimental Diabetes in Rats.

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Abstract

The present work investigated the effect of daily oral administration of CF (50 mg/kg body weight) for 21 days on blood glucose, lipid profile, glycosylated haemoglobin, total haemoglobin and plasma insulin in normal and STZ-induced diabetic rats. Chloroform fraction at a dose of 50 mg/kg showed significant reduction in blood sugar level in diabetic rat when compared with diabetic control rats ($p < 0.05$). Significant ($p < 0.05$) differences were observed in serum lipid profiles, serum insulin, glycosylated hemoglobin, body weight and hemoglobin levels in diabetic animals treated with CF compared with the diabetic control. These results demonstrated that the chloroform fraction has significant antidiabetic activity and there is need to isolate the active compounds and develop them as a potential antidiabetic compound

Key words: *Abutilon indicum*; antidiabetic; Streptozotocin; chloroform fraction

INTRODUCTION

Diabetes mellitus often referred to simply as **diabetes** is a syndrome of disordered metabolism, usually due to a combination of hereditary and environmental causes, resulting in abnormally high blood sugar levels (hyperglycemia).^[1] Diabetes mellitus, chronic metabolic disorder, has now become an epidemic, with a worldwide incidence of 5% in the general population. The number of people suffering from diabetes has soared to 246 million and the disease now kills more people than AIDS.^[2] The antihyperglycemic effect of several plant extract which are used as antidiabetic remedies has been confirmed.^[3-6] Compared with synthetic drugs, drugs derived from plants are frequently considered to be less toxic with fewer side effects.^[7] Furthermore, after the recommendations made by WHO on diabetes mellitus, investigation on hypoglycemic agents from medicinal plants have become more important.^[8]

Abutilon indicum (Linn.) Sweet (Malvaceae) is a shrub distributed throughout India. The various parts of the plant (leaves, roots, seeds and seed oil) are widely used by various tribal communities and forest dwellers for the treatment of variety of ailments. The plant has been used by tribal community of Melghal forest of Amravati district for diabetic disease.^[9] A scrutiny of literature revealed some notable pharmacological activities of the plant such as analgesic,^[10] antimalarial,^[11] antifertility,^[12] hepatoprotective,^[13] hypoglycemic,^[14] and wound healing.^[15] The plant contains sesquiterpene lactones, alantolactone and isoalantolactone^[16] which were reported to be

hypoglycemic^[17] in the plant extract of some plants. The present work was therefore undertaken to study the Antidiabetic effects of the chloroform fraction of whole ethanolic extract of plant *Abutilon indicum* in Streptozotocin-induced types-I diabetic rat to correlate the antidiabetic activity with the chemical constituents present therein and verify the claims of the tribal's.

METHODS

Collection of plant material: *Abutilon indicum* (Malvaceae) was collected from surrounding local areas and identified by Department of Botany, Kurukshetra University, Kurukshetra, Haryana, India. A voucher specimen (Sr. No. KUK/IPS/2008/AI-106) was deposited in the herbarium of the Botany Department, Kurukshetra University, Kurukshetra, Haryana, India.

Chemicals: STZ was obtained from Sigma Co, St. Louis, Mo, USA. Glipizide as a gift sample from Oyster lab, ambala. All other chemicals and reagents used were of analytical grade (Ranchem, S.D fine, India).

Preparation of extract: The dried and coarsely powdered plant material was extracted with petroleum ether (60-80°) by hot percolation in soxhlet apparatus until it become colorless. The defatted plant material was then extracted with alcohol until it become colorless. The extract was concentrated under reduced pressure to yield a crude semi-solid mass. This material was stirred with 6% acetic acid and kept overnight, and then extracted with chloroform. The chloroform fraction (CF) was suspended in water using Tween 80 as a suspending agent for the purpose of oral administration.

Table.1 Effect of CF on blood Glucose levels in rats with STZ induced diabetic

Groups	Treatment	Blood Glucose Levels (mg/dl)			
		0 day	07 day	14 day	21 day
I	Normal control	95.1±6.6 ^{b,c,d}	98.1±7.0 ^{b,c,d}	95.5±5.7 ^{b,c,d}	98.1±4.1 ^{b,c,d}
II	Diabetic control	383.6±8.7 ^a	391.0±5.8 ^{a,c,d}	391.8±2.9 ^{a,c,d}	390.8±2.8 ^{a,c,d}
III	CF+ STZ	373.6±4.9 ^a	251.0±1.4 ^{a,b}	242.0±1.8 ^{a,b,d}	195.1±3.7 ^{a,b,d}
IV	Glipizide + STZ	367.1±4.2 ^a	221.1±5.9 ^{a,b}	181.8±5.3 ^{a,b,c}	140.0±1.3 ^{a,b,c}

Each value represents as the mean ± S.E.M of six observations (Anova followed by Dennett's test)

a p<0.01 when compared with normal control

b p<0.01 when compared with Diabetic control(STZ)

c p<0.01 when compared with CF (50 mg/kg) +STZ

d p<0.01 when compared with Standard drug (glipizide)+ STZ

Table.2 Effect of CF on lipid profile

Groups	Treatment	Time(days)	HDL	LDL	TG	CHL
I	Normal control	0	43.3±1.0 ^{b*,d}	44.3±0.6 ^{d*}	62.3±0.6	57.5±0.7 ^{b,c}
		7	43.1±0.8 ^{b*}	43.1±0.6 ^{b,d*}	63.3±1.2 ^b	56.8±1.1 ^{b,c,d}
		14	42.0±0.6 ^b	41.0±3.8 ^{b,d*}	63.6±0.6 ^{b,c,d}	56.8±1.1 ^{b,c}
		21	42.8±0.7 ^b	43.6±0.7 ^{b,d}	61.6±0.4 ^{b,c}	56.3±1.1 ^{b,c}
II	Diabetic control	0	50.8±0.9 ^{a*,c}	57.1±5.8	56.1±2.7	111.1±1.6 ^a
		7	51.3±2.6 ^{a,c}	87.1±4.3 ^{a,c,d}	95.5±0.9 ^{a,c,d}	115.6±4.1 ^{a,c,d}
		14	53.0±3.1 ^{a,c,d}	93.1±1.8 ^{a,c,d}	105.5±1.2 ^{a,c,d}	123.3±3.5 ^{a,c,d}
		21	59.0±3.7 ^{a,c,d}	103.3±3.1 ^{a,c,d}	123.3±2.2 ^{a,c}	133.3±3.8 ^{a,c,d}
III	CF + STZ	0	42.1±2.5 ^{b,d}	57.5±4.5 ^{a*}	60.6±3.7	109.5±1.9 ^a
		7	40.3±1.9 ^b	54.1±5.0 ^{b,d}	59.0±3.9 ^b	103.0±1.9 ^{a,b,d}
		14	37.3±2.0 ^b	45.8± 2.3 ^{b,d}	54.0±2.3 ^{a,b}	98.8±3.4 ^{a,b,d}
		21	35.5±1.0 ^b	42.6±2.8 ^{b,d}	55.0±0.5 ^{a,b}	86.6±5.8 ^{a,b,d}
IV	Glipizide + STZ	0	51.1±1.4 ^{a,c}	59.6±3.8	60.1±2.3	112.8±2.2 ^a
		7	45.5±2.3	30.0±0.8 ^{a,b,c}	57.8±3.1 ^b	91.8± 1.8 ^{a,b,c}
		14	39.8 ± 2.6 ^b	31.0±1.3 ^{a*,b,c}	53.5±0.8 ^{a,b}	57.1± 2.3 ^{b,c}
		21	36.3±1.9 ^b	26.8±1.1 ^{a,b,c}	44.3±0.4 ^{a,b,c}	51.0±5.0 ^{b,c}

Each value represents as the mean ± S.E.M of six observations (Anova followed by Dennett's test)

a p<0.01 when compared with normal control

b p<0.01 when compared with Diabetic control(STZ)

c p<0.01 when compared with CF (50 mg/kg) +STZ

d p<0.01 when compared with Standard drug (glipizide)+ STZ

Experimental animals: Male Sprague-Dawley rats (200-250 g) were used. They were kept at 25 ± 2°C in a 12 h light dark cycle with lights on at 07:00h and fed the standard pellet rat diet (Ashirwad Industries, Tirpari,Ropar(Punjab))and water ad libitum. Institutional Animal Ethics Committee, constituted under the guidelines of CPCSEA, Ministry of Environment, Govt. of India, New Delhi, Approved all the animal experimental protocols(Register Number:562/02/a/CPCSEA)

Induction of experimental diabetes: Diabetes was induced in rats by a single intraperitoneal injection of a buffered (0.1 M citrate, pH 4.5) solution of streptozotocin at a dose of 60 mg/kg, body weight. The animals were considered diabetic if their blood glucose values were between 350 and 400 mg/dl on 3rd day of streptozotocin treatment.

Experimental design: After induction of diabetes, all rats were divided into four groups of six animals each

Group-I normal untreated control rats and received distilled water daily for 21 days.

Group-II was the diabetic control rats and received distilled water daily for 21 days.

Diabetic rats of Group III were treated orally with 50 mg/kg CF of *Abutilon indicum* suspended in Tween 80 (5%).

Group IV was treated orally with glipizide, 350 mg/kg/day for 21 days.

Blood was withdrawn from retro orbital sinus on day 0, day 7, and 21 from control and experimental animals. Blood samples were centrifuged at 3000 rpm for 20 min. Serum was separated and was used for determination of biochemical parameters

Biochemical analysis: Glucose^[18], cholesterol^[19] and triglycerides^[20] were analyzed spectrophotometrically using the diagnostic kits (ERBA Diagnostics Mannheim, Germany). Serum insulin levels were estimated by radio immunoassay method^[21]. Glycosylated hemoglobin as

Table.3 Effect of CF on Serum Insulin, Glycosylated hemoglobin, Hemoglobin levels

Groups	Treatment	Serum insulin	Glcosylated hemoglobin(%)	Hemoglobin
I	Normal control	94.3±0.6**	6.12±0.15**	14.6±0.6**
II	Diabetic control	0.03±.01	9.50±0.18	5.0±0.5
III	CF+ STZ	33.3±0.8**	8.92±0.19**	8.0±0.6**
IV	Glipizide + STZ	51.4±0.9**	7.92±0.08**	11.0±0.4**

Each value represents as the mean ± S.E.M of six observations. * $p < 0.05$, ** $p < 0.01$ vs normal control (Anova followed by Dennett's test)

Table.4 Effect of CF on body weight

Group	Treatment	Body weight (gm)			
		Day 0	Day 7	Day14	Day 21
I	Normal Control	246.67±4.4	247.17±3.8 ^b	251.67±4.9 ^b	252.17±4.9 ^{b,c,*}
II	Diabetic control	248.33±4.8	211.83±2.5 ^{a,c,d}	187.33±3.7 ^{a,c,d}	183.50±3.7 ^{a,c,d}
III	CF + STZ	237.17±4.5	238.33±5.8 ^b	240.33±5.8 ^b	241.67±5.8 ^{a*b}
IV	Glipizide + STZ	239.17±6.1	243.33±5.2 ^b	245.00±6.5 ^b	248.50±6.5 ^b

Each value represents as the mean ± S.E.M of six observations. (Anova followed by Dennett's test)

^a $p < 0.01$ when compared with normal control

^b $p < 0.01$ when compared with Diabetic control(STZ)

^c $p < 0.01$ when compared with CF (50 mg/kg) + STZ

^d $p < 0.01$ when compared with Standard drug (glipizide)+ STZ

described by Nayak S S, Pattabiraman TN^[22] and normal hemoglobin by Sahil's method^[23].

STATISTICAL ANALYSIS

Data are expressed as mean ± S.E.M. Stastical evaluation was performed with student's t test for paired data or ANOVA followed by Dunett's t test. Values of $P < 0.05$ were considered stastically significant

RESULTS AND DISCUSSION

CF at a dose of 50 mg/kg showed significant reduction in blood sugar level in diabetic rat when compared with diabetic control rats ($p < 0.05$) (Table 1). Significant ($p < 0.05$) differences were observed in serum lipid profiles (Table 2), serum insulin, glcosylated hemoglobin and hemoglobin levels (Table 3) in diabetic animals treated with CF compared with the diabetic control. Changes in body weight of CF treated diabetic animals are presented in (Table 4).

Streptozotocin-induced diabetes mellitus causes the destruction of beta cells of the islets of Langerhans^[24] which leads to a reduction in insulin release. An insufficient release of insulin causes high blood glucose, namely hyperglycemia, which results in oxidative damage by the generation of reactive oxygen species (ROS)^[25] and the development of diabetic complications.^[26] STZ-induced diabetic animals may exhibit many other diabetic complications such as myocardial, cardiovascular, gastrointestinal, nervous, and urinary bladder dysfunctions.^[27] The oral glucose tolerance tests showed that the CF at a dose level of 50 mg/kg caused within 30 min a reduction in blood glucose level compared with glucose-loaded control.

Normoglycemic studies revealed its capacity to lower blood glucose levels. Diabetic rats treated with the CF revealed a significant reduction in blood sugar levels compared with the diabetic control group at the end of a 21-day experimental period. This decrease in the blood sugar levels may be attributed to the stimulation of the residual pancreatic mechanism or to a probable increase in the peripheral utilization of glucose.^[28] Treated diabetic rats showed a marked increase in serum insulin levels thereby suggesting that the hypoglycemic activity of *Abutilon indicum* is related to insulin secretion. The increase in serum triglycerides and cholesterol observed in diabetic rats is in agreement with the findings of Nikkila and Kekki.^[29] The most common abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia.^[30] Hypertriglyceridemia is also associated with the metabolic consequences of hypercoagulability, hyperinsulinemia, insulin resistance, and insulin intolerance.^[31] In our study, administration of the CF to the STZ-induced diabetic rats significantly ($p < 0.05$) improved these parameters. The observed hypolipidemic effect may be because of decreased cholesterologenesis and fatty acid synthesis. Significant lowering of total cholesterol and rise in HDL-cholesterol is a very desirable biochemical state for the prevention of atherosclerosis and ischemic conditions.^[32] The characteristic loss of body weight associated with STZ-induced diabetes is due to increased muscle wasting in diabete.^[33] The significant fall in glycosylated hemoglobin indicated the efficiency of the CF in glycemc control. Our studies have shown that the plant *Abutilon indicum* is endowed with marked antidiabetic activity, with minimal toxicity. Its potent

antidiabetic activity may be attributed to the lactones present therein. However, longer duration studies of *Abutilon indicum* and its isolated compounds on chronic models are necessary to develop a potent antidiabetic drug.

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