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Abstract

In light of traditional claim of *Cajanus scarabaeoides* (L) in the treatment of diabetes, we studied the effects of different solvent extracts in normal, glucose over loaded normal rats and streptozotocin-induced diabetic rats. The methanolic extract (500 mg/kg orally) was produce significantly reduce blood glucose level at normal, glucose over loaded normal rats, and streptozotocin-induced diabetic rats after 15 days treatment; whereas petroleum ether and chloroform extract (500 mg/kg orally) did not exhibit any significant effect on three groups of rats. Histopathology studies on pancreas of streptozotocin-induced diabetic rats shows inflammatory changes in pancreatic islets, results from selective destroy of insulin producing β -cells. These changes are inhibited by *C. scarabaeoides* methanolic extract and gliclazide. The antidiabetic activity of methanolic extract may be due to the presence of flavonoids.

Introduction

Diabetes is the world's largest endocrine disease with deranged carbohydrate, fats and protein metabolism. As per WHO report, approximately 150 million people have diabetes mellitus world wide, and this number may be double by 2025. Statistical projection suggests that the number of diabetics will rise from 15 million in 1995 to 57 million in 2025, making India apart the country with the highest number of diabetics in the world. Although many drugs and interventions are available to manage diabetics, these are expensive for a developing country like India apart from their inherent adverse effects. Therefore, it is necessary to look for new avenues to manage this major health problem (Shalam et al., 2006; Nanu et al., 2008). The plants kingdom has become a target for the search by multinational drugs and biological active lead compound. Ethnobotanical information indicates that a large number of

plants show antidiabetic activity (Bandara et al., 2009; Bhowmik et al., 2009; Prajapati et al., 2008; Saha et al., 2009).

From the literature survey conducted it was found that *Cajanus cajan* Millsp (Family: Fabaceae) seed shows hypoglycemic and antidiabetic activity on alloxan-induced diabetic mice (Amalraj and Ignacimutu, 1998). By correlating the activity present study was undertaken to explore antidiabetic activity of *C. scarabaeoides* (L) of different extracts on normal and streptozotocin-induced diabetic rats. *C. scarabaeoides* (Family: Fabaceae) commonly known as Rantur or Banna adhaki, is traditionally used in the treatment of diarrhea in cattle and sterility of women (Kritikar and Basu, 2003; The Wealth of India, 2007). The whole plant extract have also been found to exhibit antimicrobial and anthelmintic activity (Pattanayak et al., 2009).



Materials and Methods

Plant materials

The whole plant of *C. scarabaeoides* was collected in September 2008 from Midnapur, West Bengal, India. The whole plant material was taxonomically identified by Dr. S. C. Majumdar, Taxonomist, Botanical Survey of India, Koregaon Road, Pune 411 001. The whole plant were dried under shade with occasional shifting and then powdered with a mechanical grinder and stored in an airtight container.

Preparation of extracts

The powder obtained was subjected to successive soxhlet extraction with the solvents with increasing order of polarity i.e. petroleum ether (50°C), chloroform (50°C) and methanol (60°C).

Drugs and chemicals

The following drugs and chemicals were used with their sources: streptozotocin (Sigma-Aldrich Co. USA), glucose kit (Ranbaxy Diagnostics, India), petroleum ether (SD Fine, Mumbai), chloroform (SD Fine, Mumbai) and methanol (SD Fine, Mumbai).

Preliminary phytochemical screening

Results of preliminary phytochemical tests suggest that petroleum ether extract shows the presence of alkaloids and glycosides, chloroform extract shows the presence of glycosides and steroids and methanol extract shows the presence of glycosides and flavonoids (Khandelwal, 2005; Mukherjee, 2005)

Animals

Male Wister rats weighing 150-200 g and male Swiss albino mice (25-30 g) were used. Animals were fed a standard pellet (Lipton India, Ltd) and water *ad libitum* and maintained at 24-28°C temperature, 60-70% relative humidity, and 12 hours day and night cycle. Animals described as fasted were deprived of food for 16 hours but had free access to water.

Acute oral toxicity study

The acute oral toxicity study was done according to OECD 423 guidelines. Administration of the stepwise doses of petroleum ether, chloroform, methanolic extracts of *C. scarabaeoides* from 5 mg/kg up to the dose 5,000 mg/kg cause no considerable signs of toxicity in the tested animals. One tenth and one twentieth of the half dose of the lethal dose were selected as the levels for examination of antidiabetic (OECD: Guideline 423, 2000).

Streptozotocin-induced diabetic rats (Babu et al., 2003)

Diabetes was induced in night fasted rats by intraperitoneal injection of streptozotocin (50 mg/kg, i.p.) dissolved in 0.1M citrate buffer, pH 4.5. Forty eight

hours after streptozotocin administration blood samples were drawn by retro-orbital puncture and glucose levels determined to confirm diabetes. The diabetic rats exhibiting blood glucose levels in the range of 200 and 300 mg/100 mL were selected for the studies. These diabetic rats were sub-divided into 5 groups, each group consisted of 6 animals for further study.

Histopathological studies (Rajesh et al., 2005)

At the end of the study, all the surviving animals of the respective groups were sacrificed by an overdose of ether anesthesia. After exsanguinations of the animal's pancreas were removed immediately and washed with ice-cool saline. The pancreas was fixed in 10% of neutral formalin. The sections of 3-5 mm thickness were stained with hematoxylin and eosin for histopathological studies.

Statistical analysis

The data obtained in the studies were subjected to one-way analysis of variance (ANOVA) for determining the significant difference. The intergroup significance was analyzed using Dunnett's t-test. P values <0.05 were considered to be significant. All the values were expressed as mean \pm SEM.

Effect of different solvent extract of *C. scarabaeoides* on oral glucose tolerance (Jafri et al., 2000)

To perform glucose tolerance test, overnight fasted rats were used. Rats were divided into five groups, each of six animals. Group I was kept as control which received 1 mL of 2.5% Tween 80 per oral and Group V received gliclazide (25 mg/kg) per oral suspended in vehicle. A dose 500 mg/kg a petroleum ether, chloroform, methanolic extracts of *C. scarabaeoides* suspended in vehicle was administered orally, to the Groups II, III and IV respectively. All the animals were given glucose (3 g/kg orally) 30 min after dosing. Blood was collected by retro-orbital puncture for glucose estimation 0 min and at 30, 90 and 150 min after drug administration.

Effect of different solvent extract of *C. scarabaeoides* on blood glucose level in normal fasted rats (Tepne et al., 2007)

Fasted rats were divided into five groups of six rats of each. Group I received only vehicle (Tween 80 in distilled water 2.5% v/v) per oral. Group V received gliclazide (25 mg/kg per oral). Group II, III and IV received dose 500 mg/kg a petroleum ether, chloroform, methanolic extracts of *C. scarabaeoides* suspended in vehicle was administered to the animals. Blood was collected by retro-orbital puncture for glucose estimation just prior to and at 1, 2, and 3 hours after dosing.

Effect of different solvent extract of *C. scarabaeoides* on streptozotocin-induced diabetes in rats (Babu et al., 2003)

Diabetes was induced in night fasted rats by intraperitoneal injection of streptozotocin (50 mg/kg, *i.p.*) dissolved in 0.1M citrate buffer, pH 4.5. (One group of 6 identical rats was kept without streptozotocin administration as normal control, Group I). Forty eight hours after streptozotocin administration blood samples were drawn by retro orbital puncture and glucose levels determined to confirm diabetes. The diabetic rats exhibiting blood glucose levels in the range of 200 and 300 mg/100 mL were selected for the studies. These diabetic rats were sub-divided into 5 groups as follows: Group II, (untreated rats) given 0.5 mL of 5% Tween 80; Group III, diabetic rats given (500 mg/kg) *C. scarabaeoides* (L) petroleum ether extract in 0.5 mL 5% Tween 80; Group IV, diabetic rats given (500 mg/kg) *C. scarabaeoides* chloroform extract in 0.5 mL 5% Tween 80; Group V diabetic rats given (500 mg/kg) *C. scarabaeoides* methanolic extract in 0.5 mL 5% Tween 80; Group VI diabetic rats given 0.5 mL of 5% Tween 80 containing gliclazide (25 mg/kg per oral). The dose (25 mg/kg) of gliclazide was selected based on previous reports (Babu et al., 2003) the normal control group of rats (Group I) were given 0.5 mL of 5% Tween 80. Each group consisted of 6 animals. The treatments were continued daily for 15 days. Blood was collected by retro-orbital puncture for glucose estimation just before drug administration on the 1st day and 1 hour after drug administration on days 4, 7, 10 and 15 as described earlier.

Results

Different solvent extract of *C. scarabaeoides* was prepared by continuous hot extraction method using soxhlet apparatus with the solvents of increasing order of polarity i.e. petroleum ether (50°C), chloroform (50°C) and methanol (60°C) yielded 3.5, 1.2, and 8.9% respectively.

The color of the extract respectively was dark green, greenish, yellowish brown. Results of preliminary phytochemical tests suggest that petroleum ether extract shows the presence of alkaloids and glycosides, chloroform extract shows the presence of glycosides and steroids and methanol extract shows the presence of glycosides and flavonoids.

The methanolic extract at 500 mg/kg and standard drug gliclazide (25 mg/kg) produced significant ($p < 0.01$) hypoglycemic effect in the fasted normal rat after 2 hours and 3 hours of oral administration, when compared with normal group (Table I). The pet ether and chloroform extract at 500 mg/kg per oral dose did not exhibit any significant hypoglycemic effect on rat.

The *C. scarabaeoides* methanolic extract 500 mg/kg reduced the blood glucose level, (hyperglycemia due to glucose load 3 g/kg per oral) significantly and

Table I

Effects of *Cajanus scarabaeoides* on oral glucose tolerance test on rats

Group	Blood glucose (mg/dL) concentration at different time (min)				
	0	30	60	90	120
Vehicle	88.5 (3.1)	144.5 (2.8)	136.1 (2.0)	126.3 (2.0)	122.0 (2.6)
Ether extract 500 mg/kg	87.0 (2.4)	145.8 (1.9)	138.2 (1.9)	127.8 (2.1)	119.5 (1.7)
Chloroform extract 500 mg/kg	86.17 (4.4)	138.6 (3.6)	127.5 (2.4)	124.2 (2.8)	118.5 (2.2)
Methanol extract 500 mg/kg	86.17 (4.4)	128.6 (3.6) ^a	117.5 (2.4) ^b	104.2 (2.8) ^b	98.5 (2.2) ^b
Gliclazide 25 mg/kg	87.17 (3.0)	115.5 (3.9) ^b	103.0 (2.5) ^b	94.6 (2.8) ^b	85.5 (4.1) ^b

Value are given as mean (\pm SE); n = 6; Values are statistically significant compared to normal Group at ^a $p < 0.05$, ^b $p < 0.01$ respectively

Table II

Effects of *Cajanus scarabaeoides* on blood glucose level in normal fasted rats

Group	Blood glucose (mg/dL) concentration at different time (hour)				
	0	1	2	3	3
Vehicle	75.0 (3.8)	80.2 (3.2)	78.8 (2.9)	75.8 (3.2)	75.8 (3.2)
Ether extract 500 mg/kg	80.3 (3.9)	79.5 (2.5)	77.8 (3.2)	76.1 (2.2)	76.1 (2.2)
Chloroform extract 500 mg/kg	75.5 (2.2)	77.5 (2.1)	75.6 (1.9)	78.5 (1.1)	78.5 (1.1)
Methanol extract 500 mg/kg	75.5 (2.2)	77.5 (2.1)	63.6 (1.9) ^a	58.5 (1.1) ^a	58.5 (1.1) ^a
Gliclazide 25 mg/kg	75.8 (2.9)	63.1 (1.2) ^a	54.2 (2.1) ^a	48.6 (1.9) ^a	48.6 (1.9) ^a

Value are given as mean (SE); n = 6; Values are statistically significant compared to normal Group at ^a $p < 0.01$ respectively

gliclazide (25 mg/kg) after 60 min of oral administration, when compared to control group (Table II). The petroleum ether and chloroform extract at 500 mg/kg per oral dose did not exhibit any significant effect on oral glucose tolerance test in rat.

Methanolic extract of *C. scarabaeoides* reduced elevated blood glucose level in streptozotocin-induced diabetic rats. At testing period of 15 days similar results also have been found with gliclazide. The ether and chloroform extract at 500 mg/kg per oral dose did not exhibit any significant antidiabetic effect (Table III). The streptozotocin-induced diabetic rats inflammatory changes were detected in pancreatic islets results from

Table III

Effects of *Cajanus scarabaeoides* on blood glucose level of streptozotocin-induced diabetic

Group	Blood glucose (mg/dL) concentration at different time (day)				
	0	4	7	10	15
Vehicle	242.5 (1.7)	240.6 (6.4)	235.7 (7.4)	224.7 (5.2)	220.0 (8.9)
Ether extract 500 mg/kg	225.0 (3.6)	236.2 (7.2)	232.5 (3.0)	226.2 (9.9)	221.0 (4.4)
Chloroform extract 500 mg/kg	227.5 (9.0)	235.2 (10.9)	228.2 (1.8)	216.2 (3.6)	202.0 (2.3)
Methanol extract 500 mg/kg	227.5 (9.0)	185.2 (10.9) ^a	168.2 (1.8) ^b	136.2 (3.6) ^b	102.0 (2.3) ^b
Gliclazide 25 mg/kg	229.5 (7.4)	171.2 (6.2)	152.5 (6.1) ^b	100.2 (2.5) ^b	88.7 (3.2) ^b

Value are given as mean (±SE); n = 6; Values are statistically significant compared to normal Group at ^ap<0.05, ^bp<0.01 respectively

selective destroy of insulin producing β -cells. These changes are inhibited by *C. scarabaeoides* methanolic extract and gliclazide. The antidiabetic activity of numerous herbal extract observed based on histopathology of the pancreas.

Discussion

The present study was an effort to investigate the effect of different solvent extract of the *C. Scarabaeoides* on normal and streptozotocin-induced diabetic rats.

Different mechanism of action to reduce the blood glucose levels with the help of plant extract already exists. Some plant exhibits properties similar to well known sulfonylurea drug like gliclazide; they reduce the blood glucose in normoglycemic animals (Ivorr et al., 1988; Davis, 2006). Some other plant act like biguanides such as metformin which is an antihyperglycemic compound; they don't affect blood glucose level in normal state (Baily et al., 1985). We hypothesized that methanolic extract of *C. scarabaeoides* could have sulfonylurea like mechanism which is responsible to decrease blood glucose level in normoglycemic rats such as gliclazide. The hypoglycemic effect detected in the present study is a direct evidence of the methanolic extract of *C. scarabaeoides* in stimulation of insulin secretion. The activity was comparable to that of the standard drug which reinforces the insulin secretagogue action of the extract.

C. scarabaeoides methanolic extract also improved oral glucose tolerance in glucose fed rats. These results may be obtained due to promotion of disposal of glucose by enhancing translocation of glucose transporter to the

plasma membrane as the sulfonylurea acts in similar manner.

It is also known that streptozotocin destroys insulin secreting β -cells in the islets of Langerhans and their effect is irreversible (Fisher, 1985). Methanolic extract of *C. scarabaeoides* reduced blood glucose level in streptozotocin-induced diabetic rats. At testing period of 15 days similar results also have been found with gliclazide. The histopathological investigation along with the biochemical evaluation suggests the possibility of the islets regeneration and recovery of normal carbohydrate metabolism in treated group of methanolic extract of *C. scarabaeoides*. Whereas the petroleum ether and chloroform extract did not exhibit any significant effect on blood glucose level in rat.

Conclusion

Methanolic extract of *C. scarabaeoides* at 500 mg/kg dose was found antihyperglycemic in streptozotocin-induced diabetic rats and oral glucose tolerant rats, and also found hypoglycemic in fasted rats.

Ethical Issue

The study was approved by the Institute Animal Ethics Committee (1197/C/08/CPCSEA).

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