Antihyperglycemic activity of aqueous extract of leaves of *Cocculus hirsutus* (L.) Diels in alloxan-induced diabetic mice

S. Badole, N. Patel, S. Bodhankar, B. Jain, S. Bhardwaj*

Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Erandwane, Pune-411 038
*Clinical Research, Serum Institute of India, Hadpsar, Pune-411 007. India

Received: 16.5.2005
Revised: 7.6.2005
Accepted: 23.11.2005

Correspondence to:
Subhash Bodhankar
E-mail: sbodh@yahoo.com

ABSTRACT

Objective: To evaluate the antihyperglycemic activity of aqueous extract of leaves of *Cocculus hirsutus* (L.) Diels in alloxan-induced diabetic mice.

Materials and Methods: Alloxan-induced (70 mg/kg, i.v.) diabetic mice were given aqueous leaf extract (250, 500, and 1000 mg/kg, p.o., *n*=6) of *C. hirsutus* or vehicle (distilled water, 10 ml/kg, p.o.) or standard drug glyburide (10 mg/kg, p.o.) for 28 days. Blood samples were withdrawn by retro-orbital puncture and were analyzed for serum glucose on 0th, 7th, 14th, 21st, and 28th days by glucose oxidase/peroxidase method. In oral glucose tolerance test, glucose (2.5 g/kg, p.o.) was administered to nondiabetic control, glyburide (10 mg/kg, p.o.), and aqueous extract of *C. hirsutus* (1000 mg/kg, p.o.) treated mice. The serum glucose level was analyzed at 0, 30, 60, and 120 min after drug administration.

Results: The aqueous leaf extract of *C. hirsutus* (250, 500, and 1000 mg/kg, p.o.) showed significant (P<0.01) reduction of serum glucose level in alloxan-induced diabetic mice at 28th day. In oral glucose tolerance test, aqueous extract of *C. hirsutus* increased the glucose tolerance.

Conclusion: It is concluded that *C. hirsutus* has significant antihyperglycemic activity as it lowers serum glucose level in diabetic mice and significantly increases glucose tolerance.

KEY WORDS: Antidiabetic, glucose tolerance test, tana, vasanvel

Introduction

Diabetes mellitus is a metabolic disorder in which the body does not produce or properly use insulin. It causes disturbances in carbohydrate, protein, and lipid metabolism and complications such as retinopathy, microangiopathy, and nephropathy.[1]

The currently available oral antihyperglycemic agents for clinical use have characteristic profile of side effects.[2,3] Management of diabetes with agents devoid of any side effects is still a challenge to the medical system. This has led to an increase in the demand for natural products with antihyperglycemic activity having less side effects. Indian traditional medicine is one of the richest medicinal systems among those available around the world. Long before the use of insulin, since the time of Charaka and Sushruta (sixth century BC, 400 BC), indigenous remedies have been used for the treatment of diabetes mellitus. In accordance with the recommendations of the WHO[4] expert committee on diabetes mellitus, an investigation of antihyperglycemic agents of plant origin used in traditional medicine seems important. Many herbs and plant products have been shown to have antihyperglycemic action.[5–8]

The roots of *Cocculus hirsutus* (L.) Diels (Menispermaceae family) (locally called Vasanvel or Tana) have been mentioned as bitter, acrid, alterative, laxative, demulcent, and antiperiodic in fever, tonic, and diuretic. The juice of leaves coagulates in water and forms mucilage, which is used externally as a cooling and soothing agent in prurigo, eczema, and impetigo.[9]

The roots of *C. hirsutus* have been mentioned to possess antiinflammatory and analgesic properties.[10] The objective of this study was to evaluate the antihyperglycemic activity of aqueous extract of leaves of *C. hirsutus* in alloxan-induced diabetic mice.

Materials and Methods

Animals

Swiss albino mice (National Toxicology Centre, Sinhagad Road, Pune) weighing between 30 and 35 g of either sex were used. Animals were housed under standard conditions of temperature (24±2°C) and relative humidity (30–70%) with a 12:12 light:dark cycle. The animals were fed with standard pellet diet (Chakan Oil Mills, Sangli) and water *ad libitum*. Animal handling was performed according to *Good Laboratory Practice* (GLP).

The Institutional Animal Ethics Committee (IAEC) of the

Poona College of Pharmacy approved the proposal.

Preparation of the extract

The plant *C. hirsutus* was collected from Katraj Ghat, Pune, and authenticated by an expert taxonomist of Agharkar Research Institute, Pune (museum sample no. WP-021).

The leaves were separated, shade-dried and powdered in a grinder. The powder was extracted successively with petroleum ether (60–80°C) and absolute alcohol (ethanol) using Soxhlet apparatus. The residue was macerated overnight with water and filtered. The filtrate was dried on a tray drier at 60°C and was used for study purpose.

Preparation of the drug solution

An aqueous extract (100 mg) of leaves of *C. hirsutus* was dissolved in 10 ml of distilled water to prepare stock solution of 10 mg/ml. Appropriate dilutions were made to prepare lower doses for administration according to the body weight of mice.

Determination of LD_{50} of C. hirsutus

For acute oral toxicity and LD_{50} determination, the Organization for Economic Co-operation and Development (OECD) guideline 425[14] was followed.

Induction of experimental diabetes

Diabetes was induced in Swiss albino mice of either sex by a single intravenous injection of aqueous alloxan monohydrate (70 mg/kg, i.v.) by the method described by Kameswara Rao *et al.*[11] After 48 h, animals with serum glucose level above 200 mg/dl (diabetic) were selected for the study. The animals were allowed free access to tap water and pellet diet and maintained at room temperature in plastic cages.

Collection of blood and determination of serum glucose

Blood samples from mice were collected by retro-orbital puncture (ROP) technique. Serum glucose levels were determined by glucose oxidase and peroxidase method as described by Abdel-Barry *et al.*[12] using commercially available kit (Accurex Biomedical Pvt. Ltd., Mumbai). Determination of serum glucose was done at 505 nm on Jasco UV-visible spectrophotometer (Model V530) and expressed as mg/dl.

Effect of aqueous extract of leaves of C. hirsutus on serum glucose in alloxan-induced diabetic mice

The method described by Dunn *et al.*[13] was adopted. The diabetic mice were fasted overnight and dived into five groups (n=6). Group I—vehicle (distilled water, 10 ml/kg, p.o.), group II—glyburide (10 mg/kg, p.o.), groups III–V—*C. hirsutus* extract (250, 500, and 1000 mg/kg, p.o., respectively).

Study for the acute antihyperglycemic activity involved withdrawal of blood at 0, 2, 4, 6, and 24 h after administration of vehicle, glyburide, or aqueous extract of *C. hirsutus*. The blood samples were centrifuged and serum obtained to determine the glucose level.

Subacute study involved administration of vehicle, glyburide, or different doses of aqueous extract of *C. hirsutus* for a period of 28 days. Serum glucose levels were estimated on days 7, 14, 21, and 28. Mean change in serum glucose were calculated.

Effect of aqueous extract of leaves of C. hirsutus on body weight in alloxan-induced diabetic mice

During the study period of 28 days the mice were weighed daily and the mean change in body weight calculated.

Effect of aqueous extract of leaves of C. hirsutus on oral glucose tolerance test (OGTT)

Normal mice were divided into three groups (n=6), viz, group I—only glucose (2.5 g/kg, p.o.), group II—glyburide (10 mg/kg, p.o.), and group III—*aqueous extract of C. hirsutus* (1000 mg/kg, p.o.). The animals were fasted overnight before commencing the experiment. All mice were loaded with 2.5 g/kg, p.o., *d*-glucose solution (S.D. Fine-Chem. Ltd, Mumbai) after 0.5 h of drug administration. Blood samples were collected by the ROP method just prior to drug administration and 30, 60, and 120 min after glucose loading. Serum glucose level was measured immediately.

Statistical analysis

The results are expressed as mean±SD. Comparison between the groups was made by analysis of variance (ANOVA), followed by Dunnett's test as per suitability. $P < 0.05$ was considered significant.

Results

Effect of aqueous extract of leaves of C. hirsutus on serum glucose level in alloxan-induced diabetic mice

Single administration (single dose) of aqueous extract of leaves of *C. hirsutus* (250, 500, and 1000 mg/kg, p.o.) in diabetic Swiss albino mice, showed reduction in serum glucose level after 2, 4, and 6 h interval. Maximum reduction in serum glucose level was seen at doses of 250, 500, and 1000 mg/kg (38.33%, 32.72%, and 16.54% decrease, respectively) after 6 h of *C. hirsutus* extract administration. Glyburide (10 mg/kg, p.o.) showed maximum reduction (44.33% decrease) after 6 h [Table 1].

On repeated administration (subacute treatment) of either vehicle or glyburide or aqueous extract of *C. hirsutus* for 28 days, a significant ($P<0.01$) decrease in serum glucose of the diabetic mice were seen at a dose of 250, 500, and 1000 mg/kg, p.o., in dose-dependent manner as compared with vehicle-treated group. On the other hand, glyburide showed a significant ($P<0.01$) decrease in serum glucose at a dose of 10 mg/kg, p.o., (58.57% decrease) as compared with vehicle-treated group.

Maximum activity of *C. hirsutus* was seen with a significant decrease ($P< 0.01$) in serum glucose levels at the dose of 1000 mg/kg [Table 2].

Effect of aqueous extract of leaves of C. hirsutus on body weight in alloxan-induced diabetic mice

Administration of vehicle (distilled water, 10 ml/kg, p.o.) in alloxan-induced diabetic mice resulted in gradual decrease in body weight during the period of 28 days. Aqueous extract of *C. hirsutus* in all doses except 250 mg/kg did not cause any decrease in body weight.

Effect of aqueous extract of leaves of C. hirsutus on OGTT in normal mice

The aqueous extract of leaves of *C. hirsutus* (1000 mg/kg, p.o.) and glyburide (10 mg/kg, p.o.) significantly depressed the peak of serum glucose level at 30 min after glucose loading [Table 3].

Determination of LD_{50} of aqueous extract of leaves of C. hirsutus in mice

Administration of a single dose of *C. hirsutus* (175 mg/kg, body weight, p.o.) did not produce mortality in mice. The

Table 1

Effect of *C. hirsutus* on serum glucose level in alloxan-induced diabetic mice (acute study)

Groups		*0 h (mg/dl)*	*2 h (mg/dl)*	*4 h (mg/dl)*	*6 h (mg/dl)*	*24 h (mg/dl)*
Vehicle (distilled water,10 ml/kg)		530.10 ± 25.46	534.70±26.46	543.04 ± 32.23	552.43±33.88	558.09±35.95
Glyburide (10 mg/kg)		569.84 ± 48.67	458.21±39.53*	419.00 ± 38.06*	358.86±9.65*	521.71± 83.96
C. hirsutus (250 mg/kg)		601.63 ± 230.19	577.03±28.04**	438.73 ± 69.57*	371.58±69.11*	597.57±28.59
C. hirsutus (500 mg/kg)		593.15 ± 4.27	587.20±16.32*	516.05 ± 34.89*	398.82±47.64*	570.45±23.11
C. hirsutus (1000 mg/kg)		594.16 ± 4.89	592.93±7.99*	542.98 ± 4.75*	405.69±33.65*	568.58±47.28
One-way	F		28.003	9.566	19.303	1.893
ANOVA	df		4,25	4,25	4,25	4,25
	P		<0.01	<0.01	<0.01	<0.01

Values are mean±SD, n = 6 in each group. *P<0.01. **P < 0.05 when compared with vehicle-treated group (Dunnett's test).

Table 2

Effect of *C. hirsutus* on serum glucose in alloxan-induced diabetic mice (subacute study)

Groups		*Day 0 (mg/dl)*	*Day 7 (mg/dl)*	*Day 14 (mg/dl)*	*Day 21 (mg/dl)*	*Day 28 (mg/dl)*
Vehicle (distilled water,10 ml/kg)		530.10 ± 25.46	574.38±16.09	579.23 ± 11.95	593.95±29.34	603.26±20.86
Glyburide (10 mg/kg)		569.84 ± 48.67	467.54±35.54*	397.61 ± 42.99*	384.85±36.32*	286.25±54.23*
C. hirsutus (250 mg/kg)		601.63 ± 23.19	457.33±15.71*	438.38 ± 26.56*	404.33±32.70*	367.22±35.66*
C. hirsutus (500 mg/kg)		593.15 ± 4.27	450.26±44.60*	392.14 ± 47.03*	377.87±41.48*	365.98±41.55*
C. hirsutus (1000 mg/kg)		594.16 ± 4.89	467.74±1.34*	340.40 ± 39.67*	323.16±28.39*	300.31±48.86*
One-way	F		21.049	38.009	55.631	55.846
ANOVA	df		4,25	4,25	4,25	4,25
	P		<0.01	<0.01	<0.01	<0.01

Values are mean±SD, n = 6 in each group. *P<0.01 when compared with vehicle-treated group (Dunnett's test).

Table 3

Effect of *C. hirsutus* on OGTT in normal mice

Groups		*Narmal*	*0 min*	*30 min*	*60 min*	*120 min*
Glucose (2.5%, p.o.)		124.90 ± 18.46	270.88±65.85	374.64 ± 69.44	171.62±32.93	138.72±34.78
Glyburide (10 mg/kg)		104.53 ± 15.11	182.17±55.05*	237.99 ± 34.26*	164.71±12.88	147.85±31.95
C. hirsutus (1000 mg/kg)		106.05 ± 13.21	328.92±30.62*	252.98 ± 30.25*	172.31±33.45	143.58±17.84
One-way	F		4.270	4.693	5.421	9.149
ANOVA	df		4,25	4,25	4,25	4,25
	P		<0.01	<0.01	<0.01	<0.01

Values are mean±SD, n = 6 in each group. *P < 0.05 when compared with vehicle-treated group (Dunnett's test).

animals were alive, healthy, and active during the observation period of 14 days. Use of AOT 425 software was made to obtain higher doses for LD_{50} determination as per OECD guidelines. In case of *C. hirsutus,* the computer program suggested doses 550, 1750, and 2000 mg/kg. Results indicated that doses upto 2000 mg/kg were nonlethal. All animals were found to be alive, healthy, and active during the observation period of 14-day postadministration of highest dose. The computer program showed LD_{50} > 2000 mg/kg.

Discussion

The plant of *C. hirsutus* has been reported to contain essential oil, ß-sitosterol, ginnol,[15] glycosides, sterols, and alkaloids.[16] Preliminary phytochemical analysis of the leaves showed presence of alkaloids, phenolic compounds, flavonoids, glycosides, and carbohydrates.

The alkaloids reported to be present in the plant are shaheenine,[17] cohirsinine,[18] hirsutine,[19] jamtinine,[20] jamitine-*N*-oxide,[21] cohirsine,[22] corsitinine,[23] and haiderine.[24] The alkaloids present in the leaves of *C. hirsutus*

Table 4

Effect of *C. hirsutus* on body weight (g) in alloxan-induced diabetic mice

Groups		Day 0	Day 7	Day 14	Day 21	Day 28
Vehicle (distilled water,10 ml/kg)		32.00 ± 1.41	28.00±3.03	26.67 ± 2.42	25.00±2.61	23.33±2.34
Glyburide (10 mg/kg)		36.17 ± 1.33	34.37±.88*	36.00 ± 4.69*	36.33±5.85*	37.00±4.9*
C. hirsutus (250 mg/kg)		33.17 ± 2.04	31.83±3.54	31.00 ± 4.24	30.33±2.94	29.50±2.43
C. hirsutus (500 mg/kg)		30.33 ± 2.58	31.17±3.49	31.17 ± 4.58	32.00±3.69*	31.83±3.92*
C. hirsutus (1000 mg/kg)		35.50 ± 1.64	33.33±2.66*	33.17 ± 2.79*	34.00±6.19*	34.33±6.19*
One-way	F		11.840	14.629	0.05656	0.1474
ANOVA	df		2,25	2,25	2,25	2,25
	P		<0.05	<0.01	<0.05	<0.05

Values are mean±SD, n = 6 in each group. *P < 0.05 when compared with vehicle-treated group (Dunnett's test).

are D-trilobine and DL-coclaurine.[25] The leaves also contain isotrilobine, (+)-syringaresinol and protoquericitol.[26]

Alloxan, a ß-cytotoxin, induces "chemical diabetes" in a wide variety of animal species including mice by damaging the insulin-secreting ß-cells of the pancreas. Alloxan causes time- and concentration-dependent degenerative lesions of the pancreatic ß-cells. The dose of alloxan required to produce diabetes varies with the species.

In the present study, the antihyperglycemic activity of aqueous extract of leaves of *C. hirsutus* was evaluated in alloxan-induced diabetic mice. Single-dose study with 250, 500, and 1000 mg/kg showed significant ($P<0.01$) decrease in serum glucose level at 2, 4, and 6 h. Continuous treatment with the aqueous extract of leaves of *C. hirsutus* (250, 500, and 1000 mg/kg) for a period of 28 days showed a significant decrease ($P<0.01$) in the serum glucose level in diabetic mice. Maximum reduction of serum glucose level occurred at the dose of 1000 mg/kg, p.o.

There was a significant weight loss in the vehicle-treated diabetic mice, whereas treatment with the aqueous extract of leaves of *C. hirsutus* at the doses of 500 and 1000 mg/kg, p.o., showed improvement in their body weights, indicating that the aqueous extract had beneficial effect in preventing loss of body weight of diabetic mice [Table 4].

Antihyperglycemic activity of methanol extract of roots of *C. hirsutus* is reported. Total alkaloid fraction prepared from methanol extract showed considerable antihyperglycemic activity in diabetic rats. Total alkaloids reduced the blood sugar level of diabetic rats significantly. Hence, the alkaloids in the roots of *C. hirsutus* have been reported to be responsible for the antihyperglycemic activity.[27]

It may be said that the aqueous extract of leaves of *C. hirsutus* decreased the serum glucose level and improved glucose tolerance owing to the presence of alkaloids. LD_{50} determination (>2000 mg/kg) indicated safety profile of the drug.

Conclusion

The aqueous extract of leaves of *C. hirsutus* has antihyperglycemic activity as it lowers serum glucose level in diabetic mice and significantly increases glucose tolerance. The extract also prevents loss of body weight in diabetic mice.

Acknowledgment

The author would like to thank Dr. S. S. Kadam, Principal, Poona College of Pharmacy, Pune, India, for financial support, keen interest, and valuable guidance to carry out the work.

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