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Research Article

ANTI-DIABETES AND HYPOGLYCAEMIC PROPERTIES OF *HEMIGRAPHIS COLORATA* IN RATS

GAYATHRI.V£*, LEKSHMI. P#, PADMANABHAN R.N*

*** Department of Biochemistry, Government Medical College, Trivandrum, Kerala, India 695011 , # Department of** Pathology**, Government Medical College, Kottayam, Kerala, India 686008. £Email: haricharueaswer@gmail.com**

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ABSTRACT

Hemigraphis colorata Blume [Family:Acanthaceae]. This ethno-medicinal plant was evaluated for its hypoglycaemic and anti-diabetic properties using rats. Glucose lowering effect and anti-diabetes activity were studied using glucose tolerance test in normal rats and alloxan diabetic rats, respectively. When different extract were tested, the n-hexane and, to some extent, the ethanol extracts were found to lower the levels of blood glucose in glucose fed rats. The n-hexane extract showed optimum activity at 100 mg/kg. The extract exhibited only marginal hypoglycaemic activity in overnight fasted normal rats and it was devoid of any conspicuous toxic symptoms in sub-acute toxicity evaluation in mice. When the n-hexane extract was fractionated by sequential solvent extraction, the activity was found in ethyl acetate fraction (12.5mg/kg). This fraction containing steroids and coumarins showed anti-diabetes activity in alloxan diabetic rats as judged from serum glucose levels, liver glycogen content and body weight. This fraction is an attractive material for further research vis-à-vis drug development.

Keywords: *Hemigraphis colorata*; Alloxan diabetic rats; Anti-hyperglycaemic activity, Glucose, Alloxan, n-hexane

INTRODUCTION

Hemigraphis colorata Blume [Family:Acanthaceae]. This plant possesses various medicinal properties, only a few are reported like, the whole plant or leaves are used to treat fresh wound, cuts, ulcers, inflammation and in folk medicines, it is used internally to cure anemia.

Since ancient times, plants have been an exemplary source of medicine. Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. India has about 45000 plant species and among them, several thousands have been claimed to possess medicinal properties. Research conducted in last few decades on plants mentioned in ancient literature or used traditionally for diabetes has shown anti-diabetic property[1].

Diabetes mellitus is an important medical syndrome rather a problem and it is on the raise[2]. The available oral hypoglycaemic agents are insufficient and there is a need for discovering more effective and safe oral hypoglycaemic agents[3]. Diabetes mellitus is known from ancient time onwards and numerous medicinal plants are used to control diabetes in traditional medicine.

In South India, many medicinal plants are traditionally used to treat Diabetes mellitus. Some of them were scientifically verified[4]. Many anti-diabetes plants used in folk and tribal medicine in remote village in Kerala state, India are not known to the main stream population. In ethno-medical practices, *Hemigraphis colorata* plant is also used to treat wounds and burns[5].

Assay methods used to screen plants for hypoglycemic activity are varied and not directly comparable. *In vivo* techniques include animals with normoglycemia or induce hyperglycemia (alloxan, streptozotocin, various hormones, or surgery) as well as diabetic human subjects. Natural compounds with antidiabetic activity, in descending frequency of occurrence, include complex carbohydrates, alkaloids, glycopeptides, terpenoids, peptides and amines, steroids, flavonoids, lipids, coumarins, sulfur compounds, inorganic ions and others[6] .The present study was undertaken to scientifically verify the traditional claim using experimental (normal and diabetic) rats.

MATERIALS AND METHODS

Collection of plant materials

Hemigraphis colorata was collected from the wild area at, Trivandrum district and some temperate habitat identified by the taxonomists of TBGRI and voucher specimen (specimen no. 0001) has been deposited in the herbarium of TBGRI.

Chemical and reagents

All chemicals and reagents used were analytical grade and purchased from E. Merck India Ltd., Mumbai and Ranchem, India, SRL, India.

Preparation of *Hemigraphis colorata* extracts

The whole plants were cleaned, dried and powdered. To prepare water extract, the powder was extracted with distilled water (5g/100 ml) with constant stirring for 4 h and then filtered through a filter paper. Residue was again extracted as above with water. The combined filtrate was freeze-dried in a lyophilizer. The yield of the water extract was approximately 20% of the plant powder. (Since the heat sensitivity of the extract with reference to bio-activity is not known, the extraction was carried out at low temperature without using rigorous extraction procedures.) The alcohol extract of the plant powder was prepared similarly using ethyl alcohol instead of distilled water.

However, in this case the combined filtrate was evaporated to dryness in a rotary evaporator under reduced pressure at 40^0C.

The yield of the alcohol extract was approximately 12% of the plant powder. The n-hexane extract of the powder was prepared as above using n-hexane instead of alcohol. However, to ensure complete extraction, 2 g powder was extracted with 100 ml hexane and the process was repeated three times.

The filtrates from the extractions were combined and dried in a rotary evaporator under reduced pressure at 40^0C. The yield of the hexane extract was approximately 2% of the powder.

Animals

Our animal house and breeding facility have been registered with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. Inbred Wistar rats (150-200 g weight) and Swiss albino mice (6-7 weeks old), reared in Vivarium, Medical College Trivandrum, and were used.

Animals were caged in uniform hygienic conditions and fed with standard pellet diet (Lipton, India Laboratories, Bangalore) and water *ad libitum* as per the guidelines of College Animal Ethics Committee, CPCSEA guidelines were followed [IAEC approval obtained].

Glucose tolerance test

This was done as described elsewhere[7]. Rats were divided into indicated number of groups. Control group received the vehicle (2% gum acacia and 5% Tween 80; 1 ml, p.o.). The experimental groups received the herbal drug (water suspension or extracts or fractions) at indicated doses in an identical manner. In the screening study, a relatively high dose [500 mg (dry weight)/kg] of the water suspension of the plant powder was taken to detect activity, if any.

The rats of all the groups were loaded with 60% glucose (3g/kg, p.o.) 30 min after herbal drug administration.

Blood samples were collected by retro-orbital puncture, just 1 min prior to drug administration, and at 30, 90 and 150 min after glucose loading under mild ether anesthesia. Serum glucose levels were measured immediately. Six overnight fasted animals were used in each group.

Hypoglycaemic study in normal fasted rats

To investigate hypoglycaemic effect, if any, of the n-Hexane extract, the overnight fasted rats were divided into two groups of six each. Control group received 1 ml of 5% Tween 80 and the experimental group received 100 mg/kg n-hexane extract. Blood samples were collected at 0,120 and 180 min after the extract administration and glucose levels measured as described above.

Alloxan-induced diabetic rats

Rats were injected with alloxan (60 mg/kg) through tail veil. Five days later, blood samples were drawn and glucose levels were determined to confirm induction of diabetes. The diabetic rats showing blood glucose levels in the range of 400-450 mg/dl were selected for the efficacy evaluation of the herbal drug[8].

Isolation of an active fraction

The n-hexane extract from the plant was suspended in water and sequentially extracted with hexane, chloroform, ethyl acetate and butanol; each fraction was tested for activity using glucose tolerance test. The active ethyl acetate fraction was subjected to chemical analysis to determine the classes of compounds present in it [9].

The fraction was tested for the presence of alkaloids (Dragendorff reagent and Mayer's reagent), coumarins (Borntrager reagent), flavonoids (Shinoda test), steroids (Liebermann- Buchard test), and terpenes (vanillin-sulphuric acid reagent). The yield of this fraction was 10% of the n-hexane extract.

Determination of the efficacy of the active fraction in alloxan diabetic rats

The alloxan diabetic rates were divided into three groups of six each. The control group was given 1 ml of 5% Tween 80 p.o. daily. The test group was given daily dose of the active fraction (25 mg/kg). The third group received insulin (5 IU/kg, i.p.; Knoll Pharmaceuticals Ltd., India) daily. Weight and sex matched six normal rats were kept as a normal control group. The treatment was continued for 12 days. (Death started in the diabetic control group of rats on the 12th day.) Blood samples were collected on days 1, 4, 8 and 12. On day 12 animals were killed after blood collection and liver samples were removed for glycogen estimation.

Estimation of blood glucose and liver glycogen

Serum glucose was estimated spectrophotometrically using a commercial assay kit (Monozyme, India Ltd.). Liver glycogen was estimated by the method of Carroll *et a1*[10].

Toxicity evaluation in mice

To study sub-acute (short term) toxicity, four groups of mice each containing six male mice (20-25 g body weight) were used. One group was kept as control and groups 2, 3 and 4 received 100, 200, 400 and 800 mg/kg n-hexane extract, respectively. The drug was administered daily for 30 days (p.o.). Control group received the vehicle in an identical manner.

The behaviour of the animals was observed daily for 1 h for 29 days. Initial and final body weights, water and food intake, state of stool and body temperature were observed. The animals were killed on the 30th day. Hematological and serum biochemical parameters were determined. Important organs were dissected out, weighted and observed for pathological and morphological changes.

Hemoglobin was measured using hemoglobinometer with comparison standards. Glutamate pyruvate transaminase (GPT) and Glutamate oxaloacetate transaminase (GOT) were measured by the method of Reitman and Frankerl[11] and Alkaline phosphatase by determining hydrolysed phenol with antipyrine[12]. Urea and cholesterol were determined by conventional methods[13]. The peritoneal macrophages and total leucocytes were counted as described elsewhere[14].

Statistical treatment

Statistical comparison was done using one-way ANOVA followed by Dunnet's post hoc comparison when more than two groups were involved. p values <0.05 were considered significant. When the number of groups was 2, Student's t-test was used for comparison.

RESULTS AND DISCUSSION

As shown in Table 1, in glucose tolerance test, the crude water suspension of *Hemigraphis colorata* (500 mg/kg), when administered 30 min before glucose loading, scientifically reduced the rise in blood glucose levels at 90 and 150 min after glucose administration and % reduction was more at 150 min compared to that at 90 min. When different extracts of the plants was tested for their glucose lowering effects, using glucose tolerance test, the n-hexane (100 mg/kg) showed significant activity at 90 and 150 min after glucose loading whereas the water extract showed some level of activity at 150 min and not at 90 min.

The n-hexane extracts was devoid of significant activity (Table 2). The n-Hexane extract showed optimum activity at 100mg/kg and at a higher dose (200 mg/kg) it did not show a matching increase in activity; at 100mg/kg it did not show significant effect (Table 2).

As given in Table 3, in fasted rats, the extract (100 mg/kg) showed marginal decrease in glucose levels at 3 h, and not at 2h, after the administration.

An active ethyl acetate fraction was isolated from the n-hexane extract by sequential extraction with chloroform, ethyl acetate, Butanol and Water. The yields of hexane, chloroform, ethyl acetate, butanol and water fractions were approximately 32, 14, 14, 13, 21 and 19% respectively of the n-hexane extract.

The glucose lowering effects of different fractions in glucose tolerance test is given in Table 4.The presence of steroids and coumarins in the ethyl acetate fraction was identified by qualitative tests.

As given in Table 5 and 6 the active fraction showed significant antidiabetic activity against alloxan-induced diabetic rats. The daily drug administration at a dose of 12.5mg/kg gradually decreased the blood glucose levels from 23.12 to 15.42mmo1/1 in 12 days while in the untreated control rats the glucose levels increased from 22.83 to 51.12 mmo1/1 during the same period. However, 5IU/kg of insulin was found to be slightly better than the herbal drug (Table 5).

The body weight loss and drastic reduction in liver glycogen, observed in the diabetic animals, were prevented to a large extent by the drug administration and this drug's effect on these parameters is almost comparable to that of insulin (Table 6).

Table 1: Effect of water suspension of *Hemigraphis colorata* on glucose tolerance (serum glucose levels (mmol/l) in fasted and glucose loaded normal rats

	Time (min) after glucose administration			
	0	**30**	**90**	**150**
Control (2% gum acacia)	3.45+0.13	8.12+0.23	5.62+0.20	5.52+0.27
500 mg/kg (water suspension)	3.00+0.05	5.87+0.20	4.13*+0.25	3.98**+0.18

Values are mean + S.D.; n=6.

*P<0.05, ** P<0.01(compared to control).

Table 2: Effect of different extracts of *Hemigraphis colorata* on glucose tolerance (serum glucose levels (mmol/l) in fasted and glucose loaded normal rats

Treatments	Time (min) after glucose administration			
	0	30	90	150
Control (5% Tween 80)	3.43+0.20	7.68+0.20	5.85+0.18	4.79+022
n-Hexane extract (100mg/kg)	3.08+0.05	6.40+0.20	4.85*+0.13	4.18**+0.21
n-Hexane extract (200mg/kg)	3.45+0.05	6.55+0.13	5.18+0.07	4.65+0.13
n-Hexane extract (400mg/kg)	3.55+0.13	6.99+0.25	5.68+0.11	4.78+0.27
Ethanol extract (100mg/kg)	3.70+0.08	7.00+0.18	5.93+0.12	5.60+0.08
Water extract (200mg/kg)	3.61+0.16	7.01+0.25	5.76+0.25	4.47*+0.16
F	3.53	3.77	117.25	95.41
d.f.	5,30	5,30	5,30	5,30
P	NS	NS	<0.001	<0.001

Values are mean + S.D.; n=6. NS, not significant; *P<0.05, **P<0.001 (compared to control).

Table 3: Effect of n-Hexane extract of *Hemigraphis colorata* on blood glucose levels (mmol/l in fasted normal rats)

Treatments	Time (min) after drug administration		
	0	120	180
Control (5% Tween 80)	3.89+0.20	4.56+0.11	3.76+0.16
n-Hexane extract (100mg/kg)	3.33+0.23	3.62+0.23	3.42+0.25*

Values are mean + S.D.; n=6, *P<0.05 (compared to control).

Table 4: Effect of different fractions of n-hexane extract of *Hemigraphis colorata* on glucose tolerance (serum glucose levels (mmol/l) in fasted normal rats

Treatments	Time (min) after glucose administration			
	0	30	90	150
Control (5% Tween)	2.73+0.18	8.21+0.16	6.78+0.18	6.00+0.18
Chloroform fraction (50mg/kg)	2.68+0.20	6.53+0.20	4.96*+0.16	4.53*+0.16
Ethyl acetate fraction(12.5mg/kg)	2.62+0.20	6.08+0.14	4.30**+0.27	4.15**+0.16
Ethyl acetate fraction (25mg/kg)	2.58+0.18	5.75+0.31	4.76*+ 0.07	4.29*+0.10
Ethyl acetate fraction(50mg/kg)	2.76+0.20	6.32+0.16	5.82+0.08	5.35+0.13
Butanol fraction (75mg/kg)	2.71+0.11	7.00+0.20	5.30+0.26	4.62+0.22
Water fraction (60mg/kg)	2.75+0.13	7.01+0.14	5.31+0.16	5.20+0.24
F	0.77	13.79	35.11	33.91
d.f.	6,35	6,35	6,35	6,35
P	NS	<0.001	<0.001	<0.001

Values are mean + S.D.; n=6. The concentrations of various fractions tested were fixed based on their yield from the n- hexane extract. [Since 25 mg/kg ethyl acetate fraction was active, a lower dose (12.5mg/kg) was also tested.]

*P<0.05., **P<0.001 (compared to control).

Table 5: Effect of the ethyl acetate fraction of n- hexane extract of *Hemigraphis colorata* on serum glucose levels (mmol/l) in alloxan diabetic rats

Groups	1st day	4th day	8th day	12th day
Normal	5.42+0.12	5.64+0.23	5.79+0.21	6.56+0.27
Diabetic control	22.83+1.12	30.94+0.57	38.46+0.54	51.12+0.99
Diabetic+ EA fraction (12.5mg/kg)	23.12+0.54	23.02**+0.74	17.23**+0.27	15.42**+0.24
Diabetic insulin (5 IU/kg)	23.34+0.75	22.39**+0.82	15.62**+0.58	12.68**+0.61
F	955.86	1452.72	4213.96	4861.28
d.f.	3,20	3,20	3,20	3,19
P	<0.001	<0.001	<0.001	<0.001

Values are mean + S.D.; n=6 except in diabetic control group where n=5 on the 12th day (one animal died on the 12th day). EA fraction, ethyl acetate fraction [50mg/kg dose of this fraction was found to be the optimum dose in glucose tolerance test].

** P<0.001 (compared to diabetic control).

Table 6: Effect of ethyl acetate fraction of n-Hexane extract of *Hemigraphis colorata* on body weight, liver weight and liver glycogen in alloxan diabetic rats

Groups	Body weight(g)		Liver weight (g)	Liver glycogen (mg/g) wet tissue
	Initial day	Final day		
Normal control	155.0+9.6	200.0+8.4(+49)	7.8+1.41	14.4+2.8
Diabetic control	151.0+8.6	144.0**+6.5(-7)	6.0+1.5	3.0**+1.2
Diabetic+EA fraction (12.5mg/kg)	155.0+9.2	185.0+7.1(+30)	7.4+1.2	10.7*+2.4
Diabetic+insulin (5 IU/kg)	150.0+6.9	188.0+7.2(+38)	7.5+1.6	12.1+1.9
F	0.37	57.62	1.61	25.73
d.f	3,19	3,19	3,19	3,19
P	NS	<0.001	NS	<0.001

Values are mean + S.D,; n=6 in each group except diabetic control group where n=5 (one animal died on the 12th day). EA fraction, ethyl acetate fraction. The values in parenthesis are differences in initial and final body weights.

*P<0.05, **P<0.001 (compared to normal control).

Table 7: Effect of n-hexane extract of *Hemigraphis colorata* on serum biochemical and hematological parameters in short term (30 days) toxicity study

Groups	Biochemical parameters					Hematological parameters			
	SGPT (IU/l	SGOT(IU/L	ALP(KAU	Urea(mmol/l)	Cholesterol (mmol/l	Glucose(mmol/l)	Hb(mg%)	WBC(10^6/ml	Macrophages (10^6/mouse)
Control	21.3+2.2	79.5+4.9	9.5+1.8	7.35+0.46	3.40+0.21	6.65+0.27	13.3+0.6	11.5+1.0	8.6+0.2
100mg/kg	27.0+4.2	71.8+2.6	12.6+1.6	5.83+1.16	3.56+0.25	5.43**+0.25	13.2+0.4	12.8+0.5	8.7+1.1
200mg/kg	25.4+4.2	72.6+3.6	9.7+1.9	6.00+0.60	3.26+0.22	5.30**+0.20	13.2+0.5	12.0+0.4	8.1+0.1
400mg/kg	19.2+2.6	71.0+2.3	10.9+1.8	6.35+0.70	3.26+0.15	5.93*+0.25	13.3+0.6	13.2*+0.8	8.9+0.8
F	3.88	5.98	2.73	3.21	3.68	37.32	0.092	6,71	1.37
d.f.	3,20	3,20	3,20	3,20	3,20	3,20	3,20	3,20	3,20
P	NS	NS	NS	NS	NS	<0.001	NS	<0.05	NS

SGPT, serum glutamate pyruvate transaminase; SGOT, serum glutamate oxalate; ALP, alkaline phosphatase; NS, not significant. Values are mean + S.D.; n=6 in each group.

*P<0.05, ** P<0.01 (compared to control).

The n-Hexane extract of *Hemigraphis colorata*, when fed daily for 30 days at a dose of 2 or 4 times higher than the therapeutic dose, did not exhibit any conspicuous adverse toxic effects (Table 7). The drug administration did not change body weight as well as food and water intake (data not given). Further, it did not result in any gross behavioral changes. As shown in Table 7, peritoneal macrophage count and serum biochemical parameters (except glucose which showed a decrease) were unaltered. Total WBC count showed a marginal increase at the highest dose (400 mg/kg) suited.

It appears that, unlike insulin, the herbal drug does not have severe hypoglycaemic action. When the dose of the n-Hexane extract was increased from 100 to 200 mg it did not result in a proportional decrease in serum glucose. Further, the drug had only marginal hypoglycaemic will not result in hypoglycaemic shock. Insulin and sulfonylurea drugs cause hypoglycemia when taken in excessive doses and severe hypoglycemia is the most worrisome effect of these drug [15].

Hemigraphis colorata is a commonly occurring plant in most of the temperate climatic conditions. So the plant material can be cheaper and easily available. Besides, it can be easily cultivated. So there cannot be shortage of raw materials for phytomedicine development. Further, biotechnological intervention can lead to the production of the plant mass in the laboratory by tissue culture.

The n-hexane extract of *Hemigraphis colorata*, when fed daily for 30 days at a dose of 2 or 4 times higher than the therapeutic dose, did not exhibit any conspicuous adverse toxic effects (Table 7). The drug administration did not change body weight as well as food and water intake (data not given). Further, it did not result in any gross behavioral changes. As shown in Table 7, peritoneal macrophage count and serum biochemical parameters (except glucose which showed a decrease) were unaltered;

Total WBC count showed a marginal increase at the highest dose (400 mg/kg) studied.

Anti-diabetic plants have often been used by practitioners of herbal medicine in treating individuals with non-insulin-dependent (type 2) diabetes. In such cases patient response must be carefully monitored and significant benefit can be gained from such therapies. Consultation with the prescribing physician is necessary and an integrative management of the case by conventional and herbal practitioners working together would be preferred.

The shared goal would be to regulate the dosage of both types of medication and enable a smooth transition to lower dependence on insulin in cases where such is desirable and attainable. While hypoglycemic herbs may offer promise in the treatment of diabetes in their combined effect with insulin, treatment is inherently disruptive and extreme caution must be exercised in order to promote a smooth transition, maintain suitable blood sugar levels and avoid insulin shock[16, 17].

Naturally occurring hydroxycinnamic acid derivatives, such as *p*-coumaric acid (CA), ferulic acid (FA), and caffeic acids, act as anticancer, antiinflammation, antihepatoxicity, antibacterial, antimutatio, and antioxidation agents . Several hydroxycinnamic acids and their derivatives have been found to possess strong antioxidant activities as radical scavengers against reactive oxygen species and to have considerable tyrosinase inhibitory activities . Oxidation of low density lipoprotein (LDL) has been suggested to play a key role in the development of atherosclerosis and DM and as a consequence an important role in the prevention of LDL oxidation has been attributed to the antioxidants contained within LDL and Plasma[18].

This model is almost comparable to type one diabetic model with near complete β-cell destruction[19].

So the drug may be mimicking one or more actions of insulin at the insulin receptor level or/and it may be influencing one or more post receptor evens.

The control of blood glucose levels is critical in the treatment of diabetes mellitus. α-Glucosidase inhibitors are of great importance in reducing hyperglycemia, and plants have provided many of these agents[20].

Two stilbenes, lonchocarpene and 3,5-dimethoxy-4'- O-prenyl-TRANS-stilbene (DPS) from the plants Gnidia glauca and Dioscorea bulbifera,on were tested for their efficiency to inhibit α-amylase and α-glucosidase activity and on mice postprandial hyperglycemia[21].

Literatures reveal that many plants are pharmacologically screened for theiranti diabetic activity eg: Extracts of Acacia arabica bark, Benincasa hispida fruit, Tinispora cordifolia stem, Ocimum sanctum areal parts and Jatropha curcus leaves, ameliorate the derangements in lipid metabolism caused by diabetes mellitus in alloxan induced diabetic rats towards normal level[22].

Petroleum-ether, ethyl acetate and chloroform fractions isolated from ethanolic extract of the leaves of Coccinia cordifolia Ln showed antidiabetic and hypolipidemic effects on normal and streptozotocin (STZ)-induced diabetic rats[23].

From various studies it is reported that HFD/STZ in rats causes hyperglycemia, hyperlipidemia accompanied by the presence of oxidative damage in the pancreas. Moreover, treatment with BM, by virtue of its antioxidant potential significantly ameliorated HFD/STZ-induced alterations and also morphological changes in rat pancreas. The beneficial effects of BM possess a vast ethnomedical history and represent a phytochemical reservoir of heuristic therapeutic value8 and exhibit hypoglycemic and high antioxidant potential[24].

When the n-hexane extract was fractioned, the major activity came in the ethyl acetate fraction; but some level of activity was present in the chloroform fraction ion also, but not in the butanol and water fractions. This suggests the presence of more than one active compound in the n-hexane extract. Studies are in progress in this laboratory to identify the active compound present in the ethyl acetate fraction.

CONCLUSION

The present study shows for the first time the anti hyperglycaemic and anti-diabetes property of *Hemigraphis colorata* in rats. The

active fraction obtained from this plant is an attractive material for further studies leading to possible drug development. This fraction can be used as such for phytomedicine development with further studies leading to possible drug development. This fraction can be used as such for phytomedicine development with further studies to establish safety and efficacy.

In the present study, severe alloxan diabetic model was used where the initial blood glucose level was more than 450mg/dl. Since one of the animals died in the control diabetic group on the 12th day, the treatment period was not continued beyond that.

Studies are in progress in this laboratory to elucidate the mechanism of action of this herbal drug. Development of phytomedicines is relatively inexpensive and less time consuming; it is more suitable to our economic conditions compared to allopathic type of drug development. However, ecotype, genotype and seasonal variations in efficacy and safety, if any, have to be determined in phytomedicine development.

Our preliminary analysis suggests than the active compound in the ethyl acetate fraction is a steroid or coumarin.

The plant is ground into a paste with water and consumed as folk medicine for various ailments in certain remote village in Trivandrum district, Kerala state without any known or recorded adverse effects. Such herbal drugs may be used directly for clinical trials.

However, when an extract or active fraction is used it is better to evaluate for possible toxicity. In the present study, the alcohol extract was evaluated for its toxicity and it did not exhibit any toxic symptoms in the limited short term toxicity evaluation in mice. (Toxicity evaluation was carried out before the isolation of the active fraction.) Toxicity evaluation of the active fraction remains to be done. Literature search shows that pharmacological and phytochemical studies were not carried out on this plant for its anti diabetic activity.

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