Research Article

Hypoglycaemic Activity of Root Extracts of *Cocculus hirsutus* (L.) Diels In Streptozotocin induced Diabetic Rats

Digbijay Kumar[1]*, GK. Dash[2] and NK. Tripathy[3]

[1]C. U. Shah College of Pharmacy and Research, Wadhwan , Gujarat-363030, India.
[2]Faculty of Pharmacy and Health Sciences, Universiti Kuala Lumpur Royal College of Medicine Perak, Ipoh-30450, Malaysia.
[3]Department of Zoology, Berhampur University, Berhampur, Odisha-760007, India.

ABSTRACT

The present investigation was carried out to study the effect of chloroform, methanol and aqueous extracts of the root of Cocculus *hirsutus* (L.) Diels for its hypoglycaemic activity in adult Wistar albino rats at 100, 200 and 400 mg/kg p.o. respectively using normoglycemic, glucose loaded and streptozotocin induced hyperglycaemic rat models. Metformin (250 mg/kg, p.o.) was used as reference standard for the activity comparison. Among the tested extracts, the methanol and aqueous extracts showed significant reduction in blood glucose levels in normal, glucose loaded and streptozotocin induced diabetic rats that is comparable to metformin, with more promising decrease in glucose concentration observed in methanol extract than the aqueous extract. The chloroform extract on the other hand, did not elicit significant reduction of blood glucose concentration. The hypoglycaemic activity produced by the extracts may be due to by promoting the insulin release from the undestroyed β-cells or its action may be insulin like The preliminary phytochemical screening of root extracts revealed presence of alkaloids, steroids and sterols, triterpenoids, tannins and phenolic compounds, flavonoids, carbohydrates, gums and mucilages, proteins and amino acids. The results obtained from this study are quite promising and comparable with metformin. The study further provided a scientific validation of the folklore use of the *C. hirsutus* and suggested that this plant (root) has promising therapeutic activity for the maintenance of diabetes mellitus.

Keywords: Cocculus *hirsutus,* Streptozotocin, Hyperglycaemic, Normoglycemic, OGTT.

INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2DM) is approaching an epidemic proportion that affects people of all ages[1]. The International Diabetes Federation in its Global Diabetes Plan 2011-2021 estimates that there are already, 366 million diabetes people and another 280 million are at identifiably high risk of developing diabetes in the world. If nothing is done, by 2030 this number is expected to rise to 552 million with diabetes and an additional 398 million people at high risk [2]. Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides and glinides. Many of them have a number of serious adverse effects; therefore, the search for more effective and safer hypoglycemic agents is one of the important areas of investigation [3]. Traditionally, many indigenous plants have been used successfully for the management of the disease throughout the world, some of them have been evaluated experimentally and their active ingredients have been isolated [4,5]. Antihyperglycemic activity of the plants is mainly due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. More than 400 plant species having hypoglycemic activity have been available in literature, however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which demonstrate alternative and safe effects on diabetes mellitus [6].

Cocculus hirsutus (L.) Diels (Fam. Menispermaceae) is a climbing under shrub, often densely velvety, grows all over India especially in dry region from East and

Southern Africa eastward to India, Myanmar, Thailand and southern China[7]. Traditionally, the plant is reported to be used for the treatment of a variety of ailments. The decoction of the fresh roots used to treat rheumatism and fever [8]. It is further believed to be useful in treating *'Kapha'* and *'Vata'* conditions, poisonous bites, leprosy, skin diseases, pruritis, dyspepsia, bronchitis, gout, spermatorrhoea, hypertension and as antiperiodic [9,10]. The leaves are used in rheumatism and gonorrhoea [11,12,13] and as blood purifier, analgesic and antiallergic [14,15]. The tribes of Bangriposi in Simlipal Wild Life Century in Mayurbhanj district of Odisha use orally, the freshly prepared root paste for treatment of diabetes since ancient times and claim for its promising activity.

Several phytochemicals have been reported from different parts of *C. hirsutus*. The plant is rich with isoquinoline alkaloids. Presence of cohirsine, jamtine-N-oxide cohirsinine, jamtine and haiderine were identified in the aerial parts[16] DL-coclaurine, daphonoline, magnoflorine, isotriboline, coclaurine, 2-N-Oxy cocsuline, triobine, isotriboline and 2-O-demethyltriboline were reported from the roots and stem [17,18]. Presence of (+) Syringaresinol, a lignan has been identified in the leaves [19] .

Many reports on pharmacological activities on this plant are available in the literature. Different extracts of the roots have been studied for anti-inflammatory and analgesic [20], diuretic [21], anticonvulsant, antispasmodic, CNS depressant, hypotensive, laxative, smooth muscle relaxant and uterine relaxation [22] effects and reported significant activities. The diuretic and laxative [23], antidiabetic and spermatogenic [24] activities of the aerial parts are also reported. In the present paper, we report the antidiabetic activities of different extracts of the roots of *C. hirsutus*.

MATERIALS AND METHODS

Plant Material and Preparation of Extracts

Fresh root of *C. hirsutus* were collected from the Simlipal forest of Mayurbhanj districts of Odisha during July 2011 and authenticated. After authentication, the plant material was collected in bulk, shade dried and pulverized in a mechanical grinder to obtain coarse powder. The dried powdered plant material (1.5 kg) was defatted with petroleum ether (40^0–60^0 C) and extracted successively with chloroform, methanol and water using a soxhlet extractor. Following extraction, the liquid extracts were separately concentrated under vacuum and extractive value calculated (yield: Chloroform extract-1.5 %, methanol extract- 5.5% and aqueous extract-7.8% w/w with respect to the dried plant material). Qualitative phytochemical studies were performed on the extracts to study the nature of phytoconstituents they contain [25,26].

Animals

Healthy adult Wistar rats (150-200 g) of either sex were used. The animals were kept in standard polypropylene cages at room temperature (30 ± 2^oC, 66-65% RH). The experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and the experimental protocols were duly approved by the Institutional Animal Ethics Committee.

Acute Oral Toxicity Study

Toxicity studies of the test extracts were evaluated as per OECD guideline 423. The test extracts were suspended in 0.5% w/v sodium carboxymethyl cellulose in distilled water. The animals were fasted overnight, provided only water, after which the test extracts were separately administered to the respective groups orally at the dose level of 5 mg/kg and the groups of animals were observed carefully and continuously for any behavioural changes for 72 h and for mortality if any up to a period of 14 days. Since no mortality was observed in any of the groups of animals, the procedure was repeated for further higher doses such as 50, 300, and 2000 mg/ kg. The animals were observed for toxic symptoms such as behavioural changes, locomotion, convulsion, and mortality for 72 h[27,28].

Screening for Antidiabetic Activity

Screening for antidiabetic activity of the test extracts was assessed using normoglycemic, glucose loaded and streptozotocin induced hyperglycaemic rats. Metformin (250 mg/kg p.o.) was used as reference standard for activity comparison. All the test samples were suspended in 0.5% w/v sodium carboxymethyl cellulose in distilled water. The selected animals were divided in to different groups comprising of six rats in each group in different models adopted.

Using normoglycemic rats

For the normoglycemic study, rats were divided into eleven groups. The animals were allowed free access to food and water before and throughout the duration of experiment. At the beginning of the experiment, taken as zero time (0 h), blood was withdrawn from the tip of the tail of each rat and the blood glucose was estimated with a prestandardized Acu-Chek Active glucometer, Roche Diagnostics, Germany using standard strips. Control was

designated as Group I and received vehicle (2 ml/kg) through oral route. Group-II animals received metformin (250 mg/kg p.o.). Group-III, IV and V received 100, 200 and 400 mg/kg of chloroform extract, Group-VI, VII and VIII received 100, 200 and 400 mg/kg of methanol extract, Group-IX, X and XI received 100, 200 and 400 mg/kg aqueous extract of *C. hirsutus* in a similar manner. After 1, 2, 4 and 8 h of administration of single dose of test samples, a drop of blood was collected from tip of the tail of each animal and blood glucose concentrations were measured[29]. The results are presented in Table 1.

Oral glucose tolerance test (OGTT)

Healthy overnight fasted rats were divided into eleven groups. Thirty minute following the various treatment schedules, each rat was given an oral glucose load of 2 g/kg. Group I served as a control and received only vehicle (2 ml/kg) through oral route. Group-II received metformin (250 mg/kg p.o.). The other groups received 100, 200 or 400 mg/kg of chloroform, methanol and aqueous extracts in a similar manner. Blood samples were collected before and at 30, 60, 150 and 180 min after glucose loading and blood glucose levels were estimated [30]. The results are depicted in Table 2.

Streptozotocin Induced hyperglycaemic rats

After overnight fasting, experimental diabetes was induced in rats by single intraperitoneal injection (65 mg/kg) of streptozotocin in citrate buffer (pH 4.4, 0.1M). The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia. The blood glucose level was checked before streptozotocin administration and 72 h after streptozotocin induction by withdrawing blood from the tip of the tail of each rat. Animals were considered diabetic when the blood glucose level was raised beyond 200 mg/100ml of blood. This condition was observed at the end of 72 h after streptozotocin induction. The diabetic animals were then placed in different groups consisting of six animals in each. Group-I which served as diabetic control received vehicle (2 ml/kg) orally. Metformin (250 mg/kg p.o.), was received by Group-II. Chloroform, methanol or aqueous extracts at doses of 100, 200 or 400 mg/kg were received by the other groups of animals in a similar manner. After 1, 2, 4 and 8 h of administration of single dose of test samples, blood glucose levels were measured [31]. The results are tabulated in Table 3.

Statistical analysis

All values are expressed as mean ± SEM. Statistical analysis was performed by One-way Analysis of Variance (ANOVA) followed by Dunnet's t-test. A 'p' value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

The root of *C. hirsutus* has been used by the local tribes of Mayurbhanj district of Odisha for the treatment of diabetes mellitus since time immemorial and they claim for its promising activity. The preliminary phytochemical screening of root extracts revealed presence of alkaloids, steroids and sterols, triterpenoids, tannins and phenolic compounds, flavonoids, carbohydrates, gums and mucilages, proteins and amino acids. In acute oral toxicity study, no mortality and sign of toxicity were observed at the dose of 2000 mg/kg. Results of antidiabetic activity of *C. hirsutus* root extract established the scientific basis for the utility of this plant in the treatment of diabetes. Among the tested extracts, the methanol and aqueous extracts showed significant reduction in blood glucose levels in normal, glucose loaded and streptozotocin induced diabetic's rats at the tested dose levels with more promising decrease in glucose concentration with the methanol extract in a dose dependant manner. The chloroform extract on the other hand, did not elicit significant reduction of blood glucose concentration (Table 1 to 3).

Results of the normoglycaemic study of *C .hirsutus* (Table 1) indicated that in normal rats, metformin improved glucose tolerance (48.24% maximum reduction versus control) followed by methanol extract (31.95%) and aqueous extract (25.14%) at the highest dose tested (400 mg/kg) at the end of 8 h of the study.

The OGTT is well accepted and frequently used assay procedure to screen for hypoglycemic activity. *C. hirsutus* might enhance glucose utilization in significantly reduce blood glucose level in normal rats. From the data (Table 2) obtained with the OGTT, it is evident that blood glucose level reached a peak (124.25 mg/dl) and returned near to initial normal value (86.25 mg/dl) after 180 min in glucose loaded rats under methanol extract treatment at the dose of 400 mg/kg. The activity of the methanol and aqueous extracts was observed to be dose dependant. The methanol extract at the dose of 200 mg/kg showed percentage reduction in blood glucose concentration of 14.91 and 26.30 at the end of 150 min. Among all the test extract studied percentage reduction of blood glucose concentration of the methanol extract at the

end of 180 min was found to be 13.95, 19.11 and 30.58 at 100, 200 and 400 mg/kg. However, the aqueous extract showed percentage reduction of 17.54 and 22.36 at the dose of 200 mg/kg and 400 mg/kg respectively at the end of 180 min, after glucose administration. The standard drug metformin showed maximum lowering glucose concentration of 38.98% at 180 min.

In streptozotocin induced diabetic rats, single administration of methanol extract at (200 and 400 mg/kg, p.o) showed significant reduction in blood glucose level after 2, 4 and 8 h time interval (Table 3). The percentage reduction of blood glucose concentration at the end of 8 h was found to be 16.92, 30.60 and 55.79 at 100, 200 and 400 mg/kg. Metformin (250mg/kg, p.o) shows 61.86% decrease as compared with vehicle treated group. The chloroform extract at dose of 400 mg/kg showed percentage reduction of 15.04 in blood glucose concentration, whereas aqueous extract treated groups revealed significant percentage reduction of 15.57 and 27.38 in blood glucose concentration at the dose of 200 mg/kg and 400mg/kg respectively at the end of 8 h.

Streptozotocin is widely used to induce experimental diabetes in animals. The mechanism of action on β-cells of the pancreas has been intensively investigated and now is quite well understood. The deleterious effect of streptozotocin results from the generation of highly reactive carbonium ions (CH_3^+) that cause DNA breaks by alkylating DNA bases at various positions, resulting in activation of the nuclear enzyme, poly(ADP-ribose) synthetase, thereby depleting the cellular enzyme substrate (NAD^+), leading to cessation of NAD^+ -dependent energy and protein metabolism. This in turn leads to reduced insulin secretion [32]. It has been suggested that free radical stress occurred during β-cell destruction mediated by mononuclear phagocytes and cytokines [33,34] .

In our present study, we have observed that the methanol and aqueous extracts of *C. hirsutus* could reverse the hyperglycaemic condition in diabetic rats and brought about hypoglycaemic action because blood glucose once lowered by the extracts did not increase again throughout experiment when compared to untreated control. The possible mechanism of action of the test extracts may be due to by promoting the insulin release from the undestroyed β-cells or its action may be insulin like [35] (Chandola et al., 1980). Further studies are in progress to find out the possible mode of action.

CONCLUSION

The results obtained from this study are quite promising and comparable with metformin. The study further provided a scientific validation of the folklore use of the *C. hirsutus* and suggested that this plant (root) has promising therapeutic activity for the maintenance of diabetes mellitus.

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Table 1: Effect of different extracts of the roots of *C. hirsutus* on the blood glucose level in normal rats

Groups	Treatment	Dose	Blood glucose concentration (mg / dl) Time (h) after treatment				
			Fasting	1 h	2 h	4 h	8 h
I	Control	2 ml/kg	79.43 ± 2.35	77.45 ± 2.17	77.25 ± 2.25	76.46 ± 2.28	74.23 ± 2.11
II	Metformin	250 mg/kg	80.45± 2.45	67.33 ± 3.48* (16.30%)	62.5 ± 3.52 (22.31%)	55. 4 ± 3.20** (31.13%)	41.64 ± 3.12** (48.24%)
III	Chloroform extract	100 mg/kg	78.53 ± 2.47	75.4 ± 2.23 (3.94%)	74.26 ± 2.53 (5.40%)	72.4 ± 2.80 (7.77%)	70.46 ± 2.23 (10.31%)
IV		200 mg/kg	77.28 ± 2.54	74.3 ± 2.4 (3.85%)	72.14 ± 2.45 (6.47%)	70.25 ± 3.45 (9.06%)	69.45 ± 2.56 (10.10%)
V		400 mg/kg	79.25 ± 2.84	74.24 ± 2.42 (6.31%)	73.74 ± 2.52 (6.94%)	70.36 ± 2.52 (9.78%)	69.52 ± 3.52 (12.62%)
VI	Methanol extract	100 mg/kg	81.25 ± 2.02	76.16 ± 2.28 (6.15%)	73.85 ± 2.52 (9.11%)	70.45 ± 2.12 (13.30%)	65.14 ± 2.24* (19.77%)
VII		200 mg/kg	78.87 ± 3.09	72.23 ± 2.54 (8.37%)	70.1 ± 2.41 (11.40%)	64.62 ± 3.52 (18.20%)	62.75 ± 2.78** (20.43%)
VIII		400 mg/kg	81.5 ± 2.8	71.19 ± 2.5 (12.65%)	66.4 ± 2.74* (18.52%)	57.52 ± 2.30** (29.44%)	55.46 ± 2.56** (31.95%)
IX	Aqueous extract	100 mg/kg	79.6 ± 2.52	76.64 ± 2.76 (3.79%)	74.23 ± 2.52 (6.40%)	69.52 ± 2.85 (12.56%)	68.81 ± 2.53 (13.56%)
X		200 mg/kg	79.43 ± 2.20	74.37 ± 2.52 (6.29%)	73.3 ± 2.42 (7.55%)	68.5 ± 2.7 (13.72%)	65.26 ± 2.64* (17.88%)
XI		400 mg/kg	80.46 ± 3.80	75.4 ± 4.12 (6.21%)	72.16 ± 4.29 (10.31%)	65.23 ± 3.41* (18.92%)	60.23 ± 3.68** (25.14%)

Results expressed as Mean ± SEM from six observations (n = 6). *P<0.05, **P<0.01 as compared with control group (One way, ANOVA followed by Dunnet's t-test). Figures in parenthesis denote percentage reduction of blood glucose.

Table 2: Effect of different extracts of the roots of *C. hirsutus* in Oral Glucose Tolerance Test

Groups	Treatment	Dose	Blood glucose concentration (mg / dl) Time (min) after treatment				
			Fasting	30 min	60 min	150 min	180 min
I	Control	2 ml/kg	79.4 ± 2.15	118 ± 2.53	126.5 ± 7.25	133 ± 6.85	129.83 ± 7.53
II	Metformin	250 mg/kg	80.43 ± 2.29	118.73 ± 2.46	100.14 ± 4.54* (15.25%)	86.42 ± 6.52** (27.11%)	72.144 ± 6.57** (38.98%)
III	Chloroform extract	100 mg/kg	82.3 ± 2.2	123.26 ± 2.42	119 ± 4.45 (3.40%)	116.4 ± 4.49 (5.51%)	111.5 ± 4.25 (9.49%)
IV		200 mg/kg	80.5 ± 2.27	120 ± 2.36	114.65 ± 3.42 (4.5%)	112.47 ± 6.25 (6.27%)	107.8 ± 6.27 (10.16%)
V		400 mg/kg	80.58 ± 2.08	119.17 ± 2.56	111.52 ± 6.4 (6.41%)	109.5 ± 4.45 (8.11%)	106.48 ± 7.31 (11.09%)
VI	Methanol extract	100 mg/kg	83.4 ± 2.8	123.5 ± 2.2	112.88 ± 4.85 (8.59%)	111.83± 4.15 (9.44%)	106.26 ± 4.96* (13.95%)
VII		200 mg/kg	81.25 ± 2.79	122.64± 2.53	108.63 ± 6.48 (11.41%)	104.37 ± 5.89* (14.92%)	99.16 ± 8.50* (19.11%)
VIII		400 mg/kg	83.60 ± 2.70	124.25 ± 2.76	105.16 ± 3.96* (15.36%)	91.56 ± 6.72** (26.30%)	86.25 ± 7.68** (30.58%)
IX	Aqueous extract	100 mg/kg	81.42 ± 2.32	119.52 ± 3.95	114.43 ± 6.73 (4.26%)	111.4 ± 5.45 (6.77%)	107.75 ± 5.65 (9.87%)
X		200 mg/kg	83.45 ± 2.05	125.4 ± 2.32	113.23 ± 5.61 (9.72%)	106.62 ± 4.32* (14.49%)	103.4 ± 6.62* (17.54%)
XI		400 mg/kg	82.53 ± 2.46	122.53 ± 2.02	109.52 ± 4.89 (10.61%)	102.23 ± 7.92* (16.57%)	95.13 ± 6.61* (22.36%)

Results expressed as Mean ± SEM from six observations (n = 6). *P<0.05, **P<0.01 as compared with control group (One way, ANOVA followed by Dunnet's t-test). Figures in parenthesis denote percentage reduction of blood glucose.

Table 3: Effect of different extracts of the roots of *C. hirsutus* on the blood glucose level in streptozotocin induced diabetic rats

Groups	Treatment	Dose	Blood glucose concentration (mg / dl) Time (h) after treatment				
			Fasting	1 h	2 h	4 h	8 h
I	Control	2 ml/kg	228.43 ± 7.25	236.54 ± 7.14	237.46 ± 6.24	242.52 ± 7.25	246.78 ± 6.32
II	Metformin	250 mg/kg	229.25 ± 8.26	191.60 ± 9.14* (16.57%)	157.25 ± 8.81** (31.37%)	106.32 ± 10.23** (53.70%)	87.28 ± 11.52** (61.86%)
III	Chloroform extract	100 mg/kg	228.56 ± 11.27	225.4 ± 9.58 (1.38%)	221.5 ± 9.14 (3.09%)	217.84 ± 8.24 (4.69%)	210.33 ± 12.87 (7.97%)
IV		200 mg/kg	226.4 ± 10.45	220.26 ± 11.5 (2.71%)	210.4 ± 10.95 (7.07%)	209.16 ± 13.87 (7.61%)	203.4 ± 14.2 (10.16%)
V		400 mg/kg	225.25 ± 11.35	212.46 ± 11.38 (5.68%)	203.23 ± 09.52 (9.77%)	197 ± 15.29 (12.54%)	191.36 ± 15.32* (15.04%)
VI	Methanol extract	100 mg/kg	229.33 ± 09.3	215.26 ± 11.52 (6.13%)	208.66 ± 09.91 (9.01%)	198 ± 13.15* (13.66%)	190.53 ± 11.42* (16.92%)
VII		200 mg/kg	230.82 ± 09.74	212 ± 10.28 (8.15%)	193.2 ± 11.87* (16.29%)	178.62 ± 16.65* (22.61%)	160.18 ± 12.24** (30.60%)
VIII		400 mg/kg	223.56 ± 10.07	198.66 ± 10.48 (11.14%)	182.18 ± 13.54* (18.51%)	134.16 ± 14.18** (39.99%)	98.83 ± 10.62** (55.79%)
IX	Aqueous extract	100 mg/kg	227.25 ± 11.6	220.33 ± 10.45 (3.04%)	218.17 ± 09.65 (3.99%)	204.19 ± 11.54 (10.15%)	201.3 ± 14.69 (11.42%)
X		200 mg/kg	222.83 ± 12.74	210.43 ± 13.83 (5.56%)	205.38 ± 14.5 (7.83%)	195.35 ± 13.71 (12.33%)	188.14 ± 13.96* (15.57%)
XI		400 mg/kg	224.46 ± 11.58	207.83 ± 12.4 (7.41%)	201.4 ± 15.27 (10.27%)	186.83 ± 14.08* (16.76%)	163 ± 15.2** (27.38%)

Results expressed as Mean ± SEM from six observations (n = 6). *P<0.05, **P<0.01 as compared with control group (One way, ANOVA followed by Dunnet's t-test). Figures in parenthesis denote percentage reduction of blood glucose

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