

Full Length Research paper

Anti-diabetic and spermatogenic activity of *Cocculus hirsutus* (L) Diels

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Anti-diabetic effect was observed with *Cocculus hirsutus* when given as an aerial part extract in normal as well as diabetic rats. The effect, however, was more pronounced in diabetic animals in which administration for 15 days after streptozotocin (STZ)-induced diabetes, significantly reduced blood glucose levels. After STZ-induced diabetes, it was observed that both standard drug (glibenclamide) and methanolic extract of *C. hirsutus* were significantly superior to control in reducing blood sugar on long treatment (15 days). The data suggested that *C. hirsutus* could be of benefit in diabetes mellitus in controlling blood sugar. The present investigation established pharmacological evidence to support the folklore claim that it is an anti-diabetic.

Key words: *Cocculus hirsutus*, glibenclamide, hyperglycemia, spermatogenic, streptozotocin.

INTRODUCTION

Diabetes mellitus is a group of disorders with different etiologies. It is characterized by derangements in carbohydrate, protein and fat metabolism, caused by the complete or relative insufficiency of insulin secretion and insulin action (Balkau, 2002). Approximately, 150 million people world wide suffer from diabetes (WHO, 1999). The disease become a real problem of public health in developing countries, where it prevalence is increasing steadily (Djrolo et al., 1998). In those countries, adequate treatment is often expensive or unavailable. Alternative strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed (WHO, 2002), because of the inability of existing modern therapies to control all the pathological aspects of the disorders, as well as the enormous cost and poor availability of the modern therapies for many rural populations in developing countries. Many herbal products, including several metals and minerals have been described for the cure

of diabetes mellitus in ancient-literature. Herbal preparations alone or in combination with oral hypoglycemic agents, some times produced a good therapeutic response in some resistant case where modern medicines alone fail (Anturlikar et al., 1995).

Cocculus hirsutus Linn (Menispermaceae) is commonly known as Jal-jammi (Chopra et al., 1958). It is a climber found in tropical and sub-tropical regions of India. A decoction of the leaves is taken in eczema, dysentery and urinary problem. Leaves and stem are used for treating eye diseases. Roots and leaves are given for Sarsaparilla, as diuretic and in gout (Nadkarni, 1982). Ethanolic extract of whole plant showed the presence of isoquinoline alkaloid d-trilobine and dl-coclaurine (Jaganatha, 1961), Cohirsinine (Viquaruddin, 1991), Jaminine (Viquaruddin, 1992) cohirsutine (Viquaruddin, 1993). Aerial parts of the plant reported to be used as a diuretic, laxative (Ganapathy et al., 2002) and root extract showed analgesic and anti-inflammatory effect (Nayak, 1993). Leaf juice of this plant is used in the treatment of eczema (Masilamani, 1981). Hence there is a search for new anti-diabetic agent that retain therapeutic efficacy and yet are devoid of these adverse effects. Since not much study

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had been done to evaluate the biological activity of the plant, the present study is focused to evaluate the anti-diabetic activity of aerial parts of *C. hirsutus*.

MATERIALS AND METHODS

Plant material

The plant parts were collected from the foot hills of Yercaud, Salem, in the month of September 2005. The plant was identified and authenticated by the experts in the department of Botany Govt. Arts College, Salem, Tamil Nadu, India. A voucher specimen (CHL-03) has been kept in our museum for future reference. The plant material was collected and shade dried at room temperature for 10 d and coarsely powdered with the help of a hand-grinding mill and the powder was passed through sieve No.60.

Preparation of the extract

The powdered material of *C. hirsutus* was extracted separately by continuous hot extraction process using soxhlet apparatus with different solvents in increasing order of polarity from petroleum ether, chloroform, acetone, methanol, to finally chloroform : water (Kokate, 1994). After extraction, the extracts were concentrated under reduced pressure in tared vessels. The dried extracts were subjected to various chemical tests to detect the presence of different phytoconstituents like isoquinoline alkaloids, triterpens and traces of flavonoids, etc.

Animals

Male albino rats of approximately same age, weighing about 150 - 175 g were used for the study. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum*. The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each test, the animals were fasted for at least 12 h. The experimental protocols were subjected to the securitization of the Institutional Animal Ethics Committee and were cleared by same.

Toxicity evaluation in mice

The methanolic extract was tested for its acute toxicity in mice. To determine acute toxicity of a single oral administration of the methanolic extract of *C. hirsutus* in different doses (300, 600, 900 mg/kg) were administrated to different groups of mice (3 mice were used for each group, control mice received Tween 80). Mortality and general behavior of the animals were observed periodically for 48 h. The animals were observed continuously for the initial 4 h and intermittently for the next 6 h and then again at 24 h and 48 h following drug administration. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion.

Streptozotocin-induced diabetic rats

Streptozotocin (STZ), purchased from Sigma aldrich chemical Co., was dissolved in 0.9% ice-cold saline immediately before use. Diabetes was induced in rats by intra peritoneal (i.p) injection of streptozotocin at a dose of 50 mg/kg, dissolved in normal saline (Pullock, 2002). Forty eight hours after streptozotocin administration blood samples were drawn from tail and glucose levels determined

to confirm diabetes. The diabetic rats exhibiting blood glucose levels in higher than 250 mg/dl were selected for studies. These diabetic rats were divided in to 5 groups as follows. Group I, Normal control group of rats were given food and water, Group II (Un treated) diabetic rats given 0.5 ml of 5% Tween 80; Group III, diabetic rats given 0.5 ml of 5% Tween 80 containing glibenclamide (50 mg/kg), Group IV, diabetic rats given (400 mg/kg) *C. hirsutus* (methanolic extract) in 0.5 ml 5% Tween 80; Group V, diabetic rats given (800 mg/kg) *C. hirsutus* (methanolic extract) in 0.5 ml 5% Tween 80. The dose (50 mg/kg) of glibenclamide was selected based on previous report. Each group consists of 6 animals.

The treatment continued daily for 15 days. In the untreated control (diabetic) rats 3 animals died on 5th day and 3 on the 7th day. Blood drop was collected from the tail, for glucose estimation just before drug administration on 1st day and 1 h after drug administration on days 4, 7, 10 and 15. The animals were sacrificed after blood collection, under chloroform anesthesia on the 15th day and testis was removed for spermatogenesis estimation.

Glucose tolerance test

Overnight fasted rats were divided in to 5 groups. First group was kept as normal control which received 5% Tween 80 (0.5 ml, p.o), second group diabetic control and group III and IV received methanolic extract of *C. hirsutus*; 400 and 800 mg/kg, respectively. The rats of all the groups were loaded with glucose (93 g/kg, p.o) 30 min after the administration of the drug. Blood samples were collected at 30, 90 and 150 min after the glucose loading. Serum was separated and glucose levels were measured immediately. Six rats were used in each group (Agasti et al., 1996).

Gonadosomatic Index

The testis was dissected and weighed up to nearest mg on the electronic balance. The organ weights were calculated per 100 g/body weight by using the formula, Organ weight / Body weight X 100.

Biochemical estimations

The left testis was dissected out and frozen for biochemical analysis. Levels of testosterone in the serum and testes were determined using the radio immuno assay (RIA) methods (Snedecor et al., 1974). Statistical analysis was done using method described by Snedecor and Cochran.

Histopathology

The portion of testis was fixed in Bouins fluid, dehydrated in ascending grades of alcohol, embedded in paraffin wax and sectioned as 5 μ and stained with haematoxylen and eosin. The micrometric measurement such as diameter of testis was measured by the help of ocular and stage micrometers. The spermatogenic elements were counted (Abercromboc, 1946) and sperm count was made (Kempinas et al., 1987).

Statistical evaluation

All the data are presented as mean \pm SEM. The differences between group were evaluated by one-way analysis of variance (ANOVA) followed by the Dunnette multiple comparisons test. $P < 0.01$ was consider to be significant.

Table 1. Diabetic activity of methanolic extract of aerial parts of *Cocculus hirsutus* (L) Diels. on STZ induced rats.

Groups	Treatment	Changes in blood Glucose levels (mg/dl)				
		1 st day	4 th day	7 th day	10 th day	15 th day
Normal	D/W 10 ml/kg (p.o)	95.33±3.72**	95.83±2.56**	95.00±2.54**	94.50±2.26**	93.16±2.10**
Diabetic control	D/W 10 ml/kg (p.o)	356.17±4.21	369.33±4.10	381.33 ± 3.31	403.67±7.30	431.30±3.89
Positive Control	Glibenclamide (50 mg/kg) p.o	360.83±3.66	343.17±3.80**	278.33±3.70**	199.17±2.08**	122.17±2.75**
Methanolic extract	400 mg/kg p.o	353.50±3.45	345.83±3.42**	271.83±3.60**	230.50±3.10**	173.00±2.79**
Methanolic extract	800 mg/kg p.o	353.33±3.45	340.50±3.12**	256.50±3.47**	223.83±2.98**	165.33±2.698**

Values are mean±SEM, n= 6.

When compared with diabetic control *= p<0.05, **p<0.01 (One way ANNOVA Followed by Dunnette multiple comparison test).

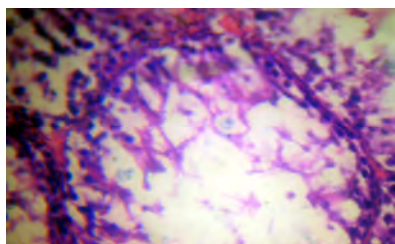


Figure 1. Normal testis and *Cocculus hirsutus* treated diabetic rats.

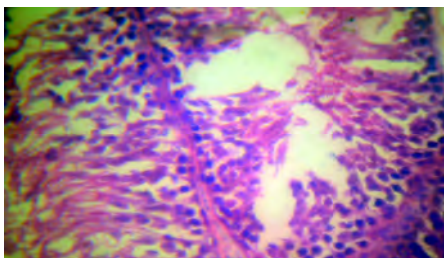


Figure 2. Methanolic extract (Lower dose) treated testis and *Cocculus hirsutus* treated diabetic rats.

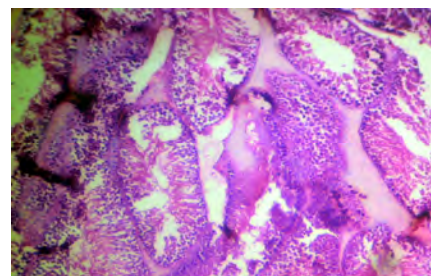


Figure 3. Methanolic extract (Higher dose) treated testis and *Cocculus hirsutus* treated diabetic rats.

and 800 mg/kg) treated rats, the blood glucose levels steadily decreased and it was 165 mg/100 ml on the 15th day. Thus treatment restored the serum glucose levels almost nearer to normal values. The effect of the alcoholic extract of *C. hirsutus* is comparable to that of standard (glibenclamide).

Testosterone levels in the testes were significantly higher in methanolic extract (both 400 and 800 mg/kg) of *C. hirsutus* treated rats after 15th days of treatment compared to the control group.

RESULTS

The hyperglycaemic animals showed significant decrease in the glucose level on long term treatment for 15 days model at the doses of 400 and 800 mg/kg (Group-IV, V). Untreated control (Diabetic) group out of 6 animals 3 animals died on 5th day and 3 on the 7th day. The body weight was slightly increased in the normal control rats compared to initial body weight whereas in the diabetic control rats there was a significant decrease in the body weight. Glibenclamide (50 mg/kg) as well as the methanolic extract (400 and 800 mg/kg) treatment significantly prevented this reduction in body weight.

The effect of the extract of *C. hirsutus* on glucose levels in streptozotocin induced diabetic rats is shown in Table 1. The initial blood glucose levels of the diabetic rats selected for the study were in the range of 280 to 350 mg/100 ml on the fourth day. The methanolic extract (400

DISCUSSION

From the above result, we can confirm that the methanolic extract of *Cocculus hirsutus* at doses of 400 and 800 mg/kg possesses significant anti-hyperglycemic activity on long term (15 days) treatment in rats. The anti-hyperglycemic activity of *C. hirsutus* could be due to insulinogenic activity of the extract. It indicates that *C. hirsutus* extract may stimulates insulin secretion from the remnants β cells or/and from regenerated β cells. The drug showed optimum activity at 800 mg/kg.

There will be an increase in body weight after attaining normal glycemic levels in the diabetes, particularly after treatment with sulfonyl urea or insulin or both. Weight gain is not seen normally with the use of metformin or glibenclamide, in part because these anti-diabetic agents do not stimulate the pancreas and do not elevate circulating insulin levels. Treatment with extract of *C. hirsutus*

Table 2. Effects of *Cocculus hirsutus* on oral GTT in STZ induced diabetic rats.

Groups	Treatment	Changes in blood Glucose levels (mg/dl) in mints			
		0	30	60	120
Normal	D/W 10 ml/kg (p.o)	97.16±2.42**	169.83±2.91**	135.83±2.41**	115.33±4.18**
Diabetic control	D/W 10ml/kg (p.o)	432.83±6.94	440.50±6.78	444.67±6.859	436.00±6.71
Positive Control	Glibenclamide (50 mg/kg) p.o	200.00±2.53**	213.33±2.29**	202.0±20436**	213.50±2.363**
Methanolic extract	400 mg/kg p.o	229.87±3.14**	238.63±4.11**	228.83±3.87**	243.8±4.183**
Methanolic extract	800 mg/kg p.o	224.83±2.93**	233.5±3.67**	223.0±3.12**	236.33±4.03**

Values are mean ± SEM, n= 6.

When compared with diabetic control * = p<0.05, **p<0.01 (One way ANNOVA Followed by Dunnette multiple comparison test).

Table 3. Changes in weight of body and testis, level of testosterone in serum and testis after treatment of methanolic extract of *Cocculus hirsutus*.

Groups	Treatment	Body weight (g)		Weight of testes (mg/100 g)	Testosterone	
		Initial	15 th day		Serum (mg/ml)	Testis (mg/g)
Normal	D/W 10 ml/kg p.o	150±0.0	186.67±1.05	1053±32.83	0.59±0.04	3.90±0.15
Methanolic Extract (Lower dose)	400 mg/kg p.o	150±0.0	174.17±2.38**	1227.7±23.46**	4.55±0.17**	8.18±0.20**
Methanolic Extract (Higher dose)	800 mg/kg p.o	150±0.0	181.67±2.47**	1255.5±14.97**	5.00±0.12**	8.90±0.20**

Values are mean ± SEM n = 6.

When compared with normal * = p<0.05, ** = p<0.01 (One way, ANNOVA Followed by Dunnette multiple comparison test).

Table 4. Effect of spermatogenic elements in rats after treatment of methanolic extract of *Cocculus hirsutus*.

Groups	Motility (%)			Sperm count
	Active	Sluggish	Immotile	
Normal	46.16±1.70	26.00±1.65	29.16±0.70	74.83±1.97
Methanolic extract 400 mg/kg (Lower dose)	78.33±1.64**	15.83±1.04**	26.50±0.76	102.83±1.85**
Methanolic extract 800mg/kg (Higher dose)	87.50±1.17**	12.83±0.70**	20.33±0.88**	117.83±3.49**

Values are mean ± SEM n = 6.

When compared with normal * = p<0.05, ** = p<0.01 (One way, ANNOVA Followed by Dunnette multiple comparison test).

induced an increase in the body weight in the diabetic animals, probably due to improvement in insulin secretion and glycemic control. A similar kind of effect, that is, body weight gain was previously reported with other plant such as *Trigonella greacum* known for their anti-diabetic activity (Sheeja, 1995).

In this study, the chronic treatment of the extract for 15 days has increased in the weight of testis, its diameter and seminiferous tubules. There is also a progress in spermatogenesis and increase in *cauda epididymal* sperm count, which may be due to the availability of pituitary FSH during the entire experimental period. The significant increase in the weight of reproductive organs is also indirectly supports the increase availability of androgens. Increased weight and high protein concentration of the testis indicates the enhancement of testicular growth as FSH is essential for protein synthesis in gon-ads (Means et al., 1975).

The experimental result indicated that methanolic extract of *C. hirsutus* exhibited a potent blood glucose lowering property in STZ induced diabetic rats. The exact

mechanism underlying the glucose lowering efficacy of extract is not yet be established. A further exploration of the bioactive molecule responsible for the activity is under investigation in our research laboratory. On the basis of the current investigation it was noted that that the methanolic extract of *C. hirsutus* acted in a similar fashion to glibenclamide (standard drug) and it can be suggested that these results provide pharmacological evidence for its folklore claim as an anti-diabetic agent.

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