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ANTIDIABETIC ACTIVITY OF HYDRO-ALCOHOLIC EXTRACT OF *CISSAMPELOS PAREIRA* LINN. LEAVES IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract

The objective of the study was to investigate the antidiabetic action of hydro-alcoholic extract of *Cissampelos pareira* linn. leaves in streptozotocin induced diabetic rats. The antidiabetic activity of hydro-alcoholic extract of *Cissampelos pareira* linn. leaves was evaluated by using normal and streptozotocin-induced diabetic rats. After the oral administration of hydro-alcoholic extract at doses of 200 mg/kg and 400 mg/kg body weight, blood glucose levels and body weights were monitored at specific intervals. In chronic model of diabetic, hydro-alcoholic extract of *Cissampelos pareira* linn. leaves at a dose of 200 mg/kg, 400 mg/kg and glibenclamide (5 mg/kg) were administered for 21 days. In our study, both glibenclamide and HAACP significantly decreases fasting blood glucose and increases the body weight in streptozotocin induced diabetic rats as compared to the animals in the diabetic control group. The antidiabetic activity of HAACP was comparable to that of standard drug glibenclamide at a dose of 5 mg/kg. Our results suggest that *Cissampelos pareira* linn. leaf have potent antidiabetic property, justifying the use of drug for the treatment of diabetes mellitus.

Keywords: Anti diabetic, *Cissampelos pareira* linn., Hydro-alcoholic extract, Streptozotocin.

Introduction

Diabetes mellitus is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar. Defective insulin secretion is the major cause for chronic hyperglycemia resulting in impaired function or serious

damage to many of the body's systems, like eyes, kidneys, nerves, heart and blood vessels¹. Type-I (IDDM) diabetes is an auto immune disease caused by the destruction of pancreatic islets cells². Whereas, type-II (NIDDM) is due to the combination of Insulin resistance and a loss of secretory function by pancreatic β -cells.

In modern medicine, no satisfactory effective therapy is still available to cure the diabetes mellitus. Though insulin therapy is also used for the management of diabetes mellitus, but there are several drawbacks like insulin resistance, anorexia nervosa, brain atrophy and fatty liver after chronic treatment^{3,4}. Besides the use of insulin for the treatment of insulin dependent diabetes mellitus (IDDM), other approaches for the control of hyperglycemia include the use of amylin analogues. Sulphonylureas, the most widely used class of drugs act by closure of ATP dependent channel. Metformin, a biguanide oral antidiabetic limits intestinal glucose absorption. These drugs have certain effects like causing hypoglycemia at higher doses, liver problems, lactic acidosis and diarrhea⁵. It is apparent that due to the side effects of the currently used drugs, there is a need for a safe agent with minimal adverse effects, which can be taken for long durations. Recently, there has been increasing interest in the use of medicinal plants. The use of medicinal plants in modern medicine suffers from the fact that though hundreds of plants are used in the world to prevent or to cure diseases. Recently search for appropriate antihyperglycemic agent has been focused on plants used in traditional medicine because of leads provided by natural products that may be better treatment than currently used drugs.

Cissampelos pareira Linn. belongs to Menispermaceae family. The plant is a climbing shrub distributed throughout warm parts of Asia, East Africa, and America. A novel tropoloisoquinoline alkaloid named pareirubrine-A was reported for antileukemic activity⁶. Cissamperine and other four bisbenzylisoquinoline alkaloids isolated from *C. Pareira* were found to show significant and reproducible inhibitory activity against human carcinoma of the nasopharynx cell culture⁷. The roots are used as a diuretic and febrifuge, as a remedy for heart trouble, dysentery and soars⁸. Furthermore, the roots are also used to prevent a threatened miscarriage and the herb is used to stop uterine hemorrhage⁹. The plant is judged to be good against muscle relaxant properties, antifertility, dyspepsia, diarrhea,

dropsy and cough, urinary difficulties like cystitis, dysentery, asthma and heart diseases^{10,11,12,13}. In the simplest cases

leaves are good as an antiseptic against inflammation and can be put on wounds in order to heal sores¹⁴.

Materials and Methods:

Animals:

Healthy adult albino wistar rats of either sex weighing 150-200 gms from our breeding stock were used in this study. Animals were maintained under controlled condition of temperature at $27^{\circ} \pm 2^{\circ}$ C and 12 hrs light-dark cycles for one week. They had a free access to standard pellets and water *ad libitum*. The study was conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Gautham College of Pharmacy, Bangalore, Karnataka, India. Registration No. 491/01/c/CPCSEA.

Plant Material:

Fresh leaves of *Cissampelos pareira* Linn. was collected from the forest near to Chittoor district (Andhra Pradesh) and authenticated by Dr. K. Madhava Chetty, Assit. Professor, Dept. of Botany. Sri Venkateswara University, Tirupathi.

Preparation of Extract:¹⁵

About 250 gms of powdered shade dried leaves of *Cissampelos pareira* Linn. was extracted with 70% v/v of ethyl alcohol by continuous extraction in a soxhlet extractor for 24 hours. The extract was concentrated to small volume under reduced pressure and stored in desiccator.

Acute Toxicity Studies:¹⁶

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n=6) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for overnight providing only water, after which the extract were administered orally at the dose level of 100 mg/kg body weight by intragastric tube and observed for 14 days. If mortality was observed in 2 - 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated

again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 200, 500 and 2000 mg/kg body weight.

Induction of Diabetes:¹⁷

The animals were overnight fasted for 16 hrs before the induction of diabetes. Diabetes was induced in rats by intraperitoneal (i.p.) injection of streptozotocin (STZ) at a dose of 50 mg/kg body weight, dissolved in 0.1 M cold citrate buffer (pH= 4.5). After a period of 3 days blood glucose levels were checked by snipping the tail of STZ treated fasted rats. Rats showing the blood glucose levels more than 200 mg/dl is taken into the study. To prevent the hypoglycemia which occurred during the first 24 hrs following the STZ administration, 5% glucose solution was orally given to the diabetic rats.

Experimental Design:¹⁸

Diabetes was induced in fasted Albino wistar rats (150-200 gms) by intraperitoneal injection of 50 mg/kg body weight of STZ except Group-I. After 72 hrs, animals with fasting blood glucose levels higher than 200 mg/dl were selected and used.

Group-I: Animals received distilled water and served as normal control.

Group-II: Animals received distilled water and served as diabetic control.

Group-III: Animals received a dose of 5 mg/kg of Glibenclamide¹⁹ (Ranbaxy, India) p.o. and served as standard.

Group-IV: Animals received a dose of 200 mg/kg of HAECp p.o.

Group-V: Animals received a dose of 400 mg/kg of HAECp p.o.

Fasting blood glucose levels was measured before the administration of extracts. It was recorded as 0th day. The 200 mg/kg and 400 mg/kg doses of the extract along with the standard (Glibenclamide) were given daily to the animals for 21 days. The blood glucose levels were checked on 0th, 7th, 14th, and 21st day of the treatment period. Blood was collected from snipping of the rat tail. Blood glucose levels were measured by using the glucose oxidase-peroxidase reactive strips and a glucometer (Sugarchek, Wockhardt, India).

Statistical Analysis:

The mean \pm S.E.M values were calculated for each group. The data were analyzed using one way ANOVA followed by Dunnett's multiple comparison tests. $P < 0.05$ was considered to be statistically significant.

Results

In acute toxicity study the hydroalcoholic extract of *Cissampelos pareira* Linn. leaves did not produce lethality up to the dose level of 2000mg/kg. In the antidiabetic activity, the effects of hydroalcoholic extract of *Cissampelos pareira* Linn. leaves on body weight is measured on 0th, 7th, 14th and 21st day of post induction and were compared with normal and diabetic control groups. The values are shown in Table No-1. Streptozotocin induced diabetic rats showed a significant decrease ($P < 0.05$) in body weight compared to normal rats. Oral administration of leaf extract at the dose of 200 mg/kg showed a significant increase ($P < 0.01$) in body weight on 21st day and oral administration of leaf extract at the dose of 400 mg/kg showed a significant increase ($P < 0.01$) in body weight on 14th day and very significant increase ($P < 0.001$) in body weight on 21st day of post induction when compared to untreated diabetic rats.

Table-1: Effect of *Cissampelos pareira* Linn. leaves extract on body weight in STZ induced diabetic rats.

Groups	Treatment	Body weight (gms)			
		0 th Day	7 th Day	14 th Day	21 st Day
Group-I	Saline	181.0 \pm 7.53	186.0 \pm 6.807**	189.25 \pm 7.36**	192.75 \pm 7.09***
Group-II	Saline + STZ (50 mg/kg)	173.25 \pm 7.54	159.25 \pm 2.35	153.5 \pm 3.96	150.75 \pm 1.70
Group-III	Glibenclamide (5 mg/kg) + STZ (50 mg/kg)	190.5 \pm 5.86	186.2 \pm 3.32**	191.0 \pm 2.97***	194.25 \pm 3.56***
Group-IV	HAECP (200 mg/kg) + STZ (50 mg/kg)	191.0 \pm 7.56	179.0 \pm 5.43	180.75 \pm 5.55*	184.0 \pm 5.33**

Group-V	HAECP (400 mg/kg) + STZ (50 mg/kg)	183.0 ± 7.03	181.25 ± 6.94*	183.0 ± 6.75**	187.25 ± 5.77***
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Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** represent very significant P<0.001, **represent significant at P<0.01, * represent significant at P<0.05 and ns represents not significant. All the values are compared with the diabetic control group.

The effects of hydroalcoholic extract of *Cissampelos pareira* Linn. Leaves on fasting blood glucose level is measured on 0th, 7th, 14th and 21st day of post induction and compared with normal and diabetic control groups. The values are shown in Table No-2. Streptozotocin induced rats showed a significant increase (P<0.05) in fasting blood glucose level compared to normal rats. Oral administration of leaf extract at the dose of 200 mg/kg body weight showed a significant decrease (P<0.01) in blood glucose level on 21st day of treatment. The fasting blood glucose level on 21st day of post induction was 175.25 ±17.22 mg/dl compared to fasting blood glucose of diabetic control animals 299.75 ±34.78 mg/dl and oral administration of leaf extract at the dose of 400 mg/kg body weight showed a significant decrease (P<0.01) in blood glucose level on 14th day of treatment and very significant decrease (P<0.001) in blood glucose level on 21st day of treatment. The fasting blood glucose level on 14th and 21st days of post induction was 191.75 ±12.57 and 139.75 ±10.42 compared to fasting blood glucose of diabetic control animals 295.5 ±21.61 and 299.75 ±34.78 respectively. The group treated with Glibenclamide 5 mg/kg showed fasting blood glucose level of 125.5 ±13.86 mg/dl on 21st day of post induction.

Table No-2: Effect of *Cissampelos pareira* Linn. leaves extract on blood glucose levels in STZ induced diabetic rats.

Groups	Treatment	Blood glucose levels (mg/dl)			
		0 th day	7 th day	14 th day	21 st day
Group-I	Saline	81.25 ± 6.60	87.75 ± 7.66***	83.0 ± 5.78***	86.0 ± 5.43***
Group-II	Saline + STZ (50	265.5 ± 11.39	293.75 ± 22.87	295.5 ± 21.61	299.75 ± 34.78

	mg/kg)				
Group-III	Glibenclamide (5 mg/kg) + STZ (50 mg/kg)	303.75 ± 22.31	208.5 ± 18.37**	151.25 ± 17.52***	125.5 ± 13.86***
Group-IV	HAECP (200 mg/kg) + STZ (50 mg/kg)	260.0 ± 19.90	250.5 ± 16.15	220.75 ± 16.37*	175.25 ± 17.22**
Group-V	HAECP (400 mg/kg) + STZ (50 mg/kg)	277.25 ± 29.07	213.0 ± 12.02*	191.75 ± 12.57**	139.75 ± 10.42***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett’s test. Where, *** represent very significant P<0.001, **represent significant at P<0.01, * represent significant at P<0.05 and ns represents not significant. All the values are compared with the diabetic control group.

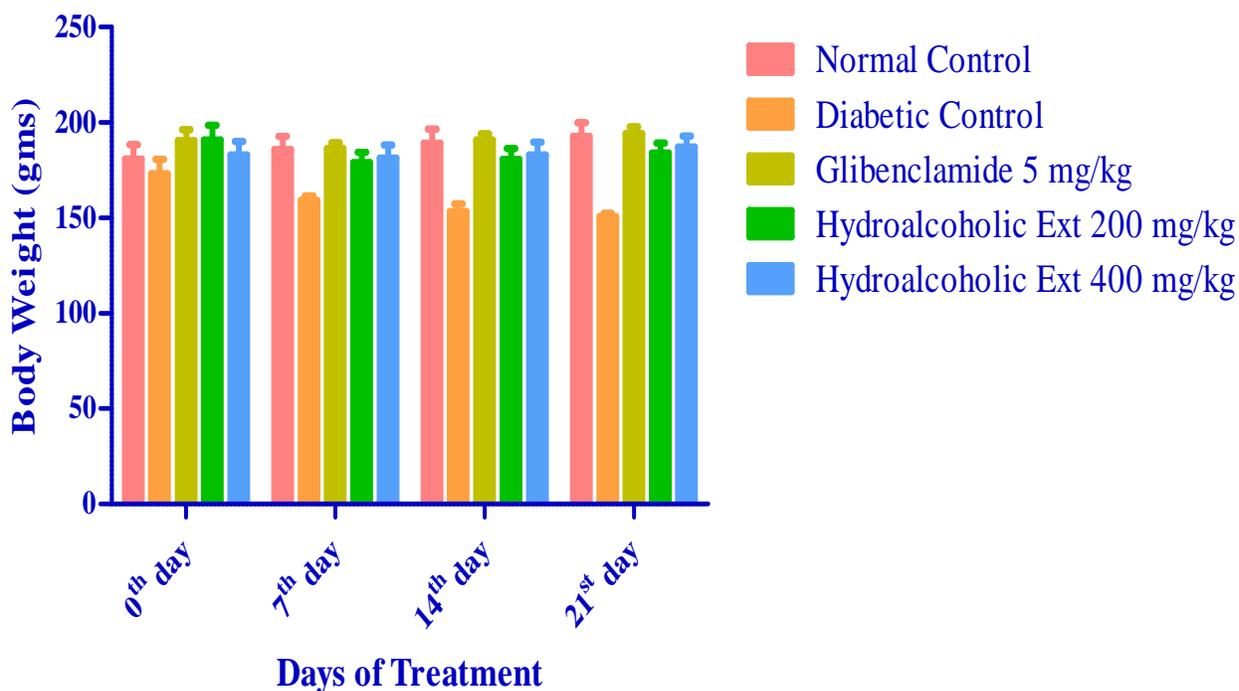


Figure No-1: Effect of *Cissampelos pareira* Linn. leaves extract on body weight of STZ induced diabetic rats.

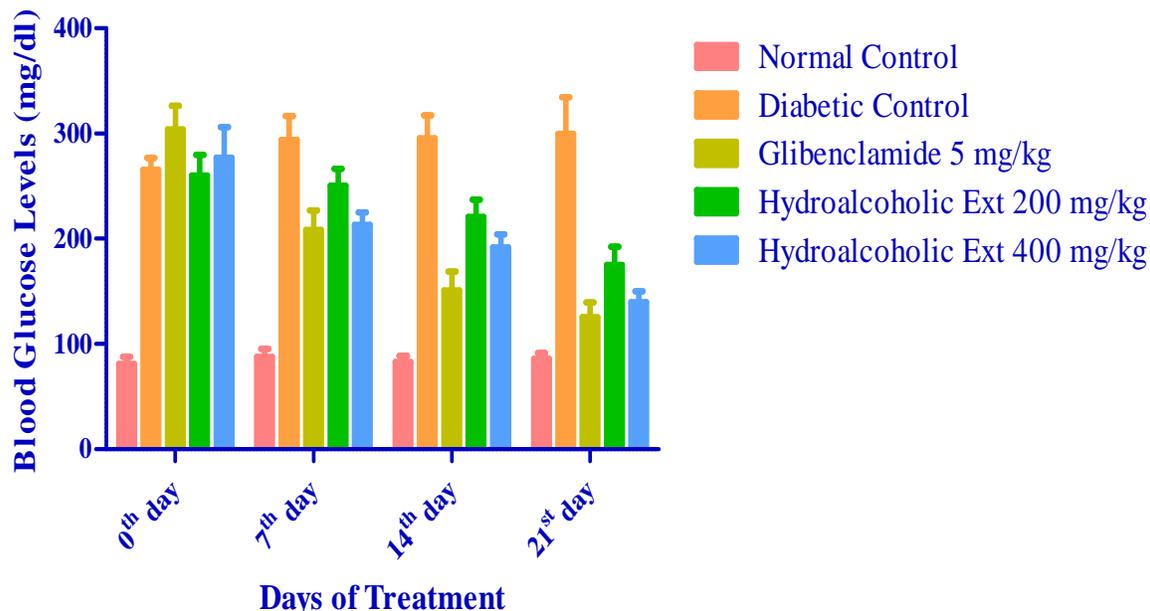


Figure No-2: Effect of *Cissampelos pareira* Linn. leaves extract on blood glucose levels in STZ induced diabetic rats.

Discussion

The antidiabetic effect of herbs has been paid more attention gradually because of increasing incidence of diabetes and predominance of traditional herbs in therapy. The effective components of herbs that have hypoglycemic effect include flavonoids, oligosaccharides, Polysaccharides, alkaloids and organic acids etc²⁰. Streptozotocin induced diabetes has been described as a useful experimental model to study the activity of antidiabetic agents²¹. Streptozotocin induced diabetes is characterized by severe loss in body weight²². Hence, the weight gain after administration of the extract in severely diabetic rats is simply due to the ability of the extract to reduce hyperglycemia. In the present study the antidiabetic activity of hydroalcoholic extract of *Cissampelos pareira* Linn. leaves was evaluated in Streptozotocin induced diabetic rats. The continuous treatment of leaf extract for a period of 21 days produced a significant decrease in blood glucose level in diabetic rats which is comparable to that of standard drug Glibenclamide which is used in treatment of type-II diabetes mellitus.

The standard drug Glibenclamide stimulates insulin secretion from beta cells of islets of langerhans. From the study, it is suggested that the possible mechanism by which the plant extract decreases the blood glucose level may be by potentiation of insulin effect either by increase in pancreatic secretion of insulin from beta cells of islets of langerhans or by increase in peripheral glucose uptake. The above experimental model is quite sensitive and relatively specific to all major classes of oral hypoglycemic drugs including sulfonylureas, biguanides, meglitinide analogues, thiazolidinediones and α -glucosidase inhibitors.

Conclusion

Thus, it may be concluded that *Cissampelos pareira* Linn. produced significant antidiabetic activity in streptozotocin induced diabetic rats. The efficacy of the *Cissampelos pareira* Linn. was comparable to that of Glibenclamide. Further work was necessary to elucidate the mechanism of action involved in the antidiabetic activity of *Cissampelos pareira* Linn. with special references to phytochemicals.

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