Anti-diabetic Activity of *Diplocyclos palmatus* Linn. in Streptozotocin-Induced Diabetic Mice

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ABSTRACT

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The anti-diabetic activity of methanolic extract of seed of *Diplocyclos palmatus* Linn. (Cucurbetaceae) was evaluated in streptozotocin-induced diabetic mice. Methanolic extract of the seed (150 mg/kg body weight), was administered orally to male Swiss albino mice. Streptozotocin was used to induce diabetes mellitus. The anti-diabetic potential was assessed by determining oral glucose tolerance, fasting blood glucose, urine glucose, liver glycogen content, serum lipid profile, change in body weight and histopathology. Methanolic seed extract was administered to normal and experimental diabetic mice for 15 days. Significant ($p < 0.001$) reduction in fasting blood glucose levels was observed in the treated diabetic animals from day 7 onwards. In oral glucose tolerance test, reduction of fasting blood glucose levels was noted after 60 min of extract administration. After 15 days of treatment with extracts the maximum reduction in fasting blood glucose (53.87%) was observed in diabetic mice treated with methanolic extract 150 mg/kg. Serum lipid levels reversed towards near normal and the loss of body weight was controlled in treated mice as compared to diabetic control. The extract treatment also showed a significant increase in the liver glycogen content and gradual decrease in level of urine sugar level. Microscopically examined pancreas section of mice treated with 150 mg/kg methanolic extract showed normal architecture of pancreas. The results demonstrate that seed of *Diplocyclos palmatus* possesses significant anti-diabetic activity. The results suggest that *Diplocyclos palmatus* has anti-diabetic activity, thereby justifying its traditional use. The plant may be used in present day systems of medicine as an anti-diabetic drug.

Keywords: *Diplocyclos palmatus*, Seeds, Streptozotocin, Diabetes.

INTRODUCTION

Diabetes is a chronic metabolic disorder characterized by abnormalities in carbohydrate and lipid metabolism[1], which leads to postprandial and fasting hyperglycemia, dyslipidemia and hyperinsulinemia[2]. Although many synthetic drugs show significant therapeutic potential, their use has already been restricted due to several undesirable side effects such as hepatotoxicity, cardiomegaly and hemotoxicity[3, 4]. Despite the presence of known anti-diabetic medicines in the market, screening for new drugs from plants is still attractive because of their safety and efficacy. A number of plant species are known worldwide to have hypoglycaemic potential[5]. *Diplocyclos palmatus* Linn. (Cucurbetaceae), is annual slender herb. Its leaves are palmately 5-lobed, scabrous along with smooth beneath, denticulate margin. Its peduncle male flowers contain calyx tube 2-4×3-6 mm, spreading lobes; greenish-yellow corolla, shortly papillose, ovate, acute; lobes. Female flowers are fasciculate. The fruits are spherical, yellowish-green, six striped. Its seeds are grey belted, attenuated with raised projections on both faces**.** This species is widely distributed throughout India and globally distributed in tropical and sub tropical region of Asia, Africa and India. Leaves are used in inflammations and impotency[6] and to treat malarial fever and chronic colitis[7]. Fruits contain bitter bryonin and are used in bilious attack, flatulence and inflammation. Its roots with roots of *Michelia champacais* are used in asthma and to promote conception[8]. It has likewise been employed in more recent times in convulsions due to the presence of worms in the intestine, as a cathartic in dropsy, and in cases of chronic inflammations, attended with glandular enlargements, or serous effusions. Literature survey reveals its use as an anti-inflammatory, anti-malarial, anti-ulcer, anti-viral and anti-diabetic agent. However, sufficient scientific data to support these claims are still not available. Therefore, it seemed worthwhile to assess anti-diabetic potential of seeds of *Diplocyclos palmatus*. There is no scientific evidence to support its use as anti-diabetic drug hence, the objective of this study was to establish the scientific basis of the use of seeds of *Diplocyclos palmatus* in the management of diabetes using streptozotocin-induced diabetic mice.

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MATERIAL AND METHODS

Collection of plant materials: Seeds of *Diplocyclos palmatus* were collected from Haridwar Uttrakhand (India) and authenticated by Dr. Anjula Pandey, Principal Scientist at

Herbarium of National Bureau of Plant Genetic Resources (NBPGR) Pusa Campus, New Delhi. Seeds of plant were air dried in the shade and coarsely powdered in a grinder.

Preparation of extract:: The aqueous, methanolic and petroleum ether extracts were prepared by extracting 100 gm of air dried powder in a Soxhlet apparatus (Perfit, India) which were further subjected to successive extraction using petroleum ether (40-60°c), methanol and distilled water. Subsequently, the extracts were filtered; concentrated and dried using Rotary Vacuum Evaporator (Perfit, India) under reduced pressure at ⩽ 50°c temperature (yield 2.88, 3.62, 4.33 % respectively) and the residue was stored in desiccator till subsequent use.

Preliminary phytochemical screening: Preliminary phytochemical screening was carried out according to the method described by Kokate, et al[8].

Animals: Swiss albino mice of either sex, weighing 25-30 g were procured from Indian Toxicological Research Centre, Lucknow. They were housed individually in polypropylene cages, maintained under standard conditions (12 hours light/ 12 hours dark cycle, 25 ±1, 45-55% relative humidity). The animals were fed with standard rat pellet chow (Ashirwad Animal Feed Industries, Punjab, India) and tap water *ad libitum.* All the animals were acclimatized for seven days before the study. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) 1279/ac/09/CPCSEA/05.

Sample collection: After completing the treatment of 2 weeks, the mice were anesthetized by diethylether and sacrificed. Blood samples were collected by cardiac puncture method and intermediately by tail vein method and blood glucose levels were estimated using Dr. Morepen Glucometer (Tai Doc Technology Corporation, Taiwan). For histopathological studies, pancreas and the liver were dissected out immediately and transferred into 10% formalin.

Experimental design: All the animals were randomly divided into the four groups with six animals in each group. Group I, II, III were administered vehicle (10 ml/kg distilled water), diabetic agent {streptozotocin (60 mg/kg, i.p.)} + vehicle (10 ml/kg, p.o. distilled water), standard {glibenclamide (10 mg/kg, p.o.)} respectively. Pilot study was carried out for selection of dose of methanolic extract. Group IV was treated with STZ (streptozotocin) + MESDP (methanolic seeds extract of *Diplocyclos palmatus*). The experimental group was subdivided in such a manner that all sub groups concurrently received STZ (60 mg/kg, i.p.) and either methanolic extract of *Diplocyclos palmatus* seeds (150 mg/kg, p.o.) or the glibenclamide (10 mg/kg, p.o.) or vehicle. The above treatment was given daily for 2 weeks.

Drug solution: The extract was emulsified in 0.5% w/v aqueous solution of tween-80. Glibenclamide (Aventis Pharma Ltd. Goa) was used as a standard drug. Streptozotocin was dissolved in citrate buffer (pH 4.5).

Induction of non-insulin dependent diabetes mellitus (NIDDM): NIDDM was induced in overnight fasted mice weighing 20-30 g by intraperitoneal administration of streptozotocin (Sigma chemical Co. USA) solution prepared in 0.1 M citrate buffer pH = 4.5 at the dose of 60 mg/kg body weight. Diabetes was confirmed by the determination of fasting glucose concentration on the third day post administration of streptozotocin. Blood samples were collected after 1h of administration of streptozotocin on 1st, 4th, 7th, 10th and 15thday. Elevation in blood glucose level was found to be constant throughout 15 days. Serum glucose level was determined by glucometer. Mice having serum glucose level between 300-400 mg/dl were selected for further study.

Oral glucose tolerance test: The oral glucose tolerance test was performed in overnight fasted (18 h) normal mice. Mice divided into three groups, each consisting of six mice were administered distilled water (10 ml/kg), glibenclamide (10 mg/kg, p.o.), MESDP (150 mg/kg, p.o.). Glucose (Central Drug House New Delhi) (2.5 g/kg, p.o.) was fed 0.5 h after the administration of extract. Blood samples were collected by the tail-vein method just prior to the drug administration (normal fasting) and at the time intervals of 0, 30, 60 and 120 m after glucose loading. Blood glucose level was measured immediately by using glucose oxidase-peroxidase reactive strips and a glucometer (Dr. Morepen, Tai Doc Technology Corporation, Taiwan).

Assessment of anti-diabetic activity of methanolic seeds extract of Diplocyclos palmatus: Mice were made diabetic by intraperitoneal administration of streptozotocin at the dose of 60 mg/kg. Treatment with plant extract was started 48 h after streptozotocin injection. Blood samples were withdrawn at three day intervals till the end of study (i.e. 2 weeks).

Effect of methanolic seeds extract of Diplocyclos palmatus (MESDP) on lipid profile: Blood samples were collected by the cardiac puncture method, in the centrifuge tubes and allowed to clot for 30 m at room temperature. Blood samples were centrifuged (R-8C Laboratory centrifuge, Remi India) at 3000 rpm for 20 m. Serum was separated as supernatant and stored at – 20°c until analysis.

determination of triglyceride levels: Triglyceride was estimated by method of Wako and the modifications by McGowan and Fossati method using Accurex, triglyceride determination kit. Working reagent was prepared by dissolving contents of reagent 2 enzymes [lipoprotein lipase, glycerol kinase, glycerol-3-phosphate oxidase, peroxidase, 4-

aminoantipyrine and adenosine tri phosphate (ATP)] into one bottle of reagent 1 buffer (3, 5 dichloro-2-hydroxybenzene sulphonate, pH 7.0). It was swirled to dissolve and allowed to stand for 10 m at room temperature. Serum triglycerides were hydrolyzed to glycerol and fatty acids by lipase enzyme. In the presence of ATP and glycerol-kinase, glycerol was converted into glycerol-3-phosphate and adenosine di phosphate (ADP). Glycerol-3-PO_4 oxidase dissociates glycerol-3-phosphate into dihydroxy-acetone phosphate and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with 4-aminoantipyrine to form a coloured complex. The intensity of the colour developed was proportional to the triglycerides concentration and was measured photometrically at 505 nm. The instrument was adjusted to zero with distilled water. 10µl sample was taken with the help of pipette into a cuvette. It was mixed and incubated for 5 m. at 37°c . The absorbance of the samples and standard of 200 mg/dl concentration was read out against the blank. The colour should be stable for 30 m. Triglyceride was estimated by using the following formula:

$$\text{Triglyceride}\left(\frac{\text{Mg}}{\text{dl}}\right) = \frac{\text{Absorbance of Samples}}{\text{Absorbance of standard}} \times \text{Conc. of standard (mg/dl)}$$

estimation of total serum cholesterol (STC): Total cholesterol was estimated by CHOD-POD enzymatic colorimetric (Photoelectric colorimeter-114, Syntronics Ahmedabad) end point method[9] using Accurex, cholesterol determination kit. For this method, 0.01 ml each of serum as a test, standard sample and distilled water as blank along with 1.00 ml reaction solution were pipetted into the reaction vessels using a micropipette.

estimation of serum lipids: Phosphotungstate method[10] was used to estimate the serum lipids like very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol level. The clear supernatant after removal of VLDL and LDL, containing HDL was used for determination of HDL-Cholesterol (HDLc). The VLDL and LDL from serum were precipitated by phosphotungstate in the presence of magnesium ions.

$$\text{Serum/Plasma} \xrightarrow{\text{Phosphotungustate,mg}^{2*}} \text{HDL+(LDL+VLDL+Chylomicrons)}$$

VLDL-Cholesterol (VLDLc) and LDL-Cholesterol (LDLc) were respectively calculated by using Frederickson-Friedwald's[11] formula as follows:

$$\text{LDL Cholesterol} = \text{Total Cholesterol} - \frac{\text{Trigyceride}}{5} - \text{HDL Cholesterol}$$

$$\text{VDDL Cholesterol} - \frac{\text{Trigyceride}}{5}$$

For this 0.5 ml of serum was taken in a test tube and 0.5 ml of precipitation reagent was added. The mixture was shaken thoroughly and left to stand for 10 m at 25 to 30°c and then centrifuged for 20 m at 4000 rpm. Within 2 h after centrifugation, the clear supernatant was used for the determination of HDL-Cholesterol. The supernatant containing 0.05 ml was taken in a test tube and 1 ml reaction solution was added to it. In another test tube, 0.1 ml distilled water was taken and 1 ml reaction solution was added.

The mixtures were mixed thoroughly, incubated for 5 m at 37°c and measured for the absorbance of the sample against blank reagent at 510 nm in Biochemistry Auto analyzer (STAR 21 plus, RAPID Diagnostic Pvt. Ltd.).

Change in body weight: Body weight was taken before and after experiment at the intervals of 1st, 4th, 7th, 10th and 15th day of study with the help of single pan balance. The change in the body weight was noted.

Urine glucose estimation: The urine glucose levels were estimated by "Benedict's (Rankem, RFCL Ltd. New Delhi) test for glucose as reducing sugar in urine method". The urine was collected from the STZ-induced diabetic mice individually in a clean beaker on the day 1, 4, 7, 10 and 15 and the glucose level was determined.

Liver glycogen estimation: The determination of glycogen in liver was done by solution of "anthrone reagent[12]". Purified anthrone (500 mg), thiourea (10 g) and 1 liter of 72 % sulfuric acid were placed in a flask. The mixture was heated up to 80-90°c . The flask was occasionally shaken to mix the contents. The mixture was cooled and stored in a refrigerator. Stock solution of standard was prepared by dissolving 100 mg of dry glucose in 100 ml of saturated benzoic acid solution. 5 ml stock solution was placed in a 100 ml volumetric flask and the volume was made up with saturated benzoic acid solution.

Liver was blended by blender under trichloroacetic acid (TCA) and homogenized for 3 m. The homogenate was poured into a centrifuge tube. The supernatant fluid was centrifuged and decanted upon an acid-washed filter paper placed in a funnel and drained into a graduated cylinder. The residue was quantitatively transferred to the blender with TCA and homogenized again for 1 m. The mixture was centrifuged and the supernatant fluid was poured through the same filter. Two more extractions were made in the same manner. The desired volume was made up with 5 percent TCA and the solution was mixed thoroughly. 1 ml of the TCA filtrate was pipetted into a 15 ml Pyrex centrifuge tube. Duplicate samples of each unknown were analyzed to obtain

the most reliable results. To each tube, 5 volumes of 95 percent ethanol were added with careful blowing. This was checked by noting the absence of an interface. The tubes were capped with clean rubber stoppers and allowed to stand overnight at room temperature. After precipitation was completed, the tubes were centrifuged at 3000 r.p.m. for 15 m. The clear liquid was gently decanted from the packed glycogen and the tubes were allowed to drain in an inverted position for 10 m.

The glycogen was dissolved by adding 2 ml of distilled water, the water being added in a manner that was washed down the sides of the tube. Blank reagent was prepared by pipetting 2 ml of water into a clean centrifuge tube. A standard was prepared by pipetting 2 ml of standard glucose solution, containing 0.1 mg of glucose, into a similar tube. At this point 10 ml of anthrone reagent was delivered into the centre of the each tube with vigorous, but consistent, blowing to ensure good mixing. As each tube received anthrone reagent, it was tightly capped with an air condenser and placed in a cold tap water bath. After the temperature of all tubes had reached the temperature of cold water, they were immersed in a boiling water bath to a depth a little above the level of the liquid in the tubes for 15 m and then removed from water bath and cooled to room temperature. The tubes and stoppers were wiped dry and the contents of each tube were transferred to a calorimeter tube and the absorbance was read at 620 nm after adjusting the calorimeter with the blank reagent. Care was taken to avoid introduction of lint or contaminating carbohydrate into the anthrone reaction. The calculation of glycogen content was done by using the following formula[13].

$$\frac{DU}{DS} \times 0.1 \times \frac{\text{Volume of Extract}}{\text{100gm. of Tissue}} \times 100 \times 0.9 = \text{mg. of glycogen per 100gm. of tissue}$$

Where, DU = Optical density of the unknown, DS = Optical density of the standard, 0.1 = mg of glucose in 2 ml of standard solution, 0.9 = factor for converting glucose value to glycogen value.

Histopathological studies: Isolated pancreas was preserved in 10 % formalin for 24 h. Pancreas was fixed in Bouin's fluid and cut in section of 3–5 μm thickness and stained by hematoxyline-eosin stain. The photomicrographs of each tissue section were taken using electron microscope.

Statistical analysis: Values are presented as mean ± standard deviation for groups of six animals. The results were analyzed by one way analysis of variance (ANOVA) followed by post hoc Dunnett's multiple comparison test. Differences between means were considered to be statistically significant at ($p \leq 0.05$).

RESULTS

Phytochemical testing: Preliminary phytochemical screening revealed that saponins were present in all extracts while alkaloid, carbohydrates, flavanoids, triterpenoids and phenolic compounds were present in methanolic extract. Amino acids were present only in aqueous extract. Fat and fixed oils were found only in petroleum extract.

Fasting blood glucose determination: The effect of treatment of the extracts on fasting blood glucose levels is depicted in Table No. 1. Glibenclamide (GBC) treated diabetic mice of standard group III showed significant reduction in blood glucose values on day 1, 4, 7, 10 and 15 respectively in comparison to diabetic control group II. This indicated that the GBC treatment successfully reduced the blood glucose levels in the diabetic mice towards the normal level in 15 days. Similarly, MESDP treated diabetic group IV showed significant reduction in blood glucose values on day 1, 4, 7, 10 and 15 respectively as compared with diabetic control group II. This indicated that the MESDP treatment could reduce the blood glucose levels in the diabetic mice towards the normal level in the 15 days of study.

Table 1: Effect of MESDP on fasting blood glucose level in diabetic mice

Groups	Treatment	Fasting blood glucose level(mg/dl)				
		Day 1	Day 4	Day 7	Day 10	Day 15
I	Distilled water (10 ml/kg, p.o.)	120.83± 12	113.16± 9.19	107± 15.53	116.83± 16.26	118.83± 14.02
II	STZ (60 mg/kg, i.p.) + distilled water (10 ml/kg, p.o.)	312.67± 34.46	325.17± 40.93	326.4± 37.48	308.5± 51.86	305± 47
III	STZ (60 mg/kg, i.p.) + GBC (10 mg/kg, p.o.)	363.5± 35.55**	215± 30.96***	168.17± 40.65***	150.5± 27.04***	129.33± 19.21***
IV	STZ (60 mg/kg, i.p.) + MESDP (150 mg/kg, p.o.)	320.67± 28.82^{ns}	264.17± 27.20**	202.17± 29.52***	171.67± 17.60***	140.67± 21.79***

***p< 0.001 vs. diabetic control group II, **p< 0.01 vs. diabetic control group II, MESDP= methanolic extract of *Diplocyclos palmatus* seed, GBC= glibenclamide.

Biochemical parameters

oral glucose tolerance test (OGTT) in normal mice: Treatment with GBC significantly improved the glucose tolerance at normal fasting levels at 0, 30, 60 and 120 m, respectively. Further, treatment with MESDP significantly reduced sugar glucose level at 120 m compared to normal control. These data suggested that treatment with MESDP showed tolerance to glucose administration (Table No. 2).

***urine glucose estimation*:** The urine sugar levels in normal and diabetic group of mice are given in Table No. 3**.** The normal control mice showed absence of sugar in urine. The urine sugar levels of the different groups of diabetic animals treated with standard drug (glibenclamide) and MESDP for 15 days decreased towards the normal level.

estimation of liver glycogen content: There was significant increase in liver glycogen level to 473.25 ± 64.91 ($p< 0.001$) on day 15 in glibenclamide treated diabetic control group III. Similarly MESDP treatment significantly ($p< 0.001$) increased the glycogen content to 383.85 ± 42.96 ($p< 0.001$) in STZ-induced diabetic group IV (Table No.4).

estimation of lipid profile parameters: MESDP exhibited significant reduction ($p< 0.001$) in all tested lipid parameters. A marked increase in total cholesterol and decrease in HDLc were observed in untreated diabetic mice. MESDP administration decreased serum triglycerides (STG); total cholesterol (STC), LDL and VLDL levels and increased HDLc level. The markers of dyslipidemia such as STC/HDLc and LDLc/HDLc ratios were found to be significantly elevated in the diabetic group (Table No. 5).

Table 4: Effect of MESDP on liver glycogen content in STZ-induced diabetic mice

Groups	Treatment	Liver glycogen content on day 15 (mg/100 gm)
I Normal control	Distilled water (10 ml/kg, p.o.)	542.86 ± 31.64
II Diabetic control	STZ (60 mg/kg, i.p.) + Distiled water (10 ml/kg p.o)	263.95 ± 8.62
III Standard	GBC (10 mg/kg, p.o.)	473.25 ± 64.91***
IV Treated	MESDP(150 mg/kg, p.o.)	383.85 ± 42.96***

*** p< 0.001 compared to diabetic control, MESDP= methanolic extract of *Diplocyclos palmatus* seed, STZ= streptozotocine, GBC= glibenclamide

Table 2: Effect of MESDP on OGTT in normal mice

Groups	Treatments	Normal fasting values(mg/dl)	Blood glucose concentration(mg/dl)			
			0 m	30 m	60 m	120 m
I	Glucose(2.5 g/kg)	124.66 ± 5.92	269.83± 3.43	373.66± 4.08	250.33± 4.5	174.5± 4.5
II	GBC(10 mg/kg)	104.83 ± 2.31***	181.83± 5.07***	237.5 ± 2.73***	165.16± 3.31***	147± 2.82***
III	MESDP (150 mg/kg)	108.67 ± 4.93***	190.33± 4.13***	266.67± 4.55***	186.5± 5.43***	156.17± 8.30***

***p<0.001 vs. normal control, MESDP= methanolic extract of *Diplocyclos palmatus* seed, OGTT= oral glucose tolerance test, GBC= glibenclamide

Table 3: Effect of MESDP on urine glucose level in STZ-induced diabetic mice

Groups	Groups Treatments	Intensity of glucose in urine (colour change of the precipitate)				
		1 (days)	4 (days)	7 (days)	10 (days)	15 (days)
I Normal control	Distilled water(10 ml/kg, p.o.)	Nil	Nil	Nil	Nil	Nil
II Diabetic control	STZ (60 mg/kg, i.p.) + Distilled water(10 ml/kg p.o)	+++	+++	+++	+++	++++
III Standard	GBC (10 mg/kg, p.o.)	+++	++	++	+	+
IV MESDP	MESDP(150 mg/kg, p.o.)	+++	+++	+++	++	+

Keys: (+) = mild, (++) = moderate, (+++) = higher, (++++) = severe, MESDP = methanolic extract of Diplocyclos palmatus seed, STZ = streptozotocine, GBC= glibenclamide.

Table 5: Effect of MESDP on lipid profile in STZ- induced diabetic mice

Treatment groups	STG (mg/dl)	STC (mg/dl)	HDLc (mg/dl)	LDLc (mg/dl)	VLDLc (mg/dl)	STC/HDLc ratio	LDLc/HDLc ratio
I Normal control	90.67± 4.5	82.33± 3.79	38.87± 1.02	25.33± 2.27	18.13± 0.9	2.11± 0.06	0.67± 0.02
II Diabetic control	132.67± 4.16	157± 5.29	18.67± 1.52	111.8± 6.6	26.53± 0.83	8.45± 0.89	6.03± 0.8
III MESDP (150 mg/kg)	117± 2.64*	133.33± 5.51*	33.67± 3.05**	76.27± 4**	23.4± 0.52*	3.97± 0.23**	2.27± 0.19**

*p< 0.05, **p< 0.01 compared to diabetic control, MESDP= methanolic extract of *Diplocyclos palmatus* seed, STZ= Streptozotocine, STG =total serum triglyceride, STC=Total serum cholesterol**,** HDLc **=** high density lipoprotein cholesterol**,** LDLc= low density lipoprotein cholesterol**.**

Change in body weight: The body weight was slightly increased (28 ± 1.22 g) in the normal control group I as compared to initial body weight. Whereas in diabetic control group II, there was marked decrease (24.5 ± 0.5 g) in the body weight. Group III treated glibenclamide and the group IV pretreated with MESDP increased body weight significantly to 27.5 ± 1 g ($p< 0.01$) and 27.58 ± 0.58 g ($p< 0.05$) respectively. Although there was a marginal reduction in the body weight of animals in these groups, compared to the final weight of normal control mice (Table No.6).

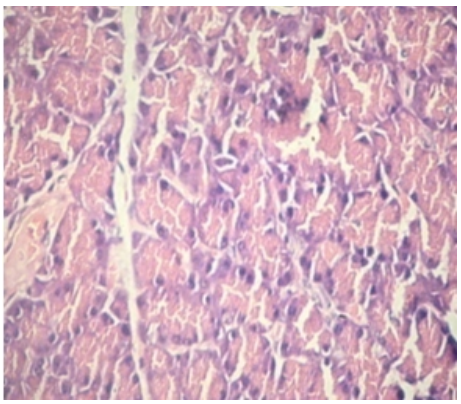
Histopathology: Photomicrographs showed normal architecture of pancreas with acini of serous epithelial cells along with nest of endocrine cells separated by fibrocollagenous, stroma into lobules of vehicle-treated mice (Fig.1a). Extensive damage to the acini of serous epithelial cells and islets of langerhans (Fig.1b), restoration of normal architecture of pancreas with acini of serous epithelial cells by glibenclamide (Fig.1c) are also shown. The partial restoration of normal cellular population and normal architecture of pancreas with acini of serous epithelial cells along with nest of endocrine cells separated by fibrocollagenous, stroma into

Table 6: Effect of MESDP on body weight (gm) in STZ-induced diabetic mice

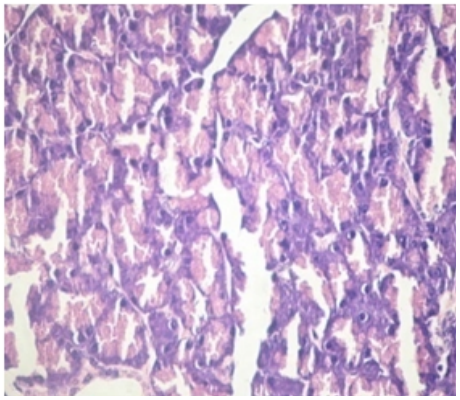
Groups	Treatments	Body weight(g)	
		Day 1	Day 15
INormal Control	Distilled water(10 ml/kg, p.o.)	26.83 ± 0.98	28 ± 1.22
II Diabetic Control	STZ (60 mg/kg, i.p.)+ Distiled water(10 ml/kg p.o)	27.91 ± 0.66	24.5 ± 0.5
III Standard	GBC (10 mg/kg, p.o.)	28.91 ± 0.86	27.5 ± 1**
IV Treated	MESDP(150 mg/kg, p.o.)	29.08 ± 0.92	27.58 ± 0.58*

**p< 0.01,*p< 0.05 compared to diabetic control, MESDP = methanolic extract of *Diplocyclos palmatus* seed, STZ= streptozotocine GBC= glibenclamide

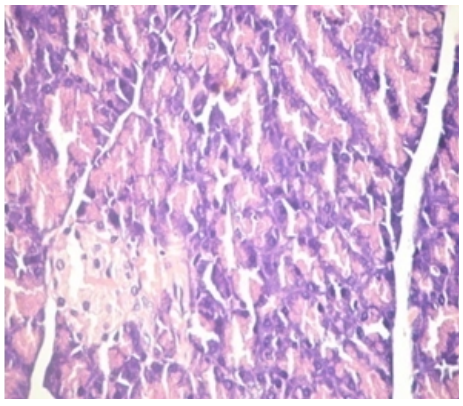
Fig.1: Histological study of pancreas of isletes of Langerhans



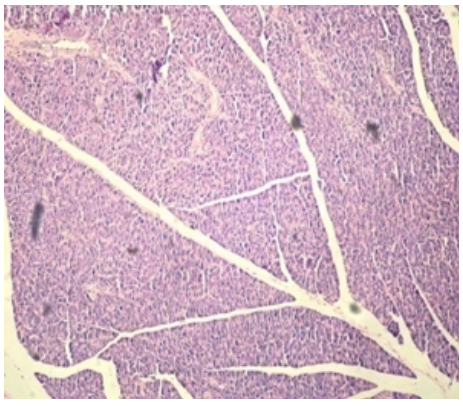
a. Control: Distilled water (10 ml/kg, p.o)



b. Diabetic control: STZ (60 mg/kg, i.p.)



c. Standard: Glibenclamide (10 mg/kg, p.o)



d. Treated: MESDP (150mg/kg, p.o)

lobules was shown by methanol extract. No fibrosis or inflammation was noted (Fig. 1d).

DISCUSSION

The aim of present study was to investigate the anti-diabetic potential of methanolic extract of seed of *Diplocyclos palmatus* (MESDP), using STZ-induced diabetic mice model. Hyperglycaemia produced by STZ exhibited marked increase in serum triglycerides and total cholesterol. Under normal conditions, the enzyme lipoprotein lipase hydrolyses triglycerides. Diabetes mellitus results in failure to activate this enzyme thereby causing hypertriglyceridemia. Elevated serum total cholesterol, triglycerides and decreased high density lipoprotein level were observed in diabetic control mice. Chronic administration of the extract for 15 days to the STZ-induced diabetic mice significantly ($p < 0.05$) produced a fall in blood glucose level and lipid profile. Hence the methanolic extract may be considered to have good anti-hyperglycemic activity and did not cause any hypoglycemic effect unlike insulin and other synthetic drugs. Normalization of the blood glucose level resulted in significant reduction in the level of serum cholesterol and triglycerides. The anti-hyperglycemic activity caused by glibenclamide and MESDP in streptozotocin-induced diabetic mice indicates normalization of serum lipid profile and stimulation of insulin secretion from beta cells. The observed hypolipidaemic effect may be because of decreased cholesterogenesis and fatty acid synthesis. Significant lowering of total cholesterol and elevation of HDL cholesterol are very desirable biochemical states for prevention of atherosclerosis and ischemic conditions.

In diabetic control group, the characteristic loss of body weight is caused by an increase in muscle wasting and loss of tissue proteins[14]. The difference in the body weight observed during the period of treatment of the mice treated with MESDP was less as compared to the diabetic control group, which may be due to its protective effect in controlling muscle wasting, i.e. reversal of gluconeogenesis and may also be due to proper glycemic control. Based upon these results it can be hypothesized that MESDP probably act by releasing insulin from pancreatic β cells. The hypothesis is further supported by the pancreatic histology which showed protection of pancreatic β cells from toxic effect of STZ. The difference observed between the initial and final fasting blood glucose levels of different groups under investigation revealed a significant elevation in blood glucose in diabetic control group at the end of the 15th day experimental period. When MESDP was administered to glucose loaded normal mice fasted for 18 h, reduction in blood glucose levels was observed after 60 m. The decline in the level of blood glucose reached its maximum at 120 m. Administration of the extract to diabetic mice showed a significant decrease in the fasting blood glucose. Hence, the possible mechanism of anti-hyperglycemic action of MESDP is the potentiation of the insulin effects of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form.

The decrease in hepatic glycogen content in diabetes is probably due to lack of insulin in the diabetic state which results in the inactivation of glycogen synthase enzyme[15]. The significant increase in the glycogen content of the treated groups may be because of reactivation of the glycogen synthase enzyme. Hence, improvement of glycogenesis may be another probable way of anti-diabetic action[16]. The anti-hyperglycemic activity caused by glibenclamide and MESDP in streptozotocin-induced diabetic mice indicates normalization of serum lipid profile and stimulation of insulin secretion from beta cells. Flavonoids, sterols/triterpenoids, alkaloids and phenolic compounds are known to be bioactive anti-diabetic principles[17]. Flavonoids are known to regenerate the damaged beta cells in the alloxan-induced diabetes in rats[18]. Phenolic compounds are found to be effective anti-hyperglycemic agents[19]. The anti-diabetic effect of MESDP may be due to the presence of more than one anti-hyperglycemic constituent and their synergistic properties.

CONCLUSION

It is thus concluded that *Diplocyclos palmatus* (MESDP) has promising anti-diabetic effect, which potentially improved abnormalities of diabetic conditions in streptozotocin-induced diabetic mice. The probable hypoglycemic effect of MESDP may be attributed to increase in serum and pancreatic insulin levels. However, longer duration studies on chronic models are required to elucidate the exact anti-diabetic mechanism of action. As well as there is a need to isolate bioactive principles which can be developed as potent anti-diabetic drug.

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