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Original Article

Antidiabetic Activity of an Alkaloid (4a-Methyl-5-(6-Methylhept-5-En-1-Yl)Octahydro-1*H*-Cyclopenta[*A*]Pyridazine) Isolated From *Lumnitzera racemosa* in Streptozotocin-Induced Diabetic Wistar Rats

Ranjana[1,*], Deepak Kumar Singh[2], Bhaskar Laxman Jadhav[1]
[1]Department of Life Sciences, University of Mumbai, Vidyanagari Campus, Santacruz (East), Mumbai, Maharashtra, INDIA.
[2]University School of Biotechnology, Guru Gobind Singh Indraprastha University, Dwarka, New Delhi, INDIA.

ABSTRACT

Background: Diabetes mellitus is a metabolic disorder that causes millions of deaths throughout the world every year, and today also its incidence is on the increase. Many antidiabetic drugs are available in the pharmaceutical market, but drawbacks related to them had forced the researcher's attention to switch towards naturopathy. The study aimed to isolate and evaluate the antidiabetic principle from the mangrove plant *Lumnitzera racemosa* leaves. **Materials and Methods:** The active principle was isolated using column chromatography and identified with high-performance liquid chromatography, and further structure elucidation was done by FT-IR, LCMS, NMR, and elemental analysis. The antidiabetic activity of that isolated compound was monitored using *in vitro* α-amylase and α-glucosidase inhibition and *in vivo* STZ-induced diabetic rat models. **Results:** The isolated compound showed potent antidiabetic activity by inhibiting α-amylase and α-glucosidase with IC_{50} values of 30.23 and 0.022 mg/ml, respectively. Furthermore, the isolated compound administration (250 and 500 mg/ml BW) in STZ-induced diabetic rats has exhibited a significant dose-dependent decrease in blood glucose levels. Besides this, the haematological findings, biochemical, and histopathology of the isolated compound showed comparable results to that of standard glibenclamide, indicating the protective role of the compound against any damage to the pancreas, liver and kidney. HPLC and different spectroscopic analyses revealed that the isolated compound is 4a-methyl-5-(6-methylhept-5-en-1-yl)octahydro-1*H*-cyclopenta[*a*] pyridazine, which belongs to the alkaloid class of the secondary metabolites. **Conclusion:** Data obtained states that the alkaloid isolated from *L. racemosa* leaves possess significant antidiabetic activity in both *in vitro* and *in vivo* models.

Keywords: Antihyperglycemic, *In vivo*, Mangroves, Secondary metabolite.

Correspondence

Dr. Ranjana
Department of Life Sciences, University of Mumbai, Vidyanagari Campus, Santacruz (East)-400098, Mumbai, Maharashtra, INDIA.

Email id: ranjanaram987@gmail.com

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INTRODUCTION

Diabetes is one of the most common endocrine metabolic disorders, which results in significant morbidity and mortality because of micro-vascular and macro-vascular complications. Mainly there are two types of diabetes, viz. type 1 and type 2. In type 1 (Insulin-dependent diabetes mellitus), which accounts for 5% to 10% of total diabetes cases, the body does not produce any insulin, so the patients need daily insulin injections to stay alive. While in type 2 (Non-insulin dependent diabetes mellitus), which accounts for 90% to 95% of total diabetes cases, either the body fails to produce enough insulin or is unable to use it properly.

It is the fourth leading cause of death in most developed countries.[1] As per the 10th edition of IDF Diabetes Atlas, 2021, approximately 537 million people have diabetes and it is expected to reach 643 and 783 million by 2030 and 2045, respectively. Strategies employed for the management of diabetes are targeted toward different oral hypoglycemic drugs. However, there is growing evidence of adverse effects such as weight gain, flatulence, diarrhoea, gastrointestinal disturbances, and abdominal bloating associated with various antidiabetic drugs.[2] This prevailing condition has forced researchers to search for natural antidiabetic compounds with less or no toxicity from plant sources. World Health Organisation expert committee on diabetes also suggested that medicinal plants can be further investigated as they are repetitively considered less toxic. Indeed, experimental proofs have demonstrated that medicinal plants possess chemicals with antidiabetic activity, which is not restricted to a particular class of phytochemicals.[3-4] There are diverse classes of secondary metabolites that have proved to possess potent antidiabetic activity like flavonoids,[5] saponin,[6] sterol/triterpenoids,[7] alkaloids.[8] *Lumnitzera racemosa* (*L. racemosa*) is an Indo-West Pacific genus in the family Combretaceae. It is a small to a medium-sized evergreen tree, growing to a maximum of 37 m (121 ft) in height. The leaves are arranged spirally at the shoot tips, they are simple and ovate, with slightly toothed margins. This plant has antibacterial,[9] hepatoprotective, antioxidant,[10] cytotoxic,[11] and antidiabetic activities.[12] Based on our *in vitro* antidiabetic activity of *L. racemosa* leaves,[12] the present study's main objective was to isolate the antidiabetic principle from *L. racemosa* leaves and to check its antidiabetic efficacy *in vivo*.

MATERIALS AND METHODS

Plant Material

The whole plant of *L. racemosa* was collected in May from the Bhatye beach area located at Ratnagiri coast, Maharashtra (Co-ordinates: 16°58'44.0691"N, 73°17'38.7499"E), India, and identified by an expert taxonomist (voucher specimen bearing reference no. NIO/DOD/DIO-1466). The leaves were washed thoroughly under running tap water to free them from dust and other contaminants, oven-dried at 40°C to

remove the moisture content, ground, and resultant powder was sieved through a muslin cloth.

Extraction and Fractionation

The leaves powder (500 g) was extracted with hexane (620 ml) at 50°C for 4 h. The extract was evaporated to dryness (14.5 g) and further reconstituted in hexane followed by column chromatography (300 mm x 15 mm) on silica gel (60-120 mesh, supplied by Merck). The column was plugged with cotton at the blunt end and filled with hexane. The lower end of the column was kept open, silica gel was added from the top, and simultaneously hexane fractions were collected from down the column continuously. It was successively eluted with a stepwise gradient of methanol (50 ml) and chloroform (80 ml). A total of 38 fractions were collected. The eluent 1-16 showing similar compounds in it, therefore, pooled together and designated as hexane fractions. Similarly, the eluent 17-29 and 30-38 are designated as methanol and chloroform fractions respectively. The purity of the isolate (potent fraction in the *in vitro* antidiabetic study) was detected by HPLC analysis.

HPLC Analysis and Characterization

The separation of the antidiabetic principle was performed with a Nova-Pak C18 Prep Column, 60Å, 5 µm, 250 mm × 4.6 mm at a column temperature of 30°C with mobile phase Acetonitrile: Water (50:50) with isocratic elution at a flow-rate 1mL/min, injection volume was 10µL, and UV detection at λ 254nm was carried out.

The compound was further characterized by spectroscopy techniques like FT-IR using KBr pellets on a SHIMADZU instrument, LC-MS (Phenomenex, Luna C_{18}), CHN Analyzer (Thermo Finnigan), and ^{1}H-NMR spectra were recorded in $CdCl_3$ on a JNM model, Jeol 400 MHz.

In vitro Antidiabetic activity

All three fractions were tested for their *in vitro* antidiabetic activity, which was estimated by α-amylase and α-glucosidase inhibition assays by following the method mentioned in our previous report.[12]

Animal Experiments

Wistar rats were procured from *in vivo* Biosciences, Karnataka, India. The animals were housed in a standard polypropylene cage size Length x Breadth x Height (430 × 270 × 150 mm) under standard laboratory conditions. The room temperature was maintained between 20.2°C to 22.8°C and the relative humidity at 45-69 %, with 12hr light and 12hr dark cycle. They were fed with a standard rodent pellet diet (Pranav Agro Industries Limited, Sangli, Maharashtra) and water *ad libitum* throughout the acclimatization period and study period.

Acute toxicity study

Acute oral toxicity of compounds was performed on Wistar rats according to OECD (2001) (Organization of Economic Cooperation and Development) Guideline 407.

Induction of Diabetes in rats

Rats were fasted overnight and injected intraperitoneally with a freshly prepared solution of streptozotocin (STZ, Sigma Aldrich, USA) dissolved in 0.1 M citrate buffer (pH 4.5) at a dose of 60 mg/kg body weight to induce diabetes. Streptozotocin-injected rats were provided to drink 5% glucose solution overnight in order to overcome hypoglycemic mortality. Blood glucose level was checked using a Glucometer (ACCu-Chek active, Roche, Diagnostics USA) by glucose oxidase-peroxidase method using strips, 72 hr after being injected with streptozotocin. The rats with fasting blood glucose above 200 mg/kg were enrolled in the study.

Experimental Design

Group I: Animals were treated as normal control

Group II: Animals were treated as a positive control, Glibenclamide (5 mg/kg BW) subcutaneously.

Group III: Animals orally received test sample (250 mg/kg BW)

Group IV: Animals orally received test sample (500 mg/kg BW)

All the animals in the group received the treatment by the above schedule for 14 days. Blood samples were collected by the End tail vein cutting method one hour after drug administration to determine blood glucose levels. At the end of the experimental period, the animals fasted overnight, and blood was collected for various haematological and biochemical parameters.

Haematological Analysis

Blood samples were collected by retro-orbital puncture technique using a capillary tube containing disodium ethylene diamine tetraacetate (an anti-coagulant). The haematological parameters evaluated are red blood corpuscles (RBCs) count, white blood corpuscles (WBCs) count, Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), neutrophils, lymphocytes, monocytes using an automated hematology analyser (Sysmex KX-21, Japan).

Biochemical Analysis

Biochemical parameters such as serum lipid profiles like total cholesterol, triglycerides, and high-density lipoprotein (HDL-C), were estimated using commercially available kits (Agappe diagnostics kit). Low-density lipoprotein (LDL-C) and very-low-density lipoprotein (VLDL-C) levels were calculated by the Friedewald formula: VLDL= TG/5; LDL= TC-(HDL+VLDL).[14] Renal function tests, including blood urea nitrogen (BUN), urea, and creatinine, were estimated using a commercial kit (Agappe diagnostic kit). Liver function test viz. aspartate aminotransferase (AST), alanine aminotransferase (ALT),[13] alkaline phosphatase (ALP),[14] creatinine kinase, total protein, globulin, and albumin were also analyzed by using a commercial kit (Agappe diagnostic kit).

Histopathological Studies

At the end of the experiments, the kidney, liver, and pancreas from all experimental groups of rats were subjected to histopathological studies. They were cut into small pieces (1mm x 1mm x 1mm), preserved in 10% normal saline for 48h, followed by dehydration by passing successively in different mixtures of ethyl alcohol-water (50%, 80%, 95%), and final incubation in alcohol. It was cleared in xylene and embedded in paraffin. In order to perform a microscopic study of cells, samples were first cut down into ultra-thin sections by Ultra-Microtome, then stained with hematoxylin and eosin dye, followed by mounting in neutral deparaffinated xylene (DPX) medium. The Micrographs (Canon10.1megapixel digital camera, Japan) were captured using an Axiostar plus microscope (Zeiss-Germany).

Ethical Aspects

Permission and approval were obtained from CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) (Reg. No. 1165/PO/RcBit/S/08/CPCSEA) Government of India through the Bangalore-based Institutional Animal Ethical Committee.

Statistical Analysis

Statistical analysis was performed using SPSS (version 21.0 IBM Japan Ltd., Tokyo, Japan). All the results were prepared in triplicate, and

expressed as mean±SD. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Duncan's multiply comparison test. The values of $p<0.05$ were considered statistically significant.

RESULTS

Identification of Isolated Compound

Column chromatography separation of the *L. racemosa* leaves powder produced a total of 38 fractions, which were pooled into three major fractions, including methanol (3.9 g), hexane (1.9 g), and chloroform fraction (1.7g). Based on the α-amylase and α-glucosidase inhibition assays, the methanol fraction exhibited the highest antidiabetic activity than the other two fractions, therefore the methanol fraction was subjected to HPLC. Thus, a bioactive phytochemical compound was isolated from *L. racemosa* leaves and characterized as an alkaloid, named 4a-methyl-5-(6-methylhept-5-en-1-yl)octahydro-1*H*-cyclopenta[*a*] pyridazine (abbreviated as MOCP in further text for convenience). The MOCP was quantified using HPLC chromatography and its yield was found to be 1.2 g (Figure 1). It was crystalline yellow with 145 °C as its melting point, ≥90% as its purity, and insoluble in water. Structural elucidation of MOCP was done using various spectroscopic techniques (Graphs not given).

Chemical formula: $C_{16}H_{30}N_2$

Molecular weight: 250

^{1}HNMR (δ value ppm): 0.85 (s, 1H),1.26 (s, 1H),1.70 (s, 1H)

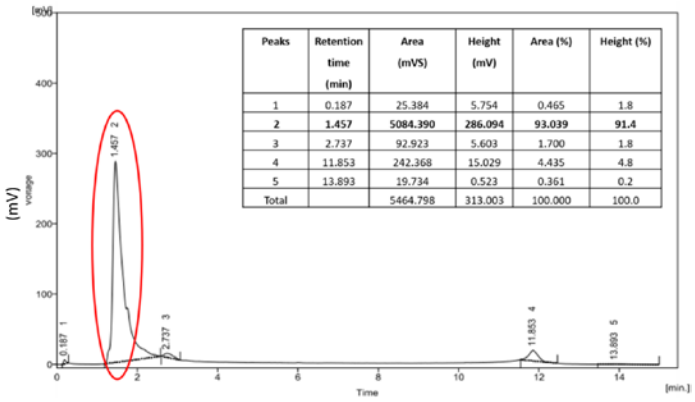


Figure 1: HPLC Chromatogram of Methanol fraction showing various peaks and other characteristics of purified fraction (Detector A Ch1 254 nm).

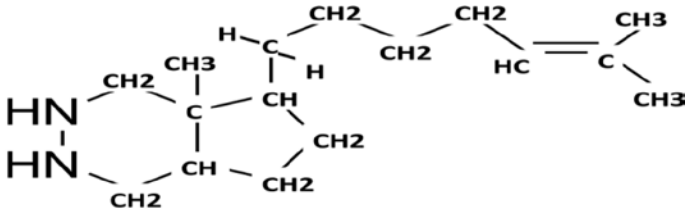
^{13}C NMR (δ value ppm): 125.03, 135.22, 60.43, 41.36, 36.08, 11.45, 14.14, 18.76

LC-MS [m/z value]: 273 $[M+Na]^+$ (molecular ion peak)

FTIR: showed wave number peak at 648.08 cm^{-1}, 835.18 cm^{-1}, 1033.85 cm^{-1}, 1055.06 cm^{-1}, 1375 cm^{-1}, 2345.44cm^{-1}, 2370.51 cm^{-1}, 2848.86 cm^{-1}, 2916.37 cm^{-1} and 2958.8 cm^{-1}

CHN Analysis: Nitrogen- 12.65%, Carbon-71.85% and Hydrogen-11.43%

Based on the above-mentioned spectral data, MOCP was confirmed to be an alkaloid. Its chemical structure is given below:



The MOCP exhibited antidiabetic activity by inhibiting α-amylase and α-glucosidase enzymes with IC_{50} 30.23 and 0.022 mg/ml, respectively (Table 1).

In vivo antidiabetic activity on Wistar rats

In the acute toxicity study, oral administration of MOCP at a dose of 2000 mg/kg BW did not produce any signs of toxicity and there was no mortality up to 14 days. It showed that MOCP was non-toxic in rats up to an oral dose of 2000 mg/kg BW. Therefore, further investigation was carried out using 250 and 500 mg/kg BW dose levels.

After the induction of diabetes, a significant increment in body weight was noticed in both MOCP-treated (G3 & G4) and glibenclamide (G2) groups at the end of the 7th day. A similar trend of increase in body weight was observed for the next seven days (8th to 14th day) also. 2.19 to 3.66 % enhancement in body weight was imparted by MOCP at all-day intervals.

The blood glucose level was noted on 0, 1, 3, 5, 7, and 14th day of treatment with MOCP and standard drug (Glibenclamide). On the very 1st day, a steep increase was noticed in the blood glucose level of the diabetic rats (G2, G3, and G4). Later on, a significant dose-dependent decrease in blood glucose level was observed in the diabetic treated groups, from an initial level of 322.66 mg/dL to 283.33 mg/dL in the G2, from 329.0 mg/dL to 296.75 mg/dL in the G3 and from 328.40 mg/dL to 291.60 mg/dL in G4. The MOCP at its highest dose (500 mg/kg BW)

Table 1: α-Amylase and α-Glucosidase Inhibition activity of MOCP.

α-Amylase					α-Glucosidase			
Acarbose			MOCP		Acarbose		MOCP	
Conc. (mg/ml)	Inhibition (%)	IC_{50} (mg/ml)	Inhibition (%)	IC_{50} (mg/ml)	Inhibition (%)	IC_{50} (mg/ml)	Inhibition (%)	IC_{50} (mg/ml)
0.5	50.32±0.292	0.095±0.007	49.35±0.145	30.234±0.013	50.61±0.429	0.059±0.353	50.69±0.191	0.022±0.004
1.0	57.1±0.165		54.90±0.191		56.86±0.494		57.45±0.270	
2.0	70.25±0.16		70.25±0.125		70.23±0.399		70.45±0.092	
3.0	80.22±0.113		79.66±0.180		80.00±1.525		80.28±0.188	
4.0	88.09±0.037		86.99±0.222		86.67±0.156		84.89±0.107	
5.0	89.39±0.87		88.72±0.051		89.26±0.156		89.16±0.162	

Data were expressed as mean ± SD, n=03. Statistical significance was determined by one-way ANOVA followed by Duncan's multiply comparison test, $p<0.05$ compared with control.

Table 2: Effect of MOCP on blood glucose level of different rat groups.

Groups	Blood glucose level at different intervals (in days) post 14days of STZ induction (mg/dL)					
	0	1	3	5	7	14
G1	269.21±8.21	269.83±8.4	270.02±8.02	270.15±8.05	270.19±7.09	270.23±7.23
G2	269.32±9.2	322.66±7.3	312.17±7.1	308.02±8.02	295.33±7.2	283.33±8.0
G3	268.54±8.3	329.00±7.0	321.75±6.25	313.00±6.0	305.40±6.4	296.75±6.25
G4	268.11±7.11	328.40±6.4	319.00±7.0	311.60±6.3	299.98±7.3	291.60±6.2

Data were expressed as mean ± SD, *n*=03. Statistical significance was determined by one-way ANOVA followed by Duncan's multiply comparison test, $p<0.05$ compared with control.

Table 3: Effect of Isolated Alkaloid on Haematology parameters of rat groups.

Group	Treatment and Dose (mg/kg BW)	WBC (10^3 cells/µL)	RBC (10^6 cells/µL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)
G1	Control	7.51±0.16	6.36± 0.11	37.02±1.02	54.14± 1.6	20.48± 0.9	33.21± 1.21	13.52± 0.8	74.24±3.24	2.73± 0.13
G2	Positive Control	8.49±0.2	7.07± 0.2	31.69±1.19	48.91± 1.8	19.21± 0.8	34.54± 1.34	14.8±0.8	84.06± 3.06	2.41±0.11
G3	Low dose 250	7.63±0.23	5.83± 0.23	37.53±1.2	37.24± 1.14	17.58± 1.1	29.88± 1.1	13.0±0.54	83.1± 3.1	2.28± 0.13
G4	High dose 500	7.35±0.2	4.9± 0.15	31.7± 1.08	46.45± 1.45	19.01± 1.01	30.89± 1.2	14.4±0.44	82.7± 2.2	3.28±0.13

Data were expressed as mean ± SD, *n*=03. Statistical significance was determined by one-way ANOVA followed by Duncan's multiply comparison test, $p<0.05$ compared with control.

Table 4a: Effect of Isolated Alkaloid on Lipid Profile and Kidney of different rat groups.

Group	Treatment and Dose (mg/kg BW)	Triglyceride (mg/dL)	Total Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	Creatinine (mg/dL)	BUN (mg/dL)	Urea (mg/dL)
G1	Control	148.89±1.0	74.90±0.6	20.54±0.2	24.58±0.2	29.78±0.2	0.75±0.05	15.17±0.12	35.46±0.3
G2	Positive Control	10.20±0.75	63.15±0.45	20.57±0.15	20.54±0.15	22.04±0.15	0.79±0.04	17.61±0.11	38.41±1.3
G3	Low dose 250	85.80±0.5	59.79±0.3	21.83±0.1	20.80±0.91	17.16±0.1	0.77±0.04	15.89±0.09	36.49±1.1
G4	High dose 500	54.20±0.25	54.56±0.15	22.85±0.05	20.87±0.05	10.84±0.05	0.73±0.03	14.56±0.08	34.43±0.4

Data were expressed as mean ± SD, *n*=03. Statistical significance was determined by one-way ANOVA followed by Duncan's multiply comparison test, $p<0.05$ compared with control.

was efficient enough to reduce the blood glucose level and to a far extent comparable to standard (283.33 mg/dL) (Table 2).

Effect of MOCP on Haematological and Biochemical parameters

Leucocyte counts of G3 and G4 groups ($7.63*10^3$ cells/µL and $7.35*10^3$ cells/µL) were slightly lower than G2 ($8.49*10^3$ cells/µL). The highest value of RBC was observed in the G2 group (7.07×10^6 cells/µL), while treated groups G3and G4 recorded $5.83x\ 10^6$ cells/µL and 4.9×10^6cells/µL respectively. Other haematological parameters were either insignificantly changed or increased in some cases (Table 3). The data from Table 4A illustrates that the MOCP-treated rats exhibited a significantly increased TG level (85.80 mg/dL and 54.20 mg/dL in G3 and G4 respectively, as compared to 10.20 mg/dL in G2), and decreased Cholesterol (59.79 mg/dL and 54.56 mg/dL in G3 and G4 respectively, as compared to 63.15 mg/dL in G2) and VLDL level (17.16 mg/dL and 10.84 mg/dL in G3 and G4 respectively, in comparison to 22.04 mg/dL in G2). While HDL and LDL did not show much variation and were near normal when compared to the glibenclamide. The renal functions, including BUN (15.89 mg/dL and 14.56 mg/dL in G3 and G4 respectively, as compared to 17.61 mg/dL in G2) and urea concentration (36.49 mg/dL and 34.43 mg/dL in G3 and G4 respectively, as compared to 38.41 mg/dL in G2) were significantly reduced in the MOCP-treated rat groups and exhibited better results than standard. The activities of liver marker enzymes and proteins are presented in Table 4B. Total protein, albumin, and globulin were found to show insignificant changes compared to the glibenclamide group while, level of AST, ALT, and ALP and creatinine kinase marker enzymes upon the oral administration of MOCP was reduced in treated groups.

Histopathological studies

Pancreatic tissue at a low dose (G3) of MOCP shows a focal decrease in islet size, and cells focally demonstrate swelling (increase in cell size), nucleomegaly (increase in nuclei size), and cytoplasmic vacuolations. While at a higher dose (G4), acini and islet of Langerhans were noticed healthier with fewer vacuolations and swellings (Figure 2A). The MOCP-treated rats showed a typical architecture of liver parenchyma cells. As the dosages were increased, moderate to mild portal inflammation was noticed in treated rats. Central veins and sinusoids showed moderate dilation; Hepatocytes appear unremarkable. While in glibenclamide-treated rats, the central vein appeared to be mildly dilated. At the highest dose (G4) of the MOCP, the histopathological degenerations turned out to be mild and comparable to the standard (Figure 2B). The

Table 4b: Effect of Isolated Alkaloid on Liver of different rat groups.

Group	Treatment and Dose (mg/kg BW)	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Creatinine Kinase (IU/L)
G1	Control	6.84± 0.12	4.22±0.05	2.62±0.07	58.7±1.08	139.84±3.4	129.06±4.06	193.13±6.13
G2	Positive Control	5.92±0.2	3.61±0.09	2.31±0.11	52.0±1.04	147.77±3.2	136.87±3.4	199.02±5.02
G3	Low dose 250	5.67±0.2	3.45±0.1	2.22±0.1	46.0±1.02	143.98±3.4	131.42±2.3	189.45±5.2
G4	High dose 500	5.86±0.12	3.55±0.06	2.31±0.06	45.3±1.05	141.22±2.2	128.8± 2.6	187.68±4.08

Data were expressed as mean ± SD, n=03. Statistical significance was determined by one-way ANOVA followed by Duncan's multiply comparison test, p<0.05 compared with control.

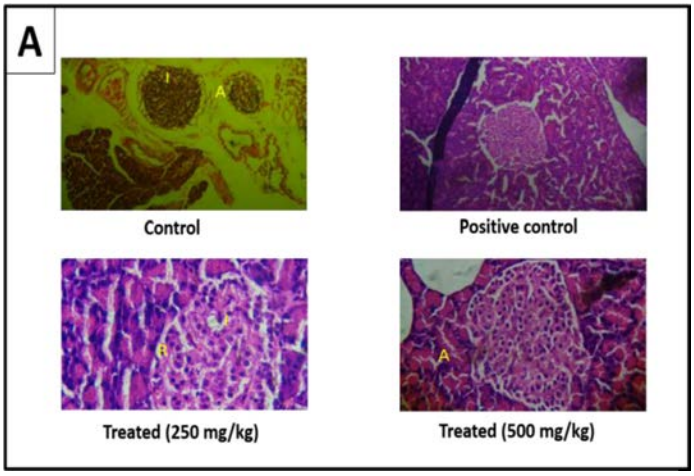


Figure 2A: Effects of MOCP on Rat's Pancreas.
[Acinar cells (A), Islet of Langerhans (I) of the pancreas, Regeneration (R)]

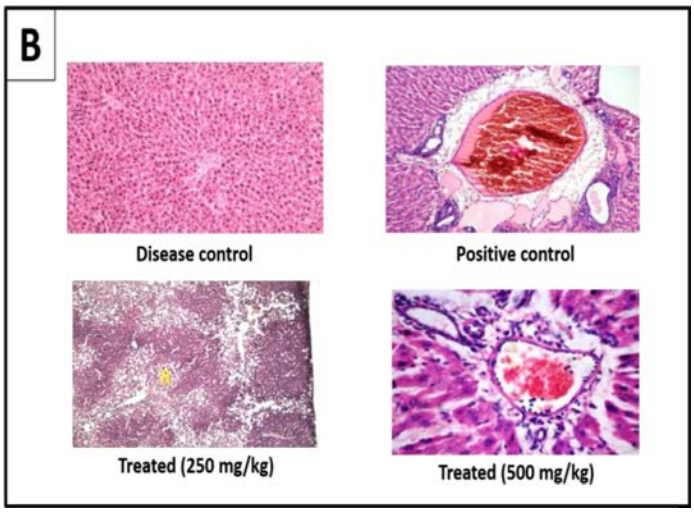


Figure 2B: Effects of MOCP on Rat's Liver.
[Regeneration (R)]

histopathology of the kidney exhibited renal parenchyma with normal tubules and glomeruli in rats in G4 comparable to glibenclamide-treated rats. However, a mild focal enlargement and sclerosis were observed in the glomeruli; the interstitium showed few congested blood vessels in G3 (Figure 2C).

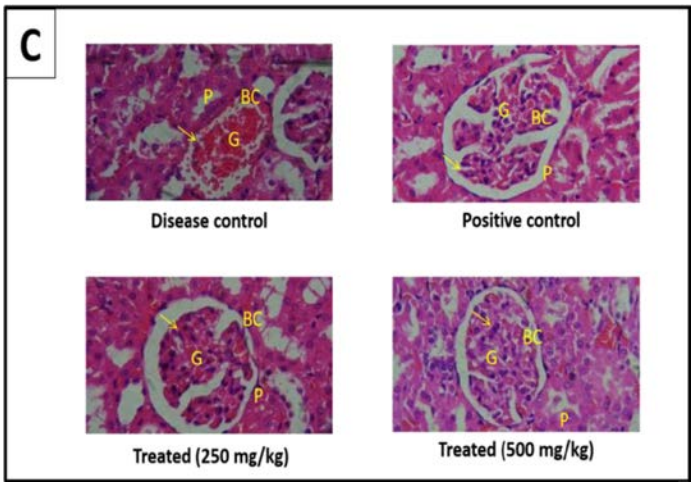


Figure 2C: Effects of MOCP on Rat's Kidney.
[Glomerulus (G), Bowman's capsule (BC), Proximal convoluted tubule (P), Regeneration (R)]

DISCUSSION

The ethnobotanical information of about 1200 plant species has been reported to possess anti-diabetic properties.[15] Various secondary metabolites isolated from different medicinal or mangrove plant species have been proved to possess antidiabetic activity. Out of various classes of secondary metabolites, alkaloids are the compounds with a diverse range of structure that shows an array of pharmacological activities like anti-diabetic properties,[8] anticancer activity,[16-17] acetylcholine esterase activity,[18] antibacterial,[19] anti-rheumatic activity,[20] anti-inflammatory,[21] antinociceptive effects,[22] antiprotozoal, and cytotoxic activity.[23] Several alkaloids from natural sources have been proved efficacious to cure various ailments. Indole alkaloids from *Catharanthus roseus*,[24-25] carbazole alkaloids from *Murraya koenigii*,[26] isoquinoline alkaloids,[27] etc. are some of the examples which are useful in the treatment of diabetes.

In our earlier study, we found *L. racemosa* to be a potent inhibitor of α-amylase and α-glucosidase enzymes *in vitro* and a rich source of secondary metabolites like alkaloid, flavonoid, and saponin as well which are proved to possess antidiabetic activity.[12] So in continuation of our previous work, the present study has been undertaken to isolate an antidiabetic principle from *L. racemosa* leaves and evaluate its antidiabetic efficacy *in vivo* on Streptozotocin-induced Wistar rats. Thus, a bioactive compound was isolated from *L. racemosa* which is characterized as an alkaloid, named 4a-methyl-5-(6-methylhept-5-en-1-yl)octahydro-1*H*-cyclopenta[*a*] pyridazine (MOCP). MOCP was also evaluated for its *in vitro* antidiabetic activity before the commencement of *in vivo* studies. MOCP significantly inhibited α-amylase and α-glucosidase activities in a

dose-dependent manner. The α-amylase and α-glucosidase are two vital carbohydrate metabolizing enzymes that are majorly involved in the degradation of oligosaccharides and disaccharides into monosaccharides. The inhibition of these enzymes increases carbohydrate digestion and reduces glucose absorption,[28] hence thought to be beneficial in treating diabetes.

Based on the above *in vitro* findings, MOCP was further tested for its *in vivo* efficacy against STZ-induced diabetic rats. Oral administration of MOCP to these rats significantly improved most of the parameters, and the respective values were found to be comparable to the standard drug. STZ-induced diabetes is characterized by severe loss in body weight,[29] which might be because of protein wastage due to carbohydrates' unavailability as an energy source.[30] In the present study, MOCP which might impart the preventive effect on protein degradation was found to improve the body weight in diabetic rats, resulting from an improvement in glycemic control. Similarly, the blood-glucose-lowering effect of MOCP was observed against STZ-induced diabetic rats, which might be due to stimulation of surviving β-cells of islets of Langerhans leading to more insulin release.[1]

The assessment of haematological parameters is a useful guide for determining the extent of deleterious effects of foreign compounds, including plant extracts.[31-32] There is marked alteration in the haematological values in the G3 and G4 compared to the standard (G2) suggesting that the MOCP recovers the abnormal values up to some extent which might help in protecting the susceptible tissues under chronic diabetic conditions. The prevalent lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia.[33] In the present study, MOCP-induced reduction of TG, TC, and VLDL may result in a presumption of the fact that MOCP is responsible for the enhancement of the transcription of lipoprotein lipase similar to that of insulin.[34] The above action could help in preventing diabetic complications such as coronary heart diseases and atherosclerosis. The most common way to evaluate kidney functions is to measure creatinine, BUN, and urea concentrations. An elevation in the BUN level indicates renal dysfunction,[35] while creatinine is a noticeable index assessing glomerular filtration rate.[36] A slight decrease in these parameters upon the treatment of MOCP suggests its curative effect on renal injury in STZ-induced rats. The presence of alkaloid MOCP in *L. acemose* might have contributed to the regeneration of islet of Langerhans in diabetic rats as reported earlier.[37-38] The liver which is the vital organ for metabolism, detoxification, and storage, possesses marker enzymes like ALT, AST, and ALP as an increase in their activities reflects active liver damage.[39] The G4 was observed to reduce the values of these marker enzymes making MOCP responsible for protecting cellular damage in conformity with similar results of Eliza *et al.*[40] In diabetes, reduction in protein and albumin might be due to proteinuria, albuminuria, or increased protein catabolism, which are the clinical markers in diabetic nephropathy.[41] In the present study, a slight increase in G4 protein levels as compared to G3 is possible through an increase in the insulin-mediated amino acid uptake, enhancement of protein synthesis/or inhibition of protein degradation.[42]

In the histopathological studies, there is no significant hepatocellular damage observed except for small areas of focal degeneration and sinusoidal dilation in the MOCP-treated liver, whereas STZ-induced rats showed fatty change with widespread hepatocellular necrosis and centrilobular necrosis suggesting MOCP's role comparable to glibenclamide.

CONCLUSION

Current study demonstrates the antidiabetic activity of an isolated compound which is an alkaloid, named MOCP [4a-methyl-5-(6-methylhept-5-en-1-yl) octahydro-1*H*-cyclopenta[*a*]pyridazine)] from *L. racemosa* leaves collected from the Konkan region of Maharashtra. The possible mechanisms of MOCP's antidiabetic action include inhibition of carbohydrate hydrolytic enzymes, reduction in glucose levels by increasing glucose uptake by tissues and adipocytes, and stimulation of pancreatic insulin release. In addition, MOCP treatment also showed normalization of serum biochemical and haematological parameters, and histopathological observations as well in STZ-induced diabetic rats which signifies its protective action against dyslipidemic complication that occurs in diabetes. It is also inferred from our previous study that *L. racemosa* leaves possess various phytochemicals that are responsible for its antidiabetic activity. Thus, we propose the alkaloid MOCP as an antidiabetic agent because of its well-documented significant role in the management of diabetes and its associated complications in our present findings. Furthermore, comprehensive pharmacological investigations are needed to elucidate the exact mechanism behind the action of the antidiabetic effect.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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