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EVALUATION OF ANTI-DIABETIC ACTIVITY OF *CARALLUMA ADSCENDENS* WHOLE PLANT IN DITHIZONE INDUCED DIABETIC RATS

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ABSTRACT: This study was aimed to evaluate the anti-diabetic activity of Ethanolic extract of the whole plant administered at different dosages (100 mg/kg and 200 mg/kg) for 21 days in Dithizone-induced diabetic rats using Glibenclamide as a standard drug (hypoglycemic drug). For the study, rats were divided into five groups of six animals each. Group, I served as control, Group II served as diabetic control received Dithizone, Group III diabetic rats were served with Glibenclamide while Group IV and V diabetic rats were received 100 mg/kg and 200 mg/kg of Ethanolic extract of *Caralluma adscendens* whole plant. The antidiabetic potential of the whole plant extract was undertaken in Dithizone induced hyperglycemic models by comparing biochemical parameters like blood glucose levels, OGTT and lipid profile (total cholesterol, triglycerides, LDL, and HDL), total protein and liver function tests (Total bilirubin, ASP, ALT, and AST) along with the liver antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), malondialdehyde (MDA), glycogen content and Glycogenic enzymes were quantified using standard experimental procedures. Administration of the extract at 100, 200 mg/kg body weight significantly decreased blood glucose levels, increased glucose tolerance, and improved imbalance in lipid metabolism in diabetic rats. These are indications of antidiabetic property of *Caralluma adscendens* with 200 mg/kg body weight of the extract showing the best hypoglycemic action by comparing favorably well with Glibenclamide. This investigation clearly showed that the extract is endowed with hypoglycemic activity; *Caralluma adscendens* may also protect the liver against impairment due to diabetes.

INTRODUCTION: Diabetes mellitus is a chronic metabolic disease, occurs when the pancreas is not producing insulin or produced insulin cannot be used by the body; these may lead to rising blood glucose levels. Hyperglycemia for the long term is associated with damage to the various organs and tissues.

The number of people living with diabetes is expected to rise from 366 million in 2011 to 552 million by 2030. IDF also estimates that as many as 183 million people are unaware that they have diabetes ¹.

It can be predicted that by 2030, India, China, and the United States will have the largest number of diabetic patients ². There are two types of diabetes: type 1 diabetes mellitus and type 2 diabetes mellitus. Despite the great interest in developing new drugs to reduce the burden of this disease, the scientific community has raised the interest to evaluate either raw or isolated natural products in experimental studies; few were tested clinically in

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humans³. The ayurvedic medicinal plant plays a vital role in healing various diseases⁴. Identification and preparation of medicament from the natural origin is a crucial phenomenon and challenges the research for curing the disease without side effects. This study evaluates the anti-diabetic activity of Ethanolic extract of *Caralluma adscendens* whole plant on dithizone-induced diabetic rats. The efficacy was compared with Glibenclamide (a standard hypoglycemic drug).

MATERIALS AND METHOD:

Collection of Plant Material and Plant Extraction: The whole plant of *Caralluma adscendens* was collected from the roadsides of Military Diary farm Road, Trimulgherry, Secunderabad, situated in the state of Telangana (India). The botanist of Osmania University authenticated the plant specimen, and authenticated voucher specimen Number 203 of the plant has been preserved in the department for future reference. The dried plant was then milled to coarse powder mechanically and successively extracted with Petroleum ether, Ethanolic, Ethyl acetate and Ethanol using a soxhlet extractor. Method of maceration was followed for water for 72 h. The rotary evaporator was used for concentrating the extracts, dried in vacuum desiccators, properly labelled and weighed, stored after that in the refrigerator until further use. Preliminary Phytochemical screening for the above plant Extracts was conducted. Based on the presence of phytoconstituents, chloroform extract was selected for the screening of anti-diabetic activity

Animals: The protocol was approved by the Institutional Animal Ethical Committee (IAEC Approval no: CPCSEA/IAEC/JLS/11/11/19/14). Albino rats with an average body weight of 150 to 250 g were utilized in this study. They were procured from Sanzyme Bio-analytical lab, Plot no. 8 Sys.No.542, Kothur(V), Shameerpet, R. R. dist. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles at 25 ± 3 °C and 35-60 % humidity). Standard pelletized feed and tap water were provided *ad-libitum*.

Experimental Design: The rats were divided into two sets, each comprising five groups ($n = 6$ in each group): one for anti-diabetic study and the

other for the evaluation of glucose tolerance. All groups (except group I) were made diabetic by injecting 50 mg/kg body weight of Dithizone intraperitoneally. Development of diabetes was allowed for 3 days. Group I served as control receiving normal saline (1ml/kg p.o.) as a vehicle for a period of 21 days, Group II served as diabetic control, were administered with dithizone (50 mg/kg) intraperitoneally, Group III, Group IV and Group V animals received an oral daily dose of Glibenclamide (10 mg/kg), 100 and 200 mg/kg body weight/day of *Caralluma Adscendens* Ethanolic extract (CAEE) respectively.

Estimation of Blood Glucose Level: On 22nd day, Blood samples (3 ml) were collected from the tail vein of the experimental animals after overnight fasting for the estimation of blood glucose level using a glucometer (Accu-Chek, Roche Products (Pty) Ltd., South Africa) at 0, 1/2, 1, 2, 4, 6, 8 h.

Analysis of Lipid Profile and Total Protein: The serum concentrations of total cholesterol, triglycerides, HDL cholesterol and LDL-cholesterol were determined by automatic analyzer technique (Beckman Coulter Inc., Ireland). Total protein in the serum was estimated using bovine serum albumin as standard⁵.

Liver Function Tests: The concentrations of hepatic markers like total bilirubin⁶, alkaline phosphatase (ALP)⁷, aspartate and alanine transaminases (AST and ALT)⁸ were determined in the serum using Randox Assay kits.

Biochemical Estimation of Markers of Oxidative Stress: After sacrificing the animals on 22nd day, the liver tissue from various groups of animals were removed carefully followed by washing thoroughly with ice-cold saline, 0.5 gms of the wet tissue was weighed exactly and homogenized in 0.1M Tris-HCl buffer, pH 7.4 at 4 °C in a Remi homogenizer with a Teflon pestle rotated at 600 rpm for 30 min. The homogenate was centrifuged at 2500 rpm for 10 min at 4 °C using a refrigerated centrifuge. The supernatant was used for the assay of lipid peroxidation products and antioxidant enzymes such as malondialdehyde (MDA)⁹, reduced glutathione (GSH)^{10, 11} superoxide dismutase (SOD)¹², catalase (CAT)¹³.

Oral Glucose Tolerance Test: On day 22, the rats in groups 1 to 5 (from the second set) were given glucose (2 g/kg body weight; p.o.) 30 min after administration of the extract/drug¹⁴.

Blood samples were collected from the tail vein prior to glucose administration and at 30, 60 and 90 min after glucose loading for immediate measurement of blood glucose levels.

Estimation of Glycogen Content and Gluconeogenic Enzymes: Hepatic glycogen content was estimated by Carroll *et al.*¹⁵. Gluconeogenic enzyme activities in the liver were assayed using the following procedures: glucose-6-phosphatase was estimated by the method described by Koide and Oda¹⁶, succinate dehydrogenase was estimated by the method described by Slater *et al.*¹⁷.

Statistical Analysis: Data were expressed as mean \pm SEM of six replicates and subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests to determine significant differences in all the parameters. Values were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSIONS:

Determination of Blood Glucose Level: The continuous administration of Ethanolic extract of *C. adscendens* was found to significantly reduce the blood glucose level in diabetic rats at the end of the experiment **Table 1** and **Fig. 1**.

The effect was more pronounced in the rats treated with 200 mg/kg body weight of the extract, and it compared favorably well with Glibenclamide treated rats.

TABLE 1: EFFECT OF ORAL ADMINISTRATION OF CARALLUMA ADSCENDENS ON BLOOD GLUCOSE LEVELS IN DIABETIC RATS (n = 6, MEAN \pm SEM)

Group	Blood Glucose Level (mg/dL)						
	0 H	½ H	1 H	2 H	4 H	6 H	8 H
Control	88.2 \pm 2.4	88.8 \pm 3.2	87.5 \pm 3.2	87.5 \pm 2.7	88.2 \pm 2.5	88.3 \pm 2.5	87.4 \pm 2.5
Diabetic control	286.6 \pm 3.4	286.1 \pm 3.2	285.5 \pm 3.8	285.1 \pm 3.5	283.8 \pm 3.2	283.9 \pm 2.9	281.8 \pm 3.4
Diabetic + Glibenclamide	287.7 \pm 5.2	272.8 \pm 3.8	260.8 \pm 2.6	224.6 \pm 2.6	200.2 \pm 2.4	190.2 \pm 2.6	180.3 \pm 2.4
Diabetic + CA extract(100mg/kg)	290.3 \pm 2.4	278.6 \pm 3.4	269.4 \pm 3.5	262.8 \pm 2.6	233.5 \pm 3.5	227.4 \pm 3.7	219.3 \pm 4.6
Diabetic + CA extract (200mg/kg)	289.8 \pm 2.3	275.5 \pm 3.8	263.6 \pm 3.5	249.5 \pm 3.7	222.5 \pm 3.9	216.8 \pm 3.7	208.7 \pm 3.5

TABLE 2: EFFECT OF ORAL ADMINISTRATION OF CARALLUMA ADSCENDENCE ETHANOLIC EXTRACT ON SERUM LIPID PROFILE AND TOTAL PROTEIN IN DIABETIC RATS (n = 6, MEAN \pm SEM)

Group	Cholesterol (mg / dL)	Triglycerides (mg / dL)	HDL (mg/dL)	LDL (mg/dL)	Total Protein (g/L)
Control	109.26 \pm 0.78	85.1 \pm 0.86	35.5 \pm 0.98	43.19 \pm 0.01	88.60 \pm 0.34
Diabetic control	136.03 \pm 0.70 [#]	132.1 \pm 0.50 [#]	24.5 \pm 0.49 [#]	58.19 \pm 0.40 [#]	76.60 \pm 0.69 [#]
Diabetic + Glibenclamide	112.9 \pm 0.170 ^{***}	87.2 \pm 0.158 ^{***}	32.8 \pm 0.42 ^{***}	49.8 \pm 0.54 ^{***}	83.4 \pm 0.21 ^{***}
Diabetic + CA extract(100mg/kg)	129.5 \pm 0.28 [*]	104.2 \pm 1.56 [*]	29.7 \pm 0.12 [*]	52.4 \pm 0.42 [*]	79.8 \pm 0.34 [*]
Diabetic + CA extract(200mg/kg)	120.3 \pm 0.12 ^{**}	93.2 \pm 0.19 ^{**}	31.9 \pm 0.34 ^{**}	50.3 \pm 0.85 ^{**}	82.9 \pm 0.13 ^{**}

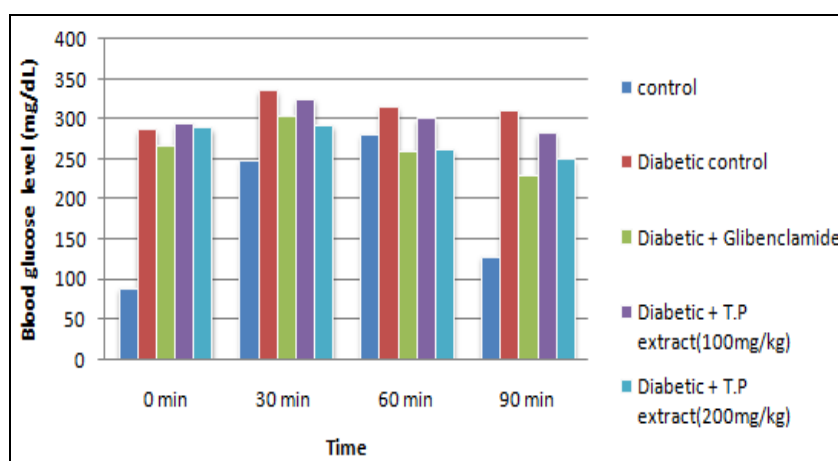


FIG. 1: EFFECT OF ORAL ADMINISTRATION OF CARALLUMA ADSCENDENS ON BLOOD GLUCOSE LEVELS IN DIABETIC RATS (n = 6, MEAN \pm SEM)

Serum Lipid Profile and Total Protein: There was a significant elevation in the levels of serum cholesterol, triglycerides and LDL and reduced HDL and protein concentrations in diabetic rats when compared with the control group **Table 2**. The Ethanolic extract of *C. adscendence* and Glibenclamide significantly reduced the levels of serum cholesterol, triglycerides and LDL and increased HDL to near normalcy as observed in control after 21 days of treatment.

Liver Function Parameters: The untreated diabetic rats exhibited a significant increase in serum activities of ALP, ALT, AST and bilirubin concentrations when compared with the control **Table 3**. Continuous administration of Ethanolic extract of *C. adscendence* to diabetic rats for 21 days was able to restore all the liver function indices back to normal.

TABLE 3: EFFECT OF ORAL ADMINISTRATION OF CARALLUMA ADSCENDENCE ETHANOLIC EXTRACT ON SOME LIVER FUNCTION PARAMETERS OF DIABETIC RATS (n = 6 ± MEAN ± SEM)

Groups	Total bilirubin (µmol / L)	ALP (U/L)	ALT (U/L)	AST (U/L)
Control	0.48 ± 0.11	12.7 ± 0.21	16.4 ± 0.12	12.2 ± 0.13
Diabetic control	1.38 ± 0.46 [#]	45.4 ± 0.19 [#]	34.3 ± 0.17 [#]	24.4 ± 0.14 [#]
Diabetic + Glibenclamide	0.65 ± 0.31 ^{***}	27.2 ± 0.15 ^{***}	21.6 ± 0.34 ^{***}	17.2 ± 0.26 ^{***}
Diabetic + CA extract(100mg/kg)	1.03 ± 0.7 [*]	34.1 ± 0.8 [*]	27.2 ± 0.6 [*]	21.2 ± 0.4 [*]
Diabetic + CA extract (200mg/kg)	0.96 ± 0.8 ^{**}	31.1 ± 0.4 ^{**}	25.4 ± 0.4 ^{**}	19.5 ± 0.5 ^{**}

Biochemical Estimation of Markers of Oxidative Stress: The results of the study are shown in **Table 4**. The estimated concentrations of liver MDA on 21st day of the study of both test and standard drug indicated that the MDA levels are declined in the case of 100 mg/kg dose and 200 mg/kg extracts with statistical significance. Similarly, the standard drug also showed a reduction with statistical

significance in the same experiment. The enzymes like GSH, SOD and CAT values are lowered significantly in diabetic rats as compared with normal control rats.

The standard drug, the test extracts (100 mg/kg and 200 mg/kg) showed an elevated value of these enzymes with statistical significance.

TABLE 4: EFFECT OF ORAL ADMINISTRATION OF CARALLUMA ADSCENDENCE ETHANOLIC EXTRACT ON ANTIOXIDANT PROFILES

Groups	MDA(µM/ 100g wet Tissue)	GSH(µM/ g wet Tissue)	CAT(Units/ mg Protein)	SOD(Units/mg Protein)
Control	0.85 ± 0.03	22.4 ± 1.2	8.42 ± 0.6	9.2 ± 0.5
Diabetic control	1.38 ± 0.5 [#]	13.5 ± 1.4 [#]	4.98 ± 0.7 [#]	5.3 ± 0.7 [#]
Diabetic + Glibenclamide	0.91 ± 0.05 ^{***}	18.6 ± 1.3 ^{***}	7.81 ± 0.5 ^{***}	8.8 ± 0.4 ^{***}
Diabetic + CA extract(100mg/kg)	1.08 ± 0.05 [*]	14.75 ± 0.3 [*]	5.46 ± 0.4 [*]	6.97 ± 0.5 [*]
Diabetic + CA extract(200mg/kg)	0.95 ± 0.03 ^{**}	16.86 ± 0.61 ^{**}	6.98 ± 0.3 ^{**}	7.9 ± 0.3 ^{**}

Oral Glucose Tolerance Test: **Table 5** and **Fig. 2** represent the blood glucose levels of the rats after oral administration of glucose.

peak increase in blood glucose concentration was observed after 30 min and remained high over the next 60 min.

The level in the control rats rose to the peak 30 min after glucose load and decreased to near normal levels at 90 min. In the untreated diabetic rats, the

Caralluma adscendence and glibenclamide-treated diabetic rats showed a significant decrease in blood glucose concentration than diabetic control rats.

TABLE 5: EFFECT OF ORAL ADMINISTRATION OF CARALLUMA ADSCENDENCE ETHANOLIC EXTRACT ON BLOOD SUGAR LEVELS IN GLUCOSE-LOADED DIABETIC RATS (n = 6, MEAN ± SEM)

Group	Blood Glucose Level (mg/dL)			
	0 Min	30 Min	60 Min	90 Min
Control	88.2 ± 2.4	247.8 ± 3.2	280.8 ± 3.2	128.5 ± 2.7
Diabetic control	286.6 ± 3.4	336.1 ± 3.2	315.5 ± 3.8	310.1 ± 3.5
Diabetic + Glibenclamide	267.7 ± 5.2	302.8 ± 3.8	260.8 ± 2.6	228.6 ± 2.6
Diabetic + CA extract(100mg/kg)	290.3 ± 2.4	325.1 ± 3.4	300.4 ± 3.5	288.8 ± 3.6
Diabetic + CA extract (200mg/kg)	289.8 ± 2.3	292.5 ± 3.8	271.6 ± 3.5	269.5 ± 3.7

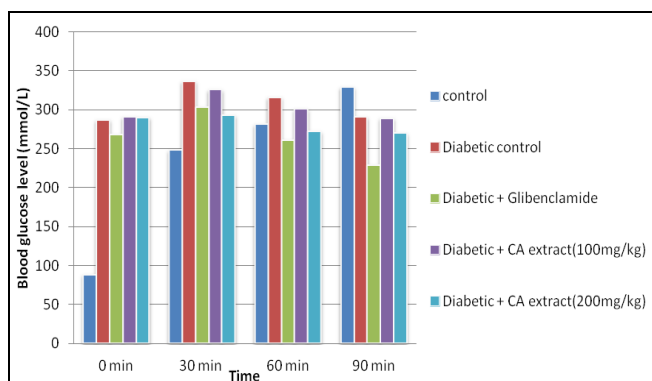


FIG. 2: EFFECT OF ORAL ADMINISTRATION OF *CARALLUMA ADSCENDENCE* ETHANOLIC EXTRACT ON BLOOD SUGAR LEVELS IN GLUCOSE-LOADED DIABETIC RATS ($n = 6$, MEAN \pm SEM)

Estimation of Glycogen Content and Gluconeogenic Enzymes: Table 6 represents the effect of *Caralluma adscendence* on Glycogen content and Gluconeogenic enzymes. Glycogen content in diabetic rats was found to be

TABLE 6: EFFECT OF *CARALLUMA ADSCENDENCE* ON GLYCOGEN CONTENT AND GLUCONEOGENIC ENZYMES

Groups	Glycogen (mg/g wet Tissue)	Glucose -6- phosphatase (nmol NADP+ Reduced/min/mg Protein)	Succinate Dehydrogenase (nmol Pot. Ferricyanide Reduced/min/mg Protein)
Control	4.85 \pm 0.76	19.33 \pm 1.29	5.16 \pm 0.8
Diabetic control	2.64 \pm 0.6 [#]	14.53 \pm 1.09 [#]	2.92 \pm 0.56 [#]
Diabetic + Glibenclamide	4.58 \pm 0.42 ^{***}	17.96 \pm 0.54 ^{***}	4.74 \pm 0.4 ^{***}
Diabetic + CA extract (100mg/kg)	3.67 \pm 0.63 [*]	15.12 \pm 0.65 [*]	3.87 \pm 0.64 [*]
Diabetic + CA extract (200mg/kg)	4.09 \pm 0.64 ^{**}	17.12 \pm 0.64 ^{**}	4.14 \pm 0.65 ^{**}

CONCLUSION: Oral administration of Ethanolic extract of *Caralluma adscendens* whole plant shown significant hypoglycemic activity in Dithizone-induced diabetes using Glibenclimide as standard in experimental Wistar rats, which could be attributed to its possible action on lipid metabolism as evidenced by antioxidant defense properties. The rise in total cholesterol, total bilirubin, and hypoproteinemia are also key features of liver damage. The results also revealed the beneficial effects of this medicinal plant in improving the imbalance in lipid metabolism experienced during diabetes. It can, therefore, be concluded from this study that the Ethanolic extract of *Caralluma adscendens*, besides its hypoglycemic action, could protect the liver against impairment due to diabetes.

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significantly reduced compared with the control. Treatment with standard *Caralluma adscendence* enhanced the glycogen storage efficiency of the liver of treated diabetic rats compared with diabetic control animals. The activity of glucose-6-phosphatase, the first regulatory enzyme of the pentose phosphate pathway, was found to be decreased in diabetic animals and increased in standard *Caralluma adscendence* extract-treated animals and the activity was higher in comparison to untreated diabetic animals indicating improvement in glucose utilization by this pathway. Succinate dehydrogenase activity was decreased in diabetic animals and increased in standard, *Caralluma adscendence* treated animals. An increase in succinate dehydrogenase activities in treated animals indicates better utilization of energy-yielding intermediates by the TCA cycle.

CONFLICTS OF INTEREST: There is no conflict of interest.

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