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#### ANTI-DIABETIC AND ANTI-INFLAMMATORY ACTIVITY OF CARALLUMA ADSCENDENS VAR. ADSCENDENS

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## ABSTRACT

The plant *Caralluma adscendens var. adscendens* belongs to Asclepiadaceae family was taken for the evaluation of anti-diabetic and anti-inflammatory activity. Diabetes mellitus is a chronic disorder characterized by hyperglycemia with a disturbance of carbohydrate, protein and fat metabolism. Phytochemical screening of *C. adscendens* extract indicated the presence of several bioactive compounds like alkaloids, saponins, tannins, steroids, terpenoids, flavonoids and proteins. Anti-diabetic study was carried out by using the butanone extract and investigated using alloxan induced diabetic rats. The oral administration of extract at dose 500 mg/kg led to a significant blood glucose reduction. The anti-inflammatory activity of *C. adscendens* against carrageenan induced paw edema in albino rats was also carried out. The anti-inflammatory activity is more effective in carrageenan induction with oral administration of *C. adscendens* extract at dose 500 mg/kg body weight and the study was compared with standard drug Indomethacin (10mg/kg). The results showed that *C. adscendens* is having significant anti-diabetic and anti-inflammatory activity.

**KEY WORDS:** *Caralluma adscendens*, Diabetes mellitus, Hyperglycemia, Phytochemistry, Anti-inflammatory activity

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## INTRODUCTION

Diabetes mellitus is a major global health problem throughout the world. In the year 2011, 366 million people worldwide had diabetes, and this will have risen to 552 million. More than 80% of diabetes deaths occur in lowand middle-income countries. Type 2 diabetes comprises 90% of people with diabetes around the world<sup>1</sup>. Diabetes mellitus is a chronic disorder characterized by hyperglycemia with a disturbance of carbohydrate, protein and fat metabolism resulting from a deficiency in the activity of insulin. Diabetes mellitus can lead to the dysfunction, failure and damage of multiple organs<sup>2</sup>. Insulin is a complex protein containing 51 amino-acids and is produced and released by the  $\beta$  cells of the pancreas. Insulin is secreted when blood glucose values rise and is stimulated by the amino acids, fatty acids, and gastrointestinal hormones that are released while eating and by parasympathetic nervous system<sup>3</sup>. The main function of insulin is to lower the blood glucose concentration in several ways. It stimulates glucose uptake in muscles and adipose tissue cells and stimulates the formation of glycogen, proteins and fat in the liver and in muscle and adipose tissue cells. In addition, insulin inhibits the formation of glucose through the inhibition of glycogenolysis and gluconeogenesis in the liver<sup>4</sup>. When a deficiency in insulin secretion or activity occurs, blood levels of glucose remain in after a meal resulting in hyperglycemia and abnormality of lipid profile. These lead to series of secondary complications including polyuria, polyphasia, ketosis, retinopathy as well as cardiovascular disorder<sup>5</sup>.

Currently available therapy for diabetes includes insulin and various oral hypoglycemic agents such as sulfonylureas, metformin, glucosidase inhibitors, troglitazone, etc. But, these are reported to produce serious adverse side effects such as liver problems, lactic acidosis and diarrhea<sup>6</sup>. Moreover, increased oxidative stress and generation of excessive free radicals in diabetic patients are thought to be the etiology of chronic diabetic complications. Increased reactive oxygen species and oxidative stress are observed in type 1 and type 2 diabetes mellitus<sup>7, 8</sup>. This abnormal high level of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. These consequences of oxidative stress can promote the development of complications of diabetes mellitus<sup>9</sup>. So, if any medicinal plant can work as a potential antioxidant together with having anti-diabetic property, then it could prevent or reduce diabetic complication more effectively than the conventionally used anti-diabetic drugs. Many anti-diabetic drugs have already available commercially and none of these have the dual property of reducing blood glucose level and scavenging free radicals. Moreover, the side effects and cost of the drugs are not affordable<sup>10</sup>.

Hence, there is a need to search for newer anti-diabetic agents that retain therapeutic efficacy and reduce the side effects and also risk factor like hyperlipidemia, hypertension and so on. There is an increased demand by patients to use natural products with anti-diabetic activity. Hence, today there is need for finding the alternatives which will minimize the side effects and cost of drug. Therefore, it becomes necessary to make use of vast reserves of plant origin for medicinal purposes which will help to search effective as well as safer drug remedy for diabetes mellitus<sup>11</sup>. Inflammation is responsible for tissue damage. It involves a complex array of enzyme activation, mediator release, extravasation of fluid, cell migration, tissue breakdown and repair. Inflammation had become the focus of global scientific research because of its implications in virtually all human and animal diseases<sup>12, 13</sup>. The attention of pharmacologists throughout the world has been focused on finding out safer and potent anti-inflammatory drugs. The natural products today symbolize

safety in contrast to the synthetic drugs that are regarded unsafe to humans as and environment. So, people are returning to the natural products with the hope of safety and security. However, so far there is no systematic study on anti-inflammatory activity has been reported in the literature. Hence, the present study focuses on evaluating the antidiabetic and anti-inflammatory activity of whole plant of C. adscendens.

Caralluma a genus of the family Asclepiadaceae is an important medicinal plant. It is a widely distributed succulent plant found in India, Africa, Arabia, southern Europe and Afghanistan. C. adscendens is a dry herb growing in the dry parts of India. Ethnobotanically it is being used to cure diabetes and fat accumulation or as vegetable in different regions of India (Amrutha Audipudi). The key phytochemical constituents of the herb are pregnane glycosides, flavone glycosides, megastigmane glycosides and saponins<sup>14</sup>. species Caralluma have shown antiinflammatory<sup>15</sup>, hypolipidemic, antioxidant<sup>16</sup>. anti-hyperglycemic<sup>17</sup> and anti-diabetic properties<sup>18</sup>. This study was designed to examine the anti-diabetic and anti-inflammatory effects of C. adscendens on alloxan and carrageenan induced rats respectively.

## MATERIALS AND METHODS

#### (i) Plant materials

Fresh whole plant of *C. adscendens* was collected from hill region of Anaikatty, Coimbatore district (Tamil Nadu, India) in Aug 2005 and authenticated by a botanist at the Botanical survey of India, Coimbatore. A voucher specimen has been deposited at the museum of our college.

#### (ii) Extraction Procedure

The fresh collected whole plants of *C. adscendens* were shade dried and then coarsely powdered in a blender. The coarse powder was successively solvent extracted in a soxhlet extractor using diethyl ether, ethylacetate, butanone, and n-butyl alcohol as

solvents. The extracts so obtained were further dried in vacuum desiccators. The residue obtained from various extracts was used for further studies by preserving it in refrigerator.

#### (iii) Phytochemical screening

Qualitative chemical test<sup>19, 20</sup> was performed for the presence of different class of constituents in plant extracts; these include alkaloids, flavonoids, saponins, tannins, etc.

#### (iv) Animals

Adult Wistar Albino rats (190-220 g) of either sex were employed in this study. The rats were maintained under standard laboratory conditions at 25  $\pm$  2°C, relative humidity 50  $\pm$  15% and normal photo period [12 hrs dark/12 hrs light] were used for the experiment. Rats were fed with standard dry pellet diet (Goldmohur Brand, M/s Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. The experimental protocol has been approved by the Institutional Animal Ethics committee and by the Regulatory body of the government.

#### (v) Acute toxicity study

Acute oral toxicity was performed by following OECD-423 guidelines (Acute toxic class method), albino rats (n=5) of either sex selected by random sampling were used for acute toxicity study<sup>21</sup>. The animals were kept fasting for overnight and provided only with water, after which the extracts were administrated orally at 5 mg/kg body weight by gastric incubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, and then the same dose repeated again to confirm the toxic dose. The test drug was found to be safe up to the dose 5000 mg/kg body weight and hence 1/10th of dose was taken as an effective dose (500 mg/kg).

#### (vi) Alloxan induced hyperglycemia

Diabetes mellitus was induced by single intraperitoneal injection of freshly prepared alloxan monohydrate (140 mg kg<sup>-1</sup>b.wt;

5% w/v in normal saline; Sigma chemicals, USA)<sup>22</sup>. Rats were supplied with 5% glucose solution for 48 hrs after alloxan injection in order to alloxan injection to prevent hypoglycemia. After 24 hrs of alloxan administration, blood samples were drawn and glucose levels were determined to confirm development of diabetes  $(>250 \text{ mg dL}^{-1}).$ The diabetic rats were divided into four groups, each containing 5 animals. Groups I, II and IV served as Control, Diabetic and Standard drug (Glibenclamide 5 mg kg<sup>-1</sup>, Alembic Ltd., Baroda, India). Group III butanone extract of were treated with C. adscendens at dose of 500 mg kg<sup>-1</sup>, respectively. Fasting serum glucose was estimated by glucose oxidase method<sup>23</sup>.

# (vii) Anti-inflammatory activity (Carrageenan induced rat paw edema)

Adult Wistar Albino rats (190-220 g) of either sex were divided into three groups containing five animals each. The dosage of the drugs administered to different groups was as follows. Group I - Control (normal saline0.5 ml/kg), Group II - carrageenan (0.1 ml of 1% carrageenan), Group - III (500 mg/kg, p.o) and Group IV - Indomethacin (5 mg/kg, p.o), acute inflammation was induced according to edema assay<sup>24</sup>. All the drugs were administered orally. Indomethacin was served as the reference standard anti-inflammatory drug. After 1 hr of administration of the drugs, 0.1 ml of 1% w/v carrageenan solution in normal saline was injected into the sub-plantar tissue of the left hind paw of the rat and the right hind paw was served as the control. The paw volume of rats were measured in the digital the plethysmograph (Ugobasile, Italy), at the end of 0 min, 60 min, 120 min, 180 min, 240 min, 360 min, and 480 min. The percentage increases in paw edema of the treated groups were compared with that of the control and inhibitory effect of the drugs were studied. The percentage of inhibition of edema was calculated. The relative potency of the drugs under investigation was calculated based upon the percentage inhibition of inflammation.

% inhibition of edema = Vc-Vt Vc

Where

Vt = Paw volume in test group animals Vc = Paw volume in control group animals

#### (viii) Statistical analysis

All the values were expressed as mean  $\pm$  standard error mean (SEM). Statistical significance was determined by one way ANOVA (Analysis of Variance) followed by Dunnett's multiple comparison test. *p* values <0.05 were considered as significant.

## **RESULTS AND DISCUSSION**

Phytochemical screening of *C. adscendens* extract indicated the presence of several bioactive compounds like alkaloids, flavonoids, tannins, terpenoids, saponins and steroids, which could be responsible for the versatile medicinal properties (Table.1).

Table 1Preliminary phytochemical screening of the various extracts of C. adscendens



Diabetes is a disorder of carbohydrate, fat and protein metabolism attributed to reduced circulating concentration of insulin, poor insulin sensitivity or poor glucose tolerance resulting in high sugar level<sup>16</sup>. Blood sugar levels measured in four different groups are given in the Fig (1).



Effect of C. adscendens extract on Blood glucose level in rats

#### Figure 1 Mean error bars in the graph represent the ± standard error. Significance of differences: p<0.05 compared to error.

The significant changes in the blood glucose levels were compared between the treated group and control group. The alloxan induced diabetic rats showed significant increase in the level of blood sugar. Plant extract at the dose of 500 mg/kg body weight showed significant decrease (p<0.05) on blood sugar level in 24 hrs of treatment and also comparable to that of the standard Glibenclamide. The exact mechanism by which the plant extract lowered the blood glucose level is not yet clear. The

standard drug Glibenclamide has been used to treat diabetes, which stimulate insulin secretion from pancreatic  $\beta$  cell, it may be suggested that the mechanism of action of butanone extract of *C. adscendens* is similar to Glibenclamide. The possible mechanism by which the plant extract decreases the blood sugar level may be potentiation of insulin effect either by increasing the pancreatic secretion of insulin from  $\beta$  cells of islets of langerhans. Medicinal plants and herbal extracts containing glycosides, flavonoids,

tannins. been reported etc., have to activity<sup>25</sup>. anti-diabetic demonstrate The phytochemical study of C. adscendens contains all the above phytoconstituents. It is therefore possible that the phytochemicals may be responsible for the observed anti-diabetic activity. The increasing peripheral blood glucose uptake and oxidative stress plays a major role in diabetes<sup>26</sup>. In the present study the antiinflammatory activity of butanone extract of C. adscendens was evaluated in carrageenan induced rats (Table 2). The extract found to significantly inhibit the carrageenan induced rat paw edema, a test which has significant predictive value. The butanone extract of C. adscendens decreased the paw edema significantly (p < 0.05) when compared to the control group. Anti-inflammatory agents act by inhibiting the mediators of acute inflammation. Carrageenan induced inflammation is useful in detecting orally active anti-inflammatory 28. agents<sup>27,</sup> Carrageenan edema is а phenomenon that liberates multimediated diversity of mediators. It is believed to be biphasic; the first phase (1 hr) involves the release of serotonin and histamine while the second phase (over 1 hr) is mediated by prostaglandins, the cyclooxygenase products, and the continuity between the two phases is provided by kinins<sup>29, 30</sup>. Butanone extract of C. adscendens produced time-dependent and significant inhibition of carrageenan induced paw edema at dose 500 mg/Kg when compared with control. Anti-inflammatory activity of many plants has been attributed to their high sterol/triterpene or flavonoid contents<sup>13</sup>.

## Table 2Anti-inflammatory activity of C. adscendens extract on Carrageenan induced Paw Edema in rats

	% increase in paw volume (minutes)						
Treatment	0	60	120	180	240	360	480
Control							
(Group-I)	64.5±3.4	73.7±4.2	85.9±4.4	98.6±5.2	106.4±5.6	108.2±5.8	110.5±6.2
Carrageenan							
(Group-II)	68.5±3.6	76.6±4.2	89.8±4.4	102.4±5.8	110.2±5.4	103.5±5.6	104.4±5.4
Plant extract							
(Group-III)	62.2±3.2	60.8±3.8*	64.8±3.5*	68.5±4.1**	72.6±3.8**	74.4±4.4**	76.5±4.6**
Indomethacin							
(Croup IV/)	60 0+0 5	E0 6+4 0*	62 6+4 4*	61 6+5 2**	60 0+1 1**	70 5+4 6**	74 0+4 0**

 $(Group-IV) \qquad 62.8\pm3.5 \qquad 58.6\pm4.9^* \qquad 62.6\pm4.4^* \qquad 64.6\pm5.2^{**} \qquad 69.8\pm4.4^{**} \qquad 72.5\pm4.6^{**} \qquad 74.9\pm4.8^{**}$  *Mean error bars in the table represent the* ± *standard error.* Significance of differences: \*p<0.05 & \*\*p<0.01 compared to control.

Since carrageenan-induced inflammation is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation<sup>31</sup>. The results of this study are an indication that, butanone extract of С. adscendens has potent anti-inflammatory activity. This anti-inflammatory property may provide additional benefits to use of C. adscendens in diabetes.

## CONCLUSION

The results of the present study support the traditional use of *C. adscendens* extract, possessing significant anti-diabetic and anti-

inflammatory activity. This may be due to the presence of saponins, tannins, phytosterols, terpenoids and flavonoids. It might be possible having dual property that due to of anti-diabetic and anti-inflammatory activity, this natural medicine can better manage the diabetes diabetes and associated complications, which usually accompanied by increased oxidative stress. However, further study is required to study the effects on lipid profile. isolation and characterization of anti-diabetic bioactive compound and establishment of exact mechanism of action. This might lead to the development of new drugs.

#### CONFLICT OF INTEREST

I declare no conflict of interest.

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