Antioxidant and Hypolipidemic Effect of
*Caralluma adscendens* Roxb. in Alloxanized Diabetic Rats

R.C. Patel Institute of Pharmaceutical Education and Research,
Shirpur-425405, Dist. Dhule, Maharashtra, India
Department of Pharmacognosy, R.C. Patel Institute of Pharmaceutical Education and Research,
Shirpur-425405, Dist. Dhule, Maharashtra, India

Abstract: In the present study, various extracts of *Caralluma adscendens* were evaluated by alloxan induced (150 mg kg⁻¹ i.p.) diabetic rats and oral glucose tolerance test. Fasting blood glucose estimation was done at 0, 30, 90 and 150 min after treatment. Lipid profile and body weight measurements were done on day 0, 7, 14 of the study. Antioxidant effects were also evaluated using Diphenyl-1-Picrylhydrazyl (DPPH), *in vitro* lipid peroxidation and reductive ability methods. The treatment showed significant lowering of blood glucose in the treated diabetic rat from 273 ± 4.01 to 82.1 ± 4. *mg dl⁻¹* by butanolic extract of *C. adscendens*, 150 min after the treatment (*p<0.01*). It also showed significant decrease in total cholesterol, LDL, triglyceride and TC /HDL and an increase in HDL in the treated diabetic animal group. Glucose tolerance was also improved. *In vitro* antioxidant activity showed that the butanolic extract exhibited potent free radical scavenging effects. All the results were compared with standard drug Glibenclamide

Key words: *Caralluma adscendens*, antidiabetic, hypolipidemic, alloxan, oral glucose

INTRODUCTION

Diabetes is a chronic metabolic disorder that continues to present a major worldwide health problem. The prevalence of diabetes for all age groups worldwide is projected to rise from 171 million in 2000 to 366 million in 2030 (Amos et al., 1997). Diabetes mellitus is characterized by hyperglycemia and alterations in carbohydrate, fat and protein metabolism, associated with absolute or relative deficiencies in insulin secretion and/or insulin action. Various studies have shown that diabetes mellitus is associated with increased formation of free radicals and decrease in antioxidant potential. This leads to oxidative damage of cell components such as proteins, lipids and nucleic acids. In both insulin dependent (type 1) and non-insulin-dependent diabetes (type 2) there is increased oxidative stress (Naziroglu and butterworth, 2005; Paolilloso et al., 1993). Earlier studies confirmed the efficacy of several medicinal plants in the modulation of oxidative stress associated with diabetes mellitus (Amos et al., 1997; Ansari et al., 2005). Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, there is increasing demand by the patients to use natural products with antidiabetic activity (Holman and Turner, 1991; Rao et al., 1997; Valiathan, 1998). *Caralluma adscendens* is succulent plant found wild in Africa, the Canary Islands, India, Arabia, southern Europe, Ceylon and Afghanistan. In India, it is found in the dry hills of Andhra Pradesh, Warangal and some other district.

*Caralluma adscendens*, family: Asclepiadaceae commonly known as makadushing. The Phytochemistry of genus *Caralluma* is characterized by many pregnane glycosides, while recently megastigmane glycosides also have been isolated from *Caralluma negevensis* (Jayakar et al., 2004) with few flavones (Bader et al., 2003; Gencor). As *Caralluma atteenuata* Wight. the fresh whole plant contains luteolin-4-O-neohesperidoside, a flavonoid (flavones glycoside) identified as the major chemical constituents of the plant (Ramesh et al., 1998). *Caralluma adscendens* is stated to contain saponin glycosides, bitters, pregnane glycosides (caratubersides A and B and various boucerosides).

*Caralluma adscendens* is a traditional food consumed in the form of a pickle and vegetable and is also eaten during famines (The Wealth of India Raw Material, 1992). Traditionally, the juice of the plant is combined with black pepper (*Piper nigrum* L., Piperaceae) in treating migraine. The plant is also eaten raw as a treatment for

Corresponding Author: A.U. Tatiya, R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur-425405, Dist. Dhule, Maharashtra, India
diabetes (Venkatesh et al., 2003). Caralluma species have been used for centuries in semi-arid areas of Pakistan as emergency food (Bnouhaim et al., 2003). Caralluma species have shown anti-inflammatory (Ramesh et al., 1998; Zakaria et al., 2001), gastric mucosa protecting and antiulcer properties (Al-Harbi et al., 1994). Caralluma edulis is known for its antidiabetic and antioxidant properties (Rao et al., 1997; Wadood et al., 1989) and Caralluma attenuata for their antihyperglycemic activity (Jayakar et al., 2004; Venkatesh et al., 2003) however, no scientific investigation has so far been conducted on the antidiabetic activity of Caralluma ascendenens. The present study was undertaken to verify the claim and evaluate the antidiabetic property of stem of Caralluma ascendenens var. fimbriata.

MATERIALS AND METHODS

Plant material: Fresh whole plant of Caralluma ascendenens, Aselepiadaceae (freely available) was collected (Year 2006) from Satara District and Laling Ghat of Dhule District (India) and authenticated by Dr. D.A. Patil, Botanist, SSVPS Science College, Department of Botany, Dhule (MS), India. A voucher specimen (RCP/07 C) of plant material kept at Institute level.

Preparation of extracts: The dried plant material was subjected to size reduction to a coarse powder by using pulveriser and passed through sieve (40#). This powder was packed into soxhlet apparatus and extracted successively with petroleum ether (60-80°), n-butanol, methanol and distilled water (yield 5.79, 13, 6.2 and 7.1%, respectively. Kokate (2006), Treese and Evans (1983) and Bruneton (1999). All extracts were filtered and concentrated under reduced pressure using rotary evaporator (Roteva Equitron, Mumbai, India) and dried in vacuum dryer till semisolid to solid mass was obtained and were stored in airtight containers in refrigerator below 10°C. The suspensions of petroleum ether, n-butanol and methanol extracts were prepared by using 0.5% Tween-80 (SD Fine Chemicals, Mumbai, India) in normal saline and solution of aqueous extract was prepared by using normal saline as solvent.

Phytochemical screening: Qualitative chemical test (Trease and Evans, 1983; Bruneton, 1999) was performed for the presence of different class of constituents in plant extracts, these include alkaloids, flavonoids, saponins, tannins, etc.

Animals: Three month old male Wistar albino rats (180-240 g) were obtained from Toxicology Center, Pune (Nov., 2006). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days they were kept in a 12:12 h light: dark cycle, temperature (22±2°C) and relative humidity (30-70%) controlled conditions. They were fed with a standard diet (Gold Moher, Lipton India Ltd.) and water ad libitum. The experimental protocol was approved by Institutional Animal Ethical committee. Animals described as fasted were deprived of food for 16 h but allowed free access to water.

Experimental design

Oral Glucose Tolerance Test (OGTT): Fasted rats were divided into six groups of six rats each. Group I served as control received distilled water. Group II received Glibenclamide at dose of 5 mg kg⁻¹ b.wt. as reference drug (Bnouhaim et al., 2003). Groups III-VI were treated with petroleum ether extract, methanol extract and aqueous extract of C. ascendenens at dose of 500 and 300 mg kg⁻¹ b.wt. for butanolic extract, respectively as a fine suspension orally. Thirty minutes after administration of the drug, all animal groups received glucose (2 g kg⁻¹ b.wt., p.o.) as per method of Syiem et al. (2002). Blood samples were collected from retro orbital plexus method just prior to glucose administration and at 30, 60, 90, 150 min after glucose loading. Serum was separated and blood glucose levels were measured immediately by glucose oxidase method of Trinder (1969).

Alloxan-induced hyperglycemia: Rats were made diabetic by single i.p., injection of 150 mg kg⁻¹ b.wt. of alloxan monohydrate (Sigma chemicals, USA; 5% w/v in normal saline) by method of Nagappa et al. (2003). Since, alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were kept for next 24 h on 5% w/v glucose solution to prevent hypoglycemia as per Gupta et al. (1984). Five days later blood samples were drawn and glucose levels were determined to confirm development of diabetes (>250 mg dl⁻¹). The diabetic rats were divided into seven groups, each containing 5 animals. Group I, II and III served as saline, diabetic and standard drug (glibenclamide 5 mg kg⁻¹, Alembic Ltd, Baroda, India) (Sokeng et al., 2005), control, respectively. Groups IV, V, VI and VII were treated with C. ascendenens extracts at dose of 500 mg kg⁻¹ for petroleum ether extract, methanol extract and aqueous extract and 300 mg kg⁻¹ for butanolic extract, respectively. Treatment with drugs started on 6th day of alloxan treatment (i.e., day 1) and was continued for 2 weeks. No sign of toxicity was
noticed on behavior and general health of the animals when exposed to extracts. Blood samples were drawn at weekly intervals till end of study. Fasting blood glucose estimation, lipid profile and body weight measurements were done on day 0, 7, 14 of the study.

**Biochemical analysis:** Fasting serum glucose was estimated by glucose oxidase method by Trinder (1969). Serum was separated and analyzed for serum Total Cholesterol (TC) (Roeschlau et al., 1974), triglycerides (TG) (Muller et al., 1977), High Density Lipoprotein (HDL) (Allain et al., 1974) and Total Cholesterol/ High Density Lipoprotein (TC/ HDL). By using Friedwald formula the concentration of Low Density Lipoprotein (LDL) in serum was calculated (Friedewald et al., 1972).

**Free radical scavenging activity in DPPH assay:** The antioxidant activity of the plant extracts and the standard (Ascorbic acid) was assessed on the basis of the radical scavenging effect of the stable DPPH free radical by Velazquez et al. (2003). The antioxidant activity of each extract was expressed in terms of IC$_{50}$ (µg mL$^{-1}$) concentration required to inhibit DPPH radical formation by 50%, calculated from the log-dose inhibition curve (Blois, 1958).

**In vitro lipid peroxidation scavenging:** The degree of lipid peroxidation in rat liver homogenate was assayed by estimating the thioabarbituric acid-reactive substances (TBARS) using the standard method with minor modifications by Ohkawa et al. (1979), Kumar et al. (2005) and Mondal and Muzzundar (2006). The antioxidant activity of each extract was expressed in terms of IC$_{50}$. The percentage of anti-lipid per oxidation effect (%ALP) was calculated by Feldman et al. (1999).

**Reductive ability:** In this method antioxidant compound forms a coloured complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, which is measured at 700 nm. Increase in absorbance of the reaction mixture indicates the reducing power of the extracts as per Oyaizu (1986).

**Statistical analysis:** Data was expressed as Mean±standard error mean (SEM). Statistical analysis was done by one-way ANOVA and post hoc Dunnet test, with p<0.005 considered as significant difference.

**RESULTS AND DISCUSSION**

**Alloxan induced hyperglycemia:** Alloxan induces diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycemia (Szadelski, 2001). Administration of alloxan (150 mg kg$^{-1}$, i.p.) led to elevation of fasting blood glucose level, along with significant decrease in body weight which was maintained over a period of 2 weeks and it was partially restored or improved upon administration of C. ascendentens extract. The antidiabetic effect of extracts of C. ascendentens on fasting blood glucose level is shown in Fig. 1. Two weeks of daily treatment of various extracts of C. ascendentens led to fall in blood glucose level by 30-70%. Butanol, methanol, aqueous and petroleum ether extract significantly (p<0.01) decreased the elevated blood glucose level in comparison to untreated diabetic rats. Treatment with glibenclamide and C. ascendentens extract on alloxan induced diabetic rats produced significant reduction in total cholesterol, triglyceride and LDL levels. HDL levels were significantly increased by Glibenclamide, n- butanol and methanol extracts while aqueous and pet ether extract have little effect on lipid profile, as shown in Table 1. The n-butanol extract significantly reduced total cholesterol from 174.9±2.6 to 94.2±2.3* mg dL$^{-1}$, triglyceride level from 168.5±5.6 to 100±1.8* mg dL$^{-1}$ and LDL level from 116.1±1.9 to 25±3.1* mg dL$^{-1}$ in diabetic group treated with C. ascendentens (*p<0.05).

Alloxan caused body weight reduction, which was reversed by n-butanol from 201.9±1.7 to 222±0±8* g, by methanol extract from 201.9±1.7 to 222±0±1.4* g and by aqueous extracts from 201.9±1.7 to 218±6±1.6* (p<0.01) but petroleum ether extract failed to cause such reversal (p<0.05), as shown in Table 2.
Table 1: The effect of 2-week treatment with various extracts of C. adscendens on blood lipid profile after alloxan (150 mg kg⁻¹ p.) induced diabetes in rats

| Groups | Treatment                  | Dose (mg kg⁻¹ b.w.) | Average serum lipid profile (mg dl⁻¹) | Total cholesterol | Triglycerides | HDL cholesterol | LDL cholesterol | TC/HDL |
|--------|----------------------------|---------------------|--------------------------------------|-------------------|---------------|-----------------|-----------------|--------|-------|
| I      | Vehicle control            | 0.2 mL²             | 78.4±0.92                            | 84.7±0.77         | 52.4±0.87     | 9.3±0.61        | 1.5±0.02        |        |
| II     | Diabetic control           | 0.2 mL²             | 174.5±2.69                           | 51.0±0.71         | 116.1±1.91    | 7.0±0.19        |                  |        |
| III    | Gibellenicamide control   | 5                   | 113.4±2.52                           | 78.9±0.46         | 91.4±1.76     | 10.4±0.65       |                  |        |
| IV     | Diabetic+CAP               | 164±0.03            | 153±0.33                             | 126±1.68          | 94±0.19**     | 15.7±0.89       |                  |        |
| V      | Diabetic+CAM               | 500                 | 117±0.40                             | 110±0.25          | 110±0.40      | 5.7±0.25        |                  |        |
| VI     | Diabetic+CAA               | 120±0.20            | 128±0.20                             | 110±0.40          | 94±0.19**     | 15.7±0.89       |                  |        |
| VII    | Diabetic+CAP               | 500                 | 94±0.23                              | 106±0.20          | 126±1.68      | 94±0.19**       |                  |        |

Values are expressed as Mean±SEM (n = 5). *Vehicle (Normal saline). **Alloxan single dose of 150 mg kg⁻¹ i.p., in normal saline. CAP: Petroleum ether extract, CAM: Methanol extract, CAA: Aqueous extract CAB: Butanol extract. *p<0.01, **p<0.05 compared with diabetic control. TC/HDL: Total Cholesterol/HDL Cholesterol.

Table 2: The effect of 2-week treatment with various extracts of Caralluma adscendens on body weight (g) after (150 mg kg⁻¹ p.) induced diabetes in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose (mg kg⁻¹ b.w.)</th>
<th>Body weight (g)</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle control</td>
<td>0.2 mL²</td>
<td>211±0.97</td>
<td>224±1.70</td>
<td>229±2.60</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>0.2 mL²</td>
<td>212±0.58</td>
<td>201±0.58</td>
<td>201±1.73</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Gibellenicamide control</td>
<td>5</td>
<td>214±0.68</td>
<td>222±1.40</td>
<td>222±0.85</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Diabetic+CAP</td>
<td>500</td>
<td>214±0.68</td>
<td>206±1.12</td>
<td>206±1.05**</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Diabetic+CAM</td>
<td>500</td>
<td>214±0.68</td>
<td>219±1.77</td>
<td>222±0.65</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>Diabetic+CAA</td>
<td>500</td>
<td>213±4.85</td>
<td>217±4.58</td>
<td>218±4.60</td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>Diabetic+CAP</td>
<td>500</td>
<td>214±0.69</td>
<td>220±1.16</td>
<td>220±0.64**</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM (n = 5). *Vehicle (Normal saline). **Alloxan single dose of 150 mg kg⁻¹ i.p., in normal saline. CAP: Petroleum ether extract, CAM: Methanol extract, CAA: Aqueous extract CAB: Butanol extract. *p<0.01, **p<0.05 compared with diabetic control.

Table 3: Comparative effect of different extracts of C. adscendens on blood glucose level in orally glucose fed rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose (mg kg⁻¹ b.w.)</th>
<th>Blood glucose (mg dl⁻¹)</th>
<th>0 min</th>
<th>30 min</th>
<th>90 min</th>
<th>150 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose</td>
<td>2000</td>
<td>63±0.75</td>
<td>149±0.99</td>
<td>124±4.13</td>
<td>91±3.67</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Glucose+Gibellenicamide</td>
<td>500</td>
<td>63±0.80</td>
<td>128±1.01</td>
<td>108±2.16</td>
<td>77±0.70</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Glucose+CAM</td>
<td>500</td>
<td>70±0.61</td>
<td>144±1.09**</td>
<td>118±0.74**</td>
<td>88±0.84**</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Glucose+CAA</td>
<td>500</td>
<td>63±0.77</td>
<td>130±1.66**</td>
<td>117±1.19**</td>
<td>75±0.88**</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Glucose+CAB</td>
<td>500</td>
<td>70±0.76</td>
<td>145±1.38**</td>
<td>118±1.09**</td>
<td>88±1.88**</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Glucose+CAP</td>
<td>500</td>
<td>70±0.43</td>
<td>120±0.68*</td>
<td>87±0.110*</td>
<td>72±0.64*</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM (n = 6). CAP: Petroleum ether extract, CAM: Methanol extract, CAA: Aqueous extract CAB: Butanol fraction. *p<0.01, **p<0.05 compared with Glucose treated rats.

Oral glucose tolerance test: Administration of the crude extracts, orally 30 min prior to glucose load showed improved glucose tolerance in normal rats. The maximum glucose tolerance was noted at the 30th min. The blood glucose levels were reduced considerably within 90 min of the drug administration. In glucose fed rats with glibenclamide, butanol and methanol extract significantly increased (p<0.01) tolerance for glucose, as shown in Table 3.

Antioxidant activity: Table 4 shows antioxidant effect of various extracts of C. adscendens. Butanol extract showed a decrease in absorbance at 517 nm. The IC₅₀ values were found to be 157.62 and 8.70 µg ML⁻¹ for butanol extract and ascorbic acid, respectively.

The butanol extract also has moderate lipid peroxidation scavenging activity (IC₅₀ 201.28 µg mL⁻¹). It offered protection against the lipid peroxidation in rat liver homogenate.

Table 4: IC₅₀ values of various extracts of C. adscendens and standard ascorbic acid tested against DPPH radical and TBARS assay

<table>
<thead>
<tr>
<th>Drugs</th>
<th>TBARS</th>
<th>DPPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAB</td>
<td>201.28</td>
<td>157.62</td>
</tr>
<tr>
<td>CAM</td>
<td>277.16</td>
<td>225.63</td>
</tr>
<tr>
<td>CAA</td>
<td>543.47</td>
<td>258.26</td>
</tr>
<tr>
<td>CAP</td>
<td>985.99</td>
<td>458.71</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>159.77</td>
<td>8.70</td>
</tr>
</tbody>
</table>

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing power of C. adscendens is increased with increasing amount of sample. The reductive ability was also found to be increasing in a dose dependent manner, with butanol extract showing the maximum absorbance as shown in Fig. 2.
Fig. 2: The reductive ability of *C. adscendens* extracts

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 levels. The results of present study showed that *C. adscendens* extracts exert anti-hyperglycemic effect in glucose loaded and in alloxan induced diabetic rats. In glucose loaded animals, it is possible that the extract may act by potentiating the pancreatic secretion or increasing the glucose uptake as per Venkatesh et al. (2003).

Hyperlipidaemia is a recognized consequence of diabetes mellitus according to Waddood et al. (1989), Yokozawa et al. (1998) and Zakaria et al. (2002). Diabetes-induced hyperlipidaemia is attributable to excess mobilization of fat from the adipose tissue due to the under utilization of glucose according to Krishnakumar et al. (2000) and Momo et al. (2006). In alloxan-induced diabetic rats, rise in blood glucose level was accompanied with marked increase in TC, LDL-C, TG and reduction in HDL-C. Repeated oral administration of *C. adscendens* extracts normalized these effects possibly by controlling selective uptake of lipoproteins or their metabolism by different tissues. This implies that *C. adscendens* can prevent or be helpful in reducing the complications of lipid profile seen in whom hyperglycemia and hypercholesterolemia coexist quite often. Alloxan has been shown to induce free radical production and can cause tissue injury. Pancrease is especially susceptible to the action of alloxan induced free radical damage (Joy and Kuttan, 1999). From *in vitro* antioxidant assay, butanolic extract of *C. adscendens* has potent radical and lipid peroxide scavenging activity. This antioxidant property may provide additional benefits to use of *C. adscendens* in diabetes. The phytochemical screening revealed the presence of flavonoids, sterols/terpenoids and saponins, which play a major role in controlling free radicals and diabetes (Ivorra et al., 1989; Rahman and Zaman, 1989; Rao et al., 1997). Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats (Nagappa et al., 2003). The anti-diabetic effect of *C. adscendens* extracts may be due to the presence of more than one anti-hyperglycemic principle and their synergistic properties. In this study, the anti-hyperglycemic activity caused by glibenclamide in alloxan-induced diabetic rats is an indication of the presence of some beta cells, as glibenclamide is known to stimulate insulin secretion from beta cells. The *C. adscendens* may have stimulating effect on the remnant beta cells. Thus present study supports the traditional claim, however, pharmacological studies required to evaluate the exact mechanism of action and components responsible for. Therefore, further work is in progress for isolation and identification of the components from *C. adscendens*.

ACKNOWLEDGMENTS

Authors are grateful to Dr. D.A. Patil, Botanist, SSVP'S Science College, Dept. of Botany, Dhule (MS), India, for the identification of the plant material and also Department of Pharmacology, R.C. Patel College of Pharmacy, Shirpur for providing facilities to carry out pharmacological activities.

REFERENCES


