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Hypoglycemic activity of *Caralluma attenuata* extract on alloxan induced diabetic rat

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ABSTRACT

Caralluma atteunata extract have been reported to have antihyperglycemic effect. White albino rats were rendered diabetic by intraperitoneal administration of alloxan (120 mg/kg body weight). Oral administration of ethanolic extract of *Caralluma atteunata* 500 mg/kg body wt was given orally for 30 days. Experimental results showed that, alloxan significantly elevated the blood sugar level whereas treatment with ethanolic extract of *Caralluma atteunata* (500 mg/kg body wt.) depressed the alloxan induced high blood sugar level and also it shows the marked changes in the level of Insulin, Hemoglobin, Glycosylated hemoglobin, urea, protein, Hexokinase, Pyruvate kinase, Glucose-6-phophatase, Fructose 1, 6 biphosphatase and glucose 6 phosphate dehydrogenase. This study strongly suggests that the ethanolic extract of *Caralluma atteunata* attributed its prominent hypoglycemic activity on experimental diabetic rats through suppression of gluconeogenesis and stimulation of glucose oxidation using the pentose phosphate pathway.

Introduction

Diabetes is the most common of the endocrine disorders and posses a serious challenge to health care world wide. A type of diabetes varies in different parts of the world. However it is projected that by 2010 at least 239 million people will the affected b y the disease. (Mandrup *et al., 1998)* Diabetes is a disorder of mechanism due to absolute deficiency on diminished effectiveness of insulin. Different types of oral hypoglycemic agents treatment of diabetes mellitus, there is increasing demand by patients to use natural products with anti diabetic activity. Insulin cannot be used orally and continuous use of the synthetic anti diabetic drugs causes side effects and toxicity (Yamamoto *et al*., 2001) (Tolman and Chandramouli, 2003).Herbal treatments are becoming increasing by popular as the herbal preparations have no least side effects (Raja sekaran *et al*., 2005).Many herbs and plant products have been shown to have anti hyperglycemic action. (Ajgaonkar, 1979)

The whole plant of *Caralluma attenuata* belonging to the Asclepiadaceae family. It is widely distributed in India (Tamilnadu) and other countries. In India Herbal drugs are based on ayurvedha is more commonly practiced from long past years and it is less expensive. In accordance to recommendations by the WHO expert committee on diabetes mellitus investigations on hypoglycemic agents from medicinal plant have become more important (WHO, 1980). *Caralluma* is found in dry regions of the world has significant anti inflammatory and anti tumor activity (Deepak *et al*., 1993) (Ramesh *et al., 1999*). The extracts of *Caralluma attenuata, Caralluma edulis* had hypoglycemic properties. *Caralluma sps* have been extensively used for paralysis, joint pain and fever. (Khan and Khatoon, 2008).

The objective of this study is to investigate the pharmacological effects of *Caralluma attenuata* plant extracts on Blood glucose, Insulin, Urea, Hemoglobin, Protein, Glycosylated hemoglobin, Hexokinase, Pyruvate kinase, glucose -6- phosphatase, Glucose-6- phosphate dehydrogenase and fructose 1,6 bis phosphatase in alloxan induced diabetic rats. The effect *Caralluma attenuata* plant extracts was compared to Glibenclamide used as a reference hypoglycemic drug.

Materials and methods:

Alloxan was purchased from ponmani chemicals Pvt Ltd., Trichy. All other chemicals and reagents used were of analytical grade. The fresh plant was identified authenticated and the voucher specimen has been kept in our laboratory for future reference. The plants were shade dried powdered and passed through a 40- mesh sieve and kept well closed container for further extraction 500g of dried powdered plant material were extracted successively with ethanol using soxhelt apparatus. The residual extract was suspended in water for overnight and filtered. The filtrate was dried and was stored at 4°c until used. A known volume of the residual extract is suspended in distilled water and was orally administrated to the animals during the experimental period. Male albino rats of the Wister strain weighing about 160-220 gm were used for this study. The rats were 10-12 weeks of age at the time of this study. They were acclimatized to the animal's house conditions at least for one week before carrying out any experimental work. The rats were fed ad libitum with normal pellet. (Hindustan lever Ltd., Bangalore, India) and water. The experiments were designed and conducted in accordance with the ethical norms approved by ministry of social justice and empowerment, Government of India and International animal ethics committee guidelines for the investigation of experimental pain in conscious animals. Diabetes was induced by single IP injection of 120 mg/kg of alloxan mono hydrate in sterile saline.(Ravi vijayavargia *et al*.,2000).After 72 hrs alloxan injection, the diabetic rats

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(glucose level >250mg/dl) were separated and used for the study. (Perfumi and Tacconi, 1996). The method described by (Pari and Satheesh, 2004) was adopted. In the experiment a total of 30 rats (18 diabetic surviving rats and 12 normal rats) were used. The rats were divided in to 5 groups (6 rats/ group) after the induction of alloxan.

Group I: Normal untreated rats

Group II: Normal rats were given CAEt 500mg / kg body weight in aqueous solution daily for 30 days.

Group III: Diabetic control

Group IV: Diabetic rats were given CAEt 500mg / kg body weight in aqueous solution daily for 30 days.

Group V – Diabetic rats were given glibenclamide 600µg/kg body weight (Pari and Uma,1999) in aqueous solution daily for 30 days.

On completion of 30 days of experimental period the 18 hours fasted rats were anaesthetized and sacrificed by cervical dislocation. Blood was collected with anticoagulant and used for the preparation of plasma. Blood collected without anti coagulant was used for serum separation.

Biochemical estimation:

Blood glucose was determined by the method (Sasaki *et al*., 1972) using ortho toludine reagent. Glycosylated hemoglobin content was assayed by (Nayak and Pattabiraman, 1981) method. Hemoglobin content was estimated by cyan methemoglobin method of (Drabkin and Austin, 1932). Protein was determined by (Lowry *et al*., 1951) method. Blood urea was determined by the method of DAM (Natelson *et al*., 1951). Insulin content was estimated by using RIA kits (for rats) supplied by Linco research Inc. (Stat diagnostics, Mumbai). Amount of Hexokinase was assayed by the method of (Branstrup *et al*., 1957). Glucose-6- phosphatase was estimated by (Koide and Oda, 1959) method. Pyruvate kinase activity was assayed by method of (Pogson and Denton, 1967). Activity of Fructose1,6 bis phosphatase estimated by (Gancedo and Gancedo,1971) method. Glucose-6-phosphate dehydrogenase activity was assayed by the method of (Ell and Kirkman, 1961).

Stastical analysis

The values are expressed as mean ±SD for six rats in each group. All other data were analyzed with SPSS / 15.0 student software. Hypothesis testing method included one way analysis of variance (ANOVA) followed by post hoc testing performed with least significant difference (LSD). Test. The" P" □ value of less than 0.05 was considered indicate statistical significance.

Results:

(Note : Values are given as Mean + SD of six rats from each group , values are statistically significant at *p<0.05; (a) Normal + CAEt rats were compare with normal rats; b) Diabetic control rats were compared with normal rats (c) CAEt treated rats were compared with diabetic control and glibenclamide (d) Glibenclamide treated rats were compared with diabetic control).

Table 1shows that the level of blood glucose, insulin and urea in control and experimental animals in each group. The level of blood glucose and urea was significantly increased and insulin level was decreased in alloxan induced diabetic rats (group III) as compared to control (group I) and normal animals treated with *Caralluma attenuata* plant extract (group II).However the level of blood glucose, insulin and urea was returned to near normal concentrations in diabetic rats treated with CAEt and glibenclamide. CAEt showed comparable effect to that of glibenclamide.

Table 2 indicates the amount of Hemoglobin, protein and Glycosylated hemoglobin in serum of the control and experimental animals in each group. A significant decrease in Hemoglobin, protein and increase in Glycosylated hemoglobin level in serum of diabetic rats as compared to control animals. Oral administration of CAEt and glibenclamide to diabetic animals revert back to normal concentrations.

Table 3 shows the level of serum hexokinase, pyruvate kinase and glucose-6-phosphate dehydrogenase in control and experimental animals in each group. The level of Hexokinase, Pyruvate kinase and Glucose-6-phosphate dehydrogenase were significantly decreased in diabetic animals as compared to control animals. However, oral administration of CAEt and glibenclamide in diabetic rats increase the level of Hexokinase, Pyruvate kinase and Glucose-6-phosphate dehydrogenase.

Table 4 shows that the level of glucose – 6- phosphatase and fructose 1,6 bis phosphatase in control and experimental animals in each group. The activity glucose -6-phosphatase, fructose 1,6 bis phosphatase was increased in diabetic control animals as compared to control animals . However, oral administration of CAEt and glibenclamide to diabetic animals revert back to normal conditions.

Discussion:

Diabetes mellitus is a chronic disease characterize by high blood glucose levels due to an absolute or relative deficiency of circulating insulin level. Although various types of oral hypoglycemic agents are currently available along with insulin for treating Diabetes mellitus, there is a growing interest in herbal medicines due to side effect associated with the existing therapeutic hypoglycemic agents (Kameswara and Appa, 2001). Animal models have extensively been used to study diabetes mellitus (Namila *et al*., 2000).

In the present study diabetes was introduced in rats using intra peritoneal administration of alloxan and the hypoglycemic effect *Caralluma attenuata* was investigated. Blood sugar level increased in alloxan induced diabetic rats. Since alloxan causes a massive reduction in insulin release by the destruction of ß cells of the islets of langerhans (Goldner and Gomari, 1943). We have observed a significantly decrease in blood glucose in glibenclamide and *Caralluma attenuata* treated diabetic rats, when compared with diabetic control. The possible mechanism of *Caralluma attenuata* action may be through potential of pancreatic secretion insulin from ß-cells of islets or due to enhanced transport of glucose to the peripheral tissue. In our results showed an increased in the level of insulin in diabetic rats treated with *Caralluma attenuata* and also we proved an increase in the immune reactivity of insulin present in the ß-cells of pancreas.

The literature survey indicates a number of other plants have also been reported to have hypoglycemic and insulin release stimulator effects (Prince *et al*., 1997). In our study the high level of Glycosylated hemoglobin was reduced after one month of treatment by *Caralluma attenuata* indicate the long lasting effect of this plant extract as a hypoglycemic agent. Associated with the changes in insulin are changes in hepatic enzyme activity. Glycosylated hemoglobin comprises about 3.4-5.8% total hemoglobin in normal red cells, but it is increased in patients of overt Diabetes (Arora *et al*., 2009). Hexokinase is a glycolytic enzyme whose activity is induced by insulin (Mayes, 1983).As expected the activity of this enzyme is reduced in non

treated diabetic rats compared to control rats. The activity of this enzyme had increased after one month of treatment by *Caralluma attenuata,* as a result that would be consequent to the increase in insulin concentration. Glucose-6-phosphatase and fructose 1, 6 bis phosphatase are enzymes of gluconeogenesis, whose activity are repressed by insulin (Mayes, 1983). As expected the activity of these enzymes are increased in non treated diabetic rats compared to control rats. The activities of these enzymes are decreased after one month of treatment by *Caralluma attenuata,* which would be consequent to the increase in insulin concentration. Decrease in plasma protein may be due to micro protein urea, which is important clinical markers of diabetic neuropathy and are may be due to increased protein catabolism in diabetes (Almdal and Nilstrys, 1988). In our present study plasma total protein level have increased after one month of treatment by *Caralluma attenuata*.This could be due to increase in concentration of insulin, which is improvement in renal function. Serum urea level was higher in diabetic control than in control groups. The level of the substance had reduced after one month of treatment by *Caralluma attenuata*.

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Table: 1 Blood glucose, Plasma Insulin and Blood urea level, in control and experimental groups of rats

Group	Blood Glucose (mg/dl)	Plasma Insulin (mg/dl)	Blood Urea (mg/dl)
I	90.31 ± 3.21	94.17 ± 2.31	35.32 ± 1.45
II	93.15 ± 4.30 [a]	91.05 ± 9.52 [a]	34.43 ± 2.82 [a]
III	231.31 ± 2.41 [b*]	58.71 ±10.32 [b*]	53.26 ± 1.31 [b*]
IV	107.31 ±8.42 [c*]	89.35 ± 4.65 [c*]	38.66 ± 3.24 [c*]
V	91.10 ± 2.61 [d*]	91.52 ± 5.81 [d*]	35.58 ± 5.65 [d*]

Values are given as mean ± SD of 6 rats from each group.
* Values are statistically significant * P<0.05.
a) Normal + CAEt rats were compared with normal rats.
b) Diabetic rats were compared with normal rats.
c) CAEt treated diabetic rats were compared with diabetic rats and glibenclamide treated diabetic rats.
d) Glibenclamide treated diabetic rats were compared with diabetic rat

Table: 2 Protein, Hemoglobin and Glycosylated Hemoglobin levels in control and experimental groups of rats

Group	Protein (mg/dl)	Hemoglobin(mg/dl)	Glycosylated Hemoglobin(mg/dl)
I	6.71 ± 1.32	11.72 ± 0.83	4.23 ± 1.03
II	6.82 ± 1.44 [a]	10.76± 0.58 [a]	4.63 ± 0.82 [a]
III	4.16 ± 0.71 [b*]	7.21 ± 0.53 [b*]	9.81 ± 0.41 [b*]
IV	6.62 ± 0.62 [c*]	9.4 ± 0.69 [c*]	5.72 ± 0.92 [c*]
V	7.37 ± 0.21 [d*]	11.9 ± 2.01 [d*]	4.69 ± 0.23 [d*]

Values are given as mean ± SD of 6 rats from each group.
* Values are statistically significant * P<0.05.
a) Normal + CAEt rats were compared with normal rats.
b) Diabetic rats were compared with normal rats.
c) CAEt treated diabetic rats were compared with diabetic rats and glibenclamide treated diabetic rats.
d) Glibenclamide treated diabetic rats were compared with diabetic rats.

Table: 3 Hexokinase, Pyruvate kinase and Glucose-6- phosphate dehydrogenase levels in control and experimental groups of rats.

Group	Hexokinase(IU/L)	Pyruvate kinase (IU/L)	Glucose-6-phosphate dehydrogenase(IU/L)
I	128.30 ± 2.21	213.65 ± 3.5	68.52 ± 6.12
II	136.42 ± 2.61 [a]	209.43± 2.6 [a]	68.76 ± 7.45 [a]
III	80.12 ±1.36 [b*]	160.23 ± 2.1 [b*]	46.95 ± 7.65 [b*]
IV	124.43 ±1.80 [c*]	204.61 ± 2.2 [c*]	64.96 ± 3.64 [c*]
V	127.52 ±2.75 [d*]	206.23 ± 3.1 [d*]	67.69 ± 3.21 [d*]

Values are given as mean ± SD of 6 rats from each group.
* Values are statistically significant * P<0.05.
a) Normal + CAEt rats were compared with normal rats.
b) Diabetic rats were compared with normal rats.
c) CAEt treated diabetic rats were compared with diabetic rats and glibenclamide treated diabetic rats.
d) Glibenclamide treated diabetic rats were compared with diabetic rats.

Table: 4 Glucose-6-phosphatase and Fructose 1,6 bis phosphatase level in control and experimental group of rats

Group	Glucose-6-phosphatase (IU/L)	Fructose1,6bisphosphatase (IU/L)
I	52.4± 9.35	32.64 ± 3.06
II	51.1± 10.65 [a]	31.89 ± 3.13 [a]
III	96.9 ±10.25 [b*]	58.82± 8.21 [b*]
IV	61.2 ± 10.76 [c*]	36.12± 2.01 [c*]
V	52.8 ±10.68 [d*]	31.21± 3.45 [d*]

Values are given as mean ± SD of 6 rats from each group.
* Values are statistically significant * P<0.05.
a) Normal + CAEt rats were compared with normal rats.
b) Diabetic rats were compared with normal rats.
c) CAEt treated diabetic rats were compared with diabetic rats and glibenclamide treated diabetic rats.
d) Glibenclamide treated diabetic rats were compared with diabetic rats.