Original Article

Effect of Leaves of *Carissa spinarum* Linn. on Blood Glucose and Lipid Profile in Alloxan Induced Diabetic Rats

Amreen Fatima*, Prem Prakash Singh, Raghuveer Irchhaiya & Parul Agarwal

Institute of Pharmacy, Bundelkhand University, Jhansi 284121, U.P, India

Address for Correspondence

Department of Pharmacognosy, Institute of Pharmacy, Bundelkhand University, Jhansi 284121, U.P, India
Tel: +919795950163.

E-mail: amreenfatima070@gmail.com

ABSTRACT

Objective: The aim of the study is to analyze the antidiabetic effect of oral administration of acetone extract of *Carissa spinarum* (*C. spinarum*) leaves on blood glucose, haemoglobin, plasma insulin and lipid peroxidation to alloxan-induced diabetic rats. **Method:** Diabetes mellitus was induced by injection of alloxan (150mg/kg body weight), via the intraparitoneally. The extract was administered orally at 200, 400 and 600 mg/kg body weight (both to normal and diabetic rats), and glimepiride at 2g/kg body weight. **Results:** Acute toxicity study shows acetonic extract is safe or non toxic even at the dose of 2000 mg/kg body weight. The effect of the extract at a dose of 600 mg/kg body weight was highly significant than 200mg/kg, 400 mg/kg and Glimepiride 2 g/kg body weight. Administration of the extract at various doses and glimepiride tends to significantly bring the FBG level to normal. Urine sugar also tends to decrease at different doses of acetonic extract of *C. Spinarum*, The effect of *C. spinarum* extract on Cholesterol, Triglyceride, LDL and VLDL values increased significantly in diabetic rats, whereas HDL values decreased significantly in diabetic rats. *C. spinarum* extract treatment decreased the Cholesterol, Triglyceride, LDL and VLDL values and increased the HDL values significantly in diabetic treated rats. **Conclusions:** Our results suggest that acetonic extract of *C. spinarum* exhibit antihyperglycemic as well as antihyperlipidaemic effects on alloxan-induced diabetic rats.

Keywords: *Carissa spinarum*, Hyperglycemia, Blood Glucose, Lipid Profile, Alloxan.

INTRODUCTION

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects[1]. Diabetes mellitus (DM) is a major health problem all over the world. Globally, the number of people that has been

diagnosed with diabetes has exploded in the past two decades. In 2000, 151 million people in the world were diabetic. With the current rate of increase (6% per annum), it has been projected that 221 million people will be diabetic in 2010 and 324 million by 2025[2]. Diabetes mellitus is an endocrine disorder that is characterized by hyperglycemia[3]. Diabetes is a metabolic disorder of carbohydrate, fat and protein, affecting a large number of populations in the world[4]. Diabetes mellitus has caused significant morbidity and mortality due to microvascular and macrovascular complications[5]. Diabetes is mainly attributed to the rapid rise in unhealthy life style, urbanization and aging[6]. Diabetes mellitus is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fat and protein[7]. The growing public interest and awareness of natural medicines have led the pharmaceutical industry and academic researchers to pay more attention to medicinal plants[8]. The role of traditional medicines in the solution of health problems is invaluable on a global level. This is all the more striking when we consider the fact that approximately 80% of the people living in less developed countries rely exclusively on traditional medicines for their healthcare needs[9].

Carissa spinarum (Apocynaceae – the oleander family) is most often found in semi-arid coastal areas of the tropical regions around the Indian Ocean, on fine-textured soils such as clays and clay-loams[10]. *C. spinarum* is often discussed under its many synonyms that are *Carissa ovata*, *Carissa brownii*, *Carissa diffusa*, *Carissa edulis and Carissa opaca*. It is also called Wild Karaunda.

Carissa spinarum is an evergreen shrub which can grow up to 3.5 m tall, branches glabrous or puberulous, leaves glabrous, opposite and ovate, spines arising between the petiole, hard and sharp, 2.5–3.5 cm long. Flowers white, corolla tube slender 8–12 mm long. Fruit berry somewhat ellipsoid, dark purple when ripe, with milky juice, edible. The plant is distributed in drier parts of India and Pakistan (from Punjab-Himalayas up to 6000 ft, in Murree) Burma and Sri Lanka[11].

In traditional system of medicine the plant is used as purgative, for the treatment of rheumatism, cleaning worm infested wounds of animals and in snake bite[12,13]. Earlier studies have shown that the extract of the plant possesses Cardiotonic[14], Anticonvulsant[15], Hepatoprotective[16], Antiarthritic[17], Antibacterial[18], Potent Antioxidant[19] and CNS depressant activity[20]. Various cardiac glycosides[21], caffeic acid[22], ursolic acid, naringin[18], germacrane sesquiterpene and lignans[19] were reported from this plant.

Roots and leaves are rich in tannins, carissone, palmitic acid, benzyl benzoate, farnesene, stigmasterol, ursolic acid, lupeol, campesterol,17-hydroxy-11-oxo-nor-β-amyrone and urs-12-ene-3β, 22β-diol-17-carboxylic acid[23]. The plant has many medicinal properties viz. roots are bitter, stomachic, antihelmintic and are used to treat malaria, wound, inflammation, stomach ache, bleeding after delivery, muscle cramps, dysentery, ulcer, diabetes, male and female weakness, skin disorders and antidote for snake bite. Leaves are used to treat remittent fever, jaundice, hepatitis and chest pain[24-26].

MATERIALS AND METHODS

Collection and authentication of plant material

The leaves of *Carissa spinarum* was collected from village amarpur near Jhansi, (U.P) India and authenticated by Dr. Gaurav Nigam, Department of Botany, Bundelkhand University, Jhansi. A voucher specimen has been deposited at the department of botany,

bundelkhand university, Jhansi. (BU/M.pharma/601)

Plant Material

Leaves of Carissa spinarum was air dried in the shade and coarsely powder by using motar pistle. The powder plant material (100g) was packed in the Soxhlet apparatus and continuously extracted by acetone at the temperature 60°C. The percentage yield was calculated against 100 g of powder drug. It was 14.1%.

Chemicals and drugs

All the chemicals and solvents were of analytical grade and were procured from Loba Chemie Pvt. Ltd. 107, Wodehouse Road, Mumbai, India. Alloxan was procured from Qualikems Fine Chem Pvt. Ltd., Nandesari, Vadodara, India. Glimepiride sample is taken from Indian Drug Pharmaceutical Pvt. Ltd., Rishikesh.

Animals

Adult male Wistar albino rats, weighing 150-250g were used for the studies. The animals were maintained under standard hygienic conditions. The animals were given food and water and were exposed to proper light and dark cycle (12 hours each of light and darkness). The experimental protocol was approved by the Institutional Animal Ethics Committee of the Bundelkhand University (Reference number:BU/Pharm/IAEC/12/019).

Acute toxicity study

The study was carried out to determine the therapeutic dose of the acetonic extract. For acute toxicity study evaluation of *Carissa spinarum* acetonic extract was aseptically suspended in 0.5% Tween 80 in distilled water and administered through oral route by gavage at dose of 200, 500, 1000, and 2000mg/kg. Body weight. The general activity of rats was monitored for 1 h after dosing periodically during first 24 hours and then daily after for total of 14 days. Changes in normal behaviour of rats and their weights were monitored and time at which sign of toxicity or death appeared. The OECD guidelines were followed for the study[27].

Anti-diabetic activity (Alloxan induced model)

A freshly prepared solution of alloxan monohydrate (150 mg/kg body weight), in sterile normal saline solution, was injected intraparitoneally to overnight fasted rats[28,29]. Blood glucose was measured after 72 hours of alloxanisation by Glucometer, accucheck-COMFORT (Roche-Diagnostics)[30]. Rats showing fasting blood glucose (FBG) levels > 250mg/dL were selected for the study.

The rats were divided into six groups with six rats in each group as follows; Group I: Normal control rats; Group II: Diabetic control rats; Group III: Diabetic rats that received *C. spinarum* extract (200 mg/kg body weight); Group IV: Diabetic rats that received *C. spinarum* extract (400 mg/kg body weight); Group V: Diabetic rats that received *C. spinarum* extract(600 mg/kg body weight); Group VI: Diabetic rats that received Glimepiride (2g/kg body weight) for a period of 14 days orally.

After the experimental regimen, the animals were fasted overnight and sacrificed by cervical dislocation under mild anaesthesia. Blood was collected on decapitation in two different tubes, one with anticoagulant for plasma and another without anticoagulant for serum separation. Serum and plasma was separated by centrifugation at 2,500rpm for 15 min, and utilised for biochemical studies. Blood glucose was estimated by an enzymatic glucose oxidase peroxidase (GOD-POD) method using a commercial kit (Span Diagnostics, Surat India). Plasma insulin was assayed by Axsym auto analyser (Abbott Laboratory, Abbott Park, IL, USA). TC, TG and HDL were analysed by kits (Roche Diagnostics GmbH,

Mannheim, Germany) on Hitachi auto analyser. LDL, very low density lipoproteins (VLDL) and were calculated using the formula of Friedewald et al, Haemoglobin was estimated by the method of Drabkin and Austin[31]. Values reported are mean of six experiments ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA). Tukey's test was used for multiple comparisons. The values were considered to be significantly different when $p < 0.05$.

RESULTS

Acute toxicity study

There was no mortality amongst the graded dose groups of animals and they did not show any toxicity or behavioural changes at a dose level of 2000mg/kg. This finding suggests that the acetonic extract is safe in or non-toxic to rats and hence dose of 200, 500, 1000 and 2000mg/kg, p.o. (per oral) were selected for the study.

Anti-diabetic activity

Table 1 shows the effect of *C. spinarum* extract on FBG in control and diabetic animals. Alloxan caused a significant increase in the FBG of experimental animals compared with control ($p<0.05$).The FBG was significantly reduced after 14 days of treatment in all animals except non diabetic control animals. The effect of the extract at a dose of 600mg/kg body weight was more highly significant than 200 mg/kg, 400 mg/kg and glimepiride 2g/kg body weight. There was significant decrease in total haemoglobin, plasma insulin and total protein levels in alloxan-induced diabetic rats, when compared to normal control rats. Administration of the extract at various doses and glimepiride tends to significantly bring the level to normal. In diabetic control rats, the urine sugar was (+ + +) but in the treatment group at 200mg/kg and 600mg/kg body weight urine sugar was decreased to (+ +) and (+), respectively. But the *C. spinarum* extract at 400mg/ kg body weight showed no urine sugar as compared with glimepiride.

Table 2 shows Lipid profile of normal, diabetic and treated diabetic rats. The effect of *C. spinarum* extract on Cholesterol, Triglyceride, LDL and VLDL values increased significantly in diabetic rats, whereas HDL values decreased significantly in diabetic rats. *C. spinarum* extract treatment decreased the Cholesterol, Triglyceride, LDL and VLDL values and increased the HDL values significantly in diabetic treated rats.

DISCUSSION

Diabetes mellitus induced by alloxan, is usually characterized by decreased insulin level, hyperglycemia, elevated triglycerides and total cholesterol, and decreased high density lipoprotein[32]. The high percentage reduction in plasma glucose levels, produced by the extract in this study, supports the use of the plant in the management of diabetes mellitus. The hypoglycemic effect of the extract may have been produced by the flavonoids, saponins and tannins present in the leaves[17,19]. The flavonoids, saponins and tannins are families of compounds with established hypoglycemic activity[33-38]. Thus, anyone or a combination of some or all of the above mentioned components could have been responsible for the hypoglycemic effect of the extract, observed in this study.

Hyperglycemia and hyperlipidaemia are important characteristics of diabetes mellitus; an endocrine disorder is one of the most common chronic diseases worldwide. Alloxan, a β-cytotoxin, induces diabetes mellitus by damaging the insulin secreting β-cells of the pancreas, resulting in decreased endogenous insulin release. Alloxan-administered rats become hyperglycaemic in a short period of time, followed by hepatic glucose over production[39]. Intraperitoneal administration of alloxan (150 mg/kg body

weight) effectively induced diabetes mellitus in normal rats as reflected by glycosuria, hyperglycemia, polyphagia, polydipsia and body weight loss compared with normal rats. The aim for the present work is to explore the scientific basis of the utility of the Acetonic extract of *C. spinarum* for correction of hyperglycemia and hyperlipidaemia in diabetes mellitus.

It was evident from the results that *C. spinarum* extract reduced the FBG level in alloxan-induced diabetic rats. The antihyperglycaemic effect of *C. spinarum* extract could be linked to more than one mechanism. The possible mechanism includes the stimulation of β-cells and subsequent release of insulin and activation of the insulin receptors. The plant's antihyperglyacemic action may be by potentiation of pancreatic secretion of insulin, which was clearly evidenced by the increased level of insulin in diabetic rats treated with *C. spinarum* extract. *C. spinarum* also acts as a hepatoprotective agent[16], so this evidently improves the function of the liver and maintains glucose uptake, enhances the transport of blood glucose to peripheral tissues and its utilization, which may be another mechanism of action.

Oral administration of *C. spinarum* extract for 14 consecutive days to diabetic rats decreased their food consumption and improved body weight. This could be due to a better control of the hyperglycaemic state in the diabetic rats. Decreased FBG could improve body weight in alloxan-induced diabetic rats[40,41]. The total haemoglobin level was found to be decreased in diabetic animals. During diabetes mellitus, the excess glucose present in the blood leads to glycation of tissue proteins[42]. Administration of extract to diabetic rats significantly increased the level of total haemoglobin and this might be due to the decreased level of blood glucose.

Diabetes mellitus is also associated with hyperlipidaemia with profound alteration in the concentration and composition of lipid[43]. Changes in the concentrations of the lipid with diabetes mellitus contribute to the development of cardio vascular diseases[44,46]. It is often associated with hypertension[47], abnormal lipoprotein metabolism, obesity, insulin resistance and diabetes mellitus[46-49].

CONCLUSION

The results of the present investigation clearly indicate that the extract of *C. spinarum* have a glucose lowering effect on alloxan-induced diabetic rats. It was also found to be highly effective in managing the complications associated with diabetes mellitus, such as haemoglobin, plasma insulin and hyperlipidaemia and prevents the defects in lipid metabolism. Therefore *C. spinarum* show therapeutic promise as a protective agent against the development and progression of atherosclerosis and possible related cardiovascular complications in diabetes mellitus. Further studies are in progress to isolate the active principle and elucidate the exact mechanism of action of *C. spinarum* leaves.

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Table 1. The Dose response effects of *Carissa spinarum* on FBG, haemoglobin, plasma insulin and urine sugar levels in normal and alloxan-induced diabetic rats

Groups	Fasting Blood Glucose (mg/dl) [a]		Haemoglobin (g/dl) [a]		Plasma insulin (µu/ml) [a]		Urine sugar
	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14	
Normal control	86.0±2.31	87.5±2.18	14.78±1.12	15.21±1.37	23.58±0.32	24.98±0.78	Nil
Diabetic control	232.2±0.76*	245.6±1.20*	12.81±0.61*	11.98±0.68*	19.76±0.55*	16.11±0.44*	+++
Diabetic + *C.spinarum* (200mg/kg)	243.0±1.70**	134.5±1.94**	12.11±0.63**	13.10±0.80**	14.77±0.34**	17.28±0.89**	++
Diabetic + *C.spinarum* (400mg/kg)	235.8 ± 0.89**	126.8± 0.95**	12.21±0.83**	14.66±0.63**	14.78±0.94**	20.11±0.58**	Nil
Diabetic + *C.spinarum* (600mg/kg)	239.1 ± 2.11**	97.4 ± 1.01**	12.98±0.76**	15.31±0.80**	15.12±0.35**	18.76±0.73**	+
Diabetic + Glimepiride	239.0 ± 1.93**	106.1 ± 1.21**	12.86±0.53**	15.64±0.41**	18.21±0.72**	21.22±0.56**	Trace

Statistical analysis a values are expressed as mean ± S.E.M. (n= 6) the results were considered statistically significant if $p < 0.05$ Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Student T-test. * $p< 0.05$, when compared to corresponding value of normal control ** $p < 0.05$, when compared to corresponding value of diabetic control. (+++) - shows more effective, (++) - shows effective, (+) - shows less effective.

Table 2. Effect of *Carissa spinarum* leaves extract on plasma cholesterol, triglyceride, LDLs, HDLs and VLDL in alloxan-induced diabetic rats

Group	Total cholesterol (mg/dl)	Triglyceride[a] (mg/dl)	LDLs[a] (mg/dl)	HDLs[a] (mg/dl)	VLDL[a] (mg/dl)
Normal control	87.9± 0.90	86.0 ±2.31	45.0± 1.63	29.2 ± 1.55	1.3± 1.35
Diabetic control	121.2± 0.48*	210.8 ±1.25*	36.4 ± 1.05*	68.2 ± 2.10*	28.9 ± 0.33*
Diabetic + *C.spinarum* (200mg/kg)	89.1± 1.23**	112.5± 0.98**	44.4±0.42**	43.4±2.95**	18.7±1.21**
Diabetic + *C.spinarum* (400mg/kg)	62.3± 3.29**	105.6 ± 1.20**	46.7±1.43**	41.6±2.25**	18.3±0.89**
Diabetic + *C.spinarum* (600mg/kg)	57.2 ±2.01**	100.5 ± 1.22**	49.2±2.01**	42.5±2.87**	15.2 ±1.21**
Diabetic + Glimepiride	59.6 ±2.69**	101.6± 1.91**	45.7±1.31**	37.5±2.12**	20.1±0.69**

Statistical analysis a values are expressed as mean ± S.E.M. (n= 6) the results were considered statistically significant if $p < 0.05$ Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Student T-test. * $p< 0.05$, when compared to corresponding value of normal control ** $p < 0.05$, when compared to corresponding value of diabetic control.