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Research Article

HEPATOPROTECTIVE AND ANTIDIABETIC EFFECT OF METHANOL EXTRACT OF *CARALLUMA FIMBRIATA* IN STREPTATOZOCIN INDUCED DIABETIC ALBINO RATS

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ABSTRACT

Objective: Effect of methanol extract of *Caralluma fimbriata* (MCF) on streptatozocin (STZ) 50 mg/kg b.w. induced diabetic rats was studied. Methods: Oral administration of methanol extract of *Caralluma fimbriata* (MCF) to diabetic rats at a dose of 100 and 200 mg/kg body weight resulted in a significant reduction in blood glucose in STZ induced diabetic rats at different treatment period (0^{th}day, 7^{th}day, 14^{th} day and 21^{st} day). Results: The MCF treated diabetic rats were significantly recovered from hepatotoxicity, diabetic and renal toxicity by analyzing the factors such as, body weight, Glycosylated hemoglobin, Plasma insulin, Total protein, SGOT, SGPT and ALP. Further the histopathology results of MCF treated rats also confirmed the significant recovery of liver and kidney destruction. Conclusion: Our study reveals the therapeutic effect of MCF for diabetes and its related complications.

Keywords: Anti diabetic, *Caralluma fimbriata*; Streptatozocin, Hepatoprotective, MCF.

INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by increased blood glucose levels, resulting from inadequate or absence of pancreatic insulin. It is estimated that 3% of the world's population have diabetes and the prevalence is expected to double by the year 2025 to 6.3% according to WHO [1,2]. As the prevalence of this disease increases, there is a need to look for more efficient drugs with fewer side effects. A wide variety of pharmacological drugs are being used for Diabetes' treatment. However currently available drugs may lead to obesity and hyperandrogenemia while reducing blood glucose.

Traditional medicinal plants are used throughout the world for the treatment of Diabetes mellitus, because the plants are considered to be less toxic, low cost and free from side effects than the synthetic medicines [3]. Most of the medicinal plants are sufficiently validated for their therapeutic efficiency [4]. Therefore investigation of drugs from traditional medicinal plants has become more important. Several such herbs have shown antidiabetic activity when assessed using presently available experimental techniques [5-8].

Caralluma fimbriata is an endemic, succulent cactus and wild medicinal plant in the family Apocynaceae, growing in dry places, used by tribal Indians to suppress hunger and enhance endurance. It has been used as chutneys, pickles and vegetable for many centuries [9]. *C. fimbriata* reported for appetite control, antiobesogenoic and other metabolic regulations [10]. In this study, we made an attempt to evaluate the hepatoprotective activity of *C. fimbriata* along with antidiabetic effect in Streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Plant collection and Authentication

Caralluma fimbriata was collected from Pudhukkottai, Tamil Nadu and authenticated (No. BSI/SRC/5/23/09-10/Tech.-1569) by Botanical survey of India (BSI), Southern Circle, Coimbatore, Tamil Nadu, India.

Preparation of methanol extract of Caralluma fimbriata (MCF)

The whole parts of *Caralluma fimbriata* were washed in tap water and powder after drying in blotted paper. The powdered material (100 g) of the plant was extracted with methanol in a soxhlet extractor and the methanol extract was dried in Rotary evaporator (Yamato, Japan).

Animals

Adult male albino rats of Wistar strain weighing approximately 150-200 g were procured from Tamil University, Thanjavur. They were acclimatized to animal house conditions, fed with standard rat feed supplied by Hindusthan Lever Ltd., Bangalore. All the animal experiments were conducted according to the ethical norms approved by the Institutional ethical committee of PRIST University, Tamil Nadu, India (Ref.: IAEC/Ph.D1/2011-12).

Induction of experimental diabetes

The animals were fasted overnight and then prepared diabetic by a single intraperitoneal injection of Streptozotocin (50 mg/kg body weight). Streptozotocin was prepared just prior to injection in 0.1 N cold sterile sodium citrate buffers [11]. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia. Control rats were injected with citrate buffer alone.

Three days after Streptozotocin injection, plasma blood glucose level of each animal was determined. After the development of diabetes, the rats with moderate diabetes having glycosuria and hyperglycemia were considered as diabetic and used for the drug treatment.

Experimental design

In the experiment, the rats were divided into 5 groups for the evaluation of histopathological and biochemical parameters with six animals in each group.

Group I: Normal control rats.

Group II: Diabetic control rats

Group III: Diabetic rats given MCF (100 mg/kg b.w./Rat/day) for 21 days.

Group IV: Diabetic rats given MCF (200 mg/kg b.w./Rat/day) for 21 days.

Group V: Diabetic rats given Glibenclamide (600 µg/kg b.w./Rat/day) for 21 days.

Every 7 days the body weight and blood glucose were monitored and after 21 days of treatment, the rats were sacrificed by cervical dislocation. Blood glucose was determined by the method [12] using ortho toludine reagent. Serum separated from blood of each animal and analyzed the biochemical parameters such as, Glycosylated hemoglobin [13], Plasma insulin (Boehringer–Mannheim kit), total protein [14], SGOT [15], SGPT [15] and ALP [16]. The liver and kidney were collected in ice cold containers containing 10% formaldehyde for histopathological analysis.

Statistical Analysis

Results were expressed as means±SD and the difference between the groups were tested by one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test using the software "GraphPad Instat". The $p<0.05$ were considered as statistically significant.

RESULTS

Administration of STZ results in a significant increase in blood glucose level and also reduces the body weight. However, after the treatment of diabetic rats with 100mg/kg b.w and 200mg/kg b.w of MCF for 21 days, the blood glucose level significantly decreases and body weight increases compared with the levels of untreated diabetic rats as shown in Table 1 & Table 2.

Table 1: Effect of MCF on body weight in STZ-induced diabetic rats

Groups	Body weight (g/day)			
	0 days	7th day	14th day	21st day
Group I	156.17±4.21	156.50±4.19	160.5±10.93	163.17±12.61
Group II	147.33±3.63#	136.67±8.55#	136.12±11.34#	133.83±10.40#
Group III	152.83±2.12*	153.10±8.02*	153.77±9.65*	153.4 ±13.12*
Group IV	154.02±3.54*	155.83±11.56*	158.44±8.45*	160.57±7.62*
Group V	156.65±2.43*	157.53±7.43*	157.94±6.73*	158.83±13.83*

Results are expressed as mean±SD; n=6; *and #=p<0.05

Table 2: Effect of MCF on plasma glucose levels in STZ-induced diabetic rats

Groups	Plasma glucose levels (mg/dl)			
	0 day	7th day	14th day	21st day
Group I	84.91±6.62	88.22±6.27	80.60±6.75	84.54±6.63
Group II	185.38±7.44##	217.36±9.47##	243.32±11.30##	267.51±9.76##
Group III	182.53±13.42NS	149.74±8.26**	138.63±12.41**	125.31±10.72**
Group IV	181.75±14.63NS	145.21±6.68**	133.04±4.45**	121.82±8.95**
Group V	183.82±13.15NS	134.56±6.82**	123.32±8.83**	119.22±8.34**

Results are expressed as mean±SD; n=6; **and ##=p<0.0001; NS=Not Significant

Serum insulin level was decreased significantly in untreated diabetic rats compared with control rats (p≤ 0.0001). Treatment with 100mg/kg b.w and 200mg/kg b.w of MCF for 21 days increases the serum insulin levels comparatively. MCF administration results in the restoration of biochemical parameters to normal levels. There is a significant increase in glycosylated hemoglobin and decrease in protein levels in diabetic rats when compared to normal rats. Administration of Glibenclamide (600 µg/kg b.w./day/rat) to diabetic rats revert back the levels to normal conditions (Table 3).

Table 3: Effect of MCF on glycosylated hemoglobin, plasma insulin levels and total protein in STZ-induced diabetic rats

Groups	Glycosylated hemoglobin (% total Hb)	Plasma insulin (µU/ml)	Total protein (g/dL)
Group I	0.41±0.05	18.35±1.11	8.23±0.41
Group II	0.83±0.03##	6.39±1.82##	3.35±0.62##
Group III	0.48±0.05**	11.03±2.59*	6.03±0.32**
Group IV	0.36±0.07**	13.99±1.43*	6.83±0.58**
Group V	0.52±0.03**	12.16±1.42*	7.56±0.45**

Results are expressed as mean±SD; n=6; **and ##=p<0.0001; *=p<0.05

Serum transaminases SGOT, SGPT and ALP levels were significantly increased in the diabetic control rats. After treatment with 100mg/kg b.w, 200mg/kg b.w of MCF and Glibenclamide (600 µg/kg b.w./day/rat), the serum transaminases level was brought back to almost normal levels (Table 4).

Table 4: Effect of MCF on SGOT, SGPT, and ALP in STZ-induced diabetic rats

Groups	SGOT(U/L)	SGPT(U/L)	ALP(U/L)
Group I	63.45 ± 4.65	48.38 ± 2.12	56.22±7.24
Group II	128.53 ± 7.14##	55.24 ± 1.42##	88.47±4.86##
Group III	65.83± 4.97**	44.28 ± 8.25*	73.06±3.18**
Group IV	67.24 ± 6.02**	46.81 ± 1.64*	68.22±2.18**
Group V	69.54 ± 4.76**	47.95± 3.13*	65.83±4.87**

Results are expressed as mean±SD; n=6; **and ##=p<0.0001; *=p<0.05

Histopathalogy studies of liver and kidney of MCF treated diabetic rats

Histopathology of liver and kidney was studied in normal, diabetic and treated groups. The normal histological liver section shows the well arranged cells and clear central vein. In the diabetic group, it shows the complete destruction of hepatocytes degeneration of central vein, fatty degeneration and also shows the damaged hepatocytes and various size vacuoles. Histopathological changes are restored near to normal in the *C. fimbriata* treated group (Fig. 1).

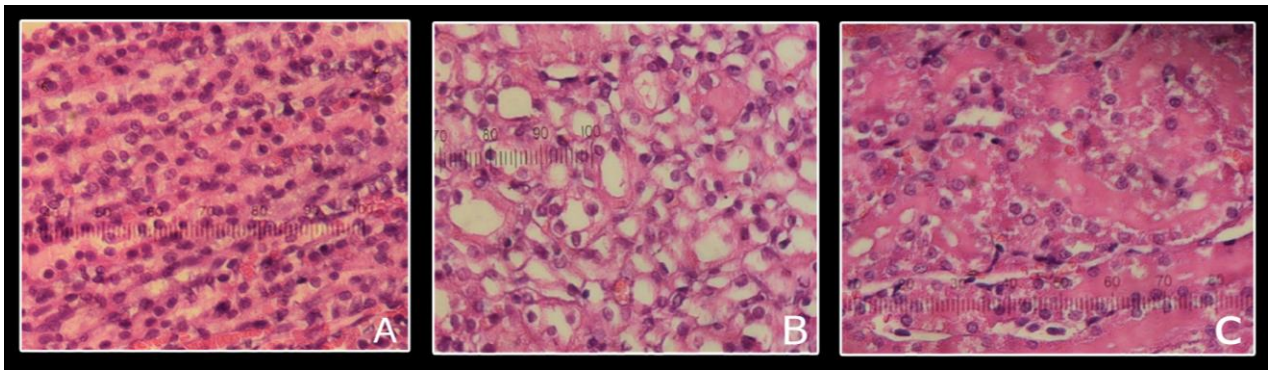

Fig. 1: Histopathological changes in liver of MCF treated diabetic rats

1. Normal histological section of rat liver
2. Histological section of diabetic rat liver showing destruction of hepatocytes & various vacuoles
3. Histopathological changes are restored near to normal in the MCF (200mg/kg b.w.) treated group

The normal kidney section shows the well arranged cells. The glomerular basement membrane is compact. Diabetic groups showed that the endocytic vacuoles as characteristically seen in the proximal tubules and the thickening of the glomerular basement with glomerulosclerosis. The damage is recovered with the treatment of *Caralluma fimbriata* (Fig. 2).

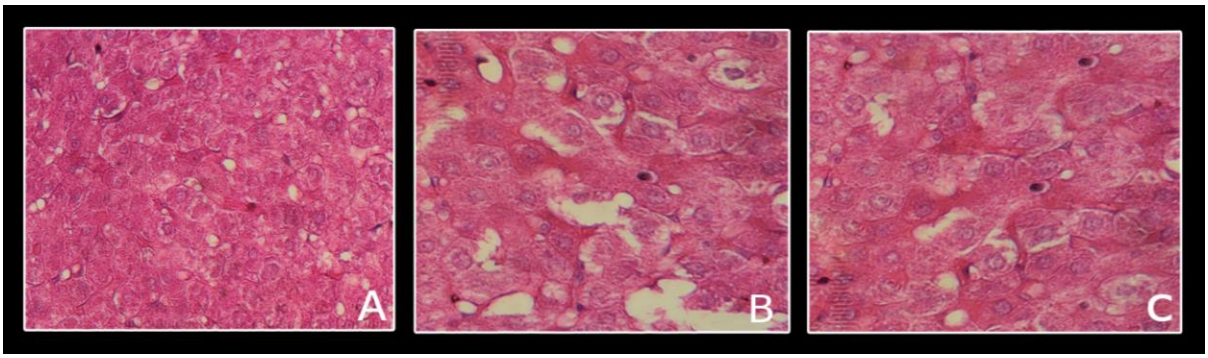

Fig. 2: Histopathological changes in kidney of MCF treated diabetic rats

1. Normal histological section of rat kidney
2. Histological section of diabetic rat kidney showing damaged cells & various size vacuoles
3. Histopathological changes are restored near to normal in the treated (200mg/kg b.w.) group of *C.fimbriata*

DISCUSSION

Diabetes Mellitus is a metabolic disorder characterized by a loss of glucose homeostatis with the disturbance of carbohydrates, fat, protein metabolism resulting from defects in insulin [17]. In our study, diabetes was induced in rats by single intraperitoneal injection of STZ (50mg/kg b.w) and the hepatoprotective, antidiabetic activity of MCF was determined.

Treatment of Diabetes mellitus with oral hypoglycemic agents like sulphonylurea and biguanide is associated with severe adverse effects [18]. Therefore, herbal drugs are gaining importance in the treatment of various diseases. The administration of STZ to the normal rats results in the destruction of beta cells of Islets of Langerhans and malfunctioning of the pancreas. This results in the diabetic condition leading to the increase in the blood glucose levels and decreased body weight in the untreated diabetic rats. Due to the action of STZ, the beta cells undergo destruction of necrosis [19]. The elevation of blood glucose in STZ induced diabetic rats may be due to lower levels of plasma insulin [20]. We observed a significant reduction of blood glucose in MCF and glibenclamide treated diabetic rats was compared to diabetic control. This is due to the pancreatic secretion insulin from beta cells of the Islets of Langerhans. Increased insulin levels in MCF treated rats showed the possible mechanism of glucose uptake. The obtained result is similar to *Caralluma edulis* [21], *Calocybe indicia* [22], *Helianthus annuus* [23], *Swertia chirayita, Andrographis paniculata* [24] and *Xanthosoma sagittifolium* [25] treated diabetic rats.

The blood glucose level was estimated in both normal and STZ induced diabetic rats. After treatment with STZ, the fasting blood glucose level was significantly increased in the range of 100-200mg/dl and it was significantly ($p< 0.05$) reduced by 14 days treatment with MCF. On the progression of treatment with MCF, fasting blood glucose reduced from 7th day (Table.2). In streptozotocin induced diabetic rats, MCF treatment significantly ($p<0.05$) increased the body weight and reduced plasma glucose levels. The results showed that the decreased postprandial glucose in animals may be correlated with decreased gluconeogenic activity [26]. So, this may be the reason for the increased body weight in MCF treated rats [27]. Serum transaminases are responsible for producing ketone bodies from amino acids and produce increased concentration of glucose levels [28]. Increase in SGOT and SGPT results in increased glucose levels. After the treatment with MCF, SGPT, SGOT levels was brought back to normal suggesting the regeneration process. Similar results found in *Xanthosoma sagittifolium* treated diabetic rats [25]. Reduction in ALP shows its stability of biliary function against the damage caused by STZ. Histopathogical studies of liver and kidney in diabetic and treated groups are determined to show the protective action of the MCF.

CONCLUSION

The methanolic extract of *Caralluma fimbriata* significantly controlled the diabetic condition including oxidative stress in liver and kidney. Further investigations regarding the synergistic activity of the extract and phytochemical isolation are in the process.

REFERENCES

1. Andrade-Cetto A, Heinrich M. Mexican plants with hypoglycemic effect used in the treatment of diabetes. J Ethnopharmacol 2005; 73:325-348.
2. Attele AS, Zhou Y, Xie J, Wu JA, Zhang L, Dey L, Pugh W, Rue PA, Polonsky KS, Yuan C. Antidiabetic effects of *Panax ginseng* Berry extract and the identification of an effective component. Diabetes 2002; 51:1851-1858.
3. Prince PSM, Menon VP, Pari L. Hypoglycaemic activity of *Syzygium cumini* seeds: effect on lipid peroxidation in alloxan diabetic rats. J Ethnopharmacol 1998; 61:1-7.
4. Babu V, Gangadevi T, Subranabuab A. Anihyperglycemic effect of *Cassia keinii* leaf extract in glucose feed normal rats and alloxan induced diabetic rats. Indian J Pharmacol 2002; 34:409-415.
5. Saifi AQ, Shinde S, Kavishwar WK, Gupta SR. Some aspects of phytochemistry and hypoglycemic actions of *Pterocarpus marsupium*. J Res Indian Med 1971; 6 (2):205-207.
6. Mukherjee K, Ghosh NC, Datta T. *Coccinia indica* Linn. as potential hypoglycemic agent. Indian J Exp Bio 1972; 10 (5):347-349.
7. Coimbra TC, Danni FF, Blotta RM, Da-Periara CA, Guedes MD, Graf RG. Plants employed in the treatment of diabetes mellitus results of an ethnopharmacological survey in Porto Alegre, Brazil. Fitoterapia 1992; 63(4):320-322.
8. Ajit K, Choudhary BK, Bandhopadhyay NG. Preliminary studies on the inorganic constituents of some indigenous hypoglycemic herbs on oral glucose tolerance test. J Ethnopharmacol 1999; 64:179-184.
9. Zakaria MNM, Islam MW, Radhakrishnan R, Chen HB, Kamil M, Al-Gifri AN, Chan K, Al-Attas A. Anti-nociceptive and anti-inflammatory properties of *Caralluma Arabica.* J. Ethnopharmacol 2001; 76:155-158.
10. Kamalakannan S, Rajendran R, Venkatesh R, Clayton P, Akbarsha AK. Anti-obesegenic and Anti-Atherosclerotic Properties of *Caralluma fimbriata* Extract. J Nut Metabolism 2011; Article ID 285301:6.
11. Sekar N, Kanthasamy S, William S, Subramanian S, Govindasasamy S. Insulinic actions of vanadate in diabetic rats. Pharmocology Res 1990; 22:207-217.
12. Sasaki T, Matsy S, Sonae A. Effect of acetic acid concentration on the color reaction in the O-toluidine boric acid method for blood glucose estimation. Rinsho kagaku 1972; 1:346-353.
13. Sudhakar NS, Pattabiraman TN. A new colorimetric method for the estimation of glycosylated hemoglobin. Clin Chin Acta 1981; 109:267-274.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein Measurement with the Folin Phenol Reagent. J Biol Chem 1951; 193:265-275.
15. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957; 28:56-63.
16. Bessey OA, Lowery DH, Brock MJ. A method for the rapid determination of alkaline phosphatase with five cubic meters of serum. J Biol Chem 1964; 164:321.
17. Barcelo A, Rajpathak S. Incidence and prevalence of diabetes mellitus in the Americas. Am J Public Health 2001; 10:300-308.
18. Weidmann P, Boehlen LM, De-courten M. Pathogenesis and treatment of hypertension associated with diabetes mellitus. Am Heart J 1993; 125:1498-1513.
19. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 2001; 50(6):537-546.
20. Maiti R, Jana D, Das UK, Ghosh D. Antidiabetic effect of aqueous extract of seed of *Tamarindus indica* in streptozotocin induced diabetic rats. J Ethnopharmacol 2004; 92: 85-91.
21. Sayantan R, Abhishek S. Antidiabetic activity of *Caralluma edulis* bark and leaf extract against streptozotocin induced diabetic rats. NSHM J Pharm Healthcare Management 2012; 03:76-81.
22. Paramasivam R, Shanmugasundaram K. Potent antihyperglycaemic activity of *Calocybe indica* in streptozotocin induced diabetic rats antihyperglycemic activity of *Calocybe indica*. Int J Pharm Pharm Sci 2013; 5(2):512-515.
23. Shivani S, Sunil S. Antidiabetic effect of *Helianthus Annuus* L., seeds ethanolic etract in streptozotocin-nicotinamide induced type 2 diabetes mellitus. Int J Pharm Pharm Sci 2013; 5(2):382-387.
24. Vinod KV, Khomedndra KS, Kamaruz ZMD. Antihyperglycemic activity of Swertia *chirayita* and *Andrographis paniculata* plant extracts in streptozotocin induced diabetic rats. Int J Pharm Pharm Sci 2013; 5(3):305-311.
25. Shajeela PS, Kalpanadevi V, Mohan VR, Potential antidiabetic, hypolipidaemic and antioxidant effects of *Xanthosoma sagittifolium* extract in alloxan induced diabetic rats. Int J Pharm Pharm Sci 2013; 5(1):27-31.
26. Oliveira HC, Dos SMP, Grigulom R, Lima LL, Martins DTO, Lima JCS, Stoppiglia LF, Lopes CF, Kawashita NF, Antidiabetic activity of *Vatairea macrocarpa* extract in rats. J Ethnopharmacol 2008; 115:515-519.
27. Pandikumar P, Prakash BN, Ignaciumuthu S. Hypoglycemic and antihyperglycemic effect of *Begonia malabarica* Lam. In normal and streptozotocin induced diabetic rats. J Ethnopharmacol 2009; 124:111-115.
28. Lemieux J, Pichette C, Vinay P, Gougoux A. Cellular Mechanism of antiammoniagenic effect of ketone bodies in the dog. Am J Physiol-Renal 1980; 239(5):420-426.