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Anti-hyperglycemic Activities of Aqueous and Ethanolic Extracts *Cynodon dactylon* (Linn) Streptozotocin-induced Diabetic Rats

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Abstract: A study was undertaken to evaluate the anti-hyperglycemic and anti-hyperlipidemic activity of ethanolic and aqueous extract of *Cynodon dactylon* (Linn), respectively. The various parameters studied included fasting blood sugar levels, serum lipid levels, total hemoglobin and glycosylated hemoglobin in diabetic and normal rats. Streptozotocin was used to induce diabetes. Treatment with the extract at single dose levels showed a significant increase in the liver glycogen and a significant decrease in fasting blood glucose and glycosylated hemoglobin levels. The total cholesterol and serum triglycerides levels were also significantly reduced and the HDL cholesterol levels were significantly increased upon treatment with the extract thus proving the potent anti-diabetic and modify effect of anti-hyperlipidemic property of the plant. Present results clearly indicate that *Cynodon dactylon* (Linn), has potent for anti-hyperglycemic effects in Streptozotocin induced diabetic rats.

Key words: *Cynodon dactylon*, anti-hyperglycemic, anti-hyperlipidemic

Introduction

According to *Ayurveda*, India's traditional pharmacopoeia, *Cynodon dactylon* plant is pungent, bitter, fragrant, heating, appetizer, vulnerary, anthelmintic, antipyretic, alexiteric. It destroys foulness of breath, useful in leucoderma, bronchitis, piles, asthma, tumors and enlargement of the spleen. According to Unani system of medicine, *Cynodon dactylon* plant is bitter, sharp hot taste, good odor, laxative, brain and heart tonic, aphrodisiac, alexipharmic, emetic, emmenagogue, expectorant, carminative and useful against grippe in children and for pains, inflammations and toothache (Agharkar, 1991). Virus-affected discolored leaves of *Cynodon dactylon* are used for the treatment of liver complaints. In Homoeopathic systems of medicine, it is used to treat all types of bleeding and skin troubles (Ghosh, 1988; Oudhia *et al*., 1998).

Present number of diabetics worldwide is 150 million and this is likely to increase to 300 million or more by the year 2025 (King *et al*., 1998). Reasons for this rise include increase in sedentary lifestyle, consumption of energy rich diet, obesity, higher life span, etc. (Yajnik, 2001). Many herbal medicines have been recommended for the treatment of diabetes (Marles and Farnsworth, 1995; Alarcon-Aguilara *et al*., 1998). Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones (Pari and Umamaheswari, 2000). Furthermore, after the recommendation made by WHO on diabetes mellitus investigation on hypoglycemic agents from medicinal plants have become more important (WHO, 1980).

Experimental type I diabetes induced with streptozotocin (STZ) in rats display many of the features seen in human subject with uncontrolled diabetes mellitus (Chattopadhyay *et al*., 1991). The development of new therapies that are able to improve glycemia management and even to cure diabetes is of great interest.

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There is no scientific evidence to support the anti-diabetic effect of *Cynodon dactylon* (Linn). The objective of this study was to as certain the scientific basis for the use of this plant in the management of diabetes using STZ- induced diabetic rats.

Materials and Methods

Plant Material

The plant was *Cynodon dactylon* collected from Trichirapalli district, Tamilnadu, India. The plant was authenticated by the botanical survey of India, Coimbatore, Tamilnadu, India.

The aerial plant was dried in shade, pulverized by a mechanical grinder and passed through a 40 mesh sieve to get the fine powder and stored in an airtight container.

Preparation of Aqueous Extract

The aqueous extract was prepared by cold maceration of 150 g of the shade dried plant powder in 500 mL of distilled water for 7 days. After extraction, the solvent was filtered and then evaporated by Rota vapor. The obtained *Cynodon dactylon* aqueous extract was stored at -20°C until being used.

Preparation of Cold Extract

Cynodon dactylon plant was air dried for 3-5 days in the shade. Five hundred grams were macerated with ethanol (80%, v/v) and kept for 48 h at room temperature (28-30°C). The extraction was filtered and the filtrate was evaporated to dryness under reduced pressure at 50°C (yield 15.6%, w/w, dry weight basis) and stored at 4°C until use. After extraction, the solvent was filtered and then evaporated by Rota vapor. The obtained *Cynodon dactylon* ethanolic extract was stored at -20°C until being used.

Chemicals

Streptozotocin was obtained from Sigma Chemicals. All other chemicals used were of analytical grade.

Animals

Healthy adult male Wistar Albino rats between 2 and 3 months of age and weighing 250-280 g were used for the study. Housed individually in polypropylene cages, maintained under standard conditions (12 h light and 12 h dark cycle, 25±30°C, 35-60% humidity), the animals were fed with standard rat pellet diet and water *ad libitum*.

Induction of Diabetes

Streptozotocin was prepared in freshly prepared citrate buffer (0.1 mol L^{-1}, pH 4.5) and injected intraperitonially at a concentration of 65 mg kg^{-1} of body weight to the experimental animals.

Experimental Design

Animals were divided into seven groups of six rats each. The extract was administered for 45 days.

Group I: Normal rats administrated with normal saline (0.5 mL).

Group II: Normal control rats administered with single intraperitoneal injection of Streptozotocin (65 mg kg^{-1}).

Group III: Normal Control rats administered *Cynodon dactylon* aqueous extract (400 mg kg^{-1}).

Group IV: Normal Control rats administered *Cynodon dactylon* ethanolic extract (400 mg kg^{-1}).

Group V: Diabetic rats administered *Cynodon dactylon* aqueous extract (400 mg kg^{-1}).
Group VI: Diabetic rats administered *Cynodon dactylon* ethanolic extract (400 mg kg^{-1}).
Group VII: Diabetic rats administered reference drug glibenclamide (5 mg kg^{-1}).

The effects of administration of *Cynodon dactylon* ethanolic and aqueous extract to normal and diabetic rats were determined by measuring fasting plasma glucose levels, serum lipid profiles, liver glycogen levels (Nicholas, 1956), total hemoglobin, glycosylated hemoglobin level and initial and final changes in body weight. All other biochemical parameters were determined on day 45 after the animals were sacrificed by decapitation.

All parameters were studied in the Research Laboratory School of Chemical and Biotechnology, SASTRA Deemed University, Thanjavur, Tamilnadu, India.

Blood Sampling

In all these experiments, approximately 0.5 mL blood was drawn each time from retro orbital sinus using aseptic precautions and plasma was separated immediately by centrifuging at 3000 rpm for 10 min.

Isolation of Tissues and Preparation for Biochemical Analysis

At the end of the experimental period, *Cynodon dactylon* ethanolic and aqueous extract treatment, the animals were sacrificed after an overnight fast and EDTA blood samples from all groups of animals were collected. Following perfusion of the animal with physiological saline, the liver, kidney, testis and brain were removed. The tissues were washed with ice cold saline (0.9% NaCl) and portions weighing about 500 g were homogenized in 4 mL of 100 mM potassium phosphate buffer (pH 7.4), containing 150 mM KCl and 0.1 mM EDTA.

Oral Glucose Tolerance Test (OGTT)

The oral glucose tolerance test (Bonner-Weir, 1988).The glucose concentration in the plasma samples was analyzed immediately by the glucose oxidase method using Randox assay kit (Randox Laboratories Ltd., UK) analyzed by autoanalyzer.

Effect of Cynodon Dactylon on Liver Glycogen

Glycogen content was determined using a spectrophotometric method as described in detail by Borst *et al*. (2000).

Effect of Cynodon Dactylon on Glycosylated Haemoglobin

Glycosylated Haemoglobin was estimated by the method Bannon (1982) and Sudhakar and Pattabiraman (1982). The values were expressed as HbA1%.

Measurement of Lipid Profiles

Triglycerides, total cholesterol and high density lipoprotein (HDL) was measured in serum from all animals, using appropriate Randox assay kit (Randox Laboratories Ltd., UK), analyzed by autoanalyzer.

Statistical Analysis

The experimental results were expressed as the mean±SEM Data were assessed by the method of analysis of ANOVA followed by student's t-test. $p<0.05$ were considered as statistically significant.

Results and Discussion

The non-toxic nature of the aqueous and ethanolic extract of *Cynodon dactylon* (Linn), There was no lethality or any toxic reactions found at the dose selected until the end of the study period. In OGTT, the aqueous and ethanolic extract, from 30 min onwards showed significant reduction in plasma glucose levels.

Induction of diabetes in the experimental rats was confirmed by the presence of a high fasting plasma glucose level are presented in Table 1. The effect of aqueous and ethanolic extract of *Cynodon dactylon* on fasting plasma glucose levels are presented in Table 2. The difference between the experimental and control rats in lowering the fasting plasma glucose levels was statistically significant ($p<0.05$) in diabetic rats. Significant difference was also observed liver glycogen levels total hemoglobin and in glycosylated hemoglobin levels estimated in diabetic rats (Table 3). A decrease in the serum triglycerides and total cholesterol levels and an increase in the HDL cholesterol levels were observed (Table 4). Diabetic rats treated with CD also showed an increase in body weight as compared to the diabetic control group (Table 5).

The present paper discussed about the anti-diabetic effect of the aqueous and ethanolic extract of on *Cynodon dactylon*, streptozotocin - induced diabetic rats. The knowledge regarding diabetes existed since Brahmic period and treatment of diabetes has been mentioned in Sushruta Samhita (Dhanukar and Thatte, 1989). In this ancient text, two form of diabetes were described; one genetically based and other as a result of dietary indiscretion (Dhanukar and Thatte, 1989). Which may be due to the destruction of β cells of the islet of langerhans of the pancreas (Kavalali *et al*., 2002).

Table 1: Effect of *Cynodon dactylon* (CD) extract on oral glucose tolerance test (OGTT)

	Treatment plasma glucose concentration (mg dL^{-1})		
Groups	30 min	60 min	120 min
I	115.00±1.03	105.23±2.65	98.12±2.36
III	70.25±0.76*	116.58±4.56	88.52±2.55*
IV	80.12±1.73*	100.52±2.74*	83.26±1.60*

Values are mean±SE, n = 6 animals in each group.*$p<0.05$ when compared to control

Table 2: Effect of *Cynodon dactylon* (CD) extract on fasting plasma glucose level in diabetic rats

	Fasting plasma glucose concentration (mg dL^{-1})			
Groups	0th day	20th day	40th day	45th day
I	74.43±1.720	75.81±3.64	76.34±4.82	78.25±4.98
II	212.35±10.41	228.56±2.35	245.56±2.53	251.58±3.11
III	76.52±1.250	77.20±2.30	78.00±1.96	79.85±3.62
IV	77.65±1.860	76.98±3.21	78.25±3.88	80.86±2.66
V	205.05±2.450	170.43±2.81	149.36±2.13*	118.75±2.63*
VI	202.37±5.840	165.89±1.53	143.45±1.53*	120.23±1.41*
V II	200.72±9.270	155.45±4.61	120.25±1.49*	101.56±1.26*

Values are mean±SE, n = 6 animals in each group. * $p<0.05$ when compared to control

Table 3: Effect of *Cynodon dactylon* (CD) extract on liver glycogen, total hemoglobin and glycosyated hemoglobin levels in diabetic rats

Groups	Liver glycogen (mg g^{-1})	Total hemoglobin (g dL^{-1})	Glycosyated hemoglobin (%)
I	14.54±2.60	14.28±1.21	2.86±0.18
II	5.25±0.12	8.92±0.78	5.99±0.61
III	14.48±0.58	14.38±1.61	2.80±0.22
IV	14.14±0.62	14.42±1.52	2.76±0.15
V	16.82±0.35	13.29±1.51	3.21±0.37
VI	19.14±0.55	13.58±1.22	3.06±0.22
VII	13.31±0.72	13.4±1.140	3.39±0.26

Values are mean±SE, n = 6 animals in each group

Table 4: Effect of *Cynodon dactylon* (CD) extract on serum lipid profile levels in diabetic rats

Groups	Triglycerides (mg dL^{-1})	Total cholesterol (mg dL^{-1})	HDL (mg dL^{-1})
I	89.9±6.30	76.5±5.20	95.09±2.76
II	149.8±10.7	153.9±10.9	58.9±1.710
III	86.8±5.90	80.1±7.20	98.98±1.91
IV	87.2±5.90	78.4±6.10	99.4±1.710
V	101.3±9.20	90.8±7.80	94.58±2.54
VI	98.6±7.50	96.2±6.40	110.34±1.63
VII	104.2±1.20	92.1±8.90	107.41±1.84

Values are mean±SE, n = 6 animals in each group

Table 5: Effect of *Cynodon dactylon* (CD) extract on body weight changes in diabetic rats

	Body weight (g)	
Groups	Initial	Final
I	184.2±13.1	220.9±16.7
II	188.6±14.2	157.6±14.2
III	183.5±11.5	217.9±19.6
IV	181.7±11.8	216.5±17.4
V	186.3±13.8	183.2±13.9
VI	182.5±10.9	179.9±15.8
VII	185.8±10.9	179.9±15.8

Values are mean±SE, n = 6 animals in each group

Over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues are the fundamental basis of hyperglycemia in diabetes mellitus (Latner, 1958). When *Cynodon dactylon* aqueous and ethanolic extract was administered to glucose loaded normal rats fasted for 18 h, hypoglycemia was observed after 30 min. The decline in blood sugar level reached its maximum at 2 h.

In this study the difference observed between the initial and final fasting plasma glucose levels of different groups under investigation revealed a significant elevation in blood glucose in diabetic control group as compared with normal animals at the end of the 45-day experimental period. Our investigations indicate the efficiency of the aqueous and ethanolic extract in the maintenance of blood glucose levels in normal and streptozotocin-induced diabetic rats. Administration of aqueous and ethanolic extract of *Cynodon dactylon* to diabetic rats showed a significant decrease in the levels of blood glucose. A marked increase in total cholesterol and decrease in HDL cholesterol have been observed in untreated diabetic rats. Under normal circumstances insulin activates enzyme lipoprotein lipase and hydrolyses triglycerides. Insulin deficiency results in failure to activate the enzymes thereby causing hypertriglyceridemia. The significant control of the levels of serum lipids in the aqueous extract treated diabetic rats may be directly attributed to improvements in insulin levels upon *Cynodon dactylon* therapy. Induction of diabetes with STZ is associated with the characteristic loss of body weight, which is due to increased muscle wasting (Swanston-Flat *et al*., 1990) and due to loss of tissue proteins (Chatterjea and Shinde, 2002). Diabetic rats treated with the *Cynodon dactylon* showed an increase in body weight as compared to the diabetic control, which may be due to its protective effect in controlling muscle wasting i.e., reversal of gluconeogenesis and may also be due to the improvement in insulin secretion and glycemic control. The decrease seen in hepatic glycogen content in diabetes has been observed in earlier studies (Whitton and Hems, 1975) and in this study is probably due to lack of insulin in the diabetic state which results in the inactivation of glycogen synthase systems.

The significant increase in the glycogen levels of the aqueous extract treated diabetic animals may be because of the reactivation of glycogen synthase system. This focuses the one possible way of anti-diabetic action of this extract by improvement of glycogenesis process (Maiti *et al*., 2004). Glycosylated hemoglobin levels were found to be increased in the untreated diabetic control group.

Increased non-enzymatic and auto-oxidative glycosylation is one of the possible mechanisms linking hyperglycemia and the vascular complications of diabetes (Hall *et al*., 1984). Diabetic rats showed higher levels of glycated hemoglobin indicating their poor glycemic control. Treatment with *Cynodon dactylon* aqueous and ethanolic extract showed a significant decrease in the total hemoglobin and glycosyated hemoglobin levels, which could be due to an improvement in insulin secretion.

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