ORIGINAL ARTICLE

CURATIVE EFFECT OF *CYNODON DACTYLON* AGAINST STZ INDUCED HEPATIC INJURY IN DIABETIC RATS

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ABSTRACT

The aim of the study was to ascertain the role of ethanolic extract of *Cynodon dactylon* against hepatic complications in streptozotocin (STZ) induced type 2 diabetic models. Effect of the pre identified most effective dose of 500 mg/kg body weight was studied on hepatic injury caused by chemically induced diabetes by 55 mg/kg body weight i.p. injection of STZ in male Wistar rats. The dose of 500mg/kg body weight given once daily for 14 days reduced the levels of serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase, creatinine and urine sugar significantly ($P<0.05$) with increase in total protein, haemoglobin and body weight was increased. High LD_{50} validates its high margin of safety.

KEY WORDS

Antidiabetic effect, *Cynodon dactylon*, Diabetes, Hepatoprotective.

INTRODUCTION

The popularity of plant-based drug in the modern system of medicine is growing day by day as they are claimed to be safe, economical and yet efficacious (1-3). The adverse drug reaction of synthetic medicines in the treatment of diabetes mellitus has restricted in a growing demand for the use of herbal drug or Phytomedicines (4). However, there is still an unmet need for improved oral drugs from medicinal plants for Diabetes Mellitus.

The *Cynodon dactylon* (Family: Poaceae) commonly known as "Doob; Hindi" "Aroogum pillo; Tamil", "Garike; Telugu" and "Bermuda grass; English" in India is a creeper. It is a weed and has been regarded to possess varied medicinal properties (5). The aqueous fluid extract of the rhizome is used as anti-

inflammatory, diuretic, antiemetic, antidiabetic and blood purifying agent (6). Since, high potential of hypoglycemic, hypolipidemic and antioxidant activities of both aqueous and ethanolic extracts have already been reported by our research group (7-9) therefore, the present study was taken into consideration for evaluating the hepatoprotective effect of ethanolic extract of *Cynodon dactylon* on STZ induced hepatic injury in severely diabetic animal models. This extract can be treated as new addition to therapeutic armamentarium of drugs to combat the rapidly increasing number of diabetic patients in this country especially when the existing drugs are becoming very expensive.

MATERIALS AND METHODS

Reagents: Streptozotocin was purchased from Sigma-Aldrich Co. USA. Commercial kits were purchased from Bayer Diagnostics, India Ltd for enzymatic assays. One touch (Accu-chek sensor) of Roche, Germany for BGL and glucose based Uristix, from Bayer Diagnostics India Ltd for urine sugar were used.

Extract : *Cynodon dactylon* was collected from the campus of University of Allahabad, Allahabad, India in the month of August. It was identified and authenticated by Botanical Survey

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Table 1: Effect of administration of aqueous extract of *Cynodon dactylon* on SGOT and SGPT levels in normal and severely diabetic rats

Groups	Treatment	SGOT (U/L)		SGPT (U/L)	
		Before treatment	After 14 days treatment	Before treatment	After 14 days treatment
Group I	Distilled water	66.7 ± 3.2	65.8 ± 3.8	29.2 ± 1.4	28.8 ± 2.4
Group II	Extract	73.7 ± 2.8	58.2 ± 3.4*	27.9 ± 1.2	20.8 ± 1.8*
Group III	Distilled water	103.8 ± 2.8	120.5 ± 3.5*	51.7 ± 6.5	63.5 ± 5.1*
Group IV	Extract	122.6 ± 4.2	85.5 ± 4.1*	52.2 ± 7.3	44.5 ± 1.5*

* P < 0.05 as compared to initial value. Group 1 – Normal Control (Treated with distilled water), Group 2 – Normal (Treated with 500 mg/kg extract), Group 3 – Diabetic Control (Treated with distilled water), Group 4 – Diabetic (Treated with 500 mg/kg extract).

of India, Allahabad branch and Prof. B.D. Singh, taxonomist, Allahabad Agriculture Institute, Naini, Allahabad. A voucher specimen (AA518) has been submitted. The whole green plant was washed with water and shade dried. About 500 g of shade-dried plant was treated with hexane (2_2.5 l) to defat it and then extracted with ethanol (3 l) for 48 h. The resulting extract was filtered and concentrated in rotary evaporator under reduced pressure to give a residue (yield about 10.8% w/w) for further exploration.

Animals: More than fifty albino Wistar rats of body weight 180-220 g selected for the experiment were kept in standard conditions of temperature (23±5°C) and relative humidity (55±5%) with a 12h each of dark and light cycle. Animals were fed with commercial pellet diet (Golden feed, New Delhi) and water ad-libitum.

Diabetes was induced to a group of 14 overnight fasted rats by a single intraperitoneal injection of freshly prepared solution of streptozotocin (55 mg/kg body weight) in 0.1M citrate buffer (pH= 4.5). After one week of STZ administration animals with marked hyperglycemia (FBG>250 mg/dl) were selected for the study considering severely diabetic.

Experimental Design: Pre identified most effective dose of 500 mg/kg of *Cynodon dactylon* (7-8) ethanolic extract was

selected for the study. All biochemical parameters were estimated in blood serum. Four groups of six rats each were designed: Group I- Normal control (received vehicle); Group II-Normal treated (received 500 mg/kg body weight extract); Group III- Diabetic control (received vehicle); Group IV-Diabetic treated (received 500 mg/kg body weight extract)

Group I and III served as normal and diabetic control received vehicle (distilled water) only, whereas, groups II and IV were treated once daily for 14 days with the dose of 500 mg/kg body weight of ethanolic extract *Cynodon dactylon*. Various biochemical parameters like BGL, Hb, US, ALP, CREA, SGOT, SGPT and TP along with body weight were taken initially and then weekly up to 2 weeks.

Biochemical parameters: Blood glucose (10), Alkaline phosphatase (ALP) (11), glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT) (12), creatinine (CREA) (13), total protein (TP) (14) and haemoglobin (Hb) (15) were estimated. All the kits used were purchased from Bayer Diagnostic, India, Ltd. Urine sugar was detected by uristix. All the parameters were measured initially before the treatment and then monitored every week upto 2 weeks inclusive of body weight.

LD₅₀ Experiment: Four groups having six normal healthy rats

Table 2: Effect of administration of aqueous extract of *Cynodon dactylon* on serum alkaline phosphatase and serum creatinine levels in normal and severely diabetic rats

Groups	Treatment	ALP (U/L)		CREA (mg/dl)	
		Before treatment	After 14 days treatment	Before treatment	After 14 days treatment
Group I	Distilled water	77.1 ± 5.6	78.2 ± 5.2	0.98 ± 0.14	1.10 ± 0.16
Group II	Extract	72.9 ± 6.4	48.8 ± 7.2*	1.07±0.18	0.88 ± 0.15*
Group III	Distilled water	147.6 ± 3.2	154.8 ± 7.2*	1.70 ± 4.2	2.10 ± 3.4*
Group IV	Extract	145.2 ± 6.5	102.5 ± 8.2*	1.61 ± 2.2	0.94 ± 3.8*

* P < 0.05 as compared to initial value. Group 1 – Normal Control (Treated with distilled water), Group 2 – Normal (Treated with 500 mg/kg extract), Group 3 – Diabetic Control (Treated with distilled water), Group 4 – Diabetic (Treated with 500 mg/kg extract).

Table 3: Effect of administration of aqueous extract of *Cynodon dactylon* on haemoglobin and protein levels in normal and severely diabetic rats

Groups	Treatment	Total haemoglobin (mg/dl)		Total protein (mg/dl)	
		Before treatment	After 14 days treatment	Before treatment	After 14 days treatment
Group I	Distilled water	13.2 ± 1.6	13.5 ± 1.7	7.5 ± 3.2	7.8 ± 2.3
Group II	Extract	12.8 ± 2.1	14.6 ± 2.4	6.8 ± 5.6	7.4 ± 3.2
Group III	Distilled water	12.1 ± 3.5	10.5 ± 1.6	7.8 ± 3.4	7.1 ± 6.4
Group IV	Extract	12.5 ± 6.5	14.8 ± 4.1*	7.5 ± 1.2	8.9 ± 4.2*

*P < 0.05 as compared to initial value. Group 1 – Normal Control (Treated with distilled water), Group 2 – Normal (Treated with 500 mg/kg extract), Group 3 – Diabetic Control (Treated with distilled water), Group 4 – Diabetic (Treated with 500 mg/kg extract).

in each were treated orally with a single dose of 5, 10, 15 or 20 times the effective dose of ethanolic extract of *Cynodon dactylon*. The rats were observed for gross behavioral, neurologic, autonomic and toxic affects continuously upto 24 hours.

Statistical analysis: Data were statistically evaluated using one-way ANOVA, followed by a post hoc Scheffe's test using the SPSS computer software, version 7.5. The values were considered significant when P<0.05.

RESULTS

Liver Function Tests (SGOT, SGPT and ALP): Table 1 indicates the significant decline of 30.28 and 14.75% in the raised levels of SGOT and SGPT respectively of severely diabetic rats on 14 days treatment indicating thereby its hepatoprotective effect. This effect was further confirmed by a fall of 33.05% in ALP levels of severely diabetic rats shown in Table 2.

Creatinine (CREA): Increased creatinine levels of severely diabetic animals was an indication of diabetes induced mal functioning of kidney. However, Table 2 shows the sharp

decrease of 41.61% in its level of diabetic animals as compared to initial values as an additional advantage of treatment with ethanolic extracts of *Cynodon dactylon*.

Total haemoglobin and total protein (Hb and TP):

Moreover, Table 3 observes that the decrease in haemoglobin and total protein levels of diabetic rats was also raised by 15.54 and 15.73% on extract treatment for 14 days.

Urine sugar and body weight (US and body weight):

Table 4 clearly shows the decrease of 75% in urine sugar level as it comes down from +4 to +1 in case of treated severely diabetic rats. Moreover, the result given in Table 4 also reveals that the body weight (body weight) in case of treated diabetic groups with ethanolic extract for 14 days increased by 17.4 g.

LD₅₀ : No toxic effect was reported at doses upto 15 and 20 times the effective dose as the behavior of experimental animals was normal and no mortality was observed in any of these groups.

DISCUSSION

Cynodon dactylon is traditionally used in India as a therapeutic agent to control diabetes mellitus. Our earlier reports on

Table 4: Effect of administration of aqueous extract of *Cynodon dactylon* on body weight and urine sugar levels in normal and severely diabetic rats

Groups	Treatment	Body weight (g)		Urine sugar (g/lit)	
		Before treatment	After 14 days treatment	Before treatment	After 14 days treatment
Group I	Distilled water	190.0 ± 8.6	220.8 ± 9.4	Nil	Nil
Group II	Extract	198.6 ± 7.8	224.4 ± 8.2	Nil	Nil
Group III	Distilled water	216.5 ± 8.2	165.2 ± 5.2	+4	+4
Group IV	Extract	188.2 ± 6.2	205.6 ± 5.8*	+4	+1**

*P < 0.05 as compared to initial value; ** P < 0.01 as compared to initial value. Group 1 – Normal Control (Treated with distilled water), Group 2 – Normal (Treated with 500 mg/kg extract), Group 3 – Diabetic Control (Treated with distilled water), Group 4 – Diabetic (Treated with 500 mg/kg extract).

aqueous and ethanolic extracts of *Cynodon dactylon* (8, 9) also reveal its antidiabetic effect in severely diabetic models on 2 weeks treatment. Lowering of high lipid profile of diabetic rats with increase in cardioprotective lipid (HDL) was an additional observation of these reports. The present scientific investigation not only supports but validates too the traditional use of *Cynodon dactylon* as antidiabetic agent of high potential in diabetic models.

The elevated level of SGOT, SGPT and ALP in severely diabetic experimental rats was observed after one week of STZ administration as an indication of STZ induced hepatic injury (16). The data suggested that the 14 days long administration of ethanolic extract of *Cynodon dactylon* improves the liver function by decreasing the raised levels of SGOT, SGPT and ALP of severely diabetic rats. Hence, the present study reconfirms our previous observations about antidiabetic effect by showing improvement in enzymatic assays affected due to hepatic injury caused by STZ induced diabetes. The serum creatinine level of severely treated diabetic rats was also found to decrease on treatment suggesting thereby an improvement in kidney function also in addition to liver function (17).

Levels of total haemoglobin, total protein and urine sugar and body weight were additional parameters taken into consideration for validating the effect of extract treatment by finding improvement in all of them. Since, high LD₅₀ indicates high margin of safety therefore the extract can be effectively used to control diabetes mellitus and its related complications.

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