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Non-Retinal Histological Changes And Antihyperglycemic Activity of Cynodon Dactylon Extract In Streptozotocin Induced Diabetic Male Rats

KEYWORDS: Antihyperglycemic, Streptozotocin, Cynodon dactylon, Retinal histology

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ABSTRACT The onset of diabetes mellitus at young age is increasing worldwide. Traditional Medicinal plants forms potential source of therapeutic agents. Cynodon dactylon possess anti-inflammatory, anti-ulcer and few researchers reported its Antihyperglycemic activity. The aim of this study is to determine the anti-hyperglycemic activity of Cynodon dactylon extract, and to observe any histological retinal changes in normal and streptozotocin induced diabetic male rats.The aqueous extract of Cynodon dactylon significantly ($P<0.001$) lowered the plasma glucose levels in STZ induced diabetic male rats, and also the histological observation of the retinal tissues of the normal, streptozotocin induced diabetic and diabetic treated groups, did not show any marked observable changes. These clearly indicate the efficacy and safety of Cynodon dactylon aqueous extract in streptozotocin induced diabetic male rats.

INTRODUCTION

Diabetes mellitus is one of the most common metabolic disorders with a prevalence estimated to be between 1% to 5% of the world population (1). In the year 2000, 171 million people suffered from diabetes, which is expected to double by the year 2030 (2). Oral synthetic hypoglycemic agents have failed to manage and control long term micro vascular and macro vascular complications (3). Alternative medicines particularly herbal medicines are available for diabetic treatment, as they are effective, low cost with less or no toxic side effects. More commonly juice of Cynodon dactylon are taken by the people of Tamilnadu. The objective of this study is to evaluate the Antihyperglycemic effect of Cynodon dactylon aqueous extract in streptozotocin induced diabetic rats, and also to observe any retinal changes in the diabetic rats histologically.

Cynodon dactylon belonging to family poaceae commonly known as Doob in Hindi and Arugum pillu in Tamil, Bermuda grass in English, a perennial weed, grass native to east africa,Asia,Australia and southern Europe (4). It has been found to possess various medicinal properties. The aqueous fluid extract of the rhizome is used as anti-inflammatory, diuretic, antiemetic, antidiabetic and blood purifying agent (5). The Cynodon is rich in glycosides, saponins, tannins, flavonoids and carbohydrates, variable amount of proteins, minerals and oil triticin (6, 7).

MATERIALS AND METHODS

This study was approved by the institutional animal ethical committee, under CPCSEA, India (Ref.45/IAEC/2011).

Chemicals used

Chemicals used in this study were of analytical grade.

Plant extract preparation

The whole plant of Cynodon dactylon was washed with tap water, air dried, and grinded in a mechanical blender. The dried powder (100 g) of Cynodon dactylon was extracted with distilled water in a soxhlet extractor and the resultant extract was concentrated in a rotary vacuum evaporator, the concentrated dark extract stored in an air tight container.

Animals used

Adult male albino wistar rats (aged 10 weeks, weighing 150-200 g) approximately were acclimatized and housed in the central animal house of our institute. All animals were kept in 12:12 hr light: dark cycle, at a room temperature of 22±2°C. Rats were fed with standard rat pellet supplied by Provimi animal nutrition India ltd, Bangalore, India, were also allowed free access to water. Animal experimentation was carried out under the supervision of on duty veterinary medical officer in accordance to the ethical norms approved by the Institutional animal ethical committee (IAEC).

Induction of diabetes

Animals were fasted overnight and diabetes was induced by single intraperitoneal injection of streptozotocin (45mg/kg body weight) prepared in 0.1 M Citrate buffer at pH 4.5 (8). To overcome drug induced hypoglycemia, animals were allowed to drink 5% glucose solution overnight. Citrate buffer alone injected to control rats. After 72 hours of STZ injection, (taken as 0^{th} day) fasting blood glucose levels of each animal were analyzed. Animals with fasting blood glucose levels > 200 mg/dl were considered as diabetic and taken for the study.

Experimental Design

The rats were randomly divided into 5 groups of 6 rats in each group.

Group I: Normal control rats fed with distilled water only for 45 days.

Group II: Diabetic control rats fed with distilled water only for 45 days.

Group III: Diabetic rats fed with Glibenclamide (5 mg/kg/bodyweight/rat/day) for 45 days.

Group IV: Normal rats fed with aqueous extract of Cynodon dactylon (500 mg/kg bodyweight/rat/day) for 45 days.

Group V: Diabetic rats fed with aqueous extract of Cynodon dactylon (500 mg/kg/bodyweight/rat/day) for 45 days.

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Fasting plasma glucose levels were measured on 0th day and 45th day. Plasma glucose was determined by ortho toluidine reagent method (9). Blood collected from the retro-orbital plexuses of the rats of all groups, under light ether anesthesia.

Collection of Retina tissue samples

After 45 days of experiment, animals were sacrificed, following the guidelines of animal ethical committee. The eyeball were dissected out, a incision was made at the equator of the eyeball, using hand lens, Lens and cornea of eyeball, was separated out, choroid removed and the posterior pole of the eyeball, containing the retina tissue were fixed in 10% neutral buffered formalin (NBF) solution for histological analysis.

Histological preparation of retinal tissue

The fixed retinal tissues were sectioned with Leica rotary microtome to produce serial sections of 6 micron thickness. Retinal sections were stained with routine Hematoxylin and Eosin (H&E) stains. The stained slides were then photomicrographed with APCAM -5 USB 2 digital camera attached to a computer monitor, supplied by ADELTAVISION OPTEC India microscope Ltd.

Statistical Analysis

Results were expressed as Mean ± S.E.M and the data were tested by one way analysis of variance (ANOVA) followed by the student-Newman-Keuls post-hoc test using the software "Graphpad Instat". The $p<0.05$ were considered as statistically significant.

Toxicity and LD50

No toxic effects were observed with the doses up to 2000mg/kg body weight of aqueous extract of Cynodon dactylon, as the behavior of the treated groups appeared normal and no signs of mortality seen (10).

Results

Table 1: Effect of aqueous extract of Cynodon dactylon on plasma glucose values in normal & streptozotocin induced diabetic rats

Groups	Plasma glucose levels in mg/dl	
	0 day	45th day
Group I	98.16±2.22	95.5±2.12
Group II	271.33±8.80**	371.83±11.85**
Group III	266.66±8.53#	120.5±2.95**
Group IV	89.50±0.76	87.16±0.70**
Group V	264.50±7.02#	117.5±2.39**

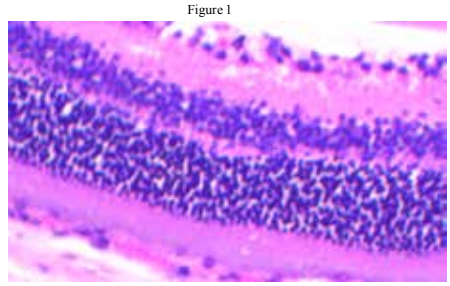
Results are expressed as mean ±SEM; n=6; **=p<0.001 and # =not significant.

Table 1 shows the results of the effect of aqueous extract of Cynodon dactylon on fasting plasma glucose levels. Plasma glucose levels in streptozotocin-induced diabetic group were significantly higher (P< 0.001) than control group and decreased significantly (P< 0.001) in all treated diabetic groups, when compared to streptozotocin-induced diabetic group.

Histological assessment of the retina

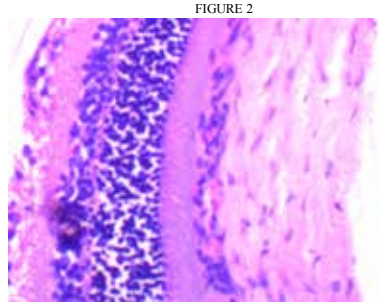
Figure 1, shows the stained sections of the retina of STZ diabetic rats , revealed no changes in any of the layers of retina, which was also similar to control groups, that was shown in figure 2, The retinal cytoarchitecture of diabetic rats treated with aqueous extract of Cynodon dactylon, alsos as shown in figure 3.

Figure 1



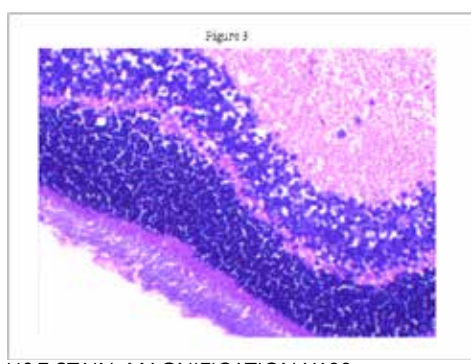
H&E STAIN, MAGNIFICATION X100

FIGURE 2



H&E STAIN, MAGNIFICATION X100

Figure 3



H&E STAIN, MAGNIFICATION X100

Discussion

Antihyperglycemic activity of plants has been paid more attention, because of increasing incidence of diabetes and predominance of traditional plants in the therapy (11). Streptozotocin used to develop animal models of diabetes, by exerting cytotoxic effect on pancreatic β-cells, possibly by generating lipid peroxides and excess reactive oxygen species (ROS), interfering with glucose transporter GLUT-2 and causing DNA damage either by alkylation or peroxynitrite formation (12). In the present study the aqueous extract of Cynodon dactylon reduced the plasma glucose levels in STZ induced-diabetic rats. No significant alteration was found in the plasma glucose level of control rats which justifies the antidiabetic activity of this plant, as the extract did not show much reduction in the plasma glucose level of normal rats. It has been hypothesized that bioactive compounds from plant sources having Antihyperglycemic activities might act by various mechanisms such as stimulating insulin secretion, increasing repair or prolifera-

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tion of beta cells and enhancing the effects of insulin and adrenalin (13, 14). Antihyperglycemic activity of Cynodon dactylon extract in our study reduced the plasma glucose levels in streptozotocin induced diabetic rats, which may be due to regeneration or proliferation of pancreatic islet beta cells.

Diabetic retinopathy remains a major cause of morbidity in diabetic patients. Retinal ganglion cell loss or atrophy demonstrates the retinal damage in almost all strains of diabetic rats induced by the toxic chemical streptozotocin. The reduction in the thickness of inner plexiform layer of retina also proves the retinal ganglion cell loss or damage. Various researchers have demonstrated retinal ganglion cell death or loss in diabetic rats, the duration of their studies extended from 8weeks to 1year (15). The histological observation made in our study revealed no retinal changes in the streptozotocin induced diabetic rats, which were almost similar to control rats; as well the diabetic rats treated with Cynodon dactylon extract also exhibited no significant histologically demonstrable retinal changes. This may be because the period of 45days is too short to cause rat's retinal tissue damage, and the treatment of diabetic rats with the extract of Cynodon dactylon did not show any adverse reactions in the retinal tissue, which proves its safety and non toxic nature of the plant. our study further indicate that the continuous tight control of hyperglycemia with the aqueous extract of Cynodon dactylon for a longer period of time, may definitely prevent diabetic retinopathy, which usually develops in long term diabetic patients.

Conclusion

Our study demonstrated the aqueous extract of Cynodon dactylon possessing Antihyperglycemic potential in streptozotocin induced diabetic rats, which also showed non-retinal histological changes. The results of the present findings will have a great effect in the treatment of diabetes mellitus, if tested in human beings. Further more studies are needed to confirm these findings.

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