Nephroprotective Effects of Cynodon dactylon Aqueous Extract in STZ Induced Diabetic Male Rats – Histological Study

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ABSTRACT
Diabetes mellitus is the world’s most common endocrine disorder, characterized by hyperglycaemia and impaired glucose tolerance. The aim of the present study was to evaluate the effect of aqueous extract of Cynodon dactylon on renal function in Streptozotocin induced diabetic rats. STZ induced diabetic male rats showed significant decrease in the levels of serum total protein, which lead to the reduction in their body weight, and significant elevation in the levels of blood urea and serum creatinine were observed, when compared to normal rats. These levels were reverted in the STZ induced diabetic rats, treated with Cynodon dactylon extract and in those treated with glibenclamide, which was also demonstrated and correlated with the histopathological findings of the kidney tissue. According to the results of our study, Cynodon dactylon aqueous extract effectively prevented the nephropathic changes induced by diabetes and this is the first study to report on nephroprotective effect of Cynodon dactylon with histological correlations.

Keywords: Cynodon dactylon, Nephropathy, Creatinine, Histopathology, Total Protein.

INTRODUCTION
Diabetic nephropathy is a major cause of end stage renal disease and one of the most serious micro vascular complications of diabetes mellitus1, which occurs approximately in one -third of diabetic patients and is on rise continuously2. Kidneys are one of the important vital organs in the body, which eliminates urea, uric acid, creatinine etc., and also maintains the water balance to the optimal levels. In diabetes, the toxic concentration of blood glucose levels, damages the renal tissue, which lead to altered renal function, causing diabetic nephropathy. Elevated blood glucose and glycosylated protein levels, associated with increased oxidative stress produces haemodynamic changes within the renal tissue, thereby leading to altered kidney function in patients with diabetes mellitus3. Plants and herbs are treasures of wide amount of bioactive phytochemicals that might serve as a lead for the development of cheap, safe, novel effective drugs, which provide an alternate to modern system of medicine, as several medicinal plants widely used worldwide in the treatment of diabetes mellitus and its complications3. Many ethno botanical surveys on medicinal plants used by the local population have been performed in different parts of the world including Morocco, Saudi Arabia, Taiwan and Trinidad and Tobogo. Several plant species have been described as hypoglycaemic, which include Opuntia streptacantha Lem, Trigonella foenum graecum L., Momordica charantia L., Gymnema sylvestre R, etc3. The ethno botanical information reports that about 800 plants possess antidiabetic potential4. Many Indigenous Indian medicinal plants have been found to be useful in the management of diabetes, which are easily available and have least side effects3. The grass Cynodon dactylon is also known as the Bermuda grass, crouch grass, Grama, Handjes grass. Vernacularly, it is called as dhub or doob in Hindi; durba in Bengali; durva in Sanskrit; Arugampullu in Tamil; garikagoddi in Telugu. It is a creeping grass, very tough, light green in color and has a coarse texture, drought resistant, fast growing, 3 to 20 mm long, and 2-4 mm in diameter. It is odourless and has a sweet mucilaginous taste4. Chemical constituents of Cynodon dactylon are glycosides, Saponins, tannins, flavonoids and carbohydrates. It also contains agropyrene, arunodin, and furfural. Furfural alcohol, s β ioine, 2-(4’ hydroxyl phenyl) propionic acid, 2 – (3’ – methoxy – 4’ – hydroxy benzoic acid, phytol, β – Sitosterol – D – glucoside, stigmasterol acetate, phrgostimulant phytone (6,10 – 14 – trimethyl pentadecane – 2- one). It also contains essential oil triticin 12.4%. Cuticular wax in it contains triacontane, docosanol, tetracosanol, hexacosanol, octacosanol, eicosanic acid and docosanoic acid5,6.

MATERIALS AND METHODS
Experimental Animals
Adult male albino wistar rat (Rattus norvegicus) weighing approximately 150-200g (housed 3 per cage) were acclimatized and housed in the central animal house of SRM medical 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 Provim 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. (Ref: 45/IAEC/2011)

Chemicals and Reagents
Reagents were obtained from Thermo Fischer scientific India and Merck (India) and were of the highest commercial grade available.

Preparation of the Aqueous Extract
*Cynodon dactylon* was collected from kanniyakumari district of Tamilnadu, south India and authenticated by Dr Manian Director of plant sciences, Bharathiar University, Coimbatore, Tamilnadu, south India. 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 air tight container. The resultant yield of this extract was 6.6%.

Induction of Experimental Diabetes
Animals were fasted overnight and diabetes was induced by single intraperitoneal injection of Streptozotocin (Sisco research laboratories, Mumbai, India) at a dose of 45mg/kg body weight, prepared in 0.1 M Citrate buffer at pH 4.5 (Siddique et al., 1987). 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, 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 the following five groups with six rats in each group:
Group I: Normal control rats received only vehicle solutions.
Group II: Diabetic control rats served as positive control group received single dose of STZ (45 mg/kg, intraperitoneal.).
Group III: Diabetic rats received glibenclamide (5mg/kg, per oral).
Group IV: Normal control rats received the aqueous extract of *Cynodon dactylon* (500 mg/kg, per oral).
Group V: Diabetic rats received the aqueous extract of *Cynodon dactylon* (500 mg/kg, per oral).

Animals were treated by oral gavage once a day, preferably in the morning for a period of 45 days. At the end of the experimental period, the animals were fasted overnight; blood was collected by Retro-orbital puncture and then sacrificed under ether anesthesia.

Analytical Procedures
Serum total protein, urea, and creatinine estimated with appropriate standard biochemical laboratory kits.

Collection of Tissue Samples for Histopathology
The Kidneys were dissected out, fixed in 10% neutral buffered formalin solution and embedded in paraffin wax for routine histological procedure. Sections of 5-6 µm thickness were cut on a Leica rotary microtome and stained with hematoxylin and eosin (H&E) for general histological study (Bancroft and Gamble, 2002). The stained slides were then photomicrographed with APCAM -5 USB 2 digital cameras attached to a computer monitor, supplied by ADELTAVISION OPTEC India microscope Ltd.

Statistical Analysis
Statistical analysis was done with Statistical package for social sciences software (SPSS) version 21. Results are expressed as mean ± standard error of the mean. Comparison of the means between groups was performed by one-way analysis of variance (ANOVA) followed by post hoc of Tukey’s test, all statistical tests were two-tailed and a p value of 0.05 or less was considered statistically significant.

RESULTS
Table 1 shows the effect of *Cynodon dactylon* aqueous extract on body weight in normal and experimental group of rats. In this study, diabetic control group rats showed significant loss of body weight when compared with the normal group. All animals treated with *Cynodon dactylon* extract and the standard drug glibenclamide showed significant prevention in the loss of body weight. Table 2 depicts the effect of *Cynodon dactylon* aqueous extract on serum total protein, urea and creatinine levels in the normal and experimental group of rats. Diabetes induced rats showed significant decrease in the levels of serum total protein, and a significant increase in the levels of blood urea, and serum creatinine when compared to normal rats. The reduced levels of total protein were reverted back to normal levels in both the *Cynodon dactylon* extract treated and glibenclamide treated group of rats. Diabetic rats treated with *Cynodon dactylon* extract and with glibenclamide, showed significant reduction in the levels of blood urea and serum creatinine when compared with diabetic rats. However, a non-significant change in the levels of blood urea and creatinine in the normal rats treated with *Cynodon dactylon* aqueous extract denotes the nontoxic nature of the administrated extract.

Histopathological findings
Figure 1-5 depicts the histopathological features of kidney tissue in normal and experimental rats. From the figures, it is very clearly evident that, Group I control rats showed normal renal parenchymal structure. Group II diabetic induced rats, showed enlarged renal corpuscle, mesangial thickening, interstitial nodular sclerosis and fibroplasia. Group III and V diabetic rats treated with glibenclamide and aqueous extract of *Cynodon dactylon* showed reverted pattern of renal architecture, hence proving their potential effect in diabetic nephropathy. Group IV normal rats treated with aqueous extract of *Cynodon dactylon* showed no adverse histological changes, and exhibited normal renal parenchymal architecture.

DISCUSSION
Induction of diabetes with STZ was related with the reduced rate of body weight gain and the increased food and water intake. Treatment with *Cynodon dactylon* extract prevented the reduction in body weight in diabetic rats, which also lowered food and water intake. Body weight is one of the general indicators of metabolic
regulation for diabetes: Gluconeogenesis in cells is stimulated to compensate for the reduced level of glucose, which results in a decrease in the body weight. Induction of diabetes with STZ is associated with the characteristic loss of body weight, which is due to increased muscle wasting and to the loss of tissue proteins. Diabetic rats treated with aqueous extract of *Cynodon dactylon* showed improvement in body weight as compared to the diabetic control, which may be due to its protective effect of controlling muscle wasting, i.e., reversal of gluconeogenesis and may also be due to the improvement in insulin secretion, thereby possible glycaemic control. Distinct metabolic renal alterations are demonstrable in experimental diabetes, leading to a negative nitrogen balance, enhanced proteolysis and lowered protein synthesis. Reduction in the levels of total protein in diabetic rats may be attributed to decreased amino acid uptake, increased conversion rate of glycogenic amino acids to carbon dioxide and water and reduced protein synthesis secondary to decreased amount and availability of mRNA. Increased protein catabolism in diabetes may have direct adverse effect in the synthesis of albumin. The restoration of protein levels, which was lost initially in the diabetic rats, treated with *Cynodon dactylon* aqueous extract and with glibenclamide might be due to stimulation of insulin, which in turn activated protein synthesis via oxidative phosphorylation. The major function of the kidneys is to excrete the waste products of metabolism and to regulate the body’s water and salt concentration. Serum creatinine levels serve as a marker to assess renal function; as high levels of creatinine indicate several disturbances in the function of kidneys. In the present study, the changes observed in STZ diabetic rats were associated with significant increase in the levels of urea, and creatinine, indicating impaired renal function of diabetic rats. Hyperglycaemia induced elevation of serum levels of urea and creatinine which are considered as significant markers of renal dysfunction. *Cynodon dactylon* aqueous extract treatment significantly reduced the levels of blood urea and serum creatinine in diabetic induced rats, which could be due to the prevention of protein and nucleic acid degradation. Similar results were also observed in the diabetic rats treated with the standard drug glibenclamide, whereas no significant change in these levels were observed in the rats treated with *Cynodon dactylon* aqueous extract alone. Renno et al. demonstrated that green tea extract provides a beneficial effect on long term diabetic nephropathy via suppressing hyperglycaemia and preventing glycogen accumulation in the proximal tubules. The diabetic hyperglycaemia induced elevation of plasma levels of urea and creatinine which are significant markers of renal dysfunction and reflecting a decline in the glomerular filtration rate. In the present study, histopathological findings also provided supportive evidence for the antioxidant potential of *Cynodon dactylon* aqueous extract during diabetes. In STZ-induced diabetic rats, the kidneys undergo pathological changes and the marked pathologic features of glomerular hypertrophy, tubular dilatation, mesangial thickening with increased Bowman’s space and tubulointerstitial fibrosis. All structural changes in kidneys resulting from STZ administration in rats can be attributed to altered metabolism in diabetes. Mesangial thickening and tubulointerstitial fibrosis increases with the duration and the severity of hyperglycaemia. As this study was done for only 45 days, mild nephropathic changes were observed, which is in contrast to other studies reported on ameliorative effects of various plant extracts in the prevention and treatment of diabetic nephropathy, hence it is very clear from this study, that severe nephropathic tissue changes can occur only in prolonged diabetes and not in early stages. Progressive glomerulosclerosis associated with decreased kidney function, resulting in end stage renal failure is the major finding in diabetic nephropathy. Treatment with aqueous extract of *Cynodon dactylon* and glibenclamide, reduced cell infiltration, improved tubular necrosis, showed normal glomerulus and maintain the near normal renal architecture. In diabetes mellitus, hyperglycaemia increases the generation of free radicals by glucose auto-oxidation and the increment of free radicals may lead to kidney damage. Aqueous extract of *Cynodon dactylon* treated rats, proved that the renal damage might be protected by their potent antioxidant property and by inhibition of lipid peroxidation in renal tissues, and possibly by the stimulation of insulin secretion from the regenerated beta cells of pancreatic islets. Moreover, the significant nephroprotective activity of aqueous extract of *Cynodon dactylon* in STZ induced diabetic rats may be attributed to the presence of biologically active compounds such as flavonoids, saponins tannins and terpenoids. The biochemical findings of this study were correlated with the histopathological changes in the renal parenchyma of normal and experimental animals, which reveal that treatment of diabetic rats with 500 mg/kg, bodyweight of aqueous extract of *Cynodon dactylon* showed substantial recovery of damaged renal architecture. The observed histopathological findings were similar to the findings, reported by very few authors, with other plant extracts. The aqueous extract of *Cynodon dactylon* exhibits ameliorative effects almost similar to the standard group (glibenclamide 5 mg/kg, bodyweight). Histopathological observations made in this study, also warrant that aqueous extract of *Cynodon dactylon* is effective in preventing the renal tissue damage induced by Streptozotocin (STZ).
Figure 1a: Histopathological section of Renal Cortex of control rats (H&E, 400X) showing normal Renal Corpuscle (RC), Proximal Convoluted Tubule (PCT) and Distal Convoluted Tubule (DCT).

Figure 1b: Histopathological section of Renal Medulla of control rats (H&E, 400X) showing normal pattern of loop of Henle (LH) and Collecting Duct (CD).

Figure 2a: Histopathological section of renal cortex of STZ induced diabetic rats (H&E, 400X) showing enlarged Renal Corpuscle (RC) with progressive Glomerular Sclerosis (Asterisk), Tubular lipid deposition (Arrows) and Tubular dilatation (Arrow heads).

Figure 2b: Histopathological section of Renal Medulla of STZ induced diabetic rats (H&E, 400X) showing tubular dilatation (Arrow), interstitial nodular sclerosis and fibroplasia (Asterisks).

Figure 3a: Histopathological section of Renal Cortex of STZ diabetic rats treated with Glibenclamide (H&E, 100X) showing normal size Renal Corpuscle (RC) with clear urinary space (Asterisks), Bowman’s capsule and less tubular lipid deposition.

Figure 3b: Histopathological section of Renal Medulla of STZ diabetic rats treated with Glibenclamide (H&E, 100X) showing recovering pattern of thin tubules (Asterisk) and Collecting Duct (Arrow).
CONCLUSION

The present study, clearly indicates that *Cynodon dactylon* aqueous extract exhibits nephroprotective effect against STZ induced diabetic male rats, which may be attributed to the synergistic action of various active compounds present in this plant extract, which has been clearly demonstrated both biochemically and histologically. However, more warrant studies are necessary to find out

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<th>Groups</th>
<th>Total Protein (gm/dl)</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
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<td>I</td>
<td>7.6±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.55±1.24&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0.835±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
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Values are expressed as Mean ± SEM for each six rats. Values not sharing a common superscript letter differ significantly at p < 0.05 (Tukey’s HSD). Here all the p values are < 0.001.
the exact mechanism of action of this plant extract in ameliorating diabetic nephropathy.

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REFERENCES