

Research Article

Protective Effect of *Cynodon dactylon* Aqueous Extract in Streptozotocin Diabetes Induced Liver Damage in Rats – Histological Study

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ABSTRACT

The purpose of this study is to investigate the protective effect of *Cynodon dactylon* aqueous extract (500mg/kg) in the liver tissue of streptozotocin induced diabetic rats. Thirty male albino wistar rats were divided into five groups. Diabetes was induced by single intraperitoneal injection of streptozotocin (45mg/kg). *Cynodon dactylon* aqueous extract were administered via oral gavage (500mg/kg) for 45 days. Liver damage caused by diabetes was evaluated by an increase in the levels of intracellular hepatic enzymes activity. Histopathological observation showed marked disturbance in the hepatic cellular structure, when compared to normal control liver sections. Treatment with aqueous extract of *Cynodon dactylon* significantly ($p < 0.001$) reduced the levels of serum transaminases and alkaline phosphatase and also showed demonstrable histopathological changes. The observed histopathological changes correlated with the biochemical results, confirmed the protective effect of liver tissue in streptozotocin diabetes induced liver damage in Wistar rats.

Keywords: *Cynodon dactylon*; Streptozotocin; Histopathology; Hepatocytes, Diabetes

INTRODUCTION

The liver is a vital organ, having crucial and vital role in carbohydrate metabolism regulation. The normal functioning of the liver is essential for the continued supply of glucose energy source to various organs, besides its great detoxifying capacity^{1,2}. This function is impaired by frequent exposure to drugs, toxins, infections, which affect the basic cellular structures and consequently induce hepatocytes damage, followed by necrosis and finally lead to fibrosis³. The regenerating capacity of the liver is so tremendous, as it has a large reserve capacity, and the liver produces symptoms only after extensive damage⁴. Hepatic dysfunction such as elevation in the levels of serum transaminases and alkaline phosphatase are associated with diabetes⁵. It has been shown that many herbal plants found to exhibit protective effect in the liver tissue, such as *Hibiscus sabdariffa L*, *Tamarindus indica*, *Phyllanthus amarus*, *Rosmarinus officinalis*^{6,7,8,9}.

Cynodon dactylon is a perennial weed, belongs to the family poaceae, widely distributed throughout the world. It possess various medicinal properties like antihelminthic, anti diuretic, anti-inflammatory and hepatoprotective activity, traditionally used in the treatment of diabetes, jaundice and renal problems¹⁰. Phytochemical studies have shown that *Cynodon dactylon* is rich in glycosides, saponins, tannins, flavonoids, proteins and steroids¹¹. Although curative effect of *Cynodon dactylon* against streptozotocin induced hepatic injury in diabetic rats have been reported¹². There are no documented studies,

available in the literature that showed the histopathological changes in the liver tissue of streptozotocin induced diabetic rats treated with aqueous extract of *Cynodon dactylon*, which made us to conduct this study.

MATERIALS AND METHODS

Plant material and Extract preparation

Cynodon dactylon was collected from kanniyakumari district of Tamilnadu, India and authenticated by the Director of plant sciences, Bharathiar University, Coimbatore, Tamilnadu, 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 was stored in an air tight container.

Experimental animals

Male albino wistar rats (aged 10 weeks, weighing 150-200 g) approximately were acclimatized, and housed in the central animal house of SRM medical college hospital and research centre, SRM university campus, Kattankulathur, Tamilnadu. All animals were kept in 12:12 hr light: dark cycle, at a room temperature of $22 \pm 2^\circ\text{C}$. Rats were fed with standard rat pellet supplied by Provimi animal nutrition India Ltd, Bangalore, India, were also allowed free access to water. Animal

Table 1: Effect of Aqueous extract of *Cynodon dactylon* on SGOT, SGPT and ALP levels in Normal & Experimental Groups.

Groups	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)
Normal control	67.33±0.66	61.25±1.03	77.43±0.80
Diabetic control	247.83±2.05 ^a	225±3.50 ^a	209±1.15 ^a
Diabetic + Glibenclamide (5mg/kg)	95.83±1.07 ^a	101±1.29 ^a	94.16±1.49 ^a
Normal + Extract (500mg/kg)	67.83±1.30 ^a	60.33±0.61 ^a	78.5±0.99 ^a
Diabetic + Extract (500mg/kg)	74±2.11 ^a	86.35±3.34 ^a	80.16±0.87 ^a

Data expressed in mean ±SEM, ^ap<0.001 in comparison to diabetic control group.

experimentation were 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) of SRM Medical College, Tamilandu, India. (Ref: 45/IAEC/2011)

Experimental Diabetes Induction

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. 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 0th 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.

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.

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 (p.o) only for 45 days.

Group II: Diabetic control rats fed with distilled water (p.o) only for 45 days.

Group III: Diabetic rats fed with Glibenclamide 5 mg/kg (p.o) for 45 days.

Group IV: Normal rats fed with aqueous extract of *Cynodon dactylon* 500 mg/kg (p.o) for 45 days.

Group V: Diabetic rats fed with aqueous extract of *Cynodon dactylon* (500 mg/kg (p.o) for 45 days.

Biochemical Investigations

On 45th day, Blood collected from the retro-orbital plexuses of the rats of all groups, under light ether anesthesia, serum then separated from the whole blood, were analyzed for serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) levels by using automatic biochemical analyzer with commercially available biochemical kits.

Collection of tissue samples

After 45 days of experiment, animals were sacrificed, following the guidelines of animal ethical committee. The liver tissues were excised and fixed in 10% Neutral buffered formalin (NBF) solution for histological analysis.

Histological examination of liver tissue

The fixed liver tissues were sectioned with Leica rotary microtome to produce serial sections of 5μ thickness. Liver sections were stained with Gordon and Sweets reticulin stain and with Masson trichrome 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) using the software "Graphpad Instat". The p<0.05 were considered as statistically significant.

RESULTS

Biochemical assay

The biochemical parameters SGOT (serum glutamate oxaloacetate transaminase), SGPT (serum glutamate pyruvate transaminase) and alkaline phosphatase (ALP) were elevated significantly (P<0.001) in diabetic control group, when compared to normal control group. Aqueous extract of *Cynodon dactylon* showed significant (P<0.001) reduction in the levels of SGOT, SGPT and ALP, when compared to diabetic control group as shown in Table 1.

HISTOLOGICAL CHANGES

Histological sections of the liver tissue of streptozotocin induced diabetic group, showed hepatocytes degeneration; breakdown of reticulin fibres (Figure 1 E) accumulation of collagen fibres in the areas of reticular fibers (Figure 1 B) clearly demonstrates the disturbance in the architecture of hepatocytes, when compared to normal control group (Figure 1 A & D). Diabetic group treated with extract of *Cynodon dactylon* showed marked reversal in these changes (Figure. 1 C & F) when compared to diabetic group (Figure. 1B & E). However normal rats with the extract showed no changes in the liver tissue.

DISCUSSION

In the present study, protective effect of aqueous extract of *Cynodon dactylon* was evaluated in the liver tissue of streptozotocin diabetes induced liver damage in Wistar rats. Liver enzymes SGOT, SGPT are present in high concentration in the normal hepatocytes and these enzymes leaks into the circulation due to damage to hepatocytes cell membrane¹³. In our study, levels of hepatic enzymes were elevated in the diabetic group showing hepatocytes damage, by means of elevation of endotoxins which pass through the intestinal wall into the

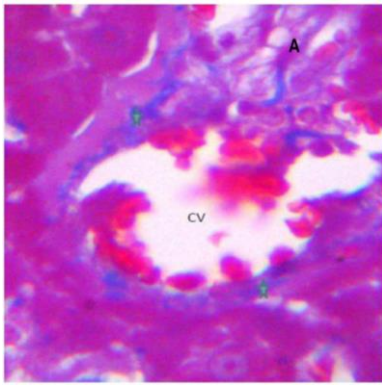


Figure 1 A: Photomicrograph of liver section of normal control group stained with Masson's trichrome stain shows moderate areas of collagen staining (Green star) around central vein (CV). (Magnification 100X)

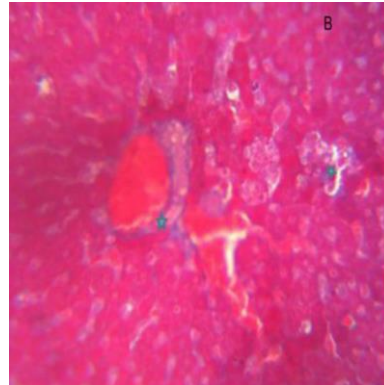


Figure 1 B: Photomicrograph of liver section of Diabetic control group stained with Masson's trichrome stain shows extensive areas of collagen staining (Green star) around central vein (CV). (Magnification 100X)

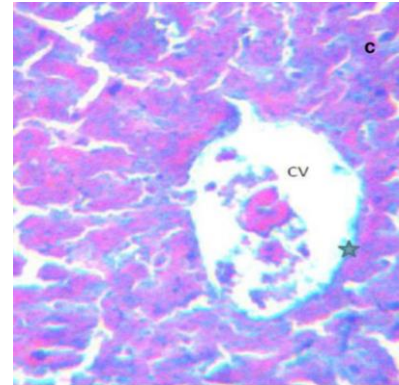


Figure 1 C: Photomicrograph of liver section of Diabetic group treated with extract of *Cynodon dactylon* stained with Masson's trichrome stain shows mild to moderate areas of collagen staining (Green star) around central vein (CV). (Magnification 100X)

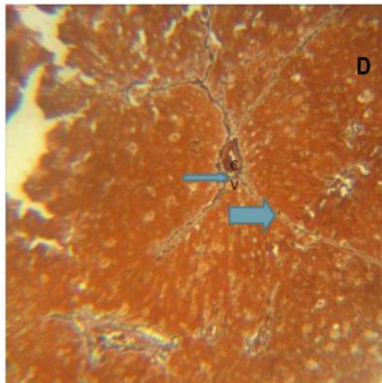


Figure 1 D: Photomicrograph of liver section of normal control group stained with Gordon and Sweets reticulin stain shows dense network of reticular fibres (green arrow) around the central vein (CV). (Magnification 100X)

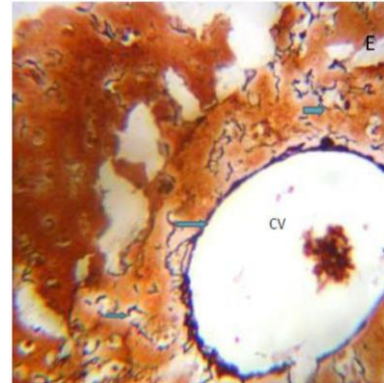


Figure 1 E: Photomicrograph of liver section of Diabetic control group stained with Gordon and Sweets reticulin stain shows poor staining areas of reticular fibres (green arrow) between sinusoids, except around the central vein (CV). (Magnification 100X)

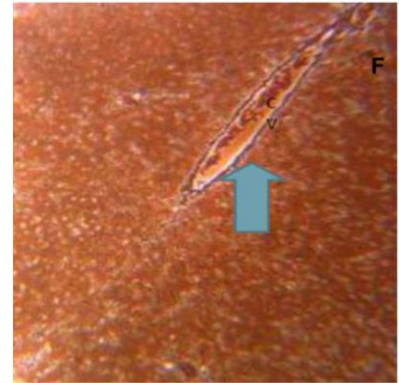


Figure 1 F: Photomicrograph of liver section of Diabetic treated group stained with Gordon and Sweets reticulin stain shows dense network of reticular fibres (green arrow) around the central vein (CV). (Magnification 100X)

portal blood, thereby enter into the liver, these endotoxins stimulate Kupffer cells to produce reactive oxygen species (ROS) and pro-inflammatory cytokines such as TNF- α AND IL-1 β , both cytokines are important mediators in inflammation, leading to cell death¹⁴. The significant decrease in the levels of hepatic enzymes observed in the present study, when treated with aqueous extract of *Cynodon dactylon* in diabetic group, exhibited its protective effect in the liver tissue, which indicates the membrane stabilizing action of the extract that further prevented leakage of intracellular hepatic enzymes. Histopathological observation has shown the restoration of normal liver morphology in the *Cynodon dactylon* treated diabetic rats, regeneration of reticular fibers as well as reduction in the collagen fibers in the liver section of the extract treated diabetic rats. In the diabetic condition, hepatic stellate cells are activated, resulting in increased accumulation of collagen fibers in the hepatic stellate cells¹⁵. After activation of hepatic stellate cells,

matrix metalloproteinase 2 (MMP-2) and tissue inhibitor metalloproteinase 1, 2 (TIMP-1, 2) increased with simultaneous decrease in matrix metalloproteinase 1 (MMP-1). The elevated MMP-2 activates the breakdown of collagen fibers type III & IV in the normal liver, elevated TIMP-1, 2 and decreased MMP-1, inhibit the degradation of collagen type I fiber in the scar tissue, consequently the accumulation of numerous collagen fibers may lead to liver fibrosis and cirrhosis^{16, 17, 18, 19}. The Phytochemical present in the *Cynodon dactylon* may be responsible for the protective effect of liver tissue of streptozotocin diabetic induced liver damage in wistar rats, possibly through their anti-oxidant activity. To conclude, this study showed that *Cynodon dactylon* aqueous extract has protective effect that were proven by histopathological and biochemical analysis. Also, the extract showed its non toxic nature in normal treated rats. Further studies are warranted to confirm these findings.

CONFLICT OF INTEREST STATEMENT

None Declared

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