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Original Research Article

Histological and Immunohistochemical Study of the Beta Cells In Streptozotocin Induced Diabetic Rats Treated with Cynodon Dactylon Aqueous Extract

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ABSTRACT

Background: Diabetes mellitus is a chronic metabolic disorder that affects human body in terms of physical, psychological and social health. Traditional medicines have been widely used in treatment of diabetes and its complications. After administrating the aqueous extract of *Cynodon dactylon* in streptozotocin induced diabetic rats, we evaluated the histological and immunohistochemical effects of the β – cells in the pancreatic islets.

Objectives: The objective of the present study is to investigate the antidiabetic effect of *Cynodon dactylon* aqueous extract in β – cells of islets, against streptozotocin induced diabetic rats.

Materials and Methods: In this study adult male albino rats were used. Aqueous extract of *Cynodon dactylon* (500 mg/kg.bw/day) administered orally for 45 days. At the end of experimental period, animals were sacrificed, pancreas was isolated and processed for histological and immunohistochemical analysis.

Results: The pancreas of *Cynodon dactylon* aqueous extract treated diabetic rats showed improved islet morphology; and the pancreatic islets of the *Cynodon dactylon* aqueous extract treated diabetic rats showed consistent elevation in the insulin levels from the β – cells. Therefore, the antidiabetic activity of *Cynodon dactylon* aqueous extract might be due to the compounds present in the *Cynodon dactylon* aqueous extract which stimulate the insulin secretion and protect the intact functional β – cells from streptozotocin induced destruction.

Conclusion: *Cynodon dactylon* aqueous extract increased the insulin secreting β – cells in the pancreatic islets and protecting the intact β – cells from oxidative damage.

KEYWORDS: *Cynodon dactylon,* streptozotocin, Pancreatic islets, oxidative stress.

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INTRODUCTION

Diabetes mellitus (DM) is considered as a significant disorder worldwide in both mortality and morbidity affecting several demographics despite of topographical locality, age, gender, ethnicity. DM is a sort of metabolic disorder characterized by chronic hyperglycaemia that affects Globally, 10.5% of the population [1]. Hyper glycemia produces oxidative stress that can cause cellular and tissue damage and chronic DM leads to microvascular complications in the kidney and other organs due to strong linkage with reactive oxygen species (ROS) that induce oxidative damage [2]. DM is classified as type 1 and 2, type 1 diabetes occurs due to death of pancreatic beta cells which is autoimmune, but Type 2 diabetes has genetic reason and is greatly associated to obesity and a sedentary lifestyle with increase in insulin resistance [3,4]. Histopathological considerations, such as quantity of cell damage and accumulation of fat in pancreatic tissues in animal models are the novel factors to know crucial information about the mechanisms of action and therapeutic agents that act against type 2 DM [5].

Streptozotocin (STZ) is an antibiotic produced by the reliable strains of the gram-positive bacteria Streptomyces achromogenes [6]. STZ has cytotoxic activity on insulin-producing pancreatic islets beta cells [7].

STZ is a glucosamine nitrosourea compound, so it expresses very high levels of GLUT2 and thus are preferentially sensitive to STZ's cytotoxicity [8]. STZ has been widely used in biomedical research, as a drug of choice in DM animal models selectively for destruction of beta cells in the pancreatic islets [9].

Ethnopharmacological research propose that 1,200 plants have been used in traditional medicines for their antidiabetic and hypoglycaemic activities throughout the world [10]. Cynodon dactylon is the weed, which belongs to Poaceae family, it is distributed widely in India, which has been exhibited various medicinal properties like antiviral, antimicrobial, antioxidant, antidiabetic activities and used to treat urogenital infections such as renal calculi, urinary tract infection and prostatitis [11]. Cynodon dactylon plant contains compounds like terpenoids, vitamin C, palmitic acid and alkaloids, flavonoids [12, 13].

The present study aimed to establish allocated immunohistopathological profile of the beta cells (β) in pancreatic tissue in STZ-induced rat model treated with Cynodon dactylon aqueous extract.

MATERIALS AND METHODS

Chemicals and Plant extract

Streptozotocin was procured from Sisco research laboratory Mumbai. Glibenclamide was purchased from Aventis pharma India Ltd. All other chemicals and reagents used in this study were of analytical grade procured from Thermofischer scientific India Ltd, Mumbai. *Cynodon dactylon (L.)* was collected from Kanniyakumari district of 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 vaccum evaporator. The concentrated dark extract was stored in an airtight container.

Experimental animals

Albino male Wistar rats of 10 weeks of age, weighing approximately 150-200 g were acclimatized and caged in the SRM medical college hospital and research centre, central animal house, Tamilnadu. All animals were kept in 12:12 hr light: dark cycle, at a room temperature of 22±2ÚC. Rats were provided with pellets of standard size and weight, procured from Provimi animal nutrition India ltd, Bangalore, India, were also allowed free access to water. Animal experimentations were carried out under the supervision of on duty veterinary medical officer in accordance with the ethical norms approved by the Institutional animal ethical committee (IAEC) of SRM Medical College, Potheri, Tamilnadu, India. (Ref: SRM/45/IAEC/2011).

Experimental Design

Grouping of the rats were done randomly into 5 groups of 6 rats in each group. Group 1

control rats were treated with normal water, group 2 diabetic control rats, group 3 diabetic induced rats treated with Glibenclamide (5 mg/kg body weight) for 45 days, via Oral gavage, group 4 diabetic induced rats treated with aqueous extract of *Cynodon dactylon* (ACD) (500 mg/kg body weight) for 45 days, via oral gavage.

Experimental Diabetes Induction

Diabetes was induced by the intraperitoneal administration of single dose of streptozotocin (45mg/kg body weight prepared in 0.1 M Citrate buffer at pH 4.5, after overnight fasting of experimental animals.

Collection of Tissue Samples

On completion of 45 days of experimental period, animals were sacrificed, following the guidelines of animal ethical committee. The Pancreas was excised and fixed in 10% neutral buffered formalin (NBF) solution for histological and immunohistochemical analysis.

HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDIES

Immunohistochemical study of pancreatic islets

Immunohistochemical expression of Insulin in the β cells of pancreatic islets of normal and experimental groups of animals, carried out with Primary monoclonal mouse anti-insulin antibody, purchased from Biogenex, USA, Catalog no.MU029-UC. Immunohistochemical procedure was done using Ultravision Quanto-Detection system, from Thermofischer scientific, USA. Photomicrography of the stained slides were done by utilising the APCAM-5 USB 2 digital camera attached to a computer monitor, supplied by ADELTAVISION OPTEC India.

Procedure

Paraffin sections of 5-micron thickness were mounted on positively charged slides and subjected to the immunohistochemical procedure, using an Ultravision Quanto-Detection system (Thermofischer scientific, USA), following the manufacturer's instructions.

Gomori chrome alum haematoxylin stain

To histologically demonstrate the β - cells in the islets of pancreas, pancreatic tissue sections were stained with Gomori chrome alum haematoxylin stain, a beta cell specific stain. Photomicrography of the stained slides were done.

RESULTS

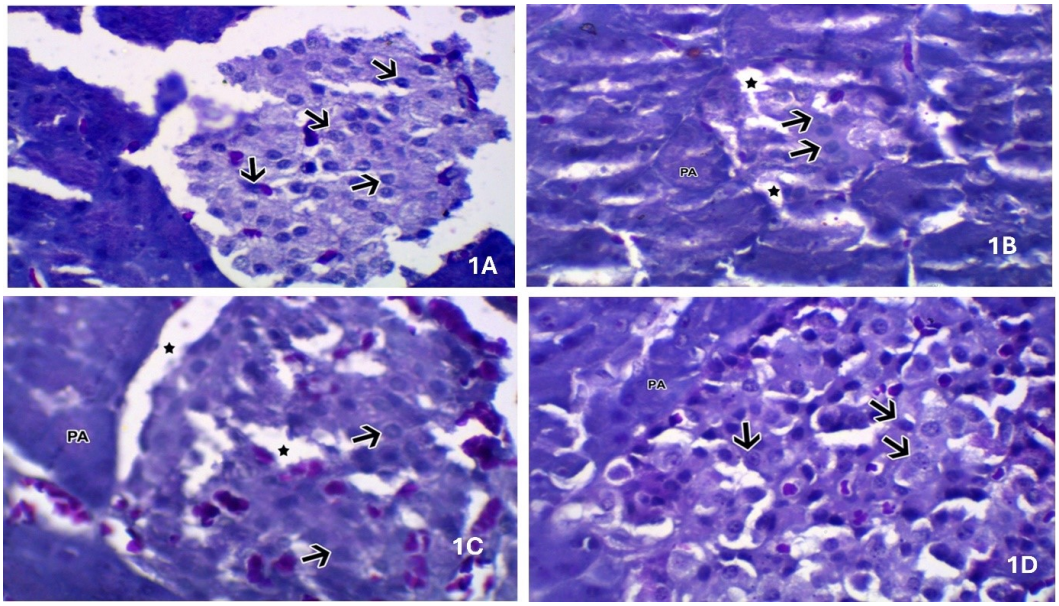

Fig. 1: Photomicrographs of sections of rat pancreas stained by Gomori chrome alum haematoxylin (400X). **1A**. Control group showing the normal distribution of β- cells (black arrow) in the islets. **1B**. Diabetic control group showing Degenerating β –cells, with very few surviving cells (black arrow), Vacuolation in islets also shown (black star). **1C**. Diabetic + Glibenclamide treated group shows regeneration of β–cell, (black arrow) with vacuolation in islets (black star), **1D.** Diabetic +ACD treated group shows regeneration of β–cell with few Vacuolation in islets. Regeneration well appreciated than glibenclamide treated group.

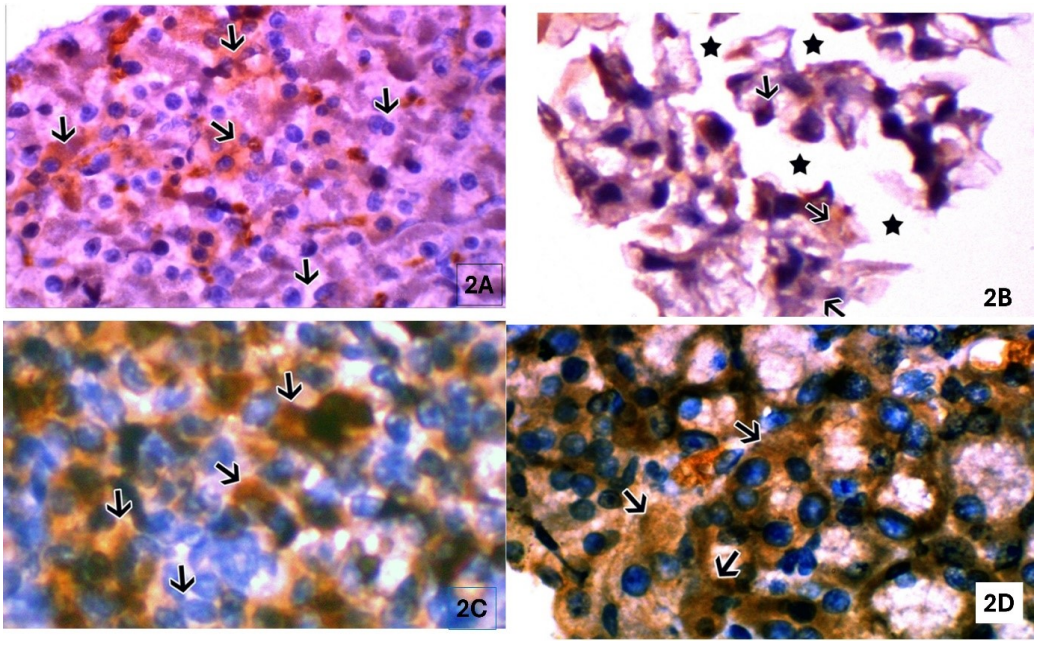

Fig. 2: **Photomicrographs of insulin immunohistochemical staining of pancreatic islets (400X)**. **2A**. Control group showing strong immunoreactivity of insulin in the β– cells (black arrow). **2B.** Diabetic control group showing significant reduction in the immunohistochemical expression of insulin in β–cells (black arrow), vacuolations also seen (Black star) **2C**. Diabetic + Glibenclamide treated group shows apparent increase in the immunohistochemical expression of insulin in β– cells (black arrow), **2D**. Diabetic +ACD treated group shows significant increase in the immunohistochemical expression of insulin in β cells (black arrow), with normal density, when compared to Diabetic control group.

Histological analysis of the rat pancreas stained with Gomori chrome alum haematoxylin stain (400X)

The Control group showed the normal distribution of β- cells in the islets (Figure 1A) Diabetic control group showed degenerating β cells, with very few surviving cells, Vacuolation in islets is also seen (Figure 2B). The Diabetic + Glibenclamide treated group showed regeneration of β–cell, with vacuolation in islets (Figure 1C). The Diabetic +ACD treated group showed regeneration of β–cell with few vacuolation in islets. Regeneration is well appreciated than glibenclamide treated group (Figure 1D).

Immunohistochemical observation of the insulin staining in the pancreatic islets (400X).

The Control group showed strong immunoreactivity of Insulin in the β– cells (Figure 2A). A significant reduction in the immunohistochemical expression of insulin in β– cells (Figure 2B) was seen in Diabetic control group. Diabetic + Glibenclamide treated group showed apparent increase in the immunohistochemical expression of insulin in β– cells (Figure 2C). A significant increase in the immunohistochemical expression of insulin in β cells, with normal density was seen in Diabetic +ACD treated group, which was identical when compared to the Diabetic + Glibenclamide group (Figure 2D).

DISCUSSION

Diabetes mellitus is associated with progressive metabolic derangement, worsening glycaemic control and morphological changes in the pancreas, liver and other organs. The release of free radicals due to oxidative stress play a vital role in the development of morphological changes in various tissues. Reserve amount of pancreatic insulin is a prominent sign of islet function with serious linkage between insulin secretion and production which is essential for prompt functioning of pancreatic β- cells. Insulin deficiency in diabetes mellitus leads to accumulation of lipids especially triglycerides and total cholesterol in diabetic patients [14]. Aqueous extracts of *Cynodon dactylon* analyzed by HPLC–ESI MS have identified the presence of apigenin, luteolin, 6-*C*-pentosyl-8-*C*-hexosyl apigenin and 6-*C*-hexosyl-8-*C*-pentosyl luteolin [15]. Mammalian cells are constantly exposed to ROS as result of normal metabolic process occurring during aerobic respiration. STZ is

taken up by the pancreatic β cells via glucose transporter GLUT-2. The main cause of STZ induced β cell death is via formation of extensive poly ADP ribose, depletion of cellular nicotine adenine dinucleotide and ATP and generation of ROS [16]. Diabetes arises from irreversible destruction of the â islet cells in the pancreatic islets, causing deregulation or insulin reduction [17].

In this study, 45 days of treatment with aqueous extract of *Cynodon dactylon* at a dose of (500 mg/kg, body weight) had significantly shown the antidiabetic effect in STZ induced diabetic rats. Blood glucose lowering potential of aqueous extract of *Cynodon dactylon* might be due to activation of β - cells giving insulinogenic effect through the stimulation of regeneration process and reactivation of β cells, and the presence of flavonoids in the extract might be responsible for the stimulation of glucose uptake in peripheral tissues and regulation of activity and/or expression of the rate limiting enzymes involved in carbohydrate metabolism [18]. In the present study we observed that, the pancreas of aqueous extract of *Cynodon dactylon* treated diabetic rats showed improved islet morphology; and the pancreatic islets of the aqueous extract of *Cynodon dactylon* treated diabetic rats showed significant increase in the insulin secreting β cells, which was similar to one of the study where the diabetic rats were treated with high dose of caffeine [19].

Therefore, the antihyperglycaemic activity of aqueous extract of *Cynodon dactylon* may be due to the substances present in the aqueous extract of *Cynodon dactylon* which stimulate insulin secretion and / or protect the intact functional â cells from STZ induced destruction.

Histopathological observations shown in one of the studies confirmed that aqueous extract of *Cynodon dactylon* treated diabetic rats were found to be efficient in reducing the islet cellular toxicity induced by Streptozotocin (STZ) [20]. In the previous study one of the flavonoid compound quercetin was shown to increase in the â cells numbers stained with gomori chrome alum haematoxylin stain in STZ induced diabetic rats [21].

An another immunohistochemical study demonstrated that, administration of high dosage genistein resulted in partial improvement of the β -cells associated with increased insulin immunoreactivity in STZ induced diabetic rats [22]. One of the histopathological study results of aqueous extract of *Cynodon dactylon* treated diabetic rats also confirmed the significant recovery of liver damage in STZ induced diabetes and exhibited antidiabetic effect [23].

CONCLUSION

Aqueous extract *of Cynodon dactylon* in diabetic rats revealed prompt increase in the insulin secreting β-cells in the islets, which was clearly demonstrated by both histological and immunohistochemical analysis in our study. However ultrastructural changes in the β-cells are necessary to document further mechanism of action of this extract.

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Author Contributions

M K S J: Designed, performed the experiments, wrote the manuscript. Analysed the image.
R S: Analysed the data and assisted in manuscript preparation.
R S K: Guidance in manuscript preparation
G P: Reviewed the manuscript and language editing.
M M: Editing and formatting the manuscript

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