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Research Article

Histological Studies on Islets of Langerhans of Pancreas in Diabetic Mice after Curcumin Administration

Walvekar M V*, Potphode N D, Desai S S, Deshmukh V M

Department of Zoology, Shivaji University, Kolhapur, India.

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ABSTRACT

The present study was carried out to determine the effects of curcumin on blood glucose level and histology of pancreas in alloxan induced diabetic mice. 15 male albino mice weighing 26-30 gm were used. They were divided into 3 groups, control group received subcutaneous injection of 0.15 M acetate buffer for 15 days, alloxan induced group received subcutaneous injection of alloxan 150 mg/kg body weight to induce diabetes and recovery group received intraperitoneal injection of curcumin 100 mg/kg body weight per day for 15 days. At the end of experiment body weight, pancreatic gland weight, blood glucose level was determined and histology of pancreas was studied by HE and PAS technique. In diabetic mice there was decrease in body weight, pancreatic gland weight and increase in blood glucose level but after treatment with curcumin significant increase in body weight and pancreatic gland weight and decrease in blood glucose level was observed. In histology there was decrease number and size of islets which was again increased after curcumin administration. In conclusion curcumin can be useful as curative agent in diabetes.

Keyword: curcumin, diabetes, islets of Langerhans, histology.

INTRODUCTION

Diabetes mellitus (DM) is considered as one of the major health concerns all around the world today[1,2]. It is a metabolic disorder featured by hyperglycemia and alteration in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency of insulin secretion and / or insulin action[3]. DM is classified into two types, insulin dependent diabetes mellitus (IDDM Type 1) and non insulin dependent diabetes mellitus (NIDDM, Type 2). Type I diabetes is an autoimmune disease characterized by a local inflammatory reaction in and around islets that followed by selective destruction of insulin secreting β cells[4,5]. Type II diabetes is characterized by peripheral insulin resistance and impaired insulin secretion. There is growing consensus that diabetes is usually accompanied by an increased production of free radicals or by impaired antioxidant defenses which is accompanied by development and progression of diabetic complications[6-9]. Alloxan is a chemical compound used to induce experimental diabetes by leading the β cells islets of the Langerhans to swell and finally degenerate. Alloxan diabetic mice have been reported to have increased vascular permeability; with no recorded fiber loss[10]. Various drugs presently available to reduce diabetes associated hyperglycemia are associated with several side effects. Hence, in the recent years, there is growing interest in herbal medicine all over the world, as they have little or no side effects[11]. Here we have selected one such important popular ayurvedic herb, *Curcuma longa* (Zingiberacae) commonly known as turmeric. Curcumin is the major pigment from dried rhizome of the plant *Curcuma longa* Linn, that has been used as spice and traditional medicine in Asia for centuries to treat gastrointestinal upset, arthritic pain, parasites, inflammation and other diseases. Studies have shown the potent antioxidative activity of curcumin may be one of the mechanisms of antiaging. Curcumin extends life span in *Drosophila* by reducing oxidative stress and increasing locomotive activity[12]. The present study was conducted to investigate the curative effect of curcumin in diabetes mellitus.

MATERIAL AND METHODS

Materials

Animals

Male albino mice (*Mus musculus* Linn.) of age 3 months and weighing between 28-30 gm were used for present investigation. All the animals were maintained under controlled condition with 12 hr light and 12 hr dark cycles at temperature of $26^0C \pm 2^0C$ in departmental animal house (1825/PO/EReBi/S/15/CPCSEA). Mice were divided into groups such as control and experimental and were caged separately. The animals were housed in plastic cages having dimensions of 29×22×14 cm and allowed to live in groups of 4 to 5 per cage with rice husk bed and under proper condition of light, temperature and humidity. The animals were supplied with standard 'Amrut Mice Feed' (Pranav Agro Industries, Pvt. Ltd. Sangli, Maharashtra, India) and water was given *ad libitum.*

Experimental design

Mice were divided into 3 groups

**Author for Correspondence: madhuri_walvear@rediffmail.com*

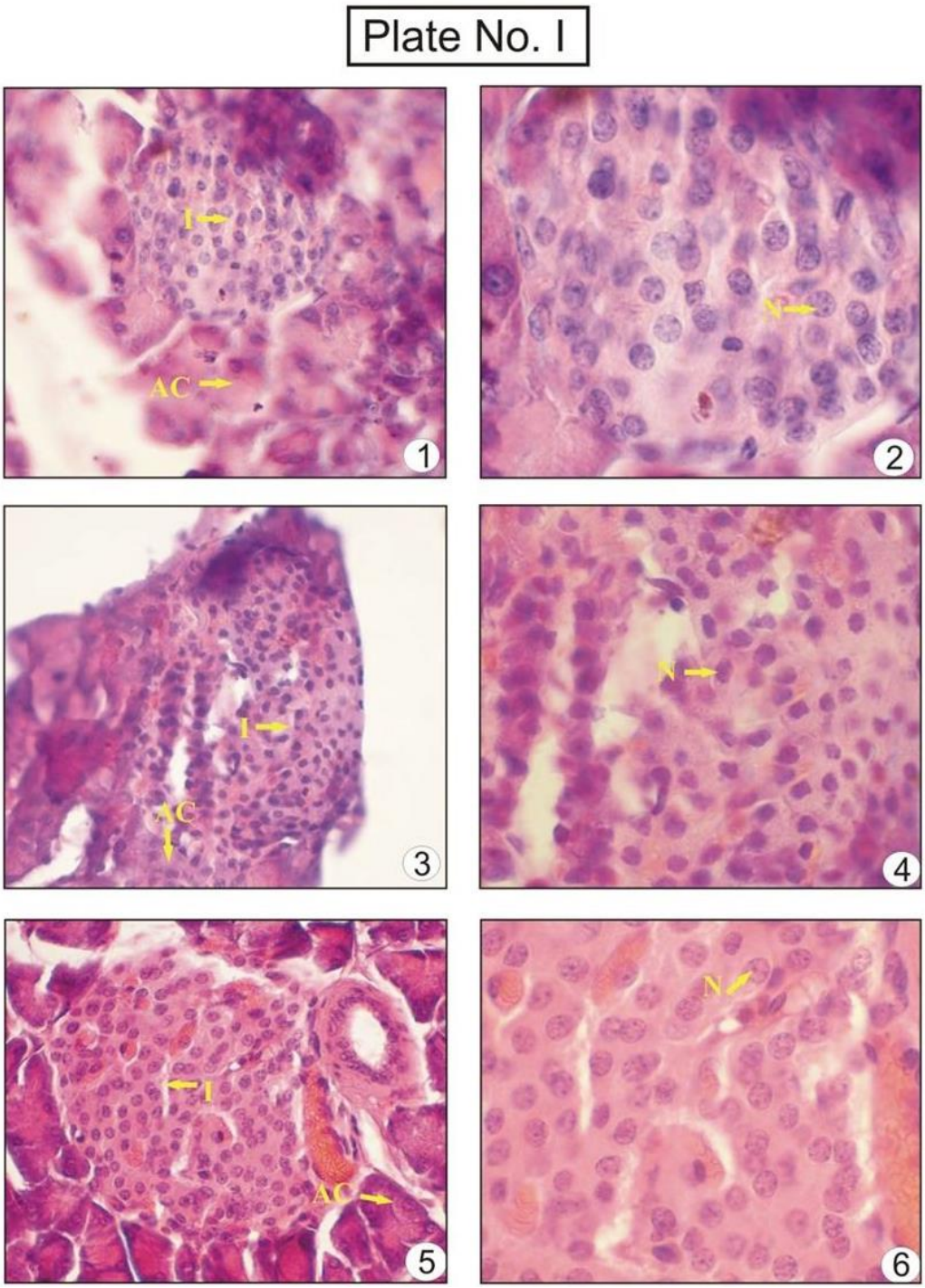

Plate 1: Islets of Langerhans of pancreas stained by HE.
Figure 1 and 2: Control mice pancreas showing normal structure of Islets of Langerhans at 400X, 1000 X respectively.
Figure 3 and 4: Diabetic mice pancreas showing degenerative and necrotic changes, reduced dimension of Islets of Langerhans at 400X, 1000 X respectively.
Figure 5 and 6: Diabetic mice treated with curcumin showing marked improvement of Islets of Langerhans at 400X, 1000 X respectively.
Captions: AC- Acinar cells, N-Nucleus, I- Islets of Langerhans

Control Group
Three months' male mice were given subcutaneous injection of 0.15M acetate buffer pH 5.4 for 15 days.
Diabetic Group
Three months' male mice were given single subcutaneous injection of alloxan 150 mg/kg body weight[13].
Recovery group

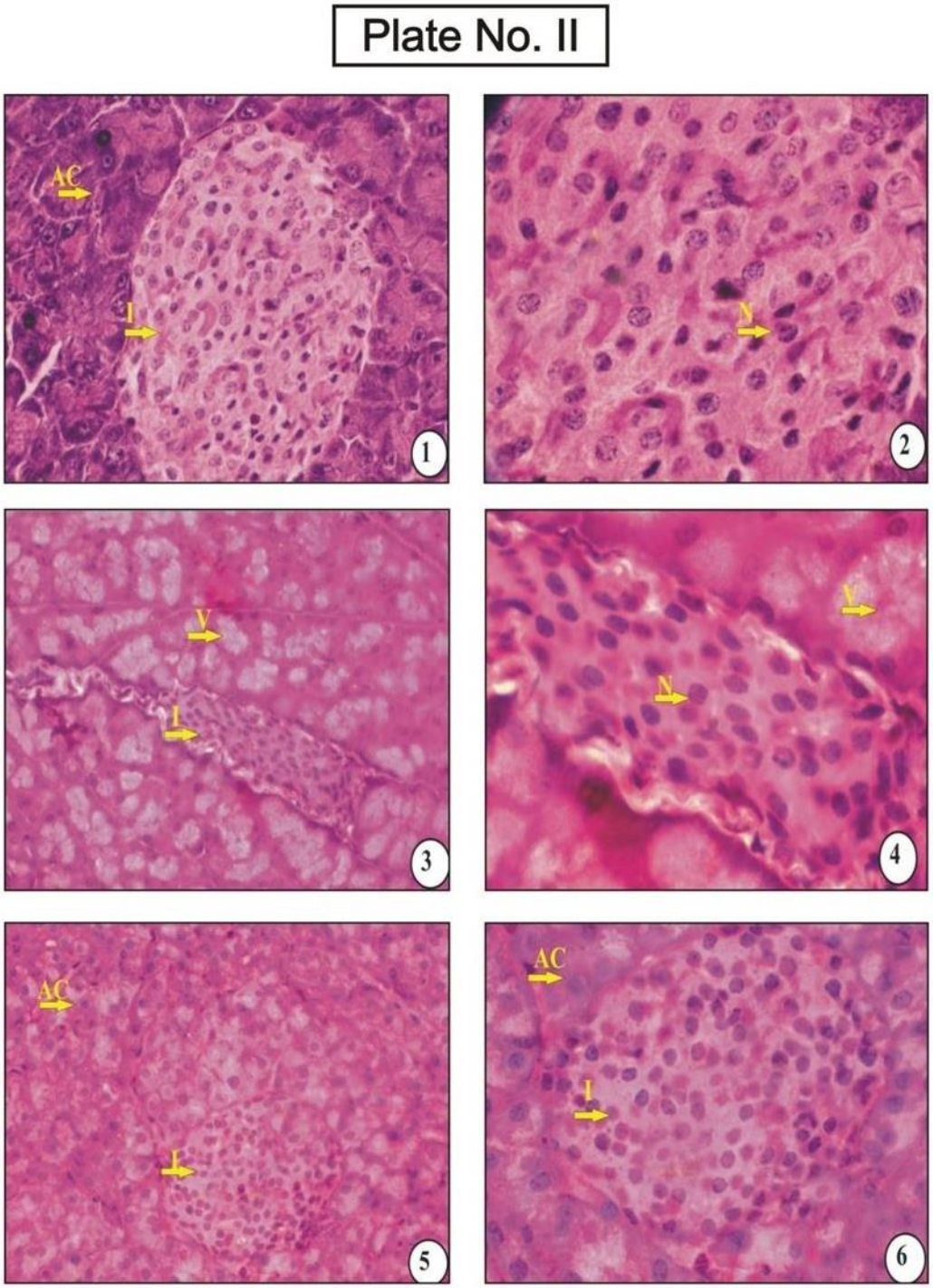

Plate II: Islets of Langerhans of pancreas stained by PAS
Figure 1 and 2: Control mice pancreas showing normal structure of Islets of Langerhans at at 400X, 1000 X respectively
Figure 3 and 4: Diabetic mice pancreas showing degenerative and necrotic changes, reduced dimension of Islets of Langerhans at 400X, 1000 X respectively
Figure 5 and 6: Diabetic mice treated with Curcumin showing marked improvement of Islets of Langerhans at 400X, 1000 X respectively.
Captions: AC- Acinar cells, N-Nucleus, I- Islets of Langerhans.

Three months' male mice were given intraperitoneal injection of curcumin at a dose of 100 mg/ kg body weight to diabetic mice daily for 15 days[14].

Body weight of mice

Animals were weighed before starting experiment, during respective treatment and also after completion of each treatment. The record of these observations was maintained.

Weight of Pancreas

The animals from respective groups were killed by cervical dislocation after completion of treatment. Pancreas dissected out, washed in ice cold saline (0.09 %

Table 1: Curcumin effect on body weight (gm), weight of pancreas (mg) and blood glucose level of alloxan induced diabetic mice. Values are mean ± S.D. (Numbers in parenthesis denotes number of animals) P<0.01= Significant, P> 0.5 non-significant

S. no.	Treatment (n=5)	Weight of animal (gm)	Statistical significance	Weight of pancreas (mg)	Statistical significance	Blood glucose	Statistical significance
1	Control	30.424 ± 1.3395	1:2 P<0.01	108.8 ± 4.4385	1:2 P<0.01	105.4 ± 5.0794	1:2 P<0.01
2	Diabetic	18.048 ± 1.2401	2:3 P<0.01	83.8 ± 2.3875	2:3 P<0.01	265.8 ± 47.0287	2:3 P<0.01
3	Recovery	23.31 ± 1.055	1:3 P<0.01	94.2 ± 2.5884	1:3 P<0.01	137.4 ± 8.2644	1:3 Non-significant

NaCl), dried with the help of blotting paper and wet weight of gland was measured using digital scale balance. The record of these observations was maintained.

Blood glucose

Fasting blood glucose was measured by collecting a drop of blood from the tail after incision with a sharp blade. The blood glucose level was determined by using a rapid glucose analyzer with a glucose strip inserted in Accuchek blood glucose monitoring glucometer (Roche diagnostics India Pvt. Ltd.). The results were expressed in terms of milligram per deciliter of blood[15].

Histopathological study

After the completion of dose, mice from all groups were killed by cervical dislocation and pancreas were dissected out quickly and fixed in 2% CAF fixative. Tissue were processed and embedded in paraffin wax. Sections were cut at 5µ thickness and stained with Hematoxyline-Eosin (HE)[16] and Periodic Acid-Schiff reaction (PAS)[17] technique for histochemical studies. After completion of staining sections were observed under microscope for histogical change

Statistical Analysis

All values were expressed as mean ±SD. Statistical analysis was carried out by one-way ANOVA, Turkey's HSD test. There was a significant reduction in body weight and pancreatic gland weight of the mice in diabetic group in comparison to control. After administration of curcumin for 15 days, the body weight and pancreatic gland weight was recovered significantly (P<0.01) with respect to diabetic mice. Fasting blood glucose level in all mice in control group before treatment was within the normal levels and it was significantly elevated after 5 days of alloxan treatment with respect to control level. Treatment of curcumin resulted restoration of fasting blood glucose level near too normal when compared with diabetic mice. Histological examination of slides of pancreas stained with HE and PAS of control group showed normal pancreatic islets with rich vascular supply while in diabetic group (fig. 1,2), alloxan administration led to shrinkage of normal architecture of the pancreatic islets. The number and size of islet of Langerhans was decreased as compared to control. The number of cells in each islet was also reduced drastically (fig. 3,4). Curcumin treatment showed recovered architecture of the pancreatic islets. The number and size of islet of Langerhans was increased as compared to diabetes. The number of cells in each islet was also increased (fig.5,6).

DISCUSSION

In this study alloxan induced diabetes produced marked loss in body weight and pancreatic gland weight. Diabetes is usually associated with weight loss; this is because the body switches to burning fatty acids due to insulin shortage. However significant increase in body weight and pancreatic gland weight in curcumin treated mice may be due to improved level of insulin in these groups. In the present study the diabetic mice showed increase in blood glucose level as compared to control and after treatment with curcumin there was significant decrease in blood glucose level (P<0.01). This clearly indicated the hypoglycemic activity of curcumin. This result is in agreement with research work by some scientist[18,19]. Curcumin showed an anti-hyperglycemic effect[20,21]. Moreover, curcumin antagonizes the deficit of glucose energy metabolism or oxidative stress related to cognitive impairment associated with diabetes[22]. Alloxan induces damage to β -cell DNA[23], mitochondria[24], lysosomes[25], and plasma membrane[26]. It was noted that the destruction of 90% insulin secreting β cells of islets of Langerhans was caused by alloxan and hence high blood glucose level was detected27. Curcumin might aid in the recovery of β cells to secrete insulin; therefore, the blood glucose level was decreased after treatment. The present study showed increase in PAS staining intensity after curcumin treatment which indicates increase in neutral type of glycoproteins indicating increase in insulin secretion i.e. may be due to increase in β cell or recovery of β cells. The histological studies of pancreas of the diabetic mice showed shrinkage of islets of Langerhans, reduction in size and number of islets while recovery group showed restoration of number and size of islets of Langerhans. Islets cells of recovery group treated with curcumin have regenerated considerably suggesting the presence of stable cells in the islets with the ability of regenerating[28,29]. In conclusion, this study investigated the effect of alloxan on a β cells and threw light on the potential of curcumin in the prevention or treatment of diabetes. This also suggests that the curcumin at 100 mg /kg dose has the ability of inducing the quiescent cells to proliferate to replace the lost cells of islets of Langerhans.

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