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Induced Experimental Diabetes and Its Impact on the Rabbit Brain

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Abstract : Diabetes mellitus in rabbits was permanently established by intraperitoneal administration of alloxan @ 80 mg/kg.b.w. at weekly intervals following twelve hours fasting. The diabetic rabbits exhibited a significant increase in blood sugar level (F) ($P<0.001$) and a significant decrease in body weight ($P<0.50$) upto six weeks in contrast to control rabbits. After twenty six weeks the diabetic rabbits showed histomorphological disturbances in brain that included edema, degeneration of neurons and degenerative changes in purkinji cells in cerebellum. Further, a decrease in beta cell number was observed in diabetic rabbits compared to control. It is concluded that with the progress of untreated diabetes, the subsequent effects of hyperglycemia alter the histomorphology of brain in alloxan induced diabetic rabbits.

Key words : Diabetes, rabbits, brain, histopathology

Introduction

Diabetes mellitus is one of the most common metabolic disorders with a worldwide prevalence estimated to be between 1% and 5% (Meral *et al*., 2004). In India, over 20 million people are affected by diabetes and the numbers are expected to increase to 57 million by 2025 (Arvind *et al*., 2002). The World Health Organization (WHO) has declared India as the country with the largest no of diabetic subjects in the world.

Greater than half of all patients with diabetes develop neuropathy, a progressive deterioration of nerves resulting in peripheral and autonomic nerve dysfunction. As a result, diabetic neuropathy is the most common cause of nontraumatic amputations and autonomic failure (Feldman *et al*., 2002; Vinik *et al*., 2002). A diabetic patient with neuropathy has a 15% chance of undergoing one or more amputations (Feldman *et al*., 2001).

Due to meager information regarding the histomorphology of diabetics, the present study was undertaken in a view to observe the subsequent effects of prolonged hyperglycemia on histomorphology of brain in alloxan induced diabetic rabbits.

Materials and Methods

An experiment was conducted in 12 male New Zealand white rabbits of almost uniform age. The rabbits were divided into two groups of six each. Group I was made diabetic by four doses of intraperitoneal administration of alloxan @ 80 mg/kg. b.w.

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(Baqui *et al*., 2005; Mir *et al*., 2006) following twelve hours of fasting where as Group II was kept as control which received an equal volume of isotonic saline without alloxan. The animals were fed on green vegetables and commercial pelleted diet, and received human care according to the guidelines outlined in the "Guidelines for the Care and Use of Animals in Scientific Research" prepared by the Indian Science Academy, New Delhi (Anonymous, 2000).

Development of induced diabetes mellitus was confirmed by examining the fasting glucose level in the blood taken from marginal ear vein. The rabbits were observed for twenty six weeks and periodical blood sugar level was monitored. The rabbits were latter sacrificed for histological examination of pancreas and brain.

Analytical Procedure

The blood sugar of rabbits was estimated by Glucometer Gx (Bayer Diagnostic India, Ltd.). Histological examination was done by fixing the pancreas and brain of rabbits in 10% formalin, and processed and embedded in paraffin wax. Tissue blocks were sectioned 5 micron thick and stained with Harris Haematoxylin and Eosin (Luna, 1968). However, to demonstrate pancreatic islet cells, Gomori's modified stain (Halami, 1952) was used with a modification of substituting the Lugol's iodine by equal parts of 0.5% KMNO4 and 0.5% sulphuric acid, and sodium thiosulphate by 2% sodium bisulphite respectively.

For quantitative assessment of beta cells in islets of Langerhan's, cells of approximately four islets on each tissue and forty islets of each group were counted.

Results and Discussion

The establishment of diabetes mellitus in Group I rabbits was observed after first week of alloxan administration by increased fasting blood glucose levels (149 ± 6.26). The periodical monthly estimation of blood glucose indicated highest values (292.75 ± 8.87) in Group I rabbits during the sixth week, plateaued on the seventh week and latter on showed a fluctuation. The blood glucose levels were however, significant in Group I rabbits in contrast to Group II rabbits which showed normal values (105.25 ± 4.65) throughout the experimental period. Furthermore, high blood glucose levels were observed in Group I rabbits upto the rest of the experimental period indicating the permanent nature of the disease induced by alloxan.

Table I summarizes the body weight profile of Group I and Group II rabbits. In Group I rabbits, a significant decrease in body weight from 1.68 0.11kg to 1.46 0.02 Kg was recorded after the first week of alloxan administration followed by fluctuations. However, in Group II rabbits a steady increase in body weight was observed throughout the experimental period.

Pancreatic sections stained with Haematoxylin and Eosin showed that alloxan caused severe necrotic changes of pancreatic islets in Group I rabbits. Using modified Gomori's aldehyde fuchsin staining techniques (Halami, 1952) for pancreatic sections, nuclear changes, karyolysis, disappearing of nucleus and rarefaction of nuclear contents were visible (Fig. 1) in comparison to Group II rabbits.

Haematoxylin and Eosin stained sections of brain in Group I rabbits showed edema, degenerative changes in neurons and

Table-I: Changes in Blood Glucose (F) and Body Weight Profile of Alloxan-induced Diabetic Rabbits

Parameters	Initial Value		WEEKS											
			1st		2nd		3rd		4th		5th		6th	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T
Blood Glucose (F) (mg/dl)	103.25 ± 3.19	103.75 ± 3.63	106.75 ± 4.71	149 ± 6.26	98 ± 5.18	192.5 ± 6.88	101.5 ± 4.97	195.25 ± 7.41	94.5 ± 5.28	234.75 ± 8.68	99.5 ± 4.80	257.5 ± 9.19	105.25 ± 4.65	292.75 ± 8.87*
Body Weight (Kg)	1.15 ± 0.09	1.68 ± 0.11	1.21 ± 0.07	1.46 ± 0.02	1.29 ± 0.06	1.56 ± 0.04	1.35 ± 0.07	1.52 ± 0.02	1.45 ± 0.07	1.55 ± 0.03	1.51 ± 0.06	1.63 ± 0.01	1.58 ± 0.05	1.65 ± 0.02**

C = Control (Saline-treated normal rabbits); T = Treated (alloxan-induced diabetic rabbits). Values are mean SEM; *p < 0.001, **p < 0.50 compared to control.

purkinji cells in cerebellum [Fig. 2 to 4] in contrast to normal histologic sections of Group II rabbits.

Discussion

The increase in blood glucose levels of rabbits after first week of alloxan administration as observed in the present study can be attributed to the fact that alloxan is rapidly taken up by the beta cells of the islets of Langerhan's (Boquist *et al*., 1983) and subsequently cause hyperglycemia by inhibiting the insulin secretory mechanism (Grodsky *et al.*, 1982). The minimum defining characteristic feature to identify diabetes mellitus has been reported to be chronic and substantiated elevation of circulating glucose concentration (Keen and NgTang, 1982). The persistent hyperglycemia during the entire experimental period observed in the study might be attributed to specific irreversible toxic effects of alloxan on cells of pancreas (Dunn *et al*., 1943; Lukenes, 1948). The relative reduction in the number of beta cells of diabetic rabbits, confirmed histologically with Gomori's modified aldehyde fuchsin stain, is also an evidence of alloxan diabetogenecity.

The reduction in the body weight as observed in the Group I rabbits might be due to insulin insufficiency leading to decreased accumulation of body reserve and an increased mobilization of endogenous energy store particularly fat (Edward, 1977).

The present experiment demonstrated degenerative changes in neurons and edema in brain sections of alloxan-induced diabetic rabbits. A great number of anatomical, functional and biochemical alterations have been described in the nervous system of diabetic animals (Tomlinson *et al*., 1992;

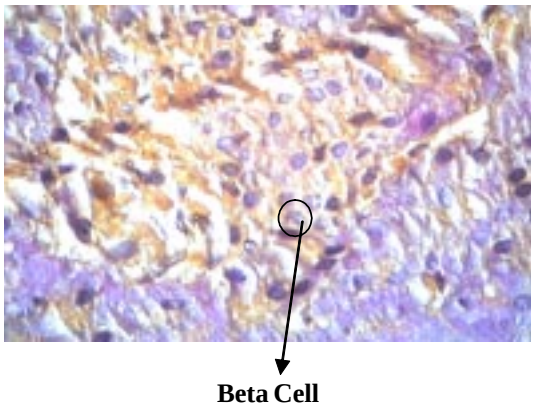

Fig. 1: Pancreatic islet section showing degenerative changes and reduction in beta cells(Purple) (Halami, 1952 ×1000).

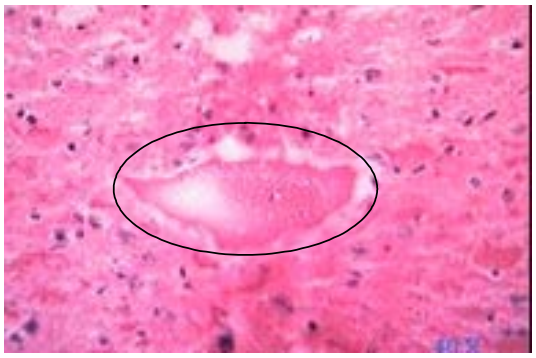

Fig. 2: Brain section showing edema (H&E ×400).

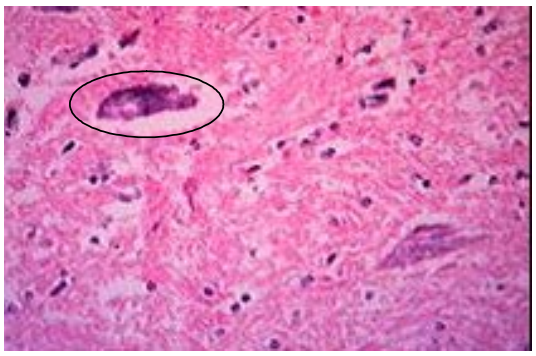

Fig. 3: Brain section showing degenerative changes in neurons (H&E ×400)

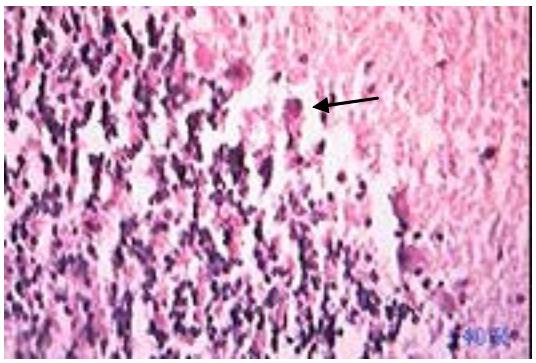

Fig. 4: Brain section showing degeneration of purkinji cells in cerebellum (H&E ×400)

Ozturk *et al*., 1996). The variety of alterations, called diabetic neuropathy, affects the brain, spinal cord and peripheral nerves (Gallego *et al*., 2003). Diabetes aggravates brain damage in experimental and clinical subjects, accelerates maturation of neuronal damage, increases infarct volume and induces post-ischemic seizures (Muranyi *et al*., 2003). Diabetic neuropathy has been related to excessive generation of sorbitol by aldose reductase due to maintained hyperglycemia, altered metabolism of phosphoinositides and reduced Na/K-ATPase activity (Greene *et al*., 1987; Tomlinson *et al*., 1992). Animal and *in vitro* experiments have implicated four major

pathways of glucose metabolism leading to neuronal damage in hyperglycemia (Stevens *et al*., 2002). 1) Excess glucose is diverted away from glycolysis by the polyol pathway that depletes dihydronicotineamide adenine dinucleotide phosphate (NADPH) and cellular antioxidant capacity. 2) Glucose also may become oxidized and form advanced glycosylation end products (AGEs) that alter extracellular matrix, activates receptors that produce reactive oxygen species (ROS) intermediates, and alter intracellular protein function. 3) Protein kinase C (PKC) becomes activated either directly by glycolytic intermediates or indirectly as a second messenger for stress hormones, leading to increased vascular disease, inflammation and oxidative stress. 4) Partial glycolysis causes accumulation of glycolytic intermediates and leads to escape of fructose-6-phosphate along the hexoseamine pathway that increases vascular disease and further ROS generation (Windebank and Feldman, 2001; Brownlee, 2001; Cameron *et al*., 2001; Stevens *et al*., 2002). The combined effect of these mechanisms is responsible for overproduction of mitochondrial superoxides causing cellular stress and damage.

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