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RESEARCH ARTICLE

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DIABETOGENIC ACTION OF ALLOXAN ON LIVER HISTOPATHOLOGY

ABSTRACT

The cytotoxic alloxan is the most prominent diabetogenic chemical agent in experimental diabetes research. Alloxan has been found to be selectively toxic to liver hepatocytes. The experiments aim to understand the effect of alloxan on cytomorphology of liver in rat. Treatment with alloxan caused central vein congestion with significant dilatation of sinusoidal spaces. The liver of the alloxan treated diabetic group showed hydropic degeneration and mild infiltration and small necrosed areas in some sections. Alloxan hepatocytes showed cloudy swelling and pyknosis of nuclei. Some sections showed beginning of pyknosis in hepatocytes and kupffer cells. The morphological changes that we observed in the livers of alloxan treated rat suggest impairment of the function of this tissue. Further research on the causes of liver damage will help us to unravel the pathogenesis of diabetes and its complications.

Keywords: Diabetes, Alloxan, Liver Diseases, Hepatocytes, kupffer cell

1. INTRODUCTION

Diabetes Mellitus is a complex metabolic disorder in which the pancreas produces insufficient amounts of insulin, or in which individual's system fail to respond appropriately to insulin. The disease is ranked seventh among the leading causes of death and third in terms of its complications and is a major health problem in developed and developing countries [1]. The number of diabetic patients is increasing globally because of diverse changes in diets in all cultures. It has been predicted that the number of diabetic patients will double from 143 million in 1997 to about 300 million by 2025 largely because of dietary intake and other lifestyle factors [1].

The cytotoxic alloxan is the most prominent diabetogenic chemical agent in experimental diabetes research. They are selectively toxic to beta cells of pancreas because they preferentially accumulate in beta cells as glucose analogues through uptake via the GLUT2 glucose transporter [2]. Alloxan, in the presence of intracellular thiols, especially glutathione, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid [3]. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction. Autoxidation of dialuric acid generates superoxide radicals ($O2^{\cdot -}$), hydrogen peroxide (H_2O_2) and, in a final iron catalysed reaction step, hydroxyl radicals ($OH^{\cdot}$). These hydroxyl radicals are ultimately responsible for the death of the beta cells [4].

Accumulated literature reviews suggest that cellular oxidative stress plays a crucially important role in the genesis of hyperglycemia related tissue damage [5, 6]. Clinical and experimental findings suggest that the liver may also be affected by diabetes mellitus (DM) in the long-term [5,6,7,8]. Increased production of reactive oxygen species (ROS) in diabetes would be capable of initiating tissue damage in liver, along with pancreas [9, 10]. Liver damage by diabetes is known as non-alcoholic fatty liver disease (NAFLD), which, histologically, cannot be distinguished from ethanol-induced hepatic steatosis [11]. NAFLD is characterized by being a pathological clinical syndrome with a broad spectrum of histological manifestations that can take from a benign course to more severe forms of chronic liver disease, such as hepatic cirrhosis and, occasionally, liver carcinoma [12]. In our previous study it has been established that alloxan rat developed atrophy of pancreatic islets and pyknosis of islets cells [13]. This study aims to understand the effect of alloxan on cytomorphology of liver in rat.

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2. MATERIALS AND METHODS

Young adult male rats weighing about 100 g were divided into experimental and their respective control groups. Each group contained 6 individuals. Rats were acclimatized for 7 days before experiments. They were treated to standard environmental conditions for temperature, relative humidity and dark/light cycle. Group I animals were treated with intra peritoneal injection of alloxan with a concentration of 10% solution in 0.9% NaCl, at the dose of 100 mg/kg of body weight for 21 days [14]. The animals were fed standard rodent diet and water was provided *ad libitum*.

Glucose tolerance test (GTT) was performed in control and alloxan treated rats. Blood was collected from the tail veins of rats after 18 hr of fasting followed by challenge with glucose (25 mg glucose/100 g body weight) and at the following time point after glucose infusion: 1.5 hr, 2.5 hr and 24 hr, blood glucose was measured using a blood glucose monitoring system (glucometer).

Rat Liver was fixed in Bouin's fixative. After routine processing the tissues were embedded in paraffin wax. The sections of liver were stained with Haematoxyline- Eosin (HE) stain for histological analysis. Liver stained with periodic acid Schiff (PAS) method for glycogen analysis.

3. RESULTS AND DISCUSSION

3.1. Blood Glucose Level

In the control rats the blood glucose level returned to the normal level after 24 hr of glucose feeding. Like control rats, in alloxan treated rats glucose level increased after 1.5 hr of glucose challenge but the elevated glucose didn't return to control level even after 24 hr of glucose challenge establishing their diabetic in nature (Table1).

3.2. Histopathological findings

The histological examination of the H-E stained control liver tissues showed normal cytoarchitecture of liver with visible central veins with radiating cords of hepatocytes (Fig.1a). Treatment with alloxan caused central vein congestion with significant dilatation of sinusoidal spaces. Few uniform sized cell bodies stained in eosin were found in the central hepatic venule. These may be periportal fatty infiltration (PFI) (Fig.1b and c).

Control liver showed hepatocytes showing normal arrangement in hepatic cords (HC), hepatocytes with granular cytoplasm (Cy), prominent nucleus (Nu) with prominent nucleolus (nl) and prominent Kupffer cells (Kc) (× 1000) (Fig. 2a and b). The liver of the diabetic group showed hydropic degeneration and mild infiltration and small necrosed areas in some sections. Alloxan hepatocytes showed cloudy swelling (CS) and pyknosis of nuclei (Pc) (× 1000) (Fig. 2c). Liver section of diabetic group showed significant dilatation of sinusoidal spaces (Fig. 2d). Some hepatic sections showed beginning of pyknosis (Bp) in hepatocytes and kupffer cells (Fig. 3a and b). Treated liver showed lower PAS response (Fig 4b).

The present study examined the effects of alloxan on liver. In our previous study it has been established that alloxan rat developed atrophy of pancreatic islets and pyknosis of islets cells [13]. In this study treatment with alloxan caused central vein congestion of liver with significant dilatation of sinusoidal spaces, pyknosis of nuclei of hepatocytes. In alloxan treated rat increments of blood glucose levels were observed after GTT and the hyperglycemia persisted even 24 h after glucose load. Moreover, in contrast to unusually high level of glucose, the hepatic glycogen content in alloxan-induced diabetic rat was not increased compared to control rat. The lack of increase in glycogen levels may be to decreased glucokinase activity in the liver and altered cytomorphology of liver parenchymatous tissues [15].

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In our study alloxan treated liver was associated with partially low hepatic glycogen levels as indicated by PAS response. This condition may provide an explanation for the decrease of glucose tolerance. Alloxan damages liver by destroying parenchymatous tissues and may hamper the process of glycogenesis [15]. Further, level of glycogen, the primary intracellular storable form of glucose in various tissues is a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthetase and inhibiting glycogen phosphorylase [15]. In conclusion, the results showed that alloxan diabetes influence pancreatic islets morphology [13] concomitant with cytomorphology of liver. But further experiments are in progress to solve this paradox.

4. CONCLUSION

This article decisively highlights about the action of alloxan in liver hepatocytes architechture and histopathology. The result indicated that administration of alloxan to rats have adverse effects on the liver cytoarchitecture that may play a relevant role in the genesis of the diabetic chronic liver disease, including the non-alcoholic fatty liver disease and its occasional progression to cirrhosis. Animals with severe liver damage consequently exhibit a diabetic type of glucose tolerance, as was confirmed in our experiments. But further pharmacological and biochemical investigations are needed to clearly elucidate the relation of alloxan diabetes and liver damage.

5. DECLARATION OF CONFLICT OF INTEREST

The authors wish to declare that there is no conflict of interest.

Table1. Blood glucose level (mg/dl) during glucose tolerance test in control and alloxan treated rats. Values are the blood sugar level (mg/dl) expressed as mean ± SE. P-value < 0.05 is considered to be statistically significant.

Group	0hr	1.5hr	2.5hr	24hr
Control	40.31±1.60	72.12±1.83	52.19±2.19	41.66±1.94
Alloxan treated rats	38.70±2.04	63.30±2.49	49.80±1.18	47.30±1.94

Fig. 1 a

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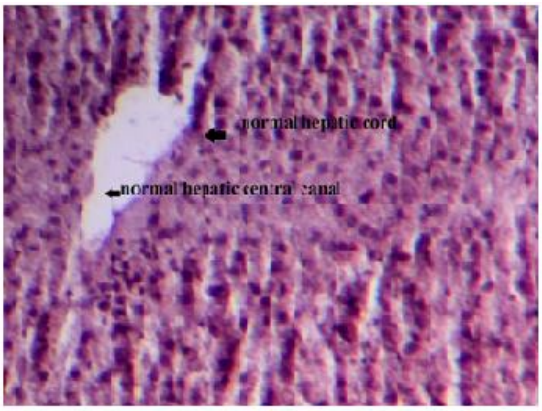

Figure.1.a

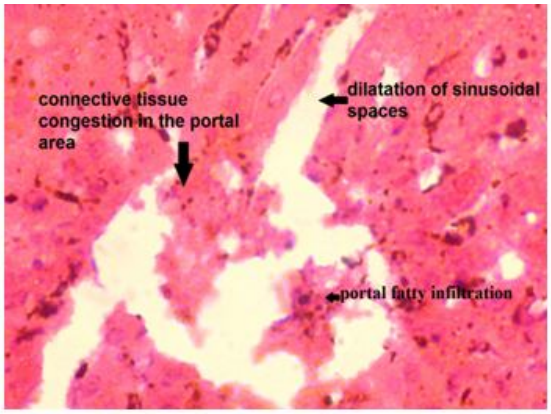

Figure.1.b

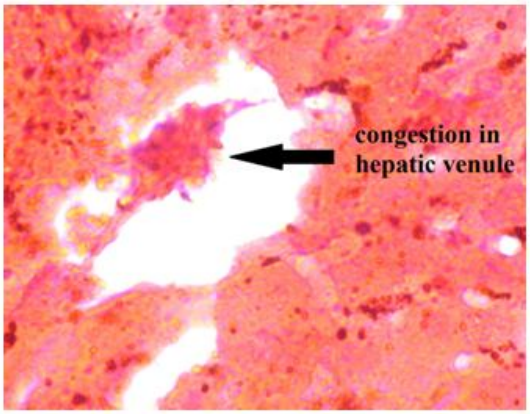

Figure.1.c

Figure 1: (a) Rat liver with normal hepatic architecture (x400),

(b & c) Alloxan treated diabetic liver (x1000)

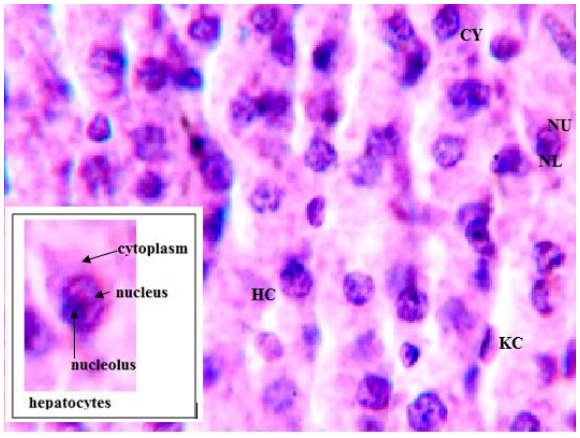

Fig. 2a

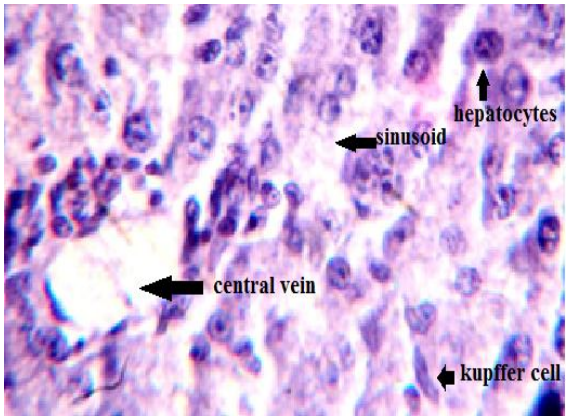

Fig. 2b

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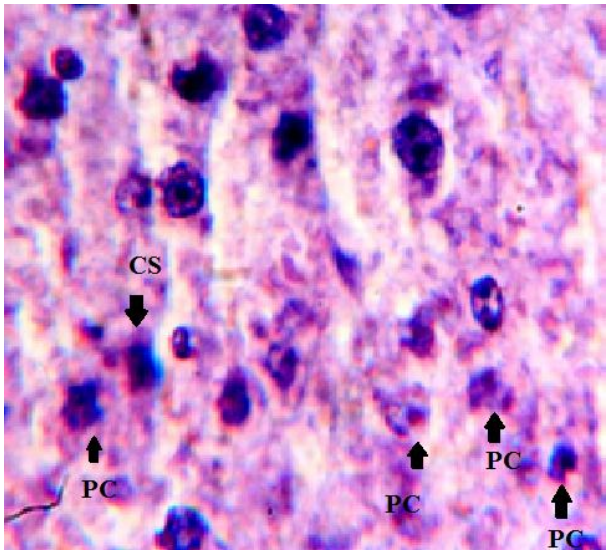

Fig. 2c

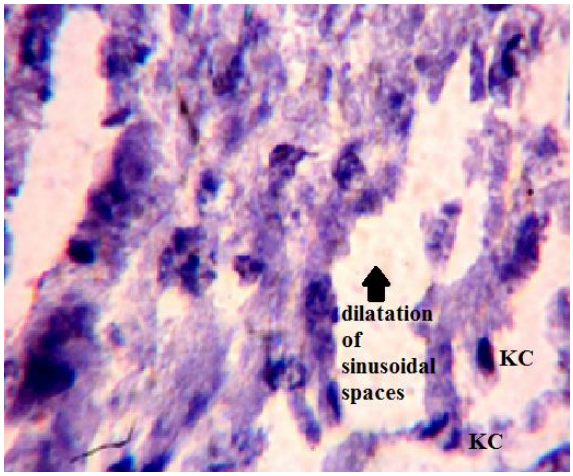

Fig. 2d

Figure 2: (a & b) Rat liver with normal hepatic architecture (x1000), (c & d) Alloxan treated diabetic liver (x1000). Notice normal hepatocyte cell (lowerinset) (x1000) (2a)

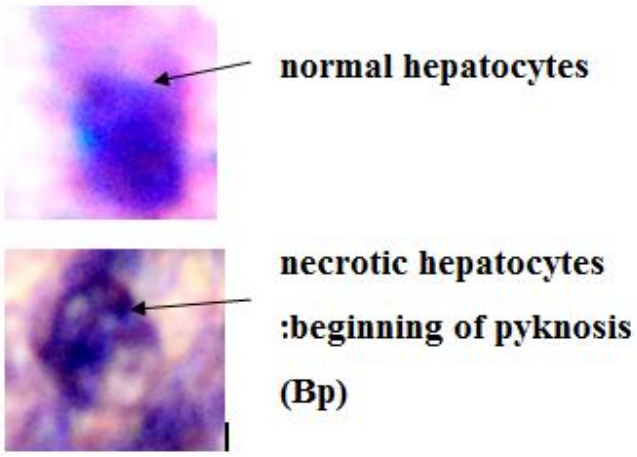

Fig. 3a

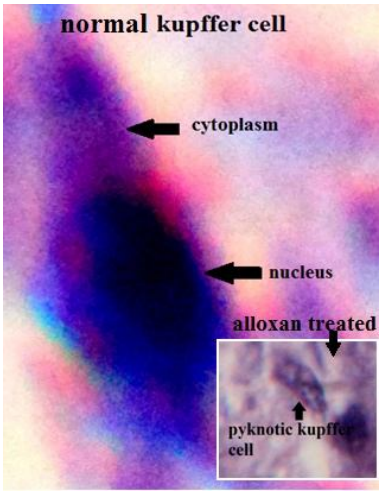

Fig. 3b

Figure 3: (a) Normal and necrotic hepatocytes (x1000), (b) Normal kupffer cell (x1000). Notice necrotic kupffer cell (lower inset) (x 400) (3b).

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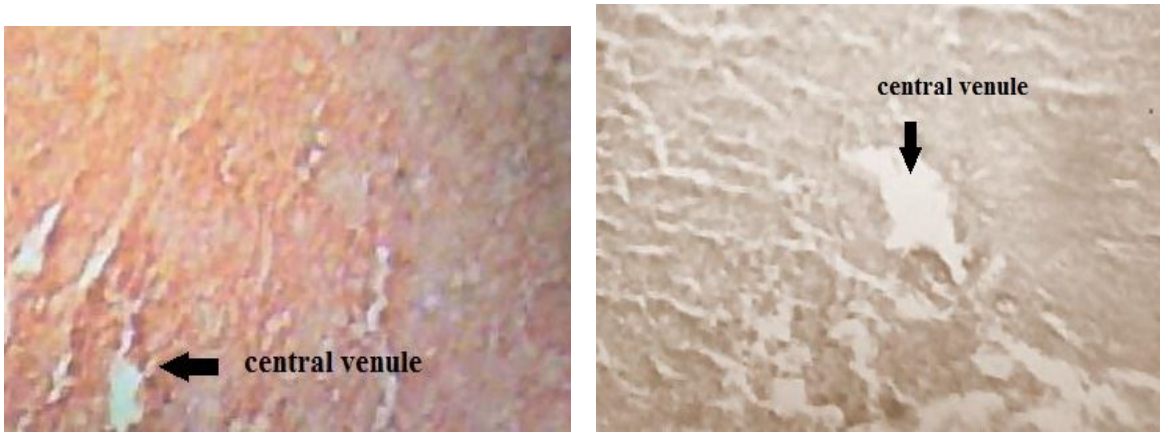

Fig. 4a **Fig.4**

Figure 4: (a) PAS stained normal rat liver section (x 400), (b) PAS stained alloxan treated liver section (x 400).

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