

HISTOMORPHOLOGICAL CHANGES OF LIVER IN DRUG INDUCED DIABETIC RATS

*A.O. IBEGBU¹; S.P. SINGH¹; J.O. HAMBOLU² AND S.A. OJO²

¹ Department of Human Anatomy.

² Department of Veterinary Anatomy
Ahmadu Bello University, Zaria, Nigeria.

Abstract

The study of the changes in the histomorphology of the liver induced by the administration of diabetogenic dose of alloxan was undertaken using one hundred and twenty male Albino (Wistar) rats. The animals were randomly separated into four groups. A, B, C and D, of thirty rats each. The first group (A) was used as the primary Control group, the second group (B), the third group (C) and the fourth group (D) were used as test groups. The test groups were administered with 200mg per kg body weight of alloxan which is the diabetogenic dose. The second group (B) was used to study the direct effect of alloxan on the histology of the liver. The third group (C) was used to study effect of diabetes mellitus on the morphology of the liver. The fourth group (D) was used to study the effect of the therapeutic agents on the liver. Histological examination of the liver of the test groups showed various accumulation of degenerative fatty vacuoles and areas of necrosis while the control showed normal histology of the liver.

Key words: Histomorphology, Liver, Diabetic Rats, Alloxan, Degeneration, Fatty Vacuoles

Introduction

Diabetes mellitus can be defined as a chronic disorder characterized by a raised level of glucose in the blood (Guyton and Hill, 1996; Gale, 2001). Diabetes can be the outcome of many different causes, some hereditary, some environmental and some hormonal (Greenspan and Baxter, 1994; Granner, 1995; Biesenbach, et al; 2000). The aetiology of the commonest forms of diabetes is unknown and these are referred to as primary diabetes. These include, type I or juvenile-onset diabetes; type II or maturity-onset diabetes and the much rarer types of diabetes which are usually associated with other hereditary disorders (Bloom and Ireland, 1980; Edwards, et al., 1995; Agarwal, et al., 2001).

The cause of some forms of diabetes is known and are classified as secondary diabetes. These include diabetes due to destruction of the pancreas by drugs, disease, autoimmunity or surgery and diabetes due to hormonal imbalance (Tierney, et. Al., 1996; Greenspan and Baxter, 1994; Balsells, et.al; 2000; Berger, et.al. 2001).

Diabetes has been known to mankind since time immemorial and was described as a disease with "honeyed urine" (Turner, 1966). The first clear account of diabetes was given by Aretaeus in about 170 AD. He described it as "This mysterious affection, being a melting down of flesh and limbs in urine, thirst unquenchable, and death inevitable" (Blood and Ireland, 1980).

It has been shown that intravenous or intraperitoneal injection of alloxan or streptozotocin into experimental animals, causes degenerative lesions in various organs but with proper dosage, it produces prompt and selective coagulative necrosis of the beta cells of the islets of Langer-

hans resulting in a syndrome resembling clinical diabetes mellitus (Barnes and Eltherington, 1964; Steiner and Freinkel, 1972; Govan, 1992; Yala, et.al; 2003)

Since the liver is the site of metabolism and detoxification in the body, any derangement of the liver tissue may lead to impaired body function. The present study examines the significant histological changes in the liver in drug-induced diabetes rats.

Materials and Methods

The experimental drug was alloxan crystals contained in a 250gms bottle. The alloxan crystals weighing 1.2gms were dissolved in 10mls of injection water amounting to 1.2gm/10ml. 0.3ml of the 1.2gm/10ml solution which is equivalent to 40mg of alloxan was administered as the diabetogenic dose according to Barnes and Eltherington, (1964); Barbato and Landau, (1977); Bowman and Rand, (1985); Greenspan and Baxter, (1994).

The test animals were 120 male Albino (Wistar) rats which were separated into four groups A, B, C, and D of thirty rats each. The first group (A) was the primary control group. The second, third and fourth groups (B, C and D) were used as the test groups and were administered with 0.3ml of 1.2gm/10ml solution of alloxan equivalent to 200mg per kg body weight which contained 40mg of alloxan. This single diabetogenic dose of alloxan was administered to the test animals through the intraperitoneal route (IP) while the Control group received normal saline through the same route.

The group B animals were the first test group and were used to study the effect of alloxan on the liver during diabetic induction. The second test group C. was used to study

the effect of diabetes mellitus on the histology of the liver, while the third test group (D) was used to study the influence of therapeutic agent, insulin in the resuscitation of the liver.

Animals from each of the groups were anaesthetized and their abdomen opened by a midline incision and part of the liver was excised. These tissues were placed in cold normal saline until excision of the tissues were completed. The tissues were fixed using 10% neutral formalin and Bouins fluid. The technique of tissue processing used were both manual and automatic tissue processing techniques using Histokinette bench model tissue processor obtainable from the Department of Human Anatomy, ABU., Zaria.

The tissues were embedded in paraffin wax and sections between 5-8 microns were made using the Rotary microtome. The tissues were stained using Haematoxylin and Eosin (H and E) method and Periodic Acid Schiff (PAS) methods. These methods were used as outlined by Drury and Wallington, (1973); Gurr, (1992); Culling (1993).

Results

The results of the microscopic examination of the liver, show the normal liver architecture with cord arrangement of cells from group A animals as shown in plates 1 and 2. Animals in group B, C, and D, show fatty vacuoles in some hepatocytes as in plate 3 while some in addition to fatty vacuoles, contain inflammatory cells as in 4 and small area of necrosis as in plate 5. The liver from group D animals treated with insulin, show plant-like enlarged hydropic cells as shown in plate 6.



Plate 1: A section of liver from the Control group (A), Showing normal liver architecture with cord radiating from the Central Vein (V). H and E x100.

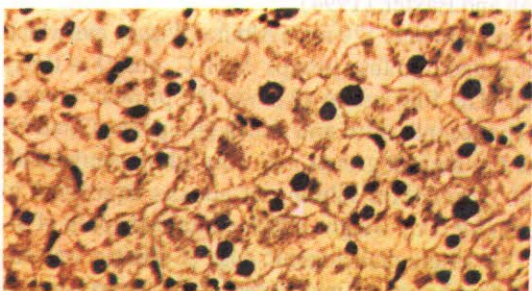


Plate 2: A section of liver with higher mag. from group A, showing normal hepatocytes. The cellular components are highly PAS positive indicating high glycogen content. PAS x400

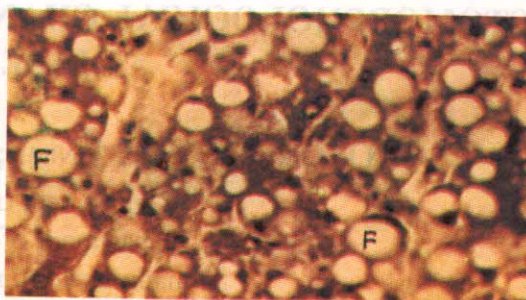


Plate 3: A section of liver from the test group (B), showing fatty infiltration resulting in vacuolation of some hepatocytes (F). H and E x 400.

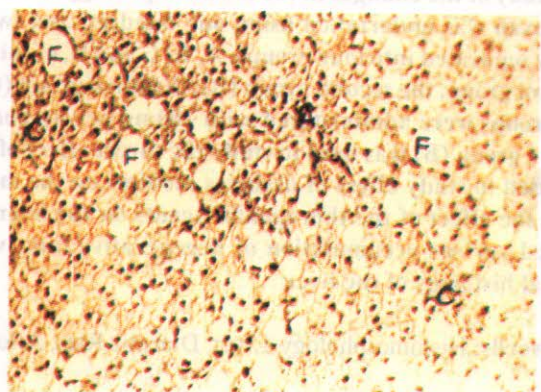


Plate 4: A section of Liver from the test group (C), showing fatty infiltrated hepatocytes causing vacuolation of some hepatocytes (F) and presence of inflammatory cells (C) in the liver. H and E x 400.

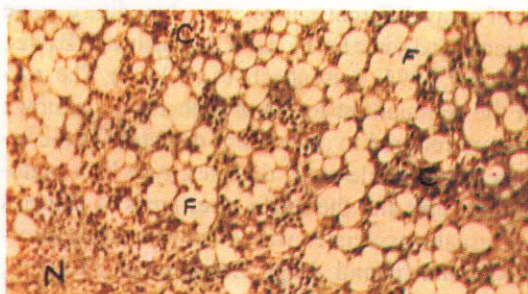


Plate 5: section of liver from the test group (D), showing fatty infiltrated and vacuolated hepatocytes (F), small area of necrosis (N) and inflammatory cells (C) in the liver. H and E x400.

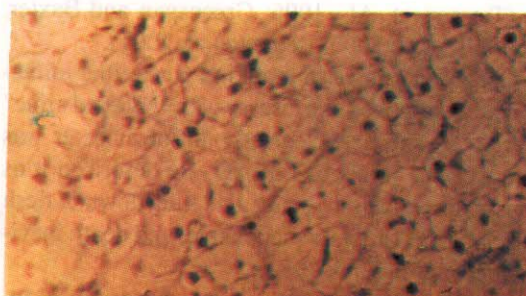


Plate 6: section of liver from the test group (D), treated with insulin, showing plant-like cell pattern with hepatocytes overladen with glycogen. H and E x400.

Discussion

The liver of rats in the test groups showed **degenerative fat deposits** in the form of fatty vacuoles which appear as single vacuoles termed univacuolar fatty infiltration or numerous vacuoles in a cell called multivacuolar fatty infiltration.

It has been shown that some agents inhibit protein synthesis, thereby producing fatty liver while others does not inhibit hepatic synthesis of protein but leads to fatty liver by interfering with egress of lipids from the cells (Zimmerman, 1982). Damage to the plasma membrane may contribute to the accumulation of fat in the cells (Trump and Arstila, 1975). The degenerative fatty changes in the liver may not be unconnected with the direct cytotoxic effect of alloxan in the test animals or the indirect cytotoxic effect of diabetes mellitus caused by the alloxan (Lazarus and Volk, 1962; Sterner and Freinkel, 1972). The cytotoxic agents may have affected the plasma membrane which led to the interference with the movement of the low density lipoprotein out of the cell and their effect on the synthesis of very low density lipoprotein may be responsible for the accumulation of lipid in the liver cells (Grundmann, 1980; Zimmerman, 1982). Also manifested was necrosis of liver cells in the test groups. Keele and Neil, (1964) had shown that most toxicity on the liver manifested mainly in fatty liver which showed diffuse hepatic fibrosis called cirrhosis while gross chronic fatty liver leads to death of many liver cells and their replacement by scar tissue (Ritchie, 1990; Scheuer, 1973).

Zimmerman (1982) has explained that some toxic agents which lead to necrosis. The role of the injury to the plasma membrane was the pathogenesis of the necrosis. This may lead to rapid loss of intracellular electrolytes, enzymes and coenzymes and entry of extra cellular electrolytes. Thus the entry and intracellular accumulation of calcium ions has been considered by Zimmerman, (1982) to be responsible for triggering of necrosis. It has also been shown that the inability to synthesize protein that have been destroyed by the direct toxic action of alloxan may conceivably contribute to necrogenesis (Grundmann, 1980; Zimmerman, 1982). The toxic activity may be responsible for the initiation of necrogenesis as well as degenerative fatty infiltration present in the test groups (Wood, 1965; Wepler and Wildhirt, 1972; Alagozlu, *et. al.*; 2000; Yala, *et. al.*; 2003).

However, Grundmann, (1980); Patrick and McGree, (1980) have shown that in all cases the nature of the cytotoxic agent is less influential on the causation of liver injuries than the personal and individual susceptibility. Some hepatocytes showed hydropic changes, referred to as toxic swelling of cells and this may be due to the abnormal storage of glycogen due to degradation or a considerable dilatation of the endoplasmic reticulum cisterns resembling the pattern found in chronic alcoholic livers (Grundman, 1980; Ritchie, 1990, Scheuer, 1973; Wepler and Wildhirt, 1972). Plea, (1975) has shown that the permeability of mitochondria and the activities of the membrane-bound proteins were altered while, Wills, (1971) showed that the

loss of Glucose - 6- phosphatase activity was due to the distortion of endoplasmic reticulum membrane caused by oxidative toxicity of the toxic agents.

Duve, (1970); Wood (1965) have shown that membrane damage increases membrane permeability and cell leakage results, posing a serious damage to lysosomes. In that lysosomes has been shown to participate in the production of numerous pathological disorders (Cheville, 1989). It has also been shown that various lipid-soluble compounds become incorporated in the lysosomal membrane and renders it more fragile. These groups include a number of steroids and vitamins as well as several drugs (Alagozulu, *et. al.*, 2000). Also stimulating lysosomal ruptures but by mechanisms that are not understood is pathological conditions like traumatic shock, anoxia, exposure to hyper basic oxygen, endotoxin poisoning and viral infections (Duve, 1970; Biesenbach, *et. al.*; 2000).

Fatty liver has been shown to be characteristic of the mild obese diabetics; similar changes can be induced in poorly controlled insulin - dependent diabetic patients (Keele and Neil, 1964; Ganong, 1995; Gale, 2001). The insulin - dependent diabetic may develop glycogen infiltration in circumstances of chronic insulin overdosage (Gale, 2001). Excess insulin will stimulate the pathways of glycogen storage and patients who swing between poor control and insulin excess have been reported to show a mixed picture of fatty infiltration and glycogen storage (Scheuer, 1973; Zimmerman, 1982; Hanninen, *et. al.*; 2001; Alagozulu, *et. al.*; 2000).

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