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# IMPROVEMENT OF GLUCOSE LEVEL, LIPID PROFILE, SOME ENZYME ACTIVITIES AND STRUCTURE OF PANCREAS AND LIVER IN DIABETIC RATS TREATED WITH GLIBENCLAMIDE

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## التحسن فى مستوى السكر والمحتوى الدهني والنشاط الإنزيمي وتركيب الكبد والبنكرياس المصاحب لعلاج الفئران المصابة بالسكرى بعقار الجلابينكلاميد

**ملخص: خلال هذه الدراسة** تم بحث التأثير العلاجى لعقار الجلابينكلاميد على مستوى السكر والـدهون والجلسريدات الثلاثية والكولسترول والإنزيمات الناقلة للأمين وإنـزيم الفوسـفاتيز القاعـدي بالإضـافة للتركيب النسيجي للبنكرياس والكبد فى الفئران المصابة بمرض السكر المعاملة بمركب الألوكسان. تبين أن الجلابينكلاميد أدى إلى خفض الإرتفاع الحادث فى مستوى سكر الدم من 8و377% فى دم الفـران المصابة بمرض السكر ليصبح 3و60% فى دم المجموعة التجريبية وذلك مقارنة بالمجموعة الضابطة. وعند دراسة مستوى زيادة الدهون الكلية فى الحيوانات المعاملة بمركب الألوكسان وجدت أنها وصـلت إلى 8و103% فى السبوع الثامن من التجربة وقد أدت المعالجة بالجلابينكلاميد إلى خفض تلك الزيـادة لتصبح 19و7% مقارنة بالمجموعة الضابطة. كما زاد مستوى كل من الجلسريدات الثلاثية والكولسترول وكذلك نشاط الإنزيمات الناقلة للأمين ونشاط إنزيم الفوسفاتيز القاعدي فى الفئران المصابة بالسكرى ولكن عند المعالجة بالجلابينكلاميد تراجعت تلك المستويات بشكل عام لتعادل مستواها فى حيوانات المجموعة الضابطة تقريبا. وكذلك لوحظت تغيرات نسيجية فى بنكرياس وكبد الفئران المعاملة بمركب الألوكسان. وقد أدت المعالجة بالجلابينكلاميد إلى إعادة ظهور كمية محدودة من جزر لانجرهانز فى البنكرياس وإلى استشفاء أنسجة الكبد بشكل كبير.

**Abstract**: The therapeutic effect of glibenclamide on serum values of glucose, total lipids, triglycerides, cholesterol, transaminases and alkaline phosphatase as well as on the histological structure of pancreas and liver were examined in alloxan diabetic rats. Glibenclamide decreased the elevation of serum glucose from 377.8% in the diabetic sub-group to be 60.3% in the treated diabetic sub-group as compared to the control. Total lipid content in diabetic sub-group was gradually increased to reach a percentage increase of 103.8% in the eighth week as compared to control. Glibenclamide administration decreased the elevation of total lipids to 7.2% in treated diabetic animals as compared to control. Triglyceride and total cholesterol concentrations, transaminases and alkaline

phosphatase activities were increased in diabetic rats. However these increments were, in general, backed near to normal levels after glibenclamide administration. Histopathologic changes in pancreas and liver of alloxan diabetic rats were manifested. On glibenclamide treatment, the islets of Langerhans started to re-exist but still with limited pattern. The existing cells themselves seemed to regain near to normal distribution. Also, glibenclamide treatment stimulates considerable recovery to liver damaged tissues.

**Key words:** Diabetes, rats, glibenclamide, pancreas, liver, lipid, enzyme.

## Introduction

Blood glucose concentration is normally tightly regulated by the coordinated action of insulin and counter regulatory hormones. A balance is maintained between glucose production by the liver, and glucose clearance into peripheral tissues, primarily muscles. Insulin released from beta cells of the pancreatic islet is constantly adjusted so that normoglycemia is maintained. The failure of diabetic patients to maintain normal blood glucose concentrations is due to either a defect in insulin release or the ineffective utilization of insulin resulting in hyperglycemia (1).

In hyperglycemia there are decrease in cellular glucose uptake and storage as glycogen and fat, excess glucose breakdown from storage, defective glycolysis, and excess fat breakdown. All these actions result in cellular starvation and accumulation of glucose and fat in blood. Fat breakdown results in increased levels of free fatty acids in blood (2). Insulin deficiency causes hypertriglyceridemia due to decreased lipoprotein lipase activity that results in accumulation of chylomicrons and very-low-density lipoproteins (VLDL) in the plasma (3). Associated changes in plasma lipid and lipoprotein levels in diabetes remain important in terms of explaining the accelerated atherosclerosis (4). Plasma values of cholesterol, triglycerides, and their low density lipoprotein (LDL) and VLDL subfractions were found to increase with the severity of hyperglycemia (5).

Alteration of enzyme activities during hyperglycemia was the subject of concern for many authors. Elevated levels of serum aminotransferases (aspartate transaminase, AST and alanine transaminase, ALT) and alkaline phosphatase (ALP) were recorded in diabetic animals (6,7,8).

Diabetes mellitus is characterized by wide spread pattern of damage in almost every tissue of the body including pancreas and liver. Histopathologic changes including vacuolar degeneration, reduction of β-cells and pancreatic

necrosis were demonstrated in the pancreas of diabetic animals (9,10,11). Liver lesions of diabetic animals included hydropic degeneration in hepatocytes, increase in Kupffer cells, necrosis and presence of inflammatory cells (12,13,14).

Glibenclamide stimulates insulin secretion from pancreatic β-cells principally by inhibiting ATP-sensitive $K^+$ ($K_{ATP}$) channels in plasma membrane (15). Serum glucose was unchanged after glibenclamide administration but plasma insulin rose by 36% and 15% after the 15 and 100 μg/min infusions, respectively (16).

Although diabetes mellitus has become a common chronic disease in Gaza strip, only few reports addressed the problem (17). The current investigation is aimed to highlight the alterations of glucose level, lipid profile and of some enzyme activities in alloxan diabetic rats. Also, changes in pancreas and liver structure are investigated. Glibenclamide was the drug of choice as it is commonly used for the treatment of diabetes mellitus in Gaza Strip.

## Materials and Methods

### Experimental Animals

Animals used in the current investigation were young adult male albino rats, weighing 90-110 gm. They were purchased from the breeding unit of Biology department, Faculty of Science, Islamic University of Gaza. Animals were housed in well-aerated cages and left for one week before experimentation to adapt to laboratory conditions. Commercial balanced diet and water were continuously and regularly supplied *ad libitum* all over the experimental period.

### Induction of diabetes and treatment

Animals were divided into two major groups. One group was used as normal control. The other group of animals were fasted 24 hours then rendered diabetic by a single intraperitoneal injection of a freshly prepared alloxan monohydrate solution at a dose of 150 mg/kg body weight (18). Rats with glucose level >200 mg/dl were used. The diabetic animals were subdivided into two sub-groups. Animals of the first sub-group remained without treatment and considered as a diabetic control group, while the second group was given glibenclamide and considered as a treated diabetic sub-group. Glibenclamide (Commercially known as Glucocare) was purchased from local pharmacies as tablets and then grinned using a mortar. The powder was dissolved in water and orally administrated at a dose level of 36 mg/kg body weight, daily for eight weeks, which represents the

overall experimental duration. This was done by special stomach tube with a smooth tip to protect the interior lining of the oral and buccal cavity from injury. The dose of glibenclamide was estimated (19).

**Physiological Studies**

Animals from both normal control and experimental sub-groups were decapitated weekly for eight weeks. Blood was collected in 10 ml plain tubes for serum preparation. Clear serum samples were separated by centrifugation at 3000 r.p.m. for 20 min. Serum glucose was determined (20). The kits used purchased from Randox lab LTD, U.K. Serum total lipids were determined by using the colorimetric method (21). Serum triglyceride concentration was determined enzymatically (22). Serum total cholesterol levels were determined following instruction manuals of Randox reagent kits (23). Enzyme activities were measured by using Boehringer reagent kits. The activity of aspartate aminotrasferase (AST) and alanine aminotrasferase (ALT) were determined (24). The activity of alkaline phosphatase (ALP) was measured (25).

**Histological Studies**

Following decapitation at weekly basis for eight weeks, the pancreas and liver were dissected freed from the surrounding connective tissues and organs, then excised. They were immediately immersed in saline solution for blood removal. The preparations were then fixed in 10% buffered formaline (26). Fixed tissues were dehydrated, cleared with xylene and completely impregnated with paraffin wax. The tissues were then sectioned by a rotary microtome at a thickness of 3 μm, mounted and affixed to slides and later used for the routine haematoxylin and eosin stain (27).

**Statistical Analysis**

All the experimental data were statistically analyzed (28).

## Results

**Physiological studies**

The data in Table 1 shows the average of glucose, total lipid, triglyceride and total cholesterol levels in the serum of both control and experimental groups. It is obvious that there is a progressive increased in the levels of serum glucose in alloxan diabetic rats, commencing from the first week. The rate of increase is

amounted to 377.8% at the end of the experiment as compared to control group. However, on glibenclamide administration, serum glucose levels were significantly decreased recording 60.3% of change as compared to control group. A progressive increase in serum total lipid was observed for diabetic rats which became more pronounced during the last three weeks with a maximium value of 103.8% as compared to normal controls. However, non-significant increase in serum total lipid levels was recorded in the rats treated with glibenclamide, where it reached a maximum of 7.2% as compared to control. Serum total cholesterol level was generally increased among diabetic animals. This increase became more pronounced commencing from the third week till the end of the study, showing a maximum value of 47.6% as compared to the control. However, the treated diabetic sub-group showed that the total cholesterol level was more or less near to control level. A substantial increase in serum triglyceride levels of diabetic rats was recorded with a percentage increase of 107.1% at the end of the experiment as compared to controls. On the other hand, there was no significant change of serum triglyceride concentration in the treated diabetic sub-group when compared to the control group and managed to stimulate more or less constant figures on triglyceride concentration showing a percentage increase of 6.3% as compared to control group. The activities of serum AST, ALT and ALP for both control and experimental animals are presented in Table 2. A general increase in AST activity was found in the diabetic sub-group. This elevation reached a significant value commencing from the third week. The percentage was estimated to be 39.1% as compared to control group.

On the other hand, diabetic rats treated with glibenclamide evidenced a non-significant increase in the enzyme activity recording a percentage increase of 2.3% as compared to normal control group. Serum ALT activity in diabetic rats showed a highly significant increase during all time periods examined with a percentage increase of 200.3% at the end of the experiment as compared to control group. On glibenclamide treatment, ALT activity recorded a non-significant increase of 13.3%. For alkaline phosphatase, a highly significant increase in the enzyme activity in diabetic sub-group was detected. This increase began from the first week recording a percentage increase of 148.6% as compared to control group. Moreover, serum ALP activity still increased but not significantly in rats from the treated diabetic sub-group with a percentage increase of 9.5% as compared to control group.

**Histological studies**

**Pancreas**

Fig. 1 represents pancreatic section from normal control albino rat showing islets of Langerhans containing normal α–and β–cells. The histology of pancrease of diabetic rats showed intensive histopathologic changes (Fig. 2). These investigated as vacuolar degeneration and loss of regular pattern of islets; reduction in size and number of β-cells; hydropic and fatty degeneration; necrotic and faintly stained, of specific granules of β-cells: Haemorrhagic infiltration and scar tissue in between pancreatic lobules. The distribution of the different types of cells within each islet was disrupted where mainly α-cells were present. The walls of pancreatic ducts were thickened. A large portion of the pancreatic acini acquired pyknotic nuclei with limited secretory intity. In glibenclamide treatment (Fig. 3), the earlier weeks still showed some disturbances in the distribution of cells within islets of Langerhans; hydropic degeneration of some islet cells; scar tissue in between pancreatic lobules denoting areas of degenerated islets. The exocrine part (acini) suffered from early degenerative changes. At the late weeks, the islets of Langerhans started to re-exist but still with limited pattern. The existing cells themselves seemed to regain near to normal distribution.

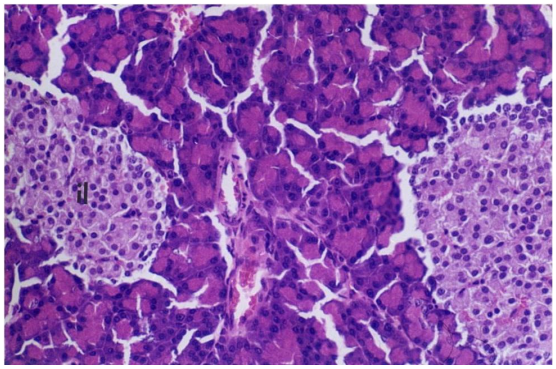


**Fig.1:** Photomicrograph of a section of the pancreas of normal control rat showing islets of Langerhans (il) containing normal α and β cells (HX – E; X 132).

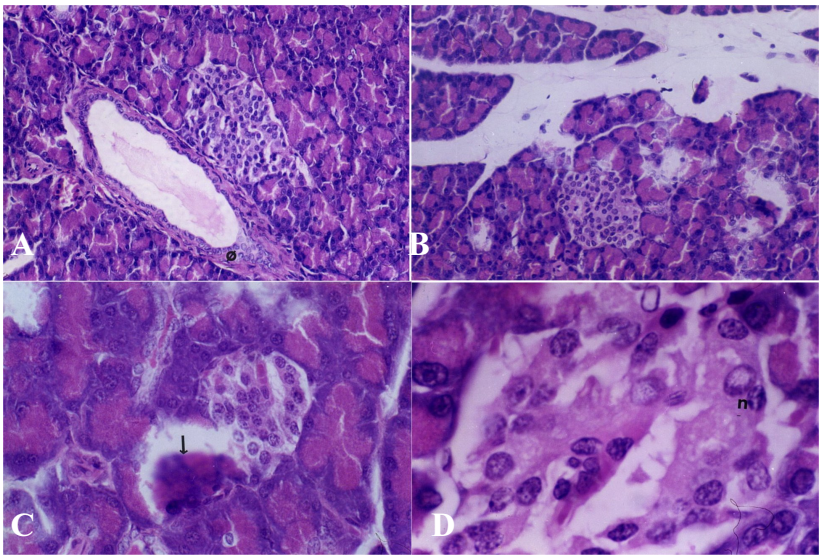


**Fig. 2:** Photomicrograph of pancreatic tissue of diabetic rats showing, (A) thickening in the walls of the pancreatic duct (Φ ). [HX – E; X132]; (B) scar tissue replacing necrotic islets of Langerhans. [HX – E; X132]; (C) blood clot (↓) replacing haemorrhagic lesions. [HX – E; X330]; (D) pyknotic nuclei ( n ) with necrotic cells. [HX – E; X330].

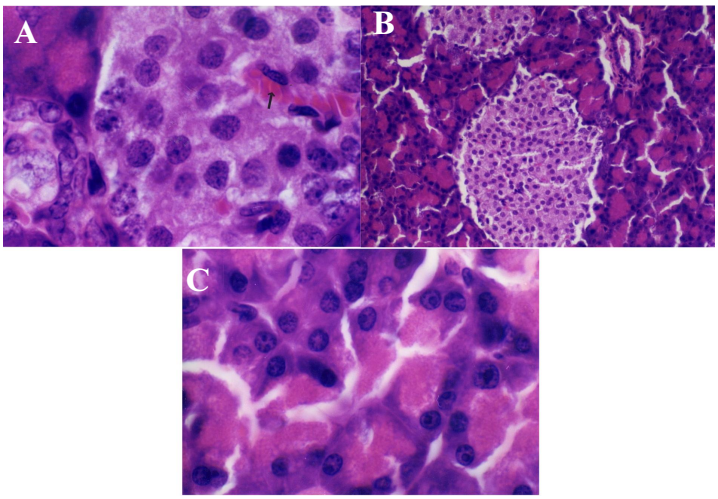


**Fig. 3:** Photomicrograph of pancreatic tissue of diabetic rats treated with glibenclamide showing, (A) hydropic degeneration with haemorrhagic infiltration (↑) in the islet. [HX – E; X330]. (B), reexistance of islets of Langerhans with variable sizes and degenerative pancreatic acini. [HX–E; X132]. (C) near to normal pancreatic architecture in cells of pancreatic acini.[HX – E; X330].

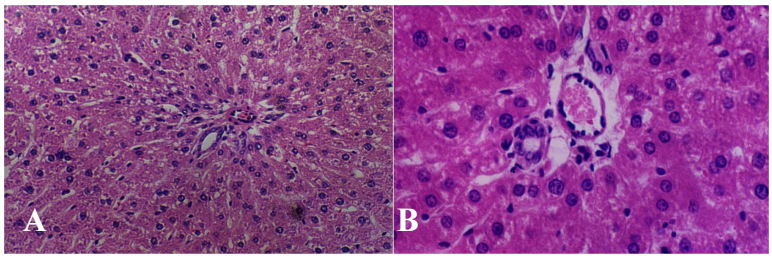


**Fig. 4:** Histological examination of the liver of normal albino rats showing. (A) normal hepatic pattern.[HX - E; X 66]. (B) portal area with hepatic vein and bile duct . [HX – E; X 132].

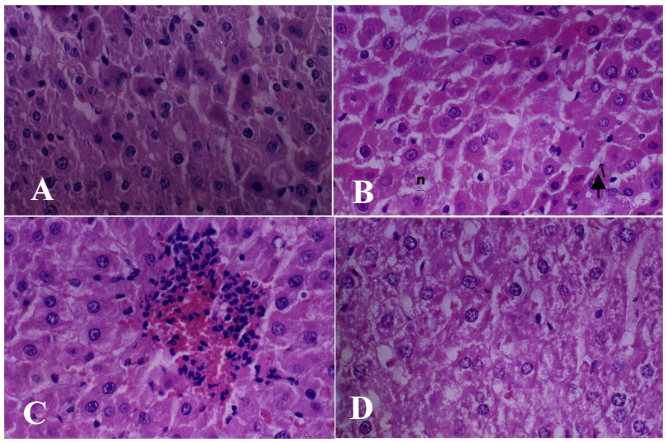


**Fig. 5:** Photomicrograph of sections of the liver of diabetic rat showing, (A) degenerative cells; granulated & vacuolated cytoplasm and dilated sinusoids. [ HX-E; X 66]; (B) showing necrotic area (n) invaded hepatic lobules and dilated sinusoidal spaces ( ) . X E; X 132]; (C) increase in Kupffer cells; chronic ononuclear l phoc tes and $RBC_s$ . X E; X132]; (D) necrotic changes with vacuolar c toplas abnor al hepatic architecture. X E; X132].

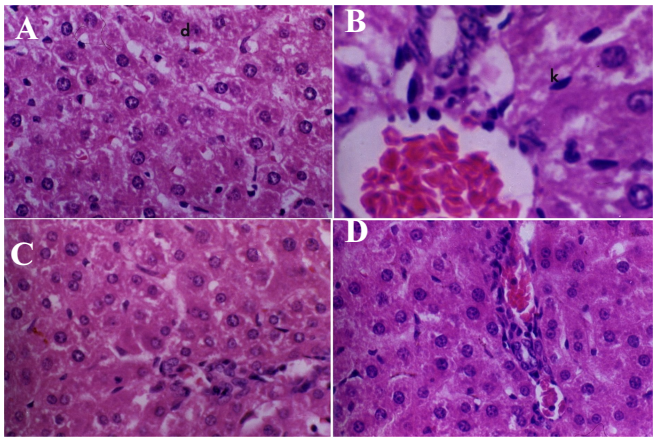


**Fig. 6:** hoto icrographs of sections of the liver of diabetic of glibencla ide treated rats showing ( ) degenerative hepatoc te (**d** ) X E; X132 ]. (B) portal tract and blood vessels with near to nor al argins and presence of Kupffer cells ( **k** ) X E; X330]. (C) reduction of dilated sinusoids and existence of nor al level of Kupffer cells x E; X 66]; (D) showing near to nor al hepatic architecture with its lobular pattern and portal tracts X E; X 132].

### Liver

Section of the liver from normal control albino rat showing normal hepatic pattern is illustrated in Fig. 4. Histological lesions of liver from diabetic rats are shown in Fig. 5. They were present in the form of hydropic degeneration in hepatocytes; faintly stained cytoplasm; more or less shrunken nuclei; increase in Kupffer cells: dilated sinusoids; wider portal tracts with loose edematous connective tissues; thickining of blood vessel walls and bile canaliculi; inflammatory cells mainly lymphocytes: necrosis within hepatocytes and portal tracts and the end result was the loss of normal hepatic architecture in most of the liver tissue. Treatment of diabetic rats by glibenclamide was found to stimulate considerble recovery to liver damaged tissues (Fig 6). Dilatations within sinusoids were reduced and Kupffer cell presence returned to near to normal levels. Hepatocytes retained their cytoplasmic integrity. The end result was a near to normal hepatic architecture with its lobular pattern and portal tracts. However, limited areas were still slightly affected. Hepatocytes were probably undergoing recovery but requiring longer duration of treatment. The data showed clearly that many physiological and histological aspects could be altered in diabetes and glibenclamide return most of such changes to near normal.

## Discussion

### Physiological Investigation

Most of rats developed diabetes within twenty-four hours of alloxan injection and it persisted throughout the whole experimental duration. The hyperglycemia is suggested to be due to lack of insulin, increased gluconeogenesis, and/or glycogenolysis (29,30). Such suggestions come as a normal sequel to decreases and inhibition of pancreatic β-cell activities of alloxan diabetic rats. Glibenclamide provoked a strong hypoglycemic action in the diabetic rats. This action is in concomitant with the findings of several investigators (7,31,32,33). Glibenclamide may potentiate insulin effects, either by increasing insulin secretion (15,16,34), increasing release of bound insulin (35), enhancing transport of blood glucose to peripheral tissues (36) or inhibiting the degradation of insulin in the vascular endothelial cells (37).

Serum total lipids were found to be significantly increased in diabetic rats. This result is in accord with other studies (38,39). However, administration of glibenclamide to diabetic rats retarded the total lipid levels to almost normal

situation. Induction of diabetes by alloxan managed to stimulate a double fold increase in triglyceride leading to hypertriglyceridemia. This result is in agreement with the findings of., (38,40). Feeding of glibenclamide to diabetic rats induced regression in triglyceride figures which become more pronounced in the last four weeks of the study. Similar increase in total cholesterol levels was detected following alloxan injection to rats. Such enhancement is in accordance with several authors (41,42,43), A common cause of elevated cholesterol is the development of diabetic nephropathy and nephrotic syndrome. Again, glibenclamide potentiated attenuations in cholesterol concentrations that were more visualized in the last four weeks of the study. It seems reasonable to conclude that glibenclamide takes a longer mode of action that may be encountered after four weeks treatment.

The significant increase in serum AST & ALT activities observed in rats after alloxan injection is in agreement with other findings (8,44,45). After a pathologic disorder, serum transaminase levels rise. AST showed a notably higher increase than did ALT, which is in conformity with the present findings and thought to be consistent with the greater need for gluconeogenic substrates and for hepatic and tissue damage (6). ALP is present in several kinds of tissues including liver, bone and intestine (46). The elevated level of this enzyme found in diabetic animals is in accordance with that reported (7). In general, the significant increase in AST, ALT & ALP activities occurred in alloxan diabetic rats could be due to the toxicity of alloxan, extensive tissue distructions, disturbances in the transphosphorylation and in the general metabolism of the different cells and tissues of diabetic rats. On the other hand, administration of glibenclamide to the diabetic rats reduces the activities of these enzymes. Such reduction could be attributed to increased insulin secretion, hepatic uptake of glucogenic amino acids and stimulation of amino acid incorporation into protein.

**Histological Investigation**

**Pancreas**

The observed intensive histopathologic changes in pancreas of alloxan diabetic rats including vacuolar degeneration, loss of regular pattern of islets, reduction in size and number of β-cells and pancreatic acini acquired pyknotic nuclei with limited secretory intity are in accordance with other findings (10,11). The release of pancreatic enzymes, particularly lipase and trypsin in an active form into the pancreas itself cause necrosis (9). Glibenclamide was found to stimulate

considerable recovery to pancreatic damaged tissue. Glibenclamide has been found to stimulate insulin secretion. A correlation was observed between insulin secretion with glibenclamide and the histological changes of islet cells which may suggest also their stimulation of the reserve cells to differentiate into β-cells or enhancement of the multiplication of the remaining living cells. This effects may be due to the ability of glibenclamide to undergo oxidation reduction very easily in biological systems to give disulphide compounds that yielded the most described physiological effects or on their direct interactions on carbohydrate metabolism in association with their effect on activating β-cells formation (11). It is worth mentioning that the histological features coincided with the reported physiological ones. The recovery within islets of Langerhans and the newly formed health β-cells from differentiation of blood cells activated a more normalized pattern of secretion. This is mainly attributed to the close relation between the islets and blood circulation.

**Liver**

The observed histopathologic alterations of liver in alloxan diabetic rats represented by vacuolar and hydropic degeneration, increase in Kupffer cells with hepatic sinusoids, thickening of blood vessel walls and bile canaliculi and necrosis of hepatic lobules are similar to that found in liver of different animals subjected to various agents (47,48). Cytoplasmic vacuolation may be attributed to be formed as a result of breakdown of lipoprotein complexes in the affected cells (49). The vacuolations are mainly a consequence of considerable disturbances in lipid inclusions and fat metabolism occurring under pathological cases (50,51). The degenerative changes in the liver were attributed to the disturbances including the increase in the level of cholesterol which predisposed the cell membrane to degeneration (52). Vacoular degeneration may also be a normal sequence to disruption of lysosomes. Their hydrolytic enzymes under pathological conditions are liberated bringing about considerable autolysis of various cellular parts (53). Also, inhibition of both ATP-synthetase and ATP-ase activities in mitochondria of rat liver may be held responsible for degenerative and necrotic changes (54). Again the necrosis was attributed to depletion of glutathione in their tissue (13). A frank indication of cytotoxic injury induced by drugs and chemicals is the chronic inflammatory response (12). However, the administration of glibenclamide to alloxan diabetic rats managed to amend most of the previous lesions. The end results was a near to normal hepatic architecture with its lobular pattern and portal

tracts. In accordance improved patterns have been reported following glimepiride (a sulfonylurea agent) treatment of type II diabetes mellitus (14). It was recorded that metformin have the same effect (55).

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