COMPARATIVE HISTOPATHOLOGICAL EFFECTS OF METFORMIN AND GLIBENCLAMIDE ON LIVER, KIDNEY & PANCREAS IN ALLOXAN INDUCED DIABETIC ALBINO RATS

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ABSTRACT

Introduction: Diabetes mellitus is a systemic metabolic disorder characterised by elevated blood glucose levels due to absolute or relative deficiency of insulin secretion from pancreatic β-cells. Diabetes is responsible for causing impairment in functions of liver, kidney & pancreas. Oral hypoglycemic drugs like metformin and glibenclamide improved functions of liver, kidney & pancreas. Hence in this study we evaluated the comparative histopathological effect of metformin and glibenclamide on liver, kidney & pancreas in Alloxan induced diabetic rats. Material and Methods: The present study was conducted on 24 experimental animals which were divided into four groups of 6 rats in each group and were treated accordingly Group 1: Healthy control (HC) rats, Group 2: Diabetic control (DC) rats. Group 3: Diabetes mellitus (DM) + Metformin (M) rats, Group 4: Diabetes mellitus (DM) + Glibenclamide (G) rats. After 28 days of treatment rats were sacrificed and blood glucose and body weight was determined along with histopathological study of liver, kidney & pancreas. Result: The results showed that both Metformin and Glibenclamide reversed Alloxan induced diabetic histopathological changes in liver, kidney & pancreas. Conclusion: The present Results demonstrate that normoglycemia with metformin and glibenclamide ameliorates diabetic induced histopathological lesions in liver, kidney & pancreas.

KEYWORDS: Diabetes, Histopathology, Liver, Kidney, Pancreas, Metformin, Glibenclamide.

INTRODUCTION

Diabetes mellitus is a major worldwide health problem involving endocrine pancreas characterised by elevated blood glucose levels due to absolute or relative deficiency of insulin secretion from pancreatic β-cells. It is also characterized by excessive disturbance of carbohydrates, proteins and lipid metabolism and long term complications which affect eyes, kidneys, nervous system and circulatory system. According to WHO, the prevalence of diabetes in adults worldwide was estimated to be 4.0% in 1995 and is to rise to 5.4% by the year 2025.

Histopathological studies have revealed that the alloxan-induced diabetic rats, display feathery degeneration, micro and macro cellular fatty changes and inflammatory cells around portal tract in liver tissue.

However, treatment with metformin showed normal architecture of liver with visible veins, decrease in hepatocyte degeneration, vacuolation and pyknosis of nuclei as compared to diabetic control group.

Similarly, glibenclamide treated albino rats showed protection from alloxan-induced histopathological changes in liver.

Histopathological studies have revealed that the alloxan-induced diabetic rats, display feathery degeneration, thickening of glomeruli, inflammatory cells and severe congestion in kidney tissue.

Whereas metformin and glibenclamide treated albino rats showed protection from alloxan-induced changes in kidney.

Histopathological study of pancreas in alloxan-induced diabetic rats showed pancreatic lobules separated by connective tissue septa. The islets appear reduced in number with extensive necrotic changes followed by fibrosis and atrophy of pancreas.

However, treatment with metformin showed increase in number and size of islets.
Similarly, glibenclamide treated albino rats showed protection from alloxan-induced change in pancreas.

AIMS AND OBJECTIVES
To Compare the Histopathological effects of Metformin and Glibenclamide on Liver, Kidney & Pancreas in Alloxan-Induced Diabetic Albino Rats.

MATERIALS AND METHODS
The present study is based on the findings carried out on a total of 24 albino rats weighing between 120-160gm. The rats were procured from the Central Animal House, Department of Pharmacology, Govt. Medical College, Jammu. The study was conducted after getting clearance from Institutional Animal Ethics Committee (IAEC).

24 experimental animals were divided into four groups of 6 rats each and each group was administered drugs as follows:-
**Group 1:** Healthy control (HC) rats served as controls and were administered only Normal saline (0.5ml/day) orally.
**Group 2:** Diabetic control (DC) rats were induced with diabetes using alloxan and were not given any form of treatment throughout the study.
**Group 3:** Diabetes mellitus (DM) + Metformin (M) rats were induced with diabetes by alloxan and treated with standard drug metformin orally for 28 days.
**Group 4:** Diabetes mellitus (DM) + Glibenclamide (G) rats were induced with diabetes by alloxan and treated with glibenclamide for 28 days orally.

The animals were kept in clean plastic cages in a well ventilated room and were maintained at room temperature of (25±2°C). Rice husk was used as bedding material. All animals were fed with rat feed and water ad-libitum throughout the experimental period. Their cages were cleaned of waste daily.

The animals were weighed and injected alloxan (150mg/kg) dissolved in distilled water using insulin syringe via intraperitoneal route. Diabetes mellitus was confirmed after 75 hours of alloxan injection by testing the blood glucose levels using glucometer and glucose test strip. Animals with blood glucose level of 250mg/dl and above were considered diabetic and were given metformin (500mg/kg) and glibenclamide (10mg/kg) orally for 28 days after dissolving these drugs in distilled water.

Albino rats of all groups were sacrificed after 28 days by keeping them in an inverted glass jar containing a large piece of cotton soaked in chloroform, so that the process can occur without pain and discomfort as recommended by Laboratory Animals Information Service Centre.

OBSERVATIONS
Blood glucose and body weight of albino rats of 4 different groups were observed on zero, 7, 14 and 28 day of experimental study shown below.

![Fig. 1: Bar chart showing mean blood glucose(mg/dl) of experimental groups (Group no. 1, 2 and 3).](image1)

![Fig. 2: Bar chart showing mean blood glucose(mg/dl) of experimental groups (Group no. 1, 2 and 4).](image2)
Fig. 3: Bar chart showing mean body weight (gms) of experimental groups (Group no. 1,2 and 3).

Fig. 4: Bar chart showing mean body weight(gms) of experimental groups (Group no. 1,2 and 4).

MICROSCOPIC OBSERVATIONS
Light Microscopic Examination of Liver
Group 1 (Healthy control)
Architecture of liver (Fig. A)
Light microscopic examination of Hematoxyline and Eosin stained liver sections of Group 1(Healthy control) rats revealed the normal basic structure of liver, showing hepatic lobules with central veins located in the centre of the lobule and portal areas containing portal triad formed by portal vein, hepatic arteriole and bile ductule surrounded by connective tissue. The cords of the hepatocytes which were one cell thick were found to be radiating from the central veins towards the periphery of the lobule which contain portal areas.

Hepatocytes were polygonal, stained pink in colour and had centrally placed spheriodal, euchromatic nucleus which stained light blue in colour and contained one nucleolus. Two nucleoli were seen in the nuclei of some hepatocytes.

Group 2 (Diabetic control)
Architecture of liver (Fig. B)
On histological examination of liver of Group 2 rats, the basic architecture of liver was found disturbed. The central veins were dilated and congested over wider areas. The sinusoids radiating from the congested veins were also dilated and congested in these areas and some containing focal inflammatory infiltrate. The liver tissue showed distortion in the arrangement of hepatocytes around the central veins. Dilated and congested portal veins were present in the portal areas. Inflammatory cell infiltration around the portal areas was also observed. Surrounding the portal areas there was focal hepatocyte degeneration at the limiting plates at some places accompanied with inflammatory cell infiltrate, thereby breaching the limiting plates.

The hepatocytes and their nuclei were pleomorphic in appearance. At certain areas enlarged hepatocytes were seen containing enlarged nuclei with prominent nucleoli known as karyomegaly, while at other areas degeneration and necrosis of hepatocytes was seen with small condensed nuclei known as Karyopyknosis.

Group 3 (Diabetic control + Metformin)
Architecture of liver (Fig. C)
On histological examination the basic architecture of the liver was found to be normal. The central veins were dilated and congested at certain places. The sinusoids radiating from the central veins were dilated at certain places. The cords of hepatocytes were arranged radially around the central vein. Liver tissue exhibited mild degeneration of hepatocytes as compared to diabetic control group. The normal lobular architecture was maintained. Portal areas containing the portal tracts were normal in size. The bile ductules and hepatic arterioles
were found to be normal. Mild inflammatory cell infiltrate of the portal areas was also seen.

Group 4 (Diabetic control + Glibenclamide).
Architecture of liver (Fig. D)
In group 4 rats the architecture of the hepatic lobule appeared more or less like normal control. The central veins were dilated with mild congestion at few places only & surrounded by radially arranged hepatocytes. Sinusoids were dilated, congested and filled with inflammatory infiltrate. Portal areas were also dilated at certain places with mild inflammatory changes.

Fig.D: Photomicrograph of the section of the liver of Diabetic mellitus+glibenclamide treated group rat. Haematoxylin and Eosin 100X.

LIGHT MICROSCOPIC EXAMINATION OF KIDNEY
Group 1 (Healthy control)
Histologically, kidney of control rats of Group 1 contained numerous nephrons consisting of the renal corpuscle, the proximal convoluted tubule, thin and thick limbs of loop of Henle and distal convoluted tubules. The renal corpuscle contained a tuft of capillaries, the glomerulus surrounded by Bowman’s capsule. In between glomerulus and Bowman’s capsule, there was urinary space. The proximal convoluted tubule was lined by simple cuboidal epithelium with brush border, where as the distal convoluted tubule was lined by simple cuboidal epithelium (Fig.A).

Group 2 (Diabetic control)
Kidneys of Alloxan induced diabetic rats of Group 2 showed glomerular alterations. The glomerular size was expanded and congested in diabetic rats resulting in reduction in Bowman’s space (Fig.B). Glomeruli were infiltrated by inflammatory cells. Degenerated tubules were observed in cortex. In some tubules, the cells had separated from the basement membrane and collected in the center. Many tubules had completely sloughed off epithelium. Cells at other places were separated from one another also and were shed into the lumina of tubules. The lumina contain the cellular debris.

Group 3 (Diabetic control+Metformin)
Kidney of Alloxan induced diabetic rats treated with metformin showed normal sized glomerulus with normal Bowman’s space and mild degeneration of renal tubules (Fig.C).

Group 4 (Diabetic control + Glibenclamide)
Kidney of Alloxan induced diabetic rats treated with glibenclamide showed normal architecture of the cortex and medulla. In the cortex, the renal corpuscles appeared normal with mild congestion in the glomeruli. Renal tubular degeneration was also seen (Fig.D).
Fig.(A): Photomicrograph of the longitudinal section of kidney of Healthy control group 1 rat showing normal architecture of cortex containing glomeruli, PCTs, DCTs and interstitial tissue containing blood vessel. Haematoxyline and Eosin 100X.

Fig.(B): Photomicrograph of the longitudinal section of kidney of Diabetic control group 2 rat showing congested and expanded glomerulus with decreased Bowman’s space, degenerated renal tubules. Haematoxyline and Eosin 100X.

Fig.(C): Photomicrograph of the longitudinal section of kidney of Diabetic mellitus+metformin group rat showing normal sized glomeruli with normal Bowman’s space, degeneration of renal tubules. Haematoxyline and Eosin 100X.

Fig.(D): Photomicrograph of the longitudinal section of kidney of Diabetic mellitus+glibenclamide group 4 rat showing normal size glomeruli with normal Bowman’s space, tubular degeneration. Haematoxyline and Eosin 100X.

LIGHT MICROSCOPIC EXAMINATION OF PANCREATE

Group 1 (Healthy control)
Architecture of pancreas: Light microscopic examination of Hematoxyline and Eosin stained pancreatic sections of Group 1 (control) rats revealed the normal basic structure of pancreas, showing the exocrine part made up of serous acini lined by pyramidal cells with basal round nuclei. The endocrine part of pancreas was made up of many rounded microscopic elements called the pancreatic islets of langerhans. There were small isolated masses of cells distributed throughout the pancreas (Fig.5).

Group 2 (Diabetic control)
Architecture of pancreas: On histological examination of pancreas of Group 2 rats revealed decreased diameter and number of islets of langerhans. At some places islets were irregularly shaped, relatively small and atrophic. Severe degeneration of islet cells and presence of several cavities were also observed between serous acini. The inflammatory cell infiltrate were also present around the affected area (Fig.6).

Group 3 (Diabetic control + Metformin)
Architecture of pancreas: Histological examination of pancreas of Group 3 rats revealed restorative effect on pancreatic tissue with abundant islet cell mass. Few cavities were found between serous acini (Fig.7).

Group 4 (Diabetic control + Glibenclamide)
Architecture of pancreas: Histological examination of pancreas of Group 4 rats shows marked improvement of cellular injury as evident from normal pancreatic acini and lobules, restoration of normal cell population of islet cells, with few cavities between acini (Fig.8).
Fig. 5: Photomicrograph of the section of the pancreas of Healthy control group rat showing exocrine part made up of serous acini and endocrine part consisting of many rounded islets. Haematoxylin and Eosin 100X.

Fig. 6: Photomicrograph of the section of the pancreas of Diabetic control group rat showing presence of several cavities between acini, atrophic islets, Haematoxylin and Eosin 100X.

Fig. 7: Photomicrograph of the section of the pancreas of diabetic mellitus+metformin group3 showing regenerating islets between acini. Haematoxylin and Eosin 100X.

Fig. 8: Photomicrograph of the section of the pancreas of diabetic mellitus+glibenclamide group 4 rat showing regenerating large islet cell mass. Haematoxylin and Eosin 100X.

DISCUSSION

Diabetes mellitus is a systemic metabolic disorder characterized by elevated blood glucose levels due to absolute or relative deficiency of insulin secretion from pancreatic β-cells.

Increase in blood glucose level leads to structural and functional changes in target organs of diabetic patients. In the present study alloxan monohydrate, a toxic glucose analogue, was used for induction of diabetes in albino rats.

The present study is based on the observations made on 24 albino rats, weighing 120-160 gm to determine the comparative histopathological effect of metformin and glibenclamide on liver, kidney & pancreas in alloxan induced diabetic rats.

The rats were housed in the cages under standard laboratory conditions and divided into four groups. The body weight and blood glucose was measured on day 0, 7, 14, 21, 28 after treatment was started.

It was observed that diabetes induced by alloxan caused a significant decrease in the body weight throughout the study as compared to the healthy control group (p<0.05). Also diabetic rats treated with metformin showed a significant reduction in body weight in comparison to healthy control group (p<0.05). Whereas diabetic rats treated with glibenclamide showed increase in body weight throughout the study period in comparison to diabetic rats treated with metformin which is statistically significant (p<0.05).

Similarly, diabetes induced by alloxan caused a significant increase in the blood glucose level throughout the study compared with the healthy control group (p<0.05). However, after treatment with metformin and glibenclamide there was significant reduction in blood glucose level in comparison to diabetic control group (p<0.05).
After sacrificing the animals, tissue processing was done on the specified organ of liver, kidney & pancreas and slides were prepared for histopathological study.

In the present study it was observed that the liver tissue of Alloxan induced diabetic albino rats showed central vein congestion, degeneration and necrosis of hepatocytes with inflammatory cells around portal tract. Similar findings were revealed by Koyaguru et al (2013), Sunil C et al (2009) and Prakash D et al (2012) which is in accordance with present study.

In present study we observed that after daily administration of metformin (500mg/kg) in alloxan induced diabetic rats for 28 days liver exhibited an apparent decrease in hepatocyte degeneration, as compared to diabetic control group. Further liver cords arrangement appeared normal. The results of present study are in agreement with Khadre SEM et al (2011) and Brantley AU et al (2015).

In present study after daily administration of glibenclamide (10mg/kg) in alloxan induced diabetic rats for 28 days the architecture of the hepatic lobule appeared normal. The central veins were surrounded by radially arranged hepatocytes. Sinusoids were slightly dilated, congested and filled by inflammatory infiltrate. These findings are in consonance with previous studies done by Sangeetha MS et al (2015) and Murali R et al (2013)

In the present study we observed that kidney of alloxan induced diabetic albino rat showed healing features, treatment with glibenclamide kidney tissue of alloxan induced diabetic rats showed expanded glomerular size with hypercellularity, sloughing of epithelial cells of proximal convoluted tubule, degenerated renal tubules, and cellular debris in the lumen of tubules. The present study is in agreement with Sunil C et al (2009). Similar study was conducted by Khadre SEM et al (2011) who observed similar findings except for cellular debris. Hence our study is in partial agreement with Khadre SEM et al (2011).

In the present study daily administration metformin (500mg/kg) for 28 days in alloxan induced diabetic rats showed normal sized glomerulus with normal Bowman’s space and mild degeneration of renal tubules. The present study is in agreement with Nasri H and Rafieian-Kopaei M (2014) and Emam HT (2015).

In present study daily administration glibenclamide (10mg/kg) for 28 days reversed diabetic induced changes which was revealed by normal appearance of the renal corpuscle with mild congestion in the glomeruli and degeneration of tubules at certain areas. A similar study done by Mahood AKS (2012) revealed that after treatment with glibenclamide kidney tissue of alloxan induced diabetic albino rat showed healing features, which resembles that of normal kidney. The present study is in agreement with Mahood AKS (2012).

Further in the present study pancreatic tissue of diabetic induced albino rats showed decreased diameter and number of islets of langerhans. However, after treatment with both metformin and glibenclamide regeneration of islets was observed.

CONCLUSION
In conclusion the present Results demonstrate that normoglycemia with metformin and glibenclamide ameliorates diabetic induced histopathological lesions in liver, kidney & pancreas. Thus the frequent biochemical and laboratory analysis is important to check the occurrence of complications during the course of treatment.

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REFERENCES


