



Comparative Histopathological Effects of Metformin and Glibenclamide on Liver 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 liver functions. Oral hypoglycemic drugs like metformin and glibenclamide improved functions of liver. Hence in this study we evaluated the comparative histopathological effect of metformin and glibenclamide on liver in Alloxan induced diabetic rats.*

Material and Methods: *The present study was conducted on 24 experimental animals which were divided into four groups of with 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*

Result: *The results showed that both Metformin and Glibenclamide reversed Alloxan induced diabetic histopathological changes in liver in Albino rats*

Conclusion: *The present Results demonstrate that normoglycemia with metformin and glibenclamide ameliorates diabetic induced histopathological lesions in liver*

Keywords: *Diabetes, Histopathology, Liver, Metformin, Glibenclamide.*

Introduction

Diabetes mellitus is a major worldwide health problem involving endocrine pancreas characterized 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.

Aims and Objectives

To Compare the Histopathological effects of Metformin and Glibenclamide on Liver 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, and 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 $(25\pm 2^{\circ}\text{C})$. 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

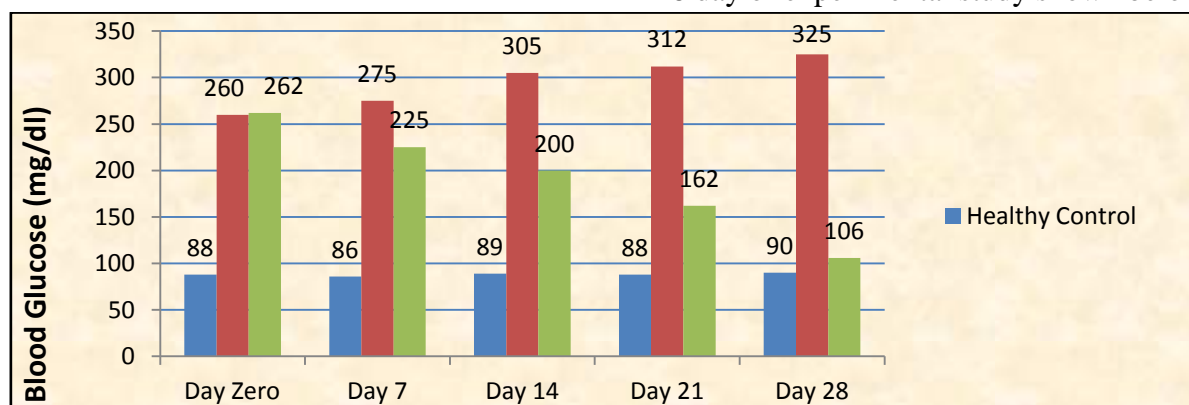


Fig 1: Bar chart showing mean blood glucose (mg/dl) of experimental groups (Group no. 1, 2 and 3).

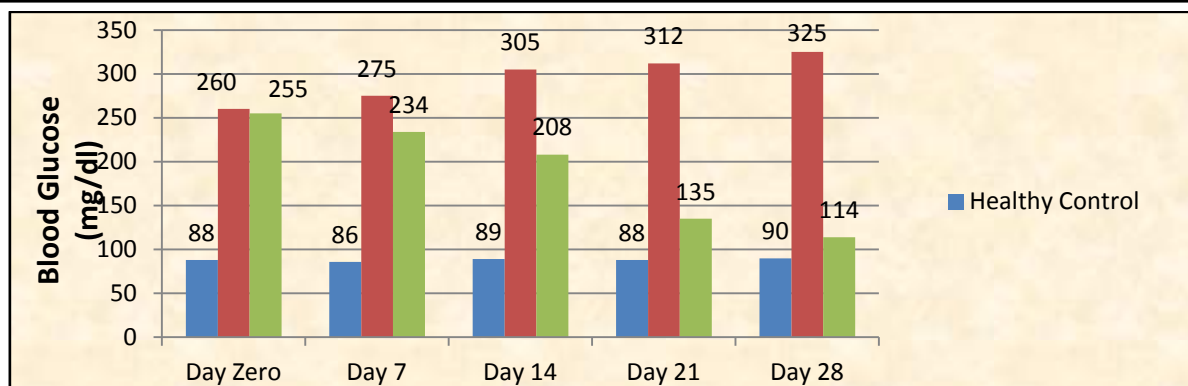


Fig 2: Bar chart showing mean blood glucose (mg/dl) of experimental groups (Group no. 1, 2 and 4)

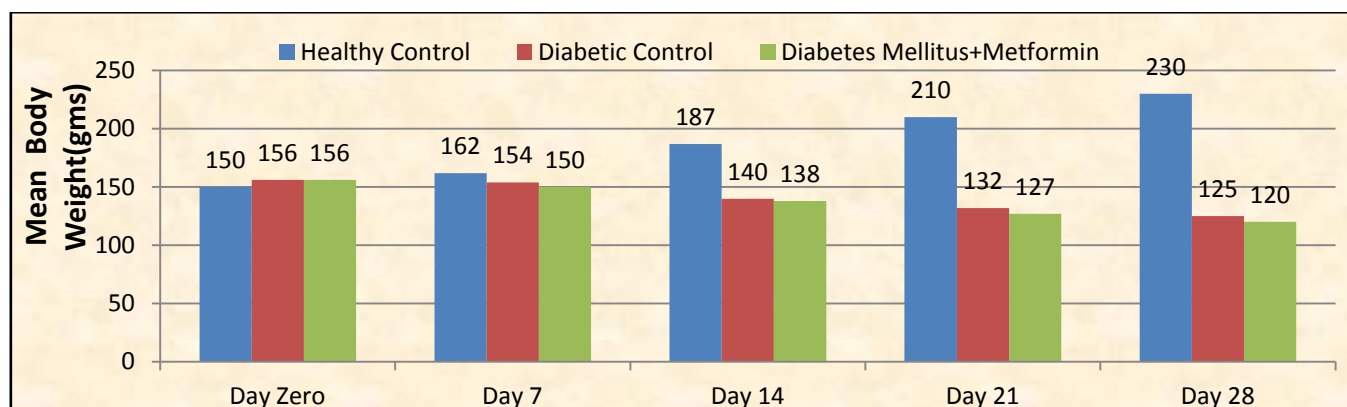


Fig 3: Bar chart showing mean body weight (Gms) of experimental groups (Group no. 1, 2 and 3)

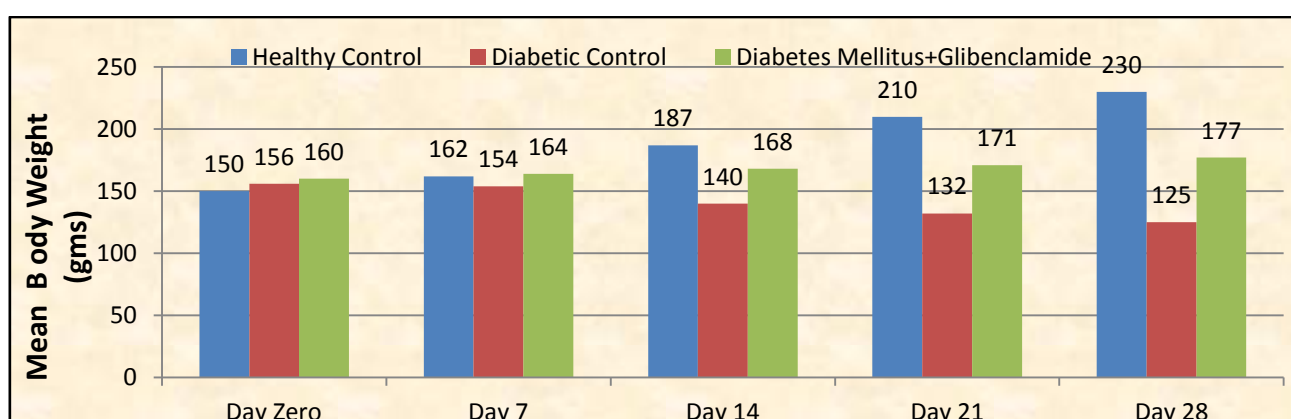


Fig.4: Bar chart showing mean body weight (Gms) of experimental groups (Group no. 1, 2 and 4).

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 spheroidal, 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.

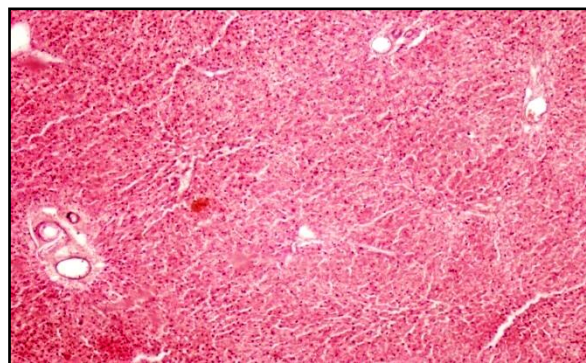


Fig A: Photomicrograph of the section of liver of Healthy control rat. Haematoxyline and Eosin 100X.

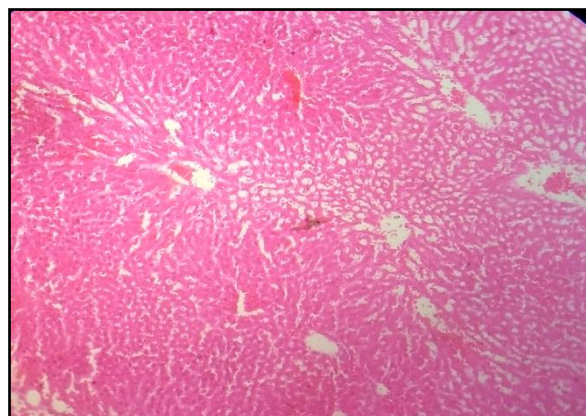


Fig B: Photomicrograph of the section of the liver of Diabetic control group rat .Haematoxylin and Eosin 100x

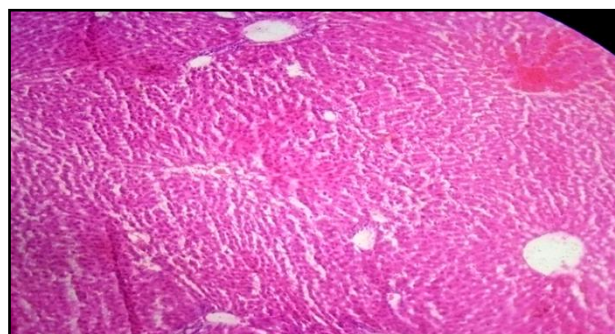


Fig C: Photomicrograph of the section of the liver of Diabetic mellitus+metformin treated group rat .Haematoxylin and Eosin 100X.

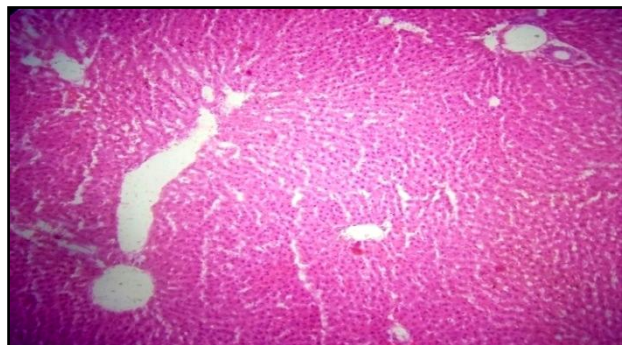


Fig. D: Photomicrograph of the section of the liver of Diabetic mellitus+glibenclamide treated group rat. Haematoxylin and Eosin 100X.

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 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, 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)

Conclusion

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

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