



**COMPARATIVE HISTOPATHOLOGICAL EFFECTS OF METFORMIN AND GLIBENCLAMIDE ON 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  $\beta$ -cells. It is implicated in oxidative stress which induces insulin resistance in the peripheral tissue and impairs insulin secretion from pancreatic beta cells. In this study we evaluated the comparative histopathological effect of Metformin and Glibenclamide on 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 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 pancreas. **Result:** The results showed that both Metformin and glibenclamide reversed Alloxan induced diabetic histopathological changes in Pancreas in Albino rats. **Conclusion:** The present results demonstrate that normoglycemia with metformin and glibenclamide ameliorates diabetic induced histopathological lesions in Pancreas.

**KEYWORDS:** Diabetes, histopathology, 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  $\beta$ -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 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 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.

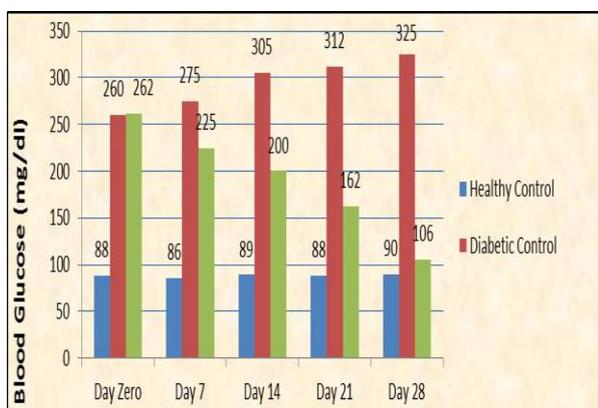
The animals were kept in clean plastic cages in a well ventilated room and were maintained at room temperature of (25±2<sup>0</sup>c). Rice husk was used as bedding material. All animals were fed with rat feed and water ad-libitum throughout the experimental period. Their cages were cleaned of waste 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.

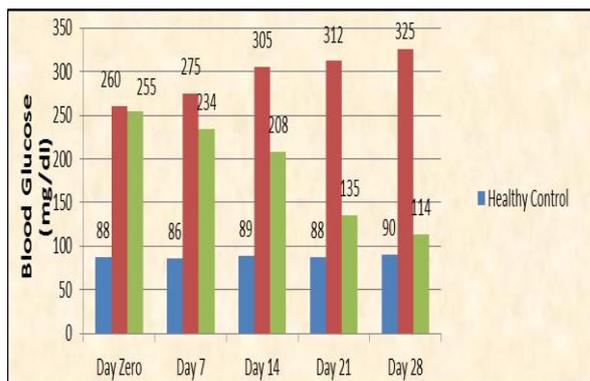
Albino rats of all groups were sacrificed after 28 days by keeping them in an inverted glass jar containing a large piece of cotton soaked in chloroform, so that the process can occur without pain and discomfort as recommended by Laboratory Animals Information Service Centre.

**OBSERVATIONS**

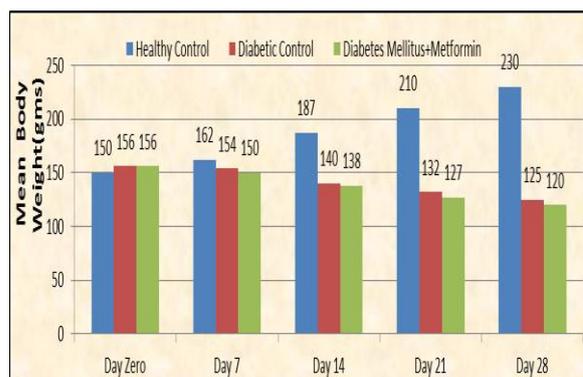
Blood glucose and body weight of albino rats of 4 different groups were observed on zero, 7,14 and 28 day of experimental study shown below.



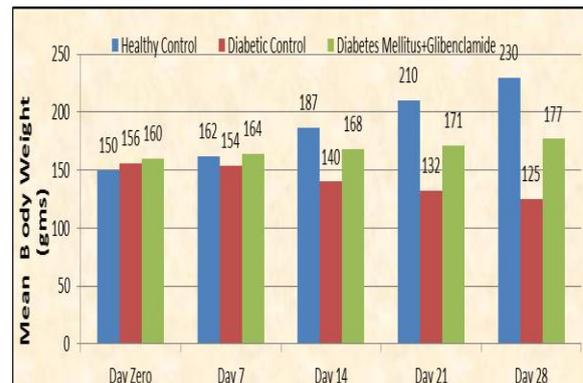
**Fig. 1:** Bar chart showing mean blood glucose(mg/dl) of experimental groups (Group no. 1,2 and 3).



**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 Observation**

**Light Microscopic Examination of Pancreas**

**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)**

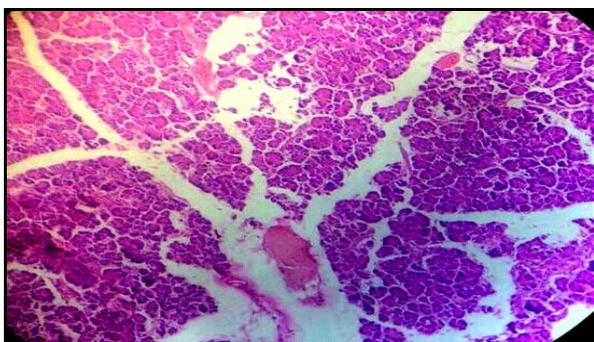
**Architecture of pancreas:** On histological examination of pancreas of Group 2 rats revealed decreased diameter and number of islets of langerhans. At some places islets were irregularly shaped, relatively small and atrophic. Severe degeneration of islet cells and presence of several cavities were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

**Group 3 (Diabetic control + Metformin)**

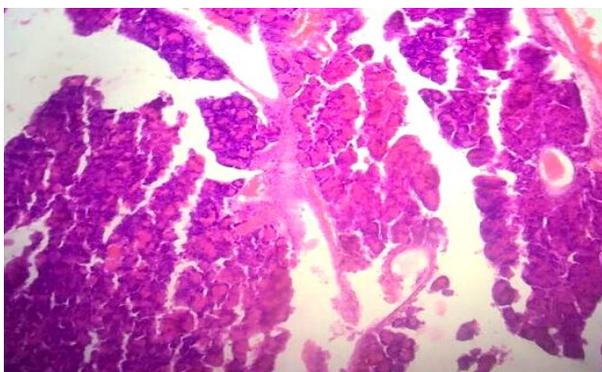
**Architecture of pancreas:** Histological examination of pancreas of Group 3 rats revealed restorative effect on pancreatic tissue with abundant islet cell mass. Few cavities were found between serous acini (Fig.7).

**Group 4 (Diabetic control + Glibenclamide)**

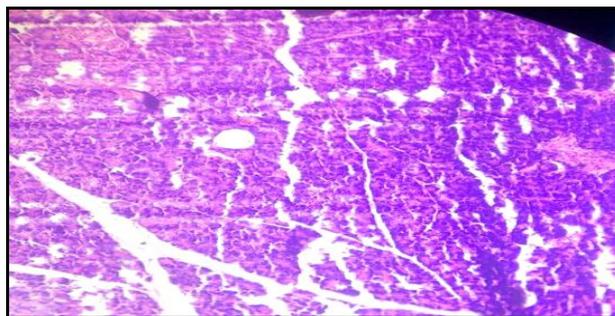
**Architecture of pancreas:** Histological examination of pancreas of Group 4 rats shows marked improvement of cellular injury as evident from normal pancreatic acini and lobules, restoration of normal cell population of islet cells, with few cavities between acini (Fig.8).



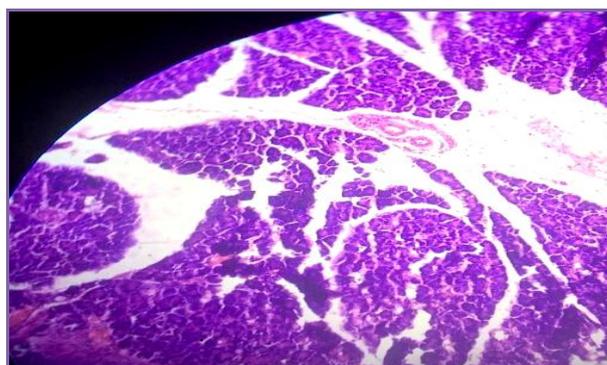
**Fig.5 :**Photomicrograph of the section of the pancreas of Healthy control group rat showing exocrine part made up of serous acini and endocrine part consisting of many rounded islets . Haematoxylin and Eosin 100X



**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  $\beta$ -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 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 pancreas, and slides were prepared for histopathological study.

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 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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