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Original Article

The Effects of Metformin Treatment on Diabetic Albino Rats' Pancreas, Liver, and Kidney Histology

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Abstract

Metformin, an oral hypoglycemic drug, has traditionally been considered the standard therapy for hyperglycemia. Metformin's several modes of action include inhibition of hepatic gluconeogenesis, anti-glucagon activity, and insulin-sensitizing effect. This study aims to assess the effectiveness of Metformin on the liver, pancreatic, and kidney tissues of alloxan-induced diabetic albino rats. Twenty mature albino white male rats were allocated at random into two groups. Intraperitoneal injections of alloxan monohydrate were utilised to induce diabetic Mellitus type II in the first ten rats. The second group of rats were injected intraperitoneally with normal saline. Both groups were then separated into four subgroups: Group 1 consisted of non-diabetic rats that were only administered distilled water (control), Group 2 consisted of non-diabetic rats that were administered metformin at a dose of 1000 mg/kg/day, and Group 3 consisted of diabetic control animals that were administered alloxan intravenously and distilled water orally, but were not given any medications. After seven days of DM induction, diabetic rats were administered Metformin at a dose of 1000 mg/kg/day orally. After one month of therapy, the animals were slaughtered and their organs were harvested. Compared to the control group, the histological results of pancreatic tissue were normal in the treatment groups. In contrast, liver and kidney sections from non-diabetic control, non-diabetic, and diabetic animals given 1000 mg/kg/day of Metformin had normal histology. Still, both tissues of untreated diabetic control mice exhibited lymphocyte infiltration. Metformin has been found to have significant blood glucose lowering properties and the capacity to protect several organs from the negative consequences of diabetes.

Keywords: Metformin, Alloxan-induced, Albino rats, Pancreas, Liver, Kidney

1. Introduction

In the absence of insulin, the liver increases glucose absorption, whereas the kidney, an insulin-independent tissue, seeks to combat the rising glucose levels in diabetes (1). Diabetes mellitus type 2 (T2DM) is a serious global public health challenge affecting millions of people. This condition is associated with insulin resistance (IR), which contributes to dyslipidemia and alterations in the profiles of gene regulation (2). An ageing population, a sedentary lifestyle, growing obesity trends, and a poor diet are significant contributors to the global increase in diabetes (3). Insulin is the principal hormone that regulates the production and use of glucose by human tissues and organs.

It was unknown if the histological changes observed in the livers of alloxan-induced diabetic rats were due to the toxicity of the medication or the diabetes state. Alloxan is toxic to the pancreatic beta cells that cause diabetes, as well as the kidneys and livers of different animal species (1, 4). On the other hand, the harmful effects of these treatments are largely reliant on the animals' species, age, and body weight, as well as

their hydration status, method of administration, and infusion rate (4).

Metformin reduces hepatic insulin resistance and hyperglycemia, and is the first-line treatment for type 2 diabetics, especially those with hyperlipidemia and obesity (5). To effectively regulate glucose, it functions by reducing glucose absorption in the gastrointestinal tract, boosting cellular glucose uptake, and decreasing glycogen production in the liver. Metformin was used to manage blood glucose levels and avoid complications such as diabetic cardiomyopathy and retinopathy in diabetic individuals. Even though Metformin affects other T2DM complications, no reports of Metformin treatment for testicular dysfunction exist, and the testicular tissue mechanisms remain unclear (6).

Metformin is the most often given glucose-lowering medication for T2DM due to its safety, efficacy, and beneficial cardiovascular and metabolic activity when paired with lifestyle modifications. Metformin has pleiotropic effects on skeletal muscles, adipose tissue, and the endothelium, which are all affected by insulin resistance (IR) and hyperinsulinemia (2). Metformin is effective as a single agent or in combination with other drugs, such as insulin secretagogues or insulin sensitizers, according to Adeniji, Lawal (7).

This study aimed to estimate the efficiency of Metformin on liver, pancreas, and kidney tissues of diabetic albino rats induced by alloxan.

2. Materials and Methods

20 adult white male albino rats (8-10 weeks old) weighing 170-200 g were obtained from the house of animals in the University of Babylon/ college of science. The rats were given two weeks to acclimate before beginning the experiment. These animals were divided into two main groups. The first ten rats were used to develop diabetes mellitus (type II) by injecting alloxan monohydrate as a single dosage of 130 mg/kg body weight intraperitoneally after 72 hours. Their fasting blood sugar levels were evaluated using a glucometer after 9-12 hours of fasting. The second set of ten rats received intraperitoneal injections of normal saline. Then both groups were split into four groups: Group 1: In the control group, as an alternative to the treatment given to the other groups, non-diabetic rats were given DW orally using an orogastric tube. Non-diabetic animals in Group 2: were given 1000 mg/kg/day of Metformin, administrated orally by oral gavage tube. Group 3: Animals in the diabetic control group were given alloxan i.p. and administrated distilled water orally but have not been given any medications. Groups 4: Diabetic rats with Metformin after seven days of DM induction, diabetic rats received Metformin 1000 mg/kg/day orally. After 1 month of treatment, the animals were sacrificed, and the liver, pancreas, and kidney were collected for histological studies according to Bancroft's procedure (8).

3. Results and Discussion

Figure 1 depicts that the histology of pancreatic tissue was normal in treatment groups as compared to the control group (A, B, C, D). Regarding liver tissue, all non-diabetic controls had normal hepatic tissue; non-diabetic animals got 1000 mg/kg/day of Metformin, while diabetic animals received 1000 mg/kg/day of Metformin (Figure 2A, 2B, 2D). Figure 2 illustrates lymphocyte infiltration in the livers of diabetic control subjects who did not take any medication (C). Figure 3 shows the kidney histology of non-diabetic control, non-diabetic, and diabetic rats receiving 1000 mg/kg/day of metformin (A, B, D). Figure 3C depicts the existence of lymphocyte infiltration in the kidneys of diabetic control mice not given treatment.

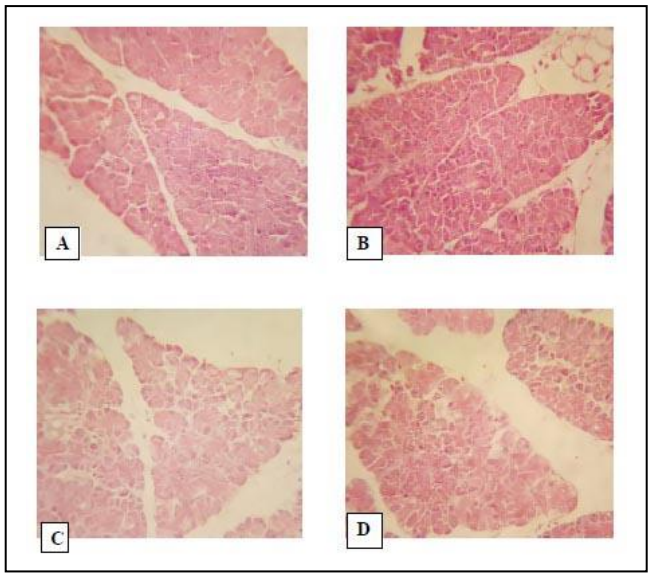

Figure 1. Cross-section of the pancreas showing normal histology in all of **A:** non-diabetic rats (control group), **B:** non-diabetic rats treated with Metformin 1000 mg/kg/day, **C:** diabetic rats, **D:** diabetic rats treated with Metformin 1000mg/kg/day, Stained with H&E 400×

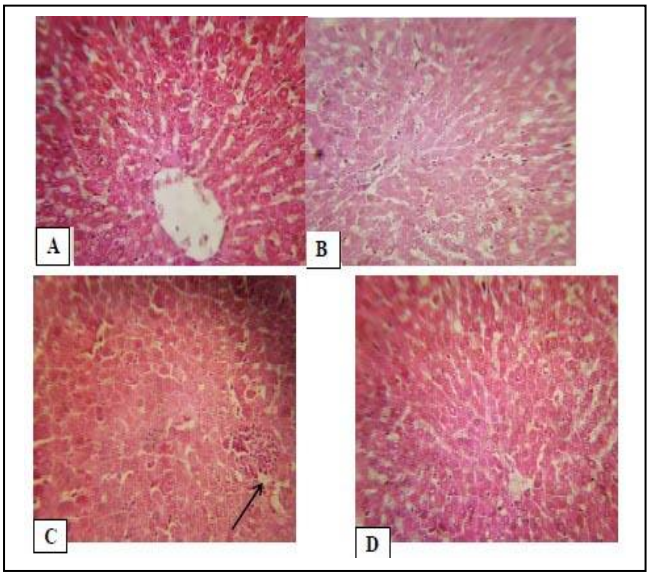

Figure 2. Cross-section of the liver of **A:** non-diabetic control rats showing normal histology, **B:** non-diabetic rats treated with Metformin 1000 mg/kg/day showing normal histology, **C:** diabetic rats showing lymphocyte infiltration (black arrow), **D:** diabetic rats treated with Metformin 1000mg/kg/day showing normal histomorphology, stained with H&E 400×

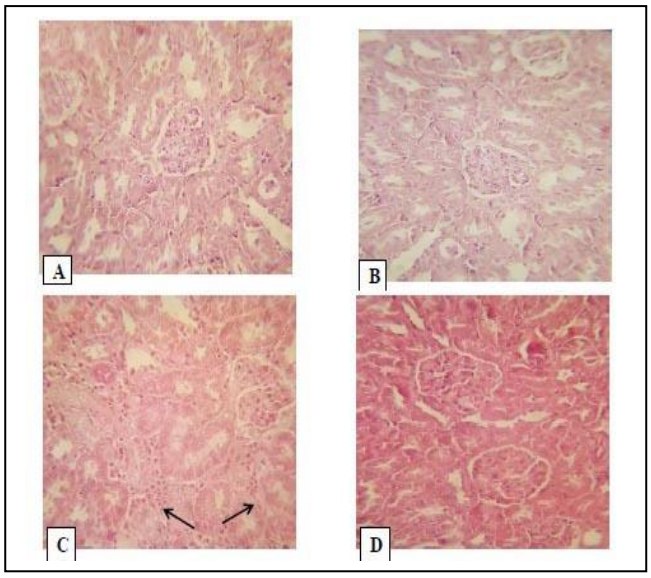

Figure 3. Cross histological section of the kidney of **A:** non-diabetic control rats showing normal histology with typical structure, **B:** non-diabetic rats receiving metformin 1000 mg/kg/day showing normal histology, **C:** diabetic rats showing lymphocyte infiltration (black arrow), **D:** diabetic rats were given Metformin 1000mg/kg/day showing normal histology, stained with H&E 400×

Due to increased glycosylation, which affects protein structure, hyperglycemia results in a number of biochemical and morphological problems. Microvascular complications, such as retinopathy, nephropathy, and capillary basement membrane thickening, are critical complications of diabetes mellitus. If diabetes therapy is not initiated quickly, prolonged hyperglycemia will lead to serious consequences, including permanent hepatic and renal damage (1). Type 2 diabetes is characterised by growing cell destruction due to chronic IR as well as reduced cell mass and function (1).

Metformin has a high rate of blood glucose reduction, up to 40.7%, and is often referred to as the "preferred medicine" for type 2 diabetes (7). Metformin therapy significantly altered the insulin signalling pathway in a rat model of type 2 diabetes (2). Metformin inhibits gluconeogenesis and enhances glucose elimination mediated by insulin. Enhanced insulin-receptor affinity or post-receptor effects may be the molecular

foundation for Metformin's insulin-like action, say some researchers (9).

Individuals with T2DM are unable to create sufficient insulin to compensate for decreased insulin sensitivity, mostly due to insulin release failure and a considerable decrease in the number of functional cells (10). According to a research by Bunnag, Warner (11) the beta-cell degranulates after 6 hours. However, microcirculation changes did not emerge until 24 hours after beta cell loss and did not appear to play a substantial role in beta cell death. In the present investigation, we did not discover significant abnormalities, damage, or modifications in the rat islet or beta cells of the pancreas after injecting diabetogenic dosages of alloxan followed by therapy with metformin 1000 mg/kg/day. In accordance with the findings of Balamash, Alkreathy (3), no degenerative alterations were observed in the diabetic rat group, nor were there any significant changes, damage, or alterations in the islet or beta cells of the pancreas of rats after administration of diabetogenic doses of alloxan followed by treatment with metformin 1000 mg/kg/day.

In the current work, histological assessment of diabetic liver cells revealed lymphocyte infiltration between hepatic cells. It is widely established that the absence of insulin induces structural and biochemical abnormalities in the liver. The liver structure of alloxan-induced diabetic rats following metformin treatment was equivalent to that of the control group. Yanardag, Ozsoy-Sacan (9) investigation demonstrated that there are large histological changes in the liver due to the absence of insulin in diabetes; in the diabetic group, metformin medication resulted in a partial decrease of degenerative modifications. In various research, long-term metformin therapy hasn't been proved to protect the liver histologically. On the other side, Metformin may induce hepatotoxicity, such as acute hepatitis and cholestasis (5).

Kidney sections of alloxan-induced diabetic control rats show lymphocytic infiltration (12). A research done by Zafar (13) revealed that streptozotocin-induced diabetes causes kidney structural changes with varied degrees of lymphocyte infiltration (13). After 30 days of daily treatment of 1000 mg/kg of Metformin to alloxan-induced diabetic rats, the tissue architecture of the kidneys seemed normal in the current investigation. The outcomes of this investigation concur with Zhang, Xu (14), who proved that Metformin could greatly ameliorate renal lesions and lower kidney GBM thickness, is supported by these data.

Metformin-induced normoglycemia can protect several organs from the deleterious effects of diabetes. Metformin would decrease and heal the inflammatory damage to the liver and kidneys in diabetic patients.

Authors' Contribution

Study concept and design: R. S. A.
Acquisition of data: R. S. A.
Analysis and interpretation of data: R. S. A.
Drafting of the manuscript: R. S. A.
Critical revision of the manuscript for important intellectual content: R. S. A.
Statistical analysis: R. S. A.
Administrative, technical, and material support: R. S. A.

Ethics

All the ethical approvals were approved from the ethical committee of the University of Babylon, Babylon, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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