Ministry of Higher Education and Scientific Research

University of Babylon – College of Pharmacy

Oral Hypoglycaemic Agent Repaglinide's Effect on the Liver and Kidney Histology of Diabetic Albino Rats

A Graduation Project Submitted to the Council of College of Pharmacy,University of Babylon as Partial Fulfillment of the Requirement for a BSc Degree in Pharmacy

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وَالرَّاسِخُونَ فِي الْعِلْمِ يَقُولُونَ آمَنَّا بِهِ كُلٌّ مِّنْ عِندِ رَبِّنَا ۗ

صدق الله العلي العظيم

سورة ال عمران اية 7

اهداء

هنيئا لنا ولكل زميل وزميلة لم يقف او يستسلم بل قاوم بصبره وسعيه واثقاً بربه وبنفسه، وها نحن اليوم ننوج لحظاتنا الأخيرة في ذلك الطريق الذي كان تحمل في باطنه العثرات والاشواك ورغما عنها ظلت اقدامنا تخطو بكل صبر وطموح، وكم من أيام مرت شعرنا بثقلها ومرارتها ولكن لم تعيقنا بل كانت ذكرى تمر لتثير احلامنا بقلوبنا ، وها نذا اكتب وللدمع بصمة تشهد فرحتها لكل تلك الكتب التي جففت مدمعها ،،

اهدي هذا النجاح لنفسي اولاً ثم الي كل من سعى معي لإتمام هذه المسيرة، دمتم لي سنداً لا عُمر له . .

أهدي تخرجي إلي من أحمل أسمه بكل فخر إلي من حصد الأشواك عن دربي ليمهد لي طريق العلم بعد فضل الله ما أنا فيه يعود إلي أبي الرجل الذي سعى طوال حياته لكي نكون افضل منه "أبي الغالي"

إلي اليد الخفيه التي أزالت عن طريقي الأشواك، ومن تحملت كل لحظة ألم مرت بها وساندتني عند ضعفي وهزلي "أمي الحبيبة"

شكرا لدكتورة رفاه وكل أعضاء الهيئة التدريسية . . .

CONTENTS

1. Introduction

1.1 Diabetes mellitus

Diabetes mellitus is a complex metabolic disorder characterized by hyperglycemia , pancreatic beta (β) cells dysfunction and abnormal lipid profile that result from metabolic deregulations, impaired insulin secretion and action, and inappropriate consumption of glucose [1].

It is one of the most prevalent chronic diseases and leads towards severe complications such as increase in production of reactive oxygen species (ROS), impairment of antioxidant enzymes[2] hyperglycemia [3], dislipidemia [4], alteration in insulin signaling pathway, and ROS-induced cellular damage [5].

All these changes will result in diabetes-associated secondary complications like nephropathy, retinopathy, neuropathy and cardiovascular morbidity [6]. The incidence of insulin resistant/type 2 diabetes in our time related to the luxurious lifestyle and the high consumption of high-fat diet, which is the main causal aspect (Amin at al., 2014).

2.1 Alloxan

Can elevate blood glucose levels and interfere with the manufacture of insulin because it damages pancreatic cells. High blood glucose levels are caused by disruptions in the uptake of glucose into cells [7]. A study by Lucchesi et al. showed that rats with type II DM caused by injected ALX had chemical anomalies in their blood in addition to microscopic changes in the liver's morphology that were strikingly similar to human chronic liver disease [8].

3.1 Repaglinide

Is an oral antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). It belongs to the meglitinide class of short-acting insulin secretagogues, which act by binding to β cells of the pancreas to stimulate insulin release. Repaglinide induces an early insulin response to meals decreasing postprandial blood glucose levels . It should only be taken with meals and meal-time doses should be skipped with any skipped meal. Approximately one month of therapy is required before a decrease in fasting blood glucose is seen. Meglitnides may have a neutral effect on weight or cause a slight increase in weight. The average weight gain caused by meglitinides appears to be lower than that caused by sulfonylureas and insulin and appears to occur only in those naïve to oral antidiabetic agents. [10].

Due to their mechanism of action, meglitinides may cause hypoglycemia although the risk is thought to be lower than that of sulfonylureas since their action is dependent on the presence of glucose. In addition to reducing postprandial and fasting blood glucose, meglitnides have been shown to decrease glycosylated hemoglobin (HbA1c) levels, which are reflective of the last 8-10 weeks of glucose control. Meglitinides appear to be more effective at lowering postprandial blood glucose than metformin, sulfonylureas and thiazolidinedione. Repaglinide is extensively metabolized in the liver and excreted in bile. Repaglinide metabolites do not possess appreciable hypoglycemic activity. Approximately 90% of a single orally administered dose is eliminated in feces and 8% in urine [10].

Repaglinide is an insulin secretagogue, meaning it binds to receptors on pancreatic beta cells and stimulates insulin release. Repaglinide binds to an ATP-dependent potassium channel on beta cells, known as SUR1, bringing about its closure. This mechanism of action is similar to that of the sulfonylureas, and as a result, the concurrent use of these therapies is contraindicated. Repaglinide is rapidly absorbed, with a half-life of fewer than 60 minutes. The fast onset and short duration of action of repaglinide have made it one of the most appropriate therapies for managing postprandial glucose spikes, and as such, it is recommended approximately 30 minutes before an intended meal.[11][12]

Repaglinide may be used as monotherapy (supplemental to exercise and diet control) or in combination with other antihyperglycemic agents (metformin or others, if metformin is not tolerated/contraindicated). The exception is usin sulfonylureas (e.g., gliclazide, glipizide), which is a contraindication for dual therapy with meglitinides. Research has suggested that repaglinide is a favorable treatment choice in patients with chronic kidney disease and end-stage renal disease . Repaglinide is FDA class C during pregnancy, meaning its use requires caution. There has been no evidence to support its safe use during breastfeeding, and thus an alternative is recommended ,The safety of repaglinide in children under the age of 18 has not been established and, as such, is not recommended, Repaglinide should be taken shortly before meals and should be omitted when skipping meals.[13][14][15][16][17][18].

The most commonly reported side effect associated with repaglinide monotherapy is hypoglycemia (7% in a study of 76 patients), followed by weight gain (1.8 kg / 16 weeks)[19]. Contraindications does include[20][6] ; Hypersensitivity to any of the components of the oral preparation ,Diabetes mellitus type 1 and diabetic ketoacidosis, Severe liver dysfunction.

2.Materials and Methods

In this investigation, twenty male white adult albino rats, aged 2-3 months and weighing between 155-170 g, were employed. These animals were separated into two groups after a period of adaption. The first group of ten rats was used to induce diabetes mellitus (type II) by injecting alloxan monohydrate as a single dosage of 130 mg/kg body mass intraperitoneally after 72 hours, and fasted blood sugar levels were evaluated using a glucometer after 9-12 hours of fasting. The second set of ten rats got intraperitoneal injections of normal saline. Male albino diabetic and normal rats were separated into four groups: Group 1: Control group Non-diabetic rats were administered DW orally through an orogastric tube as an alternate to the therapy provided to the other groups. Group 2 non-diabetic animals were treated with repaglinide 4mg/kg/day, administrated orally by oral gavage tube. 3rd Group 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 Repaglinid after seven days of DM induction, diabetic rats treated with Repaglinide 4mg/kg/day orally. The animals were sacrificed after a 6-week treatment period, and their liver and kidneys were collected for histological analysis.

3. Histological sections examination

According to Bancroft's idea (11), histological sections of the liver and kidney (5 thick) were generated to evaluate the alterations that may be discovered in the treatment animal groups compared to the control group.

4.Results and discussion

As demonstrated in the figures (A, B,D) all of non-diabetic control, non-diabetic rats received repaglinide And in diabetic rats treated with repaglinide had normal histology of liver. While, Liver section of diabetic Control showed some hepatocytes degeneration in addition to the presence of inflammatory cells infiltration (lymphocyte infiltration) as shown in the figure ©. The kidney section of non-diabetic control, non-diabetic animals treated with repaglinide and in diabetic Animals treated with repaglinid (figures A, B,D) showed normal renal construction. While, in the kidney of Diabetic control (figure C) showed lymphocyte infiltration.

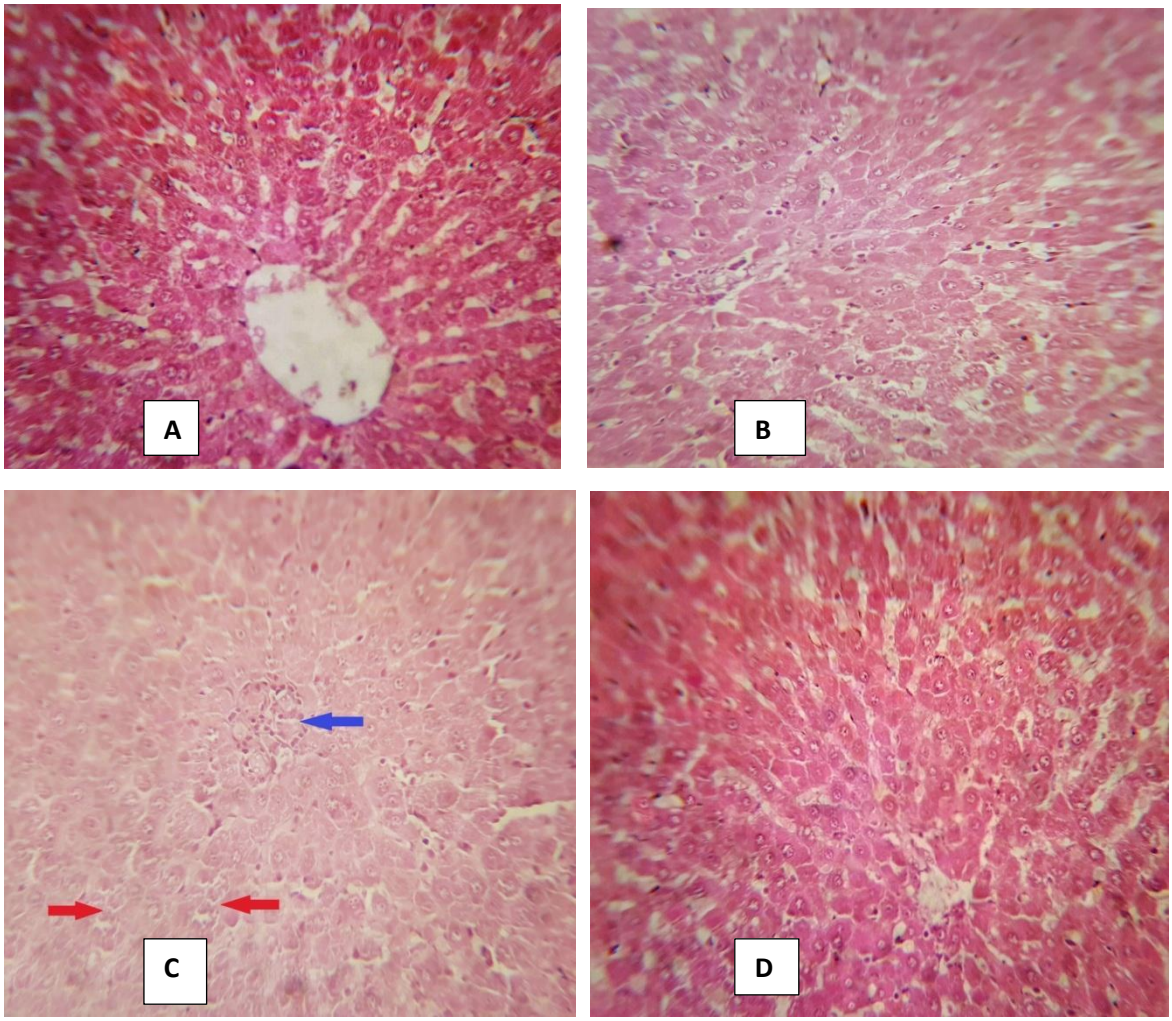

Figure 1: Cross Section of the Liver of (A) Non-Diabetic Control Rats Showing Normal Histology , (B) Non-Diabetic Rats treated with Repaglinide showing normal histology, (C) Diabetic Rats showing lymphocyte infiltration (blue arrow) and hepatocytes degeneration (red arrows), (D) Diabetic Rats treated with Repaglinide showing normal histomorphology , Stained with H&E 400x.

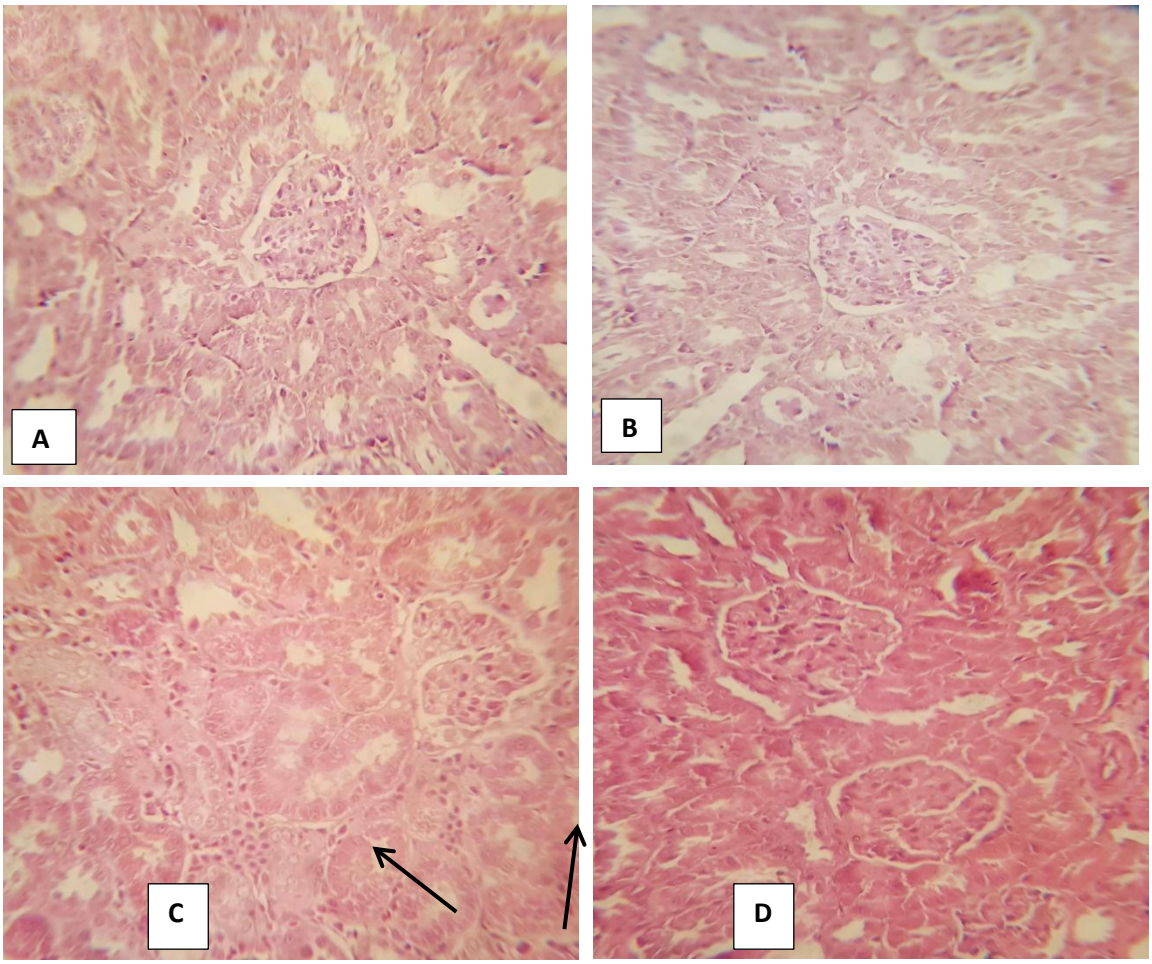

Figure2: Cross histological section of the kidney of (A) non-diabetic control rats showing normal histology with normal structure , (B) non-diabetic rats receiving Repaglinide showing normal histology, (C) diabetic rats showing lymphocyte infiltration (black arrow), (D) diabetic rats treated with Repaglinide showing normal histology , Stained with H&E 400x.

Many studies have shown lesions in the livers and kidneys of alloxan-treated diabetic rats that demonstrate significant hepatocyte degeneration with many vacuolations that may affect all organ tissues, involving portal regions and sinusoids, as well as hepatocytes, nuclei, and intracytoplasmic organelles [8], [24] and [26]. The kidneys of alloxan-induced diabetic rats reveal significant glomerular degeneration, glomerular atrophies, and extensive vacuolations [24]. The harmful effects of alloxan on the body are commonly noticed within the first two weeks of diabetes induction [26]. [9]found cellular infiltration and renal hypertrophy, as well as tubular damage and Bowman's space bleeding owing to glomerular injury.

Our results showed no deleterious effects of alloxan induced diabetic rat on the liver and kidney histology of all DM groups. There was only lymphocytic infiltration, some hepatocytes degeneration in liver which might be due to the direct influence of alloxan on the body's inflammatory processes, or it could be linked to another factor that causes infiltration in the diabetic rat's liver and kidney. However, rats treated with repaglinide illustrated a noticeable recovery of liver and kidney tissues.[21]

According to published data on the effect of repaglinide administration that has antioxidant properties independent of its action on hyperglycemia, repaglinide's significant antioxidant activities create good effects at therapeutic levels and also it can make a significant contribution to its efficacy in the treatment of type 2 diabetes [22], [23], and [25]. due to its insulinotropic action, which is referred to as insulin exocytosis and an increase in the sensitivity of its receptors [27].

Furthermore, RG therapy has been shown to reduce the levels of lipid hydroperoxide (LPO) and enhance the activities of superoxide dismutase (SOD) in diabetic nephropathy kidneys [22]. Repaglinide, according to has a favorable safety and efficacy benefit in type 2 diabetes patients with impaired renal function, and is a suitable therapy option, especially for those with more severe degrees of renal impairment [28].

5. Conclusion

Our study demonstrated that repaglinide's antioxidant activity was connected with its protective effect on liver and kidney lesion of alloxan induced diabetic rats , suggesting that it might be a suitable agent for treating and preventing the deleterious changes in diabetic rats' liver and kidney tissue.

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