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Biochemical Studies on Benzimidazole Compounds as Anti- diabetic Agents.

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ABSTRACT

Background: Diabetes mellitus (DM) is a collection of metabolic illnesses marked by a persistent hyperglycemic state caused by insulin production, insulin action, or both. **Objectives**: Present Study is designed to evaluate the anti-diabetic activities of some new heterocyclic compounds in albino rat. **Methods**: A total of 50 adult male albino rats were classified into five groups. Group I (negative control group, rats orally administered 1 ml saline daily).Group II (diabetic group, animals were injected intraperitoneally with 60 mg/kg b.wt streptozotocin).Group III (compound 1 group, diabetic animals treated with 50 mg/kg b.wt of Potassium salt of benzimidazole-2-carboxaldehyde semicarbazone for 40 day orally).Group IV (compound 2 group, diabetic rats treated with 50 mg /kg b.wt of Potassium salt of benzimidazole-2-carboxaldehyde thiosemicarbazone for 40 day orally).Group V (metformin group, diabetic rats treated with 100 mg /kg b.wt of metformin for 40 day orally). **Results:** Oral administration of new synthesized benzimidazole derivatives compounds ameliorated all biochemical parameters (ALT, AST ALB, T.Bili, urea, creatinine, CK-MB and LDH) and enhanced activity of antioxidant enzymes. Also, decrease Myostatin levels when compared with diabetic rats. Molecular docking studies confirmed binding of compounds with Myostatin protein in terms of energy and revealed of the existence of hydrogen bond, hydrophobic interaction. Our results were confirmed by histopathological examination of liver, kidney and pancreatic tissues. **Conclusion:** this study suggests that new synthesized benzimidazole derivatives compounds exhibit anti diabetic and antioxidant activity in streptozotocin- induced diabetic rats.

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Introduction:

Diabetes is a collection of metabolic illnesses characterized by a long-term hyperglycemic state caused by insulin production, insulin action, or both. Glucokinase deficiency causes permanent neonatal diabetes, which is an inborn defect in the glucose-insulin signalling pathway **[1].** Frequent urination, weight gain, increased fatigue, irritability, and sexual dysfunction are all common signs of the disease **[2]**.

A heterocyclic aromatic organic molecule, benzimidazole is a heterocyclic aromatic organic chemical. In medicinal chemistry, it is a significant pharmacogical and privileged structure. This bicyclic molecule is formed when benzene and imidazole are fused together. Nowadays, is a popular moiety with a wide range of pharmacological characteristics **[3].**

Myostatin is a member of the TGF-superfamily and was previously known as growth and differentiation factor 8 (GDF-8). Myostatin has such a strong influence on skeletal muscle growth and development that genetic deletions or mutations in the myostatin gene result in a substantial increase in skeletal muscle mass. The absence of myostatin causes muscular growth in a variety of mammalian species, including rodents, farm and domestic animals, and humans, emphasising the importance of myostatin in muscle **[4].**

Materials and methods

Chemicals

All chemicals were purchased from Sigma Aldrich Chemical Co., St. Louis, Mo, U.S.A. It was the source of the following chemicals: Streptozotocin & Metformin (Glucophage 500 mg).

Animal management

Adult male albino rats weighing 180-200 g were procured from Cairo University's Experimental Animal Care Center and housed in cages at experimental animal house of faculty of Science, Zagazig University for 7 days before the experiment under regulated environmental conditions (25°C and a 12 h light/dark cycle).

All methods followed the guidelines for animal subject care and use outlined in the Guide for the Care and Use of Laboratory Animals, as well as the protocol authorised by the Ethics Committee.

Diabetic model

STZ was given at a single dose of 60 mg/kg b.wt i.P in 16 hour fasting rats for DM induction. STZ was dissolved in a newly made cold citrate buffer (100 mM, pH= 4.5) and used within five minutes **[5].** After 72 hours of medication treatment, all animals' blood glucose levels were tested, and rats with fasting blood glucose levels of more than 250 mg/dl were declared diabetic and used in the study.

Toxicity Study:

Determination median lethal dose (LD_{50}) of a new synthesized benzimidazole derivatives compound1:[Potassium salt of benzimidazole-2-carboxaldehyde semicarbazone] .compound 2:[Potassium salt of benzimidazole-2-carboxaldehyde thiosemicarbazone] (Figure 1&2) **[6].**

Experimental design:

After a 7-day acclimation period on a conventional basal diet, a total of 50 adult male albino rats were divided into five groups, each with ten animals, to achieve the study's final purpose.

Group I (negative control group): Rats were orally administered with 1 ml saline daily).

Group II (diabetic group): Rats received STZ (60 mg/kg b.wt) i.P in 16 hour fasted rats. **Group III (compound 1 group):** Rats were induced for DM. After 1 week of DM induction, animals were post treated with compound 1 (Potassium salt of benzimidazole-2-carboxaldehyde

semicarbazone) at a dose of (50 mg/kg) dissolved in water distilled daily for 40 days orally.

Group IV (compound 2 group): Rats were induced for DM. After 1 week of DM induction, animals were post treated with compound 2 (Potassium salt of benzimidazole-2-carboxaldehyde thiosemicarbazone) at a dose of (50 mg/kg) dissolved in water distilled daily for 40 days orally.

Group V (metformin group): Rats were induced for DM. After 1 week of DM induction, animals were post treated with metformin (100 mg/kg) dissolved in water distilled daily for 40 day orally **[7]**.

Doses of a new synthesized benzimidazole derivatives and metformin were adjusted every week according to any change in body weight to maintain the same dose per each kg body weight of rat during the entire period of study for each group.

Collection and sampling of blood:

Rats were starved for 12 hours at the conclusion of the research and after the last treatment, and blood samples were taken from the retro-orbital venous plexus under light ether anaesthesia. Blood samples were taken in three tubes: one containing sodium fluoride for blood glucose determination, another empty tube for serum extraction after centrifugation at 4000 rpm for 20 minutes. Serum and plasma were transferred into eppendorff tubes and kept frozen at -20^{o}C until biochemical assays were performed.

Tissue sample:

Animals were killed by cervical decapitation after blood collection, and different tissues (liver, kidney, and pancreas) were excised and rinsed in ice-cold phosphate-buffered saline (pH 7.4) to drain away any blood.

First part of different tissue samples were homogenized with ice-cold phosphate-buffered saline (pH 7.4) to prepare a 10% (w/v) tissue. Second part of different tissue samples were used for histopathological study.

Physiological parameters

Body weights of all the rats were documented before the drug treatment and at the end of study.

Biochemical analysis

Estimation of biochemical parameters

Plasma glucose:

Determination of plasma glucose was performed by glucose oxidase peroxidase activity using a commercial kit derived from Elitech clinical systems, France **[8].**

Liver Function Tests:

Serum ALT and AST activity were determined using assay colorimetric kit **[9]**.Serum Albumin concentration was determined by colorimetric method according to modified bromcresol green binding assay (BCG) **[10].** Serum total bilirubin concentration was determined by bilirubin is converted to colored diazotized sulfanilic acid and Measured photo metrically **[11].**

Kidney Function Tests:

Serum Urea concentration was measured by Berthelot enzymatic colorimetric method using a commercial kit derived from Diamond Diagnostic Company, Germany **[12]**.Serum creatinine was measured by Buffered kinetic jaffe reaction without deproteinization, using a commercial kit derived from Spin react Company, Spain **[13].**

Determination of Creatine Kinase (CPK):

Serum CPK concentration was determined by enzymatic colorimetric method using a commercial kit derived from spectrum company, Egypt **[14]**.

Determination of Lactate Dehydrogenase (LDH):

Serum LDH concentration was measured by enzymatic colorimetric method using a commercial kit derived from spectrum company, Egypt**[15].**

Estimation of antioxidant parameters:

The tissue level of MDA was determined using kits purchased from Biodiagnostic Company (Biodiagnostic, Egypt) **[16]**.

The tissue activity of catalase was determined using kits purchased from Biodiagnostic Company (Biodiagnostic, Egypt) **[17].**

The tissue activity of GST was determined using kits purchased from Biodiagnostic Company (Biodiagnostic, Egypt) **[18].**

The tissue level of GSH was determined using kits purchased from Biodiagnostic Company (Biodiagnostic, Egypt) **[19].**

Estimation of Myostatin:

Myostatin was determined by enzyme – linked immunosorbant assay (ELISA). Rat Angiopoietin Like Protein 8 Immunoassay Kit, (Catalog Number 201-11-1071) purchased from SunRed Biotechnology Company.

Molecular Docking:

Molecular docking studies was performed to investigate the binding modes of compound 1 (Potassium salt of benzimidazole-2-carboxaldehyde semicarbazone) and compound 2 (Potassium salt of benzimidazole-2-carboxaldehyde thiosemicarbazone) targeting the crystal structure of myostatin using Autodock vina 4. 2 **[20]**. The 3D structures of compound 1& 2 were obtained by ChemBioDraw Ultra 14.0 andChemBio3D Ultra 14.0 software's. The 3D structures of myostatin were obtained from protein data bank (http://www.rcsb.org//pdf). The MMFF94 force field **[21]** was used for energy minimization of ligand molecule. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Affinity (grid) maps of 20×20×20 Å grid points and 0.375Å spacing were generated using the Autogrid program **[22]**. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method **[23]**

Histopathological examination

Different tissues were then immersed with molted paraffin wax, then embedded and blocked out. Paraffin sections (4–5 um) were stained with hematoxylin and eosin then examined through light electric microscope **[24].**

Statistical Analysis

All statistical analyses were performed using the statistical package "SPSS" 22.0 for Microsoft Windows, SPSS Inc, and statistical significance was determined at a two-sided p0.05. The mean standard error of the mean (SEM) was used to express numerical data. The levels of markers were analyzed by ANOVA **[25].**

Results

Toxicity studies

It was found that a new synthesized benzimidazole derivatives (compounds 1 and 2) being safe until 2000 mg/kg; as the selective dose was 50 mg/kg**.**

Effect of different chemical compounds on Body weight:

Results in Table 1 showed that there was significant decrease in body weight in diabetic group which amounted to -31.2 % in compared to control group (P>0.001). While in groups treated with compound 2 and metformin decleared that there was significant decrease which amounted -14.3 % (P> 0.01), -18.8 %, (P> 0.001) respectively in

compared to control group. But there was non-significant decrease in mean of bod weight values in compound 1 which amounted to -0.79 % in compared to control group (P< 0.05).

Effect of different chemical compounds on level of glucose:

Results in Table 2 showed that there was significant increase in mean level of glucose in diabetic group which amounted to 222.3 % in compared to control group (P>0.001).While in groups treated with compound 1,2 decleared that there was significant increase which amounted 27.9% (P> 0.001) , 35.8%, (P> 0.001) respectively in compared to control group. Also it was found that metformin showed significant increase in mean of glucose which amounted to 47.1% in compared to control group (P> 0.001).

Effect of different chemical compounds on mean values of ALT, AST, ALB and T.Bilirubin in all studied groups:

Results in Table 3 showed that there was significant increase in mean of ALT activity in diabetic group which amounted to 62.7 % in compared to control group (P< 0.001). While in groups treated with compound 1,2 decleared that there was significant increase which amounted to 17.9 % (P< 0.05) , 21.2 % , (P< 0.01) respectively. Also it was found metformin group significant increase in mean of ALT activity which amounted to 32.3 % in compared to control group (P< 0.001).

There was significant increase in mean of AST activity in diabetic group which amounted to 33.4 % in compared to control group (P< 0.001). While in groups treated with compound 1,2 decleared that there was significant increase which amounted to 11.9 % (P< 0.01) , 13.3 % , (P< 0.001) respectively.

Also it was found metformin group significant increase in mean of AST activity which amounted to 9 % in compared to control group (P< 0.01).

There was non-significant decrease in mean of ALB values in diabetic group , compound 1 , 2 and metformin group which amounted to -6.9 % , -4.6 % , -5.9 % , -4.4 % respectirely in compared to control group (P< 0.05)**.**There was significant increase in mean of T.Bilirubin values in diabetic group which amounted to 30.3 % in compared to control group (P< 0.001). While in groups treated with compound 1,2 decleared that there was significant increase which amounted to 24.2 % (P< 0.01), 24.2 %, (P< 0.001) respectively. Also it was found metformin group significant increase in mean of T. Bilirubin values which amounted to 21.2 % in compared to control group (P< 0.001).

Effect of different chemical compounds on Mean level of urea and creatinine in all studied groups:

Results in Table 4 showed that there was non-significant decrease in mean of Urea values in diabetic group , compound 1 , 2 and metformin group which amounted to 2.9 % , -4.2 % , -7.7 % , -5.4 % respectively in compared to Control group (P< 0.05).

There was significant increase in mean of Creatinine values in diabetic group and metformin group which amounted to 41.7 % (P< 0.05) , 35 % (P< 0.05) respectively in compared to control group. While in groups treated with compound 1 and 2 decleared that there was non-significant increase which amounted to 26.6 % , 10 %

respectively in compared to Control group (P< 0.05).

Effect of different chemical compounds on mean values of CK-MB and LDH in all studied groups:

Results in Table 5 showed that there was significant increase in mean of CK-MB values in diabetic group which amounted to 25.7 % in compared to control group (P< 0.001). While in groups treated with compound 1,2 decleared that there was significant increase which amounted to 6.9 % (P< 0.05) , 14.8 % (P< 0.001) respectively. Also it was found metformin group significant increase in mean of CK-MB value which amounted to 9.4 % in compared to control group (P< 0.05).There was significant increase in mean of LDH values in diabetic group , compound 1 , 2 and metformin group which amounted to 158.7 % (P> 0.001) , 76.5 % (P< 0.001) , 84.0 % (P< 0.001) , 92.7 % (P< 0.001) in compared to control group.

Effect of different chemical compounds on mean of MDA level, CAT activity , GST activity and GSH level in liver tissue of all studied groups:

Results in Table 6 showed that there was non-significant increase in mean of MDA level in diabetic group and compound 2 which amounted to 22.9 % , 4.5 % (P< 0.05) % respectively in compared to control group. There was significant increase in mean of MDA level in compound 1 and metformin group which amounted to 10.2 % (P< 0.001) , 11.9 % (P< 0.001) respectively in compared to control group. Also there was significant decrease in mean of CAT activity in diabetic group , compound 1 , 2 and metformin group which amounted to -9.0 % (P< 0.01) , -7.3 % (P< 0.01) , -6.6 % (P< 0.05) , -5.8 % (P< 0.05) respectively in compared to control group. There was non-significant decrease in mean of GST activity in diabetic group , compound 1 , 2 and metformin group which amounted to -22.2 %, -9.4, -8.8 %, -8.2 % (P< 0.05) respectively in compared to control group. There was significant decrease in mean of GSH level in diabetic group which amounted to-7.9 % in compared to Control group (P< 0.05). But in compound 1 , 2 and metformin are non-significant decrease which amounted to -3.9 , -4.3 % , -4.4 % (P< 0.05) respectively in compared to control group.

Effect of different chemical compounds on mean of MDA level, CAT activity, GST activity and GSH level in kidney tissue of all studied groups:

Results in Table 7 showed that there was significant increase in Mean of MDA level in diabetic group , compound 2 and metformin group which amounted to 55.6 % (P< 0.001) , 20.3 % (P< 0.01) , 23.7 % (P< 0.01) respectively in compared to control group. But there was non-significant increase in the mean of MDA level in compound 1 which amounted to 13.9 % in compared to Control group (P< 0.05).There was significant decrease in mean of CAT activity in diabetic group which amounted to-14.5 % in compared to Control group (P< 0.001).

But there was non-significant decrease in the mean of CAT activity in compound 1 , 2 and metformin group which amounted to -0.91 % , -5.6 % - 1.7 % (P< 0.05) in compared to control group. There was significant decrease in mean of GST activity in diabetic group which amounted to-14.2 % in compared to control group (P< 0.01).But there was non-significant decrease in the mean of

GST activity in compound 1 , 2 and Metformin group which amounted to -1.3 % , -0.81 % , - 2.9 % (P< 0.05) in compared to control group. There was significant decrease in Mean of GSH level in diabetic group , compound 1 , 2 and metformin group which amounted to -28.4 % (P< 0.001) , -5.7 % (P< 0.01) , -8.0 % (P< 0.01) , -4.5 % (P< 0.05) respectively in compared to control group.

Effect of different chemical compounds on mean of MDA level, CAT activity , GST activity and GSH level in pancreas tissue of all studied groups:

Results in Table 8 showed that there was significant increase in mean of MDA level in diabetic group which amounted to 62.4% in compared to control group (P< 0.001). But there was non-significant increase in the mean of MDA level in compound 1 , 2 and metformin group which amounted to 1.5 % , 5.4 % , 14.3 % (P< 0.05) respectively in compared to Control group.There was significant decrease in mean of CAT activity in diabetic group , compound 2 and metformin group which amounted to -29.7 % (P< 0.01) , -26.4 % (P< 0.05) , -25.6 (P< 0.05) respectively in compared to control group. But there was non-significant decrease in the mean of CAT activity in compound 1 which amounted to -3.3 % (P< 0.05) in compared to control group. There was significant decrease in mean of GST activity in diabetic group which amounted to -23.8 % in compared to control group (P< 0.001). But there was non-significant decrease in the mean of GST activity in compound 1 , 2 and metformin group which amounted to -1.8 % , -6.3 % , -5.6 % (P< 0.05) respectively in compared to Control group.

There was significant decrease in mean of GSH level in diabetic group , compound 2 and metformin group which amounted to -8.1 % (P< 0.001) , -3.5 % (P< 0.01) , -6.5 (P< 0.01) respectively in compared to control group. But there was non-significant decrease in the mean of GSH level in compound 1 which amounted to -3.2 % (P< 0.05) in compared to control group.

Effect of different chemical compounds on levels of Myostatin in serum and different tissues of all studied groups:

Results in Table 9 showed that in serum there was significant increase in mean of Myostatin values in diabetic group , compound 1 , 2 and metfomin group which amount ed to 13.4 % (P< 0.01) , 8.0 % (P< 0.05) , 7.2 % (P<0.05) , 4 % (P< 0.01) , respectively in compared to control group. In liver there was significant increase in mean of Myostatin value in diabetic group which amounted to 6.8 % in compared to control group (P< 0.05) , But there was non-significant increase in the mean of values of compound 1 , 2 and metformin group which amounted to 0.8 % , 2.4 % , 2.8 % (P< 0.05) in compared to Control group. In kidney there was significant increase in mean of Myostatin values in diabetic group , compound 1 , 2 and metfomin group which amount ed to 13.4 % (P< 0.01) , 8.0 % (P< 0.05) , 7.2 % (P< 0.05) , 4 % (P< 0.01) , respectively in compared to control group. In pancreas there was significant increase in mean of Myostatin value in diabetic group which amounted to 13.7 % in compared to control group (P< 0.01) , But there was non-significant increase in the mean values of compound 1 , 2 and metformin group which amounted to 4.6 % , 5.3 % , 2.9 % (P< 0.05) in compared to control group.

Molecular docking:

To find the correlation between the experimental and computational data,

the docking study of the synthesized compounds was performed against myostatin activities to understand the ligand –protein interaction. The results showed a possible revealed favorable interactions between the compound 1& 2 and the receptor of myostatin (Figure 3).

In agreement with our results, HB plot curves indicated that compound 1&2 bind to the protein with hydrogen bond, electrostatic and Vander- walls interaction (Figure 4). Interactions with decomposed interaction energies in kcal/mol exist between compound 1&2 and protein. Thus the decrease in binding energy due to the mutation will increase the binding affinity of the comounds towared the receptor ligand showed binding energy of - 4.82 kcal/mol and – 5.08 kcal/mol with myostatin respectively (Table 10& 11). Also, 2D plot curve of doking with the ligands showed in (Figure 5)

Histopathological examination

The histological of showed groups of compounds1 and 2 declared healthy liver parenchyma, normal hepatocytes and blood sinusoids (Figure 6). The kidney section of compounds1 and 2 showed healthy renal parenchyma normal glomeruli and renal tubules (Figure 7). The pancreatic section of compounds1 and 2 showed healthy parenchyma, normal acini and islets (Figure 8).

Discussion

Diabetes mellitus (DM) refers to a syndrome of hyperglycemia resulting from many different causes. It is broadly classified into type 1 (T1DM) and type 2 DM (T2DM) **[26].** Studies said that progress of DM was rapid due to different complications such as eye, foot, Skin c, heart problems, neuropathy, nephropathy developing in early stages of the disease **[2].**

The present work investigate the role of a new synthesized benzimidazole derivatives compound1:[Potassium salt of benzimidazole-2-carboxaldehyde semicarbazone],
compound2:[Potassium salt of benzimidazole-2-carboxaldehyde thiosemicarbazone] ,in treatment of induced type 2 diabetes by STZ in rats. Also, evaluation role of myostatin in prognosis of type 2 diabetes.

Our data illustrated that significant decrease ($p<0.001$) in finial body weight of (diabetic group) when compared with control group.While metformin and synthesized benzimidazole derivatives compound groups showed greet improvement in final body weight in compared to control group.

The results are in concordance with who indicated that diabetic condition was also evident from decrease in weekly body weight. The reduction in body weight because muscle and adipocyte tissues degenerate to compensate for the energy lost **[27].** Also, results found that STZ-induced body weight loss and hyperglycemia **[28].**

Administration of metformin as type 2 diabetic drugs. It caused diabetic to respond normally to insulin. As most diabetic drugs, it metabolic disorders of glucose and also lipids and loss protein tissue **[29].**

Synthesized benzimidazole derivatives compounds administration in diabetic rats enhanced body weight and this may be due to a good control of hyperglycaemic state **[30]**.

Results showed that streptozotocin administration indicated critical ($P< 0.001$) elevation blood glucose. These are in accordance with previous studies **[27].** Metformin and therapeutic treated groups exhibited a decline in plasma glucose when compared with control group. By regulating the level

of sugar in the blood, metformin reduces the production of the liver, reduces the absorption process in the stomach and small intestine, and helps reduce interferences and protect the body's sensitivity to insulin **[31]**. Researchers interested with heterocyclic compounds and their derivatives to design a new compound with good biological activity by modifying the heterocyclic ring. Among many other benzimidazole derivatives were found to be most significant in different biological activities such as anticancer, anti-inflammatory, antimicrobial, antihypertensive, antifungal, antidiuretic and antidiabetic. Long-term consumption of a high-fat diet induces fat build-up in organs other than the adipose tissue. Increased circulatory fatty acid absorption and reduced -oxidation in the liver cause hepatic fat build-up. Aspartate transaminase (AST) and alanine transaminase (ALT) are indicators for liver health, which is typically harmed in diabetes.

Concerning liver function indices, our results found the diabetic rats showed significantly elevated serum ALT, AST values compared with the healthy control rats.

ALT and AST which are markers of cellular leakage and loss of hepatic cell membrane functional integrity, which implies hepatocellular injury **[32]**.

Our results are in line with whom reported elevated ALT activity indicated liver cell damage caused by fatty acids uptake and glucotoxicity (chronic hyperglycemia) in rats **[33]**.

New synthesized benzimidazole derivatives decreased the elevated blood ALT & AST values but still statistically significant in case of ALTand AST in compared to healthy control rats, which clearly demonstrates the protective role of these compounds on the liver function.

Renal illness is one of the most widely recognized and extreme difficulties of diabetes **[34]**.

Our data found that diabetic group showed increase in serum urea and creatinine ($p< 0.05$) when compared to control group.

Previous investigations announced that diabetic rats indicated essentially elevated serum uric acid, serum creatinine and blood urea nitrogen levels **[35]**.

Our data illustrated that there was significant increase in mean of CK-MB and LDH values in diabetic group in compared to control group ($P< 0.001$). Also in groups treated with compound 1, 2 and metformin decleared that were significant increase which in compared to control group.

CK-MB and LDH, which leak out from damaged tissues to the blood stream when the cell membrane becomes permeable or ruptures, serve as diagnostic markers of myocardial cell injury **[36].**

In the present study, the levels of CK-MB and LDH relative index were significantly increased in the diabetic rats indicating the obvious detrimental effects of diabetes on the cardiac tissue. These results are in line with previous reports which found that serum CK-MB and LDH levels increased in diabetic group rats, possibly due to myocardial dysfunction and reported that the serum CK-MB and LDH levels were increased in diabetic rats, and may serve as a marker for cardiovascular risk and cardiac muscle damage **[37].**

The significant increase in CK-MB and LDH relative index with diabetic induction in this study suggests a cardiac source of elevated CK-MB and LDH, as previously reported by studies such as, which found that CK-MB relative index is more specific than

absolute CK-MB as a marker of myocardial damage **[38]**.

DM is linked to oxidative stress, which is caused by an increase in the production of free radicals such as superoxide (O2-) and hydroxyl (OH) radicals, as well as a decrease in the activity of antioxidant defense mechanisms **[39]**. Reactive oxygen species (ROS) can negatively affect various cell biomolecules such as protein, RNA, and DNA, causing damage to tissues and organs. To counteract ROS-induced cellular damage, the organism has a variety of antioxidative defence systems, including non-enzymatic (mainly GSH) and enzymatic antioxidant defences (GST, CAT, SOD, GR, and GPx), which consider the key enzymes in free radical elimination.

In diabetes, the antioxidant status is usually disturbed by hyperglycemia and metabolic disturbances, thus oxidative stress further exacerbates the systemic damage of tissues and organs. In our experiment, the changes in antioxidant biomarkers, such as decreased the activity of CAT , GST and GSH levels were found in different tissues of diabetic rats, compared with the healthy control rats, which clearly indicates the increased oxidative stress caused by chronic hyperglycemia. Previous studies indicated significant decrease in antioxidant GPX in diabetic group **[33] [40]**.

Our study showed the effect of treatment with metformin to ameliorate changes in body weight, blood glucose, liver, kidney, CK-MB, LDH and antioxidant parameters. These effects could be explained by several possible mechanisms: stimulation of the release of insulin, regeneration of b-pancreatic cells and increased sensitivity of target tissues to insulin. Metformin acts mainly on the liver by reducing de novo glucose synthesis (gluconeogenesis), improve hepatic steatosis by inhibiting lipid synthesis and increasing the oxidation of fatty acids and exerts its beneficial effects on the metabolism by decreasing the intracellular energy load and by activating the AMP-activated protein kinase (AMPK), a key regulator of energy metabolism **[41]**.

Our results revealed that synthesized targeted compounds produced decrease in blood glucose, liver enzyme activities, kidney, CK-MB and LDH. This.This signifies the importance of these derivatives as promising lead for the treatment of type II diabetes (DM).

These are in accordance with who found that synthesized benzimidazole targeted compounds produced a significant decrease in blood glucose levels, because our titled compounds are framed with nuclei benzimidazole which have a tendency to reduce the type 2 type of diabetes. Among the synthesized derivatives all compounds shows a significant hypoglycemic effect. Compound withelectron withdrawing group nor electron donating group makes the ring with prompt aromatic to exhibit action. Benzimidazole acts by enhancing the activity of Glucokinase enzyme **[42]**.

Our results showed that Myostatin values was high significantly increase in diabetic group of serum , liver , kidney and pancreas tissues while in treated groups showed slight increase but still statically significant in compared to control group.

These are in line with data declared that serum levels of Myostatin was higher in patients with diabetes **[43].**

When it comes to building computer-assisted medications, molecular doking is critical. The term "molecular docking" refers to the process of creating an optimum alteration for both the protein and the medication, with a relative direction between them that

minimises the framework's free energy. **[44].** Our molecular docking experiments revealed that interactions between chemicals and proteins exist with decomposed interaction energies in kcal/mol. As a result, the decrease in binding energy caused by the mutation will improve the binding affinity of the compounds to the receptor ligand. Compound 1 and 2 had binding energies of – 4.82 kcal/mol and – 5.08 kcal/mol, respectively. This indicates that the compounds have the potential to be effective inhibitors of the myostatin receptor.

Results of histopathological examination showed severe alterations of liver, kidney and pancreatic tissues were observed in diabetic group. Also, groups of synthesized compounds 1 and 2 attenuated the histopathological changes in diabetic group. Results are in accordance with who stated that pancreatic tissues of diabetic control rats showed a decrease of Langerhans islet size and multiple degeneration and injuries. In addition to, the number of β-cells was reduced, and some necrosis and destruction were found **[45].** Also, in agreement with, ultra structural findings of the liver showed significant differences between non-diabetic and diabetic animals **[46].** Also, in agreement with, diabetes induced in rats by STZ is associated with the generation of reactive oxygen species (ROS) which cause oxidative damage, particularly to heart, kidney, eyes, nerves, liver, small and large blood vessels, and immunological and gastrointestinal system **[47].**

Generally, the present obtained findings confirm that the influences of compounds 1 and 2 are attributed to the antidiabetic properties.

Conclusion

A new synthesized benzimidazole derivatives have the potential to become valuable complementary therapy in the treatment of DM and its complications. The present study evaluated the hypoglycemic activity of a new synthesized benzimidazole derivatives on diabetic male rats. Based on the present experimental data, it can be concluded that this benzimidazole derivatives improve the physiological and histological changes induced by STZ in the experimental animals.

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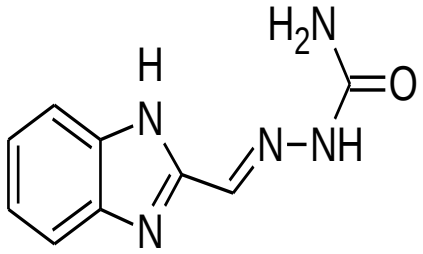

Figure 1: Compound 1(Potassium salt of benzimidazole-2-carboxaldehyde semicarbazone)

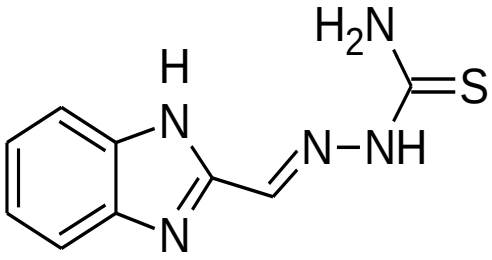

Figure 2: Compound 2(Potassium salt of benzimidazole-2-carboxaldehyde thiosemicarbazone.

Table (1): Mean values of blood Body weight in all studied groups.

Groups	Negative Control group	Diabetic group	Diabetic+ Compound 1 group	Diabetic+ Compound 2 group	Diabetic+ Metformin group
Body weight (g)	351.8±19.6	241.8±18.1***[c] -31.2 %	349±15.1 [c] -0.79 %	301.4±14.1** [b] -14.3 %	285.4±8.2*** -18.8 %

* P< 0.05 compared to control group, ** P< 0.01, *** P< 0.001 compared to control group. aP< 0.05, bP< 0.01, cP< 0.001 compared to positive control group. The mean difference is significant at P< 0.05. % change = Percent of change compared to control group.

Table (2): Mean values of blood glucose in all studied groups.

Groups	Negative Control group	Diabetic group	Diabetic+ Compound 1 group	Diabetic+ Compound 2 group	Diabetic+ Metformin group
Glucose (mg/dl)	99.6±2.0 [c]	321.1±25.6*** 222.3%	127.4±3.4*** [c] 27.9%	135.3±2.8*** [c] 35.8%	146.6±5.1*** [b] 47.1%

* P< 0.05 compared to control group, ** P< 0.01, *** P< 0.001 compared to control group. [a]P< 0.05, [b]P< 0.01, [c]P< 0.001 compared to positive control group. The mean difference is significant at P< 0.05. % change = Percent of change compared to control group.

Table (3): Mean values of ALT, AST,ALB and T.Bili in all studied groups.

Groups	Negative Control group	Diabetic group	Diabetic+ Compound 1 group	Diabetic+ Compound 2 group	Diabetic+ Metformin group
ALT (U/L)	36.8±0.0.87[c]	59.9±2.8*** 62.7%	43.4±1.5*[c] 17.9%	44.6±1.6**[c] 21.2%	48.9±0.89***[c] 32.3%
AST (U/L)	214.5±2.2[c]	286.2±8.4*** 33.4%	240±4.8**[b] 11.9%	243.1±1.6***[b] 13.3%	233.9±3.9**[b] 9%
ALB (g/dl)	3.86±0.03	3.59±0.15 -6.9%	3.68±0.04 -4.6%	3.63±0.11 -5.9%	3.69±0.06 -4.4%
T.Bili (mg/dl)	0.33±0.006[c]	0.43±0.008*** 30.3%	0.41±0.01** 24.2%	0.41±0.009*** 24.2%	0.40±0.008*** 21.2%

* P< 0.05 compared to control group, ** P< 0.01, *** P< 0.001 compared to control group. aP< 0.05, bP< 0.01, cP< 0.001 compared to positive control group. The mean difference is significant at P< 0.05. % change = Percent of change compared to control group.

Table (4): Mean values of urea and creatinine in all studied groups.

Groups	Negative Control group	Diabetic group	Diabetic+ Compound 1 group	Diabetic+ Compound 2 group	Diabetic+ Metformin group
Urea (g/dl)	16.8±0.44	17.3±0.59 2.9%	16.1±0.56 -4.2%	15.5±0.45 -7.7%	15.9±0.54 -5.4%
Creatinine (mg/dl)	0.60±0.05[a]	0.85±0.03* 41.7%	0.76±0.02 26.6%	0.66±0.04[a] 10%	0.81±0.02* 35%

* P< 0.05 compared to control group, ** P< 0.01, *** P< 0.001 compared to control group. aP< 0.05, bP< 0.01, cP< 0.001 compared to positive control group. The mean difference is significant at P< 0.05. % change = Percent of change compared to control group.

Table (5): Mean values of CK-MB and LDH in all studied groups.

Groups	Negative Control group	Diabetic group	Diabetic+ Compound 1 group	Diabetic+ Compound 2 group	Diabetic+ Metformin group
CK-MB (U/L)	20.2±0.38[c]	25.4±0.34*** 25.7%	21.6±0.21*[c] 6.9%	23.2±0.03***[c] 14.8%	22.1±0.38*[c] 9.4%
LDH (U/L)	1687.5±75[c]	4365.8±17.8*** 158.7%	2977.7±31.0***[c] 76.5%	3105.2±22.1***[c] 84.0%	3253.4±44.7***[c] 92.7%

* P< 0.05 compared to control group, ** P< 0.01, *** P< 0.001 compared to control group. [a]P< 0.05, [b]P< 0.01, [c]P< 0.001 compared to positive control group. The mean difference is significant at P< 0.05. % change = Percent of change compared to control group.

Table (6): Mean values of MDA, CAT, GST and GSH in liver tissues of allstudied groups.

Groups		Negative Control group	Diabetic group	Diabetic+ Compound 1 group	Diabetic+ Compound 2 group	Diabetic+ Metformin group
Liver	MDA (nmol/g.tissue)	46.1±0.79^{c}	56.7±0.88 22.9%	0.8±1.3***^{a} 10.2%	48.2±0.22^{c} 4.5%	51.6±0.19***^{b} 11.9%
	CAT (U/g)	190.4±4.5^{c}	173.2±1.8** -9.0%	176.4±3.8** -7.3%	177.8±3.3* -6.6%	179.3±3.2* -5.8%
	GST (U/g)	57.5±0.83	44.7±1.5 -22.2%	52.1±0.27 -9.4%	52.4±0.62 -8.8%	52.8±0.60 -8.2%
	GSH (mg/g)	244.2±4.7^{a}	224.7±1.8* -7.9%	234.6±2.0^{a} -3.9%	233.6±2.1 -4.3%	233.4±1.6^{a} -4.4%

* P< 0.05 compared to control group, ** P< 0.01, *** P< 0.001 compared to control group. aP< 0.05, bP< 0.01, cP< 0.001 compared to positive control group. The mean difference is significant at P< 0.05. % change = Percent of change compared to control group.

Table (7): Mean values of MDA, CAT, GST and GSH in kidney tissues of all studied groups.

Groups		Negative Control group	Diabetic group	Diabetic+ Compound 1 group	Diabetic+ Compound 2 group	Diabetic+ Metformin group
Kidney	MDA (nmol/g.tissue)	34.5±1.2^{c}	53.7±2.2*** 55.6%	39.3±1.3^{b} 13.9%	41.5±1.5**^{b} 20.3%	42.7±0.37**^{b} 23.7%
	CAT (U/g)	174.3±5.5^{c}	148.9±2.9*** -14.5%	172.7±3.7^{c} -0.91%	164.4±3.7^{b} -5.6%	171.3±2.2^{c} -1.7%
	GST (U/g)	36.7±1.1^{c}	31.5±0.49** -14.2%	36.2±0.62^{c} -1.3%	36.4±0.20^{c} -0.81%	35.6±0.69^{b} -2.9%
	GSH (mg/g)	251.6±3.3^{c}	180.2±4.2*** -28.4%	237.2±3.8**^{c} -5.7%	231.4±3.8**^{c} -8.0%	240.2±3.1*^{c} -4.5%

* P< 0.05 compared to control group, ** P< 0.01, *** P< 0.001 compared to control group. aP< 0.05, bP< 0.01, cP< 0.001 compared to positive control group. The mean difference is significant at P< 0.05. % change = Percent of change compared to control group.

Table (8): Mean values of MDA, CAT, GST and GSH in pancreatic tissues of all studied groups.

Groups		Negative Control group	Diabetic group	Diabetic+ Compound 1 group	Diabetic+ Compound 2 group	Diabetic+ Metformin group
Pancreas	MDA (nmol/g.tissue)	25.8±1.1[c]	41.9±1.9*** 62.4%	26.2±0.38[c] 1.5%	27.2±60[c] 5.4%	29.5±1.4[c] 14.3%
	CAT (U/g)	12.1±0.58[b]	8.5±0.10** -29.7%	11.7±0.55[b] -3.3%	8.9±0.55* -26.4%	9±0.53* -25.6%
	GST (U/g)	31.8±0.53 [c]	24.2±2.4*** -23.8%	31.2±0.76 [c] -1.8%	29.8±0.32 [b] -6.3%	30±0.70 [b] -5.6%
	GSH (mg/g)	237.9±1.7[c]	218.7±2.3*** -8.1%	230.4±3.7[c] -3.2%	229.5±1.4**[a] -3.5%	222.4±0.68** -6.5%

* P< 0.05 compared to control group, ** P< 0.01, *** P< 0.001 compared to control group. aP< 0.05, bP< 0.01, cP< 0.001 compared to positive control group. The mean difference is significant at P< 0.05. % change = Percent of change compared to control group.

Table (9): Mean values of myostatin in serum and different tissues of all studied groups.

Groups		Negative Control group	Diabetic group	Diabetic+ Compound 1 group	Diabetic+ Compound 2 group	Diabetic+ Metformin group
Myostatin (pg/ml)	Serum	2231±38.1[b]	2531±45.2** 13.4%	2410±41.5* 8.0%	2499.7±84.1* 7.2%	2425±27.6** 4%
	Liver	2133±20.4[a]	2280±31.1* 6.8%	2151±6.5[a] 0.8%	2186±26.5 2.4%	2194±52.2 2.8%
	Kidney	2082.4±26.6[c]	2299±19.4*** 10.4%	2189±22.3**[b] 5.1%	2201±15.8***[b] 5.6%	2175±22.4**[c] 4.4%
	Pancreas	2056±54.8[b]	2338±41.3** 13.7%	2150±16.3[b] 4.6%	2165.5±14.4[a] 5.3%	2117±26.5[b] 2.9%

* P< 0.05 compared to control group, ** P< 0.01, *** P< 0.001 compared to control group. aP< 0.05, bP< 0.01, cP< 0.001 compared to positive control group. The mean difference is significant at P< 0.05. % change = Percent of change compared to control group.

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Table 10: Energy values obtained in doking calculations of the new synthesized compound 1 with receptor of myostatin.

Rank	EST.Free Energy Of Binding	EST.Inhibition Constant, Ki	Vdw + H Bond + desolv energy	Electrostatic Energy	Total Intermolec. energy	Frequancy	Interact. Surface
1	-4.82 Kcal/mol	293.22 uM	-5.03 Kcal/mol	-0.39 Kcal/mol	-5.42 Kcal/mol	50%	522.239

Table 11: Energy values obtained in doking calculations of the new synthesized compound 2 with receptor of myostatin.

Rank	EST.Free Energy Of Binding	EST.Inhibition Constant,Ki	Vdw + H Bond + desolv energy	Electrostatic Energy	Total Intermolec.energy	Frequancy	Interact. Surface
2	-5.08 Kcal/mol	190.09 uM	-5.44 Kcal/mol	-0.24 Kcal/mol	-5.67 Kcal/mol	100%	539.488

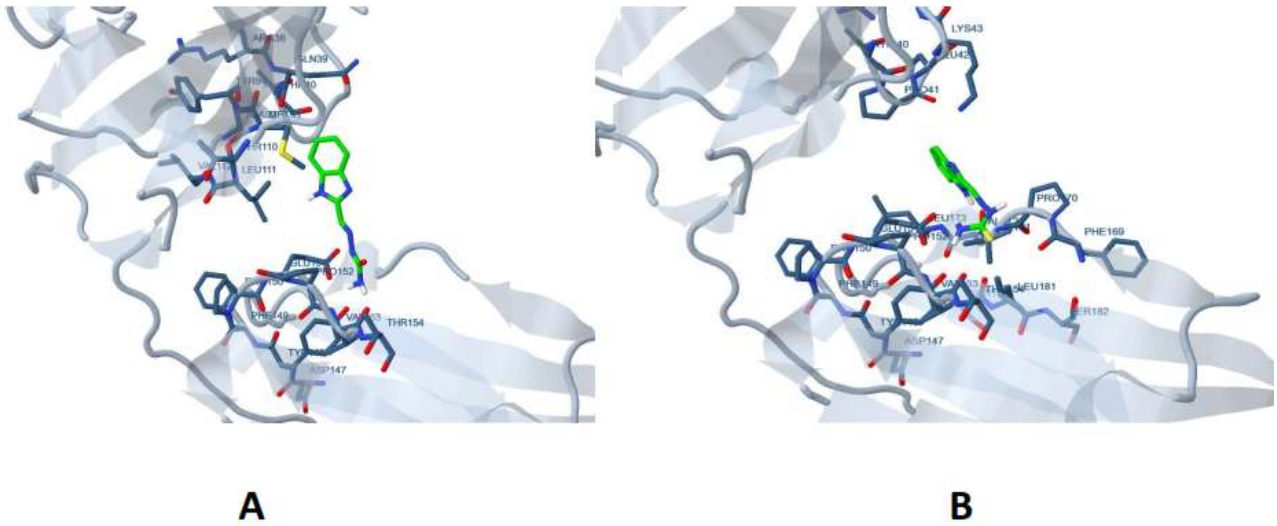

Figure 3: 3D interaction of synthesized compounds (green) (A: compound 1, B: compound 2) inside the active position of myostatin.

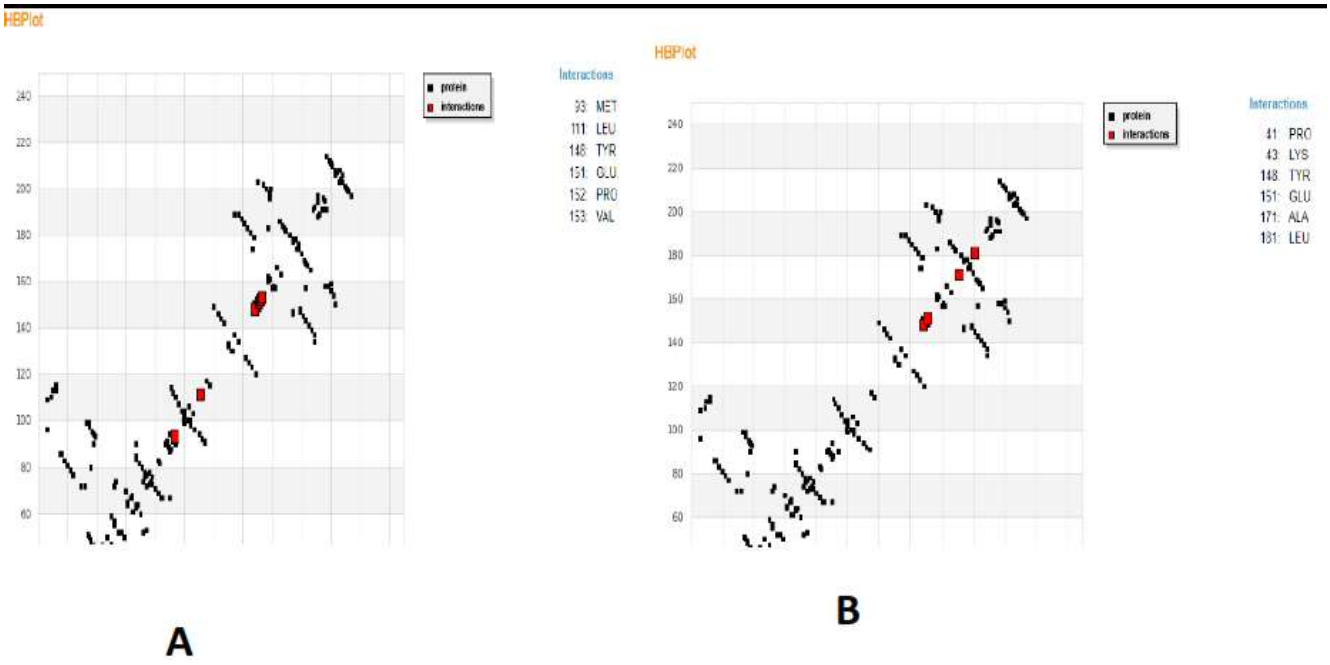

Figure 4: HB plot of the interaction of synthesized compounds (A: compound 1, B: compound 2) and receptor of myostatin.

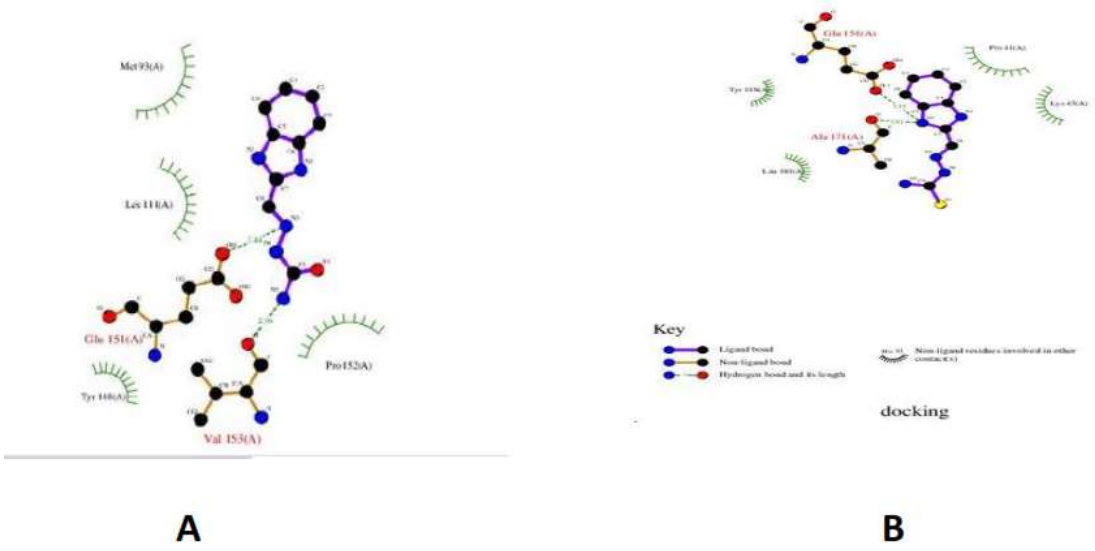

Figure 5: 2D plot interaction of synthesized compounds (A: compound 1, B: compound 2) and receptor of myostatin.

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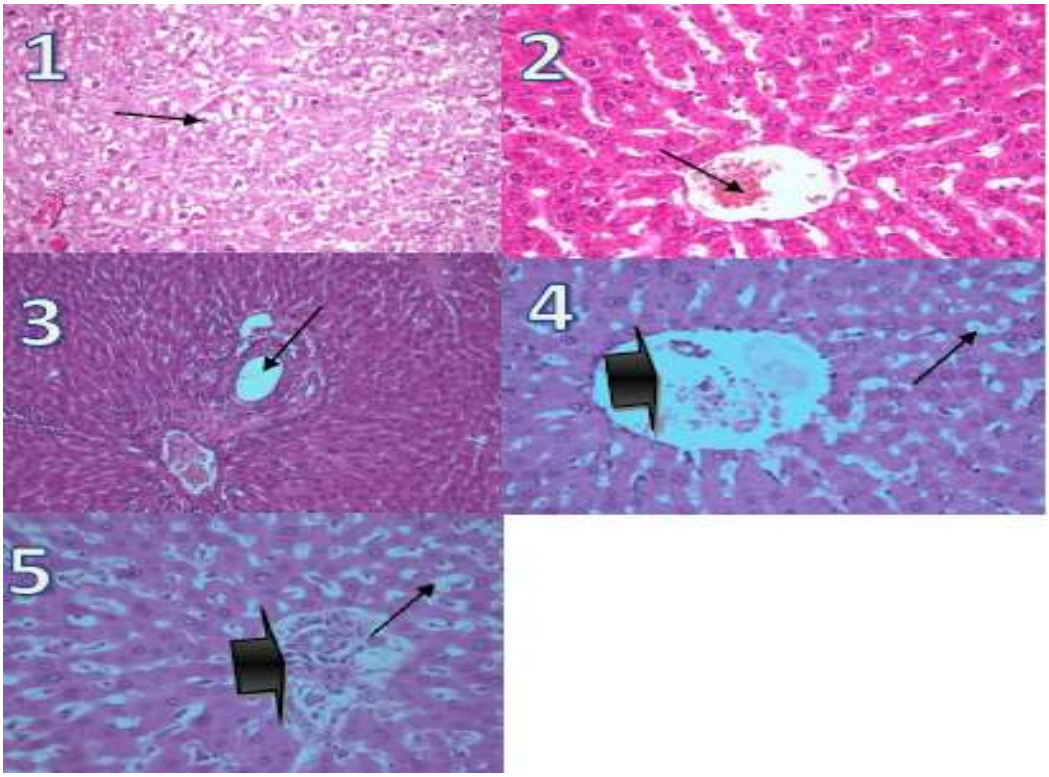

Figure 6: Histopathological examination of liver tissue.(1): Negative control showed normal hepatic parenchyma; note the normal hepatocytes, blood sinusoids, and portal area, (arrow),(H&E X200).(2):diabetic group showed vacuolated hepatocytes (arrow), Congested hepatoportal blood vessel (star), and mononuclear cells infiltrations in the portal tract (arrow head),(H&E X 200).(3):compound 1 showed normal hepatic parenchyma with normal hepatocytes, blood sinusoids, and portal area, (arrow),(H&E X 400).(4):compound 2 showed normal hepatic parenchyma with normal hepatocytes, blood sinusoids, and portal area, (arrow),(H&E X400).(5): Metformin showed normal hepatic parenchyma with normal hepatocytes, bloodsinusoids , and portal area, (arrow),(H&E X400).

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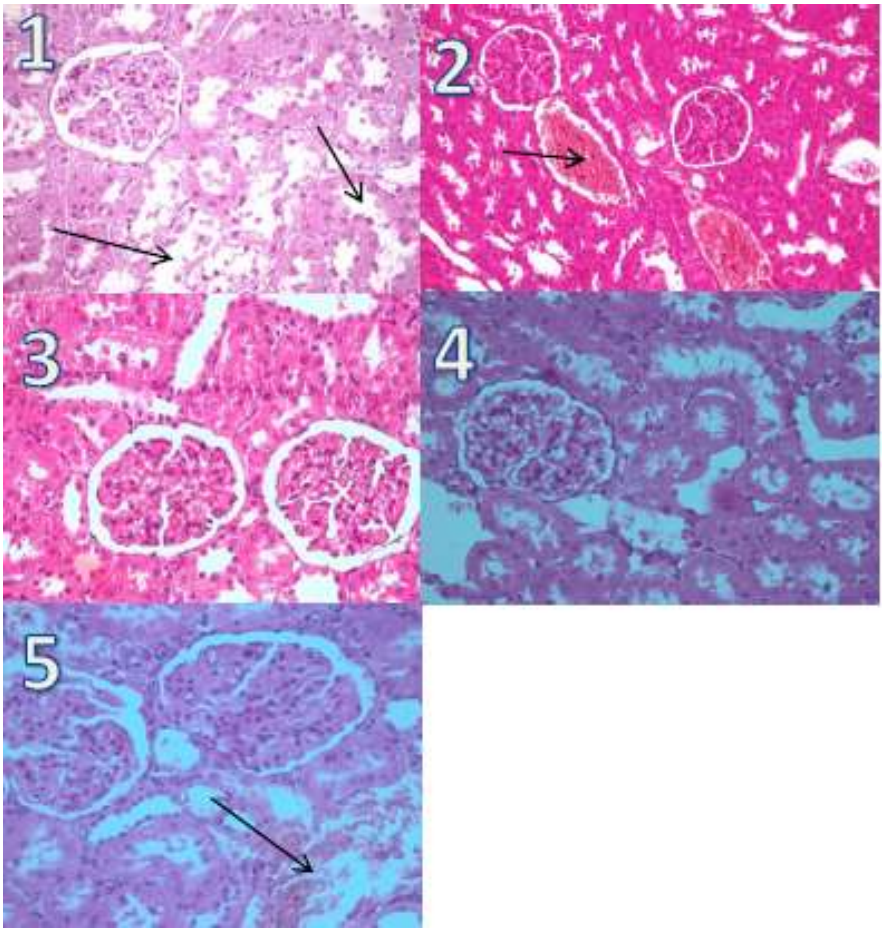

Figure 7 : Histopathological examination of kidney tissue.(1): negative control showed normal renal glomeruli and renal tubules, (arrow),(H&E X400).(2):diabetic group showed vacuolated glomerular tuft (arrows head), dilated blood vessel (star),and degenerated renal tubules (arrows), (H&E X400).(3):compound 1 showed normal renal glomeruli and renal tubules, (H&E X400).(4):compound 2 showed normal renal glomeruli and renal tubules, (H&E X400).(5):Metformin showed apparently healthy renal tubules with slight vacuolation in the renal glomeruli(arrow),(H&E X400).

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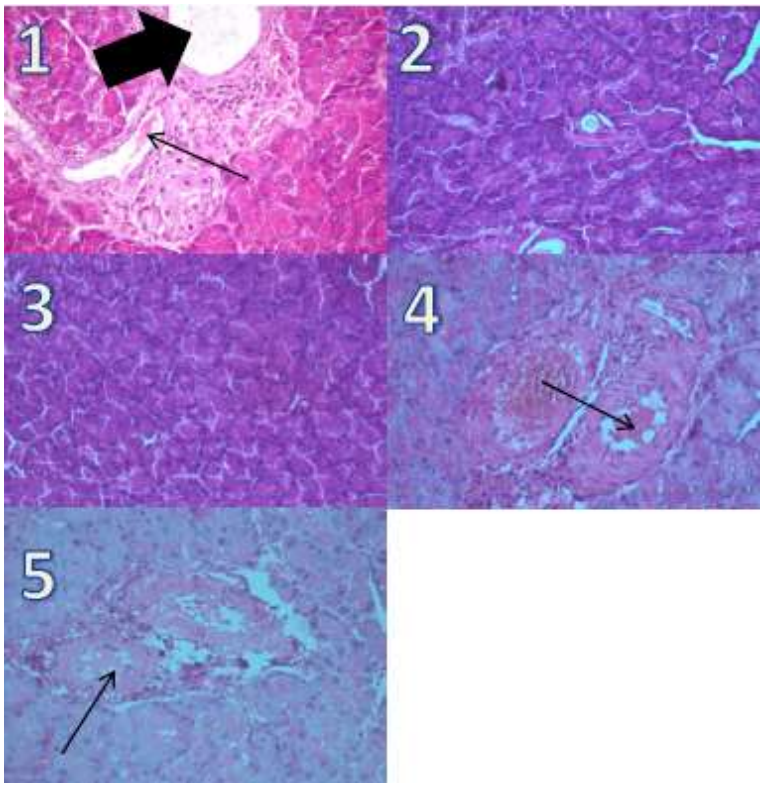

Figure 8 : Histopathological examination of Pancreatic tissue.(1): negative control showed normal pancreatic acini, islets, and duct, (H&E X 400).(2):diabetic group showed dilated and congested blood vessel (arrows), and hyperplasia of pancreatic islets,(H&E X400).(3): compound 1 showed normal pancreatic acini, pancreatic islets, and pancreatic duct (H&E X 400).(4):compound 2 showed normal pancreatic acini, islets, and duct,(arrow),(H&E X400).(5):Metformin showed normal pancreatic acini,islets,andduct,(arrow),(H&EX400).