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Biochemical and histopathological evaluation of the effect of metformin and metformin nanoparticles against alloxan-induced diabetes in rats

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ABSTRACT

Nanoparticles drug delivery systems are nanometric carriers that enhance drugs delivery and therapeutic action. The current study aimed to investigate the effect of daily oral administration of metformin and metformin nanoparticles (45 mg/kg b. w.) for 28 days on rats subjected to type 2 diabetes. 24male albino rats were divided into 4 groups, group 1 normal control, group 2 diabetic control, group 3 diabetic rats treated with metformin drug, group 4 diabetic rats treated with metformin chitosan nanoparticles drug with dose (45 mg/kg b. w.) for 28 days. Compared to diabetic control, a significant increase in body weight and decreased organs weights (liver, heart, kidneys) were recorded in diabetic rats treated. With metformin. Also, data was recorded in a significant decrease in serum glucose levels of treated diabetic rats compared to diabetic control rats. Increased serum levels of liver function (ALT, AST, ALP) and kidney function (Urea, Creatinine, Uric acid) parameters were detected in diabetic rats. Treatment with metformin and metformin nanoparticles caused a significant decrease in AST and ALT activity along with a significant decrease in creatinine and urea levels, the effects that were reversed upon metformin administration with the nanoparticles. However, the metformin nanoparticles have shown a superior effect. Various histopathological alterations were detected in the pancreas, liver, and kidney of diabetic animals. Alleviation of the histopathological changes was achieved following metformin with the nano-metformin affording better protection. In conclusion, The effect of metformin nanoparticles appears better than metformin in alloxan-induced diabetes in rats.

1. INTRODUCTION

Diabetes is a hereditary chronic metabolic disease, with hyperglycemia being the hallmark of this ailment, or in more serious cases, glycosuria can be detected (Cetin and Sahin, 2016). Diabetes is commonly associated with impaired kidney functions. First- and second-generation sulfonylureas (glimepiride, gliclazide, glipizide, glyburide, and glibenclamide), biguanides (metformin), glucosidase inhibitors (acarbose and miglitol), thiazolidinediones (pioglitazone and rosiglitazone), and meglitinide analog that primarily work by increasing pancreatic insulin secretion to reduce hepatic glucose output while increasing peripheral glucose disposal (Khadre *et al.*,2011).A previous study suggested that various classes of oral antidiabetic drugs are more effective than single drug maximum doses. Indeed, (Bailey,2005) highly suggested using combination therapy early – if not at all – in the disease process.

Metformin (N, N-dimethyl biguanide) is a commonly used drug in type 2 diabetes. (Lewis *et al*., 2016).

In diabetic rats, metformin was reported to repair renal lesions and boost superoxide dismutase antioxidant activity. A high uric acid level promoted urinary metformin excretion and decreased the plasma metformin concentration. (Zhang et *al.*,2019). A case of serious hepatotoxicity is possibly caused by metformin use at a dose of 500 mg/day for three weeks (Nathan,2008; Meligi *et al*., 2021). In diabetic rats treated with metformin, the pancreas showed that islets of Langerhans have a more- or less- normal cell population with the absence of degenerative changes, and blood sinusoids also appeared to be normal (Meligi *et al.*, 2021).

Drug nanoencapsulation arises to be an effective alternative to lessen the side effects of drug administration while achieving controlled release and site-specific delivery (Kumar *et al*., 2016). Nanoparticles drug delivery systems are nanometric carriers used to deliver drugs or biomolecules (Ab del-Hakeem *et al*., 2021).

A few nano-particle drug tablets are now available, which can help reduce the cost of diabetes management and improve adherence to the prescribed therapy (Cesur, *et al.*, 2021). Metformin and metformin nanoparticles appear to be well suited for use due to their mechanism

of action. Encapsulation of metformin in chitosan as polymeric nano capsules can provide sustained release and higher efficacy at a lower dosage. Moreover, side effects can be managed with a reduced dosage form (Kumar *et al.*,2017).
Chitosan (CTS) has previously been investigated for the effective transport of insulin to the cell. Chitosan's cationic amine groups interact with anionic groups on the epithelial cell surface, resulting in mucosal adherence and increased absorption. (Abd El-Hack *et al*.; 2020, Mohamed *et al.*, 2021). The aim of this study is to investigate the biological and histological effects of metformin and metformin nanoparticles as hypoglycemic drugs.

2. MATERIAL AND METHODS

2.1. Experimental Animals: In this experiment, 24 male albino rats with a combined total of 200 grams were fed a pelleted ration containing 20% protein, not to exceed 3% fiber, and 3% fat, all in accordance with Ain 97 NRC, as well as free access to water. Rats were acclimatized to the new environment for 14 days after being randomly assigned to groups. Rats were housed in clean cages, at room temperature. The rats fasted for 18–24 hours before drug administration by ethical number BUFVTM 03-07-21.

2.2. Chemicals: To induce hyperglycemia, alloxan monohydrate has been used in the experiment Was obtained from Sigma Aldrich, St. Louis, MO, USA, and stored at four °C. Metformin was obtained as pure powder potency (98%) from El-Nassr Co., Egypt. Metformin chitosan nanoparticles were prepared by the encapsulation method (Elkomy*et al.*, 2021).

2.3. Induction of diabetes: Alloxan was administered intraperitoneally to fasted rats at 120 mg/kg body weight dose in sterile saline. After 72 hours, fasting blood glucose (FBG) was determined using Accuchek glucometer strips (Roche Diagnostics). When the blood glucose level in the alloxan-treated rats exceeded 500 mmol/L, they were classified as diabetic. The study was carried out on uniformly diabetic rats only (Trinder, 1969).

2.4. Experimental design:
Rats were divided into four groups of 6 rats each, as following (All treatments through an intragastric tube.):
2.4.1. Group 1 (Normal Control NC): Normal control rats were given 1 ml sterile saline solution orally every day for 28 days.

2.4.2. Group 2 (Diabetic control DC): Diabetic control rats received 1 mL of normal sterile saline solution orally for 28 days after induction with alloxan.

2.4.3. Group 3 (Metformin treated group): Diabetic rats were given metformin (45 mg/kg body weight) (Nair & Jacob, 2016). in 1 ml of sterile normal saline solution orally daily for 28 days

2.4.4. Group 4 (metformin chitosan nanoparticles **treated group):** Diabetic rats were given metformin chitosan nanoparticles (45 mg/kg body weight) in 1 ml of sterile normal saline solution orally daily for 28 days.
Rat's body and organs (liver, kidney, heart) And metformin chitosan nanoparticles weights of all groups (in grams) were recorded weekly for four weeks.

2.5. Blood sampling: Blood samples were drawn further on the animal tails at 0, 30, 60, 90, 120, and 180 minutes to determine glucose and biochemical parameters measurements in serum the O-toluidine method calculated fasting blood glucose levels (Cooper,1970). Glucose level was determined using an enzymatic colorimetric method (Trinder, 1969). AST, ALT, and ALP levels were calculated using Spin react diagnostic kits (Spinreact, Gerona, Spain). (Schmidt and Schmidt, 1963). The urease-modified Berthelot reaction calculated urea (Patton and Crouch,1977). The kinetic method was used to calculate creatinine (Henry, 1974), and uric acid (Fossati *et al*., 1980).

Histopathology: kidneys, liver, and pancreas were quickly removed and fixed in Bouin's fluid for 72 hours. For microscopic inspection, tissue paraffin sections were routinely prepared and stained with hematoxylin and eosin (H and E) according to the method described by (Bancroft and Gamble 2008).

2.6. Statistical analysis:
IBM SPSS advanced statistics (Statistical Package for Social Sciences), version 24, was used to analyze the data (SPSS Inc., Chicago, IL). The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to determine the normality of the variables. Because the variables were not normally distributed, comparisons between the four groups were made using the Kruskal-Wallis test, followed by a post-hoc Mann-Whitney U test. The Bonferroni corrections were used to adjust the p-values for inflation. A p-value of 0.05 or less was considered statistically significant. All the tests were two-tailed.

3. RESULTS

3.1. Body Weight:
A significant decrease in body weight was recorded in diabetic rats (178.00 ±6.26) compared to normal rats, (240.83 ±12.42). However, Data of the present study show that metformin and metformin nanoparticles administration for 28 days resulted in a significant increase in body weight compared to that of the diabetic control group and still less than that of the normal control rat (Fig.1). Metformin nanoparticles have shown better effects than metformin. The mean values were 188.67 ±8.6 and 181.50 ±9.01 gm for the metformin and metformin nanoparticles treated group, respectively, while the normal value of body weight was 240.83 ±12 gm.

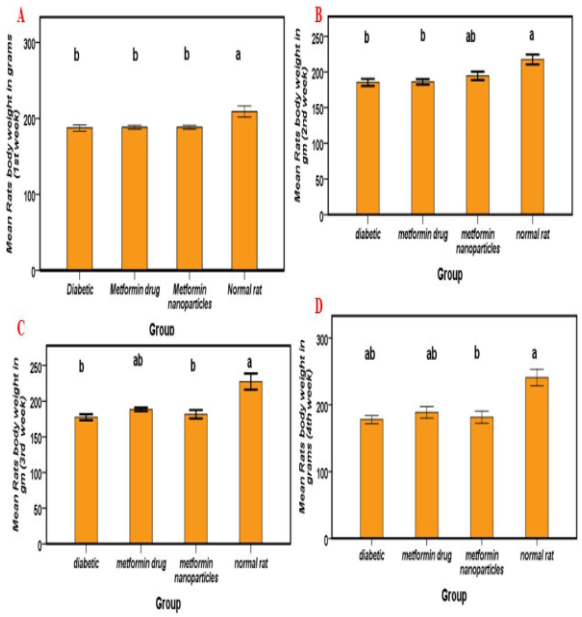

Figure 1 Effect of metformin and metformin chitosan nanoparticles on diabetic rat's body weight after oral administration (45 mg/kg b. w) for 4 weeks. (n=6, Mean ± SD). A:1st week, B: 2nd week, C: 3rd week, D: 4th week. columns shared same letter aren't statistically significant

3.2. Organ weights:

Administration of metformin and metformin nanoparticles for 28 days resulted in a significant increase in rat's liver weight: 6.42 ±0.27, 6.36 ±0.42, and 5.61±0.47 gm, Respectively compared with diabetic control group (5.61±0.47 gm)(Fig.2 A). On the other hand, the values were still less than that of the normal control rats. But in the kidneys, there was a significant difference between metformin and metformin nanoparticles compared to the control group; metformin nanoparticles significantly decreased organs weights by 1.41 ±0.2, 1.17±0.11and 1.17±0.11 gm, respectively than that of the normal control rats (Fig. 2B).

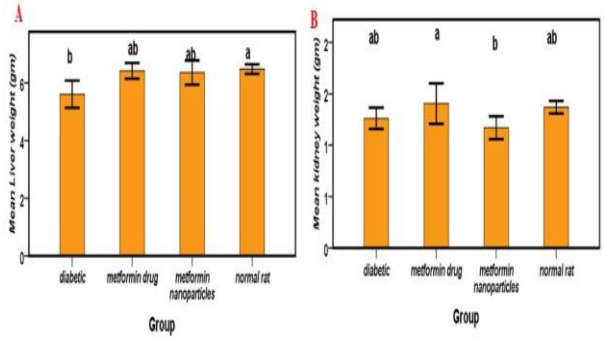

Figure 2 Effect of metformin and metformin chitosan nanoparticles on diabetic rat's liver, kidney, and heart wights (gm) after oral administration (45 mg/kg b.w) for four weeks. (n=6, Mean ± SD). A:1st week, B: 2nd week, C: 3rd week. Columns shared same letter aren't statistically significant

3.3. Biochemical parameters

3.3.1. Glucose level.

Administration of metformin and metformin nanoparticles significantly decreased the serum glucose level compared to the diabetic control group (Fig.3). However, the glucose level values were still higher than that of the normal control rats. The mean values were 98.80 ±8.35 and 93.00 ±5.61 for treatment with metformin and metformin nanoparticles respectively, compared to diabetic control (190.20 ±5.54 mg/dl).

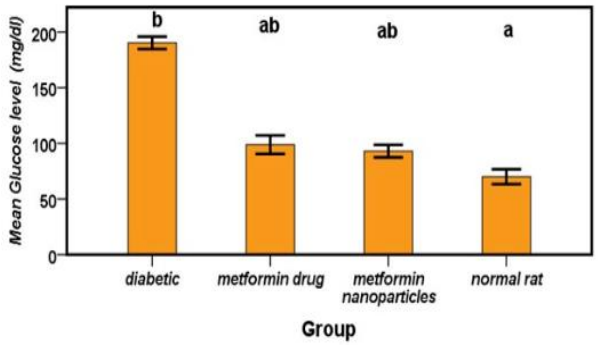

Figure 3 Effect of metformin and metformin chitosan nanoparticles on diabetic rats' serum Glycose level (mg/dl) after oral administration (45 mg/kg b. w) for four weeks. (n=6, Mean ± SD). Columns shared same letter aren't statistically significant

3.3.2. Kidney function tests (creatinine, Urea, and uric acid):

Administration of metformin or metformin nanoparticles significantly reduced serum creatinine levels in diabetic rats compared to diabetic control; these mean values for the control, diabetic control, metformin, and metformin nanoparticles drug were 0.58 ±0.05, 0.72± 0.06, 0.71± 0.06, and 0.67 ±0.05 (mg/dl), respectively (Fig. 4A).

Fig. (4B) showed that, the mean serum urea levels in diabetic rats were higher than those treated with metformin and metformin nanoparticles 33.40± 2.70, 28.00± 3.46, and 28.80 ±3.11 (mg/dl), respectively. There was no significant difference in urea level between metformin and metformin nanoparticles.

Serum uric acid levels of the diabetic rats were significantly increased compared to that of the control rats. The mean values were 3.06 ±0.23, 3.58 ±0.15, 4.92 ±0.16, and 4.48 ±0.22 (mg/dl) for the control, diabetic control, metformin, and metformin nanoparticles drug, respectively (Fig. 4C).

3.3.3. Liver function tests (AST, ALT, and ALP):

Administration of alloxan to induce diabetes in rats resulted in a significant increase in serum AST, ALT, and ALP Levels (Fig. 5) compared to the normal control group. Metformin and metformin nanoparticles treatment resulted in significant increases in serum AST, AST levels when compared to the diabetic control. Metformin nanoparticles are more effective on liver function AST levels than metformin (Fig 5). The mean values for AST levels of the normal control, diabetic control, metformin, and metformin nanoparticles drugs were 18.00±1.87, 62.60±3.58, 39.60±1.14, and 43.80±8.35 (U/L), respectively. (Fig.5A).

A significant decrease in serum ALT levels compared to the diabetic control group. However, the value was significantly higher than that of the normal control rats. The mean values for the normal control, diabetic control, metformin, and metformin nanoparticles drug were 18.80 ±1.48, 46.80 ±2.59, 31.00 ±2.74, and 32.80 ±3.27 (U/L), respectively. (Fig.5B).

Serum ALP levels of diabetic rats are significantly higher than those of control rats. There was no significant difference in serum ALP between metformin and diabetic control rats, but a significant reduction was detected in metformin nanoparticles compared to diabetic control (Fig. 5C). The mean values for the control, diabetic control, metformin, and metformin nanoparticles were 509.00± 49.08, 577.80 ±73.18, 577.00± 50.56, and 648.00± 64.97 (mg/dl), respectively (Fig.5C).

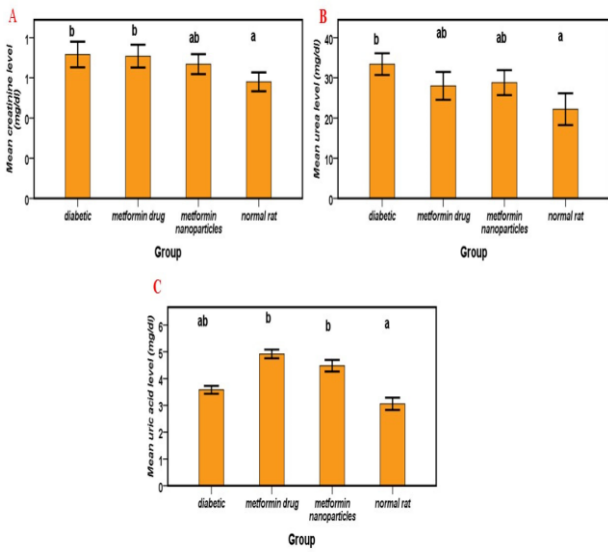

Figure 4 Effect of metformin and metformin chitosan nanoparticles on diabetic rat's kidney function parameters (urea, creatinine, Uric acid) level (mg/dl) after oral administration (45 mg/kg b.w) for four weeks. (n=6, Mean ± SD).A: urea level, B: creatinine level, C: uric acid level. Columns shared same letter aren't statistically significant

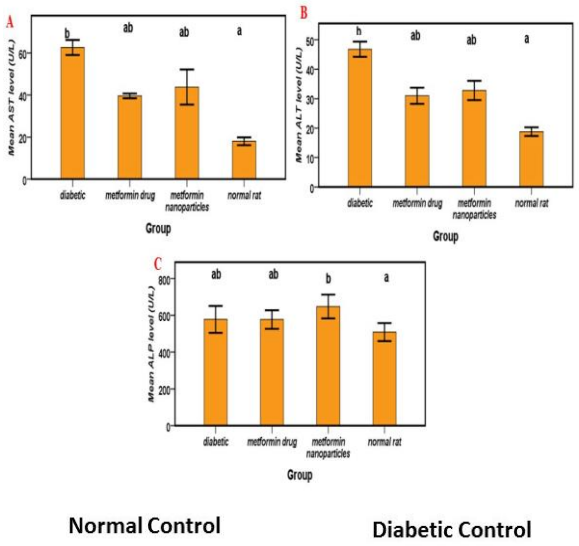

Figure 5 Effect of metformin and metformin chitosan nanoparticles on diabetic rat's liver function parameters (AST, ALT, ALP) level (U/L) after oral administration (45 mg/kg b. w) for four weeks. (n=6, Mean ± SD). A: AST level, B; ALT level, C: ALP level. Columns shared same letter aren't statistically significant

3.4. Histopathological findings:

3.4.1. Pancreas

Microscopic examination of the pancreas from the normal control group revealed the normal structure of both exocrine units and endocrine. (Fig. 6A). On the contrary, the diabetic rats showed marked histopathological alterations; islets of Langerhans were relatively few in numbers and small size (Fig.6B). The endocrine cells were necrotic. Vacuolation in the exocrine acini. Intensive mononuclear inflammatory cells infiltrations were noticed in the pancreatic tissue from this group exhibited moderate alleviation of pancreatic damage where some pancreatic acini were vacuolated. (Fig. 6C) and nano-metformin treated group showed marked improvement as both endocrine and exocrine parts were normal. (Fig. 6D).

3.4.2. Liver:

Histopathological examination of liver sections from the normal control group (Fig.7A) revealed a normal structure of hepatic parenchyma. The diabetic group (Fig.7B) showed marked mononuclear inflammatory cells infiltration in the portal areas and marked hepatocellular vacuolation. Hepatocellular necrosis was also observed associated with mononuclear inflammatory cells infiltrations. Metformin-treated group (Fig.7C) showed moderate improvement; some of the examined sections were normal, while others showed mild hepatocellular vacuolation. Likewise, Nano-the metformin-treated group (Fig. 7, D) was also improved.

3.4.3. Kidneys

Both renal cortex and medulla of rats in control group were histologically normal (Fig. 8, A). Meanwhile, kidney sections from the diabetic group showed perivascular edema with mononuclear inflammatory cells infiltration in the renal cortex (Fig. 8, B) while the renal medulla showed marked tubular damage. The metformin-treated group showed few degenerating tubules in the renal cortex (Fig. 8, C). The best protective action was detected in Nano-metformin-treated group where the renal cortex exhibited only congested blood vessels while the renal medulla was normal (Fig. 8, D

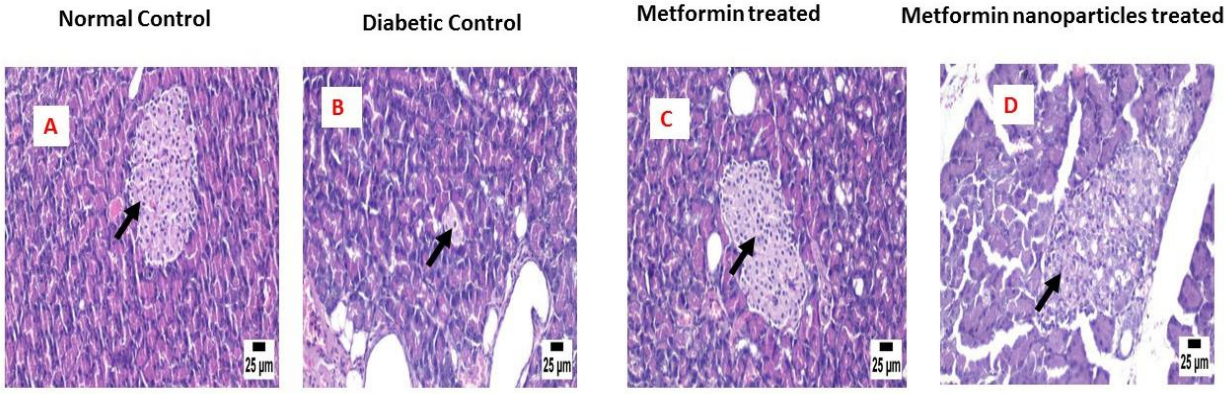

Figure 6 Photomicrograph of the pancreas, A: Normal control group showing the normal structure of both exocrine and endocrine compartments of the pancreas. B. diabetic control showing small, atrophied islets of Langerhans (arrow). C. metformin-treated group showing the normal structure of islets of Langerhans (arrow) with mild vacuolation of some pancreatic acini, D. metformin nanoparticles treated group showing normal endocrine compartment of the pancreas (arrow) (H&E).

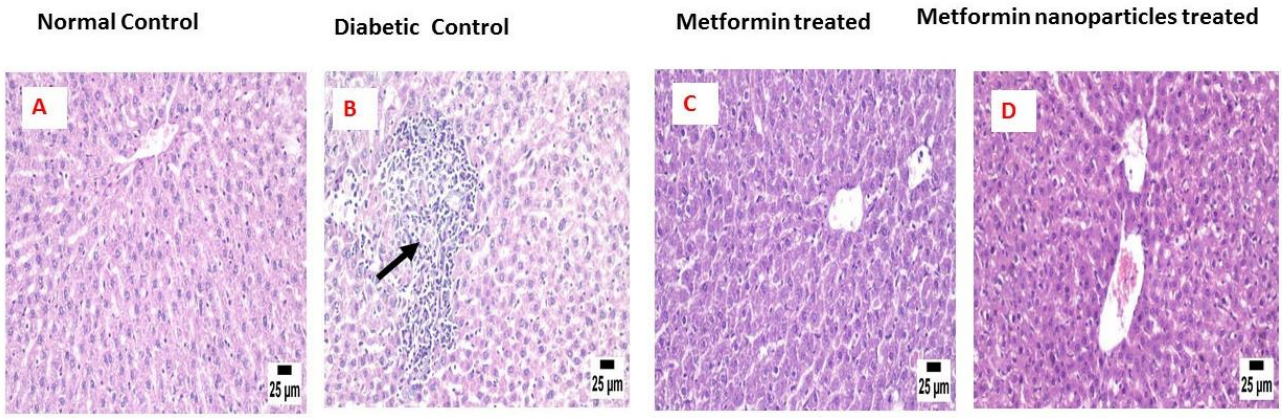

Figure 7 Photomicrograph of the liver. A. normal control showed the normal structure of hepatic parenchyma, B. diabetic control showing mononuclear inflammatory cells infiltration in the portal area (arrow), C. metformin-treated, and D. metformin nanoparticles groups showing the normal structure of hepatic parenchyma (H&E).

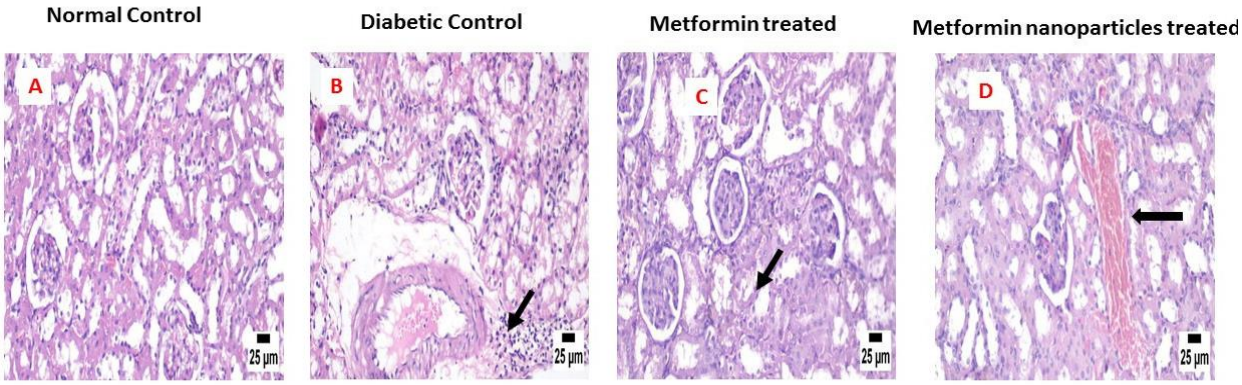

Figure 8 Photomicrograph of kidney A. normal control group showing the normal structure of renal cortex (H&E), B. diabetic control showing perivascular edema with mononuclear inflammatory cells infiltration (arrow) C. metformin-treated group showing few degenerating tubules (arrow). D. metformin nanoparticles treated group showing congested blood vessel in the renal cortex (H&E).

4. DISUCCUSION

Persistent hyperglycemia is a hallmark of diabetes mellitus (DM) resulting from impaired insulin synthesis by beta cells in the pancreatic islet and decreased insulin action in target tissue. (Thaipitakwong *et al.*, 2018)

The results of serum biochemical analysis demonstrated a significant decrease in serum glucose levels after administering metformin and metformin nanoparticles drug for 28 days to diabetic rats. This finding was consistent with several authors' previous results (Qiong Ma, *et al.*,2022).

Higher ALT levels are a risk factor for type 2 diabetes, indicating a possible role for increased hepatic gluconeogenesis and inflammation in the pathogenesis of type 2 diabetes. Parallel to preceding data (Lu *et al*., 2020), the current study revealed liver destruction in diabetic rats treated with metformin and metformin nanoparticles were decreased by AST and ALT enzymes, but ALP was increased in metformin nanoparticles than others.

Diabetic hyperglycemia raises plasma levels of urea, creatinine, and uric acid, important indicators of renal dysfunction (Khadre, *et al*.,2011). The current study's findings showed a significant increase in serum urea, creatinine, and uric acid levels in the diabetic group. Administration of metformin or metformin nanoparticles has reverted all these results. Consistent with our results, diabetic rats were treated with metformin or metformin nanoparticles (Zhang *et al.*,2017).

Diabetic rats were found to have higher levels of uric acid in their blood compared to normal control rats (Gowda *et al.*,2021).

The histopathological findings of the liver of rats in diabetic group showed marked mononuclear inflammatory cells infiltration in the portal areas and marked hepatocellular vacuolation. and necrosis. The metformin-treated group showed moderate improvement; where some of the examined sections showed normal hepatic architecture, while others showed mild hepatocellular vacuolation These hepatic changes were also recorded by Li et al., 2013. The best protective action was found in the Nano- metformin-treated group; the renal cortex had numerous congested blood vessels while the renal medulla appeared normal (El-Naggar, et al. 2021).

The reexamined pancreas of diabetic rats showed significant histopathological changes, where vacuolation of the exocrine acini was detected. With mononuclear inflammatory cellular infiltration. Metformin treatment reduced pancreatic damage These changes came in accordance with those recorded by Al-basher *et al.*, 2020.In conclusion, the combination of biochemical biomarkers and histopathological findings provides useful and sensitive tools for studying the effects of diabetes and anti-diabetic drugs (metformin and metformin nanoparticles). The obtained results revealed that metformin nanoparticles are more effective than metformin in alloxan-induced diabetes in rats.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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