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# Comparative Effects of Antidiabetic Drug, Metformin and Deferoxamine, on the Hepatotoxic and Nephrotoxic Side Effects of Streptozotocin-induced Diabetes Mellitus in Rats

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***Authors' contributions***

*The two authors made substantial intellectual contributions to this study. Both authors were involved in the conception, design and data collection as well as interpretation of results, preparation of the manuscript, revision of the article at various stages and preparation of the final draft. All authors read and approved the final manuscript.*

***Original Research Article***

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## ABSTRACT

**Aims:** The present study compared the effects of metformin (met) and deferoxamine (DFX) on the hepatotoxic and nephrotoxic side effects of streptozotocin experimental diabetes in rats using serum biochemical and histopathological indicators.
**Study Design:** Following induction of diabetes, animals were randomly and evenly distributed into four groups A, B, C and D of six rats each (n=6). Experimental diabetes was induced in overnight fasted rats by a single dose i.p each of nicotinamide and, 15min after, STZ followed by administration of the antidiabetic drug, met (os, 250mg/kgb.wt) and iron chelating drug, DFX (i.p,150mg/kgb.wt), daily for 14 days. Blood and histological samples were collected and prepared for biochemical and histopathological analysis of indicators of cytotoxic side effects.

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**Results:** STZ caused cytotoxic effects on the liver and kidney of experimental rats, indicative of cellular leakage and loss of the functional integrity of the cell membranes. Metformin and deferoxamine both effectively reversed the hepatotoxic side effects of STZ-induced diabetes as determined by serum activities of ALP, AST and ALT and histopathological presentation. However, whereas metformin effectively reversed the STZ induced side effects on the kidney as determined by serum creatinine level and histopathological indicators, DFX did not.
**Conclusion:** It is concluded that metformin has a markedly higher potency than DFX in mitigation of hepatic and renal tissue derangement, as determined by both serum biomarkers and tissue histology.

*Keywords: Type 2 diabetes; liver enzymes; renal damage; Serum ferritin.*

## 1. INTRODUCTION

Diabetes mellitus (DM), especially type 2, represents one of the most important health problems worldwide and is likely to worsen to critical levels in the next decades, with the great concern that this disease is rising rapidly in younger population groups, including children and adolescents [1]. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [2,3]. Renal impairment is one of the serious and common diabetic complications [4]. Elevated serum levels of urea and creatinine are significant markers of renal damage [5]. Hepatic abnormalities such as elevations of transaminases and alkaline phosphatase (ALP) are common in diabetes mellitus [6,7]. Elevated transaminases i.e. aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are useful biomarkers of liver injury [8]. Alkaline phosphatase is elevated in bile duct obstruction, intrahepatic cholestasis or infiltrative disease of the liver [9,10].

Increasing evidence indicates that iron overload played a pathologic role in diabetic complications [11]. Epidemiological studies have provided evidence for increased urinary iron in patients with diabetic nephropathy, and the prevention of progression either by an iron-deficient diet or iron chelators [12,13] The long term consequences of chronic iron overload include multiple organ dysfunction (heart, liver, and endocrine) and/or failure [14], [15]. Therefore iron chelation is necessary to prevent organ failure and decrease mortality.

The major goal in the treatment of diabetes has been to keep both short-term and long-term glucose levels within acceptable limits, thereby reducing the risk of long term complications [16,17]. Metformin is generally recommended as a first line medication used for treatment of type 2 diabetes as there is good evidence that it decreases mortality [18,19]. It was approved by the Food and Drug Administration (FDA) for use in the United States in 1995 [20]. Metformin is a rather safe drug and its anti-hyperglycemic property has been generally attributed to combination of a decreased rate of intestinal absorption of carbohydrate, decreased hepatic gluconeogenesis and improvement of peripheral glucose utilization [21]. Beyond its effect on glucose metabolism, metformin has been reported to reduce fatty liver, and to lower microvascular and macrovascular complications associated with T2D.

Deferoxamine is the most common iron chelator in clinical use [22,23]. The capacity of deferoxamine to chelate iron (Fe) and mediate its excretion in Fe-overloaded patients is

well documented [24]. It has been approved for use in the USA since the late 1970's [25,26]. Deferoxamine acts by binding free iron in the bloodstream and enhancing its elimination in the urine [27]. Deferoxamine works in treating iron toxicity by binding trivalent (ferric) iron (for which it has a strong affinity), forming ferrioxamine, a stable complex which is eliminated via the kidneys [28]. By removing excess iron, the agent reduces the damage done to various organs and tissues, such as the liver [27].

Apart from the expected global health care costs involved in treating and managing DM, this disease imposes additional social economic burdens from lost productivity and slow economic growth [29]. Therefore, there is a need for the development of more effective preventive and therapeutic approaches that address and abolish the reduction in life expectancy and life quality imposed by DM and its complications or comorbidities. This study was carried out to compare the effect of metformin on serum liver transaminases, alkaline phosphatase and serum creatinine with that of deferoxamine which act by a different mechanism in streptozotocin induced diabetic rats to determine their effectiveness in treating/preventing diabetes and its complications and as an effort in the search for more effective therapeutic initiatives for type 2 DM.

## 2. MATERIALS AND METHODS

### 2.1 Drugs and Chemicals

Streptozotocin (STZ), deferoxamine (DFX), nicotinamide (NA), citric acid, trisodium citrate, sodium hydroxide, sodium chloride, chloroform, and formaldehyde were purchased from Zayo-Sigma Aldrich Chemicals Ltd, St Louis, MO, USA; metformin HCl (Diabetmim, Hovid Pharmaceutical, Pune Ma Harashtra, India).

### 2.2 Experimental Animals

Thirty five male Wistar strain albino rats weighing 172-233g were used in this study. The rats were obtained from the Animal House Unit of the Federal College of Veterinary and Medical Laboratory Technology (FCVMLT), Vom. The rats were housed in clean metallic cages, kept in a well ventilated room and allowed to acclimatize to the laboratory condition of the Nigerian Institute for Trypanosomiasis Research (NITR) Vom, animal experiment room at 25±2ºC with 12 hour light/dark cycle for two weeks before being used. Animals were fed a standard animal pellet obtained from Dagwom farm of National Veterinary Research Institute (NVRI), Vom and had free access to water *ad libitum*. All animals were carefully monitored and all the experimental protocol with the animals was in accordance with the internationally accepted principles for laboratory animal use and the experimental protocols were duly approved by the Institutional Ethical Committee of NITR.

### 2.3 Experimental Induction of Type 2 Diabetes in Rats

Diabetes was induced in overnight fasted rats by a single intraperitoneal (i.p) injection of freshly prepared streptozotocin (35mg/kgb.wt) dissolved in 0.1M citrate buffer (pH 4.5), 15min after the intraperitoneal administration of nicotinamide (110mg/kg), dissolved in normal physiological saline. The control (group A) animals were injected i.p with the equivalent volume of the citrate buffer solution [30,31].

Diabetes was confirmed by the symptoms of polydipsia, polyuria, glycosuria and elevated fasting blood glucose concentration, 48 hours and then one week after STZ injection. Diabetes was developed and stabilized in STZ treated rats over a period of 7 days [32]. Following the criteria of previous workers [33,34], rats with stable glycosuria and blood glucose levels of more than 11mmol/L (>11mmol/L) were considered to be diabetic and, therefore, used for the study.

## 2.4 Animal Treatment

Following induction of diabetes, animals were randomly and evenly distributed into four groups A, B, C and D of six rats each (n=6) in clean metallic standard rat cages. Animals in group a (normal control) and those in group B (diabetic control) were given only water *ad libitum*. While those in group C (diabetic) were administered orally with antidiabetic drug metformin (Met) at a dose of 250mg/kg body weight once daily for 14 days by forceful gavage. Rats in group D (also diabetic) were administered intraperitoneally with iron-chelating drug deferoxamine (DFX) at a dose of 150 mg/kg body weight once daily for 14 days.

The dosages of drugs used in this study were pre determined in a pilot study. Baseline fasting blood glucose levels of each rat for all groups were measured before diabetes induction and at the time of grouping of the animals. Drugs were administered daily for 14 days. Blood glucose levels were also estimated on the $15^{th}$ day post treatment.

## 2.5 Monitoring of Blood Glucose Level during Treatment

All blood samples for monitoring of blood glucose level *in situ* were taken from the tail vein of the rats using 24 gauge needles. Blood glucose level was determined by the glucose oxidase method using reactive strips and a single touch glucometer (Accu-Chek Active, Roche Diagnostics, Mannheim, Germany). Results were initially recorded in mg/dl and then converted to mmol/l by dividing values in mg/dl by a factor of 18.

## 2.6 Blood Collection and Preparation

After overnight fast, the animals were sacrificed on the $15^{th}$ day under mild chloroform anaesthesia and blood was obtained via cardiac puncture. Blood sample was transferred into plain centrifuge tube and allowed to clot at room temperature. It was then centrifuged within 1 hour of collection, at 4000xg for 10min on a digital centrifuge (Biofuge 200, Bosch Medical Systems, Corinth, U.S.A.) to separate the serum from the clot. The resultant serum sample were stored frozen at -20ºC. Prior to assay, frozen sera were completely thawed and well mixed and all reagents were allowed to attain room temperature.

## 2.7 Histopathological Examination

After sacrificing the animals on the $15^{th}$ day post treatment, the liver and kidney of two animals from each group were excised and immediately fixed in 10% neutral buffered formalin solution after washing with normal saline. The resultant fixed tissue samples were used for histopathological examination in the Histopathology Laboratory of FCVMLT and Diagnostic Center of NVRI, Vom, using the routine procedures developed in the respective laboratories. The tissues were washed, dehydrated with alcohol and cleared with xylene. Serial sections of 4µm thickness were cut using a rotary microtome (ERM-200P, Erma,

Tokyo, Japan). The sections were deparaffinised with xylene and hydrated in descending grades of alcohol. The slides were then transferred to haematoxylin for 10 min, followed by rinsing with water. These were examined and later counterstained with eosin, rinsed with water, dehydrated with ascending grades of alcohol, cleared with xylene and mounted. The slides were observed using a light microscope.

## 2.8 Biochemical Analysis of Rat Serum Sample

The serum aminotransferases, alkaline phosphatase and creatinine levels were measured using Roche-Hitachi kit via Automatic Analyzer (HITACHI 902, Roche Diagnostics, Mannheim, Germany) according to the procedure described in the manufacturers' operation manual.

## 2.9 Statistical Analysis

All statistical analysis was performed with SPSS software version 16.0. Data are expressed as the means±standard error of mean (S.E.M). Student's t-test and analysis of variance (ANOVA) were employed in comparing means of continuous variables as appropriate. Differences were considered statistically significant if $p<0.05$.

## 3. RESULTS

The effect of repeated oral administration of metformin and intraperitoneal injection of deferoxamine on the blood glucose levels in streptozotocin-nicotinamide induced diabetic rats is shown in Table 1. The fasting blood glucose levels were similar in controls and experiment groups before STZ+NA injection ($P>0.05$). The diabetic control rats (group B) exhibited gradually increased hyperglycaemia. There was a significant ($p<0.05$) elevation in fasting blood glucose (FBG) level in diabetic control rats (group B) when the values were compared to normal control rats (group A). Oral administration of metformin (250mg/kg/day) (group C) significantly ($p<0.05$) reduced the elevated levels of blood glucose at the $15^{th}$ day post-treatment compared to the untreated diabetic control (group B). On the other hand, intraperitoneal administration of deferoxamine (150mg/kg/day) (group D) exhibited significantly ($P<0.05$) reduced blood glucose level at the $15^{th}$ day post-treatment compared to the diabetic control (group B). Normal control rats (group A) did not exhibit any significant alterations in blood glucose levels during the experiment.

The results of the effect of metformin and deferoxamine on the serum liver enzymes and creatinine levels are summarized on Table 2. The serum activities of three enzymic hepatic markers, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase were found to be significantly higher ($p<0.05$) in diabetic control rats (group B) than in the normal controls (group A), suggesting that STZ caused cytotoxic side effects on the liver of experimental rats, indicative of cellular leakage and loss of the functional integrity of the cell membranes. Oral administration of metformin (250mg/kg/day) and intraperitoneal deferoxamine (150mg/kg/day) produced a significant ($p<0.05$) reduction in the serum activities of all the three enzymes compared to diabetic control (group B), implying that these drugs reversed the hepatotoxic side effects produced by STZ in rats. These observations are corroborated by findings from liver histopathological studies (Plate 1). Compared to the liver of diabetic rats (Plate 1B) which revealed symptoms of serious hepatic injury, notably, distortion of hepatic lobular architecture with features ranging from diffuse chronic inflammatory hepatocyte

vacuolization, tissue sections from rats treated with metformin (Plate 1C) and with deferoxamine (Plate 1D) both showed well preserved normal-appearing hepatic lobular architecture, which suggest that treatment with either metformin or DFX reversed the hepatotoxic side effects of STZ diabetes. The serum activities of AST and ALT of metformin treated diabetic rats (group C) were significantly ($p<0.05$) lower than those of DFX treated diabetic rats (group D), implying that metformin was more effective than DFX in reducing the hepatotoxic side effects of STZ diabetes in experimental rats.

The levels of serum creatinine, a marker of kidney injury, was significantly higher ($p<0.05$) in the diabetic control (group B) than in the normal control (group A), implying that STZ (in the course of inducing diabetes) caused elevation in serum creatinine (that is, by causing adverse cytotoxic side effects on kidney of the experimental rats). Similarly, serum creatinine concentration was significantly higher in diabetic control rats (group B) than in met treated rats (group C), suggesting that treatment with metformin markedly improved the functional status of the kidney by reversing the adverse cytotoxic side effect of STZ diabetes. However, no such significant effect was observed in DFX treated rats (group D) as evidenced by the difference between group B and DFX treated group not being statistically significant ($p>0.05$). This further suggests that metformin was more effective than DFX in reversing the STZ side effects on kidney functions, a fact confirmed by the observation that serum creatinine level was significantly ($p<0.05$) higher in DFX treated (group D) than in group C (metformin treated rats). These observations were corroborated by histopathological findings on the kidney tissue (see Plate 2). Compared to the diabetic control tissue section (Plate 2B) which revealed an oedematous derangement of renal tubular structure, significant renal tubular dilation and intratubular deposition of hyaline with areas of cloudy necrosis and glomerulonephritis,-all symptoms of serious renal injury-tissue section of metformin-treated rats (Plate 2C) showed preserved renal tissue architecture but with mild tubule-interstitial nephritis. This suggests that metformin effectively reversed the nephrotoxic effect of STZ. In contrast, tissue section of DFX-treated rats (Plate 2D) showed slightly distorted renal tissue architecture, significant renal tubular dilation and moderate tubule-interstitial glomerulonephritis, suggesting that DFX treatment could not reverse nephrotoxic side effects of STZ.

**Table 1. Blood glucose levels in normal and streptozotocin induced diabetic rats following 14 days treatment with metformin and deferoxamine**

| Group | Treatment | Blood glucose level (mmol/L) | | |
|---|---|---|---|---|
| | | Before-STZ-induced | After STZ-induced | Post treatment |
| | | Baseline | Day 0 | Day 15 |
| A | Normal control | 5.19±0.10 | 5.02±0.09 | 5.15±0.09 |
| B | Diabetic control | 5.09±0.12 | 13.28±0.15 | 14.02±0.09[a] |
| C | Diabetic+Met | 5.15±0.13 | 13.18±0.12 | 8.49±0.15[a] |
| D | Diabetic+DFX | 4.96±0.12 | 12.89±0.12 | 10.75±0.13[a] |

*Values are mean±SEM for six rats in each group; Statistical significance between means was assessed using one-way analysis of variance followed by Duncans test as a post-analysis of variance test. [a]statistically significant at p<0.05 vs day 0 Abreviations: STZ=streptozotocin; Met=metformin; DFX=deferoxamine*

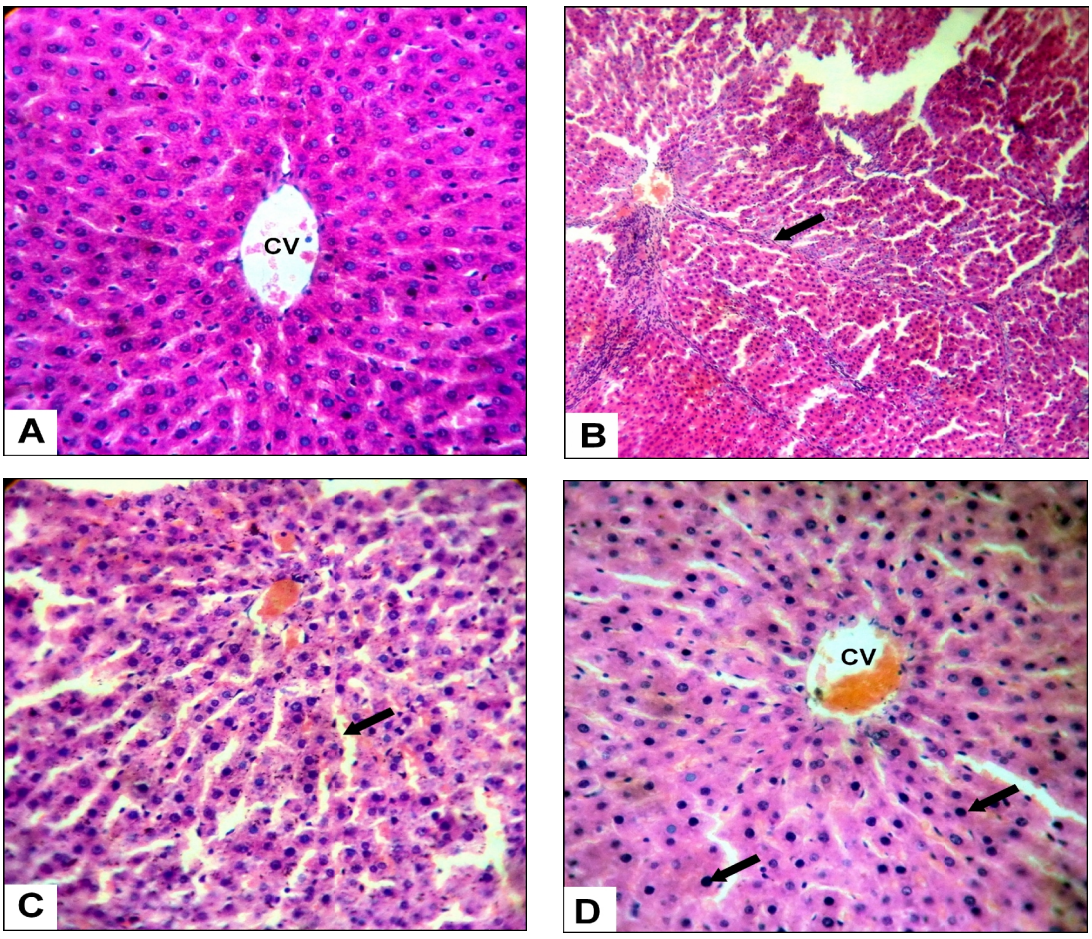


**Plate 1. Histopathological changes in liver tissue of control and experimental rats**

*Group A: Normal liver tissue showing a Central Vein (CV) with cords of hepatocytes and sinusoids radiating from the central vein; B. Group B: Diabetic Liver shows a Central-Portal bridging necrosis along the hepatic lobule (arrow); C. Group C shows normal hepatic lobule with dilated sinusoids (arrow) and suffused red blood cells; D. Group D shows a normal hepatic lobular architecture with many hepatocytes having pyknotic nuclei (arrows). H & E Stain; B (X10), A, C and D (X30)*

**Table 2. Mean serum levels of aminotransferases and creatinine in STZ diabetic rats following treatment with antidiabetic drug metformin and deferoxamine**

| Group | Treatment | Serum enzyme activity (IU/l) | | | Serum creatinine conc. (µmol/L) |
|---|---|---|---|---|---|
| | | AST | ALT | Alp. | |
| A | Normal control | 125.50±1.77 | 42.50±1.52 | 137.83±4.91 | 31.50±1.52 |
| B | Diabetic control | 153.67±2.23$^{a}$ | 112.33±2.30$^{a}$ | 169.17±4.74$^{a}$ | 47.50±2.39$^{a}$ |
| C | Diabetic+Met | 129.17±2.41$^{b}$ | 47.67±2.65$^{b}$ | 142.67±6.01$^{b}$ | 36.67±1.41$^{b}$ |
| D | Diabetic+DFX | 139.33±2.36$^{abc}$ | 63.50±1.80$^{abc}$ | 151.00±5.40$^{b}$ | 43.83±1.66$^{ac}$ |

*Tabulated values are means±S.E.M for six rats in each group; Statistical significance between means was assessed using one-way analysis of variance followed by Duncan's test as a post-analysis of variance test; $^{a}$Statistically significant at p<0.05 compared to the normal control (group A); $^{b}$Statistically significant at p<0.05 compared to the diabetic control (group B); $^{c}$Statistically significant at p<0.05 compared to the diabetic+met treated rats (group C); Abreviations: AST=aspartate aminotransferase; ALT=alanine aminotransferase; ALP=alkaline phosphatase; Met=metformin; DFX=deferoxamine*

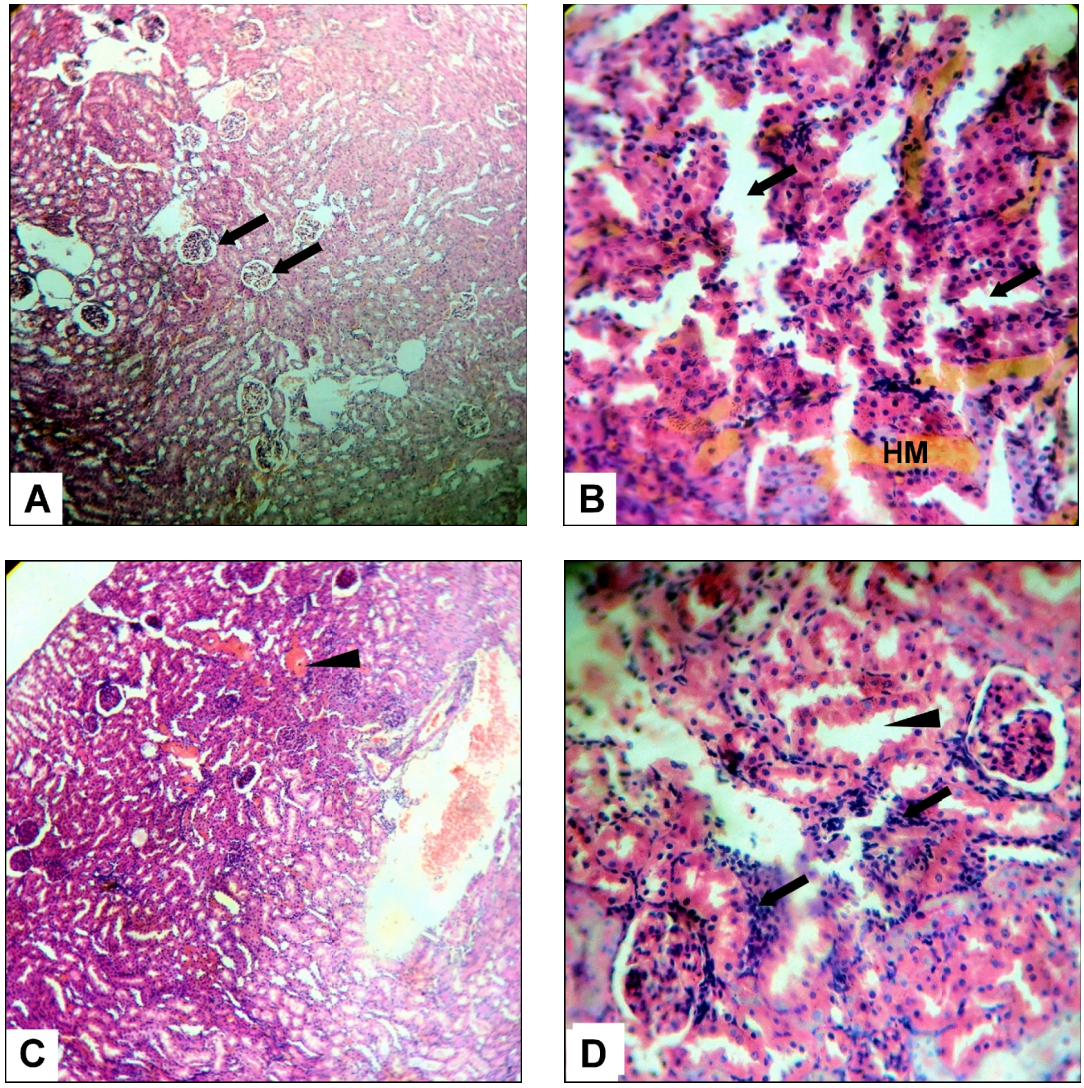


**Plate 2. Histopathological changes in kidney tissue of control and experimental rats**

*A. Group A: Normal Renal tissue architecture showing Glomeruli (arrows) and renal tubules; B. Group B shows renal tubular dilatation and damage (arrows) as well as haemorrhage (HM); C Group C: Preserved renal tissue but with areas of Hyaline deposition (arrow head); D Group D shows renal tissue with mild tubular dilatation (arrow head) and foci of inflammatory infiltrates (arrows). H & E Stain; A and C (X10), B and D (X30)*

## 4. DISCUSSION

Experimental type 2 diabetes (T2DM) in rats can be induced by several methods [35]. According to Masiello et al. [30], administration of streptozotocin (STZ) and nicotinamide (NA) produced moderate hyperglycaemia which has clinical similarities especially with respect to the insulin response to the glucose [3,36] and as such streptozotocin-nicotinamide (STZ-NA) is a method currently used to induce diabetes in animals that resemble non obese type 2 Diabetes mellitus (DM) in man [37]. Hence, STZ-NA induced

diabetes model was used in the present study. In the present study, streptozotocin injection caused β cells degeneration in rats, therefore, release of insulin by the pancreas was decreased resulting in hyperglycaemia. This confirms induction of experimental diabetes in rats .This is similar to the findings of several earlier researchers [3,17].

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) reflects the concentrations of intracellular AST and ALT that have leaked into the general circulation and thus, serves as an indicator of hepatotoxicity [34,38]. Besides, alkaline phosphatase (ALP), predominantly found in the bile duct of the liver, is considered an indicator of biliary function, cholestasis and hepatic function [10,39]. The hepatocellular damage in STZ-induced diabetic rats observed in this study corroborates those of other authors who also reported hepatotoxicity in STZ-induced diabetic rats [8,40,41]. The reversal of the hepatotoxic side effects of streptozotocin by metformin and deferoxamine administrations indicates that these drugs exert hepatoprotective effect in STZ-induced diabetic rats. The histopathological finding is in line with this observation. The actual mechanisms by which metformin or deferoxamine reversed the hepatotoxicity in STZ-induced diabetic rats remain unclear. Considering the evidence that implicates hyperglycaemia or diabetes mellitus in hepatic damage or dysfunction [42], this hepatoprotective effect may be due to hypoglycaemic effects of these drugs as reported in this study.

Renal impairment associated with elevated serum levels of urea and creatinine is one of the serious and common diabetic complications [4,5]. Catabolism of the protein and nucleic acids results in the formation of non-protein nitrogenous compounds, urea and creatinine. In diabetes mellitus, the amino acid breakdown in the liver results in an increased production of urea and creatinine [40].

Renal damage produced by STZ in this study corroborates those of other authors who also reported nephrotoxicity in STZ-induced diabetic rats [17,40]. Metformin administration was able to reverse the nephrotoxic effect of STZ in rats. This suggests that metformin may have beneficial effect in preventing renal damage, perhaps through improved glycaemic control and amelioration of oxidative stress or it could be due to decreased metabolic disturbances as the drug improved glycaemic control, which also suggested that renal dysfunction associated with diabetic condition may have been prevented by the drug at the dosage used.

## 5. CONCLUSION

The intraperitoneal injection of STZ-NAD induces type 2 diabetes in rats with instability of some biochemical parameters. STZ caused cytotoxic effects on the liver of experimental rats, indicative of cellular leakage and loss of the functional integrity of the cell membranes. It also caused adverse cytotoxic side effects on kidney of the experimental rats. The data obtained from this study show that treatment with either metformin or DFX reversed the hepatotoxic side effects of STZ diabetes. However metformin was more effective than DFX in reducing the hepatotoxic side effects of STZ diabetes in experimental rats. Treatment with metformin markedly improved the functional status of the kidney by reversing the adverse cytotoxic side effect of STZ diabetes. DFX treatment on the other hand could not reverse nephrotoxic side effects of STZ. Metformin showed markedly higher potency in mitigation of renal and hepatic tissue derangement as determined by both serum biomarkers and tissue histology.

## CONSENT

Informed consent was used in the recruitment of the participants and confidentiality was maintained in accordance with standard medical practice.

## ETHICAL APPROVAL

Ethical approval was given by the Ethics Committees of Jos University Teaching Hospital, Plateau Specialist Hospital and ECWA Evangel Hospital Jos.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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