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Streptozotocin-deoxycorticosterone Acetate-induced Haematotoxicity, Hepatorenal Damage and Oxidative Stress in Diabetic-hypertensive rats: Protective Role of Losartan, Metformin and Glibenclamide Combination Therapy

Emuesiri Goodies Moke[1,2]*, Benneth Ben-Azu[1], Emuesiri Kohworho Umukoro[3], Peace Omewomano Onyilo[4], Paul Chinwuba[5], Onos Onoriode Emoyan[6], Kenneth Kelechi Anachuna[7], Ighodaro Igbe[2], Eric Kelly I. Omogbai[2]

[1]Department of Pharmacology, Faculty of Allied Health Sciences, Delta State University, Abraka, Nigeria.
[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.
[3]Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, Delta State University, Abraka, Nigeria.
[4]Department of Anatomy and Cell Biology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria.
[5]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria
[6]Department of Chemistry, Faculty of Sciences, Delta State University, Abraka, Nigeria.
[7]Department of Physiology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria.

Corresponding author*
hiligoodies@gmail.com

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Abstract

Diabetes mellitus and hypertension frequently coexist, exacerbating organ damage. This study investigated the protective effects of combination therapy with losartan (L), metformin (M), and glibenclamide (G) against Streptozotocin-deoxycorticosterone acetate (STZ/DOCA)-induced injuries in a diabetic-hypertensive rat model. Male Wistar rats were divided into five groups: normal control, STZ/DOCA control, and three treatment groups receiving L+M (LM), L+G (LG), or L+M+G (LMG). Hematological, biochemical, oxidative stress, and histopathological analyses were performed after 8 weeks. STZ/DOCA administration induced significant hematotoxicity, hepatorenal dysfunction, and oxidative stress, evidenced by altered blood parameters, elevated liver enzymes (AST, ALT), increased urea, creatinine, and malondialdehyde (MDA), alongside reduced antioxidants (GSH, SOD). Histopathology revealed severe hepatic and renal damage. All treatments provided protection, with the LMG combination showing the most comprehensive amelioration of all measured parameters and the best preservation of liver and kidney architecture. Triple therapy with losartan, metformin, and glibenclamide demonstrated superior synergistic protection against combined diabetic-hypertensive organ damage, highlighting its potential therapeutic promise for this complex comorbidity.

Keywords: STZ/DOCA; Haematotoxicity; Hepatorenal damage; Oxidative stress; Diabetic-hypertensive; Losartan.

INTRODUCTION

Diabetes mellitus and hypertension are chronic metabolic disorders that frequently coexist, leading to adverse effects on multiple organ systems, particularly the liver and kidneys (Jovanović et al, 2017). The combination of these conditions exacerbates oxidative stress, haematotoxicity, and tissue damage, contributing significantly to morbidity and mortality worldwide (Al-Amarat et al, 2021). Experimental models that mimic diabetic-hypertensive states are essential for understanding pathophysiological mechanisms and evaluating potential therapeutic interventions (Tong et al, 2019).

Streptozotocin (STZ) is widely used to induce diabetes in animal models by selectively destroying pancreatic β-cells, resulting in hyperglycaemia (Mcneill, 1999). Deoxycorticosterone acetate (DOCA) salt administration, often combined with uninephrectomy, is a well-established model for inducing hypertension that closely resembles secondary hypertension in humans (Furman, 2021). Thus, the STZ/DOCA combination provides a relevant model for studying the interplay between diabetes and hypertension and their cumulative effects on haematological parameters, hepatorenal function, and oxidative stress (Murakami et al. 2022).

Oxidative stress plays a pivotal role in the pathogenesis of diabetic and hypertensive complications by promoting lipid peroxidation and impairing antioxidant defense systems (Caturano et al, 2023). This leads to cellular injury and organ dysfunction, particularly in the liver and kidneys, which are highly

susceptible to metabolic and hemodynamic disturbances. Hematotoxicity, characterized by alterations in blood cell counts and hemoglobin levels, further complicates the clinical picture by impairing oxygen transport and immune responses (Imenshahidi et al, 2024).

Pharmacological agents such as losartan, an angiotensin II receptor blocker, and antidiabetic drugs such as metformin and glibenclamide have been reported to exert protective effects against oxidative stress and organ damage (Mulinari-Santos et al, 2018). Losartan mitigates hypertension-induced oxidative injury and renal dysfunction by inhibiting the renin-angiotensin system (Pan et al, 2020). Metformin improves glycemic control and exhibits antioxidant properties, whereas glibenclamide, a sulfonylurea, stimulates insulin secretion and is associated with weight gain (Rahimi et al, 2021). Combination therapy with these drugs may offer synergistic benefits in attenuating the deleterious effects of diabetic-hypertensive conditions (Baker et al., 2021).

This study investigated the protective role of losartan, metformin, and glibenclamide combination therapy against STZ/DOCA-induced hematotoxicity, hepatorenal damage, and oxidative stress in diabetic hypertensive rats, aiming to provide insights into potential therapeutic strategies for managing this complex comorbidity.

MATERIALS AND METHODS

Animals

Healthy adult male Wistar rats aged 12-15 weeks and weighing 220-280 g were randomly selected for this study. Animals were obtained from the Animal Facility of the Department of Pharmacology, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The animals were acclimatized for 14 days prior to the study and fed standard animal pellets (Chikun Feed Grower Pellet, Nigeria) and clean water *ad libitum*. Guidelines followed in the handling of animals were in accordance with the ethical standards of the 'National Institute of Health Guide for the Care and Use of Laboratory Animals' as adopted by the ethical committee of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria. Ethical approval was obtained prior to commencement of the experiment (ethical approval number: EC/FP/018/01).

Drugs

Streptozotocin, STZ (Santa Cruz Biotechnology, Texas, U.S.A; sc-200719); deoxycorticosterone acetate, DOCA (Santa Cruz Biotechnology, Texas, U.S.A; sc-239659); sodium chloride, NaCl (0.1%) (LOBA Chemie PVT Ltd, India); Losartan Potassium 50 mg (Santa Cruz Biotechnology, Texas, U.S.A; sc-204796A); metformin 500 mg (Santa Cruz Biotechnology, Texas, U.S.A; sc-202000B); glibenclamide 5 mg (Santa Cruz Biotechnology, Texas, U.S.A; sc-200982A).

Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ; 45 mg/kg) in sterile citrate buffer (0.1 M, pH 4.5) to fasted male Wistar rats (Aloud et al, 2016; Zhao et al, 2024). The diabetic state of the rats was checked after 72 h using a glucometer (ACCU-CHEK Active) and compatible blood glucose test strips, and animals with hyperglycemia with a fasting blood glucose level of ≥ 200 mg/dl were selected for the study (Aloud et al, 2016; Zhou et al, 2024).

Induction of hypertension (deoxycorticosterone acetate, DOCA-salt induced hypertension model)

The DOCA model was used to mimic the secondary form of hypertension (Robles-Vera et al, 2021). The uninephrectomy (UNX) was used. The rats were anesthetized by intraperitoneal injection of ketamine (75 mg/kg body weight). The skin above the left kidney was shaved, cleaned, and treated with an iodine-based antiseptic. The kidney was visualized using a left lateral abdominal incision (1 cm long), freed from the surrounding tissues, and gently pulled out. The left renal artery and ureter were tied with silk thread, and the left kidney was removed and weighed. The muscle and skin layers were closed separately using chromic sterile absorbable sutures. The animals were allowed to recover for one week. After the recovery period, UNX animals were administered twice weekly subcutaneous injections of DOCA-salt (25 mg/kg BW) in 0.4 mL of DMF (vehicle) solution, while NaCl solution (1%) was substituted for drinking water throughout the experimental period (8 weeks) (Edosuyi et al, 2021).

Experimental design

Group 1: Normal + Distilled water (10 ml/kg)
Group 2: STZ/DOCA + Distilled water (10 ml/kg)
Group 3: STZ/DOCA + LM
Group 4: STZ/DOCA + LG
Group 5: STZ/DOCA + LMG

[L-Losartan 50 mg/kg b.wt; M-Metformin 500 mg/kg b.wt; G-Glibenclamide 5 mg/kg b.wt; dosage were according to previous studies (Alotaibi et al, 2019; Lenart et al, 2019, de Oliveira et al, 2022)]

Measurement of body weight

The body weight of animals was measured weekly in grams to estimate the effect of induced diabetes and hypertension on body composition. Weight was measured using an electronic weighing balance.

Haematological and biochemical analysis

At the end of the 8-weeks experiment period, blood and organ samples were collected for haematological (RBC, WBC, Hb, and PCV) and biochemical screening, including liver function tests (AST, ALT, ALP, albumin, total protein, bilirubin), kidney function tests (urea,

creatinine, electrolytes), and oxidative assays (glutathione, catalase, SOD, and MDA) (Reitman and Frankel, 1957; Roy, 1970; Misra and Fridovich, 1972; Sinha, 1972; Tietz 1976; Ohkawa et al., 1979; Baker, et al. 1998; Ben-Azu et al., 2022; Tekeli et al, 2023; Chidebe et al., 2024; Moke et al., 2024)

Histology of organs

Liver and kidney tissue sections from all the groups were processed for histological examination (Szunyogova, 2016).

Statistical analysis and data presentation

All data obtained were expressed as mean ± standard error of mean (SEM) and analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Analyses were performed using GraphPad Prism version 8.0.1 (GraphPad Software, San Diego, CA, USA). Statistical significance was set at $p < 0.05$. The data are presented in the tables and graphs.

RESULTS

Effect of losartan, metformin, and glibenclamide on body weight (g) of STZ/DOCA diabetic-hypertensive rats

Table 1 shows the effects of losartan, metformin, and glibenclamide on the body weight of STZ/DOCA-diabetic hypertensive rats. There was a significant ($p < 0.05$) decrease in the body weight of the DH control from week 2 to week 8 compared with the normal control. Drug treatments with LM, LG, and LMG showed a decrease in body weight throughout the treatment period; however, LG and LMG showed significance ($p < 0.05$) across weeks 3 to 8 when compared with the DH control.

Table 1. Effect of losartan, metformin, and glibenclamide on body weight (g) of STZ/DOCA diabetic-hypertensive rats

Groups	Week 0	Week 8
Normal Control	237.20 ± 3.61	317.80 ± 9.17
DH Control	273.60 ± 6.23	167.40 ± 4.45*
DH+LM	261.80 ± 7.55	200.40 ± 12.42*
DH+LG	259.60 ± 10.79	235.00 ± 13.78$^{*\#}$
DH+LMG	265.00 ± 7.76	225.80 ± 3.20$^{*\#}$

*All values are expressed as mean ± standard error of mean (SEM); n=5, *p < 0.05 when compared with normal control; #p < 0.05 when compared with diabetic-hypertensive control. (DH Control – Diabetic-hypertensive Control, DH – Diabetic-hypertensive Rats, LM – Losartan+Metformin, LG – Losartan+Glibenclamide, LMG – Losartan+Metformin+Glibenclamide).*

Effect of losartan, metformin and glibenclamide on haematological parameters of STZ/DOCA diabetic-hypertensive rats after 8 weeks of treatment

Table 2 shows the effects of losartan, metformin, and glibenclamide on the haematological parameters of STZ/DOCA-diabetic-hypertensive rats. There was a significant ($p < 0.05$) increase in the WBC count in the DH control group when compared with the normal control group. Drug treatments with LM, LG, and LMG resulted in a significant ($p < 0.05$) decrease in white blood cell (WBC) count when compared with the DH control. The red blood cell (RBC) count was significantly ($p < 0.05$) lower in the DH control group than in the normal control group. Treatment with LM, LG, and LMG increased the RBC count significantly ($p < 0.05$) when compared with the DH control.

There was a significant ($p < 0.05$) increase in platelet count in the DH control group compared with the normal control group. Treatment with LG revealed a non-significant ($p > 0.05$) decrease in platelet count, whereas LM and LMG treatments showed a significant ($p < 0.05$) decrease in platelet count when compared with the DH control. There was a significant ($p < 0.05$) decrease in packed cell volume (PCV) in the DH control group compared with the control group. LM, LG, and LMG showed a significant ($p < 0.05$) increase in PCV compared to the DH control. Haemoglobin (Hb) concentration was significantly ($p < 0.05$) lower in the DH control group than in the normal control group. Treatment with LM, LG, or LMG increased Hb concentration non-significantly ($p > 0.05$) when compared with the DH control.

Table 2. Effect of losartan, metformin and glibenclamide on haematological parameters of STZ/DOCA diabetic-hypertensive rats.

Groups	White Blood Cell Count (WBC) ($\times 10^9$/L)	Red Blood Cell Count (RBC) ($\times 10^{12}$/L)	Platelet Count ($\times 10^9$/L)	Packed Cell Volume (PCV) (%)	Haemoglobin (Hb) (g/dl)
Normal Control	5.44 ± 0.09	4.50 ± 0.15	172.00 ± 15.94	46.20 ± 2.03	14.18 ± 1.30
DH Control	8.38 ± 0.42*	3.54 ± 0.14*	239.60 ± 2.01*	32.00 ± 1.48*	8.06 ± 0.82*
DH+LM	6.12 ± 0.13#	4.32 ± 0.17#	158.80 ± 22.25#	44.60 ± 1.40#	10.36 ± 1.30
DH+LG	6.06 ± 0.07#	4.16 ± 0.10#	185.20 ± 14.57	42.00 ± 2.07#	10.20 ± 1.38
DH+LMG	6.06 ± 0.31#	4.40 ± 0.12#	156.80 ± 18.64#	42.20 ± 1.95#	10.32 ± 1.35

*All values are expressed as mean ± standard error of mean (SEM); n=5, *$p < 0.05$ when compared with normal control; #$p < 0.05$ when compared with diabetic-hypertensive control. (DH Control – Diabetic-hypertensive Control, DH – Diabetic-hypertensive Rats, LM – Losartan+Metformin, LG – Losartan+Glibenclamide, LMG – Losartan+Metformin+Glibenclamide).*

Effect of losartan, metformin and glibenclamide on liver function parameters of STZ/DOCA diabetic-hypertensive rats after 8 weeks of treatment

Table 3 shows the effects of losartan, metformin, and glibenclamide on liver function parameters in STZ/DOCA diabetic hypertensive rats. There was a significant ($p < 0.05$) increase in aspartate transaminase (AST) levels in the DH control group compared with the normal control group. Drug treatments with LM, LG, and LMG resulted in a significant ($p < 0.05$) decrease in AST compared with the DH control. Alanine transaminase (ALT) levels were significantly ($p < 0.05$) higher in the DH control group than in the normal control group. Treatment with LM, LG, and LMG significantly decreased ALT levels ($p < 0.05$) compared with the DH control. There was a significant ($p < 0.05$) increase in alkaline phosphatase (ALP) levels in the DH control group compared with the normal control group. Treatments with LM, LG, and LMG showed non-significant ($p > 0.05$) decreases in ALP compared with the DH control.

There was a significant ($p < 0.05$) decrease in albumin levels in the DH control group when compared with the normal control group. Treatment with LM, LG, and LMG showed a significant ($p < 0.05$) increase in albumin when compared with the DH control. The total protein levels were not significantly ($p > 0.05$) lower in the DH control group than in the normal control group. Treatment with LM, LG, and LMG increased total protein non-significantly ($p > 0.05$) when compared with the DH control. There was a significant ($p < 0.05$) increase in bilirubin levels in the DH control group compared with the normal control group. Treatment with LM, LG, and LMG showed a significant ($p < 0.05$) decrease in bilirubin when compared with the DH control.

Table 3. Effect of losartan, metformin and glibenclamide on liver function parameter of STZ/DOCA diabetic-hypertensive rats.

Groups	Aspartate transaminase (AST) (IU/L)	Alanine transaminase (ALT) (IU/L)	Alkaline phosphatase (ALP) (IU/L)	Albumin (g/dL)	Total Protein (g/dL)	Bilirubin (mg/dL)
Normal Control	19.40 ± 0.24	6.36 ± 0.34	48.82 ± 0.68	3.06 ± 0.02	5.18 ± 0.52	0.53 ± 0.01
DH Control	26.00 ± 0.63*	10.60 ± 0.85*	72.14 ± 3.34*	2.16 ± 0.06*	3.90 ± 0.44	0.80 ± 0.10*
DH+LM	19.20 ± 0.80#	6.04 ± 1.04#	52.42 ± 2.81	2.70 ± 0.18#	4.84 ± 0.13	0.52 ± 0.01#
DH+LG	18.00 ± 0.83#	6.84 ± 1.17#	51.02 ± 3.12	2.84 ± 0.14#	5.06 ± 0.16	0.54 ± 0.03#
DH+LMG	20.40 ± 0.67#	6.02 ± 0.41#	50.16 ± 1.52	2.78 ± 0.14#	4.80 ± 0.25	0.51 ± 0.01#

*All values are expressed as mean ± standard error of mean (SEM); n=5, *$p < 0.05$ when compared with normal control; #$p < 0.05$ when compared with diabetic-hypertensive control. (DH Control – Diabetic-hypertensive Control, DH – Diabetic-hypertensive Rats, LM – Losartan+Metformin, LG – Losartan+Glibenclamide, LMG – Losartan+Metformin+Glibenclamide).*

Effect of losartan, metformin and glibenclamide on kidney function parameters of STZ/DOCA diabetic-hypertensive rats after 8 weeks of treatment

Table 4 shows the effects of losartan, metformin, and glibenclamide on the kidney function parameters of STZ/DOCA-diabetic-hypertensive rats. There was a non-significant ($p > 0.05$) increase in creatinine levels in the DH control group when compared with the normal control group. Drug treatments with LM, LG, and LMG showed non-significant ($p > 0.05$) decreases in creatinine levels when compared with the DH control. Serum urea levels were significantly ($p < 0.05$) higher in the DH control group than in the normal control group. Treatment with LM, LG, and LMG significantly ($p < 0.05$) decreased urea levels when compared with the DH control.

There was a significant ($p < 0.05$) decrease in serum calcium levels in the DH control group compared with the normal control group. Drug treatments with LM, LG, and LMG resulted in a significant ($p < 0.05$) increase in

calcium when compared with the DH control. Serum sodium levels were not significantly ($p > 0.05$) lower in the DH control group than in the normal group. Treatment with LM, LG, and LMG did not significantly ($p > 0.05$) increase sodium when compared with the DH control. There was a significant ($p < 0.05$) increase in serum chloride levels in the DH control group when compared with the normal control group. Drug treatments with LM, LG, and LMG resulted in a significant ($p < 0.05$) decrease in chloride when compared with the DH control. Serum bicarbonate levels were not significantly ($p > 0.05$) lower in the DH control group than in the normal group. Treatments with LM, LG, and LMG did not significantly ($p > 0.05$) increase bicarbonate levels when compared with the DH control.

Table 4. Effect of losartan, metformin and glibenclamide on kidney function parameter of STZ/DOCA diabetic-hypertensive rats.

Groups	Creatinine (mg/dL)	Urea (mg/dL)	Ca^{2+} (mg/dL)	Na^{+} (mmol/L)	Cl^{-} (mmol/L)	HCO_3^{-} (mmol/L)
Normal Control	1.00 ± 0.26	24.74 ± 1.76	9.82 ± 0.37	138.40 ± 4.68	95.40 ± 2.90	26.80 ± 0.73
DH Control	1.97 ± 0.32*	36.86 ± 2.40*	6.00 ± 0.38*	130.40 ± 3.77	111.20 ± 2.93*	26.20 ± 0.73
DH+LM	1.39 ± 0.20	27.30 ± 2.29#	8.72 ± 0.72#	136.00 ± 3.67	86.00 ± 4.35#	27.40 ± 0.60
DH+LG	1.50 ± 0.16	28.02 ± 1.94#	8.98 ± 0.22#	132.40 ± 2.20	91.40 ± 0.87#	26.80 ± 0.73
DH+LMG	1.53 ± 0.38	27.45 ± 2.00#	7.94 ± 0.34#	133.80 ± 2.93	94.20 ± 1.56#	27.40 ± 0.60

*All values are expressed as mean ± standard error of mean (SEM); n=5, *p < 0.05 when compared with normal control; #p < 0.05 when compared with diabetic-hypertensive control. (DH Control – Diabetic-hypertensive Control, DH – Diabetic-hypertensive Rats, LM – Losartan+Metformin, LG – Losartan+Glibenclamide, LMG – Losartan+Metformin+Glibenclamide).*

Effect of losartan, metformin and glibenclamide on antioxidant parameters of STZ/DOCA diabetic-hypertensive rats after 8 weeks of treatment

The effect of losartan, metformin, and glibenclamide on the antioxidant parameters of STZ/DOCA-diabetic-hypertensive rats is illustrated in Fig. 1. There was a significant ($p < 0.05$) increase in malondialdehyde (MDA) levels in the DH control group compared with the normal control group. Only drug treatments with LM and LMG showed a significant ($p < 0.05$) decrease in MDA compared with the DH control. Glutathione (GSH), and superoxide dismutase (SOD) levels were significantly ($p < 0.05$) lowered in the DH control group than in the normal control group. Treatment with LM and LMG significantly ($p < 0.05$) increased GSH levels compared to the DH control. Whereas, was no significant change in catalase activity.

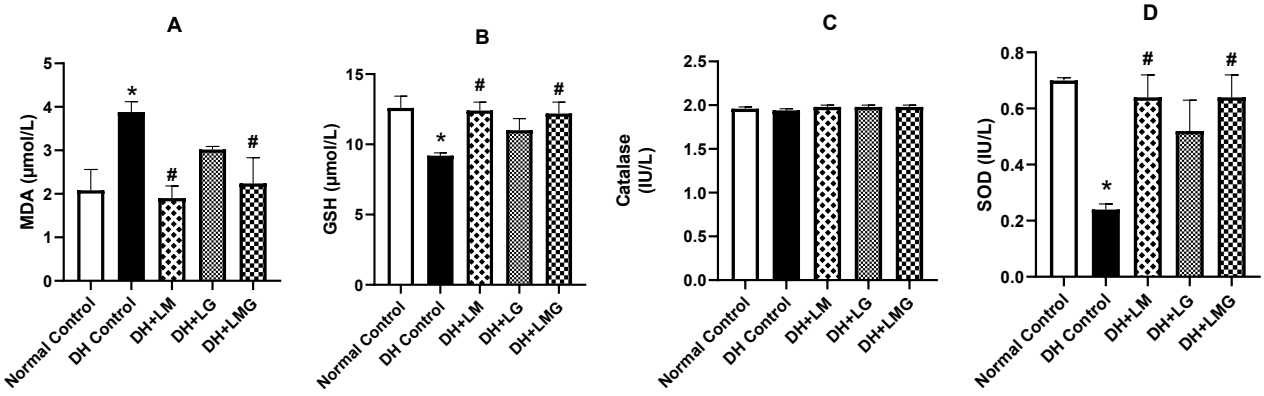

Figure 1. Losartan, metformin and glibenclamide combination therapy inhibit oxidative stress in STZ/DOCA-diabetic hypertensive rats: (A) Malondialdehyde (MDA), (B) Glutathione (GSH), (C) Catalase (CAT) (D) Superoxide dismutase (SOD). All values are expressed as Mean ± *SEM*, (n = 5), *$p < 0.05$ when compared with the normal control group while #$p < 0.05$ was significant when compared with the diabetic-hypertensive control group.

Effect of losartan, metformin and glibenclamide on the histology of the liver of STZ/DOCA diabetic-hypertensive rats after 8 weeks of treatment

Effects of losartan, metformin, and glibenclamide on the liver histology of STZ/DOCA-diabetic hypertensive rats are shown in Fig. 2. **Group 1** (normal control) showed no observable lesions of hepatocytes; **group 2** (DH control) showed severe atrophy of hepatic cords (red arrows), hepatocytes, and accentuation of sinusoids; **group 3** (LM) showed moderate centrilobular coagulation necrosis (black arrows) and Kupffer cell hyperplasia; **group 4** (LG) showed moderate centrilobular hepatocellular atrophy (black arrows) and Kupffer cell hyperplasia; **group 5** (LMG) showed mild

centrilobular hepatocellular coagulation necrosis (black arrow) and Kupffer cell hyperplasia (red arrow).

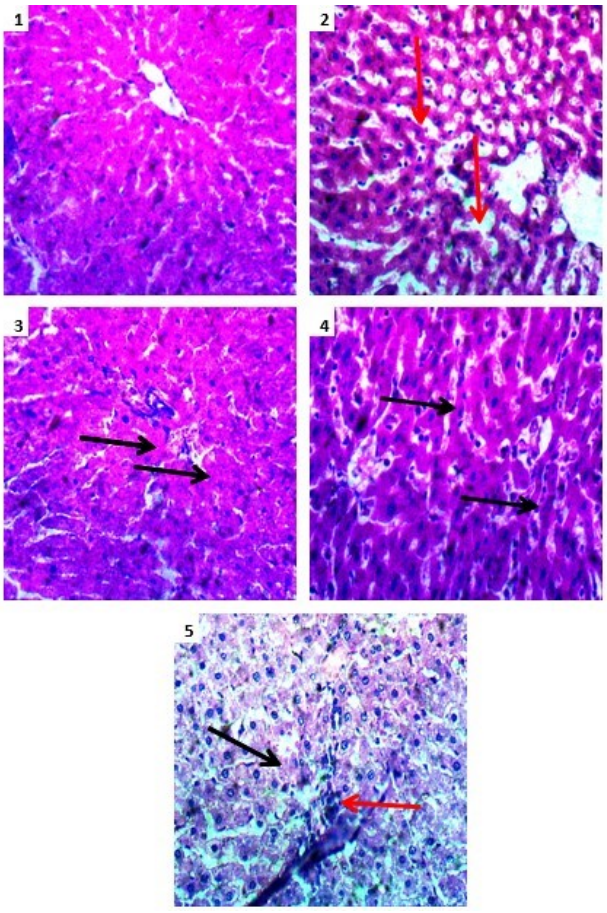

Figure 2. Effect of losartan, metformin and glibenclamide on the histology of the liver of STZ/DOCA diabetic-hypertensive rats. (Magnification = ×400) *(1 – Normal Control; 2 – DH Control; 3 – DH+LM; 4 – DH+LG; 5 – DH+LMG)*

Effect of losartan, metformin and glibenclamide on the histology of the kidney of STZ/DOCA diabetic-hypertensive rats after 8 weeks of treatment

Figure 3 illustrates the effects of losartan, metformin, and glibenclamide on the histology of the kidneys of STZ/DOCA-diabetic hypertensive rats. **Group 1** (normal control) showed no observable renal cell lesions; **group 2** (DH control) showed glomerular atrophy and patchy tubular epithelial necrosis (black arrow); **group 3** (LM) showed hyperplasia of mesangial cells (red arrow) and dilation of Bowman's space; **group 4** (LG) showed patchy tubular epithelial coagulation necrosis and inflammation (red arrows); **group 5** (LMG) showed no observable lesion of renal cells.

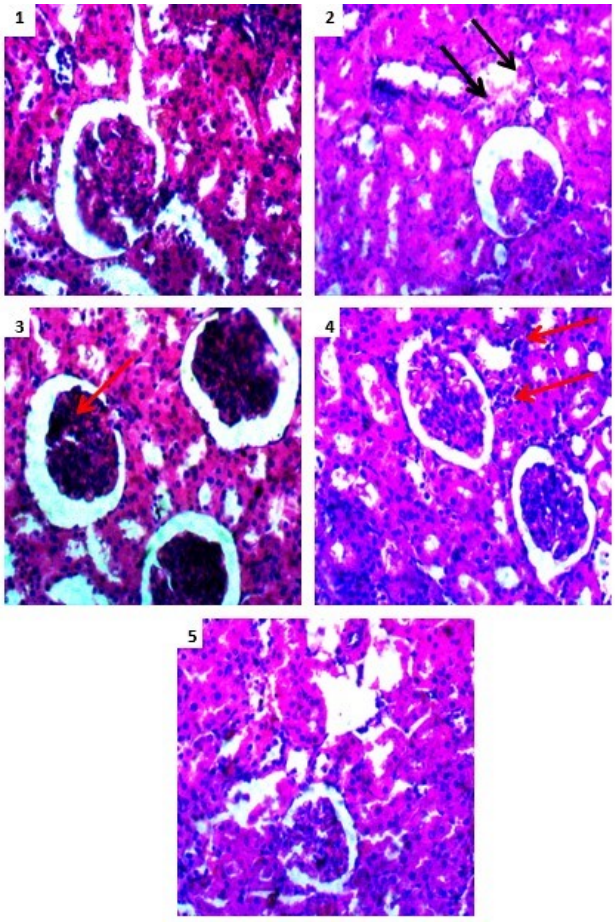

Figure 3. Effect of losartan, metformin and glibenclamide on the histology of the kidney of STZ/DOCA diabetic-hypertensive rats. (Magnification = ×400) *(1 – Normal Control; 2 – DH Control; 3 – DH+LM; 4 – DH+LG; 5 – DH+LMG)*

DISCUSSION

Experimental models that mimic diabetic-hypertensive states are essential for understanding pathophysiological mechanisms and evaluating potential therapeutic interventions (Tong et al, 2019; Moke et al, 2023a; Moke et al, 2023b). Deoxycorticosterone acetate (DOCA) salt administration, often combined with uninephrectomy, is a well-established model for inducing hypertension that closely resembles secondary hypertension in humans (Basting and Lazartigues, 2017; Furman, 2021). DOCA-salt induced hypertension in animal models is a hypertensive model most characteristic of human cardiovascular remodeling (Robles-Vera et al, 2021). DOCA induces hypertension via the endocrine system (Basting and Lazartigues, 2017). It increases blood pressure through oxidative stress and impairment of renal function by increasing mineralocorticoids, with a subsequent increase in nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and superoxide production (Li et al, 2023). DOCA increases aldosterone levels, which results in increased reabsorption of sodium ions and water from epithelial cells in the distal nephron

of the kidney to increase blood pressure (Tsilosani et al., 2022).

STZ/DOCA significantly ($p < 0.05$) decreased the body weight of DH control rats by 38.8%, indicating that it interfered with the normal body growth of rats. Drug treatments significantly ($p < 0.05$) inhibited the reduction in body weight by 9.5% in LG and 14.8% in LMG, but not in LM. The combination of glibenclamide resulted in an increase in body weight. This revealed that glibenclamide, like every other sulfonylureas antidiabetic agent, is associated with weight gain (Cheng and Kashyap, 2011).

White blood cell (WBC) counts and platelet counts of the diabetic-hypertensive control (DH control) rats were significantly increased ($p < 0.05$). Red blood cell (RBC) counts, packed cell volume (PCV), and hemoglobin (Hb) concentrations were significantly ($p < 0.05$) decreased, likely indicating an anemic-induced state. Increased WBC count is an indication of the immune response to inflammation and oxidative stress caused by STZ/DOCA administration (Almasoudi et al, 2025). Increased platelet counts in the DH control group could be attributed to platelet activation, which has been associated with cardiovascular morbidity and mortality (Qiao et al, 2020). Increased platelet activation and aggregation are involved in the pathogenesis of elevated blood pressure and diabetes (Kohlmorgen et al, 2021). Drug treatments showed a significant ($p < 0.05$) reduction in the elevated WBC count while increasing RBC and PCV, possibly by reducing erythrocyte lysis. Treatment with LM and LMG resulted in a significant ($p < 0.05$) reduction in elevated platelet levels. Metformin combinations proved to be more effective, as metformin has been reported to exhibit anti-atherogenic effects by decreasing platelet indices (such as mean platelet volume) that appear to play an essential role at the beginning of atherosclerosis development (Hlapčić et al, 2019). Drug-treated rats had no significant effect on hemoglobin concentration.

Aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) are liver-specific enzymes used to estimate the extent and type of liver damage (Chen et al, 2017; Pazoki et al, 2021). Damage to the liver architecture and vacuolation in diabetic hypertensive conditions are an indication of hepatic fatty infiltration and hepatocellular injury (Zeng et al, 2021). In this study, the serum levels of AST, ALT, and ALP were significantly increased ($p < 0.05$) in STZ/DOCA rats, which is clear evidence of liver damage. STZ/DOCA also induced hypoalbuminemia and increased the bilirubin levels. Drug treatment remarkably reduced transaminase enzymes and non-significantly reduced ALP levels. There was a reversal of serum albumin and bilirubin levels, which was evident with drug treatment. This demonstrates the repair of STZ/DOCA-induced liver damage by drug treatment combinations.

A considerable increase in serum urea and creatinine levels by STZ/DOCA reflects the altered regulation of renal function in diabetic-hypertensive states (Qiao et al, 2020). This indicated kidney damage caused by oxidative stress. Decreased serum calcium and sodium ion contents were evident in STZ/DOCA rats, with a significant ($p < 0.05$) increase in serum chloride levels. Drug treatments reduced the raised serum urea level and restored electrolyte balance by increasing serum calcium levels and decreasing chloride serum levels. No effect was observed on the serum bicarbonate level. This revealed the renoprotective activity of the combination of losartan, metformin, and glibenclamide (Alotaibi et al, 2018; Pan et al, 2020).

STZ/DOCA increased malondialdehyde (MDA) and reduced serum levels of the antioxidant enzymes glutathione (GSH) and superoxide dismutase (SOD). Increased lipid peroxidation has been reported in a DOCA-salt induction model of hypertension, resulting in increased oxidative stress (Galisteo et al, 20004). Only LM and LMG treatment inhibited the increase in oxidative stress markers. The antioxidant effects of losartan, metformin, and glibenclamide have been previously reported (Alotaibi et al, 2018).

Histopathological results showed that the diabetic-hypertensive state induced by STZ/DOCA generated oxidative stress, which led to tissue damage in the liver and kidney organs. This is depicted in severe hepatocellular degeneration and glomerular atrophy necrosis of tubular epithelial cells (Figs. 2 and 3). Treatment with losartan, metformin, and glibenclamide reduced these deleterious effects. In the STZ/DOCA model, maximal attenuation of tissue damage was more evident in the losartan combination with both metformin and glibenclamide than when the antidiabetics were used alone with losartan.

CONCLUSION

The present study underscores the significant protective efficacy of combination therapy with losartan, metformin, and glibenclamide against compounded haematological, hepatorenal, and oxidative insults induced by a diabetic-hypertensive state in an STZ/DOCA rat model. Although all dual and triple therapeutic regimens conferred measurable benefits, the triple combination (LMG) demonstrated unequivocal superiority in providing a comprehensive protective profile. LMG therapy was most effective in attenuating the model-induced pathogenic triad. It robustly countered hematotoxicity by normalizing erythrocyte and leukocyte indices, mitigating hepatorenal dysfunction by restoring near-normal levels of key enzymatic and functional biomarkers, and alleviated systemic oxidative stress by significantly enhancing antioxidant defenses while reducing lipid peroxidation. Crucially, histopathological evidence strongly supported these biochemical findings,

with the LMG group showing the most pronounced preservation of the hepatic and renal tissue architecture.

These preclinical findings suggest promising therapeutic synergy when an antihypertensive agent (losartan) is combined with antidiabetic drugs with complementary mechanisms (metformin and glibenclamide). The superior multi-organ protection offered by the LMG combination highlights its potential clinical relevance as a strategic therapeutic approach for managing the complex interplay between diabetes and hypertension, potentially targeting not only the primary metabolic and hemodynamic disorders but also their consequent oxidative and inflammatory tissue damage. Further investigation is warranted to translate these promising results into clinical applications in patients with this high-risk comorbidity.

Declarations: Before the experiment began, ethical approval was obtained from the ethical committee of the Faculty of Pharmacy at the University of Benin, Benin City, Nigeria (ethical number: EC/FP/018/01).

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Human and animal rights: Guidelines followed in the handling of animals were in accordance with the ethical standards of the 'National Institute of Health Guide for the Care and Use of Laboratory Animals' as adopted by the ethical committee of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria. Ethical approval was obtained prior to commencement of the experiment (ethical approval number: EC/FP/018/01).

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