

## Original Article



# Valsartan and Amlodipine Improved Haematological and Biochemical Indices of Vildagliptin-treated Streptozotocin-induced Diabetic Rats

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### Article info:

Received: 22 Feb 2025

Accepted: 25 Mar 2025

### Keywords:

Diabetes mellitus (DM), Hypertension, Vildagliptin, Amlodipine, Valsartan

## ABSTRACT

**Background:** Diabetes mellitus (DM) and hypertension often coexist in sufferers, which exacerbates the complications of DM. Also, the impacts of antihypertensive medications on the treatment outcomes of DM are not fully understood.

**Objectives:** Our objective was to evaluate the effect of valsartan and amlodipine on the hematological and biochemical differentials of vildagliptin-treated streptozotocin-induced diabetic rats.

**Methods:** DM was induced in male Wistar rats by a single intraperitoneal dose of streptozotocin (40 mg/kg body weight). The animals were grouped as non-diabetic rats (control), untreated diabetic rats, and diabetic rats treated with 5 mg/kg vildagliptin only, 5 mg/kg plus 30 mg/kg of vildagliptin and valsartan, respectively, and 5 mg/kg plus 2.5 mg/kg of vildagliptin and amlodipine, respectively. Drugs were administered orally, and rats were treated daily for three weeks. Then, blood samples were collected for hematological and biochemical analyses.

**Results:** The addition of amlodipine and valsartan as adjuncts to vildagliptin appeared to alter the evaluated differentials compared to vildagliptin treatment alone. Furthermore, the assessment of renal and liver functions, as well as the lipid profile, showed that treatment with valsartan as an adjunct improved the outcomes.

**Conclusion:** Our findings therefore suggest that amlodipine and valsartan brought about significant alterations in the differentials of diabetic rats treated with vildagliptin.

**Citation** Bolanle IO, Erharuyi O, Akhigbemen AM, Ugiagbe AI, Ekugum E, Nwusulor SO, et al. Valsartan and Amlodipine Improved Haematological and Biochemical Indices of Vildagliptin-treated Streptozotocin-induced Diabetic Rats. *Pharmaceutical and Biomedical Research*. 2025; 11(2):95-104. <http://dx.doi.org/10.32598/PBR.11.2.671.3>

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## Introduction

**D**iabetes mellitus (DM) is still a leading non-communicable cause of death globally, partly due to the increase in the aging population and unhealthy lifestyle changes brought on by rapid urbanization and westernization [1-3]. Since the last century, there have been advances in the management of DM; however, much more research is still required to fully comprehend the etiology of the disease, pharmacological treatment, and other associated pathologies and risk factors. Furthermore, DM and hypertension frequently co-exist in sufferers and are two leading risk factors for cardiovascular diseases, including coronary artery disease and atherosclerosis [4]. Up to 75% of adults living with DM also have hypertension, and patients with hypertension alone often exhibit insulin resistance [5]. Consequently, identifying overlapping disease mechanisms and pharmacological drug interactions will be a more proactive approach to controlling and preventing the complications of DM and hypertension.

Antihypertensive drugs can have a substantial impact on the likelihood that otherwise healthy individuals may develop type 2 DM (T2DM) or metabolic syndrome [6, 7]. For example, the prodiabetic potentials of diuretics and beta blockers have been suggested [8, 9]. Meanwhile, angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers may prevent diabetes more effectively than metabolically neutral calcium channel blockers [10, 11]. Therefore, the impact of co-administration of known antihypertensive drugs on the treatment outcomes of antidiabetic agents is not fully understood. As a proactive approach, this study assessed the effectiveness and treatment outcomes of amlodipine, valsartan, and vildagliptin combinations in the treatment of streptozotocin-induced diabetes in rats. Our focus was on the hematological and biochemical differentials, which are measures of organ function. The drugs for this study were rationally selected, with vildagliptin being the most prescribed and studied drug among the dipeptidyl peptidase-4 inhibitors [12]. Amlodipine and valsartan, on the other hand, are well-prescribed antihypertensive drugs that block calcium channels and angiotensin II receptors, respectively, and are not notorious for hypotension [13-15].

## Aim

This study aimed to evaluate the effect of valsartan and amlodipine on the hematological and biochemical differentials of vildagliptin-treated streptozotocin-induced diabetic rats.

## Materials and Methods

### Materials

#### Drugs and chemicals

Generic samples of drugs (vildagliptin, amlodipine, and valsartan) in the form of tablet dosage forms were obtained from community pharmacies in Benin City, Nigeria. The drugs were dissolved in distilled water for oral administration using a gastric tube. Sodium citrate, citric acid, and streptozotocin were acquired from Santa Cruz Biotechnology. Wet reagent diagnostic kits were obtained from Randox Laboratories Ltd (UK).

### Animals

Male Wistar rats (200 – 350 g) were obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, [University of Benin](#), Benin City, Nigeria. The rats were kept in plastic cages and housed at room temperature, with controlled humidity and a light/dark cycle of 12 hours each. They were fed dry rodent pellet feed and allowed free access to water. The bedding materials (wood shavings) in the cages were changed daily. The bedding materials (wood shavings) of the cages were changed daily.

### Methods

#### Induction of DM

The animals were fasted overnight, and DM was induced by a single intraperitoneal injection of streptozotocin at a dose of 40 mg/kg body weight, dissolved in freshly prepared 0.1 M citrate buffer, pH 4.5. After administration, the animals were allowed free access to food and water. After 48 hours, the animals were tested for DM using the Accu-Chek® Active glucometer (Roche, USA), and any animal with a blood sugar level of  $\geq 200$  mg/dL was considered diabetic [16].

#### Experimental design

The animals were divided into five groups of four rats each and treated orally for three weeks as follows;

**Group 1:** Healthy animals with no treatment.

**Group 2:** Diabetic animals treated with 5 mg/kg of vildagliptin only.

**Group 3:** Diabetic animals treated with 5 mg/kg and 2.5 mg/kg of vildagliptin and amlodipine, respectively.

**Group 4:** Diabetic animals treated with 5 mg/kg and 30 mg/kg of vildagliptin and valsartan, respectively.

**Group 5:** Untreated diabetic animals.

### Determination of lipid profile

Total plasma cholesterol, high- and low-density lipoproteins, and triglycerides were estimated by collecting blood from the abdominal aorta in the rats under chloroform anesthesia after three weeks of treatment.

### Total cholesterol

Total cholesterol levels in plasma were determined by the enzymatic method using a wet reagent diagnostic kit (Randox Laboratories Ltd, UK), which is a modification of the method of Abell et al. [17].

### Total triglycerides

Total triglyceride levels were determined by the enzymatic method using wet reagent diagnostic kits (Randox Laboratories Ltd, UK), a modification of the method by Jacobs and Vandermark [18].

### High-density lipoprotein

High-density lipoprotein levels were determined by the enzymatic method using wet reagent diagnostic kits (Randox Laboratories Ltd UK) [19].

### Hematological analysis

Following three weeks of treatment, the animals were anesthetized with chloroform, and blood samples were obtained from the aorta after dissecting the animals. The blood samples were transferred into ethylene diamine tetra-acetic acid (EDTA) sample bottles and analyzed immediately after blood collection using the Human Automated Haematology System analyzer (ERMA PCE 210, ERMA, Japan). The parameters analyzed included haemoglobin (Hb), packed cell volume (PCV), white blood cell count (WBC) and differential count (granulocyte, lymphocyte, and monocyte), platelets (PLT), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), and random blood glucose [20].

### Biochemical analyses

The assays for alanine aminotransferase, aspartate amino transaminase, and alkaline phosphatase were carried out according to previous studies [21, 22]. The assay for electrolytes (sodium, chloride, bicarbonate, and potas-

sium) was done as described by Van Slyke and Neil [23]. Chloride ions were assayed as described by Schales and Schales [24]. The assay for sodium and potassium was done according to the method by Margoshes and Vallee [25]. The assay for bilirubin was carried out according to the method described by Schmidt and Schmidt [22], and proteins were assayed using the method described by Tietz [26].

### Data collection and statistical analysis

Statistical analysis was carried out using GraphPad Prism software, version 6 (San Diego, USA). Results were expressed as Mean±SE. Statistical analyses were performed using a one-way analysis of variance followed by the Dunnett post-hoc test for multiple comparisons. A P of ≤0.05 was considered significant.

## Results

Hematological differentials of the control, treated, and untreated diabetic groups

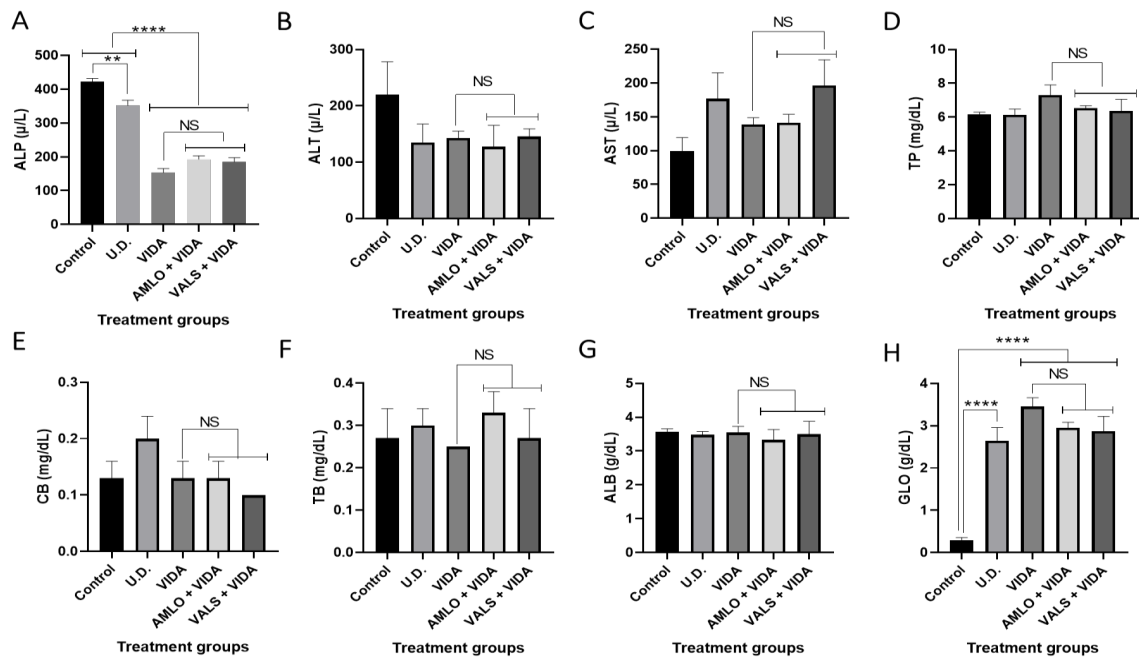
Tables 1, 2 and 3 show the mean hematological differentials of the rats in the treatment groups, the untreated diabetic rats, and the control group, compared to one another after 3 weeks of treatment.

### Effects of treatments on liver function

Figure 1 shows the mean values of some liver enzymes and indices of the rats in the treatment groups, untreated diabetic group, and control group compared to one another after three weeks of treatment. The results showed significant changes in liver function as follows: (i) a significant decrease ( $P<0.05$ ) in alkaline phosphatase in untreated diabetic rats and a decrease ( $P<0.0001$ ) in groups treated with vildagliptin, amlodipine plus vildagliptin, and valsartan plus vildagliptin, all compared to normal rats; (ii) a significant increase ( $P<0.0001$ ) in globulin in groups treated with vildagliptin alone, amlodipine plus vildagliptin, and valsartan plus vildagliptin, as well as in untreated diabetic rats, all compared to normal rats.

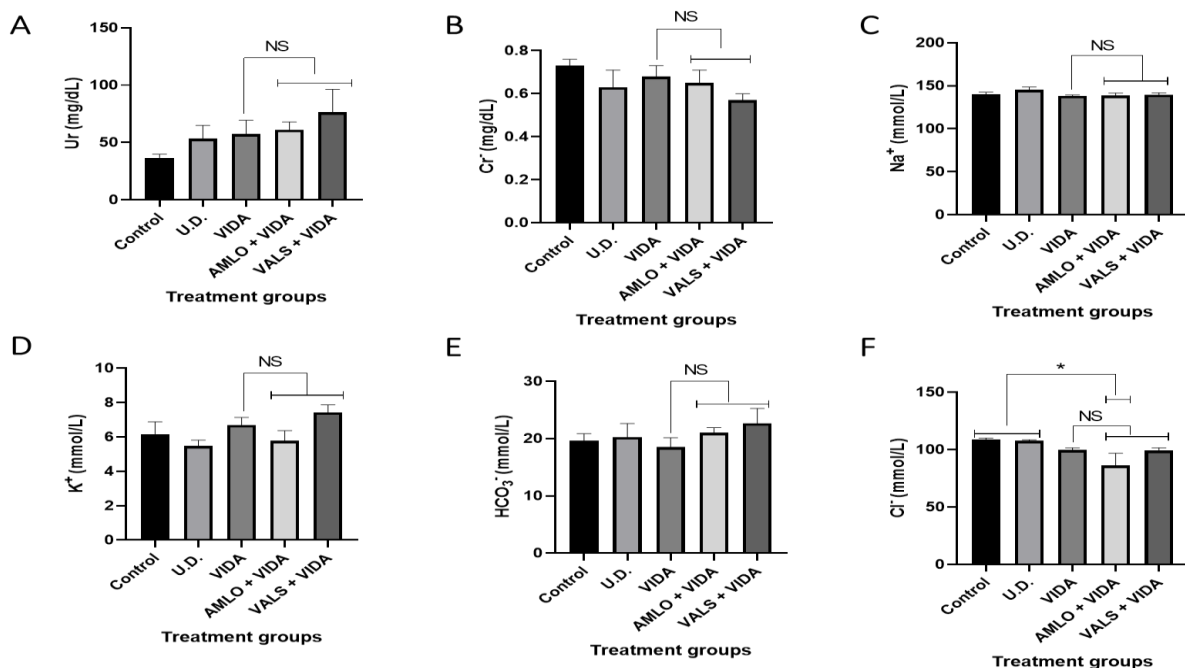
### Effects of treatments on renal function

Figure 2 shows the mean values of some renal function indices of the rats in the treatment groups, untreated diabetic group, and control group after three weeks of treatment compared to one another. The results showed a significant ( $P<0.05$ ) decrease in Cl<sup>-</sup> in the group treated with amlodipine and vildagliptin compared to both untreated diabetic and normal rats.


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**Figure 1.** Liver function indices of rats in various treatment groups, untreated diabetic rats, and normal rats compared to one another

Abbreviations: U. D: Untreated diabetic; VIDA: Vildagliptin; VALS: Valsartan; AMLO: Amlodipine; ALP: Alkaline phosphatase; ALT: Alanine transferase; AST: Aspartate transferase; TP: Total protein; CB: Conjugated bilirubin; TB: Total bilirubin; ALB: Albumin; GLO: Globulin. \*\*P<0.01, \*\*\*\*P<0.0001, n=4. Note: Values are Mean±SEM.


**PBR**

**Figure 2.** Renal function indices of rats in various treatment groups, untreated diabetic rats, and normal rats compared to one another

Abbreviations: U. D: Untreated diabetic; VIDA: Vildagliptin; VALS: Valsartan; AMLO: Amlodipine; Ur: Urea; Cr: Creatinine; Na<sup>+</sup>: Sodium; K<sup>+</sup>: Potassium; HCO<sub>3</sub><sup>-</sup>: Bicarbonate; Cl<sup>-</sup>: Chloride.

\*P<0.05, n=4. Note: Values are in Mean±SEM.

**Table 1.** Red blood cell differentials and platelet counts of the control, treated, and untreated diabetic groups

INDICES	Mean±SE				
	Control	U. D.	VIDA	AMLO+VIDA	VALS+VIDA
RBC (10 <sup>6</sup> /μL)	7.95±0.68	7.07±1.35	8.36±0.15	7.7±0.46	7.33±0.58
Hb (g/dL)	13.93±0.87	17.45±1.42	17.07±0.73	15.83±1.1	15.20±0.66
HCT (%)	42.23±3.93	48.58±1.46	51.35±2.05	42.68±1.97	48.47±1.3
PLT (10 <sup>3</sup> /μL)	383±126.06	439.25±65.01	702±122.98	543.75±89.04	423±14.29

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Abbreviations: U. D: Untreated diabetic; VIDA: Vildagliptin; VALS: Valsartan; AMLO: Amlodipine; RBC: Red blood cell; Hb: Haemoglobin; HCT: Haematocrit; PLT: Platelets. n=4.

### Effects of treatments on lipid profile

Figure 3 shows the mean values of the lipid profile of the rats in the treatment groups, the untreated diabetic group, and the control group after three weeks of treatment, all compared to one another. The results indicated a significant ( $P<0.05$ ) decrease as follows: (i) in high density lipoprotein (HDL), valsartan plus vildagliptin compared to the normal control, and (ii) in LDL, vildagliptin and valsartan plus vildagliptin, both compared to the normal control.

### Discussion

Exacerbated cardiovascular complications associated with the co-existence of DM and hypertension are major causes of the end-organ damage responsible for the mor-

talidity associated with DM [27-29]. The macrovascular and microvascular complications of DM and hypertension overlap in such a way that they may share common mechanisms [30]. Therefore, it is important to implement a multifactorial approach with concurrent emphasis on optimizing glycemic control and reducing the rate of pathological progression of diabetic complications, which will ultimately lower the mortality rate of diabetic patients. Hence, the focus of this study was to investigate the effect of valsartan and amlodipine on the hematological and biochemical differentials of vildagliptin-treated, streptozotocin-induced diabetic rats.

In this study, a single dose of streptozotocin (40 mg/kg body weight) was administered intraperitoneally for the induction of DM [7, 16, 20]. Streptozotocin is a structural analog of N-acetyl glucosamine that acts as a potent

**Table 2.** White blood cell differentials of the control, treated, and untreated diabetic groups

INDICES	Mean±SE				
	CONTROL	U. D.	VIDA	AMLO+VIDA	VALS+VIDA
WBC (10 <sup>3</sup> /μL)	12.20±2.8	10.88±1.35	7.08±0.13	11.58±2.18	7.63±0.68
LY (%)	81.4±5.31	89.3±0.64	66.73±4.69 <sup>b</sup>	86.75±2.85 <sup>**</sup>	86.63±2.05 <sup>**</sup>
MID (%)	6.80±4.08	6.8±1.43	17.3±1.07 <sup>b, a</sup>	11.10±1.45	9.1±1.36
GR (%)	9.45±1.69	3.9±1.02	14.1±3.86 <sup>b</sup>	3.55±0.48 <sup>*</sup>	5.6±0.64
LY (10 <sup>3</sup> /μL)	9.2±0.17	7.88±0.2	4.4±0.3 <sup>b, c</sup>	8.03±0.7 <sup>****</sup>	6.97±0.38 <sup>b, **</sup>
MID (10 <sup>3</sup> /μL)	1.3±0.06	0.65±0.03 <sup>i</sup>	1.23±0.09 <sup>a</sup>	1.03±0.11	0.63±0.24 <sup>i, *</sup>
GR (10 <sup>3</sup> /μL)	0.7±0.06	0.25±0.03	1.55±0.34 <sup>i, c</sup>	0.38±0.05 <sup>***</sup>	0.37±0.07 <sup>***</sup>

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Abbreviations: U. D: Untreated diabetic; VIDA: Vildagliptin; VALS: Valsartan; AMLO: Amlodipine. LY: Lymphocytes; MID: Mid-range absolute blood count; GR: Granulocytes.

<sup>\*</sup> $P<0.05$ , <sup>\*\*</sup> $P<0.01$ , <sup>\*\*\*</sup> $P<0.001$ , <sup>\*\*\*\*</sup> $P<0.0001$  compared to the group treated with vildagliptin; <sup>i</sup> $P<0.05$ , <sup>ii</sup> $P<0.01$ , <sup>iv</sup> $P<0.0001$  compared to the control group; <sup>a</sup> $P<0.05$ , <sup>b</sup> $P<0.01$ , <sup>c</sup> $P<0.001$  compared to the untreated diabetic group, n=4.

**Table 3.** Other haematological differentials of the control, treated, and untreated diabetic groups

INDICES	Mean±SE				
	Control	U. D.	VIDA	AMLO+VIDA	VALS+ VIDA
MCV (fl)	53.03±1.44	56.9±2.16	60.53±1.25	55.75±1.72	66.7±3.5 <sup>i, a</sup>
MCH (pg)	17.57±0.58	20.38±1.47	20.03±0.49	20.63±1.32	20.8±1.13
MCHC (g/dL)	33.23±1.16 <sup>a</sup>	35.75±1.95	33.63±0.28	37.03±1.75	31.33±1.3
RDW (fl)	36.5±0.58	33.83±0.92	38.83±0.75	34.15±1.24	42.27±1.93 <sup>i, b</sup>
PCT (%)	0.46±0.03	0.34±0.02	0.64±0.09 <sup>a</sup>	0.33±0.01 <sup>*</sup>	0.43±0.09
MPV (fl)	12.33±0.33	8.6±0.48 <sup>iii</sup>	10±0.27 <sup>i</sup>	7.23±0.05 <sup>iv, **</sup>	8.87±0.81 <sup>iii</sup>
PDW (%)	9.57±0.12	8.23±0.34	10.83±0.51 <sup>b</sup>	8.55±0.39 <sup>*</sup>	9.3±0.7

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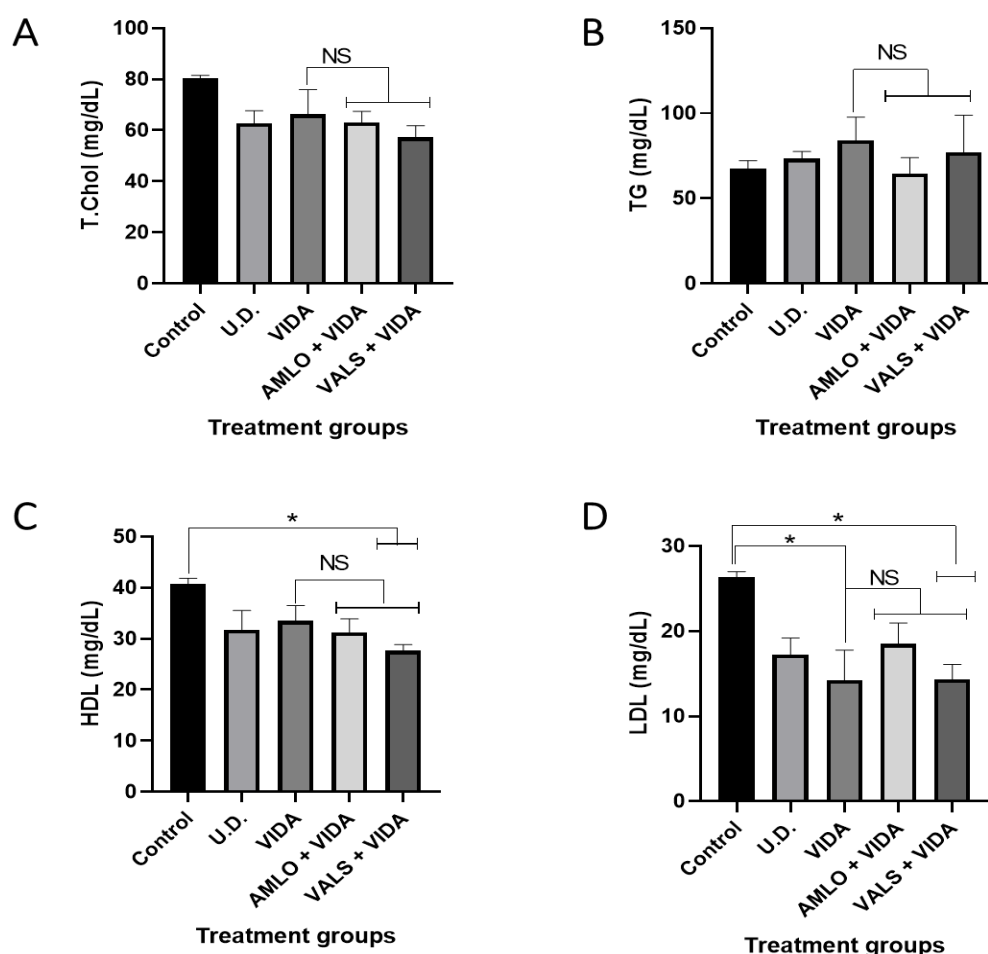
Abbreviations: U. D: Untreated diabetic; VIDA: Vildagliptin; VALS: Valsartan; AMLO: Amlodipine; MCV: Mean corpuscular volume; MCH: Mean corpuscular Hb; MCHC: Mean corpuscular Hb concentration; RDW: Red cell distribution width; MPV: mean platelet volume; PCT: plateletcrit; PDW: platelet distribution width. <sup>\*</sup>P<0.05, <sup>\*\*</sup>P<0.01 compared to the group treated with vildagliptin; <sup>i</sup>P<0.05, <sup>ii</sup>P<0.01, <sup>iii</sup>P<0.0001 compared to the control group; <sup>a</sup>P<0.05, <sup>b</sup>P<0.01 compared to the untreated diabetic group, n=4.

alkylating agent, resulting in disrupted glucose transport and glucokinase activity, as well as the breakdown of multiple DNA strands [31]. A single high dose STZ injection (>60 mg/kg body weight) leads to massive pancreatic beta cell destruction, which is a feature of type 1 DM. In contrast, intermediate dosages of STZ injections (between 40 and 55 mg/kg body weight) cause only partial impairment of insulin secretory mechanisms, leading to the hyperglycemic conditions present in type 2 DM (T2DM) [32].

Although not significant, there were marked increases in the RBC counts of the diabetic rats in the various treatment groups compared to untreated diabetic rats. Patients with diabetes over an extended period often experience anemia [33]. Hyperglycemia correlates with increased non-enzymatic glycosylation of RBC membrane proteins [34, 35]. Additionally, lipid peroxides, which cause hemolysis of RBC, are elevated due to the oxidation of RBC proteins and hyperglycemia in DM [36]. Furthermore, the hematological differentials showed that the groups treated with the antihypertensives, amlodipine, and valsartan, had slightly higher lymphocyte counts compared to those of the diabetic rats treated with vildagliptin only. This indicates that vildagliptin alone possesses good antidiabetic activity, as evidenced by a reduced lymphocyte count, suggesting regulated blood glucose control. Lymphocytes are generally increased in disease states as systemic markers of inflammation [37, 38]. In cases of poor glycemic control, as exhibited by

the untreated diabetic rats, the lymphocyte count was elevated. This can exacerbate the progression of diabetes to complications, increasing the risk of a first cardiovascular event in untreated but diagnosed diabetic subjects [39]. Also, MCV values are generally decreased in the diabetic state. This indicates abnormal Hb synthesis, failure of blood osmoregulation, and altered plasma osmolarity [40]. The increase in MCV and RDW values for the diabetic rats was more pronounced in those treated with valsartan plus vildagliptin compared to the control and untreated diabetic rats. The reason for this observation is currently unclear.

The biochemical analysis results for liver function tests showed a significant reduction in alkaline phosphatase enzymes for the groups treated with vildagliptin only, vildagliptin plus amlodipine, and valsartan plus vildagliptin compared to the control and untreated diabetic groups. Alkaline phosphatase is used as a diagnostic indicator for bone or liver disease [41]. Among the various complications associated with diabetes, diabetic liver disease, along with diabetic bone disease, indicates the severity of the condition, as revealed by another finding [42], which showed elevated serum levels of alkaline phosphatase that further validate this study. The decrease in the enzyme, as shown in this study, could be attributed to the ameliorated effects of diabetes by all the treatment groups. This suggests that the progression and complications of diabetes were slowed down. Additionally, our results indicated that globulin levels in all treatment groups and untreated dia-



**Figure 3.** Lipid profile of rats in various treatment groups, untreated diabetic rats, and normal rats compared to one another. Abbreviations: U. D: Untreated diabetic; VIDA: Vildagliptin; VALS: Valsartan; AMLO: Amlodipine. T. Chol: total cholesterol; TG: Triglyceride; HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

\*P<0.05, n=4.

Note: Values are in Mean±SEM.

abetic rats were significantly higher than those in the control group. Although the exact cause of this is unknown, live damage caused by DM may play a role, and this finding is consistent with previous studies [7, 20].

Analysis of renal function showed that chloride ion levels were reduced in the group treated with amlodipine plus vildagliptin compared to the control group. This observation may be due to the increased utilization of chloride ions in this group, with the treatment helping to ameliorate this effect. We have demonstrated similar findings in another study [7]. Additionally, lipid profile analysis showed that the addition of valsartan to vildagliptin caused a significant reduction in LDL levels when compared to the control group. The potential benefit of angiotensin receptor blockers, like valsartan as an effective adjunct therapy in the management of

hypertension with a hyperlipidemia component, as previously demonstrated [43-45], is further highlighted by this intriguing finding, although more research may be needed to validate it.

## Conclusion

Our results showed that the progression of diabetes was limited in the treated groups compared to the untreated diabetic group. Furthermore, the addition of valsartan and amlodipine, two known antihypertensives, to vildagliptin significantly improved certain hematological differentials and the lipid profile of diabetic rats. Valsartan, in particular, appeared to have a more pronounced overall effect than amlodipine when combined with vildagliptin. Therefore, valsartan may provide greater organ protection and reduce cardiovascular events in patients with T2DM,

leading to improved clinical outcomes. However, further research is strongly needed, especially concerning long-term treatment, to validate these findings.

## Ethical Considerations

### Compliance with ethical guidelines

The experiment was carried out following the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 2002. The Ethics Committee of the Faculty of Pharmacy, [University of Benin](#), granted ethical permission (reference number EC/FP/017/01) for this study.

### Funding

This work was supported by the Tertiary Education Trust Fund (TETFund) Institution-Based Research (IBR) Grant (Code: TETFund/DRSS/ESITMUSEN/2016/RP/VOL.1).

### Authors' contributions

Supervision, and project administration: Israel Olapeju Bolanle, Abigail M. Aghigbemen, Osayemwenre Erharuyi, and Abiodun Falodun; Formal analysis: Israel Olapeju Bolanle, Amenaghawon Imuetinyan Ugiagbe, Edobor Ekugum, Samuel O. Nwusulor, and Keziah E. Adudu; Conceptualization, writing the original draft: Israel Olapeju Bolanle; Review, editing: Israel Olapeju Bolanle, Abigail M. Aghigbemen, Osayemwenre Erharuyi, and Abiodun Falodun; Investigation, methodology and final approval: All authors.

### Conflict of interest

The authors declared no conflict of interest.

### Acknowledgments

The authors acknowledge the technical support of Philip Obarisiagbon and Collins Osaigbovo, both from the Department of Pharmacology and Toxicology, Faculty of Pharmacy, [University of Benin](#), Benin, Nigeria.

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