

Amlodipine and valsartan improving effect on the survival rate and deleterious pathological changes in streptozotocin-induced diabetic rats treated with metformin

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ARTICLE INFO

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Article history:

Received: Apr 18, 2019

Accepted: Jul 27, 2019

Keywords:

Type 2 diabetes,
biguanides,
hypertension,
calcium channel
blockers,
angiotensin II receptor
blockers

ABSTRACT

Diabetes mellitus (DM) and hypertension usually co-exist, and when this happens, the prognosis would be worse than each disease alone. Given this, we evaluated the possible effects of valsartan and amlodipine administration on metformin-treated diabetic rats models induced by streptozotocin.

Male Wistar rats (200–350 g) were fasted overnight. Then, we induced DM by administrating a single dose of 40 mg/kg streptozotocin (IP), which was confirmed after 48 h. Animals with blood sugar \geq 200 mg/dl were considered diabetic and divided into four diabetic groups of untreated diabetic animals (Group B), diabetic animals treated with metformin (Group C), diabetic animals treated with metformin plus amlodipine (Group D), and diabetic rats treated with metformin plus valsartan (Group E). There was also a group A, consisting of normal rats with no drug treatment. After six weeks of treatment, we sacrificed the animals under chloroform anesthesia, and their blood samples were collected for hematological and biochemical analyses.

The mortality rate in untreated diabetic rats was 100% before 6 weeks, but anti-diabetic treatment (metformin) significantly ($P < 0.05$) improved the survival rate and controlled their blood glucose level. The addition of antihypertensive drugs (amlodipine and valsartan) enhanced this curative effect. The various treated groups showed ameliorations in pathologic changes and biochemical indices, as well as, evidence of organ protection, compared with the untreated diabetic group.

The study showed that adding an antihypertensive drug (amlodipine or valsartan) to metformin regimen improved outcomes in diabetic rats compared to using metformin alone.

Citation: Pharm Biomed Res 2019;5(2): 25-31.

Introduction

Diabetes mellitus (DM) is a mixed group of disorders characterized by abnormalities in the carbohydrate, lipid, and protein metabolism. The primary problem in diabetes is an abnormality in insulin production or action or both. However, other factors can contribute to the disease, too. Hyperglycemia is the common endpoint of all types of DM, and its proper control determines the success of therapy (1). Insulin (a primary anabolic hormone) binds to specific receptors on the cells to induce several metabolic effects, and its deficiency will lead to this chronic metabolic disorder, DM, that is already a pandemic problem worldwide but affects the developing countries much more than their developed counterparts (2, 3). The prevalence of DM is increasing with the aging of the population, urbanization, and westernization (4). Diabetes Federation has reported the global number of diabetic patients as 381 million, and this number is expected to be doubled by 2030 (5). There is a significant variation across the age groups affected with DM, with the middle age groups (45-64 years) being more affected in developing countries, while the elderly age group (\geq 65 years) account for the majority of the cases in developed countries (6). DM and hypertension usually co-exist in patients and are two leading risk factors of

atherosclerosis and its complications (7). There is a substantial overlap between diabetes and hypertension with regard to their etiology and pathology. Obesity, oxidative stress, inflammation, and insulin resistance are thought to be common pathways. Prevention and treatment of these diseases would be much easier by knowing their common causes and disease mechanisms (7). In this regard, antihypertensive drugs can significantly influence the probability that otherwise, healthy individuals will develop metabolic syndrome or type 2 diabetes (8). Although inconclusive, there is some evidence that agents that interrupt the renin-angiotensin system provide greater protective effects. Given that diabetes is an important cardiovascular risk factor, it is possible to reduce this risk by lowering blood pressure, which offsets the increased risk of developing diabetes. Such concerns should be considered in the selection of antihypertensive therapy (7).

In a recent study, we reported that adding an antihypertensive drug, i.e., valsartan, but not amlodipine, as co-therapy to the anti-diabetic drug, i.e., glibenclamide, significantly improved the treatment outcome in diabetic rats (induced by streptozotocin) (9). In this study, we further aimed at evaluating the effects of antihypertensive drugs, mainly amlodipine and valsartan, combined with metformin in the management

of diabetic rats (induced by streptozotocin). The rationale implication of these findings for actual situations of comorbidity of diabetes and hypertension calls for further studies.

Materials and methods

Animals Study

Male Wistar rats weighing 200–350 g were obtained from the Animal House of 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 (24 °C) with constant humidity. They were fed with dry rodent pellet feeds (Top Feeds Limited, Ibadan, Nigeria) and allowed free access to water (borehole). The wood shavings were used as beddings of the cages that were changed daily. All experiments were carried out following the National Institute of Health Guidelines for the Care, and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 2002.

Induction of diabetes mellitus

The animals were fasted overnight. Then the diabetes mellitus was induced in them by injection of a single dose of intraperitoneal streptozotocin (40 mg/kg body weight) dissolved in freshly prepared 0.1 M citrate buffer (pH 4.5). After drug administration, the animals had free access to food and water. Forty-eight hours later, the animals were tested for diabetes using the Accu-Chek® Active glucometer (Roche, USA) and any animal with blood sugar level higher than 200 mg/dl was considered diabetic (10).

Experimental design

The animals were divided into five diabetic groups of at least 7 rats each and treated orally for 6 weeks as follow; Group A: Non-diabetic animals given 1 ml/kg distilled water daily.

Group B: Diabetic animals given 1 ml/kg of distilled water daily.

Group C: Diabetic animals treated with 500 mg/kg daily dose of metformin alone.

Group D: Diabetic animals treated with 500 mg/kg and 2.5 mg/kg daily dose of metformin and amlodipine.

Group E: Diabetic animals treated with 500 mg/kg and 30 mg/kg body dose of metformin and valsartan.

Measurement of blood glucose

Before 9 AM, the tail of the rat was cleaned with methylated spirit and allowed to dry. The lateral tail vein was pricked using a sterile lancet, and a droplet of the blood was placed on the glucose test strip and read by the Accu-chek® Active glucometer (11).

Assessment of change in body weight

The weights of the animals were measured weekly using a sensitive weighing balance, and the changes in weight during the experiment were recorded (12).

Hematological and biochemical analyses

After 6 weeks of treatment, the blood samples were obtained from the aorta of the rats under chloroform anesthesia into ethylenediaminetetraacetic acid (EDTA) and plain sample bottles for hematological and biochemical analyses, respectively. The samples were evaluated for changes in hematological parameters, liver function, renal function, and lipid profile.

Statistical analysis

The obtained data were expressed as mean ± standard error of the mean (SEM). The statistical analysis was done using 1-way analysis of variance followed by Dunnett's post hoc test (Graphpad Prism® 6, San Diego, USA). P value less than 0.05 was considered significant in all tests.

Ethical approval

The Faculty of Pharmacy, University of Benin Ethics Committee approved the study (reference number: EC/FP/017/01).

Results

Effects of treatments on survival rate

The normal control group and the untreated diabetic group had 100% and 0% survival rate, respectively while the group of diabetic rats treated with metformin plus amlodipine had the highest survival rate (38.5%) (Table 1).

Table 1 The survival rate of treated groups compared to control group and untreated diabetic groups.

Groups	n	Number of survived rats after 6 weeks	Percentage of survival (%)
Control	7	7	100
Diabetic untreated	18	NIL***	0
Diabetic treated by metformin	13	4	30.8
Diabetic treated with metformin and amlodipine	13	5	38.5
Diabetic treated with metformin and valsartan	11	4	36.4

*** Three untreated diabetic rats sacrificed in the fifth week.

Effects of treatments on blood glucose level

The result showed significant ($P < 0.05$) reduction in the blood glucose level in the various treatment groups of metformin-treated, metformin plus amlodipine-treated, and metformin plus valsartan-treated. Moreover, the blood glucose in the untreated diabetic group was consistently high while the blood glucose in the normal control remained within the normal glycemic range (Figure 1).

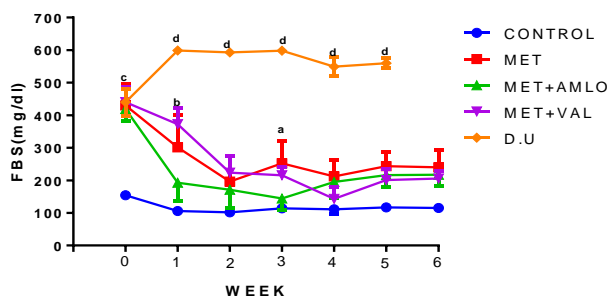


Figure 1 Blood glucose levels of treatment groups that include metformin and untreated diabetic group in comparison with the normal control group.

Abbreviation: FBS, Fasting blood sugar; D.U, diabetic untreated; MET, metformin treated; MET + AMLO, metformin, and amlodipine treated; MET + VAL, metformin, and valsartan treated. Values are presented as mean \pm SEM. ^aP < 0.05. ^bP < 0.01. ^cP < 0.001. ^dP < 0.0001. n = 4.

Effects of treatment on body weight

Treatments prevented significant ($P < 0.05$) loss in body weight in the treated groups compared with untreated diabetic rats, so there was a gradual increase in the body weight of the normal rats (Figure 2).

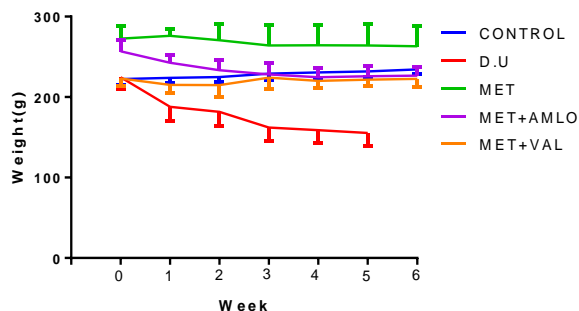


Figure 2 Weight changes of rats in treatment groups that include metformin and untreated diabetic group in comparison with the normal control group.

Abbreviations: D.U, diabetic untreated; MET, metformin treated; MET + AMLO, metformin, and amlodipine treated; MET + VAL, metformin, and valsartan treated. Values are presented as mean \pm SEM, ^aP < 0.05. n = 4.

Effects of treatments on blood differentials

Tables 2, 3, and 4 show the mean hematological values of the rats in the treated groups and untreated diabetic rats compared to the normal control for significant changes after six weeks of treatments.

Effects of treatments on liver function

Table 5 shows the mean values of some liver enzymes and indices of the rats in the treated groups and untreated diabetic group compared to the normal control group after the six weeks treatment period.

Effects of treatments on renal function

Table 6 shows the mean values of the renal function indices of the rats in the various treatment groups and untreated diabetic rats compared to the normal control

normal control group after six weeks of treatments.

Effects of treatments on the lipid profile

Table 7 presents the mean values of the lipid profile of the rats in the various treatment groups and untreated diabetic rats compared to the normal control group for significant changes after 6 weeks of treatments ($P < 0.05$).

Table 2 Red blood cell and platelet counts of the various treatment groups and untreated diabetic group compared to the control group

Groups	RBC ($10^6/\mu\text{l}$)	Hgb (g/dl)	HCT (%)	PLT ($10^3/\mu\text{l}$)
Control	10.05 \pm 1.25	14.36 \pm 0.99	48.74 \pm 5.42	341.80 \pm 57.50
D.U	8.50 \pm 0.76	17.33 \pm 1.68	58.83 \pm 6.57	297.00 \pm 86.80
MET	7.26 \pm 0.12*	15.05 \pm 0.35	55.43 \pm 2.44	432.30 \pm 69.96
MET+AMLO	4.89 \pm 0.47***	11.50 \pm 1.24	36.65 \pm 4.38	472.80 \pm 26.40
MET+VAL	7.08 \pm 0.12*	14.05 \pm 0.26	58.70 \pm 1.47	329.00 \pm 45.76

Abbreviations: D.U, Diabetic untreated; MET, Metformin; MET + AMLO, Metformin and Amlodipine; MET + VAL, Metformin and Valsartan. RBC, Red blood cell; Hgb, Hemoglobin; HCT, Haematocrit; PLT, Platelets. Values are presented as mean \pm SEM, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, n = 4.

Discussion

Diabetes mellitus prevalence is increasing globally, affecting approximately 415 million people worldwide. Type 2 diabetes accounts for the larger percentage (80%-95%) of this population, which can be attributed to increase in the aging population and massive rise in the prevalence of obesity (13). However, the association and frequent co-existence of diabetes and hypertension put the patient at higher risk of mortality compared to each one alone. Therefore, this situation calls for the development of novel and better management regimens to reduce the mortality rate, which justified conducting this study.

This study showed that the progression of diabetes mellitus was delayed in the treated groups compared to the untreated diabetic group based on the survival rate of the various groups: normal control (100%), metformin alone (30.8%), metformin and amlodipine (38.5%), metformin and valsartan (36.4%), and the untreated diabetic (0%). This study shows that the addition of one antihypertensive drug (amlodipine and valsartan) as an adjunct to the anti-diabetic drug (metformin) improves the survival rate. Also, the blood glucose level of rats reduced significantly and better controlled in the treated groups ($P < 0.05$), so that amlodipine or valsartan enhanced the metformin effect. This study also showed that anti-diabetic treatment regimens prevented a significant reduction in body weight of the rats when compared to the untreated diabetic rats ($P < 0.05$).

Table 3 White blood cell parameters of the various treatment groups and untreated diabetic group compared to the control group

Groups	Number of blood cells				Percentage of blood cells		
	WBC (10 ³ /μl)	LYMPH (10 ³ /μl)	MNC (10 ³ /μl)	GR (10 ³ /μl)	LYMPH (%)	MNC (%)	GR (%)
Control	11.26 ± 2.30	6.42 ± 1.31	1.50 ± 0.51	4.52 ± 1.89	61.30 ± 4.16	10.18 ± 0.35	28.50 ± 4.07
D.U	12.77 ± 3.39	7.17 ± 3.41	1.13 ± 0.30	3.80 ± 0.53	52.10 ± 14.27	9.37 ± 0.32	38.50 ± 14.39
MET	17.98 ± 2.27	0.50 ± 0.24**	0.60 ± 0.11	4.00 ± 0.80	56.68 ± 13.36	11.13 ± 0.70	36.70 ± 16.98
MET+AMLO	13.25 ± 1.87	1.93 ± 0.24*	2.30 ± 0.54	3.60 ± 0.67	54.40 ± 7.41	13.05 ± 2.78	30.20 ± 6.60
MET+VAL	18.98 ± 1.77	1.50 ± 0.30*	1.55 ± 0.4	3.10 ± 0.50	58.28 ± 1.40	13.33 ± 1.00	28.38 ± 1.58

Abbreviations: D.U, Diabetic untreated; MET, Metformin; MET + AMLO, Metformin, and Amlodipine; MET + VAL, Metformin, and Valsartan; WBC, White blood cell; LY, Lymphocytes; MO, monocytes; GR, granulocytes. Values are presented as mean ± SEM, *P < 0.05. **P < 0.01. n = 4.

Table 4 Other hematological values of the various treatment groups and untreated diabetic group compared to the control group

Groups	MCV (fL)	MCH (pg)	MCHC (g/dl)	RDW (%)	PCT (%)	MPV (fL)	PDW (fL)
Control	48.68 ± 1.01	16.78 ± 0.96	34.62 ± 2.57	16.3 ± 0.65	0.23 ± 0.06	8.32 ± 0.22	13.82 ± 0.79
D.U	69.63 ± 2.13	20.33 ± 0.49	29.50 ± 0.51	17.90 ± 0.15	0.21 ± 0.07	7.57 ± 0.64	10.47 ± 0.90
MET	71.23 ± 4.00*	21.98 ± 0.45	36.88 ± 8.77	17.75 ± 1.78	0.45 ± 0.09	8.70 ± 0.70	8.45 ± 2.33
MET+AMLO	58.33 ± 12.31	26.08 ± 3.00	30.73 ± 1.78	16.20 ± 0.33	0.42 ± 0.05	7.23 ± 0.73	13.48 ± 2.53
MET+VAL	79.80 ± 3.85**	21.25 ± 0.56	26.93 ± 2.12	16.48 ± 0.75	0.30 ± 0.08	9.20 ± 0.71	16.93 ± 2.82

Abbreviations: D.U, Diabetic untreated; MET, Metformin; MET+AMLO, Metformin, and Amlodipine; MET+VAL, Metformin, and Valsartan; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; RDW, Red cell distribution width. Values are presented as mean ± SEM. *P < 0.05. **P < 0.01. n = 4.

Table 5 Liver function tests of various treatment groups and untreated diabetic group compared to the control group

Indices	Control	D.U	MET	MET + AMLO	MET + VAL
ALP (u/L)	532.4±40.1	1153±261**	818.3±399.5	1428±310.9*	1515.0±499.4*
ALT (u/L)	132.00±3.56	437±28.73****	130.0±40.31	52.50±14.26	111.50±44.65
AST (u/L)	146.8±7.07	313.7±137.5	149.8±27.76	121.80±20.58	191.80±62.01
TB (mg/dl)	0.24±0.03	0.47±0.03**	0.38±0.08	0.50±0.04**	0.45±0.03**
DB (mg/dl)	0.10±0.00	0.13±0.03	0.18±0.05	0.23±0.03*	0.20±0.00
TP (g/dl)	6.26±0.15	6.6±0.21	7.63±0.35**	7.73±0.25**	8.23±0.31****
ALB (g/dl)	3.64±0.10	3.07±0.24	3.90±0.29	4.05±0.22	4.13±0.27
GLO (g/dl)	2.62±0.07	3.53±0.03	3.73±0.23	3.53±0.43	4.10±0.44**

Abbreviations: D.U, Diabetic untreated; MET, Metformin; MET+AMLO, Metformin and Amlodipine; MET+VAL, Metformin and Valsartan; ALP, Alkaline phosphatase; ALT, Alanine transferase; AST, Aspartate transaminase; TB, Total bilirubin; DB, Direct bilirubin; TP, Total protein; ALB, Albumin; GL, Globulin. Values are presented as mean ± SEM, *P < 0.05. **P < 0.01. ***P < 0.001. ****P < 0.0001. n = 4.

Table 6 Renal function tests of various treatment groups and untreated diabetic group compared to the control group

Indices	Control	D.U	MET	MET + AMLO	MET + VAL
Ur (mg/dl)	44.00 ± 2.10	42.33 ± 2.03	68.50 ± 10.3****	70.25 ± 7.03****	59.50 ± 2.78
Cr (mg/dl)	0.76 ± 0.03	0.57 ± 0.03*	0.60 ± 0.04	0.63 ± 0.03	0.68 ± 0.03
Na ⁺ (mmol/L)	142.00 ± 1.40	140.00 ± 4.36	132.80 ± 2.78*	139.50 ± 2.10	130.00 ± 1.47**
K ⁺ (mmol/L)	6.52 ± 0.39	22.33 ± 1.20****	5.78 ± 0.50	5.50 ± 0.47	6.23 ± 0.45
HCO ₃ (mmol/L)	21.00 ± 0.71	7.37 ± 0.03****	17.25 ± 1.03	20.00 ± 0.00	15.50 ± 0.65***
Cl ⁻ (mmol/L)	107.60 ± 1.47	96.00 ± 1.53**	99.75 ± 0.63*	103.80 ± 2.32	100.80 ± 2.56

Abbreviations: D.U, Diabetic untreated; MET, Metformin; MET+AMLO, Metformin and Amlodipine; MET+VAL, Metformin and Valsartan; Ur, Urea; Cr, Creatinine; Na⁺, Sodium ion; K⁺, Potassium ion; HCO₃⁻, Bicarbonate; Cl⁻ Chloride ion. Values are presented as mean ± SEM, *P < 0.05. **P < 0.01. ***P < 0.001. ****P < 0.0001. n = 4.

Table 7 Lipid profile of the various treatment groups and untreated diabetic group compared to the control group

Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control	61.80 ± 3.64	102.20 ± 15.67	32.60 ± 2.34	12.80 ± 4.74
D.U	64.67 ± 5.36	175.30 ± 5.46****	15.00 ± 1.16****	14.33 ± 5.24
MET	72.50 ± 5.24	117.30 ± 20.22	21.00 ± 1.16**	28.50 ± 5.00
MET + AMLO	67.75 ± 0.75	111.50 ± 12.89	21.75 ± 0.75**	22.50 ± 3.23
MET + VAL	64.50 ± 5.24	112.50 ± 27.38	24.75 ± 2.10*	17.00 ± 3.16

Abbreviations: D.U, Diabetic untreated; MET, Metformin; MET + AMLO, Metformin, and Amlodipine; MET + VAL, Metformin, and Valsartan. TC, Total cholesterol; TG, Triglycerides; HDL, High-density lipoprotein; LDL, Low-density lipoprotein. Values are presented as mean ± SEM. *P < 0.05. **P < 0.01. ***P < 0.001. ****P < 0.0001. n = 4.

The significant changes ($P < 0.05$) in RBC count (Table 2) agree with the results of previous studies that well established the link between chronic diseases and anemia (14, 15). The high incidence of anemia in DM has been attributed to the increased non-enzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycemia (16). Oxidation of these proteins and hyperglycemia in diabetes mellitus cause an upsurge in the production of lipid peroxides that leads to hemolysis of RBCs (17). The main pathological consequences of free radical-induced membrane lipid peroxidation are increased membrane rigidity, reduced erythrocyte survival, decreased cellular deformability, and lipid fluidity (18); all these changes end in the reduction of red blood cells in the treatment groups. Besides, there was a significant decrease in lymphocytes counts in the metformin, metformin plus amlodipine, and metformin plus valsartan treated groups ($P < 0.05$), while a significant increase in the mean corpuscular volume in the groups treated with metformin and metformin plus valsartan. Typically, DM lowers MCH, MCHC, and MCV values, which is a sign of abnormal hemoglobin synthesis, the letdown of blood osmoregulation, and plasma osmolarity (18).

MCV increase in the treated groups indicates that our treatment ameliorated the adverse effects of DM, which conforms with the findings of Ayman study (19). Significant changes in liver enzymes values suggest that type 2 diabetes (simulated in this research) is associated with a clinical spectrum of liver abnormalities collectively known as non-alcoholic fatty liver disease (NAFLD) ($P < 0.05$) (Table 5). NAFLD is a clinico-histopathological diagnosis characterized by hepatocellular steatosis (usually macrovesicular) and elevation of liver enzymes such as ALT, ALP, and ASP (20). The elevated liver enzymes and other liver function indices such as total protein, total bilirubin, and globulin were due to liver damage caused by DM. However, these indices showed ameliorations in the various treated groups compared to the untreated diabetic group. The significant changes in the renal function such as increased urea in some treated groups are a symptom of acute renal failure, which is one of the complications of diabetes mellitus (Table 6) (21). Also, the creatinine level decreased significantly in the untreated diabetic group, which is due to the chronic effect of DM (22). There was also a significant decrease in serum sodium ion level in metformin and metformin plus valsartan treated groups,

which was ameliorated in the metformin plus amlodipine-treated group. In addition, potassium ion level increased significantly in the untreated diabetic group. In the meantime, a significant decrease in bicarbonate ion and chloride ion were observed in the metformin-treated group, also improved by the addition of amlodipine. There was also a significant decrease in chloride ion level in the untreated diabetic group. Moreover, the significant reduction in the electrolytes (sodium, chloride, and bicarbonate) was seen in the untreated diabetic group because these electrolytes were used up more. However, treatment could ameliorate this condition, too.

Significant changes are seen in the lipid profile because subjects with type 2 diabetes are characterized by very high cardiovascular morbidity and mortality rates (Table 7). Plasma lipoprotein abnormalities of concentration, composition, or distribution are the main factors for the enhanced cardiovascular risk (23). Blood glucose optimization (obtained by a special diet, hypoglycemic drugs or insulin therapy) positively influences lipoprotein metabolism in type 2 diabetic patients, although a comprehensive normalization in plasma lipoprotein concentration and composition abnormalities is occasionally obtained with this type of diabetes (24). Lipoprotein disorders in type 2 diabetes affect all classes of lipoprotein and produce high levels of low-density lipoprotein, low level of high-density lipoprotein, and elevated level of triglycerides (25) with which the findings of this study agree, too. Recently, Frenais et al. reported an increase in the catabolism of HDL-apoA-I as the responsible compound for lower high-density lipoprotein concentration in non-insulin-dependent DM with pronounced diabetic dyslipidemia and hypertriglyceridemia compared to non-diabetic subjects (26). In this study, despite drug treatment, there was a progression of diabetes. However, it was delayed in the treated groups compared to the untreated diabetic group.

In summary, there was some degree of organ protection and attenuation of the changes in biochemical indices, which would be largely attributed to delay in disease progression caused by good blood glycemic control in the treated groups. The curative effect of the anti-diabetic drug (metformin) was enhanced by the addition of antihypertensive medications (amlodipine and valsartan).

Conclusion

According to our findings, adding antihypertensive drugs (amlodipine and valsartan) as co-therapy to an anti-diabetic drug, metformin, improved the disease prognosis in streptozotocin-induced diabetic rats. This effect was partly due to improved blood glucose control and partly by delaying or prevention of the disease progression, multi-organ damage, and cardiovascular complications. The result also showed the increase in the rats' survival rate.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

This study is partly funded by the Department of Pharmacology and Toxicology, University of Benin, Benin-City, Nigeria. We highly appreciate Neimeth Pharmaceutical LTD, Lagos State, Nigeria, and Edo Pharmaceuticals LTD, Edo State, Nigeria, for their support. Also, the technical assistance of Mr. Odega K. I. and Mr. Obasuyi J. O., in the Medical Laboratory Department of the University of Benin Teaching Hospital, Benin-City, Nigeria was well appreciated concerning their assistance in the hematological and biochemical analyses.

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