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Original Research Article

EVALUATING THE HISTOPATHOLOGICAL EFFECT OF AMLODIPINE AND VALSARTAN ON PIOGLITAZONE-TREATED STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Two striking factors for cardiovascular diseases, including atherosclerosis, are diabetes and hypertension. More so, there is a significant overlap in the aetiology and progression of hypertension and diabetes, and there are documented findings that drugs used in the management of either disease conditions can act as a friend or foe in the treatment outcome of the other. Therefore, the aim of this study is to evaluate the effect of the co-administration of valsartan and amlodipine on the histological features of streptozotocin-induced diabetic rats treated with pioglitazone. Albino rats weighing 205 ± 45 g (mean ± SEM) were prevented from eating and drinking overnight while diabetes mellitus was induced by administering 40 mg/kg of streptozotocin intraperitoneally. Blood samples were taken 48-hour post induction. Animals were considered diabetic if their blood sugar level was≥ 200 mg/dl. The animals were grouped into control rats without treatment, untreated diabetic rats, and treatment groups, pioglitazone only, valsartan plus pioglitazone, and amlodipine plus pioglitazone. After 3 weeks of treatment, the animals were humanely sacrificed while the brain, lung, heart, kidney, and liver were harvested for histopathological analysis. Stained tissues were viewed by means of an optical photomicroscope at 100 x magnification. From the histological study, our findings suggest that valsartan enhanced the hypoglycemic effect of pioglitazone-treated diabetic rats compared to those treated with pioglitazone alone and those treated with amlodipine and pioglitazone combination.

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INTRODUCTION

Diabetes mellitus (DM) has continued to be a major non-communicable cause of mortality globally since it was first reported in Egyptian manuscript about 3000 years ago [1]. DM, a major metabolic disease, is characterised by prolonged hyperglycaemia; implying high concentration of glucose in the blood stream, complemented by major or minor modifications in the breakdown of carbohydrates, lipids, and proteins. Insulin fuels muscle and fat cells to absorb glucose from the blood while stimulating the liver to breakdown glucose, hence, triggering the utilization of glucose by cells thus reducing blood glucose. People suffering from DM will have sustained high blood glucose levels, and while type 1 diabetes is an autoimmune disorder wherein the body's immune system alters the pancreas ability to secrete insulin. However, with type 2

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diabetes, insulin is secreted by the pancreas, but the body cells are resistant to insulin. This is often associated with obesity, gestational diabetes, which is a form of diabetes that occurs in pregnancy and other forms of diabetes which are very rare and are caused by a single gene mutation [2]. In the past, type 2 diabetes was rarely seen in young people, which is why it was given the unusual term "adult-onset diabetes." However, these days, it is increasingly being seen in both young adults and children. The rising rate of obesity in today's population seems to be the reason why diabetes is prevalent. Nowadays, maintaining a healthy weight can be quite difficult because of things like eating enough food and leading a sedentary lifestyle [3].

Complications arising from diabtetes mellitus can be grouped into microvascular complication (impairment in small blood vessels) and macrovascular complications (impairment in larger blood vessels). Microvascular complications include retinopathy which can result in blindness, nephropathy a trigger of renal failure, as well as neuropathy a major risk for impotence and diabetic foot disorder. Macrovascular complications that can arise include cardiovascular diseases ranging from heart attacks, strokes, and insufficient blood flow to the lower extremities. There is evidence from large randomized-controlled trials that good metabolic control in both type 1 and 2 diabetes can delay the onset and progression of these complications [4].

This study was embarked on to evaluate the effect of co-administering valsartan and amlodipine on the histological features of streptozotocin-induced diabetic rats treated with pioglitazone.

MATERIALS AND METHODS

Experimental Animals

Male albino Wister rats weighing 205 ± 45 g (mean ± SEM) were acquired from the Animal house domiciled in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The animals were kept in cages made of plastic with daily changes of wood-based bedding; dry rodent pellet feed for feeding, and unrestricted access to water. The National Institutes of Health's Guidelines for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) which were updated in 2002 [5] were followed for conducting the experiments. Additionally, ethical approval for all experimental protocols was requested from the Faculty of Pharmacy Ethics committee, University of Benin, and an approval number (EC/FP/017/01) was obtained.

Diabetes mellitus induction in animals

Rats were denied food and drink overnight; thereafter 40mg/kg of streptozotocin dissolved in 0.1M citrate buffer (pH 4.5) was used to induce diabetes mellitus intraperitoneally. Following induction, the animals were allowed access to nourishment and water. Forty-eight hours later, the animals were confirmed diabetes if they had a blood glucose level of ≥ 200 mg/dl [7]. The blood glucose levels of the animals were monitored with the aid of an Accu-chek active glucometer (Roche, USA).

Experimental Design

Thirty animals were grouped into five, with each group having six rats each. Daily treatments were carried out for three weeks as follows:

Group 1: healthy animals with no treatment
Group 2: diabetic animals with no treatment
Group 3: diabetic animals with pioglitazone (30 mg/kg) treatment
Group 4: diabetic animals with pioglitazone (30 mg/kg) and valsartan (30 mg/kg) treatment
Group 5: diabetic animals with pioglitazone (30 mg/kg) and amlodipine (2.5 mg/kg) treatment

Determination of Blood Glucose

Blood glucose level was determined by collecting blood from the lateral tail vein of fasted rats using sterile lancets. The tails were aseptically cleaned and allowed to dry. A single drop of the blood from the tail was smeared on a glucose test strip while readings were obtained via the Accu-chek® Active glucometer (Roche, USA).

Histopathological Study

Organs such as liver, heart, kidney, lungs, and brain were obtained after humane sacrifice of the animals. The dissected organs were fixed in 10% formal saline and entirely dehydrated in varying concentrations (70, 90, 96 and 100 %) of alcohol. The tissues were immersed in xylene to eliminate the alcohol, following impregnation and embedment with molten paraffin wax. They were allowed to solidify before sectioning into 4 µM using a microtome (Leica RM 2235, UK). The 4 µM sections were placed on slides and stained with hematoxylin-eosin dye [6]. Stained tissue was viewed using an optical photomicroscope (Leica MC170 HD, Leica Biosystems, Germany) at 100× magnification.

RESULTS

Effects on the Histology of Selected Organs

The hippocampal sections of brain of animals appear normal except the hippocampus of diabetic rats where cerebral odema, vascular ulceration and congestion were noticed (Figure 1). Figure 2 shows that the bronchioles, alveolar spaces, and bronchial arteries of rats in all treated groups were essentially normal. Photomicrographs of the heart of rats treated with amlodipine plus pioglitazone revealed active interstitial and vascular congestion similar to coronary vascular distortion seen in animals treated with valsartan and pioglitazone (Figure 3). Notably, photomicrographs of the kidney of untreated diabetic rats revealed tubular necrosis and interstitial congestion (Figure 4). The photomicrographs of the liver are represented in Figure 5 wherein the liver of all groups except the control showed sinusoidal Kupffer cell activation.

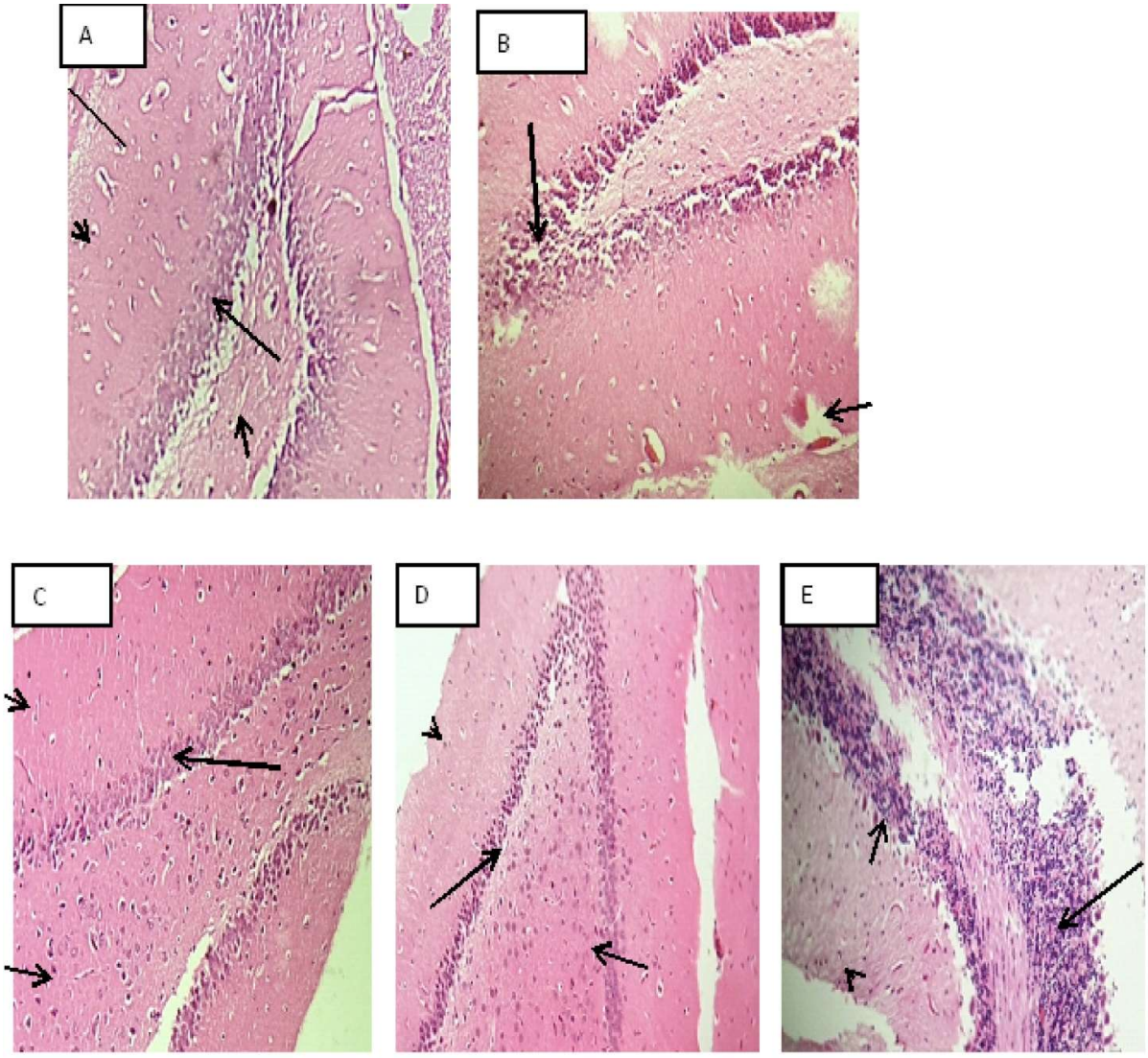

Figure 1: Photomicrographs of the brain of a rat from each treatment group X 100 magnification
A*: Rat hippocampus, Control showing of normal architecture: composed of granular cell layer (long arrow) outer molecular layer (arrow head) and pyramidal cell layer(short arrow).* ***B****: Untreated Diabetic hippocampus showing mild cerebral oedema (long arrow), vascular ulceration and congestion (short arrow).* ***C****: Pioglitazone treated rat hippocampus showing normal architecture: granular cell layer (long arrow), outer molecular layer cell, (arrow head) and pyramidal cell layer(short arrow).* ***D****: Amlodipine + Pioglitazone treated rat hippocampus showing normal architecture: granular cell layer (long arrow), pyramidal cell layer(short arrow) and molecular layer (arrow head).* ***E****: Valsartan + Pioglitazone treated cerebellum showing: granular cell layer (long arrow), Purkinje cell layer (short arrow) and molecular layer (arrow head), all normal*

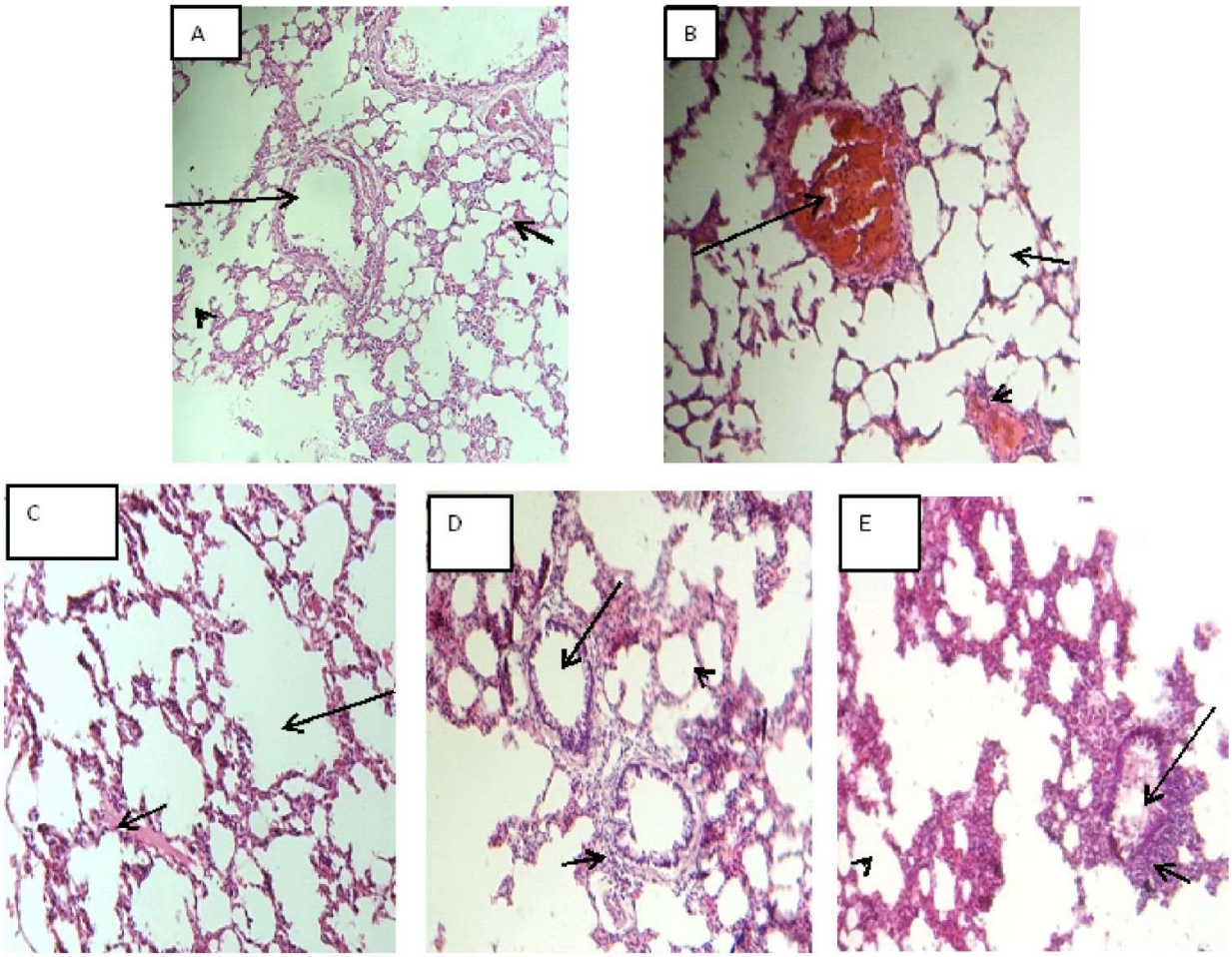

Figure 2: Photomicrographs of the lungs of rat from each treatment group X 100 magnification
A: *Control, lungs composed of normal architecture: terminal bronchiole (long arrow) , alveolar spaces (short arrow) and bronchial artery(arrow head).* ***B****: Untreated Diabetic rat lungs showing: vascular dilatation and congestion (long arrow), vascular ulceration (arrow head), the alveolar spaces appear normal (short arrow). C: Pioglitazone treated rat showing: normal alveolar spaces (long arrow) and active interstitial congestion (short arrow).* ***D:*** *Amlodipine + Pioglitazone treated rat showing: normal terminal bronchiole (long arrow) normal alveolar spaces (arrow head),activated interstitial cells of the mononuclear phagocyte system (short arrow). E: Valsartan + Pioglitazone treated rat showing: normal terminal bronchiole (long arrow) , normal alveolar spaces (arrow head)and mildly activated bronchiolo-alveolar lymphoid aggregate(short arrow)*

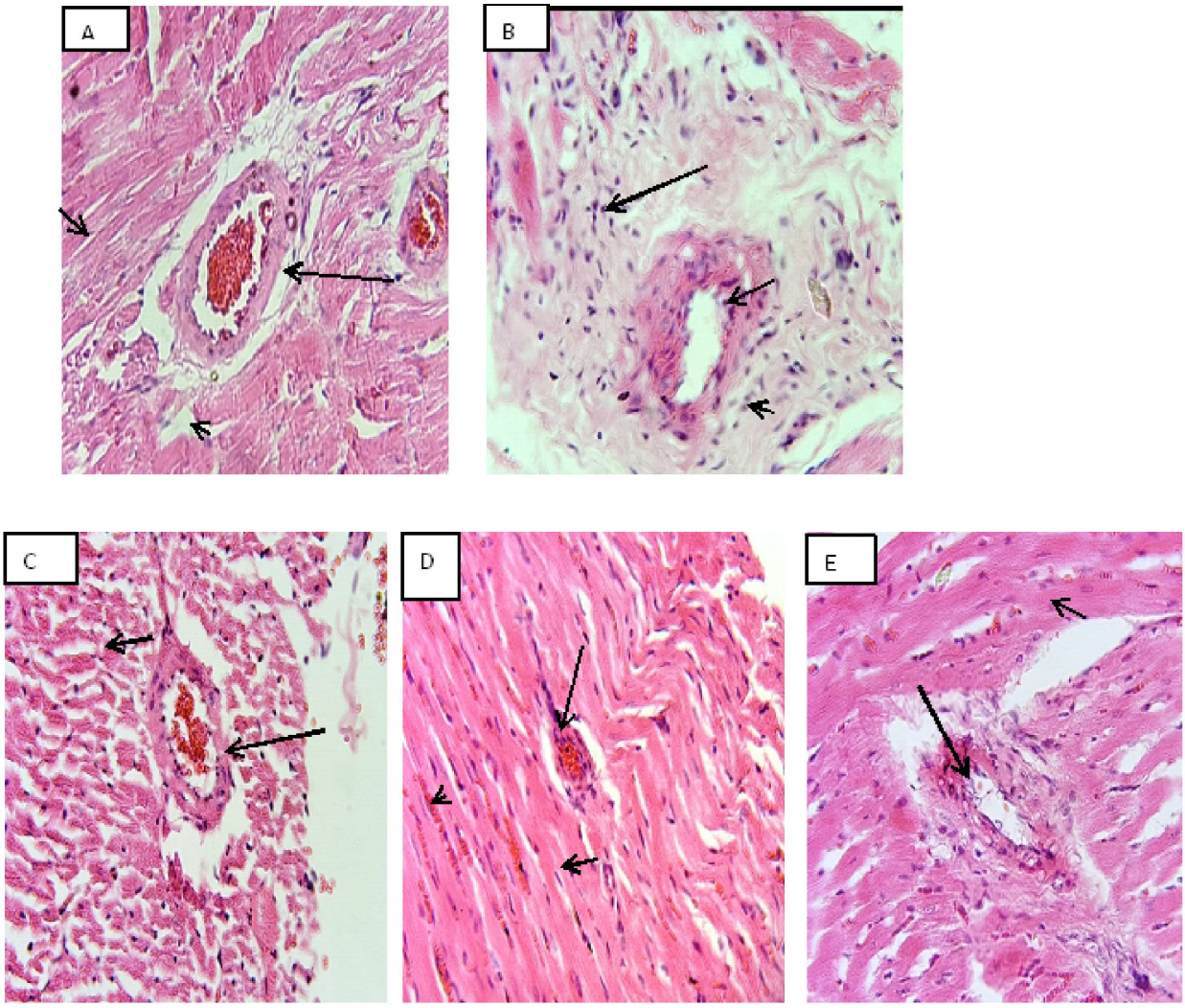

Figure 3: Photomicrographs of the heart of a rat from each treatment group X 100 magnification
***A**: Control, heart showing normal architecture: with bundles of myocardial fibres (short arrow), interstitial space (arrow head) and coronary artery (long arrow). **B**: Untreated diabetic rat showing: vascular distortion (short arrow), myocardial degeneration (arrow head)and perivascular infiltrates of inflammatory cells (long arrow). **C**: Pioglitazone treated rat showing normal architecture. **D:** Amlodipine + Pioglitazone treated rat showing: normal bundles of myocardial fibres (short arrow), active interstitial congestion (arrow head) and coronary vascular congestion (long arrow). **E**: Valsartan + Pioglitazone treated rat showing: normal bundles of myocardial fibres (short arrow) and coronary vascular distortion (long arrow)*

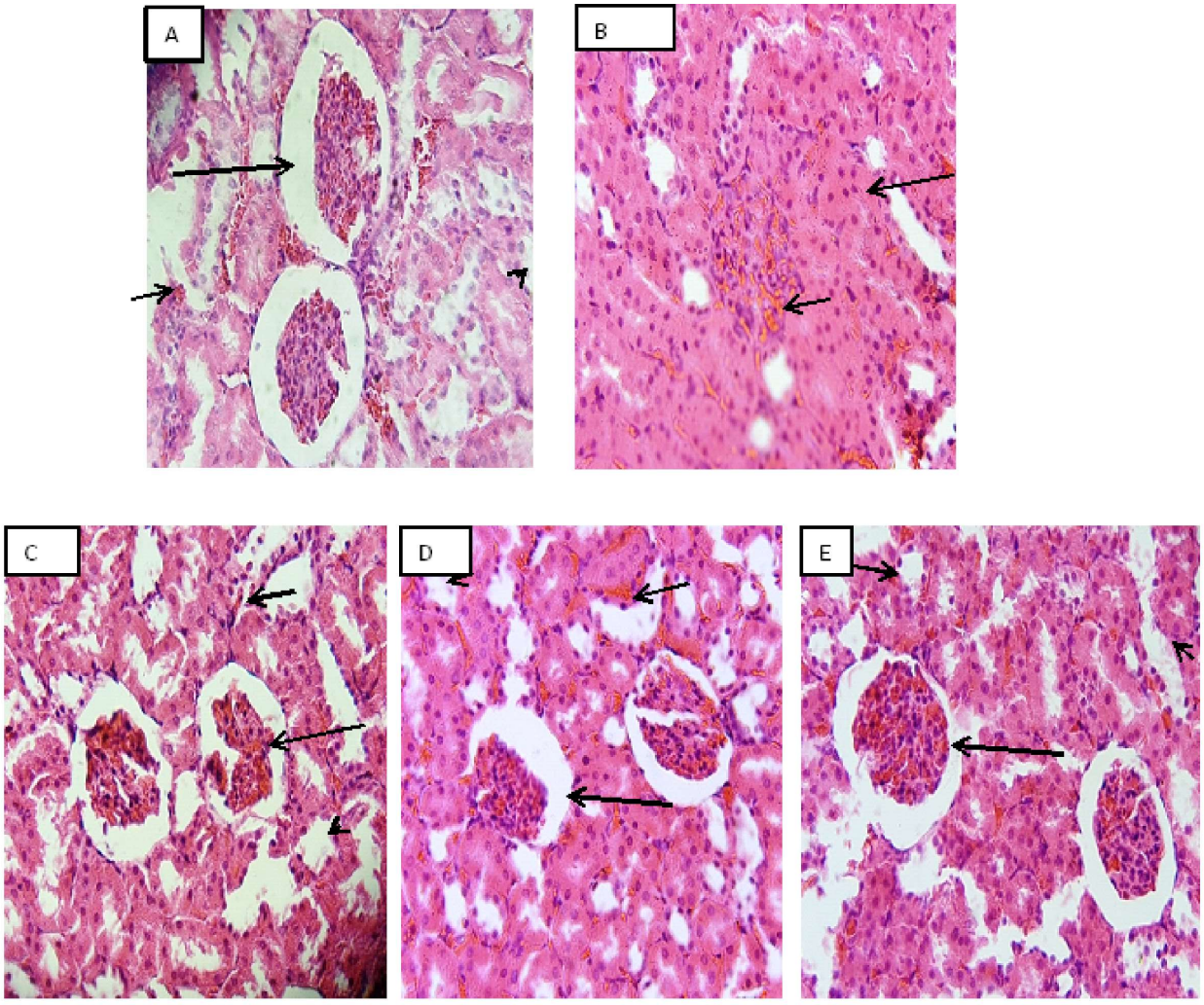

Figure 4: Photomicrographs of the kidney of a rat from each treatment group X 100 magnification
A: *Control, showing normal architecture, composed of the renal corpuscle (long arrow) , interstitial space (short arrow) and tubules(arrow head).* ***B****: Untreated Diabetic rat showing: tubular necrosis (long arrow) and interstitial congestion (short arrow).* ***C****: Pioglitazone treated rat showing normal architecture composed of renal corpuscle (long arrow) and interstitial space (short arrow) and tubules (arrow head).* ***D****: Amlodipine + Pioglitazone treated rats showing: renal corpuscle (long arrow) , interstitial space (short arrow) and tubules(arrow head) .* ***E****: Valsartan + Pioglitazone treated rats showing normal architecture: renal corpuscle (long arrow) , interstitial space (short arrow) and tubules(arrow head)*

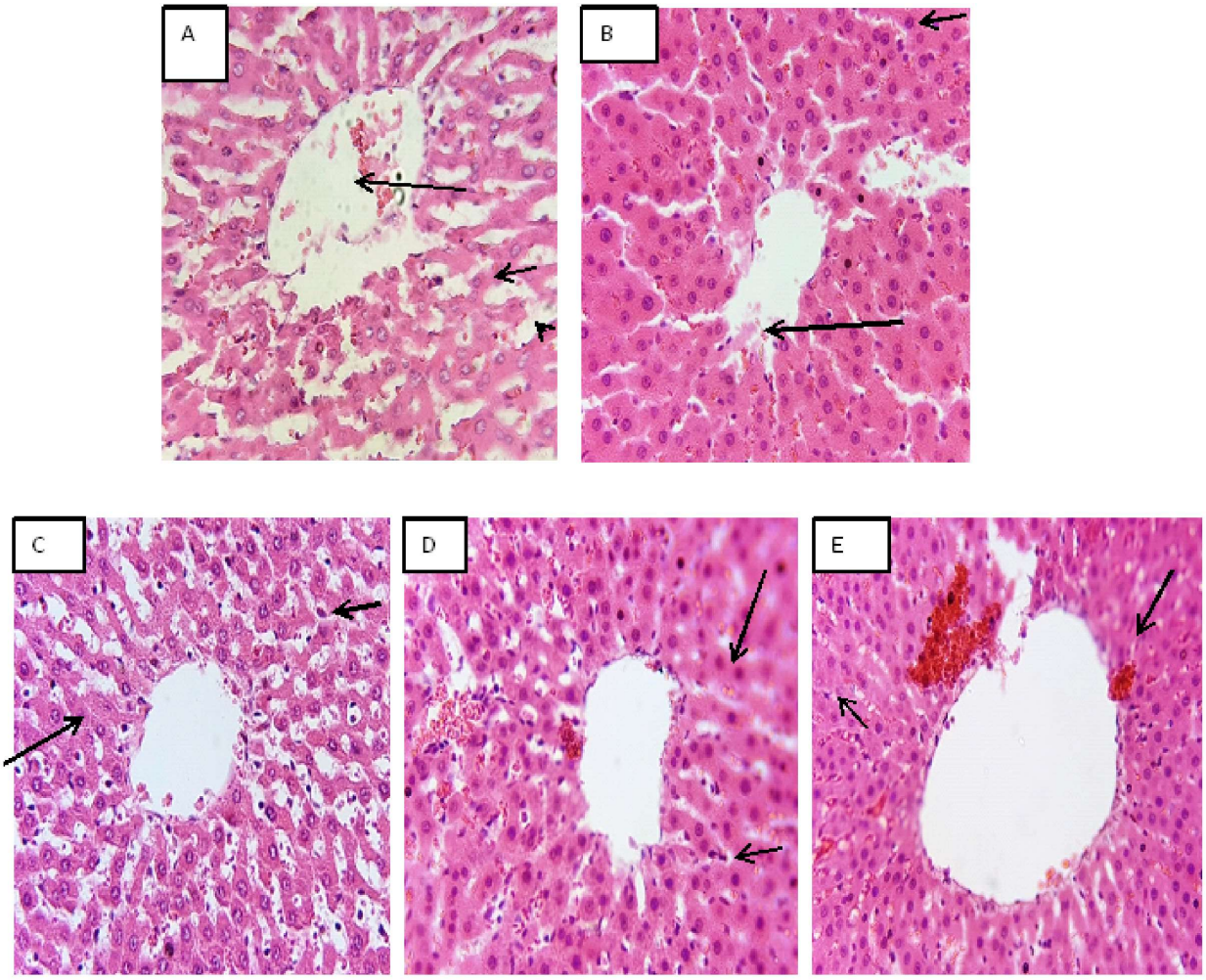

Figure 5: Photomicrographs of liver of a rat from each treatment group X 100 magnification
A: *Control rat liver showing normal architecture composed of: central vein (long arrow) hepatocytes(short arrow) and sinusoids (arrow head).* ***B****: Untreated Diabetic rat showing: central vascular ulceration (long arrow) , sinusoidal Kupffer cell activation (short arrow).* ***C****: Pioglitazone treated rat showing : normal hepatocytes (long arrow) and sinusoidal Kupffer cell activation (short arrow).* ***D****: Amlodipine + Pioglitazone treated rat showing normal hepatocytes (long arrow) and sinusoidal Kupffer cell activation with fatty changes (short arrow).* ***E****: Valsartan + Pioglitazone treated rats showing: active vascular congestion(long arrow) normal hepatocytes(arrow head) and sinusoidal Kupffer cell activation (short arrow)*

DISCUSSION

End-stage renal and vascular dysfunction amongst other cardiovascular complications account for the majority of mortalities caused by Type 2 diabetes [8]. In Africa, about 4.3% of adults have DM and this is responsible for 401,276 deaths in the continent, with Nigeria having approximately 4.7 million cases of diabetes [9]. Considering these statistics, there is a need to advance our knowledge of the factors that promote these disturbing numbers of deaths. Hence, this study has revealed the effect of known antihypertensives, valsartan and amlodipine, on the histopathological features of some vital visceral organs in streptozotocin-induced diabetic rats treated with pioglitazone.

Photomicrographs of the brain of control rats appear essentially normal, however, histology of the brain of untreated diabetic group revealed mild cerebral oedema when compared to other groups. However, this does not correspond to the work done by Reske-Nelson and Lundback [10] where the histological findings of a diabetic brain revealed thickening of cerebral cortical capillary basement membrane and possible abnormality in the blood brain barrier. It is known that cerebral oedema occurs due to ischemia–reperfusion injury, by way of inflammation as well as altered cerebrovascular self-regulation contributing to its pathogenesis [11]. A possible reason for these observable changes could be that the manifestation of

hyperglycemic effect on the brain cells takes a longer period of time though prior studies have been unable to characterize the changes that differentiate diabetic brain from non-diabetic brain [12].

The histology of the bronchioles, alveolar space, and bronchial arteries of the lungs in all treatment group showed no abnormalities. Photomicrographs of the heart of untreated diabetic rats showed vascular distortion, myocardial degeneration and the presence of inflammatory cells. These are indicative and pointer to progression of heart disease condition typically observed over time in a diabetic patient. Although, pioglitazone is a thiazolidinedione that enhances insulin uptake by body cells for utilization by binding to peroxisome proliferating activating receptor gamma, activation of this receptor causes adipokines production [13]. This increases fat oxidation, thus the release of inflammatory mediators enabling the pathogenesis of atherosclerotic vessel, however, the heart of animals that received pioglitazone was essentially normal.

Furthermore, the histology of the kidney in the treatment group revealed distinct and clear tubules when compared to healthy groups. Valsartan conferred a degree of protection to the kidney when compared to other treatment. The delay of disease complication could be due to inhibition of angiotensin II, a potent vasoconstrictor found in the kidney, implicated in most cardiovascular disease such as coronary artery disease, stroke, and hypertension. The histology of the kidney of untreated diabetic rats revealed distorted renal corpuscle with tubular necrosis which relates with the work carried out by Nosadina et al. [14] where they noticed glomerular basement membrane thickening on the histology of diabetic kidney. Histology on the kidney of amlodipine + pioglitazone treated rats revealed mildly diffused mononuclear infiltrate, evident of lesion progression, and when compared to untreated diabetic rats, there was a form of slowed progression of the disease complications. Additionally, in amlodipine + pioglitazone treated rats, the kidney showed gradual loss of muscle in renal corpuscle and diffusion of mononuclear infiltrate in the tubules which are indicative of lesions in the kidney. This illustrates how nephropathy progresses. Any kind of damage to the renal corpuscle, which serves as the nephron's filtration system, could result in kidney disease. One hypothesis is that adiponectin is released, which in turn triggers the release of inflammatory mediators. Meanwhile, pioglitazone conferred slowed advancement of the disease when compared to the result of untreated diabetic rat which showed a distortion in renal corpuscle and necrosis of the tubule.

The histology of the liver of untreated group revealed sinusoidal Kupffer cell activation which is a vital response of the liver to an injury or infection [15]. A reason for reduced diseased outcome observation in diabetic untreated animals could be that the length of time or duration of time (3 weeks) for diabetes mellitus progression was not sufficient for complete organ damage often observed in a diabetic as opposed to other literature in which duration of observation is 6 weeks [16]. In the group treated with pioglitazone and valsartan, the liver revealed Kupffer cell activation, though the effect of this medication delayed the progression of the disease complications when compared with untreated diabetic. While photograph of liver treated with pioglitazone and amlodipine revealed fatty changes and sinusoid with focal mononuclear cells which are indicative of inflammation in the liver, damage to the capillaries of hepatocytes are indicative of microvascular damage hence disease progression. In the group treated with pioglitazone alone, liver histology reveals prominent dilated sinusoid with mononuclear cells and fatty changes. This is indicative of lesions and hepatic steatosis which is consistent with the work done by Zafar et al., [17]. The significant fatty alterations may be explained by the hypoinsulinemia-induced inflow of fatty acids into the liver and the reduced liver excretion of lipoprotein secretion caused by apolipoprotein B synthesis deficiencies. Additionally, hyperlipidemia may contribute to the development of a fatty liver [18], which would explain the progression of diabetes mellitus. However, in contrast to the diabetic group receiving no treatment, there was a slowdown in the disease's progression. This could be attributed to the action of pioglitazone, a thiazolidinedione that works by improving the body's ability to absorb glucose for use in cells, lowering blood glucose levels, and delaying the development of microvascular damage.

CONCLUSION

In this study, we sought to investigate the effect of the co-administration of valsartan and amlodipine on the histological features of streptozotocin-induced diabetic rats treated with pioglitazone. From our findings, the progression of diabetes mellitus caused degenerative alterations in the histology of the visceral organs examined. Meanwhile, valsartan when combined with pioglitazone was found to protect the visceral organs, particularly the kidney, while the combination of pioglitazone and amlodipine revealed pathological deficits in the hearts and kidneys. Therefore, our study suggests that combining angiotensin receptor blockers with oral hypoglycaemic medications may produce better treatment outcomes in streptozotocin-induced diabetic rats.

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This study did not receive any external funding.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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