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Anti-diabetic effect of combined treatment with *Aloe vera* gel and Metformin on alloxan-induced diabetic rats

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ABSTRACT

Aim: The present study investigated the effects of intensive glucose regulation with combined treatment of *Aloe vera* gel (a herbal agent) and Metformin in an alloxan-induced diabetic rat model. **Methods:** Forty rats were assigned to the following groups and treated for 21 days: Group 1 (normal untreated control), Group 2 (untreated diabetic rats, DR), Group 3 (DR + 300 mg/kg PE of *Aloe vera*), Group 4 (DR + 2 mg/kg Metformin) and Group 5 (DR + 300 mg/kg PE of *Aloe vera* + 2 mg/kg Metformin). The effect of the treatments on fasting blood glucose (FBG) level, lipid profile, renal function, atherogenic index, pancreas and kidney histopathologies were assessed. **Results:** FBG level in Group 5 rats decreased by 28.4, 38.0 and 69.0% at Day 7, 14 and 21, respectively. Hyperlipidaemia, high atherogenic index, increased plasma creatinine and urea levels observed in the diabetic rats were ameliorated by the single and combined treatments with *Aloe vera* and Metformin. Reduced level of HDL-cholesterol in the untreated diabetic rats significantly improved by 160.0, 89.8 and 178.7% respectively in Group 3, 4 and 5 animals. The pancreas and kidney histopathologies indicated signs of recovery in Group 5 rats unlike those of Group 2 that had evidence of necrotic cells in both the acini and islet in pancreas and total glomeruli erosion in kidney. **Conclusion:** *Aloe vera* in combination with Metformin for treatment of diabetic patients could avert diabetes-associated dyslipidaemia, improve cellular integrity and increase, thereby preventing patients from risk of cardiovascular diseases and kidney failure.

Keywords: Diabetes mellitus, *Aloe vera*, Metformin, Hyperlipidaemia, Hyperglycaemia, Renal failure.

INTRODUCTION

Aloe vera is a xerophyte and therefore survives very well in highly dried arid conditions found mostly in African countries [1]. Aloe belonged to the Lily family (*Liliaceae*) until it was reclassified to its own family known as *Aloaceae* [2]. Nonetheless, the *Aloaceae* still shows relatedness with the *Liliaceae* e.g. garlic, onion, and asparagus which have proven pharmacological properties [1, 2]. The *Aloe* family include species with varied morphologies ranging from dwarf species e.g *Aloe variegata* a popular house-plant to species about the size of a small tree.

The plant has been reported to possess wound healing and anti-inflammatory effects due to the presence of phytochemicals such as tannic acid, glycoproteins, and sulfated polysaccharides among other complex polysaccharides [4]. *Aloe vera* is effective in treatment and prevention of scar formation. This is made possible by the ability of *Aloe vera* to stimulate cell division. Enzymes present in the plant promote regeneration at the deepest layers of the skin [4].

Diabetes mellitus is a metabolic disease characterized by insulin deficiency or insulin insensitivity resulting in hyperglycaemia [5]. Dysregulation of the insulin pathway results in abnormalities in the metabolism of lipids, proteins, carbohydrates and electrolytes.

Metformin, an anti-diabetic biguanide drug lowers blood glucose level by enhancing sensitivity to insulin. Consequently, peripheral uptake of glucose is induced, coupled with decreased hepatic glucose output [6]. Turner in 1998 demonstrated that a combination of dietary control and metformin treatment in obese patients significantly reduced their chances of developing diabetes mellitus related endpoints [6]. One major limitation of the drug, however, is that, though it is generally regarded as safe, it is not recommendable to use metformin for high-risk patients such as those with renal failure as the drug could accentuate the renal condition. Due to incidence of some challenges associated with the use of some standard anti-diabetic drugs, including but not limited to metformin, the World Health Organization (WHO) sensitised researchers on the need to develop alternative medicines for the management of diabetes mellitus [7] and that provided the impetus for research in this area.

MATERIALS AND METHODS

Chemicals and drugs

Diagnostic kits for creatinine, urea, total cholesterol (TC), high density lipoprotein (HDL) and triglyceride (TG) were purchased from Randox Diagnostics (UK). Alloxan was purchased from Sigma (St. Louis, Germany). Accu-check Glucometer was a product of Roche Diagnostics (Germany). Metformin and other chemicals used in this work were of analytical grade obtained locally.

Phytochemical analysis

Experimental animals

Male albino rats (average weight, 160 g) were used in this study. The animals were housed in the Animal Care Facility of the Department of Biochemistry, Kogi State University, Nigeria. Acclimatization of the animals was done for one week prior to experiments. The animals were fed standard, commercial rat feed and water *ad libitum* throughout the acclimatization experimental period.

Plant collection and extraction

Aloe vera leaves were collected in the month of April 2016 and identified by Prof S.M. Ayodele of the Department of Botany, Kogi State University, Nigeria. The leaves were dissected and the gel from the leaves was drained, homogenized and used fresh for experiments.

Induction of diabetes

Diabetes was induced by single intraperitoneal injection of freshly prepared alloxan [150 mg/kg body weight (BW)] to overnight fasted rats. Fasting blood glucose concentration of the rats was determined two (2) days after induction and animals with blood glucose concentration greater than or equal to 250 mg/dL were considered diabetic.

Experimental design

Survivors of alloxan-diabetes induction were allocated to five (5) groups of eight (8) rats each as follows:

Group 1: Normal untreated control rats

Group 2: Diabetic untreated rats

Group 3: Diabetic rats (DR) treated with 300 mg/kg BW Polysaccharide Equivalent (PE) *Aloe vera* gel orally

Group 4: Diabetic rats treated with 2 mg/kg BW Metformin orally

Group 5: Diabetic rats co-administered with 300 mg/kg BW PE *Aloe vera* gel and 2 mg/kg BW Metformin orally

Animals were treatment according to the respective description given above for 21 days. All animal groups were fed rat feed and water *ad libitum*.

Biochemical assays

Blood glucose concentration was monitored by the glucose oxidase method using an Accu-check glucometer. One animal was randomly selected per group, each time, for the assay. Animals were sacrificed on the 22nd day. Blood samples were collected in plain bottles and organs (namely pancreases and kidneys) where excised; preserved in 10 % formalin. Biochemical assays for creatinine, urea, total cholesterol (TC), high-density lipoprotein (HDL) cholesterol and triglyceride (TG) levels in plasma were carried out using Randox Diagnostic kits according to manufacturer's instructions.

Statistical analysis

Statistical analyses (Mean, Standard Deviation and Analysis of Variance) were performed with the Microsoft Excel (Version 2010) and SPSS 16.0.

RESULTS AND DISCUSSION

Alloxan [2,4,5,6(1H,3H)-pyrimidinetetrone], also known as mesoxalylcarbamide, has been severally reported to induce hyperglycaemia in rats in experimental models [8, 9]. Its mechanism of action involves destruction of insulin secreting β-cells of the pancreas. This results in decreased production of insulin consequently, rats administered alloxan become hyperglycemic [8].

In the present study, the effect of *Aloe vera* gel on fasting blood glucose (FBG) concentration in alloxan-induced diabetic rats is as shown in Table 1. The FBG concentration in diabetic animals treated with 300 mg/kg BW PE of *Aloe vera gel* decreased by 40.5, 47.6 and 65.5% as at the 7th, 14th and 21st days of the study, respectively; that of the animal group treated with the standard drug decreased by 33.4, 43.4 and 76.0% at Day 7, 14 and 21 respectively, while FBG concentration in diabetic animal group administered a combination of both *Aloe vera* extract and Metformin decreased by 28.4, 38.0 and 69.0% at Day 7, 14 and 21, respectively. Decrease in blood glucose level is a common generally used routine physiological marker for assessment of diabetic patients' responses to treatment of hyperglycaemia [6, 10]. Insulin, a hormone that regulates glucose uptake from the blood into body cells for metabolism, is secreted by β-pancreatic cells in the body. When alloxan and other related diabetes-inducing agents are administered or taken into the body, they attack the pancreas membrane thereby disrupting the pancreatic cells. Disruption of pancreatic cells leads to drastic reduction or cessation of insulin production which predisposes the animal to type 1 diabetes (Insulin-dependent diabetes mellitus), characterized by high blood glucose level among others [8]. The high blood glucose concentration observed in this study in the untreated diabetic animals group was significantly reduced ($p<0.05$) in the diabetic animals administered either single treatments or a combination of both 300 mg/kg BW PE of *Aloe vera* gel and 2 mg/kg BW Metformin. This indicates that the PE of *Aloe vera*, or its combined form with Metformin, was able to induce healing of wounded β-pancreatic cells thereby leading to increased production of insulin in the type 1 diabetic rats resulting in glucose uptake from the blood to body cells.

Table 1: Effect of *Aloe vera* gel on fasting blood glucose concentration in alloxan-induced diabetic rats

Groups	Blood glucose concentration post induction with alloxan			
	Day 2 (mg/dL)	Day 7 (mg/dL)	Day 14 (mg/dL)	Day 21 (mg/dL)
Normal untreated	146	140	144	130
Diabetic untreated	390	495	490	>500
300 mg/kg BW PE of *Aloe vera* gel	420	250 (40.5%)	220 (47.6%)	145 (65.5%)
2mg/kg BW Metformin	>500	333 (>33.4%)	283 (>43.4%)	120 (>76.0%)
300 mg/kg BW PE of *Aloe vera* gel + 2mg/kg BW Metformin	>500	358 (>28.4%)	310 (>38.0%)	155 (>69%)

Plasma levels of creatinine and urea are biomarkers used for assessment of kidney functions in human and other vertebrates [11]. In this study, the creatinine and urea concentrations in plasma of rats

treated with both *Aloe vera* extract and metformin singly, and in combination, are not significantly different ($p>0.05$) from those of control though slightly higher. However, plasma level of urea in the diabetic untreated group was significantly higher ($p<0.05$) than that of the control group. The elevated level of urea in the diabetic rats is an indication of glomerular infiltration in nephrons due to the toxicity effect of the induced alloxan to the kidney cells. Administration of 300 mg/kg of PE of *Aloe vera,* 2mg/kg BW Metformin, and a combination of both treatments, to diabetic rats led to percentage decreases in plasma urea concentrations of 53.2, 38.4 and 35.4% respectively, compared to the untreated diabetic animals. Prolong infiltration of the glomerula could lead to impaired kidney function which in turn could interfere with body homeostasis leading to death [11]. The subtle disturbance in the integrity of the kidney observed in the diabetic rats in this study was significantly ameliorated by the single treatment or combined treatment with 300 mg/kg of PE of *Aloe vera* and 2 mg/kg BW Metformin (Table 2).

Table 2: Effect of *Aloe vera* gel on creatinine and urea concentrations in alloxan-induced diabetic rats

Group	Serum creatinine (mg/dL)	Serum urea (mg/dL)
Normal untreated	2.06 ± 0.89	25.11 ± 5.87
Diabetic untreated	0.57 ± 0.18$^{\alpha}$	57.87 ± 2.85$^{\alpha}$
300 mg/kg BW PE of *Aloe vera* gel	1.27 ± 0.19$^{\beta}$	27.07 ± 4.52$^{\beta}$ (-53.2%)
2mg/kg BW Metformin	2.38 ± 0.52$^{\beta}$	35.65 ± 2.20$^{\beta}$ (-38.4%)
300 mg/kg BW PE of *Aloe vera* gel + 2mg/kg BW Metformin	1.93 ± 0.32$^{\beta}$	37.40 ± 3.26$^{\beta}$ (-35.4%)

α-Statistically significantly different from normal control;
β-Statistically significantly different from diabetic untreated rats.
*Values in parenthesis are percentage decrease (-) compared to the untreated diabetic group.

Induction of diabetes in rats by alloxan administration has been severally reported to culminate in alteration in body lipid profile [8, 12, 13]. The presence of the appropriate proportion of fatty acids, triglycerides, cholesterols, and lipoproteins help in maintenance of the integrity of biological membranes. Alterations in lipid contents of biomembranes and adipose tissues may sometimes be reflected in the blood as either hypolipidemia or hyperlipidemia. Significant variation in lipid profile has been implicated in onset of many physiological disorders and or diseases including, but not limited to, obesity, hypertension, arteriosclerosis and cardiovascular diseases [12, 14]. In this study, the effect of *Aloe vera* gel on lipid profile in alloxan-induced diabetic rats was also investigated (Table 3). A non-significant increase in plasma triglyceride concentration was observed in the untreated diabetic rats group compared; the triglyceride level was, however, reduced by 15.29, 0.67 and 6.89% in the diabetic animals treated with 300 mg/kg BW PE of *Aloe vera* gel, 2 mg/kg BW Metformin and both 300 mg/kg BW PE of *Aloe vera* gel and 2mg/kg BW Metformin, respectively; although the decrease was not statistically significant ($p>0.05$). Also, the significantly high plasma concentration of total cholesterol observed in the untreated diabetic rats compared to control, was significantly reduced by in diabetic animal groups treated singly with either 300 mg/kg BW PE of *Aloe vera* gel or 2 mg/kg BW Metformin. Total cholesterol was also decreased in diabetic rats treated with a combination of both therapeutic agents, but the decrease was not significant. Similarly, plasma concentration of high-density lipoprotein (HDL)-cholesterol, commonly referred to as 'good' cholesterol, was significantly reduced in the untreated diabetic rats compared to the healthy control group (Table 3). The observed reduction in HDL-cholesterol in the untreated diabetic rats was, however, significantly improved by 160.0, 89.8 and 178.7% respectively in animals treated with 300 mg/kg BW PE of *Aloe vera* gel, 2 mg/kg BW Metformin, and a combination of both agents. The synergistic effect of the combined PE of Aleo vera and Metformin treatment was 1.12 and 1.99 times more effective than the respective single treatment with 300 mg/kg BW PE of *Aloe vera* gel and 2 mg/kg BW Metformin in their improvement of HDL-cholesterol levels. Excessively high total cholesterol and low HDL-cholesterol levels have been implicated in arteriosclerosis and cardiovascular diseases [12, 14]. Though Metformin, a standard diabetic drug, is highly efficacious in treatment of hyperglycermic patients, our findings reveal that its use in combination with the PE of *Aloe vera* would aid to avert diabetes-associated decrease in HDL-cholesterol level by 1.99-fold, thereby preventing patients from risk of cardiovascular diseases.

Table 3: Effect of *Aloe vera* gel on lipid profile in alloxan-induced diabetic rats

Groups	Triglycerides (mg/dL)	Total Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Atherogenic Index
Normal untreated	107.72 ± 38.59	68.55 ±39.95	16.10 ± 8.05	30.91 ± 24.18	3.258
Diabetic untreated	118.98 ± 32.23	98.56 ±39.35$^{\alpha}$	2.54 ± 1.27$^{\alpha}$	72.23±31.63$^{\alpha}$	37.803
300 mg/kg BWPE of *Aloe vera* gel	100.79 ± 34.32 (-15.29%)	56.91 ± 5.89$^{\beta}$ (-42.26%)	6.63 ± 3.32$^{\beta}$ (+161.0%)	30.12 ±4.29$^{\beta}$	7.583
2mg/kg BW Metformin	118.15 ± 40.91 (-0.67%)	47.38 ± 19.86$^{\beta}$ (-51.93%)	4.82 ± 2.41$^{\beta}$ (+89.8%)	18.94 ±9.54$^{\beta}$	8.829
300 mg/kg BW PE of *Aloe vera* gel +2mg/kg BW Metformin	111.14 ± 9.09 (-6.59%)	73.81 ± 27.24 (-25.11%)	7.08 ± 3.54 (+178.7%)	44.50±21.88$^{\beta}$	9.425

α-Statistically significantly different from normal control;
β-Statistically significantly different from diabetic untreated rats.
*Values in parenthesis are percentage decrease (-) or increase (+) compared to the untreated diabetic group.

The results of histological analyses of the pancreas and kidneys of rats used in this study also support the synergistic use of PE of *Aloe vera* and Metformin in treatment of diabetic subjects (Figures 1 and 2). Unlike the pancreas of the control healthy rats which did not any pathological changes at the end of the study, those of the untreated diabetic rats showed evidence of necrotic cells in both the acini and islet; atrophoid of islet of langaharn was also observed, indicating severe harm to the pancreatic β-cells due to the alloxan administration (Figure 1). The micrographs of diabetic rats treated with 300 mg/kg BW PE of *Aloe vera* gel, however, showed signs of early onset recovery features which was evident by the normal of pattern of globular ducts intercalation. The metformin treated diabetic rats also showed presence of normal histological features. The pancreas of diabetic rats given combined treatment of *Aloe vera* and metformin only showed signs of a few inflammatory cells (lymphocytes) found in the tissue, which is evident of cell recovery [15].

Toxic chemicals are generally capable of not only altering biochemical parameters but also causing alterations in the histology of internal organs [16]. In this study, the distal and proximal convulated tubules in kidneys of control group rats were normal but the untreated diabetic rats' kidneys showed evidence of total glomeruli erosion, cellular distortion and tubular dissolution. The kidney microphotograph of diabetic rats treated only with *Aloe vera* showed signs of early onset

features of recovery of tubules and components of renal capsules, while those of metformin treated rats had onset evidence of regeneration and re-arrangement of the tubule with diffused fibrin deposits, an indication of wound healing. Diabetic rats administered a combined treatment of *Aloe vera* and metformin showed traceable evidence degeneration, albeit there was cell recovery from erosion of the distal and proximal tubules [16].

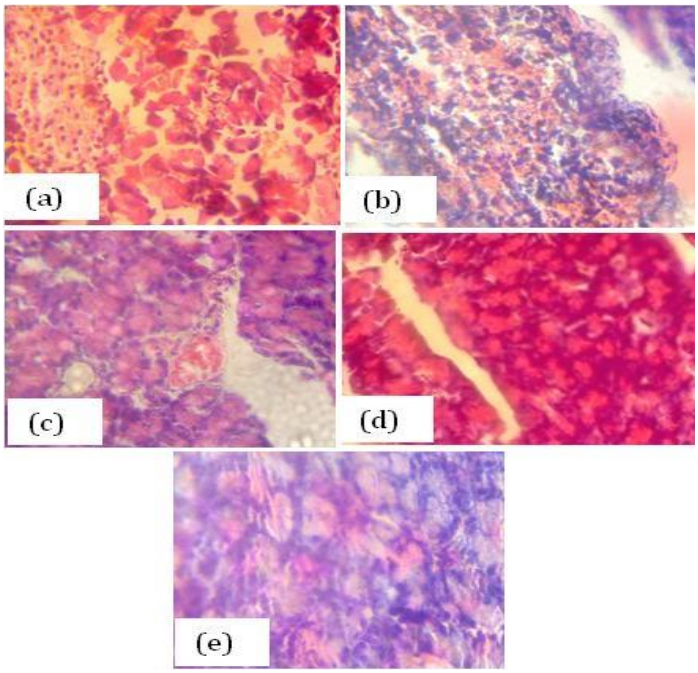

Figure 1: Microphotographs of histology of the pancreas of different groups after administration of *Aloe vera* (Eosin-Haematoxylin staining, 40x). The micrographs show pancrease from **(a)** normal control which has no pathological changes. The pancreatic tissues are filled normal cells of islets **(b)** untreated diabetic rats showing evidence of necrotic cells in both the acini and islet, the is observed atrophoid of islet of langaharn **(c)** *Aloe vera* treated rats shows of early onset features of recovery. The is evidence of normal of intercalated globular ducts **(d)** metformin treated rats, shoes onset of normal of histological features **(e)** combined treatment of *Aloe vera* and metformin showing evidence of cell recovery, a few inflammatory cells (lymphocytes) found in the tissue.

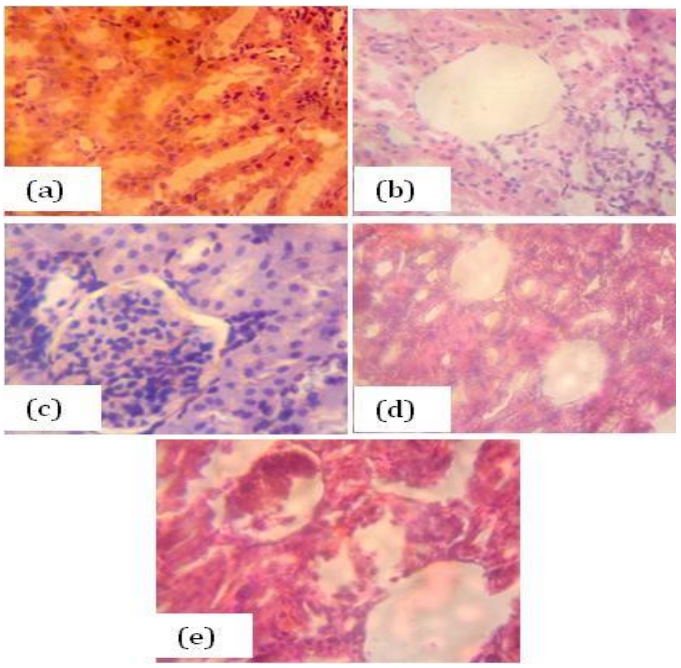

Figure 2: Microphotographs of histology of the kidney of different groups after administration of *Aloe vera* (Haematoxylin-Eosin staining, 40x). The micrographs show kidney from **(a)** normal control which has no pathological changes. The distal and proximal convulated tubules are normal **(b)** untreated diabetic rats showing evidence of total glomeruli erosion, cellular distortion and tubular dissolution **(c)** *Aloe vera* treated rats shows of early onset features of recovery of tubules and components of renal capsules **(d)** metformin treated rats, shows onset evidence of regeneration and re-arrangement of the tubule with diffused fibrin deposits, an indication of wound healing **(e)** combined treatment of *Aloe vera* and metformin showing traceable evidence degeneration albeit there is cell recovery from erosion of the distal and proximal tubules.

CONCLUSION

Combined treatment with *Aloe vera* and Metformin improved the indices of cardiovascular and renal health better than the respective single treatments with *Aloe vera* gel and Metformin. The pancreas and kidney histopathologies indicated signs of quick cell recovery in animal group given the combined treatment of *Aloe vera* gel and Metformin, unlike those of the untreated diabetic group that had evidence of necrotic cells in both the acini and islet in pancreas and total glomeruli erosion in kidney. The findings of the present suggest that use of *Aloe vera* in combination with Metformin for treatment of diabetic patients would aid to avert diabetes-associated hyperlipidaemia, improve pancreas and kidney integrity, and increase HDL-cholesterol level, thereby preventing patients from risk of cardiovascular diseases and kidney failure.

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