Evaluation of the Therapeutic Effects of Aloe Vera Gel on Alloxan Induced Diabetic animal models

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ABSTRACT

Aloe vera gel (AVG) extract is commonly used as a home remedy for some diseases, including diabetes mellitus. This study investigates the effects of pure Aloe vera (Aloe babardensis) gel on some biochemical, haematological and histological parameters in diabetic rats. The animals were divided into six groups of six rats each (n=6). Diabetes mellitus was experimentally induced in rats by intraperitoneal administration of a single dose of alloxan monohydrate (170 mg/kg BW.) following a 12 h fasting period. The Groups I and V received isotonic saline in a similar manner. The establishment of diabetes mellitus was confirmed by fasting blood glucose (FBG) levels above 350mg/dl using a GlucoMetre (AccuChek Active). The AVG extract was administered for 14 days along with the metformin and alloxan drugs administered. Blood samples were collected from fasted rats. The effect of AVG on fasting blood biochemical, haematological and oxidative stress parameters in the liver and histological examination of the liver, kidney and pancreas were evaluated. Results indicated a decrease in glucose level in the AVGtreated group and an improvement in the lipid profile, as well as some haematological parameters. In addition, oral administration of Aloebarbadensis gel decreased the level of MDA with a corresponding increase in the activities of CAT, SOD, GST and GSH levels in the liver tissue of diabetic rats. These results suggest potent hypoglycaemic, hypolipidemic, antioxidant and therapeutic effects of Aloe, beneficial as a herbal remedy for the treatment/management of diabetes mellitus.

Keywords: Diabetes; Alloxan; *Aloe babardensis g*el; Rats; hypolipidaemic

INTRODUCTION

Diabetes mellitus (DM), is still a serious health challenge all over the world, especially in developed and developing countries, with Type 2 diabetes mellitus now considered a worldwide epidemic. Recent statistics indicate that the global prevalence of diabetes mellitus for all age groups was estimated as 366 million in 2011, with a projected rise to 522 million by 2030 (Whiting et al., 2011). Diabetes mellitus is a long-term metabolic disorder of multiple etiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion and/or insulin action (ADA, 2011). The symptoms of diabetes are excessive urine production (polyuria), excessive thirst and increased fluid intake (polydypsia), blurred vision, unexplained weight loss and lethargy (Lionel, 2007).

It has been identified as one of the most important causes of complications, such as diabetic nephropathy, angiopathy, neuropathy, retinopathy, deficiency in the antioxidant defence system, and lipid profile disorders (Asburn and Villarreal, 2006; Giugliano and Ceriello, 1996).

In diabetes mellitus, chronic hyperglycemia produces multiple biochemical abnormalities, hyperlipidemia and diabetes-induced oxidative stress can play a role in the symptoms and progression of the disease (Rajasekaran et al., 2006; Baynes, 1991).

It has been reported that oxidative stress is also one of the major causes of pathophysiological conditions during diabetes. Moreover, persistent hyperglycaemia may lead to an exaggerated production of free radicals, especially reactive oxygen species (ROS), in tissues due to glucose oxidation and protein glycosylation (Oberley, 1988).

To reduce the risk of late complications and negative outcomes of diabetes mellitus such as renal failure, blindness and limb amputation, the control of both blood glucose and lipid levels is important.

The prevalence and complications of type 2 diabetes are increasing daily. The use of conventional drugs to treat metabolic disorders and the pathological consequences of diabetes further increases the complications because of the side effects and high costs of these drugs. Therefore, there is a need to develop alternative strategies for diabetes therapy.

Natural products are useful alternatives because these compounds are believed to have fewer side effects. Many minor components of foods, such as secondary plant metabolites, have been shown to alter biological processes, which may reduce the risk of chronic diseases in humans (Bailey and Day, 1989).

Many herbs, spices and other plant materials have been used to treat diabetes. A total of more than 400 species were reported to display hypoglycaemic effects, but few of these species have actually been investigated. To date, 90 plants have been screened for hypoglycaemic properties at various research institutes on Diabetes, Endocrine and Metabolic Disorders all over the world (Tiwari and Rao, 2002). These plants include *Trigonella foenum graecum*, *Allium cepa*, *Hemidesmus indicus*, *Syzigium cumini*, *Murraya koenigii* and *Aloe vera* ((Tiwari and Rao, 2002; Ugochukwu et al., 2003).

Aloes have long been used all over the world for their various medicinal properties. *Aloe barbadensis* miller (otherwise known as *Aloe vera*) is a perennial plant belonging to the family *liliaceae* and with its origin in the North Africa.
Aloe vera is one of the plants considered to have a hypoglycemic effect and many diabetic subjects take the gel because of its hypoglycaemic effect (Helal et al., 2003). It has been used for many centuries for its curative and therapeutic properties and for its ability to treat hyperlipidaemia (Helal et al., 2003; Kim et al., 2009).
Aloe vera not only possesseshypoglycaemic activity but is also hypotensive, hepato-protective and also a blood purifier (Vogler and Ernst, 1999).Moreover, *Aloe vera* reduces the intestinal absorption of water, helping to increase the occurrence of bowel movements. As a result, this plant is widely used as a laxative (Hamman, 2008).
The hypoglycemic effects of *Aloe vera* have been investigated by various researchers. Ethanolic extracts of *Aloe vera* administered to Wistar albino rats with high blood glucose levels resulted in a significant decrease in the plasma glucose levels of the rats (Abuelgasim et al., 2008).

In this study, thetherapeutic effect of *Aloe babardensis* gel was investigated on some biochemical, haematological and antioxidant parameters, as well as on the histology of the liver, and kidney in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Experimental Animals
A total of thirty-six healthy wistar albino rats of both sexes, weighing between 110 g and 200 g were used in the present study. They were purchased from the Animal Laboratory Centre, College of Medicine, University of Lagos, Nigeria. Before and during the experiment, the animals were maintained in a well-ventilated room at room temperature with natural day-night cycle in polypropylene cages lined with husk in standard environmental conditions (temperature 22 ± 5ºC, and a 12:12 light: dark cycle) at the rat room of the Animal Laboratory Centre, College of Medicine, University of Lagos. The rats were allowed free access to water (public tap) and food (standard rat chow diet purchased from Top Feed Nigeria Limited) except when overnight fasting was required according to the experimental design, and allowed to acclimatize for two weeks prior to induction. All animal procedures were conducted in accordance with the standards set forth in the guidelines for the care and use of experimental animals by the Committee for the Purpose of Control and Supervision of Experiments on Animals and the National Institutes of Health.
Rats weighing 180 – 200g were randomly divided into six groups of six animals in each group:
Group I: Normal Control group –> received0.9% saline solution;
Group II: Diabetic Untreated group–> received alloxan(170mg/kg b.w) once;
Group III: Metformin-treated group -> received Alloxan + Metformin (7.14mg/kg b.w);
Group IV: *Aloe vera*-treated group -> received Alloxan + *Aloevera* gel (300mg/kg b.w);
Group V: *Aloe vera*-Control group -> received 0.9%Salinesolution + *Aloe vera* gel (300mg/kg b.w);
Group VI: *Aloe vera* pre-treated group -> *Aloe vera* gel(300mg/kg b.w) for 2 weeeks + Alloxan.

Body weight and fasting blood glucose levels of all the rats were determined before the start of the experiment. The body weights of control and experimental groups were recorded at an interval of one week till the completion of the experimental period (14days), at day 0, 7 and 14 days. Dosages were calculated as extrapolated doses for humans.

Induction of experimental diabetes

Diabetes mellitus was experimentally induced in the rats of Groups II, III and IV and VI, by intra-peritoneal injection of a single dose of alloxan monohydrate (170 mg/kg b.w) following a 12 h fast. After injection, they had free access to food and water and were given 5% glucose solution to drink overnight to counter hypoglycaemic shock. Groups I and V received 0.9% saline solution in a similar manner. The establishment of diabetes mellitus was confirmed by fasting blood glucose (FBG) levels above 350mg/dl, using a glucometer. The drug solutions were prepared freshly each time and orally administered intragastrically for a period of 14 days. The dosing schedule used was once per day.

Blood and liver samples
At the end of the experimental period (14 days) of treatment, rats were fasted overnight and blood was drawn from the animals by retro-orbital sinus puncture method with capillary tubes. The blood was collected in heparin-coated centrifuge tubes, centrifuged at 4000rpm for 5minutes, then plasma was separated in Eppendorf tubes and stored at $-30°C$. Whole blood was also collected in EDTA bottles and used for the determination of some haematological parameters (such as Hb, RBC, WBC, etc.), whereas separated plasma was used to determine the lipid profile and renal function parameters (Electrolytes, Urea and Creatinine).
The animals were sacrificed. The liver and kidney were removed, washed with ice-cold 0.9% normal saline. The liver was divided into two parts, one part along with the other organs was used for the histological study and the other part was used for the assessment of lipid peroxidation (Malonaldehyde MDA, Gluthathione reductase GSH, GST, Superoxide dismutase SOD, and Catalase CAT).
The effect of AVG on fasting blood glucose, lipid profiles, liver and renal function parameters, haematological parameters in blood and oxidative stress parameters in the liver were evaluated.

Biochemical Analysis
Assay of lipid profile and renal function tests.
The levels of cholesterol, triglycerides, LDL, HDL, protein, creatinine and urea, were measured in the plasma samples obtained from all the groups. The measurements were performed in accordance with the manufacturer protocols of the Randox diagnostic kits, UK. The concentrations of all the parameters were evaluated by colorimetry. The intensity of colouration was measured using the UV/Visible-Model-80-2106-00 spectrophotometer, Pharmacia Biotech, Cambridge, England.

Estimation of haematological parameters
The haemoglobin (Hb), red blood cell (RBC), and white blood cell count (WBC) were measured. The Hb level was measured using a hemoglobinometer. The RBC and WBC counts were made using a haemocytometer, according to earlier described methods by Van Kampen and Zijlstra (1961). The tests were carried out within 12 hours after collection of blood.

Estimation of protein in liver
Protein content in the organ source was estimated by the method of Lowry et al. (1951),using bovine serum albumin as standard.

Estimation of lipid peroxidation
The lipid peroxidation level, or the amount of MDA in the liver, was measured by a method described by Okhawa et al. (1979). The liver tissue was homogenized in ice-cold 0.1M phosphate buffer (pH 7.2) and the absorbance was read at 532 nm. The concentration of MDA, which was expressed as nm of MDA per mg protein was determined.

Assay of hepatic reduced glutathione (GSH)
The reduced form of glutathione was determined using DTNB as the colouring reagent and following the method described by Beutler et al.(1963). The absorbance was read at 412 nm using a spectrophotometer and the GSH values were determined using the molar extinction coefficient of 13,600 $cm^{-1}M^{-1}$.

Determination of hepatic catalase (CAT) activity
The level of catalase activity was estimated in the liver homogenate by themethod (Takahara et al., 1960). The specific activity of catalase is expressed in units of moles of H_2O_2 consumed/min/mg of protein. The difference in the absorbance at 240 nm per unit time was used to determine the catalase activity. An extinction coefficient at 240nm H_2O_2 of 40.0 $M^{-1}cm^{-1}$ was used for the calculation.

Determination of hepatic superoxide dismutase (SOD) activity
Superoxide Dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as described by Misra and Fridovich (1972). Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min. $\sum = 4020M^{-1} cm^{-1}$.

Histological study

The liver and kidney samples from each animal were processed using light microscopy. The tissue sections were fixed in 10% neutral buffered formalin and embedded in paraffin. The paraffin sections were then stained with hematoxylin-eosin (H&E). Mallory Trichrome was used for detecting the collagen deposition in the hepatic tissue via a Leica DMRB/E light microscope (Heerbrugg, Switzerland).

Statistical Analysis

The statistical analysis was performed using the GraphPad Prism Program (GraphPad Software, version 5.0 Inc., San Diego, USA). The results were expressed as arithmetic mean (M) ± Standard Deviation (S.D) (n=4-6), and total variation present in a set of data was analysed through one-way analysis of variance (ANOVA). This measure was supplemented by individual comparison between the different treatments by applying Turkey's multiple tests for pair wise comparisonsat 95% ($p < 0.05$) confidence level.

RESULTS

Effect of *Aloe barbadensis* gel on the body weights and blood glucose levels in control and diabetic rats.
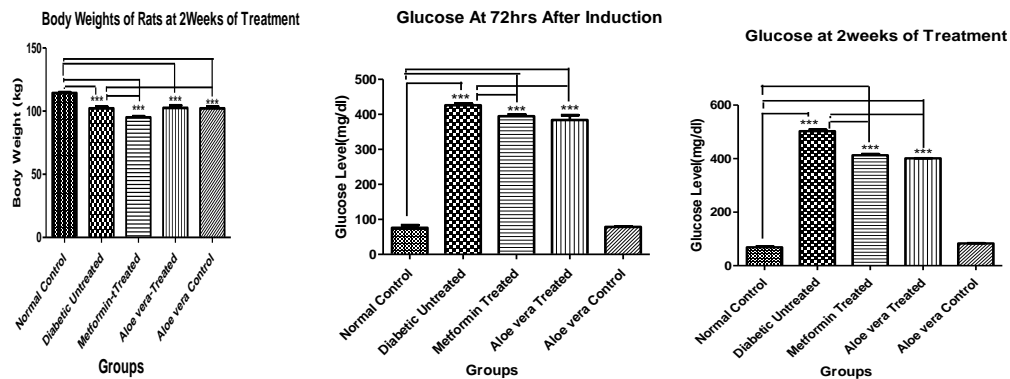

Fig. 1: The Effect of Oral Administration of Aloe barbadensis gel on body weight and fasting blood glucose

Data represents mean ± S.D. (n = 4-6). Comparison was done between the normal control and diabetic untreated rats, and between Aloe-treated and diabetic untreated rats. *p<0.05, **p<0.01 and ***p<0.001.

Effect of *Aloe barbadensis* gel on lipid profile in control and diabetic rats

The lipid profile showedan elevation in the levels of total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL-C) levels in diabetic untreated group when compared with the normal control and Aloe-treated groups, but decreased value of high density lipoprotein (HDL-C), while the *Aloe vera* showed declination in the Lipid profile levels and increased HDL-C levels denotingits hypolipidaemic effects (see Fig. 2 below).
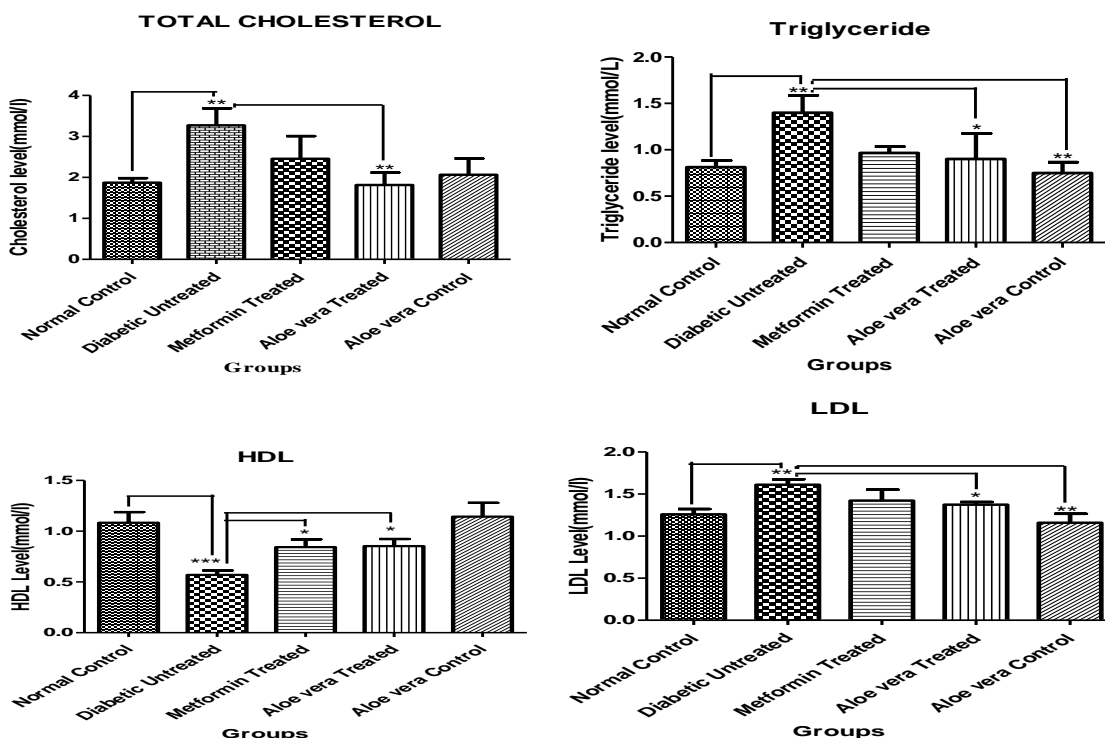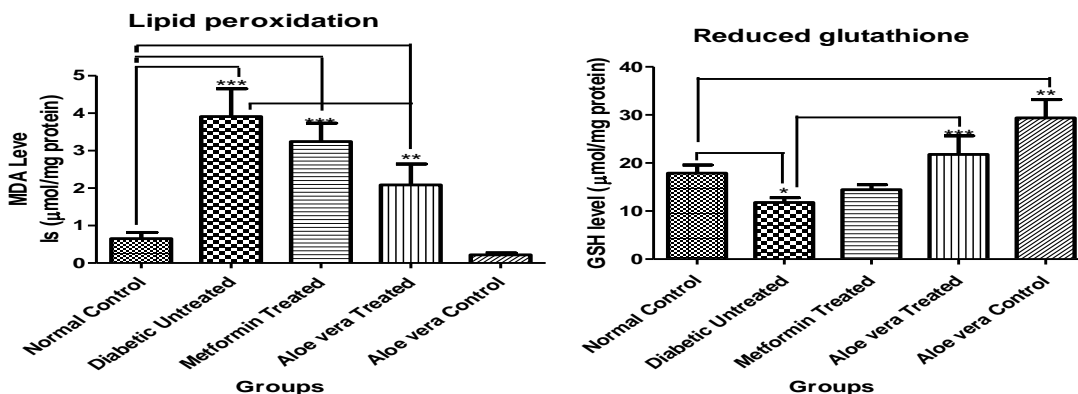

Fig. 2: The Effect of Oral Administration of Aloe barbadensis gel on Lipid profile
*Data represents mean ± S.D. (n = 4-6). Comparison was done between the normal control and diabetic untreated rats, and between Aloe-treated and diabetic untreated rats. *p<0.05, **p<0.01 and ***p<0.001.*

Effect of *Aloe barbadensis* gel on lipid peroxidation and antioxidant enzymes in control and diabetic rats

The levels of lipid peroxidation and activities of antioxidant enzymes in the liver of control and experimental rats are shown in Figure 3. A significant increase in lipid peroxidation was observed in the liver of diabetic untreated rats when compared to normal control group. The activities of SOD, GSH, CAT and GST significantly decreased in the liver of diabetic untreated rats when compared to normal control rats. Whereas in *Aloe vera* gel treated diabetic rats, lipid peroxidation levels were significantly decreased and antioxidant enzymes like SOD, GSH, CAT and GST activities were significantly increased in the liver when compared to diabetic untreated rats.
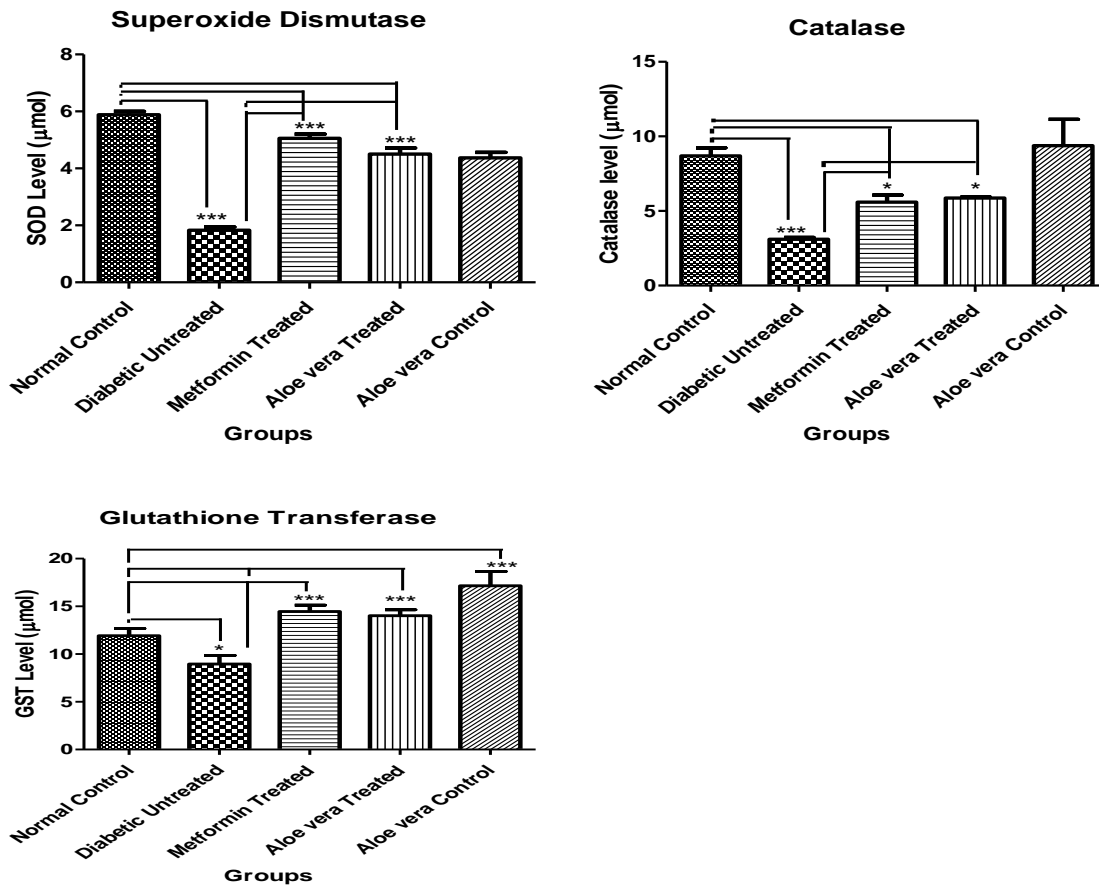
Fig. 3: The Effect of Oral Administration of Aloe barbadensis gel on lipid peroxidation and Antioxidant enzymes

Data represents mean ± S.D. (n = 4-6). Comparison was done between the normal control and diabetic untreated rats, and between Aloe-treated and diabetic untreated rats. *p<0.05, **p<0.01 and ***p<0.001.

Effect of *Aloe barbadensis* gel on renal function parameters in control and diabetic rats
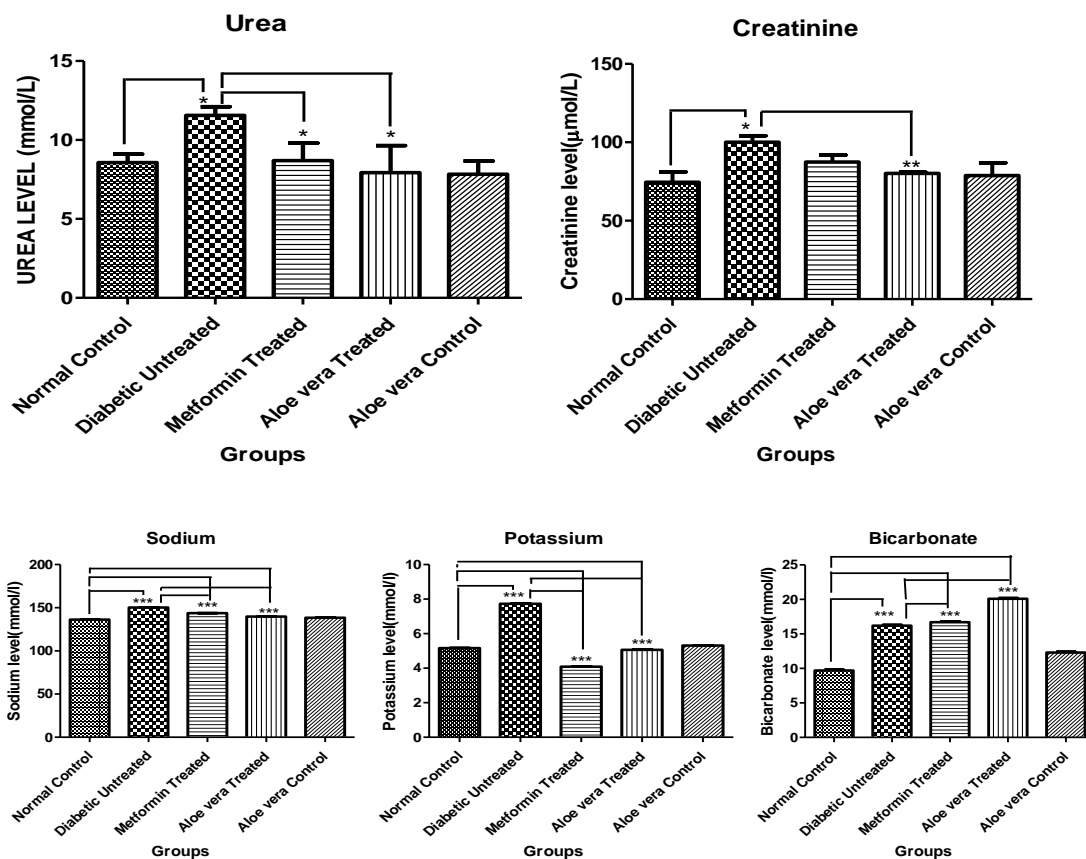

Fig. 4: The Effect of Oral Administration of Aloe barbadensis gel on Renal Function
*Data represents mean ± S.D. (n = 4-6). Comparison was done between the normal control and diabetic untreated rats, and between Aloe-treated and diabetic untreated rats. *p<0.05, **p<0.01 and ***p<0.001.*

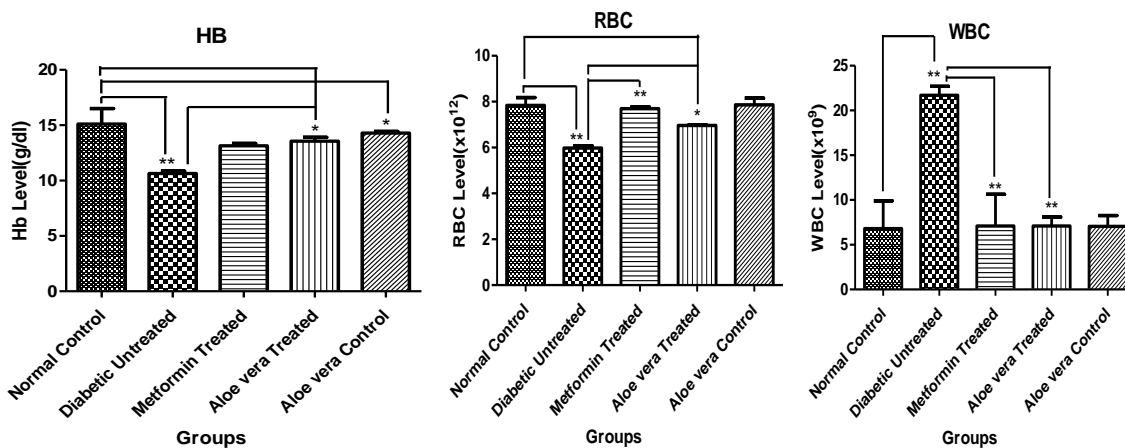**Effect of Aloe barbadensis gel on some haematological parameters in normal control and diabetic rats**

Fig. 5: The Effect of Oral Administration of Aloe barbadensis gel on Hematogical parameters, *Data represents mean ± S.D. (n = 4-6). Comparison was done between the normal control and diabetic untreated rats, and between Aloe-treated and diabetic untreated rats. *p<0.05, **p<0.01 and ***p<0.001.*

Effect of *Aloe vera* gel on histopathological changes in liver and kidney of diabetic rats
In control rat, liver cells show normal morphology. In the diabetic rats, few granular hepatocytes (arrow)were observed in the liver of diabetic untreated rats.
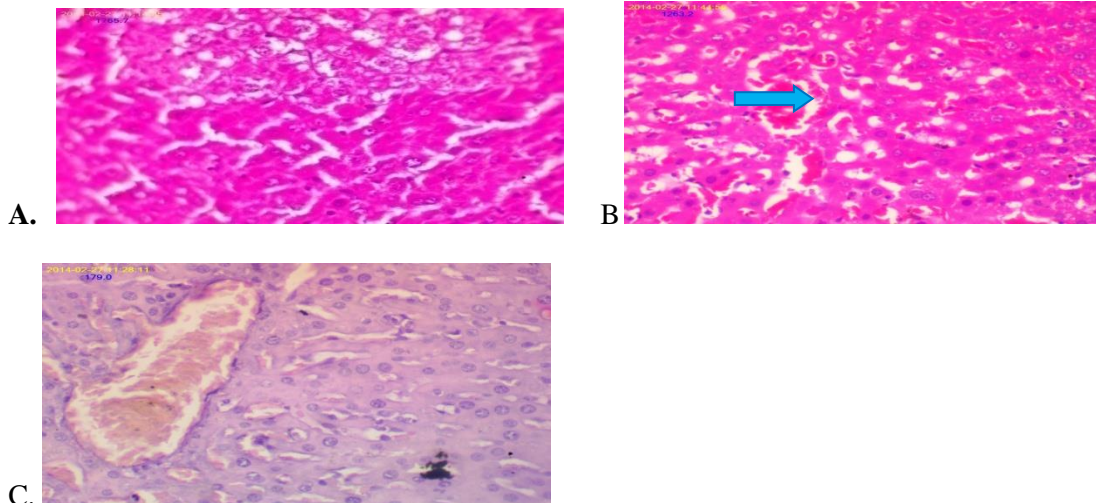
Plate 1: Histological observations of liver of control, diabetic and Aloe vera gel treated diabetic rats
(H&E ×400). A. *The liver cells of normal control rats shows normal morphology.* B. *A few granular hepatocytes (arrow) were observed in the liver of diabetic untreated rats.* C. *Moderate amount of congestion was observed in the liver of Aloe veragel treated diabetic rats.*

However, in diabetic rats treated with *Aloe vera* extract moderate amount of congestion was observed in the liver of Aloe vera gel treated diabetic rats the liver looking almost normal (Plate 1). In diabetic untreated rat, the kidney tubules show acute tubular necrosis. No abnormality was observed in *Aloe vera* extract treated diabetic rats, (Plate 2)
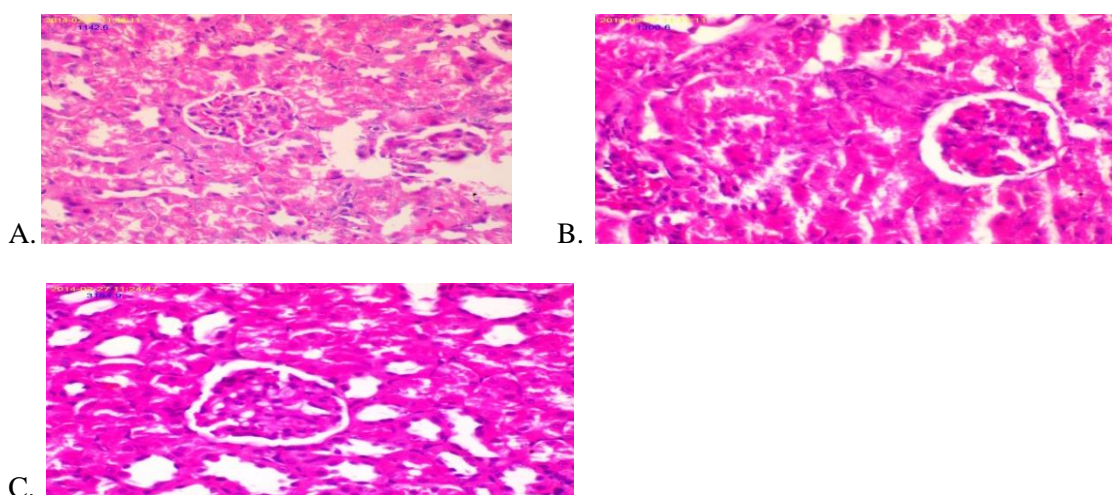

Plate 2: Histological observations of Kidney of control, diabetic and Aloe vera gel treated diabetic rats
(H&E ×400)**.** *A. The kidney cells of normal control rat shows normal architecture. B. The tubules show Acute tubular necrosis was observed in the kidney tubules of diabetic untreated rats. C. No abnormality was observed in kidney of Aloe vera gel treated diabetic rats.*

DISCUSSION
Diabetes mellitus is a multifunctional disease with several causes and complex consequences. It remains an important risk factor for cardiovascular disease and increasing rate of childhood and adult obesity. Diabetes is likely to become even more prevalent over the coming decade (Afaf et al., 2008). The currently available drug regimens for the management of DM have certain drawbacks and therefore, there is a need to find safer and more effective anti-diabetic therapies (Grover et al., 2002). DM of long duration is associated with several complications such as atherosclerosis, myocardial infarction, nephropathy etc. These complications have long been assumed to be related to chronically elevated glucose level in blood (Beppu et al., 2003).

The results obtained in this study are similar to previous findings (Chika and Bello, 2010; Jain and Arya, 2011; Maiti et al., 2004). Alloxan selectively destroyspancreatic cell, after being taken up by the pancreatic cells via GLUT-2 glucose transporters, alloxangenerates reactive oxygen species in a cyclic redoxreaction with its reduction product, dialuric acid, autoxidation of which generates superoxide radicals, hydrogen peroxide and, in

a final iron catalyzed reaction step, hydroxyl radicals. These hydroxylradicals are ultimately responsible for the death of the β-cells (Lensen, 2008).

In this study, the *Aloe vera* gel demonstrated its antidiabetic properties, significantly lowering blood glucose in the alloxan-induced diabetes to values that were comparableto those of the non-diabetic control consistently for a period of 14 days. The glucose lowering effects were even more pronounced. This result was consistent with other co-workers (Kim et al., 2009; Mohamed et al,. 2009; Rajasekaran et al., 2005).

The antidiabetic properties of *Aloe vera* is comparable to those of several plantswhich have been demonstrated to possesshypoglycaemic and antidiabetic actions, for example, significant antidiabetic and antihyperlipidaemic effect was reported with neem seed (*Azadirachta indica*) onalloxan induced diabetes in rats (Chttopadhyay, 1996),while the leavesextract was found to antagonize the glycogenolyticeffects and increased peripheral utilization of glucoseby epinephrine in alloxan and streptozocin induced diabetic and normal rats (Bopanna et al., 1997; Zhang et al., 2005). Similarly, chronic administration of crude aqueous extracts of *Momordicacharantia* and *Swertia chirayita* also showed hypoglycemic effect in streptozocin treated rats andmice. Although *S. chirayata* was more effective (Banerjee et al., 1994).

Hyperlipidemia is a known complication of DM and co-exists with hyperglycemia and is characterized by increased levels of cholesterol, triglycerides and marked changes in lipoprotein fractions. Control of hyperlipidemia is a prerequisite for the prevention of diabetic microangiopathy (retinopathy, nephropathy and neuropathy) and macroangiopathy (ischemic heart disease), cerebral vascular disease and arteriosclerosisobliterans in diabetes (Oberley, 1988). In the present study, elevation in serum lipid profile with concomitant decrease in HDL-C in alloxan-induced diabetic animals is in agreement with previous studies regarding alteration of these parameters under diabetic condition (Satyanarayana et al., 2006). The biochemistry of the movement of lipids in the blood stream and the factors that increase lipid deposition in arteries is extremely complex. As far as cholesterol is concerned, the two lipoproteins most concerned with its transport are the high density lipoproteins (HDL) and the low density lipoproteins(LDL). LDL transports cholesterol to the cells where it is deposited even though it may not be required and is therefore associated with atherosclerosis. HDL, on the other hand, transports cholesterol to the liver where it can be removed from the body (Allan et al., 2007). Normally, it is found that high cholesterol levels are associated with high LDL levels, but having a high HDL may compensate for this. In this respect, the markedly increased level of triglycerides and LDL-cholesterol in the serum of diabetic rats of the present work may be a consequence of either over production by the liver or defective removal from the circulation or both secondary to insulin deficiency (Capeau, 2008). Mechanisms by which HDL decreases in diabetes may be due to the impaired metabolism of triglycerides rich lipoprotein with decreased activity of lipoprotein lipase and impaired transfer of materials to the HDL components, in addition to the high level of hepatic lipase among diabetics (Balkis et al., 2009). Thomas showed a strong relationship between high level of total cholesterol concentration in the blood and cardiovascular disorder was shown by Thomas (2002).

Furthermore diabetics have an increased risk of coronary disorder (Stratton et al., 2000; Davis et al., 2001). However, administration of *Aloe vera* significantly reduced the lipid profile level and increased the HDL-C level. These results are similar to previous reports by Rajasekaran et al. (2006); Sharma et al. (2010).

Traditional plant remedies have been used for centuries in the treatment of diabetes, but only a few have been scientifically evaluated, especially on body tissue of diabetic patient. Therefore, investigation on the effect of *Aloe barbadensis* gel on biomarkers of oxidative stress and the antioxidant enzymes in diabetic rats was carried out. The increase in tissue MDA concentrations (end product of lipid peroxidation) in alloxan-induced diabetic rats along with a significant decrease in the antioxidant SOD activity are in conformation with previous reports documenting elevated serum lipid peroxide levels and diminished antioxidant status in diabetic subjects (Oberley, 1988). In this study, aloe supplementation results in suppressed free radical-induced oxidative damage. The reduced glutathione (GSH) levels, superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) activities were increased in the aloe-treated groups and signs of oxidative tissue damage, such as MDA, were decreased. Can et al.(2004)found similar results in the treatment of neonatal alloxan-induced type diabetic rats. Can and co-workers reported that treatment with aloe decreased damage to liver, increased glutathione and decreased lipid peroxidation. Singh et al.(2000) conducted an extensive study showing that oral dosing with aloe induced the phase II enzyme system (including SOD, CAT and GPx) of mice and significantly reduced lipid peroxidation. In 2002 at the International Aloe Science Council (IASC) Annual Conference, Vinson Joe presented evidence from a human chemical study, that the bioavailability of antioxidant supplement vitamins C and E was increased by over 200 percent when taken with *Aloe vera* gel (Vinson et al., 2005). The decreased activity of antioxidant molecules along with elevated lipid peroxide levels in diabetic rats

could probably be associated with oxidative stress and/or decreased antioxidant defense potential has also been reported (Mahdi, 2002). The reversal in their content following treatment may be due to decreased oxidative load. The *Aloe barbadensis* leaf extracts may also act by either directly scavenging the reactive oxygen metabolites, due to the presence of various antioxidant compounds or by increasing the synthesis of antioxidant molecules (Gupta et al.,2002).

CONCLUSION

The results of this study suggest that the medicinal plant, *Aloe Vera* may have nutritional remediation qualities, which when used in the right proportion could be of tremendous benefit to the body. These benefits include its usage in health challenges such as diabetes mellitus (DM) and associated complications. The Aloe gel produced a palliative and therapeutic effect as observed on blood glucose levels and other biochemical and haematological parameters examined.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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