# Anti-Atherogenicity Following Administration of Exudate from *Aloe barbadensis* Leaves in Diabetic Rats

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**Abstract**

***This study evaluated the effect of exudates from Aloe barbadensis leaves; 500 mg/kg on the lipid profiles and some atherogenic risk predictor indices in streptozotocin induced diabetes in rats. The classes of chemical components of the plant exudates were determined: Alkaloids, carbohydrates, saponins, tannins, and flavonoids were detected. Cyanogenic glycosides were absent. The acute toxicity test conducted in rats gave $LD_{50}$ of 1250.25 ± 86 mg/kg. High values of LDL – Cholesterol, triacylgcycerols levels, which are typical of the diabetic condition, were found in the rats. The exudates significantly reduced triacylglycerol levels and normalized cholesterol concentration. It also positively improved the ratio of atherogenic risk predictor indices. On this basis, we conclude that Aloe barbadensis leaf exudates have hypolipidaemic effect which when combined with the improved atherogenic risk predictor indices may be useful in controlling diseases associated with hypolipidaemic such as diabetes, ischaemia and atherosclerosis.***

**Keywords:** Diabetes, *Aloe barbadensis,* anti-atherogenicity

## Introduction

Diabetes mellitus has been defined by the world health organization (WHO), on the basis of laboratory findings, as a fasting venous plasma glucose concentration greater than 7.8 mmol/l (140mg/dl) or greater than 11.1 mmol/l (200 mg/dl) two hours after a carbohydrate meal or two hours after an oral ingestion of the equivalent of 75g glucose, even if the fasting concentration is normal. It is a metabolic disease characterized by hyperglycaemia and glycosuria due to absolute or relative lack of insulin (Aguwa, 1996).

Changes in lipid concentration and consequent disorders of lipid metabolism have been observed in diabetes mellitus (Ononogbu, 1998). With ketosis of diabetes mellitus, hyperlipidaemia and hypercholesterolemia may lead to increased level of lipid peroxidation. This enhances the oxidation of lipids and lipoproteins exposing a diabetic to dangers of atherosclerosis (Halliwell, 1990).

*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. Aloes have long been used all over the world for their various medicinal properties. *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 (Okyar *et. al.,* 2001). *Aloe vera* not only possesses hypoglycaemic activity but are hypotensive, hepatoprotective and also blood purifier (Tiwari and Rao, 2002).

In this study the possible hypocholesterolaemic potential of *Aloe barbadensis* was investigated for its possible use for prophylaxis and treatment of atherosclerosis commonly found in diabetic diseases, a fast growing plague in the world especially in Africa.

## Materials and Methods

**Plant material:** Fresh spiny leaves of *Aloe vera* were collected from aloe plant growing from the horticultural unit of Imo State University, Owerri. Botanical identify was kindly confirmed by Dr. C. I. Onuoha of Plant Science and Biotechnology Department of Imo State University, Owerri. A voucher specimen of the plant is deposited in Imo State University, Owerri.

**Preparation of the crude extract;** Fresh spiny leaves of *Aloe barbadensis* were collected and washed without squeezing to remove debris and dust particles. Large quantities of the leaves were cut open and the inside was collected/extracted, weighed and stored for use.

A measured volume of the extract (80 ml) was put in a weighed evaporating dish. The dish containing 80 ml of extract was evaporated to dryness by weight difference; the weight of the active ingredient in the 80 ml of the extract (the solid residue (2.4g) referred to as extract) was determined. Appropriate concentrations of the extract were made in distilled water for experiments and expressed as mg/ml.

**Acute toxicity test;** The acute toxicity ($LD_{50}$) of the extract was estimated in 30 Wistar albino rats by the I.P. route as described by Miller and Tainter (1944). In brief, the method involved the administration of 5 different doses of the extract to 5 groups of rats (6 rats/group). The number of deaths in each group within 24 hours was recorded. The $LD_{50}$ was estimated from the graph of percentage (%) mortality (converted to probit) against log-dose of the extract-probit 5 being 50%.

**Phytochemical tests:** The chemical classes of constituent in the freshly prepared extract were detected using standard phytochemical reagents and procedures as described by Trease and Evans (1983).

In general, tests for the presence or absence of phytochemical compound using the above methods involve the addition of an appropriate chemical agent to the crude material in a test tube. The mixture is then shaken vigorously or gently as the case may be. The presence or absence of saponins, flavonoids, tannins, alkaloids etc. was observed.

**Animals:** Wistar albino rats weighing 150 – 200 g were used in this study. All the rats were kept at room temperature of $30^0$C in the animal room of College of Medicine and Health Science, Imo State University, Owerri. They were allowed free access to water and feed diet (product of Pfizer Nigeria Ltd.) throughout the period of the experiment.

Eighteen rats, included for the study, were divided into 3 groups, 2 were made diabetic by intraperitoneal injection of 65 mg/kg body weight of streptozotocin (STZ) (Sigma, St. Louis, MO, USA) dissolved in citrate buffer (0.01M, pH 4.5). Diabetes was confirmed by the determination of fasting blood glucose concentration on the third day post administration of STZ showing fasting blood glucose levels above 250 mg/dl.

Body weight and fasting blood glucose levels of all the rats were determined before the start of the experiment. Rats were divided into the following groups.

Group 1: Control, given only the citrate buffer (0.01M, pH 4.5)

Group 2: Streptozotocin induced diabetic, made with a single dose of streptozotocin (65 mg/kg body weight) by intraperitoneal route.

Group 3: Diabetic rats treated with *Aloe barbadensis* 500 mg/kg/once a day, daily

Treatment was by oral compulsion. After 14 days of treatment the body weight and fasting blood glucose of the animals were again determined. Blood was collected and transferred in an EDTA anticoagulated tube for plasma separation.

**Experimental and analytical procedure:** Twelve hours after the last treatment and after the last feed given, 8ml of blood was collected from all the rats. The blood collected was transferred onto an EDTA anticoagulated tube.

The anticoagulated blood was then centrifuged using Wisperfuge model 1384 centrifuge (Tamson, Holland) for 15min to facilitate separation. The plasma obtained was used for lipid estimation. Total cholesterol was measured using the method of Frieldwald *et al* (1972), in which the colour intensity in acetic acid with ferric chloride and sulphuric acid gives the amount of cholesterol present. Triacylgcycerols was measured using the extraction method of Mendez *et al* (1975), while HDL-Cholesterol was measured using extraction method by Friedwald *et al*, (1972) and the amount also determined colorimetrically. LDL-Cholesterol was calculated using Friedwald formula.

**Statistical analysis:** The results were express as mean ± SD. The significance difference in mean value of tests and control was detected using Duncan multiple range test.

## Results

Table 1 showed the results of the analysis, in which there was a significant increase ($p<0.05$) in fasting blood glucose and a comparative decrease ($p<0.05$) in body weight when compared with the normal control. There was a slight increase in body weight and a significant decrease in fasting blood glucose in diabetic rats treated with *Aloe barbadensis*.

In table 2 there was a significant increase in mean plasma total cholesterol, LDL-cholesterol and triacylgcycerols levels ($p<0.05$) in streptozotocin induced diabetic rats with respect to normal controls. It also shows a statistically significant decrease in mean plasma total cholesterol, LDL-cholesterol and triacylgcycerols levels in diabetic rats treated with extract of *Aloe barbadensis* leaves. There was no significance difference of HDL-cholesterol in rats that consumed extract of *Aloe barbadensis* leaves when compared to diabetic control rats ($p<0.05$).

Table 3 showed mean ratios of the atherogenic risk predictor indices HDL-C/TC and LDL-C/HDL-C of Wistar Albino rats treated with extract of *Aloe barbadensis* leaves and controls. The mean ratio HDL-C/TC increased in rats that consumed extract of *Aloe barbadensis* leaves when compared to diabetic control rats ($p<0.05$) while LDL-C/HDL-C decreased.

## Discussion

The prevalence of atherosclerosis among diabetics is on the increase in the world and recently in Nigeria. The clinical consequences of this condition are serious and exert major research efforts to improve knowledge of its pathogenesis and thereby provide a more rationed approach to its prophylaxes and therapy (Kritchersky, 1970).

Various studies indicate that serum levels of cholesterol are strongly related to coronary atherosclerosis and increased risk of coronary heart disease. Clinical studies in humans have shown that lowering levels of serum cholesterol with diet or drugs decreases the incidence of coronary heart disease in diabetics (Gotto *et, al.*, 1990). The increase in serum triacylglycerols and LDL-cholesterol in diabetic controls are in conformation with pervious reports documenting elevated serum triacylgcycerols and lipid peroxide levels in diabetic subjects (Oberley, 1988). In this study administration of extract of *Aloe barbadensis* leaves significantly reduced serum triacylglycerols and LDL-cholesterol in diabetic.

The mean ratios of the atherogenic risk predictor indices HDL-C/TC and LDL-C/HDL-C of Wistar Albino rats treated with extract of *Aloe barbadensis* leaves and controls were also studied. The mean ratio HDL-C/TC increased in rats that consumed extract of *Aloe barbadensis* leaves when compared to diabetic control rats ($p<0.05$) while LDL-C/HDL-C decreased. There are several explanations for the interpretations of the obtained results. The mechanism for the cholesterol lowering effect of *Aloe vera* may be the inhibition of the specific activity of the enzyme HMGCoA reductase, a rate-limiting enzyme in cholesterol biosynthesis.

**Table 1: The mean values of body weight and blood glucose in both normal, diabetic and *Aloe barbadensis* treated diabetic rats. Values are expressed as mean ± SD**

| Body weight | Mean initial weight (g) | Mean final weight (g) | Mean weight gained (g) | Fasting Blood glucose (mg/dl) | |
|---|---|---|---|---|---|
| | | | | Initial | After 4 weeks |
| Normal Control | 136.32 ± 9.8 | 173.79 ± 11.8 | 38.6 ± 2.2 | 94.54 ± 2.6 | 97.42 ± 2.6 |
| Diabetic control | 138.26±10.54 | 122.48±8.5 | -14.2±2.1* | 252.22±3.0 | 261.42±5.6 |
| Treated diabetic rats | 137.8±11.8 | 133.6±12.2 | 5.5±2.23** | 255.56±7.2 | ¥107.71±4.4 |

** Significantly different from normal control group (p<0.05). ** Significantly different from normal and diabetic controls (p<0.05). ¥ Significantly different from Initial Fasting Blood glucose (p<0.05). Each group n = 6 albino Wistar strain rats. Total n = 30 albino Wistar strain rats.*

**Table 2: The mean concentrations of plasma lipoproteins in normal, diabetic and *Aloe barbadensis* treated diabetic rats. Values are expressed as mean ± SD**

| | Normal | Diabetic control | Treated diabetic rats control |
|---|---|---|---|
| Total cholesterol (mg/dl) | 115.0 ± 4.64 | 121.4 ± 4.23 | 116.42 ± 5.21 |
| HDl- cholesterol (mg/dl) | 33.86 ± 1.98 | 31.06 ± 2.27 | 33.98 ± 2.74 |
| LDl- cholesterol( mg/dl) | 61.84 ± 2.45 | *68.12 ± 1.34 | 63.11 ± 2.12 |
| triacylglycerols(mg/dl) | 96.5 ± 2.43 | *111.12 ± 3.62 | 96.78 ± 2.098 |

** Significantly different from normal control and treated diabetic rats (p<0.05); Each group n = 6 albino Wistar strain rats; Total n = 30 albino Wistar strain rats.*

**TABLE 3: Atherogenic risk predictor indices in normal, diabetic and *Aloe barbadensis* treated diabetic rats. Values are expressed as mean ± SD**

| | Normal | Diabetic control | Treated diabetic rats control |
|---|---|---|---|
| HDL/TC | 0.29 ± 0.006 | *0.24 ± 0.007 | 0.29 ± 0.007 |
| LDL/HDL | 1.82 ± 0.070 | *2.19 ± 0.070 | 1.85 ± 0.050 |

** Significantly different from normal control and treated diabetic rats (p<0.05); each group n = 6 albino Wistar strain rats; Total n = 30 albino Wistar strain rats*

This enzyme was significantly lowered after administration of *Aloe barbadensis* leaves in the rat liver microsomes (Merat and Fallahzadeh, 1996; Omkumat *et. al.,* 1991). There was no significant difference of HDL-cholesterol in rats that consumed *Aloe vera* extract when compared with the control. In contrast to the usually stated inverse correlation between levels of triglyceride and HDL-cholesterol, the significant reduction of triglyceride levels, found in this study was not associated with a significant increase in HDL-cholesterol.

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