

## Antioxidant activity of the exudate from *Aloe barbadensis* leaves in diabetic rats

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### Abstract

The effects of the exudate of *Aloe barbadensis* leaves on oxidative stress and some antioxidant status of streptozotocin induced - diabetic rats were studied. There was significant reduction in scavenging enzymes like superoxide dismutase (SOD) activity and significant increase in signs of oxidative tissue damage, such as lipid peroxidation products (plasma MDA) in streptozotocin induced - diabetic rats. Treatment with *Aloe barbadensis* (150mg/kg) increased antioxidant enzymes like SOD activities and significantly reduced lipid peroxidation products. This study shows that high blood sugar leads to increased oxidative stress and that exudates of *Aloe barbadensis* leaves possessed antioxidant activity as shown by increased scavenging SOD activity and decreases in lipid peroxidation products levels.

**Keywords:** Diabetes, *Aloe barbadensis*, oxidative stress, Antioxidant enzymes

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## INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by hyperglycaemia and glycosuria due to absolute or relative lack of insulin. Diabetes mellitus is considered a chronic disease, not yet curable by any known orthodox medicine.

Oxidative stress is reported to be increased in patients with diabetes mellitus<sup>1</sup>. Accumulating evidence suggests that oxidative cellular injury caused by free radicals contributes to the development of diabetes mellitus<sup>2</sup>. Reactive oxygen species generated in the cells are scavenged by antioxidant enzymes. Moreover diabetes also induces changes in the tissue content and activity of the antioxidant enzymes<sup>3</sup>.

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<sup>4</sup>. *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<sup>5</sup>. *Aloe vera* not only possesses hypoglycaemic activity but is hypotensive, hepatoprotective and also blood purifier<sup>6</sup>.

In view of the above consideration the present study was designed to investigate the protective effect of *Aloe vera* on plasma lipid peroxide and superoxide dismutase (SOD) activity, an antioxidant enzyme.

## 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 or extracted, weighed and stored for use.

A measured volume of the extract (80ml) was put in a weighed evaporating dish. The dish containing 80ml of extract was evaporated to dryness by weight difference; the weight of the active ingredient in the 80ml 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<sub>50</sub>) of the extract was estimated in 30 Albino rats by oral compulsion as described by Miller and Tainter<sup>7</sup>. In brief, the method involved the administration of 5 different doses of the extract to 5 groups of rats (6 rats per group). The number of deaths in each group within 24 hours was recorded. The LD<sub>50</sub> 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<sup>8</sup>.

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 150-200g were used in this study. All the rats were kept at room temperature 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 65mg/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 250mg/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 (65mg/kg body weight) by intraperitoneal route;

Group 3: Diabetic rats treated with *Aloe barbadensis* 150mg/kg/once a day, daily. Treatment was by oral compulsion.

After 4 weeks 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, 4 ml 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 thus obtained was used for malondialdehyde (MDA) (product of lipid peroxidation) estimation. Plasma MDA was measured by a thiobarbituric acid assay procedure<sup>9</sup>, which was calibrated using 1, 1, 3, 3 – tetraethoxypropane (Sigma Chemicals, St. Louis, MO, USA) as a standard. Results were expressed as nanomoles of MDA per millimetre of serum.

The remaining packed RBCs were washed thrice with normal saline. Haemolysis was performed by pipetting out 1ml of washed red blood suspension in ice-cold distilled water. Erythrocyte ghosts were sedimented in a high-speed refrigerated centrifuge at 12000 rpm for 40min. The cell content was separated out carefully and used for superoxide dismutase estimation<sup>10</sup>.

### Statistical analysis

The results were analyzed using Duncan Multiple range test. All data are expressed as mean ± SD. Differences between groups were considered significant at p<0.05.

## RESULTS

Table 1 shows 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*.

**Table 1:** The mean values of body weight and blood glucose in normal, diabetic and *Aloe barbadensis* treated diabetic rats. Values are expressed as mean ± SD. (n = 6)

Body weight	Mean initial weight (g)	Mean final weight (g)	Mean weight gained (g)	Fasting Blood glucose (mg/dl)	
				Initial	After 4weeks
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).

**Table 2:** The mean values of lipid peroxide and vitamins E and C in normal, diabetic and *Aloe barbadensis* treated diabetic rats. Values are expressed as mean  $\pm$  SD. (n = 6).

	Normal control	Diabetic control	Treated diabetic rats
MDA (nmol/ml)	3.63 $\pm$ 0.94	6.03 $\pm$ 1.51**	4.02 $\pm$ 0.62
SOD (U/mg protein)	84.3 $\pm$ 2.14	45.42 $\pm$ 1.94**	80.3 $\pm$ 3.14

\* Significantly different from normal control group ( $p < 0.05$ ). \*\* Significantly different from normal control and treated diabetic rats ( $p < 0.05$ )

In Table 2 there was a significant increase in lipid peroxide levels ( $p < 0.05$ ) in streptozotocin induced diabetic rats with respect to normal controls. It also shows a statistically significant decrease in lipid peroxide levels in diabetic rats treated with extract of *Aloe barbadensis* leaves. The activities of SOD were significantly higher in diabetic rats treated with extract of *Aloe barbadensis* leaves compared to diabetic control rats ( $p < 0.05$ ). There was no significant difference among the treated diabetic and normal groups but there was significant difference between diabetic and normal controls.

## DISCUSSION

Traditional plant remedies have been used for centuries in the treatment of diabetes<sup>4</sup>, but only a few have been scientifically evaluated. Therefore, we investigated the effect of extract if *Aloe barbadensis* leaves on biomarkers of oxidative stress and the antioxidant enzyme (SOD) in diabetic rats.

Our data showed that the extract of this plant significantly lowered the level of glucose in streptozotocin induced diabetic rats. This finding is similar to the work reported previously by Okyar *et al*<sup>5</sup>.

The increase in plasma MDA concentrations (end product of lipid peroxidation) in streptozotocin-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<sup>11</sup>. In this study, aloe supplementation results in suppressed free radical-induced oxidative damage. The superoxide dismutase (SOD) activity was increased in the aloe treated group and signs of oxidative tissue damage, such as lipid peroxide, were decreased. Can *et al*<sup>12</sup> found similar results in the treatment of neonatal streptozotocin-induced type 2 diabetic rats. Can and coworkers reported that treatment with aloe decreased damage to liver, increased glutathione and decreased lipid peroxidation. Reports of research indicating high blood sugar leads to increased oxidative stress and evidence of oxidative damage has been demonstrated in arterial samples from human diabetic subjects<sup>13</sup>. This damage is caused by reactive oxygen species that promote lipid peroxidation and play a serious role in the development of cardiovascular disease. In 2000, Singh and coworkers conducted an extensive study showing that oral dosing with aloe induced the phase II enzyme system (including SOD, catalase and glutathione peroxidase) of mice and significantly reduced lipid peroxidation<sup>14</sup>.

And finally, in 2002 at the International Aloe Science Council (IASC) Annual Conference, Vinson Joe presented evidence from a human chemical study<sup>13</sup>, that the bioavailability of antioxidant supplement vitamins C and E was increased by over 200 percent when taken *Aloe vera* gel.

The decreased activity of antioxidant molecular along with elevated lipid peroxide levels in diabetic rats could probably be associated with oxidative stress and/or decreased antioxidant defense potential<sup>15</sup>. 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<sup>16</sup> or by increasing the synthesis of antioxidant molecules.

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