Serum Glucose And Lipid Levels In Alloxan-Induced Diabetic Rats Following Oral Administration Of *Aloe Barbadensis* Miller Juice Extract.

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Abstract

Effect of *Aloe barbadensis* Miller juice extract on serum glucose and lipids in alloxan-induced diabetic rats was investigated. Diabetes was induced by intraperitoneal injection of 150mg/kg alloxan in 5% solution. Diabetes was confirmed 72 hours after alloxan injection, if fasting blood glucose (FBG) was equal to or greater than 10mmol/l.

Twenty Albino rats (*Rattus novergicus*) were divided into four groups of five rats each. Two control groups of non-diabetic control and diabetic control, received tap water. The other two groups consisting of diabetic test groups A and B, received 500mg/kg and 1000mg/kg respectively of the extracts daily for seven days. FBG was determined every 48hrs after the administration of the extract. Serum lipids were later determined, and the rats sacrificed 24hrs thereafter.

Results showed gradual but significant reduction ($P<0.05$) in blood glucose levels in diabetic test groups A and B, and this returned to normal level on the 8th day. The diabetic control rats remained hyperglycaemic throughout the experiment. There was no significant difference ($P>0.05$) in the rate of reduction of blood glucose between the two doses of the extracts. There were significant reductions ($P<0.05$) in serum LDL-cholesterol and Total cholesterol in diabetic control and diabetic test groups A and B, but no significant changes ($P>0.05$) in HDL-cholesterol and Triglyceride levels in all the groups.

The results showed that oral administration of *Aloe barbadensis* Miller juice extract to alloxanised diabetic rats has potent glucose lowering but no lipid lowering properties. The reduction in LDL-cholesterol and Total cholesterol may be ascribed to selective alloxan effect since diabetic control group which did not receive the extract also showed reduction. The mechanism of action of the extract will need further elucidation.

Keywords: Serum glucose and lipid levels, Alloxanised diabetic rats, Aloe vera juice extract.

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Introduction

Diabetes mellitus is a metabolic disorder of carbohydrate, lipid and protein caused by absence or deficiency of insulin, insulin resistance or both, ultimately leading to hyperglycaemia.[1] It is a major health problem in the developed and developing countries. The disease is ranked seventh among the leading causes of death, and third when its complications are taken into consideration.[2]

It has been predicted that the number of diabetic patients will double from 143 million in 1997 to about 300 million in 2025 largely because of dietary and other lifestyle factors.[2] In the developing countries where majority of diabetics are poor and cannot afford effective drugs, the use of medicinal plants become the option.

It is therefore imperative to evaluate one of the acclaimed herbal remedies for diabetes mellitus (*Aloe barbadensis* Miller) for its blood glucose lowering properties, more so that its safety had earlier been established.[3]

Aloe barbadensis Miller (Aloe vera) [family:Liliaceae], belongs to a class of plants called 'xeroids' which have the ability to close their stomata completely to avoid loss of water.[4] The aloe plant is the source of two herbal preparations: the aloe gel and aloe latex. The aloe latex is commonly referred to as 'aloe juice'- the bitter yellow exudate from the pericyclic tubules just beneath the outer skin of the leaves. The latex contains a series of glycosides known as anthraquinones, the most prominent of them being aloin A and B.[7] There are over 75 known components of aloe vera which are contained in about 1% of the plant while the rest is water. The components are obviously present in small amounts, hence their proportionate action is thought to arise from the synergistic effect of these substances.[4]

Many bioactive compounds have been isolated from plants and have been used as antidiabetic agents,[5] such as mucilaginous fibres of *Trigonella foenum graecum*[5], which is said to interfere with glucose absorption.[6]

Materials and Methods

The Aloe vera plant was obtained from Sango area of Ilorin and this was sent for identification at the Department of Biological Sciences, University of Ilorin, Ilorin, Ni-

geria, where voucher specimen was deposited.

White albino rats (*Rattus novergicus*) with an average weight of 150g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The rats were exposed to rat chow and water *ad libitum*. They were allowed to acclimatize in metabolic cages for two weeks and exposed to 12hrs each of natural daylight and darkness. The cages were cleaned of metabolic waste twice a day.

Alloxan monohydrate was manufactured by BDH Chemicals, Poole, England.

Assay kits for lipids were obtained from Quimica Clinical Applicada S.A (QCA), Amposta, Spain. Other reagents used were of analytical grade and were prepared in double walled glass distilled water.

Induction of diabetes

Diabetes was induced by intraperitoneal injection of 5% solution of alloxan monohydrate at a dose of 150mg/kg body weight of rat.

Administration of Aloe vera Juice

Aloe vera leaves were washed thoroughly under a running tap water, after which the leaves were cut open and the gel scooped into a muslin cloth. The cloth was squeezed to obtain the juice which was weighed, then administered orally to the rats at dosages of 500 and 1000mg/kg body weight for diabetic test group A and B respectively. This extract was prepared daily for each administration over the seven day period.

Determination of blood glucose

Medi-test Glycaemic C Test strip used for glucose determination was made by Mocherey-Nagel GMBH and Co.KG, Duren, Germany and uses glucose oxidase method.

The rats were fasted for 12hrs and the fasting blood glucose determined for all the groups before the injection of alloxan to diabetic control and test groups A and B. The tail of each rat was cut with sterile scapel blade and squeezed to obtain a sizeable drop of blood to cover the test area of the strip. The colour which developed after 60 seconds was compared with the colour chart, and the value recorded. The test was repeated every 48hrs until the termination of the experiment.

Determination of serum lipids

The rats were sacrificed 24hrs after the administration of the extract for seven day period. Blood was collected by cardiac puncture into clean dry beakers which were allowed to stand for 1hr before it was centrifuged at 1000 rpm for 15minutes. The serum was pipetted using Pasteur pipette into clean dry sample bottles. The total serum cholesterol, HDL-cholesterol, LDL-cholesterol and Triglycerides were determined using QCA high performance kits.[8] This was based on the principle of cholesterolesterase, chlesterol oxidase and PDD catalysis to produce coloured quinonic derivative that was measured by colorimeter.

Statistical analysis

The data obtained were expressed as mean ± SD and analysed by Duncan's multiple range test.[9] P values of less than 0.05 were considered statistically significant.

Results

Induction of diabetes resulted in significant elevation of fasting blood glucose concentration when com-

Table 1: Fasting blood glucose in alloxan induced diabetic rats following oral administration of Aloe vera juice extract

Blood glucose concentration (mmol/L)				
Animal groups	Day 0	2	4	6
Normal control	5.11 ± 0.25^{a}	5.11 ± 0.25^{a}	5.11 ± 0.25^{a}	5.11 ± 0.25^{a}
Diabetic control	11.32 ± 1.81^{b}	10.97 ± 1.60^{b}	10.50 ± 2.60^{b}	10.33 ± 1.20^{b}
Diabetic test Group A	13.30 ± 0.00^{c}	9.51 ± 3.41^{b}	6.50 ± 2.29^{a}	5.61 ± 1.06^{a}
Diabetic test Group B	$12.38\pm1.31^{b,c}$	9.00 ± 2.12^{b}	5.81 ± 1.03^{a}	5.51 ± 0.50^{a}

Values are mean of five determinations ±SD abc… values along same vertical column with different superscripts are significantly different ($P<0.05$)

Table 2: Serum lipid profile in alloxan induced diabetic rats following oral administration of Aloe vera juice extract.

Serium Lipid Profiles (mmol/L)				
Animal groups	LDL-Chol	HDL-Chol	Trig	Total-Chol
Normal control	1.34±0.24[a]	1.10±0.20[a]	0.46±0.11[a]	2.54±0.34[a]
Diabetic control	0.96±0.13[b]	1.20±0.19[a]	0.51±0.09[a]	2.25±0.21[ab]
Diabetic test Group A	1.00±0.07[b]	0.94±0.22[a]	0.45±0.13[a]	2.00±0.21[b]
Diabetic test Group B	0.92±0.08[b]	1.04±1.17[a]	0.44±0.13[a]	2.02±0.19[b]
Values are mean of five determinations ±SD a,b.… values along same vertical column with different superscripts are significantly different (P<0.05)				

pared to levels in normal animals (table 1). Hyperglycaemia was recorded in all the three diabetic groups.

Administration of Aloe vera juice extract resulted in reduction of fasting blood glucose levels in diabetic rats beginning from the third day of administration (table 1). As the period of administration of extract progresses, the fasting blood glucose value of treated rats declined toward normal control level. There was no significant difference (P>0.05) between the groups treated with 500mg/kg and those treated with 1000mg/kg body weight.

Table 2 did not show any increase in serum lipid levels in diabetic control when compared to normal control. However, there were significant reductions (P<0.05) in LDL-cholesterol and Total cholesterol values in diabetic control and diabetic test groups A and B when compared to the control. There were no significant differences (P>0.05) in the serum HDL-cholesterol and Triglyceride values in all the four groups.

Discussion

The hyperglycaemia (blood glucose greater than 10mmol/l) observed after intraperitoneal injection of alloxan when compared to the fasting blood glucose of 5.11mmol/l of normal control rats in this study suggests that the pancreas of the rats were functioning well before alloxan injection. This agrees with previous observations that experimentally induced diabetes is accompanied by hyperglycaemia, amongst other biochemical changes.[10,11] The hyperglycaemia observed after alloxan injection is attributable to the inability of the insulin-dependent tissues to take up plasma glucose and partly to enhanced gluconeogenesis from amino acids, especially those derivable from muscle proteins.[12]

The results showed progressive decrease in blood glucose levels as treatment progressed for both diabetic test groups A and B. This showed that the extracts has hypoglycaemic effect but there was no significant difference in the effect produced by the two doses considered. Aside from hyperglycaemia, it is also well known that diabetes is often accompanied by a concomitant increase in serum lipids.[11,13] This was not observed in this study, and may probably be due to either short duration of this study or some unknown causes. In this study, the period of monitoring was seven days as against fifteen days employed by previous ones.[11,13,14]

There were significant reductions (P<0.05) in serum levels of LDL-cholesterol and Total- cholesterol in both untreated diabetic control and diabetic test groups A and B. This reduction may be ascribed to unexplained selective alloxan effect rather than that of the extract, since untreated diabetic control also showed similar reduction. The selectivity is based on the fact that the serum levels of HDL-cholesterol and Triglycerides were not affected. The observed hypoglycaemic effect of the extract without concomitant hypolipidaemic properties showed that the mechanism of hypoglycaemic activity is not likely to be insulin-like, since insulin is known to possess hypoglycaemic and hypolipidaemic properties.[11,13] Hyperlipidaemia in diabetes mellitus is due to accelerated biosynthesis of very low density lipoprotein(VLDL) and lack of clearance by lipoprotein lipase whose activity is dependent on high insulin:glucagon ratio, which is deficient in diabetics.[10] Further studies should be carried out to elucidate on the mechanism of its hypoglycaemic ropperties.

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