Research Article

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Evaluation of Antidiabetic Effects of *Kleinia Squarrosa* on Alloxanized Diabetic Mice

*[1]Njagi Joan Murugi, [2]Ngugi Mathew Piero, [3]Kibiti Cromwell Mwiti, [2]Ngeranwa Ngari Jeremiah Joseph, [2]Njagi Eliud Nyaga Mwaniki, [4]Mbiti Wilson Njue, [5]Maina David & [6]Gathumbi Peter Karuri

[1]Department of Environmental Health, Kenyatta University, Nairobi;
[2]Department of Biochemistry and Biotechnology, Kenyatta University,Nairobi;
[3]Department of Pure and Applied Sciences , Mombasa Polytechnic University College, Mombasa;
[4]Department of Chemistry, Kenyatta University, Nairobi;
[5]Institute of Nuclear Science, University of Nairobi,
[6]Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi, Nairobi.

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Abstract: Diabetes mellitus is a disease of antiquity with a worrying global prevalence and incidence. It is conventionally managed by insulin and use of oral hypoglycemic drugs besides exercise, diet and physical intervention therapies. Use of insulin and oral hypoglycemic agents is bedeviled by the fact that they are costly and have numerous adverse effects. Medicinal plants have for long been used for treatment of many disease including management of diabetes mellitus. They habour the pros of affordability and accessibility. This study was designed to bioscreen aqueous stem bark extracts of *Kleinia squarrosa* for its blood glucose lowering potential. The three tested dose levels (50, 100, and 150mg/kg body weight) lowered blood glucose levels significantly. This study has established that the aqueous stem bark extract of *Kleinia squarrosa* has antidiabetic effects and can justifiably be used for management of diabetes mellitus.

Key words: *Kleinia squarrosa*, stem bark extract, antidiabetic activity, medicinal plants, diabetes mellitus.

INTRODUCTION:

Diabetes mellitus is an endocrinological disorders presenting with hyperglycemia. Its etiologies entail a complex interaction of genetic factors, environmental factors and lifestyle choices. It has an estimated global prevalence of 4.4% by the year 2030. The number of diabetics worldwide is expected to rise from 171 million in 2000 to 366 million in 2030 [1].

Type 1 (IDDM) diabetics lack insulin and their treatment is hormone replacement therapy with insulin. Type 2 (NIDDM) diabetics, however, often do not respond to insulin and require alternative drugs if dietary control is insufficient. There are two main classes of oral hypoglycaemic drugs, the sulphonylureas such as tolbutamide and glibenclamide, and the biguanides such as phenformin and metformin [2]. The use of sulphonylureas, the other class of hypoglycaemic agents, is also not without its problems and side effects. They have adverse effects including coma [3], particularly in the elderly and patient with impaired hepatic or renal function who are taking longer acting sulfonylureas [2].

Unfortunately, sulfonylureas and metformin are often unable to lower glucose concentrations to within the normal range, or to reinstate a normal pattern of glucose homeostasis [4, 5]. Equally, insulin therapy does not reinstate a normal pattern of glucose homeostasis in most type 2 diabetics, and over vigorous insulin treatment may carry an increased risk of atherogenesis and hypoglycaemia [6].

As a result of these numerous side effects of oral hypoglycaemic drugs, there has been an increase in research of other potential oral hypoglycaemics and renewed attention to alternative medicines and natural therapies. This has stimulated a new wave of research in traditional practices and the World Health Organization Expert Committee on Diabetes has listed these as one of the treatment for diabetes which should be further investigated [7,8].

Traditional oral hypoglycemic plants might provide a useful source of new oral hypoglycemic compounds for development as pharmaceutical entities, or as simple dietary adjuncts to existing therapies. The known use of plants for diabetes dates from the Ebers papyrus of about 1550BC and many traditional plant treatments for diabetes are used throughout the world [9]. After the introduction of insulin therapy, the use of traditional treatments for diabetes greatly declined in societies, although some plant extracts are still used as prophylactics and adjuncts to conventional medicine [2].

It is in this regard that this study was designed to bioscreen aquesous stem bark extract of a medicinal plant *Kleinia squarrosa*, for its antidiabetic bioactivity with an ultimate goal of scientifically validating its use in the management of diabetes mellitus. The plant has been in use successfully in the management of diabetes melltus in some parts of Kenya but it has not received much focus by way of medical and/or scientific scrutiny.

MATERIALS AND METHODS

Collection of Plant Materials: Stem barks of the medicinal plant *Kleinia squarrosa* were collected from their natural habitat in *Muthuari* village in Siakago division of Mbeere North district in Embu county, Kenya. The collection was based on the folklore reports from local practicing herbalists on hypoglycemic activity of the plant. An acknowledged authority in taxonomy identified the plant and voucher specimen was deposited at the National Museum of Kenya herbarium. The voucher specimen number of the plant was *Hypo 3/06*. The stem barks were cut into small pieces and air-dried at 25±3^{o}C away from direct sunlight for three weeks. The dried materials were then crushed into powder by use of an electric mill (Christy and Norris Ltd, England).

Preparation of Extracts: The aqueous stem bark extracts of *Kleinia squarrosa* were prepared by boiling 100g of crushed material in one liter of distilled water for two hours with frequent stirring. The mixture was left to cool at room temperature and then decanted into a dry clean conical flask through folded cotton gauze stuffed into a funnel. Following decantation, the extract was filtered using Whatmann no. 1 filter papers by use of a vacuum pump. The filtrate was freeze-dried for 72 hours. Afterwards the powder stored at 4^{o}C in airtight containers.

Preparation of Extracts for Injection into experimental animals: Physiological saline was prepared by dissolving 0.85g of analytical grade NaCl in 100 ml of distilled water. The extract for injection was prepared as follows: the 50mg/kg body weight dose was prepared by dissolving 12.5mg in 1ml of physiological saline; the 100mg/kg body weight dose was prepared by dissolving 25mg in 1ml of

physiological saline; and the 150mg/kg body weight dose was prepared by dissolving 37.5mg in 1ml of physiological saline. The experimental animals were given 0.1 ml of the extract solutions. Insulin was also reconstituted and animals were given 0.1 ml.

Hypoglycemic Bioscreening

Laboratory Animals: 4-6 weeks old healthy Swiss albino male mice weighing 23-27 g were used for the study. They were fed on the standard mice pellet diet and allowed free access to water *ad libitum*. Permission for use of laboratory animals in this study was sought and granted by animal rights agency in Nairobi, Kenya. For experimental purposes, the animals were fasted overnight and allowed free access to water. The animals were divided into 4 groups of four animals each. Group 1 (Non-diabetic mice) was given 0.1ml of normal saline; Group 2, (diabetic mice) was given 0.1ml of normal saline; Group 3, (diabetic mice) was given insulin at a dose of 1 IU/kg body weight; and Group 4, (diabetic mice) was given plant extracts at three dose levels (50mg/kg body weight, 100mg/kg body weight and 150mg/kg body weight). Each dose level was given to 4 animals. Group 1 and 2 served as controls whereas Group 3 served as the reference group. Diabetic condition was induced in mice by intraperitoneal injection of alloxan monohydrate (150mg/kgbw) [sourced from Fluka chemie Gmbhch 9471 Switzerland] 72 hours before the start of experiment. Before administration of the different treatments the animals were bled and blood glucose level in the animals was measured. This was the initial measurement at time zero. The animals were again bled hourly until the fourth hour.

Collection of Blood Samples: Blood samples were collected from the tails of the animals after wiping the tail with surgical spirit. The tail was nibbed by use of a pair of sharp scissors, a drop of blood was squeezed into a Glucometer. After collection of blood, the nibbled side of the tail was rubbed with cotton wool soaked in absolute ethanol to protect the animal from infection and to arrest further bleeding.

Blood Glucose Level Determination: The principle of the test is based on a glucose oxidase/peroxidase reaction, which is specific for β-D-glucose. The hypoguard machine was used together with GB Supreme blood glucose test strips. The Supreme Test Strip is a disposable plastic strip containing a chemically treated test area used to measure the amount of blood glucose. The test area is designed in such a way that when a drop of blood is placed on the top surface, color change occurs which is determined by a Supreme Hypoguard meter. The supreme Test Strip was fully inserted into the meter before applying a drop of blood to fully cover the test area inside the grey target. The test strips and the Supreme Hypoguard Glucometer were obtained from Hypoguard Ltd, United Kingdom through Chemoquip Ltd, Kenya.

RESULTS AND DISCUSION:

Effect of *Kleinia squarrosa* on Blood Glucose in Alloxan-Induced Diabetic Mice: The aqueous stem bark extract of *Kleinia squarrosa* exhibited remarkable hypoglycemic properties in alloxan-induced diabetic mice. Similar work carried showed hypoglycemic activity on streptozotocin induced diabetic mice on administration of aqueous leave extracts of *Camellia sinensis* [10].

As figure 1 shows, in the 1st hour after administration of the extract at the three dose levels (50,100, and 150mg/kg body weight), the blood glucose level was lowered by 28%, 29%, and 14% respectively. At this point, the three dose ranges significantly lowered the blood glucose levels but not as effectively as insulin ($^{a}p<0.05$; $^{b}p<0.05$) (table 1). The same trend was repeated in the second hour where the three dose levels produced hypoglycemic condition by 23%, 41%, and 21%, respectively. The percentage decrease in blood sugar level by the three dose ranges in the 3rd hour was 54%, 51%, and 32% respectively. At this hour, the 50 and 100mg/kg body weight dose range lowered blood glucose level to normal with the former being as effective as insulin. On the other hand, the 150mg/kg body weight dose range showed similar hypoglycemic action as in the second hour. In the 4th hour, the three dose ranges lowered blood glucose levels by 45%, 60%, and 32%, respectively.

It is clear that the extract demonstrated a non-dose dependent response. This trend might suggest that the extract may have been absorbed in the cell system through active transport where at a particular concentration saturation of the extract occurred resulting in the rest of the extract being excreted

The three dose ranges lowered the blood sugar levels to normal (Table 1). This is consistent with previous similar study established a significant blood glucose lowering effect in alloxan- induced diabetic mice by aqueous root extracts of *Rheum ribes* [11]. Ethanolic leaf extracts of *Cassia kleinii* were also shown to lower blood glucose in streptozotocin-induced diabetic rats to normal [12].Since the extract was administered intraperitoneally, it is postulated that the extract might have been absorbed through the cell lipids membrane through facilitated diffusion. The ions might have been transported in the direction of its electrochemical gradient. This trend is in agreement with expectations seen in administration of higher concentration of drug.

CONCUSION:

The results of this study confirm the suitability of the aqueous stem bark extract of *Kleinia squarrosa*, for the management of diabetes mellitus. However, the mode of its antidiabetic activity is still obscure. Further, it is hereby recommended that toxicity studies be carried out to establish the toxic dosage of the extracts.

Since oral hypoglycemic agents are taken orally, it is imperative to undertake studies of the plant using oral route of extract administration. This would establish whether the extracts are inactivated by the digestive enzymes of the gut. Nevertheless, the study has been justified.

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Table 1: Effects of *Kleinia squarrosa* extract on blood glucose levels in alloxan induced diabetic mice (mg/dl)

Animal Group	Treatment/Dose	0 hr	1 hr	2 hr	3 hr	4 hr
Normal	Saline	68.3±3.2	65.5±1.9	62.3±2.7	65.3±0.3	67.5± 1.0
Diabetic	Saline	177.5±9.5	178.3±10.2	182.5±12.6	191.3±13.3	199.8± 14.9
Diabetic	Insulin (1IU/kgbw)	123.5±15.2	47.3±8.3	49.8± 6.2	54.3± 2.3	62.5± 1.9
Diabetic	50mg/kgbw	142.0±14.6	102.9±15.0^{ab*}	112.3±12.9^{ab*}	62.8±6.3^{a}	75.3± 5.5^{a}
Diabetic	100mg/kgbw	161.0 ±16.3	114.8±9.4^{ab*}	92.8±3.3^{ab*}	76.3±4.7^{ab}	61.0± 7.5^{a}
Diabetic	150mg/kgbw	151.5 ±6.4	130.3±4.1^{ab*}	120.8±11.7^{ab*}	103.5±8.5^{ab*}	96.2± 6.2^{ab}

*P<0.05 with respect to normal control; ^{a}P<0.05 with respect to diabetic control; ^{b}P<0.05 with respect to insulin. The data was analyzed using student‘s ‘t’– test. All the results were expressed as mean ± SEM . All blood glucose levels were recorded in mg/dL.

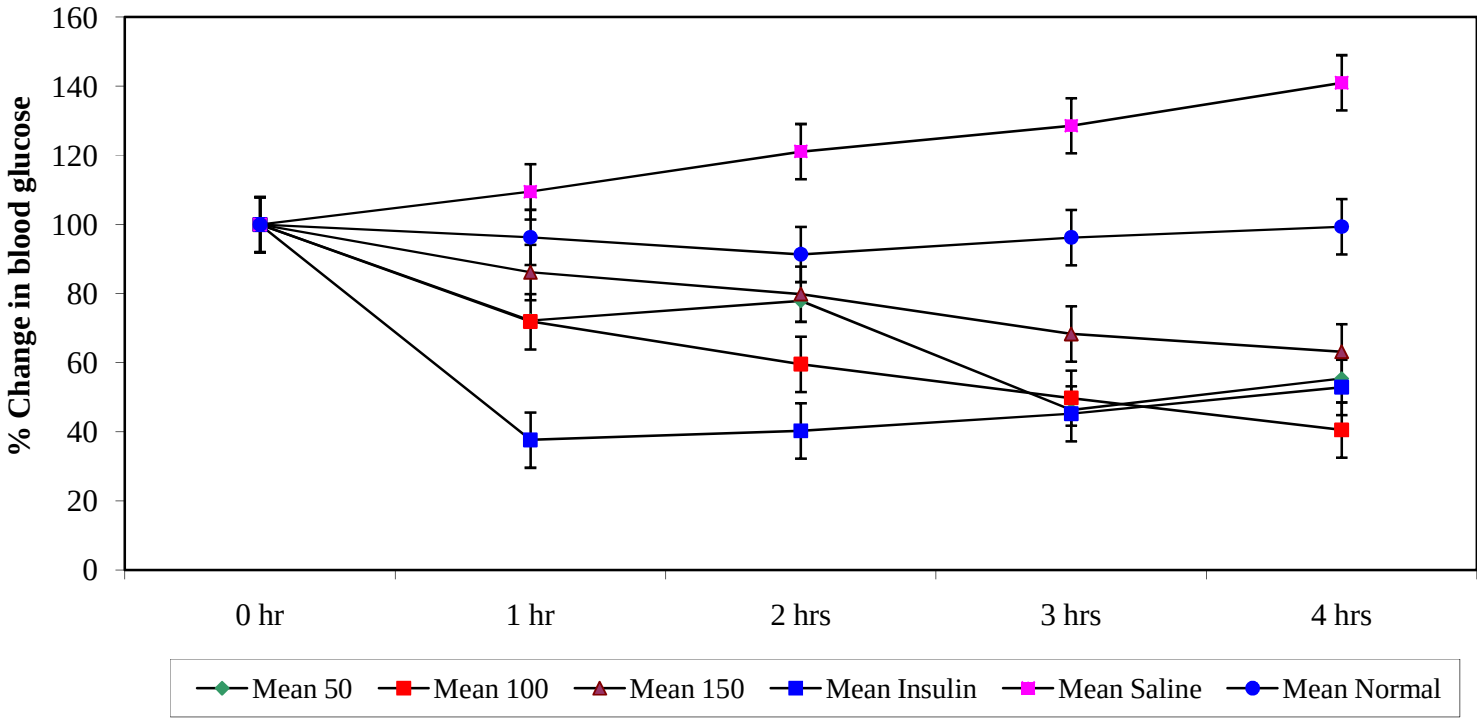

Figure 1: Percentage reduction in blood glucose by varying doses of *Kleinia squarrosa* in diabetic mice.

*P<0.05 with respect to normal control; [a]P<0.05 with respect to diabetic control; [b]P<0.05 with respect to insulin.

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***Correspondence Author:** Njagi Joan Murugi, Department of Environmental Health, Kenyatta University, Nairobi, KENYA.