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Research Article

Evaluation of the Anti-Hyperglycemic Activity of the Crude Leaf Extracts of *Sida Acuta* in Normal and Diabetic Rabbits

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ABSTRACT

The effect of the aqueous and methanol extracts of *Sida acuta* on blood glucose levels in both normal and diabetic rabbits was studied. The leaf extracts were screened for their effects on blood glucose levels in glucose overloaded rabbits. These extracts were also tested for anti-diabetic activity in alloxan-induced diabetic rabbits. Acute toxicity and preliminary phytochemical studies were performed. Results showed that both the aqueous extracts of *S acuta* (AESA) and the methanol extracts of *S acuta* (MESA) (400mg/kg) significantly increased the tolerance for glucose in glucose fed normal rabbits. Blood glucose was reduced significantly at $1^1/2$ hrs post-glucose load ($p<0.05$). This reduction was consistent and persisted to $2^1/2$ hrs. The positive control drug (glibenclamide, 0.5 mg/kg body weight, p.o) produced significant reduction on glycemia at 2 hours post glucose load ($p<0.01$). The methanol extract produced a significantly lower glucose concentration (mg.min/dl), as calculated from the area under the curve (AUC) of the glucose tolerance test, than AESA, glibenclamide and negative control respectively in the time periods 30-60 minutes, 60-90minutes and 90-150minutes ($p<0.05$; $p<0.01$). Both extracts (AESA and MESA) reduced blood glucose level in alloxanized rabbits significantly ($p<0.05$). The AESA and MESA (400mg/kg p.o) produced significant decreases in blood sugar at 4hours with percentage glycemic change of 30% and 20% respectively. The anti-hyperglycemic action of AESA and MESA were sustained up to 8hours with significant percentage glycemic change of 46% and 45% respectively ($P<0.01$). Glibenclamide (0.5 mg/kg p.o) produced significant glucose reduction in alloxanized rabbits at 2 hours with a percentage glycemic change of 24.5% ($P<0.01$) and a percentage glycemic change of 40.4% at 8hours. The crude leaf extracts of *Sida acuta* possess anti-hyperglycemic activity in diabetic and normal glucose fed rabbits.

INTRODUCTION

Sida acuta Burm F. is claimed in folk medicine to be an effective oral hypoglycemic agent. *Sida acuta* (Malvaceae) is an erect, branched small perennial herb or small shrub growing abundantly in Nigeria. It is commonly known as wire weed because of the resilience of the plant. In the Southern part of Nigeria, the plant is used to hasten delivery, treat malaria, jaundice, and as anti-inflammatory and hypoglycemic agent. Elsewhere, the decoction of the entire plant is taken orally for asthma, fever, aches and pains, ulcers and for venereal diseases.

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The alarming rate of increase in the incidence of diabetes mellitus recently has led to the anchor of herbal remedies for the treatment. As of 2000 at least 171 million people worldwide suffer from diabetes, or 2.8% of the population [1] and the number is increasing in rural and poor populations throughout the world [2]. Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underutilized, producing hyperglycemia [3]. It is characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from vascular diseases. Treatment of diabetes mellitus with medicinal plants is an alternative to allopathic treatment [4]. Medicinal plants are inexpensive and can easily be sourced locally. Medicinal plants play an important role in the management of diabetes mellitus especially in

developing countries where resources are meagre [5]. Despite efforts in the management of this dreadful condition with allopathic medicinal agents, diabetes mellitus still ravages mankind at an alarming rate. Moreover most of the drugs used in allopathic medical practice are not devoid of side effects [6]. On the other hand, several medicinal plants possess hypoglycemic properties[5, 7-11] and many plant preparations are used in folk medicine to manage diabetes mellitus. New oral hypoglycemic compounds from medicinal plants may provide a useful source for development of drugs or as a dietary adjunct to existing therapies [12]. Herbal drugs are considered to be less toxic and more free from side-effects compared to synthetic drugs [13]. Recently, a search for appropriate anti-hyperglycemic agents has focused on plants used in traditional medicine because natural products may be a better option than currently used drugs. Therefore, this study was undertaken to verify the folkloric claim on the use of *Sida acuta* for the treatment of diabetes mellitus.

MATERIALS AND METHOD

Animals

Twenty-eight (28) male rabbits weighing 1.2kg-2.0kg were obtained from the Animal House of the College of Medicine, University of Nigeria Teaching Hospital, Enugu. They were housed under standard conditions of temperature (28 ± 3^0C) and a 12 hour light/12 hour dark cycle. The animals were housed in groups and were provided with water and standard pellets (Guinea feed) *ad libitum*. The period of acclimatization was 2 weeks. All the animals were handled in this study according to Institutional guidelines on experiments involving the use of animals.

Plant Collection and Identification

The leaves of *Sida acuta* Burm F. used for this work were collected from their natural habitat in and around Enugu between the months of February and March, 2010. A specimen of the plant was identified by a taxonomist at the Herbarium section of the Department of Botany, University of Nigeria, Nsukka. A voucher specimen was deposited at the Herbarium for reference (UNH/82 b).

Plant Extraction

The leaves were air-dried under the shade to avoid decomposition of the Phytochemical constituents. They were dried for about seven days after which there observed to be dried and brittle. They were ground into fine powder with a gasoline powered grinding machine. The dry powder was stored until needed for the extraction process.

Extraction

Methanol Extraction

The powered leaves (1000 g) of *Sida acuta* was weighed out, placed in a gallon, and 2.5 liters of 80% methanol was added and left for 48hrs. The mixture was intermittently agitated during the extraction process. After 48 hrs, the mixture was filtered using a Whatman No. 1 filter paper and the filtrate was evaporated to dryness on a rotary evaporator (model 349/2 Corning Ltd, England). The residue obtained was stored in a refrigerator at 4 ± 2^oc until required. The methanol extract had a yield of 14.6% (w/w). The methanol extract (10 g) was dissolved in 3% aqueous suspension of tween 80 and made up to 100ml with the same solvent (100 mg/ml). Appropriate dilutions were made from this for the study.

Aqueous Extraction

The powdered leaves (500 g) was soaked in 250ml of portable water and homogenized using a stirring wooden rod. The homogenate was strained using a muslin cloth. The resultant filtrate was filtered through a Whatman No 1 filter paper. The filtrate was concentrated by evaporation in an incubator at 60°C. The resultant concentrate was stored in the refrigerator until required. The concentration of the aqueous extract was 100 mg/ml.

Phytohemical Test

Phytochemicals of the extracts were identified by qualitative chemical tests [14].

Acute Toxicity Test (Median Lethal Dose, Ld_{50})

This was performed on mice and the Lorke [15] procedure of LD_{50} determination was used.

Experimental Design

Glucose Tolerance Tests

Sixteen (16) male rabbits were divided into four groups (A-D) of 4 rabbits per group. Blood was collected from all the rats after an overnight fast for fasting blood sugar estimation. Group A was kept as vehicle control and received 5ml/kg of 3% aqueous suspension of Tween 80 p.o. The rats in groups B, C, and D received 400mg/kg of aqueous extract of *Sida acuta* (AESA), 400mg/kg of the methanol extract of *Sida acuta* (MESA) and

0.5mg/kg glibenclamide respectively by the oral route. The rabbits in all the groups were loaded with 60% glucose (3gm/kg, p.o) thirty (30) minutes after extract/drug administration according to the method of Babu *et al* [16] with slight modification. Blood samples were collected from the tail vein at 30, 60, 90, 120 and 150 minutes after glucose administration for glucose estimatiomn.

Alloxan-Induced Diabetic Rabbits

Rabbits were fasted for 24 hours but had access to portable water. The animals were then injected with alloxan monohydrate (prepared freshly as an 8% solution in saline), 120 mg/kg intraperitoneally, as described by Akah and Okafor [17]. After 4 days, animals with fasting blood glucose ≥250 mg/dl were selected for the study. Twenty (20) diabetic rabbits were grouped into four (4) groups of five (5) animals per group. Fasting blood samples were collected from animals in all the groups for blood glucose estimation through the ear vain. These fasting samples were labeled 0 hr samples. Animals in groups A to D received 5 ml/kg of 3% Tween 80, 400 mg/kg AESA, 400 mg/kg MESA and 0.5 mg/kg glibenclamide respectively via an oral cannula. Blood samples were collected from rabbits in all the groups from the ear vein at 2, 4 and 8 hours for glucose estimation. The percentage glycemic change was calculated using the following formula:

$$\% \text{ Glycemic change} = \frac{\text{Glucose concentration (2,4, or 8)} - \text{fasting blood glucose}}{\text{Fasting blood glucose}} \times 100$$

The areas under the curve (AUC) of changes in the blood glucose were calculated by the following formula:

$$AUC = \sum \{[(C_n - C_o) + (C_{n+1} - C_o)] \times (t_{n+1} - t_o)]\}$$

Glucose Determination

Blood glucose was measured using the One Touch-ultra blood glucose monitoring system (LifeScan, California, USA).

Statistical Analysis

Results were expressed where appropriate as Mean ± Standard Error of Mean. Differences between mean values were determined with the students't – test. $p<0.05$ was considered significant.

RESULTS

Preliminary phytochemical tests revealed the presence of abundant amounts of alkaloids and proteins, moderate amount of flavonoids, and the presence of glycosides, tannins, saponins, steroids, and terpenoids. The acute toxicity tests showed that the extract had an oral LD_{50} >5000 mg/kg. The effect of the aqueous and methanol extracts of *Sida acuta* (Malvaceae) on glucose tolerance of normal fasted rabbits is shown in figure 1. Both AESA and MESA at a dose of 400 mg/kg significantly increased the glucose tolerance. Both extracts reduced blood glucose significantly at $1^1/2$ hrs post glucose load ($p<0.05$). This reduction was consistent and persisted for $2^1/2$ hrs. However, there was marked reduction in glycemia at 2 hrs by both extracts ($p < 0.01$, table I). The positive control drug (glibenclamide) produced significant reduction in glycemia at 2 hrs ($p<0.01$).

The result of anti-diabetic study (Table 2) revealed that both extracts (AESA and MESA) reduced blood glucose of alloxanised rabbits at 4 hrs significantly ($p<0.05$). The 400 mg/kg body weight does of AESA Produced a significant decrease in blood sugar at 4 hrs with a percentage glycemic change of 30% while the 400 mg 1kg body weight does of MESA produced a percentage glycemic change of 20.5%. This implies that the 400Mg 1kg does of AESA had better anti diabetic potency than the MESA. Interestingly, the anti-hyperglycemic action of AESA and MESA were maintained at 8 hrs with a significant decrease in glycemia and a glycemic change of 46% and 45% respectively ($p < 0.01$). Glibendamide (0.5 mg/ kg) produced significant glucose reduction in alloxanised rabbits at 2 hrs with a glycemic change of 24.5% ($p<0.01$; table 2; figure 2). From these results, the extracts of *Sida acuta* had better hypoglycemic effect in the glucose tolerance studies than glibenclamide. However, glibenclamide showed superior glucose control in alloxanised rabbits.

Table 3 shows the blood glucose concentration in mg x min/dl calculated from the areas under the curve (AUC) of blood concentration at specific time periods of the glucose tolerance study.

The AUC was significantly lower in the methanol extract, glibenclamide, and negative control groups respectively in the time periods 30 – 60 minutes, 60 – 90 minutes and 90 – 150 minutes ($p<0.01$; $p<0.05$; $p< 0.01$). The total AUC was also significantly lower in the methanol extract treated group than in other groups.

Table 1: The Acute Effect (Hypoglycemlic) of *S. Acuta* on Glucose Tolerance of Normal Glucose Feed Rabbits

Group	Glucose at 0 hour	Glucose at 0.5hr	Glucose at 1hr	Glucose at 1 hr 30 minutes	Glucose at 2 hrs	Glucose at 2 hr 30 minutes
A 5ml/kg (3%Tween 80)	134.75±7.35	299.00± 10.50	339.50±19.59	286.25± 11.29	237.50±14.34*	172.75±4.13***
B 400 mg/kg (AESA)	142.50±14.75	297.50± 22.60	244.75±20.62	202.00±18.98*	181.75±14.07**	141.75± 9.41**
C 400 mg/kg (MESA)	147.50±11.96	256.75± 21.12	248.00 ± 9.98	178.25± 8.45*	131.25±5.98**	107.25± 5.34**
D 0.5mg/kg (glibenclamide)	138.00±6.26	254.00± 20.08	302.75± 8.96	207.50± 10.07	168.25 ± 9.11	137.00±11.52**

*p < 0.05; **p < 0.01; ***p < 0.001

Table 2: The Anti-Hyperglycemic Effect of S. Acuta in Mean Blood Glucose (Mg/Dl) (% Glycemic Change)

Group	Fasting Glucose 0 hr	Glucose at 2 hrs	Glucose at 4 hrs	Glucose at 8 hrs
A 5ml/kg (3% Tween 80)	277.00 ± 8.89	312.67 ± 6.89	289.33 ± 10.97	277.67± 8.95
B 400 mg/kg (AESA)	282.67 ± 15.60	305.67 ± 13.09 (+ 8.1%)	198.67 ± 10.41* (- 30%)	154.33 ± 4.70** (-46%)
C 400 mg/kg (MESA)	272.67 ± 9.33	311.00 ± 12.58 (+13.9%)	217.00 ± 10.69* (-20.5%)	149.33 ± 13.22** (-45.2%)
D 0.5 mg/kg (Glibenclamide	287.00 ± 9.84	208.67 ± 3.53** (- 24.5%)	168.00 ± 4.04** (- 39.3%)	165.00 ± 4.36*** (-40.4%)

*p < 0.05; **p < 0.01; ***p < 0.001; n =3 ;

Table 3: Blood Glucose (Mg.Min/Dl) Calculated From Areas under the Curve (Auc) Of Blood Concentration at Specific Time Periods

Time Period	3%Tween80 (Negative control)	400mg/kg AESA	400mg/kg MESA	Glibenclamide (0.5 mg/kg)
0 – 30 minutes.	6510 ± 216.90	6600 ± 193.6	6063.6 ± 187.2	5880 ± 176.3
30 -60 minutes	9577.8 ± 314.50	8133.6 ± 206.4	7571.4± 256.6[a]	8351.4 ± 214.0
60 – 90 minutes	9375 ± 296.30	6701.4 ± 198.7	6393.6+298.2 [a,b]	7653.6 ± 228.9[a]
90 – 150 minutes	13770 ± 726.50	10311±436.3[c]	8565± 321.8[a,b,d]	10335 ± 412.5 [c]
TOTAL AUC	39262.8 ± 206.5	31746 ± 217.6[a]	28593.6± 226[a,b]	32220 ± 231[a]

a = p < 0.01 with respect to negative control; b = p < 0.05 with respect to glibenclamide

c = p< 0.05 with respect to negative control; d = p< 0.01 with respect to glibenclamide

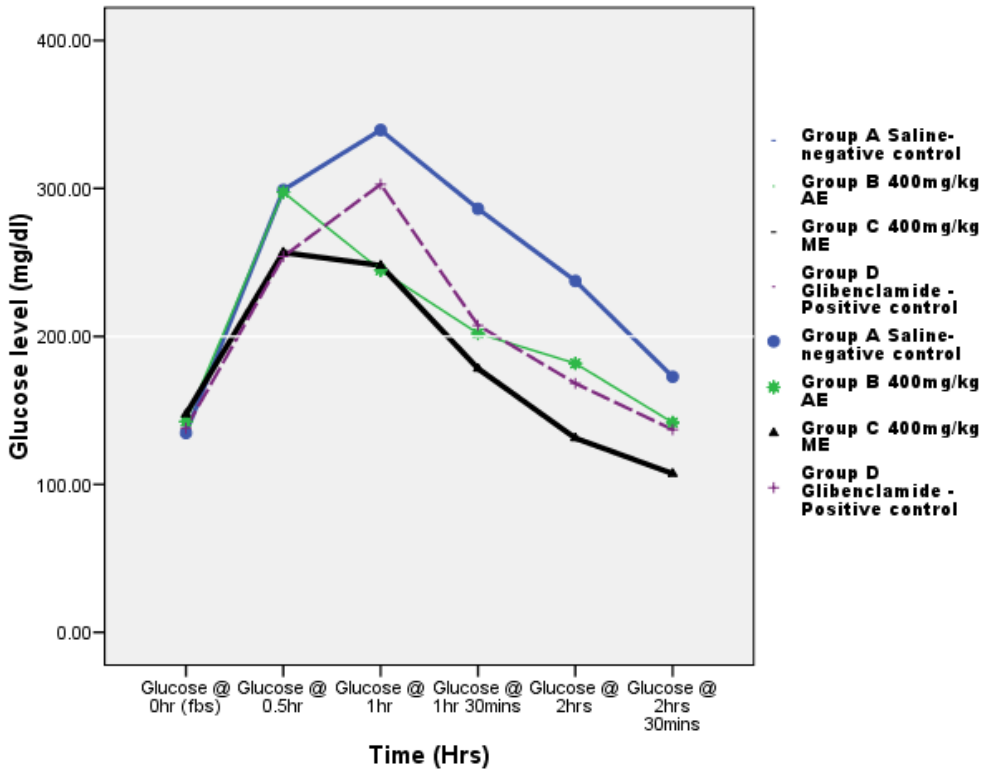

Figure 1: Glucose tolerance curve of normal fasted rabbits

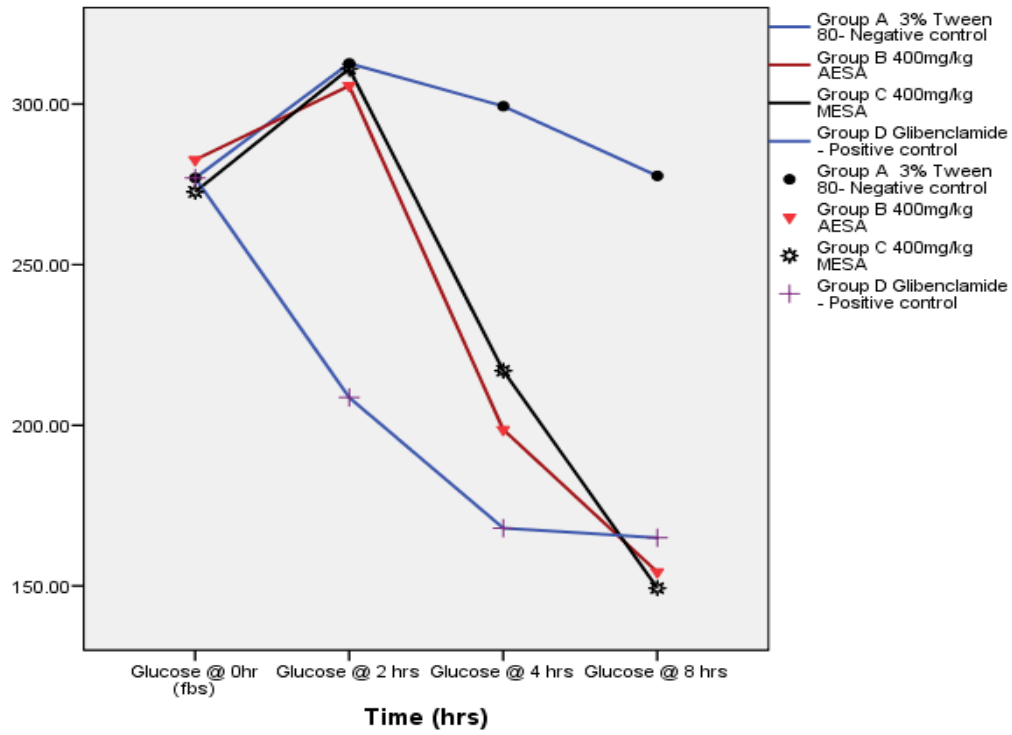

Figure 2: Glucose Vs Time curve in diabetic rabbits

DISCUSSION

Diabetes is a metabolic disorder which can be considered a major cause of high economic loss that can in turn impede the development of nations [18]. Moreover, uncontrolled diabetes leads to many chronic complications such as blindness, heart failure, and rental failure. In order to prevent this alarming health problem, the development of research into new hypoglycemic and potentially antidiabetic agents is of great interest. Alloxan induces "clinical diabetes" in a wide variety of animal species by damaging the insulin secreting pancreatic B cell, resulting in a decrease in endogenous insulin release [19, 20]. Numerous studies demonstrate that a variety of plant extracts effectively lowered the glucose level in alloxan-induced diabetic animals [21-28].

In the present study, the aqueous and methnanol extracts of *Sida acuta* effectively decreased the blood glucose in alloxan-induced diabetic rats. This reduction was not superior to that of glibenclamide, a sulphonylurea anti-hyper glycemic agent. The extracts of *Sida acuta* also enhanced glucose tolerance in normal glucose fed rabbits significantly. The precise mechanism of action of this plant was not eludicated in this work. However, three types of alkaloidal constituents, viz, beta-phenethylamines, quinazolines and carboxylated tryptamines, in addition to choline and betaine have been isolated from *Sida acuta* [29]. Other metabolites present in *Sida acuta* include heraclenol, beta-sitosterol, acanthoside B and daucoglycoside [30].

Generally, *Sida acuta* contains alkaloids, flavonoids, polyphenols, tannins, cardenolides and saponins [31]. Cryptolepine is a natural product isolated from *Cryptolepis sanguinolenta* and *Sida acuta*. A series of substituted and heterosubstituted cryptolepine analogues have been synthesized. Antihyperglycemic activity of Cryptolepine has been measured in vitro and in a NIDDM mouse model to generate the first structure bioactivity study of the cryptolepine nucleus [32]. Cryptolepine, an indoloquinolone alkaloid significantly lowers glucose when given orally to diabetic mice. The antihyperglycamic effect of cryptolepine leads to a significant decline in blood glucose concentration, associated with evidence of an enhancement in insulin-mediated glucose disposal. Cryptolepine increased glucose uptake by 3T3-L1 cells [33]. Many secondary metabolites participate in a variety of anti-diabetic functions in vivo [34]. Polysaccharides, coumarins, flavonoids, terpenoids and a host of other secondary plant metabolites, including arginine and glutamic acid, possess hypoglycemic effects in various experimental animal models [17, 35-37]. Tannin containing drugs have also been shown to demonstrate anti-diabetic activity [38-39]. Effect of the flavonoids- quercetin and ferulic acid on pancreatic cells leading to their proliferation and secretion of more insulin have been proposed by Mahesh and Menon [40] and Sribalasubashini *et al* [41] as the mechanism by which they reduced hyperglycemia caused by streptozotocin in diabetic rats. The presence of flavonoids and tannins in the crude extracts of *Sida acuta* may also be acting similarly thereby decreasing the high blood glucose levels of normal and alloxan-diabetic rabbits. The area under the plasma (Serum or blood) concentration versus time curve (AUC) has a number of important uses in toxicology, biopharmacentics and pharmacokinetics. AUC can be used as a measure of drug exposure. It is derived from drug concentration and time so it gives a measure of how much-how long a drug/substance stays in a body. A long, low concentration exposure may be as important as shorter but higher concentration. Drug AUC values can also be used to determine other pharmacokinetic parameters, such as clearance or bioavaibility. The AUC was significantly lower in the methanol extract treated group than in the aqueous extract, glibenclamide and negative control groups respectively in the periods 30-60 minutes, 60-90 minutes and 90-150 minutes. The total AUC was also significantly lower in the methanol extract treated group than in the other groups for the glucose tolerance studies. This finding is indicative that the methanol extract of *Sida acuta* decreased the bioavailability of glucose significantly and also increased clearance of glucose from the blood probably by increasing insulin sensitivity of peripheral tissues through the up-regulation of specific and non-specific insulin receptors.

The extracts showed superior glycemic control than glibenclamide in the glucose tolerance studies while glibenclamide showed better glycemic control in alloxanized rabbits. This suggests that apart from increased peripheral disposal of glucose, reduction in the bioavailability of orally administered glucose could be a major contributing factor.

This study indicate that the aqueous and methanol extracts of *Sida acuta* possess hypoglycemic and anti-hyperglycemic

properties. These results together with its phytochemical constituents may provide a basis for the pharmacological appreciation of its use in folklore medicine for the treatment of diabetes mellitus.

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