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Area Under Curve (AUC) as a Validating Index for the Hypoglyceamic Activity of S*ida Acuta* Ethanolic Leaf Extract in Experimental Diabetic Animals

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ABSTRACT

Sida acuta, used for treatment of diabetes by traditional healers is evaluated for hypoglyceamic effect of its leaf extract in alloxan-induced diabetes rats relative to metformin and to establish the predictability of Area under the Curve (AUC) as tool for monitoring its hypoglyceamic potential. Diabetic rats with blood glucose levels (BGLs) > 200 mg/dl were screened following 72 hrs post-alloxan induction (150 mg/kg/i.p). The BGLs were determined prior to *S. acuta* extract and metformin treatments. The extract and metformin were administered daily as follows: group II (induced only); group III (induced + metformin 2.57 mg/kg/p.o); group IV (induced + *S. acuta* 200 mg/kg/p.o); group V (induced + *S. acuta* 400 mg/kg/p.o); group VI (induced + metformin + *S. acuta* 200 mg/kg/p.o) and group VII (induced + metformin + *S. acuta* 400 mg/kg/p.o). BGLs were observed at 0 hr, 1 hr, 3 hr, 6 hr, 24 hr, 48 hr, 72 hr, 7 days, 14 days and 21 days and AUCs determined from pharmacokinetic plots. Significant decrease in BGLs was observed and AUC trends as IV > III > V > VI > VII establishing an evidence of reduced glucose. *Sida acuta* (200 mg/kg) showed a better hypoglyceamic property than Metformin.

Keywords: Hypoglyceamia, *Sida acuta*, Metformin, AUC.

INTRODUCTION

Diabetes mellitus is a chronic disease with an escalating prevalence worldwide and constitutes a significant health and socioeconomic burden for patients and the health care systems. It was estimated to affect 177 million people worldwide in 2000 and this figure is projected to increase to 300 million by 2025 (Porter and Barrett, 2011). The International Diabetes Federation (IDF) estimates that over 5 million people suffer from the disease in Africa and the number is expected to increase to 15 million by 2025 (IDF, 2006). The WHO suggests that Nigeria has the greatest number of people living with diabetes in Africa, with an estimated burden of about 1.7 million, which is anticipated to increase to 4.8 million by 2030. The economic burden of diabetes is enormous in terms of the direct cost of intensive monitoring and control of blood glucose and managing cardiovascular, renal, and neurological consequences (Wild *et al*, 2004; Zimmet, 2003). Africa is blessed with a wide plethora of medicinal plants used to treat several diseases. The World Health Organization (WHO) has reported that 80% of the emerging world's population depends on traditional medicine for therapy and the past decades have seen the developed world witness an ascending trend in the utilization of CAM, particularly herbal remedies (Chintamunnee, 2012).

In Africa, a large number of medicinal plants are used traditionally for the management and/or control of diabetes mellitus. Unfortunately, only a few of these African medicinal plants have received scientific scrutiny, leading to isolations and pre-clinical evaluations. In the same light, orthodox drug and herbal products administrations cum interactions are increasingly becoming a common phenomenon in the management of many disease conditions. The interactions between widely used medicinal plants and orthodox drugs may increase or decrease the pharmacological or toxicological effects of either component (Fugh-Berman, 2000).

Pharmacokinetics, which describes the disposition of a drug in the body, should be a primary consideration in the selection of a drug candidate, ultimately contributing to its eventual clinical success or failure. Recent evidences, like Lin (1999), have strengthened the role of pharmacokinetics as an integral part of drug development especially in the choice of drug candidates for disease conditions. The plant *sida acuta* Burm.f. (Malvaceae) is a medicinal plant with numerous medicinal uses (Simplice *et al*., 2007) acclaimed to have hypoglycemic activities. This study aims to draw attention to the hypoglyceamic potential of s*ida acuta* leaf extract, using AUC, a pharmacokinetic parameter, as a predictive and validating tool for a hypoglyceamic response.

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MATERIALS AND METHODS

Sample Plant Collection

The fresh leaves of *Sida acuta* were collected in and around a botanical garden near Sanyo, Ibadan, Nigeria in April 2013. Identification was carried out by a certified taxonomist, and a herbarium number LUH 5828 was assigned to the plant material with a voucher specimen deposited at the herbarium of the Department of Botany, University of Lagos, Akoka. The leaves were washed with distilled water to remove debris and shade dried at room temperature for seven days to a constant weight. The dried materials were pulverized and weighed.

Extraction of plant material

The pulverized plant material was cold-macerated in 90 % ethanol for one week. The extract was filtered and the filtrate concentrated under reduced pressure in a rotary evaporator at <50 ^{o}C, followed by freeze-drying. The freeze-dried extract was stored at 5 °C prior to bioassay.

Standard Drug

The reference drug, Metformin, (Ashpage$^{(R)}$) was dissolved in normal saline and administered orally based on body weight. A dose of 2.57 mg/kg/body weight was obtained for the study, calculated from the recommended daily dose of 1500 mg/70kg body weight for human subjects.

Experimental animals

Thirty five (35) healthy Swiss wistar rats of either sex, of average weight 150 g, in-house bred at the Animal Facility of the College of Medicine of the University of Lagos, were used throughout the study. The animals were handled in accordance with the Institute of Laboratory Animal Research (ILAR) guidelines for care and use of animals in experimental studies. The animal subjects were housed under controlled laboratory conditions, air conditioned with 12-15 filtered fresh air changes per hour, environmental temperature (24 ± 2 °C), relative humidity 30-70 %, with 12 h -12 hr light and dark cycle. The animals were randomly weighed and grouped into five animals per group. Each group was housed in standard wire mesh bottom wooden cages with hard wood chips as beddings (size: approximately L 410 x B 280 x H 140 mm), with stainless steel top grill having facilities for pellet food and Aquaguard filtered cum-purified water *ad-libitum*.

Inducement of diabetes

Following acclimatization, the animals were fasted overnight for 12 hours prior to treatments but were allowed water *ad libitum*. The fasting blood glucose levels (FBGL) were determined. Hyperglycemia was induced by a single intraperitoneal administration of freshly prepared Alloxan monohydrate (150 mg/kg) in normal saline solution. The negative control group received similar volume of vehicle, normal saline (2 ml/kg).

Experimental protocol

The experimental rats were distributed into 7 groups of 5 rats per group. Diabetes was induced in the rats in groups II - IV by administration of alloxan 150 mg/kg intraperitoneally. The rats were treated as shown below.
Group I - Negative control (food and water only)
Group II - Induced but not treated only
Group III - Induced + Metformin (2.57 mg/kg/p.o)
Group IV - Induced + *S. acuta* extract (200 mg/kg/p.o)
Group V - Induced + *S. acuta* extract (400 mg/kg/p.o)
Group VI - Induced + Metformin (2.57 mg/kg/p.o) + *S. acuta* extract (200 mg/kg/p.o)
Group VII- Induced + Metformin (2.57 mg/kg/p.o) + *S. acuta* extract (400 mg/kg/p.o)
hr, 6 hr, 24 hr, 48 hr, 72 hr, 7 days, 14 days and 21 days and the BGLs determined accordingly.

Collection of blood sample

Blood collection was done at different stages of the study through the tail vein puncture and the concentrations determined using ACCU-CHEK® active blood glucometer kit. Screening for diabetic rats was carried out between 48 and 72 hrs post-induction by selecting animals with blood glucose levels (BGLs) 200 mg/dl and above. Experimental animals considered diabetic were further selected for the treatment groups. Blood collections were also carried out post-treatment with metformin and *S. acuta* extract at regular intervals of 0 hr, 1 hr, 3

Estimation of Percentage Change in BGLs

The percentage (%) change in blood glucose level (BGLs) using post-treatment mean value of BGLs of each group and zero (0) hour post-induction mean value of BGLs were used to estimate the change (increase or decrease), which was consequently used to evaluate the hypoglyceamic activity of the *S. acuta* extract relative to the standard drug. The percentage change was calculated using the equation,

$$\% \text{ Change in BGLs} = \frac{M(BGLs)_{\text{post treatment}} - M(BGLs)_{0 \text{ hour post induction}} \times 100}{M(BGLs)_{0 \text{ hour post induction}}}$$

where $M(BGLs)_{post\ treatment}$ = Mean Value (BGLs) Post-treatment

$M\ (BGLs)_{0\ hour\ post\ induction}$ = Mean value (BGLs) 0 hour of post- induction

The percentage change was also determined by calculating the difference from the post-induction time to the post-treatment times after the 3week period, and expressed in percentages as shown in Table 1

Statistics

Data were analysed with statistical software (Graphpad Prism 5) and values were presented as Mean ± SEM. The differences between the groups were statistically determined by analysis of variance (ANOVA) and followed by the Dunnet's test. The student paired t-test was also employed to determine significance in FBGLs before and after treatment in the groups. Statistically significant levels were considered at $P < 0.05$, $P < 0.01$ and $P < 0.001$.

RESULTS

The result showed that there is no statistically significant change in the blood glucose levels of rats at every stage throughout the period of the experiment in the negative control that received normal saline. In all treatments groups, when compared with the diabetic control group, the results showed a time-dependent statistical significant reduction in the BGLs between the 0 hour post induction and 21 day post-induction times at varying levels of confidence. The extract at single daily doses of 200 mg/kg and 400 mg/kg as well as metformin 2.57 mg/kg, all resulted in significant decrease in BGLs at ($p<0.001$) by 86.25 %, 73.5 % and 80.9 % respectively at the end of the treatment. The co-administered doses of (400 mg/kg + metformin) and (200 mg/kg + metformin) caused a reduction of 41.9 % and 54.0 % respectively ($p<0.05$). The test agents did not show any significant decrease in the BGLs within the first 24 hours of administration but showed slight reduction after the first 3 days of treatment for animals that were severely diabetic at the initial post induction time. A steady reduction in BGLs on the 7th day of treatment followed by a significant increase through the 14th day of treatment was observed in the 400mg/kg + metformin and 200 mg/kg + metformin treatment group. The highest activity in this experiment, in terms of showing a steady anti-hyperglyceamic activity was observed with the single dose of 200 mg/kg with and without metformin. Figures 1 to 6 show the curves for progression of blood glucose levels in all diabetic groups.

Table 1: The blood glucose levels (mg/dl) of the groups treated with *Sida acuta* extract and Metformin

TREATMENT GROUPS	POST INDUCTION TIME										% Change / Comment at Day 21
	0 h	1 h	3 h	6 h	24 h	48 h	72 h	7 day	14 day	21 day	
Negative control	85.3±3.84	72.7±2.03	79.7±3.18	83.0±4.04	81.7 ± 5.21	85.7±0.88	89.3±1.45	85. 7 ± 1.76	88.3±3.28	81.7±3.71	4.29 %; decrease
Non-treated Diabetic	389.0 ±109.4	398±101.9	398.0±101.4	401.7±99.5	393.3±103.3	410.3±95.2	393.0 ±84.7	392.0 ±83.9	380.0 ±70.2	373.3±63.9	4.03 %; Decrease
Diabetic + Metformin	549.3 ± 50.7	548.7 ±54.3	546.0±54.0	549.0±51.0	555.3±46.7	448.3±151.7	274.3 ±81.1	141.0 ±19.7	110.7 ±15.8	$104.7\pm12.4^{a,ns}$	80.9 %; Decrease
Diabetic + *Sida acuta* extract (200 mg/kg)	598.7 ± 1.33	569. ±30.3	555.7±44.3	551.0±49.0	510.7±59.5	305.7±92.2	161.0 ±53.9	93. 7±10.4	88.0±5.20	$82.3\pm4.06^{a,ns}$	86.3 %; Decrease
Diabetic + *Sida acuta* extract (400 mg/kg)	557.3 ± 42.7	572.3 ±27.7	577.3±22.7	570.7±15.8	370.3±130.2	404.7±91.4	384.0 ±22.9	384.0 ±22.9	71. 7±9.77	$147.7\pm16.8^{b,ns}$	73.5 % Decrease
Diabetic + Metformin + *Sida acuta* extract (200 mg/kg)	363.3± 118.4	366.3 ±117	381.3± 110	387.7± 106.9	383.7± 110.0	300.3± 116.4	104.0 ±7.94	76.0± 24.8	183.0 ±30.3	$167.3\pm25.8^{b,ns}$	54.0 %; Decrease
Diabetic + Metformin + *Sida acuta* extract (400mg/kg)	265.7 ± 33.4	281.7 ±34.5	281.7± 34.5	307.3± 25.0	303.3± 28.1	261.0± 65.6	93.3± 18.0	111.3 ±9.56	169.3 ±37.2	$154.3\pm22.6^{b,ns}$	41.9 % ; Decrease

Data are expressed as Mean ± SEM (mg/dl); a = P <0.001 with respect to diabetic control; b = P < 0.01 with respect to diabetic control; ns = non-significant

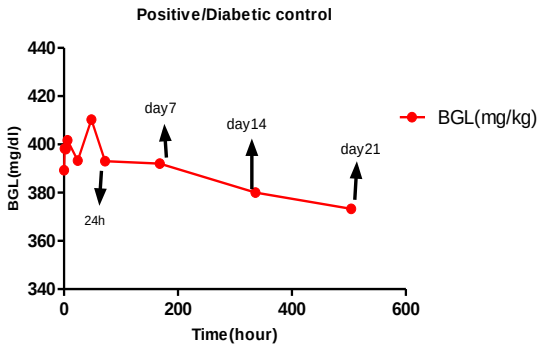

Figure 1: Curve for progression of blood glucose levels of diabetic control rats.

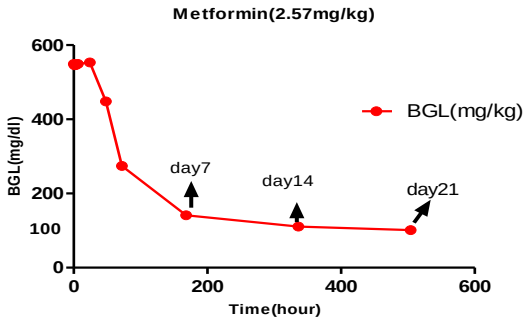

Figure 2: Curve for progression of blood glucose levels in diabetic group receiving metformin (2.57 mg/kg)

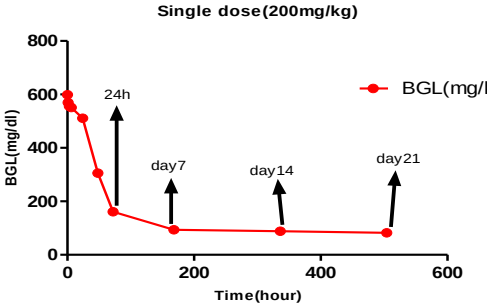

Figure 3: Curve for progression of blood glucose levels in diabetic group receiving *Sida acuta* (200 mg/kg)

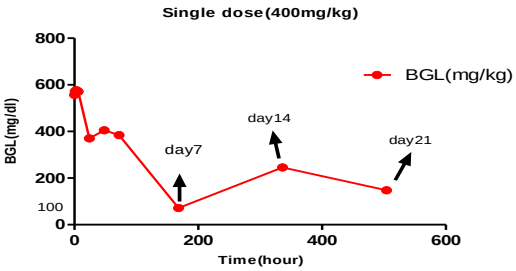

Figure 4: Curve for progression of blood glucose levels in diabetic group receiving *Sida acuta* (400 mg/kg)

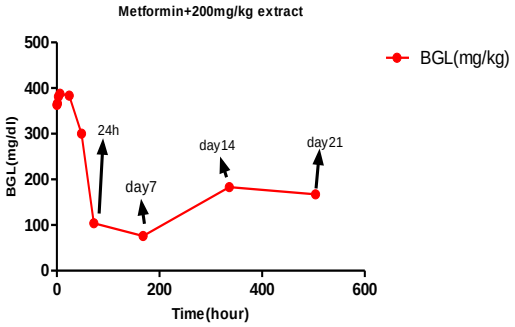

Figure 5: Curve for progression of blood glucose levels in diabetic group receiving *Sida acuta* (200 mg/kg) and Metformin (2.57 mg/kg)

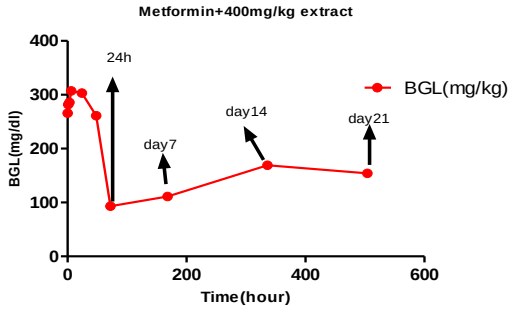

Figure 6: Curve for progression of blood glucose levels in diabetic group receiving *Sida acuta* (400 mg/kg) and Metformin (2.57 mg/kg

Area Under the Curve (AUC) Estimation of Blood Glucose

The pharmacokinetic plots (Figures 1- 6) were plotted to determine the area under the plasma concentration versus time curve (AUC) as a means to quantify the amount of blood sugar in the test animals. The AUC of each plot was verified using the trapezoidal rule for the determination of the AUCs and the obtained values presented in Table 2. The plasma concentration-time curve was considered to monitor the metabolic course of blood glucose in the experimental animals following the different treatments between 0 hour and 21 days.

Table 2a: Area under the Curves (AUCs) of Blood glucose levels at peak levels (0-24) hour and post-treatment (day 1- 24).

Treatment Groups	AUC (0 h - 24 h)	AUC (24 h – 21 days)	% Reduction in AUC after 21 days
Non-treated diabetic	9545	8087	15.3
Diabetic + Metformin	13202	4053	69.3
Diabetic + *S. acuta* extract (200 mg/kg)	12925	3347	74.1
Diabetic + *S. acuta* extract (400 mg/kg)	11905	4016	66.3
Diabetic + Metformin + *S. acuta* extract (200 mg/kg)	9209	3740	59.4
Diabetic + Metformin + *S. acuta* extract (400 mg/kg)	7226	3563	50.7

Our result showed that the single 200 mg/kg dose of *Sida acuta* extract gave a 74.1 % reduction in blood sugar level after the 21 days, while the 400mg/kg single dose, the metformin standard, 400 mg/kg and 200 mg/kg combined doses had 66.3 %, 69.3 %, 50.7 % and 59.4 % respectively.

Table 2b: Abridged table of values relating the blood glucose levels (BGLs) and the Area under the curve (AUC)

% Reduction	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
BGLs (mg/dL)	4.29 % ↓	4.03 % ↓	80.9 % ↓	86.3 % ↓	73.5 % ↓	54.0 % ↓	41.9 % ↓
AUC (mg.hr/L)	22.4	15.3	69.3	74.1	66.3	59.4	50.7

Table 3: Table of values for other pharmacokinetic parameters (Cmax and Tmax) correlating to the individual groups

Parameter	Group II	Group III	Group IV	Group V	Group VI	Group VII
Cmax (mg/dL)	410.3±95.2	598.7 ±1.33	577.3±22.1	553.3±46.7	307.3±25.0	387.7±106
Tmax (hr)	48	0	3	24	6	6

DISCUSSION

This study was carried out, not only to investigate the hypoglyceamic potential of *Sida acuta* ethanolic extract in alloxan-induced experimental diabetes but also to establish the predictability of hypoglyceamia using AUC. The study was also intended to establish credence of using derived pharmacokinetic parameters as validated indices for pharmacodynamic responses, in this case, hypoglyceamia. Despite studies that have investigated and established the hypoglyceamic activity of *S. acuta*, there is paucity of information on the drug-herb interaction and the use of AUC, a pharmacokinetic parameter, to monitor and validate reduction of sugar in the test subjects. The interaction was majorly pharmacodynamic as earlier reported by Okwuosa *et al*, (2010) but our result is a frontline data on the possible pharmacokinetic implications from the extract administration and the co-administration with metformin.

Pharmacokinetic analysis may be performed by non-compartmental or compartmental methods. The non-compartmental method usually estimates the exposure to a drug or xenobiotic by estimating the Area Under Curve (AUC) of a concentration-time graph while the compartmental methods estimate the concentration-time graph using kinetic models. Physiologically, the body produces insulin from the pancreatic beta cells to counteract the rise in glucose levels. However, in diabetes, this process does not occur and tested glucose levels consequently increases. Alloxan is a substance used for the induction of diabetes mellitus and has a destructive effect on the β-cells of the pancreas (Mohsen *et al.*,

2003), hence the use in this study. The result of the present study showed that the ethanolic extract of *S. acuta* at the two single doses used, the co-administered doses and the metformin (2.57 mg/kg) all exhibited a time-dependent significant ($p<0.05$) reduction of the blood glucose levels of the alloxan-induced diabetic rats. The significance noted was within and across the groups when compared to the negative control groups. Our results (Table 2a and 2b showed that the co-administered doses of the extract with standard metformin gave poorer blood glucose reducing effect relative to either the individual doses of the extracts or metformin administered alone. A pronounced observation in this study is the hypoglyceamic effect at the low dose of *S. acuta* i.e. the 200 mg/kg single dose. This finding is consistent with an earlier work by Ekor *et al*, (2010), while Okwuosa *et al*., (2011) reported the superior activity of glibenclamide, a sulphonylurea oral hypoglyceamic, over aqueous and methanolic extracts of *Sida acuta*, our findings showed the superior hypoglyceamic effect of the ethanolic extract over metformin, a biguanide hypoglyceamic agent (Table 1). The hypoglyceamic effect of metformin was only seen when compared with the high dose *Sida acuta* extract (400 mg/kg) and the co-administered treatments (Table 1). The implications thus, is that co-administration of *Sida acuta* with a biguanide oral hypoglyceamic does not effectively reduce blood sugar levels as would the individual components. The drug-herb combination effect would be one of antagonism, since the individual hypoglyceamic activity of *sida acuta* and metformin is reduced.

The area under the plasma concentration versus time curve (AUC) has a number of important uses in toxicology, bio-pharmaceutics and pharmacokinetic studies. The AUC, a toxicological and pharmacokinetic parameter, can be used as a measure of the body's exposure to a drug or xenobiotic following quantitation. In this work, we have used a pharmacokinetic parameter to monitor and validate a pharmacodynamic response following treatments with metformin and *Sida acuta* in experimental diabetes. The AUC determination in this study was modeled to determine the exposure of the physiological milieu of the experimental rats to blood sugar before and after treatments. This was considered since an inverse relationship exists between blood sugar levels and the time-course concentrations of a hypoglyceamic agent. It was therefore necessary to monitor the amount of blood sugar at varying pre-determined time intervals. The AUC significantly dropped in the 200 mg/kg single dose extract-treated group (74.1 %) after the 21 days of treatment than in the 400 mg/kg single dose (66.3 %) and the metformin (69.3 %) treated groups. However, with the combined extract-metformin doses, the AUC is only lowered by 50.7 % and 59.4 % after the 21 days for the 400 mg/kg and 200 mg/kg (plus metformin) respectively (Table 2). This trend in AUCs further supports the hypoglyceamic activity of *S. acuta* and also strengthens the observation of potent activity to lower dose of *S. acuta* in reducing blood sugar when used alone, while the activity of the respective agents (extract or synthetic) when combined is compromised.

CONCLUSION

This study has further added support to the ethnopharmacological use of the extract of *Sida acuta* in controlling excessive sugar rise, thus providing a preliminary scientific standpoint for its use. Thus, AUC can be used to monitor BGLs and validate the anti-diabetic potentials of *Sida acuta*. Further studies will however be necessary to isolate the principal bioactive component in the extract responsible for anti-hyperglyceamic effect and also elucidate the mechanisms for this action.

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