ORIGINAL PAPER

https://dx.doi.org/10.4314/njpr.v16i2.9S

Nig. J. Pharm. Res. 2020, S1 pp 73-84

ISSN 0189-8434 **e-ISSN 2635-3555** **Available online at http://www.nigjpharmres.com**

Evaluation of Antidiabetic Property of *Sansevieria liberica* Gerald and Labroy (Dracaenaceae) Leaf Using Alloxan Induced Diabetes Model

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: The phytochemical screening and antidiabetic activities of the methanol leaf extract and fractions of *Sansevieria liberica* Ger. and Labr. were investigated.
Objectives: To identify the phytoconstituents present and evaluate the anti-diabetic activity of the leaf of *S. liberica* Ger. and Labr.
Material and Methods: A 1.2 g of the pulverized leaf was cold macerated in methanol to obtain 240 g of the crude extract (CE), fractionation was done using n-hexane, ethylacetate and butanol. The CE and the fractions were screened for phytochemical and antidiabetic analysis using standard procedures. Diabetes was induced by intraperitonial injection of alloxan. The blood glucose levels were analyzed as indices of diabetes.
Results: After 24 hours, alloxan increased the blood glucose level of rats indicating hyperglycaemia. Treatment of the rats with the extract and fractions reduced the blood glucose level within 3 to 7 days of treatment. The standard antidiabetic, metformin (500 mg/kg) also showed similar effect. Extracts (250 and 500 mg/kg) of the crude extract decreased significantly ($p<0.05$) the blood glucose level. The antidiabetic potency of the extract and fractions were in the order of CE > ethylacetate fraction > n-Hexane fraction > butanol fraction. The phytochemical screening of the extract and fractions revealed the presence of flavonoids, steroids, alkaloids, glycosides etc.
Conclusions: From the findings, *S. liberica* leaf posses antidiabetic activity which may be due to the phytochemical constituents present, and this justifies its use by local users as traditional remedy for diabetes mellitus.
Keywords: Phytochemical constituents, *S. liberica* leaf, antidiabetic activity, Metformin

INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by high blood glucose level resulting from defects in insulin secretion, insulin action or both (Khan and Zaman, 2009). Diabetes is a disorder of metabolism—the way the body uses digested food for energy. The digestive tract breaks down carbohydrates (sugars) and starches found in many foods into glucose, a form of sugar that enters the bloodstream. With the help of the hormone insulin, cells throughout the body absorb glucose and use it for energy. Insulin is made in the pancreas, an organ located behind the stomach. According to National Diabetes Information (NDIC, 2000), as the blood glucose level rises after a meal, the pancreas is triggered to release insulin. Within the pancreas, clusters of cells called islets contain beta cells, which make the insulin and release it into the blood (NDIC, 2000). The pancreas is an organ that sits behind the stomach and releases hormones into the digestive system. In the healthy body, when blood sugar levels get too high, special cells in the pancreas

(called beta cells) release insulin. Insulin is a hormone and it causes cells to take in sugar to use as energy or to store as fat. This causes blood sugar levels to go down. Diabetes develops when the body doesn't make enough insulin or is not able to use insulin effectively, or both. As a result, glucose builds up in the blood instead of being absorbed by cells in the body. The body's cells are then starved of energy despite high blood glucose levels (NIDDK, 2006).

Typically, destruction of pancreatic β-cells progresses to absolute deficiency in insulin. This condition develops rapidly in young people and has been found to occur in any age group (Chhabra and Brayman, 2013). That is, your body no longer makes insulin or enough insulin because the body's immune system, which normally protects you from infection by getting rid of bacteria, viruses, and other harmful substances, has attacked and destroyed the pancreatic β-cells that make insuli. Hence, pancreatic β-cell destruction involves autoimmune mechanisms. Therefore, type 1 diabetes mellitus is also known as 'autoimmune' type 1 diabetes mellitus (Thompson *et al.,* 2012). In addition to insulin therapy, exercise and careful attention to diet is necessary to prevent fluctuations of blood sugar. Type 2 Diabetes Mellitus: Type 2 diabetes mellitus (formerly called non-insulin dependent diabetes mellitus (NIDDM), type II or adult-onset) is characterized by insulin resistance in peripheral tissue and an insulin secretory defect of the beta cell. Some causes of insulin resisitance are obesity or over weight, excessive glucocorticoids (in steroid therapy), pregnancy (gestational diabetes) and autoantibodies in the insulin receptors (Ozougwu *et al.,* 2013). Type 2 diabetes mellitus is characterized by derangement of carbohydrate, protein and fat metabolism. Insulin resistance and hyperinsulinemia eventually lead to impaired glucose tolerance. Defective beta cells become exhausted, further fuelling the cycle of glucose intolerance and hyperglycaemia. Type 2, diabetes usually begins with insulin resistance; a condition that occurs when fat, muscle, and liver cells do not use insulin to carry glucose into the body's cells for energy. As a result, the body needs more insulin to help glucose enter cells. (Pathak and Pathak, 2012).

S. liberca Ger. and Labr. is used in ethnomedicine to treat numerous ailments such as cough, hemorrhoids, infections, inflammation, diabetics, malaria, teething pain, fever, ulcer, headache and as a vermifuge (Bero *et al.*,2011). Antimicrobial activity of the root extract of *S. liberica* Rukiyat *et al.,* 2015), hepatoprotective (Ikewuchi *et al.,* 2011), anti-inflammatory (Chinasa *et al.,* 2011), antitrypanosomal, antileishmanial and antiplasmodial (Bero *et al.,* 2011), Hypolipidaemic (Nnodim *et al.,* 2014) activities of extracts of the plant had been reported. Due to the numerous ethnomedicinal applications and uses of the leaves of this plant, it is deemed necessary to ascertain the scientific basis for the ethnomedicinal applications and uses of its leaf extracts in hypoglyceamia, and identify the phytochemical constituents responsible for its effect.

METHODOLOGY

Materials

Drugs, chemicals, solvents and reagents

All drugs, chemicals, solvents and reagents used were commercially obtained and are of analytical grade. Methanol (BDH), n-hexane (BDH), ethylacetate (BDH), Solution of crystalline $CuSo_4$ in sulphuric acid (Fehling's solution 1), solution of Rochelle salt and potassium hydroxide (Fehling's solution II), potassium bismuth iodide solution (Dragendorff's reagent), solution of iodine in potassium iodide (Wagner's reagent), potassium mercuric iodide solution (Mayer's reagent), saturated solution of picric acid (Hager's reagent) Milton's reagent, naphthol solution in ethanol (Molisch's reagent), α-naphthol, sulphuric acid (H_2SO_4), ammonium hydroxide (NH_4OH) chloroform, sodium hydroxide (NaOH), carbon tetrachloride, ferric chloride, ethanol (70 %, 90 %), lead subacetate, glacial acetic acid, ethylacetate, aluminium chloride, olive oil, Alloxan, Metformin (Hovid), Distilled water.

Experimental animals

Adult Wistar rats (190 - 230 g) and mice (24 - 36 g) of either sex were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka. They were housed in cages; and allowed free access to drinking water and were fed *ad libituum* a standard laboratory diet (UAC feed Nigeria). They were acclimatized under standard environmental conditions. Animals described as 'fasting' was deprived of food and water for 16 hrs. Animal experiment were done in compliance with the National institute of Health Guide for care and use of laboratory Animals (Pub. No. 85 – 23, revised 1985).

METHODS

Collection and authentication of plant material

The plant material was collected from Nsukka and authenticated by Mr. Alfred Ozioko at the International Centre for Ethnomedicine and Drug Development (INTERCEDD), Nsukka, Enugu State, Nigeria; the voucher specien has been deposited in the Department of Pharmacognosy and Traditional Medicine, Nnadi Azikiwe University, Awka with voucher specimen number: PCG 474 / A / 048.

Preparation of the plant material and extraction

The plant materials were cleaned, air-dried at room temperature for one month and pulverized. A 1.2 kg of the pulverized leaves were cold macerated in methanol with intermittent shaking and exchange of fresh solvent every 24 hrs (aliquot extraction) for 72 hrs. The resulting solution was pooled and filtered. Two liters of the filtrate was concentrated in vacuum using rotary evaporator at 40 ^{o}C and thereafter, dried to a constant weight in a water bath at a temperature of 40 ^{o}C to obtain methanol extract (ME) which we also refered to as the crude extract (CE). The remaining filtrate was concentrated to one quarter ($^{1}/_{4}$) of the volume that was used in fractionation.

Determination of yield of extracts

Extracts of known volumes were evaporated to dryness in a tarred glass crucible. The weight of each extract was determined by finding the difference between the weight of empty crucible and crucible containing the semi-solid marc. The weight per ml was then calculated from the values obtained.

Liquid-liquid fractionation

The pre-concentrated filtrate of the ME was subjected to liquid-liquid partitioning successively with 2.5 L of n-hexane, ethylacetate and butanol using separating funnel to give the n-hexane, ethylacetate and butanol soluble fractions respectively. The fractions were pre-concentrated using rotary evaporated at 40 ^{o}C thereafter to dryness using open water bath at 40 ^{o}C.

Phytochemical analysis

The CE and the fractions were tested for presence of carbohydrates, alkaloids, reducing sugar, glycosides, saponins, tannins, resins, flavonoids, proteins, steroids, terpenoids, fats and oils according to Evans (2002) and Harbourne (1973).

Pharmacological evaluation

Induction of experimental diabetes

To the overnight fasted rats, 150 mg/kg alloxan were administered intraperitonieally. After a period of 24 hours, rats with marked hyperglycemia (blood glucose level >150 mg/dl) measured using a glucometer (One touch ultra, USA) were used for the study (Rees and Alcolado, 2005)

Study design for antidiabetic evaluation

The animals were divided into 5 groups of 5 animals in each group and they were treated for 7days. The doses received are shown below:

Group 1 10 ml/kg distilled water

Group 2 Standard drug (metformin) at 500 mg/kg

Group 3 250 mg/kg of crude extract

Group 4 500 mg/kg of crude extract

Group 5 500 mg/kg crude extract (Normoglycemic rats)

The doses for the crude extract (250 and 500 mg/kg) which was selected after an initial preliminary test using 100, 250 and 500 mg/kg were repeated for the fractions to evaluate the hypoglycemic activity in the following order;

Group 1 250 mg/kg of n-Hexane fraction for 7days

Group 2 500 mg/kg of n-Hexane fraction for 7days

Group 3 250 mg/kg of ethylacetae fraction for 7days

Group 4 500 mg/kg of ethylacetate fraction for 7days

Group 5 250 mg/kg of butanol fraction for 7days

Group 6 500 mg/kg of butanol fraction for 7days

Percentage glycemic reduction calculated using the formula below;

$$Percentage \text{ glycemic reduction} = \frac{\text{Mean glycemic FBGL} - \text{Mean Treatment FBGL}}{\text{Mean glycemic FBGL}} \times 100 \quad \ldots\ldots Equ7$$

Key:

FBGL – Fasting blood glucose level

Biochemical studies

The biochemical study of the crude extract was studied according to the method employed by Baheti *et al*, (2006). Blood was collected in non- heparinised tubes. The blood sample was centrifuged at the speed of 4000 rpm at room temperature for 10 min and the supernatant separated and stored in the refridgerator.

Procedure (Teco diagnostics)

Alkaline phosphate (ALP): Fresh tubes were labeled "standard", "sample" and "blank" respectively. Approximately 0.5 ml of alkaline phosphatase substrate (in the kit) was dispensed in all the tubes and was equilibrated to 37 ^{o}C for 3min using water bath. To the standard, 5 µg of the standard reagent (in the kit) was dispensed; to the sample 50 µg of the sample are dispensed and to the blank 50 µg of deionized water was dispensed. The whole tubes were mixed gently; and incubated for 10 min at 37 ^{o}C. Then 2.5 ml of alkaline phosphatase colour developer (in the kit) was dispensed in all the tubes and was mixed properly. The absorbance was read at 600 nm using spectrophotometer. Then the concentration of ALP was calculated as follow:

$$\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times Conc\ of\ stsndard \dots \dots \dots Eqn$$

Alanin aminotransferase (ALT)

R_1 and R_2 (in the kit) were mixed at the ratio of 5:1 and was termed "working reagent" .Fresh tube was labeled "sample" and "blank". To all the tubes, 1.0 ml of working reagent was dispenses and incubated for 5 min at 37 ^{o}C. To the samples, 100 µl of each sample was added respectively; and to the blank, 100 µl of distilled water was added. The absorbance was read at 1min and at 3mins using spectrophotometer at 340 nm. Then the ALT concentration calculated as follow:

$$\frac{\Delta A}{Minutes} \times 1768 \dots \dots \dots Eqn\ 9$$

Aspartate aminotransferase (AST): R1 and R2 (in the kit) was mixed at the ratio of 1:5 and was termed "working reagent". Fresh tube was labeled "sample" and "blank". To all the tubes, 1.0ml of working reagent was dispenses and incubated for 5 min at 37 ^{o}C. To the samples, 100 µl of each sample was added respectively; and to the blank, 100 µl of distilled water was added. The absorbance was read at 1min and at 3mins using spectrophotometer at 340 nm. Then the AST concentration calculated as follow:

$$\frac{\Delta A}{Minutes} \times 1768 \dots \dots \dots Eqn\ 10$$

Determination of body weight

Body weight of all the treated groups and controlled groups of rats were recorded before and during treatment periods. A properly calibrated and standardizes electronic balance was used for taking body weight of the rats and expressed as gram (g).

Statistical Analysis

The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Dunnett Multiple Comparisons Test. The values are expressed as mean ± standard mean error (SME). $P < 0.05$ is considered statistically significant.

RESULTS

Extraction and fractionation

The extraction process yielded 240 g of the Methanol Extract (ME), and the yield of the fractionation are extract = 240 (20 % w/w), N- Hexane fraction = 0.16 (0.24 % w/w), Ethylacetate fraction = 3.34 (5.06 % w/w) and Butanol fraction = 10.52 (16.0 % w/w).

Phytochemical analysis

The result of the phytochemical analysis of the crude extract (CE) and fractions (butanol fraction (BF), ethylacetate fraction (EF) and n-hexane fraction (HF)) are presented in table 1 below:

Table 1: Results of Phytochemical Analysis

S/N	PHYTOCHEMICAL CONSTITUENTS	RELATIVE ABUNDANCE CE	BF	EF	HF
1	Resins	+	+	++	+
2	Fats and Oils	+	+	+	+
3	Flavonoids	++	+	+++	-
4	Saponins	+++	+++	-	-
5	Proteins	+++	+++	-	-
6	Steroids	+	+	++	+
7	Terpenoids	+	+	++	+
8	Tannins	+++	+++	-	-
9	Reducing sugars	++	++	-	-
10	Carbohydrates	+++	+++	-	-
11	Acidic compounds	+++	+++	-	-
12	Alkaloids	++	++	-	-
13	Glycosides	++	++	-	-

The type of tannins is condensed tannins

CE = Crude extract; BF = Butanol fraction; EF = Ethylacetate fraction; HF = n-hexane fraction; -= Not present

+ = Present

++ = Present in moderately high concentration.

+++ = Present in very high concentration.

++++ = Abundantly present.

Quantitative estimation of Phytocomponents

The quantitative analysis of phytoconstituents of *S.liberica* leaves extract is shown bellow: Flavonoid (7.1 %) and Saponin (4.7 %) are the highest phytochemical component in the leave extract while alkaloids (2.5 %) and Tannins (1.5 %) have lesser phytochemical components.

Antidiabetic evaluation of *s. Liberica* leaves

Effect of crude extract on hyperglycemic rats: The crude extract of *S. liberica* in studied doses (250 and 500 mg/kg) gave a significant ($p < 0.05$, $p < 0.01$) reduction of the blood glucose levels of the Alloxan induced diabetic rats when compared to the negative control group (distilled water 10 ml\kg), the reference drug (metformin 500 mg/kg) and the normoglycemic rat which received 500 mg/kg crude extract and showed a significant reduction in their glycemic levels. The result of the effect of crude extract on blood glucose level of alloxan induced diabetic rats are shown in Tables 2 and Figure 1 below:

Table 2: Effect of Crude Extract on Fasting Blood Glucose Level of Alloxan Induces Diabetic Rats

Group	Basal	Hyper	1hr	2hrs	3hrs	6hrs	Day3	Day7
10 ml/kg Distilled. water	46.50** ±2.25	269.75 ±24.67	283.00 ns ±21.96	290.75 ns ±24.26	307.00 ns ±25.01	307.00 ns ±25.73	336.00 ns ±33.98	345.00 ns ±41.00
500 mg/kg Metformin	47.33** ±1.76	428.33 ±48.64	340.67 ns ±54.92	185.67** ±36.22	84.33** ±5.55	330.33 ns 62.43	160.33** ±45.51	53.33** ±6.40
250 mg/kg Crude Extract	46.50** ±1.44	283.50 ±26.95	302.75 ns ±39.02	277.50 ns ±11.92	286.25 ±24.42	320.50 ns ±20.17	85.00** ±10.24	48.75** ±2.21
500 mg/kg Crude Extract	49.00** ±1.53	267.00 ±26.13	269.33 ns ±23.13	269.00 ns ±25.15	225.67 ns ±21.71	290.00 ns ±20.84	179.33 ns ±35.57	85.00** ±4.36
NGR 500 mg/kg crude extract	43.00ns ±3.00	43.11 ±5.20	42.00ns ±1.90	41.50ns ±4.80	33.72* ±4.40	31.33* ±3.00	22.00* ±2.30	19.46** ±3.50

All values are expressed as mean ±standard error of mean (SEM)

ns = Non significant p ≥ 0.05

**= Significant; p ≤0.05*

***= Significant; p ≤ 0.01*

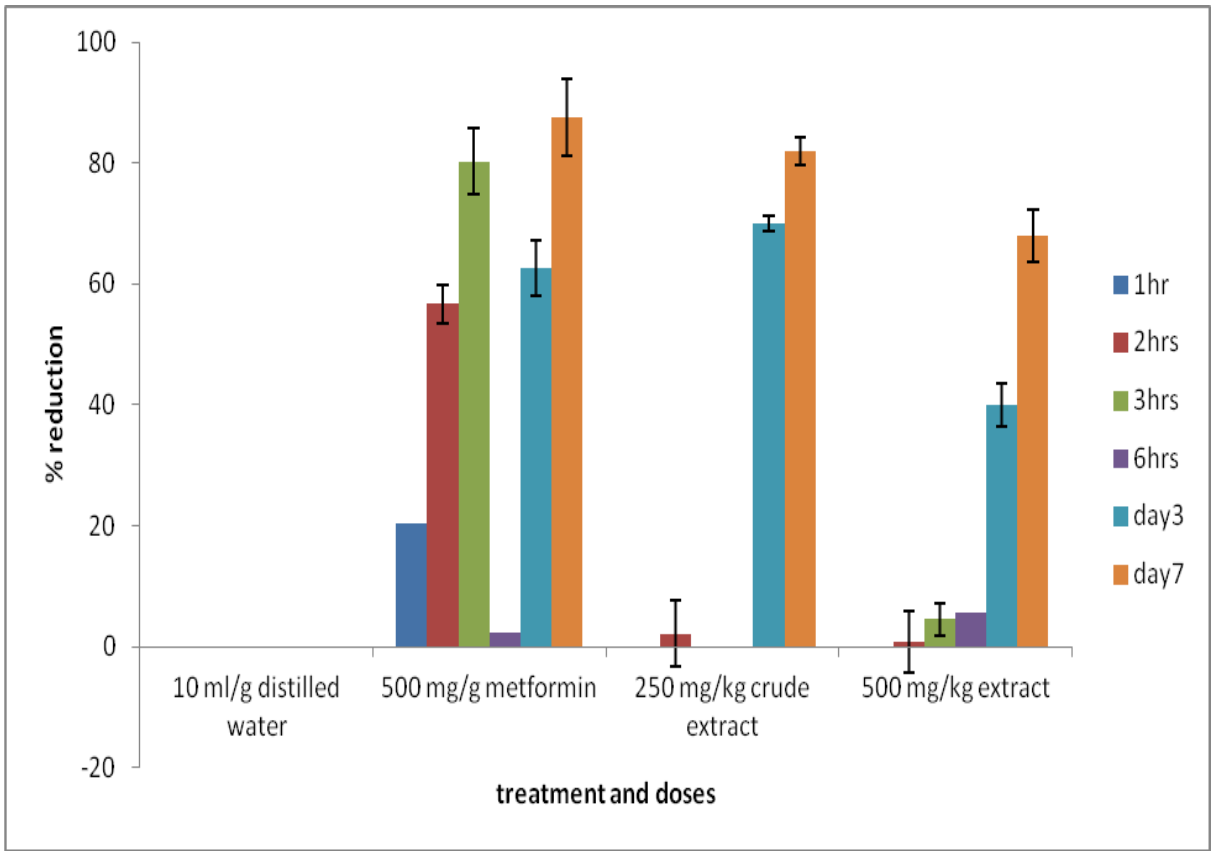

Figure 1: Mean percentage reduction of fasting blood glucose level in alloxan induced diabetic rat by the crude extract.

Effect of Fractions (ethylacetate, butanol and n-hexane) on hyperglycaemic rats.

The graph of the mean glyecmic effect of ethylacetate fraction, butanol fraction, n-hexane fraction, distilled water (negative control) and metformin (standard drug) is presented in Figures 2, 3 and 4. The ethylacetate fraction recorded a sustained marked decrease in the blood glucose level of the hyperglycaemic rats from first hour till 7th day ($P < 0.05$) while n-hexane and butanol fractions showed an insignificant ($P > 0.05$) activity.

Table 3: Effect of Ethylacetate Fraction on Fasting Blood Glucose Level of Alloxan Induced Diabetic Rats

Group	Basal	Hyper	1hr	3hrs	6hrs	3days	7days
10 ml/kg	46.50	269.57	283.00	307.00	306.00	336.00	345.00
Distilled water	±2.25	±24.67	±21.96	±25.01	±25.73	±33.98	±41.00
Metformin	47.33	428.33	340.67	84.33	330.33	160.33	53.33
500 mg/kg	±1.76	±48.64	±54.92	±5.55	±62.43	±45.51	±6.40
250 mg/kg	59.33*	242.67	223.33 ns	97.67 ns	206.67 ns	112.33*	61.67*
Ethylacetate fraction	±12.54	±64.18	±56.83	±51.21	±50.31	±15.96	±4.91
500 mg/kg	44.25	169.25	199.00	192.75 ns	200.50 ns	138.50 ns	89.25**
Ethylacetate fraction	±1.03	±10.64	±16.29	±14.29	±16.95	±12.92	±4.81

All values are expressed as mean ±standard error of mean (SEM)
ns = Non significant $p \geq 0.05$
**= Significant ; $p \leq 0.0$*

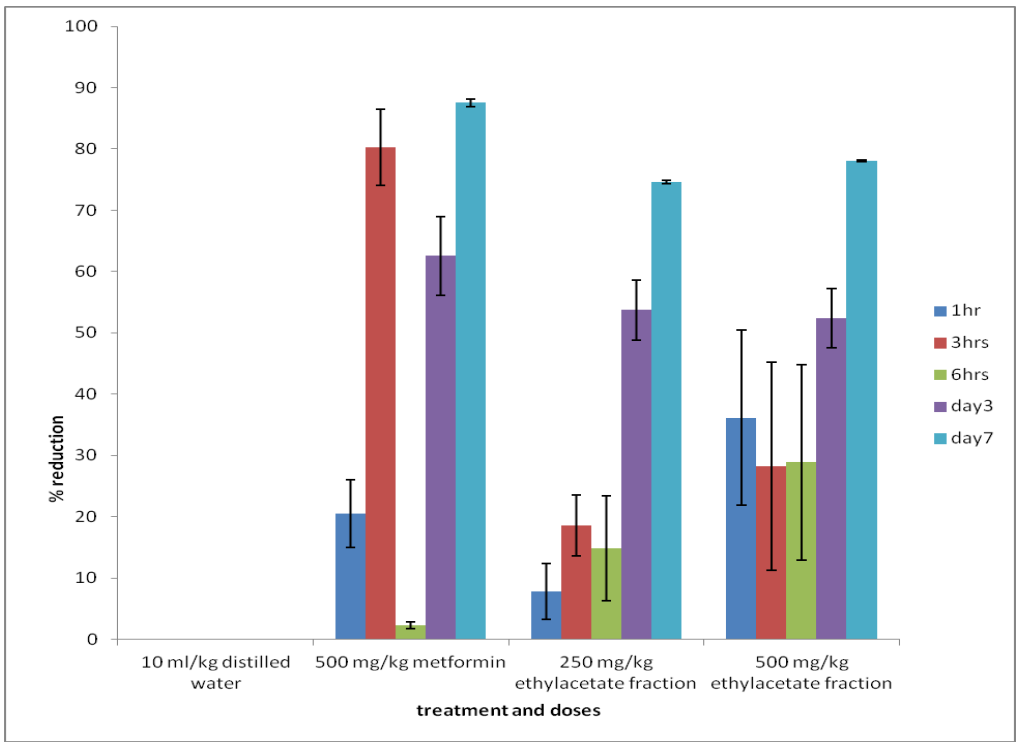

Figure 2: Mean percentage reduction of fasting blood glucose level in alloxan induced diabetic rat by ethylacetate fraction.

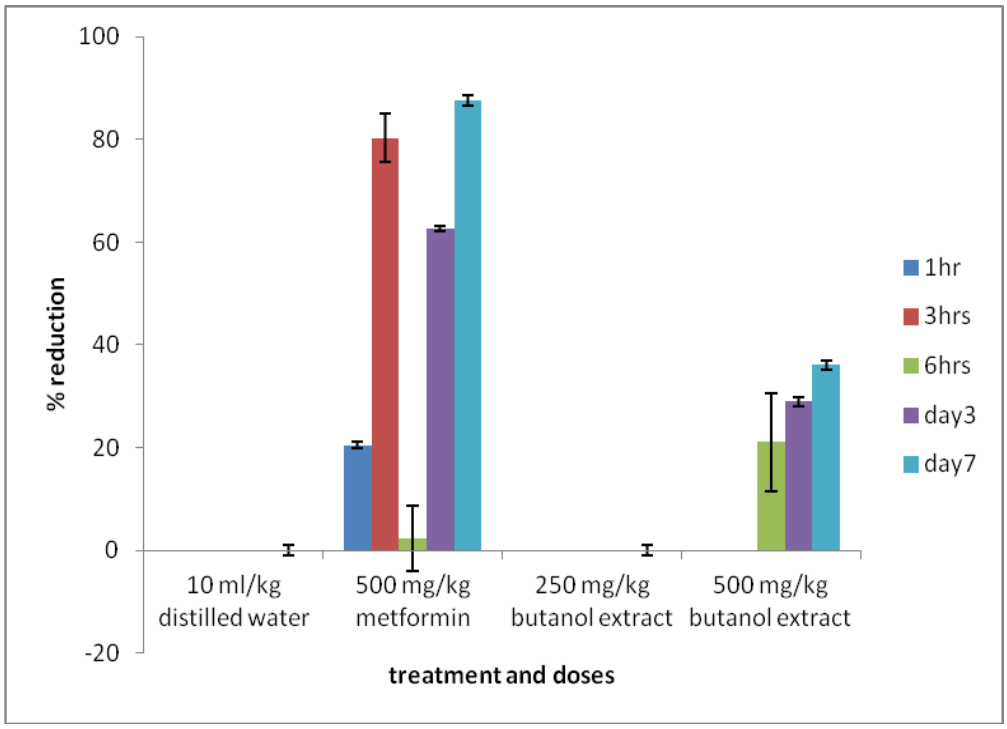

Figure 3: Mean percentage reduction of fasting blood glucose level in alloxan induced diabetic rat by butanol fraction

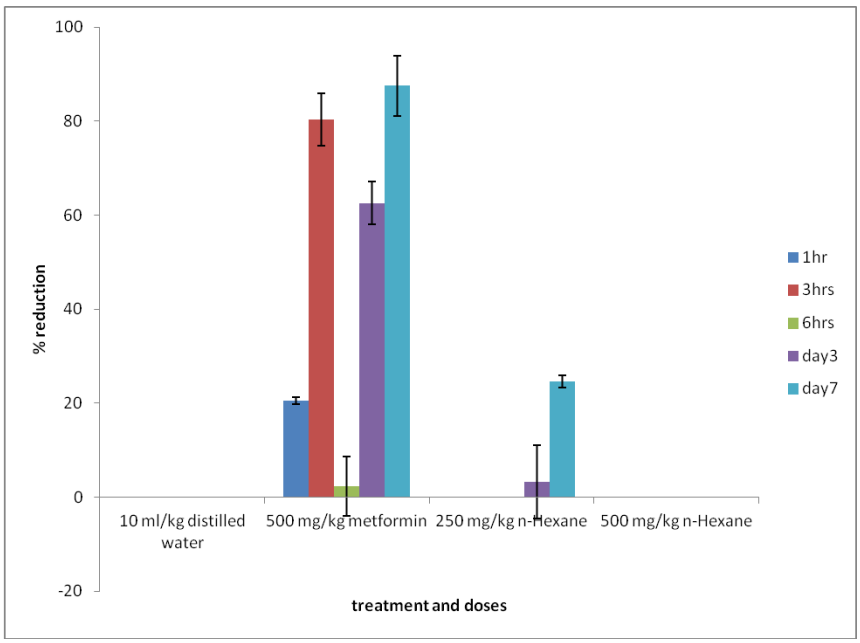

Figure 4: Mean percentage reduction of fasting blood glucose level in alloxan induced diabetic rat by n-hexane fraction.

Result of the Toxicity Assay:

The increase in AST, ALT and ALP when both the crude extract and ethylacetate fraction of *S. liberica* were not statistically different from the control group, indicating no hepatotoxic potential. The results were shown belo:

Effect of Crude Extract on Mean Aspartate Aminotransferase (AST) Level in Alloxan Induced Diabetic Rats.

(AST (U/I). The doses (mg/kg), 100 (distilled water), 500 (metformin), 250 (crude extract), 500 (crude extract) and basal for the 3 and 7 days are 50.21 ± 42.7, 62.19^{ns} ± 0.28, 62.19^{ns} ± 0.14; 64.04 ± 0.31, 71.27^{ns} ± 0.14, 69.19^{ns} ± 0.42; 72.11 ± 4.21, 78.21^{ns} ± 0.39, 62.11^{ns} ± 0.38; 52.17 ± 24.14, 59.02^{ns} ± 34.00, 58.19^{ns} ± 0.59 respectively. All values are expressed as mean ± standard error of mean (SEM) ns = Non significant p ≥ 0.05, * = Significant ; p ≤0.05, ** = Significant; p ≤ 0.01.

Effect of Crude Extract on Mean Alanin Aminotransaminase (ALT) Level in Alloxan Induced Diabetic Rats

ALT (U/I). The doses (mg/kg), 100 (distilled water), 500 (metformin), 250 (crude extract), 500 (crude extract) and basal for the 3 and 7 days are 21.41 ± 0.92, 29.04^{ns} ± 0.51, 23.84^{ns} ±1.64; 15.20 ± 2.28, 34.02*± 0.75, 12.32^{ns} ± 2.20; 11.68 ± 5.15, 28.42* ± 1.58, 19.74^{ns} ±0.54; 24.40 ± 0.59, 27.65^{ns} ±1.28, 21.21^{ns} ± 2.95 respectively. All values are expressed as mean ± standard error of mean (SEM) ns = Non significant p ≥ 0.05, * = Significant ; p ≤0.05, ** = Significant; p ≤ 0.01.

Effect of Crude Extract on Mean Alkaline Phosphate (ALP) Level in Alloxan Induced Diabetic Rats

ALP (U/I). The doses (mg/kg), 100 (distilled water), 500 (metformin), 250 (crude extract), 500 (crude extract) and basal for the 3 and 7 days are 62.00 ± 0.51, 71.00^{ns} ± 0.63, 52.00^{ns} ± 0.71; 51.68 ± 5.40, 20.64* ± 0.60, 31.66^{ns} ± 0.68; 20.52 ± 0.79, 26.48^{ns} ± 0.56, 27.12^{ns} ± 0.33; 29.26 ± 2.95, 32.43^{ns} ± 0.61, 26.54^{ns} ± 0.18 respectively. All values are expressed as mean ± standard error of mean (SEM) ns = Non significant p ≥ 0.05, * = Significant ; p ≤0.05, ** = Significant; p ≤ 0.01.

Effect of Ethylacetate Fraction on Mean Aspartate Aminotransferase (AST) Level in Alloxane Induced Diabetic Rats

AST (U/I). The doses (mg/kg), 100 (distilled water), 500 (metformin), 250 (ethylacetate fraction), 500 (ethylacetate fraction) and basal for the 3 and 7 days are 62.00 ± 0.51, 71.00^{ns}± 0.63, 52.00^{ns}± 0.71; 51.68 ± 5.40, 20.64*± 0.60, 31.66*± 0.68; 59.20± 0.57, 60.00^{ns}± 3.67, 50.20^{ns}± 0.58; 40.27± 0.24, 43.00^{ns}± 0.45, 17.01^{ns}± 0.44 respectively. All values are expressed as mean ± standard error of mean (SEM) ns = Non significant p ≥ 0.05, * = Significant ; p ≤0.05, ** = Significant; p ≤ 0.01.

Effect of Ethylacetate Fraction on Mean Alanin Aminotransaminase (ALT) Level in Alloxan Induced Diabetic Rats

ALT (U/I). The doses (mg/kg), 100 (distilled water), 500 (metformin), 250 (ethylacetate fraction), 500 (ethylacetate fraction) and basal for the 3 and 7 days are 62.00± 0.51, 71.00 ^{ns} ± 0.63, 52.00 ^{ns} ± 0.71; 51.68 ± 5.40, 20.64 *± 0.60, 31.66 *± 0.68; 21.96 ± 0.27, 28.64 ^{ns} ± 3.04, 15.65 ^{ns} ± 2.90; 17.01± 0.44, 23.78 ^{ns} ± 0.74, 21.30 ^{ns} ± 0.53 respectively. All values are expressed as mean ± standard error of mean (SEM) ns = Non significant $p \geq 0.05$, * = Significant; $p \leq 0.05$, ** = Significant; $p \leq 0.01$.

Effect of Ethylacetate Fraction on Mean Alkaline Phosphate (ALP) Level in Alloxan Induced Diabetic Rats

ALP (U/I). The doses (mg/kg), 100 (distilled water), 500 (metformin), 250 (ethylacetate fraction), 500 (ethylacetate fraction) and basal for the 3 and 7 days are 62.00 ± 0.51, 71.00 ^{ns} ± 0.63, 52.00 ^{ns} ± 0.71; 51.68 ± 5.40, 20.64 * ± 0.60, 31.66 * ± 0.68; 55.20 ± 0.49, 77.50 ^{ns} ± 0.68, 65.20 ^{ns} ± 0.69; 24.73 ± 0.80, 25.54 ^{ns} ± 0.37, 25.47 ^{ns} ± 0.59 respectively. All values are expressed as mean ± standard error of mean (SEM) ns = Non significant $p \geq 0.05$, * = Significant ; $p \leq 0.05$, ** = Significant; $p \leq 0.01$.

Effect of crude extract on body weight of alloxan induced diabetic rats.

The change in body weight of the Alloxan induced diabetic rats treated with *S. liberica* crude extract was not significant. The result of the change in body weight of the animals is presented below in Table 4.

Table 4: Effects of crude extract on mean body weight (g) of Alloxan induced diabetic rat.

Dose mg/kg	Basal	3days	7days
100	209.25	202.14 ^{ns}	306.75^{ns}
	± 10.26	±21.00	±22.30
250	201.13	202.14^{ns}	207.00 ^{ns}
	±16.10	±21.00	±21.40
500	208.25	210.75 ^{ns}	306.75
	±12.69	±06.41	±22.30
1000	210.74	223.20	241.60
	±1.25	±1.93	±0.75

All values are expressed as mean ±standard error of mean (SEM)

ns = Non significant $p \geq 0.05$

**= Significant ; $p \leq 0.05$*

***= Significant; $p \leq 0.01$*

DISCUSSION

This study investigated the phytochemical constituents and antidiabetic activity of *S. liberica* leaf extract and fractions. Plants are well known in traditional herbal medicine for their hypoglycaemic activities and available literature indicate that there are more than 800 plant species showing hypoglycaemic activity (Rajapogal and Sasikala, 2008). There is increasing demand for use of plant products with antidiabetic activity due to its low cost, easy availability and lesser side effects. Therefore plants materials are continuously examined and explored for their effect as antidiabetic agents. Plant material may vary in its chemical content and therefore in its therapeutic effect due to differences in environmental factors and species (Sahoo *et al*., 2010).

The percentage yield from the extract and fractions are 0.24 % (N-hexane), 5.06 % (Ethyl-acetate), and 16.0 % (Butanol). Plants having anitdiabetic activity have been ascertained to be rich in alkaloids, flavonoids, terpenoids, steroids and saponins (Mishra, 2010) which are known to be bioactive against diabetes. In other plants used in folk medicine for diabetes mellitus management, phytochemical constituent like alkaloids, glycosides, flavonoids, saponins and tannins have variously been reported to be contributory(Mahrkh *et al*., 2015).

Phytochemical screening of the extract and fractions of *S. liberica* showed the presence of various chemical constituents mostly saponins, proteins, carbohydrates, resins, alkaloids and flavanoids. Terpenoids and steroids were conspicuously present in considerable amount in the crude extract and fractions (Table 1). All these have potential health promoting effect, at least under some circumstances (Busa *et al*., 2007). The literature reports reveal that flavonoids and terpenoids present in the plant extract is known to possess antidiabetic activity (Sharma *et al.*, 2010). Among this bioactive component, highest concentration of flavonoids were found and thought to be the chief ingredient helping to produce antidiabetic activity (Mahrkh *et al*., 2015).

Pradeep (2013) reported that the maximum possibility of antidiabetic activity of the ethanolic extract of *Strobilanthes asperrimus* leaf was the presence of tannins, flavonoids, and alkaloids. Also the antidiabetic

effects (α- amylase and α-glucosidase inhibition activities) of tannins extracted from some cereals, legumes, oil seeds, and vegetables have been studied (Catherine *et al*., 2011) and results have shown encouraging hypoglycaemic effects. Alkaloids have also been severally reported to have antidiabetic activity. For example, alkaloids isolated from *Catharanthus roseus* leaves have shown to induce antidiabetic and antioxidant properties in mouse pancreatic β-cells (Soon *et al*., 2013). Generally alkaloids have been said to inhibit α-glucosidase and decrease glucose transport through the intestinal epithelium (Mishra *et al*., 2010; Patel *et al*., 2012). Saponin, an abundant secondary metabolite in the seed of *Entada phaseoloides* was reported (Zheng *et al*., 2012) to have dramatically reduced fasted blood glucose and serum insulin levels and alleviates hyperglycemia associated oxidative stress in type 2 diabetic. In another study, Alli *et al.,* (2012) reported that saponin extract from the root of Garcinia kola (bitter cola) demonstrated remarkable antidiabetic activity even more than a standard antidiabetic drug metformin in alloxan-induced diabetic rats. Although the mechanism of action of the extract is unknown, the effect exhibited suggests a possible stimulation of insulin release from the residual β-cells and glucagons inhibition. In addition, the extract might have insulin-like effect acting by improving the glucose uptake and metabolism or by inhibiting gluconeogensis thereby exerting the hypoglyceamic effect. Another literature also showed that saponins, resins and flavonoids are good antidiabetic metabolites (Sharma *et al*., 2010).

The result of this study indicates that *S. liberica* CE and EF decreased the blood glucose level. The CE at 250 and 500 mg/kg decreased glucose level by 82 and 68 % respectively after 7 days while the reference drug (metformin 500 mg/kg) decreased the blood glucose level by 87 %. The EF exhibited significant ($p < 0.05$) antihyperglycemic. EF of at 250 and 500 mg/kg decreased glucose level by 74.6 and 78.10 % respectively after 7days while the reference drug (metformin 500 mg/kg) decreased the blood glucose level by 62.57 %. Other fractions (BF and HF) gave a non-significant ($P > 0.05$) antidiabetic effect. The EF consistently produced the highest blood glucose reduction in the model used. This suggests that the antidiabetic constituents of this plant may reside mainly in this fraction.

Several mechanisms such as inhibition of carbohydrate metabolizing enzymes, enhancement of glycogen regulatory enzymes expression in the liver and glucose uptake by tissues and adipocytes as well as stimulation of pancreatic insulin release (Xu *et al*., 2008) have been associated with the antihyperglycemic effect of antidiabetic medicinal plants. Alloxan is a beta cytotoxin which induces chemical diabetes by selectively damaging insulin secretory beta cells, causing impaired insulin secretion and function. The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of the β-cells. The induction of diabetes with alloxan causes significant hyperglycemia. Therefore, the determination of the blood glucose level in the blood of animals among others is a useful quantitative index of diabetes. The extract might have acted through several mechanisms such as slowing down the absorption of sugar from the guts, increasing insulin production by the pancreas from possibly regenerated β-cells, decreasing the release of glucose from the liver or increasing glucose uptake by fat and muscle cells. These proposed mechanisms of action will be a subject of further study. The reduction in blood glucose level of the albino rats by CE and fractions of *S.liberica* leaves at 250 and 500 mg/kg is similar to the findings by Anaga and Opara (2009) that the methanolic extract of *C. planchonii* root at the doses of 250, 500 and 1000 mg/kg reduced the blood glucose of alloxan induced diabetic rats. The extract and fractions of *S.liberica* leaves might have insulin-like effect acting by improving the glucose uptake and metabolism or by inhibiting gluconogenesis thereby exerting the hypoglycemic effect. Further studies however need to be carried out to identify the exact mechanism of action of *S. liberica* leaves and the compound responsible for the hypoglycemic effect.

There was no significant change in the body weight of the alloxan induced diabetic rats after 7 days. The insignificant change of body weight in the rats could be attributed to the increase in metabolic activity of their body systems. This clearly indicates that the plant extract increased glucose metabolism which enhanced body weight in the rats. There were no significant difference in alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities. This is in agreement with the research done on *S. liberica* aqueous leave extract and rhizome by Ngozi and Ohaeri (2012) and Ikewuchi *et al.,* (2011) respectively which showed no significant change in ALT, AST and ALP value.

CONCLUSION

S. liberica leaves possess significant antidiabetic effect which could be attributed to the presence of phytochemical constituents present in the plant. These findings might provide the lead in the development of a novel class of therapeutics in the management of diabetes and as well justifies the folkloric use of *S. liberica* leaf in the management of diabetes mellitus.

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Conflict of Interest: None declared

Received: October 2020

Accepted: December 2020