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ACUTE TOXICITY EVALUATION AND ANTIDIABETIC EFFICACY OF *SENNA SIAMEA* LAM. METHANOL LEAF EXTRACT IN MICE

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ABSTRACT

Senna siamea Lam Irwin et Barneby is used as a medicinal plant especially its leaf, fruit, and stem bark. In this work, the leaf of *Senna siamea* was extracted using 85 % methanol by maceration method, screened, and investigated for phytochemical constituents, acute toxicity was evaluated using Lorke's method and an antidiabetic study was carried out using alloxan-induced Wistar mice. The result of the phytochemical screening revealed the presence of carbohydrates, tannins, saponins, steroid/terpenes, terpenoids, cardenolides and alkaloids, while phlorotannins and soluble starch were absent. The acute toxicity study revealed no death was recorded on the administration of 10, 100 and 1000 mg/kg dose of the leaf methanol extracts via both the oral and intraperitoneal routes in phase I. But death was recorded in phase two, when an extract dose of 5000 mg/kg of the leaf extracts was administered intraperitoneally. Thus, LD_{50} of the leaf extracts in rats administered via the oral route was ≥5000 mg/kg while the intraperitoneal route was calculated as 3807 mg/kg. The antidiabetic study revealed that the extract at doses of 200 mg/kg and 400 mg/kg bd.wt produced a significant ($p<0.05$) reduction in the fasting blood glucose (FBG) of the animals with reductions of 61.01 % and 34.84 % respectively, while both the negative and positive controls had 00.00 % and 76.68 %. The result shows that the effect of the extract was not dose-dependent since the extract of 200 mg/kg exerted a more significant antidiabetic effect on the mice. Thus, the study justified the traditional use of the plant for the management of diabetes.

Keywords: *Alloxan, Diabetes mellitus, Senna siamea, Phytochemicals*

INTRODUCTION

Medicinal Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization estimates that up to 80 % of people still rely mainly on traditional remedies such as herbs for their medicines. Plants are also the source of many modern medicines. It is estimated that approximately one-quarter of prescribed drugs contain plant extract or active ingredients obtained from modelled on plant substances (Sofowora, 2008).

A medicinal plant is defined as a plant intentionally used for the maintenance of good health of a living organism (Smith-Hall *et al*., 2012) The ability of a plant to cure diseases, prevent or manage them is attributed to chemical substances produced by the plants during secondary metabolism, and are called phytochemicals. They are generally used to describe plant compounds that are under research with unestablished effects on health and are not scientifically defined as essential nutrients (Molyneux *et al*., 2007).

Senna siamea Lam, (Fabaceae) (Jensen, 1995), is a native of Southeast Asia and better known in folklore, feeding and agriculture, and it is widely distributed in African countries which include Nigeria, Cote d'Ivoire, Eritrea, Ethiopia, Ghana, Kenya, Togo and in Latin America (Gutteridge, 1995). The plant possesses wide therapeutic value which includes mostly malaria, a tropical endemic disease with high morbimortality. In Southeastern and Sub-Saharan parts of Africa, the root decoction is used for the treatment of diabetes mellitus, amongst other ailments. Scientific studies have revealed that *S. siamea* has recently been shown to have antimicrobial, anti-malarial, anti-diabetic, anti-cancer, hypotensive, diuretic, antioxidant, laxative, anti-inflammatory, analgesic, antipyretic, anxiolytic, antidepressant, and sedative activities (Kamagaté *et al*., 2014).

Diabetes mellitus (DM) is a metabolic disease in which the person has high blood glucose (blood sugar), either because insulin production is inadequate because the body's cells do not respond properly to insulin or both. Patients with high blood sugar will typically experience polyuria (frequent urination), they will become increasingly thirsty (polydipsia) and hungry (polyphagia). Currently, available therapy for diabetes includes insulin and various oral hypoglycaemic agents such as sulfonylureas, metformin, glucosidase inhibitors, troglitazone, etc. These drugs have, however, produced serious health problems such as liver problems, lactic acidosis, and diarrhoea (Rajalakshmi *et al*., 2009). Despite advances in understanding and management of this metabolic disorder, the rate of morbidity and mortality due to this disorder is increasing every year. Globally, an estimated 415 million adults were living with DM in 2015 and this figure is expected to increase to 642 million by the year 2040 (Ogurtsova *et al*., 2017) More than 800 plants have been reported to have an anti-diabetic effect. Several plant species have been used for the prevention or management of diabetes by the Native Americans, Chinese, South Americans, and Asian Indians (Mentreddy *et al*., 2005)

The plant *Senna siemea* which is widely used for the treatment of several illnesses by the local people of northern Nigeria has little been investigated scientifically or its antidiabetic efficacy. This has searched for a novel drug for the treatment of diabetes with less or no side effects in *Senna siamea* paramount.

MATERIALS AND METHODS

Sample Collection and Identification of the Plant Materials

Fresh leaves of *Senna siamea* (Lam) were harvested handpicked from the University of Maiduguri campus and were identified by a Plant Taxonomist from the Department of Biological Science, Faculty of Science, the University of Maiduguri on 14th January 2022.

Sample Preparation

The leaves were neatly washed and then shade-dried for 96 hours before grinding into a fine powder using mortar and pestle. The powdered material was then stored in a cool, dry, and dark place until required for use.

Sample Extraction

Exactly 350g of finely powdered leaves of the *S. siamea* was weighed and 2000 ml methanol was added to the sample and was left for 72 hours with occasional shaking at room temperature. The sample mixture was then filtered using a muslin cloth and allowed dry under atmospheric pressure. The extract obtained was weighed and used for the following experiments below.

Preliminary Phytochemical Screening

The extract fraction of the leaf was screened for phytochemical constituents using procedures described by Evans (2009).

Experimental Animals

A total of forty-four (44) albino mice weighing 25-30 g of both sexes were acquired from the Animal House of the Faculty of Veterinary Medicine, University of Maiduguri, Borno State, Nigeria. The animals were handled according to the International Guiding Principle for Biomedical research involving animals.

Extract and Alloxan Preparation

The crude methanol extract and alloxan (2 g each) were well dissolved in a 10 cm^3 distilled water to give a stock solution of 200 mg/ml.

Acute Toxicity Studies (LD_{50})

The acute toxicity (LD_{50}) of the methanolic extract of *S. siamea* was determined using the protocol described by Lorke (1983). The mice used for the study were deprived of food for 16-18 hrs before administration of the extract. The study was carried out in two phases. Phase 1 consisted of three groups of three animals per group. The extract was administered orally and intraperitoneally in geometrical doses (10 mg/kg, 100 mg/kg and 1000 mg/kg). The treated animals were observed for 4 hrs post-administration for signs of toxicity. Phase 2 was initiated after no death was recorded. In phase 2, three groups of one animal each were orally given the extract at doses of 1600 mg/kg, 2900

mg/kg and 5000 mg/kg), respectively. The animals were observed for signs of toxicity for the first 4 hours and mortality for 24 hrs. the result of the study was calculated using the formula:

$LD_{50} = \sqrt{ab}$

Where - a = lowest dose that kills an animal

b = highest dose that did not kill an animal

Antidiabetic Effect Study

The mice were fasted for 12 hrs but were allowed water *ad libitum* before and throughout the experiment. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn from the lateral tail vein of each mouse under mild anaesthesia and the fasting blood glucose (FBG) was estimated with a glucometer (AccuCheck, Roche, Germany).

Evaluation of Extract Activity in Alloxan-induced hyperglycaemic Mice

The method described by Uzor *et al* (2014) and Yakubu *et al* (2020) was adopted in this study, with little modification. The animals were fasted for 12 hrs with water *ad libitum* and injected intraperitoneally with freshly prepared alloxan monohydrate (150 mg/kg) in distilled water. They were given 5 mL of 10 % dextrose solution to overcome the drug-induced hypoglycaemia and were provided with a standard laboratory diet *ad libitum* after one hour. The Fasting blood glucose (FBG) was checked before and 72 hrs after alloxan injection by withdrawing blood from the tip of the tail of each mouse. The FBG was measured as described above. The animals were considered diabetic when the FBG is greater than 200 mg/dL. They were segregated into five (5) groups of four animals in each. Group, I served as the normal i.e, animals fasted, and water was given *ad libitum*. Group II-V were all alloxan-induced diabetic mice. Group II served as the negative control and received vehicle (normal saline, 2mL/kg, p.o.), Groups III, and IV (i.e alloxan-induced diabetic mice) were administered 200 and 400 mg/kg each of the extract respectively. Group V was administered glibenclamide (2 mg/kg, p.o.). Blood glucose concentration was measured after 0, 1 hr, 6 hr, 12 hr, and 18 hr after administration of a single dose of each of the regimens.

Statistical Analysis

Results of the antidiabetic study were analysed using GraphPad Prism. 2016 Model, Version 7.0 for windows. One-way Analysis of Variance (ANOVA) test followed by Dunnet's Multiple Comparison Test was used to analyse and compare the results at a 95% confidence level.

RESULTS

PHASE 1: CHEMICAL ANALYSIS

Extraction Yield of *S. siamea* leaf

The extract of the leaf produced extract with brown colour and gummy mass in texture with a yield of 12.50 %. The result of the extraction profile is shown in Table 1.

Phytochemical Screening of the Leaf Extract

The preliminary phytochemical screening of the leaf extract revealed the presence flavonoids, terpenoids, cardiac glycosides, saponins, tannins, flavonoids and alkaloids; while phlobatannins, anthraquinones, and soluble starch were absent. The result of the study is shown in Table 2.

PHASE II: PHARMACOLOGICAL STUDY

Determination of Median Lethal Dose (LD_{50}) of Methanol Leaf Extract of *S. siamea*

Table 3 present the result of acute toxicity of methanol leaf extracts of *S. siamea* in mice. No death was recorded on administered doses of 10, 100 and 1000 mg/kg of the leaf methanol extract via both the oral and intraperitoneal routes in phase I. But death was recorded in phase two when the extract dose of 5000 mg/kg of the leaf extract was administered intraperitoneally. Thus, LD_{50} of the leaf extract in mice administered via oral and intraperitoneal routes were ≥5000 mg/kg and 3807 mg/kg respectively.

Effect of Methanol leaf extract of *S. siamea* on Alloxan-Induced Diabetic Mice

The antidiabetic effect of methanol extract on the leaf of *S. siamea* is shown in Table 4. Two doses of the 200 mg/kg and 400 mg/kg were administered orally to the alloxan-induced mice. The extract at a dose of 200 mg/kg and 400 mg/kg bd. wt produced a significant (p<0.05) reduction in the FBG of the animals with reductions of 61.01 % and 34.84 % respectively, while both the negative and positive controls had 00.00 % and 76.68 %. The result shows that the effect of the extract was non-dose-dependent since the extract of 200 mg/kg had a more significant antidiabetic effect on the mice. Although glibenclamide at 10 mg (a positive control drug) was more effective than the extract.

Table 1: The extraction profile of air-dried powdered leaf of *S. Siamea*

S/N	Sample	Mass (g)	%Yield ($^{w}/_{w}$)	Colour	Texture
1	Leaf	400.00	12.50	Greenish brown	Gummy

Table 2: Phytochemical constituents of methanol leaf extract of *Senna siamea*

S/N	Phytochemical Test	Inference
1	Test Tor Carbohydrates	+
2	Test for Tannins	+
3	Test for Phlobatannins	-
4	Test for Steroids/Triterpenes	+
5	Test for Flavonoids	+
6	Test for Saponins	+
7	Test for Soluble Starch	-
8	Test for Alkaloids	+
9	Test for Steroidal Nucleus/Cardinolides	+
10	Test for Terpenoids	+

Keys: - = not present; + = present

Table 3: Acute toxicity effect of methanol leaf extract of *S. siamea* in mice

Phase	Dose (mg/kg)	No. of mice	Mortality rate	
		Oral route	IP route	
I	10	3	0/3	0/3
	100	3	0/3	0/3
	1000	3	0/3	0/3
II	1600	1	0/1	0/1
	2900	1	0/1	0/1
	5000	1	0/1	**1/1**

ip LD_{50} = 3807 mg/kg Oral $LD_{50} \geq 5000$ mg/kg

Table 4: Effect of crude methanol Leaf Extract *S. siamea* on mice

		Fasting Blood Glucose (FBG) concentration (mg /dL) Time (hr) after treatment						
S/N	**Treatment (mg/kg)**	**Basal FBG (mg/dL)**	**0**	**1**	**6**	**12**	**18**	**% I.G**
1	Normal	73.50±5.24	87.25±2.29	88.00±2.12	87.50±2.40	88.50±2.99	89.25±2.87	-
2	D.C (100)	72.40±5.20	321.00±4.61	323.50±42.72	321.30±43.72	319.80±4.65a	321.00±41.32[a]	00.00
3	200	75.00±3.24*	274.30±50.30	225.80±47.43	160.00±25.33*	101.50±9.99*[b]	106.00±9.41*[b]	61.01
4	400	72.00±4.08*	475.50±33.50	435.00±51.74	404.80±49.10	321.80±45.17[a]	309.80±70.68[a]	34.87
5	Glibenclamide (10 mg)	73.25±1.90*	482.50±22.50	362.80±30.48	270.80±39.98[a]	190.00±42.78[c]	112.50±14.51[c]	76.68

Results are expressed as Mean ±SEM (n=4). *P<0.05 across rows and values assigned different alphabetical notations across columns are considered statistically significant when compared to the negative control (One way, ANOVA followed by Dunnet's t-test, 2-sided). % Inhibition of glycaemia denotes percentage reduction of blood glucose from 0 h. Basal FBG=FBG before induction of diabetes; DC = diabetic control; IG = inhibition of glycaemia

DISCUSSION

Plants have been used locally for the treatment of diabetes and some have been proven scientifically to have hypoglycemic activity. Researchers have shown that these plant extracts contain compounds including polysaccharides (Bhavapriya and Govindrajsamy, 2000), flavonoids (Tomada *et al*., 1985), terpenoids and tannins (Schimizu *et al*., 1984), steroids (Recher *et al*., 1991), polypeptides (Ivorra *et al*., 1989) and alkaloids (Karawya and Wahab, 1984) which are compounds implicated for the antidiabetic activity. The phytochemical studies of the methanol leaf extract of *S. siamea* revealed the presence of flavonoids, cardiac glycosides, tannins, saponins, terpenoids and alkaloids.

The present study revealed that there was no mortality amongst the mice used for the study at all dose levels of the leaf of *S. siamea* when administered orally. But there was mortality at 5000 mg/kg dose of the leaf methanol extracts when administered *i.p*. the oral and i.p LD_{50} were estimated as ≥5000 mg/kg and 3807 mg/kg respectively. Clarke and Clarke (1977) believed compounds with i.p LD_{50} of 1500 mg/kg and above have low toxicity and this could explain the safe use of the plant traditionally (Idris *et al*., 2014).

Folklore medicines are widely now used substantially by people suffering from diabetes mellitus globally (Gaikwad *et al*., 2014) and medicinal plants have been identified to be a target for scientists to come up with newer and better therapeutic options in years to come.

The observed hypoglycaemic activity of *S. siamea* could be attributed to the reported phytochemicals and phytonutrients present in the plant. These are compounds that have been known to exert pharmacological and antagonistic effects within the body (Guimaraes *et al*., 2013).

The possible mechanisms responsible for the hypoglycaemic activity exhibited by *S. siamea* include inhibition of intestinal absorption of glucose, facilitation of glucose-induced insulin release, enhancement of peripheral glucose uptake, promotion of the regeneration of β-cell of islets of langerhans and amelioration of oxidative stress (Kibiti and Afolayan, 2015) attributed to the presence of a variety of phytoconstituents present in this plant.

CONCLUSION

In conclusion, the present study revealed that *Senna siamea* has a relatively safe regimen. The methanol leaf extract of the plant was relatively effective in the management of diabetes which could be due to the presence of phytochemicals in biological activities.

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