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Antihyperglycemic and Antihyperglycemic Effect of Leaves and Stem Bark Methanol Extracts of *Senna siamea* in Alloxan Induced Diabetic Rats

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ABSTRACT

Senna siamea is traditionally used for treatment of many diseases. This study was carried out to evaluate the antihyperglycemic and antihyperglycemic effect of the methanol extracts of the leaves and stem bark of *S. siamea*. The extracts were orally administered daily at 250 and 500 mg kg⁻¹ b.wt. for three weeks. The effect of the extracts on Fasting Blood Glucose (FBG) level and body weight were determined weekly. All rats in the diabetic groups had FBG levels well within the diabetic range (>150 mg dL⁻¹) at the initial stage of the experiment but after three weeks of treatment with extracts or glibenclamide; the FBG significantly (p<0.05) dropped in dose-dependent manner, with leaves being more active. So also, the increase in triglycerides, LDL-cholesterol and total cholesterol in diabetic rats were significantly (p<0.05) prevented by the two extracts, with concomitant increase in serum HDL-cholesterol. The increase in serum total protein and decrease in urea in all treated groups was statistically (p<0.05) significant when compared with the diabetic control group. The slight increase in the levels of ALT and AST recorded in the diabetic control group were prevented in the extracts treated groups, though the decrease did not differ significant. These effects were quite comparable with the glibenclamide treated group which is a standard drug used to treat diabetes. The results suggest that the methanol extracts of the leaves and stem bark of *S. siamea* restored the metabolic changes in alloxan-induced diabetic rats.

Key words: *Senna siamea*, antihyperglycemic effect, antihyperglycemic effect, diabetes, alloxan, residual insulin

INTRODUCTION

Hyperglycaemia observed in diabetic patients arose due to impaired insulin release/insulin action which causes complications in the overall metabolism of proteins, lipids and other body organs (Kim *et al.*, 2006). The glycation of body proteins has been reported to be the main consequences hyperglycaemia which eventually result into retinopathy, nephropathy and atherosclerosis (Rao *et al.*, 1999).

The rapid increase in the number of people with Diabetes Mellitus (DM) worldwide is quite alarming (Modak *et al.*, 2007). At least, 177 million people worldwide live with diabetes and this figure is likely to increase by 2030 (WHO, 2000). The International Diabetes Federation (IDF)

estimated that 10.8 million people have diabetes in sub-Saharan Africa in 2006 and that this would rise to 18.7 million by 2025 (Haque *et al.*, 2011). In Nigeria, about 3% of adult were reported to have DM (Bakari *et al.*, 1999).

Senna siamea Lam. (Irwin and Barneby-*Cassia siamea* Lam.) (Fabaceae, Caesalpiniaceae) (Doughari and Okafor, 2008), Thailand shower or in Hausa as "Malga" (Sidi-Aliyu, 2006), is a native of tropical Asia, introduced and now naturalized in Africa (Bernard, 2005). *S. siamea* is claimed traditionally to be used in treatment of various medical conditions such as diabetes, insomnia, hypertension, asthma, constipation and diuresis (Hill, 1992).

Modern synthetic antihyperglycemic agents were reported to produce undesirable and unwanted side effects such as; weight gain, lactic acidosis and hypoglycaemia (Malviya *et al.*, 2010) and are not easily or cheaply available (Iwu *et al.*, 1999). Thus, more alternative therapy and approach with less or no side effects are needed to manage DM and its complications. Many plants derived medicines were reported to demonstrate a bright future in therapy and management of DM due to their less toxic effects and are cheaply available (Azadbakht *et al.*, 2010). Thus, this study was undertaken to evaluate the antihyperglycemic and antihyperglycemic effect of the leaves and stem bark methanol extracts of *S. siamea* in alloxan induced diabetic rats as a way of validating its traditional usage.

MATERIALS AND METHODS

Plant collection and extraction: The leaves and stem bark of *S. siamea* were harvested from gardens around Zaria, Nigeria in the month of April, 2008. The study ended in June, 2008. The plant parts were authenticated by a botanist, in the Department of Biological Sciences, Ahmadu Bello University, Zaria. Leaves and stem bark were separately washed, wiped-dry, cut into small pieces and subsequently reduced to a coarse powder. About 100 g of the leaves and stem bark were separately extracted for 24 h with 90% methanol with intermittent vigorous shaking. The extracts were filtered, concentrated with a rotary evaporator and dried over a water bath at 45°C. The residue from the plant parts were used for experimental analysis.

Experimental animals: Wistar strain rats weighing 120 to 200 g were obtained from the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. They were housed in well ventilated cages and kept in a room where a twelve-hour light/dark cycle was maintained. They were allowed free access to water and fed commercial growers' mash (Vital feeds, Jos) *ad libitum* throughout the period of the experiment. The rats were allowed to acclimatize for two weeks. The experimental rats were all handled in strict compliance with international guidelines as prescribed by the Canadian Council on the Care and use of Laboratory Animals in Biomedical Research (CCAC, 1984).

Induction of diabetes: Some of the rats were subjected to 8 h fast. Diabetes was induced with a single intraperitoneal injection of alloxan 180 mg kg⁻¹ b.wt. (Misra *et al.*, 2011; Gayasuddin *et al.*, 2011). After 72 h, fasting blood glucose levels was measured and 42 rats with fasting blood glucose concentration of more than 200 mg dL⁻¹ were considered diabetic and included in the experiment.

Experimental design: In this experiment, a total of forty nine rats (42 diabetic surviving rats, 7 normal rats) were used. The rats were divided into seven groups of seven rats each after the induction of alloxan. Group 1 and 2 were normal untreated and diabetes-induced rats, respectively

and received a normal saline throughout the study period. Group 3, 4, 5 and 6 received 250 and 500 mg kg⁻¹ b.wt. of leaves and stem bark extracts respectively, while group 7 received a standard drug glibenclamide.

Fasting blood glucose was measured at the 0, 1st, 2nd and 3rd week after first extracts administration. Blood was withdrawn from the tail vein each time. Fasting blood glucose was measured using Accu-chek advantage Glucometer; Roche Diagnostics, USA.

Sample collection and preparation: At the end of the treatment period, all the rats were starved for 8 h. Animals were humanly sacrificed. Blood samples were collected, by puncturing the jugular vein allowing free flow of blood. Serum was harvested from the blood and stored at -20°C until required.

Determination of biochemical parameters: Total cholesterol, High Density Lipoproteins (HDL)-cholesterol, triacylglycerols, alanine (ALT) and aspartate transaminase (AST) as well as urea, total protein were assayed in the serum using commercial reagent kits (Randox Laboratories, UK).

Quantitative determination of phytochemicals: Quantitative determination of phytochemicals was carried out for total phenolics (Edeoga *et al.*, 2005), flavonoids (Bohm and Koupai-Abyazani, 1974), alkaloids and saponins (Obadoni and Ochuko, 2001).

Statistical analysis: Data are presented as Means±SD and analyzed using analysis of variance (ANOVA) and Duncan *post hoc* test and significance was determined at p<0.05.

RESULTS

The result of Fasting Blood Glucose (FBG) for all the groups is presented on Fig. 1. It showed that the FBG of the normal control group remained within the normal range throughout the

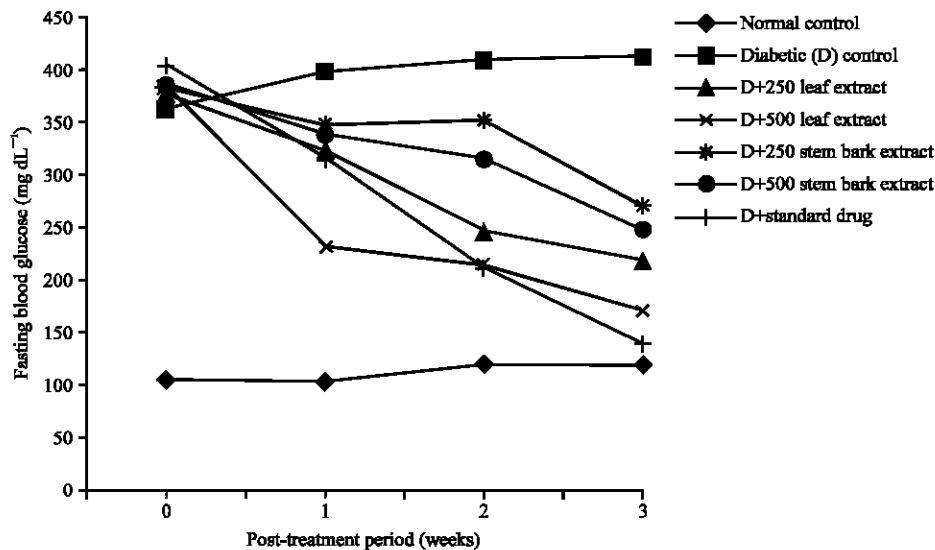


Fig. 1: Profiles of fasting blood glucose for all groups

Table 1: Effect of aqueous leaf and stem bark extract of *Senna siamea* on fasting blood glucose (FBG) of normal and alloxan-induced diabetic rats

Group	Group						
	1	2	3	4	5	6	7
Change in FBG (%)	7.35±2.11 ^a	13.50±5.30 ^b	-42.18±1.22 ^c	-55.12±15.50 ^d	-29.32±10.45 ^e	-36.01±2.01 ^e	-65.59±1.22 ^d

Values are Mean±SD of seven replicates, Values not sharing a common superscript along a row are significantly different at $p < 0.05$

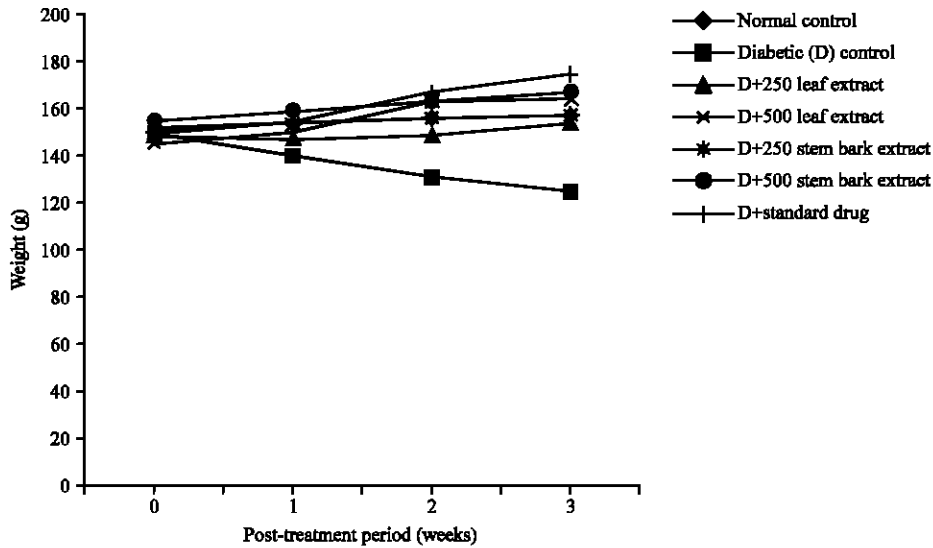


Fig. 2: Change in body weight for all groups

experimental period. On the other hand, the FBG of diabetic control group maintained their increase in FBG throughout the period. The diabetic groups treated with the 250 and 500 mg kg^{-1} methanolic leaves extracts recorded a significant ($p < 0.05$) drop in FBG levels by about 42.18 and 55.47%, respectively, while that of stem bark dropped by 29.32 and 36.01%, respectively when compared with that of diabetic control group; 13.5% (Table 1). The blood glucose-lowering effect of the extract was comparable to that of the standard hypoglycemic drug; glibenclamide.

Diabetes is characterized by weight loss and it was also seen in this study. Alloxan administration brought about marked reduction in body weight of rats. This reduction was found to be significant ($p < 0.05$) when compared with normal control group. These reduced body weights were found to be increased when compared to their respective diabetic control group and this increase was found to be statistically significant in rats treated with the leaves and stem bark extracts of *S. siamea* (Fig. 2). Percent increase in body weight for 250 and 500 mg kg^{-1} leaves extract treated groups were 5.59 and 12.77%, respectively and 5.3 and 6.33% for stem bark extract against 4.18% for glibenclamide.

Induction of diabetes significantly ($p < 0.05$) increased the levels of total cholesterol, triglycerides and LDL-cholesterol and decreased serum HDL-cholesterol of diabetic control group (Table 2). Administration of the extracts to diabetic rats significantly prevented the diabetes-induced increase in levels of serum triglycerides, total and LDL-cholesterols and also significantly ($p < 0.05$) caused an increase in HDL-cholesterol, that of the diabetic control animals. Alloxan

Table 2: Effect of methanol extracts of the leaf and stem bark on serum lipid profile of normal and alloxan-induced diabetic rats

Parameter (mg dL ⁻¹)	Group						
	1	2	3	4	5	6	7
Total cholesterol	119.81±4.81 ^a	166.65±1.12 ^b	140.01±2.84 ^c	131.95±2.72 ^c	142.17±2.66 ^c	139.66±2.93 ^c	133.24±3.08 ^c
HDL-cholesterol	77.10±3.81 ^a	61.11±3.13 ^a	70.51±2.17 ^a	72.65±3.27 ^a	69.11±2.11 ^a	71.31±4.10 ^a	86.05±3.11 ^a
LDL-cholesterol	30.78±1.76 ^a	80.74±1.98 ^b	50.49±1.02 ^b	42.50±1.11 ^b	53.91±1.87 ^b	50.45±1.65 ^b	31.62±1.79 ^a
Triglycerides	60.10±3.81 ^a	124.14±3.36 ^b	95.04±2.49 ^c	84.02±2.73 ^c	95.73±2.54 ^c	89.46±1.86 ^c	74.84±3.02 ^c

Values are Mean±SD of seven replicates, Values not sharing a common superscript along a row are significantly different at p<0.05, LDL: Low density lipoproteins, HDL: High density lipoproteins

Table 3: Effect of methanol extracts of the leaf and stem bark on serum total protein, urea and liver enzymes of normal and alloxan-induced diabetic rats

Parameter	Group						
	1	2	3	4	5	6	7
Total protein (g dL ⁻¹)	4.10±1.84 ^a	1.98±0.11 ^b	3.03±0.13 ^c	3.09±0.09 ^c	2.94±0.18 ^c	3.00±0.06 ^c	3.18±0.11 ^c
Urea (mg dL ⁻¹)	3.56±0.45 ^a	5.12±1.21 ^b	4.67±0.93 ^b	4.87±0.56 ^b	4.49±1.21 ^b	5.01±1.13 ^b	4.55±0.79 ^b
Aspartate transaminase (IU L ⁻¹)	38.12±0.34 ^a	40.12±0.54 ^a	40.03±0.91 ^a	39.12±0.32 ^a	40.12±1.01 ^a	38.22±0.76 ^a	40.17±1.11 ^a
Alanine transaminase (IU L ⁻¹)	36.89±1.34 ^a	43.87±0.98 ^a	41.34±1.23 ^a	42.55±1.45 ^a	40.33±0.78 ^a	39.56±0.69 ^a	35.76±0.45 ^a

Values are Mean±SD of seven replicates, Values not sharing a common superscript along a row are significantly different at p<0.05

Table 4: Quantitative amount of phytochemical constituents of *Senna siamea* leaves and stem bark extracts

Phytochemicals (g g ⁻¹)	Leaves	Stem bark
Alkaloids	0.10±0.02 ^a	0.05±0.02 ^b
Saponins	0.08±0.02 ^a	0.07±0.01 ^a
Polyphenols	0.95±0.02 ^a	0.92±0.05 ^a
Flavonoids	0.19±0.03 ^a	0.06±0.01 ^b

Values are Mean±SD of seven replicates, Values not sharing a common superscript along a row are significantly different at p<0.05

induction caused a significant (p<0.05) increase in serum urea levels and a marked decrease in serum total proteins (Table 3). Treatment with 250 and 500 mg kg⁻¹ of *S. siamea* leaves and stem bark extracts did prevent these abnormalities caused by the diabetes. Serum levels of ALT and AST were not affected by diabetes. This effect was the same even after treatment with the two extracts.

The results for phytochemical screening (Table 4), indicated that the amount of polyphenols and saponins in the leaves and stem bark were found not to differ significantly (p<0.05) from each other, while flavonoids and alkaloids differ significantly with leaves containing the higher amounts.

DISCUSSION

The alloxan-induced diabetic rats in this experiment were all clearly hyperglycemic; an indication of a successful induction of diabetes. The diabetic control group exhibited an increase in Fasting Blood Glucose (FBG) during the experiment, indicating that no reversion to normoglycemic status took place during the period of experimentation. The leaves extracts-treated diabetic groups at 250 and 500 mg kg⁻¹ recorded a dose-dependented drop of 42.18 and 55.47% decrease in FBG, while those of stem bark were 29.32 and 36.01% at the same doses, as opposed to the 13.5% decrease in the diabetic controls. This suggested that the extracts had hypoglycemic activity in the diabetic animals; comparable to that of the standard hypoglycemic drug (65.59%) used in another group of diabetic rats. The anti-hyperglycaemic effects of leaves extract were found to be more than that

of stem bark. This could be attributed to the higher content of some active constituents like flavonoids present in the leaves than that of stem bark (Wang *et al.*, 2011) which may have enhanced the activity of residual insulin in the alloxanic animals or promoted glucose uptake by peripheral tissues, by other means (Umar *et al.*, 2003), hyperlipidemia, a feature of diabetes mellitus, was reported affect 40% of diabetics (Wolfe *et al.*, 2003; Kim *et al.*, 2006) and is directly linked to insulin deficiency (Ramarathnam *et al.*, 1997). Increases in triglycerides, total and LDL-cholesterol were recorded in the untreated diabetic rats in this work, this is in consistent with several earlier reports (Anthony *et al.*, 2003). Since diabetic hyperlipidemia is, amongst other factors, a direct consequence of the inability of peripheral tissues to access blood glucose (hyperglycemia), hence any agent that corrects the diabetic hyperglycemia would consequently correct or at least ameliorate, the attendant dyslipidemia. Significant lowering of triglycerides, LDL and total cholesterol and rise in HDL-cholesterol is a very desirable biochemical state for prevention of hyperlipidemic condition (Fuller *et al.*, 1980).

The decrease in protein observed in diabetic control group was reported to be due to net increase in protein breakdown rather than a decline in protein synthesis (Moller and Nair, 2008). After oral administration of the *S. siamea* extracts, the alteration in protein metabolism was attenuated, as evidenced by decreased serum urea levels and increase in total protein level in diabetic rats. In all, the leaves extract was found to be more effective than that of stem bark. This also corresponds with the previous studies reported by Sini *et al.* (2011) and Salahuddin *et al.* (2010). Alloxan induction indicated no significant effect on serum ALT and AST levels. This contradicts with the earlier report by Umar *et al.* (2012) that serum AST levels were statistically significant from that of diabetic control group. But correspond with the study reported by Opajobi *et al.* (2011). Serum urea level is one the significant markers of renal function. Impaired nitrogen balance is usually linked to low protein biosynthesis which may lead to increased levels of urea in blood (Asayama *et al.*, 1994). The result on serum urea and total protein also correspond with that reported by Sriram and Subramanian (2011).

CONCLUSION

Conclusively, the present study demonstrates that methanolic extracts of leaves and stem bark of *S. siamea* possesses anti-hyperglycaemic and antihyperglycemic properties due to the presence of biologically active phyto-components present. The leaves extracts was found to be more effective than the stem bark. So also the effective dose was also found to be 500 mg kg⁻¹ b.wt. These results suggest that the product of *S. siamea* may provide a new therapeutic avenue against diabetes and its associated complications.

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