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Sub-chronic Study of Aqueous Stem Bark Extract of *Senna siamea* in Rats

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ABSTRACT

Senna siamea is a plant commonly used in traditional medicine to treat hypertension, malaria and diabetes mellitus. The aim of this study was to investigate the sub-chronic toxicity of aqueous extract from the stem bark of *S. siamea* male Wistar rats. The extract was orally administered for seven weeks at 200, 400, 800 and 1600 mg kg⁻¹ body weight. The results obtained showed that the extract significantly ($p < 0.05$) increases the body weight and feed intake of the rats. Hematological parameters (PCV, Hb, platelets, WBC and RBC) were not significantly ($p < 0.05$) affected by the extract. But the levels of serum liver enzymes (Alkaline Phosphatase (ALP), Alanine Aminotransaminase (ALT), Aspartate Aminotransaminase (AST) were significantly different ($p < 0.05$) from the normal control group. In contrast, no significant change was observed in the total protein and albumin levels in the treated group compared to the normal group. Similarly, serum glucose, triglycerides, cholesterol and the markers of kidney function (creatinine, urea, potassium, sodium and chloride) did not differ significantly ($p < 0.05$) from the normal control group. The quantitative determination of saponins, alkaloids, total polyphenolics and flavonoids in (g/g) were found to be 0.07 ± 0.01 , 0.05 ± 0.02 , 0.92 ± 0.05 and 0.06 ± 0.01 . These results may explain the use of *S. siamea* stem bark in folk medicine due its less toxic effect.

Key words: Biochemical parameters, hematological parameters, *Senna siamea*, sub-chronic effect

INTRODUCTION

Medicinal plants are extensively used in treatment and management of diseased conditions, especially in developing countries. This is due to result of their wide availability, less toxicity and more affordable alternative to synthetic drugs. Toxicity studies on medicinal plants or extracts from them usually determine the level of safety particularly during the development of drugs (Jaijoy *et al.*, 2010). *Senna siamea* Lam. (Irwin and Barneby-*Cassia siamea* Lam.) (Fabaceae, Caesalpiniaceae) (Doughari and Okafor, 2008), or "Thailand shower" (Aliyu, 2006), is a native of tropical Asia, introduced and now naturalized in Africa. This plant reaches up to 15-20 cm tall, with about 30 cm diameters. Bark is grey or light brown, smooth but becoming slightly fissured with age (Von Maydell, 1986). *S. siamea* is claimed to be used to treatment of disease conditions such as diabetes, insomnia, hypertension, asthma, constipation and diuresis (Hill, 1992). *S. siamea*, fresh young flowers and/or young leaves have been used as vegetables in Thailand (Otimenyin *et al.*, 2010). The stem bark extract was locally reported to have analgesic and anti-inflammatory effects (Ntandou *et al.*, 2010) and antimalarial effect (Odugbemi *et al.*, 2007).

Most of the toxicity studies carried out on *S. siamea* was on the leaf and root extracts, little information on *S. siamea* stem bark toxicity is currently available. The purpose of the present study is to investigate sub-chronic toxicity of the aqueous stem bark extract *S. siamea* in rats.

MATERIALS AND METHODS

Collection, identification and extraction of plant materials: The fresh stem bark of the *S. siamea* was collected from the Zaria Local Government garden, Zaria, Kaduna State, Nigeria in March, 2008. The plant was identified and authenticated by botanist in the Biological Sciences Department, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The stem bark was sorted to eliminate any dead matter and other unwanted particles. The stem bark was air-dried for 2 weeks and then ground into fine powder using pestle and mortar. A total of 400 g of the ground powder was soaked in 2 L of distilled water for 48 h at room temperature. The mixture was filtered into conical flasks with Watman filter paper (No. 1). The filtrate was dried at a temperature of 30°C for 10 h.

Experimental animals: Sixty five healthy male Wistar rats (weighing about 100-160 g) bred in the animal house of Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria-Nigeria, were used for this study. They were fed with feeds (Vital feeds, Nigeria) and tap water *ad libitum*. The experiment was approved by the ethical committee on the use of experimental animals of the Ahmadu Bello University, Zaria, Nigeria.

Methodology

Acute toxicity study: The acute toxicity (LD₅₀) was carried out by Lorke (1983) Method. The method involved two phases of which eighteen (18) rats were grouped in to three groups of six rats each. They received 10, 100 and 1000 mg kg⁻¹ body weights of the extracts. In the second phase twelve rats were grouped in to three groups of for rats each, and they received 1500, 2900 and 5000 mg kg⁻¹ body weights. The rats were observed daily for any signs of toxicity, throughout the period of study.

Sub-chronic study: Thirty five were used for sub-chronic study. The rats were randomly grouped into five groups of seven animals in each group. Group 1 served as control and received distilled water while Groups 2, 3, 4 and 5 received the aqueous extract of the stem bark of *S. siamea* 200, 400, 800 and 1600 mg kg⁻¹, respectively. The dosages used were established from the previous study. Rats received their doses once daily orally, for 7 weeks. The rats were observed daily for any signs of toxicity, throughout the period of study.

Throughout the treatment period, body weights and feed intakes were recorded weekly. At the end of the treatment, all the animals were fasted for 8 h. Blood samples were collected from rat's tail into heparinized blood sample bottles and then the rats were anesthetized with ether and sacrificed by drawing blood from the inferior vena cava. Samples collected were used for the determination of hematological and biochemical parameters.

Determination of hematological parameters: Packed Cell Volume (PCV) of each sample was determined using a Hawksley micro-haematocrit centrifuge (McGovern *et al.*, 1955). Erythrocytes (RBC) and total leucocytes (WBC) were counted using the improved Neubauer haemocytometer as describe by Otimenyin *et al.* (2009).

Determination of biochemical parameters: Biochemical analysis of serum samples were conducted using reagents kits (Randox kits) by the following methods: AST and ALT (Reitman and Frankel, 1957), ALP (King, 1965), bilirubin (Malloy and Evelyn, 1937), creatinine (Bartels *et al.*, 1972), cholesterol (Stein, 1987), triglyceride (McGowan *et al.*, 1983), total protein and albumin (Lowry *et al.*, 1951), urea (Fawcett and Scout, 1960), glucose by glucose oxidase method (Beach and Turner, 1958). Sodium, potassium and chloride were analyzed using flame photometer (model Gallen-Kamp FGA-300-C).

Quantitative determination of phytochemicals: Quantitative determination of phytochemicals was carried out for total phenolics (Edeoga *et al.*, 2005), flavonoids (Bohm and Koupai-Abyazani, 1994), alkaloids and saponins (Obadoni and Ochuko, 2001).

Statistical analysis: Data are presented as Mean±Standard deviation (SD) and analyzed using Analysis of Variance (ANOVA) and Duncan *post hoc* test and significance was determined at $p < 0.05$.

RESULTS

The results obtained from the present study indicated that the oral LD₅₀ of the aqueous extract of stem bark of *S. siamea* was found to be greater than 5000 mg kg⁻¹. The extract showed no sign of toxicity for the time periods and no death was recorded.

For sub-chronic study, the result indicated that the aqueous stem bark extract of *S. siamea* did produce significant ($p < 0.05$) increase in body weight from second week to seventh week, especially with group 5 (Fig. 1). It was also observed that all the groups significantly ($p < 0.05$) increased in their feed-intake habit when compared with that of normal control group (Fig. 2).

The results for phytochemical screening (Table 1), indicated that the amount of total polyphenolics (g/g) was the highest (0.92±0.05), while the amounts of saponins, flavonoids and alkaloids were found not to differ significantly ($p < 0.05$) from each other.

Hematological parameters analyzed (Table 2) showed that, all the treated groups did not differ significantly ($p < 0.05$) from the normal control group (group 1) up to the maximum dose of 1600 mg kg⁻¹ body weight. The PCV slightly increases by 1.7% in group 2, but decreased in other

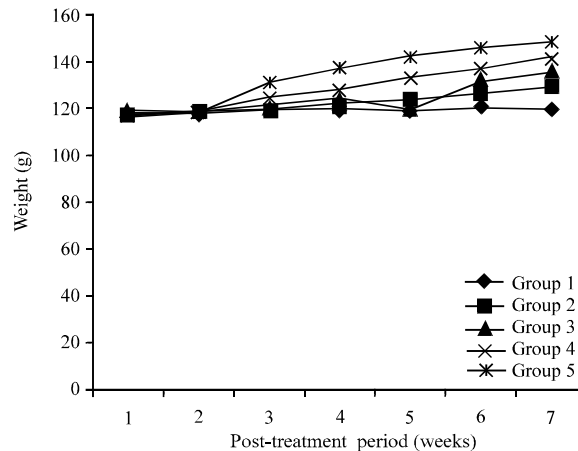


Fig. 1: Growth curves of rats receiving the aqueous stem bark extract of *S. siamea* for 7 weeks

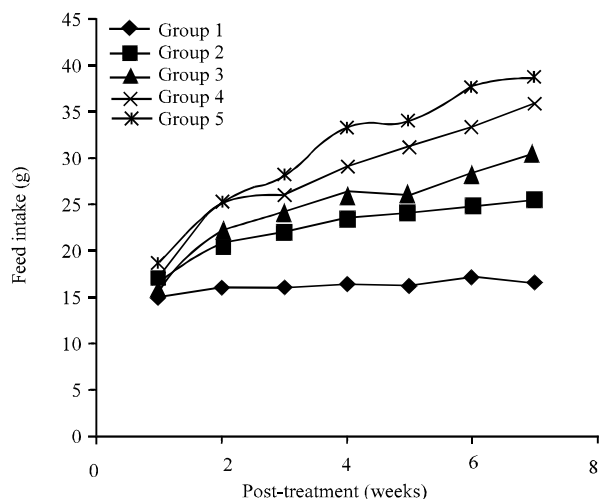


Fig. 2: Feed intake of rats receiving *S. siamea* for 7 weeks

Table 1: Quantitative amount of phytochemical constituents of *Senna siamea* stem bark extract

Sample	Alkaloids (g g ⁻¹)	Saponins (g g ⁻¹)	Flavonoids (g g ⁻¹)	Polyphenolics (g g ⁻¹)
Leaves	0.05±0.02	0.07±0.01	0.06±0.01	0.92±0.05

Values are of Mean±SD of triplicates determinations

Table 2: Effect of aqueous stem bark extract of *S. siamea* on haematological parameters in rat

Parameters	Group				
	1	2	3	4	5
PCV (%)	49.50±1.15 ^a	50.34±0.95 ^a (I = 1.7)	48.25±1.45 ^a (D = 2.5)	49.10±0.25 ^a (D = 0.8)	49.13±0.32 ^a (D = 0.75)
Hb (g dL ⁻¹)	16.34±0.50 ^a	15.36±0.21 ^a (D = 6.0)	16.82±0.82 ^a (I = 2.9)	15.37±0.75 ^a (D = 5.9)	16.39±0.22 ^a (I = 0.3)
RBC (×10 ⁶ μL ⁻¹)	7.89±0.01 ^a	6.91±0.01 ^a (D = 12.4)	6.59±0.25 ^a (D = 16.5)	8.22±0.15 ^a (I = 4.2)	7.34±0.33 ^a (D = 7.0)
WBC (×10 ³ μL ⁻¹)	15.92±0.97 ^a	16.01±0.67 ^a (I = 0.6)	18.11±0.01 ^a (I = 13.8)	17.22±0.45 ^a (I = 8.2)	17.88±0.35 ^a (I = 12.3)
Platelets (×10 ⁵ μL ⁻¹)	8.03±0.12 ^a	7.31±0.51 ^a (D = 9.0)	8.05±0.14 ^a (I = 0.2)	8.80±0.35 ^a (I = 9.6)	8.06±0.28 ^a (I = 0.4)

All values are Mean±SD of seven replicates, Values with different superscripts along a row are statistically different at p<0.05, n = 7, D: Percentage decrease compared to control, I: Percentage increase compared to control

treated groups. Group 3 recorded the highest decrease of 2.5% when compared with that of group 1. The Hb content of group 2 and 4 recorded similar percentage (%) decrease serum levels (6.0 and 5.9%, respectively) which are not statistically significant (p<0.05) from group 1. Group 3 Hb levels had an increase of 2.9%, higher than that of group 5 (0.3%). All the treated groups recorded a decrease in RBC levels, with the exception of group 4, which has an increase of 4.2% versus group 1. WBC levels of all the treated groups indicated an increase, with group 4 having the highest of about 13.8%. The platelets levels of group 2 decrease by 9.0% when compared to group 1. Unlike the other treated groups that showed an increase in platelets contents which did not differ significantly (p<0.05).

Moreover, result on serum liver enzymes showed that AST, ALT and ALP were statistically significant (p<0.05) in comparison to the normal control group (Table 3). AST and ALP levels of all the treated groups recorded a decrease, with group 2 and 3 having the highest % decrease in AST and ALP of 4.7 and 8.0%, respectively. The ALT levels of group 3 significantly increase by 1.4%, while the other treated groups recorded a decrease in ALT levels, with group 5 having the highest decrease of 7.5%. Group 3 and 4 showed an increase in bilirubin and total protein levels of 16.1 and

Table 3: Effects of the aqueous stem bark extract of *S. siamea* on serum markers of liver damage

Parameters	Group				
	1	2	3	4	5
AST (IU L ⁻¹)	119.6±36.70 ^a	114.0±7.20 ^b (D = 4.7)	117.8±7.60 ^b (D = 1.5)	116.4±8.17 ^b (D = 2.7)	117.0±8.10 ^b (D = 2.1)
ALT (IU L ⁻¹)	72.0±34.00 ^a	68.8±1.80 ^b (D = 4.4)	73.0±2.60 ^b (I = 1.4)	68.2±1.30 ^b (D = 5.3)	66.6±1.70 ^b (D = 7.5)
ALP (IU L ⁻¹)	248.0±40.00 ^a	236.8±15.90 ^b (D = 4.8)	228.2±18.90 ^b (D = 8.0)	230.0±12.70 ^b (D = 7.3)	240.2±13.00 ^b (D = 3.1)
Bilirubin (µmol L ⁻¹)	11.20±1.30 ^a	10.00±2.40 ^a (D = 10.7)	13.00±1.22 ^a (I = 16.1)	0.40±1.14 ^a (D = 7.1)	9.80±2.40 ^a (D = 12.5)
Total protein (g dL ⁻¹)	9.89±0.02 ^a	7.84±0.04 ^a (D = 20.7)	8.88±0.05 ^a (D = 10.2)	8.94±0.05 ^a (I = 9.6)	9.83±0.12 ^a (D = 0.6)
Albumin (g dL ⁻¹)	5.05±0.11 ^a	5.28±0.25 ^a (I = 4.6)	5.18±0.12 ^a (I = 2.6)	5.24±0.44 ^a (I = 3.8)	5.33±0.15 ^a (I = 5.5)

All values are Mean±SD of seven replicates, Values with different superscripts along a row are statistically different at p<0.05, n = 7, D: Percentage decrease compared to control, I: Percentage increase compared to control

Table 4: Effects of the aqueous stem bark extract of *S. siamea* on serum markers kidney function

Parameters	Group				
	1	2	3	4	5
Creatinine (mg dL ⁻¹)	0.76±0.15 ^a	0.77±0.05 ^a (I = 1.3)	0.75±0.02 ^a (D = 1.3)	0.76±0.05 ^a (D = 0.0)	0.75±0.02 ^a (D = 1.3)
Urea (mg dL ⁻¹)	7.20±0.20 ^a	7.22±0.30 ^a (I = 0.3)	7.23±0.35 ^a (I = 0.4)	7.29±0.22 ^a (I = 10.0)	6.87±0.55 ^a (D = 0.5)
Sodium (mmol L ⁻¹)	155.20±6.50 ^a	156.50±5.80 ^a (I = 0.8)	155.80±2.50 ^a (I = 0.4)	157.50±5.20 ^a (I = 1.5)	154.05±4.24 ^a (D = 0.7)
Potassium (mmol L ⁻¹)	7.25±0.55 ^a	7.82±0.50 ^a (I = 7.9)	7.88±0.54 ^a (I = 8.7)	7.29±0.70 ^a (I = 0.6)	7.35±0.25 ^a (I = 1.4)
Chloride (mmol L ⁻¹)	110.35±2.50 ^a	108.47±0.56 ^a (D = 1.5)	109.45±1.58 ^a (D = 0.8)	109.67±1.09 ^a (D = 0.6)	109.73±2.60 ^a (D = 0.6)

All values are Mean±SD of seven replicates, Values with different superscripts along a row are statistically different at p<0.05, n = 7, D: Percentage decrease compared to control, I: Percentage increase compared to control

Table 5: Effects of the aqueous stem bark extract of *S. siamea* on serum glucose, cholesterol and triglyceride

Parameters	Group				
	1	2	3	4	5
Glucose (mg dL ⁻¹)	125.91±5.11 ^a	123.57±7.52 ^a (D = 1.9)	122.73±5.55 ^a (D = 2.5)	122.20±7.65 ^a (D = 2.9)	120.26±4.86 ^a (D = 4.5)
Cholesterol (mg dL ⁻¹)	89.55±2.10 ^a	86.28±8.68 ^a (D = 3.7)	84.80±5.06 ^a (D = 5.3)	84.08±9.64 ^a (D = 6.1)	81.78±8.11 ^a (D = 8.7)
Triglyceride (mg dL ⁻¹)	120.35±5.55 ^a	119.25±4.90 ^a (D = 0.9)	121.43±5.10 ^a (D = 0.9)	118.54±6.23 ^a (D = 1.5)	120.33±3.90 ^a (D = 0.04)

All values are Mean±SD of seven replicates. Values with different superscripts along a row are statistically different (p<0.05). n = 7, D: Percentage decrease compared to control

9.6%, respectively. The remaining other groups recorded a % decrease when compared with group 1. In all the treated groups there is an increase in the levels of the albumin contents when compared to group.

Also, no significant (p<0.05) difference in the serum levels of markers of kidney functions was recorded (Table 4). In all the parameters analyzed, group 2 recorded a slight % increase while group 5 showed a % decrease in the levels of markers of kidney functions, with the exception of group serum chloride levels (1.4% increase).

In Table 5, the serum glucose, cholesterol and triglycerides levels of all the treated groups recorded a % decrease which did not differ significantly (p<0.05) from that of group 1. The highest % decrease recorded was serum cholesterol of group 5 (8.7%), while the lowest was the triglycerides levels of group 4 (0.04%).

DISCUSSION

The therapeutic effect of most medicinal plants is indisputable but their toxicities sometimes limit their clinical uses. Hence, the toxicity profile of most plants must always be considered

especially as the doses and dosing regimens of their preparations are not usually determined. The result of this finding is quite promising as the stem bark extract of *S. siamea* was relatively non toxic to rats at oral doses up to 5000 mg kg⁻¹. The increase in body weight observed, which cuts across all the groups might have resulted from the increased in the feed intake habit, probably stimulated by the extract. This contradict with that of *C. sieberiana* stem bark extract, which resulted in a slight reduction on body weight of the treated groups (Obidah *et al.*, 2009). But the result of this study agreed with the findings of Otimenyin *et al.* (2010). This might be likely due to similar content of phytochemicals, as the plant's activity depends on its chemical contents (Kwada and Tella, 2009).

The result also indicated no gross physiological alterations in the body system as no significant ($p < 0.05$) effect were noticed on the hematological parameters analyzed (Table 2). The slight physiological fluctuations on hematological parameters observed did not correspond with that of the *S. siamea* leaves extract (Chavalittumrong *et al.*, 2003). This possibly might be due short study period or the high levels of some hepatotoxicants, such as barakol, reported on the leaves extract (Padumanonda and Gritsanapan, 2006).

In detecting liver damage the determination of enzyme levels such as AST, ALT and ALP is largely used. Necrosis or membrane damage releases the enzyme into circulation and hence it can be measured in the serum. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Palanivel *et al.*, 2008). The extract has shown no much effect on the serum enzymes of liver function (Table 3), this might be attributed to the fact that, the liver was not greatly damaged by the extract due to short time period, to cause a significant increase on the serum levels of liver enzymes and bilirubin into the blood.

Serum total protein is a complex mixture containing a number of components which differ in properties and function. Liver is the organ mainly responsible for formation of plasma albumin (Obidah *et al.*, 2009). Decrease in serum total protein and increase serum urea and creatinine levels are associated with liver and renal failure (Chawla, 1999). Urea represents the major way of total urinary nitrogen excretion. The treated groups in this study exhibited no significant increase on serum urea and creatinine that might suggest the level of safety of the extract on kidney. This corresponds with the previous findings on roots extract of *S. siamea* (Otimenyin *et al.*, 2010).

The extract also showed no toxic effect on the serum levels of glucose and lipids profile throughout the experimental period. This might be associated to less toxic effect of the extract on liver, as liver is plays a significant role in metabolism of cholesterol and other lipids compounds. Many toxic effects of medicinal plants extract depend basically on many factors such as route of administration and the dose administered (Singh and Devkota, 2003). Intraperitoneal administration of extracts provides more clear toxicity levels than the oral route, usually due to some inherent factors that limit absorption in the gastrointestinal tract. Thus, further work is recommended for intraperitoneal administration of the extract and chronic toxicity study, to fully establish its safety.

CONCLUSION

In conclusion, the crude aqueous stem bark extract of *S. siamea* showed no significant effect on most of the parameters evaluated. The finding suggests that the stem bark extract of *S. siamea*, is relatively not toxic and further work should be carried out on its chronic toxic effect.

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