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Effects of sub acute oral administration of aqueous extract of *Stereospermum kunthianum* (Bignoniaceae) stem bark on body weight and haematological indices of rats

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Abstract
The study evaluates the effects of sub acute oral administration (28 days) of aqueous extract of *Stereospermum kunthianum* stem bark on the body weight and haematological indices of rats. Treatments were administered by oral gavage once daily for a total of 28 days. The first group (control) received distilled water (5 ml/kg), the second, third and fourth groups received the extract at the doses of 0.5, 1 and 2 g/kg body weight respectively. The animals were observed daily for behavioral changes and any signs of toxic manifestations. The body weight of each rat was recorded on days 0, 7, 14, 21 and 28. Standard methods were employed to assay for the haematological indices using the automated haematologic analyzer. The extract (0.5, 1 or 2 g/kg) caused no symptoms of toxicity nor death. Body weights of the treated rats were not significantly altered on day 28 compared to their corresponding body weight before treatment. However, the control rats manifested marked increase in body weight compared to their body weight at the beginning of experiment. Most of the haematological parameters evaluated were not significantly altered by the extract at the doses studied, however, the extract at 1.0 or 2.0 g/kg significantly ($P<0.05$) reduced the packed cell volume (PCV) values of the treated rats compared to that of the control. Oral administration of the extract to rats for 28 days produced no overt toxic manifestation nor marked adverse effects on the body weight and most of the haematological parameters.

Keywords: Sub acute; Toxicity; Body weight; Haematological indices, *Stereospermum*; *Kunthianum*

Introduction

The rich tropical vegetation has endowed Africa with a vast number of medicinal plants. Over 80 % of the world's populations, especially in the developing countries still rely on their indigenous systems of medicine and use herbs (Ramawat and Goyal, 2008). In Africa, the use of herbal medicines has always been prevalent and has continued to rise relentlessly (Sofowora, 1993). In many communities in Africa, the use of traditional medicine is the predominant medical care due to the non-existence of orthodox medical facilities and/or unregulated concomitant use of both types of remedy (Omogbai, 2008). While some documented

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pharmacological evidences of some Nigerian medicinal plants from 1970 – 2004 (Okujagu *et al.*; 2004) exist, not much is known of the toxicological profile of most of these medicinal plants or their products. Recently, significant pharmacological studies of *Stereospermum kunthianum* have been undertaken (Ching *et al*., 2008; Ching *et al*., 2009 a, b, c, d, and e; Falodun *et al*., 2009; Ching *et al*., 2010). As part of our toxicological evaluation of *S. kunthianum*, in the present study, we herein report the body weight and haematological effects of the sub acute oral administration of its aqueous stem bark extract in rats.

Experimental

Plant collection, preparation and extraction. The fresh stem bark of the *S. kunthianum* was collected in Ogun State, Nigeria in March, 2010. Identification and botanical authentication were done at the Forestry Research Institute of Nigeria, Ibadan where a voucher specimen (No. FHI 107277) was deposited for future reference. The stem bark was carefully separated from the woody part, cut into small pieces shed - dried and pulverized. The powdered material (400g) was macerated in 2L of distilled water at an initial temperature of 60^{o}C, allowed to cool and filtered after 24 hours. The filtrate was evaporated to dryness in an oven set at 40^{o}C until a constant weight was obtained. The yield was 26.4% with reference to the powdered stem bark. The extract obtained was stored in closed containers in the refrigerator until when required for use in experiments reported in our study.

Animals. The experimental protocols and procedures used in this study were approved by the Animal Ethics committee of the University of Benin, Benin City, Nigeria. Wistar rats of either sex obtained from the Animal House unit of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria were used. The animals were maintained under standard laboratory conditions, natural light and dark cycles, and had free access to standard chow (Bendel Feeds and Flour Mills Plc, Ewu, Nigeria) and tap water for drinking.

Sub acute toxicity studies. Sub acute toxicity studies on the extract was carried out according to the guidelines for testing of chemicals issued by the Organization of Economic Co-operation Development (OECD, 2001 a). Forty Wistar rats (130 – 150 g) of either sex were randomly allotted into four groups of ten animals per group. Treatments were administered by oral gavage once daily for a total of 28 days. The first group (control) received distilled water (5 ml/kg), the second, third and fourth groups received the extract at the doses of 0.5, 1 and 2 g/kg/ body weight respectively. The animals had free access to feed (Bendel Feeds, Plc Ltd, Ewu, Nigeria) and tap water during the study period. The animals were kept five per cage and maintained under standard laboratory conditions with natural lighting conditions. The animals were observed daily for behavioral changes and any signs of overt toxic manifestations during the 28days. The body weight of each rat was recorded on days 0, 7, 14, 21 and 28. The animals were sacrificed by excess chloroform inhalation 4 h after extract treatment on day 28. Blood samples obtained by cardiac puncture were collected into tubes containing 0.1 ml of ethylenediaminetetraacetic acid (EDTA) and were used for haematological assays immediately.

Haematological assays. The blood (5 ml) from each rat was collected into EDTA tube and was shaken to mix uniformly with the anticoagulant. The haematological assays were carried out immediately using the automated haematologic analyzer (Sysmex KX21 N, connected to the Stromatolyser ® WH and Cell pack reagent containers, Sysmex Corporation, Japan) using the

methods of Dacie and Lewis (1991). Each blood sample was held to the sample prop and the instrument aspirated the blood (0.1 ml) when switched on. The aspirated blood was automatically diluted with cell pack reagent in a white blood cell (WBC) counting container. Fixed volume of stromatolyser-WH solution (1 volume of Stromatolyser-WH to 2 volumes of cell Park reagent) was added automatically. The addition of Stromatolyser-WH caused lysis of the red blood cells (RBC); preserved WBC membrane and stabilized the cells at a level detectable by the instrument and counted (Dacie and Lewis, 1991). Haemoglobin (Hb) released when the RBC were lysed was converted to methaemoglobin. A portion of the diluted sample was transferred automatically to the haemoglobin detector where the absorbance of the red pigment was measured to give the haemoglobin level. The other haematological parameters which included red blood cell (RBC) count; packed cell volume (PCV); mean corpuscular volume (MCV); mean corpuscular haemoglobin (MCH); mean corpuscular haemoglobin concentration (MCHC); platelets (Plt); lymphocytes (LYM) and neutrophils (NEU) were determined automatically by haematologic analyzer. The results of the parameters of each blood sample were displayed on the screen and were printed.

Statistical analysis. Data were expressed as mean ± SEM and analyzed using the unpaired Student's t-test. Results were considered significant at $P<0.05$.

Results

Oral administration of aqueous stem bark extract of *S. kunthianum* (0.5, 1 or 2 g/kg body weight/day) to rats for 28 days did not cause any overt signs of toxicity and all the animals survived to the end of the experiment. The results of the effect of the aqueous extract on the body weight of the rats are shown on Table 1. The results indicate that daily treatment of the rats for 28 days with the extract did not significant affect the body weight of the rats on day 28 when compared to their corresponding body weight on day zero (before commencement of treatment). The control rats, however, showed marked increased in body weight compared to their corresponding body weight on day zero. The results of the effect of the aqueous extract of *S. kunthianum* on the haematological indices are presented in Table 2. Most of the haematological parameters evaluated were not significantly altered by the extract at the doses studied, however, the extract at higher doses (1.0 or 2.0 g/kg) significantly ($P<0.05$) reduced the packed cell volume (PCV) values compared to that of distilled water-treated rats (Table 2).

Table 1: Effect of 28 days oral administration of aqueous stem bark extract of *S. kunthianum* on body weight of rats

Treatment	Body weight (g)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Distilled water (5 ml/kg)	131.00 ± 4.07	143.50 ± 4.35	143.50 ± 4.35	146.00 ± 3.71	151.50 ± 2.69
S. kunthianum (0.5 g/kg)	119.00 ± 2.87	136.00 ± 2.67	136.00 ± 2.67	120.50 ± 2.41	120.50 ± 2.41
S .kunthianum (1 g/kg)	131.00 ± 3.55	141.50 ± 5.95	141.50 ± 5.95	131.10 ± 4.55	130.60 ± 4.67
S .kunthianum (2 g/kg)	124.00 ± 2.34	131.50 ± 3.58	131.50 ± 3.58	124.00 ± 4.34	124.00 ± 4.34

Values are mean ± SEM, (n= 10). Daily treatment with the aqueous extract at 0.5, 1.0 and 2.0 g/kg (p.o.) did not significantly alter the body weight of the rats on day 28 compared to their corresponding body weight on day zero (before commencement of the experiment).

Table 2: Effect of 28 days oral administration of aqueous stem bark extract of *S. kunthianum* on some haematological parameters in rats

Haematological parameters	Distilled water 5 ml/kg	*Stereospermum kunthianum*		
		0.5 g/kg	1 g/kg	2 g/kg
WBC (x 10^3/µl)	9.40 ± 1.54	8.34 ± 0.63	8.73 ± 0.44	8.06 ± 0.89
RBC (x 10^6/µl)	7.1 0 ± 0.41	7.04 ± 0.24	7.11 ± 0.06	6.45 ± 0.15
Hb (g/dL)	13.10 ± 0.50	11.70 ± 0.65	13.26 ± 0.15	12.39 ± 0.15
PCV (%)	43.80 ± 0.67	39.22 ± 2.43	40.40 ± 0.48*	38.07 ± 0.65*
MCV (fL)	62.06 ± 3.39	64.94 ± 2.86	63.83 ± 0.43	59.20 ± 1.08
MCH (Pg)	18.52 ± 0.24	19.38 ± 0.77	18.04 ± 0.19	19.29 ± 0.39
MCHC(g/dl)	29.96 ± 0.75	29.86 ± 0.33	32.81 ± 0.23	32.60 ± 0.46
[a]Plt (x10^3)	788.00 ±27.07	795.00 ± 25.02	796.89 ± 26.09	798.89 ± 60.86
[b]LYM (%)	78.48±2.77	77.00 ± 2.61	80.60 ± 2.30	81.56 ± 2.08
[c]NEU (%)	20.90±1.24	18.52 ± 1.23	19.21 ± 1.10	19.36 ± 1.79

Values are mean ± SEM (n=10), [a]Platelets; [b]Lymphocytes; [c]Neutrophils. Oral administration of the extract (0.5. 1.0 or 2.0g/kg) to rats for 28 days did not significantly alter most of the haematological parameters, however, the extract (1.0 or 2.0 g/kg) significantly ($P<0.05$) reduced the PCV values of the treated rats compared to that of the control.

Discussion

One of the major challenges with the use of herbal medical care by the herbal medical practitioners is the fact that very little attention is accorded to the toxic potentials of the herbal products. Since most of the products do not cause instant dead, they erroneously assume that they are safe. The increasing use of these products is most likely to cause toxic effects at short-term and chronic administration. This study evaluates the sub acute effects of oral administration of the aqueous extract of *S. kunthianum* stem bark on the body weight and haematological indices of rats. Sub acute oral administration of the aqueous extract to rats and mice for up 28 days caused no overt signs and symptoms of toxicity for example respiratory depression, salivation, aggressiveness, muscle paralysis, reduced agile movements, marked decrease in food and water intake etc nor mortalities. Acute oral and intraperitoneal administration of the aqueous extract to rats and mice at single doses up to a maximum dose of 8 g/kg had also caused no overt signs of toxicity (Ching *et al*., 2008; Ching *et al*., 2009a). The exact similarly did not significantly alter the body weight of the treated rats. Equally, the aqueous extract did not cause any significant effect on most of the haematological parameters except a significant reduction in the pack cell volume (PCV) at the higher doses and this turns to suggest that the extract is relatively safe at the doses tested. Although, a precise explanation could not be given to the reduction in the PVC values at this stage, however, it is considered to be a consequent of the treatment of the rats at the higher doses as the rats given the lower dose of the extract did not show a similar reduction in PVC values compared to the control rats.

Conclusion

The aqueous extract of *S. kunthianum* was found to be relatively safe on short-term oral administration in rats. However, biochemical and histopathological studies of *S. kunthianum* on some vital organs/ systems on short-term as well as chronic toxicity studies are required for the support of the safe use of this plant.

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