http://dx.doi.org/10.4314/jpb.v10i2.1
Vol. 10 no. 2, pp. 38-44 (September 2013)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Sub-acute toxicity studies on the aqueous extract of *Stereospermum kunthianum* (Cham, Sandrine Petit) stem bark

Stephen O. Okpo[1*] and Fidelis P. Ching[2]

[1]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City. Nigeria.*
[2]*Department of Pharmacology, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Yenagoa. Bayelsa State, Nigeria.*

Received 7th June 2013; Accepted 8th July 2013

Abstract
Stereospermum kunthianum Cham, Sandrine Petit (Bignoniaceae) is used in traditional medicine in the treatment of various diseases including bronchitis, rheumatoid arthritis, dysentery, ulcers etc. The aqueous stem bark extract of the plant was evaluated for its acute and subacute (28-day) toxicity in rats. The subacute effects of the extract (0.5, 1 and 2g/kg, p.o.) were evaluated on the following parameters, namely: alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol, low density lipoprotein (LDL) and high density lipoprotein (HDL)-cholesterol, triglycerides as well as organ weight and histopathologic changes. In the acute toxicity studies, no adverse behavioural effects or mortality were observed up to a dose of 8000mg/kg. No significant changes were observed in the serum and liver enzymes (ALT and ALP) except for an increase in the serum ALT levels at the highest dose (2g/kg) of the extract. Significant increases in triglyceride, total cholesterol, LDL- and HDL- cholesterol levels were also recorded with the dose of 2g/kg. Increases of the organ (liver, kidney, heart and spleen) weights were produced with all doses of *Stereospermum kunthianum* but histological alterations of these organs were observed with only the highest dose. From the results obtained, it is concluded that *Stereospermum kunthianum* up to a dose of 1000mg/kg may be safe for long term treatment of various ailments.

Keywords: *Stereospermum kunthianum*; Subacute toxicity; Histopathology, Lipid profile

INTRODUCTION

Stereospermum kunthianum (Cham, Sandrine Petit), family *Bignoniaceae,* also referred to as pink jacaranda, in English, is known locally as *sansami* among the Hausa of Northern Nigeria; *umana* among the Tiv of Middle Belt of Nigeria, *ayada* among the Yoruba of South West Nigeria, and *alakiriti* among the Igbo of South East Nigeria (Gill, 1992). Various morphological parts of *Stereospermum kunthianum* are used in traditional medicine to treat a wide variety of human ailments. While the pods are chewed with salt to treat coughs, ulcers, leprosy, skin eruptions and venereal diseases, the stem bark decoction or infusion is used to cure bronchitis, pneumonia, coughs, rheumatoid arthritis and dysentery (Hutchison and Dalziel, 1963; Von Maydell, 1986; Keay *et al.*, 1989). The twigs are chewed to treat toothache and clean the teeth. The roots and leaves have been found useful in treating

* Corresponding author. *E-mail*: steveokpo@uniben.edu *Tel*: +234 (0) 8064028832
ISSN 0189-8442

venereal diseases, respiratory ailments and gastritis (Gill, 1992).

Previous studies in our laboratory had confirmed that the aqueous stem bark extract of *Stereospermum kunthianum* possesses antidiarrheal (Ching *et al.,* 2008), analgesic (Ching *et al.,* 2009a), anti-inflammatory (Ching *et al.,* 2009b) and anticonvulsant (Ching *et al.,* 2009c) effects. The efficacy of the water extract of *Stereospermum kunthianum* in human complement system fixation *in vitro* has been reported (Drissa *et al.,* 2002). The antiplasmodial activity of naphthoquinones and one anthraquinone from the lipophilic extract of the root bark of *Stereospermum kunthianum* has also been reported (Onegi *et al.,* 2002).

The wide application of this plant in traditional medicine for management of various ailments makes it imperative to study its potential toxicity. The present study was, therefore, designed to evaluate the acute and sub-acute toxic effects of *Stereospermum kunthianum* in rats in order to establish its safety and/or potential long term adverse effects.

EXPERIMENTAL

Collection of plant material and extraction. The fresh stem bark of the *S. kunthianum* was collected in Idi-Okpe, Ogun State, Nigeria in the month of March. Identification and botanical authentication were done by Mr. Usang Felix Inah of the Forestry Research Institute of Nigeria, Ibadan, Nigeria 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 sun-dried and pulverized using a grinder (Lab. Mill, serial No. 4745, Christy and Norris Ltd, England). 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 h. 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 at -4^{o}C until required.

Animals. Sprague-Dawley rats (140±10g) and Swiss mice of either sex (22.5±2.5g) 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 maintained under standard laboratory conditions (12 h light and dark cycle) had free access to standard chow (Bendel Feeds and Flour Mill Plc. Ewu, Nigeria) and drinking water. Animals were accustomed to the experimental conditions prior to the experiment and were handled according to standard protocols as outlined in "*Principles of Laboratory Animal Care*" (National Institute of Health Guide for Care and Use of Laboratory Animals, Pub No. 85 – 23, revised 1985).

Acute toxicity. Acute toxicity was performed according to the Organization of Economic Co-operation Development-420 guidelines (OECD, 2001a). Swiss mice of either sex were used. The animals were administered with distilled water (10 ml/kg), the vehicle or aqueous extract (1 - 8 g/kg) of *S. kunthianum* stem bark orally (p.o). The animals were initially observed for 6 hours and then general symptoms of toxicity and mortality were recorded for 24 hours and a further 2 weeks for any delayed toxic manifestations.

Sub-acute toxicity studies. Sub acute toxicity studies on the extract were carried out according to the guidelines for testing of chemicals (OECD, 2001b). Forty rats of either sex were randomly allotted into four groups of ten animals per group. Animals were administered 0.5, 1 and 2 g/kg/body weight/day of the extract equivalent to 1/16th, 1/8th and 1/4th of the highest dose (8g/kg) used for acute toxicity studies. The control group

received distilled water (5 ml/kg). All administrations were via the oral route once daily for a total of 28 days. The animals were maintained under standard laboratory conditions including 12h light /dark cycles and had free access to food (Bendel Feeds Plc., Ewu, Nigeria) and tap water during the study period. Overt toxic manifestations and mortality were monitored during the study.

The animals were sacrificed by excess chloroform inhalation 4 hours after extract treatment on day 28. Blood was obtained by cardiac puncture, centrifuged at 3000g for 5min and the serum collected was used to assay for alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol, high density lipoprotein (HDL) - cholesterol and triglycerides immediately.

The animals were dissected after blood collection and the liver, heart, kidneys and spleen were isolated, blotted with filter paper and weighed. Each organ was examined macroscopically for colour changes and any discernible pathological lesions and sections were fixed in 10% buffered formalin for histopathological studies. Liver slices (0.97-0.99g) obtained from the second liver lobe were homogenized, centrifuged at 3000g for 10 min and the supernatant collected was used immediately for enzymes assays. Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were assayed by the colorimetric method (Varley, 1980). Total cholesterol and HDL-cholesterol were determined by the method of Searcy and Bergquist, (1960) while triglyceride was determined by the method of Tietz (1990).

Statistical analysis. Data are mean ± SEM. The INSTAT exe statistical package was employed and data analyzed using one way analysis of variance (ANOVA) followed by Turkey multiple comparison test. Values were considered significant at $p<0.05$.

RESULTS

Acute toxicity study. The observation of the animals after oral acute treatment with aqueous extract of *Stereospermum kunthianum* stem bark (1- 8 g/kg) did not reveal any signs of toxicity and no deaths were observed even at the highest dose of 8 mg/kg body weight.

Subacute toxicity study. Table 1 shows the effect of the aqueous extract of *Stereospermum kunthianum* on the weight of some selected rat organs. Daily treatment for twenty eight days with the extract significantly ($*p<0.05$) increased the organ weights of the treated rats compared to the control animals given distilled water only.

The extract (1 and 2 g/kg/day), administered for 28 days, caused a significant increase in the serum level of alanine aminotransferase without any significant effect on the serum level of ALP. The levels of both enzymes in the liver were, however, not significantly different relative to control. The effects of the aqueous extract on some lipid profiles are summarized in Table 3. While all doses of the extract caused significant increases in triglyceride levels, only the 2 higher doses significantly ($p<0.05$) increased total-, LDL- and HDL- cholesterol levels when compared to the control.

Histopathologic analysis. Histopathological examination of the various organs (Fig. 1), showed that control rats given distilled water during the 28 days study period maintained normal livers with central vein and radiating hepatocytes (Fig. 2A). The heart showed intact pericardium and myocardium (Fig. 2E) and the renal tubules (Fig. 2C) and spleen (Fig. 2G) were unaffected. Figure 2B shows the liver section of rat treated with 2g/kg of *Stereospermum kunthianum*. There was mild vascular activity as evidenced by congestion and oedema of the liver. Figures 2D, 2F and 2H show sections of the kidney, heart and spleen, respectively, of rat treated with the

extract. There was vascular congestion in the renal cortex and mild tubular injury while the heart presented with oedema and moderate degeneration of the myocardium. The spleen showed evidence of activation of the follicles and a reaction at the sinuses, an evidence of the activation of the local immune system.

DISCUSSION

In the acute toxicity study, no mortality or apparent toxic effects were observed up to the highest dose of 8000mg/kg body weight of the orally administered extract. This dose is far above the 2000 mg/kg recommended by the OECD guidelines-423 for a chemical to be categorized as non-toxic (OECD, 2000).

Oral administration of aqueous extract of *S. kunthianum* stem bark to rats at single doses up to the highest dose of 2.0 g/kg/day for 28 days did not cause any overt signs of toxicity and all the animals survived till the end of the treatment period. However, a significant increase in the weights of the liver, kidney, heart and spleen was observed in the extract treated rats. Increased organ weights in the treated rats could be a possible consequence of the daily treatment with the extract. The aqueous extract caused a significant increase in serum alanine aminotransferase level at the higher doses with no significant increase in the liver alanine aminotransferase. Serum and liver alkaline phosphatase levels were also not significantly altered at the tested doses. The liver releases alanine aminotransferase following liver injury and an elevation in plasma concentration of the enzyme could be an indication of possible liver injury. Tissue damage in the heart and kidneys also liberates alanine aminotransferase. The increased serum alanine aminotransferase observed may suggest also some damage in the liver, heart or kidney.

Table 1: Effect of *S. kunthianum* on relative organ weights of rats

Treatment	Dose (mg/kg)	Relative organ weight			
		Liver	Heart	Kidney	Spleen
Distilled water	5 ml/kg	2.65 ± 0.08	0.26 ± 0.01	0.55 ± 0.02	0.24 ± 0.02
S. kunthianum	0.5	3.13 ± 0.08*	0.32 ± 0.01*	0.63 ± 0.01*	0.50 ± 0.09*
	1.0	2.94 ± 0.09*	0.32 ± 0.01*	0.62 ± 0.02*	0.49 ± 0.01*
	2.0	2.85 ± 0.09*	0.33 ± 0.01*	0.65 ± 0.01*	0.52 ± 0.07*

Values are Mean ± SEM. *p<0.01, significantly different from control; n= 10 animals.

Table 2: Effect of *S. kunthianum* on marker enzymes in serum and liver of rats

Treatment	Dose (mg/kg)	ALT (IU/L)		ALP (IU/L)	
		Serum	Liver	Serum	Liver
Distilled water	5 ml/kg	24.20 ± 0.80	17.40 ± 5.51	49.78 ± 3.58	26.03 ± 3.33
S. kunthianum	0.5	24.80 ± 0.43	19.00 ± 3.20	49.98 ± 3.28	26.90 ± 2.99
	1.0	27.40 ± 0.98*	19.50 ± 3.61	53.42 ± 2.31	28.33 ± 3.02
	2.0	30.10 ± 1.52**	20.20 ± 3.01	56.65 ± 2.64	30.78 ± 2.64

Values are mean ± SEM. *p<0.05, **p<0.001 significantly different from control; n= 10 animals.

Table 3: Effect of *S. kunthianum* on lipid profiles of rats

Treatment	Dose (mg/kg)	TG (mMol/L)	TC (mMol/L)	LDL (mMol/L)	HDL (mMol/L)
Distilled water	5 ml/kg	6.22 ± 0.14	26.20 ± 0.93	25.34 ± 0.24	1.97 ± 0.02
S. kunthianum	0.5	10.05 ± 0.99*	26.35 ± 1.94	23.81 ± 0.21	2.03 ± 0.48
	1.0	11.53 ± 0.25**	42.28 ± 0.59**	40.87 ± 0.34**	3.83 ± 0.01**
	2.0	11.18 ± 0.26**	43.04 ± 0.54**	41.78 ± 0.22**	3.82 ± 0.01**

Values are mean ± SEM. *p<0.001, **p<0.0001 significantly different from control; n=10 animals.

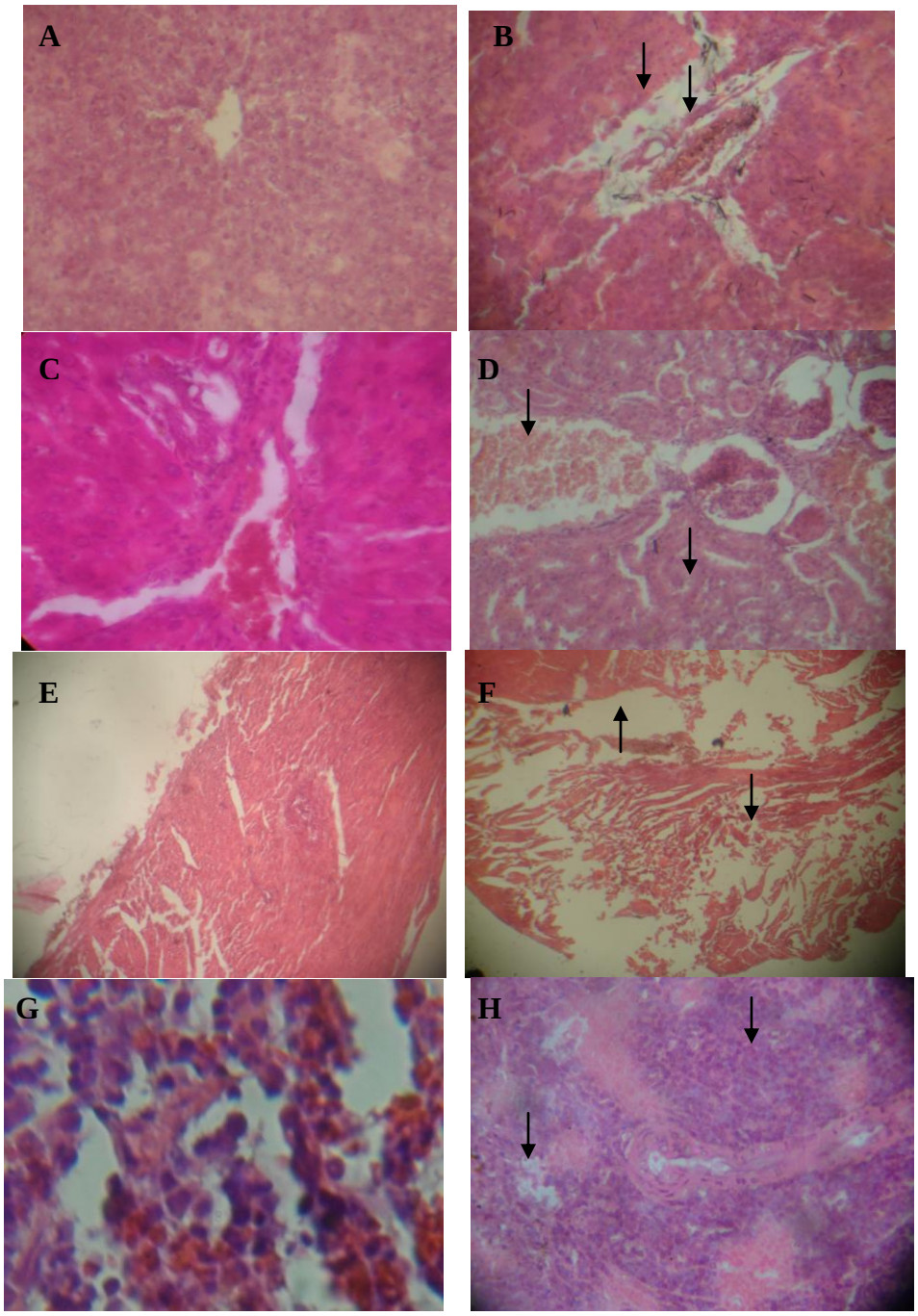

Figure 2: Histological sections of the liver (A), kidney (C), heart (E) and spleen (G) of control rats given distilled water for 28 days showing normal architecture. Aqueous extract of *S. kunthianum* (2g/kg/day) given for 28 days shows periportal congestion and oedema in the liver (B); moderate cortical tubular necrosis, and interstitial haemorrhage in the kidney (D); heart with moderate myocardial degeneration (F) and spleen with well formed follicles and sinus hyperplasia (H) [H&E x40]. Arrows (↓) depict points at which lesions occurred.

The rise in serum levels of ALT has been attributed to the damaged structural integrity of the liver (Chenoweth and Hake, 1962) since it is localized in the cytoplasm and released into circulation after cellular damage (Sallie *et al.*, 1991) hence an elevation in plasma concentration of the enzyme could be an indication of possible

liver injury. Alkaline phosphatase is a sensitive indicator of early intrahepatic and extrahepatic bile obstruction (Varley, 1980).

At the higher doses (1.0 and 2.0 g/kg), the extract significantly increased the serum levels of triglycerides; HDL-cholesterol; total cholesterol and LDL-cholesterol of the rats. Abnormalities of lipids and lipoproteins metabolism are positively correlated with heart disease (Siedel, 1987). The high levels of plasma lipids in the extract-treated rats compared to the control rats may be due to either increase in mobilization of free fatty acids from peripheral depots or secondary to a variety of disorders that may be induced by the administration of the extract. Elevated triglyceride level may promote a rapid progression of atherosclerosis. This suggests that the extract may have some harmful effects with increasing cardiovascular risk factors with repeated or prolonged ingestion.

The effect on the organs is further confirmed by the histopathological assessment of selected organs (heart, liver, kidney, spleen) from extract-treated animals which showed morphological changes and lesions. These effects were seen at the highest dose of the extract as the lower doses (0.5 and 1g/kg/day) did not show any visible changes in the normal architecture of the various organs.

It can be concluded that *S. kunthianum* aqueous extract produced significant increases in the relative organ weights, serum ALT as well as the lipid profile. However, these effects were obtained at doses far higher than those used in the pharmacological studies, suggesting that such doses may be safely used for daily administration without serious deleterious effects. It is, therefore, necessary to exercise caution in the use of the high doses of the extract in long term treatment.

REFERENCES

Chenoweth, M.B. and Hake, C. L. (1962); The smaller halogenated aliphatic hydrocarbons. *Ann. Rev. Pharmacol*. 2, 363-398.

Ching, F.P.; Omogbai, E.K.I.; Ozolua, R. I. and Okpo, S.O. (2008); Antidiarrhoeal activities of aqueous extract of Stereospermum kunthianum (Cham, Sandrine Petit) stem bark in rodents. *Afr. J. Biotech*. 7(9), 1220-1225.

Ching, F. P.; Omogbai, E. K. I.; Ozolua, R. I. and Okpo, S. O. (2009a); Analgesic Activity of aqueous extract of *Stereospermum kunthianum* (Cham, Sandrine Petit) stem bark. *Acta Poloniae Pharmaceut. Drug Res*. 66(1), 83-88.

Ching, F. P.; Omogbai, E. K. I.; Okpo, S. O. and Ozolua, R. I. (2009b); Anti-inflammatory Activity of Aqueous Extract of *Stereospermum kunthianum* (Cham, Sandrine Petit) Stem Bark in Rats. *Ind. J. Pharm. Sci*. 71, 106-110.

Ching, F. P.; Omogbai, E. K. I. and Otokiti, I. O. (2009c); Aqueous stem bark extract of *Stereospermum kunthianum* (Cham, Sandrine Petit) protects against generalized seizures in pentylenetetrazole and electro-convulsive models in rodents. *Afr. J. Trad. CAM* 6(4), 544-548.

Drissa, D.; Cecille, S.; Fatoumata, B.; Berit, S.; Paulsen, T. E. M. and Arouna, K. (2002); Wound healing plants in Mali, the Bamako Region: An ethnobotanical survey and complement fixation of water extracts from selected plants. *Pharmaceut. Biol*. 40, 117-128.

Gill, L. S. (1992); Ethnomedical uses of plants in Nigeria. Uniben Press, University of Benin, Benin City, Edo State, Nigeria. p. 223.

Hutchison, J. and Dalziel, J. M. (1963); Flora of West Africa, vol. 11, Crown Agents for Overseas, London, p. 544.

Keay, R. W.; Onochie, C. F. and Stanfield, D. P. Trees of Nigeria (formerly Nigerian trees 1960, 1964). Oxford: Oxford University Press; 1989. p. 435.

OECD (2000) Guidance Document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment, No. 24.

OECD (2001a). Organization of Economic Co-operation Development guideline for testing of chemical: 407 Repeated Dose Oral Toxicity in rodent: 28-Day or 14-Day Study. OECD, Paris, pp. 1-7.

OECD (2001b) Organization of Economic Co-operation Development guideline for testing of chemical: 420 Acute Oral Toxicity. OECD, Paris, pp.1-14.

Onegi, B.; Kraft, C.; Kohler, I.; Freund, M.; Jenett-Siems, K. and Beye-Melzig, M. F. (2002); Antiplasmodial activity of nephthoquinones and one anthraquinone from *Stereospermum kunthianum*. *Phytochemistry* 60: 39-44.

Sallie, R., Tredger, J. M. and William, R. (1991); Drugs and the liver. *Biopharmaceut. Drug Disposition* 1991, *12*, 251-259.

Searcy, R. L. and Bergquist, L. M. (1960); A new colour reaction for quantification of serum cholesterol. *Clin. Chim. Acta*. 5, 192-199.

Siedel, D. (1987); Lipoproteins in liver disease. *J. Clin. Chem. Clin. Biochem.* 25, 541-551.

Tietz, N. W. (1990); Clinical Guide to Laboratory Tests: 2nd Ed., W.B. Saunders Company, Philadelphia, p. 554.

Varley, H. (1980). Practical Clinical Biochemistry: 4th Ed., ELBS, London. p. 798.

Von Maydell H. J. (1986); Trees and shrubs of the Sahel, their characteristics and uses, p. 286, GTZ 6MBH, Eschborn.