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ANTIBACTERIAL AND TOXICOLOGICAL EFFECTS OF METHANOLIC LEAF EXTRACT OF *PYRENACANTHA STAUDTII* (ENGL.)

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ABSTRACT

Despite the widespread use of *Pyrenacantha staudtii* Engl. In the traditional medicine of Nigeria, there is scanty of information on its efficacy and toxicity. The aim of this study was to evaluate the antibacterial activity against the growth of some pathogenic microorganisms and toxicological effects of methanolic extract of the plants in male Wistar albino rats. Phytochemical screening indicated that the extract is rich in alkaloid, tannin, saponins, while anthraquinones, phlobatannins and cardiac glycosides were absent. The histological and heamatological investigations on liver, duodenum and blood samples of the rats that were administered with the extract (acute doses of 30mg/ml and 25mg/ml) showed mild toxicities. However, the control experiment that was given normal saline presented no toxicities on the liver and duodenum cells. The extracts inhibited the growth of *Bacillus cereus*, *Escherichia. coli* and *Staphylococcus aureus* while *Shigella dysentriae* and *Salmonella typhi* were resistant to the extract at both concentrations. This is an indication that the extracts may not be completely safe in male rats when continuously administered for 14 days.

Keywords: *Pyrenacantha staudtii* Engl., antibacterial, histological, haematological parameter, toxicological effect.

INTRODUCTION

Pyrenacantha staudtii Engl. (Icacinaceae) is widely distributed in the tropical Africa and Asia. In traditional African medicine, the plant is used for the treatment of malaria, hernia, antidiarrheal and intestinal pain (Oliver-Bever, 1986; Iwu, 1993; Awe *et al*., 2005, 2011). The leaf extract of the plant has been reported for its anticonvulsant and analgesic activities (Awe *et al*., 2005). In addition, the anti-inflammatory, antiulcerogenic activities and smooth muscle relaxant activity (Okunji and Iwu, 1986) have been investigated.

The ethnomedicinal utilization of *P. staudtii* among *Ekitis* in Nigeria includes the treatment of sexually transmitted diseases like gonorrhea, trichomoniasis, chlamydial infection and syphilis (Kayode and Kayode, 2002). Previous studies have revealed that various extract of this plant exhibit antimicrobial properties against some pathogens (Mitscher *et al*., 1972; Okunji and Iwu, 1986). A good number of secondary plant metabolites such as alkaloids, saponins and tannins have been found as the major constituents of *P. staudtii* (Okunji and Iwu, 1988). Notwithstanding the ethnomedicinal uses of *P. staudtii* nevertheless, no toxicological study has been undertaken on this plant. Therefore, the aim of the present study was to carry out basic toxicological studies and establish the safety of an aqueous extract of *P. staudtii*, focusing on its acute and chronic toxicity in rats.

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MATERIALS AND METHODS

Plant material

Fresh leaves of *P. staudtii* were collected during the month of January 2008 from a farm at Ifaki Ekiti in Ekiti-State Nigeria, where they were found growing naturally. The plant was authenticated by Mr. Omotayo, Department of Plant Science and Forestry, University of Ado Ekiti Ado-Ekiti Nigeria where a voucher (UNADPLT 10033) was deposited. The leaves were air dried for three weeks in the laboratory at 25-27^{0}C and was pulverized into fine powder.

Preparation of Leaf Extract

Exactly 600g of the powdered sample got from grinded dried leaves was weighed into a clean transparent plastic container and soaked with 2.5 liters of methanol. It was filtered after 72hours using a Buckner funnel and Watman No. 1 filter paper. The crude extract (75.5g) was obtained from the filtrate after drying using rotary evaporator. 72 g of the crude extract were reconstituted separately in saline solution to give the required doses of 25 and 30mg/kg body weight for the experiment.

Antibacterial assay

Pure isolates of the test organisms, *Salmonella typhi*, *Bacillus cereus*, *Staphlocccus aureus*, *Shigela dysentriae*, and *E. coli* were obtained from the Microbiology Research Laboratory of Ladoke Akintola University of Technology, Ogbomosho and maintained on sterile nutrient agar throughout the period of the

experimentation. Inoculates were prepared in the iso-sensitest broth at a density adjusted to a 0.5 McFarland turbidity standard. The bacterial were streaked on the surface of the agar using sterile swab stick, using different plates and different swab sticks for different organisms. Two wells of approximately 7mm were made at equidistant on the agar plates using sterile cork borer. 100µl of the 30mg/ml and 15mg/ml extracts/fractions were introduced into the respective wells on the agar plate. Extract diffusion time of 30 minutes were allowed and the plates were incubated at 37^0c overnight. The zones of inhibition diameter were measured with a calibrated calliper and metre rule. The same procedure was repeated for all the other fractions of the two plants. Gentamycin (250µg/ml) was used as positive control and 2% DMSO as negative control.

Experimental animal, grouping and extract administration procedure

Male Albino Rats of Wistar strain with a mean weight of 129.8 ± 3.01 g were obtained from the Experimental Animal House of Ladoke Akintola University of Technology, Ogbomosho. The animals were housed in clean metabolic cages placed in a well ventilated house with optimum condition (temperature: 23 ± 1°C; photoperiod: 12 h natural light and 12 h dark; humidity: 45–50%). They were acclimatized to animal house conditions and allowed free access to commercial pelleted and water. The cleaning of the cages was done on a daily basis. Experimental procedures and protocols for the care and use of animals in research and teaching used in this study were approved by the Ethics committee of the Ladoke Akintola University of Technology, Ogbomosho. Twenty-five male rats were randomized into five groups of five animals each. Groups 1–4 were orally treated with 25 and 30 mg/kg body weight/day of *P. staudtii* leave extract. Group 5 (control) were orally administered with distilled water. The treatment continued for 14 days and the administration was done using metal oropharyngeal cannula.

Preparation of serum and organs isolation

After 14 days of extract administration, the rats were sacrificed by ether anaesthetization and the neck area was quickly cleared of fur to expose the jugular vein. The vein, after being slightly displaced, was sharply cut with sterile surgical blade and an aliquot (5 ml) of the blood was collected and centrifuged at 1282g X 5 min. The resulting serum was carefully aspirated into sample bottles for the various biochemical assays. The rats were quickly dissected and the whole liver, duodenum were excised, freed of fat, blotted with clean tissue paper and then weighed. The organ to body weight ratio was determined by comparing the weight of each organ with the final body weight of each rat.

Hematological and histological analysis

The packed cell volume (PCV) was estimated by spinning the blood in heparinized capillary tube at 3000 rpm for 15 minutes in microhaematocrit centrifuge and values were finally determined using microhaematocrit reader. The haematological parameters (total red blood cell (RBC), HB (heamoglobin) and leukocyte (WBC) were determined using an autoanalyzer (System H1, Bayer Diagnostics). Liver and duodenum tissues were collected from animals in different groups and were fixed in 10% buffered formaldehyde solution for at least 24 h. The paraffin sections were then prepared and cut into 5 µm thick sections by a Leica RM 2016 rotary microtome. The sections were stained with hematoxylin and eosin staining and then mounted with Canada balsam (Sigma, USA). The degree of liver and duodenum damage was examined under the microscope (Leica Microsystems Digital Imaging, Germany). The images were taken using Kodak digital camera at original magnification of 10 × 10. Chronic liver and duodenum injury were then evaluated by grading the liver and duodenum sections numerically to assess their histological features.

Phytochemical analysis

The leaf extracts were subjected to qualitative phytochemical tests by adapting the methods described by Sofowora (1993) and Trease and Evans (1985). Tannins, alkaloids, cardiac glycosides, saponins phlobatanins, antraquinones and cardiac glycosides and phlobatanins were investigated.

RESULTS

Antimicrobial susceptibility screening of the test extract

The antibacterial effect of *P. staudtii* on different bacteria tested is shown in table 1. The average zone of inhibition of *P. staudtii* against *S. aureus*, *S. typhi* and *B. cereus* at the concentrations tested ranged from 10 ± 0.5 mm to 14.0 ± 1.10 mm. The positive control, gentamicin resulted in zones of inhibition ranging from 18.70 ± 2.30 mm to 20.70 ±1.90 mm (Table 1). The plants produced no zone of inhibition against *E. coli* and *S. dysentriae* (Table 1).

Table 1. Antibacterial Activity of *P. staudtii* Extract on test organisms.

Test Organism	Diameter of zones of Inhibition (mm)	
	30mg/ml	25mg/ml
B. cereus	14±1.10	11±0.7
E. coli	-	-
S. typhi	12±1.0	10±0.9
S. dysentriae	-	-
S. aureus	11±1.3	10±0.5
Gentamycin (control)	18.7±2.3	20.7±1.9

- Indicate no inhibition

Phytochemical Screening of the Extract

Phytochemical screening of extracts of *Pyrenacantha staudtii* revealed the presence of alkaloids, tannins, saponins, cardiac glycosides, while anthraquinone and phlobatanins were absent (Table 2).

Table 2. Phytochemical Components of the Extract.

Phytochemical	Result
Alkaloids	+ve
Anthraquinones	– ve
Cardiac glycosides	+ve
Phlobatannins	– ve
Saponins	+ve
Tannins	+ve

+ve and -ve mean presence and absence of phytochemical respectively

Table 3. Effect of chronic oral administration of methanolic extract of *Pyrenacantha Staudtii* on the haematological parameters of Wistar Rats.

Group	% PCV	HB (g/dl)	RBC × 10^6/µl	W.B.C/ µl
A	25*	8.2*	2.5 × 10^6*	4,740
B	26*	8.6*	2.3 × 10^6*	4,584
C	24*	8.1*	2.1 × 10^6*	4,643
D	18*	6.0*	2.00 × 10^6*	4,899
E (control)	47	15.5	4.5 × 10^6*	4, 538

The methanolic extract was given daily three times by the oral route to groups of rats (n = 10) at the following doses:
A – Rats dosed, with 30mg/ml extract 3 times a day
B – Rats dosed with 25mg/ml extract 3 times a day
C – Rats dosed with 30mg/ml extract 4 times a day
D – Rats dosed with 25mg/ml extract 4 times a day
E – Rats dosed with Normal saline (control)
Haematological parameters were measured every 30 days. The data are expressed as mean ± S.E.M.; significant differences in each group vs. the controls were as follows:
$p < 0.05$; *. Results are means of triplicates

Effect of chronic oral administration of *P. staudtii* on the haematological of Rats

The effect of chronic oral administration of *P. staudtii* extract on the haematological parameters is presented in table 3. White Blood Count (WBC) of all the treated rats remained within normal limits as compared with the control ($p < 0.05$) throughout the treatment period. Chronic oral administration of *P. staudtii* extracts (up to a dose of 30 mg/kg body weight) caused significant changes in PCV, heamoglobin (HB) and red blood cell (RBC) value.

Histological Analysis

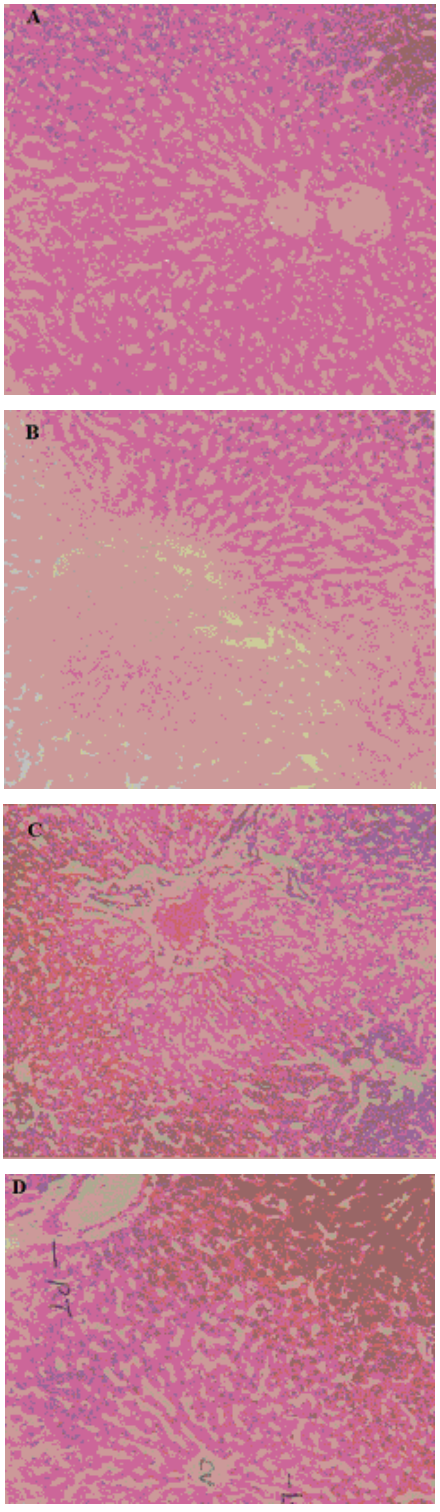

Fig. 1. Selected microphotographs of liver of rats in groups A, B, C and D revealed toxic effect of the extracts on the cells of highest dose group at 30mg/ml. Groups A, B, C and D showing degenerative changes in the liver with dilated sinusoids compared with group E (rats fed with distilled normal only).

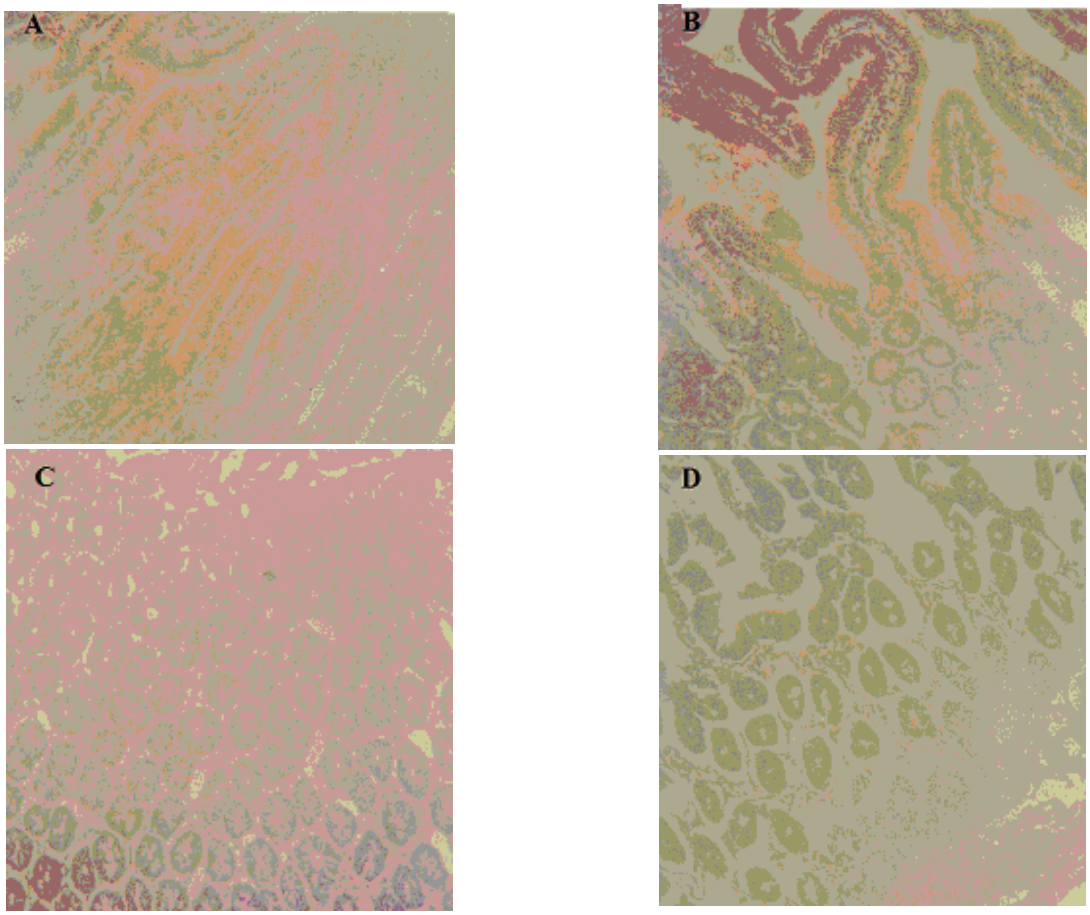

Fig. 2. Selected microphotographs of duodenum of rats in groups A, B, C and D revealed toxic effect of the extracts on the cells of highest dose group at 30mg/ml. Duodenum also revealed severe hyperplasia, which is abnormal increase in cells of the duodenum compare with the normal group of rat treated with normal saline alone.

DISCUSSION

Results of this study indicate that *P. staudtii* methanolic extract possesses significant antimicrobial activity against *Salmonella typhi* and *Bacillus cereus*. In this study, the presence of phytochemical (Table 2) in *P. staudtii* extracts could be responsible for their medicinal values though the exact mode of action is poorly understood. Some of the phytochemicals are known to stop bacterial and fungal attacks, which makes them natural antibiotics (Garrod *et al*., 1975; Okwu and Emenike, 2006). Therefore, the detection of common phytochemical in the extracts of *P. staudtii* could be a contributing factor for their antibacterial properties.

Histological finding on the liver and duodenum showed that high dose of the extract of *P. staudtii* may be toxic to the liver and cause abnormal increase in cells of the duodenum (ulceration). This toxic effect on liver alters normal morphology of hepatic lobules causing disruption of hepatocytes arrangement. These observations indicated marked changes in the hepatic histoarchitecture, which could be explained on the basis that *P. staudtii* toxic effects through the induction of oxidative stress in the liver of rat (Figs. 1 and 2). It is evident that the extract has ability to stimulate immune response in albino rats, where white blood cell counts of the experimental rats increase significantly (Table 3). The packed cell volume and red blood cell counts decrease in value which indicates anaemic condition in the experimental rats. In contrast to the observed abnormal increase in cells of the duodenum, the stimulation of immune after oral administration of *P. staudtii* extract in rats may be explained by low bioavailability of the toxic component(s) due to poor absorption from the gastrointestinal tract and rapid metabolism to non-toxic metabolites.

Aguwa and Okunji (1986) reported that *P. staudtii* exhibited anti-ulcer activity and the main constituents of the ethanol extract of the leaves were identified to be triterpenoid saponins and orally this extract was shown to protect rats from developing gastric ulcers induced by various in rat. Therefore, the presence of phytochemicals probably accounted for the medicinal efficacy of *P.*

staudtii extract. The various histopathological results evaluated in this study are useful indication that can be employed to assess the toxic potentials of botanicals in living systems. Alterations in histological of organs (liver and duodenum) function will impair the normal functioning of the organs (Latha *et al*., 1998). It can be concluded from this study that the alterations in liver and duodenum function indices are suggestive of adverse effects arising from treatment with high dose of methanolic *P. staudtii* leaves. The components of *P. staudtii* extract responsible for the toxic manifestations are not known. The toxicity of the *P. staudtii* may be due to any one or more of the phytochemicals present in the crude methanolic extract, some of which have been isolated and identified (Aguwa and Okunji; 1986; Okunji and Iwu, 1988).

However, to confirm the toxic nature of any plant product, one has to consider several factors that can alter its toxicity profile, which may include the growth stage and maturity of the plant parts of the plant used the seasonal variation in the relative abundance of phytochemicals, the storage conditions of the product (Zbenden *et al*., 1963). To balance the effects of these variables, additional long-term studies with graded doses of *P. staudtii* are needed to rule out any long-term adverse effects. In conclusion, at the oral doses tested, the extracts may not be completely safe in rats when repeatedly administered for 14 days at the investigated doses.

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REFERENCES

Aguwa, CN. and Okunji, CO. 1986. Gastrointestinal studies of *Pyrenacantha staudtii* leaf extracts. Journal of Ethnopharmacology. 15 (1):45-55.

Awe, OE., Kolawole, OS., Wakeel, OK. and Abiodun, OO. 2011. Antidiarrheal activity of *Pyrenacantha staudtii* Engl. (Iccacinaceae) aqueous leaf extract in rodents. Journal of Ethnopharmacology. 137(1):148-153.

Awe, EO., Makinde, JM., Olajide, OA., Wakeel, OK. and Adeloye, AO. 2005. Eval-uation of analgesic, anticonvulsant and hypnotic activities of *Pyrenacantha staudtii*. African Journal of Traditional Complementry and Alternative Medicine. 2:122-128.

Evans, WC. 2002. Trease and Evans Pharmacognosy (15th ed.). WB Saunders Company Ltd., London. 191-393.

Garrod, LP., Lambert, HP. and Gray, OF. 1975. Antibiotics and chemotherapy (4th ed.). Churchill Livingstone, Edinburgh London and New York, USA. 501-512.

Iwu, MM. 1993. Handbook of African Medicinal Plants. CRC Press Inc, New York, USA. pp27.

Kayode, J. and Kayode, GM. 2002. Ethnomedicinal Survey of Botanicals. J. of Res. in Ethnopharmacol. 39:70-71.

Latha, R.M., Geentha, T. and Varalashmi, P. 1998. Effect of *Vernonia cinerea* Less. Flower extract in adjuvant-induced arthritis. J. Pharmacol. 31:601-606.

Mitscher, LA., Ryey, PL., Bathala, MS., Wu-Nan, W., Deal, JL. and Roger, W. 1972. Antimicrobial agents from higher plants introduction, Rational and Methodology, Lioydia. 35(2).

Okarter, N., Liu, C., Sorrells, ME. and Liu, RH. 2009. Phytochemical content and antioxidant activity of six diverse varieties of whole wheat. Food Chem. 119(1):249-257.

Okwu, DE. and Emenike, IN. 2006. Evaluation of the phytonutrients and vitamin contents of citrus fruits. Int. J. Mol. Med. Adv. Sci. 1:1-6.

Okunji, CO. and Iwu, MM. 1986. Control of schistosomiasis using Nigerian medicinal plants. Annual Review Nutrition. 6:433-458.

Okunji, CO. and Iwu, MM. 1988. Phytochemical screening of Pyrenacantha staudtii. International Journal of Crude Drug Research. 4:246-252.

Oliver–Bever, B. 1986. Medicinal plants in Tropical West Africa. Cambridge University Press Cambridge, UK. pp126.

Saad, B., Azaizeh, H., Abu-Hijleh, G. and Said, S. 2006. Safety of traditional Arab herbal medicine. Evid. Complement. Alternat. Med. 3:433-439.

Sofowora, A. 1993. Medicinal plants and Traditional medicine in African (2nd ed.). Spectrum Books Ltd Ibadan. 172-180.

Trease, GE. and Evans, CW. 1985. A Textbook of pharmacology (13th ed.). A.E.L. B.S.I Bailliere Tandall, London. 378-387.

Zbenden, G. 1963. Experimental and clinical aspect of drug toxicity. Advances in Pharmacology. 2:1-112.

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