

SHORT TERM ACUTE AND SUBACUTE TOXICITY STUDIES ON *PILIOSTIGMA THONNINGII* LEAF EXTRACT IN RATS

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ABSTRACT

The *Piliostigma* plant, *Piliostigma thonningii* (Shum) Milne-RedHead, [Caesalpiniaceae], is widely known from ancient ages. The *Piliostigma* plant is widely distributed in Africa and Asia. The medicinal and commercial importance of the plant has been known since ancient days. In the present study, an experimental approach is applied to *Piliostigma* plant to elicit toxic responses over an exposure period of 28 days. Since no data are available in this regard, it was thought worthwhile to undertake acute and subacute toxicity studies in male and female rats. Acute toxicity determination indicated that *Piliostigma* plant has LD₅₀ values of >5000 mg/kg. The drug is practically non-toxic at oral doses. To carry out the subacute toxicity studies, the extract was administered to rats for 28 days, and the blood samples drawn from animals were subjected to full biochemical, hematological investigations. It was found that prolonged exposure of rats to *Piliostigma* plant extract exerted no toxic symptoms

KEYWORDS: *Piliostigma* plant, acute and subacute toxicity.

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INTRODUCTION

The use of herbal medicines as complements or alternatives to orthodox medicines has been on the increase. The reasons, which have given rise to this trend, include the cheapness, availability and accessibility of these natural medicines. Besides, there has been the erroneous belief that these medicines are free from adverse effects^{1,2}. On the other hand they have been rejected because many of the acclaimed medicinal values have not been scientifically evaluated and their safety profiles uncertain². It is, therefore, pertinent that safety assessments should be conducted on natural products for which certain medicinal uses have been scientifically validated.

The tree is perennial in nature and its petals are white to pinkish in colour, the fruit is a hairy, hard, flattish pod³. Different parts of *P. thonningii* have also been described as useful medicinally. Its roots and twig have been used for the treatment of dysentery, fever, wound infection, jaundice, chicken pox, respiratory ailments, snake bites, hookworm and skin diseases. In the present study, an experimental approach was applied to *P. thonningii* leaf extracts to elicit toxic response over an exposure period

of 28 days. These studies are important as they help in evaluating both the functional and morphological changes in the experimental animals. Since no data are available in this regard, it was thought worthwhile to undertake the acute and subacute toxicity studies for 28 days.

MATERIALS AND METHODS

Sample collection

The fresh leaves of this plant, *P. thonningii* were collected from Minna and Bida environment in Niger State. Identification was carried out by local people and confirmed by a Botanist and Taxonomist of the Department of soil Science, School of Agriculture and Agricultural Technology, Federal University of Technology, Minna (Prof. M. I. S Ezenwa) and authenticated by a botanist of Herbarium department of National Institute for Pharmaceutical Research and Development, Idu, Abuja (Mrs Ugbabe G.E.). In the present study, an attempt was made to determine the toxicity (acute and subacute) data of *Piliostigma* plant. The following experimental protocol was therefore designed to allow a systematic approach to the study.

Phytochemicals analysis

Presence of phytochemicals was determined using the methods of Trease and Evans⁴ and Sofowora⁵.

Acute toxicity

The method of Lorke⁶ was employed to determine the toxic level of the extracts on rats. The evaluation of the LD₅₀ was carried out in 2 stages, in the first stage, 3 groups of rats each were administered with the extract using syringe and canula. Graded doses (1,000mg, 1,500 and 2000mg/100g body weight of rats/day) orally of *P. thonningii* crude extract to determine the range of LD₅₀. In the second stage, other groups of 3 rats each were administered with the extract using syringe and canula. Graded doses (2,500mg, 3000 and 5000mg/100g body weight of rats/day) after 24hours of such ingestion the animals were observed for signs of abnormalities (such as alertness, mobility, eating/drinking, calmness, sleeping) during the period of treatment.

Subacute toxicity

Sub acute toxicity was performed on effective extract using the methods of (2005) and Lorke⁶ and Aimmanas *et al.*⁷ graded doses (1000mg, 1,500mg and 2000mg/100 g body mass of rat/day) of *P. thonningii* extract were each administered in 0.5 ml of saline to the respective groups of rats for 28 days. This was done by using a flexible catheter attached to a tuberculin syringe. The control group was given saline (0.5 ml saline/rat) only. Daily water intake, feed intake and mass gain were recorded at 7-day intervals.

Haematology and Biochemical indices

At the end of sub acute toxicity, blood was drawn from all the rats by cardiac puncture under chloroform anaesthesia and divided into 2 samples containing EDTA (1.0 mg/ml) or heparin (0.2 mg/ml blood) for cell counts and plasma biochemistry, respectively. Haemoglobin concentration (Hb) was determined by the cyanomethaemoglobin method using a Beckman model spectrophotometer. Erythrocytes (RBC) were counted with an improved Neubauer haemocytometer. Total leucocyte (WBC) count and packed cell volume (PVC) were measured with the QBCII Centrifugal Haematology System (Becton Dickinson Co., U. S. A.) The erythrocytic indices: mean corpuscular volume (MCV) were calculated. Total protein, albumin, bilirubin, alanine amino-transferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), were measured using autoanalyser (SMA 12/60 Technicon Autoanalyser, Terry-town, N. Y.).

Histopathology

The anaesthetized rats were decapitated immediately after blood sampling and eviscerated. Organs were removed, weighed fresh and fixed for 5 days in 10%

neutral buffered formalin for histopathology. Their weights were later expressed as percentages of final body mass. Tissue samples from testes, kidneys, liver, heart, lungs and brain were trimmed and processed by the paraffin wax procedure outlined below, and sections of 4µm thickness were cut and stained with haematoxylin and eosin for examination by light microscopy.

Relative organ weights

Liver, kidneys, spleen, heart, brain, stomach testis/ovary, lungs intestine were excised, mopped with filter paper, and weighed, and the relative organ weights were calculated and expressed as mg or g percent of body weight.

Statistical analysis

The data were decoded and analyzed and expressed as mean ± S.D. The differences between groups were tested by one way analysis of variance (ANOVA). A difference of P < 0.05 was considered to be statistically significant.

RESULTS

Result of biochemical and hematological parameters (hepatic and renal function tests) are presented in **Tables 1 and 2**. There were no significant increase (P<0.05) in various hematological parameters such as Hb, RBC, WBC and differential count compared to control group. The histopathologic examination of various organs such as the liver, kidney revealed normal architecture on comparison with the control. The findings suggest that *P. thonningii* is nontoxic since no marked changes in hematological, biochemical and histopathological parameters were observed.

DISCUSSION

According to organization for Economic Cooperation and Development (OECD) guidelines for acute oral toxicity, an LD₅₀ dose of 2000mg/kg and above is considered to be safe. In the present study an LD₅₀ >5000mg/kg was observed. In the present study, the extract from *P. thonningii* was found to be non-toxic to the experimental mice even at an LD₅₀ >2000mg/kg showing a high safety level of the ethnoproduct. All the parameters assessed in the subacute toxicity assay, such as effect of the extract on body and organ weight, haematology, liver and kidney functions were found not to be significantly different from the control group (Tables 1,2) also the histopathologic examination of various organs such as the liver, kidney revealed normal architecture on comparison with the control. This further confirmed the safety of the product.

CONCLUSION

The findings suggest that *P. thonningii* is nontoxic since no marked changes in hematological, biochemical parameters were observed. Further, chronic toxicity and tetragenic studies have to be conducted before the

extract could be recommended for long term treatment of infectious diseases.

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Table 1: Biochemical parameters observed in experimental animals treated with varying concentration of plant extracts

Organs(g)	Doses			
	Control	1000mg/kg	1500mg/kg	2000mg/kg
Urea	4.14±0.229 ^a	4.35±0.233 ^a	4.43±0.233 ^a	4.10±0.153 ^a
Crt	77.20±17.611 ^a	48.50±26.276 ^a	51.00±17.010 ^a	77.00±34.962 ^a
Na	114.40±3.530 ^a	118.00±4.143 ^a	115.33±4.667 ^a	109.67±4.631 ^a
K	5.18±0.128 ^a	5.20±0.173 ^a	4.83±0.033 ^a	4.93±0.033 ^a
Cl	99.80±3.891 ^a	100.50±3.617 ^a	100.67±3.180 ^a	105.33±6.333 ^a
AST	24.80±1.068 ^a	25.75±0.946 ^a	24.67±1.667 ^a	25.33±1.333 ^a
ALT	89.40±19.487 ^a	49.00±9.652 ^a	79.67±16.895 ^a	72.00±16.503 ^a
Bilrub	38.60±3.655 ^a	36.00±6.364 ^a	33.33±5.812 ^a	38.00±1.155 ^a
TP	67.00±8.325 ^a	56.25±14.517 ^a	52.67±9.905 ^a	63.33±6.936 ^a

a : Values on the same row were not significantly different from each other (P>0.05). Values are ± standard error of mean from their replicates.

KEY: Crt- Creatinine, Na- Sodium, K- Potassium, Cl- Chloride, AST- Aspartate amino- transferase, ALT-Alanine amino-transferase, Bilrub- Bilrubin, TP- Total protein.

Table 2: Haematological parameters observed in experimental animals treated with varying concentration of plant extracts

Parameters	Doses			
	Control	1000mg/kg	1500mg/kg	2000mg/kg
WBCx10 ⁹	12.06±4.215 ^a	6.03±2.861 ^a	8.37±1.330 ^a	13.95±2.811 ^a
RBCx10 ¹²	0.58± 0.102 ^a	0.38±0.118 ^a	0.58±0.169 ^a	0.63±0.085 ^a
PCV(%)	6.20±1.356 ^a	3.75±1.702 ^a	6.67±1.333 ^a	6.75±1.031 ^a
Hb(g)	9.66±1.150 ^a	7.60±1.164 ^a	8.10±1.405 ^a	11.00±0.507 ^a
MCV	86.48±19.064 ^a	107.35±2.631 ^a	106.50±3.512 ^a	108.38±2.465 ^a
N(%)	35.60±6.408 ^a	26.50±3.304 ^a	32.33±2.333 ^a	36.25 ±3.250 ^a
L(%)	73.40±3.027 ^a	67.50±4.368 ^a	71.00±4.583 ^a	73.50±3.476 ^a

A : Values with same letters on the same row were not significantly different from each other (P>0.05). Values are ± standard error of mean from their replicates.

KEY: WBC-White boold cell, RBC-Red blood cell, PCV-Packed cell volume, Hb-Haemoglobin, MCV- Mean cell volume, n-Neutrophil, L-Lymphocyte.

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