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Acute Toxicity of *Pentaclethra macrophylla* and *Psidium guajava* Use as Antiprotozoan Medicinal Plants

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Abstract

Background: Tropical protozoan diseases are currently a major veterinary and medical health problem throughout the world. Medicinal plants have long been used for the treatment of certain tropical protozoan diseases. The aim of this study was to evaluate acute toxicity effects of methanol extract of the stem bark of *Pentaclethra macrophylla* and leaves of *Psidium guajava* in rats.

Materials and Methods: The control group received 3% DMSO, while the experimental groups received a single dose of 5000mg/kg extract of the stem bark of *Pentaclethra macrophylla* and leaves of *Psidium guajava* per oral. General appearance and behavior were observed for 14 consecutive days. Effect on haematological parameters and histopathological changes were also monitored.

Results: The methanol extracts of *P. macrophylla* and *P. guajava* showed no evidence of single dose toxicity (5000mg/kg) when studying. The acute toxicity study showed no signs of toxicity, hematological or histological parameters. So the LD_{50} values of the tested extracts were more than 5000mg/kg bwt.

Conclusion: The methanol extracts of *P. macrophylla* and *P. guajava* do not produce adverse effects in rats after acute treatment. However, further studies to determine subchronic and chronic toxicity are needed to establish an antiprotozoal drug.

Keywords: *Psidium guajava*; *Pentaclethra macrophylla*; Acute toxicity; Protozoan

Introduction

Coccidiosis is an infection caused by *Eimeria spp* protozoa and this infection and disease are considered a major obstacle in raising rabbits. Coccidial infection is initiated by oral ingestion of sporulated oocysts by the susceptible host and the infection can lead to clinical coccidiosis primarily in kits, whereas adults are mostly healthy carriers [1]. Eleven distinct *Eimeria* species have been identified in rabbits (*Oryctolagus cuniculus*), with 10 species colonizing the intestinal tract and one species (*Eimeria stiedae*) infecting the biliary ducts of the liver [2]. The anticoccidiosis drug in rabbits is relatively inexpensive medicine. However, there are several weak¬nesses, such as the fecal excretion which still may pollute the environment, especially when utilized as a fertilizer. Alternative approaches to control coccidiosis by medicinal plant extracts have promising prospects for anticoccidiosis agents [3].

In Dschang (West Region of Cameroon), the stem bark of *Pentaclethra macrophylla* (Common name Capres) and the leaves of *Psidium guajava* are used in combination by farmers to treat bacterial infections as gonorrhoea, syphilis and typhoid, protozoan diseases such as coccidioses and malaria [4,5]. They are also used to treat cases of stomach-ache, waist pain and even diarrhoea. *Pentaclethra macrophylla* commonly called African oil bean belongs to the Family Fabaceae. Antimicrobial property and the oil extracted from the seeds of *P. macrophylla* are used in the preparation of drugs against pruritus, intestinal worms and dysentery [6,7]. *Psidium guajava* is a medicinal plant used in tropical and subtropical countries to treat health disorders such as diarrhea, dysentery, vomiting, sore throat and also to regulate menstrual cycles [6,7].

No drug should be used clinically without its clinical trials and toxicity studies [8]. Sub-acute oral toxicity studies of herbal medicines are essential to identify the safety and the determination of dose level that could be used subsequently. It also helps in the investigation of the therapeutic index of drugs and xenobiotics [9].

It is on the basis of the traditional use of the stem bark of *P. macrophylla* and leaves of *P. guajava* in combination as an anticoccidial agent that we found it necessary to investigate the acute toxicity effects of methanol extract of the stem bark of *P. macrophylla* and leaves of *P. guajava* in rats.

Materials and Methods

Collection and identification of plant samples

The stem bark of *P. macrophylla* was collected (March 2014) in Melong Littoral Region of Cameroon while the leaves of *P. guajava* were collected in Dschang, Western Region of Cameroon. These plants were identified by Mr. NGANSOP Eric, a Botanist at the Cameroon National Herbarium (Yaoundé) using a voucher specimen registered under the Reference No 2328/SRFCam for *P. macrophylla* and No 2884/SRF for *P. guajava*.

Preparation of organic extracts

The stem bark of *P. macrophylla* and the leaves of *P. guajava*

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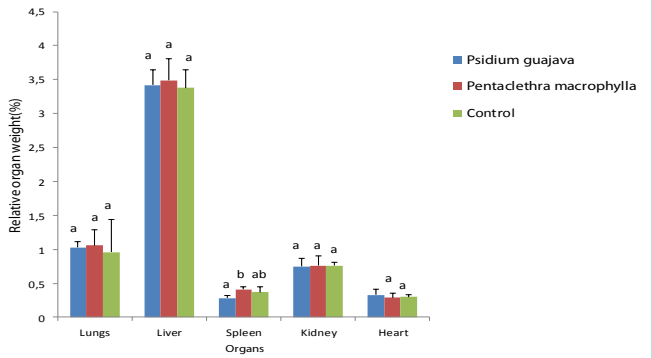


Figure1: Effect of methanolic extracts of *Pentaclethra macrophylla* and *Psidium guajava* on the relative organ weight. Values are expressed as mean ± SEM, n=5 females. Values carrying the same superscript letter are not significantly different at p≥0.05.

were air-dried at room temperature under shade in the Laboratory of Biology and Applied Ecology. The dried stem bark of *P. macrophylla* and the leaves of *P. guajava* were pulverized using an electrical grinder under strict hygienic conditions. One hundred grams of plant powder were macerated in 1.5L of methanol. This helped to extract the principal natural compounds of the plants [10]. The mixture was stirred daily and 72 hours later, the resulting solutions were then filtered using Whatman Paper No 3. The filtrate was concentrated by evaporating the solvent using a rotatory evaporator (Buchi R-200) to obtain the extracts.

Acute toxicity study

Acute toxicity of the plant extract was carried out according to the Organisation of Economic Co-operation and Development (OECD) guideline 425 [11]. A limit test was performed using healthy female albino rats weighing 175-200g of age 3 months. Prior to dosing, animals were fasted overnight and the dose for each animal was determined based on body weight. Initially, the extract was administered to one animal in a single dose of 5000mg/kg by gavage using a stomach tube. After the administration, food was withheld for a further 3-4 hours. The animal was observed once during the first 4 h after dosing, then periodically, during the first 24 hours. As the animal did not die, 4 additional animals were given the same dose and observed similarly. All the survived animals were kept for 14 days for observation. The animals were observed for general behavioral changes; symptoms of toxicity and mortality after treatment for the first four (critical) hours. The LD_{50} is greater than 5000mg/kg if three or more animals survive. The oral route was selected for use because oral route is considered to be the proposed therapeutic route.

The animals were divided into 3 groups consisting of 5 female rats of matched body weight and age in each group.

Group I: Control rats (received 3% DMSO);

Group II: 5000mg/kg, of *P. macrophylla* methanol extract;

Group III: 5000mg/kg, of *P. guajava* methanol extract.

Vital organ weight changes: At the end of the experiment, animals were sacrificed using chloroform vapors. The vital organs mainly liver, lungs, kidney, heart and pancreas were removed, cleaned with saline, weighed and preserved in 10% formalin for further histopathology observation. Relative organ weight was calculated as (weight of organ/bodyweight of rat on day of sacrifice)×100. Liver, kidney, lungs and spleen were isolated and their weights noted.

Histopathological studies: Histological cross sections of the liver and kidney were done in the Laboratory of Animal Physiology of the University of Yaounde I.

Ethical consideration

Experimental protocol used in this study strictly conformed to internationally accept standard ethical guidelines for laboratory animal use and care as described in the European Community guideline, EEC Directives 86/609/EEC, of the 24th November 1986 [12].

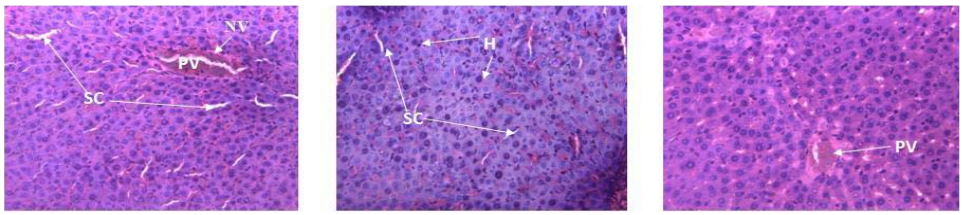
Statistical analysis

Animal toxicity results were expressed as mean±Standard Error of Mean (SEM). The data obtained from acute toxicity studies was analyzed using Student's t-test. P values less than 0.05 were considered significant.

Results

Table 1 shows general behavioral changes, symptoms of toxicity and mortality after treatment. From this Table, it appears that the oral administration of *P. macrophylla* and *P. guajava* methanol extracts at 5000mg/kg neither caused any death nor clinical sign of toxicity in rats. As there were no mortality and clinical signs of toxicity in both extracts tested, LD_{50} value of *P. macrophylla* and *P. guajava* methanol extracts were found to be greater than 5000mg/kg.

The haematological profile of treated and control groups are presented in Table 2. Analysis of blood parameters after treatment shows that all haematological parameters such as total red blood cell count, total white blood cell count, platelet count, haemoglobin, hematocrit, lymphocyte and granulocyte count are within normal

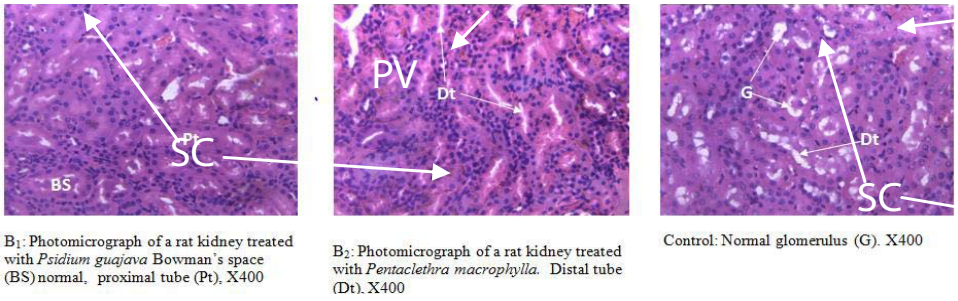


Photomicrograph of a rat liver treated with *Psidium guajava*. Sinusoidal capillaries (SC), portal vein (VP), Normal vascularised membrane (NV). X400

Photomicrograph of a rat liver treated with *Pentaclethra macrophylla*. Sinusoidal capillaries (SC), Hepatocyte (H). X400

Control X400

Figure 2: Histological examination of Liver of rats treated at a dose of 5000mg/kg and untreated (HE X400).



B_1: Photomicrograph of a rat kidney treated with *Psidium guajava* Bowman's space (BS) normal, proximal tube (Pt), X400

B_2: Photomicrograph of a rat kidney treated with *Pentaclethra macrophylla*. Distal tube (Dt). X400

Control: Normal glomerulus (G). X400

Figure 3: Histological examination of kidney of rats treated at a dose of 5000mg/kg and untreated (HE X400).

Table 1: General behavioral changes, symptoms of toxicity and mortality after treatment.

Parameters studied	*Pentaclethra macrophylla*					*Psidium guajava*				
Animal number	1	2	3	4	5	1	2	3	4	5
Locomotion	N	N	N	N	N	N	N	N	N	N
Reaction to noise	N	N	N	N	N	N	N	N	N	N
Touch response	N	N	N	N	N	N	N	N	N	N
Sleep	A	A	A	A	A	A	A	A	A	A
Convulsion	A	A	A	A	A	A	A	A	A	A
Tremor	A	A	A	A	A	A	A	A	A	A
Skin fur	N	N	N	N	N	N	N	N	N	N
Faeces	G	G	G	P	G	P	G	P	G	G
Mortality after 48 h	0	0	0	0	0	0	0	0	0	0
Mortality after 14 days	0	0	0	0	0	0	0	0	0	0
LD_{50} (mg/kg)	>5000					>5000				

N: Normal; A: Absent; G: Granular; P: Paste

range in both control and treated groups during the experimental period and did not show any significant treatment- related variation.

The effect of methanolic extracts of *P. macrophylla* and *P. guajava* on the relative organ weight of rats is presented in Figure 1. There were no significant differences (P≥0.05) in the relative organ weight between the control and treated groups.

The microscopic structures of organs shown in Figures 2 and 3 indicate unnoticeable differences between the control and test groups. The microscopic examination revealed that the liver and kidneys from the extract treated rats did not show any alteration in cell structure or any unfavorable effects when viewed under the light microscope. The liver showed normal architecture, clear lumen of central vein and no evidence of lesion Figure 2; the kidney showed adequate glomeruli and normal tubules, with no trace of focal intestinal oedema and lesion Figure 3.

Discussion

In this study, rats in the control group received 3% DMSO and treated groups were administered with crude methanolic extracts of *P. macrophylla* and *P. guajava* at a unique dose of 5000mg/kg. The rats were monitored daily until day fourteen for any toxic signs and mortality. Clinical symptoms are one of the major observations which indicate toxicity effects on organs in the treated groups [13]. During the 14 days period of acute toxicity evaluation, rats which were orally administrated *P. macrophylla* and *P. guajava* methanolic extracts at single dose 5000mg/kg showed no adverse signs of distress, and there were no observable symptoms of neither toxicity nor deaths. All the rats gained weight and displayed no significant changes in behavior. Apart from that, features of physical appearance such as skin fur and locomotion were found to be normal and whilst body weight of the rats showed an increase, this was an indication that administration of the crude extracts had negligible level of toxicity on growth of the animals.

Generally, the alterations in body weight gain and internal organ weight of rats would reflect toxicity after exposure to the toxic substances [14]. The body weight changes are indicators of adverse effects of drugs and chemicals and it will be significant if the body weight loss occurred is more than 10% from the initial weight [14,15]. Organ weight also is an important index of physiological and pathological status of animals. The relative organ weight is fundamental in diagnosing whether the organ was exposed to injury or not. The heart, liver, kidney, spleen and lungs are the primary organs affected by metabolic reaction caused by toxicants [16]. No changes were observed in gross observation of systemic organs of both control and treated groups.

In this study, relative and absolute organ weights in both *P. macrophylla* and *P. guajava* treated groups were not significantly different from those of control groups. Similarly, body weight gain was the same in both control and treated groups and difference were

Table 2: The effect of methanolic extracts on red blood cell and white blood cell indices.

Treat-ments	Red blood cell indices						White blood cell indices					
	RBC (X 10^6/µl)	HGB (g/dl)	HCT%	MCV	MCH (pg)	MCHC (g/dl)	WBC (X 10^3/µl)	LYM(%)	Monocytes (%)	Granulocytes (%)	Platelets (X 10^3/µl)	MPV (fl)
P. G	7.50±0.03	15.76±0.51	36.30±2.26	48.43±3.08	20.93±0.70	43.53±3.98	9.80±2.62	81.26±2.07	4.70±0.17	14.03±2.21	272.33±27.00	7.70±0.43
P. M	7.13±0.74	15.16±1.58	35.96±4.83	50.36±2.55	21.23±1.24	42.40±4.50	8.53±1.41	77.53±2.76	5.13±1.19	17.33±2.70	223.66±31.87	7.43±0.46
Control	7.38±0.70	15.46±0.92	36.40±3.87	49.33±0.51	20.96±1.02	42.60±2.51	7.00±1.66	79.16±4.08	4.83±1.37	16.00±2.75	255.66±19.03	7.63±0.35

P.M: *Pentaclethra macrophylla*; P.G: *Psidium guajava*; HCT: Hematocrit; HGB: Hemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration; MCV: Mean Corpuscular Volume. For the same column, values carrying the same superscript letter are not significantly different at p≥0.05.

not statistically significant. The administration of methanolic extracts did not show any adverse effect on organ weight of all important organs.

This study reckoned that *P. macrophylla* and *P. guajava* extracts do not cause acute toxicity effects with an LD_{50} value greater than 5000mg/kg. In principle, the limit test method is not intended for determining a precise LD_{50} value, but it serves as a suggestion for classifying the crude extracts based on the expectation at which dose level the animals are expected to survive [17]. According to the chemical labeling and classification of acute systemic toxicity recommended by OECD, the methanol extracts of *P. macrophylla* and *P. guajava* were assigned Class 5 status (LD_{50}>5000mg/kg) which was the lowest toxicity class. According to Kennedy et al., [18] substances with LD_{50} values higher than 5000mg/kg by oral route were regarded as being safe or practically non-toxic. Similar results were found for a single dose at 5000 mg/kg oral administration of *P. guajava* leaf extracts that was shown to be nontoxic to the tested rats [19].

Analysis of blood parameters is important in the evaluation of risks associated with test compounds under investigation as changes in the haematological system have a greater indicative value for animal toxicity [20]. In the present study, treatment with *P. macrophylla* and *P. guajava* extracts did not produce any changes in haematological parameters (i.e. haemoglobin, platelet count, white blood cell count, red blood cell count) which indicate that the extract did not affect blood cellular components or their production.

Only the liver and kidneys of the treated and untreated rats were explored for histopathological observation because of their primary function to expel toxins that result from body metabolism of food, water, drug or any other substances consumed. In acute toxicity studies, rats treated with extracts *P. macrophylla* and *P. guajava* showed normal architecture of the liver, and adequate glomeruli and normal tubules in the kidney. There was no evidence of lesion(s) due to toxic effects of *P. macrophylla* and *P. guajava* extract in the liver and kidney. This was an indication that *P. macrophylla* and *P. guajava* were safe for use without any adverse toxicological effects or up to a maximum concentration of 5000mg/kg body weight.

Conclusion

The results obtained in our study demonstrate that, the use of *P. macrophylla* and *P. guajava* does not produce any acute toxicity at the dose of 5000mg/kg, thus demonstrating their potential safety as Antiprotozoan medicinal plants. However, further studies to determine the long-term toxicity effects of *P. macrophylla* and *P. guajava* on animals are needed to establish safety and toxicity of these plant extracts.

Availability of Data and Materials

Data and material are available to other researchers upon request.

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Author's Contributions

YC, VKP, NACN, TNA, ML and JRK contributed to the design of the study, data collection, led the analysis and drafting of the manuscript. All authors read and approved the final manuscript.

Ethical Approval and Consents to Participate

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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