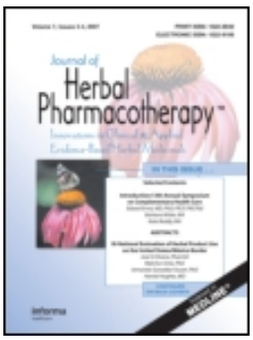

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Evaluation of Valuation of Toxicity Profile of an Alkaloidal Fraction of the Stem Bark of *Picralima nitida* (Fam. Apocynaceae)

T. O. Fakeye, BPharm, PhD
S. O. Awe, BSc, PhD
H. A. Odelola, BPharm, PhD
O. E. Ola-Davies, DVM, MSc
O. A. Itiola, BPharm, PhD
T. Obajuluwa, BPharm

ABSRACT. Dermal and acute toxicity evaluation of the basic alkaloidal fraction of the stem bark of *Picralima nitida*, which has been shown to have pronounced activity against causative organisms of dermatomycosis in man, was carried out in animals. Acute intraperitoneal toxicity tests showed a dose-dependent toxicity. There was inflammation and necrosis of liver hepatocytes accompanied by reduction

T. O. Fakeye is affiliated with Department of Pharmaceutical Microbiology & Clinical Pharmacy, University of Ibadan, Ibadan, Nigeria (E-mail: titifakeye@yahoo.com).

S. O. Awe is affiliated with Department of Pharmacology & Therapeutics, College of Medicine, University of Ibadan, Ibadan, Nigeria.

H. A. Odelola is affiliated with Department of Pharmaceutical Microbiology & Clinical Pharmacy, University of Ibadan, Ibadan, Nigeria (E-mail: akinodelola@skannet.com).

O. E. Ola-Davies is affiliated with the Department of Veterinary Physiology, Pharmacology & Biochemistry of the University of Ibadan, Ibadan, Nigeria.

O. A. Itiola is affiliated with Department of Pharmaceutics & Industrial Pharmacy, University of Ibadan, Ibadan, Nigeria (E-mail: oprofit@yahoo.com).

T. Obajuluwa is affiliated with the Department of Pharmaceutical Microbiology & Clinical Pharmacy, University of Ibadan, Ibadan, Nigeria.

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in neutrophilic count and a corresponding increase in lymphocytic count. There was no sign of reddening or irritation when applied into the eye conjunctiva. Dermal tests also showed that the fraction caused no sensitization, inflammation or death in the animal models used.

KEYWORDS. Acute toxicity, *Picralima nitida,* alkaloidal fraction, experimental animals

INTRODUCTION

Picralima nitida, a shrub-like tree containing alkaloids including akuammine, akuammicine, akuammigine, akuammidine, akuammiline and pseudo-akuammigine, is widely found and used in West Africa. Some parts of the plants such as the stem bark and seeds have been known to possess a wide range of medicinal uses which include antimalarial,[1,4] analgesic,[2,3] antipyretic,[4] anti-inflammatory,[4,5] antitrypanosomial,[6] antileishmanial,[7] antiviral,[8] antimicrobial,[9] antihypertensive[10,11] and local anaesthetic[12] activities. The alkaloidal basic fraction (BF) of the extract of the stem bark of the plant has been shown to possess *in-vivo* anti-inflammatory activity comparable with acetylsalicylic acid in our laboratory.[5] The same fraction has also been shown to possess impressive topical activity in clinical human dermatomycosis.[13] It is highly probable that this fraction or some of its constituents may be developed into useful drugs for human use.

Before this can be done, however, the toxicity profile of the plant must be established. Literature search provides little information on the toxicity profile of this important plant. Therefore, this study is necessary in order to evaluate the toxicity profile of the basic alkaloidal fraction (BF) of the stem bark that has been identified to possess some impressive medicinal activities.

METHODS

Experimental Animals

Animals were obtained from the Central Animal House of the College of Medicine, University of Ibadan, Nigeria for this study.

Dermal and oral toxicity studies of the basic fraction (BF) of the stem bark of *Picralima nitida* that was obtained as described by Fakeye et al.,[9] were carried out using established protocol.[14]

Sterile water, 80% ethanol (BDH Chemicals Ltd. Poole, UK) and propylene glycol (2.5%,v/v) were used as vehicles for the basic fraction (BF) depending on the solubility and route of administration as appropriate.[13]

Acute Limit Toxicity Tests

To two groups of five male wistar rats each were administered 2.5 g/kg or 5 g/kg body weight of basic fraction in ethanol intraperitoneally. Another group of five male rats were given an equivalent volume of the vehicle as control. Feed and water were allowed *ad libitum*. The weight of each animal was monitored over the 21-day observation period. The animals were also bled just before they were all sacrificed for the hematological examination. Histopathology of the liver tissues was carried out.

Acute IP LD_{50} Toxicity Tests

Twenty male mice were divided into four groups of five animals each. Three groups were administered 0.1, 0.2 or 0.4 g/kg body weight of BF in ethanol intraperitoneally. The fourth group was given an equivalent volume of 80% ethanol as control. Feed and water was allowed *ad libitum* while the animals were observed closely for 21 days. The animals were bled just before sacrifice for hematological tests. Histopathology of the liver tissues was carried out.

Acute Dermal Toxicity Test

BF in ethanol was applied on a shaven part of the skin of four groups of five male wistar rats per group at doses of 0.05 g/kg; 0.10 g/kg; 1.00 g/kg or 2.00 g/kg body weight each. No group received more than one dose. Ethanol at a concentration of 80% was used as control and applied on the skin of a fifth group of five male albino rats. The animals were observed for 21 days for signs of irritation, irritation on the skin and/or death. Feed and water were allowed *ad libitum*.

Dermal Irritation Test

The right hind limbs of eight male New Zealand rabbits were shaved. The basic fraction (BF), well triturated and moistened with 2.5% (v/v)

propylene glycol, was applied on the shaven skin of five rabbits with the aid of semi-occlusive patch technique.[14] The patch was removed after four hours and the site assessed for irritation and/or inflammation at 1, 4, 24, 48 and 72 hours after removal of the patch. To the other three rabbits, the shaven part was rubbed with 2.5% (v/v) propylene glycol as control. Cross sections of the skin of the two groups were thereafter prepared after the animals were sacrificed humanely for histopathological examination.

Eye Irritation Test

Single dose of one tenth of a milliliter of a 0.01% (w/v) of the BF in sterile distilled water was applied into the conjunctiva sac of the right eye of each of five male New Zealand rabbits while the left eye was instilled with 0.1 ml of sterile distilled water as control. The cornea of both eyes were observed for 21 days for signs of irritation, reddening or inflammation.

Dermal Sensitization Test

Semi-occlusive topical patch technique was used to evaluate five groups of five male guinea pigs per group. The basic fraction (BF) was applied on the shaven skin of four groups and occluded for 6 hours at induction and challenge phases. At the induction phase, 20% (w/v) or 80% (w/v) of the BF in 2.5% (v/v) propylene glycol was applied once a week to the same site of the skin of each animal of two groups per dose in the first, second and third week in an attempt to boost sensitization of the animals to the BF. Two weeks after completion of the induction phase, two groups that had received different concentrations (20% and 80%, w/w) during the induction phase were challenged with the same concentration that was applied during the induction phase at the same site. The sites were evaluated for dermal reactions such as irritation, inflammation or reddening after 24 and 48 hours. The sites were compared with the sites of the two groups that received the BF at induction phase only. The fifth group received 2.5% (v/v) propylene glycol only as control. The animals were humanely sacrificed and cross sections of the test skin sites were prepared for histopathological examination.

RESULTS

Summary of the results are shown in Table 1.

TABLE 1. Toxicity parameters of the basic fraction in animal models

Test	Dose (g/kg body weight)	Parameters			
		Mortality (%)	Inflammation	Neutrophils	Lymphocytes
Acute limit	5.0	100	NA	ND	ND
	2.5	20	NA	40.8 ± 16.4	58.6 ± 17.1
Control	0.0	0	NA	19.4 ± 6.1	76.2 ± 6.0
Acute IP	0.1	0	NA	69.3 ± 6.1	29.3 ± 5.0
	0.2	0	NA	65.3 ± 3.1	34.7 ± 3.1
	0.4	0	NA	62.5 ± 11.4	37.0 ± 10.9
Control	0.0	0	NA	12.3 ± 4.8	84.4 ± 6.0
Acute dermal	2.0	0	Absent	ND	ND
Dermal irritation	Skin patch	0	Absent	ND	ND
Eye irritation	0.1%	0	Absent	ND	ND
Sensitivity	Skin patch	0	Absent	ND	ND

Key:
NA–not applicable
ND–not done

Acute Limit Tests

There was 100% mortality at 5 g/kg body weight dose immediately after intraperitoneal administration of the fraction. The dose, when reduced to 2.5 g/kg body weight, however, produced 20% mortality. There was reduced activity of the animals 24 hours post administration in the test animals. Histopathology of the liver tissues of the test animals when compared with the control showed a few portal areas infiltrated with mononuclear cells which were predominantly lymphocytes. The few foci of inflammatory cells were mainly lymphocytes accumulating within the liver parenchyma. Hepatocytes in these areas showed mild and scanty necrotic changes.

Hematological examination two weeks post administration showed normal erythrocytes. There were scanty platelets with slight increase in the packed cell volume (PCV) and white blood cell (WBC) counts when compared with the control group. There was a significant increase in the neutrophilic count compared to the control group (control = 19.4 ± 6.1; test = 40.8 ± 16.4) with a corresponding drop in lymphocytes count (control = 76.2 ± 6.0; test = 58.6 ± 17.1).

Acute IP Toxicity Tests

There was no mortality at the highest dose of 0.4 g/kg body weight. There was no reduction in activity when compared with test animals.

Histopathology of the liver tissues showed increase in necrotic damage and congestion of the hepatic blood vessels with increase in BF dose. There was however a slight increase in white blood cell count. There was corresponding significant increase in the neutrophilic count and a significant drop in lymphocyte when compared with control animals (Table 1).

Acute Dermal Toxicity Test

The basic fraction (BF) did not produce any toxic effect on the skin of the animals. Even though a much higher dose of 2 g/kg was applied than for acute intraperitoneal toxicity test, no change in behavior was observed. Hematological tests showed no abnormality.

Dermal Irritation Test

No toxicity was observed. There was no irritation, reddening or inflammation at the test site as seen in the cross section of the skin of both the test and control animals. The eye irritation test also showed that the BF did not cause irritation to the eye conjunctiva. There was no sign of reddening throughout the 21 days of observation when compared with the control eyes.

Challenging a sensitized site of the skin of the guinea pig with either 20% (w/w, low) or 80% (w/w, high) did not lead to irritation or reddening. Cross-sections of the sensitized skin sites and the challenged skin sites at 20% (w/w) or 80% (w/w) BF showed that the BF did not cause sensitization of the skin when applied topically.

DISCUSSION

The first 24 hours of the intraperitoneal administration of the fraction led to reduction in activity and mobility of the test animals. This reduction in activity, which however became more intense with increased concentration of the fraction, may have been due to the anaesthetic effects of akuammine and akuammidine,[12] both of which are alkaloids present in the fraction.

The acute limit tests showed that the fraction may possess antiplatelet activity at very high doses as seen with scanty platelets at the 2.5 g/kg body weight dose level. This is not unusual since some non-steroidal anti-inflammatory agents possess antiplatelet activity and the fraction had been shown in a previous study to possess *in vivo* anti-inflammatory activity comparable with aspirin at 0.1 g/kg in experimental animals.[5]

Administration of the fraction in high doses led to a reduction in the lymphocytes count and an increase in neutrophilic count. Lymphocytes play an important role in humoral antibody formation and cellular immunity by synthesizing needed antibodies. Neutrophils also play a major role in reducing multiplication of pathogenic organisms and other foreign bodies in the host system thereby both play a complementary role in body defense. A corresponding increase in neutrophils, which became very pronounced at 2.5 g/kg, could indicate the host system reacting positively to the chemical intoxication. With the introduction of an antigen, there should have been an increase in both neutrophilic and lymphocytic count.[15] In this study however, there was a significant reduction in lymphocyte count and an increase in neutrophillic count which could have been due to the host body responding to make up for the temporary loss of lymphocytic activity. This could be interpreted to mean that the fraction could compromise the immune system of the host. However, further work needs to be done before a conclusion can be made.

Intraperitoneal administration of the fraction was seen to cause necrosis of the hepatocytes with accumulation of lymphocytes in few portal areas. There was also some inflammation due to lymphocytes accumulating within the liver parenchyma. This could be indicative of the response of the host body to the hepatotoxic activity of the fraction. This became more obvious with increase in dose, a reaction that was not present in dermal toxicity tests.

Acute dermal toxicity tests showed no toxicity at a high dose of 2.0 g/kg which indicated that the BF is not absorbed into the systemic circula-

tion in any appreciable quantity from the skin. It did not cause any irritation or sensitization reaction on the skin of the animals. This is not unexpected since the fraction has been established to possess *in vivo* anti-inflammatory activity.[5]

CONCLUSION

The basic alkaloidal fraction of the stem bark of *Picralima nitida* has been shown to be safe for topical application. The fact that the basic fraction did not cause topical sensitization in the animals, coupled with the absence of toxic reactions at topical high doses of the fraction makes its use as a topical preparation a great possibility in the therapy of skin ailments such as dermatomycosis in humans.

It exhibits dose-dependent toxicity after intraperitoneal administration. However, the effect of the fraction on cell immunity needs further investigation.

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