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Anti-inflammatory, antipyretic and anti-malarial activities of a West African medicinal plant — *Picralima nitida*

I.C. EZEAMUZIE, M.C. OJINNAKA*, E.O. UZOGARA** and S.E. OJI

*Department of Pharmacology, College of Health Sciences; *Department of Chemistry and **Dept of Mental Health, College of Health Sciences, University of Port Harcourt.*

Summary

A preliminary pharmacological screening of the methanolic extract of *Picralima nitida* fruit was carried out. The extract showed potent and dose-dependent anti-inflammatory, antipyretic and anti-malarial activities. Given intraperitoneally, it inhibited carrageenan-induced rat paw oedema with IC50 of 102mg/kg, and with the highest dose tested (300mg/kg) producing 72.2% inhibition. On the LPS-induced pyrexia in rabbits, 50mg/kg of the extract produced a mean percentage antipyrexia of (38.7%) compared with (29.0%) by 200mg/kg of aspirin. In a 4-day *in vivo* schizontocidal test in mice infected with *P. berghei berghei*, up to 300mg/kg daily for 4 days was ineffective in preventing the development of parasitaemia or the consequent mortality. However, marked inhibitory activity was obtained on multi-drug resistant human *P. falciparium* parasites cultured in *in vitro*. The dose causing 50% inhibition of parasite growth was 1.75µg/ml compared with (0.14µg/ml for chloroquine. The results confirm the medicinal value of this plant and thus justify its use by natives of W.Africa.

Resume

Un test preliminaire pharmacologique est effectue sur l'extrait de fruit de *Picralima nitida*. L'extrait a montre des activités anti-inflammatoire, antipyretique et anti-paludisme tres puissantes qui est dependente de la dose administree. Une administration intraperitoneale de cet extrait inhibait l'edema des pattes de rat provoquee par la carrageenan avec un IC50 de 102 mg/kg. Teste avec une dose tres fort de 300 mg/kg que est la dose la plus éleveé administree on a remarque une inhibition de 72.2%. Sur la pyrexie induite par le LPS, chez la lapin, 50 mg/kg de l'extrait produisa une pourcentage moyenne d'antipyrexie de 38.7% en comparant avec 29.0% pruit par 200 mg/kg d'aspirin administre. Une test *in vivo* chez des souris infestés des schizontes des *P. berghei berghei*, et suivi pendent 4 jours, une dose de jusqua 300 mg/kg/jour pendent 4 jours de l'extrait n'etait pas efficace dans la prevention du developpement d'une parasitemie on d'une morte consequante. Par contre, une activitee inhibitoire tres marquee etait obtenu sur les parasites de *P. falciparium* que sont culturees in vitro et qui sont des parasites tres resistantent aux medicaments chez l'homme. La dose de 1.75µg/ml avait provoquee 50% d'inhibition dans la croissance des parasites en comparant avec une dose de la 0.14 µg/ml de chloroquine. Ces resultats affirment l efficacite madicamenteux de cette plante et donc justfie son usage pas les origiaires de l'Afrique de l'Ouest.

Introduction

Picralima nitida (Apocyanease) is a medicinal plant native to West Africa. It is extensively used by natives in the treatment of a wide variety of ailments especially malaria fever. In the latter case the mesocarp of the fleshy indehiscent fruit, the size of a big orange, is scooped out and native palm wine or gin (ethyl alcohol) poured into the scoop and drunk after it has extracted the intensely bitter active principles.

The bark is used as a febrifuge while the seeds are eaten as a remedy for chest complaints, pneumonia, and acute stomach troubles. The fruit also finds application in rheumatic fever and yellow fever, as well as to generate "sexual power".

Early literature[1] reported that the seeds contain

Correspondence to: Dr. I. C. Ezeamuzie, Department of Pharmacology, College of Health Sciences, University of Port Harcourt, Port Harcourt, Nigeria.

many alkaloids the most common of which is akuammine, but that none was found to be active against avain malaria.

The continued use of the fruit in the treatment of malaria and other febrile conditions in several communities in the South eastern parts of Nigeria and its apparent success in these regards prompted us to investigate the pharmacological properties of the fruit extract which might possibly justify the traditional applications. Consequently, we have screened the methanolic extract of the fruit for three activities — anti-malarial effect on both *in vivo* rodent malaria and *in vitro* human falciparium malaria, anti-inflammatory and antipyretic effects.

Materials and methods

Plant Extract

The plant material used was the crude methanol extract of the mesocarp of the fruit. After filtration of the solvent extract, the solvent was removed by distillation under reduced pressure using a rotary evaporator (Rotavapor R). The concentrated extract was then freeze-dried to yield a dark brown solid material used for all experiments.

Animals

Male albino rats of Wistar strain, 100-200g, obtained from the University of Nigeria, Nsukka, animal house were used. Antipyretic studies were performed using 8 male white rabbits, 1.5-2.5 kg, obtained from University of Port Harcourt animal house. For the *in vivo* anti-malarial studies, albino Swiss mice of both sexes, 20-30g obtained from University of Ibadan animal house were used.

All animals were allowed at least 7 days to acclimatize in the local animal house before use and were freely supplied with food and water except otherwise stated.

Drugs and reagents

Carrageenan, indomethacin, acetylsalicylic acid (Aspirin), lipopolysaccharide of *E. coli* (LPS) were all purchased from Sigma Chemicals, U.K Chloroquine phosphate injection (Chemochin), a product of PLIVA, Zagreb, was purchased from a local Pharmacy.

Evaluation of rat paw inflammation

A total of 45 rats divided into 9 groups of 5 animals each were used. Each group received one of the 4 doses of the *Picralima nitida* extract (PNE) — (10, 50, 100, and 300 mg/kg) or indomethacin (1, 3, 10, 30 mg/kg). The 9th group received vehicle only and served as control. All drugs were given intraperitoneally 1hr. before induction of inflammation. The latter was done by injecting 0.1 ml of 1% (w/v) carrageenan in normal saline into the right hind paw. Equal volume of sterile saline was injected into the left paw. Paw size (diameter) was measured "blind" using vernier callipers that measured accurately up to 0.01mm. Measurement was done hourly for 6hr. after induction of inflammation and percentage increase determined after deducting the effect of saline injection. To quantitate the results, areas under the response-time curves were determined as index of inflammation and expressed as arbitrary square units — the scales of the curves being same in all cases. Since the responses were progressive in nature, this method was preferred to using peak responses because it took better care of any skewing in the peaking of responses. Statistical analysis of the difference between groups receiving different treatments was done using Student's t-test (unpaired).

Evaluation of antipyretic effects

The method used was similar to that of Okpanyi and Ezeukwu[2]. Eight rabbits divided into 4 groups were used and each group treated with one of the following — vehicle, acetylsalicylic acid (200 mg/kg), PNE (10mg/kg) and PNE (50mg/kg). The agents were administered intraperitoneally 30 min. before induction of pyrexia. After keeping the animals in the laboratory for 3hr. during which time, food and water were withheld, 3 rectal temperature measurements were taken at 30min. intervals and the mean formed the basal temperature for the individual animals.

Pyrexia was induced by intravenous injection of 2ıng/kg of the pyrogen (*E. coli* LPS). Half-hourly rectal temperature readings were then taken for the next 6hrs. The mean of these readings formed the "Induced temperature". In conjunction with basal temperature the change in temperature and percentage antipyrexia were calculated.

Evaluation of antimalarial effect

The *in vivo* antimalarial effect was determined in mice infected intraperitoneally with 0.2 ml inoculum containing 10 million *P. berghei berghei* parasitized erythrocytes per animal[3]. The method employed was the early infection blood schizontocidal assay the "4-day test" of Peters *et al.*, [3]. Twenty infected mice divided into 4 groups were treated on days 0, 1, 2, and 3 with intraperitoneal injection of one of the following — 50mg/kg PNE, 200mg/kg PNE, 5mg/kg chloroquine phosphate and saline. On the 4th day after infection, blood films were made from the tail of each animal and after routine staining in Giemsa, the percentage parasitaemia was assessed microscopically.

The *in vitro* effect was determined on cultured multi-drug resistant K1 strain of human *P. falciparium* malaria. This aspect was performed in the laboratories of Dr. D. Warhurst at the School of Hygiene and Tropical Medicine, London. Schizontocidal activity was assessed by the micro-technique of O'Neil *et. al.*,[4], and involves the estimation of the inhibition of the uptake of radiolabelled precursor — (hypoxanthine) by the parasites.

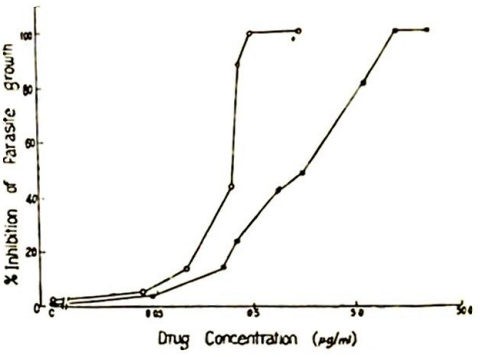

Fig. 1: Time-course of carrageenan-induced rat paw oedema in control animals (o) and in animals treated with 300mg/kg of PNE (O) or 30mg/kg indomethacin (□), in all cases, each point represents mean and s.e.m. of 5 animals.

Results

Anti-inflammatory Effects

Subplantar injection of carrageenan resulted in an intense acute paw swelling which progressed rapidly, reaching a peak of about 82% increase in 3 hr. Fig. 1 shows the time course of this response and its suppression by 300mg/kg PNE (about 72% inhibition) which is higher than that produced by a high dose (30mg/kg) of indomethacin, (57.7% inhibition). Like the latter, PNE affected only the magnitude but not the time profile.

Fig. 2 shows the full dose-related effect of the extract in comparism with indomethacin. The smallest dose of PNE tested (10mg/kg) produced a statistically significant inhibition of inflammation (137.0 ± 10.1 to 98.1 ± 3.6 sq units), $p < 0.05$. The effect increased dose-dependently with the highest dose tested (300mg/kg) producing 72.2% inhibition (137.0 ± 10.1 to 38.4 ± 9.1 sq units). Indomethacin produced similar effects but this occurred in the dose range 1 - 30mg/kg. The calculated IC50 values were 102mg/kg and 10mg/kg for PNE and indomethacin respectively.

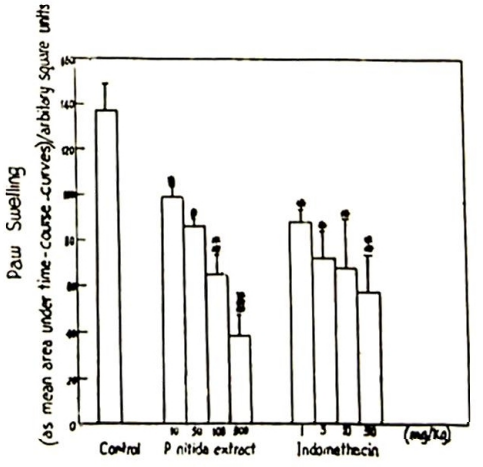

Fig. 2: Dose-related effect of PNE and indomethacin and carrageenan-induced rat paw oedema. Each value is the mean determined from 5 response-time curves representing 5 animals. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 1: Antipyretic effect of PNE and acetylsalicylic acid (aspirin) on LPS-induced pyrexia in rabbits. Drugs were administered 30min before induction.

		TEMPERATURE (°F)				
		Mean basal	Mean induced	Increase	Mean increase	Percentage Antipyrexia
Animals						
Control	A	104.0 + 0.4	105.6 + 0.3	1.6	1.55	—
	B	104.2 + 0.2	105.7 + 0.3	1.5		—
Aspirin (200mg/kg)	C	104.2 + 0.4	105.2 + 0.2	1.0	1.10	29.0
	D	103.9 + 0.4	105.1 + 0.3	1.2		
PNE (10mg/kg)	E	103.8 + 0.4	105.3 + 0.3	1.5	1.50	3.2
	F	104.5 + 0.1	106.0 + 0.4	1.5		
PNE (50mg/kg)	G	104.0 + 0.1	104.9 + 0.1	0.9	0.95	38.7
	H	104.3 + 0.3	105.3 + 0.2	1.0		

Antipyretic effect

As shown in table 1, PNE at a dose of 10mg/kg showed little or no antipyretic effect (3.2% antipyrexia), while 50mg/kg produced a marked antipyretic effect (38.7% antipyrexia). This is comparable with the 29.0% antipyrexia produced by 200mg/kg acetlysalicylic acid (Aspirin).

Table 2: Effect of 4 daily administration of PNE on the development of parasitaemia in mice infected with *P. berghei berghei*. Drug treatment was started on the day of infection.

	Mean parasitaemia (day 4)	% Suppression of parasitaemia (day 4)	Mortality (day 15)
Control Saline	9.8 + 3.6	—	5/5
PNE 50mg/kg	9.1 + 2.2	7.1	5/5
PNE 200mg/kg	8.7 + 2.3	11.2	5/5
Chloroquine 50mg/kg	0.0	100	0/5

Antimalarial effect

Table 2 shows the result of the *in vivo* anti- malarial studies in mice. In untreated animals mean percentage parasitaemia reached 9.8% 43.6 on the 4th day after infection. Daily administration of 50 or 200 mg/kg PNE produced no significant suppression of parasitaemia (7.1% and 11.2% suppression respectively), and as in the untreated group all the animals were dead by day 15. In contrast, there was a 100% suppression of parasitaemia in the group treated with 5mg/kg of chloroquine, and all the animals were alive by the 15th day. In the *in vitro* studies on cultured human falciparium malaria, PNE showed strong anti-malarial activity. Fig. 3 shows the dose-dependent effect of PNE in comparism with chloroquine. Inhibition of parasite growth was detectable with as low as 0.09 µg/ml of the extract and 99.9% inhibition was achieved at about 15µg/ml. The calculated concentration that achieved 50% inhibition was 0.75µg/ml. Chloroquine by contrast had an IC50 of 0.14µg/ml, but with a much steeper dose-response curve.

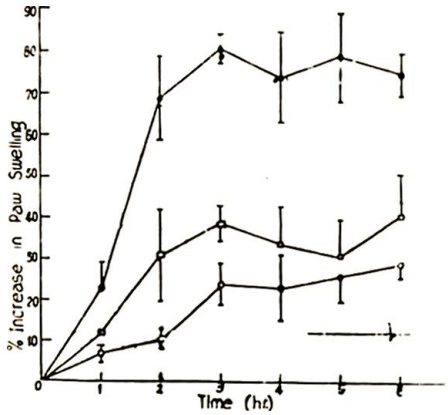

Fig. 3: Inhibitory effect of PNE (■) and chloroquine (O) on multi-resistant K1 strain of *P. falciparium* growth *in vitro*. Results are expressed as means of two experiments.

Discussion

Picralima nitida extract has in this study been shown to posses three important pharmacological activities — anti-inflammatory, antipyretic and antimalarial. In the anti-inflammatory test, it demonstrated a pronounced dose-dependent inhibition of carrageenan-induced acute paw oedema. The dose producing 50% inhibition (IC50) of 102mg/kg is only 10 times less than that of the prototype and potent anti-inflammatory drug indomethacin. This level of activity is impressive for a crude extract, and thus suggests that the active principle when eventually isolated will likely be very potent, possibly comparable in degree with indomethacin.

In the antipyretic study, the extract showed even a more striking potency. Although 10mg/kg produced virtually no effect, 50mg/kg had a significant antipyretic effect (38.7% antipyrexia) that was comparable to that due to 200mg/kg acetylsalisylic acid (29.0%). Results obtained from antimalarial studies were rather suprising. While the extract (up to 200mg/kg) showed no *in vivo* schizontocidal activity against *P. berghei berghei* in mice, it proved to be a potent schizontocide on cultured *P. falciparium* with IC50 of 1.75μg/ml merely 12.5 times that of chloroquine. Early studies of the antimalarial effect of this plant[1], showed that the alkaloids isolated from the plant had no effect on avian malaria, inspite of its intensive use by natives in malaria fever. Thus it appears that the present result has revealed a species-specific anti-malarial activity of PNE in which activity is restricted to human malaria or conceivably falciparium malaria. Such inter-species specificity is, generally-speaking, uncommon with antimalarials. However, since only the 4-day early infection test was used in our study, the possibility that it could be more active on established infection cannot be ruled out. For example, Obi and Makinde[6], have shown that the crude extracts of *Azadirachta indica* was effective in rodent early malaria infection but not on the established one. Furthermore, it might be that much higher doses than the ones used here were necessary to affect non-human malaria.

The clearly potent anti-malarial effect of PNE on *P. falciparium* tends to justify the use of this plant by natives in the treatment of malarial fever in West Africa — essentially a *P falciparium* endemic area[5]. Since acute malaria is a multifactorial disease manifesting in fever, joint pains and sometimes glomerulonephritis that is inflammatory in nature[7], it is interesting that this extract combines three activities that can be concurrently relevant. The occurrence of many pharmacological activities in one plant part *par se* is not new. Infact, in their separate studies Okpanyi and Ezeukwu[2] and Obi and Makinde,[6] have shown that the crude extract of *Azadirachta indica* leaves possessed all the three activities detected in this study. However, PNE appears to be unique in its potency. For example, the anti-inflammatory effect produced on carrageenan-induced paw oedema by 800mg/kg *A. indica* was comparable with that producible by 20mg/kg of PNE in the same model in the present study.

Although this is essentially a preliminary study, involving no purification steps whatsoever, it is nevertheless possible to conclude, on the basis of the results obtained and the level of potency exhibited by this extract, that at least some of the traditional uses of the plant such as in malaria and pyrexia of diverse eitiologies are justified. *Picralima nitida* therefore promises to be a potential source of important drugs and as such deserves detailed studies.

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