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The Anti-Inflammatory and Antinociceptive Properties of the Chloroform Fraction From *Phyllanthus niruri* Plant Is Mediated via the Peripheral Nervous System

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ABSTRACT. *Phyllanthus niruri* (Euphorbiaceae) is used folklorically for the treatment of diabetes, malaria, fever, diarrhea, liver disease, and urolithiasis. As an initial step toward isolating compounds effective against inflammation and pain, this study is aimed at providing scientific evidence for the anti-inflammatory, antinociceptive, and antipyretic properties of the chloroform soluble fraction (PNF1) of *Phyllanthus niruri* methanol extract in rats and mice. Three doses of PNF1 [25, 50, 100 mg/kg body weight (bw)] were used. Screening was done using acetic-acid-induced writhing, egg-albumin-induced pedal inflammation, Randall–Selitto test, hot-plate test, and yeast-induced pyresis as experimental models. Results show that PNF1 significantly ($p < .01$) inhibited writhing response induced by acetic acid at all doses used by 56.2%–66.7% and caused significant ($p < .05$, $p < .01$) reduction of yeast-induced pyrexia (21.6%–40.9%). Significant ($p < .01$) reduction of egg albumin-induced inflammation was observed only at a dose of 100 mg PNF1/kg bw, which was comparable with the effect produced by aspirin (100 mg/kg bw). At 50 and 100 mg/kg bw, PNF1 significantly ($p < .05$, $p < .01$) increased pain threshold of inflamed tissue in the Randall–Selitto test but did not increase response to thermally induced pain in the hot-plate test. It is concluded that PNF1 possesses antipyretic, anti-inflammatory,

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and antinociceptive effects that are peripherally mediated. This justifies its use in traditional medicine and its potential as a candidate for further development.

KEYWORDS. Anti-inflammatory, antinociceptive, antipyretic, *Phyllanthus niruri*

INTRODUCTION

Inflammation and unrelenting pain is characteristic of many debilitating and chronic diseases such as cancer, tuberculosis, and malaria, necessitating the coadministration of anti-inflammatory and analgesic drugs such as nonsteroidal anti-inflammatory drugs and opiates during management (MacPherson, 2000). The continuous use of these drugs may, however, be limited by their side effects and development of tolerance (Vongtau et al., 2004). Thus, safer, affordable, and more effective alternatives are required. About 28% of all modern drugs in use today are derived directly or indirectly from naturally occurring substances in plants (Chin, Balunas, Chai, & Kinghorn, 2006), obtained from only a small fraction of plant species that were subjected to screening for pharmacological activity. People around the world in different civilizations have used plant products to treat different diseases for several years (Calixto et al., 2000); therefore, the need to search for pharmacologically active substances from plants that are largely unexplored cannot be underscored (Hamburger & Hostettmann, 1991). A wide variety of plants are available for this purpose, including the herb, *Phyllanthus niruri* (Euphorbiaceae). It is found distributed in tropical and subtropical areas throughout the world, growing as a rainy season weed in cultivated farmlands and wastelands. Preparations of the plant have been used as traditional remedy for various ailments, especially in ayurvedic medicine for jaundice, gonorrhea, frequent menstruation, and diabetes (Bagalkotkar, Sagineedu, Saad, & Stanslas, 2006). Additionally, a decoction of aerial parts of the plant is frequently employed in the treatment of malaria fever and pain (Asprey & Thornton, 1955). Scientific validations of the pharmacological effects of *P. niruri* have also been carried out by different investigators. This includes inhibition of HIV replication (Naik & Juvekar, 2003), hepatoprotective (Shimizu, 1989), lipid lowering (Chandra, 2000), and antidiabetic (Bavarva & Narasimhacharya, 2007) and antimalarial activities (Tona et al., 2001). Research in our laboratory has shown the multiple mechanisms of antidiabetic activity of *P. niruri* methanol extract (Obidike, 2010). As part of our continued evaluation of this plant, in this study we test chloroform fraction of this extract for its efficacy against inflammation, fever, and pain, using different experimental models. This was done to provide scientific justification for the use of *P. niruri* as a remedy for painful, inflammatory, and febrile conditions.

MATERIALS AND METHOD

Plant Material, Extraction, and Fractionation

P. niruri (whole plant) was collected from an uncultivated farmland in Suleja, Niger State, Nigeria, in October 2009. It was identified by Mallam Muazzam Ibrahim, a

taxonomist of the Department of Medicinal Plant Research, National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria. Roots of the plant were cut off, and the aerial part was air-dried under shade for 1 week and pulverized to coarse powder using a mechanical grinder. Four hundred grams of the powdered material was extracted with 2.5 L methanol (98% v/v) for 48 hr with intermittent agitation and filtered. The resulting filtrate was concentrated under vacuum in a rotary evaporator and portioned between water and chloroform (4 × 600 mL), using a separatory funnel. The combined lower chloroform layer was concentrated to dryness under vacuum by rotary evaporation at a temperature of 40°C to give a sticky green fraction designated PNF1 (yield 9.09% w/w). The fraction was stored in an airtight glass bottle, and appropriate concentrations for the experiment were freshly prepared as suspensions in distilled water prior to each experiment, using 0.1% w/v tragacanth as suspending agent.

Drugs and Reagents

Aspirin, morphine (Sigma Chemical Company, St. Louis, MO, USA), and acetic acid (BDH, Poole, Dorset, UK) were used. Analar grade methanol and chloroform were used for extraction and fractionation.

Animals

Male and female albino mice (18–30 g) and Wistar rats (150–200 g) obtained from the animal facility center, NIPRD, were used in this study. They were acclimatized for 2 weeks prior to start of experiment, fed with standard rodent feed, and allowed free access to drinking water. All experiments involving the animals were done in accordance with NIH guidelines for the care and use of laboratory animals (National Institute of Health, 1985).

Acetic-Acid-Induced Abdominal Writhing Test in Mice

This test was carried out as described by Koster, Anderson, and De Beer (1959) with slight modification. Twenty-five mice were weighed and divided into 5 groups of 5 mice each. Groups 1 and 5 served as negative and positive controls and received distilled water [5 mL/kg body weight (bw)] and aspirin (100 mg/kg bw), respectively. Groups 2, 3, and 4 were administered PNF1 (25, 50, 100 mg/kg bw, respectively) orally. Thirty minutes after treatment, the mice were injected intraperitoneally (ip) with a 0.75% v/v solution of acetic acid (10 mL/kg) and transferred into a transparent plastic observation chamber. Five minutes after acetic acid administration, the number of full abdominal writhes was counted for a total of 5 min for each mouse.

Egg Albumin-Induced Paw Inflammation in Rats

The method of Akah, Okogun, and Ekpendu (1993) was adopted for this experiment. Twenty-five rats were fasted overnight prior to the study; basal thickness of the right hind paw of each rat was measured using vernier callipers. The rats were

divided into 5 groups of 5 rats each such that the mean paw thickness of the groups was close. Treatment was initiated as follows: Groups 1 and 5 served as negative and positive controls and received normal saline [10 mL/kg per os (po)] and aspirin (100 mg/kg po), respectively. Groups 2, 3, and 4 were orally administered 25, 50, and 100 mg PNF1/kg bw. Thirty minutes posttreatment, 0.1 mL of fresh egg albumin was injected into the subplantar surface of the right hind paw. Paw thickness was measured 30 min after albumin injection and subsequently at 30-min intervals for a total of 120 min.

Hot-Plate Test in Mice

The hot-plate test was performed according to the method of Abbah et al. (2010). The response time of the mice to a hot plate (Socrel model D-537, Ugo Basile, Comerio-Varese, Italy) maintained at $53 \pm 2^{\circ}$C was measured. Basal nociceptive responses were observed as hind paw lifting/licking or jumping, and the mice were randomized into 5 groups of 5 mice each. Groups 1 and 5 served as negative and positive controls and received normal saline (10 mL/kg po) and morphine (4 mg/kg ip), respectively. Groups 2–4 were treated orally with PNF1 (25–100 mg/kg bw). The response time was measured 30 min after drug or extract treatment and subsequently at 15-min intervals for a total of 120 min.

Randall–Selitto Test in Rats (Pain in Inflamed Tissue)

This test was performed following the procedure described by Vongtau et al. (2004) with minor modifications, using 30 rats. All the rats were fasted for 18 hr prior to the study. Inflammation of the left hind paw was induced by the subcutaneous injection of raw egg albumin into the subplantar surface. After 2 hr, the nociceptive threshold of the two hind paws (inflamed and noninflamed) was determined using an analgesymeter (model 7200), and the rats were randomized into 6 groups of 5 rats each. Negative control group received 10 mL/kg distilled water, while two positive control groups received morphine (4 mg/kg ip) and aspirin (100 mg/kg po), respectively. Test groups were treated orally with 25, 50, and 100 mg PNF1/kg bw. The nociceptive threshold was measured in all the groups at 30, 60, 90, and 120 min after treatment.

Test for Antipyretic Activity in Mice

The procedure used in this test was adapted from the method described by Al-Ghamdy (2001). Basal rectal temperature of 30 mice was measured using a clinical thermometer. Thirty minutes later, the mice were injected subcutaneously with baker's yeast (10 mL/kg of 15% w/v solution in pyrogen-free distilled water), and food was immediately withdrawn. Eighteen hours after yeast challenge, rectal temperatures were recorded. Measurement was done in triplicate at 30-min intervals, and mice exhibiting minimum temperature increase of 0.5°C and above were divided into 5 groups ($n = 5$), keeping the mean temperature of the groups close. Groups 1 and 5 served as negative and positive controls and received distilled water (10 mL/kg) and aspirin (100 mg/kg),

respectively, while groups 2–4 received PNF1 (25–100 mg/kg). Thirty minutes after treatment and every 30 min for a total of 2 hr, rectal temperature was recorded. Fever index was calculated as area under the fever curve for data interpretation (Vilela, Bitencourt, Cabral, & Franqui, 2010).

Statistical Analysis

Results were expressed as mean ± standard error in mean; data were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's test for posthoc comparisons. Differences were accepted as significant at $p < .01$ and $p < .05$.

RESULTS AND DISCUSSION

PNF1 elicited anti-inflammatory activity as evidenced by the reduction of paw swelling following the injection of egg albumin into the paw. Treatment with 25–100 mg PNF1/kg bw reduced paw size within 30 min and was evident up to 120 min (Figure 1). The effect of 100 mg PNF1/kg bw was significant ($p < .01$) at 60 min and comparable with the effect of the standard anti-inflammatory drug, aspirin. Egg albumin is a standard phlogistic agent, which evokes the process of inflammation by the release of inflammatory prostaglandins, histamine, serotonin, and recruitment of neutrophils to the site of inflammation (Vilela et al., 2010). This may have increased vascular permeability resulting in edema and accounted for the observed increase in paw size. Anti-inflammatory drugs like aspirin inhibit the production of prostaglandins and via inhibition of cyclooxygenase enzymes (Xu et al., 1999). The ability of PNF1 to reduce paw swelling similar to aspirin is indicative of an anti-inflammatory effect that could be mediated by an inhibition of prostaglandin synthesis.

FIGURE 1. Effect of PNF1 on egg albumin-induced hind paw inflammation in rats. **Significantly different from control at $p < .01$.

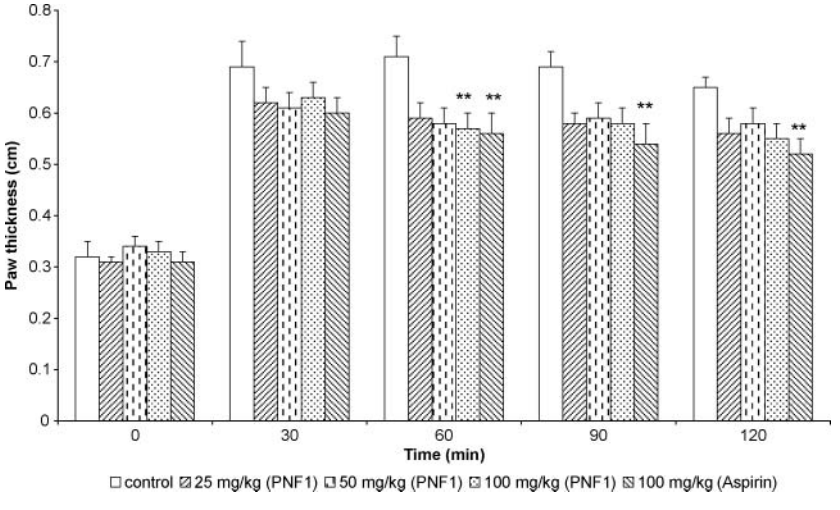


TABLE 1. Effect of PNF1 on Acetic-Acid-Induced Abdominal Writhing in Mice

Treatment	Dose (mg/kg)	Number of Writhes (mean ± *SEM*)	% Inhibition
Distilled water	–	21.00 ± 1.41	–
PNF1	25	9.20 ± 2.13[a]	56.20
	50	8.20 ± 2.40[a]	60.95
	100	7.00 ± 2.97[a]	66.67
Aspirin	100	2.80 ± 0.97[a]	86.67

[a]Significantly different from control at $p < .01$.

Treatment with PNF1 significantly ($p < .01$) reduced the number of abdominal constrictions induced by acetic acid (Table 1). At doses of 25, 50, and 100 mg/kg bw, PNF1 decreased writhing by 56.20%, 60.95%, and 66.67%, respectively, while aspirin caused 86.67% inhibition. Acetic-acid-induced abdominal writhing model is used to screen compounds, which possess anti-inflammatory and peripheral analgesic activity and is a reliable and sensitive model in the detection of anti-inflammatory and antinociceptive activity (Collier, Dinneen, Johnson, & Schneider, 1968). As an abdominal irritant, acetic acid stimulates local receptors within the peritoneum to induce pain (Vogel & Vogel, 2002), causing an increase in prostaglandin E and F2α (Deraedt, Jougney, Delevalcee, & Falhout, 1980) and lipoxygenase products (Levini, Lau, Kwait, & Goetzl, 1984). The observed activity of the extract may indicate that it elicits its action via the inhibition of prostaglandin synthesis, similar to aspirin. This finding is in agreement with the report of Bagalkotkar et al. (2006), which stated that geraniin isolated from *P. niruri* was a more potent inhibitor of acetic-acid-induced constrictions than aspirin or paracetamol.

FIGURE 2. Effect of PNF1 on the hot-plate test in mice. *Significantly different from control at $p < .05$, **significantly different from control at $p < .01$.

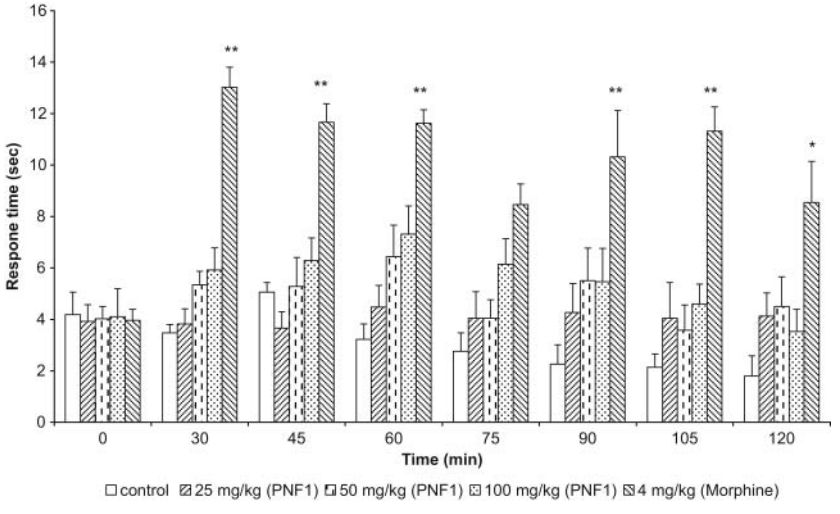
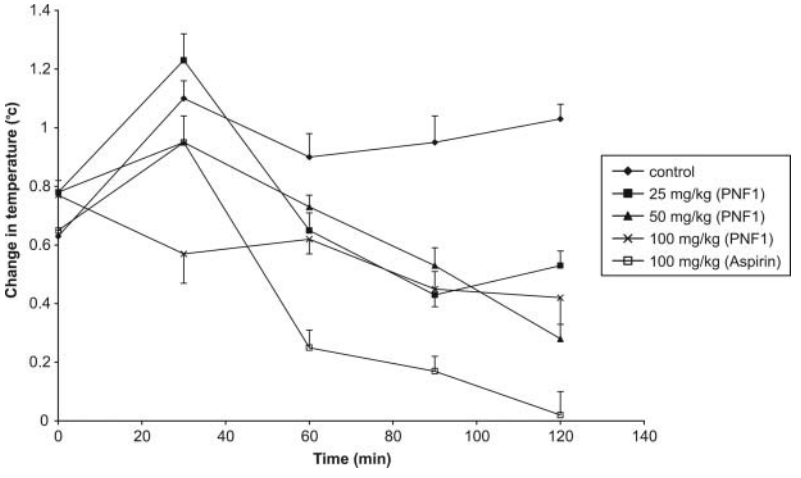


TABLE 2. Effect of PNF1 on Pain in Inflamed and Intact Tissue (Randall–Selitto Test) in Rats

Treatment	Dose (mg/kg)		Pain Threshold/Time (g/min)				
			0 min	30 min	60 min	90 min	120 min
Distilled water	–	Inflamed	9.70 ± 0.60	7.00 ± 1.03	7.80 ± 0.56	9.00 ± 0.55	8.90 ± 0.33
		Intact	7.40 ± 0.69	8.20 ± 0.20	7.80 ± 0.34	6.52 ± 0.83	4.84 ± 0.59
PNF1	25	Inflamed	9.00 ± 0.59	8.74 ± 1.11	10.68 ± 0.85	9.96 ± 0.65	10.46 ± 0.59
		Intact	9.50 ± 0.32	8.40 ± 1.68	9.82 ± 0.40	7.40 ± 0.43	8.40 ± 0.19
	50	Inflamed	7.20 ± 0.46	9.70 ± 0.98	10.64 ± 0.75[a]	10.50 ± 0.63	10.96 ± 1.41[a]
		Intact	10.10 ± 0.91	10.40 ± 0.94	11.20 ± 0.44	8.10 ± 0.66	8.70 ± 1.19
	100	Inflamed	5.00 ± 0.98	9.10 ± 1.20[a]	9.00 ± 0.65[a]	11.34 ± 1.55[b]	11.00 ± 0.61[b]
		Intact	7.30 ± 1.02	8.80 ± 0.80	7.70 ± 1.18	7.50 ± 0.98	6.70 ± 1.17
Aspirin	100	Inflamed	7.70 ± 0.75	13.62 ± 1.65[b]	14.26 ± 0.83[b]	12.80 ± 1.17[a]	12.80 ± 1.04[a]
		Intact	6.70 ± 1.19	7.40 ± 1.85	7.40 ± 1.39	8.70 ± 0.89	7.70 ± 2.03
Morphine	4	Inflamed	6.85 ± 1.11	12.38 ± 1.55[a]	12.13 ± 1.03[a]	12.38 ± 1.07[a]	12.75 ± 0.66[a]
		Intact	7.25 ± 0.92	11.63 ± 1.33[a]	11.15 ± 0.90[a]	11.78 ± 0.99[a]	11.35 ± 0.77[a]

[a]Significantly different from control at $p < .05$.
[b]Significantly different from control at $p < .01$.

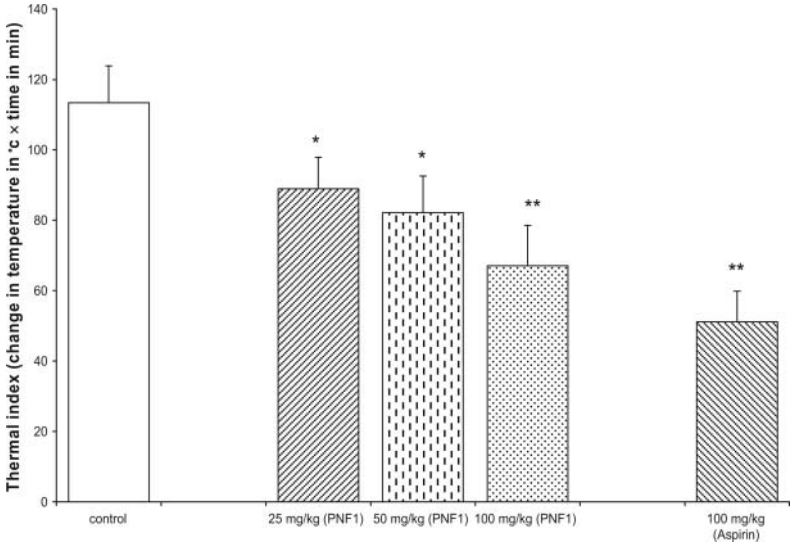
FIGURE 3 (a). Effect of PNF1 on yeast-induced pyrexia in mice. *Significantly different from control at $p < .05$, **significantly different from control at $p < .01$.



In the hot-plate test, PNF1 increased the response time to thermally stimulated pain in a dose-dependent manner up to 60 min after treatment (Figure 2). These changes were, however, insignificant ($p < .05$) compared with the negative control. Treatment with morphine significantly ($p < .05, p < .01$) increased the response time to thermal stimulus. As an opiate analgesic, morphine inhibits the simulation of sensory pain receptors by a centrally mediated action. Thus, at the doses of PNF1 used, its antinociceptive activity may not be mediated centrally.

In the Randall–Selitto test, significant reduction ($p < .05$) was observed in inflamed tissue of the group that received 50 mg PNF1/kg bw at 60 and 120 min after treatment

FIGURE 3 (b). Effect of PNF1 on fever index in mice. *Significantly different from control at $p < .05$, **significantly different from control at $p < .01$.



(Table 2). Treatment with PNF1 (100 mg/kg bw) significantly ($p < .05$, $p < .01$) increased only the threshold of the inflamed paw up to 120 min after treatment similar to the peripherally acting analgesic, aspirin, whereas the opiate analgesic (morphine) increased the threshold of both the intact and inflamed paws. These results give further evidence of the peripheral analgesic activity of PNF1. On the basis of the principle that inflammation reduces pain threshold, which is responsive to nonnarcotic salicylates and narcotic analgesics (Dubinsky, Gebre-Mariam, Capetola, & Rosenthale, 1987), this test distinguishes PNF1 as an extract, which contains principles with predominantly peripheral analgesic activity, similar to aspirin.

In yeast-induced pyrexia, fever was reduced within 30 min in the group that received 100 mg PNF1/kg bw. At doses 25 and 50 mg PNF1/kg bw, a significant ($p < .05$) decline in body temperature was observed 1 hr after treatment [Figure 3(a)], as indicated by reduction of fever indices by 21.56% and 27.51%, respectively. Treatment with 100 mg PNF/kg bw significantly ($p < .01$) reduced fever index by 40.87%, comparable to the effect of aspirin (100 mg/kg bw), which significantly reduced fever index by 54.89% [Figure 3(b)]. The subcutaneous injection of yeast in rats produces a febrile reaction due to a disturbance in hypothalamic regulation of body temperature, mediated by the production of prostaglandins (Al-Ghamdy, 2001). Fever is reduced by the administration of anti-inflammatory compounds like aspirin. Thus, the activity of the extract against fever also supports its anti-inflammatory activity.

CONCLUSIONS

This study shows that PNF1 possesses antipyretic, anti-inflammatory, and analgesic properties that are peripherally mediated and rationalize its use in febrile illnesses and pain. Further studies are ongoing in our laboratory on this fraction to isolate the compound responsible for its observed activity.

Declaration of Interest: The authors report no conflict of interest. The authors alone are responsible for the content and writing of this article.

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