

Original Research Article

In vivo Evaluation of Analgesic Activities of *Phyllanthus niruri* Leaf Methanol Extract in Experimental Animal Models.

ABSTRACT

INTRODUCTION: *Phyllanthus niruri* is a highly valued plant, commonly distributed in many countries of the tropics and subtropics. It has a wide range of uses as traditional medicine in many countries. However, this study was designed to evaluate the analgesic activity of the methanol extract of *Phyllanthus niruri* leaves in animal models.

METHODS: The Methanol extract of *Phyllanthus niruri* (PN) leaves was evaluated for analgesic action using standard hotplate test in mice and analgesy meter method in rats. The activities of the extract administered orally at different doses 300, 600, 750 mg/kg body weight doses were evaluated and compared with standard drug and control group treated with normal saline.

RESULTS: The dose 600 and 750 mg/kg b.w. of PN leaf extract exhibited significant ($p < 0.05$) analgesic effect in both hot plate and analgesy meter pressure-induced method. The activity showed a dose dependent profile.

CONCLUSION: This study showed that *Phyllanthus niruri* has strong potential analgesic activity. This supports its use as a potent analgesic drug in herbal medicine.

KEYWORDS: *Phyllanthus niruri*, Plant, leaf extract, analgesics, mice

1. INTRODUCTION

Phyllanthus niruri Linn (Family: Euphorbiaceae), is a tropical plant that is widely spread and commonly found throughout the tropical and sub-tropical areas, best known by the common names stonebreaker or seed-under-leaf. The plant belongs to the genus *Phyllanthus*, comprises over 600 species of shrubs [1]. In Nigeria, it is mostly common in raining season and locally referred to as "Eyin-olibisowo or dobisowo" in Yoruba, "Buchi oro" in Ibo, "Oyomekeso" in Efik [2]. *P. niruri* is indigenous to the rain forest of the amazon and other tropical areas. The bark is smooth and light green. It bears numerous pale green flowers which are often flushed with red. The fruits are tiny, smooth capsules containing seeds [3].

15 The plant possesses some pharmacological properties and thus used in traditional system of
16 medicine for the treatment of various diseases such as disturbances of kidney and bladder calculus,
17 intestinal infections, diabetes and hepatitis B virus [4,5]. Previous phytochemicals and
18 pharmacological investigations have shown that the main active components of *P. niruri* aerial parts
19 are Tannins [6], Alkaloids [7], Phenolic compounds and Flavonoids [4]. Studies have shown that
20 *Phyllanthus niruri* extract have many medicinal use such as, reduction of urinary calcium [3], reduction
21 of the incidence of kidney stone formation [8] and hepatoprotective action [9]. A recent study also
22 reported the interference of *Phyllanthus niruri* with many stages of stone formation, reducing crystal
23 aggregation, modifying their structure and composition as well as altering the interaction of the
24 crystals with tubular cells leading to reduced subsequent endocytosis [10]. Apart from these medicinal
25 uses, there are reports showing anti-viral effects of some *Phyllanthus* species, mainly against
26 hepatitis B virus [11,12], and *P. niruri* inhibits human immunodeficiency virus type-1 reverse
27 transcriptase (HIV-1-RT) as well [13,14].

28 In addition, western medicine is well known and in use, but at the same time it has created problems
29 due to some of its side effects such as carcinogenicity caused by synthetic drugs [15]. This has
30 entranced the interest in search of natural products with medicinal property e.g. natural occurring
31 anti-oxidant and antibiotic for use in foods and medicine. Therefore, herbal medicine has been
32 considered as an alternative to eradicate the side effects associated with synthetic drugs. This study
33 therefore, aimed to investigate the anti-nociceptive properties of methanolic leaves extract of
34 *Phyllanthus niruri* in different animal models. Although the extracts or active principles of *Phyllanthus*
35 *niruri* has been investigated in several biological models [1,12,16,17,18,19] however, further
36 investigation of the anti-nociceptive properties of the plant would be beneficial for its medicinal use.

37 2. MATERIALS AND METHODS

38 2.1 Plant materials

39 The leaves of *Phyllanthus niruri* were collected within the campus of Ekiti State University, Ado Ekiti,
40 Nigeria in May 2014. The plants was identified and authenticated at the Department of Plant Science,
41 Ekiti State University, Ado-Ekiti, Nigeria, where a voucher specimen was deposited.

43 2.2 Preparation of the Plant Extract

44 Freshly collected *Phyllanthus niruri* (PN) leaves were separated from adhering materials such as
45 weed, and rinsed with distilled water. The leaves were shade-dried for two weeks before they were
46 ground into a coarse powder with a kitchen blender. The powder was kept in a dry, cool and dark

place in an airtight container. Five hundred and ten grams (510 g) of ground *Phyllanthus niruri* leaves were mixed with 900 mL of 95% methanol in a clean, flat-bottomed glass container. The container was sealed and kept for a week with occasional shaking. This extract was filtered, and the filtrate was concentrated in vacuo to dryness at 40°C using a rotary evaporator. The extract yield was 7.2 % and was preserved at 4°C until used. The extracts were dissolved in 0.9% NaCl solution to the desired concentration just before use.

2.3 Equipment

Eddy's hot plate (Life Science No. 8 Model 39), Ugo Basile analgesy-meter (No. 7200), stop watch, Rotatory evaporator and Water bath were used in the study.

2.4 Experimental Animals

Swiss albino mice (25-30 g) and Wister rat (150-200 g) of both sexes were used for the experiment. The animal were procured from the experimental Animal House of College of Medicine, Ekiti State University, Nigeria. The animals were kept in cages within the animal house and allowed free access to water and standard livestock pellets. They were examined and found to be free of wounds, swellings and infections before the commencement of the experiment. All experimental procedures were conducted in accordance with accepted standard guidelines of National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication no. 85–23, revised 1985). All experiments were conducted in the Research Laboratory of Department of Pharmacology, College of Medicine, Ekiti State University, Nigeria.

2.5 Chemicals and reagents

All the chemicals and drugs used were of analytical grade. Methanol (Merck, Germany), Normal saline (0.9 % Sodium chloride) and Acetyl salicylic acid (Aspirin, ASA).

2.6 Experimental Design

2.6.1 Acute Toxicity Test

The method employed by Ezeja et al [20] was used for this study. Twenty mice of both sexes were randomly divided into 4 groups of 5 mice each. Group A served as control with each mouse receiving 10 mL/kg body weight (b.w.) of normal saline. Groups B, C and D were treated orally with varying doses of the *Phyllanthus niruri* extract (500, 1000 and 1500 mg/kg b.w) respectively. The doses were selected based on maximum doses in the study and from previous study [21]. Animals were fasted for 12 hr with free access to water only, during which they were observed for signs of toxicity and death for 3 days. The dose administered was considered toxic if mortality was observed in 3/5 or 5/5 animals. However, if the mortality was observed in only one mice out of five animals, then the same dose was repeated with higher doses. General behaviours such as motor activity, tremors, convulsions, straub reaction, aggressiveness, pilo erection, loss of lighting reflex, sedation, muscle relaxation, hypnosis, analgesia, ptosis, lacrimation, diarrhoea and skin colour were observed.

87

88 2.6.2 Hot plate Test

89 Mice were randomly divided into the following groups of 5 mice each: (I) Control group (Normal
90 Saline, NS), (II) aspirin group (Standard reference drug, 15 mg/kg ASA), and (III - V) three *P. niruri*
91 extract groups (300, 600 and 750 mg/kg). Anti-nociceptive drug activity was assessed using the hot
92 plate test as previously reported [22]). The temperature of the metal surface was maintained at 56±1
93 °C. The latency between the placement and shaking or the licking of the hind paws or the jumping
94 response of the animals was recorded as the latent response. The mice were screened in advance,
95 and a pre-test latency of 5–30 s qualified them for the experiment. The mice were treated with NS (10
96 mL/kg, p.o.) or PN extract (300, 600 or 750 mg/kg, p.o.) 30 min before the test. Aspirin (15 mg/kg,
97 p.o.) was used as a positive control drug and was administered 30 min before the experiment. A 60
98 second cut-off time was used to minimize tissue damage in the mice. Latencies were measured prior
99 to treatment and at 30, 60, 90, and 120 min after drug administration. This experiment was repeated
100 thrice.

101

102 2.6.3 Pressure test using Analgesy Meter

103 Wister rats were randomly separated into 4 groups of 5 as follows: Group I, the control group,
104 received 10 mL/kg of normal saline. Group II, the positive control group, was treated with standard
105 drug ASA 15 mg/kg body weight. Group III, test group, administered with 300 mg/kg b.w. PN extract.
106 Group IV, test group, administered with 600 mg/kg b.w. PN extract and Group V, test group,
107 administered with 750 mg/kg b.w. PN extract respectively. Treatments (control vehicle, extracts and
108 standard drug) were administered orally 30 min prior to pressure test.

109

110 In these experiments, different doses of the extract and standard drug were tested in rat using an Ugo
111 Basile Analgesy-Meter (N° 7200). A constant force was applied to the left hind paw of experimental
112 animals by the Analgesy-Meter plunger. Rat were restrained in the upright position while their left hind
113 paws were placed between the plinth and the plunger. Pain was determined by the physical struggles
114 of the animal to set itself free. The weight causing pain before treatment was used at time intervals:
115 0.5, 1, 1.5, 2 and 3hr after treatment of animals with the various doses. The time at which the animal
116 starts physical struggling to free itself was recorded and the experiment was repeated three times.

117

118 2.7 Statistical analysis

119 GraphPad Prism Version 5 software was used for statistical analysis. Values are expressed as
120 mean±S.E.M. Student's *t*-test was carried out to compare the results of control and test drug groups.
121 Data were considered to be significant when $P < 0.05$.

122

123 3. RESULTS

124 3.1 Acute Toxicity

125 The methanol leaves extract of *Phyllanthus niruri* (PN) was evaluated for its acute toxicity in mice.
126 The extract did not alter the general behaviour and failed to produce any mortality even at the highest

dose of 1500 mg/kg. The mice were found safe after observation for 5 days except in the group that was administered with 1000 mg/kg was found one animal dead. Previous study showed that the plant extract is safe up to 5 g/kg body weight [23]

3.2 Hot plate Test

Results of analgesic activity of acetyl salicylic acid (ASA) and treatment doses of methanol extract of *Phyllanthus niruri* measured by hot plate method is presented in Table 1. As shown in table 1 the oral administration of the methanol extract of *P. niruri* enhanced the latency time of mice 30 min after administration of the extracts and exhibited a dose dependent analgesic effect at all the three doses used as compared to control group ($p < 0.05$). Maximum analgesic effect was noted at a dose of 750 mg/kg ($p < 0.05$) after administration of the extract among the three doses but ASA (standard reference drug) exhibited significantly high effect at 30min after administration compared to control and the methanol leave extract doses.

Table 1: Anti-nociceptive effect of methanol leave extract of *P. niruri* on hot-plate test in mice.

Latency (sec) after administration					
Dose	0 min	30 min	60 min	90 min	120 min
Control (NS)	6.0±0.6	5.8±0.5	5.4±0.4	5.8±0.4	5.7±0.3
ASA 15 mg/kg	5.8±0.6	12.5±0.3	13.1±0.6	12.4±0.8	13.9±0.2
Extract 300 mg/kg	8.0±2.3	9.2±0.3*	10.2±0.9	10.7±0.8	10.8±0.4*
Extract 600 mg/kg	5.5±0.6	10.7±0.4**	10.9±0.7*	11.7±0.9	12.2±0.9
Extract 750 mg/kg	7.2±1.1	11.5±0.2*	13.2±0.3 [#]	14.4±0.4** [#]	14.6±0.1 [#]

Vehicle control mice were administered with normal saline, and ASA (15 mg/kg) was used as the positive control. [#]comparison between doses of extract. Results are expressed as the mean ± SEM (n= 5) of the reaction time in seconds. * $p < 0.05$, ** $p < 0.01$ were considered significant when compared at the same time with ASA after administration.

3.2 Analgesy Meter Test.

The analgesic activity of the methanol leaves extract of *P. niruri*, normal saline, ASA on mechanical pain induced by the plinth plunges of Analgesy meter was evaluated. The result is summarized in Figure 1 and 2. The extract 600 and 750 mg/kg exhibited good analgesic effect throughout the study period when compared with the standard reference drug or positive control, ASA group. The group administered with 600 mg/kg extract showed significantly high activity at 0.5, 1.5 and 2hr ($p < 0.05$) respectively. Likewise, the group that was administered with 750 mg/kg showed high activity 0.5 and 1.5hr ($p < 0.05$), then at 1 and 2 hr ($p < 0.001$) when compared with the ASA group. However, the analgesic effect of the extract is dose dependent (Figure 1 and 2). The maximal pain tolerance by the animal with the extract were observed 2hr after drug administration. The activity of 300 mg/kg was lower when compared with ASA ($p > 0.05$) but higher than the negative control group administered with NS.

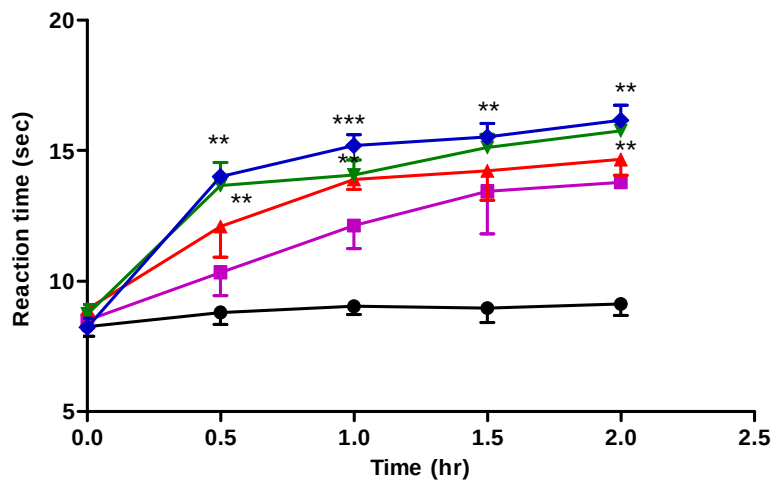


Figure 1: Effects of methanol extracts of *Phyllanthus niruri* at 300 mg/kg, 600 mg/kg, 750 mg/kg, 15 mg/kg b.w. ASA and Control on pressure induced pain using Analgesy-meter in mice (n=5). *** $p < 0.001$ or ** $p < 0.05$ was considered significant when compared at the same time with the ASA.

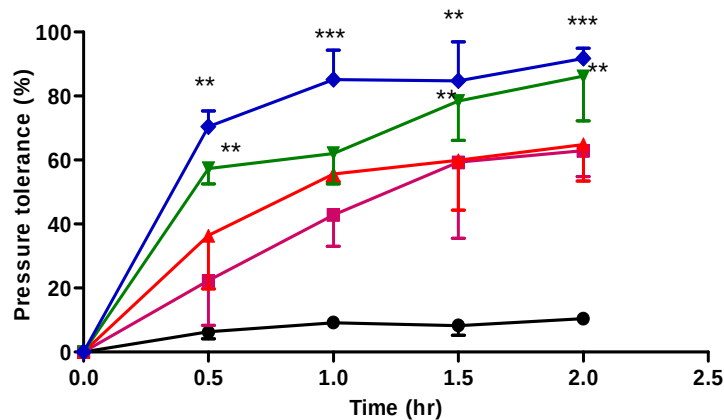


Figure 2: Anti-nociceptive effects of methanol extract of *Phyllanthus niruri* (PN) at 300 mg/kg, 600 mg/kg, 750 mg/kg, 15 mg/kg b.w. ASA and Control in pressure-induced pain (n=5). *** $p < 0.001$ or ** $p < 0.05$ was considered significant when compared at the same time with the ASA.

4. Discussion

This study investigated the analgesic effect of methanol leaves extract of *Phyllanthus niruri* in animal models. The methanol leaves extract of PN produced highly significant analgesic effect on the experimental animals.

The present study employed two different models for investigation of the analgesic effect of *Phyllanthus niruri* methanol leaves extract. The use of hot plate-induced pain and analgesy-meter

pressure tests were selected to investigate peripheral and central anti-nociceptive effects of the plant extract. The eddy's hot plate test is commonly used in elucidating the centrally mediated anti-nociceptive effects of substances based on significant increase in the latency time. ASA and all three doses of PN displayed marked anti-nociceptive effects. The analgesic effect of ASA was stronger than that of PN at 0.5 and 1 hr post-treatment. However, at 1.5 and 2 hr post-treatment, the highest and medium doses of PN displayed a stronger effect on the nociceptive threshold than ASA, and the effects of PN showed a dose-dependent response.

Generally, heat induces low pain reaction threshold (latency time) that is elevated by the use of analgesic agents. In this study, marked elevation in latency time was exhibited by PN similar to ASA during the hot plate test. The latency time is related to the response of nociceptors, it is therefore, possible that PN interferes with the synthesis of prostaglandins. In addition previous study had suggested that the anti-nociceptive effects of hydroalcoholic extract of *P. urinaria* and *P. niruri* did not depend on the endogenous release of glucocorticoids [24]. The results of this study are similar to the earlier reports that NSAIDs and plant derived products elevates the latency time during hot plate test in animals [25,26].

The use of analgesy meter is known to be a more sensitive and an observational method of measuring analgesic effect on mechanically-induced pain in animal model. This induced-pressure method is most sensitive to centrally acting analgesics, and pain induced by the analgesy-meter also provides a model for the study of non-inflammatory pain [27]. The oral administration of ASA and methanol extracts of *P. niruri* test showed increase in nociceptive threshold in rat and this was observed throughout the period of the experiment especially in the highest dose of PN. Although at up to 60min, there was no significant difference between the tolerance of ASA and 600mg/kg dose group, and thereafter the extract administered groups especially the highest dose expressed high tolerance time. The anti-nociceptive effect of the plant appears more slowly and strengthens over time. This could be explained that the plant extract releases the active component slowly into the systemic circulation. This is an indication that the plant could be useful in alleviating long term pain especially in terminal diseases. Similarly, a study conducted by Santos et al., showed that the genus *Phyllanthus* exhibits long lasting systemic analgesic effect especially in neurogenic pain [28]. In addition, significant reduction in the animal sensitivity to pressure-induced pain showed that the central protecting effect of methanol extracts of *P. niruri* were comparable to ASA. Acetylsalicylic acid is a known NSAIDs that has been shown to respond strongly to secondary pain responses during phase II (inflammatory phase), a combination of pain-induced central effect and peripheral inflammation [29,30].

In addition, the results also strongly suggest that the extracts possess peripheral analgesic activity and their mechanisms of action may be mediated through inhibition of local peritoneal receptors or arachidonic acid pathways, involving cyclo-oxygenases and/or lipoxygenases [31,32]. The significant analgesic effects observed on analgesy-meter test buttresses the effect of the extracts on peripheral local receptors. These findings strongly suggest that the analgesic effects of the active principle(s) present in these plants might result, at least in part, from their interactions with the tachykinin system. Previous studies have shown that the anti-nociception effects of hydroalcoholic extract of *P.*

urinaria and *P. niruri* did not depend on the endogenous release of glucocorticoids. In peripheral tissues, prostaglandins and kinins are suggested to play an important role in the pain process [33]. These results suggest that this plant may exercise their pain killing effect by the prostaglandins synthesis inhibition, as peripheral pain inducers and injury-like stimuli induce the release and/or increase the production of a wide variety of chemicals and cytokines in peripheral tissues. However this study also confirms previous study on potent analgesic activity possessed by the plant against pain models in rats although different anti-nociceptive models were employed [24].

Phytochemical screening of the crude extract of *P. niruri* revealed the presence of alkaloids, lignans, flavonoids, terpenoids, polyphenols, tannins, coumarins and saponins from various parts of the plants [1]. Analgesic and anti-inflammatory actions in some natural products have been attributed to the presence of alkaloids [34,35,36,37]. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammations and pain perception [37]. In addition, there are few reports on the role of tannins and saponins in anti-nociceptive and anti-inflammatory activities [38,39]. It is therefore possible that the presence of these biological principles may responsible for the anti-analgesic activity of *P. niruri*.

CONCLUSION

Methanol leaves extract of *Phyllanthus niruri* exhibit a distinctive analgesic property in experimental animal models. Thus, the present study validate the traditional use of *P. niruri* and previous studies in treatment of pain related disorder. However, further studies are warranted to know the exact active compound(s) eliciting this effect and the main mechanism responsible for the analgesic properties

ETHICAL APPROVAL

The author declare that this work was not against public interest. Animal experiments were conducted in accordance with NIH guidelines for care and use of Laboratory animals (Pub. No. 85– 23, Revised 1985).

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