Acute toxicity, antinociceptive and anti-inflammatory activity of the essential oil of fresh fruits of *Piper guineense* Schum & Thonn (Piperaceae) in rodents

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*Piper guineense* is a popular herbal medicine used to manage pains and arthritis among other indications in South-West Nigeria. Previous biological studies report anti-oxidant, anti-microbial and antidiabetic activities for the essential oil of the plant while studies on its acute toxicity profile, potential analgesic and anti-inflammatory activities were unavailable. This study investigated the antinociceptive and anti-inflammatory effect of the plant fruit volatile component and determines its acute toxicity profile in rodents in an attempt to rationalize the use of the plant in folkloric medicine. Essential oil of fresh fruits of *P. guineense* obtained by hydrodistillation was emulsified with Tween 80 and evaluated for acute toxicity test (LD₅₀) through the oral (p.o.) and intraperitoneal (i.p.) routes in mice. The oil (50 to 200 mg/kg, i.p.) was tested for anti-nociceptive activity on the hot plate and acetic acid-induced writhing models in mice, while the anti-inflammatory activity was assessed on the egg albumin-induced rat paw oedema. The LD₅₀ values obtained were 693 mg/kg, i.p. and 1265 mg/kg, p.o. The oil dose-dependently caused significant (p<0.01) prolongation of reaction time on the hot plate comparable to positive control, morphine signifying central antinociceptive effect, significantly (p<0.01) inhibited writhings induced by acetic acid analogous to diclofenac suggesting peripheral mechanism and caused significant (p<0.01) reduction in egg albumin-induced rat paw oedema comparable to dexamethasone, indicating anti-inflammatory activity. This study shows that the essential oil of *P. guineense* was moderately toxic, possessed significant antinociceptive and anti-inflammatory activities which can be used to rationalize the use of the plant in ethnomedicine.

**Key words:** *Piper guineense*, volatile oil, acute toxicity, hot plate, egg albumin.

**INTRODUCTION**

*Piper guineense* otherwise known as West African Black Pepper is a herbaceous climber commonly found in African tropical forest zone, with more than 700 species found in many tropical and sub-tropical regions of the world (Olonisakin et al., 2006). In Nigeria, it is known with different vernacular names such as *Užiza* and *Iyere* among
the Igbos and Yorubas, respectively. The fruits or berries and leaves are usually sold in Southern Nigerian markets as condiments and for food flavouring agent. The plant is one of the highly valued spices across West African countries where its fruits and leaves (Figure 1) form important ingredients in domestic cooking and commercial cuisines. In Southern Nigeria, *P. guineense* is popular in folkmedines for the management of several health-related conditions including respiratory disorders, infections, infertility, pain, rheumatism and as an aphrodisiac (Ekundayo et al., 1988; Burkill, 1995; Ekanem et al., 2010; Tankam and Ito, 2013).

Chemical composition of *P. guineense* varies from one geographical region to another and even within the same region. For example, Ekundayo et al. (1988) reported myristicin, safrole, sarisan and elemicin as major components of its fruits while Oyedeji et al. (2005) reported β-pinene, α-pinene and germacrene-B as the major components. Further studies also reported β-pinene, D-Limonene, Caryophyllene and car-z-ene as the main constituents (Olinsakin et al., 2006), yet another report indicate β-pinene, α-pinene, 1,8-cineole and γ-terpinene as major constituents of the plant fruit essential oil from Nigerian species (Oboh et al., 2013).

Biological studies on the essential oil of *P. guineense* include fertility enhancing (Mbongue et al., 2005), anti-fertility (Ekanem et al., 2010), anti-oxidant (Etim et al., 2013), hypolipidemic and hypokalaemic (Nwaichi and Igbinobaro, 2012), insecticidal (Madubuike et al., 1990; Adewoyin et al., 2006), anti-microbial (Oyedeji et al., 2005), anti-diabetic and antioxidant (Oboh et al., 2013), sedative (Tankam and Ito, 2013) and larvicidal (Ohaga et al., 2007) among numerous activities.

Preliminary enquiries (field study) from herbalists in some communities within Ondo State (South West Nigeria) indicate that fruits of *P. guineense* are indicated for muscular pain, rheumatism and as an aphrodisiac either as a single agent or in combination with other herbal agents (oral communication). Since essential oil constitute a major active components of medicinal plants with considerable bioactivities, the essential oil of this plant was evaluated for antinociceptive and anti-inflammatory activity as a preliminary screening process for validation of the folklic use of the plant in managing pain-related ailments. Furthermore, there is no data available to our knowledge concerning the acute toxicity profile of the essential oil of the fresh fruits of the plant; hence we also evaluated the oil for acute toxicity (LD50) test in order to determine its relative toxic profile orally and parenterally.

**MATERIALS AND METHODS**

**Plant identification and authentification**

The Fruits of *P. guineense* were authenticated by Mr. G. Ibhanesebor, the Herbarium Officer of the Department of Botany, Faculty of Science, Obafemi Awolowo University (OAU) Ile-Ife, Osun State. The voucher specimen of the plant and its fruits were deposited at the herbarium unit of the Department of Botany, Faculty of Sciences, OAU, Ile-Ife. The Voucher specimen of the plant and its fruits were deposited at the Herbarium Unit of the Department of Botany, Faculty of Sciences, OAU, Ile-Ife, as voucher NO. IFE 16772 was issued. Fresh fruits were purchased from the Central Market, Ondo Town, Ondo State between October and December, 2012.

**Extraction of the essential oil**

Hydrodistillation of the fruit of *P. guineense* was carried out using cleveger-like apparatus (BP 1988). Fresh fruits of *P. guineense* (400 g) were slightly crushed to open the fruits before hydrodistilled for 4 h. The essential oil obtained was dried over magnesium sulphate crystal and stored in an air-tight bottle, refrigerated until use.

**Laboratory materials and equipment**

Plexiglass cage, hot plate machine, digital thermometer, fresh egg, methylated spirit, Tween 80 and other reagents were of analytical grade.

**Drugs**

Morphine (Sigma, St. Louis, USA), acetic acid (BDH Chemicals Ltd, Poole, England), diclofenac (Supreme Pharm. Nig. Ltd., Lagos, Nigeria), dexamethasone (Hubei Tianyo pharmaceuticals, China) and other chemicals and drugs used were of analytical grade.

**Laboratory animals**

Swiss albino mice (18 to 25 g) of both sexes and rats (150 to 250 kg) of both sexes were obtained from the Animal House, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. The animals were kept under standard laboratory conditions and fed with animal pellets and they had free access to water *ad libitum*. The study was approved by the Faculty of Pharmacy Postgraduate Committee and all animal experiments were carried out in strict compliance with the guideline of the National Institute of Health (NIH, 1985) as being implemented by the Obafemi Awolowo University Research Committee.

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Acute toxicity study

The method used was described by Lorke (Lorke, 1983). This involved using the 13-animal model for rapid determination of LD$_{50}$. The method involves two phases. In the first phase of both the oral and i.p. route three increasing doses of 10, 100 and 1000 mg/kg of emulsified EOPG were administered to three different groups of mice (n = 3). In the second phase, doses of 1000, 1600, 2900 and 5000 mg/kg were used for the oral route, while doses of 400, 600, 800 and 1000 mg/kg were used for the i.p route in 4 groups of mice (n = 1). The animals were monitored for 2 h and mortality was recorded after 24 h.

Pharmacological study

Experimental design

In all the tests, animals were randomly selected into 5 different groups (n=5) as follows:

- Group I: Negative control treated with vehicle (5% Tween 80, 10 ml/kg).
- Group II-IV: test groups treated with the essential oil 50, 100 and 200 mg/kg, respectively.
- Group V: positive control treated with appropriate standard drug.

All treatments were by intraperitoneal route.

Antinociceptive test

Hot plate test

Vehicle, essential oil (50, 100 and 200 mg/kg) and morphine (10 mg/kg) were administered to different groups of mice and after 30 min, each mouse was placed on the hot-plate pre-set at 55°C and the time taken by the mouse to lick the fore/hind paw was taken as the reaction time in s. The cut off time was set at 15 s to avoid tissue damage. The test was repeated and reaction time recorded at 60, 90 and 120 min post-treatment (Silva et al., 2003).

Acetic acid-induced writhings

Different groups of mice were pretreated for 30 min with vehicle, essential oil (50, 100 and 200 mg/kg) and diclofenac (100 mg/kg) before intraperitoneally administration with 10 ml/kg of 1% acetic acid (Hajhashemi et al., 2003). The number of writhings (abdominal constriction) displayed by each mouse was counted and recorded over a period of 20 min starting from 5 min post-acid injection (Yin et al., 2003).

Antiinflammatory test

The anti-inflammatory activity was studied using egg albumin-induced paw oedema acute inflammation methods in rats previously described by Olajide et al. (2000) with minor modification. Different groups of rats were pretreated with vehicle, essential oil (50, 100 and 200 mg/kg) and dexamethazone (1 mg/kg) for 30 min prior to injection of 100 µl of undiluted fresh egg albumin into the sub-planter surface of right hand paw of the rats. Measurement of the rats’ paw sizes were carried out by measuring the circumference of the oedematos paw with thread wrapped round the paw and then placing the thread on a meter ruler to determine the diameter in mm. The circumference (mm) of the oedematos paw equates inflamed paw of the rat (Olajide et al., 2000).

The measurement of paw size was repeated at 1, 2 and 4 h post injection of the egg albumin and the % inhibition calculated for each treatment group using the formula:

\[
\text{% inhibition} = \frac{[(C_t - C_o) \text{ control} - (C_t - C_o) \text{ treated}]}{(C_t - C_o) \text{ control}} \times 100
\]

Where Ct and Co are paw sizes (mm) at different time after and before egg-albumin injection (Olajide et al., 2000).

Statistical analysis

Results are expressed as mean ± standard error of mean (SEM) and analysed by one way analysis (ANOVA), followed by Dunnett’s post-hoc test with level of significance set at p < 0.05.

RESULTS

Essential oil obtained

The yield of the essential oil was 1.25% w/w. The oil was colourless with characteristic pungent aromatic odour and its relative density was determined to be 833 mg/L (0.88 g/L). The essential oil was readily soluble in Tween 80 and was therefore used to emulsiify the oil prior to each test at concentration ≤5% v/v.

Acute toxicity (LD$_{50}$)

The results of the acute toxicity study indicate that the LD$_{50}$ of the oil was calculated to be 693 and 1265 mg/kg for the intraperitoneal and oral routes, respectively.

Effect of the essential oil on the hot plate and acetic acid-induced writhings

The result of the hot plate test is presented in Table 1. Essential oil of P. guineense (50, 100 and 200 mg/kg) and morphine induced significant (p < 0.01) increase in reaction time to thermal stimulation on the hot plate test at 30 [F(4,20) = 13], 60 [F(4,20) = 3.5], 90 [F(4,20) = 6] and 120 [F(4,20) = 54] min post-treatment. The mean reaction time (s) at 30 min for the oil (50, 100 and 200) were 9.64, 9.80 and 16.35, respectively compared to 5.7 for vehicle and 16.4 for morphine; at 60 min, for the oil (50, 100 and 200) were 12.9, 15.8 and 13.8, respectively compared to 9.7
Figure 1. *Piper guineense* plant. Panel A shows the leaves and unripe fruits and panel B the ripe fruits.

Figure 2. Effect of the essential oil of *P. guineense* on acetic acid-induced writhings in mice. VEH, EO and DFC represent vehicle (5% Tween 80), essential oil of *P. guineense* and diclofenac respectively. **p<0.01, statistically lower than vehicle (ANOVA, Dunnett’s test).

for vehicle and 21.2 for morphine; at 90 min, the oil (50, 100 and 200) were 14.8, 22.2 and 17.6, respectively compared to 10.1 for vehicle and 25.2 for morphine; and at 120 min, for the oil (50, 100 and 200) were 18.4, 16.8 and 23.0, respectively compared to 10.7 for vehicle and 13.6 for morphine. All values above 15 s were equalized to 15 as the maximum cut-off point for all reaction time. The result of the acetic acid induced writhings (Figure 2) showed that the oil (50, 100 and 200 mg/kg) and diclofenac (100 mg/kg) significantly (p < 0.01; $F_{(4,20)} = 90$)
Table 1. Effect of essential oil of *P. guineense* on the hot plate test in mice.

<table>
<thead>
<tr>
<th>Treatment (i.p.) (n = 5)</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>5.68±0.22</td>
<td>9.70±1.09</td>
<td>10.20±0.38</td>
<td>10.72±0.40</td>
</tr>
<tr>
<td>Essential oil, <em>P. guineense</em> 50 mg/kg</td>
<td>9.64 ±1.55*</td>
<td>12.92±1.96*</td>
<td>14.8±1.97**</td>
<td>15.00±0.00**</td>
</tr>
<tr>
<td>Essential oil, <em>P. guineense</em> 100 mg/kg</td>
<td>9.80±0.66*</td>
<td>15.00±0.00*</td>
<td>15.00±0.00**</td>
<td>15.00±0.00**</td>
</tr>
<tr>
<td>Essential oil, <em>P. guineense</em> 200 mg/kg</td>
<td>12.10±1.26**</td>
<td>13.8±1.32</td>
<td>15.00±0.00**</td>
<td>15.00±0.00**</td>
</tr>
<tr>
<td>Morphine 10 mg/kg</td>
<td>15.00±0.00**</td>
<td>15.00±0.00*</td>
<td>15.00±0.00**</td>
<td>13.6±0.40**</td>
</tr>
<tr>
<td>P value</td>
<td><em>p&lt;0.05,</em> **p&lt;0.01</td>
<td>*p&lt;0.05</td>
<td>**p&lt;0.01</td>
<td>**p&lt;0.01</td>
</tr>
<tr>
<td>F value</td>
<td>F (4,20) = 13</td>
<td>F (4,20) = 3.5</td>
<td>F (4,20) = 6</td>
<td>F (4,20) = 54</td>
</tr>
</tbody>
</table>

Vehicle is 5% Tween 80. Each value represents mean ± SEM of reaction time (s). *p<0.05, **p<0.01; statistically different from vehicle (ANOVA, Dunnett’s test).

...reduced the number of writhes induced by acetic acid. The writhes was reduced from 84 (vehicle) to 31, 9 and 4 by the oil (50, 100 and 200 mg/kg) representing 62, 89 and 95%, respectively, while diclofenac reduced writhes to 11, producing 87% inhibition.

**Effect of the essential oil on egg albumin-induced inflammation**

Rats in the negative control group showed progressive increase in paw sizes throughout the observation period of 4 h while the essential oil of *P. guineense* significantly inhibited the oedema induced by the egg albumin in a dose-dependent manner which was comparable to the potent steroidal anti-inflammatory drug, dexamethasone (1 mg/kg). The reduction in oedema sizes varies from 46 to 89% for the oil and 77 to 94% for demxamethasone from half ½ to 4 h post induction of inflammation. The reduction in oedema sizes expressed in mean ± SEM and percentage inhibition by the oil and dexamethasone with their statistical values were presented in Table 1.

**DISCUSSION**

Antinociceptive and antiinflammatory activities of the essential oil of *P. guineense* was evaluated in addition to determination of its acute toxicity profile in this study. The antinociceptive test was evaluated on two models (the hot plate test and the acetic-acid induced writhings) in mice, while the antiinflammatory test was evaluated on egg albumin-induced rat paw oedema in rats. Results obtained from this study showed that the oil of this plant fruits demonstrated significant effects in all the models used while the acute toxicity results indicate different toxicity outcome depending on the route of administration.

The Lorke’s method (Lorke, 1983) has gained wide acceptance in preliminary screening of new agents for acute toxicity evaluation due to the use of minimal number of animals, its reliability and rapidness, and generally more economical than the previously used methods. Furthermore, the protocol is likely to be more tolerable by the Animal Rights groups agitating against the arbitrary use of animals in experiments. The LD$_{50}$ values obtained for the oil was 1265 and 696 mg/kg for oral and intraperitoneal routes, respectively indicating that the oil may be moderately toxic orally and more toxic parenterally (Rodricks, 1992). The LD$_{50}$ of the oral route of administration was quite higher than that of i.p. route due majorly to first pass effect that is, hepatic metabolism of drug when absorbed and delivered through portal blood (Pond and Tozer, 1984; Gavhane and Yadav, 2012). Metabolism of drug in the gastrointestinal tract (GIT) by the acidic and enzymatic contents and slower absorption rate can lead to lower bioavailability (Tracy et al., 2004). The intraperitoneal route gave faster and more consistent results which are readily reproducible (de Carvalho et al., 2001); hence it was used in this study. The LD$_{50}$ values obtained in this study show that this oil is relatively safer than those obtained from *Eucalyptus* species which have been reported to possess analgesic activities and whose LD$_{50}$ ranges from 190 to 353 mg/kg (Silva et al., 2003).

The hot plate test measures the complex responses to a non-inflammatory, acute nociceptive impulse and is one of the models normally used for studying central antinociceptive activity (Ranjit et al., 2006). In all the groups including vehicle, the reaction time on the hot plate were progressively prolonged with time (Table 1). This is in consonance with previous reports (Imam and Sume, 2014) which confirm prolongation of reaction time on the hot plate with subsequent tests probably due to adaptation or learning during observational periods of 120 or 240 min (Espejo et al., 1994; Casarrubea et al.,
Table 2. Effect of essential oil of *P. guineense* on egg albumin-induced paw oedema in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>2.6±0.3 (0)</td>
<td>3.8±0.2 (0)</td>
<td>3.6±0.3 (0)</td>
<td>3.4±0.3 (0)</td>
</tr>
<tr>
<td>Essential oil, 50 mg/kg</td>
<td>1.4±0.3* (46)</td>
<td>1.2±0.4** (68)</td>
<td>1.8±0.2* (50)</td>
<td>2.1±0.6 (38)</td>
</tr>
<tr>
<td>Essential oil, 100 mg/kg</td>
<td>0.8±0.2** (69)</td>
<td>1.2±0.4** (68)</td>
<td>0.8±0.2** (78)</td>
<td>1.4±0.4** (59)</td>
</tr>
<tr>
<td>Essential oil, 200 mg/kg</td>
<td>0.8±0.2** (69)</td>
<td>1.0±0.0** (74)</td>
<td>0.4±0.3** (89)</td>
<td>1.0±0.0** (71)</td>
</tr>
<tr>
<td>Dexamethasone 1 mg/kg</td>
<td>0.6±0.2** (77)</td>
<td>0.8±0.1** (79)</td>
<td>0.2±0.2** (94)</td>
<td>0.4±0.2** (88)</td>
</tr>
</tbody>
</table>

*P value* *p<0.05, **p<0.01; statistically lower than vehicle (ANOVA, Dunnette's test)***

Vehicle is 5% Tween 80. Values are expressed as Mean±SEM and % Inhibition in parenthesis. *p<0.05, **p<0.01; statistically lower than vehicle (ANOVA, Dunnette’s test).**

The results of the hot plate test show that the essential oil dose-dependently caused prolongation in the reaction time compared to vehicle at 30, 60 and 90 min post treatment (Table 1) almost comparable to morphine (potent opioid agonist), signifying central antinociceptive activity (Silva et al., 2003; Al-Nagger et al., 2003).

Pain sensation in acetic acid-induced writhing model is elicited by the triggering of local inflammatory responses leading to the release of free arachidonic acid from tissue phospholipid via cyclooxygenase and prostaglandin biosynthesis and has been associated with increased level of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products (Al-Nagger et al., 2003; Riberio et al., 2000), thereby facilitating inflammatory pain arising from enhanced capillary permeability. The effect of the essential oil on the acetic acid-induced writhing test (Figure 2) showed that it dose-dependently caused significant (p < 0.01) reduction in the number of writhes with the highest dose (200 mg/kg) producing 95% analgesia as against 87% for the standard nonsteroidal anti-inflammatory drugs (NSAIDs), diclofenac (100 mg/kg, i.p.) indicating peripheral antinociceptive activity (Silva et al., 2003), however the mechanism is not specific because acetic acid-induced writhings has been reported to lack specificity for either central or peripheral mechanism (Melo et al., 2013).

The egg albumin-induced paw oedema model has been used extensively for evaluating antinflammatory effect of medicinal plants (Anosike et al., 2012; Singh et al., 2012). The oil at all the doses used significantly inhibited the paw oedema significantly (p < 0.05 to 0.01) throughout the experimental period of 4 h (Table 2). The decrease in oedema varies between 46 and 89%. The highest inhibition caused by the oil (200 mg/kg) was comparable to the potent steroidal drug, dexamethasone (1 mg/kg), which caused 94% inhibition. The molecular mechanisms of dexamethasone have been proposed to be inhibition of leukocyte infiltration into the inflammatory site (Tsurufuji et al., 1984), thus it can be suggested that the oil’s antinflammatory activity may be mediated similarly to dexamethasone or through inhibition of pro-inflammatory mediators or inhibition of prostaglandin synthesis at the level of phospholipase A2 and cyclooxygenase/PGE isomerase (Goppelt-Streebe et al., 1989; da Silva et al., 2014). These results serve as the preliminary screening data and further studies are imperative to isolate the active components of the oil in subsequent study in order to decipher the mechanism(s) involved in these activities.

In summary, results obtained in this study suggested that essential oil of *P. guineense* exhibits significant analgesic property (centrally and peripherally) in mice and displayed impressive antiinflammatory effect on the egg albumin-induced oedema in rats comparable to the standard drugs used in these models. The results of the acute toxicity profile of oil showed that it is moderately toxic when administered orally but more toxic when administered intraperitoneally. These results provide scientific and pharmacological basis for the use of *P. guineense* in ethnomedicine to manage pains, rheumatism and related ailments.

**Conclusion**

From the results obtained in this study we conclude that essential oil of *P. guineense* demonstrated significant antinociceptive and antiinflammatory activities in rodents, thus providing supporting evidence for the potential use of the plant in ethnomedicine as well as serving as a clue in drug discovery.

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