Original Article

Antinociceptive and antiulcer activities of *Pycnanthus angolensis*

Margaret Oluwatoyin Sofidiya*, Adepero Olubukola Awolesi

Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Nigeria

A B S T R A C T

Pycnanthus angolensis (Welw) Warb., Myristicaceae, is used in Nigeria folk medicine to treat complaints such as toothache, headache, sore throat, ulcers and wounds. The aim of the study was to investigate the antinociceptive and antiulcer activities of the stem bark extract of *Pycnanthus angolensis*. Acute toxicity was conducted with a single oral dose of 5 g/kg. Antinociceptive activity was evaluated in acetic acid-induced writhing, formalin and tail immersion tests in mice while antiulcer activity was evaluated in ethanol and indomethacin-induced models in rats. In acetic acid-induced writhing test, the extract (50, 100 and 150 mg/kg, *p.o.*), significantly reduced the number of writhes (46.75%, 57.28% and 75.69%) respectively, compared to control. The extract significantly ($p < 0.001$) reduced the time spent in licking the hind paw at both phases, in formalin test. In tail immersion test, significant antinociceptive effect was only observed with the dose of 150 mg/kg, with peak effect at 90 min (43.38%). There is no significant change in the spontaneous locomotor activity of animals in the open field. The extract prevented the gastric ulceration caused by ethanol and indomethacin treatments compared to control. The results showed that *P. angolensis* extract possesses antinociceptive and antiulcer activities supporting the traditional use for relieving pain and ulcers.

© 2015 Sociedade Brasileira de Farmacognosia. Published by Elsevier Editora Ltda. All rights reserved.

Introduction

Pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (IASP, 1994). It is a disabling accompaniment which in many cases represents the only symptom for the diagnosis of several diseases. In relieve of pain, classical analgesic drugs notably opiates and non-steroidal anti-inflammatory drugs (NSAID) are used (García et al., 2011). The use of these drugs especially NSAID is considered to be the major risk factor in gastric ulcers. In this context, focus on plant research has increased worldwide and several studies had showed immense potential of medicinal plants as alternative remedies in the management of pain and gastric ulcers.

Pycnanthus angolensis (Welw) Warb., Myristicaceae, commonly known as 'African nutmeg' or 'false nutmeg', is used in a variety of herbal recipes for the treatment of chest pain, headache, various skin diseases and gastrointestinal ailments in Nigeria (Omobuwajo et al., 1992; Oladimeji et al., 2006). The plant contains a variety of chemical constituents, such as allantoin (Prista et al., 1960), kombic acid (Lok et al., 1983), isoflavones-7,4′-dimethoxy-2′ hydroxylisoflavone and 2′-hydroxyformononetin (Omobuwajo et al., 1992), dihydroguaiacetic acid (Njoku et al., 1997), pycnanthu-quinone A and B (Fort et al., 2000) and pycnanolide A and B (Onocha and Ali, 2011).

Biological activities of *P. angolensis* reported in literature include antihelmintic (Gbolade and Adeyemi, 2008), anticancer (Njoku et al., 1997), antihyperglycemic (Luo et al., 1999; Fort et al., 2000), cytotoxicity and antileishmanial activities (Onocha et al., 2008). The plant has also been screened by a number of authors for malaria and antimicrobial activities, with proven efficacy (Ancolio et al., 2002; Oladimeji et al., 2006; Abrantes et al., 2008; Onocha and Otunla, 2010). Agyare et al. (2009), in an attempt to investigate the antiulcer potential of the plant screened the stem bark extract against *Helicobacter pylori*. Aside these reports and to the best of our knowledge, the pharmacological effect of the plant on painful processes and gastrointestinal system has not been reported. Therefore, the objective of the study was to investigate the antinociceptive and antiulcer properties of the ethanolic extract of *P. angolensis* in experimental animal models.

Materials and methods

Plant material

The stem bark of *Pycnanthus angolensis* (Welw) Warb., Myristicaceae, was collected from Olokemeji forest, Ibadan (7°25′ N, 3°31′ E), Oyo State, Nigeria in February, 2011 and authenticated by

* Corresponding author.
  E-mail: msofidiya@unilag.edu.ng (M.O. Sofidiya).

Mr. T. K. Odewo at the herbarium unit of the Department of Botany, University of Lagos, Lagos, Nigeria. Voucher specimen (LUH 4588) was deposited in the herbarium.

Extraction procedure

The stem bark was cut into pieces and dried at room temperature for three weeks. The air dried material (500 g) was powdered using Christy and Norriis 8′ Lab Milling Machine (serial No. 50158) and extracted twice by maceration with 95% ethanol (3 l) at room temperature for 48 h. The combined ethanolic extract was filtered with double-layered muslin cloth and concentrated on a water bath at 45 °C to yield a reddish-brown solid (1.32%, w/w).

Animals

Male Albino Wistar rats (130–150 g) were obtained from a private vendor, Research Enterprise, Ibadan, Nigeria. The animals were maintained under standard laboratory conditions in the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos, Nigeria. The animals were kept in cages at the room temperature and fed with food and water ad libitum. They were allowed to acclimatize for two weeks before the commencement of experimental procedures. The experimental procedures adopted were in accordance with the provisions of the Experimentation Ethics Committee on Animal Use of the College of Medicine, University of Lagos, Lagos, Nigeria (CM/COM/08/VOL.XXV) and the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals (NIH, 1985).

Acute toxicity

The acute toxicity study of *P. angolensis* was performed in a single oral dose administration of 5000 mg/kg to seven male mice (Jaijoy et al., 2010). Mice were fasted for 12 h before the administration of the extract. Behavioral parameters including convulsion, hyperactivity, sedation, grooming, increased or decreased respiration were observed for, over a period of seven days.

Antinociceptive studies

Acetic acid-induced writhing test

The acetic acid-induced writhing test was done as previously described by Arslan et al., 2010. The extract (50, 100 and 150 mg/kg), distilled water (10 ml/kg) and acetylsalicylic acid (ASA, 100 mg/kg), were administered orally to each group of mice 1 h before intraperitoneal injection with 0.6% acetic acid (10 ml/kg, body weight) to induce writhings. The number of abdominal constrictions with stretching of the hind limbs was counted cumulatively over a period of 30 min. The percentage of inhibition of the number of writhings was then calculated.

Formalin test

The method used was as previously described by Gomes et al. (2007), with slight modification. Twenty microliters of formalin (1%, v/v) was injected subcutaneously into the right hind paw of mice. The time (in second) spent in licking and biting of the injected paw was taken as an indicator of pain response. Responses were measured for 5 min (first phase) and 15–30 min (second phase) after formalin injection. The extract (50, 100 and 150 mg/kg, *p.o.*), distilled water (10 ml/kg, *p.o.*) and morphine (3 mg/kg, *s.c.*) were administered 60 and 30 min, respectively, before formalin injection.

Tail immersion test

Prior to the experiment, the animals were screened for a sensitivity test. The mouse was gently handled and one third of the tail was immersed into a water bath set at 55 ± 1 °C (Agrahari et al., 2010). The mice which withdrew the tail within 5 s were selected for activity. Each animal served as its control. The reaction time for the same group was taken at intervals 60, 90, 120, and 150 min after a latency period of l h following the oral administration of the extract at the dose of 50, 100 and 150 mg/kg. Control groups received morphine (3 mg/kg, *s.c.*) and distilled water (10 ml/kg, *p.o.*) respectively. A cut off period of 10 s was observed, to avoid damage to the tail.

Open-field test

To evaluate the effect of the extract on spontaneous locomotor activity, mice were subjected to open-field test as reported by De Mattos et al. (2007). Groups of mice ($n = 5$) received vehicle, diazepam or extract (50, 100 and 150 mg/kg, *p.o.*) 1 h before the test. Each animal was placed in the middle of the open field for a 5 min session during which the following parameters were registered: crossing (the number of squares crossed with all paws), rearing (rising on hind paws) and time of immobility.

Antiulcer studies

Ethanol-induced gastric ulcer model

The experiment was done as described by Akindele et al. (2012), with slight modification. Fasted male rats were distributed into five groups consisting of seven animals each. Group I served as control and received distilled water (10 ml/kg, *p.o.*); groups II, III and IV received the extract orally at doses of 50, 100 and 150 mg/kg, body weight, respectively. Group V received misoprostol (100 μg/kg, *p.o*) which served as the standard drug. Gastric ulcer was induced in the rats by administration of l ml of absolute ethanol. Animals were then sacrificed 1 h after by cervical dislocation. The stomachs were removed, cut along the greater curvature, washed with normal saline (0.9%), and ulcer index scored using Magistretti scoring scale (Magistretti et al., 1988). Gastric tissue samples from each group of ethanol induced model were fixed in 10% formalin. Then, the formalin fixed specimens were embedded in paraffin and sectioned (3–5 cm) and further stained with hematoxylin and eosin dye. The sections were evaluated by light microscopy and photographed.

Indomethacin-induced gastric ulcer model

The experiment was performed as described by Singh and Majumdar (1999). Male Wistar rats (130–150 g) fasted for 24 h were treated orally with the extract (50, 100 and 150 mg/kg) or omeprazole (40 mg/kg). Distilled water was used in the control group. One hour later, the animals were administered with indomethacin (40 mg/kg, *p.o.*). After 6 h of indomethacin administration, animals were sacrificed, and their stomachs removed, cut along the greater curvature, washed with normal saline and ulcer index scored.

Statistical analysis

The results were presented as the mean $\pm$ S.E.M. Statistical significance between groups was calculated by analysis of variance (ANOVA), followed by Tukey's or Bonferroni's test.

$p < 0.01$, 0.05 and 0.001 were considered significant. Statistical analysis was carried out using the instant statistical package (Graphpad software, Inc., USA).
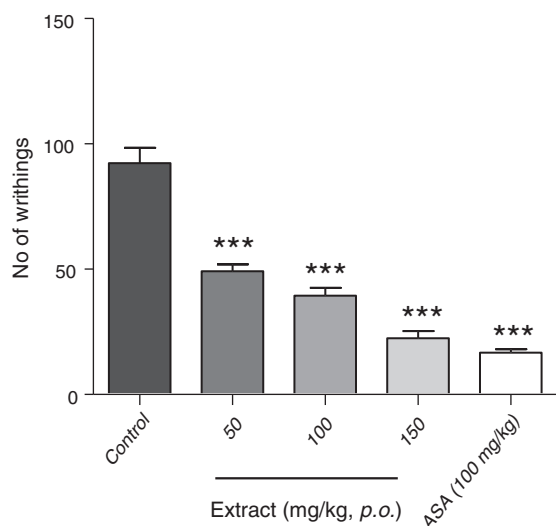
Fig. 1. Effect of *Pycnanthus angolensis* on acetic acid-induced writhing in mice. Each column represents the mean ± S.E.M. ($n = 7$). Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test ***$p < 0.001$, compared with control.

Results

Acute toxicity study

A single oral administration of *P. angolensis* extract (5000 mg/kg) did not produce any visible signs or symptoms of toxicity, abnormal behavior and mortality after 24 h and seven days of observation.

Antinociceptive studies

Writhing test

The result of the acetic induced-writhing in mice is presented in Fig. 1. The extract demonstrated a dose dependent reduction in acetic acid-induced writhing in mice. The average no of writhes for this effect was 49.14 ± 2.82, 39.43 ± 3.13, 22.43 ± 2.84 which corresponds to 46.75%, 57.28%, 75.69% inhibition for 50, 100 and 150 mg/kg, respectively. The effect was statistically significant

($p < 0.001$) relative to control. The standard drug, acetylsalicylic acid (ASA, 100 mg/kg) showed a reduction in the no writhes with percent inhibition of 81.89, compared to control.

Formalin test

The effect of ethanolic extract of *P. angolensis* on formalin induced pain in mice is shown in Table 1. The extract in a dose dependent manner reduced the time spent in licking the hind paw at both phases. In the first phase, the extract at the dose of 50, 100 and 150 mg/kg reduced the licking time with inhibition percent of 35.06, 44.47 and 60.55, respectively. The second phase showed a marked reduction in the average licking time (82.11 ± 5.30, 68.17 ± 5.47 and 47.28 ± 3.30 s) at the dose of 50, 100 and 150 mg/kg, respectively. The average licking time in the second phase was relatively lower than that of the first phase. The standard reference, morphine, produced a higher inhibition than the extract at both phases.

Tail immersion

The result of the tail immersion test with ethanolic extract of *P. angolensis* is presented in Table 2. The effect of the extract was not dose dependent at all doses tested. However, significant antinociceptive effect ($p < 0.01$ and $p < 0.001$) was observed with the dose of 150 mg/kg, at 60, 90, and 120 min, compared to control. The peak effect (43.38%) was at 90 min. Morphine exhibited a maximum peak of analgesic effect (94.11%) at 60 min after administration.

Open field test

Mice treated with the extract (50, 100 and 150 mg/kg, *p.o.*) did not produce significant effect on the number of square crossings, rearing, number of fecal boluses and immobility when compared to the control group (Table 3). However, diazepam significantly ($p < 0.05$) decreased the locomotor activity compared to control.

Anti ulcer activity

Ethanol-induced ulcer in rats

The treatment of rats with ethanol produced extensive gastric lesions in the glandular mucosa of stomach in control animals (Figs. 2 and 3). On the other hand, the pretreatment of the animals

Table 1
Effect of *Pycnanthus angolensis* on formalin induced hind paw licking in mice.

Treatment	Dose (mg/kg)	5 min	15–30 min
Extract	50	85.94 ± 5.31 (35.06%)***	82.11 ± 5.30 (37.43%)***
	100	73.12 ± 3.26 (44.74%)***	68.17 ± 5.47 (48.05%)***
	150	52.19 ± 2.71 (60.55%)***	47.28 ± 3.30 (63.97%)***
Morphine	3	0.00 ± 0.00 (100%)***	0.14 ± 0.05 (99.92%)***
Control	10 ml/kg	132.33 ± 12.91	131.22 ± 14.74

All values given in mean ± S.E.M. ($n = 7$). Data were analyzed using two-way ANOVA followed by Bonferroni's test.
*** $p < 0.001$, when compared with control.

Table 2
Effect of *Pycnanthus angolensis* on nociceptive responses in tail immersion test in mice.

Treatment	Dose (mg/kg)	Latency period (min)				
		0	60	90	120	150
Extract	50	3.36 ± 0.34	7.51 ± 1.96 (24.95%)	7.37 ± 1.53 (24.07%)	6.15 ± 1.22 (16.74%)	4.44 ± 0.71 (6.91%)
	100	3.84 ± 0.41	5.84 ± 0.66 (12.39%)	6.12 ± 0.39 (14.12%)	5.08 ± 0.38 (7.71%)	3.62 ± 0.5 (−1.31%)
	150	3.03 ± 0.44	8.43 ± 1.99 (31.84%)**	10.39 ± 2.00 (43.38%)***	7.73 ± 1.30 (27.69%)**	5.78 ± 1.08 (6.24%)
Morphine	3	3.64 ± 0.47	19.04 ± 0.51 (94.11%)***	17.50 ± 1.04 (84.74%)***	16.74 ± 1.14 (80.07%)***	14.01 ± 1.35 (63.36%)***
Control	10 ml/kg	2.66 ± 0.52	2.86 ± 0.46	2.57 ± 0.38	2.46 ± 0.36	2.10 ± 0.28

All values given in mean ± S.E.M. ($n = 7$). Data were analyzed using a two-way ANOVA followed by Bonferoni's test.
** $p < 0.01$.
*** $p < 0.001$ when compared with control.

Table 3
Effect of *Pycnanthus angolensis* on mice in open field test.

Treatment	Dose (mg/kg)	Number of square crossing	Number of center square crossing	Number of rearing	Immobility (s)
Control	–	66.40 ± 8.80	1.80 ± 0.58	19.80 ± 2.82	76.60 ± 14.42
Extract	50	62.20 ± 5.14	2.20 ± 0.49	18.00 ± 1.67	35.20 ± 9.46
	100	64.20 ± 2.06	1.80 ± 0.49	16.40 ± 3.39	40.00 ± 8.96
	150	58.20 ± 5.45	2.40 ± 0.51	13.80 ± 1.56	103.20 ± 11.59
Diazepam	1	33.60 ± 9.14[*]	0.80 ± 0.37	1.00 ± 0.45[***]	198.60 ± 9.45[***]

Data are mean ± S.E.M. ($n = 5$). Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test.
[***] $p < 0.001$.
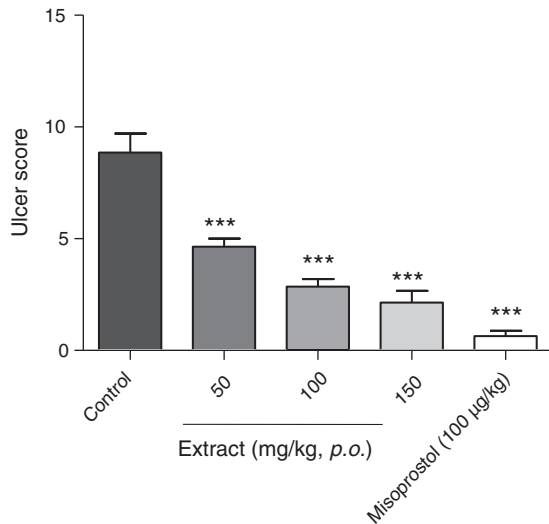[*] $p < 0.05$ compared with control.

Fig. 2. Effect of *Pycnanthus angolensis* in ethanol induced ulcer in rats. Each column represents the mean ± S.E.M. ($n = 7$). Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test ***$p < 0.001$, compared with control.

with *P. angolensis* extract (50, 100 and 150 mg/kg) exhibited significant ($p < 0.001$) reduction of gastric lesion with percentage protection of 47.58%, 67.74% and 79.26%, respectively.

The effect of the extract at 150 mg/kg dose was comparable to the group treated with misoprostol. Histological observation of stomach sections of the control group showed congested necrotic mucosal surface and infiltration of acute inflammatory cells into the mucosal (Fig. 3). However, stomach tissues of rats pretreated with the dose of 50, 100, and 150 mg/kg of *P. angolensis* extract demonstrated mild lesions of the mucosa and mild effects of hemorrhage, in a dose dependent manner.

Indomethacin induced ulcer in rats

The antiulcer effect of ethanolic extract of *P. angolensis* was also assessed in indomethacin induced ulcer model in rats and the result is presented in Fig. 4. The extract produced a significant dose-dependent reduction in the gastric lesion with ulcer index of 2.70 ± 0.10, 2.60 ± 0.21 and 2.40 ± 0.56 for 50, 100 and 150 mg/kg, respectively. Omeprazole used as reference, produced a percentage protection of 71.93%.

Discussion

The present study was undertaken to evaluate the antinociceptive and antiulcer activities of *P. angolensis*. The antinociceptive effect of the extract was evaluated on three classical nociception models in mice: acetic acid-induced writhing test, formalin test and tail immersion test, which are useful methods for screening prospective antinociceptive compounds or plant extracts.

In the acute toxicity study, the ethanolic extract of *P. angolensis*, at the dose of 5 g/kg exhibited no signs of toxicity, indicating that the extract might have a reasonably low toxicity profile, and could be regarded as relatively safe at the tested dose (Gregory et al., 2009). However, further toxicity studies; sub-acute and chronic toxicity tests are necessary in order to determine the long-term effects of the extract.

The acetic acid-induced abdominal constriction is a visceral pain model employed as a screening tool for the assessment of
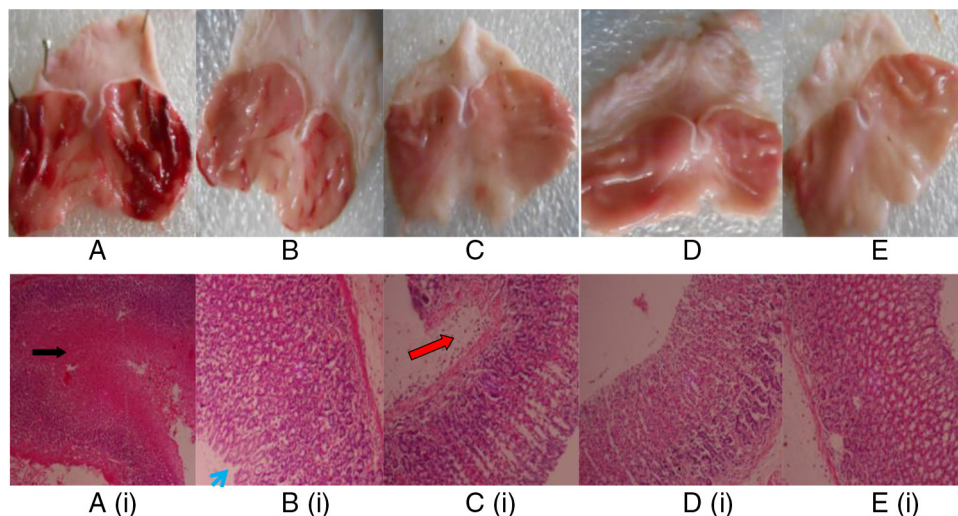

Fig. 3. Histological examination (20× magnifications) of the ethanol-induced gastric mucosal damage in rats. (A and A(i)) control group; (B and B(i)) 50 mg/kg; (C and C(i)) 100 mg/kg; (D and D(i)) 150 mg/kg; (E and E(i)) misoprostol (100 μg/kg). Black arrow: congested, necrotic mucosal surface. Blue arrow: mild mucosal damage. Red arrow: mild leukocyte infiltration of the submucosal layer.
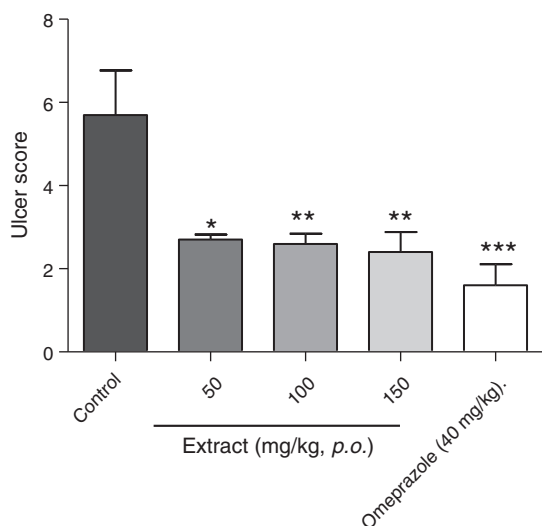
Fig. 4. Effect of *Pycnanthus angolensis* on indomethacin induced ulcer. Each column represents the mean ± S.E.M. ($n = 5$). Data were analyzed using one-way ANOVA followed by Tukey's Multiple Comparison Test, $p < 0.05$, compared with control.

antinociceptive or anti-inflammatory activity of new analgesic agent (De Souza et al., 2009). Acetic acid causes algesic response which involves the interperitoneal liberation of several mediators such as neurotransmitters and neuromodulators, kinnis, histamine, acetylcholine, substance P and prostaglandins. These mediators increase vascular permeability, reduce the threshold of the nociception and stimulate the nervous terminal of nociceptive fibers (Gorzalczany et al., 2011; Pinheiro et al., 2012). In this model, the extract exhibited a significant and dose dependent reduction in the average number of writhes in mice, compared to control. This implies that the extract had peripheral analgesic effect which may be associated with the reduction of the liberation of these inflammatory mediators into the peritoneal cavity or by direct blockage of its receptors resulting in an antinociceptive effect (Loganayaki et al., 2012).

The subcutaneous injection of formalin in the mice induces a biphasic nociceptive response (Ramirez et al., 2010). The first phase which corresponds to the neurogenic pain is caused by the direct effect of formalin on the sensory C-fibers, and followed by the second phase or the inflammatory phase that is associated with the development of an inflammatory response and the release of nociceptive mediators such as prostaglandins, serotonin and bradykinin in the peripheral tissues and from functional changes in the spinal dorsal horn (Reynoso et al., 2013). The extract significantly inhibited both phases of the formalin-induced pain, indicating that the extract has antinociceptive effect in both phases, significantly attenuating the pain response.

The tail immersion model is used to determine acute pain and it is a useful test to differentiate central opioid like analgesics from peripheral analgesics (Le Bars et al., 2001). The antinociceptive effect of the extract in this model was very low. Moreover, comparison of the effect of the extract with that of morphine showed that the extract was a weaker opioid receptor agonist.

The open field test was used to exclude the possibility that the anti-nociceptive action of extract could be related to nonspecific disturbances in the locomotor activity of the animals (Gonçalves et al., 2013). The extract at all doses tested caused no significant change in the locomotor coordination activity of the animals. The results therefore, eliminate the possibility of locomotor impairment in the antinociceptive activity of the extract.

Gastric mucosa damage as a result of treatment with non-steroidal anti-inflammatory drugs is recognized as most serious

adverse reaction to this class of compounds and has been the stimulus behind much of the research aimed at developing new and effective gastroprotective compounds. Hence, the antiulcer activity of ethanolic extract of *P. angolensis* on the mucosal of the rat was evaluated using ethanol and indomethacin induced models.

Ethanol induced ulcers are due to direct necrotizing effect of ethanol on gastric mucosa (Dharmani et al., 2004). Studies suggest that the ethanol damage to the gastrointestinal mucosa starts with micro vascular injury, namely disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting (Szabo et al., 1995). Hence a cytoprotective agent, which increases mucus secretion, will be effective in this model. Cytoprotection has been considered to be due to the capacity of some compounds/drugs to induce prostaglandin production, which in turn simulates mucus and bicarbonate synthesis (Robert et al., 1983 and Deshpande et al., 2003). In this study, the extract at the dose of 50, 100 and 150 mg/kg exhibited a dose-dependent gastro-protective activity. The effect at the dose of 150 mg/kg was comparable to that of misoprostol. The ability of the extract to reduce the ethanol induced gastric ulcer may be an indication of its cytoprotective activity.

To further confirm the cytoprotective effect of *P. angolensis*, the extract was evaluated against indomethacin induced ulcer model. Indomethacin, a non-steroidal anti-inflammatory drug, is known to cause ulcer by inhibiting prostaglandin synthetase through cyclooxygenase pathway (Oyagi et al., 2010). Prostaglandins functions to protect the stomach from injury by stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal turnover and repair (Hiruma-Lima et al., 2006). The extract significantly reduced gastric mucosal damage compared to control. The ability of the extract to reduce indomethacin induced gastric ulcer further supports cytoprotective effect and suggests the possible mobilization and involvement of prostaglandins in the anti ulcer effect of the extract.

In conclusion, the results obtained in this study showed that *P. angolensis* bark extract possesses antinociceptive and antiulcer activities, thereby providing pharmacological reference for the traditional uses of the plant in Nigeria folk medicine. The mechanism involved was not elucidated in the present study and need further investigations. Moreover, activity guided isolation of the compounds responsible for these activities need to be carried out.

Authors' contributions

AOA contributed in collecting plant sample and identification, running the laboratory work and analysis of the data. MOS designed the study, supervised the laboratory work and wrote the manuscript. All the authors have read the final manuscript and approved submission.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

References

Abrantes, M., Mil-Homens, T., Duarte, N., Lopes, D., Cravo, P., Madureira, M., Ferrira, M.J., 2008. Antiplasmodial activity of lignans and extracts from *Pycnanthus angolensis*. Planta Med. 74, 1408–1412.

Agrahari, A.K., Khaliquzzama, M., Panda, S.K., 2010. Evaluation of analgesic activity of methanolic extract of *Trapa natans* L. var. *bispinosa* roxb. roots. J. Curr. Pharm. Res. 1, 8–11.

Agyare, C., Asase, A., Lechtenberg, M., Niehues, M., Deters, A., Hensen, A., 2009. An ethnopharmacological use of medicinal plants used for wound healing in Bosomtwi-Atwima-Kwanwoma area, Ghana. J. Ethnopharmacol. 125, 393–403.

Akindele, A.J., Aigbe, F.R., Olowe, J.A., Oduntan, B.O., Adeyemi, O.O., 2012. Gastroprotective effects of DAS-77 (a phytomedicine) in ulcer models in rats. Trop. J. Pharm. Res. 11, 783–791.

Ancolio, C., Azas, N., Mahiou, V., Ollivier, E., Di Giorgio, C., Keita, A., Timon-David, P., Balansard, G., 2002. Antimalarial activity of extracts and alkaloids isolated from six plants used in traditional medicine in Mali and Sao Tome. Phytother. Res. 16, 646–649.

Arslan, R., Nurcan Bektas, N., Ozturk, Y., 2010. Antinociceptive activity of methanol extract of fruits of Capparis ovata in mice. J. Ethnopharmacol. 131, 28–32.

De Mattos, E.S., Frederico, M.J.S., Colle, T.D., de Pieri, D.V., Peters, R.R., Piovezan, A.P., 2007. Evaluation of antinociceptive activity of Casearia sylvestris and possible mechanism of action. J. Ethnopharmacol. 112, 1–6.

De Souza, M.M., Pereira, M.A., Ardenghi, J.V., Mora, T.C., Bresciani, L.F., Yunes, R.A., Delle Monache, F., Cechinel-Filho, V., 2009. Filicene obtained from Adiantum cuneatum interacts with the cholinergic, dopaminergic, glutamatergic, GABAergic, and tachykinergic systems to exert antinociceptive effect in mice. Pharmacol. Biochem. Behav. 93, 40–46.

Deshpande, S.S., Shah, G.B., Parmar, N.S., 2003. Antiulcer activity of Tephrosia purpurea in rats. Indian J. Pharmacol. 35, 168–172.

Dharmani, P., Kuchibhotla, V.K., Maurya, R., Srivastava, S., Sharma, S., Palit, G., 2004. Evaluation of anti-ulcerogenic and ulcer-healing properties of Ocimum sanctum Linn. J. Ethnopharmacol. 93, 197–206.

Fort, D.M., Ubillas, R.P., Mendez, C.D., Jolad, S.D., Inman, W.D., Canney, J.R., et al., 2000. Novel antihyperglycemic terpenoid-quinones from Pycnanthus angolensis. J. Org. Chem. 65, 6534–6539.

García, P.G., García, S.E., Martínez, G.I., Scior, T.R.F., Salvador, J.L., Martínez, P.M.M., Del Río, R.E., 2011. Analgesic effect of leaf extract from Ageratina glabrata in the hot plate test. Rev. Bras. Farmacogn. 21, 928–935.

Gbolade, A.A., Adeyemi, A.A., 2008. Investigation of in vitro anthelmintic activities of Pycnanthus angolensis and Sphenocentrum jollyanum. Fitoterapia 79, 220–222.

Gomes, N.M., Rezende, C.M., Fontes, S.P., Matheus, M.E., Fernandes, P.D., 2007. Anti-nociceptive activity of Amazonian copaiba oils. J. Ethnopharmacol. 109, 468–492.

Gonçalves, G.M., Marinho, D.G., Almança, C.C.J., Bruno Guimarães Marinho, B.G., 2013. Antinociceptive and anti-oedematogenic properties of the hydroethanolic extract of Sidastrum micranthum leaves in mice. Rev. Bras. Farmacogn. 23, 836–843.

Gorzalczany, S., Marrassini, C., Miño, J., Acevedo, C., Ferraro, G., 2011. Antinociceptive activity of ethanolic extract and isolated compounds of Urtica circularis. J. Ethnopharmacol. 134, 733–738.

Gregory, M., Vithalrao, K.P., Franklin, G., Kalaichelavan, V., 2009. Anti-ulcer (ulcer preventive) activity of Ficus arnottiana Miq. (Moraceae) leaf methanolic extract. Am. J. Pharmacol. Toxicol. 4, 89–93.

Hiruma-Lima, C.A., Santos, L.C., Kushima, H., Pellizzon, C.H., Silveira, G.G., Vasconcelos, P.C., et al., 2006. Qualea grandiflora, a Brazilian Cerrado medicinal plant presents an important antiulcer activity. J. Ethnopharmacol. 104, 207–214.

IASP Task Force on Taxonomy, 1994. Part III. Pain terms: a current list with definitions and notes on usage. In: Merskey, H., Bogduk, N. (Eds.), Classification of Chronic Pain. IASP Press, Seattle, pp. 209–214.

Jaijoy, K., Soonthornchareonnon, N., Lertprasertsuke, N., Panthong, A., Sireeratawong, S., 2010. Acute and chronic oral toxicity of standardized water extract from the fruit of Phyllanthus emblica Linn. Int. J. Appl. Res. Nat. Prod. 3, 48–58.

Le Bars, D., Gozariu, M., Cadden, S.W., 2001. Animal models of nociception. Pharmacol. Rev. 53, 597–652.

Loganayaki, N., Siddhuraju, P., Manian, S., 2012. Antioxidant, anti-inflammatory and anti-nociceptive effects of Ammannia baccifera L. (Lythracceae), a folklore medicinal plant. J. Ethnopharmacol. 140, 230–233.

Lok, C.M., Groenewegen, A., Stromk, J.B.A., Ward, J.P., 1983. Kombic acid, a hydroquinine polyisoprenoic carboxylic acid from Pycnanthus kombo seed fat. Phytochemistry 22, 1973–1976.

Luo, J., Cheung, E.M., Clark, J.P., Issai, J., 1999. Novel terpenoid – type quinnones isolated from Pycnanthus angolensis of potential utility in the treatment of type 2 diabetes. J. Pharmacol. Exp. Ther. 288, 529–534.

Magistretti, M.J., Conti, M., Cristoni, A., 1988. Antiulcer activity of an anthocyanidin from Vaccinum myrtillus. Arzneim. Forsch. Drug Res. 38, 686–690.

NIH, 1985. Guide for the Use of Laboratory Animals DHHS, PHS. NIH Publication No 85-23, rev. ed. NIH.

Njoku, C.J., Hopp, D.C., Alli, F., Asuzu, I.U., McLaughlin, J.L., 1997. Dihydroguaiaretic acid: a bioactive component of the stem bark of Pycnanthus angolensis. Planta Med. 63, 580–581.

Oladimeji, O.H., Ubulom, P.M.E., Igboasoiyi, A.C., Ndukwe, K., Nia, R., 2006. Some biological activities of Pycnanthus angolensis (Welw.) Warb. J. Pharm. Biores. 3, 49–55.

Omobuwajo, O.R., Adesanya, S.A., Babalola, G.O., 1992. Isoflavonoids from Pycnanthus angolensis and Baphia nitida. Phytochemistry 31, 1013–1014.

Onocha, P.A., Ali, M.S., 2011. Pycnanolide A and B. New lignan lactones from the leaves of Pycnanthus angolensis (Myristicaceae). Res. J. Phytochem. 5, 136–145.

Onocha, P.A., Otunla, E.O., 2010. Biological activities of extracts of Pycnanthus angolensis (Welw.) Warb. Arch. Appl. Sci. Res. 2, 186–190.

Onocha, P.A., Ajaiyeoba, E.O., Ali, M.S., 2008. In vitro antileishamanias, phyto and cytotoxicity of Pycnanthus angolensis methanolic extract. J. Med. Sci. 2, 178–181.

Oyagi, A., Ogawa, K., Kakino, M., Hara, H., 2010. Protective effects of a gastrointestinal agent containing Korean red ginseng on gastric ulcer models in mice. BMC Complement Altern. Med. 10, 45.

Pinheiro, M.M., Boykin, F., Dias Fernades, P., 2012. Antinociceptive effect of Orbignya speciosa Babassu leaves, evidences for involvement of apigenin. J. Life Sci. 10, 1013–1016.

Prista, L.N., Alves, A.C., Araujo, M.F.C., 1960. Allantoin in some plants used in the treatment of wounds. Garcia Orta 8, 315–326.

Ramirez, M.R., Guterres, L., Odila, E.D., Micheli, R.D., Henriques, A.T., De-Souza, M.M., Barros, D.M., 2010. Preliminary studies on the antinociceptive activity of Vaccinum ashei berry in experimental animals models. J. Med. Food 13, 336–352.

Reynoso, M.A., Vera, N., Aristimuño, M.E., Daud, A., Sánchez Riera, A., 2013. Antinociceptive activity of fruits extracts and "arrope" of Geoffroea decorticans (chañar). J. Ethnopharmacol. 145, 355–362.

Robert, A., Nezamis, J.E., Lancaster, C., Davis, J.P., Field, S.O., Hanchar, A.J., 1983. Mild irritants prevent gastric necrosis through adaptive cytoprotection mediated by prostaglandins. Am. J. Physiol. 245, 113–121.

Singh, S., Majumdar, D.K., 1999. Evaluation of the gastric antiulcer activity of fixed oil of Ocimum sanctum (Holy Basil). J. Ethnopharmacol. 65, 13–19.

Szabo, S., Kusstatscher, S., Sakoulas, G., Sandor, Z., Vincze, A., Jadus, M., 1995. Growth factors: new endogeneous drug for ulcer healing. Scand. J. Gastroenterol. 210, 15–18.