Anti-nociceptive effects of geraniin and an aqueous extract of the aerial parts of Phyllanthus muellerianus (kuntze) exell. in murine...

Iranian Journal of Pharmaceutical Sciences
2016: 12 (3): 17-30
www.ijps.ir

Original Article

Anti-Nociceptive Effects of Geraniin and an Aqueous Extract of the Aerial Parts of *Phyllanthus muellerianus* (Kuntze) Exell. in Murine Models of Chemical Nociception

Eric Boakye-Gyasi [a]*, Ella Anle Kasanga [a], Robert Peter Biney [a], Kwesi Boadu-Mensah [a], Christian Agyare [b], Eric Woode [a]

[a] *Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana*
[b] *Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana*

Abstract

Plants from the genus Phyllanthus like *Phyllanthus muellerianus* have long been used in folk medicine for several ailments including fevers, toothache, dysmenorrhea, anemia, and paralysis. Despite these folkloric uses, there are little scientific data supporting the use of this plant in the management of pain. The purpose of this study is to evaluate the aqueous extract of the aerial parts of *Phyllanthus muellerianus* and its dominant secondary metabolite, geraniin for potential anti-nociceptive effects. The acetic acid-induced abdominal writhing and formalin–induced nociception tests were used to assess the anti-nociceptive effects of the aqueous extract and geraniin. Morphine and diclofenac were used as standard anti-nociceptive agents. The involvement of opioidergic, adrenergic, muscarinic, adenosinergic, serotonergic and nitric oxide pathways in the anti-nociceptive effects of the extract and geraniin were evaluated by selective antagonism of these pathways. Additionally, the effects of voltage-sensitive Ca^{2+} channels and ATP-sensitive K^+ channels were also assessed by selective blockade with nifedipine and glibenclamide respectively.Oral administration of the aqueous extract (30, 100, 300 mg kg^{-1}) and geraniin (3, 10, 30 mg kg^{-1}) produced significant anti-nociceptive effects in both models of chemical nociception. The anti-nociceptive effects of both the extract and geraniin were not antagonized by L-NAME (10 mg kg^{-1}), yohimbine (3 mg kg^{-1}), atropine (5 mg kg^{-1}), theophylline (5 mg kg^{-1}), ondansetron (0.5 mg kg^{-1}) glibenclamide (8 mg kg^{-1}) or nifedipine (10 mg kg^{-1}). Naloxone (2 mg kg^{-1}), however, reversed the anti-nociceptive effects of only geraniin. In conclusion, the aqueous extract and geraniin obtained from the aerial parts of *Phyllanthus muellerianus* possess both peripheral and central anti-nociceptive effects in murine models of chemical nociception with the anti-nociceptive action of geraniin involving possibly the opioidergic pathways.

Keywords: nociception, acetic acid, aerial, formalin, muellerianus, naloxone.

Corresponding Author: Eric Boakye-Gyasi, Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana
Tel: (+233)-244635194
E-Mail: ebgyasi.pharm@knust.edu.gh
Cite this article as: Boakye-Gyasi E, Anle Kasanga E, Peter Biney R, Boadu-Mensah K, Agyare C, Woode E, Anti-Nociceptive Effects of Geraniin and an Aqueous Extract of the Aerial Parts of Phyllanthus muellerianus (Kuntze) Exell. in Murine Models of Chemical Nociception.Iranian Journal of Pharmaceutical Sciences, 2016, 12 (3): 17-30

1. Introduction

Pain forms part of most diseases and it is usually the major factor of the disease that alerts the patient to seek medical treatment [1]. Pain is known to be a significant health problem which costs society at least $560-$635 billion annually [2]. Despite the frequency of pain symptoms, individuals often do not obtain satisfactory relief of pain, and this is attributable to inappropriate or insufficient use of existing therapies, [3, 4]. Inadequate use of current therapies can be due to the numerous and life-threatening side effects associated with the use of most of these agents [5, 6]. There is therefore still the need to search for more effective agents to manage pain.

Phyllanthus muellerianus (Kuntze) Exell. (Euphorbiaceae), a monoecious, scandent shrub with numerous stems from the base, is distributed widely in tropical and subtropical countries in Africa [7] as well as Brazil and the Caribbean [8]. Traditionally, the leaf sap is applied as eye drop to treat pain in the eyes and for fevers. Also, the freshly ground leaves are applied to boils and wounds and also used for treatment of menstrual disorders, fevers and skin eruptions in Sierra Leone, Ghana, Nigeria, and Cameroon [9, 10]. Several secondary metabolites have been identified in this plant of which geraniin is one of the most dominant in the aqueous extract of the aerial parts [11]. The anti-inflammatory activity of the aqueous leaf extract has been reported [12]. Various species in the Phyllanthus genus have been reported to possess anti-nociceptive activity [13].

Considering the ethnopharmacological use of this plant for painful disorders, this study seeks to determine if an aqueous extract of the aerial parts of *Phyllanthus muellerianus* as well as its main biological constituent, geraniin, exhibit anti-nociceptive properties in mice as well as the possible mechanism of action.

2. Materials and Methods

2.1. Preparation of Extract

Fresh matured aerial parts of *Phyllanthus muellerianus* were identified and collected from uncultivated fields around the Kwame Nkrumah University of Science and Technology (KNUST) in February 2015. The plant was authenticated by Mr. Asare of the Department of Herbal Medicine, KNUST. The fresh aerial parts were washed with water and air dried at room temperature (25 – 28 ºC) for seven (7) days. The dried aerial parts were then powdered with a hammer mill. Five hundred grams (500 g) of the powdered aerial parts were suspended in 5 L of

sterile distilled water and heated at 90 °C for 15 min. The mixture was centrifuged at 6000 ×*g* for 10 min and the supernatant was lyophilized to obtain a crude extract (yield: 12.08 $\%^{w}/_{w}$). The extract was then stored at 4 – 8 ºC in a refrigerator in an air tight container. This crude extract is subsequently referred to as PME in this study.

Geraniin (Figure 1) (96 $\%^{w}/_{w}$ HPLC grade) isolated from the aqueous extract of the aerial parts of *Phyllanthus muellerianus* (Kuntze) Exell was kindly provided by Prof. Andreas Hensel, Institute of Pharmaceutical Biology and Phytochemistry, University of Muenster, Muenster, Germany.

2.2. Animals

ICR mice (25-30 g) were obtained from the Noguchi Memorial Institute for Medical Research, Ghana and housed in the *vivarium* of Department of Pharmacology, Kwame Nkrumah University of Science and Technology, KNUST. They were housed in stainless steel cages (34 × 47 × 15 cm^3) in groups of five-six (5-6) animals per cage with soft wood shavings as bedding in a 12 h light-dark cycle. Food (normal mice chow: Agricare Ltd, Kumasi, Ghana) and water (tap water) were *ad libitum*. All procedures and techniques used in these studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, 1985, revised 1996) and were approved by the Departmental Ethics Committee.

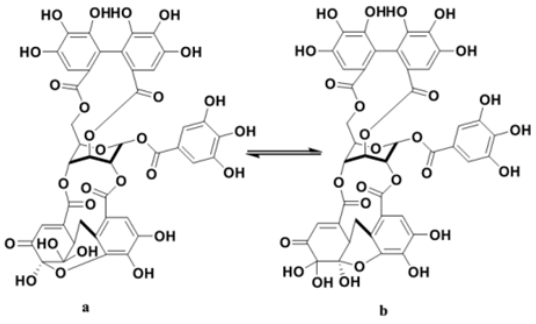

Figure 1. Chemical structures of the two isomers of geraniin in solution [11].

2.3. Drugs and Chemicals

Diclofenac sodium was purchased from Troge Medical GmbH, Hamburg, Germany; morphine hydrochloride was obtained from Phyto-Riker, Accra, Ghana; formalin, acetic acid and theophylline were purchased from British Drug Houses, Poole, England; N^{G}-Nitro-L-arginine methyl ester (L-NAME), yohimbine, glibenclamide, ondansetron, nifedipine, naloxone and atropine were also obtained from Sigma-Aldrich Inc., St. Louis, MO, USA.

2.4. Methods

2.4.1. Acetic Acid-Induced Writhing Test

The test was conducted as previously described [14]. Eleven (11) groups of mice (n = 5) received either vehicle (10 ml kg^{-1} of normal saline, i.p.), the extract (10-300 mg kg^{-1}, *p.o.*), morphine (1-10 mg kg^{-1}, i.p.) or diclofenac (10-100 mg kg^{-1}, i.p.) 60 min or 30 min before the intraperitoneal injection of 0.6 % acetic acid (10 ml kg^{-1}). Drugs were prepared such that no animal received more than 0.5 ml either orally or

intraperitoneally. Mice were placed individually in a Perspex testing chamber (15×15×15 cm) and the response, a contraction of the abdominal muscle, together with a stretching of the hind limbs, of the mice after intraperitoneal injection of acetic acid was recorded for 30 min. Tracking of frequency and duration of writhes per 5-min time block was done using the public domain software JWatcher™, Version 1.0 (University of California, LA, USA, and Macquarie University, Australia).

2.4.2. Formalin-Induced Nociception

The formalin test was carried out as previously described [15]. The mice were acclimatized to the test chambers (15×15×15 cm) for thirty minutes before formalin injection. Thirteen groups of mice (n = 5) were then pre-treated with vehicle, the extract (30-300 mg kg^{-1}, *p.o.*), geraniin (3-30 mg kg^{-1}, *p.o.*), morphine (1-10 mg kg^{-1}, i.p.) or diclofenac (10-100 mg kg^{-1}, i.p.) 60 min (*p.o.*) or 30 min (i.p.) before intraplantar injection of 10 µl of 5 % formalin. The mice were returned individually into the testing chamber after the formalin injection and their nociceptive behaviors captured for 1 h for analysis. Tracking of the behavior was done using the public domain software JWatcher™, Version 1.0. The average nociceptive score for each time block was calculated by multiplying the frequency by time spent in biting/licking and data were expressed as the mean ± SEM of scores between 0–10 min (phase I) and 10–60 min (phase II) after formalin injection.

2.4.3. Assessment of the Mechanism of Anti-Nociception in the Formalin Test

The formalin-induced nociception model [16] was used to determine the possible pathways involved in the mechanism of action of the crude extract and geraniin. The mice were pre-treated with the various antagonists and after the appropriate time intervals, received PME (100 mg kg^{-1}), geraniin (10 mg kg^{-1}) or vehicle (10 ml kg^{-1}). The doses of drugs were selected on the basis of previous literature data and from pilot experiments in the laboratory [17, 18].

Antagonists used included: the opioid antagonist, naloxone (2 mg kg^{-1}, i.p.), Nitric oxide synthase inhibitor, N^G-L-nitro-arginine methyl ester (10 mg kg^{-1}, i.p.), ATP-sensitive potassium (K^+) channel blocker, glibenclamide (8 mg kg^{-1}, *p.o.*), non-selective adenosine receptor antagonist, theophylline (5 mg kg^{-1}, i.p.), α_2-adrenergic receptor antagonist, yohimbine (3 mg kg^{-1}, *p.o.*), $5HT_3$ antagonist, ondansetron (0.5 mg kg^{-1}, i.p.), voltage gated calcium channel antagonist, nifedipine (10 mg kg^{-1}, *p.o.*), and anti-muscarinic agent, atropine (5 mg kg^{-1}, i.p.)

2.5. Data Analysis

All data are presented as mean ±S .E.M (n=5). GraphPad Prism for Windows version 6.0 (GraphPad Software, San Diego, CA, USA) was

used for all statistical analyses and ED_{50} determinations. $P \leq 0.05$ was considered statistically significant. Time-course curves were subjected to two-way (dose × time) repeated measures analysis of variance (ANOVA) with Dunnett's multiple comparisons test. Total anti-nociceptive effect for each treatment was calculated in arbitrary unit as the area under the curve (AUC). Differences in total anti-nociceptive effect were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test with drug treatment as a between-subject factor for data which were normally distributed. For data which were not normally distributed, differences in total anti-nociceptive effect were analyzed using Kruskal-Wallis test followed by Dunn's multiple comparison tests. Dose-response relationships were generated by iterative curve fitting using computer least squares method, with the following nonlinear regression (three parameter logistic) equation

$$Y = \frac{a + (b - a)}{(1 + 10^{(LogED_{50} - x)})}$$

Where x is the logarithm of dose and Y is the response. Y starts at a (the bottom) and goes to b (the top) with a sigmoid shape. The fitted midpoints (ED_{50}s) of the curves were compared statistically using *F* test.

3. Results and Discussion

3.1. Results

3.1. 1.Acetic Acid-Induced Writhing Test

PME, morphine and diclofenac significantly reduced the writhing in the mice as shown by the time-course curve (Figure 2a, c and e). Two-way ANOVA (treatment x time) revealed a significant (PME: $F_{4, 20} = 1.397$, $P = 0.1367$; morphine: $F_{3, 15} = 6.519$, $P < 0.0001$ and diclofenac: $F_{3, 15} = 5.221$, $P < 0.0001$) effect of drug treatments on the acetic acid-induced abdominal constrictions. PME (10 – 300 mg kg^{-1}) significantly ($P= 0.0156$) reduced the number of abdominal writhes over 30 min with the highest dose of 300 mg kg^{-1} giving an increase in total anti-nociceptive effect of 86.67 % compared to the control (Figure 2b). Morphine and diclofenac also produced a significant and dose-dependent ($P = 0.0095$; $P = 0.0036$ respectively)(Figure 2d, f) increase in total anti-nociceptive effects with the highest doses of 10 mg kg^{-1} and 100 mg kg^{-1} producing significant increases of 92 % and 94.1 % respectively in total anti-nociceptive effect. Morphine (ED_{50}: 0.11 mg kg^{-1}) was however, the most potent of the three agents used, followed by diclofenac (ED_{50}: 4.24 mg kg^{-1}) and then PME (ED_{50}: 22.60 mg kg^{-1}) (Figure 6a and Table 1).

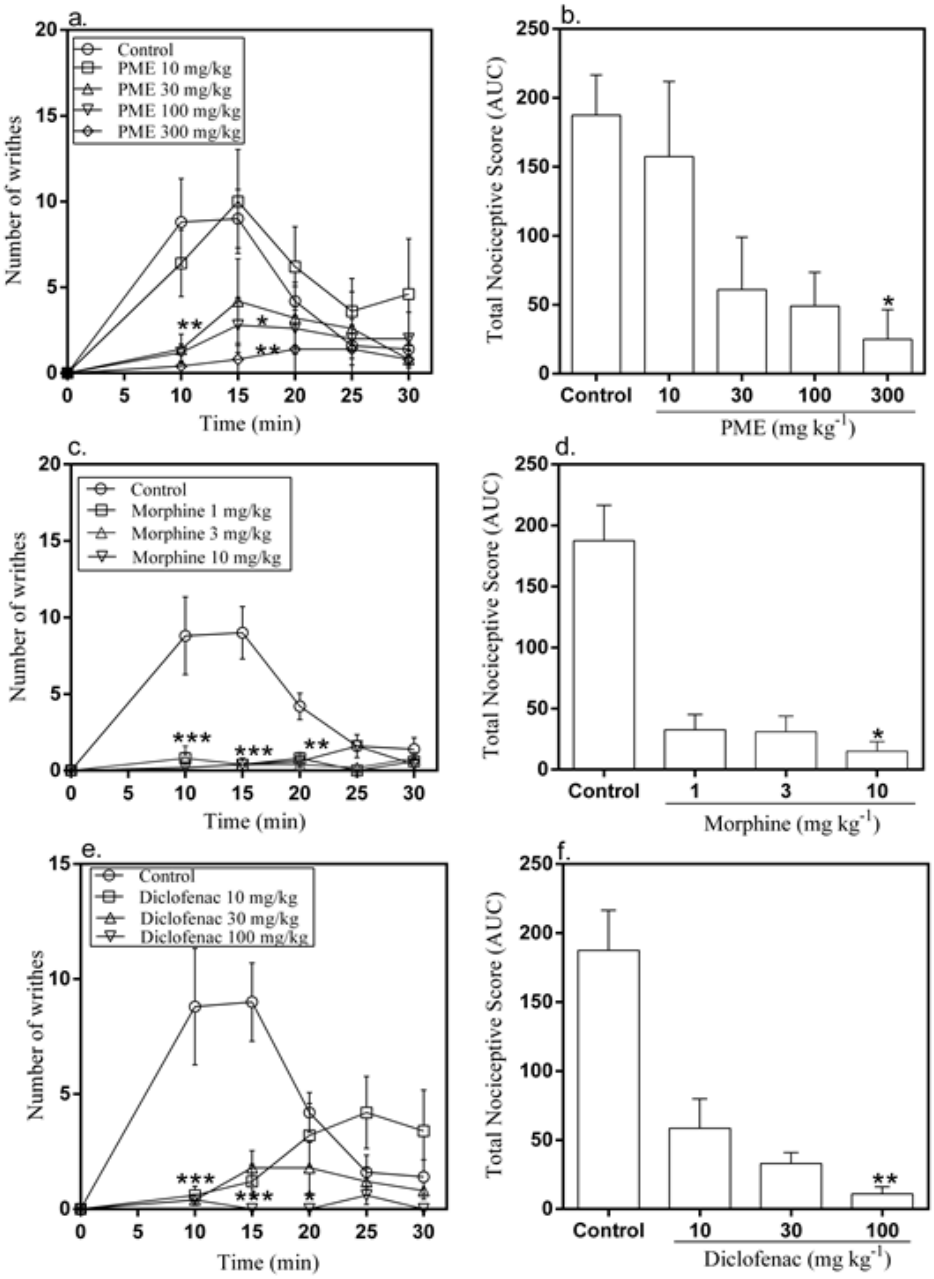

Figure 2. Effect of PME (10 – 300 mg kg^{-1}) (a,b), morphine (1- 10 mg kg^{-1}) (c, d) and diclofenac (10 – 100 mg kg^{-1}) (e, f) on acetic acid-induced writhing test. Data points are group means ± SEM. Significantly different from control: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. 2 way ANOVA followed by Bonferroni posthoc test and differences in AUCs analysed by Kruskal-Wallis test followed by Dunn's multiple comparison test.

3.1.2. Formalin-induced Nociception

Injection of formalin produced a biphasic nociception (Figures. 3 - 5). Administration of PME (30 – 300 mg kg^{-1}), geraniin (3-30 mg kg^{-1}), diclofenac (10-100 mg kg^{-1}) and morphine (1 –10 mg kg-1) to the mice significantly attenuated the nociception induced by formalin as shown by the time course curves (Figure 3).

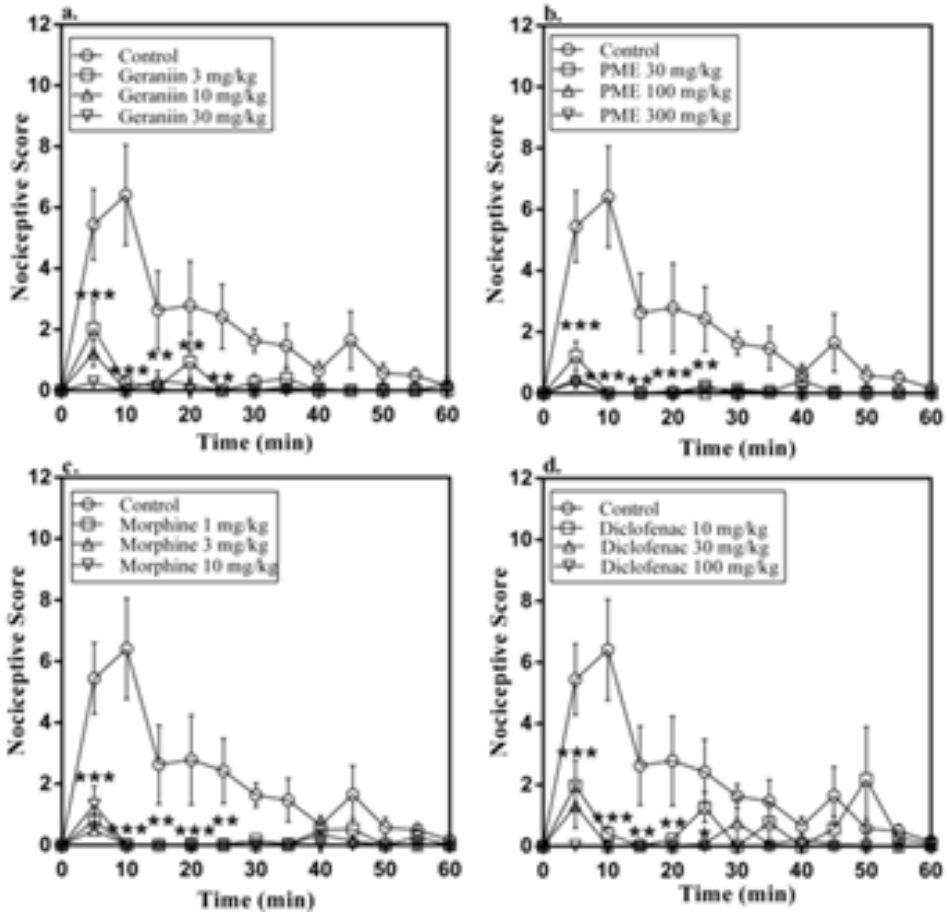

Figure 3. Effects of geraniin (3 – 30 mg kg^{-1}), PME (30 - 300 mg kg^{-1}), morphine (1 - 10 mg kg^{-1}) and diclofenac (10 – 100 mg kg^{-1}) on formalin-induced nociception shown as time course curves. $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$.

Two-way ANOVA (treatment x time) revealed a significant (PME: $F_{36,\ 204} = 3.427$, $P < 0.0001$; geraniin: $F_{36,\ 204} = 2.962$, $P < 0.0001$; morphine: $F_{36,\ 204} = 3.352$, $P < 0.0001$; diclofenac: $F_{36,\ 204} = 2.897$, $P < 0.0001$) effect of drug treatments on the formalin-induced nociception. PME dose-dependently and significantly suppressed paw licking time in both the first phase ($P = 0.0032$) and second phase ($F_{3,\ 15} = 29.79$, $P < 0.0001$) (Figure 4b and 5b). However, the highest increase in anti-nociception of 99.68 % as compared to the control was observed in the second phase at a dose of 300 mg kg^{-1} (Figure 5b). Geraniin also significantly and dose-dependently suppressed paw licking time in both the first phase ($P = 0.0041$) and second phase ($P = 0.0046$) (Figure 4a and 5a). Percentage increases in antinociceptive effects of 98.45 % and 99.24 % as compared to the control were observed in the first and second phases respectively at a dose of 30 mg kg^{-1} (Figure 4a and 5a).

In a similar manner, morphine administration resulted in a significant reduction of response time in the early ($P = 0.0059$) and the late ($F_{3, 13} = 26.76$, $P < 0.0001$) phases of formalin-induced licking with maximal inhibitions of 99.02 % and 99.88 % of the first and second phase respectively (Figure 4c and 5c). Diclofenac also significantly suppressed paw licking time in both the first phase ($P = 0.0012$) and second phase ($F_{3, 15} = 22.66$, $P < 0.0001$) (Fig. 4d and 5d). Percentage increases in anti-nociceptive effects of 99.86 % and 99.87 % as compared to the control were observed in the first and second phases respectively at a dose of 100 mg kg^{-1} (Figure 4d and 5d). In both phases, morphine was however the most potent, followed by geraniin, PME and then diclofenac (Figure 6b, 6c and Table 1).

Table 1. The ED_{50}s of the various agents used in the various models of nociception.

Drugs	**ED_{50}s (mg kg^{-1})**		
	Acetic acid-induced writhing	Formalin-induced nociception	
		Phase I	Phase II
Morphine	0.11	0.14	0.33
Diclofenac	4.24	14.51	21.30
PME	22.60	10.13	4.05
Geraniin	****	0.94	1.02

****: Geraniin was not used in the acetic acid-induced writhing test.

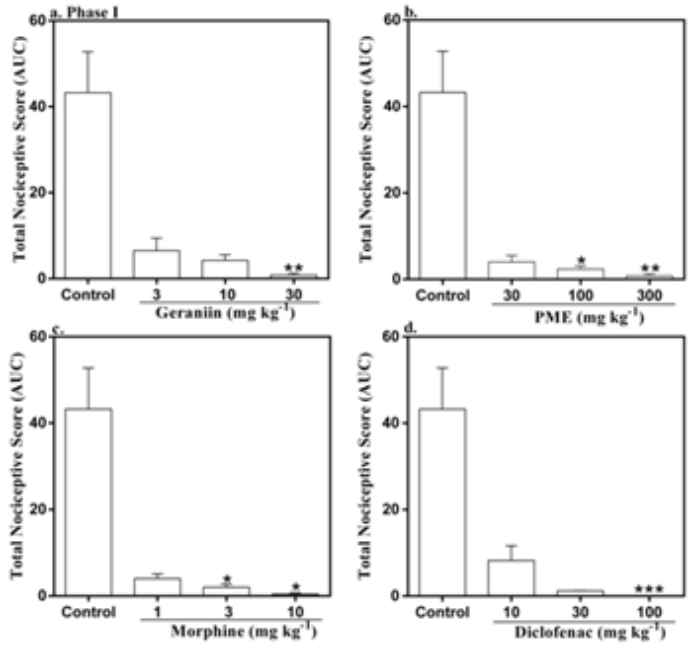

Figure 4. Effects of geraniin (3 – 30 mg kg^{-1}), PME (30 - 300 mg kg^{-1}), morphine (1 - 10 mg kg^{-1}) and diclofenac (10 – 100 mg kg^{-1}) on formalin-induced nociception shown as total anti-nociceptive effect in the first phase of the formalin test. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

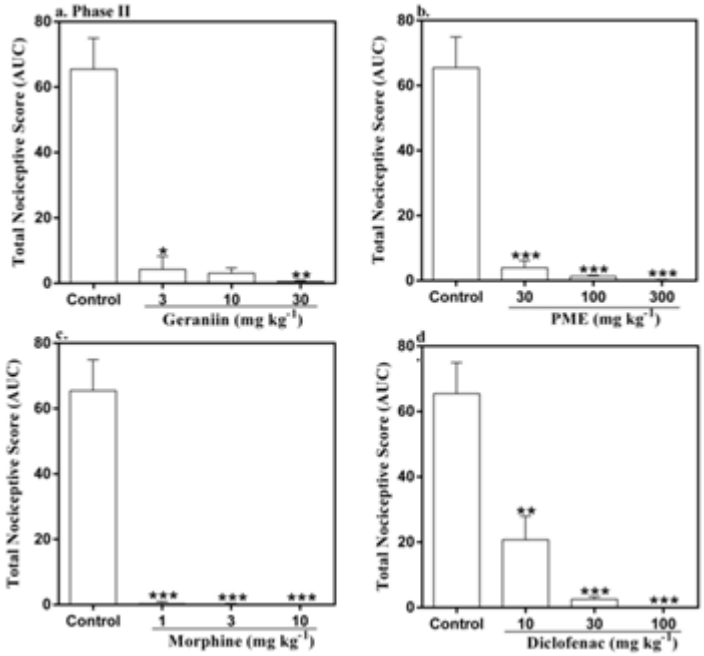

Figure 5. Effects of geraniin (3 – 30 mg kg^{-1}), PME (30 -300 mg kg^{-1}), morphine (1 -10 mg kg^{-1}) and diclofenac (10 – 100 mg kg^{-1}) on formalin-induced nociception shown as total anti-nociceptive effect in the second phase of the formalin test. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

3.1.3. Mechanism of Action

Results presented in (Figure 7a - 7b) show the effect of naloxone, glibenclamide, L-NAME, yohimbine, atropine, theophylline, ondansetron, and nifedipine on the anti-nociceptive effects of the extract (Figure 7a) and geraniin (Figure 7b). All the antagonists except naloxone did not seem to affect significantly the anti-nociceptive activity of PME and geraniin. In the presence of naloxone, the percentage change in nociception is 55.42 % in the second phase suggesting a reversal in the anti-nociceptive effect of geraniin and not the first phase.

3.2. Discussion

This present study has demonstrated that the oral administration of the aqueous extract of the aerial parts of *Phyllanthus muellerianus* as well as geraniin exerts significant anti-nociceptive activity against chemical (acetic acid and formalin)-induced nociception in mice. This anti-nociceptive effect of geraniin was reversed by the intraperitoneal administration of naloxone. Theophylline, atropine, glibenclamide, ondansetron, yohimbine, nifedipine and L-NAME, however, did not significantly block the anti-nociceptive effect of geraniin while all the antagonists did not seem to have any significant effect on the extract.

Abdominal writhing as a result of the intraperitoneal injection of acetic acid is due to increased level of prostanoids; particularly PGE_2, PGF_2, bradykinin, serotonin and lipoxygenase products which are released in

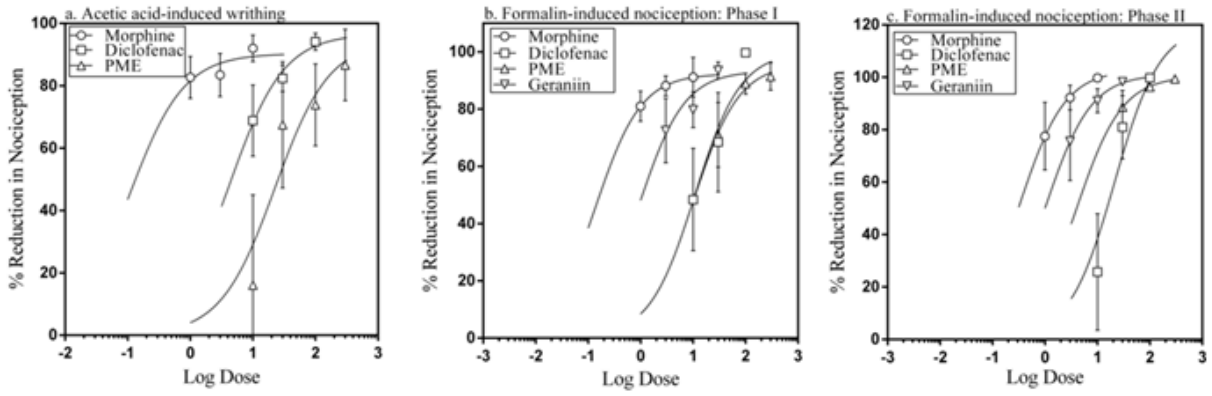

Figure 6. Dose-response effects of PME (30 - 300 mg kg^{-1}), morphine (1 - 10 mg kg^{-1}) and diclofenac (10 – 100 mg kg^{-1}) in different models. Data points represents mean ± SEM of n = 5-6 mice.

response to activation of chemosensitive nociceptors [19]. The nociceptive activity of acetic acid has also been attributed to the reduction in pH as well as the release of cytokines like TNF-α, IL-1β, IL-8 by macrophages and mast cells present in the peritoneum [20]. Diclofenac, an NSAID can inhibit both the number and duration of writhes in this model by inhibiting cyclooxygenase in peripheral tissues, thus interfering with the mechanism of transduction in primary afferent nociceptors by blocking the synthesis and/or release of inflammatory mediators [21]. The anti-nociceptive effects of morphine in this model appear to be mediated both centrally and peripherally [22]. This test is a very sensitive model as it allows for evidence to be obtained for all major and minor analgesics but it lacks specificity [16]. However, as the extract also showed anti-nociceptive effect in the formalin model, the positive results from this test can be said to be an analgesic effect. From the results obtained from the acetic acid-induced writhing model, the anti-nociceptive effects of the extract could be either centrally or peripherally mediated. The action could be due to an interaction with some of the mediators released such as prostaglandins or cytokines like TNF-α, IL-1β, IL-8.The action could also be through a novel mechanism yet to be elucidated.

The results also demonstrated that PME and geraniin significantly decreased the duration of licking and biting in both neurogenic (first phase) and inflammatory (second phase) pain responses of the formalin test in a dose-dependent manner, with the inhibition of the second phase observed to be more notable as compared to the first phase for the extract. The formalin test, a tonic model of continuous pain resulting from formalin-induced tissue injury, is a useful model, particularly for the screening of novel compounds, since the nociception produced in this test involves inflammatory, neurogenic, and central mechanisms [23]. Again, this test was employed to evaluate the anti-nociceptive properties of the extract and

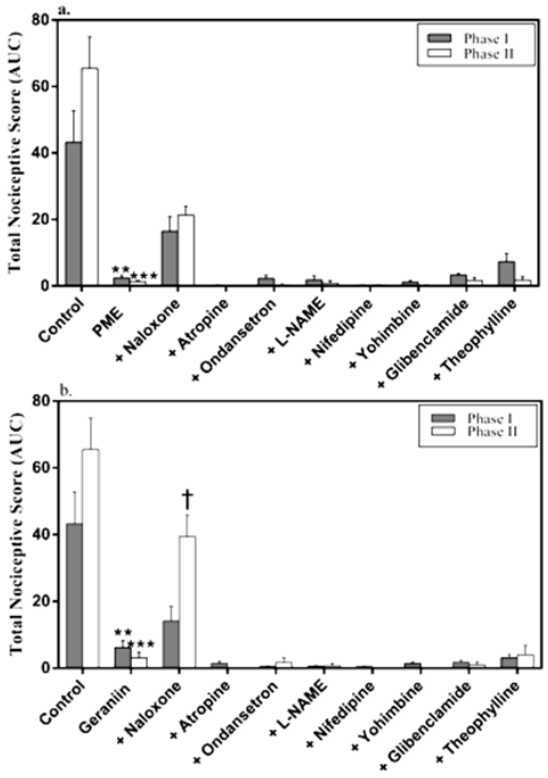

Figure 7. Effect of different antagonists on the anti-nociceptive effect of (a) extract (100 mg kg^{-1}) and (b) geraniin (10 mg kg^{-1}) for phase I and phase II of formalin-induced nociception. Each column represents the mean ± S.E.M. $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$, $\dagger P \leq 0.05$, $\dagger\dagger P \leq 0.01$, $\dagger\dagger\dagger P \leq 0.001$, compared to respective controls.

geraniin because it is considered the most predictive of acute pain and is believed to be a more valid model for clinical pain [15, 24]. The first phase which is transient is caused by the direct effect of formalin on transient receptor potential ankyrin subtype 1 receptor (TRPA 1) [25] while the second prolonged phase is associated with the combination of an inflammatory reaction in the peripheral tissue. This reaction causes a release of nociceptive mediators such as serotonin, histamine, bradykinin and prostaglandins which subsequently cause sensitization of the central neurons leading to changes in central processing of pain [26]. While it is a well-known fact that centrally-acting drugs such as narcotics inhibit nociception in both phases equally [27] some studies have also shown that diclofenac can also inhibit both phases of the formalin-induced nociception [28, 29] as it was observed in this study.

Taking together the ability of the extract and geraniin to produce anti-nociceptive effects in the acetic acid-induced abdominal writhing test and in both phases of the formalin-induced paw licking test showed that the two agents may be act both centrally and peripherally. It also implies that it possesses not only anti-nociceptive but also anti-inflammatory activities thereby confirming the finding that PME has anti-inflammatory properties [12].

Endogenous opioid system is largely involved in the central regulation of pain, as well as in the action of opioid-derived analgesic drugs [30]. This was illustrated in the present study when in the experiment to elucidate the mechanism of action, the anti-nociceptive effect of geraniin was reversed by the non-selective opioid receptor antagonist, naloxone. All the other agents did not seem to significantly reverse the effects of either the extract or geraniin while naloxone did not seem to have a significant effect on the activity of the extract. This result is not so surprising as previous studies on other species of Phyllanthus have shown that the anti-nociceptive action of the extract are unrelated to

the interaction with opioid, serotonin or L-arginine-nitric oxide pathways [31]. A possible reason why naloxone may not have had a significant effect on the effect of the extract is that the extract is known to have several components apart from geraniin such as furosin, corilagin, gallic acid and caffeic acid [11] which may also contribute to the analgesic activity of the extract but may not mediate their effects through the pathways investigated. For instance, it has been demonstrated that the antinociceptive response caused by gallic acid ethyl ester depends on the activation of Gi/o protein sensitive to pertussis toxin and involves both small- or large-conductance Ca^{2+}-activated K^+ channels and ATP-sensitive K^+ channels mechanisms [31]. Hence, the mechanism of action of the extract could not have been determined as it may have numerous sites of action due to its various constituents or even through a novel mechanism.

4. Conclusion

The aqueous extract and geraniin obtained from the aerial parts of *Phyllanthus muellerianus* possess both peripheral and central anti-nociceptive effects in the animal models of chemical nociception. It has also been shown that the effect of geraniin may be mediated through the opioidergic pathway.

Acknowledgements

The authors are grateful for the contributions and technical assistance offered by Mr. Edmund Dery and Mr. Thomas Ansah of the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi.

References

[1] Schim J D, Stang P. Overview of pain management. Pain Pract 4 Suppl (2004b): 1: S4-18.

[2] Institute of Medicine of the National Academies Report: Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research, 2011. The National Academies Press, Washington DC.(page 5) *http://books.nap.edu/openbook.php?record_id=13172&page=5* (Accessed September 5, 2015).

[3] Mcmahon S B and Koltzenburg M. Wall and Melzack's textbook of pain. 5th ed., Elsevier/Churchill Livingstone: Edinburgh (2006).

[4] Chen C H and Tang S T. Meta-analysis of cultural differences in Western and Asian patient-perceived barriers to managing cancer pain. Palliat. Medicine (2011)35(3): 125-133.

[5] Mirshafiey A, Cuzzocrea S, Rehm B H, Matsuo H. M2000: A revolution in Pharmacology. Med Sci Monit (2005) 11(8): PI53-63.

[6] Wolfe M M, Lichtenstein D R, Singh G. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. N Engl J Med (1999) 340(24): 1888-1899.

[7] Radcliffe-Smith A. *Phyllanthus muellerianus* Kuntze Exell [family EUPHORBIACEAE]. FZ, Vol 9, Part 4, Royal Botanic Gardens: Kew (1996).

[8] Calixto J B, Santos A R S, Filho V C and Yunes R A. A Review of the Plants of the Genus Phyllanthus: Their Chemistry, Pharmacology and Therapeutic Potential. Med. Res. Rev. (1998)18(4): 225–258.

[9] Burkill, H M. The useful plants of West Tropical Africa, vol 2, 5 Royal Botanic Gardens: Kew (2000).

[10] Agyare C, Asase A, Lechtenberg M, Niehues M, Deters A and Hensel A. An ethnopharmacological survey and in vitro confirmation of ethnopharmacological use of medicinal plants used for wound healing in Bosomtwi-Atwima-Kwanwoma area, Ghana. J Ethnopharmacol. (2009)125(3): 393-403.

[11] Agyare C, Lechtenberg M, Deters A, Petereit F and Hensel A. Ellagitannins from *Phyllanthus muellerianus* (Kuntze) Exell: Geraniin and furosin stimulate cellular activity, differentiation and collagen synthesis of human skin keratinocytes and dermal fibroblasts. Phytomedicine (2011)18(7): 617-624.

[12] Boakye Y D, Agyare C, Abotsi W K. Anti-inflammatory activity of geraniin and aqueous leaf extract of *Phyllanthus muellerianus* (Kuntze) Exell. Planta Med. (2013)79:1142.

[13] Santos A R S, De Campos R O P, Miguel O G, Filho V C, Siani A. C, Yunes R A, Calixto J B. Anti-nociceptive properties of extracts of new species of plants of the genus Phyllanthus (Euphorbiaceae). J Ethnopharmacol. (2000) 72:229–238.

[14] Amresh G, Singh P N, Rao Ch V. Anti-nociceptive and anti-arthritic activity of *Cissampelos pareira* roots. J Ethnopharmacol (2007)111(3): 531-536.

[15] Dubuisson D and Dennis S G. The formalin test: A quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain (1977)4(2): 161-174.

[16] Le Bars D, Gozariu M, Cadden S W. Animal models of nociception. Pharmacol Rev (2001) 53(4): 597-652.

[17] Woode E, Poku R A, Ainooson G K, Boakye – Gyasi E, Abotsi W K M, Mensah T L, Amoh-Barimah A K. An evaluation of the anti-inflammatory, antipyretic and anti-nociceptive effects of *Ficus exasperata* (Vahl) leaf extract. J. Pharmacol. Toxicol. (2009)4: 138-151.

[18] Woode E and Abotsi W K. Anti-nociceptive effect of an ethanolic extract of the aerial parts of *Hilleria latifolia* (Lam.) H. Walt. (Phytolaccaceae). J. Pharm. Bioallied Sci (2011)3: 384-396.

[19] Bhattacharya A, Agrawal D, Sahu P K, Kumar S, Mishra S S, Patnaik S. Analgesic effect of ethanolic leaf extract of *Moringa oleifera* on albino mice. Indian J Pain. (2014)28:89-94.

[20] Ribeiro R A, Vale M L, Thomazzi S M, Paschoalato A B, Poole S, Ferreira S H, and Cunha FQ. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. Eur J Pharmacol. (2000): 387:111-8.

[21] Panthong A, Norkaew P, Kanjanapothi D, Taesotikul T, Anantachoke N, Reutrakul V. Anti-inflammatory, analgesic and antipyretic activities of the extract of gamboge from *Garcinia hanburyi* Hook f. J Ethnopharmacol (2007)111(2): 335-340.

[22] Smith T W, Buchan P, Parsons D N, Wilkinson S. Peripheral Anti-nociceptive effects of N-methyl morphine. Life Sci. (1982)31:1205-1208.

[23] Ellis A, Benson N, Machin I and Corradini L. The rat formalin test: Can it predict neuropathic pain treatments? Proceedings of the Measuring Behavior (Maastricht, The Netherlands), August (2008) 324-325.

[24] Tjolsen A, Berge O G, Hunskaar S, Rosland J H, Hole K. The formalin test: an evaluation of the method. Pain (1992)51: 5 - 17.

[25] McNamara C R, Mandel-Brehm J, Bautista D M, Siemens J, Deranian K L, Zhao M, Hayward N J, Chong J A, Julius D, Fanger C M. TRPA1 mediates formalin-induced pain. Proceedings of the National Academy of Sciences, USA, (2007)104: 13525–13530.

[26] Santa-Cecília F V, Freitas L A S, Vilela F C, Veloso C, Q. da Rocha C, Moreira M E C, Dias F D, Giusti-Paiva A, and H. dos Santos M. Anti-nociceptive and anti-inflammatory properties of 7-epiclusianone, a prenylated benzophenone from *Garcinia brasiliensis*. Eur. J. Pharmacol. (2011) 670: 280–285.

[27] Hunskaar S and Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain (1987) 30: 103–114.

[28] Asomoza-Espinosa R, Alonso-López R, Mixcoatl-Zecuatl T, Aguirre-Bañuelos P, Torres-López J E, Granados-Soto V. Sildenafil increases diclofenac anti-nociception in the formalin test. Eur. J. Pharmacol. (2001) 418 (3):195–200.

[29] Santos A R S, Vedana E M A, De Freitas G A G. Anti-nociceptive effect of meloxicam in neurogenic and inflammatory nociceptive models in mice. Inflamm. Res. (1998) 47 (7): 302-307.

[30] Sakurada T, Komatsu T, Sakurada S. Mechanisms of nociception evoked by intrathecal high-dose morphine. Neurotoxicology (2005)26: 801–809.

[31] Calixto J B, Beirith A, Ferreira J, Santos A R S, Filho V C and Yunes R A. Review article: Naturally Occurring Anti-nociceptive Substances from Plants. Phytother. Res. (2000)14: 401–418.