



Anti-inflammatory and Analgesic Effects of an Aqueous Extract of *Lannea acida* Stem Bark

George Owusu^{1,2*} and Jones Ofori-Amoah^{2,3}

¹*Department of Pharmacology, School of Medicine and Health Sciences, University for Development Studies, UDS, Tamale, Ghana.*

²*Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, KNUST, Kumasi, Ghana.*

³*Department of Pharmacology, School of Medicine, University of Health and Allied Sciences, UHAS, Ho, Ghana.*

Authors' contributions

This research work was a collaborative effort of both authors. Preparation of the extract, laboratory experiment, data collection and analysis were carried out by both authors. Both authors conducted the literature research. Author GO wrote the first manuscript which was read and approved by both authors.

Article Information

DOI: 10.9734/BJPR/2017/33266

Editor(s):

(1) Cheng Wang, Division of Neurotoxicology, National Center for Toxicological Research (NCTR), Food and Drug Administration (FDA), USA.

Reviewers:

(1) Mostafa Abbas Shalaby, Cairo University, Egypt.

(2) A. O. Sijuade, Ekiti State University, Nigeria.

(3) Chaiyong Rujjanawate, Mae Fah Luang University, Thailand.

Complete Peer review History: <http://www.sciencedomain.org/review-history/19268>

Original Research Article

Received 6th April 2017

Accepted 15th May 2017

Published 31st May 2017

ABSTRACT

Background: Different parts of *Lannea acida* A. Rich (fam: Anarcadiaceae) are used traditionally to treat various ailments including inflammation, facial pain, schistosomiasis, haemorrhoids and toothache.

Objective: This study was carried out to investigate the anti-inflammatory and analgesic effects of an aqueous extract of the stem bark of *Lannea acida*.

Methodology: Rats were given sub-plantar injection of prostaglandin E2 to induce oedema, which was measured using calipers over 2½ hours at 30 min interval. In the acetic acid-induced abdominal writhing test, mice were given intraperitoneal injection of acetic acid and writhing movements were recorded. Oedema test was run for both prophylactic and curative protocols. In both paw oedema and writhing test models, inhibitory effects of the plant extract were compared

*Corresponding author: E-mail: georgeowusu.uds@yahoo.com;

with diclofenac.

Results: Aqueous extract (30 – 300 mg/kg) of *Lannea acida* stem bark significantly reduced prostaglandin E2-induced paw oedema in both prophylactic and curative protocols. The extract also significantly inhibited acetic acid-induced abdominal writhing movement in Imprint Control Region (ICR) mice.

Conclusion: The aqueous extract of *Lannea acida* stem bark inhibited prostaglandin E2-induced paw oedema in rats and acetic acid-induced writhing behaviour in ICR mice; indicating a possible anti-inflammatory and analgesic activities.

Keywords: *Lannea acida*; aqueous extract; anti-inflammatory; Prostaglandin E2; writhing test.

1. INTRODUCTION

Lannea acida A. Rich (fam. Anarcadiaceae) is a deciduous tree with a dense rounded crown that usually grows from 1.5 to 10 meters tall. It is found widely in Western tropical Africa, spanning from Senegal to Nigeria. It derives well on wooded savanna, often gravelly deep soils, rocky places and hills. In Senegal, the bark is used to ensure easy childbirth [1]. It is also mixed with other plants to treat sterility [1]. Poultice of the stem bark is known to be applied on the face to treat facial pain [2]. In Ghana, the bark is used internally to treat colic, eye inflammation, beriberi, schistosomiasis and haemorrhoids [1]. Sarwar et al. [3] reported on the antibacterial, hypoglycemic and antioxidant effects of the stem bark extract of the plant. Ouattara et al. [4] confirmed the anti-*Mycobacterium tuberculosis* H37Rv activities of hydro-alcoholic extract of the plant. In 2013, Imoro et al. [5] reported that the plant is used in Northern Ghana to treat convulsions. Hitherto, there is no scholarly report on the anti-inflammatory effect of the stem bark extract of the plant to support the traditional claim; hence the need to carry out this investigation to provide a valid scientific data on its anti-inflammatory and analgesic effects.

Currently, conventional drugs used to treat inflammation are either steroidal anti-inflammatory drugs, non-steroidal anti-inflammatory drugs or disease-modifying anti-rheumatic drugs [6]. Though these conventional drugs offer symptomatic relief of the disease, they are often not without some constraints such as high cost of the drugs, availability to the rural folks as well as adverse effects which may sometimes be life-threatening. As a result of these, new compounds with high efficacy and less adverse effects are being explored as alternative remedy for inflammation and pain. This research was carried out to investigate the

anti-inflammatory effect of the aqueous extract of the stem bark of *Lannea acida*.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extraction

The stem bark of *Lannea acida* was collected from the Dungu community in the Northern region of Ghana in October, 2016. It was authenticated by the curator of the herbarium of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, KNUST, Kumasi, Ghana. The stem bark was chopped into pieces and sun-dried for seven days. The dried pieces were pulverized in a hammer-mill to powder. Four hundred grams of the powder was infused with 3.0 L of distilled water and warmed for 60 minutes at 90°C. The infusion was filtered to obtain a dark-brown filtrate which was evaporated over a hot water-bath and later in oven at 55°C to obtain a solid extract. The yield was 10.8% ^w/_w. The extract was labelled as LAE and kept in a refrigerator for use. LAE in this report refers to the aqueous extract of *Lannea acida* stem bark.

2.2 Drugs and Chemicals

The following drugs and chemicals were used: acetic acid (British Drug Houses Ltd., Poole, UK); diclofenac (Troge, Hamburg, Germany); Prostaglandin E2 (Sigma-Aldrich Inc., St. Louis, MO, USA). The plant extract was dissolved in 2% tragacanth mucilage to obtain a uniform suspension and diclofenac was dissolved in normal saline (0.9% saline solution).

2.3 Experimental Animals

Male Sprague–Dawley rats (150-220 g, 12-16 weeks old) and ICR mice (20-25 g, 6-8 weeks old) of either sex were used in the experiments.

The animals were purchased from Noguchi Memorial Institute for Medical Research, Legon-Accra, Ghana, and kept at the laboratory for Department of Pharmacology, KNUST, Kumasi. Each experimental group consisted of at least five animals, housed in stainless steel cages (34×47×18 cm) with soft wood shavings as bedding. The animals were maintained under laboratory conditions (temperature 24–25°C, 12-hour light-dark cycle) with free access to pellet diet (GAFCO, Tema, Ghana) and water *ad libitum*.

2.4 Phytochemical Screening

Preliminary phytochemical tests were performed on the extract using methods described by Trease and Evans [7].

2.5 Prostaglandin E2-Induced Paw Oedema in Rats

Paw oedema was induced in healthy rats, as previously described by Mazumder et al. [8]. Briefly, rats ($n = 5$) were injected with 50 μL of 1 nm of prostaglandin E2 at the sub-plantar surface of the right hind paw of rats. In the prophylactic protocol, rats received the extract (30, 100 and 300 mg/kg *p.o.*) or diclofenac (3, 10 and 30 mg/kg *p.o.*) one hour before prostaglandin E2 challenge. In the curative protocol, rats received the prostaglandin challenge thirty minutes before they were orally treated with either the extracts (30–300 mg/kg) or diclofenac (3 – 30 mg/kg). In each protocol, paw oedema was measured with digital calipers (Milomex Ltd, Pulloxhill, Bedfordshire, UK; model No. Z22855, 11/2010) at thirty minutes interval for 150 minutes. The control animals in each protocol received 1 ml/kg *p.o.* normal saline (0.9% saline solution). Percentage changes in paw volume were calculated as:

$$\text{Percentage change in paw volume} = [(V_t - V_o)/V_o] \times 100$$

(Where V_t = the paw volume at a particular time after prostaglandin E2 challenge and V_o = the paw volume before prostaglandin E2 challenge).

2.6 Acetic Acid-Induced Writhing Test

Acetic acid-induced writhing test was also performed, as previously described by Witkin et al. [9], and Woode et al. [10]. Briefly, ICR mice of either sex ($n = 5$) received prophylactic treatment

of the extract (30, 100 and 300 mg/kg *p.o.*) or diclofenac (3, 10 and 30 mg/kg *p.o.*). One hour after administration of diclofenac and the extract, mice were given intraperitoneal injection of 10 ml/kg of 0.6% acetic acid. Writhing movements such as stretching of hind limbs, contraction of the abdominal muscles, or bending of the trunk were counted for thirty minutes following acetic acid induction [11]. Data collected represent the average number of writhing movements of the group [12].

2.7 Statistical Analyses

All data were presented as mean \pm S.E.M. The time-course curves were subjected to two-way (*treatment* \times *time*) repeated measures analysis of variance (ANOVA) with Bonferroni's *post hoc* test. Total oedema score for each treatment was calculated in an arbitrary unit as the area under the curve (AUC). Differences in AUCs were analyzed using one-way ANOVA followed by the Newman-Keuls posttest. GraphPad Prism for Windows Version 5 (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses and $p < 0.05$ was considered statistically significant in all analyses.

3. RESULTS

3.1 Phytochemical Screening

The preliminary phytochemical screening indicated that the plant extract contained alkaloids, glycosides, flavonoids, saponins, steroids and tannins.

3.2 Prostaglandin E2-Induced Paw Oedema

Intradermal injection of 50 μL of 1 nm of prostaglandin E2 at the sub-plantar surface of hind paw of rat produced a time-dependent oedema which peaked at 60–90 minutes after induction (Fig. 1a). Two-way ANOVA (*treatment* \times *time*) from the time-course curves and one-way ANOVA from the area under the time course curves (AUCs) showed that the extract and the reference drug dose-dependently inhibited the paw oedema in both protocols. In the prophylactic protocol, the extract inhibited paw oedema significantly ($F_{(3, 96)} = 25.02$; $p < 0.05$) and ($F_{(5, 96)} = 16.46$; $p < 0.01$) at the doses of 100 mg/kg and 300 mg/kg respectively. However, the extract did not show any significant inhibitory effect at 30 mg/kg ($F_{(15, 96)} = 1.12$; $p = 0.3505$) as

shown in Fig. 1b. Comparatively, the reference drug (diclofenac) showed dose-dependent inhibitory effect ($F_{(15, 96)} = 1.76$; $p < 0.05$, $F_{(4, 96)} = 40.04$; $p < 0.001$ and $F_{(3, 96)} = 21.94$; $p < 0.0001$) at 3, 10 and 30 mg/kg respectively, as shown in Fig. 2 (c and d). The percentage total inhibitory effects of the extract and diclofenac were 68.358% and 73.045% respectively.

In the curative protocol, the plant extract dose-dependently inhibited paw oedema (Fig. 3e, 3f). The statistical values $F_{(15, 96)} = 3.02$; $p < 0.0002$, $F_{(5, 96)} = 21.43$; $p < 0.0001$, $F_{(3, 96)} = 33.25$; p

< 0.0001 for 30, 100 and 300 mg/kg respectively for the extract were very significant. Similarly, diclofenac (3, 10 and 30 mg/kg) dose dependently and significantly inhibited rat paw oedema (Fig. 4g, h), with critical values $F_{(15, 96)} = 3.89$; $p < 0.0001$, $F_{(5, 96)} = 21.91$; $p < 0.0001$ and $F_{(3, 96)} = 21.94$; $p < 0.0001$ respectively. The percentage maximal inhibitory effects of the extract (at 300 mg/kg) and reference drug (at 30 mg/kg) as calculated from area under the time course curves were 67.1% and 68.66% respectively.

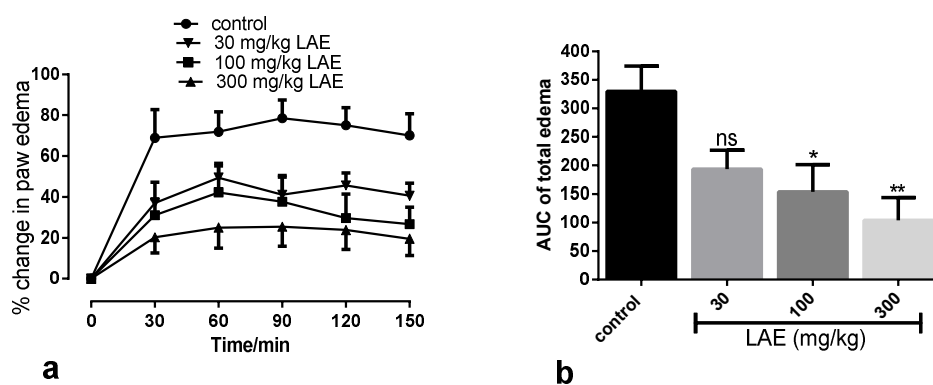


Fig. 1. The effects of LAE on prostaglandin E2-induced paw oedema in rats. Rats received sub plantar injection of 50 μ L of 1 nm of PGE2. Rats were prophylactically treated with LAE (30-300 mg/kg p.o) one hour before oedema was induced. Oedema was measured at 30 min intervals for 2½ hours using digital calipers. Percentage change in paw thickness (a) and AUC of total oedema (b) were calculated

ns implies $p > 0.05$; * implies $p \leq 0.05$; ** implies $p \leq 0.01$. Values were presented as mean \pm SEM (n=5)

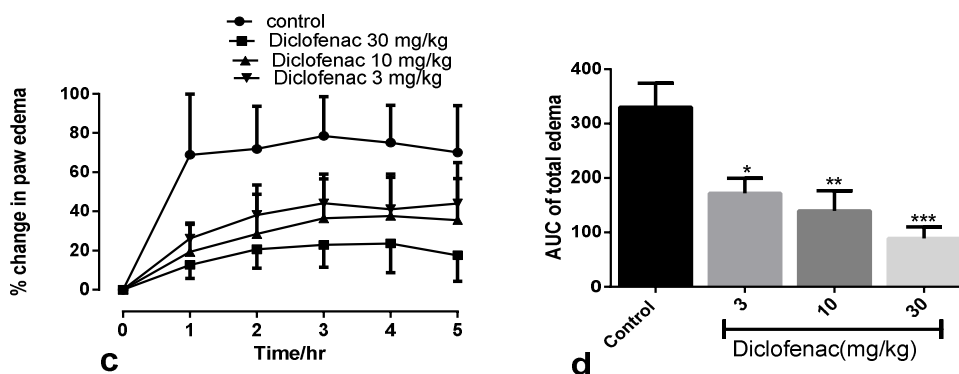


Fig. 2. The effects of diclofenac on PGE2-induced paw oedema in rats. Rats prophylactically received diclofenac (3-30 mg/kg p.o) one hour before PGE2 challenge. Oedema was measured 30 min after PGE2 injection at 30 min interval for 2½ hours. Percentage change in paw oedema (c) and total oedema as AUC (d) were determined

* implies $p \leq 0.05$; ** implies $p \leq 0.01$; *** implies $p \leq 0.001$. Values plotted are means \pm SEM (n = 5)

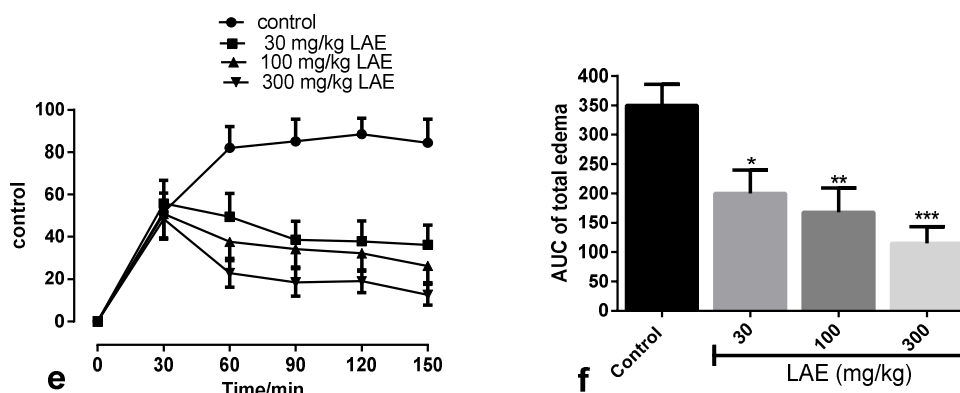


Fig. 3. Curative effects of LAE on Prostaglandin E2-induced paw oedema in rats. Rats were challenged with 50 μ L of 1 nm of PGE2 at the sub-plantar surface 30 min before treatment with LAE (30-300 mg/kg). Percentage change in paw oedema (e) and total oedema calculated as AUC (f) were determined for 2½ hours at 30 min interval

*implies $p \leq 0.05$; ** implies $p \leq 0.01$; *** implies $p \leq 0.001$. Data were presented as mean \pm SEM (n=5)

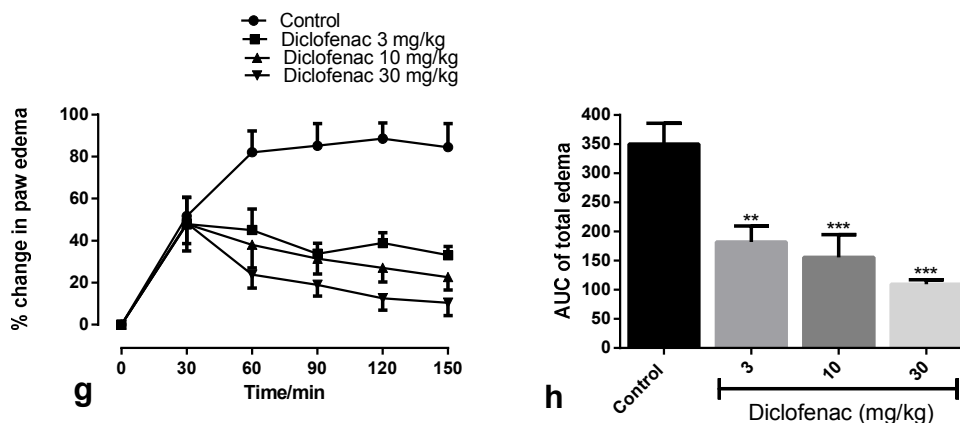


Fig. 4. Curative effects of diclofenac on PGE2-induced paw oedema in rats. Rats were challenged with 50 μ L of 1 nm of PGE2 at the sub-plantar surface of the hind paw. Treatment with diclofenac (3-30 mg/kg p.o) started 30 min after PGE2 challenge. Percentage change in paw oedema (g) and total oedema as AUC (h) were determined for 2½ hours

*implies $p \leq 0.05$; ** implies $p \leq 0.01$; *** implies $p \leq 0.001$. Data were presented as mean \pm SEM (n=5)

3.3 Acetic Acid-Induced Writhing Test

Intraperitoneal injection of 10 ml/kg of 0.6% acetic acid in mice induced a writhing movement characterized by contraction of the abdominal muscles, bending of the trunk or stretching of the hind limbs. LAE (30, 100 and 300 mg/kg) dose-dependently reduced the writhing movement in mice. The statistical values $F_{(15, 96)} = 3.573$, $p < 0.0001$; $F_{(5, 96)} = 49.36$, $p < 0.0001$ and $F_{(3, 96)} = 73.33$, $p < 0.0001$ were very significant. Similarly, diclofenac (3, 10 and 30 mg/kg) also dose-

dependently inhibited the writhing movement of the mice. The percentage maximal inhibition for the extract (at 300 mg/kg) and the reference drug (at 30 mg/kg) were 76.90% and 80.92% respectively.

4. DISCUSSION

Inflammation is a complex pathophysiological process which results from tissue injury [13]. Its cascade is driven by both chemical and cellular mediators. Some of these signaling pro-

inflammatory mediators include cytokines such as tumour necrosis factor- α (TNF- α), interferon (INF) and interleukins (IL-1, IL-6, IL-12) [13]. Tissue injury or release of these signaling molecules speeds up conversion of arachidonic acids into prostanoids (i.e. thromboxanes, prostacyclins and prostaglandins) [14]. Prostaglandins (PGE2, PGI2 and PGD2) are potential vasodilators and also synergize with other inflammatory vasodilators such as histamine and bradykinin [15]. This augmented dilator effect on the precapillary arterioles results in increased vascular permeability and blood flow, which are sometimes seen as redness

during acute inflammatory episode [16]. Similarly, injection of histamine, bradykinin, serotonin and prostaglandin E2 into the sub plantar side of rats evokes inflammatory response in experimental models [17]. In this research, injection of 50 μ L of 1 nM of PGE2 in the sub-plantar side of hind paw of rats resulted in inflammation of the paw which was characterized by oedema. The peak of oedema was recorded at the 60th minute similar to earlier reports by other investigators [18,19]. In addition to inflammation, prostaglandin E2 indirectly causes hyperalgesia by sensitizing nociceptive nerve fiber endings to inflammatory and pain mediators such as

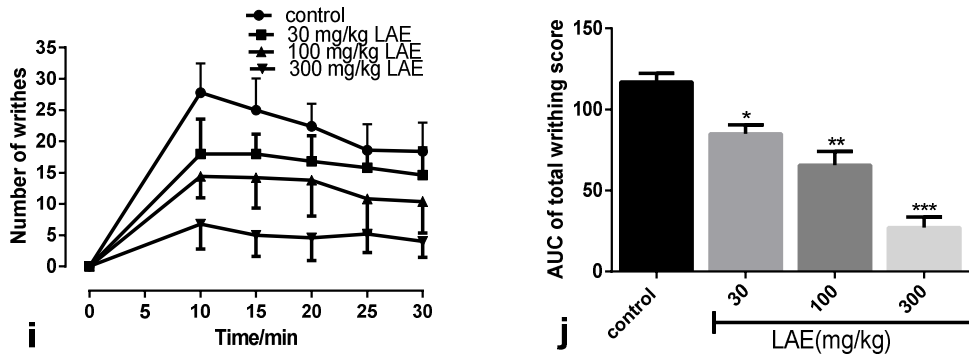


Fig. 5. Effect of LAE on acetic acid-induced writhing test in mice. ICR mice were prophylactically treated with LAE (30-300 mg/kg *p.o*) 1 hour before acetic acid challenge (10 ml/kg of 0.6% acetic acid *i.p*). Writhing behaviour were recorded at 5 min intervals for 30 min starting from 10 min after acetic acid injection. Mean number of writhes (i) and total writhing score as AUC (j) were determined

*implies $p \leq 0.05$; ** implies $p \leq 0.01$; *** implies $p \leq 0.001$. Data were presented as mean \pm SEM (n=5)

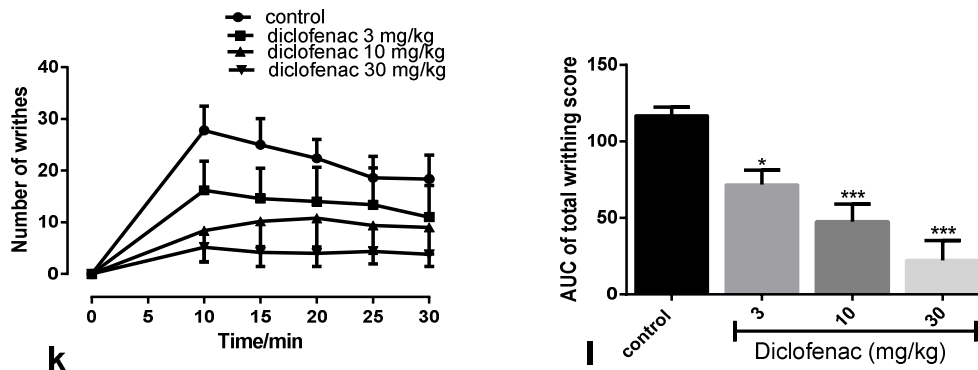


Fig. 6. Effect of diclofenac on acetic acid-induced writhing test. Mice were prophylactically treated with diclofenac (3-30 mg/kg *p.o*) 1 hour before acetic acid (10 ml/kg of 0.6% *i.p*) injection. Writhing behaviour were recorded for 30 min at 5 min intervals starting from 10 min after acetic acid injection

*implies $p \leq 0.05$; *** implies $p \leq 0.001$. Data were presented as mean \pm SEM (n=5)

bradykinin, histamine and serotonin [20,21]. Potential anti-inflammatory and analgesic compounds such as non-steroidal anti-inflammatory drugs inhibit synthesis and release of arachidonic acid metabolites such as prostaglandins [22,18]. Natural products have also been reported to exhibit potent inhibitory effect on synthesis and release of pro-inflammatory and pain mediators comparable to NSAIDs [18].

Reduction of PGE2-induced paw oedema by the *Lannea acida* extract in this experiment could be due to its ability to obliterate the important roles such as vasodilatation and vascular permeability of pro-inflammatory mediators including prostaglandins, histamine and bradykinin. It could also be due to inhibition of one or more of the cognate receptors of PGE2. Also, further synthesis of PGE2 via the cyclooxygenase pathway could have been inhibited by the extract.

The acetic acid writhing test is a convenient model often used as a stimulus for screening compounds with analgesic and anti-inflammatory effects [23,24]. In addition to directly stimulating the nociceptive nerve endings, it also offers the advantage of having rapid onset and shorter duration of analgesic action and thus minimizes the length of discomfort to which the animals are exposed. Initial writhing response following administration of acetic acid is a neurogenic pain response due to direct activation of nociceptive fibres [24]. The subsequent writhing response is sustained by synthesis and release of pro-inflammatory mediators including prostaglandins, bradykinin and cytokines [25]. It is reported that prostaglandin increases sensitization of nociceptive nerve fibres to pain mediators such as bradykinin and serotonin [26,27]. It is also a report that the prostacyclin released following intraperitoneal injection of acetic acid in mice is involved in the writhing movement [27,28] Based on these findings, we could suggest that the ability of the extract to significantly reduce the writhing response could be due to inhibition of direct activation of central and/or peripheral nociceptive nerve fibres or inhibition of synthesis and release of prostaglandins. Although the actual mechanism of anti-inflammatory and analgesic actions of the extract were not elucidated, its effects could be attributed to the various phytoconstituents (such as alkaloids, glycosides, steroids, saponins, flavonoids and tannins) identified in the plant extract; as these compounds have been reported

to exhibit inhibitory effects on the synthesis and release of many pro-inflammatory mediators [29].

5. CONCLUSION

We conclude based on these findings that the aqueous extract of *Lannea acida* stem bark significantly inhibits prostaglandin E2-induced paw oedema and acetic acid-induced writhing movements in animal models. The extract could therefore be considered as a potential source of remedy for inflammation and pain.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH Publication No. 85-23, 1985, revised 1996) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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