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Original Research Article

Aqueous extract of *Lannea microcarpa* attenuates dextran sulphate-induced paw oedema and xylene-induced ear oedema in rodents

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ABSTRACT

Background: *Lannea microcarpa* Engl. and K. Krause (Family Anacardiaceae), a tropical tree is used traditionally used in Africa for both medicinal and non-medicinal purposes. Folkoric uses of the plant include wound healing, conjunctivitis, stomatitis and gingivitis among others. Inflammatory reactions are involved in several diseases which this plant is traditionally used to manage. This study was therefore aimed at investigating the antiinflammatory effects of the aqueous extract of *Lannea microcapa*.

Methods: Oedema was induced in the right hind paws of Sprague Dawley rats (200-250g, 12 weeks old, n=5) using dextran sulphate solution whiles ear oedema was induced in ICR mice (25-30g, 6 weeks old, n=5) using xylene solutions. Aqueous extracts of ALM (30, 100 and 300mg kg-1) were administered in a set of rats and mice for both prophylactic and therapeutic studies. In the dextran sulphate-induced paw oedema, rats (200-250g) were treated orally with ALM (30, 100 and 300 mg kg-1) for both prophylactic and therapeutic studies. The paw thickness of the rats was measured before and after dextran sulphate injection at an hourly interval for 5 h. For xylene-induced ear oedema, ICR mice (25-30g) were given the same doses of the ALM and the ear weight of mice were measured after 2 h.

Results: In the dextran sulphate-induced paw oedema, the ALM reduced the mean maximal paw oedema significantly (P ≤0.05) to $36.392\pm9.207\%$ and $26.050\pm3.396\%$ at 100 and 300 mg kg^{-1} (prophylaxis) and $32.192\pm5.670\%$, $31.398\pm6.921\%$ and $31.593\pm5.841\%$ at 30, 100 and 300 mg kg-1 (therapeutic) in dose dependent manner when compared to the control respectively. Similarly, the ALM dose dependently showed a significant (P ≤0.05) reduction of percentage mean oedema in xylene-induced ear oedema by 43.56%, 59.63% and 68.07% at 30, 100 and 300 mg kg^{-1} when compared to the control respectively.

Conclusions: Aqueous extract of *Lannea microcapa* (30 -300 mg kg-1) caused significant reduction of oedema in both dextran sulphate-induced paw oedema and xylene-induced ear oedema.

Keywords: Anti-inflammation, Dextran sulphate, *Lannea microcarpa*, Oedema, Xylene

INTRODUCTION

Inflammatory response is usually stimulated by the action of various immune capable cells such as lymphocytes, platelets, mast cells and macrophages. Mast cells play a key role in the generation of inflammation via the release of inflammatory mediators including preformed soluble mediators such as histamine and serotonin.[1,2] Others include metabolites of arachidonic acid metabolism, pro-inflammatory cytokines and chemokines which cause inflammation and further act to enhance inflammatory response.[3] Pharmacological intervention is therefore required to regulate the activities of these inflammatory responses and also to manage their effects. Conventional drugs used in the management of inflammation include non-steroidal antiinflammatory drugs (NSAIDs), glucocorticoids and the disease modifying antirheumatic drugs.[4] These drugs have been reported to be associated with numerous adverse effects and therefore natural products such as plants are being considered as

alternative source of pharmacological remedy of inflammation. One of such plants is *Lannea microcarpa* Engl. and K. Krause (Family Anacardiaceae), a deciduous tree of the savannah, that grows up to about 15 m tall and 70 cm in diameter. It is located in most regions of West Africa and largely distributed in the dry forest regrowth areas. The leaves usually alternate and imparipinnate up to about 25 cm long with two to three pairs of leaflets. The flowers are green-yellowish, unisexual and regular; and the fruits are ellipsoid and glabrous drupes containing a single seed.[5] Most parts of *Lannea microcarpa* such as the bark and leaves are used traditionally in treating numerous medical disorders including swellings, gonorrhea, respiratory disorders, blisters, sore throat and rheumatism.[5-9] It has also been reported to possess wound healing ability, antimalarial, antidiarrheal, antigastroenteritis, cutaneous antiirritating effects as well as antibacterial and antihypertensive effects.[10-18] Quantitative determination of phytochemical composition of the plant has been reported and this revealed the presence of numerous phytochemical constituents.[14,18-21] Due to these numerous positive reports on this plant, it is therefore imperative that we carry out a scientific research on the antiinflammatory effect of aqueous extract of this time tested plant to provide a valid scientific data to support its traditional uses.

METHODS

Plant collection

The stem bark of *Lannea microcarpa* was collected from New Edubiase, Ashanti Region of Ghana in April, 2016 and it was authenticated by Dr. Henry Sam of the Department of Herbal Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. The plant material was chopped and air-dried for 14 days under room temperature.

Extraction

The chopped stem bark was milled into coarse powder using a hammer mill (DF-19, DADE, 20 kg/h-101v, HXJQ, China). Six hundred grams (600g) of the powder was mixed with 2.0 L of water and warmed for 90 min at 90 °C. The mixture was filtered to obtain a dark-brown filtrate which was evaporated over a hot water-bath followed by drying in an oven at 60°C. The final concentrate was cooled in a desiccator for 60 minutes to yield a dark-brown semi-solid *Lannea microcarpa* extract (refer to as ALM). The final yield was 12.4%. The extract was reconstituted as an emulsion in 2% tragacanth mucilage.

Chemicals

Dextran sulphate powder (Sigma-Aldrich, St. Louis, MO, USA), Diclofenac (Trogue, Hamburg, Germany), xylene solution (Shire Pharmaceuticals Inc., MA, USA).

Animals

Sprague Dawley rats (200-250g) and ICR (Imprint Control Region) mice (25-30g) were purchased from Centre for Scientific Research into Plant Medicine, Mampong-Akropong, Ghana. The animals were grouped into treatment and control groups (n =5); and kept in stainless steel cages (34cm×47cm×18 cm) in the animal house of Department of Pharmacology, KNUST. The animals were maintained at a room temperature of 26±27°C and incandescent illumination was provided on 12-h light-dark cycle. The animals were fed with commercial diet (GAFCO, Tema, Ghana) and water *ad libitum*. The animals were ethically handled throughout the experiment in consonant with internationally accepted principles for laboratory animals' use and care (EEC Directive of 1986: 86/609 EEC). Also, all animals and inflammatory protocols used in the experiment were approved by the Department of Pharmacology, KNUST Ethics Committee. Each animal was used only once and at the end of each experiment animals were euthanized.

Phytochemical analysis

The aqueous extract was analyzed using simple qualitative analysis previously described.[22]

Dextran sulphate-induced paw oedema

Paw oedema was induced by a method previously described.[23] Briefly, 0.1 ml of 1% $(^w/_v)$ dextran sulphate dissolved in saline solution was injected into the subplantar region of the right hind paw of rats (200-250g). The paw thickness was measured before oedema induction and then at hourly intervals for 5 h post injection using a digital caliper (VC1346i, MP Lab Equip, U.S.A). Increase in paw thickness was expressed as the mean percentage change of paw thickness using the formula:

$$\% \text{ change in paw thickness} = \left[\frac{\text{PT}o - \text{PT}t}{\text{PT}o}\right] \times 100$$

Where PTt is the Paw thickness (at various time points) post dextran sulphate injection and PTo is the Paw thickness before dextran sulphate injection. Total oedema was determined as the area under the time course curve (AUC) during the 5 h of dextran sulphate injection. The percentage inhibition of the total oedema was calculated using the formula

$$\% \text{ Inhibition of edema} = \left[\frac{AUC\ control - AUC\ treated}{AUC\ control}\right] \times 100$$

In the prophylactic protocol, the test animals were treated with ALM (30, 100, 300 mg kg⁻¹, 1 h, p.o) or diclofenac (30 mg kg⁻¹, 30 min, i.p) before dextran sulphate injection. In the therapeutic protocol, drugs were administered 1h post subplantar injection of dextran sulphate. Control rats received 1 ml of normal saline.

Xylene-induced ear oedema

Ear oedema was induced by a method earlier described[24] with slight modification. Briefly, 0.03 ml (50 μl) of xylene solution was topically applied on both posterior and anterior surfaces of the right ear. Mice (25-30g) in test groups (I, II and III) were treated with a single dose of ALM (30, 100 and 300 mg kg^{-1}, p.o, 1 h) and groups (IV, V and VI) received a single dose of diclofenac (1, 10 and 30 mg kg^{-1}, i.p, 30 min) post xylene injection over an experimental period of 2 h. The left ear was used as a control. The mice were sacrificed after 2 h by cervical dislocation and the ears (left and right) were excised and weighed on an electronic balance (Model: XPE10001L, METTLER TOLEDO, Switzerland). The degree of ear oedema was determined as the percentage difference between the right and left ears.

Determination of ED$_{50}$

ED$_{50}$ (dose responsible for 50 % of maximal effect) was determined for the *Lannea microcarpa* extract (30, 100 and 300 mg kg^{-1}) in the xylene-induced ear oedema model.

Statistical analysis

Data obtained was presented as mean±SEM. Statistical analysis was done by using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. GraphPad Prism for Windows, Version 6 (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. Probabilities less than 0.05 (P ≤0.05) was considered statistically significant between means of treated groups and the control. The ED50 (dose

responsible for 50 % of maximal effect) was determined by using an iterative computer least square method, with the non-linear regression (three-parameter logistic) equation.

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

Where X is the logarithm of dose and Y is the response. Y begins at a (the bottom) and goes to b (the top) with a sigmoid shape.

RESULTS

Phytochemical analysis

Qualitative phytochemical constituents test revealed the presence of steroids, glycosides, tannins, saponins, triterpenoids, alkaloids, and flavonoids.

Dextran sulphate-induced paw oedema

Injection of dextran sulphate (0.1 ml of 1 % in saline) resulted in paw swelling of the hind paw of the rats peaking between 2-3 h. ALM administered pre-emptively reduced the mean maximal oedema significantly to 36.392±9.207 % and 26.050±3.396 % at 100 and 300 mg kg^{-1} respectively (Figure 1A) when compared to the inflamed control response (73.607±4.990 %) while the total paw oedema induced over 5 h (measured as the area under the time course curve, AUC) was also significantly (P ≤ 0.05) reduced by 40.93±13.83 % and 64.17±5.738 % at 100 and 300 mg kg^{-1} in a dose dependent manner (Figure 1B) when compared to the inflamed control response (264.7±10.94 %).
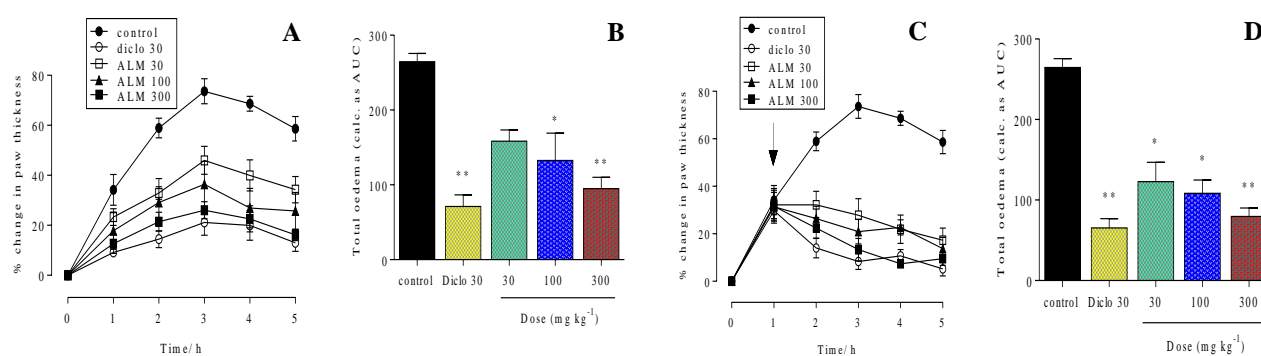

Figure 1: Effect of *Lannea microcarpa* extract on the maximal (A and C) and the total oedema responses (B and D) in the prophylactic and therapeutic studies of dextran sulphate-induced paw oedema in rats respectively (n=5). ↓ denotes time of drug administration.

The same doses of ALM (30, 100 and 300 mg kg^{-1}) administered after dextran sulphate paw oedema induction (therapeutic) reduced the mean maximal paw oedema to 32.192±5.670 %, 31.398±6.921 % and 31.593±5.841% at 30, 100 and 300 mg kg^{-1} respectively

when compared to the control inflamed response of 73.607±4.990% (Figure 1 C). The total paw oedema induced over 5 h was also reduced significantly (P ≤0.05) by 53.61±9.126%, 59.09±6.220% and 69.97±4.028% at 30, 100 and 300 mg kg^{-1} (Figure 1 D) when compared to

the inflamed control response. Diclofenac (30 mg kg^{-1}), the reference drug used, significantly suppressed all the parameters evaluated (Figure 1 A-D).

Xylene-induced ear oedema

ALM (30, 100 and 300 mg kg^{-1}, p.o) administered before the induction of xylene ear oedema caused the mean percentage ear oedema to be reduced significantly (P ≤0.05) by 43.56±9.183%, 59.63±13.500% and 68.07±14.98% at 30, 100 and 300 mg kg^{-1} respectively when compared with the inflamed control response of 82.33±11.77% (Figure 2 B). Diclofenac (1-30 mg kg^{-1}), the reference drug used, suppressed the mean percentage ear oedema significantly (Figure 2 A).
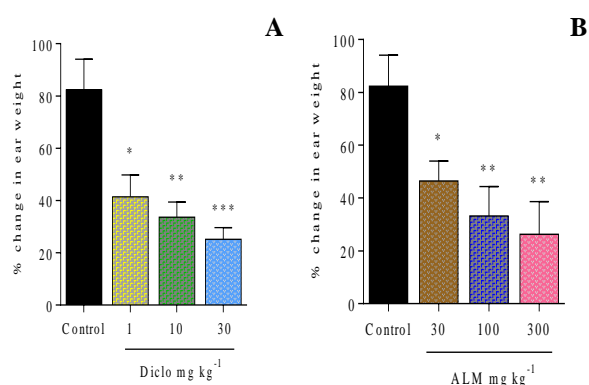

Figure 2: Effect of *Lannea microcarpa* extract on the total oedema responses (B) in xylene-induced ear oedema in mice respectively (n=5).
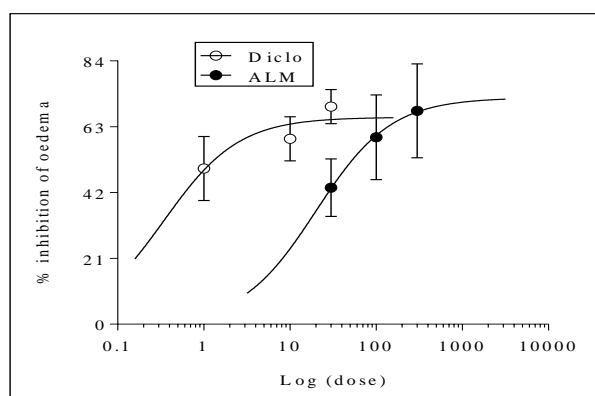

Figure 3: Dose response curves for *Lannea microcarpa* extract (30, 100 and 300 mg kg-1) and diclofenac (1, 10 and 30 mg kg-1) in xylene-induced ear edema in mice (n=5).

ED$_{50}$ for *Lannea microcarpa* extract (ALM)

Dose response curves for percentage inhibition of oedema is shown in Figure 3. ALM displayed a sigmoidal curve dose response relation with ED$_{50}$ of 20.04±2.71 whiles the ED$_{50}$ for diclofenac was 2.13±0.344.

DISCUSSION

In this study, the aqueous extract of the stem bark of *Lannea microcarpa* was considered as a potential agent for antiinflammatory activity based on its folkloric use in traditional medicine for treatment of pain, wound, swelling and other many medical disorders. Phytochemical constituents such as steroids, glycosides, tannins, saponins, triterpenoids, alkaloids, and flavonoids were present in the extract of the stem bark of *Lannea microcarpa* which is in consonant with the earlier phytochemical screening conducted.[14,16,18-20] In this study, dextran sulphate injected in the rats induces anaphylactic reaction characterized by extravasation and oedema formation as a result of histamine and serotonin release from the mast cells.[25] Dextran is known to be a potent osmotic agent which causes significant increase in vascular permeability and blood flow to the inflammatory site.[26] Oedema formation is usually caused by certain mediators such as prostaglandins, nitric oxide, histamine, serotonin and bradykinin due to the activity of cyclooxygenase enzyme.[27] The inhibition of these proinflammatory mediators provides a competent pharmacological remedy to inflammatory disorders. In the present study, *Lannea microcarpa* extract (ALM) administered either before or after dextran sulphate paw oedema induction inhibited the activities of histamine, bradykinin and serotonin during inflammatory response. It is therefore not surprising that the extract induced a more potent reduction of oedema formation and suppressed cyclooxygenase activity hence reducing the synthesis of prostaglandins and other proinflammatory mediators.

Xylene-induced ear oedema is widely employed in screening antiinflammatory agents capable of altering the activity of phospholipase A$_2$ enzyme.[28] Xylene usually initiates inflammatory response resulting in oedema, warmth and redness of the skin of the ear of mice due to the release of histamine, kinin, fibrinolysin and substance P.[29-32] In this investigation, *Lannea microcarpa* extract (ALM) significantly inhibited xylene-induced ear oedema in a dose related manner and ultimately decreased irritation, oedema and erythema effects and so it could be suggested that the extract inhibited the activities of the proinflammatory mediators including histamine, kinins and fibrinolysin. Although the actual mechanism of action of *Lannea microcarpa* extract (ALM) was not demonstrated in this research, its anti-inflammatory effects may be attributed to one or more of the phytochemicals constituents such as flavonoids, steroids saponins and tannins which were found to be present in the extract. From the study, it could be deduced that the effective dose of maximal inhibition for *Lannea microcarpa* extract (ALM) was higher than diclofenac which indicated relatively less potency when compared to diclofenac.

CONCLUSION

We therefore conclude that aqueous extract of the stem bark of *Lannea microcarpa* possesses antiinflammatory effect on acute inflammation mediated through the inhibition of proinflammatory mediators such as histamine, serotonin and other cytoplasmic enzymes.

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