

Analgesic and Anti-Inflammatory Properties of the Methanol Stem Bark Extract of *Lannea acida* (A.Rich) in Laboratory Animals

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ABSTRACT

Lannea acida is a medicinal plant used for the treatment of a wide range of ailments including haemorrhoid. In the present study, the phytochemical constituents present in the methanol stem bark extract of *Lannea acida* were determined using standard protocol. The analgesic and anti-inflammatory activities of the extract were evaluated. Acetic acid-induced writhing and hot plate-induced tests in mice were used to evaluate the analgesic activity, while Egg albumin-induced paw edema in rats was used to evaluate the anti-inflammatory activity. Median lethal dose of the extract was estimated using Lorke's method. For each of the tests, thirty animals were divided into five groups of six mice each. Groups I – V received normal saline (10 ml/kg), *Lannea acida* extract (20, 40, and 80 mg/kg *i.p.*), and piroxicam (20 mg/kg) for acetic acid-induced writhes/egg albumin-induced paw edema and pentazocine (20 mg/kg) for hot plate induced pain tests respectively. The phytochemical screening revealed the presence of carbohydrates, flavonoids, glycosides, alkaloids, saponins and tannins. The intraperitoneal LD₅₀ value in mice was estimated to be 282.8mg/kg. The extract significantly ($P < 0.05$) inhibited acetic acid-induced writhing and also produced more than 70% inhibition at all tested doses after 1 hour against thermal induced pain in mice. The extract at all tested doses afforded 64% inhibition of paw edema at the end of the 4th hour. The result obtained lends the credence to the ethnomedical use of the plant in the management of haemorrhoids.

KEYWORDS: Analgesic, Anti-inflammatory, Acetic acid, *Lannea acida*, pain

INTRODUCTION

World Health Organisation (WHO) estimated that about three-quarters of the population living in developing countries relies upon traditional remedies (mainly herbs) for the health care of its people¹ and attributed this high patronage of traditional medicine to its easy accessibility and affordability². Therefore, the inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries has been encouraged, as they hold great potentials in combating various diseases³. This infers that a wide range of traditional medicines are found to be effective; hence it is becoming imperative to

examine these medicinal plants for their possible toxicity and potential medicinal effect.

The plant *Lannea acida* has several reported ethnomedicinal uses in different parts of the world. It is traditionally used in the treatment of haemorrhoid and stomach troubles in many parts of West Africa, particularly northern Nigeria⁴. It is used internally, in Senegal for beriberi, schistosomiasis and haemorrhoids, and externally for eye-troubles, and with other drug-plant for dysentery and sterility⁵.

Currently available conventional drugs for the treatment of pain and inflammation 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-threatening⁶. It is therefore essential that efforts to introduce new medicinal plants be intensified in order to develop more cost-effective drugs with less adverse effects. Drugs of plant origin had been used for ages without or with minimal tolerable adverse effects and are being explored as alternative remedy for inflammation and pain^{7,8}.

This present study is aimed at evaluating the analgesic and anti-inflammatory properties of methanol stem bark of *Lannea acida*.

MATERIALS AND METHODS

Animals

Adult Wistar rats (120-150 g) and Swiss Albino mice (18-24 g) of either sex were used for the experiments. All animals were apparently healthy and obtained from the Animal House Facility of Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. They were housed in standard polypropylene cages and kept in a well-ventilated area. The animals were fed on standard rodent feeds and water *ad libitum*. All experimental protocols were approved by the University animal ethics committee.

Plant collection and authentication

The stem barks of *Lannea acida* were collected in January 2011 from Ahmadu Bello University (A.B.U.) Farm, Zaria, Kaduna State. The plant was identified and authenticated at the Herbarium Section, Department of Botany, Ahmadu Bello University, Zaria, Nigeria. By comparing with existing specimen, a voucher number 480 was allotted to the plant.

Preparation of Crude Extract

The stem bark of *Lannea acida* collected were dried at room temperature and pulverised into powder. Two hundred grams (200 g) of the powder was extracted with absolute methanol by cold maceration at ambient temperature for 72 h. The resultant mixture was sieved and the resulting marc was re-extracted successively

by means of absolute methanol until the extract became colourless. The methanol extract collected were concentrated *in vacuo* at 45°C, dried extracts obtained were kept at 4° C until needed. Percentage yield of extract (% w/w) was estimated by the formula below;

$$\% \text{Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Powdered Material}} \times 100$$

Drugs

Piroxicam tablet was obtained from Hovid, Malaysia, Pentazocine injection from (Fidson Healthcare Limited) Batch no: PHR 1588, Exp date 06/12, Acetic acid solution from Searle Essex, England. Egg albumin and all other reagents and consumables were obtained from the Department Department of Pharmacology laboratory, Ahmadu Bello University, Zaria and they were of analytical grades.

Preliminary Phytochemical Screening

The stem bark extract of *Lannea acida* was screened for the presence of alkaloids, carbohydrates, flavonoids, glycosides, saponins, and tannins according to standard protocol^{9,10}. The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals

Total Phenolic Content

Total phenolic contents were evaluated with Folin-Ciocalteu's phenol reagent¹¹. 5 ml of the extract solution was mixed with 5 ml Folin-Ciocalteu reagent previously diluted with water. After 5 minutes, 4 ml of 7% Na₂CO₃ solution was added to the mixture. The tubes were shaken for 5 seconds and allowed to stand for 30 min at 40°C for color development. The absorbance of the sample was measured at 765 nm. Gallic acid will be used as standard (1 mg/ml). The test was performed in triplicate. The results were determined from the standard curves and expressed as gallic acid equivalent (mg/g of extracted compound).

Total Flavonoid Content

A 10 g quantity of the powder was taken before and after exposure and extracted using 50ml of methanol by cold maceration. It was determined by Colorimetric aluminum chloride method¹². Exactly 0.5 ml solution of plant extract in methanol was mixed with 2ml of 2% aluminum chloride. After one hour at room temperature, the absorbance was measured at 420 nm at room temperature after one hour. Quercetin was used as standard (1 mg/ml). All the tests were performed in triplicates. Flavonoid contents were determined from the standard curve and expressed as quercetin equivalent (mg/g of extracted compound)

Acute toxicity study

The method of Lorke was adopted in this study. The study was divided into two phases adopting the method¹³. In the first phase, nine mice of either sex were divided into three groups of three mice each, they were administered 10, 100 and 1000 mg/kg of extracts via the intraperitoneally respectively. The mice were observed for signs and symptoms of toxicity and death for twenty-four hours after treatment. In the second phase, based on the result of the first phase, 4 mice were divided into 4 groups of one mouse each and administered 400, 800, 1600 mg/kg extract respectively. The mice were observed for twenty-four hours for signs and symptoms of toxicity and death. The final LD₅₀ was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose or the geometric mean of consecutive doses for which 0 and 100% survival rates were recorded.

Acetic acid-induced writhing test in mice

The method as described¹⁴ was employed to assess the analgesic activity of the methanol the stem bark of *Lannea acida*. A total of 30 mice were divided into 5 groups of 6 mice each. Respectively, group I – V received normal saline (10 mL/kg of 0.6% v/v), *Lannea acida* extract (20, 40 and 80 mg/kg) or piroxicam (10 mg/kg).

Thirty (30) minutes later, mice were administered 10 mL/kg of 0.6% acetic acid. After 5 minutes of acetic acid induction,

writhing movements such as stretching of hind limbs, contraction of the abdominal muscles, or bending of the trunk were counted for a period of ten minutes. Data collected represented the average number of writhing movements of the group. Percentage inhibition of writhing was calculated using the formula:

$$\%inhibition = \frac{MNW (control) - MN (test)}{MNW (control)} * 100$$

Where; MNW = Mean number of writhes

Hot plate method

The test was carried out using a Gallenkamp (England) thermostat hot plate apparatus maintained at 50°C according to the method described by Turner¹⁵. Only mice that showed initial nociceptive response within 30 seconds were selected for the experiment. The reaction time of the mice to the thermal stimulus, taken to be the interval between the instant the animal reached the hot plate to the time it licked its paw or jump off the hot plate. Thirty (30) mice were selected and divided into 5 groups of 6 mice each. They received prophylactic treatment of (10 mL/kg of 0.6% v/v), *Lannea acida* extract (20, 40 and 80 mg/kg) or pentazocine 30 mg/kg. All treatments were administered intraperitoneally respectively.

Thirty minutes after treatment, the reaction time of each mouse was again evaluated as above. The final test mean value (Ta) for each treatment group was calculated which represented the after-treatment reaction time and was subsequently used to determine the percentage thermal pain stimulus or protection (%PaTS) by applying the formula:

$$\%PaTS = \frac{\text{Test mean (Ta)} - \text{Control mean (Tb)}}{\text{Control means (Tb)}} * 100$$

EGG ALBUMIN-INDUCED PAW EDEMA TEST IN RAT

The anti-inflammatory study was carried out using egg albumin-induced paw edema in rats as described by Winter¹⁶ and modified by Okoli¹⁷. Thirty (30) rats were divided into 5

groups of 6 mice each, group I served as control and were treated with normal saline 10 mL/kg, group II-IV were treated with the extract at 20, 40, and 80 mg/kg while group V received piroxicam 20 mg/kg.

Thirty minutes later, 0.1ml of egg albumin was injected into the sub plantar region of the left hind paw of each rat. The paw diameter was measured with a Vernier calliper at 0, 1, 2, 3, and 4 hours after the injection of egg albumin. The difference between the readings at time interval was taken as the index of the edema.

The percentage anti-inflammatory activity was calculated using the formula;

$$\frac{\text{MPD (test)} - \text{MPD (control)}}{\text{Mean MPD}} * 100$$

Where; MPD=mean paw diameter

STATISTICAL ANALYSIS

Data were expressed as mean $\pm$ standard error of mean. The results were analyzed using One Way Analysis of Variance (ANOVA) followed by Dunnett's post hoc test for multiple comparison. Differences were considered significant at $p < 0.05$.

RESULTS

Acute toxicity study

The intraperitoneal median lethal dose (LD_{50}) value of the methanol stem bark extract of *Lannea acida* in mice was 282.8 mg /kg body weight.

Phytochemical screening

The results of the phytochemical analysis revealed the presence of a number of

phytochemicals including alkaloids, glycosides, flavonoids, saponins, carbohydrate and tannins. A comprehensive list of phytochemicals present in the methanol stem bark extract of *Lannea acida* is presented in Table 1.

Acetic acid-induced writhing test in mice

The methanol stem bark extract of *Lannea acida* has significantly ($p < 0.05$) reduced the number of acetic acid-induced writhes in mice. The highest percentage inhibition of abdominal writhes is 94.7% which was observed at 80 mg/kg test extract ($p < 0.05$), this showed that the extract act in a dose dependent manner (Table 2). The percentage inhibition of the standard drug used (piroxicam) was 61.7%.

Hot Plate test in mice

The extract significantly protected the mice against thermally induced pain stimulus at all the used doses (20, 40 and 80 mg/kg). The highest percentage inhibition of the extract was 180%, observed at 40 mg/kg after 1 hour of administration of the extract indicating that the activity of the extract is non-dose dependent in this study (Table 3). The highest percentage inhibition of the standard drug used (pentazocine) was 86% observed after 2 hours of administration of the drug (Table 4).

Egg Albumin-induced hind Paw Oedema

The extract suppressed the development of paw edema induced by egg albumin in rats. Interesting, at the end of the fourth hour, all the tested doses of the extract gave a percentage inhibition of 64% that were statistically significant at $P < 0.001$.

Table 1: Phytochemical constituents of the methanol stem bark extract of *Lannea acida*

Phytochemicals	Remark
Alkaloids	Present

Tannins	Present
Flavonoids	Present
Carbohydrates	Present
Saponins	Present
Glycosides	Present
Steroids	Absent
Terpenoids	Absent
Phenols	Absent

Table 2: Effect of methanol stem bark extract of *Lannea acida* on acetic acid- induced writhing in mice

Groups	Dose(mg/kg)	Mean Writhing	Percent Inhibition
Normal Saline	10ml	15.5	
<i>Lannea acida</i>	20	0.83±0.54	71.3
<i>Lannea acida</i>	40	1.32±0.71 ^b	91.5
<i>Lannea acida</i>	80	5.33±2.65 ^a	94.7
Piroxicam	20	6.00±2.37 ^a	61.7

Values are expressed as Mean ± S.E.M. one way ANOVA followed by Dunnett post hoc test. n = 6; ^aP<0.05, ^bP<0.01

Table 3: Effect of methanol stem bark extract of *Lannea acida* on thermally induced pain. Pain latency (seconds)

Time (mins)	N/saline	<i>Lannea. Acida</i> (mg/kg)			Pentazocine (mg/kg)
		20	40	80	20
30	0.95±0.02	1.94±0.33 (104)	2.09±0.29 ^b (120)	1.73±0.34 ^a (82)	1.75±0.38 (84)
60	0.74±0.06	1.80±0.40 ^a (143)	2.12±0.37 ^b (180)	1.61±0.34 ^a (118)	0.83±0.06 (12)
90	0.99±0.06	1.99±0.44 ^a (101)	2.36±0.20 (138)	1.67±0.20 ^b (69)	1.48±0.15 (49)
120	0.90±0.03	1.89±0.16 (189)	2.51±0.27 (179)	2.16±0.22 (140)	1.67±0.23 ^a (86)

Values are expressed as Mean ± S.E.M. Repeated Measures ANOVA followed by Bonferoni post hoc test. n = 6; Values in parentheses represent percentage protection against thermal stimulus; ^aP<0.05, ^bP<0.01, ^cP<0.001.

Table 4: Effect of methanol stem bark extract of *Lannea acida* on egg albumin induced inflammation on rats. Paw edema (cm)

Time (hrs)	N/saline	<i>Lannea. Acida</i> (mg/kg)			Pentazocine (mg/kg)
	10 ml/kg	20	40	80	20
½	0.22±0.04	0.09±0.02 ^b (59)	0.11±0.02 ^a (50)	0.12±0.02 (45)	0.09±0.01 ^b (59)
1	0.19±0.02	0.08±0.02 (58)	0.11±0.02 ^b (42)	0.08±0.02 ^b (58)	0.08±0.01 ^c (58)
2	0.22±0.03	0.08±0.02 ^b (64)	0.10±0.02 ^b (55)	0.08±0.01 (64)	0.08±0.01 ^c (64)
3	0.15±0.01	0.05±0.02 ^c (67)	0.07±0.01 ^c (53)	0.05±0.01 ^c (67)	0.05±0.01 ^c (67)
4	0.11±0.0	0.04±0.01 ^c (64)	0.04±0.01 ^c (64)	0.04±0.01 ^c (64)	0.06±0.01 ^c (45)

Values are expressed as Mean ± S.E.M. Repeated Measures ANOVA. n = 6; Figure in parentheses represents percentage anti-inflammatory activity; ^aP<0.05, ^bP<0.01, ^cP<0.001.

DISCUSSION

The phytochemical screening of crude methanol extracts of stem bark of *L.acida*

revealed the presence of alkaloids, glycosides, flavonoids, saponins, carbohydrate and tannins. The presence of

phytochemicals in medicinal plants has been reported to be responsible for the several medicinal and dietary benefits of the plant like the anti-inflammatory and antioxidants activities. Alkaloid have been reported to possess anti-inflammatory¹⁸ while studies have demonstrated that systematically administered flavonoids produce significant anti-nociception¹⁹.

The result of the study showed significant analgesic and anti-inflammatory activity of *L.acida* confirming the traditional claims of the usefulness of the plant in the management of pain. Considering the socioeconomic impacts of pain and inflammation, there is need to intensify the search for more effective anti-pain and anti-inflammatory drugs with minimal untoward effects from traditional medicinal plants^{7,8}.

The acetic acid-induced writhing method is widely used for the evaluation of peripheral antinociceptive activity²⁰. It is very sensitive and able to detect antinociceptive effects at dose levels that may appear inactive in other methods like the tail-flick test²¹. Local peritoneal receptors are postulated to be partly involved in the abdominal constriction response²². The method has been associated with prostanoids in general, e.g. increased levels of PGE2 and PGF2 α in peritoneal fluids²³, as well as lipoxygenase products by some researcher^{23,24}. The ability of the extract to significantly increase the nociceptive threshold in the acetic acid induced writhing could be attributed to the flavonoid found in the extract. The result of the acetic acid-induced writhing suggests that, the extract possesses peripheral analgesic activity possibly via inhibition of lipoxygenase and/or cyclo-oxygenase enzymes.

The hot plate method is one of the most common tests of nociception that is based on a phasic stimulus of high intensity²⁵. Pain induced by thermal stimulus of the hot plate is specific for centrally mediated nociception²⁶. The extract significantly delayed the mean reaction time on the hot

plate, this denotes decrease nociception. The anti-nociceptive activity was observed in all the test doses with more than 100% protection against thermal stimulus at all the test doses. This action further suggests central analgesic activity of the extract, this signifies that the extract may possess both central and peripheral analgesic activities.

The antinociceptive evaluation methods used in the present study were chosen in order to test different nociceptive stimuli, the cutaneous thermic (hot plate), and chemical visceral (writhing) stimuli. The result indicates that intraperitoneal administration of the extract exhibits a significant and dose dependent protective action on thermic (heat) painful stimuli at all the test doses. This observation may indicate that *Lannea acida* has both peripheral (writhe reduction) and central (thermal reaction time prolongation) effects.

Egg albumin-induced paw edema in a rat is a standard experimental model for acute inflammation. The extract also caused marked inhibition of albumin-induced hind paw edema in rats. Albumin-induced edema is a biphasic response; the early phase is mediated through the release of histamines, serotonin, and kinins, while the late phase is related to the release of prostaglandins and mediated by bradykinins, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages²⁷. The anti-inflammatory activity shown by the extract over a period of 3 hours was quite similar to that exhibited by the group treated with the standard drug (Piroxicam). This implies that the action involved a complex array of enzyme activation; mediator releases extravasation of fluid, cell mitigation, tissue break down and repair²⁸. These results suggest the potential of the extract to act on all the phases of inflammation; late phase activity might probably be involved with arachidonic acid metabolites which

produce an edematous response by mobilization of the neutrophils²⁹.

The extract suppressed the development of paw edema induced by egg albumin in rats, with the maximum percent inhibition (64%) occurring 4 hours after injection of egg albumin at the lowest test dose (20 mg/kg). This revealed that the extract *Lannea acida* possesses potent anti-inflammatory activity in egg albumin-induced paw edema in rats. The extract may have inhibited the release of pro-inflammatory mediators of acute inflammation such as histamine and prostaglandin like the NSAIDs³⁰. The major therapeutic effect of NSAIDs is due to their ability to inhibit cyclooxygenase enzyme³¹.

The co-existence of both antinociceptive and anti-inflammatory effects seen with this extract is well-defined for various nonsteroidal anti-inflammatory drugs (NSAIDs), particularly the salicylates and their derivatives since the cyclooxygenase enzyme which leads to the production of prostanoids is usually inhibited³². It is therefore interesting that the extract behaved similar to NSAIDs in this study which correlates well with the traditional application of the plant in pain and inflammatory condition such as Haemorrhoids.

CONCLUSION

The methanol extract of *Lannea acida* stem bark possesses both antinociceptive and anti-inflammatory effects.

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