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# Anti-nociceptive and anti-inflammatory properties of the ethanolic extract of *Lagenaria breviflora* whole fruit in rat and mice

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**Summary:** The present study was conducted to evaluate the anti-nociceptive and anti-inflammatory properties of an ethanol extract of whole fruit of *Lagenaria breviflora* (LB) in rat and mice. Analgesic activity was measured by hot plate, formalin-induced paw licking, and acetic acid-induced abdominal writhing tests, while anti-inflammatory activity was determined by inhibition of carrageenan-induced paw oedema. Extract-treated animals exhibited significantly ($P<0.05$) higher pain threshold, lower number of licking of paws in response to formalin-induced irritation and writhing movements in response to acetic acid-induced writhing movement. There was significant ($P<0.05$) inhibition of carrageenan-induced paw oedema in rats pre-treated with the extract (50, 100, 200mg/kg) by 6.4%, 27.5%, 55.9% respectively. Analgesic effect of the extract (50, 100, 200mg/kg) in hot plate test was observable within 30 minutes of administration with maximum effect obtainable 90 minutes post-administration. Also, the effect of the extract (50, 100 and 200mg/kg) was dose dependent in both the early (88.17±6.21, 80.33±3.49 and 72.33±5.16) and late (72.50±3.95, 53.83±3.96 and 35.83±3.78) phases of formalin-induced paw licking, and in acetic acid-induced writhing with inhibition of 26.8%, 48.1% and 58.1% respectively. Its effect was comparable especially at 200mg/kg body weight to those of diclofenac, indomethacin and ibuprofen. It could be suggested from the findings of this experiment that the extract may be mediating its action as a central analgesic agent but the peripheral analgesic effect was preponderant based on its outcome from the pain models..

**Keywords***: Lagenaria breviflora, Whole fruit, Anti-nociceptive, Anti-inflammatory*

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## INTRODUCTION

*Lagenaria breviflora* belongs to the plant family Cucurbitaceae (Yasuyuki *et al*., 2005 & Hanno *et al*., 2009). *Lagenaria breviflora* is a perennial climber ascending to the forest growing canopy, occurring from Senegal to West Cameroons, and generally widespread in tropical Africa. The leaves are very scabrid and sandpapery. The stem when crushed has an unpleasant smell, and a decoction from it is said to be used in Western Nigeria for headache and as a vermifuge (Ajayi *et al*., 2002). Its seeds and fruits have been used in folk medicine since antiquity. The fruit is used for treatment of cold (Faleyimu and Oluwalana, 2008) and schistosomiaisis in man (Ajayi *et al*., 2002), and coccidiosis in birds (Elujoba *et al*., 1985). There are reports of the anti-fertility (Saba *et al*., 2008) and erythropoiesis stimulating effects (Saba *et al.* 2009) of the whole fruit of *L. breviflora* in experimental animal models. Broad spectrum antibacterial activity of the whole fruit of *Lagenaria breviflora* has also been reported (Tomori *et al*., 2007). Previous phytochemical screening of *Lagenaria breviflora* revealed the presence of triterpenoid saponins (Elijoba *et al.*, 1990). Members of Cucurbitaceae such as *Lantana trifolia*, *Cayaponia tayuya* and *Coccinia indica* have actually been reported for their anti-inflammatory and analgesic properties (Silvia *et al*., 2008, Silvia *et al*., 2009 and Junaid *et al*., 2009). There is however paucity of information on the anti-inflammatory and anti-nociceptive properties of *Lagenaria breviflora*. In the present study we investigated the analgesic and the anti-inflammatory potentials of an ethanolic extract of whole fruit of *Lagenaria breviflora* in animal models.

## MATERIALS AND METHODS

### Preparation of plant extract

Fresh fruits of *Lagenaria breviflora* (LB) were obtained from local markets, in Ibadan, Oyo State Nigeria. The fruits were washed, cut and weighed. They were tied up in small quantities in sieves and

placed in plastic containers. Sufficient ethanol covering each portion was poured into each container and left for 3-4 days. The ethanol was decanted, stored and thereafter replaced with fresh ethanol. This procedure continued for 7 days until the fruit was no longer yeilding. The filtrate was kept in refrigerator at 20°C and was thereafter concentrated in a rotatory evaporator at a reduced temperature of $40^0$C. A semi-solid greenish-brown paste was obtained and kept in the refrigerator at $4^0$C until when needed.

**Experimental animal models**

Sprague Dawley rats weighing 140-160g and mice weighing 25-35g were kept in raised mesh bottom cages in environmentally controlled rooms ($25\pm2^0$C, 12 hour light and dark cycle). Animals were fed with standard pellets diet and water *ad libitum*. Animals were deprived of food for 24 hrs before the experiment.. Experimental protocols complied with the "Principle of Laboratory Animal Care" (NIH publication No 85-23) guidelines (NIH publication revised, 1985).

**Chemicals and Drugs**

All chemicals/reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Tween 20, glacial acetic acid, formaldehyde were obtained from S.D. Fine Chemical Pvt. Ltd., Mumbai, India. Standard drugs were suspended in 2.5% Tween 80/normal saline. All chemicals used are of analytical grade.

**Anti-nociceptive activity**

**Hot plate test in mice**

Pain reflexes in response to a thermal stimulus were measured using a Ugo Basile hot plate apparatus (25.4cm x 25.4cm at $(55\pm1.0)^{\circ}$C, which is surrounded by an opened-top acrylic cage (19cm tall), with the start/stop button on the timer), as described by Eddy *et al* (1950) with slight modification (Galeotti *et al.*, (1997). Mice in the control group were pre-administered with normal saline (10ml/kg body weight) while mice in the test groups were pre-administered 50, 100 or 200mg/kg of extract. Another group of mice were given diclofenac at 100 mg/kg. All administration was done orally. The mice were pre-administered with the saline, extract or reference drug at 30, 60, 90, 120 and 150 minutes and were habituated to the apparatus for 1 minute before the start of the test. A 10-second cut-off time was used to prevent tissue damage. The reaction time to pain was recorded as the time lapse between placing the mice on the hot plate and commencement of fore paw licking, hind paw flicking or jumping.

**Formalin-induced paw licking test in rats**

Twenty microlitres of 2.5% formalin was injected into the plantar surface of the left hind-paw of mice (Hunskar and Hole, 1987) 60 minutes immediately after oral administration of saline, extract (50, 100 or 200 mg/kg) or indomethacin (10mg/kg). The test was carried out in a transparent plastic chamber (30 x 30 x 30) cm with a mirror placed at the base (bottom) of the chamber to allow an unobstructed view of the mice. Licking of the formalin injected paw was indicative of pain. The numbers of lickings within 0 to 5 minutes and 15–30 minutes after injection of formalin were counted. The initial, acute nociceptive response within 0-5 minutes after injection of formalin indicated the first phase while within 15-30 minutes indicated the chronic phase. These phases represented neurogenic and inflammatory pain responses respectively.

**Acetic acid-induced abdominal writhing test in mice**

Mice were injected intraperitonealy with 0.1ml/10g body weight of 3% acetic acid solution 60 minutes after oral pre-treatment with saline, extract (50, 100, 200mg/kg) or indomethacin at 10mg/kg (Taber *et al.*, 1969). The number of writhing was observed between 5-15 minutes. The data were collected and computed according to the following formula:

Percentage Inhibition (%) =

$$\frac{[(\text{Mean No. of writhing})_{\text{control}} - (\text{Mean No. writhing})_{\text{test}}] \times 100}{(\text{Mean no of writhing})_{\text{control}}}$$

**Anti-inflammatory activity**

**Carrageenan-induced paw oedema in rats**

Pedal inflammation in male albino rats was produced according to Winter *et al.*, (1962). An injection of 0.1ml of 1% carrageenan was delivered into the right hind foot of the rat under the subplantar aponeurosis. The rats were orally pre-treated with normal saline, extract (50, 100, 200mg/kg) or ibuprofen (100mg/kg) 60 minutes before carrageenan injection. Inflammation was quantified by measuring the volume displaced by the paw using a plethysmometer (Ugo Basile, Italy) at the zero hour and 3rd hour after carrageenan injection.

The inhibiting activity was calculated according to the formula:

$$\% \text{ inhibition} = \left[\frac{(C_t - C_o)_{\text{control}} - (C_t - C_o)_{\text{test}}}{(C_t - C_o)_{\text{control}}}\right] \times 100$$

*where* $C_o$ *= Mean paw size in the control group and* $C_t$ *= Mean paw size in the treated group*

**Statistical analysis**

All values are expressed as mean ± S.E.M. Data was analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison tests. Differences of means were considered

significant at P<0.05 using Graph-Pad Prism software version 4.00 for Windows, GraphPad Software, San Diego California USA (www.graphpad.com).

## RESULTS

### Hot Plate Test

The reaction time to pain was longer in mice pre-administered with the extract than the mice in the control group. *Lagenaria breviflora* extract dose dependently exhibited longer reaction time (P<0.05) in mice when compared with the control group mice from 30 to 120 minutes. Mice pre-administered with the extract at 200mg/kg body weight for 30, 60 or 90 minutes also exhibited longer reaction time to pain compared to the mice pre-administered with diclofenac (100mg/kg.) for the same exposure periods (Table 1).

### Formalin-induced paw licking Test

The mean number of licking of paws by mice in the test groups was consistently lower compared with control mice in the early and late phases. The effect of the extract was dose dependent. The difference of the means was significant only for the mice pre-administered with 200mg/kg b.w of the extract in the early phase but significant for doses of 100 and 200mg/kg in the late phase. Mice pre-administered with indomethacin showed significantly (P<0.05) lower mean number of paw lickings than the mice in the control group and *Lagenaria breviflora*-treated group (50 and 100mg/kg) at the early and late phases except for rats pre-administered with 200mg/kg b.w of the extract in the late phase (Table 2).

### Acetic acid-induced abdominal writhing test

The mean number of writhing movements was significantly (P<0.05) lower in the test mice pre-administered with 100 or 200 mg/kg of the extract compared with the control mice. Mice administered with indomethacin exhibited significantly (P<0.05) lower mean number of writhing movements than mice pre-administered with 50 or 100 mg/kg of the extract but not for dose of 200 mg/kg b.w. The percentage inhibition of writhing movement in this group (58.1%) was comparable to that of mice pre-administered with indomethacin (66.3%) (Table 3).

**Table 1:**
Effects of graded-doses of *Lagenaria breviflora* (LB) on hot plate test in mice

| | | Exposure time before test (minutes) | | | | |
|---|---|---|---|---|---|---|
| **Group** | **Dose (mg/kg)** | **30mins Reaction time to pain (seconds)** | **60mins Reaction time to pain (seconds)** | **90mins Reaction time to pain (seconds)** | **120mins Reaction time to pain (seconds)** | **150mins Reaction time to pain (seconds)** |
| Normal saline | 10ml/kg | 2.9±0.34[a] | 3.42±0.75[a] | 3.92±0.20[a] | 3.30±1.31[a] | 2.50±1.44 |
| LB Extract | 50 | 2.9±1.20[b]* | 4.27±1.04 | 5.50±0.92 [a]* | 4.98±1.42 [a]* | 3.08±0.35 |
| LB Extract | 100 | 3.37±0.26 | 4.80±0.65[a]* | 6.17±1.04 [a]** | 5.50±0.68 a** | 2.98±0.83 |
| LB Extract | 200 | 5.67±1.43 [a]** | 5.68±1.37[a]** | 6.88±1.02 [a]** | 5.68±1.47 [a]** | 3.32±1.29 |
| Diclofenac | 100 | 4.60±0.61[a]**,[b] | 5.62±1.42[a]**,[b] | 6.68±1.30[a]**,[b] | 7.33±1.57[a]***,[b] | 3.92±0.75 |

Values are expressed as mean ± S.E.M of 6 mice. *=P < 0.05, **=P < 0.01, ***=P < 0.001
Superscripts (a) represent comparison of mean values of mice administered with LB extract or diclofenac with control mice while (b) indicates between mice groups administered with LB extract and mice administered with diclofenac within each column

**Table 2:**
Effects of *Lagenaria breviflora* (LB) on formalin-induced paw licking in mice.

| Group | Treatment (mg/kg) | Number of lickings | |
|---|---|---|---|
| | | **Early phase (0 - 5) minutes** | **Late phase (15- 30) minutes** |
| Normal saline | 10ml/kg | 90.67 ± 2.38[a] | 71.67±3.56[a] |
| LB Extract | 50 | 88.17±6.21[b]*** | 72.50±3.95 [b]*** |
| LB Extract | 100 | 80.33±3.49[b]*** | 53.83±3.96 [a]**, [b]*** |
| LB Extract | 200 | 72.33±5.16[a]*, [b]** | 35.83±3.78 [a]*** |
| Indomethacin | 10 | 58.17±4.03[a]***,[b] | 30.83±2.06[a]***,[b] |

Values are expressed as mean ± S.E.M of 6 mice. *=P < 0.05, **=P < 0.01, ***=P < 0.001
Superscripts (a) represent comparison of mean values of rats administered with LB extract or indomethacin with control mice while (b) indicates between mice groups administered with LB extract and mice administered with indomethacin within each column.

**Table 3:**
Effects of *Lagenaria breviflora* (LB) extract on acetic acid-induced abdominal writhing test in mice.

| Group | Dose (mg/kg) | Number of writhes | Inhibition (%) |
|---|---|---|---|
| Normal saline | 10ml/kg | 37.67±2.99[a] | 0 |
| LB extract | 50 | 34.50±3.89 [b]*** | 26.8 |
| LB extract | 100 | 19.33±1.48 [a]**, [b]*** | 48.1 |
| LB extract | 200 | 15.83±1.49 [a]*** | 58.1 |
| Indomethacin | 10 | 12.67±1.33[a]***,[b] | 66.3 |

Values are expressed as mean ± S.E.M of 6 mice. **=P < 0.01, ***=P < 0.001.
Superscripts (a) represent comparison of mean values of mice administered with LB extract or indomethacin with control mice while (b) indicates between mice groups administered with LB extract and mice administered with indomethacin within each column.

**Table 4**:
Effects of *Lagenaria breviflora* (LB) extract on carrageenan-induced paw oedema in rats.

| Group | Dose (mg/kg) | **Paw edema** volume at time zero, $t_0$ (ml) | **Paw edema** volume after 3hrs, $t_1$ (ml) | Change in paw size ($t_1$-$t_0$) ml | % inhibition |
|---|---|---|---|---|---|
| Normal saline | 10ml/kg | 0.91±0.03 | 1.73±0.07 | 0.82±0.05 [a] | 0 |
| LB extract | 50 | 0.92±0.03 | 1.68±0.08 | 0.76±0.06 [b]** | 6.5 |
| LB extract | 100 | 0.97±0.04 | 1.56±0.06 | 0.59±0.07 [b]** | 27.6 |
| LB extract | 200 | 0.83±0.04 | 1.19±0.07 | 0.36±0.09[a]** | 56 |
| Ibuprofen | 100 | 0.85±0.03 | 1.18±0.04 | 0.25±0.06[a]**,[b] | 69.3 |

Oedema values are expressed as mean ± S.E.M of 6 rats. **=P < 0.01.
Superscripts (a) represent comparison of mean values of rats administered with LB extract or ibuprofen with control rats while (b) indicates between rat groups administered with LB extract and rats administered with ibuprofen within each column.

**Carrageenan-induced paw oedema test.**
The change in paw size was larger in the control rats than for the rats in the test groups. The paw sizes recorded in the test rats decreased dose-dependently. The change in paw size of rats pre-administered with 200mg/kg b.wt of the extract was significantly (P<0.05) smaller than that for the control rats and also comparable to those of rats administered with ibuprofen (Table 4).

## DISCUSSION

An ethanol extract of whole fruit of *Lagenaria breviflora* exhibited both anti-inflammatory and analgesic properties in this study. Mice pre-administered with the extract showed significantly longer reaction time to pain than mice in the control group when subjected to hot plate test. This was also confirmed by significantly lowered number of licking of paws in test rats. Formalin-induced paw licking test in rats produces a distinct biphasic response involving early and late phases. Analgesics may act in early phase differently from late phase of this test (Sheu *et al*., 2009). The test is therefore used to clarify possible mechanism of anti-nociceptive effect of a proposed analgesic (Tjolsen *et al*., 1992). Centrally acting drugs such as opioids inhibit both phases equally but peripherally acting drugs such as aspirin, indomethacin and dexamethasone only inhibit the late phase (Shibata *et al*., 1989). Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin used in this study are known to inhibit cyclooxygenase 1 and 2 enzymes which are implicated in the production of inflammation-mediating agent such as prostaglandin $E_2$ ($PGE_2$) from arachidonic acid (Moody *et al*., 2006). The abdominal writhing response induced by acetic acid is used as a sensitive procedure to establish peripherally acting analgesics which might involve local peritoneal receptors (Chakraborty *et al*., 2003). The peripheral analgesic effect of the extract was demonstrated in the acetic acid-induced abdominal writhing test in mice. Abdominal writhing movement induced by acetic acid was significantly reduced by the extract. Our findings from the study indicate that administration of whole fruit of *Lagenaria breviflora* significantly inhibited the number of writhing response in comparison with the control groups. The level of inhibition produced by 200mg/kg of LB was also comparable to that of indomethacin (100mg/kg b.w).

The extract also inhibited carrageenan-induced paw oedema in rats. Data from this study demonstrated that there was no significant difference between the anti-inflammatory effect of extract of whole fruit of LB at the dose of 200mg/kg body weight and that of ibuprofen at 10mg/kg. Quantitatively, the analgesic or inflammatory activities of the extract was found to be dose dependent in the entire test conducted. The effective minimum concentration was 30mg/kg body weight and the analgesic activity was measurable within 30 minutes of its administration with peak activity

obtainable 90 minutes post administration. The analgesic or anti-inflammatory activities of the extract was found to be comparable, especially at 200mg/kg body weight to reference drugs used such as diclofenac, indomethacin and ibuprofen. Rats pre-administered with this dose of the extract 30, 60 or 90 minutes before stimulation of pain exhibited longer reaction time than rats pre-administered with diclofenac for the same period of time in the hot plate test.

These findings strongly suggest that the plant extract has strong analgesic potentials. Further studies will elucidate the exact mechanisms of action responsible for the analgesic and anti-inflammatory activities of LB.

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