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Analgesic and Acute Anti-inflammatory Activities of Aqueous Root Extract of *Salacia lehmbachii*

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Authors' contributions

This work was carried out in collaboration between all authors. Author LPT designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author BASL performed the statistical analysis and managed the analyses of the study. Author PMU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: *Salacia lehmbachii* is used in South Eastern Nigeria folk medicine to treat abdominal pain, inflammatory disorders and malaria symptom without scientific documentation. The aim of this study was therefore, to assess possible analgesic and anti-inflammatory activities of aqueous root extract of *Salacia lehmbachii* (ARESL) in albino rats.
Place and Duration of Study: The study was done in World Bank Step B Anti-Malaria Laboratory, Department of Pharmacology, Faculty of Basic Medical Sciences, University

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of Calabar, Calabar-Nigeria, between November 2013 and January 2014.
Methodology: Analgesic and anti-inflammatory properties of ARESL were assessed in Wistar rats at doses of 200 and 400 mg/kg. To assess analgesic activity, acetic acid-induced writhing and formalin tests were used. To assess anti-inflammatory property, carrageenan and dextran-induced hind paw edema were used. Differences between group means were compared statistically by one-way analysis of variance (ANOVA) followed by Tukey test as post hoc.
Results: In acetic acid-induced writhing test, the extract, at a dose of 400 mg/kg, showed a maximum inhibition (*P*=.05) of 71.66% of writhing while the standard drug Aspirin inhibited 81.05% of writhing compared to untreated control group. In formalin test, ARESL showed a maximum inhibition (*P* =.05) of 71.77% at a dose of 400 mg/kg while standard drug Pethidine showed 76.11%. For carrageenan-induced paw edema test, ARESL at a dose of 400 mg/kg showed maximum 85.90% inhibition (*P* =.05) of inflammatory activity while dextran-induced showed 87.9%.
Conclusion: ARESL possesses analgesic and anti-inflammatory activities which corroborate the aqueous extract being used in folk medicine.

Keywords: Salacia lehmbachii; analgesic; anti-inflammatory; Celastraceae; pharmacological tests; root's extract; rats.

1. INTRODUCTION

Chronic pain is a state of complex physiological and mental condition leading to irritability, anger, depression and difficulty concentrating [1]. The debilitating state of mind is always negatively influenced resulting mostly in misuse of medications [2] and exacerbation of their side effects. Most times, the body immune system is highly interfered with, paving way for other disease manifestations. In case of inflammation, reaction occurs locally in response to a pathogen or an injury or noxious stimulus [3,4]. This is considered as a complex physiologic defense mechanism that helps the tissue to remove the injurious agent as well as start the healing process [5,6]. Although it is a defense mechanism, the complex events and mediators involved in inflammatory reaction can induce, maintain or aggravate many diseases such as vasomotor rhinnorrhoea, rheumatoid arthritis and atherosclerosis [7-9].

It is believed that current drugs available such as opioids and other non-steroidal anti-inflammatory agents are not useful in all cases of inflammatory disorders because of their side effects, economy and potency [10,11]. World Health Organization reports that about 70-80% of the world's population rely on nonconventional medicine from herbal sources in their primary health care [12,13]. This percent is increasing day by day in developing countries where cost of consulting a physician is sky-rocketing let alone medicine [14]. The use of plants to treat ailments is as old as antiquity. Records of humans using plants to treat diseases have been recorded as far back as 4000 to 6000 years ago when Ayurvedic physicians started treating tumors with extracts from *Vinca rosea* [15,16].

The plant, *Salacia lehmbachii*, belongs to the family celestraceae [17] and is a small woody tree of about 3 m height. Leaves are simple, opposite, ovate oblong, acuminate and shining. Flowers are crystal orange or yellow on woody auxiliary tubercles; fruits globose, orange, contain 1 large seed at the center and 2-4 seeds immersed almost at the periphery of the pulp [18]. It is commonly found in the tropical forest of South Eastern Nigeria and Cameroon [19]. In South Eastern Nigeria, herbalists use the root in treating malaria symptoms,

abdominal pain and inflammatory disorders. No scientific documentation exists on any of their claims including analgesic and anti-inflammatory effects of the root extract in animal model. Consequently, the present study was aimed at investigating the analgesic and anti-inflammatory activities of ARESL in albino rat model because of the great similarity and homology between the genomes of rodents and humans [20].

2. MATERIALS AND METHODS

2.1 Chemicals and Drugs

Carrageenan, pethidine and Aspirin (Sigma-Aldrich, Germany) were purchased from local Chemist in Calabar.

2.2 Plant Material and Preparation of Extract

Fresh root of *Salacia lehmbachii* was harvested from Uruk Otong village of Ukanafun Local Government Area of Akwa Ibom State, Nigeria, in March 2013. The plant was identified in Cameroon National Herbarium (CNH), Yaounde, with Voucher No. 40730/SRF/CAM the roots were dried at room temperature (25–30ºC) for two weeks. The dried root sample was then ground to coarse powder using high capacity grinding machine and preserved in air tight container. The dried and ground plant material (1 kg) was macerated with distilled water (8 litres) at room temperature for 3 days. Then, the mixture was filtered and filtrate concentrated under sun at 42±3ºC to yield 6.7% extract which was subsequently defatted to get polar extract [21].

2.3 Experimental Animals

Thirty four healthy Wistar rats of either sex weighing between 180-200 g were randomly selected from Animal House Unit, Department of Pharmacology, University of Calabar and used for the experiment. The animals were housed in polyvinyl cages of at least 4 animals per cage and maintained under standard laboratory conditions of temperature (28±2ºC), relative humidity (50±5%), a 12 hour (h) dark/light cycle and received standard pellet diet and water *ad libitum*. To keep the hydration rate constant, food and water were stopped 12 h before the experiments [22]. This animal experimentation was carried out following the guidelines of the CPCSEA [23].

2.4 Phytochemical Screening

ARESL was qualitatively screened for the presence of phytoconstituents such as alkaloids, glycosides, flavonoids, tannins, saponins, polyphenols and glycosides following standard tests procedures [24].

2.5 Acute Toxicity Study

The acute toxicity study was carried out in adult female albino rats by modified ‘Up and Down’ procedure [25]. Briefly, 10 female albino rats were divided into 2 groups of 5 rats each. The animals were fasted overnight and next day ARESL dissolved in distilled water was administered orally as a single dose at different dose levels. Then, the animals were observed for general behavioral, neurological and autonomic profiles for the next 3 h and finally after 24 h [26].

2.6 Analgesic Activity

2.6.1 Acetic acid-induced writhing test

Rats were randomized into 5 groups of 6 animals per group. Group I received saline (10 ml/kg), Group II received aspirin (100 mg/kg), Groups III-V received ARESL (100, 200 and 400 mg/kg) respectively by oral gavage. Thirty minutes post treatment, 10 ml/kg acetic acid (0.6%, v/v in saline) was injected intraperitoneally. After 5 min, the rats were observed for writhings for the next 10 min [27]. A writhe is contraction of abdominal muscles accompanied by elongation of the body and hind limb(s).

$$\% \text{ Inhibition of Writhing} = \frac{\text{MNWc} - \text{MNWt}}{\text{MNWc}} \text{x } 100$$

Where;

MNWc = mean number of writhes for control group and
MNWt = mean number of writhes for test group

2.6.2 Formalin test

Rats of either sex were randomized into 5 groups of 6 rats. Group I received saline (10 ml/kg), Group II received pethidine (5 mg/kg) as standard drug, the rest of the groups received ARESL (200, and 400 mg/kg). All the rats received 20 µl formalin (5% v/v in saline) injected into subplantar region of right hind paw 45 min post treatment. Licking score counts per unit time in response to pain was recorded for 45 min [28].

2.7 Anti-inflammatory Activity

2. 7.1 Carrageenan-induced paw edema

A total of 24 rats were divided into 4 groups consisting of 6 rats per group as follows:

Group A (standard): Carrageenan + Aspirin (100 mg/kg b.wt)
Group B (control): Carrageenan + distilled water (10 ml /kg b.wt)
Group C (test): Carrageenan + ARESL (200 mg/kg b.wt)
Group D (test): Carrageenan + ARESL (400mg/kg b.wt)

Acute inflammation was induced according to edema assay [29]. Briefly, the extract was dissolved in distilled water and administered orally to rats 1 h before Carrageenan injection. Aspirin (100 mg/kg b.wt) was given to standard group. Carrageenan, 0.1ml of 1% suspension was injected in sub-plantar region of rats in all the groups. The paw edema was measured with a Vernier Caliper initially before carrageenan injection. Paw diameter was measured at 0, 1, 2, 3, 4, 5 and 24 hours post injection. The difference between the initial and subsequent values gave the actual edema which was compared with the control animals. The percent inhibition of inflammation was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{C} - \text{T}}{\text{C}} \text{x } 100$$

Where, 'C' represents mean edema in control and 'T' represents mean edema in group treated with standard and test.

2.7.2 Dextran-induced paw edema test

Experimentation was carried out by the same procedure as in carrageenan-induced test above except that instead of carrageenan, 0.1 ml of dextran (1.0 % w/v in saline) was used as phlogistic and only one dose [30] of AESL (400 mg/kg) was administered.

Group I (Control): Dextran + saline (10 ml /kg b.wt)
GroupII (Standard): Dextran + aspirin (100 mg/kg b.wt)
GroupII: Dextran + ARESL (400 mg/kg b.wt)

2.8 Statistical Analysis

Values were expressed as means ± SEM (n = 6). Differences between group means were compared statistically by one-way analysis of variance (ANOVA) followed by Tukey test as post hoc. *P* =.05 were considered statistically significant [31].

3. RESULTS

Preliminary phytochemical screening of ARESL revealed the presence of terpenoids, alkaloids, glycosides, flavonoids, tannins, anthraquinones, steroids and saponins in varying concentrations. Acute toxicity study of the extract at up to a dose of 4000 mg/kg revealed no observed toxic effect.

Analgesic activities of ARESL in acetic acid-induced writhing test is shown in Table 1. Standard drug Aspirin and extract (400 mg/kg) showed maximum analgesic (inhibitory) activities of 81.05 and 71.66% respectively as compared to control. Table 2 shows analgesic activity of ARESL in formalin-induced hind paw licking test. Analgesic activity was maximum (*P* =.05) with 76.11% for standard drug Aspirin and 71.77% for extract (400 mg/kg).

Anti-inflammatory activities ARESL in carrageenan and dextran-induced paw edema for 5 h are shown in Figs. 1 and 2 respectively. In both figures, after injecting phlogiston, inflammatory activities reach peak at 3 h while maximum inhibition (*P* =.05) of hind paw diameter was reached after 5 h. In Fig. 1, maximum inhibition was 93.9% for the standard drug Aspirin and 85.9% for ARESL (400 mg/kg) as compared to untreated control group. In Fig. 2, maximum inhibition for standard drug was 95.1% while ARESL (400 mg/kg) was 87.9%.

Table 1. Analgesic activity of aqueous root extract of *Salacia lehmbachii* in acetic Acid-induced writhing test in albino rats

Group	Treatment	Dose (mg/kg)	Mean no. of Writhing	% Writhing	% inhibition of Writhing
I control	Saline	10 ml/kg	21.21±1.1	100.00	00.00
II standard	Aspirin	100	4.02±0.12*	18.95*	81.05*
III test	ARESL	200	10.13±0.30	47.76	52.24
IV test	ARESL	400	6.01±0.12*	28.34*	71.66*

*Each value represents the mean ± SEM (6 animals in each group). *P =.05 is statistically significant from normal control*

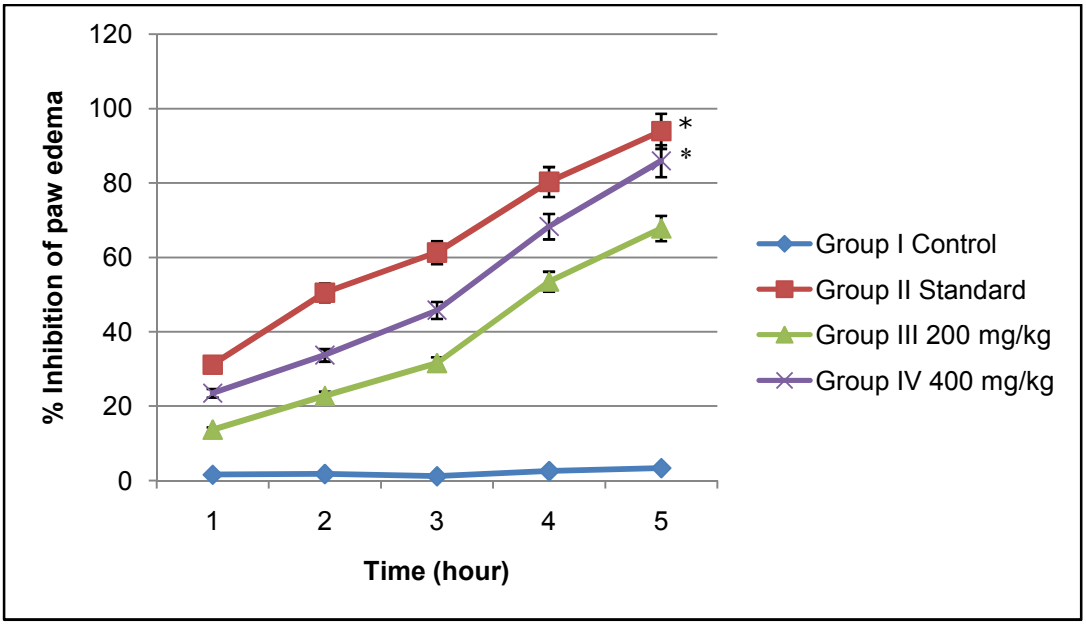

Fig. 1. Anti-inflammatory activity of aqueous root extract of *Salacia lehmbachii* in carrageenan-induced paw oedema in rats. The data were presented as mean ± SEM (6 animals in each group). *P =.05

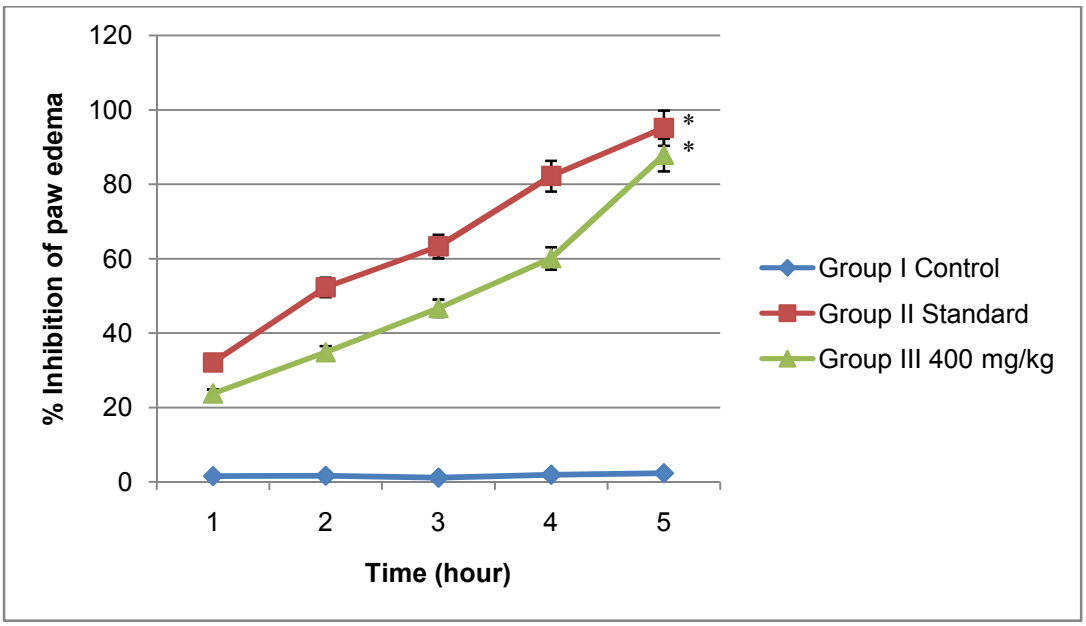

Fig. 2. Anti-inflammatory activity of aqueous root extract of *Salacia lehmbachii* in dextran-induced paw edema in rats. The data were presented as mean ± SEM (6 animals in each group). *P =.05

Table 2. Analgesic activity of aqueous root extract of *Salacia lehmbachii* in formalin-induced hind paw licking test in albino rats

Group	Treatment	Dose (mg/kg)	Mean no. of licks	% licks	% Inhibition of licks
I control	Saline	10 mg/kg	46.12±1.2	100.00	00.00
II standard	Pethidine	5	11.02±0.12*	23.89*	76.11*
III test	ARESL	200	24.13±0.30	52.32	47.68
IV test	ARESL	400	13.01±0.12*	23.64*	71.77*

*Each value represents the mean ± SEM (6 animals in each group). *P =.05 is statistically significant from normal control*

4. DISCUSSION AND CONCLUSION

Carrageenan and dextran are strong inflamagens used for the release of inflammatory and proinflammatory mediators in experimental animal models. The induction of rat paw edema is usually a biphasic process [32] with the first phase being the release of histamine, serotonin and kinin in the damaged tissue surroundings about 1 hour post injection of inflamagen. The second phase is associated with the production of bradykinin, neutrophil, protease, oxygen free radical, prostaglandin and lysosome in 2-3 hours [33,34]. ARESL maintained inhibition of paw edema diameter over the biphasic process. Inhibition in the first phase suggests that the extract might have blocked histamine release since carrageenan causes microvascular leakage [35]. Histamine blocking is evident in decrease in paw diameter in the course of the experiment suggesting that the extract has anti-permeability property since histamine causes vascular permeability through endothelial cells. In the second phase of the biphasic process, ARESL (400 mg/kg) significantly inhibited paw diameter suggesting that the extract down-regulated cyclooxygenases and peroxidases from converting omega-6 fat into prostaglandin-H_2 which is similar to that produced by non-steroidal anti-inflammatory agents [36] like aspirin.

In dextran-induced paw edema, it is known that the mediators are histamine and serotonin released from mast cells [37] and they cause vascular vasodilatation, increased permeability and reduced blood flow resulting in increased paw diameter. ARESL significantly inhibited paw diameter.

In accessing central analgesic activity, the spinal cord and the brain play a paramount role in pain mechanism. Major targets for pain and inflammation are found in the dorsal horn of spinal cord and they include neurotransmitters [38] and receptors [39]. Acetic acid-induced writhing response is a known model for evaluating peripherally acting analgesics mediated by peritoneal mast cells, acid sensing ion channel and prostaglandin pathways [40,41]. ARESL significantly inhibited mean number of writhes suggesting that the extract which contains flavonoid might have interfered in writhes excitation by producing free radical scavenging activity since free radicals have been implicated in pain stimulation. Formalin test was considered to be selective in accessing centrally acting compounds through opioid receptors. ARESL significantly inhibited the mean number of licks suggesting that the extract was centrally acting and might have increased the threshold of pain.

From the present investigation, ARESL has analgesic and anti-inflammatory properties which could be harnessed to substitute narcotic analgesics since it is non-toxic.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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