# ALKALOIDAL ROOT EXTRACT OF *NAUCLEA LATIFOLIA* (RUBIACEAE) INHIBITED INDUCED ACUTE AND CHRONIC INFLAMMATIONS IN WISTAR RATS

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**Abstract**

Background: The Plethora of adverse reactions associated with the long-term use of most Non-Steroidal Anti-inflammatory Drugs (NSAIDs) are not unconnected to their prostaglandin biosynthesis inhibition mechanism of action. NSAIDs of alternative mechanism of action could therefore be better substitutes. Indole alkaloids are both biogenetically and structurally related to serotonin, an alternative inflammation mediator, and are therefore a possible chemical repertoire explorable for the discovery of new NSAIDs. The present research was aimed at evaluating an alkaloidal root extract of an indolealkaloid-rich plant, *Nauclea latifolia* (Rubiaceae), for its potential inhibitory activities on acute and chronic inflammations, for an ultimate possible discovery of such indole-based NSAIDs.

**Methods**: Alkaloidal components of a crude methanol extract of *Nauclea latifolia* root was obtained by acid-base treatment and concentrated to dryness. Effects of the alkaloidal extract on acute inflammation was evaluated *in vivo* in a carrageenan-induced paw edema rat model, measuring edema size at 30min intervals over a 3hr time-course, while a cotton pellet-induced granuloma rat model was used to evaluate effects of the extract on chronic inflammation.

**Results**: The extract significantly reduced the induced edema to varying degrees compared to the negative control ($p < 0.05$, 0.01, 0.001 and 0.0001) over the 3hr time-course of observation, producing a maximum % inhibition (41.97%) 3hrs after induction. It also significantly inhibited the development of the cotton-pellet induced granuloma after seven days compared to the negative control ($p < 0.05$), in the chronic inflammation experiment.

**Conclusion**

The results show that alkaloids of *Nauclea latifolia* have anti-inflammatory activities and could therefore be explored as templates in the discovery of new anti-inflammatory agents.
Key Words: *Nauclea latifolia*, inflammation, Indolealkaloids, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs).

## 1. Introduction

The mechanism of action of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) largely involves inhibition of prostaglandin biosynthesis, leading to an indiscriminate interference with the rather diverse prostaglandin homeostatic functions and, hence, the plethora of adverse reactions associated with these drugs[1-3].This has long warranted the search for new NSAIDs with selective prostaglandin biosynthesis inhibition or, better still, alternative mechanisms of action[4-5]. An attempt at the latter approach based on the conjecture that indole-containing substances could inhibit the biosynthesis and/or inflammation mediation of the indole-containing inflammation mediator, serotonin, ledto the discovery of the indole-based

NSAID, indomethacin[6]. Though prostaglandin biosynthesis inhibition contributes largely to the anti-inflammatory activity of indomethacin,

it, nevertheless, possesses other anti-inflammatory mechanisms, including its hypothesized serotonin inhibition[6]. Indomethacin's multiple mechanisms of action implies interaction with diverse macromolecular targets, the biochemical attribute of molecular species now, in medicinal chemistry parlance, referred to as privileged structures[7-9], and amongst which the indole organic moiety is key[10]. This multiple anti-inflammatory mechanismpotential of the indole nucleus notwithstanding, there is a paucity of indole-based anti-inflammatory agents in clinical medicine; whilethe dream of discovering NSAIDs with little or no dependency on prostaglandin biosynthesis inhibition and, hence, possible better safety profiles, remains largely unattained. Exploring sources rich in indole-containing compounds for possible anti-inflammatory activities, is therefore a right step towards the discovery of new and possibly safer NSAIDs.

Indolealkaloids are a ready source of indole-containing compounds as they either contain the intact indole moietyorits slightly modified form. They are widely distributed in nature, cutting across plant and animal kingdoms[11]. Plant chemotaxonomy has particularly established the copious presence of indole alkaloids in the terrestrial plant families Loganiaceae,Apocynaceae and Rubiaceae[12]. *Nauclea latifolia* is one tropical member of the family Rubiaceae with diverse ethnomedicinal uses including its antimicrobial, anti-diabetic, antihypertensive, antidyslipidaemic and anti-inflammatory uses[13-15].A number of indolealkaloids have been reported for *N. latifolia* which include the Nucleamides A, B, C, D, E and the latifolamides A, B, C, D and E[16-17].

In the light of the above, we evaluated the anti-inflammatory activities of an alkaloidal extract of *Nauclea latifoliain-vivo* in rat models of acute and chronic inflammation, to assess the possible utility of its indolealkaloids as templates for the discovery of new NSAIDS. Carrageenan-induced paw edemaand Cotton pellet- induced granuloma rat models were used for acute and chronic inflammations respectively[18-19].

## 2. Materials and Methods

2.1. Materials, animals and reagents

Rootbarks of *Nauclea latifolia* were obtained fresh fromIkire, Osun State, Nigeriaand authenticated at the Botany Department of the University of Lagos. All solvents and chemicals used were obtained from Sigma-Aldrich (Taufkirchen, Germany) and were of at least analytical standards. Wistar albino rats (of both sexes), weighing between 100 and 200gwere used in the two experiments. The animals, obtained from the Central Animal House, University of Ibadan Oyo State, Nigeria, were kept in well-ventilated, hygienic compartments under standard environmental conditions. They were fed with Pfizer branded rodent feed, and had free access to water. All animal experiments were carried out in the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos, Nigeria, in accordance with the guiding principles of the Animal and Ethics Committee of the University of Lagos.

### 2.2 Extraction of *Nauclea latifolia* root bark

The root barks of the plant were dried at room temperature for 7 days and pulverized in a mechanical grinder before extracting with methanol by cold maceration. The methanol extract was then concentrated to dryness using a rotatory evaporator. The dried extract (40g) was soaked in 1N HCl and filtered. The acidic aqueous filtrate was basified using 5N NaOH to precipitate out alkaloids. Solvent extraction was carried out using Chloroform and the chloroform extract (25g)concentrated to dryness using a rotary evaporator and the dried extract subjected to standard tests for alkaloids[20].

### 2.3 Carrageenan-induced paw edema inhibition assay

Wistar rats (25) of both sexes, weighing 122-197g, were randomly sorted into five groups of five animals per group and given the following treatments orally. Group 1 (control) received distilled water (10ml/kg), groups 2, 3 and 4 received 50mg/kg, 100mg/kg, 200mg/kg extract suspension in distilled water respectively, while group 5 (reference standard) received 10mg/kg indomethacin. One hour after each of the afore-listed administrations, inflammation was induced by the subplantar administration of 0.1ml 1%w/v carrageenan in the right hind paw of the rats. Linear paw diameter was measured immediately after the induction, and at 30 minutes intervals, up to three hours after carrageenan injection, using digital vernier caliper[21]. Edema size was assessed in terms of the difference in the zero time linear diameter of the injected hindpaw and its linear diameter at 30, 60, 90,120,150 and 180 minutes following carrageenan administration. Mean edema size for each group was computed and its corresponding percentage edema inhibition calculated as follows:

$$\% \text{ inhibition} = 100 \times \left(\frac{V_c - V_t}{VC}\right)$$

Where $v_c$ and $v_t$ represent mean edema of control and test groups respectively[22].

### 2.4 Cotton pellet-induced granuloma (chronic inflammation) inhibition assay

Wistar rats(30) of both sexes and weighing between100 and 200ghad their backs skin-shaved and disinfected with 70% ethanol. Two sterilized cotton pellets (20mg each) were implanted subcutaneously in the lumbar region, one on each side, using blunt forceps. The animals were then divided into five groups of six rats each and treated orally for 7 days as follows:

Group 1(the control) received20ml/kg of distilled water, groups 2,3 and 4

received 100mg/kg, 200mg/kg and 400mg/kg extract respectively while group 5 (standard reference) received 50mg/kg celecoxib. All administrations were done same time daily for 7 days.The animals were then fasted overnight and sacrificed the next day by cervical dislocation. The pellets along with the granuloma tissue were removed and dried in an oven at 60°c until weights remained constant.

The granuloma tissue formation was calculated as follows:

Measure of granuloma tissue formation = constant dry weight –initial weight of pellet.

The level of inhibition of granuloma tissue development was calculated using the expression:

$$\% \text{ inhibition} = 100 \times \left(\frac{WgrC - WgrT}{WgrC}\right)$$

Where *W grC* is the weight of granuloma tissue of control, *W grT* is weight of granuloma tissue of test group

**2.5 Statistical analysis**

All Results were expressed as Mean ± S.E.M (standard error of mean).For each experiment, One-way ANOVA analysis followed by turkey's multiple comparison was used to compareeach group mean with that of its corresponding control. p values < 0.05 were considered significant.

## 3. Results

### 3.1. Results of alkaloidal tests.

The extract tested positive to the three standard alkaloidal tests carried out as depicted in **Table 1**

**Table 1**: Results of the standard tests for alkaloids carried out on the alkaloidal extract of the root of *Nauclea latifolia.*

| Alkaloidal Test | Result |
|---|---|
| Wagner's test | Positive |
| Drangendorff's test | Positive |
| Mayer's test | Positive |

### 3.2. Effect of extract on acute inflammation

The extract at the various administered doses produced significant reduction in edema size compared to the control (p < 0.05, 0.01) at various points in the 3-hour time-course of observation (**Table 2**). Each extract dose showed an hourly cyclic rise in % edema inhibition while the standard showed a somewhat steady rise that almost plateaued half-way into the 3hr time-course of observation as depicted in **Figure 1**. The highest % edema inhibition recorded (41.98%) was produced by the 400mg/kg extract dose 3hr after inflammation induction. This was observed to be higher than the highest % edema inhibition (37.85%) produced by the reference standard (10mg/kg indomethacin) after the same period of time.

**Table 2: Time course effects of alkaloidal extract of Nauclea latifolia on mean edema size and % edema inhibition after carrageenan paw edema induction in wistar rats.**

| Time (mins) | Mean ± S.E.M (% edema Inhibition) | | | | |
|---|---|---|---|---|---|
| | Control | 100mg/kg | 200mg/kg | 400mg/kg | Standard (indomethacin) |
| 30 | 6.23±0.27 | 5.83 ± 0.39 (6.39%) | 5.06 ±0.19* (18.75%) | 4.90 ±0.21** (21.39%) | 5.38 ±0.07 (13.8%) |
| 60 | 6.26 ±0.21 | 6.14 ±0.50 (1.92%) | 5.74 ±0.30 (8.27%) | 5.1 ±0.18 (18.53%) | 5.09 ±0.08 (18.63%) |
| 90 | 7.60 ±0.31 | 6.12 ±0.33* (19.4%) | 5.91 ±0.20** (22.14%) | 5.71 ±0.30** (24.80%) | 4.97 ±0.27** (34.65%) |
| 120 | 7.28 ±0.24 | 6.19 ±0.19* (15.03%) | 5.82 ±0.21** (20.11%) | 5.90 ±0.27** (18.87%) | 4.86 ±0.06** (33.27%) |
| 150 | 7.14 ±0.17 | 5.31 ±0.10** (25.59%) | 5.20 ±0.18** (27.19%) | 5.22 ±0.05** (26.79%) | 4.81 ±0.12** (32.54%) |

Data presented as mean ± S.E.M (n=5). *p< 0.05 significantly reducedmean edema size compared to the control; **p<0.01 significantly reduced mean edema size compared to the control; (One-Way ANOVA followed by Tukey's multiple comparison).

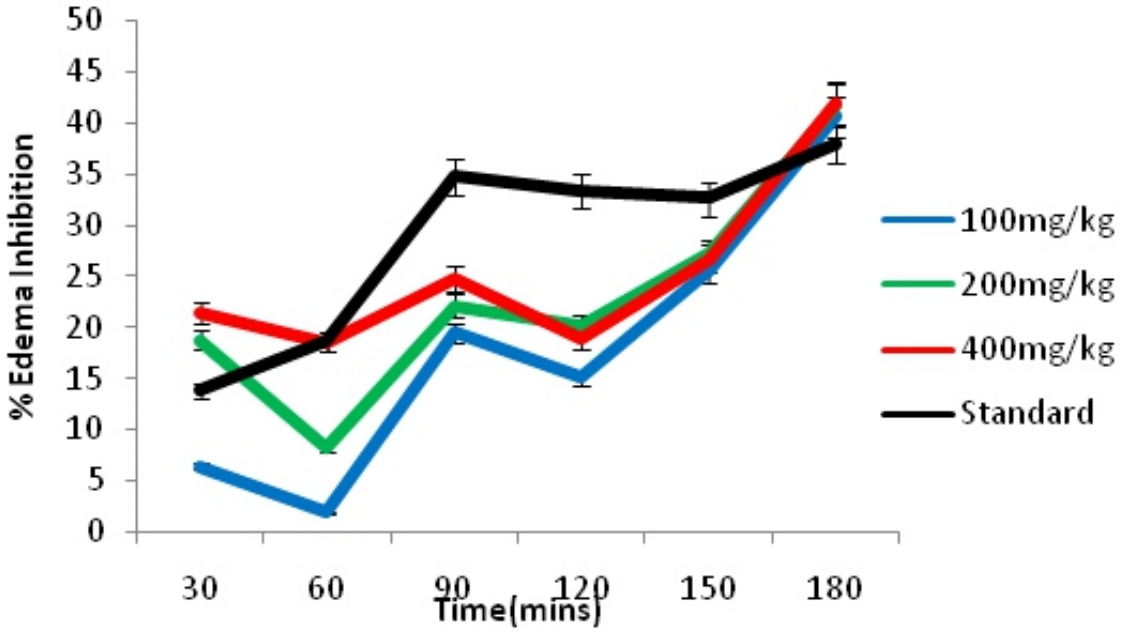


**Figure 1**: Comparison of the time-course effects of various doses of the alkaloidal root extract of *Nauclea latifolia* and a standard reference (50mg/kg indomethacin) on carrageenan-induced paw edema in wistar rats.

### 3.3. Effect of extract on chronic inflammation

A dose-dependent granuloma weight reduction by the extract is manifest from the cotton-pellet induced granuloma experiment (**Table 3**). Compared with the negative control, the inhibitory effects of the 200 mg/Kg and 400 mg/Kg doses of the extract on chronic inflammation is significant ($p < 0.05$) and comparable to that of celecoxib, an established NSAID for chronic inflammatory conditions [23].

**Table 3: Granuloma Mean Weight Reduction by various doses of alkaloidal root extract of Nauclea latifolia and 50 mg/Kg celecoxib.**

| Group | Mean± SEM | % Granuloma Inhibition |
|---|---|---|
| Control | 0.14±0.017 | — |
| 100mg/kg | 0.10±0.005 | 29.00% |
| 200mg/kg | 0.08±0.007* | 44.99% |
| 400mg/kg | 0.05±0.004* | 62.20% |
| Standard (50 mg/Kg celecoxib) | 0.06±0.005* | 59.85% |

* Significantly reduced granuloma weight compared to the control, $p < 0.05$

## 4. Discussion

The classical acid-base extraction procedure employed in the study is both historical and specific for alkaloids[24-26]. The positive standard alkaloidal tests results (**Table 1**) could therefore confirm the chloroform extract as truly alkaloidal. The indole nature of this alkaloidal extract was, however, assumed on account of chemotaxonomy[27-28] and previous phytochemical works on the plant that show its chemistry to be largely that of indole alkaloids[29-32].

Results of the anti-inflammatory evaluations showed that the alkaloidal extract demonstrated activities against both acute and chronic inflammations. Though the activities of the NSAIDs used as standards in the investigations are proves of prostaglandin involvement in the establishment of the two inflammations, serotonin inhibition as a contributing mechanism of these observed anti-inflammatory effects could be conjectured on account of the structural and biogenetic similarities of indole alkaloids and serotonin, an inflammation mediator[33]. This conjecture is particularly supported by the established involvement of serotonin in one of the three phases associated with carrageenan-induced acute inflammation[34].

The net reduction in paw edema size by each extract dose at each point of observation, relative to the control (**Table 2**), is suggestive of the extract's inhibitory activity against the carrageenan-induced acute inflammation and the presence of anti-

inflammatory principles capable of interfering with at least one of the mediators involved in its three phases[34].The cyclical (as opposed to steady) nature of edema inhibition (**Fig. 1**), however, could be attributed to variation in the sensitivity of different phases of the inflammatory process to different anti-inflammatory components of the extract which are, expectedly, present at varying concentrations. Evaluating each component in its pure form would give a better picture of its anti-inflammatory effects as could be attested to by the steady nature of the activity of the reference standard (indomethacin) over the time course of observation (**Fig.1**). The % edema inhibition (41.98%) demonstrated by the extract at the end of the 3-hour time-course of observation is higher than that of the standard (37.85%) at the end of the same period,an indication that the anti-inflammatory activity of the extract is longer-sustained compared to that of the standard (indomethacin).

Therefore, the folkloric anti-inflammatory use of *Nauclea latifolia*is at least, herein, partly attributed to itsindole alkaloidconstituents, thereby providing a platform for their employment as templates for the ultimate discovery of new Non-Steroidal Anti-inflammatory Drugs (NSAIDs) with possible alternative mechanism of action to prostaglandin biosynthesis inhibition.

**5. Conclusion**

Alkaloidal extract from the *Nauclea latifolia* has demonstrated significant activities *in vivo* in acute and chronic models of inflammation. Further works aimed at the isolation and development of the responsible suspect indole alkaloid constituents into new Non-Steroidal Anti-inflammatory Drugs (NSAIDs) are hereby recommended.

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