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AQUEOUS ROOT BARK EXTRACT OF *NAUCLEA LATIFOLIA* PREVENTS INFLAMMATION AND REDUCES PAIN IN MICE

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ABSTRACT

Nauclea latifolia Smith is a valuable medicinal plant used in the traditional medicine and reported to possess a wide spectrum of medicinal properties. The focus of this study was to evaluate the anti-nociceptive and anti-inflammatory properties of the aqueous root bark extract of N*auclea latifolia,* in mice after determining its median lethal dose. The anti-inflammatory activity was investigated using a phlogistic agent (egg albumin) and xylene-induced ear edema, while the anti-nociceptive activity was evaluated using the acetic acid induced mouse writhing and the hot-plate test. These activities were compared to those of a standard, acetylsalicylic acid (100 mg/kg). *Nauclea latifolia* root bark extract (150, 299 & 449 mg/kg) inhibited xylene-induced ear edema in mice (inhibition ratio: 3.5 %, 27.6 %, and 69.0 %, respectively) and dose dependently decreased the hind paw edema with a percentage inhibition of 10 %, 63 % and 90 % respectively. There was a significant amelioration of acetic acid increased vascular permeability in mice (inhibition ratio: 47.9 %, and 72.0 %) pretreated with 299 & 449 mg/kg of the aqueous root back extract respectively. A significant increase in pain reaction time (17.5s, 44.2s) was observed in animals treated with 299 mg/kg and 449 mg/kg of the extract in the hot-plate test when compared to the control group treated with distilled water whose pain threshold was seen within 7.7 seconds. In each of the different models used, the 449 mg/kg dose of the extract showed a greater anti-inflammatory as well as anti-nociceptive effect than 100 mg/kg of acetylsalicylic acid. The *Nauclea latifolia* root bark extract if exploited may be a better alternative for the management of pain and inflammation.

KEYWORDS: *Nauclea latifolia,* phlogistic agent.

INTRODUCTION

It is alarming to note that the quality of life of the work force of a nation is jeopardized largely due to pain and inflammation, the king of human miseries.[1] Drugs that are used to arrest these conditions such as non-steroidal anti-inflammatory drugs (NSAIDs) exhibit adverse effects including gastric irritation, peptic ulcers, liver toxicity, delayed muscle regeneration, kidney failure and cardiac abnormalities. These untoward effects re-affirm the wise saying of William Osler that "The person who takes **medicine must recover twice"** once from the disease and once from the medicine". The reality of this fact pulls more attention to plants, an effective alternative with less toxicity.

Nauclea latifolia Smith (family; Rubiaceae), popularly known as African peach, is a spreading, evergreen, multi-stemmed shrub or small tree native to tropical Africa and Asia.[2] Various extracts of this plants have been reported to possess anti-malarial activity, anti-hypertensive[3] as well as activity against cough, gonorrhea, stomach disorders, dysentery, ulcers, and liver ailments.[4] Although *Nauclea latifolia* has been widely used in traditional medicine for treatment of several disease conditions, and different decoctions of the plant in different solvents have been investigated for various medicinal activities; an extensive literature search reveals limited data on the root bark of the plant. This research aimed to investigate the pain relieving and anti-inflammatory properties of the aqueous root bark extract of *Nauclea latifolia*.

MATERIALS AND METHODS

Preparation of plant materials

The roots of *Nauclea latifolia* used in this study were collected from Itak, in Ikono local government area of Akwa Ibom State, Nigeria. The plant was identified and authenticated by a taxonomist in the Department of Botany, University of Uyo, Sample roots were then cleaned with tap water to remove any dirt before peeling the bark by scraping with a clean knife. The scrapes were shade dried for two weeks at room temperature and pulverized into fine powder using laboratory mechanical grinder.

Extraction

A 1.2 kg weight of powdered root bark was soaked in 4.0 litres of distilled water with regular agitation at an hour interval to uniformly mix the sample within the first 10 hours and left to stand for 24 hours. Filtration was performed using Whatman‟s filter paper No.1 (Sigma-Aldrich). The filtrate was concentrated using a water bath at a temperature of 49°C under reduced pressure. A concentrate of about 76 g was obtained and stored in a sealed container at 4°C until use for bioassay.

Experimental Animals

Swiss albino mice of both sexes (6-8 weeks old) were used in this study. All the experimental animals were purchased and housed in the animal house, Pharmacology and Toxicology Department, University of Uyo. They were housed in standard cages at room temperature for 12 hours in darkness followed by 12 hours light cycles throughout the experimental period and fed with rodent pellet diet and water ad libitum. Ethical guidelines and procedures were followed while handling the laboratory animals[5]; approval was obtained from the Faculty of Pharmacy institutional animal care and use committee, University of Uyo, Nigeria.

Acute toxicity of aqueous root bark extract of *Nauclea latifolia*

The median lethal dose (LD_{50}) of the root bark extract of *Nauclea latifolia* was determined using Lorke's method[6]. Briefly, mice of either sex were divided into six (6) groups of 3 animals each based on the graded concentration (800, 1000, 1200, 1400, 1600, and 1800 mg/kg). The animals were deprived of feed 12 h prior to treatment, but had free access to water before intraperitoneal administration of single doses of the root bark extract of *Nauclea latifolia*. The general behaviors of the mice were observed continuously for 1 h after the treatment and then intermittently for 4 h, and thereafter over a period of 24 h.[7-8] They were observed for any sign of toxicity and deaths. Adverse events, such as hypoactivity, salivation, convulsion, sedation, grooming, aggressiveness, writhes, sniffing, etc., were observed within 24h of administration of *Nauclea latifolia* extract. The no-observed adverse-effect level (NOAEL) as well as the lowest observed adverse- effect level (LOAEL) was noted[9] and the LD_{50} value was determined using:

$$LD50 = \sqrt{D0\ X\ D100} \text{ ----------(1)}$$

where D0=Dose at which all tested animals survived and D100=Dose at which all tested animals died

The low dose, medium and high doses used in the study were determined as 10 %, 20 % and 30 % of the LD_{50}.

Evaluation of anti-inflammatory activity of the extract

Xylene induced ear edema

This experimental procedure was performed using the method of Hosseinzadeh *et al*.[10] The mice were divided into five groups, comprising five animals each. Group I received distilled water while groups II, III and IV received the root bark extract of *Nauclea latifolia* at doses of 150, 299 and 449 mg/kg, respectively and group V received acetylsalicylic acid (100 mg/kg). All the animals were pretreated before induction of inflammation. Edema was induced in each mouse by applying 0.2 ml dose of Xylene to the inner surface of the right ear. Thirty minutes after Xylene daubing, the mice were executed by cervical dislocation and both ears were removed and weighed. The difference between the right and left ears was determined for each group, and the percentage inhibition was determined for each group (equation 2).

$$\% \text{ inhibition} = \frac{\Delta\, control - \Delta\, treatment}{\Delta\, control}\ x\ 100 \text{ ------(2)}$$

where Δ control = the difference of ear weight in control group and

Δ treatment = the difference of ear weight in treated groups

Egg albumin induced inflammation

A phlogistic agent (egg albumin) – induced mouse hind paw edema was utilized as a model of acute inflammation.[11] The mice were grouped into five (n = 5) groups. Group I animals, which served as control, were pretreated intraperitoneally with 10 mL/kg of distilled water while groups II–IV received graded doses (150, 299, 449 mg/kg i.p.) of the extract and group V animals received the standard drug, acetylsalicylic acid (100 mg/kg). Thirty minutes (30 min) following the administration of *Nauclea latifolia* extract, inflammation of the hind paw was induced. A 0.1 ml of fresh egg albumin was injected into the subplantar surface of the right hind paw of the mice. Paw diameters were measured immediately before the administration of the phlogistic agent and 5 hours thereafter using a vernier calliper. An increase in paw diameter 5 hours post-administration of the phlogistic agent was used as the parameter for measuring inflammation. Hence, inflammation was assessed as the difference between zero time paw diameter and 5 hours post-administration of the phlogistic agent.[12] Paw edema was measured and recorded every 60 minutes for at least 300 minutes. Average edema (Ct - Co) and percentage inhibition of edema were calculated for each dose.

Evaluation of analgesic effect of *Nauclea latifolia*

Test on acetic acid-induced abdominal constrictions

The plant decoction (150, 299 and 449 mg/kg, i.p.), was administered to the animals in the treatment groups of II, III and IV. Group 1 animals were treated with distilled water while group V received acetylsalicylic acid 30 minutes prior to acetic acid treatment. Following treatment, each animal was injected intraperitoneally with 0.2 mL of 3% v/v of aqueous solution of acetic acid. Each mouse was then placed in a transparent observation box and the number of abdominal constrictions (writhes) for each mouse was counted for 30 minutes, which commenced 5 minutes after intraperitoneal injection of

acetic acid. For scoring purposes, a writhe was indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. The percentage writhing inhibition was then calculated using the formula (equation 3) described by Ezeja *et al*.[13];

$$\% \text{ writhing inhibition} = \frac{C-T}{C} X\ 100 \text{ ----------(3)}$$

where;
C- mean of control
T - mean of treated group

Thermally induced pain (Hot-plate test) in mice

The Thomas scientific hot-plate/stirrer apparatus (USA model D-537), maintained at 45±0.5˚C was employed. Each animal was placed in a glass beaker, 50 cm diameter on the heated surface and the time between placement and shaking, or licking of paw or jumping was recorded as the index of response latency. Mice that showed these responses within 20 seconds were selected and randomly divided into five groups (n = 5). Group I served as control in which the mice were treated with only distilled water while groups II–IV received graded doses (150, 299 & 449 mg/kg i.p.) of the extract. Mice in group five were administered acetylsalicylic acid at 100 mg/kg i.p. Pain reaction times were measured 30 minutes post-treatment.

Statistical analysis

Data obtained from this study were subjected to descriptive statistics and the results were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA). The results among the groups were analyzed for statistical significance using one way ANOVA followed by Dunnett's multiple comparison post hoc tests to check for significant difference between treatment and control. The level of significance was set at < 0.05.

RESULTS AND DISCUSSIONS

Acute Toxicity Studies of *Nauclea latifolia* root bark extract

The LD_{50} determined by administration of different doses of the aqueous root bark extract of *Nauclea latifolia* to Swiss albino mice in six groups of 3 mice each was 1496.66 mg/kg. Zero percent mortality was observed in the 1400 mg/kg extract group whereas the minimum dose producing 100 % mortality was 1600 mg/kg. The no-observed adverse-effect level (NOAEL) for the intraperitoneal dose was 1200 mg/kg, while the lowest observed-effects level was 1400 mg/kg.[9] Adverse events including hypoactivity and shivering/jerking movement were seen shortly after intraperitoneal injection of 1400 mg/kg. One hundred percent mortality was observed within 24 h of administration of 1600 mg/kg and 1800 mg/kg of the extract.

From the obtained LD_{50} of 1496.66 mg/kg, the working concentrations (low dose, medium dose and high dose) used throughout this study was 150, 299 and 449 mg/kg respectively.

Anti-inflammatory activity on xylene-induced ear edema in mice

The control group which was treated with distilled water showed an increase in ear weight up to 0.058 ± 0.005 g but the administration of 449 mg/kg aqueous root bark extract of *N. latifolia* significantly inhibited this weight gain by 68.97 % (0.018 ± 0.00 g). The standard drug, acetylsalicylic acid (100 mg/kg) showed 37.93 % (0.036 ± 0.005 g) inhibition which was lower compared to the effect produced by 449 mg/kg extract (Figure 1).

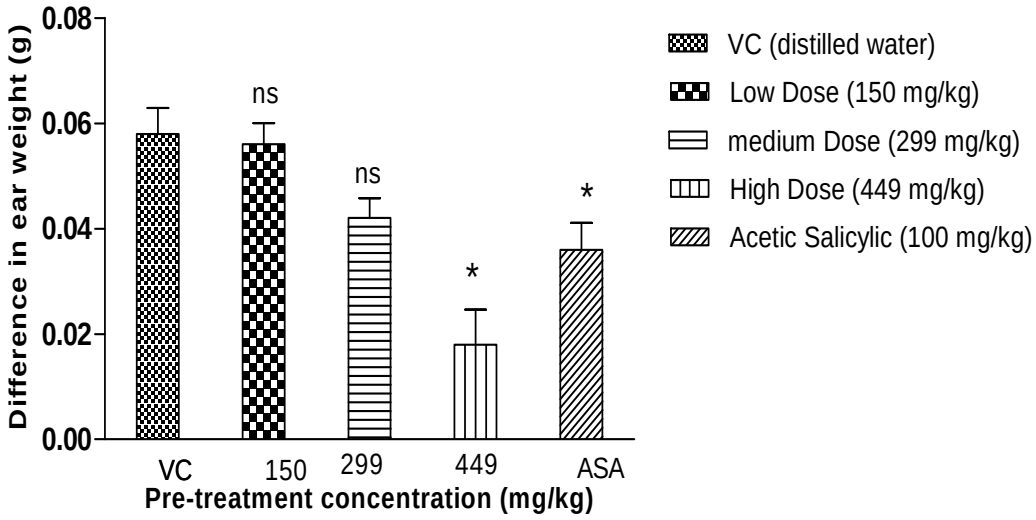

Figure 1: Reduction in Xylene-induced ear edema in mice treated with different concentration of the agueous root bark extract of *N. latifolia*. Data is expressed as mean ± SEM of five mice. *p<0.05 indicates significant difference when compared with control using one way ANOVA followed by Dunnett's post hoc test. VC = vehicle control; ASA = acetylsalicylic acid.

There was no significant difference in the low dose group and the medium dose group when compared with the control group which was treated with distilled water (Figure 1). The anti-inflammatory effect was observed to be dose dependent. Xylene-induced ear edema causes severe vasodilation and increases vascular permeability associated with substance P[14], a neurotransmitter abundant in the central nervous system. When stimulated peripherally, substance P generates neurogenic inflammation. Xylene-induced inflammation is followed by an innate immune response of the skin, a cytotoxicity reaction of activated T cells and then migration of polymorphonuclear leucocytes which augment swelling and heaviness of the ear.[15] This result, suggests that *N. latifola* inhibits this xylene-induced inflammation by suppressing the release or action of neuro-mediators, especially substance P.

Anti-inflammatory Activity on Egg Albumin-Induced inflammation in Mice

Table 1: The effect of N*auclea latifolia* root bark extract on fresh egg albumin - induced inflammation in mice.

Treatment groups	Average paw diameter (cm)	Percentage inhibition
Vehicle control (distilled water)	0.462 ± 0.072	
Low dose (150 mg/kg)	0.42 ± 0.083	10 %
Medium dose (299 mg/kg)	0.168 ± 0.063*	63 %
High dose (449 mg/kg)	0.046 ± 0.080*	90 %
Acetylsalicylic acid (100 mg/kg)	0.114 ± 0.070*	75 %

Results are expressed as mean ± SEM (n=5) *P<0.05 significantly different from control.

From Table 1, the percentage inhibition of mice hind paw edema increased dose dependently in the extract and acetylsalicylic acid treated groups. However, the effect was greater (p<0.05) in 299 mg/kg and 449 mg/kg treatment groups than the control group. Notably, the 449 mg/kg extract produced a greater percentage inhibition of inflammation than 100 mg/kg of acetylsalicylic acid. Egg Albumin induced inflammation has been reported to stimulate the release of vasoactive substances like histamine and serotonin. *N. latifolia* possibly acted by inhibiting the release and/or actions of these vasoactive substances as well as prostaglandins.[16,17]

Effect of aqueous root bark extract of *N. latifolia* on acetic acid induced abdominal writhing

The root bark extract of *N. latifolia* demonstrated analgesic activity in acetic acid-induced pain in mice by reducing the number and percentage of abdominal writhes in comparison to the control group (Figure 2).

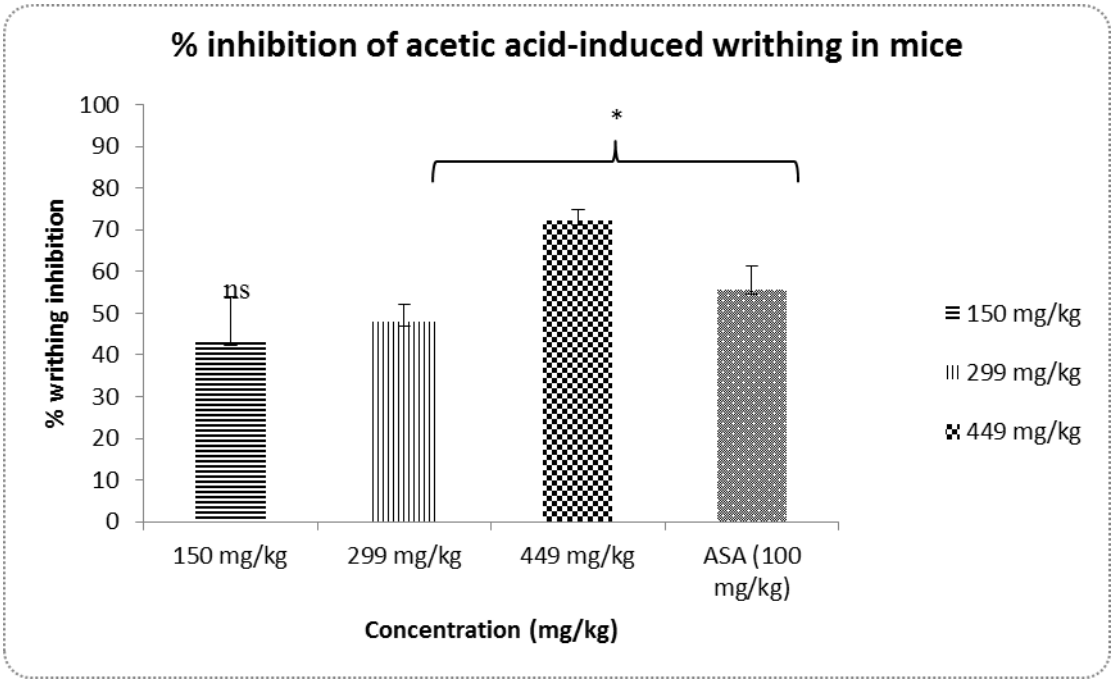

Figure 2: Percentage inhibition of acetic acid-induced writhing response in mice on various doses of root bark extract of *N. latifolia* and acetylsalicylic acid (100mg/kg). Data are expressed as mean±SEM of five mice. *p<0.05 indicates significant difference when compared with control by using one way ANOVA followed by Dunnett's Test.

The acetylsalicylic acid treated group showed a moderate inhibition (55.6 %) as the number of writhes were significantly reduced compared to the control group (p<0.05). Similarly, a greater inhibition (71.9 %) was

observed in the group treated with 449 mg/kg of the extract with significant reductions in the number of writhes compared to the control group. There was no significant difference ($p > 0.05$) in the number of writhes in the low dose extract group (150 mg/kg) with a percentage inhibition value of 43.8 %. Administration of acetic acid induces pain through activation of chemosensitive nociceptor[18] or irritation of the visceral surface, which provokes the release of histamine, bradykinin, prostaglandins and serotonin.[19] The writhing assay had been reported to be sensitive to Z-opioid and NSAIDs which primarily mediate their effect via a central and peripheral mechanism, respectively. The control drug used, mediates its analgesic activity via the inhibition of cyclooxygenase in peripheral tissues, thus interfering with the mechanism of transduction in primary afferent nociceptors by blocking the effect or inducing the release of anti-inflammatory mediators.[20] Findings from this study suggest that *Nauclea latifolia* root bark extract exerts its anti-inflammatory action by inhibiting the effect of inflammatory mediators and/or inducing the release of anti-inflammatory mediators.

Effect of aqueous extract of *N. latifolia* on thermally induced pain in mice

To demonstrate analgesic activity of *N. latifolia*, a thermal induced mouse model of pain was also exploited. The administration of aqueous root bark extract of *N. latifolia* elicited a dose dependent increase in the pain reaction time (enhancing the pain threshold) in the hot plate test. This analgesic effect as shown in Figure 3 below was statistically significant ($P<0.05$) when compared to the control group.

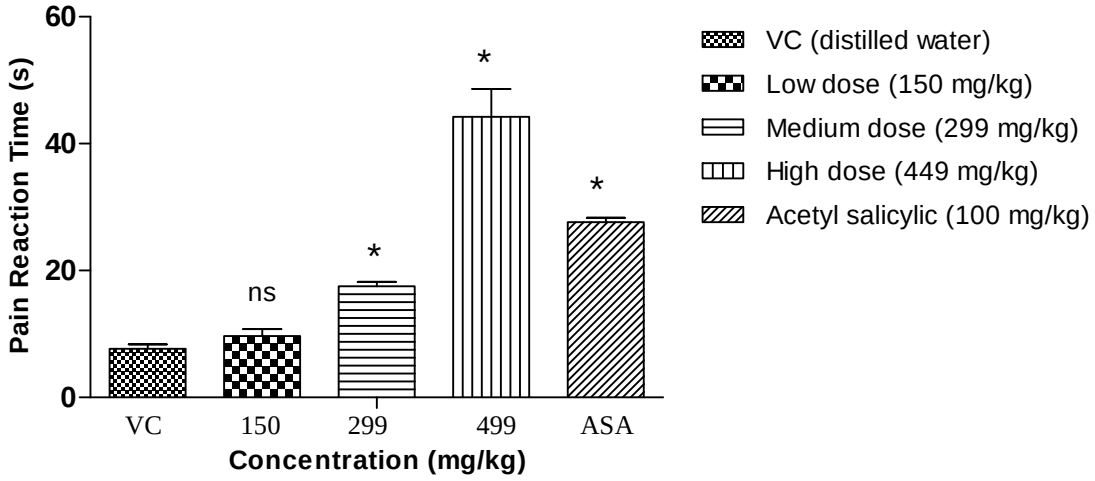

Figure 3: Effect of *N. latifolia* on thermally induced pain in mice. Data are expressed as mean±SEM of five mice. *p<0.05 indicates significant difference when compared with control using one way ANOVA, followed by Dunnett's test. VC = vehicle control; ASA= acetylsalicylic acid.

N. latifolia aqueous root bark extract (150, 299, & 449 mg/kg) exhibited a significant dose dependent increase in the latency response on thermally induced pain in mice. The acetic acid induced abdominal writhing is a visceral pain model that provokes the release of arachidonic acid via cyclooxygenase.[21] It distinguishes between central and peripheral pain. This result suggests that the extract may be exerting its action partly through the cyclooxygenase system.

This model of pain has been reported to be selective for centrally acting analgesics.[22] An increase in the reaction time as observed in this study is considered an important parameter for evaluating central anti-nociceptive activity.[23] Hence, *Nauclea latifolia* root bark extract also has a central action with a likelihood of the involvement of supraspinal and spinal components[24-25] as earlier observed.[3]

CONCLUSION

In conclusion, the root bark aqueous extract of *Nauclea latiifolia* showed promise in the management of pain and inflammation. Investigation of the underlying mechanisms of these activities and subsequent formulation of the extract into suitable dosage formation are required.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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