

## A Study of the Anti-Inflammator Y and Analgesic Activities of Aqueous Extract of *Nauclea latifolia* Leaves in Rodents

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**Abstract:** This study investigates the anti-inflammatory and analgesic activity of the leaves extract of *Nauclea latifolia* in formalin induced oedema and acetic acid induced writhing in Rodents. The aqueous extract of the leaves of *Nauclea latifolia* of the family of Rubiaceae was evaluated for its analgesic and anti-inflammatory activities. The extract exhibited significant  $p < 0.05$  and dose dependent analgesic activities between 50, 100 and 150 mg/kg body weight i.p. in acetic acid induced writhing and anti-inflammatory effects between 50 and 100 mg/kg body weight i.p. in formalin induced oedema in rats. The activities of the extract were comparable to that of the reference non-steroidal analgesic and anti-inflammatory drug: *piroxicam*.  $LD_{50}$  value was calculated to be 226.3 mg/kg body weight intraperitoneally. The extract revealed the presence of chemical constituents such as flavonoid, tannins alkaloid and saponins when phytochemically investigated. The results obtained from the present studies supported the claims of the use of the plant in the management of dysentery and pain in the throat in human.

**Key words:** Acetic acid, analgesic, anti-inflammation, formalin, *Nauclea latifolia*, piroxicam

### INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Farnsworth, 1989; Eisner, 1990). The study of plant species that traditionally have been used as pain killers should still be seen as a fruitful and logical research strategy in search for new analgesic drugs. (Elisabetsky *et al.*, 1995).

*Nauclea latifolia* belongs to the family Rubiaceae. It is a straggling shrub or small spreading tree. Tree to 3.5-5 m high with horizontal branches. It is commonly known as pin cushion tree; as tafashiya amongst the Hausa's in the northern part of Nigeria; as uche amongst the Igbo in the eastern part of Nigeria and as Itu amongst the itsekiru.

*Nauclea latifolia* herbal remedies have been commonly seen in various cultures throughout recorded history and still serve as the main means of therapeutic medical treatment. Part of the plant is commonly prescribed as a remedy for diabetes mellitus. The plant is also used in the treatment of ailments like, malaria gastrointestinal disorder, sleeping sickness and as a chewing stick (Gidado *et al.*, 2005).

There are no reports in the literature on the analgesic and anti-inflammatory effect of this plant. The present study therefore was undertaken to evaluate the effect of this plant on pain and inflammation.

### MATERIALS AND METHODS

**Plant material:** Samples of the leaves of *Nauclea latifolia* were collected within the main campus of Ahmadu Bello

University in October, 2008. The plant was identified and authenticated by M. Musa of the herbarium section in the Department of Biological Science, Ahmadu Bello University, Zaira. A voucher specimen was prepared and deposited there with a voucher number of 1268.

**Preparation of the extract:** Fresh leaves of *Nauclea latifolia* were collected, air dried at room temperature and pulverized with pestle and mortar. 200 g of the powder was macerated with 150 mL of distilled water. The extract obtained was concentrated and evaporated to dryness on a water bath at a temperature of 60°C to obtain a brownish mass of 26 g.

**Experimental animals:** Adult Wistar rats of both sexes weighing 180-220g and Swiss albino mice of both sexes weighing 18-25g were used for the experiment. They were kept under well ventilated condition, fed on standard feed (Excel feeds PLC) and allowed water *ad libitum*.

**Phytochemical screening:** The aqueous leaves extract of the plant was screened phytochemically at the preliminary level to identify its chemical constituents. This was carried out using the standard methods of analysis by Ciulei (1994), Evans (1996) and Brain and Turner (1975).

**Acute toxicity testing:**  $LD_{50}$  determination was conducted using the method of Lork (1983). In the initial phase Mice were divided into 3 groups of three mice each and treated with aqueous extract at Doses of 10, 100 and 1000 mg/kg body weight i.p and observed for 24 h. In the final Phase 4 mice were divided into 4 groups of one mouse each and aqueous extract administered at doses of

Table 1: Mean number of Writhings and percentage Inhibition of the aqueous leaves extract of *Nauclea latifolia* on Acetic acid induced Writhing in Mice

Treatment	Dose mg/kg	Mean no. of Writhings	Inhibition (%)	p-value
Normal saline	1.0ml/kg	24.3±0.6	-	
Extract	50	1.73±1.06	92.79	<0.05
Extract	100	1.5±0.96	93.83	<0.05
Extract	150	0.75±0.48	96.91	<0.05
Piroxicam	20	8.0±0.3	67.10	<0.05

Table 2: Effect of aqueous leaves of *Nauclea latifolia* on formalin induced oedema in Rats

Treatment	Paw volume (mL) at various time (min)			
	30	60	90	120
Normal saline 1.0ml/kg i.p	3.11±0.14	3.09±0.06	2.99±0.16	2.99±0.15
Extract 50mg/kg b.w i.p	2.09±0.06 <sup>a</sup>	2.14±0.09 <sup>a</sup>	2.03±0.02 <sup>a</sup>	2.04±0.04 <sup>a</sup>
Extract 100mg/kg b.w i.p	1.78±0.16 <sup>a</sup>	1.85±0.14 <sup>a</sup>	2.17±0.21 <sup>a</sup>	2.24±0.16 <sup>a</sup>
Extract 150mg/kg b.w i.p	2.68±0.20 <sup>ns</sup>	2.69±0.20 <sup>ns</sup>	2.64±0.18 <sup>ns</sup>	2.73±0.21 <sup>ns</sup>
Piroxicam 20mg/kg i.p	1.43±0.05 <sup>a</sup>	1.39±0.03 <sup>a</sup>	1.38±0.02 <sup>a</sup>	1.36±0.04 <sup>a</sup>

<sup>a</sup>: p<0.05 (Significant); ns = Not significant

Table 3: Percentage of Oedema inhibition expressed by the aqueous extract of *Nauclea latifolia* on formalin induced oedema in Rats

Treatment	Inhibition (%) at various time (min)				Mean of % Inhibition
	30	60	90	120	
Extract 50mg/kg b.w i.p	14.	10	13.5	11.71	31.7712.0±1.21
Extract 100mg/kg b.w i.p	42.95	40.51	27.42	25.08	33.99±4.52
Extract 150mg/kg b.w i.p	33.01	31.19	32.11	8.69	32.02±0.38
Piroxicam 20mg/kg b.w i.p	54.2	55.3	53.3	54.52	55.2±0.81

80, 160, 320 and 640 mg/kg body weight i.p and the final LD<sub>50</sub> value calculated.

**Acetic acid- induced Writhing in Mice:** This test was conducted employing the method described by Koster *et al.* (1959). Swiss albino mice were divided into 4 groups of 5 mice each. Group I served as negative control (normal saline: 1.0 ml/kg i.p) while group II, III and IV received the extract at doses of 50, 100 and 150 mg/kg i.p and group V (positive control) was given *piroxicam* at a dose of 20 mg/kg i.p. Thirty minutes later all the groups were treated with acetic acid (0.06% 1.0 mL/100 g i.p) Mice were placed in individual cages. The numbers of abdominal contractions were counted 5 min after acetic acid injection for a period of 10 min. Percentage inhibition of Writhings was obtained using the formula:

Inhibition (%) =

$$\frac{\text{Mean No. of Writhings (control)} - \text{Mean No. of Writhings (test)}}{\text{Mean of No. of Writhings (control)}} \times 100$$

**Anti-inflammatory studies:** The method used to conduct this experiment was that described by Koster *et al.* (1959) as modified by Sayyah *et al.* (2003). Formaldehyde 2.5% was used as inflammagen. It was injected in 50 µL volume in the sub plantar region of the left paw of the rats. The rats were divided into 5 groups of 5 rats. Thirty minutes before injection of formalin, the groups were treated as follows: Group I (normal saline: 1.0mg/kg as negative control); Group II, III and IV received extract at doses of 50, 100 and 150 mg/kg and Group V (positive control) was given *piroxicam* at a dose of 20mg/kg. Paw

volume (mL) was measured at 30, 60, 90 and 120 min after formalin injection using a digital Plethysmometer (Ugo basile LE7150):

$$\text{Inhibition (\%)} = \frac{\text{Mean paw volume (control)} - \text{Mean paw volume (treated)}}{\text{Mean paw volume (control)}} \times 100$$

**Statistical analysis:** The results of the experiment were expressed as Mean ± SEM. Data was analysed using one ANOVA to determine whether results in a particular group was significantly different from those in the corresponding control groups. Results were statistically significant when p<0.05 in accordance with Duncan *et al.* (1977).

## RESULTS

**Phytochemical screening:** Preliminary phytochemical analysis of the extract indicated the presence of alkaloids, saponins, carbohydrates, tannins, and flavonoid.

**Acute toxicity studies (LD<sub>50</sub>):** The LD<sub>50</sub> (median lethal dose) in mice was calculated to be 226.7 mg/kg body weight intraperitoneally.

**Acetic acid- induced Writhings in Mice:** The extract was found to have a significant (p<0.05) and dose dependent analgesic effect at doses of 50, 100 and 150 mg/kg body weight i.p as it decreased the number of acetic acid- induced writhes in mice (Table 1). The highest percentage inhibition (96.91%) displayed by the extract at a dose of 150 mg/kg body weight i.p was comparable to that of *piroxicam* (67.10%) at a dose of 20 mg/kg body weight i.p. All values obtained were

significant ( $p < 0.05$ ) in comparison with the negative control.

**Anti-inflammatory studies:** The extract was found to cause a significant ( $p < 0.05$ ) and dose dependent inhibition of the formalin- induced oedema in rats over a period of 120 min (Table 2). Highest percentage of the inhibitory effect of the extract (33.99%) was observed at a dose of 100 mg/kg body weight i.p. This inhibitory effect was compared to that exhibited by *piroxicam* (55.2%) at 20 mg/kg body weight. All results were significant compared to the negative control (Table 3).

## DISCUSSION

The leaves aqueous extract of *Nauclea latifolia* had demonstrated a significant ( $p < 0.05$ ) and dose dependent analgesic effect. This was evident as it reduced the number of abdominal constriction induced by acetic acid in Mice. This activity resides more at the higher dose of the extract (150 mg/kg body weight i.p) that was comparable to that of *piroxicam* (20 mg/kg body weight i.p). The acetic acid-induced writhing method, also called the abdominal constriction response, according to Koster *et al.* (1959) was a very sensitive one as it can detect anti-nociceptive effects of compounds (substances) at a dose that may be inactive in other methods such as tail-flick test (Collier *et al.*, 1968; Bentley *et al.*, 1981). In addition, Bentley *et al.* (1981) postulated that abdominal constrictions response partly involves local peritoneal receptors. Furthermore, significant anti-nociceptive activity demonstrated by the extract was supported by the fact that, the extract was a flavonoid- rich one, when phytochemically tested at preliminary level. According to Ahmadiani *et al.* (1998, 2000), flavonoid and tannins were found to possess analgesic and/or anti-inflammatory activities. This shows that this plant extract may serve as a potential source of analgesic.

The extract demonstrated ( $p < 0.05$ ) and dose dependent anti- inflammatory activity against formalin-induced oedema in rats. The highest inhibitory effect (33.99%) of the extract against formalin-induced oedema at a dose of (100 mg/kg body weight i.p) was similar to that obtained (55.2%) for *piroxicam* at a dose of 20mg/kg body weight i.p. The inhibitory effect of the extract was maintained throughout the time (120 min) of the experiment. As the plant contains flavonoids and tannins, this finding was supported by Duke (1992) that flavonoids of medicinal plant origin were found to possess analgesic and/or anti-inflammatory effects. According to Manthey (2000), prostaglandins, a group of powerful pro-inflammatory signaling molecules have been proven to be potentially inhibited by flavonoids.

Flavonoids have been found to inhibit phosphodiesterases, which are involved in cell activation, whose effect depends upon biosynthesis of protein cytokines, which mediate adhesion of circulating

leucocytes to the sites of injury. Hence, this might have suggested a possible mechanism of the inhibitory effect of this extract. Therefore, the presence of flavonoid and tannins in the extract tested is in support of the analgesic and anti-inflammatory activities observed.

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