

Anti-inflammatory Activities of the leaf chloroform extract of *Palisota hirsuta*.

ABSTRACT

Objective: To investigate the anti-inflammatory activity of the chloroform leaf extract of *Palisota hirsuta* (CLEPH). **Methods:** CLEPH was evaluated for anti-inflammatory activity using standard experimental models of inflammation namely: croton oil-induced mouse ear edema, carrageenan-induced rat paw edema and cotton pellet granuloma test. The activities of the extract at different doses were compared to indomethacin, a standard anti-inflammatory drug. **Results:** In all the experiments, the chloroform extract of *P. hirsuta* showed in a dose-dependent manner, anti-inflammatory effects which were significant ($p < 0.05$) and comparable to those of indomethacin. **Conclusion:** The results showed that CLEPH possess significant anti-inflammatory activity. This supports its use as an anti-inflammatory recipe in folk medicine.

Keywords: *Palisota hirsuta*, leaf extract, inflammation, croton oil, carrageenan, granuloma.

1. Introduction

Inflammation may be defined as the complex pathophysiologic response of vascularised tissue to injury[1] and it is common to almost all diseases that involve microbiologic, chemical or physical injury to living tissue[2]. The inflammatory response is typically characterised by five clinical signs namely: heat, redness, swelling, pain and loss of function[3]. The most commonly used drug for management of inflammatory conditions are non steroidal anti-inflammatory drugs (NSAIDs)[4], which have several adverse effects especially gastric irritation leading to formation of gastric ulcer[5]. Thus, there is a need to search for new anti-inflammatory agents with few, or no side effect. Natural products of plant, animal, or microorganism origin have been good sources of new bioactive compounds[6]. *Palisota hirsuta* belongs to the family *Commelinaceae*. It is a robust herb, growing up to 3 – 4m in the tropical rain forest. The leaves are traditionally used to treat both systemic and localised

inflammatory conditions in South Eastern Nigeria, where the plant is known as 'ikpere aturu'. The ethanolic leaf extract of *P. hirsuta* has demonstrated anxiolytic and antidepressant effects in mice[7], while its ethanol root extract has shown anti-inflammatory and antipyretic activities in chicken and rats respectively[8]. Woode *et al* , demonstrated anti-arthritis effects of the leaf extract of the plant in Freund's adjuvant-induced arthritis in rats[9]. The present study was therefore designed to investigate the anti-inflammatory activity of the chloroform leaf extract of *Palisota hirsuta* leaves in experimentally - induced inflammation.

2. Materials and methods

2.1 Collection and identification of plant materials

Fresh leaves of *Palisota hirsuta* were collected from Nsukka in Enugu State, Nigeria and identified in the Department of Botany, University of Nigeria, Nsukka by Mr. A. Ozioko.. A voucher specimen (UNN/VPP/2004/156) was deposited in the University of Nigeria, Nsukka herbarium.

2.2 Preparation of plant extract The leaves were dried under mild sunlight and pulverized into coarse powder using an electric blender. Extraction was done by cold maceration in absolute chloroform for 48 h with intermittent shaking every 2 h and later filtered with Whatman filter papers (NO 1). The filtrate was concentrated to dryness by evaporating at room temperature and the obtained extract stored in a refrigerator at 4°C until time of use. The percentage yield of the extract was calculated using the formula below:

$$\% \text{ Yield} = \frac{\text{Weight of the extract}}{\text{Weight of plant material}} \times 100$$

2.3 Experimental animals

Swiss Albino mice (25 – 30 g) and Wister rats (180 – 200 g) of both sexes were used for the experiments. The animals were procured from the Laboratory Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were kept in stainless steel cages with adequate supply of feed and water. The animals were

handled in accordance with international principles guiding the use and handling of experimental animals and were approved by the College Ethics Committee.

2.4 Chemicals and Drugs

Acetone, Carrageenan, Croton oil, Chloroform (Sigma-Aldich, Germany), Indomethacin, Ketamin HCl (Rotexmedia, Germany).

2.5 Acute Toxicity Test

The method employed by Ezeja *et al*[10] was used for this study. Twenty mice of both sexes weighing between 23 g and 36 g were randomly divided into 4 groups of 5 mice each. Group A served as control with each mouse receiving 10 ml/kg of distilled water. Groups B, C and D were treated orally with varying doses of the CLEPH (500, 1000 and 2000 mg/kg) respectively. The animals were allowed free access to food and water for 48 hours during which they were observed for signs of toxicity and death.

2.6 Effect of varying doses of CLEPH on croton oil-induced mouse ear edema

Cutaneous inflammation was induced on the inner surface of the right ear (surface about 6 mm) of anaesthetized mice (Ketamine HCl, 145 mg/kg, i.p.) following the method described by Junping *et al*[11]. Thirty six mice were divided into six groups of six mice per group. Varying doses of the extract (100, 200, 300, 400 µg/ear) and indomethacin (100 µg/ear) were applied together with the irritant (75 µg of croton oil dissolved in 15 µl of acetone). Animals in the control group received only the irritant. Six hours later, all the animals were sacrificed and 6 mm diameter plugs were obtained from both ears and weighed with a Metler analytical balance. The edematous response was quantified as weight difference between the right (treated) and the left (untreated) ears. The anti-inflammatory activity was evaluated as percent edema reduction in the animals treated with the substances under test with respect to the control animals, treated with the irritant alone. This was calculated as follows:

$$\text{Percent Inhibition} = 100(1 - \frac{x}{y})$$

Where:

x is the mean edema of treated group

102 y is the mean edema of the control group.

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105 **2.4 Effect of CLEPH on carrageenan-induced paw edema in rats**

106 Carrageenan foot edema model of inflammation demonstrated by Magaji *et al*[12] was
107 used for this study, with slight modifications. Thirty six rats were weighed and divided
108 into 6 groups (A – F) of 6 rats each. Acute inflammation was induced by injecting 0.05
109 ml of 0.6% carrageenan in normal saline, in the plantar region of the right hind paw of
110 the animals. Edema formation was quantified as foot volume increase and measured
111 by water displacement using a calibrated glass tube mounted on a stand.

112 Group A (positive control) was given 10 mg/kg indomethacin orally, one hour before
113 induction of inflammation. Groups B, C, D and E were treated with 100, 200, 400 and
114 800 mg/kg respectively of the extract, one hour before induction of inflammation.
115 Group F (negative control) received equivalent volume of distilled water. In all the
116 animals, the baseline paw volume was determined. After carrageenan injection, the
117 paw volume was measured at one hour interval for six hours. The anti-inflammatory
118 activity was calculated at each time of observation as percent inhibition of edema in
119 the animals treated with the test substances in comparison with the control animals.
120 This was calculated using the following formular:

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$$122 \text{ Percent Inhibition} = 100\left(1 - \frac{a - x}{b - y}\right)$$

123

124 Where:

125 b is the mean paw volume of the control animals after carrageenan injection

126 y is the mean paw volume of the control animals before carrageenan injection

127 x is the mean paw volume of the treated animals before carrageenan injection

128 a is the mean paw volume of the treated animals after carrageenan injection

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130 **2.5 Effect of CLEPH on cotton pellet-induced granuloma in rats**

131 Thirty-six rats were weighed and divided into 6 groups (A – F) of 6 rats each. Dental
132 cotton rolls were cut into 5 mm sections, each weighing 30 mg and sterilized in boiling
133 water. Under chloroform anaesthesia, the cotton pellets were introduced
134 subcutaneously through a skin incision in the back of the animals. Animals in groups

135 A, B, C, and D were treated with the extract (50, 100 , 200 and 300 mg/kg, p.o.
136 respectively) once daily for 5 consecutive days. Group E received indomethacin (10
137 mg/kg, p.o.) for 5 days while group F received distilled water only.
138 On the 5th day, the animals were sacrificed. The cotton pellets were removed, dried for
139 24 hours at 60 °C and the dry weights were determined. The differences between the
140 initial and final dry weights were considered to be the weight of granulomatous tissue
141 produced. The anti-inflammatory activity was quantified as percent reduction in
142 granulomatous tissue formed in the treated groups with respect to control[13].

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144 **2.6 Statistical analysis**

145 Statistical analysis was performed with analysis of variance (ANOVA) followed by
146 multiple range tests (least-square difference (LSD) test). Differences were considered
147 significant at P < 0.05.

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149 **2.7 Preliminary phytochemical analysis of the chloroform extract of CLEPH.**

150 The test was carried out according to procedures outlined by Soni *et al* [14].

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155 **3. Results**

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157 **3.1 Extraction and acute toxicity**

158 The yield of the extract was 1.60 % w/w dry matter and the acute toxicity test of the
159 extract produced no death or signs of toxicity after 48 h.

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161 **3.2 Effect of varying doses of the chloroform extract of CLEPH on croton oil-** 162 **induced mouse ear edema**

163 The chloroform extract significantly inhibited the mouse ear edema induced by croton
164 oil. The inhibition of the edema increased with the dose of the extract, the highest dose
165 (400 µg/ear) provoking a 60.43 % inhibition of edema (Fig. 1).

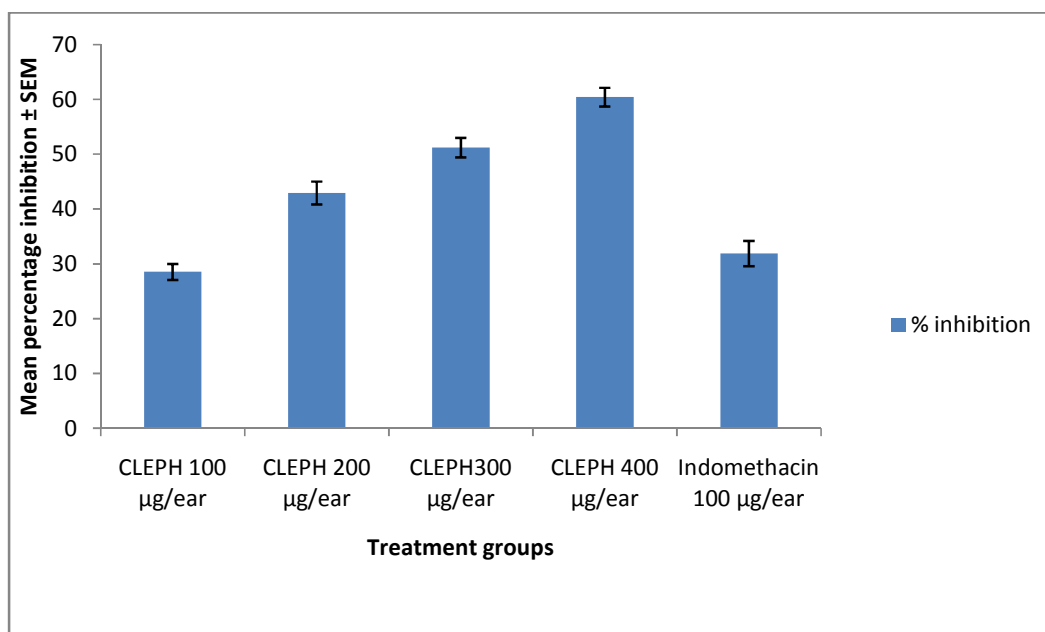


Figure 1: Percentage inhibition of edema by varying doses of CLEPH using the mouse ear edema model

3.3 Effect of CLEPH on carrageenan-induced paw edema in rats

The chloroform extract showed remarkable activity against acute inflammation by suppressing the paw edema in a dose-related manner. In the animals treated with 400 and 800 mg/kg of the extract, and in those treated with indomethacin (10 mg/kg), the reduction in edema was significant 3 hours after carrageenan administration. When compared with the control, 100 and 200 mg/kg of the extract did not show any significant edema inhibition throughout the duration of the experiment. The maximum inhibition (55 %) was achieved with 800 mg/kg of the extract within 4 hours of induction of inflammation (Table 1). In the control group, there was a progressive increase in paw edema after injection of carrageenan, which reached maximum intensity within 3 hours (Figure 2).

Group Treatment	Percentage Inhibition					
	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours
CLEPH 100 mg/kg	-	5	10	10	17	17

CLEPH 200 mg/kg	-	11	20	27	16	15
CLEPH 400 mg/kg	-	20	20	30	26	30
CLEPH 800 mg/kg	29	32	36	55	45	48
Indomethacin 10 mg/kg	29	30	43	48	45	48

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185 **Table 1 :** Effect of CLEPH on carrageenan-induced paw edema in rats.

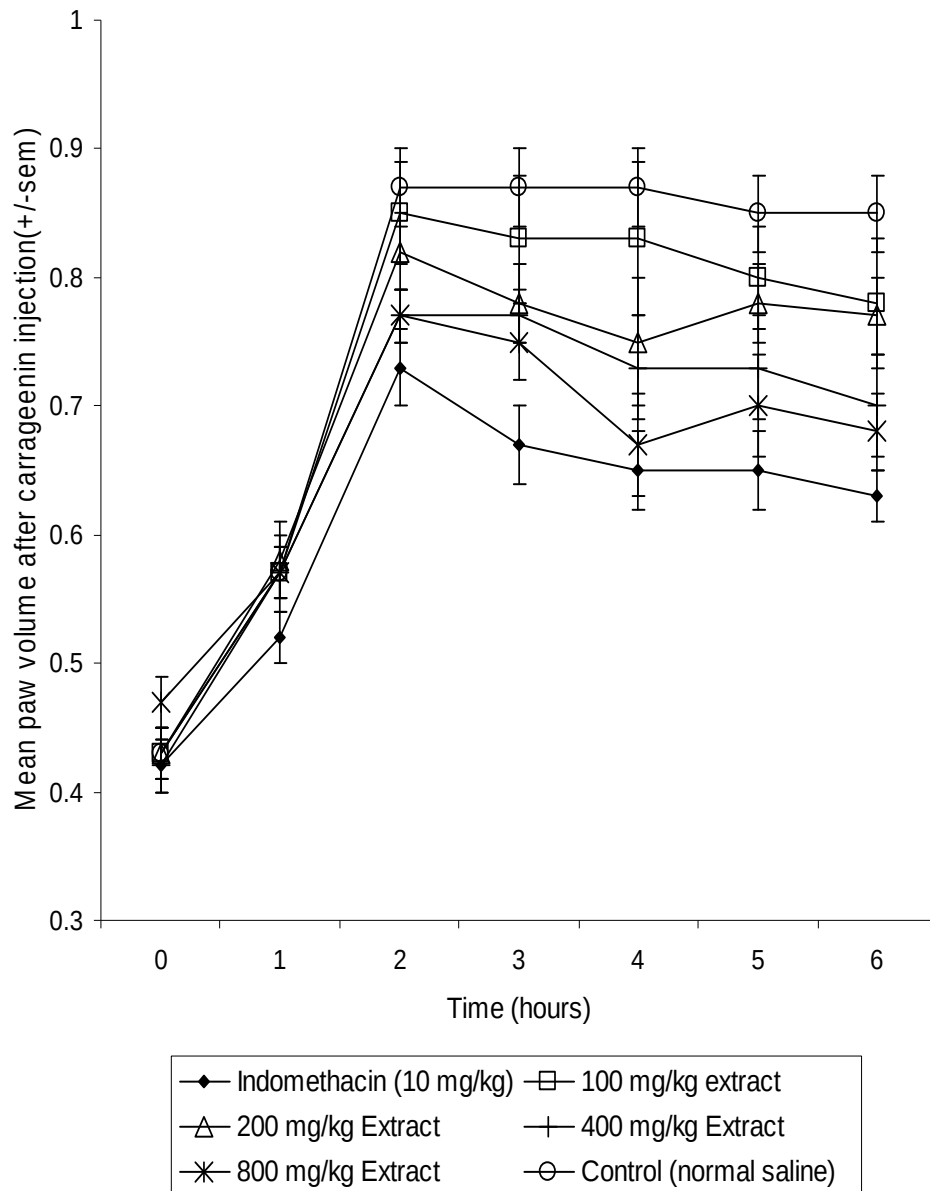


Figure 2: Effect of CLEPH on carrageenan-induced paw edema in rats.

3.4 Effect of CLEPH on cotton pellet-induced granuloma in rats

The oral administration of CLEPH inhibited the development of granulomatous tissue induced by cotton pellets inserted subcutaneously. Only the higher doses of the extract 200 and 300 mg/kg produced significant effects (19.0 % and 25.4 % inhibition respectively). The activity of the extract was dose-dependent (Figure 3).

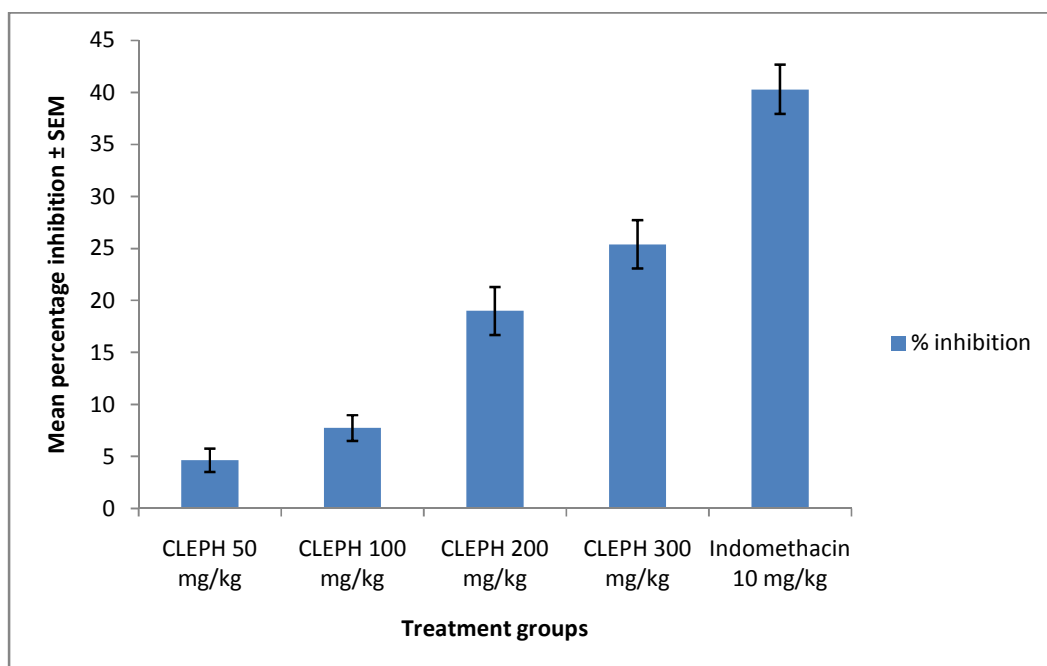


Figure 3: Percentage inhibition of cotton pellet-induced granuloma in rats by varying doses of CLEPH.

3.5 Preliminary phytochemical analysis

A preliminary phytochemical analysis of the CLEPH revealed that it contains the following: carbohydrates, alkaloids, glycosides, resins and flavonoids. Whereas carbohydrates and alkaloids were present in small concentrations, glycosides and flavonoids were present in moderately high concentrations. Resins were present in high concentrations.

4. Discussion

The models used in this study provide broad spectrum for evaluation of anti-inflammatory activity. The croton oil – induced mouse ear edema was used to test the ability of the extract to overcome acute inflammation following local application. The carrageenan-induced rat paw edema, which is widely used as a working model of inflammation in the search of new anti-inflammatory agents[15] tested for activity against acute inflammation, the extract being administered orally. The results obtained with these two models indicate that *Palisota hirsuta* leaves possess significant activity against acute inflammation following topical and oral administration.

217 The cotton pellet-induced granuloma method has been widely employed to assess the
218 transductive, exudative and proliferative components of chronic inflammation[16].
219 The fluid absorbed by the pellets greatly influences the wet weight of the granuloma
220 and the dry weight correlates well with the granulomatous tissue formed [17]. The
221 ability of the extract to significantly inhibit formation of granulomatous tissue shows
222 that the plant, apart from having effect against acute inflammation, could also exert
223 significant activity against the proliferative stage of inflammation. In all the
224 experiments, CLEPH exhibited effects which were dose-related and comparable to that
225 of the non-steroidal anti-inflammatory drug, indomethacin.

226 Although the mechanisms by which CLEPH exerts its anti-inflammatory properties
227 are not known, inhibition of the cyclooxygenase pathway of arachidonic acid
228 metabolism, which is the general mechanism of action of NSAIDs [18] could be
229 suggested. Also, scavenging of free radicals could be hypothesized, since the extract
230 contains flavonoids which have been reported to be strong scavengers of free
231 radicals[19].

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233 In conclusion, the chloroform leaf extract of *Palisota hirsuta* exhibited significant
234 anti-inflammatory properties after topical and oral administrations, confirming the
235 validity of its local use for medicinal purposes.

236 **ETHICAL APPROVAL**

237 The authors declare that this work was not against public interest. Animal experiments
238 were conducted in accordance with NIH guidelines for care and use of Laboratory
239 animals (Pub. No. 85 – 23, Revised 1985).

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