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Anti-inflammatory Activities of the Leaf Chloroform Extract of *Palisota hirsuta*

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Authors' contributions

This work was carried out in collaboration among all authors. Author IUA designed the study, wrote the protocol and did the literature search as well as wrote part of the manuscript. Author KGM managed the animals, collected all data, performed the statistical analysis, and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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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.

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Keywords: Palisota hirsute; 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 *Commelinaeceae*. 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. [9] demonstrated anti-arthritic effects of the leaf extract of the plant in Freund's adjuvanted-induced arthritis in rats. 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 48h with intermittent shaking every 2h 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 4ºC 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 (25–30g) and Wister rats (180–200g) 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 23g and 36g 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 48hours 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 6mm) 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 (75µg of croton oil dissolved in 15µl of acetone). Animals in the control group received only the irritant. Six hours later, all the animals were sacrificed and 6mm 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:

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

Where:

x is the mean edema of treated group
y is the mean edema of the control group

2.7 Effect of CLEPH on Carrageenan-induced Paw Edema in Rats

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

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

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

Where:

b is the mean paw volume of the control animals after carrageenan injection
y is the mean paw volume of the control animals before carrageenan injection
x is the mean paw volume of the treated animals before carrageenan injection
a is the mean paw volume of the treated animals after carrageenan injection

2.8 Effect of CLEPH on Cotton Pellet-induced Granuloma in Rats

Thirty-six rats were weighed and divided into 6 groups (A–F) of 6 rats each. Dental cotton rolls were cut into 5mm sections, each weighing 30mg and sterilized in boiling water. Under chloroform anaesthesia, the cotton pellets were introduced subcutaneously through a skin incision in the back of the animals. Animals in groups A, B, C, and D were treated with the extract (50,100,200 and 300 mg/kg, p.o. respectively) once daily for 5 consecutive days. Group E received indomethacin (10 mg/kg, p.o.) for 5 days while group F received distilled water only.

On the 5th day, the animals were sacrificed. The cotton pellets were removed, dried for 24 hours at 60ºC and the dry weights were determined. The differences between the initial and final dry weights were considered to be the weight of granulomatous tissue produced. The anti-inflammatory activity was quantified as percent reduction in granulomatous tissue formed in the treated groups with respect to control [13].

2.9 Statistical Analysis

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

2.10 Preliminary Phytochemical Analysis of the Chloroform Extract of CLEPH

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

3. RESULTS

3.1 Extraction and Acute Toxicity

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

3.2 Effect of Varying Doses of the Chloroform Extract of CLEPH on Croton Oil-induced Mouse Ear Edema

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

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 200mg/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 (Fig. 2).

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

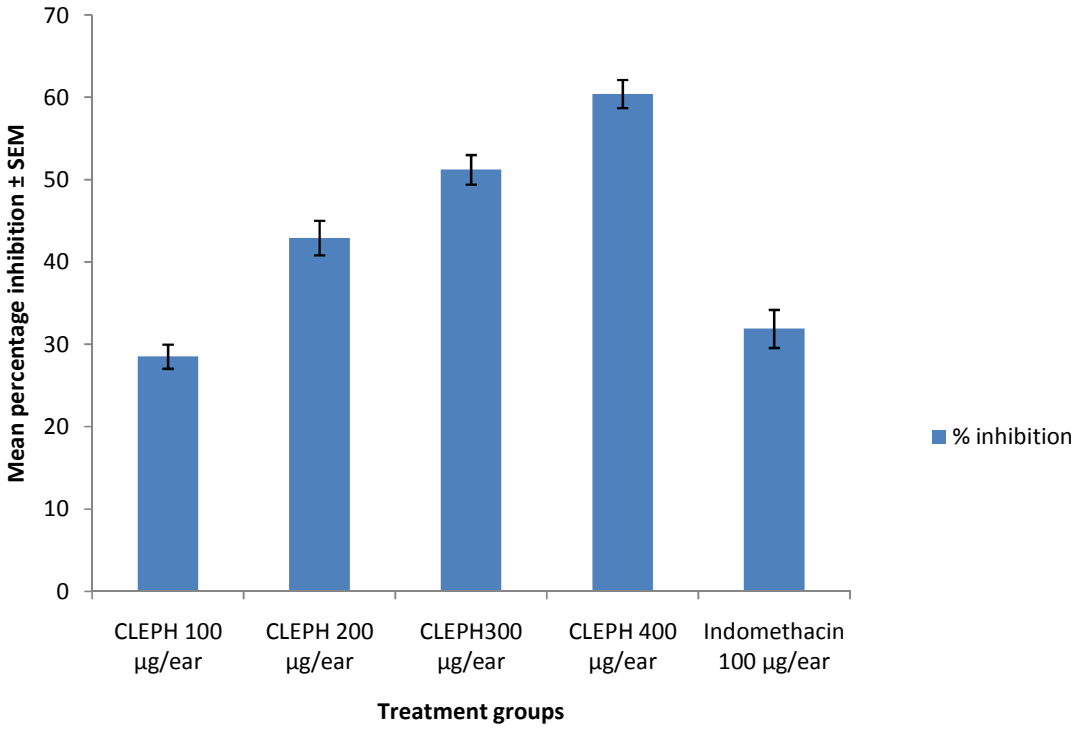

Fig. 1. Percentage inhibition of edema by varying doses of CLEPH using the mouse ear edema model

produced significant effects (19.0% and 25.4% inhibition respectively). The activity of the extract was dose-dependent (Fig. 3).

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

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

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

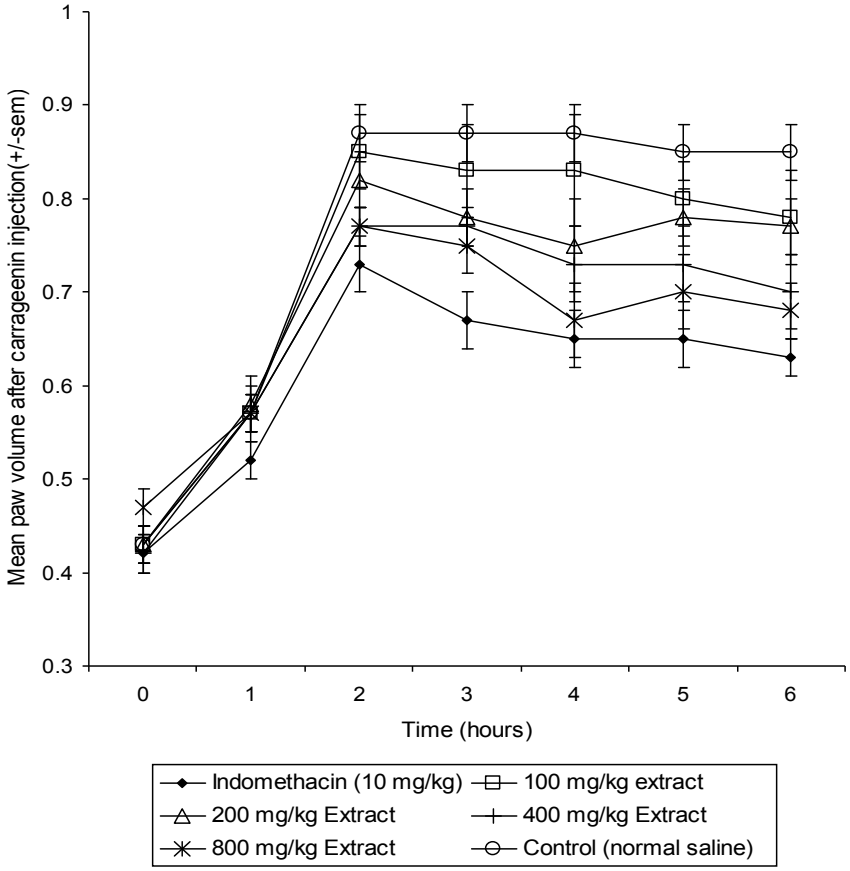

Fig. 2. Effect of CLEPH on carrageenan-induced paw edema in rats

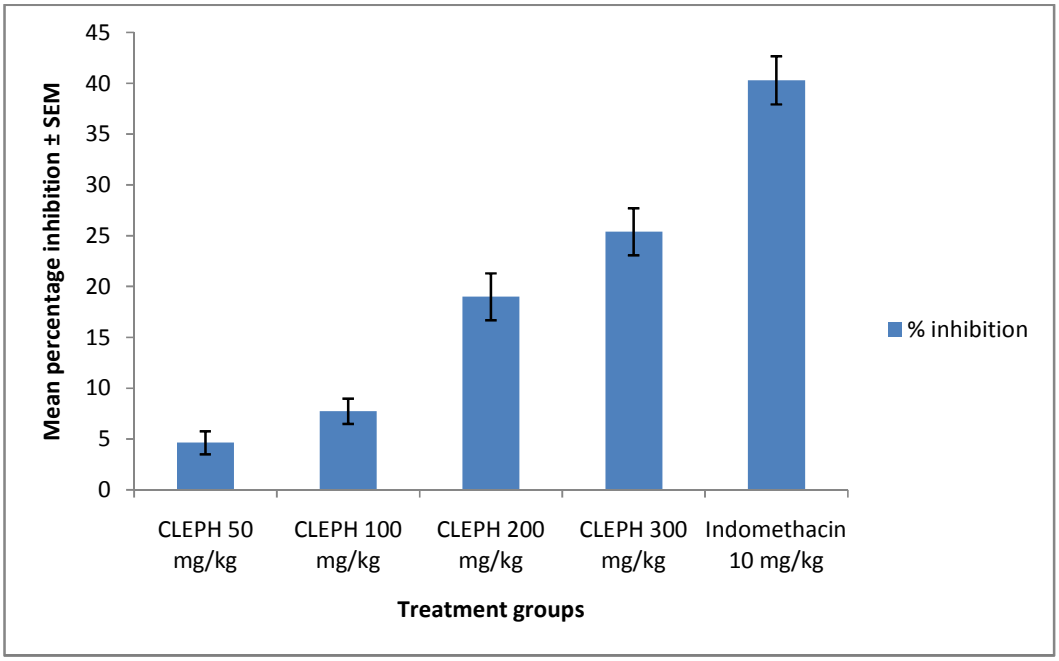

Fig. 3. Percentage inhibition of cotton pellet-induced granuloma in rats by varying doses of CLEPH

these two models indicate that *Palisota hirsuta* leaves possess significant activity against acute inflammation following topical and oral administration.

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

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

5. CONCLUSION

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

ETHICAL APPROVAL

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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