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Evaluation of the Anti-inflammatory Properties of *Chlorophora excelsa* Stem Bark Extract

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Abstract

A methanol extract of the stem bark of *Chlorophora excelsa* (Welw.) Benth and Hook was evaluated for anti-inflammatory activity in different models. Acute inflammatory effects were studied in the carrageenan-induced rat paw edema, and the effect of the extract in chronic inflammation was evaluated using the cotton pellet granuloma test. The effect of the extract on topical inflammation induced with croton oil was also tested in mice. The extract produced a significant ($p < 0.05$) and dose-dependent inhibition of the carrageenan-induced pedal edema, as well as granuloma tissue formation in rats. Topical anti-inflammatory effect was only evident with 400 mg/kg of the extract. This study demonstrated acute, chronic, and topical anti-inflammatory properties of the methanol extract of *C. excelsa*.

Keywords: Acute inflammation, anti-inflammatory, *Chlorophora excelsa*, chronic inflammation, extract, topical inflammation.

Introduction

Chlorophora excelsa (Welw.) Benth and Hook is a large tall tree, growing to about 60 m high. It belongs to the family Moraceae. It is also known as the African oak and popularly referred to as the "Iroko tree" in the southwest of Nigeria.

The plant has been employed in West Africa over many years, in spite of its popularity as a mystical tree. The plant parts are used in cough associated with bronchitis, venereal sores, and as an antibacterial. The plant also finds application in fevers, lumbago, and rheumatism (Oliver-Bever, 1986; Iwu, 1993).

In our first attempt at establishing the anti-inflammatory profile of the plant, a methanol extract of the stem bark was tested on carrageenan-induced rat paw edema, cotton pellet granuloma in rats, and croton oil–induced ear edema in mice.

Materials and Methods

Plant material

Chlorophora excelsa stem barks were collected from a tree growing around Amina Way, University of Ibadan campus, University of Ibadan, Nigeria. The plant sample was identified in the Department of Botany, University of Ibadan, where voucher specimens were kept.

Shade-dried, powdered material was extracted with methanol for 6 h in a Soxhlet extractor. Solvent removal afforded the solid extract (yield: 8.4%). The extract was stored in a dessicator and prepared fresh in 0.9% saline for pharmacological studies.

Animals

Male Swiss mice (20–25 g) and male Wistar rats (180–210 g) were used. The animals bred and housed under normal laboratory conditions of humidity and temperature and light (12 h day:12 h night). They were allowed free access to drinking water and animal pellets. The Principles of Laboratory Animal Care (NIH Publication

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No. 85–23) guidelines and procedures were used in this study (NIH, 1985).

Carrageenan-induced rat paw edema

To test the effect of the extract on acute inflammation, the method described by Winter et al. (1962) was used. Carrageenan suspension (0.1 ml, 1%) was injected in the right hind paw of each rat under the subplantar region. Rats were orally administered with the extract (100–400 mg/kg) 1 h before carrageenan injection. Control rats received an equal volume (10 ml/kg) of saline, and indomethacin (5 mg/kg) was used as standard drug. Increase in linear paw circumference was taken as a measure of edema (Hess & Milonig, 1972; Olajide et al., 1999).

Cotton pellet granuloma formation in rats

Sterile cotton pellets were implanted subcutaneously in the groin region of rats (Olajide & Alada, 2001). Animals then received the extract (100–400 mg/kg), hydrocortisone (15 mg/kg), or saline (10 ml/kg) orally once a day for 7 consecutive days. On the eighth day, the rats were sacrificed and the cotton pellet was removed, dried for 18 h at 60°C, and weighed (Olajide et al., 1998).

Croton oil–induced mouse ear edema

Mice were anesthetized with ketamine. Thereafter, croton oil (5 μg/ml) and the extract (10–40 mg/ear) were applied to the inner surface of the right ear of each mouse. The left ear was untreated. Control animals received only croton oil, and indomethacin (100 μg/ear) served as a reference. The animals were sacrificed 6 h later and 6-mm plug removed from both the treated and the untreated ears. The difference in weight between the two plugs was taken as a measure of edematous response (Zitterl-Eglseer et al., 1997).

Statistical analysis

Values are represented as mean ± SEM. Statistical significance was determined using the Student's *t*-test; values with $p < 0.05$ were considered significant.

Results and Discussion

The methanol extract of *C. excelsa* exhibited anti-inflammatory effect by producing significant ($p < 0.05$) and dose-dependent inhibition of the carrageenan-induced rat paw edema 3 h after carrageenan injection. Development of edema formation was inhibited by 37.0%, 44.1%, and 56.7% with 100, 200, and 400 mg/kg of the extract, respectively. At 5 mg/kg, indomethacin, a nonsteroidal anti-inflammatory drug, inhibited edematous response in rats by 72% (Table 1).

Table 1. Effect of the methanol extract of *Chlorophora excelsa* on carrageenan-induced rat paw edema.

Group	Dose (mg/kg)	Paw size[a] (cm) Before carrageenan	Paw size[a] (cm) 3 h after carrageenan	Inhibition (%)
Control	Saline	2.00 ± 0.03	3.27 ± 0.05	—
C. excelsa	100	2.02 ± 0.06	2.82 ± 0.04[b]	37.0
C. excelsa	200	1.97 ± 0.04	2.68 ± 0.06[b]	44.1
C. excelsa	400	2.00 ± 0.05	2.55 ± 0.07[b]	56.7
Indomethacin	5	2.10 ± 0.03	2.45 ± 0.04[b]	72.4

[a]Values are mean ± SEM (n = 6).
[b]Values are statistically significant at $p < 0.05$, comparing each extract or drug treatment value with saline (control) values; Student's *t*-test.

The inflammatory granuloma is a typical feature of established chronic inflammatory reaction (Spector, 1969). The cotton pellet granuloma method is used to evaluate the exudative and proliferative components of chronic inflammation. The dry weight of the pellets correlates well with the amount of granulomatous tissue (Swingle & Shideman, 1972). Table 2 shows that at all the doses studied, the extract of *C. excelsa* exhibited significant ($p < 0.05$) and dose-dependent reduction of dry weight of cotton pellets.

The croton oil test has a good predictive value for screening compounds and extracts for topical anti-inflammatory activity. Table 3 shows that *C. excelsa* extract (400 mg/kg) produced significant inhibition of ear edema with topical application of croton oil, an inflammatory irritant. This result suggests the potential topical anti-inflammatory property of the plant.

This study confirms the use of *Chlorophora excelsa* in traditional medicine for various inflammatory

Table 2. Effect of the methanol extract of *Chlorophora excelsa* on cotton pellet granuloma in rats.

Group	Dose (mg/kg)	Granuloma weight[a] (mg)	Inhibition (%)
Control	Saline	52.8 ± 3.1	—
C. excelsa	100	43.0 ± 2.1[b]	18.6
C. excelsa	200	34.5 ± 1.6[b]	34.7
C. excelsa	400	24.2 ± 1.9[b]	54.2
Hydrocortisone	15	19.2 ± 1.5[b]	63.6

[a]Values are mean ± SEM (n = 6).
[b]Values are statistically significant at $p < 0.05$, comparing each extract or drug treatment value with saline (control) values; Student's *t*-test.

Table 3. Effect of the methanol extract of *Chlorophora excelsa* on croton oil–induced ear edema in mice.

Group	Dose	Edema[a] (mg)	Inhibition (%)
Control	Saline	10 4 ± 0.8	—
C. excelsa	10 mg/ear	8.5 ± 1.0	14.4
C. excelsa	20 mg/ear	8.6 ± 1.3	17.3
C. excelsa	40 mg/ear	2.7 ± 0.5[b]	74.0
Indomethacin	100 μg/ear	2.2 ± 0.2[b]	78.9

[a]Values are mean ± SEM (n = 6).
[b]Values are statistically significant at $p < 0.05$, comparing each extract or drug treatment value with saline (control) values; Student's *t*-test.

conditions. We are in the process of studying further the anti-inflammatory property of the plant with the aim of elucidating the mechanism(s) involved in this action.

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