



Anti-inflammatory activity of *Drymaria cordata* extract

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Abstract

The anti-inflammatory activity of the aqueous extract of *Drymaria cordata* was evaluated using the carrageenan, egg albumin, xylene induced oedema models and pleurisy test. The extract (100 - 800mg/kg) administered 1 h before induction of swelling in rat paw, by carrageenan and egg albumin injection, produced a significant ($P < 0.05$) dose dependent inhibitory effect. This effect was greatest at the dose of 400mg/kg giving 73.66% and 63.69% levels of inhibition for the carrageenan and egg albumin models, respectively. In the xylene test, *Drymaria cordata* also elicited a dose dependent inhibition of ear oedema development, which reached a peak (61.39%) at the dose of 800mg/kg. The effect of indomethacin (10mg/kg; p.o) was lower in this respect (55.45%). The extract (400 and 800mg/kg) reduced the volume of pleural exudates and number of migrated leukocytes in the carrageenan induced pleurisy test. Greater inhibitory effect was observed at the dose of 400mg/kg (53.74% and 44.00% decrease in exudates volume and leukocytes count, respectively). The effect of indomethacin was higher in respect of volume of exudates (65.99%) but same in the case of leukocytes count (44.00%). The results obtained in this study suggest that the aqueous extract of *Drymaria cordata* possesses anti-inflammatory activity mediated possibly by the inhibition of one or a combination of mediators like histamine, serotonin, kinins and prostaglandins.

Keywords: *Drymaria cordata*; anti-inflammatory activity; oedema.

1. Introduction

Drymaria cordata (Linn.) Willd (Family: Caryophyllaceae) is a straggling procumbent herb with slender stems, broad and opposite leaves, few and small flowers, and bluntly tubercled and membranous seeds. It is widely dispersed in the tropics and sub-tropics of Africa, Asia and America [1, 2], where it occupies grassland, forest margins, roadsides, cultivated areas, often under shade at mid to higher elevations.

Many of the plants of the family Caryophyllaceae, including *Drymaria cordata*, are said to be rich in saponins especially the pentacyclic triterpenoid types [3]. Murdiati and Stoltz [4] detected the presence of alkaloid-like (pyrrolizidine) compounds in the plant, among other weeds. The presence of alkaloids and saponins was also reported by Volponi [5]. Cordatamine and the anti-leukemic substance,

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cordacin I [6], have also been isolated and characterized from the plant. An extensive work by Hu *et al.* [7] detected the presence of succinic acid (crystal 28), alpha-spinasterol (crystal 1), and a mixture containing caproic, caprylic, capric, lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic fatty acids (ether-insoluble residue) in *Drymaria cordata*.

The plant has been used in traditional medicine by people of diverse tribe and nationality for numerous purposes. As variously reported, it is used for treatment of respiratory chest ailments in Zaire, Rwanda and Tanzania [2]; eye troubles in Tanzania [8]; as cerebral stimulant, especially for children, in Madagascar [9]; as laxative and anti-febrile [10]. Further more it is used in the treatment of oedema of the feet, leprosy and yaws eruptions in Congo, Tanzania, and Gabon [8, 11, 12]; sore in West Indies and South America [13]; tumour in Mexico [14]; snake bite in China and South East Asia [15,16]; menstrual illness, pain, swellings, gout etc.

In Nigeria, the plant (commonly called Calabar woman's eye) is said to be used in folk medicine to treat or cure convulsions, febrile conditions and sleeping disorders in children.

Various studies have been conducted to scientifically verify some of these claims. For example, Mukherjee *et al.* [17] reported the antitussive activity of the methanol extract of the plant on a cough model induced by sulfur dioxide gas in mice. However, this particular study is aimed at investigating the anti-inflammatory activity of the plant based on reported traditional use.

2. Materials and methods

2.1 Plant material

The fresh plant was collected from Grailland near Iju Water Works in Lagos State of Nigeria. Botanical identification was done at the Forestry

Research Institute of Nigeria (FRIN) Ibadan, by Mr. T.K. Odewo, a Senior Superintendent. It was also confirmed by Prof. J. D. Olowokudejo of the Department of Botany, Faculty of Science, University of Lagos, Nigeria. A Voucher specimen was deposited in the herbarium of the Institute (FHI 106624).

2.2 Preparation of extract

The plant material was homogenized and extracted with distilled water by maceration at room temperature for 1 h. The solution was filtered and the filtrate was evaporated to dryness at 40°C. The yield (w/w) of the extract was 4.74% with reference to the starting material.

2.3 Animals

Adult albino rats (150 - 200g) and mice (20 - 25g) of either sex used in this study were obtained from the Laboratory Animal Center of the College of Medicine, University of Lagos, Nigeria. The animals were maintained under standard environmental conditions and had free access to standard diet and water *ad libitum*.

2.4 Chemicals

Carrageenan, egg albumin, indomethacin, xylene (Sigma Chemical Company, St. Louis, USA), chlorpheniramine (Evans Medical PLC, Nigeria) and sodium chloride (May and Baker PLC, Nigeria).

2.5 Carrageenan induced rat paw oedema

Distilled water (10ml/kg), indomethacin (10mg/kg), and extract (100, 200, 400 and 800mg/kg) were administered orally to different groups of rats. Oedema was induced in the rats 1 h after by injection of 0.1ml freshly prepared carrageenan (1% w/v in normal saline) into the sub-plantar tissue of the right hind paw [18]. The linear paw circumference was measured using the cotton thread method [19]. Measurements were made immediately before injection of carrageenan and hourly for 6 hours.

The paw swelling at each time was calculated as the difference between the linear circumference at time 't' (Ct) and that at 0 h (Co).

2.6 Egg albumin induced rat paw oedema

Inflammation was induced in rats by injection of 0.1ml egg albumin (1% w/v in normal saline) into the sub-plantar tissue of the right hind paw [18]. Extract (100, 200, 400 and 800mg/kg), chlorpheniramine (100mg/kg), and distilled water (10ml/kg) were administered orally 1 h before the induction of inflammation. The linear paw circumference was measured, as described earlier, before the injection of egg albumin and at 15 min. interval for 2 hours.

2.7 Xylene induced ear oedema

The method of Nunez Guillen *et al.* [20] was modified in this test. Thirty minutes after oral treatment of mice with extract (100, 200, 400 and 800mg/kg), indomethacin (10mg/kg), and distilled water (10ml/kg), oedema was induced in each mouse by applying 30 μ L of xylene to the inner surface of the right ear. The animals were sacrificed under ether anaesthesia 15 min. after and both ears were cut off to approximately equal size (6mm diameter disc) and weighed. The mean of the differences between the right and left ears was determined for each group.

2.8 Pleurisy induced by carrageenan

Rats were pretreated orally with distilled water (10ml/kg), extract (100, 200, 400 and 800mg/kg), and indomethacin (10mg/kg) 1 h before intrapleural injection of 0.25ml carrageenan (1% w/v in normal saline) into the right pleural cavity [20]. The animals were sacrificed 3 h after and the volume of pleural exudates was measured, and the migrated leukocytes counted.

2.9 Acute toxicity

The extract was administered orally up to 2g/kg and intraperitoneally at doses of 62.5, 125.0,

250.0 and 500.0mg/kg to groups of 5 mice each. Mortality in each group within 24 h was recorded. The LD₅₀ was determined by the method of Miller and Tainter [21].

2.10 Phytochemical screening

The presence of compounds like alkaloids, saponins, tannins, phlobatannins, anthraquinones, glycosides, phenols, simple sugars etc was investigated using the methods outlined by Odebiyi and Sofowora [22].

2.11 Statistical analysis

The results were expressed as mean $\pm$ SEM. Data were analyzed using Student's *t*-test and differences were considered significant if $P < 0.05$.

3. Results

3.1 Carrageenan induced rat paw oedema

In distilled water treated rats (control group) carrageenan induced a progressive swelling of the rat paw that reached a maximum (0.629 $\pm$ 0.047cm) in 3 h and gradually declined over the next 3 h (Table 1). The extract (100 - 800mg/kg) produced a dose dependent inhibition of oedema development, the effect increasing over the 6 h period (Table 1). The greatest effect was observed at the dose of 400mg/kg. At this dose, peak inhibitory effect (73.66%) was elicited 5 h after the injection of carrageenan, compared to a value of 89.50% for indomethacin. At the 3 h mark, when swelling reached a peak, measurements for paw oedema in respect of the extract at doses of 100, 200, 400 and 800mg/kg were 0.380 $\pm$ 0.058, 0.340 $\pm$ 0.051, 0.186 $\pm$ 0.040 and 0.320 $\pm$ 0.058cm (vs. 0.629 $\pm$ 0.047cm for control), corresponding to 39.59, 45.95, 70.43, and 49.13% inhibition, respectively. Indomethacin at this time elicited 0.071 $\pm$ 0.029cm swelling (88.71% inhibition).

Table 1. Effect of *Drymaria cordata* on carrageenan induced rat paw oedema.

Treatment	Dose mg/kg	Increase in paw oedema (cm)					
		1 h	2 h	3 h	4 h	5 h	6 h
Control	10ml/kg	0.414 ± 0.051 (-)	0.557 ± 0.048 (-)	0.629 ± 0.047 (-)	0.571 ± 0.042 (-)	0.543 ± 0.045 (-)	0.443 ± 0.048 (-)
<i>D. cordata</i>	100	0.400 ± 0.055 ^a [3.38%]	0.400 ± 0.032 ^a [28.19%]	0.380 ± 0.058 ^b [39.59%]	0.320 ± 0.037 ^b [43.96%]	0.320 ± 0.037 ^b [41.07%]	0.260 ± 0.040 ^a [41.31%]
<i>D. cordata</i>	200	0.380 ± 0.049 [8.21%]	0.440 ± 0.060 [21.01%]	0.340 ± 0.051 ^b [45.95%]	0.340 ± 0.060 ^a [40.46%]	0.220 ± 0.073 ^b [59.48%]	0.240 ± 0.051 ^a [45.82%]
<i>D. cordata</i>	400	0.300 ± 0.062 [27.54%]	0.214 ± 0.055 ^c [61.58%]	0.186 ± 0.040 ^c [70.43%]	0.214 ± 0.059 ^c [62.52%]	0.143 ± 0.037 ^c [73.66%]	0.129 ± 0.029 ^c [70.88%]
<i>D. cordata</i>	800	0.400 ± 0.045 [3.38%]	0.340 ± 0.040 ^b [38.96%]	0.320 ± 0.058 ^b [49.13%]	0.300 ± 0.032 ^c [47.46%]	0.240 ± 0.040 ^c [55.80%]	0.200 ± 0.032 ^b [54.85%]
Indomethacin	10	0.157 ± 0.030 ^c [62.08%]	0.129 ± 0.042 ^c [76.84%]	0.071 ± 0.029 ^c [88.71%]	0.071 ± 0.027 ^c [87.57%]	0.057 ± 0.020 ^c [89.50%]	0.057 ± 0.020 ^c [87.13%]

Values are mean ± SEM [n=5]. ^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.001$ vs. control [Student's t - test].

Figures in parenthesis represent level of inhibition of oedema formation.

Table 2. Effect of *Drymaria cordata* on egg albumin induced rat paw oedema.

Treatment	Dose [mg/kg]	Increase in paw oedema (cm)							
		15 min.	30 min.	45 min.	60 min.	75 min.	90 min.	105 min.	120 min.
Control	10ml/kg	0.600 ± 0.053 (-)	0.629 ± 0.029 (-)	0.600 ± 0.031 (-)	0.514 ± 0.026 (-)	0.500 ± 0.022 (-)	0.529 ± 0.036 (-)	0.500 ± 0.031 (-)	0.471 ± 0.018 (-)
<i>D. cordata</i>	100	0.540 ± 0.051 [10.00%]	0.480 ± 0.049 ^a [23.69%]	0.440 ± 0.068 [26.67%]	0.340 ± 0.040 ^b [33.85%]	0.340 ± 0.051 ^a [32.00%]	0.320 ± 0.020 ^c [39.50%]	0.260 ± 0.024 ^c [48.00%]	0.300 ± 0.000 [36.31%]
<i>D. cordata</i>	200	0.500 ± 0.000 [16.67%]	0.440 ± 0.051 ^b [30.05%]	0.400 ± 0.045 ^b [33.33%]	0.320 ± 0.037 ^b [37.74%]	0.320 ± 0.020 ^c [36.00%]	0.300 ± 0.045 ^b [43.29%]	0.240 ± 0.024 ^c [52.00%]	0.280 ± 0.020 ^c [40.55%]
<i>D. cordata</i>	400	0.486 ± 0.034 [19.00%]	0.414 ± 0.026 ^c [34.18%]	0.329 ± 0.036 ^c [45.17%]	0.243 ± 0.030 ^c [52.72%]	0.214 ± 0.026 ^c [57.20%]	0.229 ± 0.042 ^c [56.71%]	0.186 ± 0.040 ^c [62.80%]	0.171 ± 0.029 ^c [63.69%]
<i>D. cordata</i>	800	0.460 ± 0.024 [23.33%]	0.400 ± 0.032 ^c [36.41%]	0.260 ± 0.024 ^c [56.67%]	0.260 ± 0.051 ^b [49.42%]	0.280 ± 0.037 ^c [44.00%]	0.320 ± 0.020 ^c [39.51%]	0.220 ± 0.037 ^c [56.00%]	0.240 ± 0.024 ^b [49.04%]
Chlorpheniramine	100	0.600 ± 0.045 [00.00%]	0.520 ± 0.066 [17.33%]	0.420 ± 0.097 [30.00%]	0.360 ± 0.093 [29.96%]	0.360 ± 0.068 [28.00%]	0.400 ± 0.071 [24.39%]	0.320 ± 0.086 [36.00%]	0.320 ± 0.058 ^a [32.06%]

Values are mean ± SEM [n=5]. ^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.001$ vs. control [Student's *t* - test].

Figures in parenthesis represent level of inhibition of oedema formation.

Table 3. Effect of *Drymaria cordata* on xylene induced ear oedema

Treatment	Dose [mg/kg]	Weight of right ear [mg]	Weight of left ear [mg]	Difference [R-L] [mg]
Control	10ml/kg	41.2 ± 3.6	21.0 ± 1.3	20.2 ± 2.6 (-)
<i>D. cordata</i>	100	37.6 ± 1.2	22.4 ± 1.0	15.2 ± 0.4 [24.75%]
<i>D. cordata</i>	200	31.2 ± 2.6	19.0 ± 1.1	12.2 ± 1.9 ^a [39.60%]
<i>D. cordata</i>	400	30.4 ± 3.4	21.0 ± 2.7	9.4 ± 1.1 ^b [53.47%]
<i>D. cordata</i>	800	39.4 ± 2.2	31.6 ± 1.9	7.8 ± 0.7 ^b [61.39%]
Indomethacin	10	25.8 ± 1.5	16.8 ± 1.3	9.0 ± 2.1 ^a [55.45%]

Values are mean ± SEM [n=5]. ^a $P < 0.05$, ^b $P < 0.01$ vs. control [Student's *t* - test].

Figures in parenthesis represent level of inhibition of oedema formation.

Table 4. Effect of *Drymaria cordata* on pleurisy induced by carrageenan

Treatment	Dose [mg/kg]	Volume of pleural exudates [ml]	Leukocytes count [x10 ⁶]/ml
Control	10ml/kg	1.47 ± 0.22 (-)	37.50 ± 1.71 (-)
D. cordata	400	0.68 ± 0.10a [53.74%]	21.0 ± 2.86a [44.00%]
D. cordata	800	0.88 ± 0.15 [40.14%]	26.80 ± 0.66b [28.53%]
Indomethacin	10	0.50 ± 0.25a [65.99%]	21.00 ± 1.41c [44.00%]

Values are mean + SEM [n= 5]. ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$ vs. control [Student's *t* - test].
 Figures in parenthesis represent level of inhibition of exudates formation.

3.2 Egg albumin induced rat paw oedema

Swelling induced by egg albumin reached peak levels at the 30 min. (0.629 ± 0.029 cm) and 90 min. (0.529 ± 0.036 cm) marks of the 2 h period (Table 2). The extract (100 - 800mg/kg) elicited a significant ($P < 0.05$) dose dependent inhibition of oedema development with the greatest effect (63.69%) produced at 400mg/kg. This effect was greater than that elicited by chlorpheniramine (36.06%) at the same 2 h mark. Measurements for paw oedema in respect of the plant extract (100, 200, 400 and 800mg/kg) at 30 min. were 0.480 ± 0.049 , 0.440 ± 0.051 , 0.414 ± 0.026 and 0.400 ± 0.032 cm (vs. 0.629 ± 0.029 cm for control), corresponding to 23.69, 30.05, 34.18 and 36.41% inhibition, respectively. The inhibitory effect of the extract at all doses employed was greater at 90 min. than at the 30 min. mark.

3.3 Xylene induced ear oedema

Topical application of xylene increased the ear weight from 21.0 ± 1.3 mg to 41.2 ± 3.6 mg, corresponding to a change in weight of 20.2 ± 2.6 mg (Table 3). Oral administration of the extract 30 min. before xylene application produced significant ($P < 0.05$) dose dependent inhibition of ear oedema development. Increases in ear weight were 15.2 ± 0.4 , 12.2 ± 1.9 , 9.4 ± 1.1 and 7.8 ± 0.7 mg, corresponding to 24.75, 39.60, 53.47 and 61.39% inhibition for the extract at doses of 100, 200, 400 and 800mg/kg,

respectively. The effect of the extract at 800mg/kg (61.39%) was greater than that of indomethacin (55.45%).

3.4 Pleurisy induced by carrageenan

Pleural injection of carrageenan induced formation of 1.47 ± 0.22 ml of exudates containing $37.5 \pm 1.71 \times 10^6$ leukocytes/ml (Table 4). Oral treatment with the extract (400 and 800mg/kg) 1 h before carrageenan injection significantly ($P < 0.05$) reduced the exudates volume by 53.74% [0.68 ± 0.10 ml] and 40.14% (0.88 ± 1.15 ml), respectively. In same order, the number of migrated leukocytes was reduced by 44.00% ($21.0 \pm 2.86 \times 10^6$ leukocytes/ml) and 28.53% ($26.8 \pm 0.66 \times 10^6$ leukocytes/ml).

The effect of the extract at 400mg/kg was greater than at 800mg/kg on the two parameters determined. Indomethacin (10mg/kg) reduced the exudates volume and number of migrated leukocytes by 65.99% (0.50 ± 0.25 ml) and 44.00% ($21.0 \pm 1.41 \times 10^6$ leukocytes/ml) relative to control, respectively. The inhibition elicited by the extract at 400mg/kg was less than that of the standard drug in respect of exudates volume but was same for leukocytes count.

3.5 Acute toxicity

No mortality was observed when the extract was administered orally up to 2g/kg, while the LD₅₀ was 133.35mg/kg when administered intraperitoneally.

3.6 Phytochemical screening

The extract showed positive reactions for tannins, saponins, alkaloids, glycosides and phenols.

4. Discussion

Results obtained in this study suggest that aqueous extract of *Drymaria cordata* possess significant anti-inflammatory activity. Carrageenan oedema consists of three distinct phases; an initial release of histamine and 5-HT, a second phase mediated by kinins and finally a third phase, the mediator of which is suspected to be prostaglandin [23]. Pretreatment of rats with the extract inhibited carrageenan induced paw oedema development more pronouncedly at the later stages. The extract was likewise effective in the egg albumin model, indicating that it also effectively blocks the release of histamine and 5-HT, two mediators that are released by egg albumin [24]. These findings suggest a possible inhibition of cyclo-oxygenase synthesis with an effect on histamine and 5-HT release.

Drymaria cordata elicited a dose dependent effect on xylene induced mouse ear oedema test. This model is less sensitive to non-steroidal anti-inflammatory agents [25], as demonstrated by the fact that indomethacin produced a lower effect [55.45%] in this method than in the carrageenan test [89.50%]. The effect of the extract on ear oedema, a test used in the evaluation of anti-inflammatory topical steroids, suggests inhibition of phospholipase A₂ [PLA₂]. The extract also significantly prevented formation of exudates and leukocytes mobilization induced by intrapleural injection of carrageenan. Production of exudates in this model is related to a local release of vasoactive substances

(histamine and kinins) and synthesis of prostaglandins. Migration of leukocytes, though not directly related to cyclo-oxygenase products, is inhibited by non-steroidal anti-inflammatory compounds, thus indicating that many mechanisms may be implicated in its control (26, 27). The effect of *Drymaria cordata* on the pleurisy model thus supports the suggestion that inhibition of prostaglandin synthesis and blockade of histamine, 5-HT and kinins release are the underlying mechanisms for the anti-inflammatory activity of the extract.

Oral administration of the extract (up to 2g/kg) to mice did not produce visible toxic signs and mortality. This supports the safety of the extract when taken orally. Phytochemical analysis showed the presence of tannins, saponins, alkaloids, glycosides and phenols in the plant extract. Further work to determine the compound(s) that may be responsible for the anti-inflammatory activity of *Drymaria cordata* is going on in our laboratory.

5. Conclusion

The aqueous extract of *Drymaria cordata* was found to possess anti-inflammatory activity probably mediated via the inhibition of both prostaglandin synthesis and release of histamine, serotonin (5-HT) and kinins (vasoactive substances). The results of this study provide evidence to justify the use of the plant extract in inflammatory conditions.

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