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ANTI-INFLAMMATORY ACTIVITY OF *CLEOME CILIATA* LEAF SCHUMACH & THONN. IN ACUTE AND CHRONIC INFLAMMATORY MODELS USING RODENTS

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ABSTRACT

Background and aim: *Cleome ciliata* is a medicinal plant with bioactive constituents used in ethnomedicine for inflammatory conditions. The study aimed to assess anti-inflammatory activity of *C. ciliata*.
Method: The dried leaves were subjected to hot-aqueous extraction and acute toxicity testing was done at doses of 500, 1000, 2,500 and 5000 mg/kg, p.o. The anti-inflammatory effect of the aqueous extract (200, 400 and 600 mg/kg) was assessed in rats using the carrageenan assay, dextran assay and formalin-induced arthritis models.
Results: Acute toxicity tests revealed the aqueous extract to be safe at all tested doses. The extract evoked a dose-dependent reduction in paw thickness, with peak reduction at 600 mg/kg (0.9±0.0 vs 0.7±0.0 cm, $p<0.01$). Similarly, all doses also reduced dextran-induced paw thickness in a time-dependent and biphasic fashion. The 200 mg/kg dose elicited peak reduction at 2 h (0.7±0.0 vs 0.6±0.0 cm, $p<0.05$) while the 400 mg/kg elicited a late-onset effect at the 4th h (0.9±0.0 vs 0.7±0.0 cm, $p<0.0$). *C. ciliata* aqueous extract also exerted a phasic effect against formalin-induced arthritis, reducing paw size at 200 and 400 mg/kg, compared to control ($p<0.05$).
Conclusion: These results suggests that aqueous extract of *C. ciliata* leaf evoked an anti-inflammatory effect against dextran induced oedema and formalin-induced arthritis in rats.

Keywords: *Cleome ciliata,* leaf aqueous extract**,** anti-inflammatory effect, formalin-induced arthritis.

INTRODUCTION

Inflammation is a compensatory biochemical response to cell injury [1] characterized by events due to the release of local mediators such as histamine, serotonin, leukotriene, tumour necrosis factor (TNF-α), and prostaglandins [2]. Due to its pervasive action, inflammation underlines the pathogenesis of a myriad of disorders such as pain and arthritis, leading to a continuous search for ligands that can mitigate the pathological consequences of inflammation. Some of these ligands have been sourced from medicinal plants and their products [3–5].

Medicinal plants and their isolates have been documented to mitigate inflammation [6]. For instance, stems bark, leaves and isolated compounds have been demonstrated to possess anti-inflammatory action [7,8]. For instance, Igbe *et al* [5] demonstrated that the fruit pulp of *Hunteria umbellata* possessed anti-inflammatory action. They have thus served as alternative therapy to the expensive and unaffordable orthodox anti-inflammatory agents.

Cleome ciliata Schum. & Thonn (Cleomaceae) is an annual herb that grows in West Africa including Cameroon, Nigeria, Congo, Angola, Uganda, and the Sudan [9]. It can grow up to about 1 m and usually has widespread branches. *C. ciliata* has traditional names such as "*garseya*" (Hausa), "*etere"* (Yoruba) and "*alkidimmoo*" (Igbo) in Nigeria. Ethnomedicinally, it is used as an anti-inflammatory [10]. The morphological parts of the plant have been documented to possess secondary metabolites such as alkaloids, phenols, flavonoids and saponins [11], with demonstrated anti-microbial [9] and analgesic [10] activities. This present study evaluated the anti-inflammatory activity of the leaves of *C. ciliata.*

MATERIALS AND METHODS

Plant collection

Leaves of *C. ciliata* were collected from Delta State, Nigeria in August 2018. The plant was identified by Prof. Macdonald Idu of the Department of Plant Biology, Faculty of Life Science, University of Benin, Benin City and authenticated by the Forest Research Institute of Nigeria (FRIN), Ibadan, where an herbarium sample with voucher number FH12065178 has been deposited.

Extraction

Fresh leaves were sun dried to a constant weight for one week, powdered, and 400 g extracted with 2 L of water for 30 minutes. The resulting extract was filtered and concentrated to dryness at 100 ^{o}C using a rotary evaporator. The extract was then dried using a thermoregulated oven at 50 ^{o}C to yield 54.9%. The dried extract was stored in airtight container at 4 ^{o}C.

Animals

All experiment was performed using male albino mice (18-25 g) and male Wister rats (120 -200 g). The animals were obtained from the animal house, Department of Pharmacology, Faculty of Pharmacy, University of Benin. Animals were maintained under standard environmental conditions, had free access to drinking water and were fed with pelletized feed (Top feeds, Nigeria) Animals were handled according to standard experimental protocols approved by the Faculty of Pharmacy, Animal Ethics Committee, University of Benin, Nigeria (EC/FP/021/11).

Phytochemical screening

Phytochemical screening of the aqueous leaves extract was carried out using established protocols [12,13]

Acute toxicity test

Male albino mice (18-25 g) were randomly selected into five (5) animals per group. Group I received distilled water (10 mL/kg), while groups II-V-received 500, 1000, 2,500, and 5000 mg/kg extract p.o., respectively. Animals were fasted over night with only access to water, after which the aqueous leaf extract was administered orally at stated doses. Then, the animals were closely observed immediately for toxicity manifestations for 1 hour after administration of the extract and 24 hours later, animals were further observed for signs of delayed toxicity after two weeks [14].

Anti-inflammatory assays

Carrageenan-induced paw oedema: The assay was performed according to method of Winter *et al* [15]. Animals were randomly divided into five (5) groups of five (5) animals each. The basal paw thickness of the right hind paw was measured using a Vernier caliper. Groups III-V received 200, 400, 600 mg/kg extract, p.o, respectively. Standard group (II) received indomethacin (10 mg/kg, p.o) while the negative control (I) received 3 ml/kg distilled water 1 hour after treatments, 0.1 ml 0.1% w/v carrageenan was injected into the sub plantar tissue of the right hind paw. Paw thickness was measured using a Vernier caliper at hourly intervals for 5 hours.

Dextran- induced paw oedema: This assay was carried out according to the method of Lo *et al* [16]. Animals were divided into five (5) groups of five (5) rats each. The test groups (III-V) received 200, 400, 600 mg/kg, po plant extract. Standard group (II) was treated with chlorpheniramine (10 mg/kg), while the negative control group (I) received 3 ml/kg of distilled water. The basal paw size of the right hind paw was measured using a Vernier caliper. After 1 hour, 0.1 ml 1.5 % w/v of dextran was injected into the sub plantar tissue of the right hind paw. Paw thickness was measured using a Vernier caliper at hourly intervals for 5 hours.

Formalin-induced arthritis: This assay was performed according to the method of Dubuisson *et al* [17]. Animals were divided into five (5) groups (I-V) containing five (5) animals each. Inflammation was induced by subaponeurotic injection of 0.1 ml 2 % w/v formalin into the right hind paw of the rat on the first day and third day. The extract (200, 400, 600 mg/kg, po) (for groups III-V) and distilled water (3 ml/kg, po) (for group I) and Indomethacin (5 mg/kg, po) (for group II) were administered daily for 5 days. The right paw thickness was measured daily for 5 days using Vernier caliper. The percentage inhibition of the mean increase was calculated on the fifth day and compared with the control.

Statistical analysis

Data obtained from the experiment were expressed as the mean±SEM. One-way analysis of variance followed by Dunnets test for difference between two means using GraphPad Prism® 6. Data were considered significant at $p<0.05$.

RESULTS

Phytochemical screening

Preliminary phytochemical screening showed the presence of saponins, alkaloids, flavonoids, and phenols (Table 1).

Table 1: Phytochemical constituents of-*Cleome ciliata*

Phytochemical metabolites	Inference
Flavonoids	+
Phenol	+
Alkaloids	+
Saponins	+
Terpenoids	-

Key: +: Present. -: Absent.

Acute toxicity of aqueous leaf extract

Mice tested in this study manifested no physical signs of toxicity at all doses of extract administered. There were no toxic effects after one-hour, twenty-four hours and two weeks of observation. No mortality was also recorded for all tested doses.

Effects of aqueous leaf extract on carrageenan-induced paw oedema

As illustrated in Figure 1, injection of 0.1 % w/v carrageenan to the control group evoked a time-dependent increase in paw thickness. Treatment with *C. ciliata* extract caused a dose-dependent decrease in paw thickness at all doses. Extract also elicited an early-onset inhibition of paw oedema (1-3 hours) at all doses compared to the control ($p<0.05$). The peak decrease in paw thickness was observed at 600 mg/kg, in the 4th hour (0.9±0.0 vs 0.7±0.0 cm, $p<0.01$).

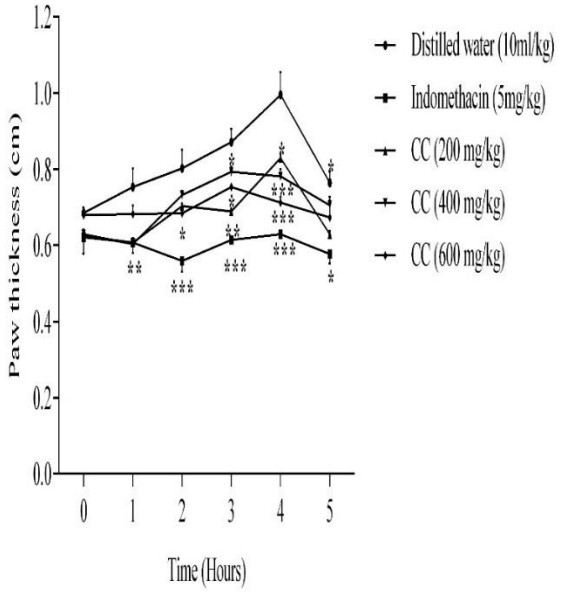

Figure 1: Effect of aqueous leaf extract of *Cleome ciliate* (CC) on paw thickness induced with carrageenan.
*p<0.05, **p<0.01, ***p<0.001 vs control group

Effects of aqueous leaf extract on dextran-induced oedema

As shown in Figure. 2, dextran (1.5 %v/v) increased paw thickness for 2-h in the negative control. All tested doses caused a time-dependent fall in paw thickness. Although all tested doses caused an initial drop in paw thickness at 1 hour, a dose of 200 mg/kg showed peak effect (0.7±0.0 vs 0.6±0.0, $p<0.05$). Similarly, although all doses caused peak fall in paw thickness in the 4th hour, 400 mg/kg dose produced the most pronounced fall in paw thickness (0.6±0.0 vs 0.5±0.0 cm, $p<0.05$). Chlorpheniramine, an antihistamine, caused a time-dependent drop in paw thickness ($p<0.05$).

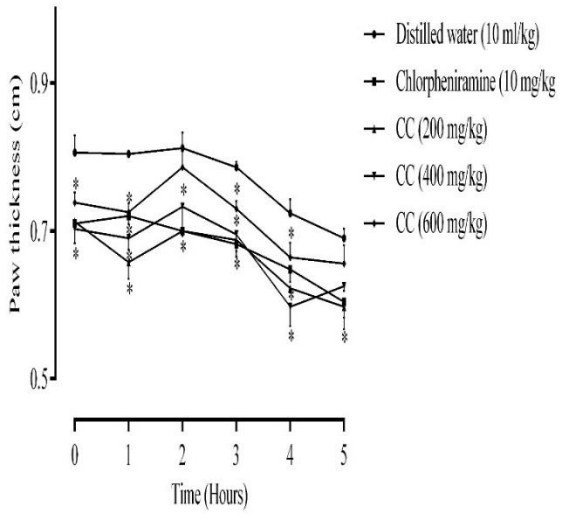

Figure 2: Effect of aqueous leaf extract of *Cleome ciliata* (CC) on dextran-induced paw thickness.
*p<0.05 vs control group.

Effects of aqueous leaf extract on formalin-induced arthritis

Table 2 shows that formalin caused a time-dependent increase in paw size in the control group. Extract evoked an early-onset effect, significantly reducing paw size at 200 and 400 mg/kg on day 2. Similarly, only 400 mg/kg significantly reduced paw size on day 4, compared to control ($p<0.01$).

Indomethacin significantly reduced paw size all through the experiment ($p<0.01$).

Group	Paw Thickness (cm)				
	Day 1	Day 2	Day 3	Day 4	Day 5
Control	0.47±0.03	0.80±0.02	0.76±0.02	0.84±0.03	0.81±0.01
CC (200 mg'kg)	0.42±0.00	0.75±0.02*	0.74±0.02	0.78±0.02	0.78±0.01
CC (400 mg/kg	0.42±0.01	0.72±0.01**	0.71±0.01	0.71±0.02**	0.72±0.02
CC (600 mg/kg)	0.46±0.02	0.82±0.00	0.76±0.01	0.75±0.01	0.73±0.01
Indomethacin (5 mg/kg)	0.48±0.02	0.72±0.00***	0.57±0.02***	0.71±0.02**	0.64±0.03***

Table 2: Effect of *Cleome ciliata* extract on paw thickness in formalin-induced arthritis

DISCUSSION

There is a continuous search for safer anti-inflammatory medications with a wider spectrum of activity and medicinal plants have been central to this search [18,19]. Hence, our study evaluated anti-inflammatory activity of aqueous leaf extract of *C. ciliata* in acute and chronic inflammatory models. Carrageenan-induced rat paw edema is used for assessing the anti-inflammatory actions of new ligands [15]. Carrageenan is an irritant that stimulates the release of pro-inflammatory mediators such as prostaglandins, substance P, histamine, bradykinin, and TNF-α. The course of carrageenan-induced inflammation is biphasic [20]. The first phase involves the release of kinins, histamine, and serotonin, while the second phase is related to the release of bradykinin and prostaglandins. There is also the release of reactive radicals (due to neutrophil infiltration) such as superoxide anion, hydroxyl radicals and hydrogen peroxide [5]. The reduction in paw thickness by *C. ciliata* (between 1-3 h) suggests that the anti-oedematous action may be due to the suppression of histamine and serotonin activities released during the first phase of carrageenan-induced inflammation. Similarly, the late-onset reduction in paw thickness is also suggestive of an inhibition in prostaglandin action because the second phase (late phase) of carrageenan-induced inflammation principally involves the actions of prostaglandins [4]. It is also necessary to state that the extract seemed to exert a consistent time-dependent reduction in paw thickness which may indicate that the action of *C. cilata* is not short-lived.

Consistent with this, the *C. ciliata* leaf extract caused a reduction in paw thickness induced with dextran. dextran-induced paw oedema is principally mediated by histamine and serotonin [16]. These mediators cause vasodilatation of the blood vessels resulting in the redistribution of blood from the vessel into the tissue space, causing fluid exudation and subsequent oedema. The extract also caused an early and late onset effect in paw thickness induced with dextran which was consistent and lasted all through the study. It also corroborates the observation of the extract in carrageenan-induced

inflammation, indicating that *C. ciliata* possibly reduces the action of histamine.

Formalin-induced rodent arthritis is a suitable model for screening for anti-arthritic agents. The pathology involved in this model mimics that of human arthritis [21,22]. Formalin has a biphasic effect; there is an early neurogenic component which is followed by a later tissue-mediated response [23]. Formalin precipitates pathological changes in connective tissue and alters the relative composition of its various constituents such as mucopolysaccharides and glycoproteins. There is also lipid peroxidation due to the reactive radicals which further intensifies the inflammatory damage. *Cleome ciliata* consistently reduced paw size in both the early and late phases of formalin-induced inflammation. This indicates that the early action of the extract observed in all models may involve a neurogenic action. Similarly, the late reduction in paw size is consistent with the extracts suppressive action on local mediators such as prostaglandins.

CONCLUSION

In conclusion, our study has shown that the *C. ciliata* leaf aqueous extract mitigated against inflammation in acute and chronic models. Mechanism of action appears to be via possible central mechanisms that may involve a suppression of inflammatory mediators. Isolation of bioactive components and mechanistic studies would be pursued in future studies to properly elucidate the actions of *C. ciliata.*

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Conflict of interest
None declared.

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