

Full Length Research Paper

Evaluation of the anti-inflammatory activity of the *Commelina ascendens* J.K Morton (Commelinaceae) aerial part extract

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This study evaluated the anti-inflammatory effect of *Commelina ascendens* using experimentally induced inflammatory models in rats. Oedema was induced on the rat hind paw by the injection of 0.1 mL of undiluted fresh egg albumin (phlogistic agent) into the subplantar surface of the right rat paw. Oedema was assessed in terms of volume of distilled water displaced by the paw. Tissue granuloma was induced in the rats by the subcutaneous implantation of two sterile cotton pellet (20 mg each) in both axillae region of anaesthetised rats. *C. ascendens* extract was orally administered to the rats for seven consecutive days. On day 8, the animals were sacrificed and the pellets surrounded by granuloma tissue were dissected out, dried and weighed. Phytochemical screening and acute toxicity test were carried out using standard procedures. The extract of *C. ascendens* significantly ($p < 0.05$) reduced the fresh egg albumin-induced rat paw oedema. The extract at 400 mg/kg significantly ($p < 0.05$) reduced the granuloma tissue formed in the treated groups as compared to the control. The extract was safe up to a dose of 5000 mg/kg and did not cause any mortality in rats, thus an indication of high safety profile. Phytochemical analysis showed the presence of tannins, saponins, glycosides, carbohydrates, proteins, alkaloids, steroids and flavonoids. This study shows that *C. ascendens* possess anti-inflammatory activity.

Key words: *Commelina ascendens*, egg albumin, cotton pellets, edema, granuloma.

INTRODUCTION

Inflammation could be defined as the complex biological response of vascular tissue to harmful stimuli such as pathogens, damaged cells or irritants (Mitchell and

Cotran, 2004). Inflammatory responses are produced and controlled by the interaction of inflammatory mediators, some derived from leucocytes, some from the damaged:

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tissues. These inflammatory mediators include histamine, platelet activating factors, kinins (bradykinin), neuropeptides (substance-P, calcitonin gene-related peptide), cytokines (e.g. interleukins), and the arachidonic acid metabolites (eicosanoids). Edema formation, leukocyte infiltration and granuloma formation are main manifestations of inflammation (Mitchell and Cotran, 2004). Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and the mediators that increase blood flow (Tian et al., 2011).

Commelina ascendens J.K. Morton belongs to the Commelinaceae family. It is found in the primary and secondary lowland rain-forest, often by rivers or streams (Morton, 1956). It is a scandent herb of 8ft length. It has stems rooting at lower nodes, lanceolate leaves up to 11 cm long and 3 cm broad, and pale blue flowers opening early in the day and fading within 3 h of dawn (about 7-9 am) (Hutchinson and Dalziel, 1954; Morton, 1956). It is traditionally used in the eastern part of Nigeria for the treatment of boils, skin ulcers, cuts, wounds, etc.

The present research aims to investigate the acute and chronic anti-inflammatory activities of the aerial part extract of *C. ascendens*.

MATERIALS AND METHODS

Chemicals

All the chemicals used in this study were of analytical grade and include: methanol (Sigma-Aldrich, Germany) and dichloromethane (Sigma-Aldrich, Germany).

Collection, identification and preparation of plant material

Fresh aerial part of *C. ascendens* were collected from Enugu, Nigeria and authenticated by Mr. A. Ozioko, a taxonomist at Bioresources Development and Conservation Programme (BDPC) Center, Nsukka, Enugu State, Nigeria. Fresh aerial parts of *C. ascendens* were cleaned, cut into smaller pieces and dried under the shade to minimize loss of volatile compound and reduced to a coarse powder using milling machine (Laboratory Mill, Serial No. 4745, Christy and Norris Limited, England). The coarse powder (3.1 kg) was stored in an air tight container before extraction.

Extraction of plant material

The powdered plant material (3.1 kg) was extracted by cold maceration in 10 L of a 1:1 mixture of methanol-dichloromethane for 72 h with intermittent vigorous shaking every 2 h. The extract was strained with a muslin cloth and filtered with Whatman No. 1 filter paper. The filtrate was concentrated using rotary evaporator set at 40°C to obtain the methanol-dichloromethane extract (MDE; 114 g, 3.7% w/w). The extract was stored in an air tight amber bottle and stored at 4°C in a refrigerator before use.

Preliminary phytochemical analysis

The extract was subjected to phytochemical analysis using standard procedures (Trease and Evans, 1983).

Animals

Adult Swiss albino rats (150-200 g) and Swiss albino mice (17-25 g) of both sexes were obtained from the laboratory animal facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. The animals were kept in individual steel cages within the facility and allowed free access to clean water and livestock pellets. They were kept in a well ventilated room with a 12/12 h light/dark conditions and ambient room temperature. Animal experiments were conducted in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub. No. 85-23, revised 1985) and in accordance with the University Ethics Committee on the use of laboratory animals.

Acute toxicity test

The mean lethal dose (LD₅₀) of the MDE of *C. ascendens* in mice was estimated using the method described by Lorke (1983). The study was carried out in two stages. In stage one, 9 mice were divided into 3 groups (n=3), received oral administration of 10, 100 and 1000 mg/kg of MDE (prepared in 3% tween 80) and were observed for 24 h for a number of deaths. At the end of 24 h, no death was recorded. Consequently, a fresh batch of mice divided into four groups (n=1) received 1600, 2900, 3600 and 5000 mg/kg of MDE in the second stage of the study and were observed for 24 h for death.

Egg albumin induced paw oedema in rat

The rat paw oedema method of Winter et al. (1962) was used. All the treatments were administered orally. Twenty-five rats were randomly divided into five groups. Group I received 2 ml/kg of 3% Tween 80 (control), Group II received 10 mg/kg indomethacin (positive control) while Groups III, IV and V received 100, 200 and 400 mg/kg MDE, respectively. Thirty minutes after the 3% Tween 80, indomethacin and MDE administration, inflammation was induced by subplantar injection of 0.1 ml of fresh undiluted egg albumin (Okoli and Akah, 2000). Edema was assessed in terms of volume of distilled water displaced by the paw before and at 0.5, 1, 2, 3, 4, 5 and 6 h after the induction of inflammation using plethysmometer. The level of inhibition of edema was calculated for each extract using the relation (Perez, 1986).

$$\text{Inhibition (\%)} = 100 \left[1 - \frac{a-x}{b-y} \right]$$

where a = mean paw volume of treated animals after egg albumin injection, x = mean paw volume of treated animals before egg albumin injection, b = mean paw volume of control animals after egg albumin injection, and y = mean paw volume of control animals before egg albumin injection.

Cotton pellet induced granuloma in rats

Cotton pellets were implanted following the method described by Rathi et al. (2004). Twenty-five rats were randomly divided into five groups of five rats. Group I (control group) was treated with 2 ml/kg of 3% Tween 80, Group II (positive control) was treated with 10 mg/kg diclofenac potassium (positive control) while groups III, IV and V were treated orally with 100, 200 and 400 mg/kg MDE, respectively.

The animals were then anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (10 mg/kg) before two sterile cotton pellets (20 mg each) were surgically implanted subcutaneously in both axillae regions of each rat following a single

Table 1. Preliminary phytochemical screening result of the extract of *Commelina ascendens*.

Phytochemical constituent	Relative abundance
Saponin	+
Reducing sugars	-
Protein	+
Tannins	+
Alkaloids	++
Glycosides	+
Flavonoids	+
Carbohydrate	++++
Steroids	++
Terpenoids	-
Resins	-
Fats and oils	+

-Not present, +present in small concentration, ++present in moderately high concentration, +++present in very high concentration, ++++abundantly present.

incision which was thereafter closed by interrupted sutures. The animals were placed individually in a metal cage after grouping them to avoid them biting each other. After the surgery, the animals were allowed to recover before they started receiving oral treatment of the *Commelina ascendens* once daily for seven days.

Calculation

The final dry weight was calculated after deducting cotton pellet weight and taken as a measure of granuloma tissue formation (Jian-Yu et al., 2011).

Wet weight of cotton pellet = Weight of the cotton pellet (wet) - Weight of the cotton pellet

Dry weight of cotton pellet = Weight of the cotton pellet (dry) - Weight of the cotton pellet.

The increase in the wet weight and dry weight of the granuloma tissue formed was calculated relative to the control (3% tween 80).

$$\% \text{ Inhibition} = \left[\frac{W_c - W_d}{W_c} \right] \times 100$$

where W_c = difference in pellet weight of the control group and W_d = difference in pellet weight of the drug treated group.

Statistical analysis

Data obtained was analyzed by one way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons post-hoc test using Graphpad Prism version 5.0. The values were expressed as mean \pm standard error of mean (SEM). $p < 0.05$ was considered statistically significant.

RESULTS

Phytochemical constituents of MDE

The phytochemical analysis showed that MDE tested

positive to alkaloids, glycosides, flavonoids, carbohydrates, tannins, steroids tannins, proteins, fats and oils (Table 1).

Acute toxicity (LD₅₀) test

MDE of *C. ascendens* caused no death in the first 24 h phase of the experiment in which extract doses of 10, 100 and 1000 mg/kg body weight were administered. Also, no death was observed at the second phase of the experiment where 1600, 2900, 3600 and 5000 mg/kg body weight of the extract were administered. Therefore, the oral LD₅₀ of the MDE of *C. ascendens* can be said to be greater than 5000 mg/kg in mice since it did cause mortality up to a dose level of 5000 mg/kg body weight (Table 2).

Effect of extract of *C. ascendens* on egg albumin induced paw oedema in rat

Oral administration of MDE (200 and 400 mg/kg) significantly ($p < 0.01$) inhibited egg albumin-induced rat paw edema when compared with the control group (Table 3). Oral administration of indomethacin (10 mg/kg) also significantly ($p < 0.01$) reduced the edema with 36.84% inhibition at 6 h. The peak inhibitory effect of the MDE was recorded with a dose of 400 mg/kg (54.39%) at 6 h. Nevertheless significant ($p < 0.01$) inhibition was observed at 6 h after treatment with MDE (200 and 400 mg/kg) and indomethacin (10 mg/kg).

Effect of extract of *C. ascendens* on cotton pellet induced granuloma in rats

Oral administration of the MDE at the dose of 100 mg/kg

Table 2. Acute toxicity (LD₅₀) test of *Commelina ascendens*.

Stage	Extract	Dose (mg/kg)	Mortality
Stage 1	MDE	10	0/3
		100	0/3
		1000	0/3
Stage 2	MDE	1600	0/1
		2900	0/1
		3600	0/1
		5000	0/1
		5000	0/1

MDE: Methanol-dichloromethane extract.

Table 3. Effect of extract of *Commelina ascendens* on egg albumin induced paw oedema in rat.

Treatment	Dose (mg/kg)	Oedema volume (ml) (% Inhibition)							
		0 h	0.5 h	1 h	2 h	3 h	4 h	5 h	6 h
Control (3%Tween 80)	2 ml/kg	0.84±0.04	1.76±0.02	1.75±0.07	1.72±0.05	1.66±0.07	1.56±0.04	1.48±0.04	1.41±0.01
Indomethacin	10	0.80±0.05	1.64±0.12 (8.70)	1.57±0.11 (15.38)	1.52±0.09 (18.81)	1.48±0.08 (17.07)	1.38±0.06 (19.44)	1.30±0.05 (21.88)	1.16±0.07** (36.84)
MDE	100	0.90±0.04	1.64±0.04 (20.00)	1.56±0.04 (27.47)	1.56±0.02 (25.00)	1.54±0.02 (21.95)	1.40±0.05 (30.56)	1.34±0.04 (31.25)	1.25±0.02 (38.60)
MDE	200	0.84±0.04	1.56±0.07 (21.74)	1.55±0.07 (21.98)	1.46±0.10 (29.55)	1.38±0.06* (34.15)	1.28±0.06** (38.89)	1.18±0.07** (46.88)	1.14±0.05** (47.37)
MDE	400	0.90±0.05	1.68±0.06 (15.22)	1.64±0.05 (18.68)	1.50±0.05 (31.82)	1.40±0.05* (39.02)	1.30±0.05** (44.44)	1.24±0.06* (46.88)	1.16±0.05** (54.39)

Values expressed as Mean±SEM; n=5 animals per group. Results were analyzed by one-way Analysis of Variance (ANOVA), followed by Dunnett' post hoc test.* $p < 0.05$, ** $p < 0.01$. MDE: Methanol-dichloromethane extract

had shown 3.68% inhibition in weight of wet cotton pellets and 14.50% inhibition in weight of dry cotton pellets, while the extract at the dose of 200 mg/kg had shown 5.79% inhibition in weight of wet cotton pellets and 11.47% inhibition in the weight of dry cotton pellets when compared with that of control group (Table 4). The extract at dose 400 mg/kg produced a significant ($p < 0.01$) anti-inflammatory activity by reducing the weight of dry cotton pellet (45.90±1.87 mg, 22.60%) when compared with the control. The standard drug diclofenac produced maximum activity by

significantly ($p < 0.05$, $p < 0.001$) decreasing the wet weight and dry weight of the cotton pellet by 26.30 and 48.74%, respectively.

DISCUSSION

Experimental animal models are one of the best strategies for the understanding of pathophysiology of any disease in order to design and develop the drugs for its treatment (Rees and Alcolado, 2005; Chatzigeorgiou et al., 2009). The evaluation of the anti-inflammatory activity of *C.*

ascendens study was carried out using the paw edema and cotton pellets induced granuloma models in rats.

The most widely used primary test to screen new anti-inflammatory agents measures the ability of a compound to reduce local edema induced in the rat paw by an injection of an irritant agent (Chakraborty et al., 2004). The extract of *C. ascendens* dose dependently inhibited acute inflammation induced by egg albumin. Maximum percentage oedema inhibition was seen at 6 h with 400 mg/kg dose level

Table 4. The effect of the extract of *Commelina ascendens* on cotton pellet induced granuloma in rat.

Treatment	Dose (mg/kg)	Wet weight of cotton pellet granuloma (mg)		Dry weight of cotton pellet granuloma (mg)	
		Wet weight	% Inhibition	Dry weight	% Inhibition
Control (3% Tween 80)	2 (ml/kg)	260.80±16.97	-	59.30±2.54	-
Diclofenac	10	192.20±18.40*	26.30	30.40±3.23***	48.74
MDE	100	251.20±13.05	3.68	50.70±3.55	14.50
MDE	200	245.70±20.47	5.79	52.50±1.61	11.47
MDE	400	228.30±4.68	12.46	45.90±1.87**	22.60

Values expressed as Mean±SEM; n=5 animals per group. Results were analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett' post hoc test. ** $p < 0.01$, *** $p < 0.001$. MDE: Methanol-dichloromethane extract.

(54.39%).

The cotton pellet test is a chronic inflammation model used for evaluating the antiproliferative effects of drugs (Panthong et al., 2004). It is also widely used to evaluate the transudative and proliferative components of chronic inflammation and can serve as a subchronic and chronic inflammatory test model for the study of anti-arthritis substances (Zhang et al., 2008). Oral administration of the MDE at the dose of 400 mg/kg gave a significant ($p < 0.01$) reduction to the weight of the dry cotton pellet (45.90±1.87 mg, 22.60%) when compared with the control. The decrease in the weight of granuloma suggests that the proliferative phase was effectively suppressed by the extract of *C. ascendens*.

The extract of *C. ascendens* showed an LD₅₀ greater than 5000 mg/kg body weight in mice indicating that it is well tolerated in the animals and may not pose any risk within these concentrations. Phytochemical screening of *C. ascendens* indicated that the plant contains biological active substances including tannins and flavonoids with potential value for the treatment of inflammatory conditions. Flavonoids have been reported to possess potent inhibitory effect on enzymes involved in the production of the chemical mediators of inflammation and metabolism of arachidonic acid (Oweyele et al., 2005; Metowogo et al., 2008; Kumar et al., 2011; Vijayalakshmi et al., 2011), thus suggesting that the flavonoids in *C. ascendens* might be part of the active anti-inflammatory constituents in the plant.

From this study results, it could be concluded that MDE of *C. ascendens* possesses anti-inflammatory activity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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