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Anti-inflammatory and analgesic activities of the aqueous extract of *Cussonia paniculata* stem Bark

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Abstract: The aqueous extract of the stem bark of *Cussonia paniculata* was investigated for its anti-inflammatory and analgesic activities in animal models. The extract at 50, 100 and 200 mg/kg body weight reduced significantly, the formation of oedema induced by carrageenan and histamine. In the acetic acid-induced writhing model, the extract showed a good analgesic effect characterized by reduction in the number of writhes when compared to the control. The extract caused dose-dependent decrease of licking time and licking frequency in rats injected with 2.5% formalin, signifying its analgesic effect. These results were also comparable to those of indomethacin and cyproheptadine, the reference drugs used in this study. Acute toxicity test showed that the plant caused 80% mortality in rats hence it is a toxic plant. Though the study has provided some justification for the folkloric use of the plant in several communities for conditions such as stomach-ache, pain and inflammations but caution should be exercised in its use for medicinal purpose.

Keywords: Analgesic, anti-inflammation, *Cussonia paniculata*, indomethacin, rats, toxicity.

1. Introduction

Despite the progress made in medical research for the past decades, the treatment of many serious diseases is still problematic. Chronic inflammatory diseases remain one of the world's major health problems [1, 2, 3]. Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair [4, 5]. Inflammation has become the focus of global scientific research because of its implication in virtually all human and animal diseases. As a result of adverse effects such as

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gastric lesions caused by non-steroidal anti-inflammatory drugs (NSAID), tolerance and dependence induced by opiates, the use of these drugs as anti-inflammatory and analgesic agents have not been successful in all cases [6, 7]. Therefore, new anti-inflammatory and analgesic drugs lacking these side effects are being researched as alternatives to NSAID and opiates [6, 8]. Attention is being focused on the investigation of the efficacy of plant-based drugs used in the traditional medicine because they are cheap, have little side effects and according to WHO, about 80% of the world population still rely mainly on herbal remedies [3, 6, 8].

Cussonia paniculata Eckl. et Zeyh. (Araliaceae) is native to tropical and southern Africa, as well as the Mascarene Islands. The genus *Cussonia* consists of 22 species most of which are indigenous and well distributed in South Africa [9, 10, 11]. *C. paniculata* is widely used in traditional medicine against pain, inflammation, infections and malaria [12, 13, 14]. The leaves provide good fodder for the stock thus, the Zulus refer to this tree as goats' food [15].

Phytochemical screenings showed that triterpene glycosides were isolated from the leaves of *C. paniculata* [16]. Anti-inflammatory activity of many plants has been attributed to their high sterol/triterpene [17] or flavonoid contents [18]. Studies have also demonstrated that flavonoids such as rutin, quercetin, luteolin, and triterpenoids produced significant antinociceptive and/or anti-inflammatory activities [19, 20, 21, 22]. It has also been shown that these pharmacological substances could exhibit anti-inflammatory activity through inhibition of cyclo-oxygenase lipo-oxygenase pathways [23].

Since the conventional drugs used to ameliorate this phenomenon are either too expensive or toxic and not commonly available to the rural folks that constitute the major populace of the world, this study therefore seeks to assess *C. paniculata* for anti-inflammatory activity and analgesic effects in experimental animal models.

2. Materials and Methods

2.1 Plant collection and extract preparation

The bark of *C. paniculata* was collected in July 2006 in the Eastern Cape Province of South Africa. The area falls within the latitudes 30°00-34° 15´S and longitudes 22° 45´-30° 15´E. It is bounded by the sea in the east and the drier Karoo (semi-desert vegetation) in the west [24]. These areas consist of villages which are generally classified as rural and poor. The plants were identified Sam Boltina by their vernacular names and later validated by Prof. Grierson at the Department of Botany, University of Fort Hare and voucher specimen (Aded Med 2006/15) was deposited in the Griffen Herbarium of the University.

The plant bark was air dried at room temperature and later ground to powder. The ground plant material (200 g) was shaken in distilled water for 48 h on an orbital shaker (Digisystem Laboratory, Germany) at room temperature. The extract was filtered using a Buckner funnel and Whatman No 1 filter paper. The filtrate was concentrated under reduced pressure at 40ºC and later lyophilized using freeze drying system for biological investigations. The extract yielded 16.02 g. Graded aqueous solutions of the extract was prepared and used for the various experiments.

2.2 Animals

The animals used in this study were male Wistar rats weighing between 100 and 250 g. They were maintained at the Experimental Animal House of the Agricultural and Rural Development Research Institute, University of Fort Hare, South Africa. They were kept in rat cages and fed on commercial rabbit cubes (EPOL Feeds, South Africa Ltd.) and allowed free access to clean fresh water

in bottles *ad libitum*. All experimental protocols were in compliance with University of Fort Hare Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care.

2.3 Chemicals and drugs

Carrageenan, acetic acid, and Tween 80 all from Sigma-Aldrich Chemie Gmbh, Steinheim, Denmark were the chemicals used. The standard drugs used were indomethacin and histamine also from Sigma-Aldrich. All the chemicals and drugs used were of analytical grade.

2.4 Acute toxicity test

The acute toxicity of *C. paniculata* aqueous extract was determined in rats according to the method of Hilaly et al. [25] with slight modifications. Rats fasted for 16 h were randomly divided into groups of six rats per group. Graded doses of the extract (200, 400, 800, 1600 and 3200 mg/kg p.o.) were separately administered to the rats in each of the groups by means of bulbed steel needle. All the animals were then allowed free access to food and water and observed over a period of 48 h for signs of acute toxicity. The number of deaths within this period was recorded.

2.5 Anti-inflammatory activities

2.5.1 Carrageenan-induced rat paw oedema

Twenty rats were used in this study and they were divided into five groups of four per group. Each group one of the following treatment: plant extract (50, 100, 200 mg/kg body weight), indomethacin (10 mg/kg body weight) or vehicle control (0.9% normal saline in 3% Tween 80 [2ml/kg]), which were administered orally. Acute inflammation was produced by the sub-plantar administration of 0.1 ml of 1% carrageenan in normal saline that contained Tween 80 in the right paw of rats. The paw volume was measured at 0, 1, 2 and 3 h after carrageenan injection using a micrometer screw gauge. Increases in the linear diameter of the right hind paws were taken as an indication of paw oedema. Oedema was assessed in terms of the difference in the zero time linear diameter of the injected hind paw and its linear diameter at time *t* (i.e. 60, 120, 180 min) following carrageenan administration. The anti-inflammatory effect of the extract was calculated by the following equation: anti-inflammatory activity (%) = (1-D/C) X 100, where D represented the percentage difference in paw volume after the extract was administered to the rats and C represented the percentage difference of volume in the control groups. The percentage inhibition of the inflammation was calculated from the formula: % inhibition = *D0-Dt*/*D0* X 100 where *D0* was the average inflammation (hind paw oedema) of the control group of rats at a given time; and *Dt* was the average inflammation of the drug treated (i.e. extracts or reference indomethacin) rats at the same time [26, 27, 28].

Table 1. Anti-inflammatory activities of aqueous extract of *C. paniculata* bark and indomethacin on carrageenan-induced oedema in the right hind-limb of rats. Data in mean ± S.D., n = 4.

Time (h)	Control	Extract (mg/kg)			Indomethacin
		50	100	200	(10 mg/kg)
1	12.7 ± 2.1	12.3±3.2 (2.7)	10.1 ± 3.1 (20.4)	6.4 ± 2.5 (49.7)	4.3±2.2 (66.4)
2	13.1±1.4	0.44±3.1 (96.6)	0.9±2.1 (93.0)	6.5 ± 0.4 (89.4)	3.4±2.1 (73.7)
3	15.5±3.2	7.4±2.3 (52.4)	5.1±2.2 (67.2)	2.6±1.2 (83.6)	0.6±1.1 (96.2)

Percentage inhibitions of the carrageenan-induced inflammation (oedema) are indicated in parenthesis.

Table 2. Anti-inflammatory activities of aqueous extract of *C. paniculata* bark and cyproheptadine on histamine-induced oedema in the right hind-limb of rats. Data in mean ± S.D., n = 4.

Time (h)	Control	Extract (mg/kg)			Cyproheptadine
		50	100	200	(10 mg/kg)
1	22.2 ± 3.2	21.3±3.1 (4.1)	19.3±2.2 (13.2)	11.2±2.1 (49.5)	12.8± 2.3 (42.6)
2	34.3 ± 1.2	19.8±3.4 (42.3)	13.8±2.5 (59.8)	7.4±2.4 (78.5)	9.8 ± 2.2 (71.3)
3	23.3 ± 1.5	19.2±2.1 (17.4)	8.5±3.2 (63.7)	1.8±1.4 (92.4)	0.9 ± 1.6 (96.3)

Percentage inhibitions of the carrageenan-induced inflammation (oedema) are indicated in parenthesis.

2.5.2 Histamine-induced rat paw oedema

Using the method of Perianayagam et al. [5], the paw oedema was produced by sub-plantar administration of 0.1% freshly prepared solution of histamine into the right hind paw of the rats. Twenty rats were divided into five groups of four rats per group and each group received one of the following treatments: plant extract (50, 100, 200 mg/kg body weight), indomethacin (10 mg/kg body weight) or vehicle control (0.9% normal saline in 3% Tween 80 [2ml/kg]), which were administered orally. The paw volume was recorded immediately before administering the histamine injection (0 h) and every hour for three hours after the histamine injection. The drug and extracts were similarly administered 1 h before eliciting paw oedema. The anti-inflammatory effect of the extract was calculated using the formula given above.

2.6 Analgesic activity

2.6.1 Formalin test

Formalin test was conducted as described by Dharmasiri et al. [6]. Male rats (n = 4/group) were treated respectively with 50, 100 and 200 mg/kg of *C. paniculata* extract, 10 mg/kg of indomethacin and 2ml/kg of normal saline. Thirty minutes later, the rats were injected with 0.05 ml of 2.5% formalin into the right hand foot pad and were immediately placed in a transparent plastic cage separately; the licking time and frequency of the injected paw were recorded for 30 min [29].

2.7 Statistical analysis

The observations were expressed as mean ± S.D. The difference in response to test drugs was determined by one-way analysis of variance followed by Duncan's test. $P<0.05$ was considered significant.

Table 3. Analgesic effect of aqueous extract of *C. paniculata* bark and indomethacin on rats using formalin. Data in mean ± S.D., n = 4.

	Control	Extract (mg/kg)			Indomethacin
		50	100	200	(10 mg/kg)
Duration (sec)	12.8 ± 2.2	5.8 ± 0.8*	9.5 ± 0.6*	6.5 ± 0.5*	5.0± 0.5*
Frequency/30 min	22.5 ± 3.5	8.3 ± 0.3*	13.3 ± 1.6*	14.8 ± 1.5*	14.3 ± 1.8*

Data are significantly different from the control at $P<0.05$.

3. Results and Discussion

Oral administration of graded doses (400, 800, 1600 and 3200 mg/kg p.o.) of the aqueous extract of *C. paniculata* to rats produce significant changes in behaviour with resulting death of all the animals in this group. No mortality was however recorded for 200 mg/kg dose after 72 h of administering the extract to the animal. The effect of indomethacin (10 mg/kg) on carrageenan-induced paw oedema was most pronounced 3 h after carrageenan injection while the 50, 100 and 200 mg/kg doses of the extract showed highest activity at 2 h. The anti-inflammatory effect of the extract was most potent with its lowest dose (Table 1). The effect of the extract (100, 200 mg/kg) and the reference drug on histamine-induced paw oedema was most pronounced at 3 h after histamine injection while the 50 mg/kg dose of the extract showed its highest activity at 2 h indicating that the extract may be more potent than the reference drug. The anti-histaminic activity of the extract also exhibited a dose-dependent trend (Table 2). Treatment with the aqueous extract at 50, 100, 200 and indomethacin at 10 mg/kg caused significant decrease in licking time and frequency of licking of the formalin-injected paw of rats (Table 3). The 50 mg/kg dose showed the highest activity.

Carrageenan oedema is a multimediated phenomenon that liberates diversity of mediators. It is believed to be biphasic; the first phase (1 h) involves the release of serotonin and histamine while the second phase (over 1 h) is mediated by prostaglandins, the cyclooxygenase products, and the continuity between the two phases is provided by kinins [5, 30, 31]. Development of oedema induced by carrageenan is commonly correlated with early exudative stage of inflammation [31, 32]. This study has shown that the aqueous extract of the stem bark of *C. paniculata* possessed a significant anti-oedematogenic effect on paw oedema induced by carrageenan. Since carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the

mediators of acute inflammation [27, 33], the results of this study are an indication that *C. paniculata* can be effective in acute inflammatory disorders.

The extract also caused pronounced reduction in the oedema produced by histamine. This result tends to suggest that the anti-inflammatory activity of the extract is possibly supported by its anti-histamine property. The antihistaminic effect of the extract increased with increase in the dose of the extract hence the effect is dose-dependent. The antihistaminic effect of the 200 mg/kg dose of the extract is comparable to cyproheptadine, an antihistaminic and antiserotonergic agent [34]. Histamine is an important inflammation mediator, potent vasodilator substance and also increases the vascular permeability [35, 36, 37]. Since the extract effectively suppressed the oedema produced by histamine, it showed that the extract exhibited anti-inflammatory actions by inhibiting the synthesis, release or action of inflammatory mediators such as histamine, serotonin and prostaglandins.

The pain in the early phase of formalin test was due to the direct stimulation of the sensory nerve fibres by formalin while the pain in the late phase was due to inflammatory mediators, like histamine, prostaglandins, serotonin and bradykinins [6, 38, 39]. This test is believed to be a more valid analgesic model which is better correlated with clinical pain [39, 40]. In this study, the extract caused a dose-dependent decrease in licking time and licking frequency by the rats injected with formalin signifying the analgesic effect of the extract.

Phytochemical screenings showed that triterpene glycosides were isolated from the leaves of *C. paniculata* [16]. Anti-inflammatory activities of many plants have been attributed to their high sterol/triterpene [17] or flavonoids contents [18, 31]. The mechanisms of anti-inflammatory activity may be related to the anti-phlogistic action of the tannins. NSAID such as indomethacin used in this study are known to inhibit cyclooxygenase enzymes I and II which are implicated in the production of inflammation-mediating agent prostaglandin E_2 (PGE_2) from arachidonic acid [28, 41, 42]. The pattern of anti-inflammatory and analgesic activities exhibited by this extract was similar to that of indomethacin which suggests that the plant's activity may be mediated by cyclooxygenase I and II inhibition.

In conclusion, this work has demonstrated that extract from the stem bark of *C. paniculata* exhibited anti-inflammatory and analgesic activities. Acute toxicity test showed that the plant caused 80% mortality in rats at the dose of 400mg/kg and above. Though the study has provided some justification for the folkloric use of the plant in several communities for conditions such as stomach-ache, pain and inflammations but caution should be exercised in its use for medicinal purpose.

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