

Full Length Research Paper

The effect of *Cordia platythyrsa* on various experimental models of pain and carrageenan induced inflammation

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Only one study has reported on the medicinal properties of *Cordia platythyrsa* (*C. platythyrsa*) though it is used in African traditional medicine for treatment of fever and pain. The current study aimed at investigating the analgesic and anti-inflammatory properties of *C. platythyrsa* using various animal models: writhing test, tail flick, thermal hyperalgesia, mechanically induced-pain, formalin-induced pain and carrageenan-induced inflammation tests. Like aspirin, the two doses of plant extracts used inhibited acetic acid-induced pain though these effects were weaker than the effects of morphine. Although, the plant extract significantly ($p < 0.01$) inhibited thermal pain, its effects were less significant compared to morphine. Celecoxib (10 mg/kg) and plant extract (100 mg/kg) significantly inhibited thermal hyperalgesia compared to indomethacin. On the other hand, both doses of plant extract significantly increased mechanical pain thresholds 30 and 90 min post treatment. The plant extract (150 mg/kg) inhibited both the neurogenic and inflammatory pain phases of formalin-induced pain as well as carrageenan-induced inflammation. This study is the first to show that *C. platythyrsa* has analgesic and anti-inflammatory properties.

Key words: Analgesia, pain, writhing, formalin, thermal hyperalgesia.

INTRODUCTION

Cordia platythyrsa (Baker) is a boraginaceae which grows up to 30 m high and about 3 m in girth. It is commonly found in secondary forests stretching from Sierra Leone to Congo. Its wood is used for furniture and building. In fishing communities, it is the wood of choice for canoe manufacturing due to its fungal and insect resistant properties (Burkil, 2004). In Cameroon, the young leaves of this plant are used for the treatment of cough and tuberculosis (Simbo, 2010). Some plants from the *Cordia* genus have been shown to have anti-inflam-

matory, antimicrobial and anti-snake venom activities (Sertie, 2005; Kloucek, 2007; Ticli, 2008). Recently, a new dioxime (cordioxime) with gamma-lactam activity was isolated and characterized from extracts of *C. platythyrsa* (Christelle et al., 2011).

Besides the aforementioned study, no other study has been reported on the medicinal properties of *C. platythyrsa*. The purpose of this study was therefore to investigate the anti-inflammatory and analgesic properties of the methanol extract of *C. platythyrsa* using

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various animal models of pain.

MATERIALS AND METHODS

Plant extract

Stem barks and plant samples of *C. platythyrsa* were collected around Yaoundé and authenticated in the National Herbarium in Yaoundé - Cameroon where a voucher specimen was deposited (YA0032566). Tree barks were air dried and then extracted in methanol for 72 h after which the solvent was recovered from the extract using a rotary evaporator. The obtained paste extract was air dried for four weeks to obtain a methanol free extract.

Animal

Wistar rats (150 to 200 g) and Swiss mice (20 to 30 g) of either sex were obtained from the South African Vaccine Producers (SAVP). Animals were housed in our animal holding facility and allowed a one week period for acclimatization during which they had free access to both food and water. Prior to treatment, experimental animals were fasted overnight but allowed access to drinking water. Ethical approval for this study was obtained from the Walter Sisulu University Ethics Committee Ref No: Ethics 0009-07.

Drugs

Morphine, aspirin and celecoxib were supplied by Pfizer Pharmaceuticals while indomethacin and carrageenan were supplied by Sigma Chemicals. All drugs were administered in 1 ml volumes orally.

Acetic acid-induced writhing response in mice

Five groups of five mice each were randomly selected for the present study. Group one received 0.09% NaCl (control group) while groups two and three received aspirin (100 mg/kg) and morphine (10 mg/kg), respectively. The last 2 groups received 100 and 150 mg/kg of *C. Platythyrsa* extract. 30 min after treatment with drugs, each rat received a 0.5 ml *ip* injection of 1% v/v acetic acid solution. Injected animals were placed individually in transparent cages and the number of abdominal contortions followed by stretching of the limbs (writhes) was counted for each animal for a period of 30 min. A reduction in the number of writhes induced by drug treatments compared to response in control animals was considered as a positive analgesic response. The percentage inhibition of writhing was then calculated using the following formula:

$$[1-(T/C)] \times 100$$

Where, T is the number of writhings of the treated group and C is the number of writhings of control group.

Thermal pain

A separate group of mice were randomly allocated to one of five groups which received treatment as described earlier. Rats were lightly restrained and posterior third of tail allowed to lie over a glass window slit of the Tail Flick meter (Ugo Basile, model: 37450-001) as previously described by Nkeh-Chungag et al. (2010). Baseline latencies for tail withdrawal from radiant light source were determined before treatment of animals with drugs and then at

various time intervals after drug treatment.

Thermal hyperalgesia

Five groups of wistar rats were randomly assigned to different treatment groups in the present study. The first group received 0.09% NaCl while groups 2 and 3 were pre-treated with aspirin (100 mg/kg) and morphine 10 mg/kg, respectively. Groups 4 and 5 were pre-treated with 100 and 150 mg/kg of methanol extract of *C. Platythyrsa*. Pre-treatment was followed 30 min later by a 50 µl sub-plantar injection of 0.5% carrageenan solution. Paw withdrawal latency to a thermal stimulus was measured using Tail Flick analgesia meter (Ugo Basile, model: 37450-001) at various time intervals after carrageenan injection. Paw withdrawal latency before carrageenan injection - Paw withdrawal latency at any given times after carrageenan injection was calculated.

Mechanical pain

Briefly, rats were kept in Perspex boxes with a metallic grid floor through which the filament was pressed onto the plantar surfaces of rat hind-paws with just enough force to bend filaments for approximately 1 s. The force applied and the withdrawal latency were determined 5 times for each rat - this constituted the baseline data. After determining the baseline, the animals were then randomly assigned to one of five treatment groups. Animals were then treated with: 0.09% NaCl, aspirin (100 mg/kg), morphine 10 mg/kg, 100 or 150 mg/kg of methanol extract of *C. Platythyrsa*. Mechanical pain thresholds were again determined at different time intervals using the same von Frey filament as earlier mentioned.

Formalin test

The formalin test was carried out as described by Prabhu et al. (2011) with some modifications. Five groups of mice were selected for the present study. Group one received 0.09% NaCl (control group) and group two and three received celecoxib (10 mg/kg) and indomethacin (20 mg/kg), respectively. The last two groups (groups 4 and 5) received 100 and 150 mg/kg of the methanol extract of *C. Platythyrsa* respectively. One hour (1 h) after treatment with the various drugs, animals were injected sub-plantarly with a 100 µl of 1% formalin solution. Animals responded to formalin injection by licking or biting the injected paw. The number of times the animal licked/bit the paw was recorded during the first 5 min (neurogenic phase) and then 20 to 30 min (inflammatory phase) after formalin injection. The percentage inhibition was then calculated using the following formula:

$$[1-(T/C)] \times 100$$

Where, T is the number of times treated mice licked/bit the injected paw; C is the number of times control mice licked/bit the treated paw.

Carrageenan-induced paw inflammation

Rats were randomly allocated to one of four groups and baseline paw volumes determined using an Ugo Basile plethysmometer (Model 7140) which determines paw volume by fluid displacement techniques. 30 min after pre-treatment with 0.09% NaCl, celecoxib 10 mg/kg, 100 or 150 mg/kg methanol extract of *C. Platythyrsa*, 100µl of 1% carrageenan in saline was injected into the right hind paw of rats. Paw volumes were again determined at various intervals after carrageenan injection. Change in paw volume was determined

Table 1. Analgesic effect of oral treatment with *C. Platythyrza* on acetic acid-induced writhing in mice.

Treatment	Dose (mg/kg)	Writhes/30 min	Inhibition (%)
Control		76 ± 2	-
<i>C. Platythyrza</i>	100	58 ± 4*	24
<i>C. Platythyrza</i>	150	55 ± 10*	27
Morphine	10	14 ± 1***	82
Aspirin	100	44 ± 2**	43

Values are mean ± SEM. (n = 5), *p<0.05, ***p<0.001.

Table 2. Antinociceptive effect of oral treatment with *C. Platythyrza* on thermal pain.

Treatment	Dose (mg/kg)	Latency (s)			
		0 min	30 min	60 min	90 min
Control		3.8 ± 0.3	4.6 ± 0.3	4.9 ± 0.4	5.1 ± 0.4
<i>C. Platythyrza</i>	100	4.4 ± 0.7	7.9 ± 0.5**	4.9 ± 0.4	4.8 ± 0.3
<i>C. Platythyrza</i>	150	4.7 ± 0.7	8.4 ± 0.4**	5.2 ± 0.3	5.3 ± 0.6
Morphine	10	4.4 ± 0.4	6.4 ± 0.5*	7.2 ± 0.6*	5.5 ± 0.5
Aspirin	100	4.3 ± 0.4	4.7 ± 0.4	4.5 ± 0.5	5.5 ± 0.6

Values are mean ± SEM. (n = 5), P<0.05, **p<0.01.

by subtracting paw volume at baseline from paw volume at specified time intervals.

Statistical analysis

GraphPad Instat[®] was used to perform ANOVA followed by Dunnet's test. Results from each treatment group were compared with results obtained from the control group at specified times. Results were reported as mean ± SEM. A p value < 0.05 was considered significant.

RESULTS

Acetic acid-induced writhing response in mice

Intraperitoneal injection of 1% v/v acetic induced abdominal contortions and stretching of hind limbs. Extracts of *C. platythyrsa* significantly reduced writhing responses in a dose dependent manner. On the other hand, aspirin reduced the number of abdominal writhes better than either doses of the plant extract. However, the analgesic effect of aspirin was weaker than the effect of morphine which was significantly better than the effect of aspirin and *C. platythyrsa*, respectively (Table 1). Whereas, morphine inhibited acetic acid induced pain by 82% and aspirin by 43%, both plant extracts inhibited this type of pain by only 24 and 27%, respectively.

Thermal pain

Paw withdrawal latencies were significantly increased in

the extract treated groups 30 min after drug administration (Table 2). Morphine on the other hand significantly increased paw withdrawal latencies during the 30 and 60 min periods. Aspirin however, did not significantly change paw withdrawal latencies from baseline values.

Thermal hyperalgesia

The difference in pain thresholds between carrageenan-injected and saline-injected paws increased in a time dependent manner in untreated animals. The lower dose of plant extract and celecoxib however, showed the smallest differences in paw withdrawal latencies between saline-injected and carrageenan-injected paws 120 and 180 min after oral treatment with drugs. Indomethacin on the other hand had very little effect on this pain model as indicated by the non-significant differences in paw withdrawal latencies of saline- and carrageenan-injected paws throughout the experimental period (Table 3).

Mechanical pain

Response to mechanical pain as tested with Von Frey filaments improved with treatment. Pain thresholds were significantly increased in all treatment groups compared to the control group. *C. platythyrsa* (100 mg/kg) showed slightly greater analgesic effects throughout the experimental period. Morphine and aspirin significantly and consistently reduce mechanical pain response with

Table 3. Effect of oral treatment with *C. platythyrsa* on thermal hyperalgesia.

Treatment	Dose mg/kg	15 min	60 min	120 min	180 min
Control		2.1 ± 0.3	1.8 ± 0.1	2.8 ± 0.3	3.1 ± 0.2
<i>C. Platythyrsa</i>	100	1.1 ± 0.2	1.2 ± 0.1	0.7 ± 0.3*	0.7 ± 0.3**
<i>C. Platythyrsa</i>	150	1.4 ± 0.4	1.5 ± 0.4	1.4 ± 0.2	1.4 ± 0.2
Celecoxib	10	1.4 ± 0.4	0.5 ± 0.4	1.3 ± 0.2*	0.8 ± 0.5*
Indomethacin	20	1.3 ± 0.3	1.7 ± 0.2	2.0 ± 0.2	1.6 ± 0.2

Values are mean ± SEM. (n = 5), *p<0.05), **p<0.01.

Table 4. Antinociceptive effect of oral treatment with *C. Platythyrsa* on mechanically-induced pain.

Treatment	Dose (mg/kg)	Force applied (g/f)			
		0 min	30 min	60 min	90 min
Control		11.5 ± 1.1	14.7 ± 1.4	15.5 ± 1.3	13.4 ± 1.1
<i>C. Platythyrsa</i>	100	16.1 ± 1.1	19.4 ± 1.5*	21.3 ± 0.9*	21.7 ± 1.3**
<i>C. Platythyrsa</i>	150	14.7 ± 1.1	18.4 ± 1.5	20.4 ± 1.4*	18.1 ± 1.1*
Morphine	10	11.5 ± 1.1	25.3 ± 1.8**	20.9 ± 1.5*	26.8 ± 2.1**
Aspirin	100	20.9 ± 1.5	29.9 ± 1.6**	33.6 ± 1.2**	21.9 ± 1.1**

Values are mean ± S.E.M. (n = 5), *p<0.05), **p<0.01.

Table 5. Antinociceptive effect of oral treatment with *C. Platythyrsa* on formalin-induced pain.

Treatment	Dose (mg/kg)	Neurogenic pain (0 to 5')		Inflammatory pain (20 to 30')	
		Number of licks/bite	Inhibition (%)	Number of licks/bites	Inhibition (%)
Control		129 ± 2	-	137 ± 2	-
<i>C. Platythyrsa</i>	100	71 ± 5*	36	54 ± 8*	45
<i>C. Platythyrsa</i>	150	79 ± 3*	39	52 ± 6**	62
Morphine	10	35 ± 4**	63	25 ± 8**	82
Aspirin	100	72 ± 6*	47	65 ± 1*	53

Values are mean ± SEM. (n = 5), *p<0.05), **p<0.01.

aspirin activity peaking at 30 and 60 min post treatment while the effects of morphine continued to increase beyond 90 min post-treatment (Table 4).

Formalin test

Typically, formalin-induced pain occurs in two phases: the first (neurogenic) phase during the first 5 min after formalin injection followed by a period of relative calm and then a second (inflammatory) phase of pain between 20 and 30 min after formalin injection. All drugs had an effect on both the neurogenic and inflammatory phases of the formalin-induced pain (Table 5). Morphine treated animals had the lowest number of licks/bites in response to formalin injection - an effect which was better in the

inflammatory phase of the response. Aspirin did not show any difference in response between the two response phases. The plant extracts however showed better analgesic properties in the inflammatory phase compared to the neurogenic phase.

Carrageenan-induced inflammation test

Carrageenan induced a steady and time dependent increase in injected paw volume in control animals. Pre-treatment with 100 and 150 mg/kg of the extract significantly inhibited inflammation from the second hour after oral dosing though the effect of the former became non-significant at the 5th h post treatment. The anti-inflammatory effects of celecoxib were significant from

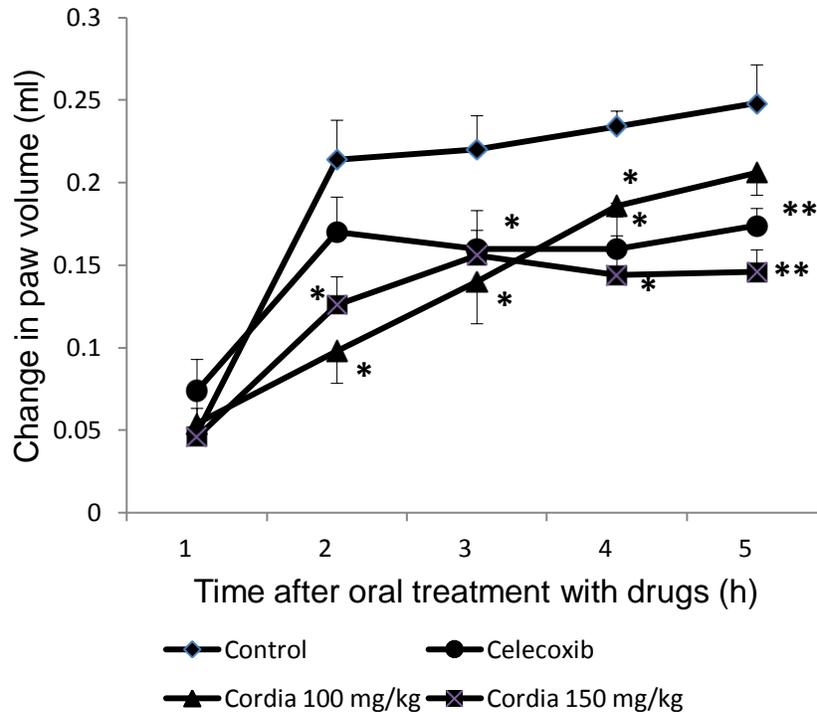


Figure 1. Change in carrageenan-injected hind paw volumes with time. (n = 5), *p<0.05, **p<0.001.

the 3rd h after drug administration and improved further during the 5th h (Figure 1). Although, the anti-inflammatory effect of 100 mg/kg was better than that of the 150 mg/kg during the first 2 h after drug administration, these effects became weaker beyond the 4th h while the effects of the latter became better and significant beyond the 5th hour.

DISCUSSION

In the present study, we investigated the analgesic and anti-inflammatory properties of methanol extract of *C. platythyrsa* tree bark. This study showed that the extract has both analgesic and anti-inflammatory properties as reported for other *Cordia* spp. We used five different models of pain to evaluate the analgesic properties of this extract: the acetic acid-induced writhing test, tail flick test, thermal hyperalgesia, von Frey mechanical pain model and formalin-induced pain test as well as the carrageenan-induced inflammatory model to show that the methanol extract of *C. platythyrsa* has both analgesic and anti-inflammatory properties. The writhing test also called acetic acid-induced abdominal writhing which is a visceral pain model is used generally for screening compounds for analgesic properties. This test is a non specific model of evaluating analgesia used to elucidate peripheral activity of tested compounds (Sánchez-Mateo et al., 2006). The nociceptive mechanism of this test

involves prostaglandin biosynthesis via cyclooxygenase from arachidonic acid (Ikeda et al., 2001; Franzotti et al., 2002). Results of the present study showed that both doses of *C. platythyrsa* used produced significant analgesic effect which might be due to inhibition of the cyclooxygenase or arachidonic acid metabolite. Previous reports however, show that the acetic acid-induced abdominal writhing test is a nonspecific test which responds to both central and peripheral analgesics (Le Bar et al., 2001; Malec et al., 2008). Therefore, because of the lack of specificity of this model, other more specific mechanisms were investigated.

The tail flick test which is based on the withdrawal latency to a thermal stimulus is sensitive to central agents such as opioid analgesics (Chakraborty et al., 2004) but not NSAIDs. In this study however, morphine failed to protect against this model of pain while the extract showed some activity. Thermal stimulus in this test is transmitted from the periphery to the spinal cord via C fibres. The methanol extract of *C. platythyrsa* may therefore inhibit transmission of pain to the spinal cord by inhibition of nerve impulses in the C-fibres. To further clarify these findings, the mechanical pain model using von Frey filaments was used. This test is used to differentiate between drugs with a central opioid-like action mechanism from peripheral analgesics. Morphine, a central acting agent, inhibited this pain model from 1 to 5 h and beyond. Like morphine, the two doses of plant extract also inhibited mechanical pain thus confirming its

central acting analgesic activity. The formalin test provides a model of behavioural pain response which closely resembles clinical pain (Doak and Sawynok, 1997; Roveroni et al., 2001). In this test, animals presented two distinct nociceptive behavioural phases, which involve different stimuli.

The first phase also referred to as neurogenic phase begins immediately after formalin injection and lasts for up to 5 min, is said to be caused by the direct stimulation of nociceptors (Abdollahi et al., 2002). The second phase also known as the inflammatory phase which lasts from 20 to 30 min after formalin injection results from the release of mediators of inflammation into peripheral tissue. Pain in this second phase is due to tissue damage and consequently sensitive to the actions of NSAIDs (Hwang et al., 2008). Opioid analgesics however, are capable of inhibiting the early and late phases of the formalin-induced pain (Vasudevan et al., 2007). In this study, *C. Platythyrsea* significantly inhibited both phases of the formalin test suggested that this extract may have an endogenous opioid-like analgesic activity on μ -opioid receptors which are tonically activated by formalin injection (Zhao et al., 2003) as well as anti-inflammatory properties. Carrageenan-induced inflammation occurs in two phases: the early phase which occurs between 1 to 2 h after injection of the phlogistic agent is mediated by the release of histamine, serotonin and bradykinin; while the late phase which may last for up to 6 h after carrageenan injection involves the active release of TNF-alpha, IL 1beta, COX-2 and prostaglandins which tend to worsen the inflammatory reaction (Gupta et al., 2006; Loram et al., 2007). The two doses of *C. platythyrsa* extract inhibited both phases of carrageenan-induced inflammation while celecoxib and other NSAIDs inhibit the second phase of this inflammatory process via cyclooxygenase inhibition.

The inhibitory activity shown by both extract doses in the first and second phases may indicate an ability of the extract to inhibit histamine and serotonin release as well as inhibit the activity of cyclooxygenase which mediates the second phase of the inflammatory response.

Conclusion

Based on the results of this study, it can be concluded that *C. Platythyrsea* possesses both analgesic and anti-inflammatory properties thus justifying its ethnomedicinal use for the treatment of fever and pain.

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