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Analgesic and Anti-inflammatory Effect of the Aqueous Extract of *Dichrostachys glomerata* (Forssk.) Hutch Fruits

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DDPD, DT and KP designed the study, wrote the protocol. Authors AAKG and DDPD wrote the first draft of the manuscript. Authors AAKG and FHS managed the analyses of the study performed the statistical analysis. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: *Dichrostachys glomerata* is a plant found in the humid areas of Africa and widely used for the treatment of many ailments including rheumatism and snake bite. The present study has been undertaken to assess the analgesic and the anti-inflammatory properties of aqueous extract *Dichrostachys glomerata* fruit.
Place and Duration of Study: Department of Animal Biology and Physiology, Faculty of Science, University of Yaounde I, Cameroon. Between January 2012 and October 2012.
Methodology: Pain was induced in mice by the intraperitoneal administration of 1% acetic acid, hot plate, formalin and tail immersion test. Carrageenan and serotonin (1%) were used to induce inflammation in rat paws.
Results: *Dichrostachys glomerata* significantly inhibited pain induced by acetic acid with a percentage inhibition of 19.4, 69.8, 33.7 and 24.3% respectively at the doses of 25, 50, 100 and 200 mg/kg. An acute pretreatment of mice with extract significantly increased reaction time in the hot plate test with a percentage inhibition more than 68%. Formalin

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induced pain was also significantly inhibited after treatment of rat with the plant extract at the doses of 25 and 50 mg/kg for the neurogenic phase with percentage of inhibition of 56.14 and 61.46% respectively. The extract significantly reduced oedema induced by carrageenan injection with a PI of 72.57 and 79.85% at the doses of 200 and 25 mg/kg respectively. In contrast, a pi of 65.03% was obtained with the plant extract at the dose of 50 mg/kg on serotonin-induced oedema.
Conclusion: The Results obtained showed that *D. glomerata* aqueous extract have both analgesic and anti-inflammatory properties and could be a potential source of new oral anti-inflammatory and/ or analgesic drug.

*Keywords: Dichrostachys glomerata; rats; inflammation; central and peripheral analgesi*c.

1. INTRODUCTION

Inflammation represent a process designed to combat infection or tissue injury. It is a physiological response of a body to stimuli, including infections and tissue injury, and protects the body from these inflammatory stimuli [1]. The complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many pathological conditions, such as bacterial sepsis, rheumatoid arthritis and skin inflammation [1]. In fact, most human diseases are associated with pain and inflammation component. That is why analgesic and anti-inflammatory drugs are among the most prescribed drugs in clinical practice [2]. One approach to discover newer anti-inflammatory agents is to search for their presence in natural sources. In fact, despite the progress in the discovery of anti-inflammatory and analgesic drugs, the chronic use of these drugs is hampered by their adverse effects such as gastro-intestinal lesions or tolerance, as seen with NSAIDs and opiate analgesics, respectively. Therefore, it is important to search for potent analgesic and antiinflammatory drugs with fewer adverse effects from plant sources [3]. *Dichrostachys glomerata* is a terrestrial herbaceous plant of the family Mimosaceae. This plant has been shown to have antiviral, anti-infectious, hypoglycemiant [4] and cicatrizing effects [5]. It is also used in some population to prevent sexual transmitted disease [6]. The main used of *D. glomerata* fruit is as spice for food and for the management of fever and headache. The aim of the present study was to evaluate the analgesic and the anti-inflammatory properties of aqueous extract *Dichrostachys glomerata* fruit in order to provide a scientific justification of it traditional use and this may be a first step of the development of a new plant derided drug.

2. MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Chemicals

Acetic acid and carrageenan were obtained from Sigma Chemical Co. (St. Louis, MO, USA), indomethacin and aspirin were obtained from Aventis Pharma (India), morphine from SIGMA Aldrich Co. (St. Louis, MO, USA), naloxone (Narcan) from Dupont de Nemours S.A. (France) and formaldehyde 40% from Biolab Centre (France).

2.1.2 Animals

The studies were carried out on female Wistar rats (90-150g) and Albino mice (18-25g). They were obtained from the Animal House of the Department of Animal Biology and Physiology, University of Yaounde I, Cameroon. They were housed in group of 5 per cages, under laboratory conditions (Natural light/dark cycle, 22-25ºC), and had free access to water and standard commercial dietfor laboratory animal, purchased from LANAVET (National Veterinary Laboratory), Garoua, Cameroon. All experiments carried out in this study were in accordance of the good laboratory practice as stated by the authorization for the use of laboratory animals (Reg. N° FWAIRD 0001954) from Cameroon National Ethical Committee. Before experiments, animals were fasted for 16 hours but had free access to water. All the procedures were in strict accordance with "Guidelines for the care and use of laboratory animals".Female rats were used in this study because they demonstrate higher pain sensitivity than do males in various nociceptive assays of inflammation [7]. Concerning mice, they were used according to the protocols described by Vogel and Vogel [8] for drug discovery and evaluation for analgesic, anti-inflammatory and antipyretic activity.

2.1.3 Plant

The fresh fruits of *Dichrostachys glomerata* were purchased from the Yaounde market, (Center, Cameroon) in 2010 and identified by a Botanist of the Faculty of Sciences of the University of Yaounde in number 8594 SRF/Cameroon. Once at the laboratory, fruits were cleaned and dried in an oven.

2.2 METHODS

2.2.1 Preparation of plant extract

One hundred and seventy five grams of oven-dried (40ºC) pulverized fruits were boiled in 3.5 L of distilled water for 2 h and kept to cool for 24 h. The extract was later filtered and the filtrate evaporated in an oven (40ºC). The yield of 170 g of crude aqueous extract was 18.57%.

2.2.2 Qualitative phytochemical determination

Qualitative phytochemical determination of the extract were tested using the following chemicals and reagents. Saponin (frothing test), tannins ($FeCl_3$), Cardiac glycosides (Salkowski test), flavonoid (NaCl and HCl), phenol ($FeCl_3$ and $K_3Fe(CN)_6$) and lipids (filter paper) [9].

2.2.3 Pharmacological activities

All reference drugs and the extract were administered by the oral route. The doses of the plant extract used in the present study were chosen following the dose used by traditional healers and after a screening. Concerning the doses of reference drugs they were used following established protocol for drug discovery and previous studies [8,10,11,12].

2.2.4 Nociceptive activity

2.2.4.1 Acetic acid writhing reflex

This was performed according to the method describe by Gaertner et al. [13]. Mice were divided in 7 groups of 5 animals each and injected with 1% acetic acid (10 ml/kg; i.p.). They were treated as follow:group 1 receiving distilled water (10 ml/kg; p.o.), groups 2 to 5 receiving aqueous extracts of *D. glomerata* at the respective doses of 25, 50, 100 and 200 mg/kg; p.o. Groups 6 and 7 received respectively morphine (5 mg/kg; i.p.) and aspirin (100 mg/kg, i.p.). Distilled water was administered 1 h before acetic acid and morphine and aspirin (used as standards) administered 15 min before chemically induced hyperalgesia (1). Mice were then introduced individually in a glass jar, and 10 min after acetic acid injection, the number of writhes (stretching of the abdomen with simultaneous stretching of at least one hind limb) was recorded during 30 min.

2.2.5 Formalin-induced pain

The procedure described by Santos et al. [14] was used with slight modifications. Briefly, 2.5% formalin (20 µl) was subcutaneously injected into the right hind paw of rats to induced pain. Animals were divided into groups of 5 and pre-treated with *D. glomerata* extract at the doses of 25, 50,100, 200 mg/kg, p.o., indomethacin (10 mg/kg), morphine (100 mg/kg), naloxone (1 mg/kg) or distilled water (10 ml/kg, p.o.) 30 min prior formalin injection. Rats were then introduced individually in a clear Plexiglas cage (20 x 30 cm) for observation. The amount of time spent by rats licking the injected paw was recorded to quantify pain behaviour. The interval from 0 to 5 min was considered as Phase I, and the interval from 15 to 30 min as Phase II. These phases represented respectively neurogenic and inflammatory pain responses [15].

2.2.6 Hot plate test

This test consists of a water bath in which was placed, a metallic cylinder. The temperature of the cylinder was set at 55ºC [16]. Each mouse (5 per group) served as its own control. The reaction time of each animal (forepaws licking or jumping response) was taken prior to treatment at 0 and 10 min interval. The average of the two readings was considered as the initial reaction time (T_i). The reaction time (T_f) following the administration of the aqueous extracts of *D. glomerata* fruits (25, 50, 100, 200 mg/kg, p.o); morphine (5 mg/kg, i.p.), naloxone + extract (1 mg/kg, i.p + 200 mg/kg) and distilled water (10 ml/kg, p.o.) was measured at 0.5, 1,2, 3, 4 and 6 h after a latency period of 30 minutes.

2.2.7 Tail immersion

Tail immersion was conducted as described by [17]. This involved immersing 3 cm length of the rat's tail in a water bath maintained at 55±2ºC. Within a few minutes, the rat reacted by withdrawing the tail. The reaction time was recorded with a stopwatch. Each animal served as its own control and two readings were obtained for the control at 0 and 10 min interval. The average of two values was the initial reaction time (T_i). The tests groups were given the aqueous extracts of *D. glomerata* fruits (25, 50, 100, 200 mg/kg, p.o.), aspirin (150 mg/kg), codeine (63 mg/kg), morphine (100 mg/kg), naloxone (1 mg/kg, i.p. + 200 mg/kg) and distilled water (10 ml/kg, p.o.). The reaction time (T_f) for the test groups was taken at intervals 0.5, 1, 2 and 4 h after a latency period of 30 min following the administration of the extract and drugs [18].

2.2.8 Anti-inflammatory activity

2.2.8.1 Carrageenan-induced oedema test

Six groups of 5 rats were given either *D. glomerata* extract at the doses of 25, 50, 100 and 200 mg/kg, (p.o.), indomethacin (10 mg/kg, p.o.) or distilled water (10 ml/kg). Acute inflammation was induced by injection of 1% carrageenan in normal saline (0.05 ml) into the plantar surface of the right hind paw. All experimental rats were treated with the plant extract or reference drug 1 hour before the administration of carrageenan [19]. A plethysmometrer (model 7150, Ugo Basil Italy) was measured the paw volume. Prior to carrageenaninjection, the average volume (Vo) of the right hind paw of each rat was calculated from 3 reading which did not deviate more than 4%. One reading was obtained for each rat (Vt) at 0.5, 1, 2, 3, 4, 5, 6 h after the injection of carrageenan. The percentage of inhibition (PI) for each rat and each group was calculated using the following formula:

$$PI(\%) = \frac{(Vt - V0)Control - (Vt - V0)Treated}{(Vt - V0)} \times 100$$

2.2.9 Serotonin-induced oedema test

The paw oedema was induced by subplantar injection of 1% freshly prepared solution of serotonin (0.05 ml) into the right hind paw of rats [20]. Prior to Serotonin injection, the average volume of the right hind paw of each rat was calculated from 3 reading which did not deviate more than 4%. 30 min and 60 min after injection of serotonin, only one reading was obtained for each animal. Rats were pretreated with *D. glomerata* extract at the doses of 25, 50, 100 and 200 mg/kg, (p.o.), promethazin (5 mg/kg, i.p.) or distilled water (10 ml/kg). The drug and extracts were administered orally 1h before eliciting paw oedema and the paw volume was measured with a plethysmometrer (model 7150, Ugo Basil Italy).

2.10 Statistical test

All data were expressed as the mean ± SEM. The results were analyzed for statistical significance ($P<0.05$) by One-way analysis of variance (ANOVA) followed by Dunnett'spost hoc test using computerized Graph Pad Prism5, Graph pad software, U.S.A.

3. RESULTS

3.1 Qualitative Phytochemical Determination

Saponin, tannins, cardiac glycosides, phenols, flavonoids and oil were identified whereas glycoside, alkaloids and anthraquinones were absent.

3.2 Analgesic Activities

3.2.1 Acetic acid writhing reflex

The aqueous extract of *Dichrostachys glomerata* significantly reduced writhing and stretching induced by acetic acid (Table 1). The reduction of writhing and stretching induced by the plant extract was 19. 4% ($P<0.001$) and 69.8% ($P<0.001$), respectively at the dose of

25 and 50 mg/kg. Aspirin and morphine used respectively as standard peripheral and central analgesic, exhibited 42.0% (p<0. 01) and 90.3% (P<0.001) respectively.

Table 1. Effect of aqueous extract of *D. glomerata*on writhing induced by acetic acid in mice

Treatments	Dose (mg/kg)	Number of writhings within 30 min	Inhibition (%)
Control	10	151.2± 8.7	-
D. glomerata	25	121.8±9.8	19.4
D. glomerata	50	43.6±12.7***	69.8
D. glomerata	100	100.213.5*	33.7
D. glomerata	200	114.4±8.5*	24.3
Aspirin	100	87.6±2.2**	42.0
Morphine	5	2.6±1.6***	90.3

*Values are expressed as mean ± SEM, n= 5 in each group. *p<0.05, **p<0,01*** p < 0.001 vs. control group*

3.2.2 Formalin-induced pain

D. glomerata extract showed analgesic effect on both first (0-5 min; neurogenic pain) and second phases (5-30 min; inflammatory pain) of formalin test as represented on (Table 2). On the neurogenic pain, a significant (P< 0.001) analgesic action of the aqueous extract of *D. glomerata* was observed at 25 and 50 mg/kg (35.9 and 38.0%, respectively). However, when compared to morphine and indometacin, *D. glomerata* extract was less potent in that phase. Indomethacin produced a significant (P< 0.001) inhibition of pain during the first and second phases (47.2% and 97.6%). The maximum effect was produced by morphine who showed 87.3 and 99.7% inhibition in first and second phases respectively. The protective effect of the aqueous extract of *D. glomerata* fruits was blocked when the by the animals were pre-treatment with naloxone.

Table 2. Effect of aqueous extract of *D. glomerata* fruits on Formalin-induced pain in rat

Treatment	Dose (mg/kg)	Total time spent in licking (s)	
		0-5 min	15-30 min
Control	0	78.5±2.0	146.6±10.3
D. glomerata	25	50.2±2.7*** (35.9±2.1)	57.4±6.2*** (38.5±3.7)
D. glomerata	50	48.6±1.6*** (38.0±1.9)	71.0±8.7*** (74.7±6.6)
D. glomerata	100	84.1±4.2 (-7.1±0.6)	4.3±1.3*** (83.9±6.9)
D. glomerata	200	99.0±22.2 (-26.1±6.1)	56.3±9.5*** (72.2±5.4)
Morphine	5	9.9±1.9*** (87.3±7.5)	0.4±0.4*** (99.7±8.1)
Indomethacin	10	41.3±1.9*** (47.2±5.5)	59.6±6.1*** (97.6±7.7)
Naloxone + *D. glomerata*	1+50	40.4±9.6 (48.5±4.6)	5.6±0.2*** (97.2±8.3)

*Values are expressed as mean ± SEM, n= 5 in each group. *** p< 0.001 vs. control group. Percentage inhibition is in bracket*

3.2.3 Hotplate

The latency of onset of reflex in mice test groups is larger compared to that of negative control group. At 30 minutes, the extract significantly (P <0.01) prolonged the average response time, from 11.0±1.8 s, in mice in the control negative group to 21.5±2.1 s in test group treated with the extract at a dose of 200 mg/kg. The highest percentage inhibition (PI) obtained with the plant extract was at the dose of 200 mg/kg (68.4%), recorded at the 4th hour (Table 3). Doses of 25, 50 and 100 mg/kg of the aqueous extract caused a significant increase in means reaction time with the percentages of inhibition of 65.7%, 68.4% and 61.2% respectively. The latency of onset of reflex was significantly (*P <0.01*) high in mice treated with morphine (5 mg/kg) with a PI of 87% obtained 2 hour following the morphine treatment. The administration of naloxone (1 mg/kg) followed by the plant extract (200 mg/kg) achieved PI ranging from 51 to 53% during the last 4 hours of experimentation.

3.2.4 Tail immersion

The aqueous extract of *D. glomerata* fruits has a significant protective effect at a dose of 25 and 50 mg/kg on pain induced by the immersion of the tails of rats in hot water. A nociceptive inhibition was observed with the plant extract at all doses, but the significant (P <0.05) inhibition (64.7%) of the thermal stimuli was noted 2h after the administration of the extract (50 mg/kg). Aspirin and morphine also had a pronounced protective effect (Table 4). The pre-treatment of the animal with naloxone (1 mg/kg) reversed the inhibitory effect of plant extract.

3.2.5 Anti-inflammatory activity

3.2.5.1 Carrageenan-induced oedema test

Inflammation increased gradually in control group rats with the maximum at the 3th hour. The extract of *D. glomerata* showed a significant (P <0.05) anti-inflammatory effect at the 3th hour (Table 5). The maximum anti-inflammatory effect (72.57%) of the plant extract was obtained after 30 minutes with the dose of 200 mg/kg. Indomethacin (10 mg/kg) significantly (P <0.001) inhibited the development of oedema during the 6 hours of the experiment but with maximal activity (87.03%) at the 4th hour.

3.2.6 Serotonin-induced oedema test

The effects of *D. glomerata* on serotonin-induced inflammation are shown in Fig. 1. The plant extract significantly (P <0.001) inhibited serotonin-induced inflammation by 57.72, 65.04, 71.51 and 60.16% at the respective doses of 25, 50, 100 and 200 mg/kg. When animals were pretreated with promethazin the percentage of inhibition was 28.45% only.

Table 3. Effect of *D. glomerata* fruits aqueous, aspirin and morphine on pain induced by hotplate in mice

Treatment	Dose (mg/kg)	Latency period (s)						
		Time (hour)						
		0	**0. 5**	**1**	**2**	**3**	**4**	**6**
Control	10	5.0±0.8	11.0±1 .8	17.4±2.5	15.2±5.1	9.3±2.3	27.8±10 .9	15.4±3.5
D. glomerata	25	8.7 ±0.3	17.7±2.9* (29.8)	16.7±4.4 (48.0)	22.7±3.2** (61.7)	17.1±2.9* (49.1)	17.8±3.7* (65.7)	16.2±4.1 (62.5)
D. glomerata	50	8.4±0.7	10.6±2.6 (34.7)	11.0±21.0 (23.5)	11.5±2.8 (27.1)	15.9±4.6 (47.0)	24.5±5.8* (65.4)	26.7±11.5 (68.4)
D. glomerata	100	9.6±0.9	18.5±6.4 (46.7)	18.0±9.0 (46.6)	17.8±7.8 (45.9)	23.9±5.9* (59.7)	24.9±4.0** (61.2)	16.6±3.0 (42.1)
D. glomerata	200	8.7±1.0	21.5±2.1*** (59.5)	18.9±4.5 (54.0)	23.1±4.8* (62.4)	16.7±1.0*** (48.0)	27.6±4.8*** (68.4)	22.9±3.9** (62.0)
Morphine	5	8.1±1.0	25.9±4.0** (68.6)	28.1±4.2** (71.1)	62.6±39 .6 (87.0)	57.6±21.0* (85.9)	63.9±26.2 (87.3)	31.3±12.3 (74.0)
Naloxone+ *D. glomerata*	50+1	6.8±0,5	11.2±0,9** (39.1)	11.1±3.8 (38.8)	14.2±4.7 (52.2)	14.0±2.8* (51.3)	14.1±5.2 (51.7)	14.7±1.7** (53.6)

*Values are expressed as mean ± SEM, n= 5 in each group. *P<0.05, **P< 0.01, *** P<0.001 compared with control.*
The percentage inhibitions are in bracket

Table 4. Effect of *Dichrostachys glomerata* fruits aqueous, aspirin and morphine on pain using tail immersion test in rat

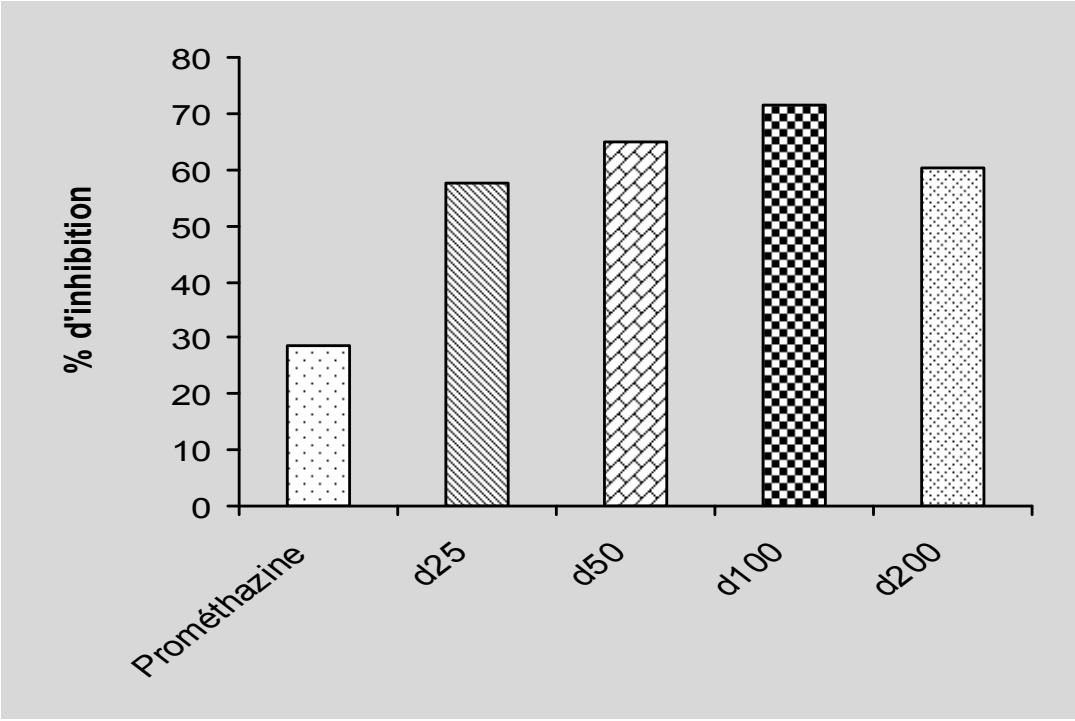
Treatment	Dose (mg/kg)	Latency period (s)					
		Time (h)					
		0	**0.5**	**1**	**2**	**3**	**4**
Control	10	1.8±0.1	3.2±0.8	3.4±0.5	3.2±0.5	3.8±0.8	2.2±0.2
D. glomerata	25	2.0±0.2	4.4±0.7 (120.0±9.3)	4.0±0.4 (100.0±8.0)	4.2±0.3* (110.0±9.9)	2.4±0.2 (20.0±2.6)	1.6±0.4 (-20.0±3.1)
D. glomerata	50	2.1±0.1	2.8±0.3 (64.7±5.9)	3.0±0.4 (76.4±6.7)	3.0±0.3* (76.4±8.6)	1.8±0.2 (5.8±1.2)	1.4±0.2 (-17.6±2.8)
D. glomerata	100	2.5±0.5	3.6±0.2 (140.0±9.8)	3.4±0.4 (126.6±11.5)	4.0±0.3 (166.6±12.6)	2.2±0.3 (46.6±6.7)	2.0±0.0 (33.3±4.4)
D. glomerata	200	2.8±0.3	4.4±0.5 (144.4±11.2)	4.6±0.2 (155.5±12.0)	2.8±0.3 (55.5±6.7)	2.2±0.5 (22.2±3.6)	3.0±1.0 (66.6±7.8)
Codéine	10	1.7±0.1	2.4±0.2 (41.1±7.2)	2.2±0.2 (29.4±3.7)	2.4±0.2 (41.1±6.6)	2.4±0.5 (41.1±5.9)	2.2±0.2 (29.4±4.6)
Morphine	5	1.6±0.1	5.4±1.0 (237.5±15.7)	4.6±1.1 (187.5±11.8)	3.8±0.8 (137.5±12.5)	3.2±0.4 (100.0±8.1)	2.2±0.2 (37.5±6.1)
Aspirin	100	1.4±0.1	2.2±0.2 (57.1±6.2)	2.6±0.2 (85.7±7.5)	2.0±0.0 (42.8±4.2)	2.0±0.3 (42.8±3.9)	2.2±0.2 (57.1±6.4)
Naloxone + *D. glomerata*	1+50	1.9±0.4	1.8±0.2 (-17.6±3.4)	2.8±0.3 (29.4±3.4)	2.6±0.2 (17.6±3.1)	2.8±0.2 (29.4±3.5)	2.2±0.2 (5.8±1.1)

*Values are expressed as mean ± SEM, n= 5 in each group. *P<0.05 compared with control.*
The percentage inhibitions are in bracket

Table 5. Effect *of Dichrostachys glomerata*on paw oedema induced by carrageenan in rats

Treatment	Dose (mg/kg)	Inflammation (mL)							
		Time (h)							
		0	**0.5**	**1**	**2**	**3**	**4**	**5**	**6**
Control	0	0.80±0.05	1.48±0.08	1.25±0.09	1.44±0.1	1.77±0.04	1.52±0.1	1.48±0.12	1.35±0.07
D. glomerata	25	0.85±0.03	0.99±0.03*** (79.85)	0.97±0.02* (74.63)	1.03±0.06** (72.35)	1.02±0.06*** (82.27)	1.02±0.1* (77.08)	0.94±0.06** (71.39)	1.2±0.03 (36.66)
D. glomerata	50	0.87±0.04	1.13±0.04** (61.65)	1.15±0.04 (38.97)	1.03±0.06** (75.71)	1.05±0.05*** (81.23)	1.28±0.07 (43 .00)	1.31±0.08 (35.69)	1.14±0.04* (51.81)
D. glomerata	100	0.8±0.04	1.06±0.03** (62.13)	1.14±0.07 (23.16)	1.23±0.1 (33.33)	1.22±0.07*** (56.10)	1.3±0.07 (51.30)	1.28±0.04 (28.60)	0.91±0.1** (80.00)
D. glomerata	200	0.84±0.08	1.03±0.05** (72.57)	1.06±0.09 (52.94)	1.03±0.04* (70.80)	1.22±0.06*** (61.10)	1.20±0.09 (50.23)	1.39±0.09 (20.29)	1.09±0.06* (54.84)
Indomethacin	10	0.70±0.02	0.82±0.03*** (85.52)	0.88±0.01** (61.39)	0.88±0.01*** (71.57)	0.9±0.02*** (79.00)	0.79±0.03*** (87.03)	0.89±0.03*** (79.70)	0.92±0.09*** (31.31)

*Values are expressed as mean ± SEM, n= 5 in each group. *P<0.05, **P< 0.01, *** P<0.001 compared with control. The percentage inhibition is in bracket*



d25= *D. glomerata*(25 mg/kg) d50= *D. glomerata*(50 mg/kg)
d100= *D. glomerata*(100 mg/kg) d200= *D. glomerata*(200 mg/kg)

Fig. 1. Effect of *Dichrostachys glomerata*on paw oedema induced by serotonin in rats
Values are expressed as mean ± SEM, n= 5 in each group

4. DISCUSSION

The effects of aqueous extract of *Dichrostachys glomerata* were evaluated on pain induced by acetic acid, hot plate, immersion test and formalin test. The intraperitoneal administration of acetic acid in mice caused abdominal cramps. It was reported that acetic acid causes inflammatory pain by inducing a permeability of capillaries [21]. The administration of aspirin, a well known steroidal anti-inflammatory drug that inhibit cyclo-oxygenase 1 and 2 (COX-1 and COX-2) inhibited the algogenic action of acetic acid. Studies have reported that aspirin relieves pain by removing the inflammatory mediators training of pain in peripheral tissues where it was shown that bradykinin and prostaglandins play an important role in the process of pain [21,22]. The contractions induced by intraperitoneal injection of chemicals are due to the chemo-sensitization of nociceptors by prostaglandins [23]. We observed that *D. glomerata* extract suppressed significantly theses abdominal contractions. The plant extract may act by the same mechanism as aspirin. Oedema due to the increase in vascular permeability in induced by training autacoids and substance P released by sensory neurons [24,25]. That release of substance P induce vasodilatation advantage and degranulation of mast led to the release of pro-nociceptive factors [25,26]. The analgesic activity of the extract could also be due to blockage of neurogenic component of oedema induced by acetic acid.

Morphine, a central analgesic, significantly inhibited the pain induced by acetic acid. The anti-nociceptive opioid agonists, opioid partial agonist, and anti-inflammatory drugs

(NSAIDs) can be evaluated by the acetic acid test [18]. The pain induced by acetic acid is inhibited by NSAIDs and morphine [10]. In different experimental conditions, opioids and drugs potentiating serotonin transmission and noradrenergic reduce inflammatory oedema [10]. These results suggest that *D. glomerata* aqueous extract could enhance the inhibitory control of pain descendant pathway where serotonin and noradrenalin acts as modulators. However, acetic acid test is non-specific models of pain study since anti-cholinergic and anti-histaminergic drugs are effective in this test [11].

Heat-induced pain was used to assess the central analgesic effect of *D. glomerata*. Our results indicated that morphine significantly inhibited the pain induced by heat stimulus. The aqueous extract of *D. glomerata* fruit significantly inhibited the pain induced by the hot plate. This inhibition was less important than that of morphine, the reference agonists used. These results suggest that the extract could act through central mechanisms and would be an agonist of opioid receptors. Some compounds of the extract are set on opioid receptors that are much more present at the central device. That may be because of their central location that needed high doses of extract as revealed by our results in the hot plate and tail immersion test. Indeed, the immersion test is specific to morphinic–agonists [18]. In the presence of naloxone the analgesic effect of the extract was not reduced. It is recognized that narcotic analgesics such as morphine exert their central action by interacting with different receptors. This interaction reduces the nociceptive influx and increases the pain threshold. The activation of these receptors also inhibits the release of neurotransmitters (acetylcholine, noradrenalin, serotonin, substance p) in the tissue and peripheral nerves [11]. Naloxone is a pure opioid antagonist with high μ receptors affinity. It was also shown that some plants as *Erigeron floribundus* have a similar central analgesic [27]. According to our results in the hot plate and tail immersion test, we can suggest that the extract of *D. Glomerata* could have a central analgesic activity. The immersion test confirms the results obtained with the hot plate. Indeed, naloxone used in this test has inhibited the action of the plant extract. It is clear from these results that our plant would consist of some compounds that act on opioid receptors. To confirm our results we performed formalin test.

Formalin test is commonly used to elucidate the mechanism of analgesia. The injection of formalin in the hind leg of the mouse causes a biphasic nociceptive response. The first phase or neurogenic phase is centrally mediated with a direct stimulation of C-fiber and the release of substance P and bradykinin. The inflammatory phase or second phase is mediated by the peripheral release of chemical mediators (histamine, serotonin, Kinins, prostaglandins) [28]. The aqueous extract of *D. glomerata* caused a decrease in pain induced by formalin during the first phase and the second phase at the doses of 25 and 50 mg/kg. The percentage of inhibition of neurogenic pain was 38.6% (50 mg/kg) versus (74.7%) for the inflammatory phase at the same dose. Morphine also inhibited the nociceptive response during the two phases. It is clear from these results that the aqueous extract of *D. glomerata* fruit may possess central analgesic activity.

Carrageenan is used in the classic model of acute inflammation in assessing the anti-inflammatory NSAIDs. The injection of carrageenan causes oedema with various types of inflammatory mediators such as free radicals, nitric oxide, prostaglandins (PG) and cytokines whose play a crucial role in the development of oedema induced by carrageenan [29,30]. Inflammation induced by carrageenan is used to determine the inhibition of COX and to establish the origin of inflammatory pain. The aqueous extract of *D. glomerata* fruit caused inhibition of oedema induced by carrageenan during all inflammatory phase, with a maximum of 81.2% at a dose 50 mg/kg at the third time versus 79% for indomethacin (10 mg/kg). This action could be through the inhibition of the mediator's secretion. When the

inflammation was induced by serotonin, the dose of 100 mg/kg of the plant extract was the most active with percentage of inhibition respectively 10.6%, versus 70.6% for promethazin, an antihistamic reference drug. It is recognized that anti-inflammatory drugs like indomethacin exert their anti-inflammatory action by inhibiting cyclo-oxygenase and/or lipoxygenase, preventing the production of serotonin, histamine, PG and LT. The presence of some phytoconstituents like flavonoids and polyphenols has been previously found to be responsible for anti-inflammatory activities in various plant extracts [12]. Theses constituents may be responsible for the anti-inflammatory activities observed in this study since they are also present in the aqueous extract of *D. glomerata* fruits.

5. CONCLUSION

The present study demonstrated that the aqueous extract of *D. glomerata* posses anti-inflammatory activity and central analgesic activity due to the present of compounds like flavonoids. These findings may scientifically justify the use of *Dichrostachys glomerata* for the treatment of pain and inflammation and shows that the plant can be source of new drug. More studies need to be done in order to isolate the active principle.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Cameroon National Ethical Committee (Reg. N° FWAIRD 0001954).

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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