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Preliminary Tests Anti-Inflammatory and Arthritis Effect of Hexanic Extracts of *Dichrostachys glomerata*

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ABSTRACT

The medicinal uses of plants are in many cases based exclusively on traditional knowledge without enough scientific evidences. Different parts of *Dichrostachys glomerata* were traditionally used for the treatment of wide variety of ailments including arthritis, inflammation and joints pain. Many extracts like aqueous and methanolic of *D. glomerata* were used to treat inflammation and pain diseases. The present study had been designed to evaluate the anti-inflammatory and anti-arthritic activities of hexanic extract of *D. glomerata* fruit.

Keywords: Inflammation, Carrageenan, Formaline, Edema, Nociception

INTRODUCTION

Non-steroidal anti-inflammatory drugs are associated with several side effects, such as gastrointestinal mucosal damage, renal toxicity and cardiovascular side effects [1]. Aiming to find a novel analgesic/anti-inflammatory drug with minimal side effects, the present study was designed to screen and evaluate some newly hexanic extract of Dichrostachys glomerata.

Method anti-inflammatory activity using carrageenan-induced rat paw edema and analgesic using acid acetic, formaline and formaline induce arthritis.

MATERIALS AND METHODS

Preparation of plant extract

Once at the laboratory, fruits were cleaned and dried in an oven. The powder was mixed with the hexane and the infusion lasted 24 h. The solvent was evaporated with the rata vapor.

Chemicals

Acetic acid and carrageenan were obtained from Sigma Chemical Co. (St. Louis, MO, USA); indomethacin was obtained from Aventis Pharma (India), morphine from SIGMA Aldrich Co. (St. Louis, MO, USA) and formaldehyde 40% from Biolab Centre (France).

Animals

The studies were carried out on female Wistar rats (90-150 g) and Albino mice (18-25 g). Prior authorization for the use of laboratory animals in this study was obtained from the Cameroon National Ethical Committee (Reg. N° FWAIRD 0001954). Prior the experiment, animals were fasted during 16 h while water was made.

Carrageenan-induced rat paw edema

Acute anti-inflammatory activity of the tested compounds was evaluated using the carrageenan-induced rat paw edema test. After 1 h of injecting the compounds as well as the control, the rats were challenged by subcutaneous injection of 0.05 mL of 1% carrageenan solution into the plantar side of the right hind paw. The paw volume is measured plethysmographically immediately after injection, after 1 and 6 h [2].

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Evaluation of the analgesic activity

Acetic acid writhing reflex: This was performed according to the method describe by Atsang et al. [2]. Briefly mice (Five mice per group) were injected intraperitoneally with 1% acetic acid (10 ml/kg; i.p.).

The animals were randomly divided into 4 groups (n=5). Group 1 was given distilled water (10 ml/kg) by oral route. The extracts of *D. glomerata* fruits (25 and 50 mg/kg; p.o.) and distilled water were administered 1 h before the nociceptive agent. Morphine (5 mg/kg; i.p.) and aspirin (100 mg/kg, i.p.) was used as standards and administered 15 min before chemically induced hyperalgesia [2]. The animals were then placed individually in a glass jar. 10 min after acetic acid administration, the number of writhes (stretching of the abdomen with simultaneous stretching of at least one hind limb) was observed for a period of 30 min.

Formalin-induced pain

The procedure described by Atsang et al. [2] was used but with slight modifications. Briefly, pain was induced by subcutaneous injection of 20 µl of formalin 2.5% in into the plantar surface of the right hind paw, using a 30 gauge needle. Rats (five per group) were pre-treated with the aqueous extracts of *D. glomerata* fruits (25 and 50 mg/kg, p.o.), indomethacin (10 mg/kg), morphine In the present study, the tests used to assess the analgesic effect of the tested compounds are based on nocifensive behavior of mice from heat applied in both hot plate and tail flick tests. Those tests have some disadvantages, since the latency may be altered by factors unrelated to nociceptor responsiveness, such as basal skin temperature. Although this is true, this factor was eliminated by taking the baseline of each mouse before the experiment and those not within the range were excluded from the experiment. In addition, it was noted that the results were better at 30 min, since those tests are prone to habituation. There was a reduction in response with repetitive stimulation [2].

Formalin-induce arthritis: The procedure described by Mendoza et al. [3] and was used but with slight modifications. Formaline was used to induce rat paw edema at 1st day (pre-treatment) except control group in a chronic arthritis experimental model, and the anti-edema effect of individual solvent extracts (post-treatment) was compared.

Rats were divided into 4 groups (n=6 per group): control group, formaline 2% (0.1 mL) only treated group (negative control), aspirin (100 mg/kg at 1st day, 1 mg/kg at 1 h) as a positive control, sample groups *D. glomerata* extracts (25 and 50 mg/kg) daily treated orally, over 9 days. Paw size was measured 1, 2, 4 h and thereafter every day.

Nitrite assay: The production of NO was measured as the nitrites that accumulated in the culture medium after colorimetric reaction with Griess reagent according to the manufacturer's manual (Cayman Chemicals, Ann Arbor, MI, USA). In brief, samples (200 mg/ml, 20 µl and dilution factor 10) were collected 24 h after treatment with cultured bovine vascular endothelial (CPAE) cells. The absorbance after 5 min at 570 nm was measured with a spectrophotometer.

Malondialdehyde (MDA) assay: The assay is by Ohkawa et al. [4]. The aldehyde has action with thiobarbituric acid for give a red color. The absorbance at 500 nm was measured with a spectrophotometer.

Protein assay: Proteins were measured by Biuret methods describe by Gornall et al. [5].

Catalase assay: The test used, according to Singh in 1972 [1]. The hydrogen peroxide is cut in presence of catalase. The absorbance at 570 nm was measured with a spectrophotometer.

RESULTS AND DISCUSSION

The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics [6]. In general, acetic acid causes pain by liberating endogenous substances such as serotonin histamine, prostaglandins (PGs), bradykinins and substance P, endings. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response [5]. The method has also been associated with prostanoids in general that is, increased levels of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products [7]. The significant increase in pain threshold produced by tests and standard in these models suggests involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic descending noradrenergic and serotonergic systems [8,9]. The analgesic effect produced by the tests and standards may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes and other endogenous substances that are key players in pain. The selective cox-2 inhibitor has high effective than the conventional NSAIDs and has low GI and high cardiovascular side effects than to the conventional NSAIDs. But the chances of side effects of combination products are more as compare to the single drug. More study on combination drug therapy may overcome. Inflammatory disease is one of the major health problems worldwide. Inflammation is the body defense mechanism. Sub-chronic inflammation is a reaction arising when the acute response is insufficient to eliminate pro-inflammation agents. Sub-chronic inflammation includes a proliferation of fibroblast and infiltration of exudation. Treatment of carrageenan induced-granuloma with extract at the doses of 25, 50, 100 and 200 mg/kg as well as indomethacine significantly reduced the volume of exudative fluid as compared to group 1 (control) and decreased the number of polymorphonoclear leukocytes indicating its effectiveness against development of

proliferate cells and infiltration of leukocytes [10]. It is well known that inhibition of edema induced by formaldehyde in rats is one of the best suitable test procedures for chronic anti-inflammatory agents as it closely resembles human inflammation [11]. Extract and reference drug significantly inhibited the inflammation on the 10th day. These results suggested that the plant extract might possess anti-proliferative and/or anti-inflammatory activities. Dimo et al. [12] obtained the similar result with the leaf extracts of *Kalanchoe crenata* Andr. They suggested that plant might act on proliferative phase of inflammation. *D. glomerata* reduce the secretion of cytokines and oxygen reactive specie and had anti-inflammatory activity and articular cartilage destruction repair, this is deemed to be the most effective agent for this purpose. *Dichrostachys glomerata* is not toxic and this plant is recommended to treatment of anti-inflammatory and pain diseases [2,13-15] **(Figures 1-3** and **Tables 1-4)**.

Table 1. Effect of hexanic extract of *D. glomerata* on writhing induced by acetic acid.

Treatment	Dose (mg/kg)	Number of writhings within 30 min	Inhibition (%)
Control	10	162.2 ± 0.7	-
D. glomerata	25	32.4 ± 1.1	80.0
D. glomerata	50	38.9** ± 2.1	76.0
Aspirin	100	87.6** ± 1.2	49.8
Morphine	5	2.6** ± 0.6	97.29

*Values expressed as mean ± SEM, n=5 in each group. Data analysis was performed using One way ANOVA followed by Dunnett's post hoc test. ** p<0.01 vs. control group*

Table 2. Effect of hexanic extract of *D. glomerata* fruits on FORMALIN-induced pain.

Treatment	Dose (mg/kg)	Total time spent in licking (s)	
		0-5 min	15-30 min
Control	0	98.00 ± 2.00	166.00 ± 1.30
D. glomerata	25	63.40 ± 1.80* (35.31 ± 1.80)	74.80 ± 0.40** (54.94 ± 2.80)
D. glomerata	50	77.20 ± 2.80* (21.22 ± 1.30)	81.00 ± 0.00** (51.20 ± 2.80)
Morphine	5	9.90 ± 1.90** (87.39 ± 7.50)	0.40 ± 0.40** (99.70 ± 0.10)
Indomethacine	**10**	41.30 ± 1.90** (47.39 ± 5.50)	59.60 ± 0.10** (97.60 ± 0.70)

*Values expressed as mean ± SEM, n=5 in each group. Data analysis was performed using One way ANOVA followed by Dunnett's post hoc test. *** p<0.001 vs. control group. Percentage inhibitions are in bracket*

Table 3. Effect of *Dichrostachys glomerata* on paw edema 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 ± 0.10	1.77 ± 0.04	1.52 ± 0.10	1.48 ± 0.12	1.35 ± 0.07
D. glomerata	25	0.76 ± 0.03	0.87 ± 0.03**	0.84 ± 0.02**	0.82 ± 0.06**	0.87 ± 0.06**	0.86 ± 0.10**	1.01 ± 0.06**	0.96 ± 0.03*
			(83.53)	(82.22)	(90.94)	(88.45)	(86.67)	(63.53)	(63.27)
D. glomerata	50	0.70 ± 0.04	0.96 ± 0.04*	0.81 ± 0.04**	0.83 ± 0.06**	0.99 ± 0.05**	0.92 ± 0.07**	0.85 ± 0.08**	0.97 ± 0.04**
			(62.06)	(76.00)	(80.31)	(70.10)	(70.28)	(77.94)	(51.64)
Indomethacine	10	0.70 ± 0.02	0.82 ± 0.03**	0.88 ± 0.01**	0.88 ± 0.009**	0.90 ± 0.02**	0.79 ± 0.03**	0.89 ± 0.03**	0.92 ± 0.09**
			(85.52)	(61.39)	(71.57)	(79.00)	(87.03)	(79.70)	(31.31)

*Values expressed as mean ± SEM. n=5 in each group. Data analysis was performed using One way ANOVA followed by Dunnett's post hoc test. *P<0.05; **P<0.01; *** P<0.001 compared with control. The percentage inhibitions are in bracket*

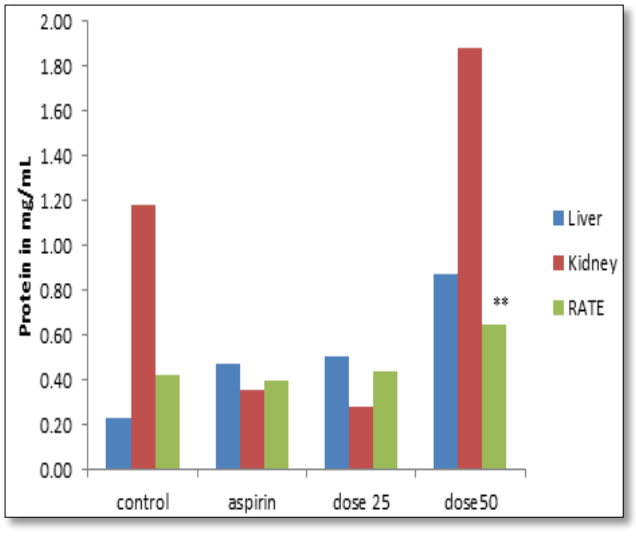

Figure 1. Effect of *D. glomerata* in secretion of protein. *Values are expressed as mean ± SEM, n=5 in each group*

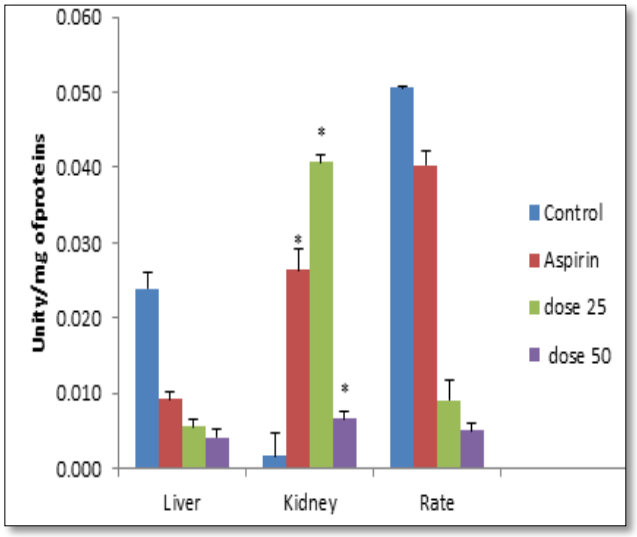

Figure 2. Effect of *D. glomerata* in secretion of SOD. *Values are expressed as mean ± SEM, n=5 in each group*

Table 4. Effect *of Dichrostachys glomerata* on paw edema induced by formaline 2%.

Treatment	Dose (mg/kg)	Inflammation (mL)							
		Time (h)				Time (days)			
		V_0	V_1	V_2	V_4	J_3	J_5	J_7	J_9
Eau	0	0.28 ± 0.00	0.59 ± 0.00	0.68 ± 0.00	0.60 ± 0.00	0.61 ± 0.00	0.69 ± 0.00	0.75 ± 0.00	0.75 ± 0.00
Aspirine	100	0.25 ± 0.01	0.41 ± 0.00** (50.65)	0.46 ± 0.00** (49.00)	0.39 ± 0.02** (57.86)	0.32 ± 0.00** (80.72)	0.56 ± 0.00** (25.98)	0.66 ± 0.00** (14.10)	0.45 ± 0.00** (59.82)
D. glomerata	25	0.21 ± 0.00	0.36 ± 0.00** (50.00)	0.34 ± 0.00** (66.50)	0.35 ± 0.00** (54.09)	0.28 ± 0.00** (77.11)	0.29 ± 0.00 (80.88)	0.38 ± 0.00 (62.39)	0.42 ± 0.00** (54.24)
D. glomerata	50	0.17 ± 0.00	0.39 ± 0.00** (27.92)	0.35 ± 0.00** (55.50)	0.43 ± 0.00** (18.87)	0.27 ± 0.00** (68.07)	0.26 ± 0.00 (77.94)	0.32 ± 0.00 (67.95)	0.46 ± 0.00** (38.14)

*Values expressed as mean ± SEM. n=5 in each group. Data analysis was performed using One way ANOVA followed by Dunnett's post hoc test. *P<0.05; **P<0.01; *** P<0.001 compared with control. The percentage inhibitions are in bracket*

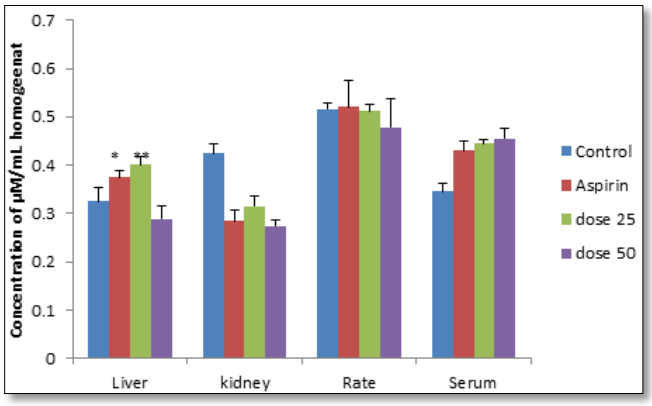

Figure 3. Effect of *D. glomerata* in secretion of No.
Values are expressed as mean ± SEM, n=5 in each group

CONCLUSION

The present study demonstrated that the hexanic extract of *D. glomerata* possess anti-inflammatory activity and central analgesic activity due to the present of compounds like flavonoids [16]. 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 [17,18].

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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