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Studies on the Anti–Inflammatory and Analgesic Properties of Chenopodium Ambrosioides Leaf Extract in Rats

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Abstract: A methanol extract of the dried leaves of Chenopodium ambrosioides was investigated for anti-inflammatory and analgesic activities. The extract (300-700 mg kg^{-1}, p.o.) produced a dose related inhibition of carrageenan-induced paw oedema and cotton pellet-induced granuloma in rats. At the same doses, analgesic effect was also observed with the hotplate device maintained at 55°C as well as on the early and late phases of formalin-induced paw licking in rats. The results of the present study further confirm the use of Chenopodium ambrosioides traditionally for the treatment of painful inflammatory conditions.

Key words: Chenopodium ambrosioides, methanol extract, analgesic, anti-inflammatory

INTRODUCTION

Chenopodium ambrosioides (Chenopodiaceae) is a common plant employed for different ailments in Africa. It is an annual herb that grows to about 1 meter in height with short leaves. The plant is used as a vermifuge (Lopez, 2001), it also has anti-tumour properties (Ruffa, 2002; Effert, 2002). The essential oil obtained from the plant has also been reported to inhibit stress as well as chemically induced ulcers (Zhang, 2003). In other in vitro studies, the activity of the essential oil has been demonstrated against a tropical parasite called Tripanozoma cruzi, it also possesses a strong anti-malarial and insecticidal actions (Johnson, 1984; Pollack et al., 1990).

Some of the bioactive compounds that have been isolated from the leaves include the flavonoids, saponins and terpinenes (Kliks, 1985). Although, Chenopodium ambrosioides has been used for along time as herbal medicine in Nigeria, no pharmacological studies in vivo have previously been conducted on the analgesic and anti-inflammatory activities of this plant. In the present study, we investigated the anti nociceptive and anti-inflammatory effects of the plant using chemical thermal tests.

MATERIALS AND METHOD

Animals: Male albino Wistar rats (180-220 g) were used for the study. They were housed and bred at the pre-clinical animal house of the College of Medicine, University of Ibadan, Nigeria where this study was carried out towards the middle of the year, 2004. The animals were fed with mouse cubes (Pfizer feeds, Ibadan) and water *(ad libitum)*.

Plant materials: Fresh leaves of the plant were obtained from the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan, where the institute's plant taxonomist, Mr. A Odewo carried out the identification. A voucher specimen of the plant with herbarium number FHI 106877 was deposited at the institute. The leaves were shade-dried and reduced to a powdery form and 500 g of the powdered sample was exhaustively extracted with 2.5 L of methanol (analytical grade) for 3 days.

The macerated mixture was filtered and evaporated in a carefully regulated water bath at 45°C to yield a green solid extract weighing 10 g. The extract was stored in a refrigerator at 4°C and dilutions of the extract were made in saline for pharmacological studies.

Carrageenan-induced paw oedema in rats: Oedema was induced by injecting 0.1 mL of 1% (w/v) carrageenan suspension into the subplantar region of the right hind paw of the rats according to the method described by Winter et al. (1962). The test groups (B-D) of the rats were treated orally with 300, 500, or 700 mg kg^{-1} of the extract respectively, 1 h before carrageenan injection. The control group (A) received 10 mL kg^{-1} normal saline orally and the reference group (E) received 10 mg kg^{-1} indomethacin (Strides, Belgium) orally.

Measurement of paw size was carried out by wrapping a piece of cotton thread round the paw and the length of the thread corresponding to the paw circumference was determined using a metre rule (Hess and Milonig, 1972; Bamgbose and Noamesi, 1981). Measurement was done immediately before and at 3 and 5 h following carrageenan injection.

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The inhibitory activity was calculated according to the following formula

$$\% \text{ Inhibition} = \frac{(C_t - C_o) \text{ control} - (C_t - C_o) \text{ treated}}{(C_t - C_o) \text{ control}} \times 100$$

where, C_t is the paw circumference at time t, C_o is the paw circumference before carrageenan injection, $C_t - C_o$ is oedema, $(C_t - C_o)$ control is oedema or paw size after carrageenan injection to control rats at time t.

In practice, carrageenan activity is maximum at 3 h and the effect of the extract at that time is accepted as the optimum inhibitory effect.

Cotton pellet granuloma in rats: The method described by Mossa *et al.* (1995) was employed. A sterilized cotton pellet weighing 30 mg was introduced subcutaneously in the groin region of the rats. They were treated orally with 300, 500, 700 mg kg^{-1} of the extract for 4 consecutive days. Animals in the control received normal saline (10 mL kg^{-1}, orally). Indomethacin (10 mg kg^{-1}, orally) was given to animals in the reference group. On the fifth day, the animals were killed with ether, the pellets removed, freed from extraneous tissue and dried overnight at 60°C and weighed.

Formalin-induced paw licking in rats: The formalin-induced paw licking was studied in the rats in groups A-E using the method of Hunskaar and Hole (1997). In this method, 20 μL of 1% formalin was injected into the dorsal surface of the left hind paw of the rats 1 h after 300, 500 700 mg kg^{-1} of the extract were administered orally to the animals. Control animals received normal saline (10 mL kg^{-1}, orally). Indomethacin (10 mg kg^{-1}, orally) was given to animals in the reference group. The animals were observed in a chamber with a mirror mounted on three sides to allow an unobstructed view of the paws. Time spent licking the injected paw (licking time) was recorded. Animals were observed for the first 5 min post formalin (early phase) and for 10 min starting at the 20th min post formalin injection (late phase).

Acetic acid – induced writhing in mice: The test was carried out using the method of Siegmund *et al.* (1957) as modified by Koster *et al.* (1959). The extract at doses of 300, 500 or 700 mg L kg was administered orally to 16 h fasted mice, divided into groups of six animals each. One hour after treatment, the mice were injected intraperitoneally with 0.2 mL of 3% acetic acid solution to induce characteristic writhing. The number of writhing occurring between 5 and 15 min after injection was

recorded. The response of the extract and Indomethacin (10 mg kg^{-1} per oral) treated groups were compared with those of animals in the control group (saline 10 mL kg^{-1}).

Hot plate test in rats: The hot plate latency assay was based on the method of Eddy *et al.* (1950). Using this method, rats in the experimental groups (B, C, D) were given 300, 500 and 700 mg kg^{-1} of the extract of Chenopodium ambrosioides respectively orally after a 12 h fast. The rats in groups A and E (control and reference groups) had 10 mL kg^{-1} normal saline and 10 mg kg^{-1} indomethacin respectively, both orally.

The rats were then placed in turn on the hot plate 30 min after the administration of either drug, extract or saline and the reaction time, which is the time taken for the animal to start licking the paw or jump from the hot plate was taken as the Ho Plate Latency (HPL). The test was carried out at the beginning of the experiment (baseline) and at 15, 30, 45 and 60 min after administration. At no time was any animal allowed to stay on the hot plate for more than 60 sec in other to avoid tissue damage. The mean HPL for each group was determined. The hot plate temperature was set at 55±2 °C.

STATISTICAL ANALYSIS

Values are expressed as mean±SEM. Statistical analysis was by unpaired comparison using the student's t-test. $p < 0.05$ were regarded as significant.

RESULTS

Carageenan-induced paw oedema in rats: Carageenan-induced rat paw oedema was markedly inhibited by oral pretreatment with either the extract (300-700 mg kg^{-1}) or indomethacin (10 mg kg^{-1}). The extract at 700 mg kg^{-1} dose level was more potent than indomethacin (10 mg kg^{-1}) (Table 1).

Cotton pellet granuloma in rats: The methanol extract dose dependently reduced granuloma tissue formation (Table 1). The reference drug, indomethacin was more potent than the extract at all dose levels.

Formalin-induced paw licking in mice: In this study, oral doses of Chenopodium ambrosioides extract decreased the time spent on licking in both the early and late phases.(Table 2). The 700 mg kg^{-1} dose level was more potent than the reference drug, indomethacin (10 mg kg^{-1}).

Acetic acid-induced writhing in mice: The results showed that the extract at the doses employed

Table 1: Effect of the methanol extract of *Chenopodium ambrosioides* leaves on Carageenan -induced paw licking in rats

Groups	Dose (mg kg^{-1}) orally	Carageenan induced paw oedema		Cotton Pellet Granuloma	
		Paw size (mm) (3 h)	Inhibit (%)	Increase in Pellet Wt (mg)	Inhibition (%)
Control (Saline)	-	2.96±0.06	-	70.50±5.65	-
C. ambrosioides	300	2.73±0.07^{NS}	7.77	50.83±3.88	27.90
C. ambrosioides	500	2.65±0.11^{NS}	10.49	37.50±3.01	46.81
C. ambrosioides	700	2.48±0.06^{+}	16.22	28.17±2.62	60.04
Indomethacin	10	2.53±0.07*	14.53	23.00±2.91	67.38

Each value is the mean±SEM of six rats., * - $p<0.05$, + - $p<0.01$ compared with control, student's t-test, NS - not significant

Table 2: Effect of the methanol extract of Chenopodium ambrosioides leaves on formalin-induced paw licking in rats

Groups	Dose (mg kg^{-1}) orally	Licking time (sec)	
		Early phase	Late phase
Control (Saline)	-	103.17±11.38	255.33±27.63
C. ambrosioides	300	97.67±7.66^{NS}	231.33±16.93^{NS}
C. ambrosioides	500	91.50±11.02^{NS}	207.67±23.37^{NS}
C. ambrosioides	700	45.67±5.78^{+}	94.50±10.14^{+}
Indomethacin	10	54.17±4.19*	159.83±9.77*

Each value is the Mean±SEM of six mice., * - $p<0.05$, + - $p<0.01$ compared with control

Table 3: The effect of the methanol of Chenopodium ambrosioides leaves on acetic acid-induced writhing in mice

Groups	Dose (mg kg^{-1}) orally	No of writhings	Inhibition (%)
Control (Saline)	-	48.83±3.67	-
C. ambrosioides	300	43.33±4.44^{NS}	11.26
C. ambrosioides	500	41.67±4.78^{NS}	14.66
C. ambrosioides	700	31.17±2.88*	36.17
Indomethacin	10	26.33±1.36^{+}	46.08

Each value is the Mean ± SEM of six mice., * - $p<0.05$, + - $p<0.01$ compared with control

Table 4: Effects of the methanol extract of Chenopodium ambrosioides leaves on hot plate latency in rats

Groups	Dose (mg kg^{-1}) orally	Reaction time (sec)			
		0 min	30 min	60 min	90 min
Control (Saline)	-	3.57±0.19	3.80±0.36	3.88±0.37	3.72±0.40
C. ambrosioides	300	3.22±0.28	3.68±0.26	3.80±0.34	3.65±0.71
C. ambrosioides	500	3.73±0.43	4.45±0.52	5.02±0.31	4.82±0.48
C. ambrosioides	700	4.97±0.41	5.58±0.30^{+}	6.43±0.23*	5.85±0.25*
Indomethacin	10	4.13±0.22	5.43±0.30^{+}	6.20±0.22*	6.08±0.23*

Each value is the Mean±SEM of six rats.
+ - $p<0.05$
* - $p<0.01$ compared with control

protected the animals against the acetic acid-induced writhing. The reference drug, indomethacin was more potent than the extract at all dose levels (Table 3).

Hot plate test: The extract was found to dose-dependently cause a prolongation of the hot plate latency (Table 4). The longest latency was obtained at 60 min post administration of the extract or the reference drug.

DISCUSSION

In the present study, the anti-inflammatory and analgesic potential of Chenopodium ambrosioides has been established. The extract was found to significantly inhibit the carrageenan-induced paw oedema, a test which has a significant predictive value for anti-inflammatory agents acting by inhibiting mediators of inflammation (Mossa *et al.*, 1995).

The test is highly sensitive to non-steroidal anti-inflammatory drugs and it has long been accepted as a useful phlogistic tool for investigating new anti-inflammatory drugs. The initial phase of carrageenan paw oedema is mediated by histamine and serotonin and the mediators in the late phase are suspected to be arachidonate metabolites producing oedema after mobilization of neutrophils. It seems that the primary effect of carrageenan as an anti-inflammatory agent is the activation of phospholipase A2 (PLA2) although its cytotoxic effect may initiate further inflammatory action. Inhibitors of arachidonate cycloxygenase are much more effective than those of arachidonate lipoxygenase in inhibiting carageenan-induced inflammation (Lo *et al.*, 1987).

Methanol extracts of the leaves of Chenopodium ambrosioides significantly inhibited this oedematous response over time at all doses assayed. However, the

greatest inhibition was obtained 3 h after carrageenan injection and this was also observed with the reference drug, indomethacin.

While the carrageenan-induced paw oedema test is a test of acute inflammation, the cotton pellet granuloma on the other hand, is a model of chronic inflammation (Ismail *et al.*, 1997) and the dry weight has been shown to correlate with the amount of granulomatous tissue formed (Swingle and Shideman, 1972). The results of these two tests showed that extracts of the Chenopodium ambrosioides leaves can effectively reduce inflammation in both the chronic and acute phases.

The analgesic activities were also studied using thermal and chemical tests. The thermal test (hot plate test) was selected because the test is sensitive to strong analgesics and limited tissue damage because of a cut off point that is usually applied to limit the amount of time the animal spends on the hot plate. The extract produced a dose dependent prolongation of hot plate latency. The hot plate test is suprspinally mediated and therefore a test of central activity. It is an established fact that any agent that causes a prolongation of the hot plate latency using this test must be acting centrally. Hence the methanol extract of the leaves of Chenopodium ambrosioides must have a central activity, this is however, subject to its not having any effect on motor activity and not causing any sedative action.

The formalin test was selected because of its advantages including its ability to mimic human clinical pain conditions, sensitivity to mild analgesics and non-steroidal anti-inflammatory agents (Prato *et al.*, 1990; Tjølsen *et al.*, 1992; Santos *et al.*, 1997; Hunskaar and Hole, 1997). The test possesses two distinct phases possibly reflecting different types of pain. The early phase reflects a direct effect of formalin on nociceptors (non-inflammatory pain), whereas the late phase reflects the inflammatory pain (Hunskaar and Hole, 1997; Elisabetsky *et al.*, 1995). The extract provided a significant inhibition in both phases, suggesting the involvement of both neurogenic and inflammatory mechanisms.

The ability of the extract to inhibit acetic acid-induced writhing in mice (a model of visceral pain) shows that it could be useful in the management of visceral pain. The test is highly sensitive and useful for analgesic drug development, but it is not a selective pain test. It gives false positives with sedations and muscle relaxation (Elizabetsky *et al.*, 1995).

In conclusion, the present study indicates that the methanolic extracts of the leaves of Chenopodium ambrosioides possesses anti-inflammatory and analgesic properties thereby validating its local use by alternative medical practitioners.

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114

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