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EVALUATION OF THE PHYTOCHEMICAL CONSTITUENTS, ANTI-INFLAMMATORY AND ANALGESIC PROPERTIES OF THE ETHANOL EXTRACT OF *ACANTHUS MONTANUS* (ACANTHACEAE) LEAF

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Abstract

The methanol leaf extract of *Acanthus montanus* was investigated for anti-inflammatory and analgesic activities in rats. The effects of the extract on acute inflammation were studied in carrageenan and dextran-induced paw oedema in rats. Analgesic effect of the extract was evaluated using acetic acid induced writhing and the hot plate method. Preliminary phytochemical analysis and acute toxicity test on the extract were also carried out. The extract at doses of 100, 200 and 300 mg./kg showed a significant ($P < 0.05$) and dose dependent inhibition of acute paw oedema-induced by carrageenan (43.01, 54.30 and 67.00 %) and dextran (39.00, 47.00 and 54.00 %) respectively. The activities were comparable to that of diclofenac sodium (25 mg/kg,) The extract at the doses of 100, 200 and 300 mg/kg produced significant ($P < 0.05$) analgesic effect against acetic acid induced writhing (44.75, 55.06 and 61.31 %) and hot plate tests (23.41, 39.65 and 46.63 %) respectively. The activity was compared with that of aspirin (standard drug). The preliminary phytochemical screening revealed the presence of alkaloids, tannins, glycosides, carbohydrates, flavonoids and steroids. The LD_{50} was estimated to be 1650. 04 mg/kg, i.p. in mice. This study justified the use of *Acanthus montanus* against pain and inflammation in herbal medical practice.

Keywords: *Acanthus montanus*, pain, inflammation, acute toxicity, analgesic.

INTRODUCTION

Inflammatory diseases such as rheumatic diseases are very common. Bacterial infections often cause inflammation and pain. In normal practice, two groups of agents (chemotherapeutic, analgesic and anti-inflammatory) are prescribed simultaneously. Compounds possessing all the three activities are not common. Most of the existing anti-inflammatory drugs have one or more deficiencies; hence search for better anti-inflammatory agents.

Acanthus montanus commonly known as bears breach, mountain thistle or alligator plant is a striking small shrub with sparse branches and soft stem. It is a native of Tropical West Africa (County and Jore, 1998) and commonly found in South –Eastern Nigeria. It grows to a maximum of 2 m in height and the leaves are lanceolate-oblong with coarse spiny margins, coriaeous semi-rigid and dark glossy green in colour. The flowers are rose or white tinted mauve in colour. Acanthus *montanus* has been found

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useful in the traditional treatment of several diseases. Igede people of Nigeria use the ground leaves of *Acanthus montanus* to treat boils, skin infections and hypertension and as an antitussive (Igoli, *et al.*, 2005). In Democratic Republic of Congo the plant is used to treat urogenital infections, urethral pain, endo-metritis, urinary diseases, cystitis, leucorrhoea (Didie, 2005). It has also been found to have intestinal smooth muscle relaxant activity (Adeyemi, *et al.*, 2004) as well as antiepileptic activity (Noumi and Fouzi, 2003).

MATERIALS AND METHODS

Plant material

The leaves of *Acanthus montanus* were harvested from Nsukka, Enugu State, Nigeria in May, 2008 and identified by Mr. A. Ezeaku in Department of Botany, Faculty of Biological Sciences, University of Nigeria, Nsukka. The voucher specimen (UNPCOG/08/392) has been preserved in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The leaves were properly dried at room temperature, pulverized and stored in airtight container.

Animals

Swiss albino mice (18-24 g) and Wistar albino rats (180-220 g) of both sexes were obtained from the Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were kept in multiple cages at room temperature and maintained on standard animal feed and water *ad libitum* at 12 h light and 12 h dark cycle. All the animals were acclimatized for a week before commencement of the experiment.

Extraction

The dried powdered leaves (500 g) were exhaustively extracted with 2.5 litres of 95 % ethanol in a soxhlet extractor. The extract was concentrated under reduced pressure using rotary evaporator at 50 ^{0}C. The extract was kept in a desiccator till needed for experimentation.

Phytochemical studies

The preliminary phytochemical screening of the extract was performed following standard qualitative chemical tests (Brian and Tuner, 1975; Clorke, 1975). The classes of phytoconstituents tested for include; alkaloids, tannins, flavonoids, saponins, glycosides, proteins, fats and oils, steroids and carbohydrates.

Acute toxicity study

The method of Lorke (1983) was adopted and a total of twenty one mice were used for this study. The animals were fasted before the study, but were allowed water *ad libitum*. In the initial phase, three groups (n=3) were given 10,100 and 1000 mg /kg of the extract intraperitoneally (i.p) respectively. They were then observed for 24 h for signs of toxicity or deaths. In the second phase, another four groups (n=3) were given 2000, 3000, 4000 and 5000 mg /kg of the extract i.p. and were observed for 24 h for signs of toxicity or deaths. The lethal dose (LD_{50}) was then calculated.

Anti-inflammatory activity

Carrageenan-induced rat paw oedema

This was performed according to the method of Winter *et al.*, (1962). Animals were divided into five groups of five each. Oedema in the right hind paws of wistar rats was induced by subcutaneously injecting 0.1 ml of 10 % (w/v) carrageenan in 1 % sodium carboxymethylcellulose. The paw volume of each rat was measured before carrageenan injection and then at 30 min intervals up to five times using a pleythysometer 7150 (UGO BASIL, ITALY). Three groups of the

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rats were treated with different (100, 200 and 300 mg / kg, i.p.) 30 min prior to carrageenan injection. The control animals were given vehicle [1% (w/v) sodium carboxymethylcellulose]. Another group of rats was administered diclofenac sodium (25 mg /kg, i.p.). The difference between the initial and subsequent reading gave the actual oedema volume. Percentage inhibition of inflammation was calculated using the formula:

$$\% \text{ Inhibition} = \frac{VC - VT}{VC} \times \frac{100}{1}$$

Where VT represents oedema volume in the treated rats and VC represents oedema volume in the untreated

Dextran-induced paw oedema

The animals were treated in a manner similar to that of carrageenan-induced paw oedema models; dextran (0.1 ml of 1% (w/v) in 1% (w/v) sodium carboxymethylcellulose) is used in the place of carrageenan (Winter, *et al*, 1963).

Analgesic activity

Hot plate reaction time in mice

The hot plate method was used to estimate the latency of responses. Twenty-five mice divided into five groups of five each were treated in the following manner: Group 1 received vehicle [1% (w/v) sodium carboxymethyl cellulose], Group 2 received aspirin (100 mg/kg) and Groups 3-5 received three different doses of the extract; 100, 200 and 300 mg/kg, i.p. Nociception (sensation of pain) was determined in all rats in terms of reaction time using the hot plate technique (Langerman, *et al.*, 1995). The animals were placed on glass funnels with the heated surface, maintained at 56 ± 3 ^{o}C with cut off time of 20 sec to avoid tissue damage. The time between placing the animals and the beginning of licking paws or jumping were recorded as latency of response. The percentage activity or inhibition was calculated using the formula:

$$\% \text{ Activity/inhibition} = \frac{MRTG - MRTCG}{MRTG} \times \frac{100}{1}$$

Where MRTG is the mean time of test group and MRTCG is the mean reaction time in control group.

Acetic acid induced writhing in mice

The method of Collier, *et al.*, (1968) was used. Five groups of five mice each were selected for the study. The animals were housed singly in a clear plastic observational chamber. Animals were pretreated with extract (100, 200 and 300 mg /kg) and a reference drug (Aspirin, 100 mg/kg) by intra-peritoneal (i.p.) injection 30 min prior to i.p. injection with 1 % acetic acid (10 ml / kg). Control animals received vehicle [1 % (w/v) sodium carboxymethyl cellulose]. The number of writhes exhibited by mice was counted 5 min after acetic acid injection for a period of 10 min. Reduction in the number of writhes by drug treatments as compared to vehicle control animals was considered as a positive analgesic response. The percentage inhibition of writhing was then calculated using the formula:

$$\% \text{ Activity/inhibition of writhing} = \frac{MC - MT}{MC} \times \frac{100}{}$$

Where, MC is the mean number of writhing in control group, while MT is the mean number of writhing in test group.

Statistical analysis

Values were expressed as mean ± S.E.M. Statistical significance was determined by student's t-test (Snedecor and Cochran, 1967).

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RESULTS AND DISCUSSION

The percentage yield of the ethanol extract was found to be 12.7% w/w. The LD_{50} was estimated to be 1650.04 mg/kg i.p. in mice. The methanol extract of *Acanthus montanus* at the doses of 100, 200 and 300 mg/kg, produced a significant ($P< 0.05$) anti-inflammatory activity against acute paw oedema-induced by carrageenan and dextran (Tables 1 and 2) compared to diclofenac sodium, a standard anti-inflammatory drug. The extract at the doses of 100, 200 and 300 mg/kg showed an inhibition (43.01, 54.30 and 67.00 %), and (39.00, 47.00, and 54.00 %) against acute paw oedema-induced by carrageenan and dextran respectively. Diclofenac sodium at the dose of 25 mg/kg showed an inhibition of 79.00 and 73.00 % against acute paw oedema-induced by carrageenan and dextran respectively,

Analgesic effect induced by different doses of the extract on the hotplate tests and, the writhing tests in mice are shown in Tables 3 and 4. At the doses of 100, 200 and 300 mg/kg, the extract exhibited significant ($P < 0.05$) inhibition (43.75, 55.06, 61.31 and 67.58 %) and (23.41, 39.65, 46.63 and 59.64 %) against acetic acid induced writhing and hot plate tests respectively.

The analgesic potency of the extract is comparable to that of Aspirin. Subcutaneous injection of carrageenan into the rat paw produces plasma extravasations and inflammation characterized by increased tissue water and plasma protein exudation with neutrophil extravasations and metabolism of arachidionic acid by both cyclooxygenase and lipooxygenase enzyme pathway (Ringbon, *et al.*, 1998; Ryu *et al.*, 2000). Carrageenan-induced inflammation is a diphasic phenomenon. The first phase begins immediately after injection and diminishes in 1 h. The second phase begins at 1 h and remains for 3 h. It is suggested that the early hyperemia of carrageenan-induced oedema results from the release of histamine and serotonin (Castro *et al.*, 1968). On the other hand, the delayed phase of carrageenan induced oedema result mainly from the potentiating effect of prostaglandins on mediator releases, especially of bradykinin and neutrophil-derived free radicals.

Dextran-induced paw oedema is known to mediate both by histamine and serotonin, which contains little protein and few neutrophils, whereas carrageenan induces protein rich exudation containing large number of neutrophils (Castro *et al.*, 1968). The extract exhibited significant ($P < 0.05$) anti-inflammatory activity in both carrageenan- induced paw oedema and dextran-induced paw oedema.

Although acetic acid-induced pain also called abdominal constriction response is a non - specific model, it is widely used for the evaluation of peripheral anti- nociceptive activity (Gene, *et al.*, 1998). It is very sensitive and able to detect anti-nociceptive effects of compounds at dose level that may appear inactive in the other methods like the tail flick test (Bentley, *et al*, 1983). Local peritoneal receptors are postulated to be partly involved in abdominal constriction response (Bentley *et al.*, 1981). The method has been associated with prostanoids (Derardt, *et al.*, 1980) as well as lipoxygenase products (Dhara, *et al.*, 2000).

The hot plate test has been found to be suitable for evaluation of centrally acting analgesics since it exerted a significant protective effect on thermic painful stimulus. Such an efficacy on this stimulus is characteristic of central analgesics like morphine (Lewis, *et al.*, 1987). The present study indicates that the extract may be a centrally acting agent.

The phytochemical analysis of the extracts revealed the presence of alkaloids, tannins, steroids,

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carbohydrates, glycosides and flavonoids. These phytochemical constituents of the extract might be responsible for the observed pharmacological activities of the extract. Analgesic and anti-inflammatory effects have been observed in flavonoids as well as tannins (Ahmadiani, *et al.*, 2000). Certain flavonoids possess potent inhibitory activity against a wide range array of enzymes such as protein kinase C, protein tyrosine kinases, phospholipase A2, phosphodiesterases and others (Midldleton, 1998). Inhibition of these key enzymes provides the mechanism by which flavonoids inhibit inflammatory processes (Manthey, *et al.*, 2001).

Table 1: Effect of the methanol extract of *Acanthus montanus* leaf on carrageenan paw oedema in rats.

Treatment	Doses (mg/kg)	Paw volume (ml) after 3 h	Percentage of Inhibition (%)
Vehicle (1 ml/kg)	-	1.00± 0.02	00.00
Diclofenac sodium	25	0.21± 0.6*	79.00
Extract	100	0.57± 0.01*	43.01
	200	0.46 ± 0.03*	54.30
	300	0.33±0.06*	67.00

*Indicate significant anti-inflammatory activity at P< 0.05 compared to control. All values are Mean ± SEM, n=5.

Table 2: Effect of the methanol extract of *Acanthus montanus* leaf on dextran induced paw oedema in rats.

Treatment	Doses (mg/kg)	Paw volume (ml) after 3 h	Percentage of inhibition (%)
Vehicle (1 ml/kg)	_	1.00± 0.01	00.00
Diclofenac sodium	25	0.27± 0.03*	73.00
Extract	100	0.61± 0.05*	39.00
	200	0.53± 0.01*	47.00
	300	0.46± 0.02*	54.00

*Indicate significant anti-inflammatory activity at P < 0.05 compared to control. All values are Mean ± SEM, n=5.

In conclusion, this study has shown that the extract does possess significant

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anti-inflammatory and analgesic effect in experimental animals at the doses investigated. The results support the traditional use of this plant in some painful and inflammatory condition and also suggest the presence of biologically active principle, which may worth further investigation and elucidation.

Table 3: Effect of methanol extract of *Acanthus montanus* leaf on hot plate reaction time in mice.

Treatment	Dose (mg/kg)	Mean reaction time (Sec)	Percentage of inhibition (%)
Vehicle (1 ml/kg)	_	9.42 ± 0.80	00.00
Aspirin	100	23.34 ± 1.40*	59.64
Extract	100	12.30 ± 0.93*	23.41
	200	15.61 ± 1.42*	39.65

Values are Mean ± S .E. M, n =5. * Denotes significant analgesic activity at P<0.05 compared to control.

Table 4: Effect of methanol extract of *Acanthus montanus* leaf on writhing induced by acetic acid in mice.

Treatment	Dose (mg/kg)	Number of writhes	Percentage activity (%)
Vehicle (1 ml/kg)	-	31.22 ± 2.15	-
Aspirin	100	10.12 ± 1.30*	67.58
Extract	100	17.56 ± 1.25*	43.75
	200	14.03 ± 1.15*	55.06
	l300	12.68 ± 1.06*	61.31

Values are Mean ± S.E.M (n = 5). * Denotes significant analgesic activity at P<0.05 compared to control.

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