ANTI-INFLAMMATORY AND ANTI-NOCICEPTIVE ACTIVITIES OF *COSTUS LUCANUSIANUS* (COSTACEAE)

*** Owolabi O.J., Nworgu Z.A.M.**

Department of Pharmacology and Toxicology, University of Benin, Benin City, Nigeria.

Summary

Costus lucanusianus J. Braun (Costaceae) is a climbing herb, found mainly in the Niger Delta region of Nigeria. This plant is locally used in situations of pain, inflammation and pyrexia. Hence the plant was screened to investigate the claimed analgesic and anti-inflammatory properties of the plant and hence validate these uses.

The aqueous extract of *Costus lucanusianus,* J. Braun (Costaceae) was evaluated for analgesic property using acetic acid-induced writhing and formalin-induced pain both in mice. Its anti- inflammatory property was also evaluated using the carrageenan- induced paw edema in rats. Acute toxicity test was also carried out using mice.

Costus lucanusianus at doses of 100, 200 and 400 mg/kg showed a dose dependent significant reduction of the number of writhes ($P< 0.05$, $P<0.05$ and $P<0.001$, respectively). The extract also showed a significant reduction of the paw licking time in the formalin-induced pain test. Its effect was more prominent on the second phase ($p<0.0001$), compared to its effect on the first phase ($p<0.05$). This effect was noted to be dose dependent as the 100 mg/kg dose had no significant effect on the first phase of formalin induced pain.

The extract exhibited an insignificant anti- inflammatory activity against the first two phases of inflammation induced by carrageenan. However, an inhibitory effect was noted at the third phase of mediator release by the 400 mg/kg dose ($P<0.05$).

The results suggest that the aqueous extract of *C. lucanusianus* has significant analgesic activity. Inhibition of the synthesis of prostaglandins and other inflammatory mediators probably account for its analgesic properties considering its significant effect on the second phase of formalin- induced pain and on acetic acid-induced writhing test.

No death was recorded during toxicity testing at all doses tested.

Key words: Acute toxicity, Anti-inflammatory activity, Central analgesic effect, *Costus lucanusianus*, Peripheral analgesic effect.

Introduction

Medicinal herbs are important due to their wide use and less side effect. In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional system (1).

More than 13,000 plants have been studied during the last 5-year period. Pain and inflammation are sensory modalities which in many cases represent the only symptom for diagnosis of several diseases. They often have protective function throughout history and man has used many different therapies for management of pain and inflammation. An example is *Papaver somniferum* from which morphine was isolated. It is regarded as prototype of opiates analgesic drugs. In the relief of inflammation and pain, opiates generally act on the central nervous system exercising their effects through three receptors (μ,κ and δ). Such drugs are especially important for the treatment of chronic pain. There are however concerns about side effects and addictive properties, which include respiratory depression, drowsiness, decreased gastrointestinal motility, nausea and several alterations of endocrine and autonomic nervous system (2). So currently used analgesic such as opiates and non-steroidal anti-inflammatory drugs are not useful in all cases, therefore the need to search for new medicinally active plant. To our knowledge there are no available reports on the bioactivity of the aqueous leaf extract of *Costus lucanusianus.*

The plant commonly called monkey sugar cane in the Niger Delta region of Nigeria grows tall reaching a full height of 12 feet. Its leaves are light green, narrowly elliptic while its base is rounded. Parts of the plants used for medicinal purpose are the leaves and stem. It is commonly used for diarrhoea, pyrexia, pain, inflammatory conditions and dysmenorrhoeal (3).

In the present study, we evaluated the aqueous extract for possible analgesic and anti-inflammatory activity in animal models.

Materials and Methods

Plant material

The leaves of *Costus lucanusianus* J. Braun (Costaceae) were collected in Amarata, Yenagoa, Bayelsa State in May, 2007. The botanical identity of the plant and its leaves was authenticated by Dr B.A Ayinde of the Department of Pharmacogonosy, Faculty of

Pharmacy, University of Benin, Benin City. Botanical authentication was confirmed at the Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria where a voucher specimen (No FHI 107855) was deposited for future reference. Immediately after collection, the leaves were air dried for one week. This was further subjected to another one week of drying in an oven maintained at 40 ºC.

Extraction

The leaves were pulverized into a smooth powder using an impact mill (Makers: Christy and Morris Ltd. Process Engineers, Chelmsford. England. Model 474/54). The pulverized material (150 g) was mixed with distilled water (3.0 L) and left for 72 h. The mixture was stirred at 6 h intervals using a sterile glass rod, and passed through a filter paper. The filtrate was concentrated *in* v*acuo* in a rotary evaporator at 40 ^{o}C, giving a yield of 5.53 %.

The concentrated extract was stored in universal bottles, labeled and refrigerated at -4 ºC prior to use.

Animals

Albino Swiss mice weighing between 20-30 g and albino rats 150-200 g of either sex fed on standard diet (Ladokun feeds, Ibadan, Oyo State) were obtained from the Animal house, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin. The animals were allowed free access to water. They were maintained in the laboratory for a minimum period of 10 days prior to experimentation. Approval for use of the animals was obtained from the Faculty of Pharmacy, University of Benin, Ethical Committee on the use of Animals for Experiments. Animals were handled according to the standard protocols for the use of laboratory animals. (National Institute of Health, USA: Public Health Service Policy on Humane care and use of Laboratory animals, 2002).

Acute toxicity study of the extract

Mice (25) were randomly selected into five (5) groups of five (5) mice each. Groups A, B, C, and D were orally administered with 1, 2, 4, and 8 g/kg of the aqueous extract, respectively. Group E was given normal saline (10 ml/kg) orally and served as the control.

General symptoms of toxicity and mortality were observed for 24 h and thereafter for 14 days (4).

Acetic acid-induced writhing test

The method of Koster (5) was used. Mice (25) were randomly selected into five (5) groups of five (5) mice each. The extract (100, 200 and 400 mg/kg, intraperitoneal) or acetylsalicylic acid (100 mg/kg i.p) was administered 30 min before intraperitoneal injection of acetic acid (0.6%, v/v in normal saline, 10 ml/kg). Normal saline was used as the control (10 ml/kg).
The number of writhes was counted immediately after acetic acid administration for 30 minutes.

Formalin-induced pain test

The method of Shibata (6) was used. Twenty microlitres of 1 % formalin was injected subcutaneously into the right hind paw of the mice.
The time (in seconds) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for 5 min after formalin injection (first phase, neurogenic pain) and 15-30 min after formalin injection (second phase, inflammatory pain). Extract (100, 200 and 400 mg/kg, intraperitoneal) and pentazocin (10 mg/kg, i.p) were administered 30 min before formalin injection.
Control animals received normal saline (10 ml/kg).

Anti-inflammatory screening

The anti-inflammatory activity was evaluated in rats according to the carrageenan-induced paw edema test described by (7). The extract (100, 200 and 400 mg/kg) were administered orally, to groups A, B and C. Group D received normal saline, 10 ml/kg orally and served as the control group, while the reference drug, indomethacin at a dose of 10 mg/kg was given to group E orally. An hour later 0.1ml of 1 % carrageenan in sterile saline was administered subcutaneously to the right hind paw.
Paw size was measured with a Vernier caliper (Tricle brand). This was done 30 mins prior to the administration of carrageenan, and at 0.5, 1, 2, 3, 4 and 5 h after the subcutaneous injection of carrageenan. The average size of the paw measured in millimeters was obtained.

Statistical analysis

All data were expressed as mean $\pm$SEM and where applicable, the data were analysed statistically by Student's t-test using Graph pad instant version 2.05a. The level of significance was from $P < 0.05$.

Results

Acute Toxicity

The doses administered are as shown in Table 1. The aqueous extract of *C lucanusianus* at all doses tested (Table 1) showed no signs of acute toxicity and there was no mortality. However, the 4 g/kg dose gave 25 % mortality on the fourth day after extract administration.

Analgesic effect

The extract produced a dose-dependent inhibition of acetic acid-induced writhing in mice. This inhibitory effect on mouse writhing was significant ($p<0.0001$) as shown in Table 2 with the 400mg/kg dose giving 29.6$\pm$ 7.31 number of writhes, which was less than that of the reference drug, acetylsalicylic acid.

The extract at 200 and 400 mg/kg significantly ($p<0.05$) inhibited the first phase of formalin-induced pain (neurogenic pain), while the second phase (inflammatory pain) was inhibited at all doses ($p<0.0001$). The effect of the extract at 400 mg/kg was noted to be better than pentazocin, the reference drug used. The data of formalin test and percentage protection is shown in Table 3.

Anti-inflammatory effect

The effect produced by the C. *lucanusianus* (Table 4) on carrageenan- induced paw edema was found not to be significant against the first two phases of induction of inflammation by carrageenan at all doses tested. However, an inhibitory effect was noted at the third phase of mediator release by the 400 mg/kg dose ($P<0.05$).

Table 1 Acute toxicity studies of the aqueous extract of *C. lucanusianus.*

Treatment(g/kg)	Log- dose	Percentage mortality (%)
Control	-	0
C.L (1)	3.000	0
C.L (2)	3.301	0
C.L (4)	3.602	0
C.L (8)	3.903	0

C.L- Costus lucanusianus, n = 5 mice per treatment.

Table 2 The effects of the aqueous extract of the leaves of C. *lucanusianus*, normal saline and aspirin on acetic acid-induced writhing test in mice.

Treatment (mg/kg)	No of writhes (per 30 minutes)	Percentage inhibition (%)
Control	77.50 ± 3.89	-
C.L(100)	32.50 ±3.18 [a]	58.1
C.L (200)	24.75 ± 9.03 [a]	68.1
C.L (400)	12.25 ± 2.69 [b]	84.2
Aspirin (100).	39.75 ±5.66 [a]	48.7

Values are mean number of writhes ± SEM. (n = 5, per group).
[a]P<0.05, and [b]P<0.0001 significantly different from control.
C.L- Costus lucanusianus

Table 3 The effect of *Costus lucanusianus* extract, pentazocin and normal saline on formalin test in mice

Groups (mg/kg)	0-5 min	% inhibition	15-30 min	% inhibition
Control	107.3 ± 4.6	-	186.0 ± 18.8	-
C.L(100)	84.0 ± 14.6	21.7	42.3 ±6.2 [a,ab]	77.3
C.L (200)	83.3 ± 8.8[a]	22.4	48.0 ± 5.8 [a,ab]	74.0
C.L (400)	74.5 ± 10.4[a]	30.6	0.75 ± 0.48[b]	99.6
Pentazocin (10)	59.3 ± 13.5[a]	44.7	3.5 ± 2.8[bs]	98.1

The values are expressed in Mean ±SEM. (n = 5 per group).
[a]p <0.05 when compared with control group.
[b]p <0.0001 when compared with control group.
[ab]p <0.05 when compared with pentazocin group.
\ *C.L- Costus lucanusianus*

Table 4 Effects of the aqueous extract of the leaves of C.*lucanusianus*, normal saline and indomethacin on carrageenan- induced paw edema in rats.

	Mean paw Sizes:						
Treatment (mg/kg)	0 min	30mins	1hr	2hr	3hr	4hr	5hr
Control	4.05±0.1	5.40±0.3	5.90±0.5	6.60±0.1	6.70±0.4	6.50±0.3	5.80±0.3
C.L (100)	4.50±0.2	5.15±0.2	6.20±0.4	6.80±0.4	7.10±0.4	6.00±0.4	5.70±0.3
C.L (200)	4.00±0.2	5.50±0.3	5.60±0.2	6.70±0.2	6.90±0.4	6.40±0.5	5.60±0.3
C.L (400)	4.90±0.4	5.60±0.3	6.10±0.6	7.60±0.1	6.00±0.3[a]	6.10±0.4[a]	5.80±0.4
Indomethacin(10)	4.90±0.3	5.60±0.5	5.40±0.6	6.20±0.1[a]	5.40±0.2[a]	5.10±0.2[a]	4.70±0.3[a]

Values are mean paw sizes (mm) in both the extract and indomethacin treated groups, (n = 5 animals per group.)
[a]$p < 0.05$ when compared with control group.

Discussion

It is necessary to apply tests which differ with respect to stimulus, intensity and duration, to obtain a picture of the analgesic properties of a substance (8). It is also necessary to apply tests that differ in order to distinguish between central and peripheral analgesic action hence two models were used. In the mouse writhing assay, the extract caused a significant ($p<0.0001$) inhibition of acetic acid-induced writhes (Table 2). Writhes can be described as a wave of constriction and elongation passing caudally along the abdominal wall with twisting of the trunk and extension of the hind limbs in mice. This is due to the nociceptive property of acetic acid (9).

Inhibition of acetic acid-induced writhing in mice, suggests that the analgesic effect of the extract may be peripherally mediated *via* the inhibition of the synthesis and release of prostaglandins (5). This method is not only reliable but also affords rapid evaluation of this type of analgesic action. It was found that C. *lucausianus* significantly inhibited the acetic acid-induced writhing responses in a dose dependent manner. The percentage of inhibition in Table 2 also indicates that the extract at 200 mg/kg and 400 mg/kg produced a higher inhibition when compared to aspirin, (100 mg/kg) a known standard analgesic.

It has been reported that in addition to NSAIDS, antihistaminics and anticholinergics can inhibit writhing response (10). Hence the extract's analgesic effect might be peripherally mediated.

Formalin produces pain by two phases: neurogenic pain releasing substance P and inflammatory pain with release of serotonins, histamine bradykinnins and prostaglandins (6). The extract of *C. lucanusianus* has inhibited both the phases of pain induced by formalin which suggest that the extract may act as both a narcotic analgesic and an NSAID. However, it was noticed that the effect of the extract on the second phase was more pronounced than on the first phase (Table 3). This further confirms that the analgesic effect of the extract may be peripherally mediated *via* the inhibition of the release of prostaglandins, histamine, bradykinins and other inflammatory mediators (5) as already pointed out by its inhibitory effect on acetic acid-induced writhing.

From the pharmacological data obtained, it shows that the plant possess analgesic property. This analgesic activity was found to be dose-dependent.

C. lucanusianus did not show significant inhibitory effect on inflammation induced by carrageenan (Table 4). Induction of inflammation by carrageenan involves three distinct phases of mediator release. The first phase involves the release of histamine and serotonin and last between the first to the second hour, the second phase is the release of kinins lasting from the second to the third hour while the third phase involves the release of prostaglandins and lasts from the third to the fifth hour (11). It was however noted that the extract had no inhibitory effect on the first two phases of inflammation induced by carrageenan. However an inhibitory effect was noted by the highest dose against the third phase of mediator release, which involves the release of prostaglandins. This again, further confirms that the effect of the extract may be peripherally mediated *via* the inhibition of the release of prostaglandins.

The extract was well tolerated by the animals as no signs of acute toxicity like rest lessness, dizziness or seizures were observed after the administration of all doses.

Most importantly, no death was recorded, after 24 h administration of extract.

The minimum effective dose of *Costus lucanusianus* that gave a response was 100 mg/kg, while the highest dose administered for the toxicity testing was 8 g/kg. This gives an idea of a large therapeutic index thus indicating the safety of the extract (12).

The research was able to produce scientific basis for the traditional use of *Costus lucanusianus* leaves as a remedy for pain. The results suggest that the aqueous extract has a potential analgesic effect that can be explored for therapeutic advantage.

To improve the safety of this traditional herbal remedy, additional research is needed to define the stability and bioactivity of this product (13).

Therefore, further studies are needed for the isolation and characterization of the active constituents.

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