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Anti-inflammatory and Anti-Nociceptive Activities of Dichloromethane Extract of *Voacanga Africana* Leaf

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ABSTRACT: In this study, we used several models for evaluation of probable anti-inflammatory and anti-nociceptive effects of dichloromethane extract from leaves of V*oacanga Africana,* using mice and rats. The extract (50 – 150mg/kg, p.o) inhibited, in a dose-related manner, carrageenan-induced paw oedema in rats. The extract caused a significant inhibition of the cotton-pellet granuloma. Vascular permeability induced by acetic-acid in the peritoneum of the animals was equally inhibited. There was reduction of writhings induced by acetic acid. In the formalin test, the extract caused inhibition of the neurogenic (first phase) and inflammatory phase (second phase) of formalin-induced pain. The extract also produced anti-nociception in the animals as assessed by the tail flick, hot-plate and limb-withdrawal tests. These findings suggest that the leaf extract of *Voacanga Africana* has potent anti-inflammatory and anti-nociceptive action.

Key Words: Medicinal plants; *Voacanga africana*; Anti-inflammatory activities; Anti-nociceptive activities.

Introduction

Voacanga Africana stapt. Ex Eliot (Apocynaceae) is a plant found in the understorey of forests. It is widely distributed from Senegal to the Sudan, and Nigeria to Angola and Zaire and within the Africa region. Extracts from this plant have found wide application in traditional African medicine. For example, in Nigeria, it is used for washing sores. A root decoction is given by mouth to women in Senegal to ward off the untoward consequences of premature or precipitant parturition. The same prescription is used for painful hernia (Thomas and Biemann, 1968). There is abundant white latex in the bark and other parts. The latex is applied to wounds in Senegal (Kerharo and Adams, 1974).

However, due to the lack of information on the biological activity of *Voacanga Africana,* we carried out studies using various models in an attempt to determine the character of any pharmacologically active ingredient of a simple dichloromethane extract of *Voacanga Africana*. These studies on the anti-inflammatory and anti-nociceptive activities of the plant extract also aim to justify the folkloric use of this plant.

Materials and Methods

Animals

All animals (Rats, weighing between 170-230gm, mice weighing between 20-28gm) of male sex used for this study were bred and housed in the pre-clinical Animal House, College of Medicine, University of Ibadan, Ibadan. The animals were kept in a photo period-controlled environment (12 hours light-dark cycle). All the animals were kept in cages with solid floors covered with wood shavings, and they were given food and water *ad libitum*.

Preparation of the plant extract

Leaves of *Voacanga Africana* tree situated at the premises of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria, were used throughout the study. The plant material was collected in the June period of the year (2000) and was authenticated and the specimens deposited in the Herbarium, Forestry Research Institute of Nigeria, Ibadan, Nigeria. The fresh leaves were oven-dried, powdered and extracted with dichloromethane. The extract was stored in the refrigerator (-20°C) for studies.

Anti-inflammatory activity

Carrageenan-induced paw oedema in rats

Acute inflammation in albino rats (10-12 weeks old) weighing 170-230gm was produced according to the method described by Winter *et al* (1962). An injection was made of 0.1ml 1% carrageenan on the sub-plantar surface of the right hind-paw of rats which had been fasted for twelve (12) hours. Oedema was assessed for three (3) hours, at 30-min interval after administration of the extract, in terms of an increase in circumference of the carrageenan injected paw compared to the non-injected one. Animals were given agents under test orally at doses of 50, 100 and 150 mg/kg of the extract one hour before carrangeenan injection.

At the same time, the control received 0.2ml normal saline and the reference group received 5mg/kg indomethacin orally. Measurement of the paw size was carried out by wrapping a piece of cotton thread round the paw and measuring the circumference with a metre rule (Hess and Milonig, 1981).

The inhibitory activity was calculated according to the following formula:

$$\text{Percentage Inhibition} = \frac{(Ct\text{-}Co)\ \text{control} - (Ct\text{-}Co)\ \text{treated}}{(Ct\text{-}Co)\ \text{Control}}$$

where Ct = Linear circumference of paw after carrageenan injection.
Co = Linear circumference of paw before carrageenan injection.

The inhibitory values at 3 hours, representing peak oedema is adopted as a measure of effect

Cotton pellet granuloma in rats

The method described by Mossa *et al* (1995) was followed. A sterilised cotton pellet weighing 20mg was introduced subcutaneously in the groin region of rats. They were treated orally with 50, 100 and 150 mg/kg of the extract for four (4) consecutive days. Animals in the control group received saline (10mg/kg). Indomethacin (5mg/kg) was given to animals in the reference group. On the fifth day, the animals were killed with chloroform, the pellets removed, freed from extraneous tissue and dried overnight at 60°C and weighed.

Acetic acid-induced vascular permeability

The method of Whittle (1964) was used. One hour after oral administration of the extract at doses of 50, 100 and 150mg/kg rats were injected with 0.25ml of 1% solution acetic acid intraperitoneally. Indomethacin (5mg/kg) served as the reference drug, while animals in the control group received saline

(10ml/kg). Immediately after administration, 10ml/kg of 10% (w/v) Evan's blue was injected intravenously through the tail vein. Thirty minutes after Evan's blue injection, the rats were killed and the viscera exposed. The abdominal wall and the viscera irrigated with distilled water over a petri dish. The exudate was then filtered and made up to 10ml. The dye leaking out into the peritoneal cavity was measured with a spectrophotometer using visible spectra at 610nm.

Analgesic activity

Formalin-induced paw

The method of Hunskaar and Hole (1987) was used. 20µl of 3% formalin was injected into the dorsal surface of the right hand paw of mice one hour after 50, 100 and 150 mg/kg of extract were orally administered to the animals. Control animals received 10ml/kg saline while 5mg/kg indemethacin was administered orally to animals in the reference group. The test was carried out in a transparent plastic chamber of 33cm by 21cm by 17cm with a mirror placed on the floor chamber, so as to have an unobstructed view of the paws. The rats were placed in the chamber one at a time for 5 minutes prior to the formalin injection. This was done to allow them to explore the chamber and get familiar with it. The amount of time spent licking the injected pore was determined with a stop clock and was considered as indicative of pain. The initial nociceptive scores normally peaked five (5) minutes after formalin injection (first phase) and 15-30 minutes after formalin injection (second phase), representing the tonic and inflammatory responses, respectively (Hunskaar and Hole, 1987).

Acetic acid-induced writhing test (chemical stimulation)

This method was based on that described by Santos *et al* (1994). Male albino mice weighing 20-28gm were divided into groups. Agents under test were administered through the intraperitoneal route 45 minutes before intraperitoneal injection of acetic acid (0.6%) solution in distilled water at a dose of 10ml/kg. Control animals received a similar volume of 0.9% Nacl (10 ml/kg). Immediately after administering the acetic acid, the number of writhings and stretchings (a syndrome characterised by a wave of contraction of the abdominal muscle together with a stretching of the hind-limbs) occuring between 5 and 15 minutes were counted. A reduction in the writhing number as compared to the control group was considered as evidence for the presence of the analgesia, which was expressed as percent inhibition of writhings.

Acetylsalicylic acid (250 mg/kg) treated group served as a positive control. Data were calculated according to the following formula:

$$\% \text{ Inhibition} = \frac{\text{Mean number of writhings In control group} - \text{Mean number of writhings In test group}}{\text{Mean number of writhings in control group}} \times 100$$

Tail-immersion method (thermal stimulation)

Male albino mice weighing between 20-28 gm were used. They were divided into groups and agents under test were administered orally, Thirty minutes later, the tail (up to 3.5 cm) was dipped in a water bath containing water maintained at 55 ± 0.7°C. The time in seconds to withdraw the tail clearly out of water was taken as the reaction time. The first reading (0 min.) was taken immediately after administration of the test drugs and readings were taken 30, 60, 90, 120, 150 and 180 minutes later.

Limb-withdrawal reflex method (thermal stimulation)

Male albino mice weighing between 20-28 gm were used. They were divided into groups and agents under test were administered orally. Thirty minutes later, the hind-limb was dipped in a water bath containing water maintained at 55 ± 0.7°C. The time in seconds to withdraw the hind-limb clearly out of water was taken as the reaction time. The first reading (0 min.) was taken immediately after administration of the test drugs, and readings were taken 30, 60, 90, 120, 150 and 180 minutes later.

Hot-plate test (thermal stimulation)

Male albino mice weighing 20-28 gm, were used. They were divided into groups and agents under test were administered orally. One hour later, the mice were placed on top of hot plate of 55 ± 0.7°C. The time in between placement and jumping was recorded as response latency. The reaction time was recorded for control mice and for animals pretreated with the extracts of the plants.

Drugs

The drugs used were Carrageenan (Marine Colloids Inc.), formalin, acetic acid, indomethacin (MSD< Canada), acetylsalicylic acid, pentazocine (Morphine-analogue), and plant extracts. All other reagents used were of a high grade in purity. All drugs and extracts were dissolved in 0.9% Nacl solution just before use.

Statistical Analysis

Results are expressed as mean ± standard error of the mean where 'n' represents the number of observations in the group. Where appropriate, comparison between groups is taken to be significant when $P < 0.05$.

Results

Carrageenan-Induced Paw Oedema In Rats:

Carrageen-induced rat paw oedema was inhibited by oral (intragastric) pretreatment with the extract (50 – 150mg/kg, p.o). The inhibitory effect was most pronounced with 150mg/kg dose (Table 1).

Cotton-Pellet Granuloma in Rats:

The extract at all doses (50 – 150 mg/kg, p.o) were found to reduce granuloma tissue formation in the animals in a dose-related manner (Table 2).

Table 1: Effect of dichloromethane extract of *Voacanga Africana* leaves on carrageenan-induced paw oedema in rats.

Group (n = 10)	Dose (mg/kg) orally	Mean paw size (cm)	Inhibition (%)
Control (saline)	-	3.91±0.05	-
V. Africana	50	2.70±0.05 *	63.40
V. Africana	100	2.61±0.04 * *	76.01
V. Africana	150	2.58±0.03 * * *	85.92
Indomethacin	5	2.52±0.03 * * *	88.73

Each group represents the mean ± s.e.m. of ten experiments. P<0.05; ** P<0.01 and *** P<0.001 compared with control values.

Table 2: Effect of dichloromethane extract of *Voacanga Africana* leaves on cotton pellet granuloma in rats.

Group (n = 10)	Dose (mg/kg) orally	Mean increase in weight of pellets (mg)	Inhibition (%)
Control (saline)	-	49.40±0.26	-
V. Africana	50	39.55±0.46 *	20
V. Africana	100	29.75±0.26 * *	40
V. Africana	150	24.95±0.23 * * *	49.5
Indomethacin	5	22.05±0.16 * * *	55.4

Each group represents the mean ± s.e.m. of ten experiments. P<0.05; ** P<0.01 and *** P<0.001 compared with control values.

Acetic acid-induced vascular permeability

There was reduction in degree of peritoneal inflammation produced by acetic acid in the animals, by extract of *Voacanga Africana.* The quantity of dye leakage was significantly reduced by all doses of the extracts investigated (50 – 150 mg/kg, p.o) (Table 3)

Table 3: Effect of dichloromethane extract of *Voacanga Africana* leaves on acetic acid-induced vascular permeability in rats.

GROUP (n = 10)	Dose (mg/kg) orally	Mean increase in weight of pellets (mg)	Inhibition (%)
Control (saline)	-	72.00±0.28	-
V. Africana	50	39.00±0.26 *	45.83
V. Africana	100	31.00±0.26 * *	57
V. Africana	150	23.00±0.26 * *	68.06
Indomethacin	5	16.50±0.37 * * *	77.08

Each group represents the mean ± s.e.m. of ten experiments. P<0.05; * * P<0.01 and * * * P<0.001 compared with control values.

Formalin-Induced Paw Licking in Mice

In the formalin test, the extracts of *Voacanga Africana* (50 – 150 mg/kg, p.o) caused marked and dose-related inhibition against both phases of formalin-induced pain (Table 4). The inhibitory effect was most pronounced with 150 mg/kg dose.

Acetic acid-induced writhing in mice:

Results shown in Table 5 indicate that oral (intragastric) administration of the extracts of *Voacanga Africana* (50 – 150 mg/kg, p.o) were effective in reducing the number of writhing and stretching

movements induced by intraperitoneally – injected acetic acid. This anti – nociceptive effect of the extracts was most pronounced at dose of 150 mg/kg.

Table 4: Effect of dichloromethane extract of *Voacanga Africana* leaves on formalin-induced paw licking mice.

Group (n = 10)	Dose (mg/kg) Orally	Mean Licking time (s)	
		Early phase	Late phase
Control (saline)	-	62.40±0.50	142.00±0.72
V. Africana	50	50.00±0.57 *	61.30±0.83 * *
V. Africana	100	40.50±0.54 * *	31.50±0.54 * * *
V. Africana	150	29.40±0.37 * * *	22.30±0.60 * * *
Indomethacin	5	18.00±0.50 * * *	10.00±0.60 * * *

Each group represents the mean ± s.e.m. of ten experiments. P<0.05; * * P<0.01 and * * * P<0.001 compared with control values.

Table 5: Effect of dichloromethane extract of *Voacanga Africana* leaves on acetic acid-induced writhings in mice.

GROUP (n = 10)	Dose (mg/kg) (I.P)	Mean number of writhings	Inhibition (%)
Control (saline)	-	39.50±0.63	-
V. Africana	50	24.50±0.50 *	38.00
V. Africana	100	18.80±0.50 * *	52.40
V. Africana	150	14.80±0.50 * *	62.53
ASA	250	11.70±0.42 * * *	70.40

Each group represents the mean ± S.E.M. of ten experiments. P<0.05; * * P<0.01 and * * * P,0.001 compared with control values.

Tail immersion Test:

The mice developed withdrawal latencies to the 56°C water bath that were significantly different from the control group. This effect was observed for all doses of the extracts tested (50 – 150 mg/kg, p.o) (Table 6).

Limb-withdrawal Test:

Table 6 also shows the response of mice to limb-withdrawal test. The latency of the hind-paw withdrawal reflex was increased on the treated groups when compared with control group.

Hot-Plate Test:

Table 6 also shows the response of mice to hot-plate test. The reaction time was increased in mice treated with extracts of *Voacanga Africana*, when administered orally at doses ranging 50 – 150 mg/kg.

Table 6: Effect of dichloromethane extract of *Voacanga Africana* leaves on tail-immersion, limb-withdrawal and hot-plate tests in mice.

Group (n = 10)	Dose (mg/kg) orally	Latency (5)		
		Tail-immersion	Limb-withdrawal	Hot-plate
Control (saline)	-	2.20±0.7	2.80±0.30	3.80±0.25
V. Africana	50	7.70±0.22 * *	9.80±0.20 * * *	10.30±0.43 * *
V. Africana	100	9.30±0.22 * * *	11.00±0.26 * * *	13.70±0.35 * * *
V. Africana	150	10.20±0.20 * * *	11.30±0.22 * * *	17.50±0.35 * * *
Pentazoain	10	13.60±0.27 * * *	12.30±0.30 * * *	17.60±0.43 * * *

Each group represents the mean ± s.e.m. of ten experiments. P<0.05; * * P<0.01 and * * * P<0.001 compared with control values.

Discussion

This study has demonstrated that extracts obtained from leaves of *Voacanga Africana* (Apocynaceae) given intraperitoneally and orally (intragastric), exhibited potent and dose – related anti – inflammatory and anti – nociceptive activity when analysed in several models of inflammation and nociception in mice and rats. Oral administration of extracts caused an inhibition of carrageenan-induced oedema assessed at 30, 60, 90, 120, 150 and 180 minutes after subplanter injection of carrageenan. Carageenan rat paw oedema is a suitable test for evaluating anti-inflammatory drugs which has been frequently used to assess the anti-oedematous effect of natural products. Dirosa *et al* (1971) earlier reported that carrageenan-induced inflammation is useful in detecting orally active anti-inflammatory agents. Vinegar *et al* (1969) reported that oedema formation due to carrageenan in the rat paw is a biphasic event. The initial phase is attributed to the release of histamine and serotonin (Crunkhon and Meacock, 1971). The second phase of oedema is as a result of liberation of prostaglandins, lysosome, bradykinins and protease (Crunkhon and Meacock, 1971; Vinegar *et al*, 1969). Dirosa *et al* (1971) had earlier stated that the second phase is sensitive to most clinically active anti-inflammatory drugs.

In a test of more chronic inflammation, such as granuloma cotton pellet, the potency of the extracts was observed. The final dry weight of the cotton pellets correlates very well with the amount of granulomatous tissue (Swingle and Shideman, 1972). The extracts of *Voacanga Africana* showed significant anti-inflammatory action in the cotton pellet test. This action showed that the extracts have the capability to reduce the synthesis of mucopolysaccharide and collagen and the number of fibroblasts, which are natural proliferative events of granulation tissue formation.

The anti-inflammatory action of *Voacanga Africana* was also shown by its ability to reduce the vascular permeability by reduction of dye leakage into the peritoneal cavity by acetic acid in the animals studied.

The extracts of *Voacanga Africana* also exhibited potent anti-nociceptive action against formalin-acetic acid-induced algesic response in mice. The formalin test in mice is a useful test for evaluating mild analgesics. The test employs an adequate painful stimulus, the animals show a spontaneous response and the test is sensitive to the commonly used analgesics. The pain stimulus is continuous rather than transient and may thus have resemblance to some kinds of clinical pain. The test have two different phases, reflecting different types of pain. The first phase reflects a direct effect of formalin on nociceptors (npn-inflammatory pain) whereas the second phase reflects inflammatory pain (Elisabetsky *et al*, 1995). All studied extracts of *Voacanga Africana* are capable of attenuating, in a dose-related fashion, both the neurogenic and the inflammatory phases of the formalin-induced pain. This probably shows that the anti-nociceptive action of the extracts was mediated by both neurogenic and inflammatory mechanisms.

The extracts show high potency in inhibiting the inflammatory components of the formalin test. This is a typical characteristic of some cyclo-oxygenase inhibitors, which are effective in inhibiting only the last phase of the formalin-induced pain.

However, these extracts , at a higher dose of 150 mg/kg (P.O) failed to abolish completely either of the phases associated with the formalin test. This suggests that part of this nociceptive response in the formalin test involves mediators that may be insensitive to active principles of *Voacanga Africana*. It has been shown that the inflammatory pain associated with the second phase of the formalin test is accompanied by release of several inflammatory mediators (Hunskaar *et al*, 1985; Abbott and Franklin, 1986; Murray *et al*; 1988; Chapman and Dickenson, 1992; Correar and Calixto, 1993).

The extracts of *Voacanga Africana* showed strong anti-nociceptive actions in mice by increasing the latencies period in the tail and limb withdrawal reflex and hot-plate test.

These findings on the extracts of *Voacanga Africana* leaves seem to, in part, justify the folkloric use of this plant. Further studies are in progress in our laboratory to confirm the identity of the bioactive compounds responsible for these actions shown by the leaves of *Voacanga Africana.*

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