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ANTINOCICEPTIVE AND ANTIINFLAMMATORY EFFECTS OF ESSENTIAL OIL OF *DENNETTIA TRIPETALA* G.BAKER (ANNONACEAE) IN RODENTS

I. A. Oyemitan[1*], E. O. Iwalewa [1], M. A. Akanmu,[1] T. A. Olugbade [2]

[1]Department of Pharmacology, [2]Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.
***Email:** oyemix @yahoo.com

Abstract

In this study we evaluated the analgesic and anti- inflammatory activities of the essential oil (EO) of the fruits of *Dennettia tripetala* in rodents. The plant is a tropical African plant and the fruits are commonly eaten as spices and consumed as a stimulant, and its various parts are used in the treatment of fever, cough and as anti-emetics.The analgesic effects of the oil was assessed in mice using the hot plate, acetic acid-induced writhings and formalin test, while carrageenan-induced paw oedema was used to study the antiinflammatory effects in rats. The EO at 25-50 mg/kg exhibited significant ($p<0.05$) antinociceptive effects comparable to a potent opioid agonist, morphine (10 mg/kg) and non-steroidal anti-inflammatory drugs such as, aspirin (100 mg/kg) and indomethacin (80 mg/kg). The antinociceptive effect of the EO was also blocked by naloxone (2 mg/kg) in all the models used. The EO demonstrated significant ($p<0.05$) anti-inflammatory effect in the carrageenan-induced paw oedema model of inflammation that is also comparable to dexamethasone (1 mg/kg) The results showed that the essential oil of *D.tripetala* possesses significant antinociceptive and antiinflammatory effects in the animal models used. The results also suggest that the analgesic effects may be mediated both centrally as well as peripherally, while the antiinflammatory activity may be effective in both early and late phases of inflammation. The results obtained may therefore be used to rationalize the use of the plant in the treatment of pain and fever in traditional medicine.

Key words: *Dennettia tripetala,* Essential Oil, Antinociceptive, Anti-inflammatory and Rodents

Introduction

The fruits of the plant *Dennettia tripetala* G.Baker (Annonaceae) are well known in many communities of some southern states of Nigeria. The plant is commonly found within cocoa plantation where it is used as means of demarcation of farm boundaries. The fruits, leaves, bark and roots of the plant possess strong pepperish and pungent taste. These various parts of the plant are popularly used as spices and condiments. The plant also possesses characteristic aroma and fragrances. The fruits are mainly chewed raw in different forms (fresh green, fresh ripened brown, black dry fruits and dry seeds). The leaves are commonly used in pepper soup delicacies, and as condiment in some special local dishes (Ejechi and Akpomedaye, 2005). The leaves are commonly used by the local herbalists in combination with other medicinal plants to treat various ailments including fever, infantile convulsion, typhoid, cough, worm infestation, vomiting, and stomach upset (Oyemitan, 2006). There are also reports that the fruits are sometimes used for masking mouth odour (Oyemitan, 2006). The fruits of the plants have been reported to be popularly used as stimulants (Aiyeloja and Bello, 2006; Ndukwu and Nwadibia, 2006; Oyemitan et al., 2006). Earlier reports showed that the estimated LD_{50} values of the oil following oral (p.o.) and intraperitoneal (i.p.) routes in rats were 1,265 mg/kg (p.o.) and 775 mg/kg (i.p.), while the values in mice were 2,150 mg/kg (p.o.) and 470 mg/kg (i.p.) respectively (Oyemitan, 2006). The reported mechanism(s) of the behavioural effects (novelty-induced and exploratory behaviours) of the essential oils of the plant have been linked to opioidergic and GABA-ergic pathways (Oyemitan, 2006). This present study was therefore carried out to further assess the activities of the essential oil of the plant for analgesic and antiinflammatory effects in mice and rats respectively as some of the ethnomedical uses are related to pain-

inflammation disorders. The results of this study may also be used to justify the ethnomedicinal uses of the plant to treat fever and cough by the people traditionally.

Materials and methods

Plant materials

Fresh fruits were purchased from Owena market, Owena Town, Ondo-East Local Government and Central market, Ondo town, Ondo-West Local Government Area of Ondo State. All collections were made within the period of April and May 2005. The fruits of *D. tripetala* G.Baker (Annonaceae) were authenticated by Mr. A. Oladele, the Herbarium officer, Department of Pharmacognosy, Faculty of Pharmacy, and Dr. H.C Illoh of the Department of Botany, Faculty of Science, Obafemi Awolowo University (OAU) Ile-Ife, Osun State. The voucher specimen of the leaves and the fruits (from Owena and Ondo) were prepared and deposited at the Herbarium of the Department of Botany, Faculty of Science, O.A.U, Ile-Ife, as voucher No. 15,356.

Essential Oil of *D.tripetala*:

Distillation of essential oils was carried out using a distillation and clevenger apparatus. Fresh fruits of *D. tripetala* were air dried at room temperature and commuted into coarse powders using pestle and mortar. Four hundred (400 gram) of the powder was hydrodistilled and it yielded 14.68 g amounting to 3.7%w/w of the characteristic pungent aromatic odour of the essential oil. The oils obtained were stored in a lightproof bottle and kept in a refrigerator until use (Vale et al., 1999; Agbakwuru et al., 1979;Trease and Evans, 1978). The relative density of the essential oil obtained was determined using the 10 ml capacity density bottle (British Pharmacopoeia, 1980). The oil was emulsified with 5% Tween 80 shortly before administration.

Animals

Swiss albino rats (both sexes) weighing 150-200 g and white albino mice (both sexes) weighing 18-25 g were obtained from the Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. The animals were kept under standard laboratory conditions and fed with animal feeds (Ladokun feeds, Ibadan, Nigeria) and given water *ad libitum* prior and throughout the period of experimentation. All experiments were carried out in accordance with NIH Guide for the care and Use of Laboratory animals.

Drugs

The following drugs were also used: Naloxone, Morphine (Sigma, St. Louis, USA), Acetic acid (BDH Chemicals Ltd, Poole, England), Aspirin (Dispirin®) (Reckitt and Colman, UK), diclofenac (Supreme Pharm. Nig. Ltd., Lagos, Nigeria), indomethacin (methacin ®) (Hovid Bhd, Perak, Malaysi) and formalin (BDH Chemicals Ltd., Poole, England).

Materials and Methods

Analgesic experiment

Hot plate test:

Twenty mice were randomly allocated to four groups (n=5). Mice in group 1 were intraperitoneally (i.p.) administered with 10 ml/kg of 5% Tween 80; mice in groups 2-4 were intraperitoneally administered with 12.5, 25.0 and 50.0 mg/kg of essential oil. Each mouse was dropped gently on the hot plate maintained at 55.0 ± 0.5 ^{O}C and the time taken for the mouse to lick the paw was recorded at time 0 second (before treatment) and at time 30, 60, 90 and 120 minutes after treatment. The cut off time was set at 15 s to avoid tissue damage. In another sets of experiment, 4 groups containing 5 mice each were randomly selected. Group 1 was administered with subcutaneous (s.c) injection of naloxone (2 mg/kg) and tested as above, groups 2 and 3 were pretreated with naloxone (2 mg/kg, s.c) 15 minutes prior to administration of 25.0 or 50.0 mg/kg oil, while group 4 was pretreated with subcutaneous administration of naloxone (2 mg/kg, s.c.) 15 minutes prior to morphine (10 mg/kg, i.p.) and the mice were tested as earlier described (Viana et al., 2000; Silva et al., 2003).

Acetic Acid-Induced writhings in mice

Four groups of 5 mice each were randomly selected (n=5). Mice in group 1 were intraperitoneally administered with 5% Tween 80 (10 ml/kg), while mice in groups 2-4 were administered with 12.5, 25.0 and 50

mg/kg of essential oil respectively. Thirty minutes after treatment, each mouse was administered 10 ml/kg of 1% acetic acid (i.p.) and allowed 5 mins delay before assessment for up to 20 mins inside the Plexiglas's cage (25 cm x 25 cm x 30 cm). The number of writhings displayed by each mouse was counted and recorded. Aspirin (100 mg/kg, i.p.) and morphine (10 mg/kg, i.p.) were administered to another groups of mice (n=5) that were used as positive controls. In another experiment, two groups of 5 mice each were pretreated with naloxone (2 mg/kg, s.c.) 15 minutes prior to administration of the essential oil (25.0 mg/kg, i.p.) or morphine (10 mg/kg, i.p.). After 30 minutes 1% acetic acid (10 ml/kg, i.p) was administered and the number of writhes displayed by the mice were counted and recorded as earlier described above for 15 mins period (N'gouemo et al., 1996; Le-Bars et al., 2001; Yin et al., 2003).

Formalin Test

The method used was that described by Elisabetsky et al. (1995) and Hunskaar and Hole (1997) with little modification. Seven groups of mice consisting of 5 mice each were randomly selected. Mice in group 1 (control) was administered with 5% Tween 80 (10 ml/kg i.p.), while mice in groups 2-4 were treated with the essential oil (12.5, 25.0 and 50.0 mg/kg, i.p.). Mice in groups 5-7 were treated with morphine (10 mg/kg i.p.), diclofenac (5.64 mg/kg i.p.) and Indomethacin (80 mg/kg, i.p.) respectively 30 minutes prior to administration of 0.02 ml of 2.5% formalin into the sub-planter space of the right hind paw and the duration of paw licking was determined 0-5 minutes (1st Phase or neurogenic phase) and 20-25 mins (2nd phase or inflammatory phase) after formalin administration. The 1st phase is regarded as the neurogenic mechanism and the 2nd phase is the inflammatory mechanism (Elisabetsky et al., 1995; Hunskaar and Hole, 1997; Yin et al., 2003). In another experiment, 3 groups of mice consisting of 5 mice each were selected and pretreated with naloxone (2 mg/kg, s.c.) 15 minutes prior to administration of the oil (25 and 50 mg/kg i.p.) and morphine (10 mg/kg i.p.) respectively. Thirty mins later, they were treated with 2.5% formalin and assessed as earlier described above.

Antiinflammatory experiment
Carrageenin-induced paw oedema in rats

The antiinflammatory activity was studied using carrageenin-induced paw oedema (acute inflammation) method in rats (Winters et al., 1962). Twenty-five rats were randomly divided into five groups (n=5). Rats in group 1 (control) were intraperitoneally (i.p.) administered with 5% Tween 80 (10 mg/kg, i.p.), while rats in group 2-4 were intraperitoneally administered with the 12.5, 25.0 and 50.0 mg/kg of essential oil and rats in group 5 were intraperitoneally administered with dexamethasone (10 mg/kg, i.p.). Thirty minutes later, 1% carrageenin (0.1 ml) was injected into the sub-planter surface of right hind paw of each of all the rats in all the groups. Measurement of paw size was done by wrapping a piece of cotton thread round the treated paw of each rat and measuring the circumference on a meter rule (Olajide et al., 2000; Yin et al., 2003). The measurement was carried out at time 0, 1, 2, 3, 4 and 5 h respectively. Inhibitory activity was calculated at 1, 2 and 3 h after carrageenan treatment (representing the peaks of oedema size), using the formula:

$$\text{Percentage inhibition} = \frac{\{(Ct\text{-}Co)\ \text{control} - (Ct\text{-}Co)\ \text{treated}\}}{(Ct\text{-}Co)\ \text{control}} \times 100$$

Where Ct is paw size after a specific time interval in hours after carrageenin injection and Co is paw size before carrageenin injection.

Statistical analysis

The results obtained were presented as means ± SEM and analyzed using analysis of variance (ANOVA) followed by Dunnet test. The level of significance was set at 95%, $p<0.05$ for all treatment carried out compared to control group using the Primer of Biostatistics by Stanton A.Glantz (version 3.01) copyright (C) 1992 by Mc Graw-Hill Inc.

Results

Analgesic effects of oil in mice
Hot-plate Test:

The essential oil at all the dose levels used induced significant ($p< 0.05$) analgesia in mice by causing an increase in the reaction time to thermal stimulus of the hot plate. Pretreatment with naloxone (2 mg/kg)

reversed the analgesia induced by the oil at these dose levels of 25.0 and 50.0 mg/kg significantly at all the time intervals of assessment (Table 1).

Acetic Acid-Induced Writhings in Mice

The essential oil dose-dependently inhibited acetic acid-induced writhes in mice significantly ($p<0.05$) compared to control, but the percentage protection was significantly ($p<0.05$) less than the standard analgesic used, aspirin (Table 2). Naloxone (2 mg/kg) alone did not inhibit acetic acid-induced writhings in mice. Pretreatment of mice with naloxone (2 mg/kg) prior to administration of the oil blocked the inhibitory effects of the oil on acetic acid-induced writhings in the mice.

Formalin test

The essential oil at 12.5, 25.0 and 50 mg/kg (i.p.) dose dependently inhibited paw-licking time at the two phases compared to control. Indomethacin (80 mg/kg, i.p.) a potent non-steroidal anti-inflammatory drug (NSAID), diclofenac (5.46 mg/kg, i.p.) another potent NSAID and morphine (10 mg/kg, i.p.) a potent opioid also showed antinociception. The essential oil at the dose of 50 mg/kg (i.p.) showed 100% inhibition in the 2nd phase as obtained with standard drugs used such as morphine (10 mg/kg), diclofenac (5.46 mg/kg) and the oil (50 mg/kg). Pretreatment with naloxone (2 mg/kg, s.c.) 15 minutes prior to injection of morphine (10 mg/kg) and oil (25.0 and 50.0 mg/kg) significantly reduced their antinociceptive effects at the two phases (Table 3)

The results of anti-inflammatory effects of the oil in rats.

The oil at the entire dose levels used inhibited significantly ($p<0.05$) carrageenan-induced rat paw oedema dose-dependently compared to the control. Dexamethasone showed greater inhibition than the oil (12.5-50 mg/kg) at 1h and 2 h but lower inhibition than the oil (25 or 50 mg/kg) at 3h (Table 4).

Table 1: The antinociceptive activity of the essential oil, morphine and their antagonism by naloxone assessed by the hot-plate test.

Treatment (N=5 per group)	Reaction Time (Second)			
	T.0 min	T.30 min	T.60 min	T.90 min
Control (10 ml/kg 5% Tween 80)	15.00 ± 0.55	13.80 ± 0.58	13.20 ± 0.55	11.50 ± 0.56
EO 12.5 mg/kg	14.10 ± 0.77	13.10 ± 0.66	18.20 ± 0.71*	18.70 ± 1.11*
EO 25.0 mg/kg	13.20 ± 0.71	22.40 ± 0.67*	22.60 ± 0.73*	17.20 ± 0.59*
EO 50.0 mg/kg	12.50 ± 0.50	17.70 ± 0.76*	19.10 ± 0.81*	18.50 ± 0.76*
NAL 2 mg/kg + EO 25.0 mg/kg	14.30 ± 0.54	11.40 ± 0.58**	9.30 ± 0.59**	10.40 ± 0.76**
NAL 2 mg/kg + EO 50.0 mg/kg	13.70 ± 0.75	11.76 ± 0.66**	10.80 ± 0.64**	9.40 ± 0.62**
MPH 10 mg/kg	12.40 ± 0.53	16.90 ± 0.48*	19.30 ± 0.45*	22.90 ± 0.65*
MPH 10 mg/kg + NAL 2 mg/kg	12.80 ± 0.55	11.58 ± 0.46#	11.60 ± 0.58#	12.20 ± 0.50#

Each value is mean ± S.E.M, n=5. EO is essential oil of *D.* tripetala, NAL is naloxone and MPH is morphine. The EO (12.5, 25.0 and 50 mg/kg, i.p) and morphine (10 mg/kg, i.p) showed significant antinociceptive effect when compared to control group. However, their antinociceptive actions were blocked by pretreatment with naloxone (2 mg/kg, s.c.) 30 minutes prior to injection of morphine (10 mg/kg, i.p.) or oil (25 or 50.0 mg/kg, i.p.). * $P < 0.05$ compared with control; **$p<0.05$ compared with corresponding dose of EO alone; #$p<0.05$ compared with morphine alone.

Table 2. The antinociceptive activity of the essential oil and morphine and their antagonism by naloxone assessed by Acetic Acid-Induced Writhings in mice

Treatment (n = 5 for each group)	Number of writhes (mean ± SEM)	Percentage Analgesia
Control (5% Tween 80) 10 ml/kg	53.40 ± 1.09	-
EO (12.5 mg/kg)	37.60 ± 0.93*	29.6%
EO (25.0 mg/kg)	16.60 ± 1.31*	64.4%
EO (50.0 mg/kg)	19.00 ± 0.42*	68.9%
Acetylsalicylic acid (ASA) (100 mg/kg)	13.90 ± 1.43*	74.0%
NAL (2 mg/kg)	55.00 ± 1.02	-3.0%
NAL (2 mg/kg) + EO (25.0 mg/kg)	55.80 ± 0.88**	0.7%
MPH (10 mg/kg)	40.60 ± 0.65*	24.0%
MPH (10 mg/kg) + NAL (2 mg/kg)	51.20 ± 1.27#	4.1%

Each value is mean ± S.E.M, n =5. EO is essential oil of *D. tripetala*, NAL is naloxone, and MPH is morphine. The essential oil at 12.5, 25.0 and 50 mg/kg, i.p. dose dependently reduced acetic acid-induced writhes in mice (29.6, 64.4 and 68.9% respectively) compared to control but aspirin (NSAID) at 100 mg/kg, i.p. showed highest analgesic activity (74.0%). Pretreatment with naloxone (opioid antagonist) completely blocked the antinociceptive effects of EO at the selected dose of 25 mg/kg and also reversed that of morphine. *P < 0.05 compared with control, **p<0.05 compared with EO at 25 mg/kg and #p<0.05 compared with morphine alone.

Table 3: The antinociceptive activity of the essential oil and morphine and their antagonism by naloxone assessed by the formalin test in mice.

Treatment (N=5 mice for each group)	**Licking time in seconds**		**Percentage inhibition**	
	1st phase	2nd phase	1st phase	2nd phase
Control (10 mg/kg 5% Tween 80)	124.2 ± 1.0	32.6 ± 1.0	-	-
EO (12.5 mg/kg)	104.6 ± 1.5*	25.0 ± 0.9*	15.8	23.3
EO (25 mg/kg)	92.0 ± 0.9*	10.0 ± 0.8*	26.0	69.3
EO (50.0 mg/kg)	65.2 ± 1.8*	0*	47.5	100
Indomethacin 80 mg/kg	90.2 ± 1.2*	18.4 ± 1.3*	27.4	43.6
MPH (10 mg/kg)	18.2 ± 1.2*	0*	85.3	100
Diclofenac (5.46 mg/kg)	97.2 ± 1.4*	0*	22.7	100
NAL (2 mg/kg) + EO (25 mg/kg)	114.6 ±1.4**	17.8 ± 0.9**	7.7	45.4
NAL (2 mg/kg) + EO (50 mg/kg)	68.8 ± 1.3	0*	43.5	100
NAL (2 mg/kg) + MPH (10 mg/kg)	133.2 ± 1.3#	36.0 ± 0.9#	-	-

Each value is mean ± S.E.M, n=5. EO is essential oil of *D. tripetala*, NAL is naloxone, and MPH is morphine. The essential oil at 12.5, 25.0 and 50 mg/kg i.p. dose dependently inhibited paw-licking time at the two phases compared to control. Indomethacin 80 mg/kg, i.p. and diclofenac 5.46 mg/kg, i.p. are NSAID agents and morphine (a potent opioid agonist) 10 mg/kg, i.p. also showed antinociception. Morphine (10 mg/kg), diclofenac (5.46 mg/kg) and the oil (50 mg/kg) showed 100% inhibition in the 2nd phase. Pretreatment with naloxone 2 mg/kg s.c. 15 minutes prior to injection of morphine (10 mg/kg) and EO (25.0 and 50.0 mg/kg) significantly reduced their antinociceptive effects at the two phases except the oil at 50 mg/kg, which was not affected at the 2nd phase. ***** P < 0.05 compared with control; **p<0.05 compared with EO at 25 mg/kg and #p<0.05 compared with morphine alone.

Table 4: The antiinflammatory effects of the essential oil using the carrageenan-induced paw oedema test in rats.

Treatment (n=5)	Oedema size (mm) and percentage inhibition of paw oedema over a period of time intervals (hrs)			
	Oedema size in mm (%) 0 h	Oedema size in mm (%) 1h	Oedema size in mm (%) 2 h	Oedema size in mm (%) 3 h
Control 5%Tween 80 (10 ml/kg)	24.8 ± 0.4	37.0 ± 0.6	40.0 ± 0.7	39.6 ± 0.5
EO (12.5 mg/kg)	23.2 ± 0.4	30.4 ± 0.7 (40.0%)	34.2 ± 0.6 (28.0%)	31.0 ± 0.5 (47.0%)
EO (25 mg/kg)	24.6 ± 0.4	26.8 ± 0.4 (84.0%)	31.6 ± 0.6 (54.0%)	29.6 ± 0.6 (66.0%)
EO (50 mg/kg)	22.2 ± 0.5	25.4 ± 0.5 (73.8%)	26.4 ± 0.5 (72.0%)	26.8 ± 0.7 (69.0%)
Dexamethasone (1 mg/kg)	24.4 ± 0.5	25.6 ± 0.7 (90.0%)	27.5 ± 0.7 (75.0%)	29.3 ± 0.5 (60.0%)

EO is the essential oil of *D.tripetala*. The oil at the entire dose levels used inhibited significantly ($p<0.05$) carrageenan-induced rat paw oedema dose-dependently compared to the control. Dexamethasone showed greater inhibition than the oil (12.5-50 mg/kg) at 1h and 2 h but lower inhibition than the oil (25 or 50 mg/kg) at 3h. *$p<0.05$ compared with control.

Discussion

The hot plate method is very effective for evaluating drugs possessing analgesic property, which act centrally (Vale et al., 1999; Haque et al., 2001; Silva et al., 2003; Al-Naggar et al., 2003). Prolongation of reaction time in hot plate test inferred possible central analgesic effects of the oil. The oil increased the reaction time significantly at the dose levels used compared to control group. Acetic Acid-induced writhing has been used to evaluate drugs possessing peripheral analgesic effects (Koster et al., 1959; Viana et al., 2000). In this study, the oil exhibited this analgesic effect in mice by inhibiting the acetic acid induced writhes, which is a model of visceral pain, however, not as much as that of the standard drug used-acetylsalicyclic acid (ASA). Acetic acid has been reported to cause hyperalgesia by liberating endogenous substances such as prostaglandins, leukotrieines, 5-HT, histamine, kinins, H^+ and K^+, etc. which have been implicated in the mediation of pain perception (Forth et al., 1986; Rang et al., 1999). Therefore, in an attempt to understand the possible mechanism of the observed analgesic action of the oil in the hot plate experiment, mice were pre-treated with naloxone, a potent opioid antagonist prior to administration of the oil (Almeida et al., 2003). From the results obtained, it was observed that naloxone blocked both hot plate and acetic acid induced writhings antinociceptive effects. Naloxone reversed the analgesic effects of the oil at the two highest dose levels chosen (Table 2). These results suggest that the oil may be exhibiting its analgesic effects in similar manner to opioids or opiates. The antagonistic effects of naloxone on the analgesic effects of the oil persist even beyond 90 minutes assessment period therefore suggesting that the analgesic effect of the oil as demonstrated is probably mediated through the opioid system in the central nervous system (CNS). This is in line with the previous reports that opioid receptors are also involved in peripheral as well as central (μ and κ-opioids and monoamines) mechanisms of analgesia in animals (Millan et al., 1994). Prolongation of reaction time in hot plate test confirmed central analgesic action that was blocked by the opioid antagonist, naloxone, a specific antagonist of opioidmimetic receptors (Almeida et al., 2003).

Yin et al (2003) reported that many studies have shown that the earlier phase (1^{st} phase) of formalin-induced pain reflects the direct effect of formalin on nociceptors whereas the late phase (2^{nd} phase) reflects inflammatory pain, which has been linked to prostaglandin synthesis (Hong and Abbot, 1995; Yin, et al., 2003). Opioid analgesics have been reported to possess antinociceptive effects in both phases having more effect at the

2nd phase (Le Bars et al., 2001). Non-steroidal anti-inflammatory drugs (NSAIDS) such as indomethacin is said to be effective only in the 1st phase especially if the formalin is injected at high concentration (Yashpal and Coderre, 1998). In this study, the oil dose-dependently inhibited nociception induced in the Formalin Test significantly compared to control group in the 1st phase (neurogenic) and 2nd phase (inflammatory). These results therefore further suggest that the oil contain constituents that exhibit anti-inflammatory properties. Commonly used Non-Steroidal anti-inflammatory Drugs (NSAID) such as aspirin and indomethacin are widely used to reduce swelling associated with pain and inflammation through inhibition of prostaglandin synthesis by direct effect on cyclo-oxygenase (COX) in the arachidonic acid (AA) metabolism (Amos et al., 2001; Nwafor and Okwuasaba, 2003). This result showed that the oil possesses an anti-inflammatory effect dose-dependently in the 1st phase including the standard drugs such as indomethacin and diclofenac when compared to control mice. Morphine, a potent opioid agonist inhibited paw licking (85%) inhibition, which was completely blocked by the opioid antagonist, naloxone. However, pretreatment with naloxone prior to administration of the oil did not block the analgesic effects in the 1st phase at a dose of 50 mg/kg, i.p. but blocked this effect with administration of the oil at 25 mg/kg, i.p. This result suggests that the oil contain constituents that have potent analgesic effects that may be acting in a similar manner to opioids. In the 2nd phase, there was a reduction in paw licking compared to control. In this phase, pretreatment with naloxone had no effect on the analgesic effect of the oil at this dose level. The results showed that the antagonist only had effect on the low dose but had no effect on the high dose of the oil administered. When the oil (50 mg/kg) was assessed, morphine and diclofenac completely blocked paw licking compared to control in the 2nd phase whereas indomethacin has lower effects at this phase. Naloxone (2 mg/kg), only reversed the analgesic effect of the oil at the dose of 25 mg/kg, while it has no effect on morphine and oil (50 mg/kg, i.p.) suggesting that both naloxone and the oils probably compete for the same receptor sites-opioidergic receptor and further suggests that opioid receptors are involved in the mechanism of action. This result showed that the oil has analgesic effects at both phases of the formalin induced paw-licking episodes in mice. This result also suggests that there may be central mediation of the anti-inflammatory effect of the oil because of its effects in the 2nd phase. There is no doubt that morphine exerts its main analgesic effects centrally, hence it appears to be more effective at the two phases (Viana et al., 2000). The results further showed that the oil has a comparable effects with the standard drugs used.

Carrageenan-induced paw oedema as an in vivo model of inflammation has been extensively used to evaluate the antiedematous effects of natural products. The EO of *D. tripetala* at all the dose levels used dose-dependently displayed significant ($p<0.05$) anti-inflammatory effects by inhibiting the carrageenan-induced paw oedema in the rats compared to control (Table 4). This result is not unusual as essential oils from other sources such as from *Eucalyptus* sp. (Silva et al., 2003), radix of *Asarum sieboldii* (Kim et al., 2003) and *Lavandula angustifolia* (Hajhshemi et al., 2003) among several reports demonstrated significantly, strong analgesic and anti-inflammatory properties. The results obtained in this study indicated that the EO of *D tripetala* may possess more lasting or prolonged anti-inflammatory effects than the standard steroidal drugs as the EO (25-50 mg/kg) inhibited the paw oedema at 3 hr more significantly than dexamethasone (1 mg/kg). However, these results are preliminary and therefore other inflammation models will be explored to further validate this anti-inflammatory property of EO and determine probable mechanism(s) involved. The EO of *D.tripetala* has been reported to contain mainly β-phenylnitroethane (80%), l-linalool (11%), β-Eudesmol and nerolidol (4%) (Agbakwuru et al., 1995). Therefore, it could be suggested that the antinociceptive and anti-inflammatory effects demonstrated by the EO may be due to one or more of these compounds. Further works are however underway to carry out activity-directed fractionation of the EO in order to determine the active compound(s) responsible for its effects. The various analgesic effects obtained in this work provide lead for detailed and comprehensive knowledge into *Dennettia tripetala* as a potential candidate for development into potent patentable analgesic drug. Combining the results from hot plate, acetic acid-induced writhes and formalin tests suggest that the oil contains constituent(s) with potent analgesic effects that are likely to be mediated both peripherally and centrally.

In conclusion, the results of the analgesic and antiinflammatory properties of the essential oil of *D.tripetala* further justify the use of the plant in ethnomedicine for treating fever, cough and vomiting and there is need for further investigation with isolated components of the oil for possible development into new class of analgesic and antiinflammatory drugs.

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