SEDATIVE, HYPOTHERMIC AND MUSCLE RELAXANT EFFECTS OF THE ESSENTIAL OILS OF *DENNETTIA TRIPETALA* G. BAKER (ANNONACEAE) AND ITS MECHANISMS IN MICE

I.A. OYEMITAN1*, E.O. IWALEWA1, M.A. AKANMU3 and T.A. OLUGBADE2

1. Department of Pharmacology, Obafemi Awolowo University, Ile-Ife, Nigeria.
2. Department of Pharmaceutical Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria.

(Received: May, 2008; Accepted: June, 2008)

Abstract

The essential oil of the fruits of *Dennettia tripetala* G.Baker (Annonaceae) has been reported to have depressant effects on the central nervous system in mice. This study was carried out to evaluate sedative, hypothermic and muscle relaxant effects of the oil and its mechanism(s) in mice.

Different groups of mice were administered varying doses (12.5-50.0 mg/kg) of the essential oils following intraperitoneal (i.p.) routes. The sedative effect was assessed by sodium pentobarbitone (50 mg/kg, i.p.) - induced sleeping time, while hypothermic effect was evaluated by estimating rectal temperature variation after administration of various doses of the oil using digital thermometer. The muscle relaxant effect was determined using the hind limb-grip test. In order to determine the mechanism(s) involved in the sedative effect of the oil, mice were pretreated with flumazenil (2 mg/kg), a specific GABA-benzodiazepine antagonist 30 minutes prior to administration of 25 mg/kg of the essential oils and its effect on sleep latency and total sleeping time recorded. The mechanism(s) of hypothermic effects of the oil was determined in another set of experiments. The results obtained showed that the essential oils of *D. tripetala* significantly (p<0.05) showed sedative, hypothermic and muscle relaxant effects. Pretreatment with flumazenil prior to the administration of essential oils (25 mg/kg, i.p.) showed that the prolongation of the total sleeping time effects of the oil was completely blocked while the reduction in rectal temperature induced by the oil in mice was blocked by both flumazenil and atropine. It is concluded that the sedative effect of the essential oils of *D. tripetala* may be mediated through GABA-benzodiazepine receptors and its hypothermic effects may be mediated through both GABA-benzodiazepine and cholinergic muscarinic pathways.

Key words: *Dennettia tripetala*, sedation, hypothermia, cholinergic, GABA pathways.

1. Introduction

The fruits of the plant *Dennettia tripetala* G.Baker (Annonaceae) are well known in many communities of some southern states of Nigeria. The fruits are commonly eaten as spices in many communities of southern states of Nigeria. Previous epidemiological studies on the habitual consumption of the fruits showed that the various parts of the plant are used for the treatment of fever and vomiting (Oyemitan et al., 2006b). The various parts of the plant are also used by the local herbalists in combination with other medicinal plants to treat various kinds of ailment including fever, infantile convulsion, typhoid, cough, worm infestation, vomiting, stomach upset among others (Oyemitan, 2006a and Oyemitan et al., 2006b). The fragrance and pungent taste qualified it to be used probably to give special taste or aroma to other medicinal preparations (Akinniyi, 2006). The abusive potential of the fruits among its habitual consumers has studied and the findings were suggestive of addiction (Oyemitan et al., 2006b). The essential oils have been reported to contain α-phenyl nitroethane (80%), linalool (11%), α-eudesmol and nerolidol (4%), α-caryophyllene and α-humuline (Ekundayo et al., 1992; Adeoti et al., 2000; NAPRALERT, 2005; Lopez et al., 2002). The present study was therefore carried out to further assess the effect of the essential oil as a sedative, hypothermic and muscle relaxant agent in mice and to postulate possible pathways involved in the mediation of those effects using various antagonists. The results of this study may also be used to validate the ethnomedicinal uses of the plant.

2. Materials and Methods

Plant material:
The fresh fruits were purchased from Owena market in Owena Town, Ondo-East Local Government and Central market, Ondo town, Ondo-West Local Government Area of Ondo State within the period of April and May, 2005. The fruits of *D. tripetala*...
G. Baker (Annonaceae) were authenticated by Mr. A. O. Adelade, 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 fruits 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.

Preparation of the essential oils:
Distillation of the oils was carried out using the Clevenger apparatus. Fresh fruits of Dennettia tripetala were air-dried at room temperature and commuted into coarse powder using pestle and mortar. The powder (400 g) was hydrodistilled to give 14.68 g (3.7% w/w) essential oils. The essential oils obtained were stored in a light-proof bottle and kept in a refrigerator until use (Agbakwuru et al., 1979).

Determination of relative density of the essential oils:
The relative density of the essential oils obtained was determined using the 10 ml capacity density bottle appropriately and the value was estimated to be 1.07 g/ml. The oil was emulsified with 5% Tween 80 shortly before administration.

Laboratory materials:
Plexiglas cage (25 cm x 25 cm x 30 cm); mice weighing balance; stop watch, digital thermometer (Thermo probe, UNESCO Instant Digital Thermometer, YPSILANTI, MI, 48197, USA), syringe and needles (1 ml, 2 ml, 5 ml), distilled water, suspended iron rod, Tween 80.

Drugs:
The following drugs were used: yohimbine, atropine, naloxone, morphine, flumazenil (Sigma), diazepam (Valium® Swipha, Nigeria), pentobarbital Sodium (BDH), ciproheptadine HCl (MSD).

Animals:
White albino mice (both sexes, weighing 18-20 g) were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. The animals were allowed to water and feed ad libitum. The ethical approval was obtained from the University Research Committee through the Faculty Postgraduate Committee of the Obafemi Awolowo University, Ile-Ife, Nigeria. All treatments were through intraperitoneal route only.

Pentobarbital-induced sleeping time
This experiment was carried out to determine the effects of the essential oils on latency and prolongation of total sleeping time induced by Pentobarbital Sodium. Seven groups of mice, consisting of 5 mice per group, were randomly selected. Group 1 was intraperitoneally (i. p.) administered with 10 ml/kg of 5% Tween 80; group 2 with diazepam (0.5 mg/kg, i.p.); groups 3-5 were administered with the oil (12.5, 25.0 and 50.0 mg/kg, i.p.), group 6 was administered with flumazenil (2 mg/kg i.p.), while group 7 was pretreated with flumazenil (2 mg/kg, i.p.) 15 minutes prior to administration of the oil (25 mg/kg, i.p.). Thirty (30) minutes later, all these groups were then administered intraperitoneally pentobarbitone sodium (50 mg/kg, i.p.). The sleep latency and total sleeping time were noted and recorded for each mouse. The sleep latency is defined as the period between the time of pentobarbital administration and observation of loss of righting reflex while the total sleeping time is the period from loss of righting reflex (loss of consciousness) and waking up (recovery of consciousness). The mean ± SEM was then calculated for each group (Dandiya and Collumbine, 1959; Hellion-Ibarrola et al., 1999; Ayoka et al., 2006).

Hypothermic effects
(a) Effects of the essential oils on body temperature of mice: Five groups of mice, each consists of 5 mice were randomly selected. Group 1 was administered with 10 ml/kg 5% Tween 80, while groups 2-5 were intraperitoneally administered with 12.5, 25.0, 50.0 and 100.0 mg/kg essential oils respectively. The rectal temperature of each mouse in all the groups were taken with a digital thermometer (thermoprobe) by inserting the probe 2 cm deep into the anus of the mice shortly before treatment time and at 30, 60, 90 and 120 minutes after treatment. The mean ± SEM were then calculated for each group treated (Al-Naggar et al., 2003 and Olajide et al., 1999).

(b) Effects of some antagonists on hypothermia induced by the essential oils in mice: Thirty mice were randomly allocated to 6 groups; each group consisted of 5 mice. Group 1 was administered 5% Tween 80 (10 ml/kg, i.p.), while mice in groups 2-6 were intraperitoneally pretreated with yohimbine (1 ml/kg), atropine (0.5 mg/kg), naloxone (2 mg/kg), ciproheptadine (2 mg/kg) and flumazenil (1 mg/kg) respectively 30 minutes prior to administration of essential oils (25.0 mg/kg, i.p.) The rectal temperature were then taken as described in (a) above. Another groups of mice, 5 mice in each group, each were also administered with yohimbine (1 ml/kg), atropine (0.5 mg/kg), naloxone (2 mg/kg), ciproheptadine (2 mg/kg) and flumazenil (1 mg/kg) and the rectal temperature was taken as described above. This experiment was carried out to determine the probable pathways involved in the oil-mediated hypothermia in mice.

Hind-limb grip test
The grip strength was used to assess the effects of the essential oils on muscle relaxation of mice. The method used was as described by Asuzu et al. (1998) and Ayoka et al. (2006) but slightly modified for quantification in this study. Six groups of mice each were randomly selected. Group 1 was administered
The results of the muscle relaxant test were analyzed using Kruskal Wallis non-parametric method. 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 McGraw-Hill Inc.

3. Results

Sedative test:
The essential oils dose-dependently reduced latency of sleep but prolongation of the total sleeping time induced by Pentobarbital sodium (50 mg/kg, i.p.) in mice when compared to control (Table 1). The essential oils at doses of 25.0 mg/kg and 50.0 mg/kg caused similar prolongation of pentobarbital-induced total sleeping time. Pretreatment with flumazenil (2 mg/kg, i.p.) 15 minutes prior to the oil (25.0 mg/kg i.p.) administration caused prolongation of pentobarbital-induced sleep latency when compared to the oil (25.0 mg/kg) alone (Table 1).

Hypothermic Effects of EO in mice:
The essential oils at all the dose levels significantly caused reduction in normal rectal temperature of mice (dose-dependently) compared to control and diazepam 1.0 mg/kg (Fig. 1). The hypothermic effects of the oil are more pronounced at T30 and T60 minutes at the doses of 12.5, 25.0 and 50.0 mg/kg. However, at a dose of 100 mg/kg, the hypothermic effect remains very significant at T30 min, T60 min, T90 min and T120 minutes.

The effects of antagonists on the hypothermic effects of EO in mice:
Pretreatment of mice with atropine 0.5 mg/kg did not reverse or block hypothermia induced by the essential oils in mice (Fig. 2). Naloxone (2 mg/kg, i.p.) prior to the oil enhanced the hypothermic effect significantly (p < 0.05) at all time intervals (Fig. 3). Pretreatment with naloxone prior to the administration of the essential oils (25 mg/kg) produces slight hypothermia at T60, T90 and T120 minutes compared to control. Flumazenil pretreatment did partially block the hypothermia induced by the oil in mice at T30 and T60 minutes time interval but slightly reversed hypothermia at T120 minutes (Fig. 4). Flumazenil alone did not produce significantly hypothermia in mice but slight hypothermia at T30

| Table 1: Effects of essential oil of Dennetta tripetala on sleep latency and total sleeping time induced by pentobarbital injection (50.0 mg/kg) in mice and its antagonism by flumazenil (a GABA-benzodiazepine antagonist) |
|-----------------|-----------------|-----------------|
| Treatment (n=5) | Sleep latency   | Total sleeping time |
| Control (5% Tween 80, 10 ml/kg) | 3.6 ± 0.3 | 71.0 ± 1.2 |
| EO (12.5 mg/kg) | 3.2 ± 0.2 | 123.0 ± 1.6* |
| EO (25.0 mg/kg) | 3.2 ± 0.4 | 125.0 ± 1.4* |
| EO (50.0 mg/kg) | 2.0 ± 0.4* | 125.0 ± 1.4* |
| FMZ (2.0 mg/kg) | 4.0 ± 0.4 | 66.6 ± 1.0 |
| FMZ (2.0 mg/kg) + EO (25 mg/kg) | 3.8 ± 0.4 | 69.8 ± 1.5 |

EO is the essential oil, FMZ is flumazenil. Each value is mean ± SEM. The EO at all doses used caused significant (p < 0.05) prolongation of the total sleeping time induced by the pentobarbital injection. The oil at 50.0 mg/kg caused significant (p < 0.05) reduction in sleep latency induced by pentobarbital injection.
Fig. 1: The effects of EO on normal rectal temperature of mice. Each line represents mean ± SEM. EO is essential oil. EO (12.5, 25.0, 50.0, and 100 mg/kg i.p) caused significant reduction in normal rectal temperature of mice dose dependently compared to control. Reduction in rectal temperature is highest after 30 minutes post treatment but reverts back to pretreatment level at 120 minutes post treatment. Diazepam (1.0 mg/kg i.p), a potent sedative and a benzodiazepine serve as a reference drug also cause a significant reduction in normal rectal temperature of mice compared to control but lesser effect than all the dose levels of EO used. The number of mice per group is 5. *P < 0.05.

Fig. 2: The effects of atropine (a muscarinic antagonist) on the reduction of normal rectal temperature of mice by EO. Each line represents mean ± SEM. Vehicle is 5% Tween 80 (10 ml/kg i.p). EO is essential oil. EO (25.0 mg/kg, i.p.) and Atr is atropine. Atropine (0.5 mg/kg, i.p.) alone did not cause significant reduction in rectal temperature compared to control. but pretreatment with atropine (0.5 mg/kg, i.p.) 30 minutes prior to EO (25 mg/kg, i.p.) caused significant reduction in normal rectal temperature of mice compared to control. Reduction in rectal temperature is highest after 30 minutes post treatment but reverts back to pretreatment level at 120 minutes post treatment. Number of mice per group is 5. *P < 0.05.
Oyemitan et al.: Sedative and muscle relaxant effects of the essential oils of Dennettia tripetala

Fig. 3: Effects of Naloxone (an opioid receptor antagonist) on the reduction of normal rectal temperature of mice by EO. Each line represents mean ± SEM. Vehicle is 5% Tween 80 (10 ml/kg i.p.). EO is essential oil and Nal is naloxone. Naloxone (2 mg/kg, i.p.) alone did not cause significant reduction in rectal temperature compared to control, but pretreatment with naloxone (2 mg/kg, i.p.) 30 minutes prior to EO (25 mg/kg, i.p.) caused significant potentiation in the reduction of normal rectal temperature of mice compared to control. Reduction in rectal temperature is highest after 30 minutes post treatment and persisted beyond 120 minutes post treatment. N=5. *P < 0.05.

Fig. 4: The effects of Flumazenil (a GABA-benzodiazepine antagonist) on the reduction of normal rectal temperature of mice by EO. Each line represents mean ± SEM. Vehicle is 5% Tween 80 (10 ml/kg i.p.). EO is essential oil and Flu is flumazenil. Flumazenil (2 mg/kg, i.p.) alone did not cause significant reduction in rectal temperature compared to control and pretreatment with flumazenil (2 mg/kg, i.p.) 30 minutes prior to EO (25 mg/kg, i.p.) have no significant effect on the reduction in normal rectal temperature of mice caused by EO (25 mg/kg, i.p.). Number of mice per group is 5. *P < 0.05.
minutes, DO pretreatment with cyproheptadine (2 mg/kg, i.p.) prior to the oil did not block or alter the hypothermic effect induced by the essential oils (25.0 mg/kg, i.p.) at all the time intervals T30, T60, T90 and T120 minutes (Fig. 5). Cyproheptadine alone produces hypothermia compared to control. Pretreatment with yohimbine (1 mg/kg, i.p.) did not reverse the hypothermia induced by the oil but rather enhanced the hypothermic effects at T30 min and T90 min. Yohimbine alone did not produce significant hypothermia compared to control (Fig. 6).

Muscle Relaxant Results
The results of the muscle relaxant effect of the oil are presented in Table 2. The results showed that the oil dose-dependently caused muscle relaxation of hind limbs of mice compared to control. The oil at lowest dose (12.5 mg/kg) caused mild muscle relaxant effect at 60 minute while at 25.0-100.0 mg/kg, the oil caused significant muscle relaxant effects at 30 and 60 minutes. Diazepam (1.0 mg/kg) also caused significant muscle relaxant effects compared to control.

4. Discussion
The essential oils (EO) of *D. tripetala* at varying doses was intraperitoneally administered to different groups of mice in order to evaluate the sedative, hypothermic and muscle relaxant properties of the essential oils in animals. Also, some antagonists were used to pretreat the animals prior to administration of the essential oils in order to determine the mechanism(s) or neuroreceptors(s) involved in the mediation of the effects of the oil. The three pharmacological parameters viz sedation, hypothermia and muscle relaxation are thought to be closely linked or related as previous works have shown that agents with significant CNS depression exhibited these effects (Vale *et al.*, 1999; Haque *et al.*, 2001; N'gouemo *et al.*, 1996; Al-Naggar *et al.*, 2003 and Asuzu *et al.*, 1998). Hence in this study, the oil was evaluated for these parameters and also efforts were made to determine the probable mechanism(s) of action. Pentobarbital-induced sleeping test is used normally to investigate sleep-inducing effect of an agent on CNS depression. Pentobarbital sodium is a short-medium acting barbiturate, which induces hypnosis in animals. The latency to sleep is defined as the time in minutes from administration time to loss of righting reflex (unconsciousness) while total sleeping time is defined as the total time in minutes from loss of righting reflex (loss of consciousness) to regain of righting reflex (recovery of consciousness) (Haque *et al.*, 2001, Ayoka *et al.*, 2006). The administration of the essential oils (12.5-25.0 mg/kg, i.p.) shortened the sleep latency time dose dependently compared to control in a non-significant manner. However, the administration of the essential oils at 50 mg/kg caused significant reduction in sleep latency compared to control. The essential oils at all the doses administered increased total sleeping time induced by pentobarbitone sodium (50 mg/kg, i.p.) significantly (p<0.05) in a dose-dependent manner when compared to control group. This result therefore suggests that the essential oils possess hypnotic effect pharmacologically by prolonging the hypnotic effect of pentobarbital. From the results obtained with administration of different doses of the essential oils, it was observed that the oil at doses of 25.0 and 50.0

---

Fig. 5: The effects of cyproheptadine (a 5-HT receptor antagonist) on the reduction of normal rectal temperature of mice by EO. Each line represent mean ± SEM. Vehicle is 5% Tween 80 (10 ml/kg, i.p.), EO is essential oil and Cyp is cyproheptadine. EO (25.0 mg/kg, i.p.) or cyproheptadine (2 mg/kg, i.p.) alone cause significant reduction in rectal temperature compared to control and pretreatment with cyproheptadine (2 mg/kg, i.p.) 30 minutes prior to EO (25 mg/kg, i.p.) cause no significant effect at 30 minutes but potentiated reduction in normal rectal temperature of mice caused by EO (25 mg/kg, i.p.) at 60, 90 and 120 minutes post treatment. Number of mice per group is 5. *P < 0.05.
mg/kg caused similar maximal effect in prolonging the total sleeping time induced by pentobarbital, it was therefore decided to use the dose of 25 mg/kg (i.p.) to study the possible mechanism of action of the oil in promoting sleep.

In an attempt to determine the involvement of GABA-benzodiazepine receptors in the observed hypnotic effect of the essential oils, a specific GABA-benzodiazepine antagonist, flumazenil (2 mg/kg i.p.) was administered 15 minutes prior to administration of the oil was observed to block the prolongation of total sleeping time induced by the oil on Pentobarbital-induced sleeping time in mice (Ayoka et al., 2006). This result showed that flumazenil, a specific GABA-benzodiazepine antagonist inhibited the potentiating effect of the oil on total sleeping time induced by the pentobarbital. This result therefore suggests that the observed hypnotic property of the essential oils or its constituents is probably mediated through potentiation of GABA-benzodiazepine neurotransmission in the CNS. It can therefore, be hypothesized that the essential oils (or its constituent(s) induced sedative effects through interaction with benzodiazepine receptors in the CNS in a similar manner to previous findings in certain plant volatile oils (Dandiya and Collumbine, 1959) and plant extract, Hellion-Ibarrola et al., 1999; Ayoka et al., 2006). The sedative effects observed in this study, also suggests that the essential oils contain compound(s) that may have interactions with GABA-benzodiazepine complex receptor system. These sedative potentials may be of advantage while using the plant to manage fever and vomiting by the local people.

The essential oils dose-dependently reduced normal rectal temperature of mice treated compared to control and diazepam 1.0 mg/kg at all the time intervals post treatment. The reduction in rectal temperature of mice was highest at 30 minutes after administration of the essential oils and thereafter gradually returning to pre treatment temperature except the oil at 100 mg/kg which persisted well beyond the last assessment time at time 120 minutes. The hypothermia observed in this study suggests an implication of both central and peripheral mechanisms. This effect may be due to the decreased
levels of metabolic heat production and or vasodilation. Thus, the preoptic anterior hypothalamus is critical in the neuronal network of thermoregulation (N'gouemo et al., 1996). The role of both histaminergic and dopaminergic systems in thermoregulation in mice has been highlighted (Colboe et al., 1982; Barros et al., 2004) and it is believed to act through $H_2$ and $D_2$ receptor sites respectively. Benzodiazepines have also been demonstrated to cause hypothermia in animals even at low doses and they are thought to act through benzodiazepine receptors (Jackson and Nutt, 1990). Hannan et al. (2004) reported that classical CNS depressants usually produce a state of hypothermia. This result therefore suggests that having shown previously in this study that the essential oils possess sedative properties it may also possess hypothermic effect in mice. The involvement of opioid system in temperature regulation has been earlier established since morphine, a potent opioid agonist had been reported to cause a significant hyperthermia (Rawls et al., 2007). Furthermore, body temperature regulation has also been linked to serotonergic system in the CNS (Morishima and Shibano, 1995; Rang et al., 1999; Conley and Hutson, 2007; da Silva et al., 2007; Gargaglioni et al., 2005). Therefore, in order to understand the involvement of various CNS neurotransmitters or their receptors in the mechanism(s) of the oil-induced hypothermia in mice we used various antagonists in this study. Mice were pretreated with intraperitoneal administration of atropine (0.5 mg/kg), naloxone (2 mg/kg), ramazemin (2 mg/kg), cyproheptadine (2 mg/kg) and yohimbine (1 mg/kg) 30 minutes prior administration of the oil (25 mg/kg). The results showed that naloxone, cyproheptadine and yohimbine potentiated the hypothermic effects of the oil in mice, while atropine and flumazenil inhibited hypothermic effects of the oil. It could therefore be suggested that the oil is exerting its hypothermic effect mainly through cholinergic muscarinic and GABA-Benzodiazepine pathways. The opioidergic, serotonergic and adrenergic receptors are also implicated in the mediation of its hypothermic effect. Therefore, our results further confirmed that body temperature can be interpreted as an index of alteration of various central neurotransmitters as reported by Al-Nagger et al. (2003).

The results obtained showed that the essential oils of $D$. tripetala caused significant muscle relaxant effect and this is comparable to the standard drug used, diazepam. The oil dose-dependently exhibited muscle relaxant effects in mice. This is in conformity with previous reports that sedative and other CNS depressants including benzodiazepine also possess muscle relaxant properties (Rang et al., 1999). This result suggests that the essential oils contain constituents or compounds that possess muscle relaxant properties, which further corroborate its sedative potentials. The results from all the experiments carried out suggest that the essential oils of $D$. tripetala contains compound(s) that caused significant sedative, hypothermic and muscle relaxant effects in mice therefore justifying the ethnomedicinal uses of the plant particularly in the management of fever among other uses.

REFERENCES
Oyemitan et al.: Sedative and muscle relaxant effects of the essential oils of *Dennettia tripetala* 9


NAPRALERT 2005. 3 part query for NAPRALERT 08/10/05.


