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Hypnotic, anticonvulsant and anxiolytic effects of 1-nitro-2-phenylethane isolated from the essential oil of *Dennettia tripetala* in mice

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ABSTRACT

This study investigated the hypnotic, anti-convulsant and anxiolytic effects of 1-nitro-2-phenylethane (BPNE) obtained from the oil of *Dennettia tripetala* G. Baker (Annonaceae) and established its mechanism of action. The essential oil (EO) from the leaf, fruit and seed was obtained by hydrodistillation, followed by isolation of BPNE purified to 99.2% by accelerated gradient chromatography on silica, and identified by NMR and GC–MS. The pure BPNE and EO of the dried seed (93.6%) were comparatively evaluated for hypnotic, anticonvulsant and anxiolytic effects in mice. The acute toxicity of BPNE was determined and the LD_{50} was 490 mg/kg, intrapritonealy. The hypnotic activities of the EO and BPNE (50–400 mg/kg, i.p.) were assessed by loss of righting reflex, while sodium pentobarbitone (PBS) and diazepam (DZM) were used as positive controls. The anticonvulsant and anxiolytic effects of the EO and BPNE were evaluated in mice. Both BPNE and EO at doses ≥100 mg/kg induced spontaneous hypnosis with loss of righting reflex, significantly decreased sleep latency (SL) and also increased total sleeping time (TST) dose-dependently. They had comparable activity with NAP in TST. The BPNE exhibited higher hypnotic potency than EO at the same dose levels. The EO and BPNE offered comparable dose-related protections against PTZ- and strychnine-induced convulsions. Flumazenil (2 mg/kg) blocked the hypnotic and anticonvulsant (PTZ-convulsions) effects of both EO and BPNE. The essential oil at 5–20 mg/kg dose levels significantly ($p < 0.05$) increased the percentage time spent and number of entries into the open arms. While at the same dose range BPNE significantly ($p < 0.05$) increased the percentage time spent and the number of entries into the open arms respectively. The study concluded that 1-nitro-2-phenylethane exhibited dose dependent significant hypnotic, anticonvulsant and anxiolytic effects and it is the compound largely responsible for the neuropharmacological effects of the oil.

Introduction

*Dennettia tripetal*a G. Baker (Annonaceae) is a medium-size tree and its common habitat is the rain tropical forest of some West-African countries, including Nigeria. The fruits when fresh are green but gradually change to light yellow when ripe. After drying, the fruits become dark-black in colour and resemble the shape of groundnuts. The fruits are eaten raw, in different forms (fresh green, fresh ripe brown, black dried fruits and dried seeds), while its leaves are used as condiments in some special local dishes (Agbakwuru et al. 1979). The leaves of the plant are used by the local herbalists in combination with other medicinal plants to treat various kinds of ailment including fever, infantile convulsions, typhoid, cough, worm infestation, vomiting and stomach upset (Oyemitan et al. 2006). The fruits of the plant contain essential oil and alkaloids (Osisiogu 1975; Ekundayo et al. 1992) and further studies reported that the essential oil contains several compounds including 1-nitro-2-phenylethane (80%), β-eudesmol and nerolidol (4%), 1-linalool (11%), β-caryophyllene and β-humuline (Agbakwuru et al. 1979). 1-Nitro-2-phenylethane (BPNE) (Fig. 1) possesses strong cinnamon-like characteristic odour, which was

Abbreviations: BPNE, 1-nitro-2-phenylethane; CNS, central nervous system; DZM, diazepam; EO, essential oil; FMZ, flumazenil; GABA, gamma amino butyric acid; IOAA, index of open arm avoidance; LRR, loss of righting reflex; NAP, sodium pentobarbitone; SL, sleep latency; TST, total sleeping time; PTZ, pentylene tetrazole; TLC, thin layer chromatography; VEH, vehicle; AGC, accelerated gradient chromatography.

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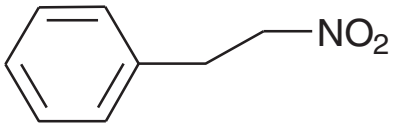

Fig. 1. β-Phenylnitroethane $C_8H_9NO_2$ [2].

reported to be responsible for the characteristic aroma of the essential oils of *Aniba canelilla* (Gottlieb and Magalhaes 1959; Vilegas et al. 1994). *Aniba canelilla* is a medicinal plant used in South America (Brazil) in the Amazon folk medicines to treat spasmodic, diarrhoea, gastrointestinal and central nervous system (CNS) disorders (de Lima et al. 2009).

The compound effects of 1-nitro-2-phenylethane, isolated from *Aniba canelilla* essential oil has been reported to be responsible for the cardiovascular and antinociceptive effects of the oil (Gottlieb and Magalhaes 1959; de Siqueira et al. 2010; Interaminense et al. 2010). 1-Nitro-2-phenylethane has also been isolated from the essential oils of dry fruits of *D. tripetala* (Okogun and Ekong 1969). In our previous studies we reported that the essential oil of the plant demonstrated significant analgesic, anti-inflammatory, hypothermic, sedative, muscle relaxant and central nervous system depressant activities (Oyemitan et al. 2008a,b, 2009). The present study was specifically aimed at establishing the contribution of 1-nitro-phenylethane (which is the major compound), isolated from the essential oil of *D. tripetala* in the mediation of its sleep-inducing, anticonvulsant and anxiolytic effects. Furthermore, the study investigated the actions of some antagonists on the hypnotic effects of the isolate and probable mechanism(s) of action of this major compound.

Materials and methods

Plant identification and authentification

The plant *D. tripetala* G.Baker (Annonaceae) was authenticated by Dr. H.C. Illoh of the Department of Botany, Faculty of Science, Obafemi Awolowo University (OAU), Ile-Ife, Osun State. A voucher specimen (IFE 15, 356), comprising the leaves and fruits, was deposited at the Herbarium, Department of Botany, Faculty of Science, O.A.U., Ile-Ife, Nigeria.

Plant collections

Fresh leaves of *D. tripetala* were collected between April and May 2007 from the plants growing within a cocoa plantation situated in Olomitoto Farm Settlement, along Ondo-Bolorunduro-Akure Road, Ondo-East Local Government Area, Ondo State, Nigeria. One batch of fresh leaves was used directly, while another batch dried under room temperature in the laboratory. Fresh fruits purchased from the Central Market, Ondo was divided into three batches; first batch was used fresh, the second batch was allowed to ripe at room temperature (seeds were remove and dried at room temperature), and the third batch was dried at room temperature before use.

Hydrodistillation of the essential oils

The dried leaves, fresh leaves, fresh fruits, dried fruits and dry seeds of *D. tripetala* were hydro-distilled using the BP method (Clevenger apparatus) for about 4 h and they yielded 0.0, 0.4, 0.9, 3.7 and 5.8% (w/w) respectively.

Isolation of 1-nitro-2-phenylethane from the crude pooled oil by chromatography

Both silica TLC (n-hexane:ethylacetate; 95:5) and NMR (1H and 13C) of the various oil samples indicated 1-nitro-2-phenylethane as the major component, hence they were pooled for further purification. However, the oil from the dried seed indicated 93 .6% content by GC and was reserved for biological testing as EO while the pooled oil (8.6 g) was adsorbed on silica gel (230–400 mesh) (8 g). The adsorbed oil was packed onto accelerated gradient chromatography column with 8 g silica in separation zone. The column was eluted with a gradient of hexane: ethylacetate to afford 32 fractions of 15 ml each. The fraction eluted with 5% ethylacetate in hexane gave pure BPNE (0.75 g), R_f 0.38, ^{1}HNMR ($CDCl_3$, 200 MHz) δ 3.3, t, 2H; 4.6, t, 2H; 7.3, m, 5H, ^{13}C NMR ($CDCl_3$, 50MHZ) δ 136.0, C-1; 129.2 × 2, C-3 and C-5; 128.9 × 2, C-2 and C-6; 127.7, C-4; 76.5, CH_2; 33.6, CH_2), EIMS *m/z* 104 (100%) (loss of HNO_2), 99.2% pure (GC).

GC–MS analysis

GC–MS data on EO of the dried seed and the isolated pure 1-nitro-2-phenylethane were obtained with Agilent GC 6890N coupled to a quadrupole MS 5973 Network set at 150 °C for quadruple while ion source was maintained at 230 °C with an ion source of 70 eV. The GC column was capillary DB-1MS 30 m coated with polysiloxane of 1 μm thickness, carrier gas He at flow rate of 1 ml/min, 0.78 PSI pressure and velocity of 37 cm/s, injection port temp of 100 °C, maintained for 2 min and set to rise at 5 °C/min up to 150 °C maintained for 10 min.

Preparation of the oil and 1-nitro-2-phenylethane for administration

The EO and 1-nitro-2-phenylethane (BPNE) were emulsified with 5% Tween 80 prior to administration. An equivalent volume corresponding to required weight was taken and emulsified with Tween 80 and then made-up with distilled water to obtain the desired concentration of the oil or BPNE in mg/ml shortly before administration.

Animals

White albino mice (both sexes) weighing between 20 and 30 g were obtained from the Animal House, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University (OAU), Ile-Ife. The animals were kept in the laboratory under standard conditions, fed with standard animal feeds prior to, and throughout the period of experimentation. The Ethical Committee of the Faculty Postgraduate Committee, Faculty of Pharmacy, OAU, approved the research work and it was finally approved by the Board of Postgraduate College on the 29th July 2010 with approval number PHA06/07/R/0098 in compliance with the EU Directive 2010/63/EU for animal experiments.

Drugs

The following drugs were used: Flumazenil, Pentylene tetrazole (PTZ), Scopolamine HCL, Cyproheptadine HCL, Yohimbine, Strychnine, Atropine HCL, Naloxone and Caffeine HCL (Sigma Chemicals Co., St. Loius, USA); Diazepam (Swipha Nig. Ltd., Lagos, Nigeria); Pentobarbitone Na (BDH chemicals Ltd, England). The volume of drug administered intraperitoneally (i.p.) was 10 ml/kg of body weight in all cases.