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Photic-Evoked Response in the Pontine Reticular Formation of Wistar Rats: Effect of Crude Methanolic Seed Extract of *Datura metel* L.

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Abstract: This work was undertaken to observe the effects of the methanolic crude extract of *Datura metel* L. seeds (family Solanaceae) on the behavioural sleep (electroencephalogram) pattern of Wistar rats. Following the oral acute toxicity study to determine the median lethal dose (LD50) of the extract in twelve (12) mice, the extract was relatively safe since when administered at the highest dose (5,000 mg/kg) no sign of toxicity and no death was recorded. In the *in vivo* sedative and anaesthetic study of the effect of *Datura metel* L in this work, the observed pharmacological effect of the extract administered orally at a dose rate of 7, 9, 11, 15, 20 and 25 g/kg to six (6) Wistar rats respectively during establishment of a suitable pilot oral anaesthetic doses, showed a graded dose response relationship. The observed effect of the extract on the behavioural sleep of 10 Wistar rats implanted with electrodes in the hyperstriatum (HS), optic tectum (OT) and reticular formation (RF) and the electromyogram from the muscle around the occipital region (EMG). Each of these rats was administered the extract orally at a dose rate of 25 g/kg. In the normal calm alert state EEG featured wave form of varying voltages and frequency characterised by desynchronisation, 0.72±0.05 μV, 0.16 wps, 2.6±0.072 μV, 0.2 wps, 2.5±0.163 μV, 0.16 wps and 1.5±0 μV, 0.2 wps of the waves emanating from HS, OT, RF and EMG, respectively. The EEG patterns observed from the treated Wistar rats showed synchronization of the waves from HS, OT and RF. With reduction of the EMG with wave pattern of 1.5±0 μV, 0.2 wps, 1.5±0 μV, 0.2 wps, 0.1±0 μV, 0.2 wps and 1.25±0.05 μV, 0.32 wps, respectively. The observed sleep pattern produced by the extract was similar to the observed sleep pattern produced by 5 Wistar rats implanted with electrodes and administered thiopental intra peritoneally with wave pattern of 1.1±0.032 μV, 0.2 wps, 1.1±0.032 μV, 0.2 wps, 1.1±0.032 μV, 0.2 wps and 1.3±0.063 μV, 0.32 wps from HS, OT, RF and EMG, respectively. This study has shown that the seed extract of *Datura metel* L. is relatively safe and induced sleep similar to that of thiopentone sodium anaesthesia.

Key words: Wistar rat · *Datura metel* L · Seed extract · Synchronisation · EEG pattern

INTRODUCTION

General anaesthesia can be compare with natural sleep. Several years have passed when the first recognition of rapid eye movement (REM) sleep and the linking of this to Moruzzi and Magoun's earlier finding in 1949 of the arousal-promoting brain stem network – the ascending reticular activating system [1]. Later, in 1962 Jouvet discovered that the pontine reticular formation played a key role in REM sleep generation [1]. Although there are serious reservations about the use of the EEG as an index of anaesthesia [2- 5]. Both halothane and natural

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sleep produce similar spindles in the cortical EEG suggesting that halothane spindles and natural sleep spindles may be generated by the same thalamocortical mechanisms [6]. It is only possible to describe the EEG changes related to anaesthesia in the most general terms. The earliest changes seen with the induction of anaesthesia are that a previously responsive α rhythm becomes unresponsive and then desynchronized and flattened, becoming replaced with high frequency activity of low or high voltage depending on the agent [7]. Further deepening of anaesthesia is associated with replacement of these waves with slow waves and the slower the frequency the deeper the level of anaesthesia [7]. ***Datura metel* L.** family Solanaceae. Common name: Thorn apple; Indigenous names: Hausa – Zakami; Yoruba – Apikan; Igbo -Myaramuo [8]. It is an annual shrub, grows erect with branches and glabrous herb sharing the sympodial growth of solanaceae attaining the height of 60-100 cm [9]. The leaves are simple, alternate, estipulate and triangular to ovate and measure about 18cm × 13cm in length. Lamina is dentate, pointed petiole and asymmetric base [10]. Inflorescence occurs as a cyne with erect nearly white flowers. Both the calyx and corolla are tubular and trumpet shaped about 26cm long [11]. Fruits are capsules, round (1.25 inches in diameter), dehiscent and covered with blunt prickles or warts, usually pale green [1,12].

In Nigeria, especially in the northern part, *Datura* is found growing as a weed in abandoned farmlands and or dumpsites. The leaves and seeds of the plant are used for several purposes and in several ways especially for its psychoactive activities [11].

MATERIAL AND METHODS

Experimental Animals: This research work was approved by the research and ethics committee of the Faculty of Veterinary Medicine Ahmadu Bello University, Zaria, Nigeria.

Twelve male laboratory mice and twenty one Wistar rats clinically healthy were purchased from the animal unit of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Nigeria. Mean weight of 22 g±1.4 g and 173 g±11.6 g, respectively. The animals were housed and acclimatised at the department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria for two weeks and fed with grower mash (Poultry feed) water was given to them *ad libitum*.

Extract Solution: For the acute toxicity test, 2% solution of Tween80 was prepared by adding 1ml of Tween80 to 49ml of distilled water. This solution was then used to prepare 200 mg/ml stock solution of *Datura metel* L seed extract by adding 2g of the extract to 10 ml of 2% solution of Tween 80.

Serial dilution was carried out using the stock solution to prepare 2 mg/ml and 20mg/ml this was then administered to the mice for the acute toxicity test. Another 2% solution of Twee80 was used to prepare 4% stock solution of *Datura metel* L seed extract. This extract solution was used for the rest of the experiments in the Wistar rats.

Plant Collection and Identification: The whole plants with fruits were collected towards the end of the rainy season (End of October – first week of November) from an old dump site behind Ameenudeen Mosque in Badawa Quarters in Nasarawa Local Government of Kano State, Nigeria. The plant was identified by Mallam Yusuf Nuhu (Chief technologist) of the Herbarium Unit and Dr. Kutama, A. S who had worked with the plant, both of the Department of Biological Sciences, Faculty of Science, Bayero University Kano, Nigeria. The plant was given a Voucher number of 325 and stored at the Herbarium for reference purpose.

Methanolic Extraction of Datura Metel L. Seeds: Plant extraction and phytochemical screening was carried out at the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello

University Zaria, Nigeria. The method used was as described by Donatus and Ephraim [10]. Two kilograms (2 kg) of the seeds was obtained from the matured fruits. The seeds were dried in the shade until a constant weight was obtained and were pounded using pestle and mortar to a coarse powder. The powder was packed into white cotton bags and put into Soxhlet apparatus and extracted exhaustively using 3.5 L of methanol at 70°C. The methanolic extract was concentrated by evaporation at room temperature on warm water bath (HH-S 21.6, double row six holes, HINOTEK China) to separate the solvent from the extract (Oily liquid) at a temperature of 93.3°C.

Phytochemical Screening of Datura Metel L Seed Extract: The "extract" in this study refers to the crude seed extract of *D metel* L.

Phytochemical screening allows the detection of secondary metabolites in a plant extract or samples. This is done as a step towards identifying potential bioactive compounds in the extract [13]. Test for reducing sugars (Fehling's test), Test for tannins (Lead sub-acetate test), Test for resins (copper acetate test), Test for saponins (Frothing test), Test for flavonoids (Shinoda test), Test for steroid glycosides (Liebermann-Burchard's test), Test for alkaloids (**Mayer's test, Dragendroff's test, Wagner's test)** [13]. Test for terpenoids, Test for anthraquinones (Borntrager's test).

Acute Toxicity Test of Datura Metel L Seed Extract: The method described by Lorke [14] was used to determine the LD_{50} of the extract as an indication of its safety.

Photic-evoked Response in the Pontine Reticular Formation of Wistar Rats: Effect of Crude Methanolic Seed Extract of Datura Metel L: The study was intended to observe the effect of the extract on behavioural sleep (CNS activity) using Wistar rats as a model, observed from the electroencephalogram (EEG) recordings of five Wistar rats.

Pilot study was carried out using randomly selected 6 male Wistar rats to establish a suitable oral anaesthetic dose. The extract was administered to the rats at the following dose rate 7, 9, 11, 15, 20 and 25 g/kg extrapolated from the previous acute toxicity study in mice. The rats were then observed for the induction of anaesthesia. The dose 25 g/kg produced desirable anaesthetic effect.

Implantations of Electroencephalogram (EEG) Electrodes: Electrodes implantations and EEG recordings were done as described by Osuide and Sokomba [15], using Grass Model 79D EEG and Polygraph Data Recording System (Grass instrument. Co, Quincy, Mass, USA. Frequency-50Hz, Volt-230V, Serial No. - 665Y1G). Calibration signals were amplified with a gain of 50µV/cm of pen deflection on standard EEG paper (Grass Inst., Mass, USA) at a speed of 2.5 mm/sec.

EEG Recordings in Wistar Rats: Prior to electrodes implantations, 10 Wistar rats were randomly allocated to 2 groups. Group 1 (5) rats and group 2 (5) rats. Group 2 were administered thiopental sodium. Prior to the administration of the extract and thiopental sodium, EEG recordings were taken for all the rats which served as base line data.

The rats were allowed to recover from chloroform anaesthesia after electrodes implantations over a period of one hour. All the 5 Wistar rats in group 1 received the extract at 25 g/kg orally. The dose was shown in previous studies to induced anaesthesia in the rats. EEG recordings were carried out in a quiet room with the rats placed in a screened cage with front netting after 15 minutes post administration of the extract. The changes in muscle tone as reflected by the electromyogram (EMG) were simultaneously observed. Group 2 rats were treated with 30 mg/kg thiopental using the intraperitoneal (Ip) route [16] and EEG recordings were as described for group 1.

RESULTS

Extraction and Phytochemical Screening of Datura Metel L Seed Extract: Two kilogram (2kg) of the dried seed powder of *Datura metel* L yielded 123.5g of the crude extract equivalent to 6.2%. The extract is a golden brown oily liquid substance. Stored at room temperature in a bottle container. Phytochemical screening results showed the extract contained alkaloids, reducing sugar, tannins, resins, Flavonoids, steroid glycosides and Terpenoids

Safety Evaluation of Datura Metel L Seed Extract: The extract at the dose rate of 10, 100 and 1,000 mg/kg did not produce any toxic effect or death in the tested mice similarly in the second phase of the acute toxicity study no toxic effect or death recorded when the extract was administered at the dose rate of 1,600, 2,900 and 5,000 mg/kg.

Effects of Different Doses of the Extract in Wistar Rats: The extract at the dose rate of 7, 9, 11, 15, 20 and 25 g/kg administered to six rats respectively showed various effects on the rats as recorded in Table 1.

In all the two groups, the normal calm alert state EEG featured wave form of varying voltages and frequency characterised by desynchronisation, 0.72±0.05 µV, 0.16 wps, 2.6±0.072 µV, 0.2 wps, 2.5±0.163 µV, 0.16 wps and 1.5±0 µV, 0.2 wps of the waves emanating from HS, OT, RF and EMG, respectively (Fig 1).

The observed effect of the extract on the behavioural sleep of 5 Wistar rats (Group 1) implanted with electrodes in the hyperstriatum (HS), optic tectum (OT) and reticular formation (RF) and the electromyogram from the muscle around the occipital region (EMG) and administered the extract orally at a dose rate of 25 g/kg. The EEG patterns observed showed synchronisation of the waves from HS,

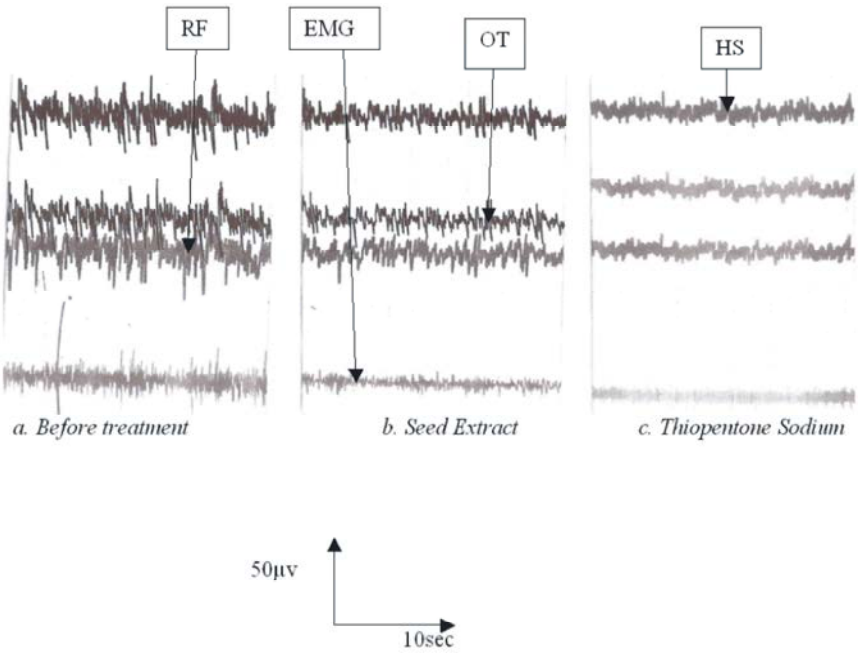

Fig. 1: EEG recordings of Wistar rats; a. before treatment, b. rats treated with *Datura metel* L seed extract and c. rats treated with thiopental.
Key: HS- hyperstriatum, OT- optic tectum, RF- mid brain reticular formation and EMG- electromyogram.

Table 1: Effects of different dose rate of *Datura metel* L. seed extract in Wistar rats.

Dose rates (g/kg)	Signs observed.
7 and 9	-Excitement and restlessness. -Indifferent to immediate environment. -Crunching at the corners of the cage (30 minutes).
11and 15	- Excitement (40 minutes) - Induction of behavioural sleep (60 minutes)
20	- Excitement (30 minutes). - Induction of behavioural sleep (65 - 70 minutes).
25®	-Excitement (5 minutes) -Induction of behavioural sleep suddenly after 7 minutes, lasting for about80 minutes

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OT and RF. With reduction of the EMG with pattern of 1.5±0 µV, 0.2 wps, 1.5±0 µV, 0.2 wps, 0.1±0 µV, 0.2 wps and 1.25±0.05 µV, 0.32 wps, respectively (Fig 1).

The observed sleep pattern produced by the extract was similar to the observed sleep pattern produced by 5 Wistar rats (Group 2) administered thiopental at the dose rate of 30 mg/kg, with wave pattern of 1.1±0.032 µV, 0.2 wps, 1.1±0.032 µV, 0.2 wps, 1.1±0.032 µV, 0.2 wps and 1.3±0.063 µV, 0.32 wps from HS, OT, RF and EMG, respectively (Fig 1).

DISCUSSION

The extract was subjected to phytochemical screening and the following secondary metabolites were detected: alkaloids, flavonoids, reducing sugars, tannins, terpenoids, resins and steroid glycosides which were also reported by Abdullahi *et al.* [8], Wannang *et al.* [9] and Kutama *et al.* [11]. The presence of the alkaloids in the seed extract could be responsible for the pharmacological effect observed in both rats and dogs in this study. Tyler *et al.* [17], reported that scopolamine (An alkaloid) content of the plant *Datura metel* L. is often associated with the CNS depression effects of the plant. Alkaloid production starts from the second week after seed germination, peaks at the tenth week [18, 19].

During the acute toxicity study in mice, the extract was observed to have a wide margin of safety which is in accordance with the international safety standard established by Lorke [14] and published by the Centre for Disease Control (CDC) United State of America, states that, when the tested substance is administered to mice at

a dose rate of 5000mg/kg and does not cause toxicity or death in the tested animal the product(s) is said to be relatively safe.

The anaesthetic effect of the extract observed in the rats increased with increased in the dose and is described as a graded dose-response, i.e. as the dose administered to a single animal increased the pharmacological response also increased in a gradual fashion, this is similar to the findings recorded in an earlier study by Kutama *et al*. [11].

In the normal, alert and calm rats, the electroencephalogram recorded showed desynchronisation. The extract, however caused synchronisation of the electroencephalogram (Sleep spindles) of the Hyperstriatum, Optic tectum and reticular formation with profound reduction of the electromyogram in the rats, these agrees with earlier description of the sleep spindles due to anaesthesia by Mori *et al*. [7]. The electroencephalograms (Sleep spindles) recorded from the rats given the extract when compared to that produced by the rats given thiopental a known general anaesthetic agent, were similar. In accordance with similar work done by Keifer *et al*. [6], comparing the sleep spindles of halothane with that of natural sleep, finds that they were similar and conclude that the halothane and the natural sleep spindles may be generated by the same thalamocortical mechanisms. Thus, since the sleep spindles of the extract were similar to that of thiopental sodium, it can be concluded that thiopental and the extract sleep spindles may be generated by the same thalamocortical mechanisms, implying that the extract and thiopental sodium may have the same central nervous stimulatory activities based on Keifer *et al*. [6] conclusion. This study has shown that the seed extract of *Datura metel* L. is relatively safe, induced sleep similar to that of thiopentone sodium anaesthesia.

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