Veterinary Research 6 (5-6): 115-119, 2013
ISSN: 1993-5412

Evaluation of the Crude Methanolic Seed Extract of *Datura metel* L. as a Potential Oral Anaesthetic in Dogs

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Abstract: This study evaluates the methanolic crude extract of *Datura metel* L. seeds (family Solanaceae) as a potential oral anaesthetic in dogs. Following the oral acute toxicity study to determine the median Lethal Dose (LD_{50}) of the extract in twelve (12) mice, the extract was relatively safe since when administered at the highest dose (5,000 mg kg^{-1}) 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 research, the observed pharmacological effect of the extract administered orally to 5 dogs separately at a dose rate of 0.6, 1.2, 1.5, 2 and 2.4 g kg^{-1}, respectively during establishment of a suitable pilot oral anaesthetic doses, showed a graded dose response relationship. The extract at an oral dose of 2.4 g kg^{-1} induced surgical anaesthesia in dogs with increased heart and respiratory rates (107-205 bpm and 36.33-41.33 cpm), respectively, normal rectal temperature (37.83°C), adequate tissue perfusion, good muscle relaxation but poor analgesia, loss of anal sphincter tone and loss of pupillary reflex. The dogs recovered without any complications. This study has shown that the seed extract of *Datura metel* L. is relatively safe, induced sleep similar to that of thiopentone sodium anaesthesia with good anaesthetic indices at the oral dose rate of 2.4 g kg^{-1} in dogs.

Key words: Dogs, *Datura metel* L., seed extract, anaesthetic, surgical anaesthesia, oral dose

INTRODUCTION

Many animals fear and resist the restraint necessary for the administration of anaesthetics thereby increasing not only the technical difficulties of administration but also the dangers inseparable from their use. A fully conscious animal forced to breathe a strange and possibly pungent vapour struggles to escape and sympatho-adrenal stimulation greatly increases the risks associated with the induction of inhalation anaesthesia (Hall *et al*., 2001). Thus, the continued developments in recent years of safe, simple, easily applied techniques of general anaesthesia are particularly welcome (Hall *et al*., 2001).

***Datura metel* L. family Solanaceae:** Common name: Thorn apple; Indigenous names: Hausa-Zakami; Yoruba-Apikan; Igbo-Myaramuo (Mann *et al*., 2003).

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 thus making the plant parts to be abused by the youths who are more prone to dangers of smoking and drug abuse (Kutama *et al*., 2010).

MATERIALS 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.

Eight clinically healthy Nigerian indigenous dogs with mean weight of 10.2±2 kg were used. The dogs were quarantined for 14 days prior to onset of study and to be acclimatised. The dogs were housed in Ahmadu Bello University Zaria, Veterinary Teaching Hospital Small Animal Kennel, food (left over from restaurants) was given twice daily and water *ad libitum*. All experimental animals were fasted 12 h for food and 6 h for water.

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

Serial dilution was carried out using the stock solution to prepare 2 and 20 mg mL^{-1} this was then administered to the mice for the acute toxicity test. Another 2% solution of Twee 80 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 dogs.

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 Okwu and Igara (2009). About 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 (Tiwari *et al*., 2011). 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), Test for terpenoids, Test for anthraquinones (Borntrager's test).

Acute toxicity test of *Datura metel* L. seed extract: The method described by Lorke (1983) was used to determine the LD_{50} of the extract as a indication of its safety.

Evaluation of the anaesthetic effect of the extract: In a preliminary study, five dogs were randomly allocated into 5 groups of 1 animal each. Groups 1-5 were given the seed extract at a dose rate of 0.6, 1.2, 1.5, 2 and 2.4 g kg^{-1} orally, respectively. These dose rates were chosen because they fall within the safety margin for the extract in the acute toxicity study. Each dog served as its own control.

The dose (2.4 g kg^{-1}) that produced the most desirable anaesthetic effect was chosen and administered to another set of 3 dogs through the oral route. Vital parameters that indicate anaesthetic activity and the effect of the extract on physiologic parameters of the dogs were monitored and recorded.

Parameters assessed: Electrocardiogram (ECG), temperature, heart rate (from the ECG), SPO_2 (tissue oxygen saturation) and respiratory rate were monitored and recorded and electrocardiogram of the hearth from Lead II with speed of 25 mm sec^{-1} and voltage of 10 mV sec^{-1}.

These were carried out simultaneously using a multipurpose patient monitoring machine (Model G3 by General Meditech, Inc., Guangdong, China).

Analgesia was evaluated using rat tooth haemostatic forcep clamp at first ratchet lock at the interdigital space and the dog evaluated for response to pain. Anal sphincter reflex and pupillary reflexes (response to the ordinary room light) was assessed visually by examining the anal sphincter for constriction or relaxation while the response to ordinary room light was used to assess the pupillary reflex.

Skeletal muscle relaxation was assessed by noting the limbs at rest as well as the presence or absence of muscular resistance when the limbs were flexed and extended.

Induction time: Time between administration and when the dog shows the first sign of reactions.

Duration of anaesthesia: Time between when the dog became recumbent, loss of consciousness and when the dog shows the first sign of environmentally conscious by lifting up its head.

Recovery time: Time from recumbency to sternal and from sternal to standing unaided.

RESULTS

Extraction and phytochemical screening of *Datura metel* L. seed extract: The 2 kg of the dried seed powder of *Datura metel* L. yielded 123.5 g 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^{-1} 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^{-1}.

Evaluation of the anaesthetic effect of the extract in dogs: At dose rate of 1.2, 1.5 and 2 g kg^{-1} the seed extract of *Datura metel* L. produced excitement followed by mild sedation in four dogs. In another set of four dogs the extract was administered at the dose rate of 2.4 g kg^{-1} and they reacted in 3 stages. The onset of the action for each of the four dogs was 5 min post administration of the extract characterised by restlessness and excitement that lasted for 30 min each. This was followed immediately by sedation that progressed to induction of anaesthesia with loss of anal sphincter tone, pupillary reflexes and protrusion of the tongue from the dog's mouth.

This stage lasted for 80 min in each of the dogs thus duration of anaesthesia for each dog was 110 min. Physiologic data (rectal temperature, heart rate, respiratory rate and tissue oxygen saturation) were recorded from the dogs before administration of the extract to form the base line data. The physiologic parameters were monitored alongside the anaesthetic indices and their electrocardiogram and recorded at these three stages 15, 45 and 75 min post administration of the extract (Table 1-9). All the dogs recovered from anaesthesia uneventfully.

Table 1: Physiologic parameters of dog 1 administered the extract at 2.4 g kg^{-1} orally

Parameters	Base line	15 min	45 min	75 min
Temperature (°C)	37.5	37.5	37.5	37.5
Heart rate (bpm)	107.0	214.0	214.0	200.0
Respiratory rate (cpm)	36.0	41.0	38.0	37.0
SPO_2 (%)	98.0	94.0	95.0	96.0

Table 2: ECG parameters of dog 1 administered the extract at 2.4 g kg^{-1} orally

Parameters	Base line	15 min	45 min	75 min
P. wave amplitude (mV)	0.1	Negligible	0.30	0.3
QRS amplitude (mV)	0.3	0.4	1.40	1.1
	0.4	0.6	1.70	1.3
T. wave amplitude (mV)	0.1	Not visible	0.20	Negligible
Q. wave amplitude (mV)	Not visible	Not visible	-0.20	Not visible
S. wave amplitude (mV)	Not visible	Not visible	-0.70	Not visible
PR interval (sec)	0.12	0.08	0.08	0.08
QRS interval (sec)	0.04	0.04	0.04	0.04
QT interval (sec)	0.16	Not visible	0.25	Not visible
RR interval (sec)	0.56	0.28	0.28	0.3

Table 3: Anaesthetic indices of dog 1 administered the extract at 2.4 g kg^{-1} orally

Parameters	Base line	15 min	45 min	75 min
Analgesia	Absent	Absent	Absent	Absent
Anal sphincter tone	Constricted	Constricted	Relaxed	Relaxed
Skeletel muscle relaxation	Not relaxed	Not relaxed	Relaxed	Relaxed
Pupillary reflex	Present	Present	Absent	Absent

Table 4: Physiologic parameters of dog 2 administered the extract at 2.4 g kg^{-1} orally

Parameters	Base line	15 min	45 min	75 min
Temperature (°C)	38	38	38	38
Heart rate (bpm)	107	188	188	188
Respiratory rate (cpm)	38	43	40	39
SPO_2 (%)	96	93	94	94

Table 5: ECG parameters of dog 2 administered the extract at 2.4 g kg^{-1} orally

Parameters	Base line	15 min	45 min	75 min
P. wave amplitude (mV)	0.1	Negligible	0.40	0.4
QRS amplitude (mV)	0.2	0.4	1.40	1.2
	0.4	0.6	1.60	1
T. wave amplitude (mV)	0.1	Not visible	0.30	Negligible
Q. wave amplitude (mV)	Not visible	Not visible	-0.10	Not visible
S. wave amplitude (mV)	Not visible	Not visible	-0.70	Not visible
PR interval (sec)	0.12	0.08	0.12	0.12
QRS interval (sec)	0.06	0.04	0.06	0.06
QT interval (sec)	0.16	Not visible	0.25	Not visible
RR interval (sec)	0.56	0.32	0.32	0.32

Table 6: Anaesthetic indices of dog 2 administered the extract at 2.4 g kg^{-1} orally

Parameters	Base line	15 min	45 min	75 min
Analgesia	Absent	Absent	Absent	Absent
Anal sphincter tone	Constricted	Constricted	Relaxed	Relaxed
Skeletel muscle relaxation	Not relaxed	Not relaxed	Relaxed	Relaxed
Pupillary reflex	Present	Present	Absent	Absent

Table 7: Physiologic parameters of dog 3 administered the extract at 2.4 g kg^{-1} orally

Parameters	Base line	15 min	45 min	75 min
Temperature (°C)	38	38	38	38
Heart rate (bpm)	107	214	188	188
Respiratory rate (cpm)	35	40	38	37
SPO_2 (%)	98	94	95	96

Table 8: ECG parameters of dog 3 administered the extract at 2.4 g kg^{-1} orally

Parameters	Base line	15 min	45 min	75 min
P. wave amplitude (mV)	0.1	Negligible	0.40	0.4
QRS amplitude (mV)	0.9	1.2	1.40	1.1
	1.2	1.4	1.70	1
T. wave amplitude (mV)	Negligible	Not visible	0.30	Negligible
Q. wave amplitude (mV)	Not visible	Not visible	-0.20	Not visible
S. wave amplitude (mV)	Not visible	Not visible	-0.80	Not visible
PR interval (sec)	0.1	0.06	0.07	0.07
QRS interval (sec)	0.04	0.04	0.04	0.04
QT interval (sec)	0.16	Not visible	0.16	Not visible
RR interval (sec)	0.56	0.28	0.32	0.32

Table 9: Anaesthetic indices of dog 3 administered the extract at 2.4 g kg^{-1} orally

Parameters	Base line	15 min	45 min	75 min
Analgesia	Absent	Absent	Absent	Absent
Anal sphincter tone	Constricted	constricted	Relaxed	Relaxed
Skeletel muscle relaxation	Not relaxed	Not relaxed	Relaxed	Relaxed
Pupillary reflex	Present	present	Absent	Absent

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.* (2003), Wannag *et al.* (2009) and Kutama *et al.* (2010). 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.* (1990) 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 2nd week after seed germination, peaks at the 10th week (Afsharypuor *et al.*, 1995; Iranbakhsh *et al.*, 2006).

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 (1983) and published by the Centre for Disease Control (CDC) United State of America, states when the tested substance is administered to mice at a dose rate of 5000 mg kg^{-1} and does not cause toxicity or death in the tested animal the product (s) is said to be relatively safe.

The observed onset of pharmacological action of the extract at 5 min post administration orally in dogs is an indication that the extract is rapidly metabolised and the duration of the first pass mechanism is relatively short as described by Aliu (2007) in the metabolism, absorption and excretion of anaesthetics.

The extract at the dose rate of 2.4 g kg^{-1} produced pharmacologic effect in the dogs in the following stages:

Stage 1 (Induction): Time from administration to when the dogs become recumbent.

Stage 2 (Excitement): The dogs though recumbent but conscious and maintenance of all the reflexes with increased heart and respiratory rates, reduced tissue oxygen saturation (SPO_2) but within the normal range and maintenance of the rectal temperature. The extract could be assumed to cause an initial peripheral vasodilatation resulting in reduced rate of blood carrying oxygen supply to the tissues. The reduction in SPO_2 triggers a compensatory mechanism by increasing the heart and respiratory rate to increase the rate of blood carrying oxygen supply to the tissues thus increase or maintain the level of the tissue oxygen saturation. This explanation agrees with the explanation giving by Aliu (2007), on the effect of anaesthetics on the cardiovascular and respiratory systems of anaesthetic patients. The maintenance of the body temperature agrees with the pharmacology of hyoscyamine as explained by Aliu (2007), one of the alkaloids content of the plant described by Van. It acts by blocking all the body secretions including the sweat glands which are responsible for the body thermal regulation. As a result, the body temperature could either be maintained or elevated depending on the severity of its action which is related to the dose ingested or injected (hyoscyamine).

Stage 3 (Surgical anaesthesia): The dogs were unconscious there was loss of anal sphincter tone, loss of pupillary light reflex, loss of laryngeal reflex, good skeletal muscle relaxation and poor analgesia. The anaesthetic indices' observed at this stage, agrees with those earlier mention by Aliu (2007) and Anonymous (2012). The three stages recorded above are also similar to the three pharmacological stages of anaesthesia as described earlier by Aliu (2007) and Anonymous (2012). The poor analgesic property observed was also reported earlier by Wannang *et al.* (2009) also Aliu (2007) state that no single drug is capable of achieving balanced anaesthesia rather several different categories of drugs are utilised.

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, Babalola (2013) with good anaesthetic indices at the oral dose rate of 2.4 g kg^{-1} in dogs.

ACKNOWLEDGEMENTS

Head of Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences ABU Zaria and their Technical Staff for their cooperation and for allowing me to use their laboratory facilities for the seed extraction. DR. S. Yaqub of Department of Veterinary Physiology, Faculty of Veterinary Medicine, ABU Zaria for helping out with the ECG. DRS. Lawal Maruf and family, Mohammed Abdulrahaman, Iliyasu Dauda, Mohammed Saidu Tanko (ST), Ebenezer Abidoye, Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine A.B.U. Zaria, Audu Solomon, Department of Veterinary Medicine, Faculty of Veterinary Medicine A.B.U. Zaria. Thank you for your moral, kind and financial support towards the success of this research.

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