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Evaluation of Anaesthetic Effects of Orally Administered Crude Methanol Seed Extract of *Datura metel* L. – Bromazepam Combination for Experimental Surgical Exploration in Dogs

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Abstract: This work was carried out to evaluate the suitability of conducting experimental surgical procedures under orally induced anaesthesia with crude methanol seed extract of *Datura metel* L. - Bromazepam combination in dogs. Three (3) apparently healthy Nigerian dogs were administered the combinations of Bromazepam at the dose rate of 2mg/kg with crude methanol seed extract of *Datura metel* L at the dose rate of 2.4g/kg. Laparatomy/gastrotomy surgical procedure was carried out during anthesis. The physiologic parameters and some anaesthetic indices of the dogs were evaluated. The induction of sedation/anaesthesia at 6 minutes, 52 seconds was smooth and devoid of excitement and restlessness, this was followed by progressive unconsciousness, loss of anal sphincter tone, loss of pupillary light reflex, good skeletal muscle relaxation and mild analgesia. The physiologic parameters assessed included the measurement of heart rate, respiratory rate; rectal temperature and tissue oxygen saturation were within the normal ranges. The combined dosages of the crude methanol seed extract of *Datura metel* L (2.4 g/kg) and Bromazepam (2 mg/kg) administered orally to the three dogs was found to be inadequate as the three dogs reacted involuntarily when incision was made on the linear alba to expose the abdominal cavity. In order to maintain humane surgical protocol in handling of the dogs in this study, this necessitated the maintenance of anaesthesia with subcutaneous administration of Ketamine at the dose rate of 15 mg/kg. There was no intraoperative complication due to the anaesthetic combinations used for the surgical procedures. All the patients recovered uneventfully from the anaesthesia.

Key words: *Datura metel* L. · Bromazepam · Dogs · Anaesthesia · Surgery · Laparatomy · Gastrotomy

INTRODUCTION

Building on the previous work carried out by Babalola *et al.* [1] and Idara [2] carried out a study "Evaluations of Orally Administered Crude Methanol Seed Extract of *Datura metel* L. - Bromazepam Combination for the Induction of Balanced Anaesthesia in Dogs". In his preliminary study, the dose of crude methanol seed extract of *Datura metel* L. was increased from 2.4g/kg [1] to 3.5g/kg (At an incremental dose of 0.2g/kg). The undesirable effects such as excitement, restlessness and phobia may be observed in the treated dogs if the depth of sedation/anaesthesia and analgesia could be increased to facilitate surgical procedure. However these were not achieved as the undesirable effects were exaggerated with increase in dose characterised by dilated pupil, foaming from the mouth that progresses to dried mouth with the dogs using their fore limbs vigorously to scratched the palate, stomach wrenching and vomiting. In another group of dogs, in which the combinations of Bromazepam at the dose rate of 2mg/kg with the crude methanol seed extract of *Datura metel* L at the dose rate of 2.4g/kg were administered orally [2], the additive sedative effects of Bromazepam were observed in the dogs and were characterised by smooth induction and mild analgesia.

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This study was undertaken to explore the possibility of conducting laparatomy/gastrotomy surgical procedures following orally administered Crude Methanol Seed Extract of *Datura Metel* L- Bromazepam Combination for the induction of anaesthesia in dogs.

MATERIALS AND METHODS

Equipments: Standard steam sterilized general surgical instruments were used and the consumables included suture materials (Catguts and Nylon) and gauze.

Patient multi parameters monitor ((Ari 800C) Ari Technology (Group) Co., Limited, The Fourth Industrial Zone, The 15th Building, Gongming Town, Shenzhen P.R. China) and Syringes and needles were used. Bathroom scale (Camry® China).

Reagents and Solvents: Tween80 (Tween® 80 (P1754 SIGMA-ALDERICH; Sigma-Alderich Chemie-GmbH Munich, Germany).

Distilled Water

Drugs: The following drugs were used in this work, Ketamine hydrochloride (KETANIR®, manufactured in India by NIRMA LIMITED (Healthcare Division) Sachana,Gujarat 382150, India). Bromazepam (Talen®, made in Nigeria by: Swiss pharma Nigeria LTD., 5, Dopemu Road, Agege – Lagos). Crude methanol seed extract of *Datura metel* L and Analgesic (Diclowin® (Diclofenac Sodium Inj. 75mg/3ml HUBEI TIANYAO PHARMACEUTICAL CO LTD. No 7 Dufu Block, Jianshe Road, Xiangyan Hubei China).

Experimental Animals: This study was performed in accordance to the institutional animal care and use Committee (IACUC) of College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. Three (3) apparently healthy male Nigerian indigenous breed of dogs of ages one to three years, sourced from the local market with mean weight of 13kg±0.365kg were used. The dogs were acclimatised for 2 week in the small animal kennel of the department of Veterinary Surgery and Radiology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike and fed on homemade food once a day and water adlibitum. During this period the dogs were treated with, Ivermectin Super® (1ml/50kg) (Merial Saude Animal Limited Brazil) for ectoparasites and were also vaccinated against rabies using the freeze-dried rabies (Low Egg Passage) Flurry vaccine (National Veterinary Research Institute Vom, Plateau State, Nigeria).

Methods: The plants collection, identification, extraction, phytochemical tests, acute toxicity test and preparation of the extract solution (40%) has been described by Babalola *et al.* [1].

Presurgical Preparation: The dogs were fasted for 6 hours by withholding food and water. Detailed presurgical evaluations of the dogs were carried out after allowing them to acclimatize for two weeks. The respiratory rate, pulse rate and rectal temperature of each dog were documented. Following the determination of the individual weights of the dogs using the bathroom scale (Camry® China) method, the dogs were prepared for laparatomy/gastrotomy surgical procedures by shaving liberally the ventral area from the xiphoid cartilage to the umbilicus of each dog.

Anaesthetic Protocol: The dogs being in stable physiologic conditions and their individual body weight already determined, each dog was administered with a mixture of 2.4 g/kg crude methanol seed extract of *Datura metel* L and 2 mg/kg Bromazepam which was earlier crushed into coarse powder in a filter paper and homogenised with the extract in a 20 ml syringe by vigorous manual shaking of the syringe before administering to dogs orally. There after the dogs were evaluated and prepared for surgery.

Surgical Procedure: Standard routine procedures for the performance of surgeries were strictly adhered to and presurgical protocols were also observed [3, 4]. The ventral abdominal area from the xiphoid process to the umbilicus was shaved and prepared aseptically for surgical procedures. The animals were placed on dorsal recumbency and skin incision made on the ventral midline from the xiphoid to the umbilicus through the subcutaneous tissues exposing the linea Alba. The linea Alba was grasped with rat toothed thumb forcep and a stab incision made on it, the incision was extended for easy access into the abdominal cavity. Exploratory laparatomy was carried out to locate and exteriorised the stomach on wet sterile gauze. About 3cm length gastrotomy incision was made to expose the gastric mucosa.

The gastrotomy incision was closed using size 2 chromic catgut with Lambert suture pattern. The linea Alba and the subcutaneous tissues were closed separately with size 2 chromic catgut using continuous suture pattern. The skin was closed with size 2 nylon using Horizontal suture pattern. The duration of the procedure by the surgeon was documented for each patient.

Parameters Assessed: Base line parameters of each of the dogs were assessed and noted before the treatment and they served for comparison with the treated. All parameters were assessed and recorded at interval of 10 minutes during the surgical procedure, thereafter at an interval of 30 minute post surgery until the animal recovered from anaesthesia.

Temperature, Heart Rate, SpO_2 (Tissue Oxygen Saturation) and Respiratory Rate: Were monitored and recorded simultaneously using a multipurpose patient monitoring machine.

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

Analgesia/Pain: This was evaluated from the interdigital web of the hind limb using a rat toothed haemostatic forcep on a simple descriptive scale (SDS) of 0 to 3 (0 – No pain (No reaction at third ratchet lock), 1 – Mild pain, 2 – Moderate pain (Reaction at second ratchet lock is scored) and 3 – Severe pain (Reaction at first ratchet lock)).

Anal Sphincter Reflex (ASR): ASR was assessed on a scale of 0 to 5 using the Digital Rectal Examination Scoring System (DRESS) (0 – No discernible pressure (Anal sphincter relaxed), 3 – Normal (Anal sphincter in mild contracture, slight digital pressure penetrates the sphincter) and 5- Anal sphincter extremely tight) [5].

Pupillary Reflexes: Pen light was used to assess the pupillary reflex, by visually observing the pupil for relaxation.

Skeletal Muscle Relaxation: It was assessed on a scale of 0 to 5 using Modified Ashworth Scale (0 – 1, No muscle resistance to flexion and extension of the limb, 2 – Mild resistance to the range of movement (ROM), 3 – Mark resistance to ROM, 4 – Considerable resistance to ROM and 5 - Muscle rigidity) [6].

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.

Duration of Recumbency: Time from recumbency to sternal and from sternal to standing unaided.

Data analyses were done using the Statistical Package for the Social Sciences (SPSS) software version 22. Results were expressed as mean±standard errors of mean. A one-way ANOVA test was used to analyze the mean differences and the significant differences between means were declared at probability level of 5 percent ($p \leq 0.05$).

RESULTS

The induction of sedation/anaesthesia at 6 minutes, 52 seconds was smooth and devoid of excitement and restlessness, this was followed by progressive unconsciousness, loss of anal sphincter tone, loss of pupillary light reflex, good skeletal muscle relaxation and mild analgesia (Table 1).

Base on the earlier findings of Idara [2] that any increase in the dose of the crude methanol seed extract of *Datura metel* L, beyond 2.4 g/kg resulted in the dogs manifesting signs of toxicity detrimental to dog, the researcher resolved not to increase the dose. In order to maintain humane surgical protocol in handling of the dogs in this study, this necessitated the maintenance of anaesthesia with subcutaneous administration of Ketamine at the dose rate of 15 mg/kg [7, 8] as the oral route could not be tolerated by the dogs because of their state of unconsciousness, abolition of the swallowing reflex and recumbency.

There was no intraoperative complication due to the anaesthetic combinations used for the surgical procedures. All the patients recovered uneventfully from the anaesthesia.

The physiologic parameters assessed (Heart rate, Respiratory rate, Rectal temperature and Tissue oxygen saturation were also within the normal ranges (Table 2).

Table 1: Anaesthetic Parameters Assessed During the Course of the Surgery

Time (Minute)	0	10	20	30	40	50	60	90	120	150	180
Analgesia/Pain	3	1	1	1	1	2	2	2	3	3	3
Skeletal Muscle Reflex	5	2	0 - 1	0 -1	0 -1	0 -1	0 -1	2	2	3	5
Anal Sphincter tone	3	2	0	0	0	0	0	1	2	3	3
Pupillary Reflex	C	MC	D	D	D	D	D	D	MC	MC	C
Time of Onset of Anaesthesia	3.50±0.34										
Induction Time of Anaesthesia	6.00±0.52										
Duration of Anaesthesia	98.33±1.41										

Different superscripts (*) in a row indicate significant differences between means at the level of probability (p = 0.05), constricted (C), Dilated (D), mild constricted (MC)

Table 2: The Physiologic Parameters Assessed During the Course of the Surgery.

Time (Minute)	0	10	20	30	40	50	60	90	120	150	180
Heart Rate (Beat/Min)	94.50 ±1.23	100.83 ±2.87	99.67 ±1.15	98.33 ±1.41	97.17 ±0.70	95.67 ±1.15	95.50 ±0.85	95.50 ±0.85	95.50 ±0.85	95.50 ±0.85	97.17 ±0.70
Respiratory Rate (Cycle/Min)	27.50 ±1.12	30.33 ±1.99	28.17 ±1.42	25.50 ±0.96	23.00 ±1.10	22.00 ±0.89	21.67 ±0.95	23.00 ±1.10	25.50 ±0.96	25.50 ±0.96	25.50 ±0.96
Temperature (Celsius)	38.28 ±0.22	38.33 ±0.22	38.22±0.17	38.33 ±0.22	38.33 ±0.22	38.33 ±0.22	38.22±0.17	38.33 ±0.22	38.22±0.17	38.33 ±0.22	38.33 ±0.22
SPO2 (%)	-	98.33 ±1.41	97.17 ±0.70	98.33 ±1.41	95.67 ±1.15	95.67 ±1.15	95.67 ±1.15	97.17 ±0.70	97.17 ±0.70	97.17 ±0.70	95.67 ±1.15

Different superscripts (*) in a row indicate significant differences between means at the level of probability (p = 0.05)

DISSCUSSION

The observed onset of pharmacological action of the combined dosages of the extract and Bromazepam at a mean time of approximately 4 minutes post administration orally in the dogs agrees with the time reported by Idara [2]. Thus, it can be said that Bromazepam relatively shortened the duration of the first pass mechanism as described by Hall *et al.* [9] in the metabolism, absorption and excretion of drugs.

The induction of sedation/anaesthesia was smooth and devoid of excitement and restlessness which also agrees with the observation by Idara [2] who reported smooth induction of sedation/anaesthesia in his own work. This was followed by progressive unconsciousness, loss of anal sphincter tone, loss of pupillary light reflex, good skeletal muscle relaxation and mild analgesia. The results of the physiologic parameters assessed were within the normal range and similar to what was reported by Babalola *et al.* [1] and Idara [2]. In another studies Healther [10] and Roche [11] also reported that Bromazepam has a complementary sedative property when combine with psychoactive drugs. It produces sedative effect without antagonizing the effects of the anaesthetic drugs and also maintains normal pulse rate, heart rate and tissue oxygen saturation.

The maintenance of rectal temperature which was recorded in this work agrees with similar observations by Babalola *et al.* [1] and Idara [2] when the extract was administered alone and when combined with Bromazepam respectively. This may be associated with one of the alkaloids contents of the plant (hyoscyamine) as described by Van [12]. The alkaloid acts by blocking the secretory glands in the body including the sweat glands which are responsible for the body thermal regulation, thus, the body temperature is either maintained or elevated depending on the quantity of the ingested or injected hyoscyamine which is responsible for the severity of its action.

The mean duration of anaesthesia was 100 minutes, same as was recorded by Idara [2] and 10 minute less than what was reported by Babalola *et al* [1].

The combined dosages of the crude methanol seed extract of *Datura metel* L (2.4 g/kg) and Bromazepam (2 mg/kg) administered orally to the three dogs was found to be inadequate as the three dogs reacted involuntarily at an average time of 10 minutes into surgery when incision was made on the linear alba to expose their abdominal cavity.

The mild analgesia observed in this study which though inadequate as the dogs reacted involuntarily at an average time of 10 minutes into the surgery as incision was made on the linea alba could be associated with additive sedative effect of Bromazepam as reported by Healther [9] and Roche [11].

CONCLUSION

Oral administration of Bromazepam at the dose rate of 2 mg/kg combined with the crude methanol seed extract of *Datura metel* L. at the dose rate of 2.4 g/kg was not adequate for the conduction of surgical procedures.

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