

Evaluation of the Effect of Crude Methanol Seed Extract of *Datura metel* L - Bromazepam Combination Orally Administered on Some Physiologic and Anaesthetic Indices in Dogs

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Abstract: This work was undertaken to evaluate the effect of crude methanol seed extract of *Datura metel* L-Bromazepam combination orally administered on some physiologic and anaesthetic indices in dogs. The extract at the dose rate of 2.4 g/kg was homogenised with the fine powder of Bromazepam at the dose rate of 2 mg/kg in a 20 mL hypodermic syringe and administered orally to six Nigerian indigenous breed of dogs of mean weight 13 ± 0.368 kg. A one-way ANOVA test was used to analyze the mean differences. Results were expressed as mean \pm standard errors of mean. The combination induced smooth sedation and anaesthesia without excitement and restlessness with initial increased heart and respiratory rate while maintaining normal rectal temperature, adequate tissue perfusion, good muscle relaxation but poor analgesia, loss of anal sphincter tone and loss of pupillary reflex. These parameters were recorded at intervals of ten minutes until the dogs recovered. All the dogs recovered uneventfully. This study showed that the combination of Bromazepam with the crude methanol extract of *Datura metel* L eliminated the excitement, restlessness and phobia exhibited by the dogs when administered the seed extract alone. When combined with crude methanol seed extract of *Datura metel* L modify the effect of crude methanol seed extract of *Datura metel* L (Solanaceae) on the physiologic parameters and some anaesthetic indices in the dogs.

Key words: Datura Metel L. • Bromazepam • Dogs • Anaesthesia • Seed Extract • Oral

INTRODUCTION

Datura metel L. family Solanaceae. Common name: Devil's trumpet. 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 [1]. The leaves are simple, alternate, estipulate and triangular to ovate and measure about 18cm \times 13cm in length. Lamina is dentate, pointed petiole and asymmetric base [2]. Inflorescence occurs as a cyne with erect nearly white flowers. Both the calyx and corolla are tubular and trumpet shaped about 26cm long [3]. Fruits are capsules, round (1.25 inches in diameter), dehiscent and covered with blunt prickles or warts, usually pale green [4]. 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 (antispasmodic, ant-tussive and bronchodilator [5, 6] and in several ways especially for its psychoactive activities [3].

Babalola [7] and Babalola, *et al.* [8] Showed that the crude methanol seed extract of *Datura metel* L exhibits the three pharmacological stages of anaesthesia with good anaesthetic indices, poor analgesia and some initial undesirable manifestations by the dogs (Hysteria, struggling, Muscle spasm and phobia) at the oral dose rate of 2.4 g/kg in dogs. An ideal general anaesthetic would possess a number of essential characteristic; it must be easily and painlessly administered, induce a rapid loss of consciousness without causing voluntary or involuntary struggling, it must give adequate analgesia and muscular relaxation at the minimum level of dosage

required to cause loss of consciousness, have a short recovery period without excitement; and be compatible with pre-medication and ancillary therapeutic [9]. However, no single anaesthetic is known to possess these entire features, but by combining drugs with complementary pharmacological actions, an approximation of the ideal one can be achieved, this combination of complementary drugs is called balanced anaesthesia [9].

Bromazepam is a benzodiazepine derivative [10, 11]. Its molecular structure composed of diazepine connected to a benzene ring and a pyridine ring, the benzene ring is having a single nitrogen atom that replace one of the carbon atoms in the ring structures [10]. The 1, 4-benzodiazepine means that, the nitrogen on the seven-side diazepine ring is in 1 and 4 positions [10, 11]. Bromazepam bind to GABA receptor, causing a conformational change and increasing the inhibitory effect of GABA. It has long acting benzodiazepine and is lipophilic and metabolized in the liver via oxidative partway [10]. Bromazepam is usually used for short term treatment of anxiety, panic disorders, pre-medication for operation, sedation for minor surgical procedure and relieve fear, anxiety and act as a general tranquilizing agent [10, 11]. A combination of bromazepam with central nervous system depressant, (alcohol, muscle relaxant, antihistamine, narcotic analgesic and anaesthetic cause additive effects [10, 11]. Therefore, this study aimed to evaluate the effect of crude methanol seed extract of *Datura metel* L-Bromazepam combination orally administered on some physiologic and anaesthetic indices in dogs.

MATERIALS AND METHODS

Animals and Experimental Design: Six apparently healthy Nigerian indigenous breed of dogs of mean age one to three years. Animals were divided equally into males and females, sourced from the local market with mean weight of $13\text{kg} \pm 0.365\text{kg}$. The dogs were housed 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 *ad libitum*. The dogs were acclimatized for two weeks after which they were dewormed and vaccinated against rabies using the freeze-dried rabies (Low Egg Passage. Flurry) vaccine (National Veterinary Research Institute Vom, Plateau State, Nigeria).

The plants collection, identification, seed extraction, phyto-chemical tests, acute toxicity test and preparation of the extract solution (40%) according to Babalola *et al*

[8]. The extract solution was prepared using 2% of Tween® 80 (P1754 SIGMA-ALDERICH; Sigma-Alderich Chemie-GmbH Munich, Germany.) by adding 1mL of Tween® 80 to 49ml of injection water) this solution was then used to prepare 40% of crude methanolic seed extract of *Datura metel* L solution.

The dosage of 2.4 g/kg crude methanol seed extract of *Datura metel* L and 2 mg/kg Bromazepam were administered dogs orally after crushing into fine powder in a filter paper, then homogenised with the extract in a 20 ml syringe by vigorous manual shaking of the syringe until form a the homogenate, then dogs were evaluated for general anaesthesia.

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 until the animals regain consciousness.

Temperature, Heart rate, SpO₂ (Tissue Oxygen Saturation) and Respiratory Rate: Were monitored and recorded simultaneously using a multipurpose patient monitoring machine (ARI- 800C Patient Monitor (Ari Technology (Group) Co., Limited. The Fourth Industrial Zone, The 15th Building, Gongmin Town, Shenzhen, P.R. China).

Induction Time: Is the time between administration and the first sign of sedation.

Analgesia/Pain: This was evaluated from the inter-digital web of the hind limb using a rat toothed haemostatic forceps on a simple descriptive scale (SDS) of 0 to 3 where the scale 0 indicates no pain (no reaction at third ratchet lock), the scale 1 indicates mild pain, the scale 2 indicates moderate pain (reaction at second ratchet lock is scored) and the scale 3 indicates severe pain (reaction at first ratchet lock).

Anal Sphincter Reflex: Was assessed on a scale of 0 to 5 using the Digital Rectal Examination Scoring System (DRESS) (0 means no discernible pressure (anal sphincter relaxed), 3 means normal (anal sphincter in mild contracture, slight digital pressure penetrates the sphincter) and 5 means anal sphincter extremely tight) [12].

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

Skeletal Muscle Relaxation: This was assessed on a scale of 0 to 5 using Modified Ashworth Scale (0 – 1 means no muscle resistance to flexion and extension of the limb, 2 means mild resistance to the range of movement (ROM), 3 means mark resistance to ROM, 4 means considerable resistance to ROM and 5 means muscle rigidity) [13].

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 Recumbence: Duration between drug-induced recumbence and assumption of sternal posture.

Data Analysis: Data analysis was 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 was declared at probability level of 5 percent ($p \leq 0.05$).

RESULTS

The combination eliminates the excitement, phobia and restlessness exhibited by the dogs when the extract was used alone with good anaesthetic indices as shown in Table 2. The combination also shortens the induction time; maintain the physiologic parameters within the normal ranges as presented in Table 1. There was no significant difference between the means.

DISCUSSION

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 is shorter than the 5 minutes reported by Babalola, *et al.* [8] when he administered the extract alone, this time was also observed by Idara [14], thus, it can be said that Bromazepam exert a synergistic effect with the extract by relatively shortening the duration of the first pass mechanism on the metabolism, absorption and excretion of drugs [9].

Contrary to the observation of Babalola *et al.* [8], the induction of sedation to anaesthesia was smooth and devoid of excitement and restlessness which was also observed by Idara [14]. The dogs progresses to unconsciousness, with loss of anal sphincter tone, loss of pupillary light reflex, good skeletal muscle relaxation and mild analgesia. The physiologic parameters assessed were maintaining within the normal ranges as also reported by Babalola, *et al.* [8] and Idara [14]. These observations are also supported by Healthier [10] and Roche [11] that Bromazepam has a complementary sedative property when combine with psychoactive drugs; it produced sedative effect without antagonist to the effect of the anaesthetic drugs and also maintains normal pulse rate, heart rate and tissue oxygen saturation.

The maintenance of the rectal temperature was also observed by Babalola, *et al.* [4] when he administered the extract alone and Idara [14] when he combined the extract with Bromazepam. This could be associated with hyoscyamine, one of the alkaloids content of the plant described by Van [15]. It acts by blocking all the body

Table 1: Effect of the Crude Methanolic Seed Extract of *Datura metel* L. (2.4g/kg) – Bromazepam (2mg/kg) Combination on the Dogs Anaesthetic Indices.

Time (Minute)	0	10	20	30	40	50	60	70	80	90	100
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	Constriction	Mild Constriction	Dilated	Dilated	Dilated	Dilated	Dilated	Dilated	Mild Constriction	Mild Constriction	Constriction
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 (*) within row indicate significant differences between means at the level of probability ($p = 0.05$)

Table 2: Effect of the Crude Methanolic Seed Extract of *Datura metel* L. (2.4g/kg) – Bromazepam (2mg/kg) Combination on the Dogs Physiologic Parameters.

Time (Minute)	0	10	20	30	40	50	60	70	80	90	100
Heart Rate (Beat/Minute)	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/Minute)	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 ($p \leq 0.05$)

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).

The mean duration of anaesthesia observed was approximately 100 minutes which was less by 10 minutes compared to the report of Babalola, *et. al.* [8]. The variation in the duration of anaesthesia could be due to some individual intrinsic or extrinsic factors which were not measured, that could be associated with age, physiologic status of the animal, health status of the animal, absorption and metabolism of the drugs and the species of the animal [16].

The poor analgesic property observed was also reported earlier by Wannang *et al.* [1] and Babalola *et al.* [8].

CONCLUSION

This research work showed that Bromazepam at the dose rate of 2mg/kg when combined with the crude methanolic seed extract of *Datura metel* L at the dose rate of 2.4 g/kg has additive effects on the seed extract of *Datura metel* L on the dogs, resulting in smooth induction of anaesthesia devoid of phobia, excitement and restlessness at the above stated oral dose rate.

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