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Quality of Anaesthesia Induced Using Diazepam-Thiopentone Sodium in Donkeys

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ABSTRACT

The anaesthetic properties of Thiopentone sodium (T) were evaluated in 12 clinically healthy male donkeys premdicated with diazepam (D). Animals were divided into two groups each of six. Donkeys were pre-medicated with diazepam (0.1 mg/kg) intravenously 10 minutes prior to the injection of the anaesthetic; and then Thiopentone sodium was injected at dose rate of 8 mg kg^{-1} body weight intravenously in the first group (TH1) and at 12 mg/kg in the second group (TH2). The quality of induction, recovery, and muscle relaxation was assessed. Respiratory and heart rate and rectal temperature were recorded using standard methods. The different anaesthetic phases (induction time, anaesthetic phase, sternal recumbency, lateral recumbency, and recovery time) were measured before, during and after induction of anaesthesia. Selected blood biochemical parameters were assayed before anaesthesia (time 0), 30, and 60 minutes after induction. Considerable sedation/tranquilization without excitement was achieved following pre-medication. Increasing the dose of thiopentone sodium from 8 to 12 mg kg^{-1} resulted in no significant decrease in induction time ($P>0.05$) and significant ($P<0.05$) increase in the duration of anaesthetic phase, sternal and lateral recumbancy and recovery phase. The use of thiopentonal sodium in both protocols tested was accompanied by significant increase ($P<0.05$) in heart rate, while rectal temperature showed no significant fluctuation. Animals anaesthetized with both protocols showed significant decrease ($P<0.05$) in respiratory rate immediately following induction of anaesthesia. There was no-significant ($P>0.05$) change in all biochemical parameters tested. In conclusion, this simple anesthetic protocol can be used in donkeys and an acceptable anaesthesia with a reasonable recovery can be expected.

INTRODUCTION

In equids, thiobarbiturates have been used for decades to induce and maintain short term (10-15 minutes) general anaesthesia. In the earlier years these ultra-short acting barbiturates were the agents of choice to induce short term recumbency in tranquilized horses. Later premedications such as guaifenesin and more recently xylazine (or other α-2 adrenoceptor agonist type drugs) were combined with or administered immediately prior to the intravenous injection of the thiobarbiturates to improve the quality of anaesthetic induction and recovery (Mama, 2000). These improved techniques of general anaesthesia facilitated surgical and other therapeutic procedures requiring more prolonged recumbency (e.g. 30-60 minutes). However, as duration of barbiturate administration increased, the duration of recovery from anaesthesia become longer and the quality of recovery become more unpredictable and in some cases dangerous to the animal and associated personnel (Mama, 2000).

In Iraq, Abd-Almaseeh (2008) compared the quality of anaesthesia produced by propofol to that of thiopentone in xylazine premedicated donkeys. He reported that Thiopentone induced anaesthesia characterized by longer narcosis and abolishment of swallowing reflex, rapid induction time, longer recumbency period than propofol. AL-Heani (2010) evaluated the anaesthetic properties of Thiopentone and Thiopentone-propofol admixture in xylazine premdicated donkeys. He concluded that both protocols produced good anaesthesia in donkeys, but Thiopentone anaesthesia may be clinically usable technique for induction of anaesthesia in donkeys. In Sudan, general injectable anaesthesia in donkeys received considerable attention from scientists in the last decade. In Sudan, Radi *et al.*, (2012b) reported adverse cardiopulmonary effects following the increase of the dose of thiopentone from 10 to 15 mg/kg in donkeys received diazepam (0.5 mg/kg) as premedication. Recently, Abakar *et al.*, (2014) evaluated different combinations of Xylazine, Ketamine, and Diazepam (XKD) in attempts to produce safe and satisfactory total intravenous anaesthesia in donkeys that could be used under field conditions. They concluded that the use xylazine at 1.5 mg/kg, ketamine at 3 mg/kg and diazepam at 0.1, 0.2, and 0.3 mg/kg would produce anaesthesia in donkeys with acceptable muscle relaxation and recovery.

The objective of the current study was to evaluate the quality of anaesthesia induced using diazepam-Thiopentone sodium in donkeys and to evaluate some of the biochemical as well as cardio-respiratory parameters during general anesthesia in donkeys.

MATERIALS and METHODS

Place of study: this study was carried out at the farm of the College of Veterinary Medicine, Sudan University of Science and Technology located in Hillat Kuku, East Nile Locality, Khartoum North, Sudan.

Experimental animals: A total of 12 male, clinically sound adult donkeys, 4-10 years of age, with body weight range of 75-135 Kg. Animals were purchased from local market (*Alkriab)*. They were clinically examined to eliminate diseases and they received an anthelmintic (Albendazole, 10mg/kg) and an antibiotic (Penicillin G Procaine & Dihydrostreptomycin Sulfate Injection (Pen Strep 1ml/20kg)) as prophylactic treatment and kept for 2 weeks for

adaptation. Animals were weighed and randomly allocated into two treatment groups; they were allowed with free excess to water and feed twice a day (morning and the afternoon).

Experimental drugs: One pre-anaesthetic medication and one anaesthetic drug were used in this study.

1. Diazepam 10 mg 0.5% "USP" 2 ml/ampoule (Ropam®, L.B.S. Laboratory LTD., PART, 602 Soi Panichanant, Sukhumvit 71 Rd., Bangkok 10110 Thailand).
2. Thiopentone sodium 5 % (Thiopental sodium "BP" 500 mg/vial NEON Laboratories limited India).

Anaesthetic protocols: Two anaesthetic protocols were used to anaesthetize donkeys as follow:-

1. Thiopentone sodium (5 %) 8 mg/kg + Diazepam 0.1 mg/kg (TH Dz1).
2. Thiopentone sodium (5 %) 12 mg/kg + Diazepam 0.1 mg/kg (TH Dz2).

Preanaesthetic preparation: Animals were fasted overnight and water was withheld for 6- 8 hours prior to anaesthesia. Animals in the two groups were injected first with diazepam 0.1 mg kg^{-1} and then after 10 minutes were either subjected to thiopentone sodium at 8 or 12 mg/kg.

Time schedule: The physiological parameters (Heart rate, respiratory rate and rectal temperature) were reported at 0 time (immediately after Thiopentone was injected) and at 10 minutes - interval until recovery. Blood samples were collected for biochemical analysis at 30 minutes interval until recovery. Anaesthetic phases and reflexes were reported immediately.

Quality of induction: Quality of induction of anaesthesia was rated as satisfactory or unsatisfactory following the description of (Matthews *et al.*, 2002).

Recovery quality: A score, ranging from 1 to 5 as per method of (Ringer *et al.*, 2007) was used for assessment of quality of recovery from anaesthesia.

Physiological Parameters: Physiological Parameters (respiratory rate, heart rate and rectal temperature) were monitored at 10 minutes intervals using standard methods as described by Kelly (1974).

Phases of anaesthesia: different anaesthetic phases were monitored following standard methods as follows: Induction phase (Jani *et al.,* 1982), Anaesthetic phase (Tamisto *et al.,* 1981), Lateral recumbancy (Thurmon *et al.,* 1996), Sternal recumbancy (Ghurashi, 2007), and Recovery (Ghurashi, 2007).

Blood samples collection: Blood samples for serum analyses were withdrawn from the jugular vein in plain vacutainer tubes. Blood was allowed to clot and centrifuged and sera were separated and stored at - 20 °C until analyzed.

Biochemical methods: some blood biochemical parameters were analyzed using standard methods as follows: Serum glucose (Barham and Trinder, 1972), Serum Urea (Fawcett and Scott, 1960), and Aspartate aminotransferaze enzyme (AST) and Alanine aminotransferaze enzyme (ALT) (Reitman and Frankel, 1957).

Statistical analysis: T- Test was used to compare data for physiological parameters (Respiratory rate, Heart rate and Rectal temperature), and also to compare between the different anaesthetic phases, using statistical package for social science (SPSS) computer package.

RESULTS

Profound tranquilization/sedation and anxiolysis were apparent in donkeys within 10 minutes of premedication.

Induction of anaesthesia was smooth and uneventful. In the current study the quality of induction was characterized by its rapidness, and free from excitement, and the donkeys lay down to the floor smoothly and adopted lateral recumbency. The induction score was satisfactory for all donkeys in the two groups. As we could observe in Table (1), recovery was scored as good (donkeys remained calm but needed more than two attempts to stand) in four donkeys and very good (donkey remained calm and needed two attempts to stand) in two donkeys in the first group (TH Dz1); and good in three donkeys and very good in the other three donkeys in the second group (TH Dz2). Although donkeys were ataxic during recovery, they were able to control their stance.

Table 1: Quality of recovery following induction of anaesthesia using thiopentone sodium with diazepam

Protocols	No. of animals	Very poor		Poor		Good		Very good		Excellent	
		No	%	No	%	No	%	No	%	No	%
TH Dz1	6	0	0	0	0	4	66.7	2	33.3	0	0
TH Dz2	6	0	0	0	0	3	50	3	50	0	0

TH Dz1 = Thiopentone sodium 8mg.kg+ Diazepam 0.1 mg/kg
TH Dz2 = Thiopentone sodium 12mg.kg+ Diazepam 0.1 mg/kg

The effects of diazepam and Thiopentone combination on heart rate (HR), respiratory rate (RR) and rectal temperature (RT) are presented in Table (2). Heart rate increased significantly ($P<0.05$) after induction and the peak rate was recorded between 20 and 30 minutes (Table 2). A significant decrease ($P<0.05$) in respiratory rate was observed during the first 10 minutes following injection of Thiopentone sodium in the second group (THDz2) (Table 2). Rectal temperature showed no significant ($P>0.05$) increase in the first group while, in the second group there was significant increase when compared with time 0 (Table 2).

Table 2: Effect of intravenous anaesthesia using Thiopentone sodium at 8 or 12mg/kg in donkeys pre-medicated with diazepam at dose 0.1 mg/kg on respiratory rate, heart rate and rectal temperature

Time	TH Dz1			TH Dz2		
	HR	RR	RT	HR	RR	RT
Base	38.40± 2.99	26.4±2.7	37.24±0.25	44.40±0.80	31.2±4.18	38.32±0.11
10Diaz	35.5±0.95	23.04±1.34	37.3±0.20	51.20±4.18	26.40±3.84	38.52±0.16
0	63.6±4.44*	10.80±0.83*	36.84±0.25	75.0±4.48*	9.60±2.27*	38.76±0.24*
10	56.40±7.93	15.20±1.32*	36.8±0.22	74.80±3.78*	13.20±1.32*	38.96±0.28*
20	72.8±3.38*	20.0±2.36	36.78±0.31	77.60±2.02*	17.20±2.76*	39.14±0.33*
30	60.4±3.37*	20.2±1.72	37.04±0.20	75.40±4.23*	19.20±1.90	39.28±0.48*
40	55.8±2.10*	20.8±1.90*	36.9±0.29	74.0±3.18*	20.0±1.15*	39.26±0.36*
50	55.6±4.16*	18.0±2.36*	37.24±0.26	74.0±5.75*	19.20±1.97	39.36±0.34*
60	49.60±4.35	22.0±2.73	37.44±0.21	74.20±3.83*	21.20±0.97	39.42±0.40*

- Mean in the same column with asterisk are significantly different ($P<0.05$) with time zero

TH Dz1 = Thiopentone sodium 8mg/kg+ Diazepam 0.1 mg/kg
TH Dz2 = Thiopentone sodium 12mg/kg+ Diazepam 0.1 mg/kg

A transient apnoea was observed in the two anaesthetic protocols following induction of anaesthesia with Thiopentone sodium. There was no significant (P>0.05) increase in apnoea duration (Table 3). There was no significant difference in induction time, lateral recumbency (Table 3). While the analgesia, sternal recumbency and recovery phase exhibited significant increase (P<0.05).

Table 3: Duration of different anaesthetic parameters (Mean ± SE) measured following injection of thiopentone sodium at dose rate 8 or 12mg/kg in donkeys pre-medicated with diazepam at dose rate 0.1 mg/kg.

Parameters	Apnoea Seconds	Induction Seconds	Analgesia Minutes	Sternal recumbancy Minutes	Lateral recumbancy Minutes	Recovery Minutes
TH Dz1	30.71±2.77	11.57±0.57	14.72±1.82*	17.73±2.61*	16.67±1.11	29.86±4.54*
TH Dz2	33.85±3.66	8.28±2.11	27.48±3.32*	29.86±5.44*	20.50±7.78	34.34±12.16*

*Means in the same column with asterisk are significantly different (P<0.05)
TH Dz1 = Thiopentone sodium 8mg/kg+ Diazepam 0.1 mg/kg
TH Dz2 = Thiopentone sodium 12mg/kg+ Diazepam 0.1 mg/kg

No significant (P>0.05) change was observed in glucose and urea concentration and AlT and AST activities during anaesthesia (Table 4).

Table 4: Effect of intravenous anaesthesia using thiopentone sodium at 8 or 12 mg/kg in donkeys premedicated with diazepam at 0.1 mg/kg on some blood biochemical

Time Min.	TH Dz1				TH Dz2			
	Glucose	urea	ALT	AST	Glucose	Urea	ALT	AST
0	4.32±0.33	6.05±0.65	2.19±0.48	48.89±4.11	3.19±0.78	4.74±1.07	1.63±0.46	49.23±18.22
30	4.20±0.38	5.66±0.49	3.14±0.7	48.9±4.11	3.47±0.43	5.98±0.60	2.21±0.40	47.02±22.28
60	3.60±0.20	6.38±0.68	1.51±0.11	40.62±13.44	4.55±0.22	6.07±0.55	24.19±11.96	56.68±12.48

TH Dz1 = Thiopentone sodium 8mg/kg+ Diazepam 0.1 mg/kg
TH Dz2 = Thiopentone sodium 12mg/kg+ Diazepam 0.1 mg/kg

Discussion

In the current study the quality of induction was satisfactory and characterized by rapidness, and free from excitement which is in agreement with previous results (Al-Heani, 2010; and Abd Almaseeh, 2008). In this study, heart rate significantly increased between 10 and 20 minutes in both anaesthetic protocols. The increase in heart rate after Thiopentone injection has been reported (Taylor 1990). Diazepam in combination with Thiopentone sodium (2.5 mg kg^{-1} + 20 mg kg^{-1}) exhibited increase in heart rate in New Zealand Rabbits (Mohammed *et al.*, 2011). Our result is also consistent with that observed in sheep where Thiopentone administration increased heart rate (Runciman *et al*., 1997 and Huang *et al.*, 1997). However, as the increase in our study began after administration of Thiopentone, it was probably as a result of this drug.

In this study, injection of diazepam and Thiopentone sodium was accompanied by a reduction in respiratory rate. Although, benzodiazepines were reported to have moderate depressant effects on respiratory system, considerable variations among species exist (Rail, 1990). The reduction in respiratory rate following diazepam injection may be attributed to its muscle relaxing properties. Barbiturates were reported to have potent respiratory depressant effects (Gaudy *et al.*, 1983). Incidence of respiratory depression as a consequence of using Thiopentone was described (Taylor 1990; Bennett *et al.*, 1998; Luna and Taylor 2001). No significant

increase in body temperature was observed in rectal temperature following diazepam-Thiopentone administration a result which is comparable with other studies (Ghurashi *et al.,* 2007, Luna and Taylor 2001, Radi *et al.*, 2011, and Mohammed *et al.*, 2011).

The occurrence of apnoea following induction of anaesthesia using Thiopentone sodium with diazepam in this study is in agreement with other studies (Ghurashi *et al.*, 2007, Radi *et al.*, 2011, 2012a,b). Those authors reported that the occurrence of apnoea following induction of anaesthesia with thiopentone sodium in goats and donkeys, respectively. In this study, the increase in the dose of Thiopentone sodium from 8 to 12 mg/kg was accompanied by no significant increase in the duration of apnoea.

The significant increase in anaesthetic phase and recovery time may be attributed to the increase in Thiopentone sodium dose. A result that was partially in agreement with that reported by Radi *et al.*, (2011) who stated that the increase in thiopentone sodium from 10 to 15 mg kg-1 was accompanied by significant increase in sternal recumbency and total recovery and prominent non significant increase in anaesthetic phase. This finding is supported by the general anaesthetic characters of thiopentone sodium described by Hall *et al.,* (2010), who stated that the anaesthetic effect of thiopentone sodium is directly affected by the dose of the drug. Our finding is also supported by Rawling and Kolata (1983) although they used doses, which different from the doses used in this study.

Previous studies demonstrated that benzodiazepines prolong barbiturate-induced anaesthesia (Chambers and Jefferson, 1977). The mechanism by which benzodiazepines and barbiturates synergistically interact is not fully understood. However, this additive effect could be attributed to the fact that both drugs interact aith γ-aminobutyric $acid_A$ ($GABA_A$) receptor/chloride ionophore complex, the chief inhibitory transmitter system in the brain (De Lorey *et al.,* 1993).The no significant prolongation of the lateral phase could be attributed to the administration of diazepam which reported to cause lateral recumbancy and more difficulty in maintaining standing position in man and horses respectively (Muir and Masonen, 1982 and Vickers *et al.,* 1984).The no significant increase in AST values in the second group are most likely to be caused by muscle damage. A similar result was obtained by Radi *et al.*, (2011) in donkeys. However, the mild relative increase of these enzymes in comparison with other reports (Duke *et al.*, 2006), and lack of any clinical signs of myositis, lameness and myoglobinuria, suggested that there was minimal muscle damage.

Although post-anesthesia blood urea nitrogen (BUN) values showed no significant increase, but they were still within the reference range, a result that is partially agree with that has been reported previously (Steffey *et al.*, 1980, Radi *et al.*, 2011).

The lack of severe cardio-respiratory changes during anesthesia was indicative of acceptable clinical levels of anaesthesia. Our results suggest that donkeys response to these protocols was considered acceptable under field conditions but further research is needed to determine how they respond to other drugs.

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