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CLINICAL STUDIES ON THIOPENTONE WITH OR WITHOUT DIAZEPAM PREMEDICATION FOR GENERAL ANAESTHESIA IN DONKEYS

M.A.A. RADI; H.I. SERI* and M.A.H. GHURASHI**

* College of Veterinary Medicine, Sudan University of Science and Technology, Sudan.
** Open University of Sudan.
Corresponding author: Dr. Hisham Ismail Seri, department of Medicine and Surger, College of Veterinary Medicine, SUST. Khartoum North, P.O. Box: 204. E-mail: hishamseri@yahoo.com

ABSTRACT

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In this study, side or toxic effects that may follow the use of thiopentone sodium with or without diazepam in donkeys were observed, through monitoring haematological serum biochemical components of donkeys. This study was conducted using 18 clinically healthy male donkeys, 4-10 years of age, weighing 80-150 kg. The animals were divided to three groups (6 animals of each), and treated with six different protocols containing thiopentone sodium alone (at 10 and 15 mg/kg) or with diazepam (at 0.25 and 0.5 mg/kg). Animals of each group were repeatedly used to study these treatments at interval of 3 weeks from the end of each treatment (as washing period of the drug). They were used alternatively in this study. Some serum biochemical parameters: Total Serum Proteins, Albumin, urea, Glucose, Phosphorus, Aspartate aminotransferase (AST), and alanine aminotransferase (ALT), were evaluated before anaesthesia and at 30, 60, and 90 minutes. Haematological parameters (haemoglobin (Hb), packed cells volume (PCV) and (WBC) count), were recorded. Animals treated with thiopentone sodium at dose rate of 10 mg/kg body weight without premedication resulted in significant increase in haemoglobin (Hb) concentration, while animals subjected to diazepam 0.25 mg/kg and thiopentone sodium 15 mg/kg expressed significant decrease in WBC count. Blood urea nitrogen concentration was significantly increased after anesthesia. Blood glucose was significantly increased; AST and ALT were significantly increased after anesthesia. Animals injected with thiopentone sodium at dose rate of 10 mg/kg body weight exhibited significant ($P<0.05$) increase in the concentration of serum glucose, serum urea, and ALT level. Increasing the dose of Thiopentone sodium to 15 mg/kg body weight resulted in non-significant ($P>0.05$) changes in all parameters tested. Animals injected with thiopentone sodium at dose rate of 10 mg/kg + diazepam 0.25 mg/ kg body weight resulted in significant decrease in serum albumin, serum phosphorus, AST, and showed significant ($P<0.05$) increase in serum ALT. Animals injected with thiopentone sodium at dose rate of 15mg/kg + diazepam 0.25 mg/kg body weight resulted in significant ($P<0.05$) increase in total serum protein (TSP), and serum ALT. Animals injected with thiopentone sodium at dose rate of 10 mg/kg + diazepam 0.5 mg/kg body weight resulted in significant increase in serum glucose. Animals injected with thiopentone sodium dose rate of 15 mg/kg + diazepam 0.5 mg/kg body weight resulted in significant decrease in serum urea. Conclusion and clinical relevance: increasing the dose of diazepam to 0.5 mg/kg is too toxic to be used with thiopentone at higher doses.

Keywords: *Thiopentone sodium, Diazepam, donkeys, biochemical constituents*

INTRODUCTION

Thiopentone sodium is used in different animal species for induction of anaesthesia and basal narcosis and maintenance of surgical anaesthesia. It was used in wide range of animal species such as dogs (Kumar *et al.,* 1983); goat kids (Ghurashi*,* 2007); horses, (Taylor, 1990); and buffaloes, (Kumar and Sharma, 1986).

Anaesthetic induction dose of thiopental is 7- 20 mg/kg in the un pre-medicated animal, but recommended initial dose is 5 – 7 mg/kg of 2.5% solution to avoid overdose in goats (Hall *et al.*, 2001).

In horses, diazepam clinically causes muscle fasiculations, weakness, and ataxia at doses sufficient to cause sedation. Doses greater than 0.2 mg/kg may induce recumbancy as a result of its muscle relaxant properties and general CNS depressant effects, severe respiratory depression or cardiovascular complications (Susan and Donald*,* 2003). When administrated alone, the drug overdoses are generally limited to: coma, decreased reflexes, hypotension, respiratory depression and cardiac arrest, drowsiness, mental confusion, impaired motor functions (impaired reflexes, impaired coordination, impaired balance), and dizziness (Bendarzewska-Nawrocka *et al.,* 1980).

Diazepam increases the central depressive effects of alcohol, other hypnotics/sedatives (e.g. barbiturates), narcotics, and other muscle relaxants. The euphoriant effects of opioids may be increased, leading to increased risk of psychological dependence (Holt, 1998).

Based on the growing population of donkeys and lack of any report on anaesthesia concerning the use of thiopentone and diazepam in this breed, our objectives were to evaluate some of the haematological and biochemical parameters during general anaesthesia in this breed.

MATERIALS and METHODS

Eighteen healthy male donkeys, aged 4-10 years were purchased from a local market in Nyala, South Darfur state, Sudan. The health status of animals was confirmed before commencement of the study by clinical examination.

Food, but not water, was withheld for 12 hours. Baseline values of haematological and biochemical parameters were recorded before injection of the drugs and in a quiet environment. Each of the donkeys was pre-medicated with an intravenous injection of 0.25 or 0.50 mg kg^{-1} diazepam 0.5% ("BP" 2ml/ampoule, 5 mg/ml, Shanghai Pharmaceutical CO., Ltd. Shanghai China, CMS Sudan) via intravenous catheter (21G) in the jugular vein. Anaesthesia was induced 10 minutes after diazepam administration with a bolus of 5% sodium-thiopental (10 or 15 mg kg^{-1}) IV; thiopental sodium "BP" 500mg /vial NEON Laboratories limited, India). 'Time zero' was set at the time the donkeys were injected with thiopentone.

Haematological (haemoglobin, packed cell volume, and total number of leukocytes and biochemical parameters (total serum protein, Albumin, Urea, Glucose, P, AST, and ALT) were analyzed pre-anaesthesia and at 30, 60, and 90 after baseline reading.

*Anaesthetic protocols***:** Six different anaesthetic protocols were tested in this investigation as follows:

1. Thiopentone sodium at dose rate of 10 or 15 mg kg^{-1} BW (without premedication).
2. Thiopentone sodium at dose rate of 10 or 15 mg kg^{-1} BW +Diazepam 0.25 mg kg^{-1} BW.
3. Thiopentone sodium at dose rate of 10 or 15 mg kg^{-1} BW + Diazepam 0.50 mg kg^{-1} BW.

Each group of animals was monitored before anaesthesia, during anaesthesia and till recovery.

*Blood collection***:** Blood samples for whole blood and serum were withdrawn from jugular vein. Whenever blood samples were taken they were divided into two portions. One portion was allowed to clot and centrifuged and sera were separated and stored at (-20) °C until analyzed. The other one was taken in a vacuntainer tube containing (EDTA) as anti-coagulant for investigating the haematological parameters.

*Haematological methods***:** In the different experiments haemoglobin packed cell volume and white blood cell count were measured using standard methods according to (Kelly, 1984).

Biochemical methods: the selected biochemical parameters were measured using standard methods as follows: Total serum protein was analyzed using Biuret method (King and Wooton, 1956). Serum albumin was measured using Bromocresol green (BCG method) according to Bartholomew and Delany (1966). Serum glucose was determined according to Barham and Trinder (1972). Serum urea concentration was measured according to Fawcett and Scott (1960). The serum inorganic phosphorus was determined according to Goldenburg and Fernandez, (1966). Aspartate aminotransferaze enzyme (AST) (Glutamic oxaloacetic transaminase "GOT"), and Alanine aminotransferaze enzyme (ALT) (Glutamic pyruvic transaminase "GPT") were measured according to Reitman and Frankel (1957).

*Statistical analysis***:** The obtained parametric data were compared statistically with pre-anaesthetic values using ANOVA for repeated measures ($P < 0.05$) using SPSS System computer package. The results are expressed as mean ± SD.

RESLUTS

Packed cell volume (PCV): As shown in Tables (1, 2 and 3) animals treated with thiopentone sodium 10 and 15 mg /kg body weight with or without premedication resulted in non-significant change in (PCV) values and this remained during the observation period of the experiments when compared with the base value at time 0.

Haemoglobin concentration (Hb): As shown on Table (1) conduction of anaesthesia in animals treated with thiopentone sodium 10 and 15 mg /kg body weight with or without premedication resulted in significant changes ($P<0.05$) in (Hb) value and this remained during the duration of the observation period of experiments when compared with the base value (0 time).

White blood cells count (WBC): As shown on Table (1) animals treated with thiopentone sodium 10 mg and 15 mg /kg body weight resulted in non-significant changes in (WBC). As illustrated in Table (2) animals treated with thiopentone sodium 10mg / kg-diazepam 0.25 mg /kg body weight resulted in non-significant changes in (WBC) when compared with the base value (0 time) and animals treated with thiopentone sodium 15 mg /kg-diazepam 0.25mg /kg showed significant ($P<0.05$) decrease in (WBC) at time points 60 and 90 minutes following induction of anaesthesia when compared with the base time (time 0).

In Table (3) animals treated with thiopentone sodium 10mg/kg-diazepam 0.5 mg /kg body weight resulted in significant ($P<0.05$) changes in (WBC) when compared with the base rate (0 time) and in animals treated with thiopentone sodium 15 mg /kg-diazepam 0.25mg /kg animals passed due to respiratory failure and hence changes in heart rate were not detected.

*Biochemical parameters***:** As shown in Table (4) animals injected with thiopentone sodium 10mg / kg body weight exhibited non-significant ($P>0.05$) changes in total serum protein, serum albumin, serum phosphorus, serum GOT, and showed significant ($P<0.05$) increase in serum glucose at time 60 and 90 minutes following injection of thiopentone sodium, significant ($P<0.05$) increase in serum urea at time 60 and 90 minutes, and significant ($P<0.05$) increase in GPT in time 30 minutes. In Table (5) animals injected with thiopentone sodium 15mg / kg body weight resulted in non-significant ($P>0.05$) changes in all parameters tested. In Table (5) animals injected with thiopentone sodium 10 mg/kg + diazepam 0.25mg/kg body weight resulted in non-significant ($P>0.05$) changes in total serum protein, serum glucose, serum urea significant ($P<0.05$) decrease in serum albumin at time 30 minute, serum phosphorus at time 30 and 90 minute, serum GOT in time 30, 60 and 90 minutes, and showed significant ($P<0.05$) increase in serum GPT in time 90 minute. In Table (7) animals injected with thiopentone sodium 15 mg/kg + diazepam 0.25 mg/kg body weight resulted in significant ($P<0.05$) increase in total serum protein (TSP) in time 90 minute, non-significant change in serum albumin, serum glucose, serum urea, serum phosphorus, serum GOT, and showed significant ($P<0.05$) increase in serum GPT at time 30 ,60 and significant decrease at 90 minutes. In Table (8) animals injected with thiopentone sodium 10 mg/ kg + diazepam 0.5 mg/ kg body weight resulted in non- significant ($P>0.05$) change in total serum protein and serum albumin, significant ($P<0.05$) increase in serum glucose at time 90 minute, non-significant changes in serum urea, serum phosphorus, serum GOT, and serum GPT.

But in Table (9) animals injected with thiopentone sodium 15mg/kg + diazepam 0.5 mg/kg body weight resulted in non- significant ($P>0.05$) change in total serum protein, serum albumin, serum glucose, significant ($P<0.05$) decrease in serum urea at time 30, 60 and 90 minute, non- significant changes in serum phosphorus, serum GOT, and serum GPT.

Table (1): Effect of thiopentone at 10 and 15 mg kg^{-1} on some haematological parameters.

Treatment	Thiopentone 10 mg Kg^{-1}			Thiopentone 15 mg Kg^{-1}		
Time	PCV	Hb	WBC	PCV	Hb	WBC
0	24.17±1.25	7.03±0.44	6.76±0.45	26.00±0.97	8.87±0.44	8.65±0.67
30	26.50±2.11	8.83±0.33*	6.51±0.10	26.00±1.69	9.07±0.35	8.49±0.56
60	24.17±1.70	7.13±0.38	6.30±0.68	25.67±0.99	8.87±0.38	8.51±0.71
90	24.83±1.74	7.40±0.31	7.13±0.11	25.67±0.71	9.37±0.46	8.21±0.57

* Mean in the same column with asterisk are significantly different ($P<0.05$) when compared with time zero.

Table (2): Effect of thiopentone at 10 and 15 mg kg^{-1} in donkeys premedicated with diazepam at dose 0.25 mg kg^{-1} on some haematological parameters.

Treatment	Thiopentone 10 mgKg^{-1}+ Diazepam 0.25 mg kg^{-1}			Thiopentone 15 mg Kg^{-1}+ Diazepam 0.25 mg kg^{-1}		
Time	PCV	Hb	WBC	PCV	Hb	WBC
0	24.17±0.54	8.90±0.36	7.72±0.29	24.00±1.57	8.97±0.72	10.11±0.96
30	24.17±0.65	9.37±0.37	7.43±0.51	24.50±0.65	10.55±0.72	8.81±0.94
60	24.17±1.08	9.08±0.34	8.13±0.70	24.75±0.85	9.85±0.78	7.51±0.33*
90	24.67±1.28	9.30±0.30	6.43±0.59	26.00±0.71	9.88±0.56	7.83±0.48*

* Mean in the same column with asterisk are significantly different (P<0.05) when compared with time zero.

Table (3): Effect of thiopentone at 10 and 15 mg kg^{-1} in donkeys premedicated with diazepam at dose 0.5 mg kg^{-1} on some haematological parameters

Treatment	Thiopentone 10 mg Kg^{-1}+ Diazepam 0.5 mg kg^{-1}			Thiopentone 15 mg Kg^{-1}+ Diazepam 0.5 mg kg^{-1}		
Time	PCV	Hb	WBC	PCV	Hb	WBC
0	25.50±1.69	8.78±0.36	8.01±0.13	23.67±0.67	8.67±0.31	7.33±0.52
30	27.33±3.28	9.47±0.87	8.50±0.21	ND	ND	ND
60	27.67±2.33	8.93±0.59	9.05±0.30	ND	ND	ND
90	30.00±3.06	9.33±0.77	10.03±0.27	ND	ND	ND

* Mean in the same column with asterisk are significantly different (P<0.05).
ND = not detected.

Table (4): Effect of thiopentone sodium at dose 10 mg kg^{-1} (without premedication) on some biochemical constituents of donkeys serum

Parameter	TSP	Albumin	Glucose	Urea	Phos	GOT	GPT
0	9.12±1.28	1.28±0.26	23.22±12.82*	56.44±10.48	4.45±1.01	8.67±7.94	66.17±8.61
30	10.03±2.35	1.52±0.51	33.90±8.58	61.30±15.08	4.37±1.08	10.00±5.51	82.67±8.53*
60	8.08±2.10	1.57±0.48	56.17±30.18*	84.45±24.66*	4.63±1.38	10.33±6.15	69.17±3.19
90	8.75±1.72	1.63±0.42	38.85±26.54	85.57±12.73*	3.25±0.88	4.33±6.98	69.50±6.12

* Mean in the same column with asterisk are significantly different (P<0.05) when compared with time zero.

Table (5): Effect of thiopentone sodium at dose 15 mg kg^{-1} (without premedication) on some biochemical constituents of donkeys serum

Parameter	TSP	Albumin	Glucose	Urea	Phos	GOT	GPT
0	11.34±1.39	1.44±0.27	24.01±9.60	33.53±7.86	4.53±1.27	18.17±7.44	13.83±7.03
30	9.50±2.45	1.60±0.24	23.98±9.33	36.27±10.18	5.16±2.35	13.00±4.74	13.20±5.72
60	9.78±2.98	1.55±0.39	34.31±10.97	36.69±6.66	4.90±2.77	15.50±6.56	10.25±5.56
90	10.90±1.93	1.78±0.05	22.95±10.20	39.93±10.43	4.22±2.39	21.40±13.22	14.60±8.23

Table (6): Effect of treatment with thiopentone at 10 mg kg^{-1} in donkeys premedicated with diazepam at 0.25 mg kg^{-1} on some biochemical constituents of donkeys serum

Parameter	TSP	Albumin	Glucose	Urea	Phos	GOT	GPT
0	6.48±0.84	1.80±0.14	68.37±20.38	63.32±22.65	5.47±1.91	35.67±14.74	15.33±6.66
30	7.15±2.19	1.25±0.39*	46.78±16.07	47.66±17.33	3.03±1.43*	14.50±4.55*	7.00±2.10
60	6.62±1.11	1.30±0.58	52.94±20.13	52.50±15.84	4.03±1.19	20.83±6.05*	6.00±3.10
90	7.74±2.62	1.24±0.22	48.82±21.42	47.75±9.67	2.78±1.06*	15.00±6.52*	43.20±35.30*

* Mean in the same column with asterisk are significantly different (P<0.05) when compared with time zero.

Table (7): Effect of treatment with thiopentone at 15 mg kg^{-1} in donkeys premedicated with diazepam 0.25 mg kg^{-1} on some biochemical constituents of donkeys serum

Parameter	TSP	Albumin	Glucose	Urea	Phos	GOT	GPT
0	8.13±0.37	1.35±0.58	27.84±13.65	86.76±12.88	2.23±0.60	11.50±3.87	70.75±2.50
30	9.30±2.40	1.00±0.00	43.51±20.66	75.63±10.10	2.50±0.28	10.00±0.00	77.00±0.00*
60	7.83±0.12	1.23±0.67	33.23±22.54	78.01±12.40	2.17±0.25	15.00±1.73	84.67±2.89*
90	10.60±0.69*	1.73±0.45	58.24±28.63	67.86±9.23	2.50±0.40	17.67±8.08	19.67±4.62*

* Mean in the same column with asterisk are significantly different (P<0.05) when compared with time zero.

Table (8): Effect of treatment with thiopentone at 10 mg kg^{-1} in donkeys premedicated with diazepam 0.5 mg kg^{-1} on some biochemical constituents of donkeys serum

Parameter	TSP	Albumin	Glucose	Urea	Phos	GOT	GPT
0	7.00±0.83	2.00±0.50	25.88±12.68	40.17±11.50	5.22±1.96	22.75±9.43	6.60±6.47
30	6.77±0.60	1.73±0.46	32.47±9.40	42.46±23.08	4.70±1.74	35.00±21.17	12.33±4.51
60	7.27±1.01	1.63±0.42	30.44±8.66	46.81±7.33	3.53±1.55	31.67±8.08	13.00±3.61
90	7.27±1.11	1.87±0.25	52.35±26.60*	55.06±19.17	6.47±3.11	31.00±14.42	14.33±9.29

* Mean in the same column with asterisk are significantly different (P<0.05) when compared with time zero.

Table (9): Effect of treatment with thiopentone at 15 mg kg^{-1} in donkeys premedicated with diazepam 0.5 mg kg^{-1} on some biochemical constituents of donkeys serum

Parameter	TSP	Albumin	Glucose	Urea	Phos	GOT	GPT
0	10.90±0.96	2.03±0.58	12.17±1.47	84.94±4.15	3.43±0.50	14.00±1.73	12.00±0.00
30	10.87±0.31	1.70±0.17	9.67±4.16	53.82±15.93*	2.40±0.17	13.00±3.00	6.00±2.00
60	8.90±3.11	1.80±0.14	18.76±0.14	40.67±19.36*	5.55±4.17	23.00±5.66	12.50±6.36
90	10.73±3.33	1.67±0.74	13.76±4.10	40.33±14.95*	4.27±2.96	31.67±18.45	10.33±6.51

* Mean in the same column with asterisk are significantly different (P<0.05) when compared with time zero.

DISCUSSION

This investigation is directed towards testing the merits and advantages to be gained by using thiopentone sodium in combination with two different doses of diazepam (0.25 mg/kg and 0.5mg/kg) with emphasis on toxicodynamics of tested anaesthetic protocols on haematological and biochemical values together with the dose of anaesthesia as well as study of some undesirable side effects of these protocols in donkeys.

Induction of anaesthesia with thiopentone sodium at dose 10 mg/kg resulted in significant increase in (Hb) at time 30 minutes in this study and using of thiopentone sodium at dose 10 or 15 mg/kg with diazepam resulted in non significant change in (Hb), packed cell volume (PCV), and White blood cell count (WBC) these findings are in agreement with findings of Edjtehadi (1978) who reported that Packed cell volume (PCV) and haemoglobin (Hb) were significantly decreased in thiopentone anaesthetized sheep, but not in animals anaesthetized by thiopentone/halothane. White blood cell count (WBC) was significantly decreased only in thiopentone/methoxyflurane anaesthetized sheep, but was not remarkable in thiopentone anaesthetized animals (Edjtehadi, 1978).

The induction of anaesthesia with thiopentone sodium with or without diazepam resulted in significant increase in serum urea, serum glucose, and serum aspartate aminotransferase (GOT) and these results were in agreement with the results reported by Seddighi and Mohri (2007), who reported that diazepam-acepromazine, thiopentone, and halothane anaesthesia in Caspian ponies resulted in considerable sedation/tranquilization without excitement, significant increase in serum urea nitrogen, and significant increase in serum glucose and significant increase in aspartate aminotransferase (AST) were also observed.

The increase in AST values are most likely to be caused by muscle damage. 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 BUN values were still within the reference range, they increased significantly as has been reported previously (Steffey *et al.,* 1980). This increase could be as a result of a mild renal hypoperfusion but resolved rapidly and did not appear to be of clinical significance.

CONCLUSION

Although, the changes in the different serum biochemical parameters tested expressed statistical difference, at some time points, but they were still within the reference normal values of Sudanese donkeys.

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دراسات اكلينيكية حول استخدام عقار الثيوبنتون منفردا ومسبوقا بعقار استخدامه مع الديازيبام لاحداث التخدير العام في الحمير

محمد عبد الله عبد النعيم راضي ، هشام اسماعيل سري ، محمد أحمد حسن قرشي

في هذه الدراسة تمت ملاحظة بعض الآثار السامة التي قد تعقب استخدام الثايوبنتون صوديوم مع أو من غير الديازيبام (الفاليوم) لاحداث التخدير العام في الحمير، وذلك من خلال مراقبة بعض مكونات الدم وأيضاً مكونات مصل الدم الكيموحيوية. اجريت هذه الدراسة علي ١٨ من ذكور الحمير الخالية اكلينيكيا من الامراض تراوحت اعمارها بين ٤-١٠ سنوات واوزانها بين ٨٠ – ١٥٠ كجم. قسمت الي ثلاثة مجموعات ٦ حمير في كل مجموعة وقد تم استخدام ستة بروتوكولات لاحداث التخدير كالآتي:- عقار الثايوبنتون صوديوم بجرعة مقدارها ١٠ و ١٥ مجم/كجم (من غير علاج تمهيدي) . وعقار الثايوبنتون صوديوم بجرعة مقدارها ١٠ و ١٥ مجم/كجم + ديازيبام بجرعة مقدارها ٠.٢٥ و ٠.٥٠ مجم/كجم. تم استخدام الحيوانات في المجموعات المختلفة بصورة متكررة و ذلك بعد فترة ثلاث اسابيع من اعطاء العقارات (كفترة للتخلص من الدواء). حيث تم استخدام المجموعات بالتعاقب. تم قياس بعض محتويات المصل الحيوية (بروتين المصل، الالبيومين، اليوريا، الجلكوز، والفسفور وانزيمي AST و ALT). أيضاً تم قياس محتويات الدم (الهيموجلوبين، حجم خلايا الدم المتكدسة وعدد خلايا الدم البيضاء). في هذه الدراسة وجد ان استخدام عقار الثايوبنتون صوديوم بجرعة ١٠ مجم/كجم أدي الي زيادة معنوية في تركيز الهيموقلوبين بينما استخدام الديازيبام كعلاج تمهيدي بجرعة مقدارها ٠.٢٥ مجم/كجم مع الثايوبنتون بجرعة ١٥ مجم/كجم أدي الي انخفاض معنوي في عدد خلايا الدم البيضاء. أيضاً وجد في هذه الدراسة ان استخدام عقار الثايوبنتون صوديوم بجرعة ١٠ مجم/كجم ادي الي زيادة معنوية في قياسات اليوريا والجلوكوز وانزيم ALT. كما أن زيادة الجرعة لعقار الثايوبنتون صوديوم الي ١٥ مجم/كجم لم تؤد الي تغيير معنوي في قياسات محتويات المصل الحيوية. أيضاً وجد في هذه الدراسة ان استخدام عقار الثايوبنتون صوديوم بجرعة ١٠ مجم/كجم الديازيبام بجرعة ٠.٢٥ مجم/كجم ادي الي زيادة معنوية في انزيم AST ، أما استخدام عقار الثايوبنتون صوديوم بجرعة ١٠ مجم/كجم و١٥ مجم/كجم مع الفاليوم بجرعة ٠.٥ مجم/كجم لم تؤد الي تغيير معنوي في قياسات محتويات الدم الحيوية وادت الي انخفاض معنوي في مستوي اليوريا وزيادة معنوية في قياس الجلوكوز.