

Short-term anaesthesia using Propofol, Xylazine, Diazepam with or without Ketamine combination in Miniature donkeys

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Abstract: To determine the most reliable safe and satisfactory short term anaesthesia in donkeys; two combinations of anaesthetic agents were assessed. Ten donkeys were randomly divided into two groups (five animals for each). Group(I) was allotted for protocol (I) (Xylazine (X), Diazepam (D), Ketamine (K) and Propofol (P) ; Group (II) for protocol (II) (Xylazine (X), Diazepam (D) and Propofol (P). Donkeys in protocol (I) were anaesthetized with diazepam(D) (0.1mg/kg), ketamine (K) (2.2mg/kg) propofol(P) (2mg/kg), while those in protocol(II) was anaesthetized with diazepam (D) (0.1mg/kg), and propofol (P) (2mg/kg) intravenous respectively five minutes later of premedication with xylazine(X)(0.5mg/kg) in both groups. Respiratory rate (RR), heart rate (HR) and rectal temperature (RT) were measured before and 10 minutes intervals throughout the duration of anaesthesia. Induction, duration of anaesthesia, selected reflexes and recovery were qualitatively and quantitatively assessed. Blood samples were collected from each animal before, one hour and 24 hours of anaesthesia to test the effect of both protocols on liver and kidney functions. It could be concluded that, addition of ketamine in protocol I provide a relative good anaesthetic effect in donkeys.

Keywords: Short term anaesthesia, Propofol, Xylazine, Diazepam, Ketamine, donkeys

INTRODUCTION

No injectable anaesthetic produces all the components of general anaesthesia without depressing some vital organ function. Because the available drugs have rather selective actions within the central nervous system, combinations of drugs are necessary to provide surgical anaesthesia without depressing vital functions. Total intravenous anaesthesia refers to the production of general anaesthesia with injectable drugs only. The advantage of total intravenous anaesthesia is its facility to provide its component of anaesthesia with a dose of a specific drug [1]. Due to meager facilities available for field veterinarians in Egypt, intravenous anaesthetics are preferred because of their early and safe induction. Choral hydrate has been the popular sole anaesthetic agent so far relied upon by the veterinarians for most surgical interventions in equine. Its unavailability or irregular availability in local market renders the veterinary surgeons incapable of performing any interventions on equine surgical patients. This situation compels the veterinary surgeons to look for some other suitable and safe alternative readily procurable from the market having better or an equivalent spectrum of anaesthesia.

Ketamine is classified as a dissociative anaesthetic, it can be injected intravenously [2]. It is composed of two isomers and it produces anaesthetic and analgesic effects and the analgesic effect seems to be greater than the anaesthetic effect [3]. The effect of ketamine became apparent rapidly after its injection and the drug produces profound analgesia with poor muscle relaxation and muscle tone is often increased [2]. The drug is described as unique drug because it has hypnotic, analgesic and amnesic effect [4].

Diazepam is a popular benzodiazepine derivative for use in different animal species [5]. The drug has a dose dependent effect [6]. The drug was reported to have minimum effect on respiratory system, heart rate and rectal temperature [2]. The drug was reported to cause good muscle relaxation and can be used in cure convulsions [7].

Propofol is a unique non-barbiturate, non-steroid, short-acting general intravenous anaesthetic agent [8]. It is associated with a rapid smooth induction and a rapid recovery. Anaesthetic stage duration of propofol could be enhanced if used in combination with ketamine hydrochloride [9]. The drug has been used in

equine species, and was found to have a desirable pharmacokinetic profile in horses, i.e., rapid onset of action, short duration of anaesthesia and prompt recovery, even following continuous infusion or supplementary dose administration. Studies of combination of propofol with alpha 2- agonist (xylazine or detomidine) [10] and benzodiazepine [11] or ketamine [12], reported to have additive anaesthetic effects and to decrease the dose of propofol required to maintain surgical anaesthesia in human beings and animals. Premedication with either xylazine or detomidine improved the quality of anaesthesia produced by a single bolus of propofol 2mg/kg [13,14].

MATERIALS AND METHODS

Ethical approval

The study conducted complies with the guidelines laid down by the Faculty of Veterinary Medicine, South Valley University Ethical committee.

Site of study

This study was conducted at the premises of the Department of Surgery, Anaesthesiology and Radiology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.

Drugs

Diazepam: “Bp” 2ml/ampoule (10mg/ml, Memphis Co. for Pharm. & Chemical Ind. Cairo – Egypt) was used as premedication.

Propofol 10mg/ml, 200mg propofol in 20ml/ampoule, Astra Zeneca 2007-2009 manufactured by Cordon Pharma SpA, Caponago, Italy).

Ketamine 50mg (as hydrochloride) Produced by Sigma-Tec Pharmaceutical Industries, Egypt-S.A.E.)

Xylazine HCL 23.3mg Eq.to 20mg xylazine base. ADWIA Co. S.A.E. 10th of Ramadan City. Egypt)

Experimental animals

A total of 10 apparently healthy female miniature donkeys, 1-7 yrs old, 80 – 150 kg body weight, were purchased from Local Market at Qena city. Animals were clinically examined and received anthelmintic (Albendazole, 10 mg/kg) and antibiotic (Penicillin G Procaine & Dihydrostreptomycin Sulphate) as prophylactic treatment and kept for 15 days for adaptation. Animals were randomly allocated into two different groups (I and II); they were allowed free excess to water and feed twice a day.

Experimental design

The animals were randomly allocated into two equal groups (five each). Water and food were withheld for 24 hours prior to induction of anaesthetic drugs. Intravenous catheter (21) was used for injecting the drugs through the jugular vein. Animals in group (I) were treated with (Xylazine 0.5 mg/kg slowly intravenous, followed after 5 minutes by diazepam 0.1 mg/kg, ketamine 2.2 mg/kg and Propofol 2.0 mg/kg), while animals in the other treatment group (II) received (Xylazine 0.5 mg/kg slowly intravenous, followed after 5 minutes by diazepam 0.1 mg/kg, Propofol 2.0 mg/kg) . Anaesthetic and some physiological parameters were investigated immediately. Each group of animals was monitored before anaesthesia, during anaesthesia and until complete recovery.

Quality of induction of anaesthesia:

The quality of induction of anaesthesia was evaluated as follows:

Satisfactory: rapid and smooth with little danger to animal or personal. Unsatisfactory: prolonged period of in-coordination muscle fasciculation [15].

Quality of recovery

A score ranging from 1 to 5 according to [16] was used for assessment of quality of recovery from anaesthesia as follows:

Table-1: A score used for assessment of quality of recovery from anaesthesia

Score	Quality	Character
1	Excellent	Donkey capable of standing at first attempt
2	Very good	Donkey remain calm and need two attempt to stand
3	Good	Donkey remain calm and need more than two attempt to stand
4	Poor	Excitement during recovery with danger of injury and needed more than two attempt to stand
5	Very poor	Severe excitement during recovery with danger of injury

Physiological parameters

Respiratory rate, heart rate and rectal temperature were monitored and recorded at 10 minutes intervals using standard methods as described by [17].

Anesthetic phases

The animals were monitored closely and remarks were reported immediately and phases of anaesthesia were determined as follows:

Induction phase

It is the time elapsed following injection of the anaesthetic to the state or condition in which the animal becomes unconscious, respond negatively to painful stimuli with disappearance of selected reflexes [18].

Anesthetic phase

This is considered as the period during which the animal shows signs of unconsciousness, no reflexes, responds negatively to painful stimuli [19].

Lateral recumbence

This is considered as the duration at which the reflexes are regained but it is incapable of adopting sternal position [20].

Sternal recumbence

It is considered as the period during which the animal could adopt sternal recumbency without falling to lateral recumbency and without adopting standing position [21].

Standing phase

It is the stage at which the animal stood but unable to walk ten steps [22].

Recovery

The animal was considered to be recovered from anaesthesia when it is capable of supporting itself in standing position and walk for ten steps without falling down [21].

Total recovery

Total recovery time was considered as the total time calculated from the time of induction of anaesthesia until recovery was attained [23].

Reflexes

The following reflexes were monitored closely and remarks were reported immediately as follows:

Tongue reflex

It was assessed by pulling the tongue outside the mouth, when the animal was capable to retract its tongue into the mouth, the reflex was considered positive [23].

Palpebral reflex

The reflex was assessed by digital touch on the canthus or eyelashes, if purposeful motor reflex observed, the reflex was considered positive [24].

Corneal reflex

Was assessed when blinking induced by gently touching the cornea and is usually lost after the palpebral reflex [25]

Swallowing reflex

External digital pressure on the larynx was used to assess swallowing reflex. Positive response was considered when swallowing or laryngeal movements were observed [26].

Pedal reflex

Pedal reflex was assessed by pinprick on the coronary band of the digit. If the animal moves its leg or leg muscle, the reflex was considered positive [27].

Anal reflex

Anal reflex was assessed by inducing tension of anal sphincter with two fingers. Positive response was

considered when the movement of the anal sphincter was noticed (Subjective).

Blood sampling and Serum collection:

At zero, 1 hr and 24 hrs post anaesthetic recovery, blood samples were collected via jugular puncture into clean dry test tubes, then left to clot at room temperature, and then centrifuged at 1200 r/m for 15 minutes. Sera were then, separated and stored at -20°C as aliquots for further biochemical analysis.

Serum biochemical analysis

Freshly separated sera were used to test the effect of the anesthetic drugs of both combinations on liver and kidney functions. Liver function was evaluated by measuring the levels of serum alkaline phosphatase (AP), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) enzymes in sera of experimental animals. The serum ALT and AST activities were measured according to [28], ALP according to [29]. The kidney function was evaluated by monitoring the urea and creatinine serum levels. Renal products; creatinine was determined according to [30] and urea was determined according to [31].

RESULTS

The pulse rate was significantly increased at 10 and 20 minutes intervals after induction of anaesthesia in both protocols (Table. 2), and then gradually decreased nearly to approach the baseline values at 50 minutes. The respiratory rate was significantly decreased in both protocols throughout the anaesthesia up till 50 minutes. These values were subsequently increased at 20 minutes to approximate the baseline values in both protocols (Table. 2). Similarly, the rectal temperature was significantly decreased in both protocols throughout the anaesthetic time to return nearly to the baseline values in both protocols (Table. 2). All animals were showing lowering the head, dropped ears, lower lip and abduction of the legs with some ataxia following xylazine injection. After diazepam injection falling down of all studied animals was noted and these animals were assumed lateral recumbency immediately post intravenous injection of propofol with snoring sound 10 minutes later from its injection. Severe lacrimation was observed during the anaesthetic time in animals treated with ketamine in protocol I. In three animals, falling down was characteristic in which the animal exhibit incomplete recumbence with rigid and extended legs along with twisted neck and raised head. The quality of induction in other animals in both protocols was satisfactory. Regarding to corneal reflex, it was disappeared in both studied groups after propofol injection and reappeared in time ranged from 15-20 minutes (mean time 19.6 minutes). After administration of respective anaesthetic agents, tongue reflex was absent in both two groups after propofol injection and still absent in group (II) in time ranged from 23-35 minutes (mean time 26.6 minutes), but in group (I), it

still absent in time ranged from 23-25 minutes (mean time 24 minutes). Swallowing reflex was reappeared after 40 minutes in both groups. The duration of different anaesthetic phases following induction of anaesthesia was significantly variable between two protocols (Table 3). The onset of anaesthesia in protocol I was more rapid than in protocol II, the anaesthetic phase, lateral and sternal recumbancy phases were also significantly increased ($p > 0.05$). Thus, the total recovery time was longer in protocol I (55.16 ± 1.55) versus (37.964 ± 1.39) in protocol II. (Table 3). The quality of recovery was similar in both protocols as it records very good in two animals (0.4%) and excellent in three (0.6%).

Effects on the Hepatic and Renal Systems

Serum biochemical analysis

Changes in serum biochemical values of AP, ALT and AST as well as Creatinine and Urea following (Xylazine- diazepam – propofol) and (Xylazine - diazepam - Ketamine - propofol) mixtures anaesthesia in donkeys at Zero time, 1hr and 24 hrs post anaesthesia recovery are summarized in fig. 1 (A, B, C, D, E & F) and fig. 2 (A, B, C & D), respectively. In (Xylazine-

diazepam – propofol) mixture anaesthetized donkeys, the serum biochemical analysis showed that, blood serum AP and ALT were significantly ($P \leq 0.05$) elevated at 1hr and 24 hrs, while AST was significantly ($P \leq 0.05$) increased at 24 hrs post anaesthesia recovery (Fig 1. A, C & E).

Regarding creatinine and urea serum blood levels, the obtained results displayed that creatinine was not significantly ($P \leq 0.05$) elevated at any time point, meanwhile blood urea nitrogen was significantly ($P \leq 0.05$) increased at 1hr but not significantly ($P \leq 0.05$) changed at 24 hrs post recovery (Fig. 2 A & C). In (Ketamine- xylazine- diazepam – propofol) mixture anaesthetized donkeys, the serum biochemical analysis showed that, blood serum AP and ALT were significantly ($P \leq 0.05$) elevated at 1hr, while not significantly ($P \leq 0.05$) changed at 24 hrs post recovery. On the contrary, AST was not significantly ($P \leq 0.05$) altered at any time point post recovery (Fig. 1 E & F). Concerning creatinine and urea serum blood levels, the obtained data clarified that creatinine was not significantly ($P \leq 0.05$) changed at any time point, while blood urea nitrogen was significantly ($P \leq 0.05$) increased at 1 hr 24 hrs post recovery (Fig. 2 B & D).

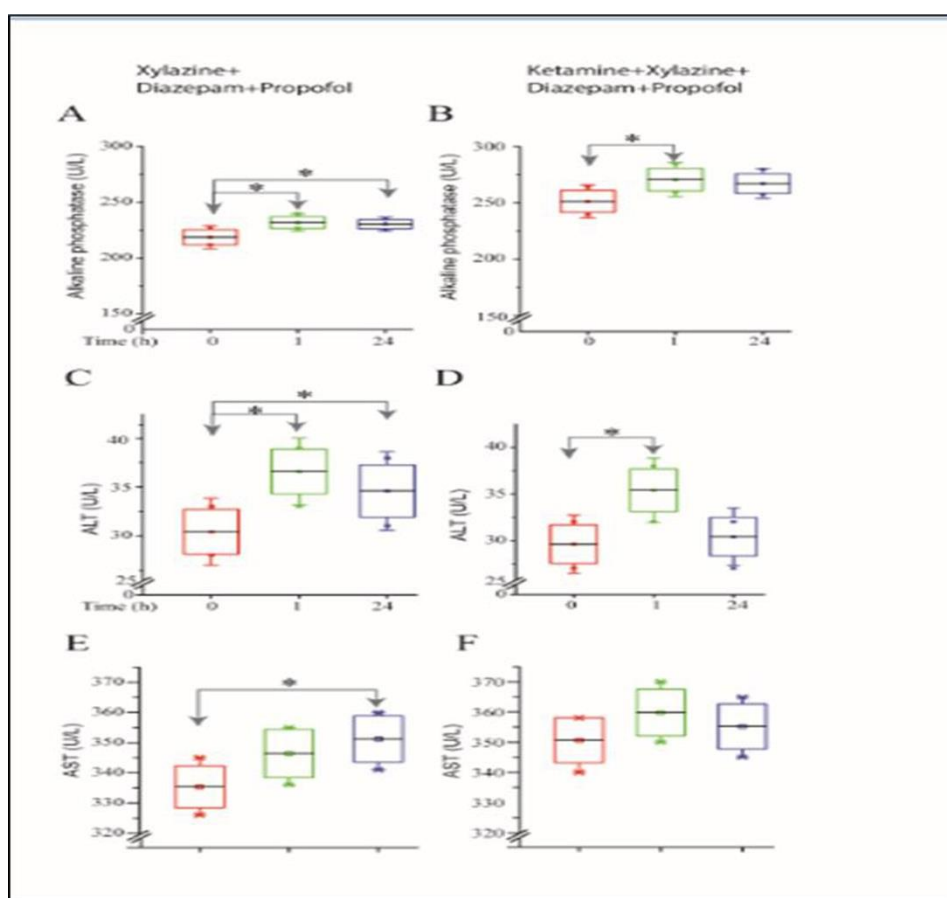


Fig-1: Alkaline phosphatase (U/L) (A & B), ALT (U/L) (C & D) and AST (U/L) (E & F) serum levels of (Xylazine + diazepam + propofol) and (Ketamine + Xylazine + diazepam + propofol) treated donkeys, respectively at zero time, 1 hr and 24 hrs post anesthetic recovery).

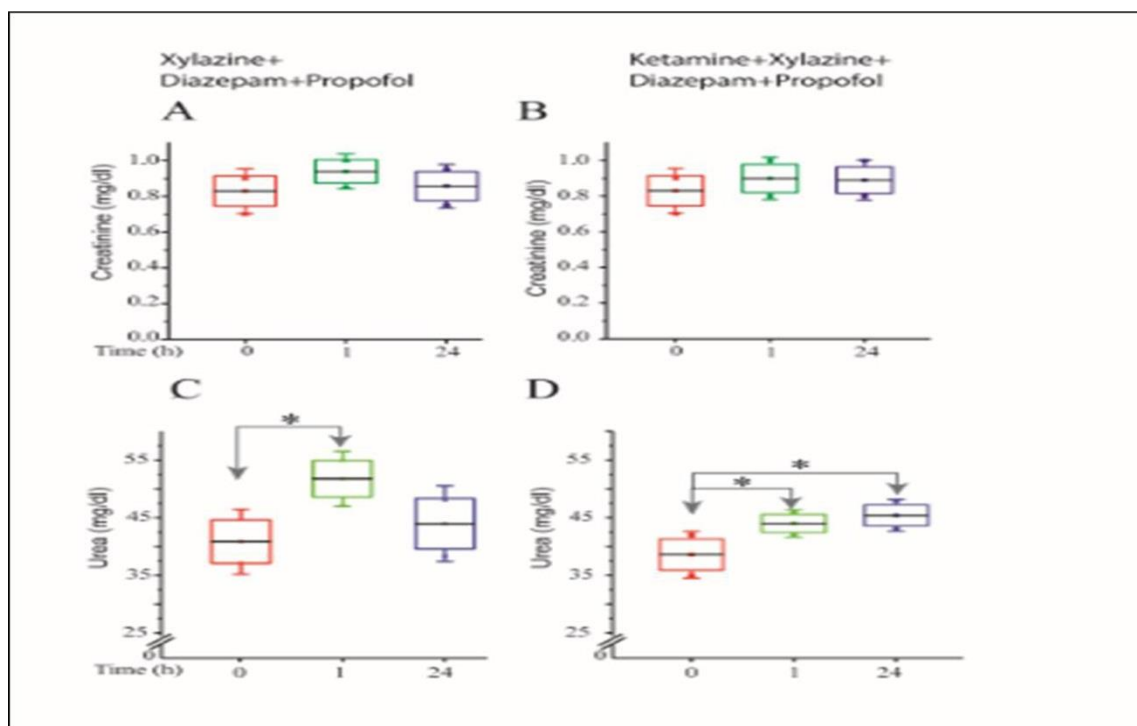


Fig-2: Creatinine (mg/dl) (A & B) and Urea mg/dl) (C & D) serum levels of (Xylazine + diazepam + propofol) and (Ketamine +Xylazine + diazepam + propofol) treated donkeys respectively at zero time, 1 hr and 24 hrs post anesthetic recovery).

Table-2: Effect of the two studied protocols on pulse rate, respiratory rate and temperature in donkeys at specified time points

Time point		Zero time	10 min.	20 min.	30 min.	40 min.	50 min.	60 min.
Protocol I X, D, P, K	Pulse rate	49.33±2.07	62.00±3.41*	63.50±1..76*	55.33±4.97	53.00±2.0	50.67±2.88	58.5
	Resp. rate	28.67±4.13*	13.83±4.62*	16.50±4..59*	15.17±5.31*	20.17±2.71*	26.83±1.47	20.00
	temperature	37.58±0.53	37.20±0.67*	36.93±0..57*	36.55±0.31*	36.70±0.31*	37.00±0.37	-
Protocol II X, D, P	Pulse rate	48.50±2.66	58.5±2.35*	55.33±5.47*	52.67±5.01	52.33±2.83	49.83±2.71	-
	Resp. rate	25.5±3.02*	14.00±1.41*	14.50±3.45*	15.17±1.47*	20.83±5.66	22.17±2.99	-
	temperature	37.07±0.35	36.84±0.32*	36.68±0.37*	36.82±0.92*	36.30±0.14*	36.98±0.22	-

Table-3: Duration of the different anaesthetic phases following induction of anaesthesia using Propofol, Xylazine, Diazepam Ketamine in donkeys:

Protocol	Onset	Anaesthetic phase	Lateral recumbency	Sternal recumbency	Standing	Total recovery
Propofol, Xylazine, Diazepam Ketamine	0.96*±0.062	35.6±1.16	6.2*±0.37	6*±0.31	6.4*±0.40	55.16*±1.55
Propofol, Xylazine, Diazepam	1.064±0.039	24.8±1.39	4.1±0.33	4±0.15	4±0.41	37.964±1.39

Values are mean ± Standard error of mean of six replicates

Values with superscript are significantly (P ≤ 0.05) different between two protocols

DISCUSSION

This study is aimed at comparing the anaesthetic effects that may follow the use of two protocols; one of them comprising propofol in combination with xylazine, diazepam and ketamine (protocol I) and the other without ketamine (protocol II) as well as the study of some hepatic and renal parameters effects of both protocols in donkeys. Although diazepam is used as a muscle relaxant in horses, but the level of sedation produced is not profound enough to be useful for restraint [32]. Sedation with α -2-adrenergic agonist is characterized by profound depression, with the horse assuming head-down posture. The eyelids and lips droop, and the horse may sway because of the muscle relaxation and ataxia produced. The muscles of the nostrils relax, which may lead to snoring or potentially obstruction in predisposed horses. [33]. Similar signs were observed in xylazine premedicated donkeys in this study. Falling down of the donkeys was noted after intravenous injection of diazepam which could be attributed to the synergistic muscle relaxant and sedative effect of diazepam [32]. Ketamine (following sedation with an alpha-2 agonist works well, but is metabolized more rapidly in donkeys and mules than in horses so higher doses or shorter dosing intervals must be used. This is especially important in miniature donkeys where a surgical plane of anesthesia will not be achieved with horse doses of xylazine and ketamine [34]. This in agreement with the present study, while donkeys required large doses of ketamine and strongly supported by the use of xylazine and ketamine in local donkeys in Sudan [35] as they revealed lots of muscle rigidity and excitatory effects. Selection of anaesthetic agent depend on species or breed of the animal, nature of surgical operation, susceptibility of the patient to the action of anaesthetic drug and health status of the animal to be anaesthetized [36]. Propofol, xylazine, diazepam and ketamine are anaesthetic combination, which was evaluated in our study in two different protocols. In our study, donkeys of group I showed rapid and smooth induction. All body reflexes were disappeared and animals were in perfect surgical stage for short duration. One of disadvantage of propofol is occurrence of apnea [9]. This was observed for about 1-2 minutes in one donkey of less than one year old age and one hindered kg body weight after propofol injection in protocol I. Absence of reflexes of the head region is previously reported by many workers [10, 9], which was observed in some selected reflexes in our study. However, the tongue and swallowing reflex was the latest one to reappear. According to the present investigation, Body temperature was decreased in all donkeys of both studied groups within 10 minutes after administration of respective anaesthetic agents and this in agreement with [37]. Pulse rate was increased in all donkeys of both studied protocols after administration of respective anaesthetic agents. This increase was noted within 10 minutes and was continued for 60 minutes post administration, these findings are in accordance with [38]. The reduction of

the respiratory rate observed in both protocols was supported in other studies [39] who attributed this reduction in respiratory rate to the effect diazepam which potentiate the respiratory depressant effect of ketamine, added to that the effect of recumbancy results from combined sedation of both drugs [18, 40]. The onset of anaesthesia was more rapid in protocol I while the anaesthetic phase, lateral and sternal recumbancy phase, standing position were longer and consequently, the total recovery time was significantly increased in protocol I. Previous studies on propofol combinations in dogs showed rapid and smooth induction, disappearance of body reflexes and animals were in perfect surgical stage for a short duration [41] and also the recovery was smooth and rapid in propofol administered animals [10, 9]. These results were in accordance with that observed in our study in particular with protocol I as the administration of ketamine with propofol avoids respiratory depression, which was seen when propofol used alone [41]. In donkeys, to our knowledge, no adequate research work pertaining to the hepato - renal effects of injectable anaesthesia had been done, and usually donkeys are considered like horses although they seem slightly different. However, in this study, the administration of various anaesthetic mixtures used in the two protocols to donkeys were not found to pose risks of renal and hepato-toxicity in donkeys, since the biochemical parameters of liver function (AST, ALT and ALP) activities and kidney function (urea and creatinine concentrations) were slightly affected and transient. In (X-D-P) anaesthetized donkeys, the blood serum ALP and ALT were significantly ($P \leq 0.05$) increased (at 1hr and 24 hrs), AST (at 24 hrs), blood urea nitrogen (at 1hr) post anaesthetic recovery, meanwhile creatinine was not significantly ($P \leq 0.05$) increased at any time point. In (K-X-D-P) protocol anaesthetized donkeys, the blood serum ALP and ALT levels were significantly ($P \leq 0.05$) elevated (at 1hr), blood urea (at 1 hr 24 hrs) post recovery. On the contrary, AST and creatinine were not significantly ($P \leq 0.05$) altered at any time point. The increase in serum level of these liver function marker enzymes is mainly owing to leakage of these enzymes from liver cells cytoplasm into the blood stream as a consequent of oxidative tissue damage produced by diazepam [42] or may be attributed to increased permeability of AST and ALT through cell membrane of liver cells in diazepam-ketamine anaesthetized donkeys which might have considered as a result of oxidative biotransformation of these drugs in the liver during the elimination phase [43]. Increased activity of these two hepatic enzymes was also detected during maximal depth of ketamine-diazepam anaesthesia in dogs [44] and in ketamine-xylazine and ketamine-diazepam treated rabbits [45]. The activities of ALP and AST are increased significantly ($P < 0.05$) in propofol treated intravenously rabbits [46]. On contrary, no significant change in serum BUN and creatinine levels is recorded during diazepam-propofol-ketamine anaesthesia in goats [47], during propofol alone and in

combination with xylazine or acepromazine anaesthesia in dogs [46] and during xylazine–ketamine–diazepam anaesthesia in sheep and goats [47].

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