Bulgarian Journal of Veterinary Medicine, 2022 ONLINE FIRST
ISSN 1311-1477; DOI: 10.15547/bjvm.2021-0131

Original article

COMPARATIVE EVALUATION OF THE INTRAVENOUS EFFECT OF MEDETOMIDINE, TRAMADOL AND MEDETOMIDINE/TRAMADOL COMBINATION ON TEAR PRODUCTION IN CLINICALLY HEALTHY DONKEYS (*EQUUS ASINUS*)

M. HAMED[1], A. SAMY[2], S. A. EL-KHODERY[3] & M. A. RIZK[3]

[1]Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Aswan University, Aswan, Egypt; [2]Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt; [3]Department of Internal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt

Summary

Hamed, M., A. Samy, S. A. El-Khodery & M. A. Rizk, 2022. Comparative evaluation of the intravenous effect of medetomidine, tramadol and medetomidine/tramadol combination on tear production in clinically healthy donkeys (*Equus asinus*). *Bulg. J. Vet. Med.* (online first).

Various ophthalmic disorders (conjunctivitis, corneal wounds, keratitis) have been reported in donkeys. There are no studies on the effect of medetomidine or tramadol on Schirmer tear test (STT) readings in donkeys. This prospective study investigated changes in STT readings in 24 clinically healthy donkeys (*Equus asinus*) (14 geldings and 10 mares) treated with commonly used doses of medetomidine hydrochloride and tramadol hydrochloride as mono- or combined therapy. Analgesia, sedation, ataxia, and STT readings were measured before treatment (baseline) and at different periods after administration (5–120 min) of the specific drug in each group. Tramadol monotherapy induced a mild analgesic effect (score 1) at 10 min post-administration. All treated donkeys exhibited mild to moderate ataxia. Medetomidine alone or in combination with tramadol induced a significant decrease ($P<0.05$) in the STT readings in both right and left eyes at 5, 15, 30, and 60 min relative to baseline, and the lowest values were observed 60 min after drug administration in both groups. Intravenous administration of medetomidine alone or in combination with tramadol induced a significant reduction in STT readings in clinically healthy donkeys. Therefore, in donkeys, the ocular surface treated with these sedatives should be carefully examined and adequately covered by an artificial tear solution or ophthalmic gel.

Key words: combination therapy, donkey, medetomidine, tear production, tramadol

INTRODUCTION

Donkeys (*Equus asinus*) are used on a wide scale by farmers in Egypt. Humans have relied on donkeys as workhorses for various activities, such as cultivating land and transporting humans and goods (Monti *et al.*, 2012). Owing to their small size, capacity to survive on poor-quality diets, and reduced demand for feed and drink, donkeys are the best draught animals (Gilger & Stoppini, 2005). Globally, in recent years, focus on the welfare of donkeys has been growing as donkey's milk is being given to children with intolerance to cow's milk (Monti *et al.*, 2012).

Generally, a good performance by animals requires them to have normal vision. In donkeys, various ophthalmic disorders, including conjunctivitis, corneal wounds, and keratitis, have been reported (Leonardi *et al.*, 2018).

Tears are critical for the eyes as they extract and remove debris and bacteria mechanically and lubricate the conjunctiva (Misk, 1990). The Schirmer tear test (STT) is one of the most effective eye tests used for accurate diagnosis of dry eyes in horses (Gilger & Stoppini, 2005) and is considered the most widely used method for evaluating basal and reflex tear production in horses without eye anaesthetic administration (Borhani *et al.*, 2021). STT readings lower than 10 mm/min are considered pathological. However, values higher than 35 mm/min are not pathological (Hendrix, 2005). The STT should be performed before eye manipulation during an eye examination to avoid a tearing reflex (Gilger & Stoppini, 2005). The higher corneal sensitivity in horses is vital to assess the equine eye but with suitable restraint (Alizadeh *et al.*, 2021). Thus, sedation may be necessary, and the effectiveness of sedation on STT readings should be considered (Hendrix, 2005).

Generally, standing sedation greatly helps in proper eye checkup and several eye procedures in horses and increases protection for both the equine patient and the inspector. In horses, medetomidine is an alpha-2-adrenoceptor agonist commonly used as a sedative for a thorough eye examination (Holve, 2012). Medetomidine hydrochloride tranquilisation is often preferred for an eye checkup in horses as it offers a powerful sedative and analgesic effect compared to xylazine and detomidine (Verbruggen *et al.*, 2000; Creighton *et al.*, 2012). Because of its long half-life, medetomidine is widely used as a premedication before general anaesthesia (Kanda *et al.*, 2015). For a painful eye, combining an alpha-2-adrenoceptor agonist with an opioid to obtain a superior analgesic effect is recommended (Hendrix, 2005; Muir, 2009).

In dogs, medetomidine alone or in combination with butorphanol results in a substantial reduction in tear production 15 min after sedation (Di Pietro *et al.*, 2021). Intramuscular medetomidine induces a significant decline in STT readings in both eyes in pigs (Kanda *et al.*, 2019a), cats (Kanda *et al.*, 2019b), and rats (Kanda *et al.*, 2020). Many alpha-2-adrenoceptor agonists used alone or in combination with other medications substantially decrease tear flow in horses (Ghaffari *et al.*, 2017; Leonardi *et al.*, 2018), dogs (Dodam *et al.*, 1998; Leonardi *et al.*, 2019), and cats (Di Pietro *et al.*, 2016).

Tramadol is a synthetic analgesic medication that is a codeine equivalent. Because of its analgesic effect, it was recently used to treat extreme post-operative pain in veterinary medicine (Pypendop *et al.*, 2009). Tramadol has minor effects on

gastrointestinal motility and cardiorespiratory function and the same analgesic effects as morphine (Natalini & Robinson, 2000). Tramadol has no analgesic effect on horses at any of the doses assessed (Dhanjal *et al.*, 2009; Franco *et al.*, 2014). A combination of xylazine or detomidine with tramadol has sedative or analgesic effects and can be used in standing horses for diagnostic and simple operations, with careful monitoring for shortly excited behaviour (Seo *et al.*, 2011; Kim *et al.*, 2012). Tear production in dogs is not affected by tramadol (Santos *et al.*, 2013; Ruiz *et al.*, 2015). Some studies have identified the effect of medetomidine on STT readings in pigs (Kanda *et al.*, 2019a), cats (Kanda *et al.*, 2019b), rats (Kanda *et al.*, 2020), and dogs, either alone or in combination with opioids (Sanchez *et al.*, 2006), but there is no documentation of the effect of medetomidine or tramadol on STT readings in donkeys. We hypothesised that our results would be in a close agreement with previous studies; medetomidine alone or in combination with tramadol substantially decrease tear flow in donkey. Thus, this prospective study examined changes in STT readings associated with clinically current doses of medetomidine, tramadol, and the medetomidine/tramadol combination in clinically healthy donkeys and reported these changes within 120 min of administration.

MATERIALS AND METHODS

Ethical statement

Before the trial, a full physical examination (heart rate, respiratory rate and rectal temperature) and regular haematological (CBC) and biochemical tests (ASAT, ALAT, total protein, creatinine) were performed (Table 1) to prove the normal status of the donkeys. Donkeys included in the current study had no history of ocular abnormalities upon ophthalmic examination including indirect ophthalmoscopy and fluorescein staining test. Donkeys with abnormalities of the ocular surface or with STT I values lower than 15 mm/min were excluded from our study. This study was approved by the Animal Care Committee of Mansoura University, Egypt, in compliance with Egyptian ethical codes for studies on animals (approval no. 04-

Table 1. The range of physical and haematobiochemical parameters apparently healthy donkeys receiving medetomidine, tramadol, medetomidine/tramadol combination or normal saline 0.9% (10 mL)

Parameters	Groups			
	Normal saline (n=6)	Tramadol (n=6)	Medetomidine (n=6)	Combination (n=6)
Heart rate (min^{-1})	44–47	45–48	47–49	47–50
Respiratory rate (min^{-1})	19–21	17–22	16–23	20–22
Rectal temperature (°C)	37.5–37.6	37.6–37.8	37.5–37.6	37.7–37.9
Erythrocytes (T/L)	4.37–6.51	5.7–7.22	4.5–6.55	4.51–7.1
Packed cell volume (%)	33–41	28–31	25–36	29–40
Haemoglobin (g/L)	92–126	102–146	98–136	112–141
ALAT (U/L)	16–23	9–23	11–27	11–22
ASAT (U/L)	281–320	251–410	290–324	259–411
Total protein (g/L)	61–76	54–78	64–68	59–71
Creatinine (μmol/L)	74–97.2	88.4–105	70.7–92.7	79.5–114

017). All experiments were conducted in accordance with the Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Egypt. An informed consent was obtained from the owner.

Animals

This study was performed on 24 clinically healthy adult donkeys (14 males and 10 females; age 5–8 years, weight 200–250 kg) reared in the Faculty of Veterinary Medicine, Mansoura University, Egypt.

Sedation protocol

The donkeys were kept indoors with access to food and water *ad libitum* under normal environmental conditions. During the preceding 2 weeks, none of the donkeys underwent any ocular therapy. The donkeys were divided randomly into four groups (n=6 per group) using online software (https://www.graphpad.com/quickcalcs/randomize1.cfm) and intravenously sedated with relevant medication: group M, medetomidine hydrochloride (1 mg/mL of Domitor; Orion Pharma Animal Health, Kvistgard, Denmark) at a dosage rate of 0.007 mg/kg (Arıcan *et al.*, 2015; Hamed *et al.*, 2018); group T, tramadol hydrochloride (100 mg/2 mL of Koralodol; Amriya Pharm, Egypt) at a dosage rate of 2 mg/kg (Dhanjal *et al.*, 2009; Hamed *et al.*, 2018); and group MT, a mixture of both drugs at the same respective dosage levels. Group C received 10 mL of 0.9% normal saline (AL-octahedron Pharma Co., Sharkia, Egypt) as a placebo control. Both drugs were balanced with saline solution to a final volume of 10 mL. Medetomidine was administered as a bolus, while tramadol was slowly injected for at least 2 min per the manufacturer's instructions. Antinociception was verified at a variety of points using a 22-gauge, 2.5-cm-long hypodermic needle via deep muscle pinpricking. This technique involved inserting the needle serially into the underlying tissues through the skin of the neck region, shoulder, the coronary band, paralumbar fossa, and the hip region. Progressive pain responses were described as recurrent movements of the head, neck, trunk, limb, and tail to avoid the needle and attempts to kick and to turn the head to the painful site. The needle was inserted bilaterally for each test, from caudal to cranial, at slightly different locations. The wounds created by the pinpricking were washed and soaked with a topical antiseptic solution. The time from drug injection to sensation loss was labeled as the time of impact onset. Furthermore, the period between the abolishment and reappearance of a reaction to pinprick stimuli was labeled as the antinociceptive duration.

The degree of analgesia was graded from 0 to 3 as follows (Hamed *et al.*, 2017): 0, no analgesia (strong reaction to noxious stimulation, such as kicking); 1 = mild analgesia (moderate response, such as rotating the heads toward the stimulus site); 2 = moderate analgesia (low and intermittent reaction); and 3 = full analgesia (no response to noxious stimulation). The degree of sedation was graded from 0 to 3 as follows (Hamed *et al.*, 2017): 0 = no sedation (donkeys remained unchanged from the initial attitude, prone to noise and environmental stimulation); 1 = mild sedation (reduced alertness with small responses to an external stimulus, occasional stumbling, and effortless ability to start walking again); 2 = moderate sedation (sleepiness, lassitude, and intermittent response to external stimuli; minor drooping of the head, lips, and upper eyelids;

and marked stumbling and walking); and 3 = deep sedation (clear lethargy, head droop, and failure to respond to external stimuli; recumbence or falling during walking). The degree of ataxia was ranked from 0 to 3 as per Hamed *et al.* 2017): 0 = normal; 1 = unimportant (minor stumbling but easily able to walk again); 2 = moderate (noticeable stumble and clear ataxic walk); and 3 = extreme (recumbency or falling while walking). In all cases, the same investigator measured antinociception, sedation, and ataxia but was ignorant of the medication used. The STT readings, degree of antinociception, and sedation were measured before sedation baseline (T0) and then at 5 (T5), 15 (T15), 30 (T30), 60 (T60), 90 (T90), and 120 (T120) min post-sedation. The experimental donkeys were clinically monitored for 1 week post-treatment.

Schirmer tear test

STTs were carried out under the same environmental conditions in indoor-inaudible traditional housing locations. Measurements were taken by the same investigator at a set time of the day (8:00–11:00 a.m.) to eliminate human and diurnal variations, and he was blinded from the treatment being performed (Piccione *et al.*, 2018). An individual, sterile, graduated 5 mm × 35 mm STT strip (I-DEW Tear Strips, Schirmer Strips, 100 Sterile Strips; Entod Research Cell UK Ltd., London, UK) was inserted into the lower eyelid around one-third of the distance between the temporal canthus and the nasal canthus for 1 min without any topical anaesthetic. The investigator's hands were clean and dry when taking the strip out of its box. The length of the moistened area on the strip was recorded in mm/min. For every donkey, the STT was performed randomly (right eye versus left eye).

Statistical analysis

Data processing was conducted using SPSS Statistics (SPSS for Windows, version 16.0; SPSS Inc., Chicago, IL, USA). Data for normal distribution were initially analysed using the Kolmogorov–Smirnov method. Typically, data were distributed; thus, the mean and standard deviation (SD) for each variable were determined at each point in time. Analysis of variance (ANOVA) was conducted to assess the key effects of time and treatment using a general linear model of repeated steps. Wilks's lambda test was performed to determine interactions within the group and evidence of seven time interactions in the treatment; when the test showed a statistically significant difference between groups, a *t*-test was performed to determine which group was statistically different at each point in time. The difference between the means at $P<0.05$ was considered statistically significant.

RESULTS

In this study, after intravenous (IV) administration of medetomidine and tramadol, whether mono- or combination therapy, the clinical parameters and mean STT readings in the right and left eyes were determined in clinically healthy donkeys. Following-up the cases did not prove any signs of ocular abnormalities upon ophthalmic examination including indirect ophthalmoscopy and fluorescein staining test. No harmful or neurological symptoms were noted in the donkeys 1 week post-administration.

Antinociception onset was noted in groups M and MT within 5 min post-administration compared with groups T and C. Complete analgesia was induced from T5 to T30 (score 3) and mild to

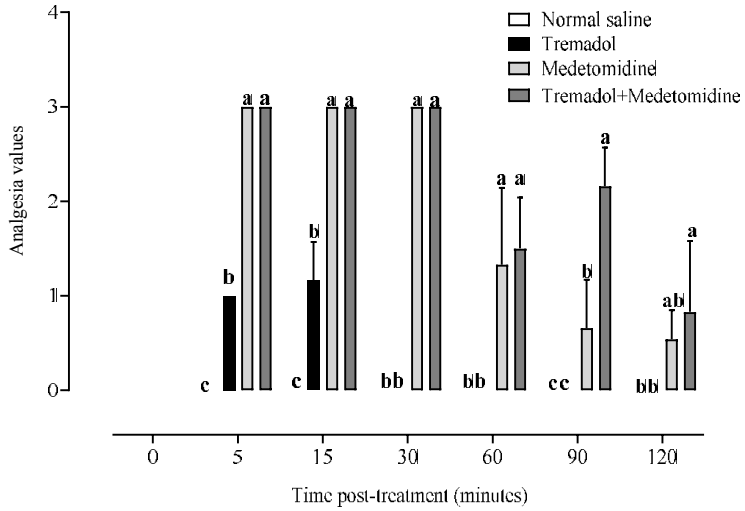

Fig. 1. Analgesia values (mean±SD) after intravenous administration of medetomidine (0.007 mg.kg^{-1}), tramadol (2 mg.kg^{-1}), medetomidine/tramadol combination, and normal saline 0.9% in clinically healthy donkeys. Variables with different superscripts in the same column are significantly different at P<0.05.

moderate analgesia – from T60 to T90 (Fig. 1). Groups M and MT had a significantly higher (P=0.017) analgesic score from T5 to T120 compared with groups T and C. Group T showed a mild analgesic effect, based on the pinprick test (score 1), at ~10 min post-administration. However, during the whole study, group C did not exhibit analgesia (Fig. 1).

Once the donkeys received normal saline, none of the sedation signs (score 0) were observed. The sedative effect was evident in groups M and MT within 5 min compared to groups T and C. The sedation score was approximately deep (score 3) in the first 15 min, moderate (score 2) from T30 to T60, mild at T90, and returned to T0 at T120. Groups M and MT showed a significantly higher (P= 0.004) sedation score from T5 to T90 compared to groups T and C (Fig. 2). Of note, for ~10 min after tramadol administration, a mild sedative effect was observed (score 1) (Fig. 2). Donkeys in groups M, MT, and T showed mild-to-moderate ataxia, which was observed from T5 to T30 and persisted in groups M and MT for T60. Conversely, group C did not show any signs of ataxia during the study (Fig. 3).

The STT (mm/min) mean ± SD baseline readings for all groups for both eyes were approximately identical and within the reference range. STT readings (mean ± SD) for both eyes of groups M and MT at T5, T15, T30, T60, and T90 showed a substantial decrease compared to baseline (P<0.0001, Wilks's lambda test for drug × time interaction). Notably, the largest decrease in STT readings in groups M and MT was detected in both eyes at T60 (12.10 ± 0.7 and 11.0 ± 0.8 mm/min for group M and 12.0 ± 0.6 and 11.10 ± 0.7 mm/min for group MT for the right and the left eye, respectively) (Table 2). At T20, the decrease in the STT reading for both eyes began to slowly increase toward

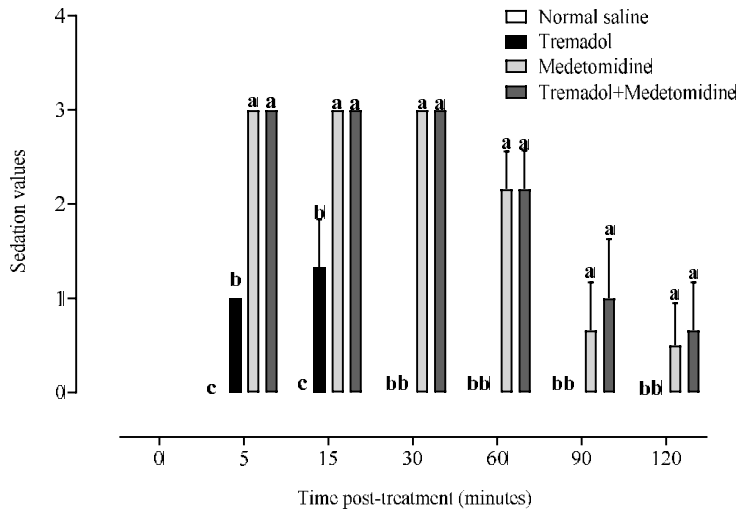

Fig. 2. Sedation values (mean±SD) after intravenous administration of medetomidine (0.007 mg.kg^{-1}), tramadol (2 mg.kg^{-1}), medetomidine/tramadol combination, and normal saline 0.9% in clinically healthy donkeys. Variables with different superscripts in the same column are significantly different at P<0.05.

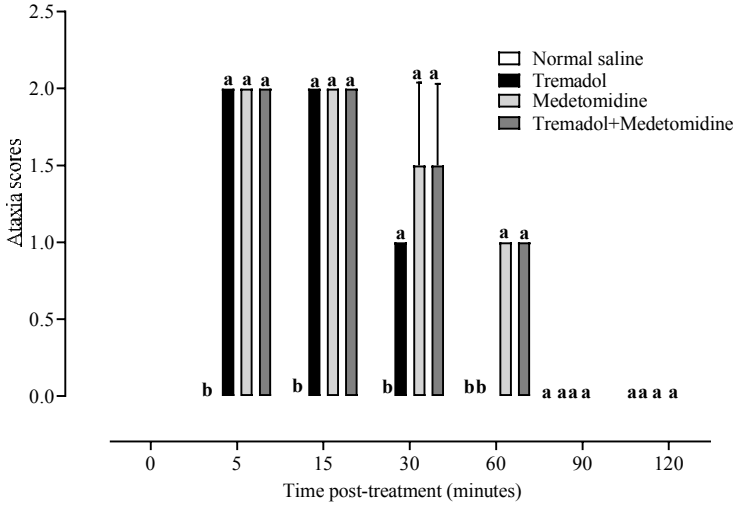

Fig. 3. Ataxia values (mean±SD) after intravenous administration of medetomidine (0.007 mg.kg^{-1}), tramadol (2 mg.kg^{-1}), medetomidine/tramadol combination, and normal saline 0.9% in clinically healthy donkeys. Variables with different superscripts in the same column are significantly different at P<0.05.

Table 2. Schirmer tear test results in the left and right eyes after intravenous administration of medetomidine (0.007 mg kg^{-1}), tramadol (2 mg kg^{-1}), medetomidine/tramadol combination, and normal saline 0.9% in clinically healthy donkeys. Data are presented as mean ± SD (n=6)

Group	Time post-treatment (minutes)						
	0	5	15	30	60	90	120
Left eye							
Normal saline	19.0± 1.7 ^{ab}	18.8± 1.7 ^{a}	19.5± 2.07 ^{a}	18.8± 2.1 ^{a}	19.3± 1.03 ^{a}	19.3± 1.3 ^{a}	20.3± 2.06 ^{a}
Tramadol	18.6± 0.8 ^{ab}	19.1± 0.7 ^{a}	20.16± 1.9 ^{a}	20.6± 1.9 ^{a}	20.0± 1.4 ^{a}	19.5± 0.8 ^{a}	19.3± 0.8 ^{ab}
Medetomidine	20.3± 1.2 ^{a}	15.8± 0.7 ^{b}	13.0± 0.8 ^{b}	12.5± 1.04 ^{b}	11.0± 0.8 ^{b}	14.1± 1.1 ^{b}	19.0± 1.09 ^{ab}
Combination	18.1± 0.7 ^{b}	16.16± 0.7 ^{b}	12.5± 1.2^{b}	11.8± 0.7 ^{b}	11.1± 0.7 ^{b}	12.8± 0.7 ^{b}	17.5± 1.5 ^{b}
Right eye							
Normal saline	19.5± 1.37 ^{b}	19.8± 1.7 ^{a}	19.6± 1.03 ^{a}	20.0± 1.8 ^{a}	20.1± 1.3 ^{a}	19.8± 0.7 ^{a}	20.0± 1.6 ^{a}
Tramadol	19.16± 1.1 ^{bc}	20.0± 0.4 ^{a}	19.8± 1.7 ^{a}	21.1± 1.1 ^{a}	19.5± 1.0 ^{a}	19.0± 0.8 ^{a}	20.0± 1.0 ^{a}
Medetomidine	21.5± 0.8 ^{a}	17.6± 1.6 ^{ab}	12.6± 1.2 ^{b}	12.3± 0.6 ^{b}	12.1± 0.7 ^{b}	13.5± 0.5 ^{b}	20.5± 0.8 ^{a}
Combination	17.5± 0.54^{c}	16.0± 1.09 ^{b}	12.3± 1.6 ^{b}	12.6± 1.2 ^{b}	12.0± 0.6 ^{b}	13.3± 0.8^{b}	16.3± 0.8 ^{b}

Variables with different superscript letters in the same column are significantly different at P<0.05. MANOVA fit, P<0.0001. Wilks' Lambda test for drug × time interaction, P<0.0001.

baseline (Table 2). In group T, there was no substantial differences (P>0.05) in the STT readings for both eyes compared with baseline and group C (Table 2). In group S, there was no substantial difference (P≥0.05) between baseline and post-treatment STT readings (Table 2). Unfortunately, some donkeys exhibited side effects post-administration in groups M (one salivation and two penile prolapses), MT (one salivation, two penile prolapses, and one excitation), and T (two excitations and two muscle tremors).

DISCUSSION

Appropriate chemical restraint is vital to perform an STT and a proper eye check-up. It increases protection, particularly in the case of a painful eye, for both the equine patient and the investigator (Hendrix, 2005). Choosing the most appropriate alpha-2-adrenoceptor agonist for sedation is crucial to the treatment of problems associated with equine dry eye, as such medications can affect STT readings (Hendrix, 2005). Medetomidine is often recommended for an eye checkup in horses as it has a strong sedative and analgesic effect compared with xylazine and detomidine (Creighton *et al.*, 2012). To obtain a superior analgesic effect for a painful eye, it may be possible to combine an alpha-2-adrenoceptor agonist with opioids (Hendrix, 2005; Muir, 2009), and the combination of medetomidine (α-2

agonist) and tramadol (opioid) may enable satisfactory ocular surgery in a standing position in donkeys.

In normal horses, the mean STT readings range from 12.7 to 24.8 mm wetting/min (Williams *et al.*, 1979). In this study, the baseline STT readings for all treated donkeys were within the reference range (17.5–21.5 mm/min). IV administration of medetomidine resulted in a statistically significant decrease ($P<0.05$) in STT readings in donkeys. This decrease was evident from 5 min and was maintained for up to 90 min post-administration. The peak reduction in STT readings was observed 5 min after the IV administration of detomidine. Our findings were in agreement with those of similar studies following medetomidine administration in dogs and cats (Ghaffari *et al.*, 2010; Sanchez *et al.*, 2006).

The exact technique by which medetomidine can reduce tear production is still unclear. There are three possible explanations: (i) increased evaporation due to inadequate blinking resulting from the effect of sedation (Crispin, 2000); (ii) postsynaptic motivation of alpha-2-adrenoceptors in the central nervous system (CNS), which may play a vital role in lowering the production of basal tears (Dodam *et al.*, 1998; Leonardi *et al.*, 2018) and a decrease in the output of reflex tears mediated by reduced nociceptive transmission, organised by the alpha-2-adrenoceptor; and (iii) alpha-2-agonist-induced adequate hypotension, leading to reduced tear gland perfusion, followed by a consequent fall in STT readings (Muir, 2009; Leonardi *et al.*, 2018).

In this study, donkeys treated with medetomidine/tramadol combination therapy showed a significant ($P<0.05$) decrease in tear production. This finding is consistent with that recorded in dogs (Dodam *et al.*, 1998; Leonardi *et al.*, 2019; Sanchez *et al.*, 2006) and horses (Leonardi *et al.*, 2018). The evaporative loss caused by the sedative effects of medetomidine/tramadol combination is an obvious cause of decreased measurable tear production, which decreases blinking. Consequently, the tear film's aqueous layer can evaporate more (Sanchez *et al.*, 2006). A previous study (Leonardi *et al.*, 2018) added that reducing STT readings is a possible alteration in the metabolism of lacrimal glands caused by a combination of detomidine and butorphanol triggered by opioids.

IV administration of tramadol (2 mg/kg) did not affect the STT readings in donkeys. This may be due to a lack of the antinociceptive effect of tramadol in donkeys, as antinociception has also been identified as a possible cause of decreased tear production (Dhanjal *et al.*, 2009; Dodam *et al.*, 1998). Similar results were observed following doses of 2 mg/kg (Santos *et al.*, 2013) and 4–6 mg/kg tramadol in dogs (Ruiz *et al.*, 2015). However, Santos *et al.* (2013) reported that the tramadol/acepromazine combination significantly reduces tear production in dogs. On the contrary, tear production declined significantly in dogs after intramuscular administration of 1 mg/kg of morphine compared to baseline (Mouney *et al.*, 2011).

Donkeys treated with medetomidine and medetomidine/tramadol combination showed satisfactory analgesia. Medetomidine is a potent and complete alpha-2-adrenoceptor agonist, and its selectivity to α-2/α-1 is greater than that of xylazine (Creighton *et al.*, 2012). The antinociceptive effect of medetomidine is triggered by a diminishing release of epinephrine and norepinephrine, which play a vital role in pain sensation by inhibiting the sympathetic nervous system through their action

on α-2 receptors (Ambrisko & Hikasa, 2002). In group T, tramadol induced a minor analgesic effect for up to 10 min post-administration, as described by Seo *et al.* (2011) and Kim *et al.* (2012). There was no noticeable difference in the duration of analgesia in groups M and MT. Conversely, in horses, the addition of tramadol to xylazine or detomidine has a longer effect compared to any sole drug (Seo *et al.*, 2011; Kim *et al.*, 2012). Studies have shown that there is no analgesic effect of tramadol in horses (Dhanjal *et al.*, 2009; Franco *et al.*, 2014).

In this study, groups M and MT showed moderate-to-deep sedation of equal duration. The sedative effect of medetomidine is due to a reduction in sympathetic CNS outflow (Toutain *et al.*, 1982). These findings correlate with those of Kim *et al.* (2012), who noted that the sedative effect of the detomidine/tramadol combination in horses is similar to the effect of detomidine alone. Another study reported that the xylazine/tramadol combination has a longer sedative effect on horses than xylazine alone (Seo *et al.*, 2011). However, tramadol has a mild sedative effect for a short period on horses (Seo *et al.*, 2011; Kim *et al.*, 2012). Furthermore, injections of tramadol at a dose of 2 mg/kg do not induce sedation in horses (Dhanjal *et al.*, 2009). Despite the reduced sedative and analgesic effects of IV administration of tramadol, its epidural injection provides prolonged analgesia without CNS agitation and motor activity and behavioural changes (Natalini & Robinson, 2000).

Groups M, MT, and T showed mild to moderate ataxia. This could be attributed to the effect of medetomidine (Ambrisko & Hikasa, 2002) and tramadol (Dhanjal *et al.*, 2009; Franco *et al.*, 2014). On the contrary, no ataxia was recorded after IV administration of tramadol in horses (Kim *et al.*, 2012). In horses, IV administration of tramadol does not produce the locomotor motivation noted with other opioids, but other CNS excitations, such as more excited temper and behaviour, increased sensitivity to noise and stimulation, tremor, and head nodding, are observed (Dhanjal *et al.*, 2009). However, in this study, tramadol had no undesirable effects on donkeys. Giorgi *et al.* (2007) reported that increasing the tramadol dose up to 5 mg/kg IV prompts tremor, confusion, excitement, and tachycardia.

This study had several limitations. First, future studies are required to evaluate the effects of kinetic analyses using various doses of medetomidine and tramadol on tear production in donkeys. We selected approximately current doses based on the previous veterinary literature on horses and donkeys (Dhanjal *et al.*, 2009; Arıcan *et al.*, 2015; Hamed *et al.*, 2018). Second, the sample size was small, which may not allow a definitive conclusion. Subsequently, more research using large samples of donkeys is needed. Third, we did not examine the effect of medetomidine and tramadol, whether mono- or combination therapy, on the STT readings of donkeys with ophthalmic diseases. Therefore, further research is required to investigate this shortcoming.

CONCLUSIONS

IV administration of medetomidine either alone or in combination with tramadol can be used for diagnostic techniques and minor ophthalmic surgeries in standing donkeys. Such combination therapy shows a significant decline in STT readings in clinically healthy donkeys. Accordingly, the ocular surface of donkeys treated with these sedatives should be carefully in-

spected and satisfactorily covered by an artificial tear solution or ophthalmic gel.

ACKNOWLEDGEMENTS

This study is supported by the Ministry of Higher Education of Egypt.

REFERENCES

Alizadeh, A., S. S. Ozmaie, A. Asghari & E. Torki, 2021. Determination the reference value for measuring tear production by using the STT1 and STT2 in Caspian horse. *Veterinary Researches & Biological Products*, **75**, 63–68.

Ambrisko, T. D. & Y. Hikasa, 2002. Neurohormonal and metabolic effects of medetomidine compared with xylazine in beagle dogs. *Canadian Journal of Veterinary Research,* **66**, 42–49.

Arıcan, M., H. Erol & E. Esin, 2015. Clinical comparison of medetomidine with isoflurane or sevoflurane for anesthesia in horses. *Pakistan Veterinary Journal,* **35,** 474–478.

Borhani, S., S. Ozmaie, A. Asghari & A. Jahandideh, 2021. Determination of reference value for Schirmer Tear Test (STT) results in caspian horse. *Journal of Veterinary Research*, **75**, 469–474.

Creighton, C. M., K. A. Lemke, L. A. Lamont, B. S. Horney & C. B. Riley, 2012. Comparison of the effects of xylazine bolus versus medetomidine constant rate infusion on cardiopulmonary function and depth of anesthesia in horses anesthetized with isoflurane. *Journal of the American Veterinary Medical Association,* **240**, 991–997.

Crispin, S. M., 2000. Tear‐deficient and evaporative dry eye syndromes of the horse. *Veterinary Ophthalmology,* **3**, 87–92.

Dhanjal, J. K., D. V. Wilson, E. Robinson, T. T. Tobin & L. Dirokulu, 2009. Intravenous tramadol: effects, nociceptive properties, and pharmacokinetics in horses. *Veterinary Anaesthesia and Analgesia,* **36**, 581–590.

Dodam, J., Branson, K., Martin, D., 1998. Effects of intramuscular sedative and opioid combinations on tear production in dogs. *Veterinary Ophthalmology,* **1**, 57–59.

Di Pietro, S., F. Macrì, T. Bonarrigo, E. Giudice, A. P. Piccionello & A. Pugliese, 2016. Effects of a medetomidine-ketamine combination on Schirmer tear test I results of clinically normal cats. *American Journal of Veterinary Research*, **77**, 310–314.

Di Pietro, S., C. Giannetto, A. Falcone, G. Piccione, F. Congiu, F. Staffieri & E. Giudice, 2021. Dexmedetomidine and tear production: Evaluation in dogs as spontaneous model for ocular surface disorders. *Veterinary Sciences,* **8**, 28.

Franco, L. G., J. C. D. Moreno, A. R. Teixeira Neto & L. A. F. D. Silva, 2014. Tramadol effects on clinical variables and the mechanical nociceptive threshold in horses. *Ciência Rural,* **44**, 517–523.

Ghaffari, M. S., D. E. Brooks, A. Sabzevari, S. M. Ghamsari, H. M. Lakooraj & H. Shad, 2017. Effects of intravenous detomidine on Schirmer tear test results in clinically normal horses. *Journal of Equine Veterinary Science,* **55**, 97–99.

Ghaffari, M. S., A. Malmasi & S. Bokaie, 2010. Effect of acepromazine or xylazine on tear production as measured by Schirmer tear test in normal cats. *Veterinary Ophthalmology,* **13**, 1–3.

Gilger, B. & R. Stoppini, 2005. Diseases of the eyelids, conjunctiva, and nasolacrimal system. In: *Equine Ophthalmology,* Elsevier Saunders, pp. 107–156.

Giorgi, M., G. Soldani, C. Manera, P. Ferrarini, M. Sgorbini & G. Saccomanni, 2007. Pharmacokinetics of tramadol and its metabolites M1, M2 and M5 in horses following intravenous, immediate release (fasted/fed) and sustained release single dose administration. *Journal of Equine Veterinary Science,* **27**, 481–488.

Hamed, M. A., K. S. Abouelnasr, H. M. Ibrahim & S. A. El-Khodery, 2017. Comparative, sedative, and analgesic effects of epidural dexmedetomidine and xylazine in donkeys (*Equus asinus*). *Journal of Equine Veterinary Science,* **59**, 104–109.

Hamed, M. A., M. A. Rizk, A. Hafez, A. S. El–Mahdi & S. A. El-Khodery, 2018. Comparative effect of intravenous administration of medetomidine, tramadol, and medetomidine/tramadol combination on intraocular pressure (IOP) in clinically healthy donkeys (*Equus asinus*). *Journal of Equine Veterinary Science,* **69**, 16–20.

Hendrix, D. V., 2005. Eye examination techniques in horses. *Clinical Techniques in Equine Practice,* **4**, 2–10.

Holve, D. L., 2012. Effect of sedation with detomidine on intraocular pressure with and without topical anesthesia in clinically normal horses. *Journal of the American Veterinary Medical Association,* 240, 308–311.

Kanda, T., A. Iguchi, C. Yoshioka, H. Nomura, K. Higashi, M. Kaya, R. Yamamoto, T. Kuramoto & T. Furukawa, 2015. Effects of medetomidine and xylazine on intraocular pressure and pupil size in healthy Beagle dogs. *Veterinary Anaesthesia and Analgesia,* **42**, 623–628.

Kanda, T., A. Kajiyama, W. Morimitsu, Y. Nishino, Y. Oishi, Y. Shimizu, N. Maeta, K. Furumoto, Y. Itoh & T. Furukawa, 2019a. Effect of medetomidine on tear flow measured by Schirmer tear test I in normal pigs. *Journal of Veterinary Medical Science*, **81**, 538–540.

Kanda, T., Y. Shimizu, C. Hanazono, S. Maki, N. Maeta, T. Itoi, K. Furumoto, Y. Okamura, Y. Itoh & T. Furukawa, 2019b. Effect of intramuscular administration of medetomidine and xylazine on tear flow measured by the Schirmer tear test I in healthy cats. *Journal of Feline Medicine and Surgery,* **21**, 788–792.

Kanda, T., Y. Mizoguchi, K. Furumoto, Y. Shimizu, N. Maeta & T. Furukawa, 2020. Effect of intramuscular medetomidine administration on tear flow in rats. *Veterinary Sciences,* **7**, 42.

Kim, T., M. Jang, , H. Lee & I. Lee, 2012. Sedative and analgesic effects of intravenous detomidine and tramadol on horses. *Journal of Veterinary Clinics*, 29, 226–232.

Leonardi, F., G. Costa, M. Dubau, A. Sabbioni, B. Simonazzi & M. Angelone, 2018. Effects of intravenous romifidine, detomidine, detomidine combined with butorphanol, and xylazine on tear production in horses. *Equine Veterinary Education,* **32**, 53–57.

Leonardi, F., G. L. Costa, A. Stagnoli, E. Zubin, P. Boschi, A. Sabbioni & B. Simonazzi, 2019. The effect of intramuscular dexmedetomidine-butorphanol combination on tear production in dogs. *The Canadian Veterinary Journal,* **60**, 55–59.

Misk, N., 1990. Ocular diseases in donkeys. *Equine Practice,* **12**, 20–27.

Monti, G., S. Viola, C. Baro, F. Cresi, P.A. Tovo, G. Moro, M. Ferrero, A. Conti & E. Bertino, 2012. Tolerability of donkey's milk in 92 highly-problematic cow's milk allergic children. *Journal of Biological Regulators and Homeostatic Agents*, **26**, 75–82.

Mouney, M. C., P. J. Accola, J. Cremer, M. K. Shepard, C. Rodriguez Guarin & E. H. Hofmeister, 2011. Effects of acepromazine maleate or morphine on tear production before, during, and after sevoflurane anesthesia in dogs. *American Journal of Veterinary Research,* **72**, 1427–1430.

Muir, W. W., 2009. Anxiolytics, nonopioid sedative-analgesics, and opioid analgesics, In: *Equine anesthesia,* Elsevier, pp. 185–209.

Natalini, C. C. & E. P. Robinson, 2000. Evaluation of the analgesic effects of epidurally administered morphine, alfentanil, butorphanol, tramadol, and U50488H in horses. *American Journal of Veterinary Research,* **61**, 1579–1586.

Piccione, G., C. Giannetto, F. Fazio, & E. Giudice, 2008. Daily rhythm of tear

production in normal horse. *Veterinary Ophthalmology,* **11**, 57–60.

Pypendop, B. H., K. T. Siao & J. E. Ilkiw, 2009. Effects of tramadol hydrochloride on the thermal threshold in cats. *American Journal of Veterinary Research,* **70**, 1465–1470.

Ruiz, T., T. P. Da Silva Peres, W. N. Da Silva Campos, E. D. C. B. Sorte & A. P. Ribeiro, 2015. Efeitos do tramadol sobre a produção lacrimal, a pressão intraocular e o diâmetro pupilar de cães: estudo clínico. *Ciência Rural,* **45**, 724–729.

Sanchez, R., D. Mellor & J. Mould, 2006. Effects of medetomidine and medetomidine ‑ butorphanol combination on Schirmer tear test 1 readings in dogs. *Veterinary Ophthalmology,* **9**, 33–37.

Santos, P. H. d. A., K. C. d. S. Pontes, R. Pinto, P. B. d. S. Rios, G. M. M. da Silva, A. P. B. Borges & L. S. C. Favarato, 2013. Comparison of pupil diameter and tear production in dogs treated with acepromazine, tramadol and their combination. *Revista Ceres,* **60**, 166–172.

Seo, J.-P., W.-G. Son, S. Gang & I. Lee, 2011. Sedative and analgesic effects of intravenous xylazine and tramadol on horses. *Journal of Veterinary Science,* **12**, 281–286.

Toutain, P., M. Zingoni & Y. Ruckebusch, 1982. Assessment of alpha-2 adrenergic antagonists on the central nervous system using reticular contraction in sheep as a model. *Journal of Pharmacology and Experimental Therapeutics,* **223**, 215–218.

Verbruggen, A.M., L. Akkerdaas, L. Hellebrekers & F. Stades, 2000. The effect of intravenous medetomidine on pupil size and intraocular pressure in normotensive dogs. *Veterinary Quarterly,* **22**, 179–180.

Williams, R., J. Manning & R. Peiffer, 1979. The Schirmer tear test in the equine: Normal values and the contribution of the gland of the nictitating membrane [Diagnosis of keratoconjunctivitis sicca]. *Journal of Equine Medicine and Surgery,* **3**, 117–119.

Paper received 02.12.2021; accepted for publication 03.05.2022

Correspondence:

Mohamed Abdo Rizk, Ph.D.
Department of Internal Medicine
and Infectious Diseases,
Faculty of Veterinary Medicine,
Mansoura University, Mansoura 35516, Egypt,
tel.: +201122245151; fax: + 20502379952;
e-mail: dr_moh_abdo2008@mans.edu.eg