

**Short Communication**

**EFFECTS OF DIFFERENT COMBINATIONS OF ACEPROMAZINE, DETOMIDINE, XYLAZINE AND KETAMINE ON SERUM CORTISOL LEVEL OF STANDING SEDATED HORSES**

H. B. Rashid<sup>1\*</sup>, M. S. Khan<sup>1</sup>, M. Chaudhry<sup>2</sup>, M. A. Khan<sup>1</sup> and M. Ashraf<sup>3</sup>

<sup>1</sup>Department of Clinical Medicine and Surgery, <sup>2</sup>Department of Epidemiology and Public Health, Faculty of Veterinary Science, <sup>3</sup>Department of Pharmacology & Toxicology, Faculty of Biosciences, University of Veterinary and Animal Sciences, Abdul Qadir Jilani Road, Lahore, Pakistan.

\*Corresponding Author Email: hamad.chaudhry@uvas.edu.pk

**ABSTRACT**

This study was performed to evaluate the sedative and analgesic effects of different combinations of Acepromazine, Detomidine, Xylazine and Ketamine intravenously (IV) administered to 25 horses at stance during minor surgical procedures and clinical examinations. Blood samples were collected from the jugular vein of horses at before sedation and post sedation at 15 minutes, 30 minutes, and 60 minutes. The serum cortisol level were evaluated. Analysis of variance (one-way ANOVA) were performed with  $p < 0.05$ . Serum cortisol mean values showed initial decline in all groups except group C (Acepromazine + Ketamine), which showed increase in mean values at 15 minutes post drug administration. The effect of drug combination of Acepromazine and Ketamine (Group C) was significantly different from drug combination of Acepromazine, Detomidine and Ketamine (Group D) on serum cortisol mean values. However, there was no statistical difference between pre-sedation and post-sedation cortisol values at 15, 30, and 60 minutes.

**Key Word: Acepromazine, Detomidine, Ketamine, Xylazine, Cortisol, Standing Sedation**

**INTRODUCTION**

Often under field conditions the availability of anaesthetic equipment is limited. No provision is made for the administration of lengthy general anaesthetics. Under field conditions, the use of drugs that produce minimal side effects becomes important, as the availability of medical care is limited. Few analgesics relieve pain without producing side effects. The ideal analgesic provides good analgesia and sedation without any side effects. Combined with sedation, analgesia aids in the handling of animals and reduces the danger to attendants (Joubert, 1999). Sedatives and analgesics have been widely used for diagnostic procedures and minor surgery in standing horses (LeBlanc, 1991). In small animals morphine-like drugs, opioids, are often used pre-, peri, and post-operative to minimize pain and to reduce the use of anaesthetic drugs. Opioids have also been administered to horses for more than 70 years by practicing veterinarians but the use in horses is not as simple as in small animals. This is because there is a small window between pain relief and excitation and therefore horses are more prone to side effects from the opioids than small animals (Bennett & Steffey, 2002). To evaluate the analgesic effect of a drug different parameters in the blood are often used as indicators of stress and pain. The generalized stress produced by sedative drugs may also cause alteration in biochemical indices (Kinjavedekar *et al.*, 2007). The cortisol

concentration in the blood is an example of an indicator often used for stress and pain evaluation in animals (Ayala *et al.*, 2012; Mircean *et al.*, 2007). Opioids themselves have been shown to affect the cortisol concentration in the blood by stimulating or inhibiting the release of hormones from the hypothalamus, pituitary gland or adrenal glands (Pechnick, 1993). Alpha-2 agonists, e.g. xylazine, clonidine, romifidine, detomidine, medetomidine, and dexmedetomidine are effective analgesics which also produce physiological and behavioural changes, e.g. bradycardia, hypertension, atrio-ventricular block, excessive sedation and ataxia, all of which can potentially limit their systemic use as analgesics in some clinical cases (Valverde, 2010). Amongst these, xylazine, detomidine, dexmedetomidine and romifidine are the four alpha-2 agonists, which are frequently used. Activity of these agents is dependent upon their specificity for the alpha-2 and alpha-1 receptors. Ketamine has been used as an anesthetic agent in equine medicine since the mid-70s (Muir *et al.*, 1977). Initially, ketamine was applied just as an induction agent, producing amnesia, loss of consciousness, analgesia and immobility. In later years, based on these properties, the application of ketamine in equine anaesthesia was extended by using in different total intravenous anaesthesia protocols (Taylor & Luna, 1995; Mama *et al.*, 2005).

The aim of current study was to determine changes in serum cortisol levels produced by various

combination of sedative drugs used to sedate horses during routine clinical procedures and minor surgeries, which required standing sedation.

## MATERIALS AND METHODS

**Animals:** A total of 25 horses, which were brought to the Indoor Hospital, Department of Clinical Medicine and Surgery (CMS), Faculty of Veterinary Science, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan, were included in current study. Among these 25 horses, 6 were brought for the treatment for thrush, exostosis, hygroma and tendinitis, while 1 horse was operated for standing castration. Antiseptic dressing (ASD) for wounds at various sites and thrush was applied to 16 horses and 3 horses were admitted for tooth rasping. All these horses were administered with various combinations of sedative drugs to provide a safe and comfortable environment to the practitioner to conduct the examination and minor surgeries.

These horses were randomly divided into 5 treatment groups and each group comprised of 5 horses. Group A was sedative with combination of Acepromazine and Detomidine, Group B was administered with combination of Detomidine and Ketamine. Group C was given combination of Acepromazine and Ketamine, while Group D was injected with Acepromazine, Detomidine and Ketamine. Group E was given combination of Acepromazine, Xylazine and Ketamine. All drugs used were injected with doses and routes recommended by the manufacturers. Acepromazine (Sedastress; Farvet Laboratories B.V.), Ketamine (Calypsol Gedeon Richter Ltd.) and Xylazine (Xylaz; Eurovet); were acquired from the local market, while Detomidine: (Detogesic; Forte Dodge) was graciously granted by a fellow veterinarian to be used in the study.

**Collection of Samples and Laboratory Analysis:** The blood samples were collected in 10cc sterilized disposable syringe (BD) via jugular vein of horses puncturing with 23 G needle before sedation, 15 minutes, 30 minutes, and 60 minutes after drug administration. Approximately 3ml blood was collected in a vacutainer and was allowed to form serum at room temperature. Samples were transported to commercial laboratory on ice and serum was harvested by centrifugation. Serum Cortisol level was determined from the serum at the

commercial laboratory using Elecysis analyser 1010 with Roche cobas<sup>®</sup> Cortisol kit as per manufacturer's instructions.

**Statistical Analysis:** The data were presented as means with standard deviation (mean±S.D), parametric data were analyzed by one ways analysis of variance (ANOVA) continued with Post hoc Tukey test and  $p \leq 0.05$  was considered to be significant. Analysis was performed using Minitab software Release 15 (State College, Pennsylvania, USA).

**Ethical Considerations:** Advance Studies and Research Board, UVAS, Lahore, Pakistan approved the research work and methodology of the study and during conduct of study, measures were adopted to minimize the pain and discomfort to the animals.

## RESULTS

The horses of Group A (Acepromazine + Detomidine) and Group E (Acepromazine + Xylazine + Ketamine) showed a gradual increase in the cortisol mean level after injection. Horses of Group B (Detomidine + Ketamine) exhibited a decrease in the mean level of cortisol and remained at a lower level till the last measurement at 60 minutes. The horses of Group C (Acepromazine + Ketamine) revealed increased mean level after 15 minutes, whereas showed decreased levels at the 30 and 60 minutes post-injection. Horses of Group D (Acepromazine + Detomidine + Ketamine) showed a general decline in the cortisol mean level, which was still lower at 60 minutes post-injection (Figure). The results of analysis of variance using Generalized Linear Model showed a significant effect ( $p < 0.05$ ) of drug groups on cortisol level, while no significant difference ( $p > 0.05$ ) of measurement times and the combined effect of drug groups and measurement times was seen. The results of post hoc statistical analysis showed no significant difference between group A and groups B, C, D & E. Group B also exhibited no significant difference with groups A, C, D & E. Group C showed significant difference with group D, while a non-significant difference with groups A, B & E was seen. Group D was significantly different from group C, while it showed non-significant difference with groups A, B & E. Group E exhibited no significant difference with groups A, B, C & D. The results are shown in table.

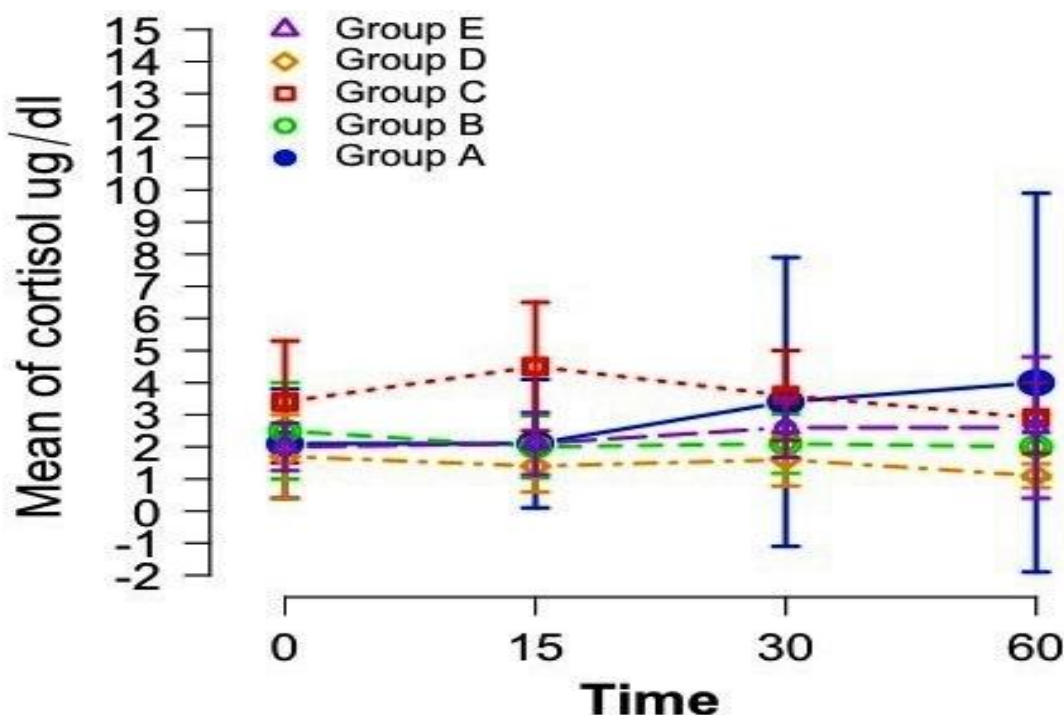


Figure . Mean ( $\pm$  SE) values of serum cortisol ( $\mu\text{g/dl}$ ) of horses over time following administration of Acp + Deto (group A) [blue circle with solid line], Deto + Keta (group B) [green hollow circle with short dashed line], Acp + Keta (group C) [red square with dotted line], Acp + Deto + Keta (group D) [yellow diamond with short dashed and dot line], and Acp + Xyla + Keta (group E) [purple triangle with long dashed line].

Table. Effect of Various Drug Combinations on the Hematology (Cortisol Level) of Horses [Mean Values]

Each value is the mean of 5 horses.

Group		Cortisol Level					
		A <sup>ab</sup>	B <sup>ab</sup>	C <sup>a</sup>	D <sup>b</sup>	E <sup>ab</sup>	
Time (Mins.)	0	Mean $\pm$ S. D.	2.1 $\pm$ 2	2.5 $\pm$ 2	3.4 $\pm$ 2	1.7 $\pm$ 1	2.1 $\pm$ 1
	15	Mean $\pm$ S. D.	2.1 $\pm$ 2	2.0 $\pm$ 1	4.5 $\pm$ 2	1.4 $\pm$ 1	2.1 $\pm$ 1
	30	Mean $\pm$ S. D.	3.4 $\pm$ 5	2.1 $\pm$ 1	3.6 $\pm$ 1	1.6 $\pm$ 1	2.6 $\pm$ 2
	60	Mean $\pm$ S. D.	4.0 $\pm$ 6	2.1 $\pm$ 1	2.9 $\pm$ 1	1.2 $\pm$ 0.4	2.6 $\pm$ 2

Normal Values

1.3 to 2.9 ( $\mu\text{g/dl}$ )

Radostists, 2007

<sup>a, b</sup>Drug groups with different alphabetic in superscript were statistically different from each other at  $p \leq 0.05$  based on Post Hoc Tukey's test.

<sup>\*, †</sup>Measurement time with different symbols in superscript were statistically different from each other at  $p \leq 0.05$  based on Post Hoc Tukey's test.

## DISCUSSION

The current study was conducted to evaluate the effect of different combinations of sedative drugs (Acepromazine, detomidine, ketamine, and xylazine) on cortisol levels in serum of horses. The main objective was to find out a drug combination with minimum side effects but maximum sedative effects. Selection of drug combinations was made on clinical expertise of research team and local availability of drugs.

Cortisol secretion rises in response to any stress to body such as illness, trauma, surgery, temperature

extremes, and transportation (Hucklebridge *et al.* 1999). Cortisol may improve the stress response by energy mobilization and behavioral changes (Schmidt *et al.* 2010). The anaesthetic drugs can inhibit the release of cortisol and decrease the cortisol level in the blood (Fragen and O'hara 1987; Sanhoury *et al.* 1992). In the current study, the serum cortisol levels compared with basal level were examined. Overall no significant difference ( $p > 0.05$ ) between pre-sedation and post-sedation serum cortisol levels were seen. All groups failed to significantly alter the serum cortisol level at given doses from the basal values. The possible

explanation why cortisol levels were not decreased may be that the dosage, used for study were not sufficient to decrease cortisol release (Maze *et al.* 1991). Similarly Ambrisko and Hikasa, (2002) also reported that medetomidine and xylazine failed to significantly alter the plasma cortisol level at the examined dosages. Contrary to the results of current study, Khan *et al.* (2006) reported increase in the serum cortisol levels after 60 minutes of administration of detomidine in donkeys with colic signs. They attributed this finding to stress induced during severe abdominal pain and post detomidine injection, while in current study horses were without any signs of colic and severe pain.

Although cortisol values were not significantly decreased from basal values but a significant difference ( $p < 0.05$ ) in serum cortisol levels among drug combinations was seen. Group C (Acepromazine-Ketamine) showed an increase in cortisol value at 15 minutes and then gradual decrease at 30 and 60 minutes while group D (Acepromazine-Detomidine-Ketamine) showed a general decline in the cortisol mean level, which continued at 60 minutes post-injection. Both C & D groups were significantly different from each other. A decrease in serum cortisol values of horses sedated with detomidine-ketamine combination (group B) and acepromazine-detomidine-ketamine combination (group D) was documented though not statistically significant. Similarly group A (Acepromazine-Detomidine) and group E (Acepromazine-Xylazine-Ketamine) showed an increase in serum cortisol levels at 15 minutes post-sedation and maintained that level at 30 and 60 minutes post-sedation.

**Conclusion:** The results of this study showed effect of drug combination on serum cortisol values. All drug groups failed to significantly alter the serum cortisol level at given doses from the basal values, which indicate that these combinations failed to significantly decrease the release of cortisol in blood during minor surgeries; clinical examinations etc. Although, mean cortisol values of drug combination with Acepromazine, Detomidine and Ketamine (Group D) were significantly lower from drug combination with Acepromazine and Ketamine only (Group C), however they were not different from basal values. It is therefore concluded that any drug combination with Acepromazine, Detomidine, Xylazine and Ketamine can safely be used for inducing standing sedation in horses.

**Acknowledgments:** The author(s) thank Dr. Tanveer Khaliq for providing Detomidine (Detogesic; Forte Dodge) to be used in the study.

**Authors Contributions:** Each author contributed significantly in the design of the study, conduct, data collection and analysis of results and compilation of manuscript.

**Conflict of Interest:** The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

## REFERENCES

- Ambrisko, T. D., Y. Hikasa (2002). Neurohormonal and metabolic effects of medetomidine compared with xylazine in beagle dogs. *Can. J. Vet. Res.*, 66(1): 42-49.
- Ayala, I., N. F. Martos, G. Silvan, C. Gutierrez-Panizo, J. G. Clavel, J. C. Illera (2012). Cortisol, adrenocorticotropic hormone, serotonin, adrenaline and noradrenaline serum concentrations in relation to disease and stress in the horse. *Res. Vet. Sci.*, 93, 103-107.
- Bennett, R. C., E. P. Steffey (2002). Use of opioids for pain and anesthetic management in horses. *Vet. Clin. Equine*, 18, 47-60.
- Fragen, R. J, D. A. O'hara (1987). A comparison of intramuscularly injected ketorolac and morphine in postoperative pain. Abstracts in Proceedings of American Society for Clinical Pharmacology and Therapeutics. Vol: 41. Pp. 249-258. DOI: 10.1038/clpt.1987.25
- Hucklebridge, F., A. Clow, T. Abeyguneratne, P. Huezodia, P. Evans (1999). The awakening cortisol response and blood glucose levels. *Life sciences*. 64 (11): 931-937.
- Joubert, K. E., P. Briggs, D. Gerber, R. G. Gottschalk. 1999. The sedative and analgesic effects of detomidine-butorphanol and detomidine alone in donkeys. *J. S. Afr. Vet. Ass.*, 70: 112-118.
- Khan, M. A., A. K. Mahmood, H. B. Rashid (2006). Analgesic and sedative effects of detomidine in experimentally designed colic model in donkeys. *Ind. J. Vet. Surg.*, 27(2);87-90.
- Kinjavdekar, P., G. R. Singh, Amarpal, H. P. Aithal, A. M. Pawde (2007). Clinicophysiological effects of spinally administered ketamine and its combinations with xylazine and medetomidine in healthy goats. *Vet. Res. Comm.*, 31: 847-861.
- LeBlanc, P. H. (1991). Chemical restraint for surgery in the standing horse. *Vet. Clin. North Am. Equine Pract.*, 7(3):521-533.
- Mama, K. R., A. E. Wagner, E. P. Steffey, C. Kollias-Baker, P. W. Hellyer, A. E. Golden, L. F. Brevard (2005). Evaluation of xylazine and ketamine for total intravenous anesthesia in horses. *Am J. Vet Res.*, 66: 1002-1007.
- Maze, M., R. Virtanen, D. Daunt, S. J. Banks, E. P. Stove, D. Feldman (1991). Effects of dexmedetomidine, a novel imidazole sedative-anesthetic agent, on adrenal steroidogenesis: In vivo and in vitro studies. *Anesth Analg.*, 73: 204-208.

- Mircean, M., G. Giurgiu, V. Mircean, E. Zinveliu (2007). Serum cortisol variation of sport horses in relation with the level of training and effort intensity. *Bulletin USAMV-CN*, 64, 488-492.
- Muir, W. W., R. T. Skarda, D. W. Milne (1977). Evaluation of xylazine and ketamine hydrochloride for anesthesia in horses. *Am. J. Vet Res.*, 38: 195-201.
- Pechnick, R. N., (1993). Effects of opioids on the hypothalamo-pituitary-adrenal axis. *Annual Reviews Pharmacology and Toxicology.*, 32, 353-382.
- Radostits, O. M., C. C. Gay, K. W. Hinchcliff, P. D. Constable. 2007. *Veterinary Medicine: A textbook of diseases of cattle, horses, sheep, pigs and goat*. 10<sup>th</sup> Ed., W. B. Saunders Co., Philadelphia, USA.
- Sanhoury, A., R. Jones, H. Dobson (1992). Effects of xylazine on the stress response to transport in male goats. *British Vet. J.*, 148 (2): 119-128.
- Schmidt, A., E. Möstl, C. Wehnert, J. Aurich, J. Müller, C. Aurich (2010). Cortisol release and heart rate variability in horses during road transport. *Hormones and behavior*. 57 (2): 209-215.
- Taylor, P. M., S. P. Luna (1995). Total intravenous anaesthesia in ponies using detomidine, ketamine and guaiphenesin: pharmacokinetics, cardiopulmonary and endocrine effects. *Res Vet Sci.*, 59: 17-23.
- Valverde, A., (2010). Alpha-2 agonists in pain therapy in horses. *Vet Clin Equine*. 26: 515-532.