Response to Oxidative Stress during Surgery under Ketamine/Propofol Anaesthesia in Acepromazine-Xylazine Premedicated Horses

R. Gnanasekar and R. Vijayalakshmi

Division of Animal Husbandry, Faculty of Agriculture, Annamalai University,
Annamalai nagar – 608002, Tamilnadu, India

***Abstract*-** The study was conducted in twelve clinical cases of horses undergoing diagnostic and surgical procedures warranting general anaesthesia. The cases were randomly divided into two groups: group I and group II, each consisting of six horses. Pre-anaesthetic medication done with Xylazine hydrochloride @ 0.5mg/kg and Acepromazine maleate @0.03 mg/kg body weight in both the groups. Ketamine @ 2.20 mg/ kg body weight and 0.05mg/kg/min was used as induction and maintenance agent in group I and Propofol @ 2.0 mg/kg body weight and 0.15mg/kg/min was used as induction and maintenance agent in group II. The total serum protein showed no significant reduction after sedation, after induction, during maintenance and after recovery in both the groups. There was no significant difference in the neutrophil, lymphocyte, eosinophil, and monocyte counts in both the groups. The magnitude of blood glucose level was lower in group II compared to that of group I. Group I exhibited an increase in plasma cortisol level, when compared to that of group II.

***Index Terms*-** Horse, Oxidative stress, Ketamine/Propofol, Acepromazine, Xylazine

I. INTRODUCTION

Horses are the most challenging species to anaesthetize and are more prone for potential complications during induction, maintenance and recovery. During anesthesia and perioperative period of surgery, it is important to prevent undue excitement and anxiety. It is also important to maintain normal cardiac output, blood pressure and acid-base balance by ensuring adequate ventilation and oxygenation. The factors responsible for triggering stress and release of oxygen free radicals have to be minimized (Wagner, 1991).Oxidative stress occurred when the production of oxygen free radicals (RSO = Reactive oxygen species) and nitric oxide radicals (NOR) exceeded the scavenging capacity of systemic endogenic antioxidants through enzymatic and non enzymatic pathways (Basu *et al*., 2001).

The reactive oxygen species were hydrogen peroxide, superoxide anion, peroxide radicals, alkoxy radical, peroxy radical and nitric oxide radicals were nitric oxide and peroxynitrite (Brasil *et al*., 2006). The production of the free radicals was favoured directly by reduced perfusion and issue anoxia and indirectly by anaesthetic and ancillary drugs (Basu *et al*., 2001 and Ozer and Kaman, 2007). Free radicals induce stress, inflammation, delay in wound healing, prolonged recovery from anaesthesia and post anaesthetic complication on the functional ability of heart, lung, spleen, liver, kidney, red blood corpuscles and muscles (Brasil *et al*., 2006). Free radicals also interferes with the regeneration of tissues by inducing single bond breakage of DNA in morphology of cells. Xylazine, an alpha 2 adrenergic agonist and Acepromazine, a phenothiazine derivative are commonly used in horses as sedatives as well as pre-anaesthetic agents. Ketamine and propofol are the common induction agents and also useful for maintenance in the field of ambulatory equine practice. The aim of the study was to assess the magnitude of physiological stress in terms of plasma cortisol and oxidative stress in terms of enzymatic and non enzymatic antioxidants. This assay will reflect on the quantum of oxygen free radical in a directly proportional manner.

II. MATERIALS AND METHODS

Study Animals

Horses admitted for various ailments warranting surgery in Teaching Hospital, Madras Veterinary College, Chennai were utilized for the study. 12 horses were randomly selected and grouped into two (group I and group II) with each group consisting of six animals.

Pre-anesthetic medication

Xylazine hydrochloride @ 0.5mg/kg and Acepromazine maleate @ 0.03 mg/kg body weight by jugular vein was given to all the horses as premedicants.

Anesthesia

Ketamine @ 2.20 mg/ kg body weight and 0.05mg/kg/min was used as induction and maintenance agent for group I and Propofol @ 2.0 mg/kg body weight and 0.15mg/kg/min was used as induction and maintenance agent for group II horses.

Collection of samples and Analysis of data

All the blood samples were collected around 10 am to avoid diurnal variation (Taylor, 1989). Blood samples were collected before sedation, after sedation, after induction, during maintenance and after recovery in both the groups. Heparinized blood samples were centrifuged (1500rpm, 10 min) and supernatants were immediately stored at -80°C until analysis. Plasma cortisol was measured quantitatively using competitive Enzyme Linked Immuno Sorbent Assay-ElAgen Cortisol Kit. The activity of superoxide dismutase (SOD) was determined

using the method of Marklund and Marklund (1974). The assay is based on the ability of SOD to exhibit the autooxidation of pyrogallol in the presence of EDTA. The values were expressed as Units/mg Hb. The plasma glutathione peroxidase (GSH-Px) activity was determined according to the method of Hafeman *et al*. (1974). The rate of oxidation of GSH by H_2O_2 was used as measure of GSH-Px activity and expressed as Units/mg Hb. The plasma catalase (CAT) activity was measured as per the method described by Aebi (1983). 20 µl of 1% erythrocyte lysate was incubated in 1.0 ml of 30 mM H_2O_2 at 37°C and decrease in absorbance was noted at every 10 sec interval for one min. at 240 nm in a UV spectrophotometer (Schimadzu UV-1208 UV-VIS, Japan). 1 unit catalase activity was defined as the amount of enzyme that decomposes 1 µM of H_2O_2/min. and expressed as Units/mg Hb. The glutathione level in plasma was measured by the method of Moron *et al.* (1979**).** This method was based on the reaction of reduced glutathione with 5-5 dithiobis-2- nitro-benzoic acid (DTNB) to produce a compound that absorbs light at 412 nm. The values are expressed as µgm/ml plasma.

The data were analysed using Statistical Package for Social Sciences (SPSS - 19).

III. RESULTS AND DISCUSSION

The mean plasma cortisol level before sedation, after sedation, after induction, during maintenance and after recovery were shown in Table 1. The mean plasma cortisol level (ng per ml) in group I was 17.50 ± 0.76, 105.83 ± 2.30, 117.67 ± 2.83, 134.17 ± 2.06 and 83.17 ± 1.19 for before sedation, after sedation, after induction, during maintenance and after recovery, respectively. The respective mean cortisol level in group II was 16.50 ± 0.76, 102.33 ± 1.67, 110.33 ± 2.11, 121.33 ± 0.99 and 79.83 ± 1.19. Statistical analysis revealed a significant increase in the plasma cortisol level after sedation, after induction, during maintenance and after recovery in both the groups. However, the mean plasma cortisol level was found to be higher in group I. This is in accordance with the findings of Clarke *et al*. (1970), Traynor and Hall (1981) and Davis (1990). Increase in plasma cortisol level following surgical trauma and anaesthesia was reported by Clarke *et al*. (1970) and the reasons were decreased breakdown of plasma cortisol due to reduction in hepatic blood flow during surgery and elevation of plasma half-life of cortisol. Traynor and Hall (1981) and Davis (1990) reported that the plasma cortisol levels were elevated due to stress responses following impulses arising from afferent nerve fiber both systemic and autonomic from the surgical wounds and also by the release of humoral factors such as prostaglandin, histamine, kinins and leukotriens. The correlation of these diverse signals occurred in the hypothalamus.

In the present study, the elevation of plasma cortisol level following administration of xylazine as premedicant could be attributed to its $alpha_2$ adrenergic agonistic action, though acepromazine decreased the plasma cortisol concentration. This was in accordance with the findings of Bettschart-Wolfensberger *et al.*,(1996) and Montane *et al*.,(2003). Ketamine by its sympathotonic effect and activation of pituitary adrenocortical axis stimulated the plasma cortisol level (Bettschart-Wolfensberger *et al*., 1996 and Mahalingam *et al*., 2014). During propofol induction and maintenance, the plasma cortisol level was elevated significantly and reduced after recovery, but the magnitude was less than ketamine anaesthesia (Sankar *et al*., 2010). It could be concluded that propofol induced less stress in terms of protein, blood glucose, percentage of neutrophils, lymphocyte, eosinophil, monocyte and plasma cortisol.

Antioxidants

The mean Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Catalase (CAT) and Reduced Glutathione (GSH) activity for group I and group II were shown in Table 2. The mean SOD (U/mg Hb) values ranged between 2.50 ± 0.32 and 3.06 ± 0.13 in group I and 1.89 ± 6.21 and 2.14 ± 0.15 in group II following the different stages of study. Statistical analysis revealed no significant difference between the mean values of the group I and group II at different stages of the study. However, the magnitude of SOD enzyme activity was less in group II when compared with group I.

The mean GPx enzyme activity (U/mg Hb) in group I and group II ranged between 0.56 ± 0.02 and 0.85 ± 0.01 and 0.64 ± 0.02 and 0.80 ± 0.03 respectively. Statistical analysis revealed significant increased activity of GPx in both the groups. However the activity of GPx was less in group II when compared with group I.

The mean CAT enzyme activity (U/mg Hb) values ranged between 25.31 ± 0.78 and 30.76 ± 0.89 in group I and 27.18 ± 1.12 and 29.97 ± 1.10 in group II. Statistical analysis revealed significant increase in CAT enzyme activity in both the groups. However, the activity of CAT was higher in group I when compared with group II.

The mean GSH activity (µg/ml plasma) in group I ranged between 85.12 ± 2.67 and 106.11 ± 2.45 and 98.71 ± 2.81 and 107.23 ± 3.00 in group II. Statistical analysis revealed a significant increased activity of GSH in both the groups after sedation. The activity of non enzymatic antioxidant GSH was less in group II when compared with group I.

Basu *et al*., (2001) reported that oxidative stress occurred when the production of oxygen free radicals (RSO = reactive oxygen species) and nitric oxide radicals (NOR) exceeded the scavenging capacity of systemic endogenic antioxidants through enzymatic and non enzymatic pathways. The reactive oxygen species were hydrogen peroxide, superoxide anion, peroxide radicals, alkoxy radical, peroxy radical and nitric oxide radicals were nitric oxide and peroxynitrite (Brasil *et al*., 2006). Xylazine and acepromazine reduced tissue perfusion (Steffey *et al*., 1977 and Wagner *et al*., 1991) and depended on hepatic cytochrome P450 for metabolism leading to elevated free radical formation which resulted in high antioxidant activity after premedication.The production of these free radicals was favoured directly by reduced tissue perfusion and tissue anoxia and indirectly the anaesthetic and ancillary drugs as reported by Basu *et al*., (2001) and Ozer and Kaman, (2007). The free radicals were released from cytochrome P450, endothelium, granulocytes, macrophages and red blood corpuscles. Free radicals caused stress, inflammation, delay in wound healing, prolonged recovery from anaesthesia and post anaesthetic complications due to the insult on the functional ability of heart, lung, spleen, liver, kidney, red blood corpuscles and muscles (Brasil *et al*., 2006). Delogu *et al*., (2004) reported that the half-life of free radicals is 10^{-6} to 10^{-9} seconds at 37^0C except

hydrogen peroxide and concluded that the level of free radicals was indirectly assessed by the directly proportional activity of enzymatic antioxidants namely SOD, GPx and CAT and non enzymatic antioxidant -GSH.

The sum of total activity of antioxidants namely enzymatic antioxidants - SOD, GPx and CAT and nonenzymatic antioxidant -GSH significantly increased after premedication with xylazine and acepromazine and further progressively increased during maintenance with ketamine. The activity started declining after recovery. Between the groups, antioxidants activity was increased more during ketamine induction and maintenance when compared to propofol induction and maintenance. The reduction in the antioxidants activity during propofol anaesthesia could be attributed to the direct scavenging of free radicals by propofol due to the structural similarity with natural antioxidant alpha tocopherol (Aarts *et al*., 1995, Sagara *et al*., 1999; Peter *et al*.,2001 and Basu *et al*., 2001). The production of free radicals was less as the biotransformation of propofol was not totally depended on hepatic cytochrome P450 but also on the other body tissues (Aubin and Mama,2002). Propofol anesthesia reduced the oxidative stress and enhanced the antioxidant defense mechanisms expressed by larger concentrations of free radical scavengers (Allaouchiche *et al*., 2001, Lee and Kim, 2012 and Lee, 2012). Further Peter *et al*., (2001) observed that propofol reduced the metabolic oxygen requirement of the tissues by reducing the metabolic rate and thereby minimized tissue anoxia and the production of free radicals, also provided neuroprotective effect against injuries caused by ischemia/reoxygenation. Runzer *et al*., (2002) reported that propofol enchanced tissue and RBC antioxidant capacity.

Ketamine due to increase in sympathetic tone increased the vascular resistance leading to tissue anoxia and release of free radicals. As ketamine depended on hepatic cytochrome P450 for detoxification and biotransformation (except 4 per cent excreted as whole) the release of free radicals was more which inturn resulted in higher antioxidant activity. Alva *et al*., (2006) reported that ketamine induced moderate oxidative stress by increasing the level of plasmatic nitric oxide and change in acid-base balance and metabolic acidosis. The results of the present study was supported by the findings of Mahalingam *et al*., (2014), that the xylazine - ketamine anaesthesia in dogs caused an increase in lipid peroxidation. This was accompanied by enhanced antioxidant status and also the superoxide dismutase and catalase provided the first line of defence against the damages induced by the reactive oxygen species.

Akin *et al*. (2015) reported that propofol activated the antioxidant system against the oxidative stress by inhibiting the lipid peroxidase production by binding with the membrane phospholipids and capturing of free radicals.

IV. CONCLUSION

The plasma cortisol level significantly elevated during maintenance and recovery in ketamine induction and maintenance when compared to propofol. The antioxidant level was significantly higher in ketamine anaesthesia when compared to propofol, revealing higher production of oxygen free radicals due to less tissue perfusion and activation of hepatic cytochrome P450. It was concluded that ketamine as the effective drug as induction and maintenance agent for short duration and propofol for long duration anaesthetic procedures in the field ambulatory equine practice.

Table 1. Mean ± SE value of Plasma cortisol in group I and group II

Parameters	Group	Before sedation	After sedation	After induction	During maintenance	After recovery
Plasma cortisol (ng/ml)	I	17.50^{a}± 0.76	105.83^{c}± 2.30	117.67^{d}± 2.03	134.17^{e}± 2.06	83.17^{b}± 1.19
	II	16.50^{a}± 0.76	102.33^{c}± 1.67	110.33^{d}± 2.11	121.33^{e}± 0.99	79.83^{b}± 1.19

Mean values bearing different superscripts in a row differ significantly($P<0.05$).

Table 2. Mean ± SE value of antioxidants in group I and group II

Parameters	Group	Before sedation	After sedation	After induction	During maintenance	After recovery	Summation of activity
Super oxide disamutase (U/mg Hb)	I	2.50^{a}±0.32	2.64^{b}± 0.22	2.88^{c}±0.23	3.06^{d}±0.13	2.72^{b}±0.11	13.8
	II	1.89^{a}±0.21	1.93^{b}±0.19	1.99^{c}±0.28	2.14^{d}±0.15	2.02^{c}±0.17	9.97
Glutathione peroxidase (U/mg Hb)	I	0.56^{a}±0.0.2	0.61^{b}±0.01	0.78^{c}±0.03	0.85^{d}±0.01	0.65^{b}±0.02	3.45
	II	0.64^{a}±0.02	0.72^{c}±0.03	0.78^{d}±0.02	0.80^{d}±0.03	0.69^{b}±0.02	3.63
Catalase (U/mg Hb)	I	25.31^{a} ± 0.78	27.01^{b} ± 1.32	28.90^{c} ± 1.09	30.76^{d} ± 0.89	27.43^{b} ± 1.09	139.41
	II	27.18^{a}±1.12	28.81^{bc}±1.01	29.35^{cd}±0.83	29.97^{d}±1.10	28.45^{b}±0.95	143.76

Reduced glutathione (µg/ml plasma)	I	98.84^{d}±3.43	106.11^{e}±2.45	90.76^{b}±2.43	85.12^{a}±2.67	93.43^{c}±2.74	474.26
	II	101.13^{b}±2.99	107.23^{d}± 3.00	102.62^{c}± 3.12	98.71^{a}± 2.81	99.12^{a}± 2.45	508.81

Mean values bearing different superscripts in a row differ significantly($P<0.05$).

REFERENCES

[1] Aarts, L., R. Van der Hee, I. Dekker, J. De Jong, H. Langemeijer and A. Bast, 1995. The widely used anaesthetic agent propofol can replace α-tocopherol as an antioxidant. FEBS letters. 357: 83-85.

[2] Aebi, H. 1983. Catalase. In Methods Enzymology, Ed. H. U. BergMeyer, Academic Press, New York. pp. 276-286.

[3] Akin, M., H. Ayoglu, D. Okyay, F. Ayoglu, A. Gur, M. Can, S. Yurtlu, V. Hanci, G. Kucukosman and I. Turan, 2015. Effects of various anesthesia maintenance on serum levels of selenium, copper, zinc, iron and antioxidant capacity. Rev Bras Anestesiol. 65(1): 51-60.

[4] Allaouchiche, B., R. Debon, J. Goudable, D. Chassard and F. Duflo, 2001. Oxidative stress status during exposure to propofol, sevoflurane and desflurane. Anesth Analg. 93: 981–985.

[5] Alva, N., J. Palomeque and T. Carbonell, 2006. Nitric oxide induced by ketamine/xylazine anaesthesia maintains hepatic blood flow during hypothermia. Nitric oxide. 15: 64-69.

[6] Aubin, M.L. and K. Mama, 2002. Field anaesthetic techniques for use in horses.Compendium @ medimedia.com. 24: 411- 415.

[7] Basu, S., D.K. Mutschler, A.O. Larsson, R. Kiiski, A. Nordgern, and M.B. Eriksson, 2001. Propofol (Diprivan-EDTA) counteracts oxidative injury and deterioration of the arterial oxygen tension during experimental septic shock. Resuscitation. 50: 341-348.

[8] Bettschart-Wolfensberger, R., P.M. Taylor, J.W. Sear, M.R. Bloomfield, K. Rentsch and Dawling, 1996. Physiologic effects of anaesthesia induced and maintained by intravenous administration of a climazolam-ketamine combination in ponies premedicated with acepromazine and xylazine. Am. J. Vet. Res. 57: 1472-1477.

[9] Brasil, L.J., B.S. Miguel, N.A. Kretzmann, J.L. Gomesdo , C.G. Zettler, N. Marroni, J.G. Gallego and M.J. Tunon, 2006. Halothane induces oxidative stress and NF-KB activation in rat liver: Protective effective of propofol. Toxicology. 227: 53-61.

[10] Clarke, R.S., H. Johnson and B. Sheriden, 1970. The influence of anaesthesia and surgery on plasmacortisol, insulin and free fatty acids. Br. J. Anaesth. 42: 295-299.

[11] Davis, C.A. 1990. The human stress response to surgical trauma and anaesthesia. Equine Vet. J. 22.304-305.

[12] Delogu, G., A. Antonucci, S. Moretti, M. Marandola, G. Tellan, M. Signore, and G. Famularo, 2004. Oxidative stress and mitochondrial glutathione in human lymphocytes exposed clinically relevant anaesthetic drug concentration. J. Clinical Anaesth. 3: 189-194.

[13] Hafeman, D. G., R. A. Sunde and W. G. Hoekstra. 1974. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rats. J. Nutr. 104: 580-587.

[14] Lee, J.Y, 2012. Oxidative Stress Due to Anesthesia and Surgical Trauma and Comparison of the Effects of Propofol and Thiopental in Dogs. J. Vet. Med. Sci. 74(5): 663–665.

[15] Lee and Kim, 2012. Effect of propofol on oxidative stress status in erythrocytes from dogs under general anaesthesia. Acta Veterinaria Scandinavica. 54: 76.

[16] Mahalingam, A., N. Kumar, S.K. Maiti, A.K. Sharma, U. Dimri, M. Kataria, D.D. Mathew, V. Remya and A. Mohsina, 2014. Laparoscopic vasectomy vs laparoscopic sterilization in dogs: A comparision of two techniques. World J. Lap. Surg. 7(1): 7 -15.

[17] Marklund, S.L and G. Marklund, 1974. Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur.J. Biochem. 47: 469-474.

[18] Montane, J., I. Marco, J.L. Olvera, D. Perpinan, X. Manteca and S. Lavin, 2003. Effects of acepromazine on capture stress in Roe deer (Capreoluscapreolus). J. Wildlife disease. 39: 375-386.

[19] Moron, M.S., J.W. Depierre and B. Mannervik, 1979. Level of glutathione, glutathione reductase and glutathione-s transferase in rat lung and liver. Biochem. Biophys. Acta. 582: 67-78.

[20] Ozer, A.B. and D. Kaman, 2007. Effects of epigallocatechin 3-gallate in rat cardiac tissue on oxidant and antioxidant system exposed to sevoflurane anaesthesia. Firat Medical J. 12: 93-96.

[21] Peter, C.E., J. Korcok, A.W. Gelb and J.X. Wilson, 2001. Anaesthetic concentration of propofol protect against oxidative stress in primary astrocyte cultures. Anaesthesiol. 94: 313-321.

[22] Runzer, T.M, D.V. Ansely, D.V. Godin and G.K. Chambers, 2002. Tissue antioxidant capacity during anaesthesia: Propofol enchances in vivo red cell and tissue antioxidant capacity in a rat model. Anesth.Analg. 94: 89-93.

[23] Sagara, Y., S. Hendler, S. Khoh-Reiter, G. Gillenwater, D. Carlo, D. Schubert, and J. Chang, 1999. Propofol hemisuccinate protects neuronal cell death from oxidative injury. J. Neurochem. 73: 2524-2530.

[24] Sankar, P., B.J. William, G.D.J. Rao, S. Prathaban, R.S. Kumar and V. Leela, 2010. Oxidative stress response during surgery under ketamine/propofol anaesthesia in acepromazine-xylazine premedicated horses. Ind. J. Anim. Res. 44(2): 113 – 117.

[25] Steffey, E.P., J.D. Wheat, D.M. Meagher, R.D. Norrie, M. Brown and J. Arnold, 1977. Body position and mode of ventilation influences arterial pH, oxygen and carbondioxide tensions in halothane anaesthetized horses. Am. J. Vet. Res. 38: 379-382.

[26] Taylor, P.M. 1989. Equine stress response to anaesthesia. Br. J. Anaesth. 63: 702-709.

[27] Taylor, P.M. and S.B. Watkins, 1992. Stress response during total intravenous anaesthesia in ponies with detomidine-guaiphenesin-ketamine. J. Vet. Anaesth. 19: 13-17.

[28] Traynor, C. and G.M. Hall, 1981. Endocrine and metabolic changes during surgery: Anaesthetic implications. Br. J. Anaesth. 53: 153-160.

[29] Wagner, A.E., 1991. Stress associated with anaesthesia and surgery. In: Equine anaesthesia, Monitoring and emergency therapy, Muir, W.W. and J.A.E. Hubbel, Edt., Mosby year book, St. Louis, pp.105-113.

[30] Wagner, A.E., W.W. Muir and K.W. Hinchdiff, 1991. Cardiovascular effects of xylazine and detomidine in horses. Am. J. Vet. Res. 52: 651-657.

AUTHORS

First Author – R. Gnanasekar, Division of Animal Husbandry, Faculty of Agriculture, Annamalai University, Annamalai nagar – 608002, Tamilnadu, India.
Second Author – R. Vijayalakshmi, Division of Animal Husbandry, Faculty of Agriculture, Annamalai University, Annamalai nagar – 608002, Tamilnadu, India.