COMPARATIVE EVALUATION OF XYLAZINE-KETAMINE ANAESTHESIA WITH AND WITHOUT ORAL HALOPERIDOL PREMEDICATION FOR VASECTOMY IN SPOTTED DEER (*Axis axis*)

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "**Comparative evaluation of xylazine-ketamine anaesthesia with and without oral haloperidol premedication for vasectomy in Spotted Deer (*Axis axis*)**" is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title of any other University or Society.

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JOJU JOHNS

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1. INTRODUCTION

From the roaming hunter-gatherer to the settled agriculturist to the tech-savvy jet-setting modern human, we have taken the fast track of evolution, enjoying special advantages that have helped us assert ourselves as the most dominating species in the scheme of life on planet earth. Whether crossing our paths on a day-to-day basis during our early days in the forests or coming across them occasionally in an urban zoo setting or more frequently as in the case of a modern wildlife veterinarian, wild animals have always caught the imagination of every single human being that have lived on earth. Animal behaviour of various sorts has always enchanted us, whether we were looking at them as prey or source of food, as a competitor for food, looking out for them as predators, collaborating with them for draught purposes, working for them as professionals as in the case of veterinarians, simply looking at them for a pastime or stalking them with binoculars as ethologists.

One of the most enigmatic peculiarities of wild animals being chased or restrained for various reasons has been uncontrollable stress amounting to distress and the subsequent, often lethal, consequence of capture myopathy. Spiralling stress leading to capture myopathy has been recognised in all classes of animals and birds with only reptiles being the exception (Paterson, 2007). Wild ungulates, especially deer species, have been identified to be most susceptible to the condition compared to carnivores. Humans must have been awed by this phenomenon from time immemorial. This cervid predicament has been used to their advantage by our forefathers while hunting deer for food, as have many other four legged predators. On the other hand, scientists and anaesthesiologists have toiled for years to find means to reduce the effects of uncontrolled stress and improve the outcome of activities involving restraint of deer, for various purposes like clinical examination, treatment of injuries, surgical procedures, translocation, radio collaring operations, research *etc*.

Protocols for prevention, reduction or management of stress related complications associated with handling or physical restraint operations and chemical restraint of wild animals have been constantly developed and tested for effectiveness since a very long time. In fact, this has become something like an obsession with a part of humanity ever since humans stopped looking at deer only as predators would and started looking up ways to improve their welfare as considerate members of the dominant species on earth.

Reduction of chase time, fasting, blind folding, proper positioning, oxygen supplementation *etc.* have been suggested to reduce stress and related complications associated with chemical restraint of wild ungulates (Caulkett and Haigh, 2004). Over the years, several classes of drugs like neuromuscular blocking agents, sedative/tranquilisers, general anaesthetics and antagonists have been tried to reduce stress and prevent complications of handling in wild ungulates.

Many of these drugs and their beneficial effects in wild ungulates have been well studied and documented. However, opportunities to scientifically study the effectiveness of many of these drugs and protocols are limited in free-ranging as well as captive wildlife. Even though commendable attempts to study safe methods for chemical restraint of deer and other ungulate species have been made in Europe, Africa and the western world, standard scientific studies in Indian species have been meagre. An earnest attempt to review literature associated with chemical restraint methods of Spotted Deer (*Axis axis*), an extremely common deer species of India, emphasised this situation. A few reports of successful chemical restraint of Spotted Deer demonstrated a wide variation in dosages of common veterinary anaesthetic drugs and no attempt at premedication (Kreeger, 1996; Sontakke *et al.,* 2007; Venugopal *et al.,* 2013), though the latter is widely recognised as a very valuable means to reduce stress and complications in anaesthetised animal patients.

Under these circumstances, the present study was conducted in 20 adult Spotted Deer stags reared in captivity at the Zoological Gardens, Thiruvananthapuram, to evaluate the effectiveness of a chosen dose combination

of xylazine and ketamine with and without oral premedication with haloperidol. The animals were randomly selected from a herd of deer that had to be anaesthetised to undergo routine vasectomy procedure as per directions of the Central Zoo Authority of India as part of population control measures in the zoo.

The following were the objectives of the study.

1. To compare the quality of xylazine-ketamine anaesthesia with and without oral haloperidol premedication for vasectomy in Spotted Deer.

2. To study the physiological, haematological and biochemical changes associated with anaesthesia for vasectomy in Spotted Deer using both protocols.

2. REVIEW OF LITERATURE

2.1. IMPORTANCE OF CHEMICAL IMMOBILISATION IN WILD ANIMALS WITH SPECIAL REFERENCE TO WILD UNGULATES

Short (1969) described that the anaesthetic risk of wild animals was very high since they were prone to excitement. Physiological and psychological stress also contributed to this risk.

Kreeger (1996) descrided the use of various classes of drugs like neuromuscular blocking agents, sedatives, tranquilisers, general anaesthetics and antagonists for the chemical immobilisation of wild animals.

Williams and Throne (1996) opined that utmost care had to be taken during the handling of wild animals to prevent capture myopathy.

Ebedes and Raath (1999) noted that tranquilisation of wild herbivores helped to reduce the stress during translocation, adaptation to unnatural surroundings and also facilitated hospitalisation and treatment.

Caulkett and Arnemo (2007) observed that wild animal chemical immobilisation was challenging due to various factors like lack of knowledge about the general health status of the animal, pharmacological effects of the drug on various species, stress related complications during induction of anaesthesia *etc.* which led to high morbidity and mortality of animals.

Fahlman (2008) opined that there were limited or no proper scientific data about the physiology and anaesthetic protocols for wild animals and that the goal of anaesthesia was to reduce the physiological stress to the animal. The author also stated that improvement of the chemical immobilisation procedure of wild animals had significant role in wildlife conservation and animal welfare.

Dickens *et al*. (2010) suggested that anaesthesia and tranquilisation helped to reduce stress during handling and translocation. The perception of stressors during the procedure had to be limited and care taken to minimize stress during the sedation technique.

2.2. PRE-ANAESTHETIC CONSIDERATIONS

Caulkett and Haigh (2004) suggested that several factors had to be considered in the selection of methods and means of drug administration. Enclosing the captive wild animal to a handling facility reduced the chase time so that the chances of trauma, hyperthermia and capture myopathy could be prevented.

Caulkett and Haigh (2007) opined that ruminal tympany was a complication during anaesthesia in cervids and that ideally they had to be fasted for 24 hours prior to anaesthesia. Elective procedures were recommended to be performed during the cool hours of the day to prevent hyperthermia.

Wolff (2009) suggested that during chemical immobilisation of captive exotic small ruminants, visual and auditory stressors had to be minimised and the person involved in darting had to remain outside the flight distance of the animal to prevent excitement of the animal and the herd.

2.3. PREMEDICATION

Presidente *et al*. (1973) reported that administration of acepromazine maleate orally in White-tailed Deer fawns helped to reduce stress prior to blood sampling.

Pusateri *et al.* (1982) described the effectiveness of diazepam administered orally in Pronghorn as a tranquiliser. The authors reported that the adequate dose range of diazepam for captive adult Pronghorn was 18 to 23 mg/kg body weight.

Neuroleptic drugs like haloperidol, azaperone, perphenazine enanthate *etc.* have been recognised as being capable of reducing aggression, excitement, fear of humans and other stress related complications in both free-ranging and captive wild animals (Blumer, 1991; Mikota *et al.*, 1999).

2.3.1. Haloperidol

Hofmeyr (1981) reported that haloperidol was effective in controlling psychological stress and physical injuries during handling and transportation of most of the African antelope species and the survival rates were enhanced in these species during translocation operations. The author suggested that animals which were subjected to haloperidol therapy had to be kept calm to produce the desirable effects. Abnormal feeding behaviour had to be expected and hence access to foreign bodies needed to be prevented. It was also noted that administration of xylazine hydrochloride could enhance the soporific (sleep inducing) effect of haloperidol in Hartmann's Zebra and Black-faced Impala.

Administration of high doses of haloperidol or prolonged use of the drug resulted in incidence of extrapyramidal signs like opisthotonos, torticollis, rolling of tongue, chewing, Parkinsonism *etc.* (Blumer, 1991).

Mikota *et al.* (1999) reported that oral dosing of haloperidol at the rate of 1 mg/kg body weight in Bongo Antelope affected the central nervous system with the drug attaining peak serum concentration by 10 hours post oral dosing. The behavioural responses were scored from good to excellent from 3 to 10 hours post oral dosing, which provided sufficient tranquilisation for physical examination, rectal temperature recording, blood collection *etc.*

Read (2002) opined that haloperidol could be administered orally and it produced dopamine antagonist action. Since dopamine was one of the five main neurotransmitters involved in behavioural modification, the tranquilisation effects were produced by blocking dopamine receptors, leading to calming of the animal during stressful situations without causing major cortical depression.

Walsh and Wilson (2002) noted that haloperidol produced an excellent calming effect in Fallow Deer, lasting 8–10 hours and in some small and medium-sized antelope species, especially Red Hartebeest, Springbok, Duiker, Steenbok and Dik Dik. Haloperidol was also reported to be effective for post capture tranquilisation in antelope.

Wolff (2009) opined that oral administration of haloperidol could be practised in captive ruminants prior to transportation, introductions, close examination *etc.*

Mentaberre *et al.* (2010) observed that parenteral administration of haloperidol after drive-net capture in Southern Chamois helped to inhibit the sympathetic-adrenal-medulla axis to some extent and were reflected in the serum creatinine, chloride and potassium levels due to improved renal function during stress response.

2.4. ANAESTHETIC DRUGS

Caulkett and Arnemo (2007) recommended that an ideal drug combination used for chemical immobilisation of wild animals should have most of the qualities like rapid onset of action, high margin of safety, small volume of delivery, drug stability, versatility, handler safety and should provide analgesia and narcosis.The authors also observed that the combination of xylazine and ketamine has been used for many years and was effective in many ungulate species.

Sontakke *et al.* (2007) noted that anaesthetic drugs like xylazine hydrochloride, medetomidine, ketamine hydrochloride, tiletamine *etc.* were routinely used for wild animal chemical immobilisation.

2.4.1. Xylazine Hydrochloride and Ketamine Hydrochloride Combination

DelGiudice *et al.* (1988) described xylazine as a non-narcotic compound which was used effectively in combination with ketamine to immobilise wild animals.

Stewart and English (1990) reported that a combination of xylazine and ketamine at the rate of 4 mg/kg body weight each, administered intramuscularly, provided sufficient chemical restraint for 15 to 30 minutes in adult Fallow Deer. The onset of sedation and time for recumbency varied with the excitability of the animal.

Xylazine and ketamine combination has been effectively used for the immobilisation of adult female Moose, which provided adequate induction time, sufficient level of anaesthesia, wide range of dose tolerance and total survival rate (Gamer *et al.*,1994).

Kreeger (1996) reviewed that a combination of xylazine and ketamine at the rate of 4 mg/kg body weight each was used for chemical immobilisation of Axis Deer.

A combination of xylazine and ketamine was found to provide sufficient anaesthesia for restraint, transportation and jugular catheterisation in White-tailed Deer. An additional dose of ketamine at the rate of 2 mg/kg body weight intravenously had to be administered in a few animals to facilitate orotracheal intubation (Posner *et al.*, 2005).

Sontakke *et al.* (2007) reported that the use of xylazine-ketamine combination at the rates of 0.5 and 2.5 mg/kg body weight respectively in male Spotted Deer produced good level of anaesthesia for electroejaculation without any sudden recovery and adverse effects of anaesthesia. The authors also reported that the anaesthesia was reversed using yohimbine intravenously.

Wolff (2009) suggested that a combination of an alpha$_2$-adrenergic agonist (xylazine) with cyclohexamine (ketamine) helped to reduce the dose requirement of ketamine and nullified most of the adverse effects of ketamine like convulsion, muscle rigidity, excitement *etc.* in exotic hoofed stock.

Venugopal *et al.* (2013) reported that a total dose of 150 mg of xylazine and 100 mg of ketamine per animal were used effectively for chemical immobilisation for translocation of adult male and female Spotted Deer.

2.4.2. Yohimbine Hydrochloride

Bubenik and Brown (1989) reported that yohimbine at the rate of 0.3 mg/kg body weight intravenously reversed the immobilisation produced by xylazine in Axis Deer.

Wallingford *et al.* (1996) observed the effectiveness of intramuscular administration of yohimbine to antagonise xylazine induced immobilisation in White-tailed Deer.

Galka *et al.* (1999) reported that the use of an alpha$_2$-adrenergic antagonist like yohimbine helped to shorten the recovery time from anaesthesia produced by a combination of xylazine and ketamine in Fallow Deer. The authors observed instances of rougher recovery due to the residual dissociative anaesthetic effect.

Read (2003) reviewed that the hypoventilation induced by xylazine administration in calves was reversed after the administration of alpha$_2$-antagonists like yohimbine and idazoxan.

Posner *et al.* (2005) reported that administration of yohimbine hydrochloride at the rate of 0.2 mg/kg body weight intravenously effectively reversed anaesthesia induced by a combination of xylazine and ketamine hydrochloride in White-tailed Deer.

Wolff (2009) suggested that alpha$_2$-agonist should not be reversed within 30 minutes from induction of anaesthesia during chemical immobilisation of hoofed stock with a combination of alpha$_2$-agonist and ketamine combination, since time had to be given to metabolise the administered ketamine.

2.5. MONITORING OF ANAESTHESIA

Kock *et al.* (1987) suggested that post-capture status of free-ranging Bighorn Sheep could be predicted by assessing their physiological and biochemical parameters during capture.

Haulton *et al.* (2001) opined that constant monitoring of vital signs during chemical capture helped to react in a timely manner towards complications which reduced the mortality rate compared to other methods of physical capture in White-tailed Deer.

Haskins (2007) opined that anaesthetic risks were unpredictable and could be devastating and that close monitoring during anaesthesia would maximise the safety of the anaesthetic experience.

Heard (2007) suggested that monitoring of physiological parameters and reflexes had to be done during chemical immobilisation and anaesthesia to assess depth of anaesthesia, cardiopulmonary function and effectiveness of supportive care. The author also suggested that the person monitoring had to have a plan and knowledge about the normal physiology and effects of the immobilising drugs.

Kilgallon *et al.* (2008) recommended that portable blood gas analysers could be effectively used for the close monitoring of anaesthesia during field immobilisations of captive and free-ranging wild ungulates. The authors suggested that the equipment could be effectively used even by personnel who were not trained in laboratory techniques.

2.5.1. Induction of Anaesthesia

Gamer and Addison (1994) reported that induction time varied with the initial level of excitement and the pursuit time during chemical immobilisation in adult female Moose.

Storms *et al.* (2005) studied qualitative parameters like excitability, muscle rigidity and overall quality, with each parameter assigned a numerical scale, in White-tailed Deer which were immobilised with carfentanil and xylazine combination. The parameters were assessed to score the quality of induction of anaesthesia.

Monteith *et al.* (2012) described time to sternal recumbency as the time taken by the animal to lie down with inablity to rise and time to lateral recumbency as time when the animal rested the head to the ground with no response to external stimuli.

2.5.2. Physiological Parameters

Gericke *et al.* (1978) reported that 50 per cent of Springbok that were captured by drop net had rectal temperature above 42°C suggestive of hyperthermia and that there was marked increase in the heart rate and respiratory rate.

Caulkett and Haigh (2004) opined that severe hypoxemia resulted in tachycardia in animals. In adult deer, tachycardia (heart rate > 150/minute) followed by bradycardia (heart rate < 30/minute) indicated severe hypoxemia and occurrence of heart failure.

Sontakke *et al.* (2007) reported that increased doses of xylazine in the xylazine-ketamine anaesthetic combination resulted in bradycardia and respiratory depression in Axis Deer.

Susanne (2012) reported that there was increase in the heart rate in response to anthropogenic external disturbances in Red Deer and Roe Deer.

2.5.3. Pulse Oximetry

Read (2003) noted that oxyhaemoglobin saturations monitored using pulse oximeter was below 85-90 per cent in wild ruminants immobilised with alpha$_2$-adrenergic agonist based combinations.

Arnemo *et al.* (2005) observed hypoxia with SpO_2 less than 90 per cent in Hog Deer which were immobilised using medetomidine-ketamine combination.

Assessment of haemoglobin oxygen saturation by pulse oximetry was suggested to be not accurate when the actual oxygen saturation was below 80 per cent in White-tailed Deer (Muller *et al.*, 2012).

2.5.4. Blood Gas Analysis

Kock *et al.* (1987) reported that the plasma pH level was low in free-ranging Bighorn Sheep which had capture myopathy when compared to the normal free-ranging Bighorn Sheep which were captured by various methods.

Martucci *et al.* (1992) observed severe metabolic acidosis in Bighorn Sheep after helicopter aided capture and physical restraint. The authors opined that accumulation of lactic acid due to the anaerobic glycolysis during chase and restraint as the major contributing factor for metabolic acidosis.

Boyd *et al.* (2000) observed normal blood pH and oxygenation in Bongo Antelope which were manually restrained three hours after oral administration of haloperidol.

Storms *et al.* (2005) noted that excitement, struggling and pacing prior to chemical immobilisation led to metabolic acidemia in White-tailed Deer immobilised with carfentanil-xylazine combination.

Smith *et al.* (2006) reported that physical exertion prior to immobilisation resulted in elevation of lactic acid in blood and metabolic acidosis in Axis Deer.

The PaO_2 and $PaCO_2$ levels of normal animals breathing room air at sea level was described to range from 80 to 110 mmHg and 35 to 45 mmHg respectively by Haskins (2007).

Bateman (2008) opined that acid-base alterations occurred due to the variation of carbon dioxide content in the blood and abnormal cellular metabolism, which were generally known as respiratory and metabolic acid-base alterations respectively. The partial pressure of carbon dioxide, bicarbonate level and base excess values helped to assess the type of alteration. The author also opined that venous blood samples were preferred to assess the acid-base status of the animal since they contained cellular waste products and arterial blood samples were preferred to assess the respiratory gas exchange.

Kilgallon *et al.* (2008) observed that the venous pH values were similar to the arterial values in non-tranquilised Arabian Oryx and suggested that venous pH values could be substituted for arterial values.

Boesch *et al.* (2011) reported that all White-tailed Deer which were captured using traps had arterial blood pH $\leq$ 7.35, base excess $< \text{-}$ 5 mmol/L and plasma lactate level > 5 mmol/L which resulted in lactic acidemia.

2.5.5. Electrocardiography

Doherty *et al.* (1986) observed that administration of xylazine at the rate of 0.15 mg/kg body weight in sheep resulted in the occurrence of second degree heart block for a transient period.

Caulkett *et al.* (2000) opined that combined effect of catecholamines and myocardial sensitisation produced by xylazine resulted in ventricular premature contractions in deer.

Ventricular arrhythmia was described as a complication primarily due to anaesthesia and was noted to be deleterious to the patient when it affected the cardiac functions (Haskins, 2007).

Ahmed *et al.* (2009) described the use of lead II electrocardiogram to detect occurrence of cardiac arrhythmias in White-tailed Deer immobilised using acepromazine-ketamine combination.

Boesch *et al.* (2011) observed that in a comparative study of biochemical variables in White-tailed Deer after chemical immobilisation in clover traps or by ground darting using butorphanol and medetomidine combination and maintenance using isoflurane, three Deer (one trapped Deer and two ground darted Deer) showed cardiac dysrhythmias like ventricular premature contractions, atrial premature contractions and second-degree atrioventricular block. All the three Deer had received ketamine hydrochloride for supplementing anaesthesia or for endotracheal intubation.

2.5.6. Biochemical Alterations

High blood glucose levels were observed in Springbok after an episode of stress which could be considered as an indicator of alarm reaction and catecholamine release (Gericke *et al.*, 1978).

Bighorn Sheep which were stressed during capture operation had elevated blood glucose, creatine phosphokinase and aspartate aminotransferase levels when compared to normal Bighorn Sheep. Animals which had cortisol levels greater than 5 µg/dL were found to have markedly elevated creatine phosphokinase level (Kock *et al*.,1987).

Stewart and English (1990) reported that alpha$_2$-adrenergic agonist mediated hypoinsulinaemia resulted in hyperglycemia and osmotically mediated haemodilution in Fallow Deer which were chemically immobilised using xylazine-ketamine combination.

Chapple *et al.* (1991) observed elevated serum concentrations of muscle enzymes like creatine kinase (CK) and aspartate aminotransferase (AST) in untrained Chital Deer stags when compared to those trained and adapted for physical restraint and blood collection. The authors also opined that CK and AST were sensitive indicators of muscle damage.

Spraker (1993) described that prolonged hypoxia and high demand of energy led to anaerobic metabolism and resulted in decreased intracellular pH due to the accumulation of intracellular lactic acid and hydrogen ions. The decrease of intracellular pH affected the active transport of sodium and potassium, which resulted in the accumulation of sodium in the cell and potassium in the extracellular fluid. The author described that muscle fibre damage due to mechanical trauma during capture led to efflux of potassium ions from the myocytes and resulted in hyperkalaemia, which caused functional abnormalities of cardiac muscles and skeletal muscles.

Morton *et al.* (1995) reported that plasma cortisol level could be effectively used as an indicator of capture related stress in wild animals.

Williams and Throne (1996) opined that serum levels of creatine kinase and aspartate aminotransferase which were released during skeletal muscle damage could be used for the diagnosis and prognosis of exertional myopathy in wild animals. The authors also noted that the serum levels of creatine kinase rose rapidly when compared to aspartate aminotransferase after skeletal muscle damage.

Mean reference values for biochemical parameters like creatine kinase (CK), aspartate aminotransferase (AST), calcium, potassium, phosphorus, and glucose were 765 U/L, 84 U/L, 2.28 mmol/L, 5.7 mmol/L, 2.68 mmol/L and 178 mg/dL respectively ISIS (2002).

Meyer *et al.* (2008) observed that the plasma cortisol level and behavioural response of Impala to stressors were correlated with the extent of habituation and

handling when compared to “naïve animals”.

Stringer *et al.* (2011) suggested blood glucose level estimation as an alternative to cortisol estimation to evaluate stress indirectly in White-tailed Deer.

2.5.7. Haematological Alterations

Gericke *et al.* (1978) reported that haloperidol therapy was effective in controlling alarm reaction and it had significant effect on haematological parameters in Springbok subjected to net capture.

Chapple *et al.* (1991) observed that handling stress had effect on the haematological parameters and there was 10 to 20 per cent increase in the haemoglobin concentration, erythrocyte count and haematocrit values in Chital Deer during the early periods of handling.

Carragher *et al.* (1997) observed increased haemoglobin level, red blood cell count and haematocrit value after stressful handling procedure in Red Deer.

Read *et al.* (2000) suggested that the autonomic system was activated during stress which resulted in the release of catecholamines from adrenal medulla and led to the contraction of spleen which resulted in haemoconcentration.

Gupta *et al*. (2007) reported the differential leucocyte count of Chital Deer (*Axis axis*) reared in semi captivity as follows: Neutrophil (54.00–68.00 %), Lymphocyte (24.00–40.00 %), Eosinophil (1.00–4.00 %), Monocyte (3.00–5.00 %) and Basophil (0 %).

2.5.8. Recovery

Munerato *et al.* (2008) recorded the quality of anaesthetic recovery based on certain criteria like number of attempts for standing up, ataxia, excitement *etc.* and scored the same as excellent, good, satisfactory, moderate and poor in Brown Brocket Deer.

Monteith *et al.* (2012) observed lengthy recovery, ataxia and resedation in White-tailed Deer which were reversed using yohimbine after immobilising with a combination of Telazole, ketamine and xylazine.

2.6. COMPLICATIONS OF UNGULATE ANAESTHESIA

Caulkett and Haigh (2004) opined that wild ruminant anaesthesia was challenging because these animals were highly prone to stress related complications like hyperthermia, hypoxia, ruminal tympany, self-inflicting trauma, capture myopathy *etc.*

2.6.1. Stress

Breazile (1987) classified stress into eustress, neutral stress and distress, in which eustress was beneficial to the animal, neutral stress did not affect the animal's wellbeing and distress might lead to harmful effects which interfered with reproduction, comfort and wellbeing of the animal.

Kock *et al*. (1987) opined that capture and handling of wild animals were stressful events and proper knowledge about stress was essential to minimise it.

Blumer (1991) noted that free-ranging and captive wild animals were exposed to various stressors, which might result in self-inflicting trauma and stress myopathy during translocation and initial adaptation to new environment. Use of neuroleptic drugs reduced the adverse effects of stress, especially in ungulates.

Spraker (1993) suggested that catecholamine released during stress led to increased oxygen consumption, basal metabolic rate, heat production and lactic acid formation in wild animals.

Moberg (2000) defined stress as the biological response elicited when an individual perceived a threat to its homeostasis. The threat could be considered as the stressor and when it affected the wellbeing of the animal it could be

considered to be under distress.

Dickens *et al.* (2010) classified stress into acute and chronic stress. In acute stress in mammals, the fight-or-flight response was described to be activated immediately followed by the release of glucocorticoids, mainly cortisol, from the adrenal glands as a result of the hypothalamic-pituitary-adrenal (HPA) axis activation. The fight-or-flight response and the glucocorticoid response equipped the animal to physiologically overcome the acute stress. Prolonged exposure to the stressor led to chronic stress and the physiological and hormonal responses became detrimental to the animal and even led to cardiovascular pathologies and immunosuppression which resulted in the death of the animal.

Bartosová *et al*. (2012) reported that opening of pre-orbital gland could be considered as an indicator of stress during handling of farmed Red Deer.

2.6.2. Hyperthermia

Elevation of body temperature resulted in increased oxygen demand during chemical immobilisation of deer. Hyperthermia in conjunction with hypoxemia aggravated the complications due to hypoxemia (Caulkett *et al.*, 2000).

Haskins (2007) opined that body temperature above 42°C resulted in the cellular damage of kidney, liver, cardiac muscle, skeletal muscle *etc*. Increased metabolic rate and high oxygen consumption during hyperthermia resulted in hypoxemia and metabolic acidosis.

Meyer *et al.* (2008) observed that the incidence of capture-induced hyperthermia in Impala irrespective of the capture technique was influenced predominantly by the stress response to capture than the ambient temperature, physical activity and effect of immobilising drugs.

Physical exertion, psychological stress, high ambient temperature and interaction of the anaesthetic drugs on the thermoregulatory centres contributed to

incidence of hyperthermia during the immobilization of wild ruminants. Body temperature above 43°C led to other complications like capture myopathy (Wolff 2009).

Mentaberre *et al.* (2010) opined that administration of haloperidol had no effect on the thermoregulatory centre to prevent hyperthermia, but reduction in body temperature was observed at the end of the physical restraint due to vasodilation induced heat dissipation in Southern Chamois.

Monteith *et al.* (2012) opined that thermoregulation was interfered by xylazine during chemical immobilisation and the immobilised animals were susceptible to capture induced hyperthermia which occurred due to the initial physical exertion in White-tailed Deer.

2.6.3. Hypoxia

Celly *et al.* (1997) reported that administration of alpha$_2$-adrenoceptor agonists like xylazine, romifidine, medetomidine and detomidine resulted in hypoxemia in sheep.

Doherty *et al.* (1986) observed that administration of xylazine had marked effect on the rate and rhythm of respiration which resulted in hypoxemia in sheep.

Caulkett *et al.* (2000) observed hypoventilation and hypoxemia when a combination of medetomidine and ketamine was used for the chemical immobilisation of Mule Deer and Mule Deer/White-tailed Deer hybrids.

Read (2003) placed on record the contribution of recumbency, hypoventilation and alpha$_2$-adrenergic agonist induced pulmonary tissue changes for the development of hypoxemia in wild ruminants during chemical restraint.

Storms *et al.* (2006) reported that increased doses of ketamine in the anaesthetic mixture consisting of xylazine and carfentanil resulted in respiratory depression and led to hypercarbia and respiratory acidosis in White-tailed Deer.

Severe hypoxemia with SpO_2 less than 85 per cent was observed when $alpha_2$-adrenergic agonist (medetomidine) and ketamine combination was used in Hog Deer (Arnemo *et al.,* 2005) and in free-ranging Norwegian Reindeer (Arnemo *et al.,* 2011).

2.6.4. Ruminal Tympany

Caulkett and Haigh (2004) suggested that development of ruminal tympany could be prevented by placing deer in sternal recumbency during anaesthesia and recommended administration of antagonists which reversed anaesthesia and stimulated ruminal movements in severe conditions of tympany.

2.6.5. Capture Myopathy

Gericke *et al.* (1978) opined that capture myopathy occurred as a result of acute stress and hyperthermia due to physical exertion and even resulted in acute death of Springbok.

Wallace *et al.* (1987) reported that 31 per cent of anaesthetic related deaths out of 2000 immobilisations of hoof stock performed over a period of ten years at National Zoological Park, Smithsonian Institution, USA, were due to exertional myopathy.

Spraker (1993) described capture myopathy as a syndrome and as a natural mechanism that potentiated the immediate death of the prey when attacked by the predator, which helped to conserve the energy of the predator and reduced the pain of the prey. Based on the clinical signs, four clinical syndromes namely capture shock, ataxic myoglobinuric, ruptured muscle and delayed-peracute syndromes were observed in artiodactylids.

Williams and Throne (1996) noted that increased respiratory rate and cardiac rate were the earliest clinical signs of capture myopathy. Other early clinical signs observed in wild animals included elevated body temperature,

depression, ataxia, unsteady movements, shock *etc*.

Paterson (2007) opined that capture myopathy was observed in a wide range of vertebrae species, mainly in the mammals and birds. The author also noted that capture myopathy was not described in reptiles.

2.7. VASECTOMY

Jänett *et al.* (2001) observed that ejaculate collected 14 days after vasectomy from rams had no motile or viable spermatozoa.

Kumar and Raj (2011) suggested vasectomy as an easy and effective surgical procedure which checked population growth in captive wild ungulates.

3. MATERIALS AND METHODS

The study was conducted in 20 adult male Spotted Deer belonging to a herd of 150 of both sexes maintained at the Zoological Gardens, Thiruvananthapuram, Kerala, which underwent routine vasectomy procedure as a part of the zoo's deer population control programme, from November 2013 to January 2014. The animals had been housed in a standard open air enclosure of 3000 square meter area.

3.1. SELECTION OF ANIMALS

Twenty healthy adult male Spotted Deer were separated from their herd and randomly allocated into two groups of 10 each. Animals in each group were serially numbered from 1 to 10 for individual identification (*ie*. 1.1 to 1.10 in Group I and 2.1 to 2.10 in Group II).

3.1.1. Group I

All the animals were anaesthetised using a combination of xylazine hydrochloride and ketamine hydrochloride intramuscularly at the rate of 3 mg/kg and 2 mg/kg body weight respectively.

3.1.2. Group II

All the animals were premedicated with haloperidol at the rate of 1 mg/kg body weight orally. After six hours, they were anaesthetised using a combination of xylazine hydrochloride and ketamine hydrochloride intramuscularly at the rate of 3 mg/kg and 2 mg/kg body weight respectively.

3.2. SEGREGATION AND HOUSING OF SELECTED ANIMALS

Twenty four healthy adult male Spotted Deer were randomly segregated from the herd in batches of three each, a day before the procedure into a holding facility attached to the open air enclosure. This holding facility was being used

routinely by the zoo for isolation of animals for veterinary examination and care. The segregation of the deer in batches of three was intended to reduce fear and subsequent excitement, escape behaviour and self-inflicted injury during the pre-anaesthetic preparation period and also ensured that maximum number of animals could be studied per day. Each batch of three animals was randomly allocated into either Group I or Group II. Two animals each from the last batch of each group were not included in the study so that each group had 10 animals.

The holding facility consisted of two adjacent pens with solid walls and a chain link mesh door separating them. The pens were 5 × 4 × 3 (length × breath × height) meters each and were designated as “Observation Pen” and “Darting Pen”.

3.3. PRE-ANAESTHETIC PREPARATION OF ANIMALS

All the animals were fasted for 18 hours prior to administration of drugs. The size and shape of the antlers were noted during this period for individual identification.

3.4. ASSESSMENT OF BODY WEIGHT

The body weights of individual animals were tentatively assessed visually by the zoo veterinarian based on previous experience. The actual body weight was measured using a hanging digital balance[1] at the end of the surgical procedure. The doses of haloperidol and the anaesthetic combination were calculated based on the assessed body weight and the dose of reversal agent was calculated based on the actual measured body weight of the animals.

[1] Kern HCB 200K 500, Kern & Sohn, GmbH, Balingen, Germany

3.5. CORRECTION OF DOSE RATES AGAINST ACTUAL BODY WEIGHT

The doses of haloperidol and the anaesthetic drugs were corrected against the actual measured body weights for statistical evaluation later. The correction of body weight was based on the formula given below.

$$\text{Corrected dose rate} = \frac{\text{Assessed body weight} \times \text{Dose rate of the agent}}{\text{Actual measured body weight}}$$

3.6. ADMINISTRATION OF DRUGS USED IN THE STUDY

All animals that underwent the procedure on a particular day were allotted to either Group I (non-premedicated) or Group II (premedicated) to avoid interaction of tranquilised and non-tranquilised animals, to prevent the non-premedicated animals from injuring the premedicated ones.

3.6.1. Haloperidol

Haloperidol[2] was administered at the rate of 1 mg/kg body weight orally to the animals of Group II, concealed in bananas (baited bananas) by an animal keeper. The animals in Group I were provided with unbaited bananas at the same time of the day to maintain uniformity in the procedures in both groups, except premedication. The animals of each batch of Group II were premedicated one after the other with haloperidol at an interval of one hour to synchronize the anaesthesia and vasectomy at a similar interval six hours after premedication, later during the day.

3.6.2. Xylazine-ketamine Combination

Six hours after feeding of the bananas, one animal at a time, as per sequence of haloperidol administration, was moved from the observation pen to the darting pen of the holding facility. All animals were anaesthetised with a mixture of

[2] Serenace 10®, (10 mg tablets), RPG Life Sciences Ltd. Ankleshwar

xylazine hydrochloride[3] and ketamine hydrochloride[4] filled in a 3 ml plastic dart syringe and delivered remotely using CO_2 powered dart rifle[5] into the muscles of the caudal aspect of the thigh.

3.6.3. Yohimbine Hydrochloride

Yohimbine hydrochloride[6] was administered at the rate of 0.3 mg/kg body weight to reverse anaesthesia in animals of both groups after vasectomy and transportation to a new open air enclosure. Half of the calculated dose of yohimbine was administered intravenously followed by the other half intramuscularly.

3.7. PARAMETERS OF OBSERVATION

3.7.1. Behaviour

Animals in both groups were observed for selected behavioural responses to the approach to within five meters of the animal by the observer just before administration of unbaited banana in Group I and baited banana with haloperidol in Group II (Plate 1. A, B, C, D, and E). The behavioural responses were recorded in an observation chart (Annexure I). This was repeated six hours after the administration of bananas in all the animals.

[3] Ilium Xylazil-100®, 100 mg/ml, Ilium, Troy Laboratories Pty Limited, Australia

[4] Ketamil®, 100 mg/ml, Ilium, Troy Laboratories Pty Limited, Australia

[5] Dan-Inject J.M.®, Denmark

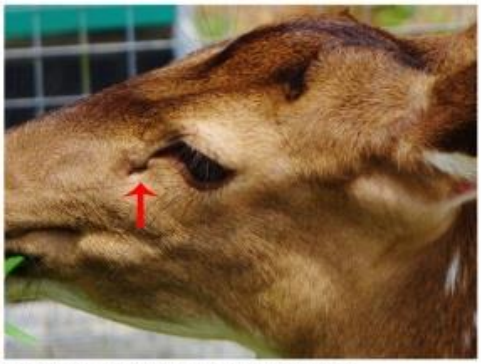
[6] Reversin™, 10 mg/ml, Bomac Pty Limited, Australia

Plate 1 . Behavioural responses

(A) Ear pricking

(B) Tail erection

(C) Stamping of fore feet



(D) Pre-orbital gland opening

(E) Anxious look

3.7.2. Quality of Darting Procedure

All the animals were darted by the same individual (zoo veterinarian) using the already described remote drug delivery equipment. The quality of darting procedure was assessed based on the easiness of darting and the response of the animal to dart placement. All observations were recorded in the observation chart (Annexure I).

3.7.3. Monitoring of Anaesthesia

All the animals were monitored for quality of induction, maintenance and recovery, time required for induction and recovery, complications during anaesthesia and physiological, haematological and biochemical variations during anaesthesia. All the observations and parameters were entered in an anaesthetic record for future reference and evaluation (Annexure II).

3.7.3.1. Induction Time

The time from darting to sternal recumbency and from darting to lateral recumbency were recorded in seconds**.**

3.7.3.2. Quality of Induction

Quality of induction was recorded by the observer based on a graded score card modified from one described by Storms *et al*. (2005).

3.7.3.3. Physiological Parameters

Rectal temperature, pulse rate, heart rate and respiratory rate were monitored and recorded every five minutes from the time of induction of anaesthesia to the end of the surgical procedure in animals of both groups (*i.e.* 0, 5^{th}, 10^{th}, 15^{th} and 20^{th} minute from lateral recumbency).

3.7.3.3.1. Rectal Temperature

Rectal temperature (°C) was monitored using a standard digital thermometer. The temperature was also recorded again just before the administration of the reversal agent.

3.7.3.3.2. Heart Rate

Heart rate (beats per minute) was monitored by auscultation in a routine manner using a standard stethoscope and the findings were recorded.

3.7.3.3.3. Respiratory Rate

Respiratory rate (breaths per minute) was assessed by observing the chest wall movements and recorded.

3.7.3.3.4. Pulse Rate

The pulse rate (pulse per minute) was assessed by palpating the facial artery.

3.7.3.4. Haemoglobin Oxygen Saturation

The haemoglobin oxygen saturation was measured using a pulse oximeter[7]. The tongue probe of the equipment was attached across the commissure of the lips throughout the surgical procedure and the readings were recorded.

3.7.3.5. Electrocardiography

All the animals were placed on right lateral recumbency over a wooden table immediately after the induction of anaesthesia. The forelimb electrodes were applied to the skin fold over the olecranon process and the hind limb electrodes were applied on the skin over the patella using crocodile clips. The Lead II electrocardiogram was monitored throughout the surgical procedure. For better

[7] Aegis, BPL Multi-parameter Monitor, BPL limited, Palakkad, Kerala, India

quality tracings, Lead I or Lead III tracings were recorded in some animals. All the ECG tracings were recorded at 25 mm/sec paper speed and at 10 mm/mV using Cardiart 6108T[8].

3.7.3.6. Blood Gas Analysis

Blood samples were collected anaerobically using 2 ml disposable syringes which were flushed with 1000 IU/ml heparin sodium. Venous blood samples were collected immediately after induction of anaesthesia and just before the administration of the reversal agent, from the jugular vein. Arterial blood samples were collected from the medial auricular artery at 10^{th} and 20^{th} minutes after the induction of anaesthesia. All blood samples were immediately stored on crushed ice and analysed within one hour.

Arterial and venous blood samples were evaluated for blood pH, partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2), bicarbonate ion concentration (HCO_3^-) and standard base excess (BE ecf) values using a portable blood gas analyser[9] and recorded. The blood gas values were corrected to the measured rectal temperature using the provision available in the equipment.

3.7.3.6.1. Arterial Catheterisation

Arterial blood samples were collected from the medial auricular artery located on the dorsal aspect of the pinna. The hair over the site was shaved and the skin prepared aseptically using 70 per cent isopropyl alcohol. Digital pressure was applied over the artery against the direction of blood flow so that the auricular artery engorged (Plate 2. A). A 22G intravenous cannula[10] was used to catheterise the artery in a standard manner (Plate 2. B). A pre-heparinized syringe was

[8] BPL limited, Palakkad, Kerala, India

[9] epoc® Blood Analysis System and epoc BGEM Test Card, Epocal, INC. Ottawa, ON Canada

[10] BD Venflon™ IV cannula, Becton Dickison India (P) Ltd.

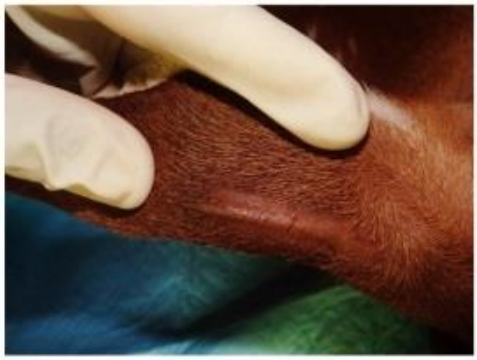
connected to the IV cannula and two milliliters of blood was withdrawn anaerobically (Plate 2. C). Air bubbles, if trapped inside the syringe, were removed by attaching a 23G hypodermic needle to the syringe, holding the syringe in an upright position and squirting out a few drops of blood along with them. To prevent further air contact of the blood, the needle was bent and cap attached. Meanwhile, a three-way stopcock[11] was attached to the cannula by another person to administer 0.5 ml of heparinized normal saline (5 IU/ml) *via.* the three way stopcock to prevent blood clot formation and maintain the patency of the cannula, which aided in sampling at the 20th minute (Plate 2. D). A rolled gauze was placed inside the concha of the ear pinna and strips of adhesive paper tape applied around the pinna including the wings of the cannula to fix the latter over the pinna (Plate 2. E). At the time of blood collection, the valve of the three-way stopcock was turned to make it patent and few drops of blood were discarded. A pre-heparinised syringe was connected to the three-way stopcock and two milliliters of blood was withdrawn anaerobically. All the blood samples were stored in the respective syringes in crushed ice and were analysed within one hour. After the 20th minute blood sampling, the cannula was removed and digital pressure was applied for two minutes to aid blood clot formation and to prevent haematoma.

3.7.3.7. Quality of Maintenance of Anaesthesia

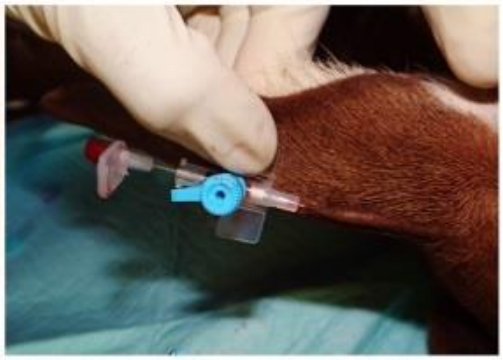
The quality of maintenance of anaesthesia was assessed by observing signs of recovery like ear twitching, head lifting, jaw movements and struggling during the surgical procedure.

[11] Tops Three-Way Stopcock, Meditop Corporation, Selangor D.E., Malayasia

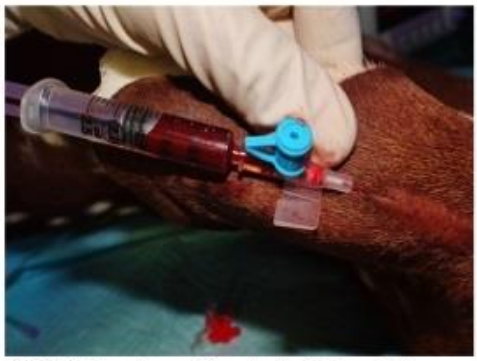
Plate 2. Auricular artery catheterisation



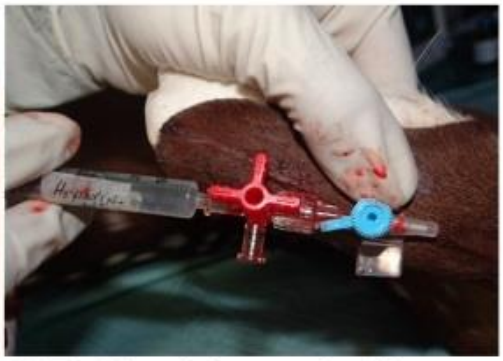
(A) Engorged medial auricular artery



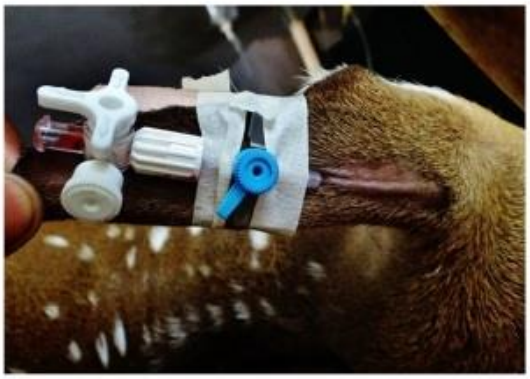
(B) Catheterisation using 22G IV cannula



(C) Collection of arterial blood to pre-heparinised syringe



(D) Flushing of the cannula with anticoagulant



(E) Fixing of three-way stopcock and cannula over the pinna

3.7.3.8. Complications during Maintenance of Anaesthesia

All animals were observed for occurrence of complications like regurgitation, aspiration, ruminal tympany *etc*., if any, throughout the period of anaesthesia.

3.7.3.9. Reversal of Anaesthesia

After the surgical procedure, all animals were translocated to a new open air enclosure while still under general anaesthesia. The average time taken for translocation was about 15 minutes. After translocation, all the animals were reversed from anaesthesia by the administration of yohimbine hydrochloride as described earlier.

3.7.3.9.1. Reversal Time

The time from the administration of reversal agent to sternal recumbency and from administration of reversal agent to standing were recorded in seconds. Those animals which failed to gain sternal recumbency on their own within 10 minutes after the administration of reversal agent were manually placed on sternal recumbency to prevent complications like ruminal tympany, regurgitation and aspiration.

3.7.3.9.2. Quality of Reversal of Anaesthesia

The quality of reversal of anaesthesia was assessed by the observer and was graded based on a score card modified from that described by Munerato *et al*. (2008) [Annexure II].

3.7.3.9.3. Resedation

All the animals were observed for resedation at 2, 6, 12 and 24 hours after recovery from anaesthesia.

3.7.3.10. Haematological Parameters

Two milliliters of venous blood were collected in K_2 EDTA vials[12] by venipuncture using 22G blood collection needles[13] just before the administration of reversal agent and were subjected to evaluation for the following parameters.

3.7.3.10.1. Total Erythrocyte Count

The total erythrocyte count (10^6/µL) was estimated using veterinary haematological analyser[14], with the pre-set reference ranges for caprine blood cells.

3.7.3.10.2. Total Leucocyte Count

Total leucocyte count (/µL) was also estimated using veterinary haematological analyser, with the pre-set reference ranges for caprine blood cells.

3.7.3.10.3. Haemoglobin Concentration

The haemoglobin concentration (g/dL) was estimated using the portable blood gas analyser from the blood sample collected for blood gas analysis just before administration of the reversal agent.

3.7.3.10.4. Volume of Packed Red Cells

The volume of packed red cells (per cent) was also assessed using the blood sample collected for blood gas analysis just before administration of the reversal agent, using the portable blood gas analyser.

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[12] BD Vacutainer®, K2 EDTA, 3.6mg, 2ml, BD Franklin Lakes, NJ, USA

[13] Eclipse™, BD Vacutainer®, Blood collection needles, 22G×1-1/4", BD Franklin Lakes, NJ, USA

[14] Exigo, Boule Medical AB, Stockholm, Sweden.

3.7.3.10.5. Differential Leucocyte Count

Blood smears were prepared from the venous blood sample collected just before the administration of reversal agent in all animals and were stained using Giemsa stain to estimate differential leucocyte count (per cent).

3.7.3.11. Biochemical Parameters

Four milliliters of venous blood was collected from the jugular vein into serum vials[15] from all animals using a 22G blood collection needle for serum biochemical analysis just before the administration of reversal agent.

3.7.3.11.1. Blood Glucose

Plasma glucose (mg/dL) was estimated from the venous blood sample collected for blood gas analysis just before the administration of reversal agent, using a portable blood gas analyser.

3.7.3.11.2. Lactate

Plasma lactate (mmol/L) was estimated from the venous blood sample collected immediately after induction of anaesthesia and just prior to administration of reversal agent and from the arterial blood sample collected at 10^{th} and 20^{th} minute immediately after induction of anaesthesia using a portable blood gas analyser.

3.7.3.11.3. Calcium

Calcium ion concentration (mmol/L) was estimated from the venous blood sample collected for blood gas analysis just before the administration of reversal agent, using the portable blood gas analyser.

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[15] BD Vacutainer® Serum, 4 ml, BD Franklin Lakes, NJ, USA

3.7.3.11.4. Phosphorus

Serum phosphorus (mg/dl) was estimated from the venous blood collected just before the administration of reversal agent by molybdenate UV kinetics method in an automated analyser[16].

3.7.3.11.5. Potassium

Potassium ion concentration (mmol/L) was estimated from the venous blood sample collected for blood gas analysis just before the administration of reversal agent, using a portable blood gas analyser.

3.7.3.11.6. Serum Cortisol

Serum cortisol (µg/dL) was estimated from the venous blood collected just before the administration of reversal agent by electrochemiluminescent immunoassay (ECLIA) method using commercially available kit[17] in an automated analyser.

3.7.3.11.7. Serum Creatine Kinase

Serum creatine kinase (U/L) was estimated from the venous blood collected just before the administration of reversal agent by IFCC without PLP (International Federation for Clinical Chemistry without Pyridoxal-Phosphate) method using commercially available kit[18] in an automated analyser.

3.7.3.11.8. Serum Aspartate Aminotransferase

Serum aspartate aminotransferase (U/L) was estimated from the venous blood collected just before the administration of reversal agent by IFCC method

[16] Cobas® 6000 analyzer series, Roche diagnostics, Mannheim, Germany

[17] Cobas ECLIA kit, Roche diagnostics, Mannheim, Germany

[18] Creatine Kinase, Custom biotech, Roche Diagnostics, Mannheim, Germany

using commercially available kit[19] in an automated analyser.

3.8. SURGICAL PROCEDURE

Immediately after induction of anaesthesia in all the animals, the eyes were protected from desiccation by instilling methyl cellulose containing eye drops and were blindfolded. The animals were placed over a wooden table on right lateral recumbency. Vasectomy was performed in both groups by caudal approach of the scrotal neck as per standard procedure. Normal saline was administered at the rate of 10 ml/kg/hr intravenously throughout the surgical procedure.

3.8.1. Surgical Site Preparation

The hair over the caudal aspect of the neck of the scrotum was shaved and cleaned with 70 per cent isopropyl alcohol. The site was painted with povidone iodine solution and draped.

3.8.2. Surgical Technique

Vasectomy was performed by caudal approach of the scrotal neck. The spermatic cord was fixed between the thumb and index finger and a small linear skin incision of one centimeter length was made using a No. 21 Bard Parker blade. The tunica vaginalis was incised and then dissected using a pair of point-point Mayo scissors. A pair of curved artery forceps was passed under the vas deferens to separate it from the other structures of the spermatic cord. The vas deferens was separated and ligatures were applied and transfixed to the testicular and proximal ends at a distance of about 2 cms from each other using No. 1 catgut. After ligation, a 1 cm segment of the vas deferens between ligatures was removed using a pair of scissors. The procedure was repeated on the other spermatic cord through a separate skin incision on the other side. The skin incisions were apposed with cruciate mattress sutures using No. 3-0 polyglactin

[19] AST, Custom biotech, Roche Diagnostics, Mannheim, Germany

910. A thin layer of cotton soaked in tincture benzoin was applied so as to form a thin protective film over the surgical site. All the animals in Group I were ear tagged on the left side and the animals of Group II were ear tagged on the right side for identification. All the deer were also micro chipped[20] for individual identification in the future.

3.8.3. Post-operative Care

A long acting preparation of enrofloxacin[21] was administered at the rate of 7.5 mg/kg body weight intramuscularly. Tetanus toxoid injection was administered intramuscularly at the rate of two ml per animal.

3.9. POST-OPERATIVE COMPLICATIONS

All the animals were observed for one week for swelling at the surgical site, scrotal edema, signs of infection of the surgical site, suture dehiscence and development of maggot wound at the surgical site. The animals were also observed for signs of capture myopathy for a period of two months post-operatively.

3.10. STATISTICAL ANALYSIS

The data obtained during the study were subjected to statistical analysis as described by Snedecor and Cochran (1994) using the statistical software SPSS version 16.0. The value of $P < 0.05$ was considered significant (Snedecor and Cochran, 1985). The behavioural observations and complications during maintenance of anaesthesia were evaluated using Chi-square test of association. The haematological, biochemical and blood gas analysis values were evaluated using and One-way Analysis of Variance to determine the level of significance. The physiological parameter values were evaluated by Two-way analysis of

[20] Trovan® ID-100, Passive transponder system, Trovan Ltd. UK.

[21] Fortivir®, 100 mg/ml, Virbac Animal Health India Pvt. Ltd.

variance. The quality of induction of anaesthesia and recovery from anaesthesia were evaluated using Wilcoxon's singed rank test. The results were expressed as Mean±Standard Error (M±SE).

4. RESULTS

Comparative evaluation of xylazine-ketamine anaesthesia with and without haloperidol premedication was carried out in 20 healthy adult male Spotted Deer which underwent routine vasectomy procedure at the Zoological Gardens, Thiruvananthapuram, and the results are summarised as follows.

4.1. BEHAVIOURAL OBSERVATIONS

The behavioural responses were studied prior to feeding of baited or unbaited bananas and prior to darting (*ie.* six hours after the administration of bananas). The observations and the results of statistical evaluation are presented in Table 1.

There was no statistically significant difference in the behavioural responses of animals of Group I between the two observations, *ie.* prior to feeding of unbaited banana and prior to darting. Statistical analysis of the behavioural responses prior to feeding of bananas and prior to darting between groups did not show any statistically significant difference in this group. In Group II, there was statistically significant difference in the behavioural responses like stamping of fore feet, pre-orbital gland opening, excitation behaviour and escape behaviour between the two observations taken prior to feeding of baited banana and prior to darting.

4.2. QUALITY OF DARTING PROCEDURE

The quality of darting procedure was evaluated based on two observations, *ie*. easiness of darting and response to dart placement. The findings and the results of statistical evaluation are presented in Table 2.

There was significant difference in the quality of darting procedure between the groups. Darting was easy in 70 per cent of animals of the Group II and was difficult in 90 per cent of animals of Group I. Only 20 per cent of Group I animals showed calm response to dart placement when compared to 80 per cent of Group II animals which showed the similar response.

4.3. ANAESTHESIA

Animals of Group I (actual body weight - 45.30±3.43 kg) were anaesthetised using a combination of 2.90±0.13 mg/kg body weight of xylazine hydrochloride and 2.00±0.09 mg/kg body weight of ketamine hydrochloride. Animals of Group II (actual body weight - 49.15±4.06 kg) were pre-medicated with 1.00±0.02 mg of haloperidol and were anaesthetised using a combination of 3.00±0.06 mg/kg body weight of xylazine hydrochloride and 2.00±0.04 mg/kg body weight of ketamine hydrochloride. There was no statistically significant difference between the assessed and actual body weights of both the groups. Thus the corrected dose of xylazine and ketamine also did not differ significantly. The anaesthetic combination provided general anaesthesia in all animals of both groups and no additional dosing of anaesthetic drugs was required in any animal. The above described data and the corrected dose with the actual body weight is represented in Table 3.

4.3.1. Induction Time

The mean±SE values for time from darting to sternal recumbency and from darting to lateral recumbency in both groups are presented in Table 4.

For the animals of Group I, the mean±SE value for time from darting to sternal recumbency was 264.80±29.90 seconds and from darting to lateral recumbency was 358.90±25.56 seconds.

For the animals of Group II, the mean±SE value for time from darting to sternal recumbency was 201.33±17.15 seconds and from darting to lateral recumbency was 300.22±32.69 seconds. There was no statistically significant difference in the induction timings between groups.

Table 1. Behavioural observations in deer subjected to xylazine-ketamine anaesthesia with and without haloperidol premedication

Observation	Group I				Group II			
	Prior to feeding of unbaited banana (n=10)		Prior to darting (n=10)		Prior to feeding of baited banana (n=10)		Prior to darting (n=10)	
	Present	Absent	Present	Absent	Present	Absent	Present	Absent
Ear pricking	10^a	0^a	10^a	0^a	10^a	0^a	9^a	1^a
Tail erection	8^a	2^a	7^a	3^a	7^a	3^a	4^a	6^a
Stamping of fore feet*	5^a	5^a	5^a	5^a	7^a	3^a	1^b	9^b
Anxious look	10^a	0^a	10^a	0^a	10^a	0^a	9^a	1^a
Pre-orbital gland opening*	10^a	0^a	10^a	0^a	8^a	2^a	2^b	8^b
Excitation behaviour*	8^a	2^a	8^a	2^a	10^a	0^a	1^b	9^b
Escape behaviour*	8^a	2^a	9^a	1^a	8^a	2^a	2^b	8^b

Frequencies (Number of animals) bearing same superscript in a row under each group does not differ significantly ($P > 0.05$)

*Observations differ significantly ($P < 0.05$) between groups

Table 2. Quality of darting procedure in deer subjected to xylazine-ketamine anaesthesia with and without haloperidol premedication

Groups	Ease of dart placement		Response to dart placement	
	Difficult	Easy	Excited	Calm
I	9[a]	1[a]	8[a]	2[a]
II	3[b]	7[b]	2[b]	8[b]
Frequencies (Number of animals) bearing same superscript in a column do not differ significantly (P > 0.05)				

Table 3. Corrected dose of drugs against actual body weight

Parameters	Mean±SE	
	Group I	Group II
Assessed body weight (kg)	43.7±3.11	49.00±4.14
Measured body weight (kg)	45.30±3.43	49.15±4.06
Corrected dose of haloperidol (mg/kg B.wt.)	Nil	1.00±0.02
Corrected dose of xylazine (mg/kg B.wt.)	2.90±0.13	3.00±0.06
Corrected dose of ketamine (mg/kg B.wt.)	2.00±0.09	2.00±0.04

Table 4. Time taken for induction of xylazine-ketamine anaesthesia with and without haloperidol premedication in deer, sec

Observations	Time (Mean±SE)	
	Group I	Group II
Time from darting to sternal recumbency	264.80±29.90[a]	201.33±17.15[a]
Time from darting to lateral recumbency	358.90±25.56[a]	300.22±32.69[a]
Means bearing same superscript in a row do not differ significantly (P > 0.05)		

4.3.2. Quality of Induction

The mean±SE values of scores obtained by the animals of Group I and Group II are presented in Table 5. The magnitude of differences of excitability and overall quality of induction differed significantly between Group I and Group II.

4.3.3. Quality of Maintenance of Anaesthesia

Animals of both groups could be maintained under general anaesthesia for a minimum duration of 35 minutes with the initial dosing of the anaesthetic combination itself. One animal each (10 per cent) of both groups showed ear twitching during maintenance of anaesthesia. All the observations and statistic test results are presented in Table 6.

4.3.4. Complications during Anaesthesia

Ruminal tympany was observed in three (30 per cent) out of ten animals of each group. One animal (Animal No. 2.6) which had ruminal tympany also showed regurgitation. In one animal (10 per cent) of Group I (Animal No. 1.8), ventricular premature contractions were observed and second-degree atrioventricular block was observed in one animal of Group II (Animal No. 2.10). There was no statistically significant difference between groups. The data pertaining to the complications during anaesthesia is presented in the Table 7.

Table 5. Quality score for induction of xylazine-ketamine anaesthesia with and without haloperidol premedication in deer

Parameters	Score (Mean±SE)	
	Group I	**Group II**
Excitability	0.80±0.02[a]	2.20±0.29[b]
Overall quality	1.10±0.28[a]	2.10±0.28[b]
Means bearing same superscript in a row do not differ significantly (P > 0.05)		

Table 6. Quality of maintenance during xylazine-ketamine anaesthesia with and without haloperidol premedication in deer

Observation	Group I (n=10)		Group II (n=10)	
	Present	**Absent**	**Present**	**Absent**
Kicking	0[a]	10[a]	0[a]	10[a]
Jaw movements	0[a]	10[a]	0[a]	10[a]
Ear twitching	1[a]	9[a]	1[a]	9[a]
Head lifting	0[a]	10[a]	0[a]	10[a]
Struggling	0[a]	10[a]	0[a]	10[a]
Frequencies (Number of animals) bearing same superscripts in a row do not differ significantly (P > 0.05)				

Table 7. Complications observed during xylazine-ketamine anaesthesia with and without haloperidol premedication in deer

Observation	Group I (n=10)		Group II (n=10)	
	Present	**Absent**	**Present**	**Absent**
Regurgitation	0^a	10^a	1^a	9^a
Aspiration	0^a	10^a	0^a	10^a
Ruminal tympany	3^a	7^a	3^a	7^a
Excessive bleeding at the surgical site	0^a	10^a	0^a	10^a
Arrhythmia	1^a	9^a	1^a	9^a
Others	0^a	10^a	0^a	10^a

Frequencies (Number of animals) bearing same superscripts in a row do not differ significantly ($P > 0.05$)

4.3.5. Reversal Time

The mean±SE values of time from the administration of reversal agent to sternal recumbency and from administration of reversal agent to standing in both groups are presented in Table 8.

For the animals of Group I, the mean±SE value from the administration of reversal agent to sternal recumbency was 824.10±121.01 seconds and from administration of reversal agent to standing was 968.30±143.21 seconds. Three out of 10 animals showed delayed recovery.

For the animals of Group II, the mean±SE value from the administration of reversal agent to sternal recumbency was 831.00±181.55 seconds and from administration of reversal agent to standing was 1095.00±184.82 seconds. One out of 10 animals showed delayed recovery. There was no statistically significant difference between the times taken for recovery between groups.

4.3.6. Quality of Recovery

The mean±SE values of scores indicating quality of recovery observed in the animals of Group I and Group II are presented in Table 9. The score for quality of recovery from anaesthesia had statistically significant difference between groups.

4.3.7. Resedation

Resedation was observed in one animal of Group I (Animal No. 1.3) and two animals of Group II (Animal No. 2.1 and 2.2) after two hours from recovery. Resedation was not observed in any of the animals after 6, 12 and 24 hours from reversal of anaesthesia.

Table 8. Time taken for recovery from xylazine-ketamine anaesthesia with and without haloperidol premedication in deer, sec

Observations	Time (Mean±SE)	
	Group I	Group II
Time from administration of antagonist to sternal recumbency	824.10±121.01[a]	831.00±181.55[a]
Time from administration of antagonist to standing	968.30±143.21[a]	1095.00±184.82[a]
Means bearing same superscript in a row do not differ significantly (P > 0.05)		

Table 9. Quality score for recovery from xylazine-ketamine anaesthesia with and without haloperidol premedication in deer

Parameters	Score (Mean±SE)	
	Group I	Group II
Recovery	3.00±0.21[a]	2.40±0.27[b]
Means bearing same superscript in a row do not differ significantly (P > 0.05)		

4.4. PHYSIOLOGICAL PARAMETERS

4.4.1 Rectal Temperature

The mean±SE values of rectal temperature (ºC) of Group I animals at 0, 5, 10, 15, 20 and 35 minutes after induction were 40.69±0.24, 40.56±0.24, 40.29±0.24, 40.05±0.24, 40.06±0.24 and 39.79±0.24 respectively.

The mean±SE values of rectal temperature (ºC) of Group II animals at 0, 5, 10, 15, 20 and 35 minutes after induction were 39.67±0.24, 39.60±0.24, 39.27±0.24, 39.50±0.24, 39.31±0.24 and 39.24±0.14 respectively.

There was no statistically significant difference within groups, but there was statistically significant difference between groups at 0, 10, 15 and 20 minutes. The mean±SE values of rectal temperature (°C) of both groups are presented in Table 10. The observations are graphically represented in Figure 1.

4.4.2 Heart Rate

The heart rates (beats per minute) did not differ significantly within the groups. There was statistically significant difference in the heart rates at 10^{th} and 15^{th} minute after induction between groups. The heart rates were 76±5 and 73±5 at 10^{th} and 15^{th} minute respectively, in animals of Group I. The heart rates were 63±5 and 63±5 at 10^{th} and 15^{th} minute respectively in animals of Group II.

The mean±SE values of heart rate (beats per minute) of both groups are presented in Table 11. The observations are graphically represented in Figure 2.

Table 10. Rectal temp. in deer subjected to xylazine-ketamine anaesthesia with and without haloperidol premedication, °C

Time (minutes)	0	5	10	15	20	35
Group I	40.69 ± 0.24^{a1}	40.56 ± 0.24^{a1}	40.29 ± 0.24^{a1}	40.05 ± 0.24^{a1}	40.06 ± 0.24^{a1}	39.79 ± 0.24^{a1}
Group II	39.67 ± 0.24^{b1}	39.60 ± 0.24^{b1}	39.27 ± 0.24^{b1}	39.50 ± 0.24^{b1}	39.31 ± 0.24^{b1}	39.24 ± 0.14^{a1}
Means bearing same alphabetical superscript in a column do not differ significantly (P > 0.05) Means bearing same numerical superscript in a row do not differ significantly (P > 0.05)						

Table 11. Heart rate in deer subjected to xylazine-ketamine anaesthesia with and without haloperidol premedication, bpm

Time (minutes)	0	5	10	15	20
Group I	83 ± 5^{a1}	77 ± 5^{a1}	76 ± 5^{a1}	73 ± 5^{a1}	72 ± 5^{a1}
Group II	66 ± 5^{a1}	62 ± 5^{a1}	63 ± 5^{b1}	63 ± 5^{b1}	63 ± 5^{b1}
Means bearing same alphabetical superscript in a column do not differ significantly (P > 0.05) Means bearing same numerical superscript in a row do not differ significantly (P > 0.05)					

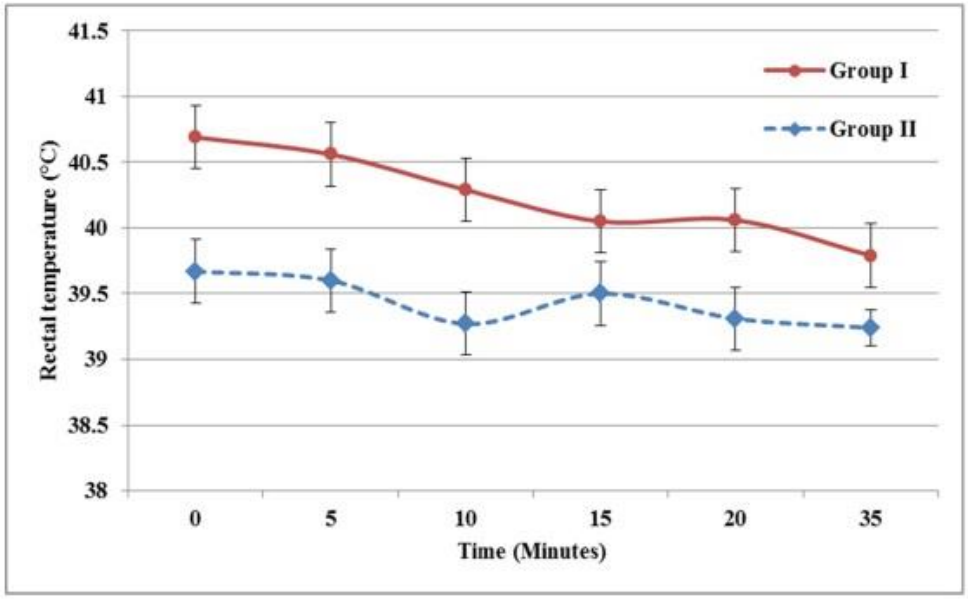

Figure 1. Comparison of rectal temperatures in deer subjected to xylazine-ketamine anaesthesia with and without haloperidol premedication

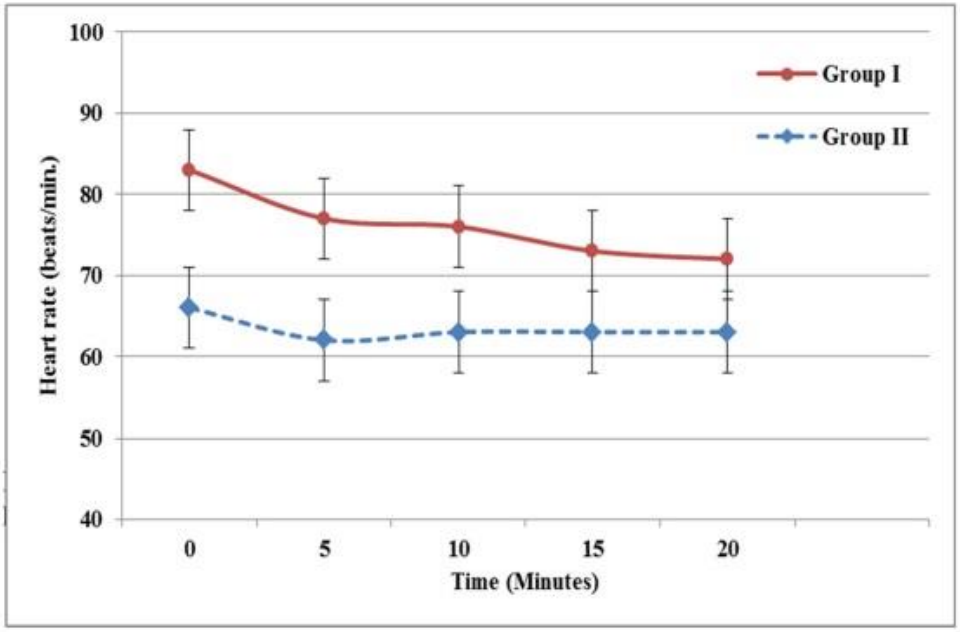

Figure 2. Comparison of heart rates in deer subjected to xylazine-ketamine anaesthesia with and without haloperidol premedication

4.4.3. Respiratory Rate

The mean±SE values of respiratory rate (breaths per minute) of both groups are presented in Table 12.

In both groups, there was a gradual reduction in the respiratory rate from induction of anaesthesia to the end of the surgical procedure. But there was no statistically significant difference between and within groups. The observations are graphically represented in Figure 3.

4.4.4. Pulse Rate

The mean±SE values of pulse rate (per minute) of both groups are presented in Table 13.

There was a gradual reduction in the pulse rate from induction of anaesthesia to the end of the surgical procedure in both groups. But the mean±SE values between and within groups did not differ significantly. The observations are graphically represented in Figure 4.

4.4.5. Haemoglobin Oxygen Saturation

The mean±SE values of haemoglobin oxygen saturation (per cent) of both groups are presented in Table 14. The mean±SE values between and within groups did not differ significantly. The observations are graphically represented in Figure 5.

Table 12. Respiratory rate in deer subjected to xylazine-ketamine anaesthesia with and without haloperidol premedication, breaths per minute

Time (minutes)	0	5	10	15	20
Group I	26 ± 2^{a1}	24 ± 2^{a1}	23 ± 2^{a1}	23 ± 2^{a1}	23 ± 2^{a1}
Group II	23 ± 2^{a1}	24 ± 2^{a1}	23 ± 2^{a1}	20 ± 2^{a1}	19 ± 2^{a1}
Means bearing same alphabetical superscript in a column do not differ significantly ($P > 0.05$) Means bearing same numerical superscript in a row do not differ significantly ($P > 0.05$)					

Table 13. Pulse rate in deer subjected to xylazine-ketamine anaesthesia with and without haloperidol premedication, pulse per minute

Time (minutes)	0	5	10	15	20
Group I	75 ± 5^{a1}	74 ± 5^{a1}	73 ± 5^{a1}	71 ± 5^{a1}	67 ± 5^{a1}
Group II	65 ± 5^{a1}	63 ± 5^{a1}	64 ± 5^{a1}	62 ± 5^{a1}	63 ± 5^{a1}
Means bearing same alphabetical superscript in a column do not differ significantly ($P > 0.05$) Means bearing same numerical superscript in a row do not differ significantly ($P > 0.05$)					

Table 14. Haemoglobin oxygen saturation in deer subjected to xylazine-ketamine anaesthesia with and without haloperidol premedication, %

Time (minutes)	0	5	10	15	20
Group I	78 ± 2^{a1}	80 ± 2^{a1}	81 ± 2^{a1}	81 ± 2^{a1}	81 ± 2^{a1}
Group II	76 ± 2^{a1}	80 ± 2^{a1}	79 ± 2^{a1}	83 ± 2^{a1}	80 ± 2^{a1}
Means bearing same alphabetical superscript in a column do not differ significantly ($P > 0.05$) Means bearing same numerical superscript in a row do not differ significantly ($P > 0.05$)					

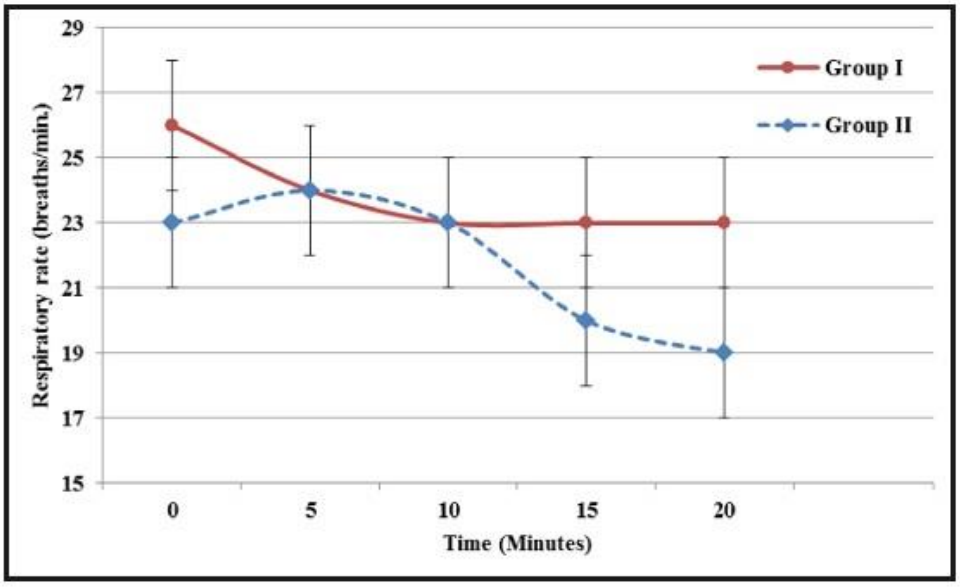

Figure 3. Comparison of respiratory rates in deer subjected to xylazine-ketamine anaesthesia with and without haloperidol premedication

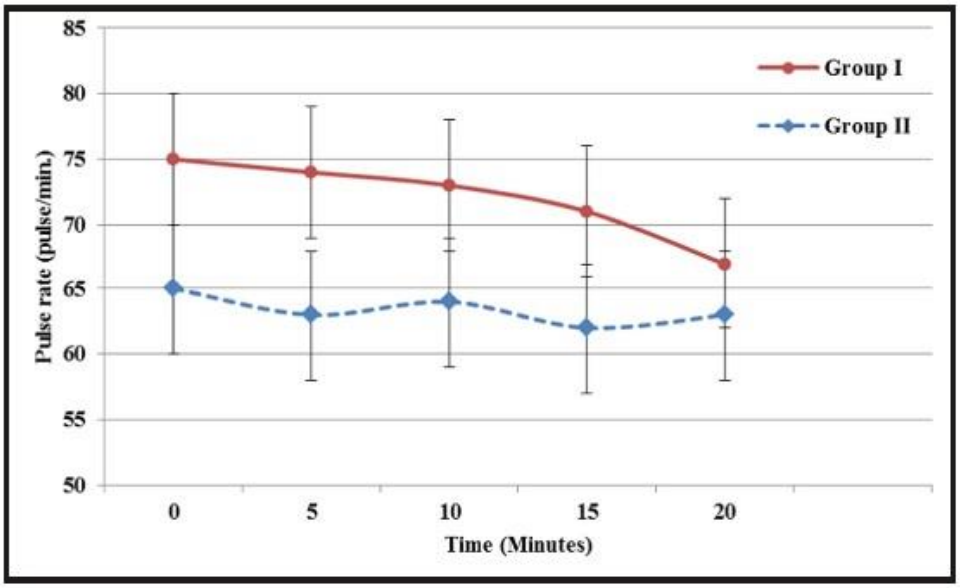

Figure 4. Comparison of pulse rates in deer subjected to xylazine-ketamine anaesthesia with and without haloperidol premedication

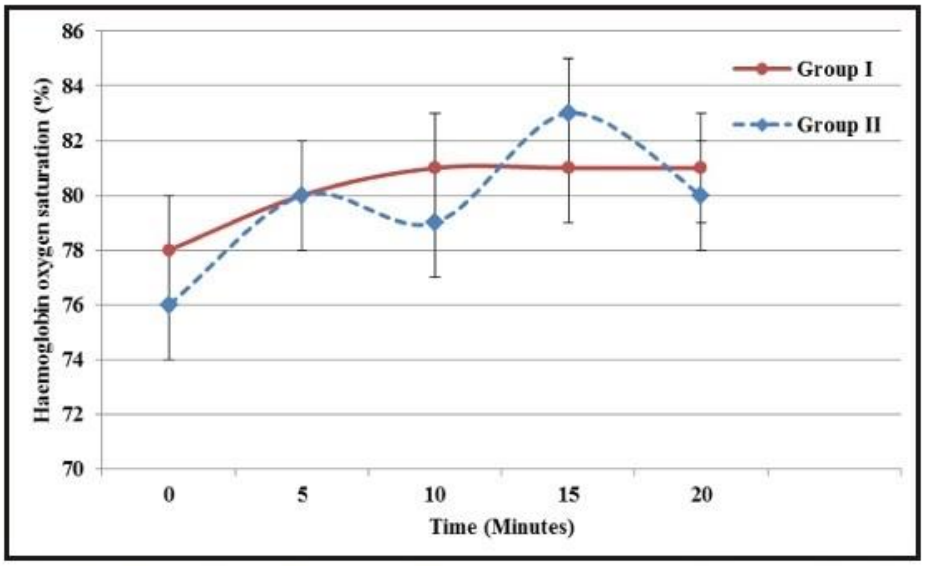

Figure 5. Comparison of haemoglobin oxygen saturation in deer subjected to xylazine-ketamine anaesthesia with and without haloperidol premedication

4.5. ELECTROCARDIOGRAPHY

The electrocardiographic tracings were recorded throughout the procedure and 90 per cent animals of each group had normal electrocardiograms.

Ventricular premature contractions were observed in one animal (Animal No. 1.8) of Group I and second-degree atrioventricular block was observed in one animal (Animal No. 2.10) of Group II. There was no statistically significant difference between and within groups.

The ECG tracings of the above mentioned arrhythmias are represented in Figure 6 and 7.

4.6. BLOOD GAS ANALYSIS

The mean±SE values of pH, partial pressure of carbon dioxide (PCO_2), partial pressure of oxygen (PO_2), bicarbonate ion concentration (HCO_3^-), standard base excess (BE ecf) and lactate concentration of both groups at 0, 10, 20 and 35 minutes post-induction of anaesthesia are represented in Tables 15.

The mean±SE values of pH, bicarbonate ion concentration (mmol/L) and standard base excess (mmol/L) immediately after induction of anaesthesia (0^{th} minute) was 7.26±0.02, 21.47±1.04 and - 4.78±1.38 respectively, for animals of Group I and 7.32±0.01, 24.70±0.83 and - 0.77±0.96 respectively, for animals of Group II. The above mentioned parameters had statistically significant difference between groups at 0^{th} minute.

The mean±SE value of lactate at 20^{th} minute was 6.45±0.90 mmol/L for animals of Group I and 3.27±0.72 mmol/L for animals of Group II. The values showed statistically significant difference between Group I and Group II at 20^{th} minute.

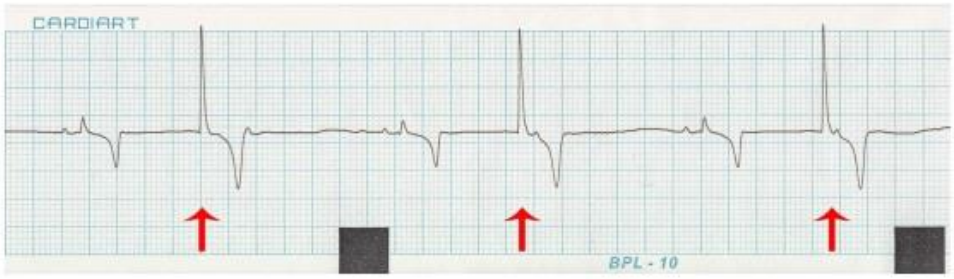

Figure 6. Electrocardiogram representing premature ventricular contractions in deer subjected to xylazine-ketamine anaesthesia without haloperidol premedication (Group I)

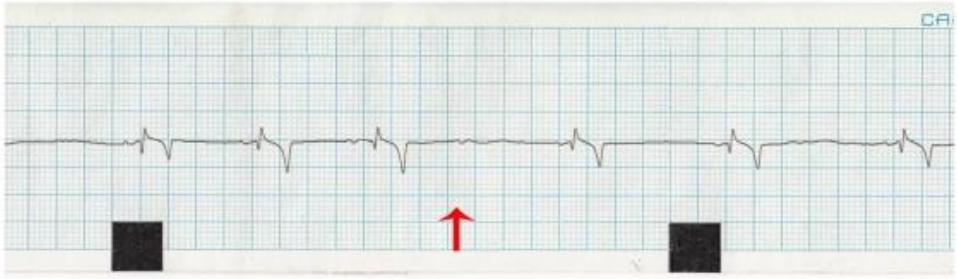

Figure 7. Electrocardiogram representing second-degree atrioventricular block in deer subjected to xylazine-ketamine anaesthesia with haloperidol premedication (Group II)

Table 15. Blood gas analysis in deer subjected to xylazine-ketamine anaesthesia with and without haloperidol premedication

Parameters	Groups	Time from induction of anaesthesia (minutes)			
		0 (Venous)	**10 (Arterial)**	**20 (Arterial)**	**35 (Venous)**
pH	Group I	7.26±0.02[a]	7.28±0.02[a]	7.30±0.02[a]	7.29±0.01[a]
	Group II	7.32±0.01[b]	7.32±0.01[a]	7.32±0.01[a]	7.32±0.01[a]
PCO_2 (mmHg)	Group I	49.73±1.08[a]	52.24±1.50[a]	52.74±1.63[a]	59.10±1.16[a]
	Group II	49.21±1.41[a]	50.65±1.42[a]	52.80±1.01[a]	56.34±1.42[a]
PO_2 (mmHg)	Group I	49.11±2.69[a]	67.79±4.62[a]	72.01±4.21[a]	44.08±2.63[a]
	Group II	41.63±1.78[b]	57.85±3.20[a]	61.88±3.16[a]	45.98±2.68[a]
Bicarbonate ion (mmol/L)	Group I	21.47±1.04[a]	23.92±1.02[a]	25.00±0.72[a]	27.65±0.56[a]
	Group II	24.70±0.83[b]	25.02±0.79 [a]	26.38±0.65[a]	28.22±0.71[a]
Base Excess (ecf) (mmol/L)	Group I	- 4.78±1.38[a]	-2.08±1.28[a]	-0.69±0.97[a]	1.70±0.66[a]
	Group II	- 0.77±0.96[b]	-0.67±0.91[a]	0.78±0.78[a]	2.63±0.82[a]
Lactate (mmol/L)	Group I	9.25±1.47[a]	7.21±1.40[a]	6.45±0.90[a]	4.62±0.71[a]
	Group II	6.88±1.24[a]	4.32±0.90[a]	3.27±0.72[b]	2.81±0.55[b]
Means bearing same alphabetical superscript under each parameter at a particular time do not differ significantly between groups (P > 0.05)					

4.7. HAEMATOLOGICAL PARAMETERS

Animals of Group I had mean±SE values of haemoglobin of 12.38±0.68 g/dL, volume of packed red cells of 36.30±1.96 per cent, total erythrocyte count of 8.95±0.55×10^6/µL and total leucocyte count of 5.00±0.53×10^3/µL. The mean±SE values of neutrophils, lymphocytes, monocytes, basophils and eosinophils were 55.80±4.02 per cent, 35.90±3.93 per cent, 6.00±0.82 per cent, 0.30±0.15 per cent and 1.00±0.15 per cent respectively.

Animals of Group II had mean±SE values of haemoglobin of 11.15±0.76 g/dL, volume of packed red cells of 33.13±2.25 per cent, total erythrocyte count of 9.75±0.60×10^6/µL and total leucocyte count of 5.81±0.47×10^3/µL. The mean±SE values of neutrophils, lymphocytes, monocytes, basophils and eosinophils were 61.70±3.46 per cent, 30.20±3.31 per cent, 6.70±0.50 per cent, 0.30±0.15 per cent and 1.10±0.23 per cent respectively. None of the haematological values showed statistically significant difference between the groups.

The data of haematological parameters is presented in Table 16.

4.8. BIOCHEMICAL PARAMETERS

The mean±SE values of biochemical parameters of animals of Group I and Group II are presented in the Table 17.

Animals of Group I had mean±SE values of serum cortisol, glucose, creatine kinase, aspartate aminotransferase, calcium, phosphorus and potassium concentrations of 2.83±0.38 µg/dL, 180.40±23.10 mg/dL, 1324.20±252.33 U/L, 59.70±10.59 U/L, 1.13±0.02 mmol/L, 6.77±0.70 mg/dL and 4.49±0.15 mmol/L respectively.

Animals of Group II had mean±SE values of serum cortisol, glucose, creatine kinase, aspartate aminotransferase, calcium, phosphorus and potassium concentrations of 1.81±0.30 µg/dL, 227.30±14.87 mg/dL, 2032.00±427.65 U/L,

76.30±7.34 U/L, 1.04±0.03 mmol/L, 7.31±0.36 mg/dL and 4.40±0.16 mmol/L respectively. The serum cortisol levels were elevated in animals of Group I and showed statistically significant difference between the groups.

4.9. POST-OPERATIVE COMPLICATIONS

One animal (1.3) of Group I developed suture dehiscence due to excessive licking of the suture line on its own. None of the animals of both groups showed signs of capture myopathy or other complications.

Table 16. Haematological parameters in deer subjected to xylazine-ketamine anaesthesia with and without haloperidol premedication

Parameter	Mean±SE	
	Group I	**Group II**
Haemoglobin Conc. (g/dL)	12.38±0.68[a]	11.15±0.76[a]
VPRC (%)	36.30±1.96[a]	33.13±2.25[a]
TEC ($\times 10^6$/µL)	9.75±0.60[a]	8.95±0.55[a]
TLC ($\times 10^3$/µL)	5.00±0.53[a]	5.81±0.47[a]
Neutrophils (%)	55.80±4.02[a]	61.70±3.46[a]
Lymphocytes (%)	35.90±3.93[a]	30.20±3.31[a]
Monocytes (%)	6.00±0.82[a]	6.70±0.50[a]
Basophils (%)	0.30±0.15[a]	0.30±0.15[a]
Eosinophils (%)	1.00±0.15[a]	1.10±0.23[a]
Means bearing same superscript in a row do not differ significantly (P > 0.05)		

Table 17. Biochemical parameters in deer subjected to xylazine-ketamine anaesthesia with and without haloperidol premedication

Parameter	Mean±SE	
	Group I	**Group II**
Serum cortisol (µg/dL)	2.83±0.38[a]	1.81±0.30[b]
Glucose (mg/dL)	180.40±23.10[a]	227.30±14.87[a]
CK (U/L)	1324.20±252.33[a]	2032.00±427.65[a]
AST (U/L)	59.70±10.59[a]	76.30±7.34[a]
Calcium (mmol/L)	1.13±0.02[a]	1.04±0.03[a]
Phosphorus (mg/dL)	6.77±0.70[a]	7.31±0.36[a]
Potassium (mmol/L)	4.49±0.15[a]	4.40±0.16[a]
Means bearing same superscript in a row do not differ significantly (P > 0.05)		

5. DISCUSSION

Chemical immobilisation of wild ungulates can be challenging as they are highly prone to stress related complications associated with the procedure resulting in high levels of morbidity and mortality among them. The present study was conducted in twenty adult male Spotted Deer to evaluate the effectiveness of oral haloperidol premedication prior to chemical immobilisation with a combination of xylazine and ketamine against the latter anaesthetic combination alone.

The observations on behavioural responses like ear pricking, tail erection, stamping of fore feet, anxious look, pre-orbital gland opening *etc.* were recorded and statistically evaluated to assess whether oral premedication helped to reduce initial stress and improve various aspects of anaesthesia produced by the xylazine-ketamine combination. The study also included monitoring the physiological, haematological and biochemical alterations during chemical immobilisation with the two anaesthetic protocols, with special consideration for changes associated with the capture myopathy syndrome. The findings are discussed below.

5.1. BEHAVIOURAL OBSERVATIONS

Behavioural responses like ear pricking, tail erection, stamping of fore feet, anxious look, pre-orbital gland opening, excitation behaviour and escape behaviour showed no statistically significant difference between the observations taken prior to feeding of unbaited banana and prior to darting in animals of Group I.

In Group II animals, behavioural responses like stamping of fore feet, pre-orbital gland opening, excitation behaviour and escape behaviour showed statistically significant difference between the observations taken prior to feeding of baited banana and prior to darting.

The opening of pre-orbital gland has already been recognized as an indicator of stress in farmed deer (Bartosová *et al*., 2012). The difference in the behavioural responses between groups could have been due to the calming effect produced by haloperidol.

The effectiveness of haloperidol in controlling psychological stress and physical injuries during handling and transportation of most of the African antelope species has been recognised since more than 30 years (Hofmeyr, 1981). According to Mikota *et al.* (1999), oral administration of haloperidol at the rate of 1 mg/kg body weight provided sufficient tranquilisation for physical examination and behavioural responses could be scored from good to excellent from three to 10 hours in Bongo Antelope. Haloperidol has been recognised to bring about a dopamine antagonist action leading to behavioural modification and the tranquilisation effects by blocking dopamine receptors (Read, 2002). The lack of statistically significant difference in the behavioural responses of animals of Group I between the two stages of observation suggests that segregation and maintenance of the animals in the holding facility did not produce any appreciable behavioural changes in them. So, the modified behavioural responses observed in premedicated animals after six hours from the administration of haloperidol just prior to darting could be due to the effect of haloperidol. This suggests that oral administration of haloperidol may help to reduce stress and excitement in captive deer.

Howerever, Blumer (1991), noted that administration of high doses of haloperidol or prolonged use of the drug could result in incidence of extrapyramidal signs in non-domestic hoof stock. But none of premedicated animals in the present study showed extrapyramidal signs. This might have been because of the fact that the dose rate selected for the study was within the normal suggested rates and because the drug was administered only once.

5.2. QUALITY OF DARTING PROCEDURE

It was observed that the non-premedicated animals were anxious and got excited on seeing the person with the darting equipment, which made the darting procedure difficult. But animals of Group II were calm and their movements were minimal which made the darting procedure easy. Eighty per cent of animals of Group II remained calm during the placement of the dart, which might have been due to the calming effect produced by haloperidol. Haloperidol has been recognised to produce excellent calming effect in wild animals including ungulates (Read, 2002; Walsh and Wilson, 2002).

5.3. ANAESTHESIA

The corrected mean dose rates of xylazine hydrochloride and ketamine hydrochloride were 2.90±0.13 and 2.00±0.09 mg/kg body weight respectively in Group I. In Group II, the corrected mean dose rates of the drugs were 3.00±0.06 and 2.00±0.04 mg/kg body weight respectively. The combination provided adequate surgical plane of general anaesthesia in all animals of both groups with no additional dose requirements. There was no statistically significant difference between the corrected mean doses of xylazine and ketamine used in both groups, which suggests that all the differences observed in Group II animals, could be due to the haloperidol premedication. The observations made during the induction, maintenance and reversal of anaesthesia in both groups are discussed below.

5.3.1. Induction Time

The mean induction time for animals of Group I and Group II were 5.98±0.45 and 5.00±0.55 minutes respectively. Lesser induction time in premedicated animals might be due to the reduction in the excitement level and ease of dart placement produced by haloperidol at the time of darting. Gamer and Addison (1994) suggested that initial level of excitement and chase time before chemical immobilisation could result in longer induction time.

5.3.2. Quality of Induction

The excitability and overall quality scores for induction were better in animals of Group II indicating that animals which were premedicated with haloperidol exhibited less excitation during the induction of anaesthesia compared with non-premedicated animals. It can be assumed that the premedication with haloperidol might have helped to reduce initial excitement which improved the quality of induction of anaesthesia. The use of the score card suggested by Stroms *et al.* (2005) was found to be effective in assessing the quality of induction of anaesthesia in captive Spotted Deer which were chemically immobilised using xylazine-ketamine combination.

5.3.3. Quality of Maintenance of Anaesthesia

The quality of maintenance of anaesthesia of animals of both groups was similar. The combination of xylazine and ketamine at the rate of 2.90±0.13 and 2.00±0.09 mg/kg body weight provided general anaesthesia for an average period of 35 minutes which was sufficient for vasectomy and translocation to a new enclosure. Xylazine-ketamine combination at the rate of 0.5 and 2.5 mg/kg body weight respectively was previously used for inducing general anaesthesia for electroejaculation (Sontakke *et al.,* 2007) and a total dose of 150 mg and 100 mg respectively was used for translocation (Venugopal *et al.,* 2013) of adult male Spotted Deer. The latter had calculated the total dose based on the assumption that adult Spotted Deer they anaesthetised weighed 50 kg and hence the approximate dose rate was 3 mg/kg body weight of xylazine and 2 mg/kg body weight of ketamine (personal commun.). In spite of using haloperidol as premedicant in Group II animals, the quality of maintenance of anaesthesia was similar in both groups. This observation may have been because of the fact that the dose of xylazine used in the present study was on a higher side, whereby the advantage of having administered haloperidol may have been overwhelmed by a higher degree of sedation caused by the higher end dose of xylazine.

5.3.4. Complications during Maintenance of Anaesthesia

The major complication observed during maintenance of anaesthesia was occurrence of ruminal tympany in three animals each of both groups. Caulkett and Haigh (2007) opined that ruminal tympany was a common complication during anaesthesia in cervids. They also recommended that, ideally, the animals should be fasted for 24 hours prior to anaesthesia. The authors also suggested that in case of severe ruminal tympany, administration of antagonists which reverse anaesthesia and stimulate ruminal movements should be resorted to.

One animal of Group II (Animal No. 2.6) which had ruminal tympany regurgitated at about 25^{th} minute post-recumbency due to the increased pressure that developed in the rumen due to tympany. The PaO_2 level, SpO_2 level and respiratory rates were lower in this animal, than the mean values of these parameters in the same group at 20^{th} minute post-induction. The PCO_2 was also elevated in this animal. This suggested that the animal might have experienced hypoxia due to increased ruminal pressure impairing the normal respiration.

5.3.5. Reversal Time

The mean time for recovery from anaesthesia for animals of Group I and Group II were 16.14±2.39 and 18.25±3.08 minutes respectively. The higher recovery time in Group II animals may have been due to augmentation of xylazine-ketamine induced CNS depression by haloperidol. This suggests that the dose of xylazine can be lowered where haloperidol is used as a premedicant. The dose of ketamine may not be altered as the dose followed in this study was towards the lower side.

5.3.6. Quality of Recovery

The quality of recovery of animals of Group I was better when compared with those of Group II. This was because of the delayed recovery of the animals of Group II from anaesthesia due to the augmentation of the sedative effect of

xylazine by haloperidol. Evaluation of quality of recovery using the modified score card was easy to perform and effective.

5.3.7. Resedation

Two animals of Group II showed resedation two hours after recovery from anaesthesia. This may have been due to the enhancement of xylazine induced sedation by haloperidol. This was in agreement with the opinion of Hofmeyr (1981) that administration of xylazine hydrochloride could enhance the soporific (sleep inducing) effect of haloperidol in Hartmann's Zebra and Black-faced Impala.

5.4. PHYSIOLOGICAL PARAMETERS

5.4.1 Rectal Temperature

Animals of Group I exhibited higher mean rectal temperatures compared to Group II animals. This could have been due to the fact that animals of Group II were less stressed and excited compared to animals of Group I before darting under the influence of haloperidol.

Four animals (1.4, 1.8, 1.9 and 1.10) of Group I and one animal (2.10) of Group II had rectal temperature above 41ºC. The overall quality of induction of anaesthesia was rough and extended in all these individuals. Increased temperature in animals of Group I may have been due to the initial psychological stress, physical exertion and interference of thermoregulatory centres by xylazine in the anaesthetic mixture during chemical immobilisation. It has already been recognised that physical exertion and psychological stress could contribute to the incidence of hyperthermia during the immobilisation of wild ruminants (Meyer *et al.,* 2008; Wolff, 2009).

Xylazine in the anaesthetic combination may also have interfered with the thermoregulatory center resulting in hyperthermia. This is in agreement with the

observations of Monteith *et al.* (2012) that thermoregulation was interfered by xylazine during chemical immobilisation. The reduction in the rectal temperatures of haloperidol premedicated animals could also have been due to the vasodilation-induced heat dissipation mediated by haloperidol, which was in agreement with the observation of Mentaberre *et al.* (2010).

5.4.2 Heart Rate

The comparison of mean values of heart rate between the groups indicated that the animals of Group II had a low heart rate throughout the procedure. Animals of Group I had an initial elevated heart rate when compared to animals of Group II. It was reported by Susanne (2012) that the heart rate of deer increased in response to anthropogenic external disturbances. Thus, the initial elevated heart rate in Group I animals might have been due to the initial level of excitement. The lower heart rate of animals of Group II may have been due to the reduced level of excitement because of the calming effect haloperidol. Animals of both groups exhibited a sharp decline in the heart rate within five minute after induction of anaesthesia which appears to be due to the influence of xylazine. Sontakke *et al.* (2007) also observed that increased doses of xylazine in the xylazine-ketamine anaesthetic combination resulted in decreased heart rate in Axis Deer. There was statistically significant difference in the heart rate at 10^{th}, 15^{th} and 20^{th} minutes post-induction between groups.

5.4.3. Respiratory Rate

The rate of respiration showed a statistically non-significant reduction with the advancement of time from anaesthesia in each group. It was also found that there was no statistically significant difference in the respiratory rates between groups. The observations are graphically represented in Figure 5.

This shows that the combination of xylazine-ketamine used in the present study produced non-significant reduction in respiratory rate in both groups during maintanence of anesthesia and that haloperidol did not have any significant effect

on the same. However, it was observed by Doherty *et al.* (1986) that administration of xylazine resulted in respiratory depression leading to hypoxemia in sheep. It was also reported that increased doses of xylazine in the xylazine-ketamine anaesthetic combination resulted in respiratory depression in Axis Deer (Sontakke *et al.*, 2007). The present findings suggest that attempts should be made to keep the dose of xylazine low in anaesthetic combinations to reduce complications associated with respiratory depression.

5.4.4. Pulse Rate

Animals of both groups showed a steady non-significant reduction in pulse rate from the time of induction of anaesthesia till the end of the surgical procedure. There was also no statistically significant difference in the pulse rates between groups.

5.4.5. Haemoglobin Oxygen Saturation

The mean haemoglobin oxygen saturation in animals of both groups were below 85 per cent which clearly suggests that all the animals were hypoxemic. The hypoxemia might have been due to the hypoventillation produced by the influence of the high dose of xylazine (alpha$_2$-agonist) in the combination. According to Celly *et al.* (1997), the hypoxemic effect of alpha$_2$-agonists in sheep was mainly due to the reduction in respiratory rate and increased pulmonary pressure. Hypoxia with $SpO_2 < 90$ per cent was also observed by Arnemo (2005) in Hog Deer and in free-ranging Norwegian Reindeer by Arnemo *et al.* (2011) during chemical immobilisation with alpha$_2$-agonist and ketamine combination. Ketamine also may have contributed to respiratory depression. Stroms *et al.* (2006) observed that ketamine can predispose to respiratory depression in high doses.

5.5. ELECTROCARDIOGRAPHY

One animal from each group had cardiac dysrhythmias. The electrocardiogram of Animal No. 1.8 indicated premature ventricular contractions. The induction of anaesthesia in this animal was scored as "rough or extended unacceptable induction" and the rectal temperature had been elevated. The premature ventricular contractions might have occurred due to the combined effect of catecholamine release due to hypercarbia observed during the study and myocardial sensitisation produced by xylazine. This observation was in agreement with the observation of Caulkett *et al.* (2000) in deer which were chemically immobilised with carfentanil-xylazine combination.

The electrocardiogram of the Animal No. 2.10 of Group II showed second-degree atrioventricular block. The overall quality of induction of anaesthesia was scored as "extremely rough, lengthy, potentially dangerous induction" and the rectal temperatures were also elevated. Administration of xylazine at the rate of 0.15 mg/kg body weight in sheep resulted in the occurrence of second degree heart block for a transient period (Doherty *et al.*, 1986). So, the second-degree atrioventricular block may have occurred due to the effect of xylazine in the combination.

5.6. BLOOD GAS ANALYSIS

The mean value of blood pH (7.26) of Group I animals clearly indicated acidemia immediately after induction of anaesthesia. Elevated lactic acid content with an apparently normal partial pressure of carbon dioxide and lowered bicarbonate concentration suggest that the acidemia was mostly due to lactic acidosis. There was statistically significant difference in the blood pH, bicarbonate concentration and standard base excess values between groups immediately after the induction of anaesthesia. The initial physical exertion and excitement in Group I animals might have lead to the increased metabolic rate and elevated oxygen demand, which resulted in anaerobic metabolism and accumulation of lactic acid.

This was in agreement with similar observations in various species (Kock *et al.,* 1987; Martucci *et al*., 1992; Storms *et al.,* 2005; Smith *et al.,* 2006).

The blood pH was acidic in animals of Group II also. However, the pH was less acidic compared with that of Group I animals. In Group II, the partial pressure of carbon dioxide in blood was elevated. However, the lactate level was less compared to that of Group I. The elevated carbon dioxide level could have been the predominant reason for acidosis in this group, which means that the animals of this group experienced respiratory acidosis. The mildly elevated lactate levels may also have contributed to the acidemia in Group II animals. The lower lactic acid content in Group II animals could be atributed to the reduced level of excitement and physical exertion in this group during induction of anaesthesia. Oral administration of haloperidol may have helped to reduce excitement in animals of this group, thus helping to reduce blood lactate induced acidemia.

The PaO_2, bicarbonate, standard base excess and lactate levels were found to be improving with time in animals of both groups. Even though metabolic acidosis was found to be resolving with time there was no marked improvement in the blood pH of animals of both groups. This could be attributed to the consistent rise in the PCO_2 levels observed with time resulting in respiratory acidosis in animals of both groups. In the six animals which had ruminal tympany there was marked rise in the PCO_2 levels towards the end of the procedure. Ruminal tympany in three animals of each group could have contributed to hypoventilation and resultant hypercarbia which contributed to hypoxemia and acidosis. The respiratory depressant effect of the combination of xylazine and ketamine could also have led to hypercarbia. So intranasal supplementation of oxygen has to be resorted during chemical immobilisation of deer.

5.7. HAEMATOLOGICAL PARAMETERS

Haematological parameters like volume of packed red cells, haemoglobin concentration, total erythrocyte count and total leucocyte count were in the

reference range (ISIS 2012). The differential leucocyte count was also in agreement with the values reported by Gupta *et al.* (2007) for Axis Deer. The haemoglobin, VPRC and total erythrocyte count mean values were higher in animals of Group I, which may have been due to higher catecholamine release and subsequent contraction of spleen leading to haemoconcentration (Chapple *et al.*, 1991; Carragher *et al.*, 1997; Read, 2000). Though statistically not significant, the lower VPRC values in haloperidol premedicated animals may have been due to the reduced stress response and the splenic sequestration of red blood cells in Group II animals.

5.8. BIOCHEMICAL PARAMETERS

The serum cortisol levels showed significant difference between the groups. The premedicated animals had a lower serum cortisol level, which suggested that the stress response of Group II animals was reduced by the premedication with haloperidol. The highest serum cortisol level estimated was 5 µg/dL in one animal of Group I (Animal No. 1.2). In this animal, the overall quality of induction of anaesthesia had been scored as "rough or extended unacceptable induction" and the initial plasma lactate level was 11.6 mmol/L.

Plasma cortisol level has already been recognized as a valuable indicator of capture related stress in wild animals (Morton *et al*., 1995). However, Meyer *et al*. (2008) had observed that the plasma cortisol level and behavioural response of Impala to stressors were correlated with the extent of habituation and handling when compared to animals that were not habituated. In the present study, in spite of the animals being used to encountering humans in close proximity being zoo animals, there was significant difference in the level of serum cortisol between animals of the two groups. This indicates how important stress is in deer species in spite of habituation. The fact that serum cortisol levels were significantly higher in the non-premedicated animals suggests the effectiveness of oral administration of haloperidol about 6 hours prior to darting in reducing stress in deer.

The blood glucose level was found elevated in animals of both groups which may have been due to the alpha$_2$-adrenergic agonist mediated hypoinsulinaemia. This was in agreement with the the observation of Stewart and English (1990) in Fallow Deer after chemical immobilisation using xylazine-ketamine combination. Gericke *et al.* (1978) had suggested that blood glucose level could be considered as an indicator of alarm reaction and catecholamine release. However, in the present study, there was no statistically significant difference in the blood glucose levels between groups.

Creatine kinase and aspartate aminotransferase, which are considered to be sensitive indicators of skeletal muscle damage, were found elevated in animals of both groups. This indicated skeletal muscle damage during the procedure. Similar findings have been reported in Bighorn Sheep (Kock *et al*., 1987) and in Chital Deer (Chapple *et al.*, 1991) during physical restraint. Creatine kinase levels were found to be higher than that of aspartate aminotransferase in both groups. However, there was no statistically significant difference in the levels of both enzymes between the groups. The rapid rise in the serum levels of creatine kinase when compared to aspartate aminotransferase during skeletal muscle damage has been already described (Williams and Throne, 1996). This could be the reason for the highly elevated levels of creatine kinase in the present study when compared to aspartate aminotransferase, which show only a gradual rise.

There was no statistically significant difference in the concentrations of calcium, potassium and phosphorus levels between the groups. According to Spraker (1993), muscle fiber damage during capture resulted in efflux of potassium ion from the myocytes resulting in hyperkalemia. However, the potassium levels were not elevated in the present study, even though some animals showed severe excitement and physical exertion.

6. SUMMARY

The study was conducted to comparatively evaluate xylazine-ketamine anaesthesia with and without oral haloperidol premedication in 20 healthy adult male Spotted Deer maintained at the Zoological Gardens, Thiruvananthapuram, which had to undergo routine vasectomy procedure as part of the zoo's deer population control programme. The animals were randomly divided into two groups of ten each. Animals of Group I were anaesthetised using a combination of xylazine hydrochloride and ketamine hydrochloride intramuscularly at the rate of 3 and 2 mg/kg body weight respectively by darting. Animals of Group II were premedicated with haloperidol at the rate of 1 mg/kg body weight orally concealed in banana (baited banana), six hours after which they were anaesthetised with the same combination of drugs used in Group I. Animals of Group I were also given plain banana (unbaited banana) at the same time of providing the haloperidol baited banana in the Group II animals to maintain uniformity in procedure between groups, except for haloperidol.

The objectives of the study were to assess the effectiveness of oral haloperidol premedication in modifying behavioural responses at the time of induction of anaesthesia, compare the quality of xylazine-ketamine anaesthesia with and without oral haloperidol premedication and to study the physiological, haematological and biochemical alterations associated with the two anaesthetic protocols.

All the animals were observed for selected behavioural responses prior to feeding of banana and prior to darting. The quality of darting procedure was also evaluated. The quality of induction, maintenance and recovery from anaesthesia and the physiological, haematological and biochemical alterations were assessed and compared between groups.

There was statistically significant difference in the behavioural responses like stamping of fore feet, pre-orbital gland opening, excitation behaviour and

escape behaviour prior to feeding of baited banana and prior to darting in Group II animals. The quality of darting procedure was found to be better in premedicated animals.

The doses of haloperidol, xylazine and ketamine were calculated based on visually assessed body weights of the selected animals. These doses were corrected against actual body weights measured using a weighing balance after the surgical procedure. Accordingly, the corrected mean dose rates of xylazine hydrochloride and ketamine hydrochloride were 2.90±0.13 and 2.00±0.09 mg/kg body weight respectively in Group I. In Group II, the corrected mean dose rates of haloperidol, xylazine and ketamine were 1.00±0.02, 3.00±0.06 and 2.00±0.04 mg/kg body weight respectively. The combination of xylazine and ketamine in the above dosage provided adequate surgical plane of general anaesthesia in all animals of both groups, with no additional dose requirements. The induction time was 5.98±0.45 minutes and 5.00±0.55 minutes for Group I and Group II animals respectively. The quality of induction was better in animals of Group II with a lesser induction time compared to those of Group I. After induction of anaesthesia, all the animals underwent vasectomy in a routine manner.

There was statistically significant difference in the rectal temperatures recorded during the surgical procedure between animals of both groups. Animals of Group I had elevated rectal temperature when compared to animals of Group II.

The heart, pulse and respiratory rates were found to be elevated in animals of Group I and showed a gradual reduction with time during the surgical procedure. There was statistically significant difference in the heart rates at 10^{th} 15^{th} and 20^{th} minute post-induction between the groups. The mean haemoglobin oxygen saturation was below 85 per cent, which is considered to be a critical lower limit in anesthetised animals, in both groups. However, there was no statistically significant difference in mean haemoglobin oxygen saturation between groups.

There was statistically significant difference in the pH, bicarbonate ion concentration and standard base excess values between the groups immediately after induction of anaesthesia. Elevated lactic acid concentration was observed in animals of Group I throughout the surgical procedure. The mean blood pH was also acidic in Group II animals, but less than that of Group I. The acidemia in Group II animals was predominantly due to the elevation of PCO_2. Hypoxemia was observed in individuals of both groups with mean partial pressure of oxygen less than 80 mmHg. However, there was no statistically significant difference between groups.

The haematological values of animals of both groups were within the reference ranges. The volume of packed red cells, total erythrocyte count and haemoglobin values of Group I animals were slightly elevated but there was no statistically significant difference between the groups.

The premedicated animals had a lesser serum cortisol level when compared to non-premedicated animals. There was statistically significant difference in the serum cortisol levels between the groups. Muscle enzymes like creatine kinase and aspartate aminotransferase were found elevated in animals of both groups. The blood glucose level was also found elevated in both groups. The concentrations of calcium, potassium and phosphorus showed no statistically significant difference between groups.

No major complications were observed during the maintenance of anaesthesia in animals of both groups. Three animals from each group had ruminal tympany and one animal of each group showed cardiac dysrhythmias.

After surgery, the animals were translocated into a new enclosure before reversal of anaesthesia using yohimbine at the rate of 0.3 mg/kg body weight, half of which was administered intravenously and the other half intramuscularly. The quality of recovery was better in Group I animals with shorter recovery time than that observed in Group II animals. The time taken for recovery was 16.14±2.39

minutes and 18.25±3.08 minutes for Group I and Group II animals respectively.

The animals were observed for a period of one week after the procedure for post-operative complications like suture dehiscence, scrotal edema, maggot wound and surgical site infection. The animals were observed for signs of capture myopathy for a period of two months. Suture dehiscence was observed in one animal. None of the animals developed complications like capture myopathy, related with stress or restraint.

The following conclusions could be drawn based on the present study.

1. A combination of xylazine hydrochloride at the rate of 3 mg/kg body weight and ketamine hydrochloride at the rate of 2 mg/kg body weight can be recommended for general anaesthesia in Spotted Deer for surgical procedures or translocation over short distances.

2. Haloperidol may be recommended as an oral premedicant prior to xylazine-ketamine anaesthesia in Spotted Deer or related species at the rate of 1 mg/kg body weight because of its advantages like calming effect favouring easy dart placement, reduction in induction time and improvement in quality of induction, ability to reduce chances of hyperthermia and excessive lactic acidosis and reduction of overall stress in treated animals.

3. Increased recovery time and poorer quality of recovery from xylazine-ketamine anaesthesia in haloperidol premedicated animals suggest that further studies may be needed to evaluate the possibility of reducing the dose of xylazine in this protocol.

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ABSTRACT

The efficacy of xylazine-ketamine anaesthesia with and without oral haloperidol premedication was evaluated in 20 captive Spotted Deer stags undergoing routine vasectomy and randomly allocated into two groups of ten each. Group I animals were anaesthetised with a combination of xylazine and ketamine at the rate of 3 and 2 mg/kg b.wt. respectively by darting. Group II animals were orally premedicated with haloperidol at the rate of 1 mg/kg b.wt., six hours prior to xylazine-ketamine anaesthesia as in Group I. Yohimbine was administered at the rate of 0.3 mg/kg b.wt., divided equally and administered IV and IM following surgery and translocation which lasted for an average of 35 minutes. Group II animals were calmer and easier to dart, showing better quality of induction of anaesthesia with shorter induction time. Group I animals showed better quality of recovery with a shorter recovery time. Rectal temperature, heart rate, serum cortisol and plasma lactate were lower in Group II animals, indicating reduced stress and physical exertion. Animals of both groups were hypoxemic throughout the study and PCO_2 increased with time in both groups. Blood pH was found to be acidic in both groups with significantly lower pH observed in Group I immediately after induction. Creatine kinase and blood glucose were elevated in both groups during the procedure but did not differ significantly between groups. Thirty percent of animals of each group showed ruminal tympany. Ventricular premature contraction and second-degree atrioventricular block were seen in one animal of Group I and Group II respectively. Haematological parameters were within the reference ranges in all animals. Overall, haloperidol premedication was observed to have several benefits in xylazine-ketamine anaesthesia in Spotted Deer and was not associated with any additional complication. However, quality of anaesthesia may be improved by reducing the dose of xylazine in haloperidol premedicated animals.

Keywords: Haloperidol premedication, xylazine-ketamine anaesthesia, Spotted Deer.

ANNEXURE I

Observation chart for behavioural responses

Animal No: **Ear tag No:** **Date:**

I. BEHAVIOURAL RESPONSES PRIOR TO FEEDING OF BANANA

Observations	Present	Not present
Ear pricking		
Tail erection		
Stamping of fore feet		
Anxious look		
Pre-orbital gland opening		
Excitation behaviour		
Escape Behaviour*		
*Animals which jumped against exit gate were considered to have shown escape behaviour		

II. BEHAVIOURAL RESPONSES PRIOR TO DARTING

Observations	Present	Not present
Ear pricking		
Tail erection		
Stamping of fore feet		
Anxious look		
Pre-orbital gland opening		
Excitation behaviour		
Escape Behaviour		

III. QUALITY OF DARTING PROCEDURE

ITEM OF OBSERVATION	COMMENTS
Ease of dart placement	**DIFFICULT or EASY**
Response to dart placement	**EXCITED or CALM**
Difficult: Animal moving very fast which leads to lengthy darting procedure **Easy: Animal movement is minimal, fast darting procedure**	

ANNEXURE II

ANAESTHETIC RECORD

I. INDUCTION OF ANAESTHESIA

TIME FOR INDUCTION	Seconds
Time taken to first sign of drug effect	
Time taken from first sign of drug effect to sternal recumbency	
Time taken from sternal recumbency to head down	
Time taken form head down to lateral recumbency	

SCORE	INDUCTION SCORING CARD	OBSERVATION
	Excitability	
3	Very calm: no excitation during induction	
2	Mild excitement: procedure-related stress evident but calming with onset of drug effects	
1	Marked excitement: frantic/racing behaviour, minimally or not reduced with onset of drug effects	
0	Extreme excitement: frantic, reckless, or violent behaviour, apparently increased by onset of drug effects	
	Overall quality	
3	Rapid, smooth, optimal induction	
2	Relatively rapid and smooth but could be improved	
1	Rough or extended unacceptable induction	
0	Extremely rough, lengthy, potentially dangerous induction	

II. QUALITY OF MAINTENANCE OF ANAESTHESIA DURING PROCEDURE

SIGNS OF RECOVERY FROM ANAESTHESIA		
Item of observation	**Present**	**Absent**
Kicking		
Jaw movements		
Ear twitching		
Head lifting		
Struggling		

III. COMPLICATION DURING ANAESTHESIA

Item of observation	Present	Absent
Regurgitation		
Aspiration		
Ruminal tympany		
Excessive bleeding at the surgical site		
Arrhythmia		
Others		

IV. RECOVERY FROM ANAESTHESIA

TIME FOR RECOVERY	Seconds
Time from administration of the antagonist to sternal recumbency	
Time from sternal recumbency to standing	

SCORES	RECOVERY SCORE CARD	OBSERVATION
4	**Excellent: Animal stood up after the first attempt without any ataxia**	
3	**Good: Animal stood up after one or two attempts with little or no ataxia**	
2	**Satisfactory: Animal stood up after one to three attempts with prolonged ataxia**	
1	**Moderate: Animal tried to stand up after three or more attempts with prolonged ataxia with or without excitation**	
0	**Poor: Animal tried to stand up after three or more attempts, with evident excitation and a high risk of injury**	

V. BIOCHEMICAL PARAMETERS

Parameter	Result
Serum cortisol (µg/dL)	
Glucose (mg/dL)	
Creatine Kinase (U/L)	
Aspartate aminotransferase (U/L)	
Calcium (mmol/L)	
Phosphorus (mg/dL)	
Potassium (mmol/L)	

VI. HAEMATOLOGICAL PARAMETER					
Parameter	**Result**				
Haemoglobin Conc. (g/dL)					
VPRC (%)					
TEC (10^6/µL)					
TLC (/ µL)					
DLC (%)	**N**	**L**	**M**	**E**	**B**

VII. PHYSIOLOGICAL PARAMETERS

Time (min)	0	5	10	15	20	25	30	35
Temp (°C)								
PR (pulse/min.)								
RR (breaths/min.)								
HR (beats/min.)								
SpO2 (%)								
Body weight (kg)	Assessed:				Actual:			

VIII. BLOOD GAS ANALYSIS

Time (min)	0 (Venous)	10 (Arterial)	20 (Arterial)	35 (Venous)
pH				
PCO2 (mmHg)				
PO2 (mmHg)				
$HCO3^-$ (mmol/L)				
BE (ecf) (mmol/L)				
Lactate (mmol/L)				

Acad. Form No. 19

KERALA VETERINARY AND ANIMAL SCIENCES UNIVERSITY
FACULTY OF VETERINARY & ANIMAL SCIENCES

PROGRAMME OF RESEARCH WORK FOR THESIS FOR MASTERS DEGREE

1. Title of thesis:

Comparative evaluation of xylazine-ketamine anaesthesia with and without oral haloperidol premedication for vasectomy in Spotted Deer (*Axis axis*)

2a. Title of the departmental/KVASU research project of which this forms a part:

Preliminary Studies on Capture Myopathy and its Control in Various Deer Species of Kerala.

2b. Code No. if any, and order by which departmental/KVASU research project is approved:

KVASU/DAR/R2/4632/2011, dated13/07/2012

3a. Name of the student

Joju Johns

3b. Admission Number

12 - MVP - 005

4a. Name of the Major Advisor (Guide)

Dr. George Chandy

4b. Designation

Assistant Professor,
Department of Veterinary Surgery & Radiology,
College of Veterinary & Animal Sciences,
Pookode, Lakkidi PO, Wayanad.673 576

5. Objectives of the study

1. To compare the quality of xylazine-ketamine anaesthesia with and without oral haloperidol premedication for vasectomy in Spotted Deer

2. To study the physiological, haematological and serum biochemical changes associated with anaesthesia for vasectomy in deer using both protocols

6. Practical/Scientific utility

A combination of xylazine and ketamine is routinely used for general anaesthesia in Spotted Deer in India. Physical and chemical restraint of deer is associated with stress induced complications like capture myopathy. Oral and parenteral use of haloperidol has been recommended as a means to reduce stress in wild ruminants (Mikota *et al.,* 1999).

Pre-anaesthetic use of oral haloperidol may help to reduce the stress at the time of darting and improve the quality of anaesthesia achieved with xylazine and ketamine.

Vasectomy is recommended as an effective procedure to control the increasing deer populations in captivity without affecting their behaviour and hierarchy in the herd.

The present study will help to assess the physiological, haematological and serum biochemical responses to chemical restraint in Spotted Deer using xylazine-ketamine with and without oral haloperidol premedication. The use of haloperidol as a premedicant in xylazine-ketamine

anaesthesia, if found effective in improving the quality of anaesthesia, may be recommended for reducing complications associated with chemical restraint in captive and free-ranging deer in future.

7. Important publications on which the study is based

Mikota *et al.* (1999) reported that oral administration of haloperidol at the rate of 1 mg/kg body weight in Bongo Antelope produced a level of tranquilization which permitted restraint for blood sampling. The authors observed that overall behavioural response remained fairly constant from 3 to 10 hours post dose.

Boyd *et al.* (2000) reported that use of haloperidol at the rate of 1 mg/kg body weight orally had reduced the stress on manual restraint for blood collection in Bongo Antelope and Eland. The authors also reported that haloperidol treated animals maintained a normal acid base balance after manual restraint.

Walsh and Wilson (2002) noted that haloperidol produced an excellent calming effect in Fallow Deer, lasting 8–10 hours and in some small and medium-sized antelope species, especially Red Hartebeest, Springbok, Duiker, Steenbok and Dik Dik. Haloperidol was also reported to be effective for post capture tranquilization in antelope.

Sontakke *et al*. (2007) reported that the use of xylazine-ketamine combination in male Spotted Deer for electroejaculation produced good level of anaesthesia without any sudden recovery and adverse effects of anaesthesia. The authors also reported that the anaesthesia was reversed using yohimbine intravenously.

Gregorio *et al.* (2010) reported that haloperidol had a protective effect against the muscular and renal damages associated with stress, thereby reducing the physio-pathological changes associated with capture myopathy in Roe Deer.

8. Outline of technical programme

A minimum of twelve healthy adult male Spotted Deer housed at the Zoological Garden, Thiruvananthapuram, will be randomly divided into two groups of six animals each.

Animals in Group I will be anaesthetised using a combination of xylazine and ketamine intramuscularly at the rate of 3 mg/kg and 2 mg/kg body weight respectively, by darting.

Animals in Group II will be premedicated with haloperidol at the rate of 1mg/kg body weight orally followed by anaesthesia using xylazine and ketamine combination at the same dose as used in Group I.

Vasectomy will be performed in both groups by caudal approach of the scrotal neck as per standard procedure. The anaesthesia will be reversed using yohimbine hydrochloride at the rate of 0.3 mg/ kg body weight half of which will be administered intravenously followed by the other half intramuscularly.

The deer will be administered long acting enrofloxacin at the rate of 7.5mg/kg body weight intramuscularly and tetanus toxoid at the time of revival.

Physiological parameters will be monitored throughout the procedure in all animals.

Blood collected at the end of procedure will be subjected to haematological and serum biochemical analysis. The results will be subjected to statistical analysis using appropriate

methods.

In case of death, if any, due to post-anaesthetic or post-operative complications like capture myopathy, the carcass will be subjected to necropsy and tissues will be subjected to histopathological examination.

9. Main items of observation to be made

1. Behavioural response after oral administration of haloperidol

2. Quality of induction, maintenance and recovery from anaesthesia using xylazine and ketamine with and without haloperidol premedication

3. Physiological parameters like rectal temperature, pulse rate, respiratory rate, SpO_2 and ECG during the period of anaesthesia

4. PaO_2, $PaCO_2$, pH and HCO_3^- values of arterial blood during the procedure

5. Serum cortisol, lactate, glucose, calcium, phosphorus, potassium, CPK and AST values from venous blood collected just before reversal

6. Haematological parameters like haemoglobin concentration, TEC, TLC, DLC and PCV from venous blood collected just before reversal

7. Post-operative complications, if any, like capture myopathy, wound dehiscence and infection at surgical site for a period of four weeks

8. Gross changes in carcasses in case of death, if any, during necropsy and histopathological changes in skeletal muscles, cardiac muscles and kidneys

10. Facilities

a. Existing: Darting equipments at Zoological Garden, Thiruvananthapuram. Patient monitoring devices at the Department of Veterinary Surgery & Radiology, CVAS, Pookode.

b. Additional facilities required:

Labwares, chemicals, suture materials, outsourcing charges for laboratory examination.

11. Duration of study

Four semesters

12. Financial estimate

Drugs & sutures materials	: Rs. 2500
Labwares and chemicals	: Rs. 2500
Out sourcing charges	: Rs. 10000
Miscellaneous	: Rs. 5000
Total	**: Rs. 20,000**

Signature of the Student:

Signature of the Major advisor:

Place: Pookode

Date:

Name and Signature of Members of the Advisory Committee

Chairman

Dr. George Chandy *MVSc, PhD*
Assistant Professor,
Department of Veterinary Surgery & Radiology,
College of Veterinary & Animal Sciences,
Pookode, Lakkidi PO, Wayanad.673 576.

Members

Co-chairman

Dr. Nigel Caulkett *DVM, MVETSc, Diplomate ACVA*
Professor
Veterinary Clinical & Diagnostic Sciences,
University of Calgary, Canada.

Dr. Ajithkumar S. *MVSc, PhD*
Professor & Head,
Department of Clinical Veterinary Medicine, Ethics & Jurisprudence,
College of Veterinary & Animal Sciences,
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Dr. Syam K. Venugopal *MVSc, PhD*
Associate Professor & Head,
Department of Veterinary Surgery and Radiology,
College of Veterinary & Animal Sciences, Mannuthy.

Dr. Sooryadas S. *MVSc, PhD*
Assistant Professor,
Department of Veterinary Surgery & Radiology,
College of Veterinary & Animal Sciences,
Pookode.

APPENDIX – I

References

Boyd, E.H., Mikota, S.K., Smith, J., Aguilar, R., Demaar, T., Hunt, D. and Hosgood, G. 2000. Blood gas analysis in Bongo (*Tragelaphus eurycerus*) and Eland (*Tragelaphus oryx*) Antelope. In: Charlotte, K.B. (ed.), *Procceedings of AAZV and IAAAM Joint Conference*; 17-21 September, 2000; New Orleans, Louisiana, USA, pp. 106-110.

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APPENDIX-II

Time frame of work

Semester I

1. Collection of literature
2. Planning of program for research
2. Discussion with zoo officials
3. Preparation of synopsis

Semester II

1. Collection of literature
2. Review of literature
3. Procurement of materials
4. Pilot study

Semester III

1. Review of literature
2. Research work

Semester IV

1. Research work
2. Writing of thesis
3. Submission of thesis

CERTIFICATE

Certified that the research project has been formulated observing the stipulations laid down under the Prevention of Cruelty to animals (Amendment, 1998).

Place: Pookode
Date:

Dr. GEORGE CHANDY
Major Advisor

CURRICULUM VITAE

Name of Candidate: Joju Johns

Date of Birth: 13/05/1987

Place of Birth: Calicut

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Major Field of Specialisation: Veterinary Surgery and Radiology

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Educational Status: BVSc & AH

Professional Experience:

Worked as Trainee Veterinary Surgeon at Zoological Gardens, Thiruvananthapuram, Department of Museums and Zoos, Government of Kerala, for 2.5 months.

Publications Made:

Numbers of published papers - 3

Number of presented papers - 7

Number of abstracts papers – 8

Membership in Professional Bodies:

Indian Society for Veterinary Surgery (ISVS)

Indian Society for Advancement of Canine Practice (ISACP)