Sokoine University of Agriculture

MSc. Dissertation

Physiological and Hemato-Biochemical Effects of Total Intravenous Administration of Ketamine, Propofol and Their Combinations in Mixed-Breed Dogs

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PHYSIOLOGICAL AND HEMATO-BIOCHEMICAL EFFECTS OF TOTAL INTRAVENOUS PHYSIOLOGICAL AND HEMATO-BIOCHEMICAL EFFECTS OF TOTAL INTRAVENOUS ADMINISTRATION OF KETAMINE, PROPOFOL AND THEIR COMBINATIONS IN MIXED-BREED DOGS

A dissertation submitted in partial fulfillment of the requirement for Master's Degree in Veterinary Surgery of Sokoine University of Agriculture, Morogoro

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EXTENDED ABSTRACT

There is increasing interest towards Total Intravenous Anaesthesia (TIVA) among veterinarians. However, information on its application in mixed breed dogs is limited. Data on the effects of ketamine, propofol and their combination 'ketofol' administered through TIVA in mixed breed dogs is also insufficient. This study assessed some of the physiological, hematological and biochemical effects of induction and maintenance of anaesthesia using ketamine and propofol individually, but also as a combination in mixed-breed dogs. Ten healthy adult mixed-breed dogs were divided into five treatment groups in a repeated crossover experiment. Treatments provided were ketamine (KK), propofol (PP) and ketamine + propofol (ketofol) at 1:1 (KP1), 1:2 (KP2) and 1:3 (KP3) ratios. The dogs were premedicated with atropine (0.04 mg/kg IM) and xylazine (2 mg/kg IM). Anaesthetic induction was through intravenous administration of 5 mg/kg body weight in KK or PP; and 4 mg/kg bodyweight ketofol i.e. 2 mg ketamine + 2 mg propofol in KP1; 1.3 mg ketamine + 2.6 mg propofol in KP2 and 1 mg ketamine + 3 mg propofol in KP3. Maintenance was by constant rate infusion (CRI) of 0.3 mg/kg/min for 60 minutes in all the five groups. In the ketofol groups, the maintenance protocol was 0.15 mg/kg/min ketamine + 0.15 mg/kg/min propofol in KP1, 0.1 mg/kg/min ketamine + 0.2 mg/kg/min propofol in KP2 and 0.075 mg/kg/min ketamine + 0.225 mg/kg/min propofol in KP3. Physiological parameters assessed were rectal temperature, respiration rate, pulse rate and oxygen saturation. Induction and recovery time and quality were also determined, and side effects observed. Hematological assessment included absolute White Blood Cell (WBC) counts; differential lymphocyte, neutrophil and monocyte counts together with total Red Blood Cells (RBC), hematocrit (HCT) and hemoglobin concentration (HB). Serum biochemical assessment included Alanine Aminotransferase (ALT), Aspartate Transaminase (AST), Blood Urea Nitrogen (BUN) and Creatinine levels. Means were compared between

protocols using Analysis of Variance (ANOVA) to detect significant differences; and within protocols using a student's t-test to compare pre-induction and post induction values. The level of significance was set at 5% Respiration rate was low in all protocols, with significant differences among protocols ($p<0.001$). KK and PP had the highest mean respiration rates (12 ± 0.5 breaths per minute, bpm) and (12 ± 0.85 bpm) respectively, while KP1 had the lowest (9 ± 2.1 bpm). Pulse rate remained within normal physiological range in all five protocols but with significant differences among protocols ($p=0.014$). KP1 had the highest mean pulse rate (121 ± 16 Beats per minute, BPM) while PP (104 ± 9.7 BPM) and KP3 (104 ± 5.3 BPM) had the lowest. Rectal temperature was within normal physiological range in all protocols. Significant differences were noted among groups ($p<0.001$), KP1 had the highest mean temperature (39.1 ± 0.05 ^{0}C) and PP lowest (38.5 ± 0.2 ^{0}C). Oxygen saturation was low in all five groups, with significant differences among groups ($p=0.002$). KP3 had the highest mean saturation (89 ± 2.2 %) while KK had the lowest (80 ± 3.8 %). The induction time was approximately 20 seconds, and the quality was good in all groups. Surgical anaesthesia lasted for an average of 58 minutes in KK, 55 minutes in PP and 60 minutes in KP1, KP2 and KP3. The recovery quality was good in all protocols except KK that had prolonged sternal recumbency and ataxia. The PP group had the shortest mean recovery time (29 ± 10 minutes) while KK the longest (67 ± 35 minutes). Side effects observed were urination, vocalization, convulsions, open eyelids and apnoea in KK; apnea, bradycardia and tachypnea in PP; apnea, urination, tachypnea, tachycardia and vomiting in KP1; tachypnea, irregular breathing and apnoea in KP2 and urination and apnoea in KP3. The absolute WBC count was significantly different among the groups. KP2 had the highest mean count (9.27 ± 2.67 x 10^3/µL) while KP1 had the lowest (4.78 ± 0.92 x 10^3/µL). The mean count at the 60th minute was less than the pre-induction count in all groups. Differences between and within protocols in the differential lymphocyte, neutrophil and monocyte counts were not significant. Differences in the RBC, (HCT) and HB were also not significant among the groups; however, within treatments, the

KP3 group had a significant decrease in the RBC count (p=0.006) and hemoglobin concentration (p=0.049) at the 60th minute. The decrease in the remaining groups was statistically not significant. Differences in the mean ALT level were significant among groups (p=0.047); KP2 had the highest mean (10.74 ± 0.24 U/L), while KP1 had the lowest (5.71 ± 0.00 U/L). Within protocols, there was a significant decrease in the PP group at the 30th minute (P<0.001). Changes in the other groups were not significant. Differences in the AST were also significant among groups (p=0.012). PP has the least mean concentration (6.06 ± 1.98 U/L), while KP2 had the highest (12.22 ± 3.1 U/L). KP2 had a significant decrease at the 60th minute (p<0.001). Changes in the other groups were not significant. Non significant differences were also noted in the creatinine and BUN concentration among groups, however there was a significant decrease in creatinine in KP2 from the 30th to 60th minute (p<0.001), and a significant increase in BUN in the PP and KP3 groups at the 60th minute (p<0.001). In general; ketamine, propofol and the 1:1, 1:2 and 1:3 Ketamine: Propofol combination ratios can safely be used in mixed breed dogs. However due to low respiration rates and oxygen saturation, coupled with decrease in red blood cell counts, hematocrit and hemoglobin concentration observed in all the protocols; it is imperative to diligently monitor the animals while under anaesthesia, but also ensure a patent airway. In addition, under the conditions of this study, the 1:3 Ketamine:Propofol combination (KP3) resulted in relatively more stable and predictable physiological effects with less side effects in comparison to the 1:1 and 1:2 ratios. Further studies with increased sample sizes and longer monitoring periods would provide additional knowledge on the short- and long-term effects of these drugs and their combinations administered through TIVA in mixed breed dogs.

Key words: Dog, Ketamine, Propofol, Total Intravenous Anaesthesia (TIVA)

IKISIRI KUU

Kuna ongezeko la nia ya madaktari wa wanyama kwa ujumla kutumia dawa za usingizi kupitia mishipa ya damu tu. Hata hivyo, taarifa juu ya matumizi yake katika jamii za mbwa mchanganyiko ni ndogo. Data juu ya madhara ya dawa aina ya ketamine, propofol na mchanganyiko wao 'ketofol' inayoingizwa kupitia mishipa ya damu katika mbwa mchanganyiko pia haitoshi. Utafiti huu ulitathmini baadhi ya athari za kifiziolojia, kihematolojia na kibiokemia za kuanzisha na kuendeleza usingizi kwa kutumia ketamine na propofol pekee, lakini pia kama mchanganyiko kwa mbwa. Mbwa kumi wakubwa wenye afya waligawanywa katika makundi matano. Matibabu yaliyotolewa yalikuwa ketamine (KK), propofol (PP) na mchanganyiko wa ketamine na propofol (ketofol) katika uwiano wa 1 kwa 1 (KP1), 1 kwa 2 (KP2) na 1 kwa 3 (KP3). Mbwa hao walipatiwa matibabu ya awali kwa kutumia atropine (miligramu 0.04 kwa kilo) na xylazine (miligramu 2 kwa kilo). Kundi la KK na PP walipatiwa dawa za kuanzisha usingizi kupitia mishipa ya damu kwa kiwango cha miligramu 5 kwa kilo ya uzito wa mwili wakati makundi ya ketofol yalipatiwa miligramu 4 kwa kilo yaani miligramu 2 za ketamine + miligramu 2 propofol katika kundi la KP1; miligramu 1.3 za ketamine + na miligramu 2.6 propofol katika kundi la KP2 na miligramu 1 ya ketamine + miligramu 3 za propofol katika kundi la KP3. Muendelezo wa usingizi ulitumia njia ya kuingiza dawa taratibu katika mishipa ya damu kwa kiwango cha miligramu 0.3 kwa kilo kwa dakika; ndani ya dakika 60 katika makundi yote matano. Katika makundi ya ketofol, muendelezo ulikuwa kwa kiwango cha miligramu 0.15 kwa kilo kwa dakika, ketamine + miligramu 0.15 kwa kilo kwa dakika, propofol kundi KP1; miligramu 0.1 kwa kilo kwa dakika, ketamine + miligramu 0.2 kwa kilo kwa dakika, propofol kundi KP2; na miligramu 0.075 kwa kilo kwa dakika, ketamine + miligramu 0.225 kwa kilo kwa dakika, propofol katika kundi la KP3. Vipimo vilivyofuatiliwa ni joto la mwili, kasi ya kupumua, kasi ya mapigo ya moyo na kiwango cha oksijeni. Muda na ubora wa kulala na kuzinduka ulitathminiwa, na madhara ya dawa pia yalifuatiliwa.Tathmini ya seli za damu ilihusisha seli nyeupe na nyekundu. Pia viashiria vya

ufanyaji kazi wa ini na figo vilitazamwa. Wastani ulilinganishwa kati ya makundi ya dawa kwa kutumia ‘ANOVA’ ili kukagua tofauti baina ya makundi, na ‘Student’s t-test’ kulinganisha vipimo kabla na baada ya dawa ndani ya makundi husika. Upumuaji ulikuwa chini katika makundi yote, tofauti kubwa ilionekana baina ya makundi. Makundi KK na PP yalikuwa na wastani wa juu zaidi wa kiwango cha upumuaji kwa dakika, wakati KP1 lilikuwa na wastani wa chini zaidi. Kiwango cha mapigo ya moyo kilisalia ndani ya wastani wa kawaida wa kifiziolojia katika makundi yote, lakini tofauti kubwa ilionekana kati ya makundi. Kundi KP1 lilikuwa na wastani wa juu zaidi wakati PP lilikuwa na wastani wa chini zaidi. Joto lilibaki ndani ya wastani wa kawaida wa kifisiolojia katika makundi yote, huku tofauti kubwa ilionekana kati ya makundi. KP1 lilikuwa na wastani wa juu zaidi wa joto na PP wastani wa chini zaidi. Kiwango cha oksijeni kilikuwa chini sana katika makundi yote matano, tofauti kubwa ikijidhihirisha baina ya makundi. KP3 lilikuwa na wastani wa juu zaidi wa kiwango cha oksijeni wakati KK lilikuwa na kiwango cha chini zaidi. Muda wa kupata usingizi ulikuwa takribani sekunde 20 na ubora ulikuwa mzuri katika makundi yote. Usingizi daraja la upasuaji ulidumu kwa wastani wa dakika 58 katika kundi KK, dakika 55 kundi PP na dakika 60 kundi KP1, KP2 na KP3. Ubora wa kuzinduka ulikuwa mzuri katika makundi yote isipokuwa KK ambapo kulikuwa na muda mrefu zaidi wa kukaa chini na kuyumba pindi wanapoamka. Kundi la PP lilikuwa na muda mfupi zaidi wa wastani wa kuzinduka (dakika 29 ± 10) huku KK muda mrefu zaidi (dakika 67 ± 35). Madhara yaliyoonekana yalikuwa kukojoa, kupiga kelele, kutetemeka, macho kutofumba na usitishwaji wa upumuaji kwa muda katika kundi KK; usitishwaji wa upumuaji kwa muda, mapigo ya moyo ya taratibu na upumuaji wa haraka katika PP; usitishwaji wa upumuaji kwa muda, kukojoa, upumuaji wa kasi na kutapika katika KP1; kupumua kwa kasi, kupumua kusiko kwa kawaida na usitishwaji wa upumuaji kwa muda katika KP2; na kukojoa na usitishwaji wa upumuaji kwa muda katika KP3. Kulikuwa na tofauti ya hesabu ya seli nyeupe za damu baina ya makundi, KP2 ilikuwa na wastani wa juu zaidi wakati KP1 ilikuwa na wastani wa chini zaidi wa hesabu ya chembe nyeupe. Hesabu

ya wastani wa makundi yote baada ya dakika 60 ilikuwa chini ikilinganishwa na hesabu kabla ya usingizi. Tofauti kati ya chembe nyekundu za damu haikuwa kubwa kati ya makundi, lakini ndani ya makundi; KP3 ilikuwa na upungufu wa chembe nyekundu dakika ya 60. Upungufu katika makundi mengine haukuwa mkubwa sana. Tofauti katika wastani wa kiwango cha vimeng'enyo aina ya 'ALT' ilikuwa kubwa kati ya makundi. Ndani ya makundi kulikuwa na upungufu ndani ya kundi PP dakika ya 30. Tofauti katika makundi mengine haikuwa kubwa sana. Tofauti ya 'AST' ilikuwa kubwa kati ya makundi, PP ilikuwa na wastani wa chini zaidi wakati KP2 ilikuwa na wastani wa juu zaidi. KP2 ilikuwa pia na upungufu mkubwa baada ya dakika ya 60. Tofauti katika makundi mengine haikuwa kubwa sana. Tofauti ya 'creatinine' na 'BUN' haikuwa kubwa kati ya makundi japo kulikuwa na upungufu mkubwa wa 'creatinine' katika kundi la KP2 kuanzia dakika ya 30 na 60; huku 'BUN' ikiongezeka katika makundi ya PP and KP3 dakika ya 60. Kwa ujumla, ketamine, propofol na mchanganyiko wake katika uwiano wa 1 kwa 1, 1 kwa 2 na 1 kwa 3 vinaweza tumika kwa mbwa mchanganyiko. Japo kwa sababu ya kiwango cha chini cha upumuaji na oksijeni, pamoja na upungufu wa chembe nyekundu za damu kilichoonekana katika makundi yote; ni muhimu kuwafuatilia wanyama kwa umakini pindi wanapokuwa katika usingizi na kuhakikisha njia ya hewa iko wazi. Kwa kuongezea, katika mazingira ya utafiti huu; mchanganyiko wa ketamine na propofol kwa uwiano wa 1:3 ulikuwa na matokeo thabiti na yanayotabirika. Tafiti zingine zenye idadi kubwa zaidi ya wanyama na muda mrefu zaidi wa kuwafuatilia zinaweza ongeza uelewa wa ziada juu ya madhara ya muda mfupi na mrefu wa hizi dawa na mchanganyiko wake zikiingizwa katika mishipa ya damu kwa mbwa mchanganyiko.

Maneno makuu: Mbwa, Ketamine, Propofol, Usingizi

DECLARATION

I **Albert Kiwale Felix**, do hereby declare to the senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

_____ _____

Albert Kiwale Felix Date
(MSc. Student)

The above declaration has been confirmed by;

_____ _____

Dr. Doreen G. Ndossi Date
(Supervisor)

_____ _____

Prof. Donald G. Mpanduji Date
(Supervisor)

LIST OF MANUSCRIPTS

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LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

^{0}C	Degrees Celsius
µ	*mu*
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
ASA	American Society of Anesthesiologists
AST	Aspartate Transaminase
BPM	Beats Per Minute
bpm	Breaths Per Minute
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CNS	Central Nervous System
CRI	Constant Rate Infusion
CVMBS	College of Veterinary Medicine and Biomedical Sciences
EDTA	Ethylene Diamine Tetracetic Acid
et al.,	*“et alia”*
HB	Hemoglobin Concentration
HCT	Hematocrit
IBM	International Business Machines
KK	Ketamine
KP1	Ketamine: Propofol (1:1)
KP2	Ketamine: Propofol (1:2)
KP3	Ketamine: Propofol (1:3)
LTD	Limited
NMDA	N-Methyl D-Aspartate
PP	Propofol
RBC	Red Blood Cells
SPSS	Statistical Product and Service Solutions
SUA	Sokoine University of Agriculture
TCI	Target-Controlled Infusion
TIVA	Total Intravenous Anaesthesia
USA	United States of America
UV	Ultra Violet
WBC	White Blood Cells

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Total Intravenous Anaesthesia (TIVA) is a practice of administering anaesthetic drugs for induction and maintenance strictly through the intravenous route without the need for any inhalation agents (Campbell *et al*., 2001). The technique often involves anaesthetic induction by administering a bolus dose aimed at achieving the required blood concentration of the anaesthetic drug, then followed by maintenance which can be through delivering intermittent boluses, Constant Rate Infusion (CRI) or Target-Controlled Infusion (TCI) (Waelbers *et al*., 2009).

TCI aims at delivering a predicted concentration of the anaesthetic agent in the specified body compartment. It uses a computer-controlled infusion pump which delivers a pre-determined drug concentration based on the patient's weight or age to achieve the required plasma or brain drug concentration. The availability of the equipment for this technique and consequently its application is, however, limited. In intermittent bolus infusion, an estimated amount of drug is administered within a short time. It is characterized by high peaks of plasma concentration and excessive depth of anaesthesia. The approach is commonly used during induction of anaesthesia but may also be applied in maintenance. This is however old technique and has been replaced largely by CRI (Seliskar *et al.,* 2007).

Constant Rate Infusion delivers a constant amount of the anaesthetic drug per unit time. The agents commonly used are short acting drugs which are to be provided over extended periods to cover the amount of time required for the animal to be under general anaesthesia. The common delivery methods are calibrated syringe pumps, but intravenous infusion sets such as buretrols may also be used (Rastabi *et al.,* 2021). CRI has a continuous steady-state concentration of anaesthetic drugs, thus producing a relatively more stable plane of anaesthesia. This

method is more likely to be adopted even in remote veterinary practices since the requirements are readily available. All it necessitates is placement of an intravenous line through which the anaesthetic drugs can be delivered to the patient. The technique has also been reported to result in less cardiopulmonary depression and other negative effects in comparison to intermittent bolus infusion, thus relatively safer for the animal (Njoku, 2015).

The main advantage of TIVA when compared to other anaesthesia modalities such as inhalational anaesthesia is that it requires relatively less expensive and sophisticated equipment for induction and maintenance (Saika *et al.,* 2022), hence can easily be applied even in field settings (Bennet, 2006). Studies have also reported better hemodynamic stability, improved quality of anaesthetic recovery (Bustamante *et al.,* 2018) and the reduction in the overall risk of exposure to volatile anaesthetic agents to practitioners (Hasei *et al*., 2003) when TIVA is used. The technique can also be used for maintenance of anaesthesia in patients that may be sensitive and react adversely to inhalational anaesthetic agents; and those that are in no position to use inhalation anaesthetic equipment such as patients undergoing diagnosis or surgeries involving the upper respiratory tract. There are also raising concerns over the negative impacts of volatile anaesthetic agents on climate change, hence TIVA offers a relatively safer option for both the practitioners and environment. For these reasons, the technique may serve as a potential alternative to inhalational anaesthesia in small animal veterinary practice; especially in short, minimally invasive surgeries or diagnostic procedures (Kennedy & Smith, 2015).

One of the commonly used drugs in TIVA is propofol (Ambros *et al*., 2008). Propofol is an alkyl phenol compound with rapid and smooth induction, short duration of action, non-cumulative properties, good muscle relaxation as well as quick, smooth and excitement-free recovery of animals (Hall *et al.,* 2001). Its mechanism of action is not fully

understood, but has been associated with positive modulation of the inhibitory function of the neurotransmitter g-aminobutyric acid (GABA) through its receptors (Ramsey, 2008). It has been the drug of choice in short procedures in animals, either used alone or in combination with other drugs (Lerche *et al.,* 2000). Propofol however has been associated with dose-dependent respiratory depression (Aguiar *et al*., 2001), apnea, little to no analgesia, decreased cardiac output and reduced arterial blood pressure (Smith *et al.,* 1993), but the effects are well tolerated by healthy dogs and cats (Berry, 2015). It is also not the preferred drug of choice for procedures necessitating prolonged anaesthesia. Propofol has shown compatibility with drugs such as xylazine and ketamine; hence the drugs may be used to potentiate its action and counterbalance its side effects (Hall *et al.,* 2001; Celestine *et al.,* 2014).

Xylazine is an alpha-2-adrenergic receptor agonist which has good analgesic properties thus may be used during premedication (Hall *et al*., 2001). The drug is thought to produce its effect by stimulating the alpha adrenoceptors in the spinal cord and brain; thus inhibiting the release of the neurotransmitters norepinephrine and substance P (Kolahian, 2014; Saha *et al*., 2005). Ketamine is a dissociative anaesthetic agent that stimulates the sympathetic nervous system, and assists on counterbalancing the depressant effects of drugs such as xylazine or propofol when used simultaneously to produce stable hemodynamics during anaesthesia (Lerche *et al*., 2000; Waelbers *et al.,* 2009). It also assists in producing analgesia through the inhibition of the N-Methyl, D-Aspartate (NMDA) receptors responsible for hyperexcitability in the thalamic and limbic systems (Hall *et al.,* 2001), and through its effect on opioid, mainly μ receptors (Lamont & Mathews, 2007). Owing to this property, ketamine administered at low doses has been used to produce analgesia in anaesthetized dogs (Slingsby & Waterman-Pearson, 2000). On the other hand, ketamine is a poor muscle relaxant. At anesthetic dosages, it has been associated with muscle stiffness, convulsions, high incidences of agitation and violent recoveries (Kennedy & Smith, 2015;

Lin *et al*., 2015); thus the use of such drugs as xylazine during premedication or propofol during induction and maintenance improves its overall effect (Hall *et al.,* 2001).

Ketamine mixed with propofol at a 1:1 ratio has been suggested for induction and maintenance of anaesthesia aiming at achieving more cardiovascular stability and less undesirable effects in comparison to the use of either drugs alone (Rastabi *et al*., 2021). When ketamine is combined with propofol, the combination assists in decreasing the dosage and cardiovascular depression caused by propofol. In the mixture, the hypnosis and cardiovascular depression produced by propofol counterbalances the psychomimetic and cardiostimulatory effects of ketamine (Intelisano *et al*., 2008). However, the 1:1 combination ratio has been associated with aggravated respiratory depression in dogs (Rastabi *et al.,* 2021). Mair *et al.* (2009) also concluded that ketamine may not be a suitable co-induction agent of anaesthesia with propofol in dogs after reporting significantly higher incidences of post-induction apnea in the group of dogs that received a relatively higher dose of ketamine for co-induction with target-controlled infusion of propofol.

Mixed-breed dogs are generally domestic dogs that do not belong to any officially recognized breed. They are estimated to be over 150 million worldwide (Morris, 2008) and appear to be the most common breed category of domestic dogs in Tanzania. The Africanis landrace is one of the most popular mixed breed type in the country. It is lightly built, with a long slender muzzle and short coat, whose colour is often fawn but may vary from white, brown, brindle to black (Maggs & Sealy, 2008). These dogs are relatively more popular due to their higher resilience and lower susceptibility to disease. They are mainly kept for security and occasionally hunting purposes.

1.2 Problem Statement

There is limited information on the physiological, hematological and biochemical effects of ketamine and propofol in the mixed-breed dogs

administered through Total Intravenous Anaesthesia. Studies on a number of officially recognized breeds have revealed breed specific metabolic and physiological peculiarities that could affect the pharmacokinetics and pharmacodynamics of drugs in different dog breeds (Fleischer *et al*., 2008). There is also inadequate information on the overall effects of their combination (ketofol) at different combination ratios in dogs, since most studies have combined the two drugs at a 1:1 ratio; which has also been reported to worsen respiratory depression in some animals (Rastabi *et al.,* 2021).

1.3 Justification

This study determined some of the physiological and hemato-biochemical effects of ketamine and propofol in mixed-breed dogs administered through TIVA. It also assessed the effect of different ratios of the ketamine-propofol combination “ketofol” on some physiological, hematological and biochemical indices in the dogs. The information may be used by small animal practitioners when planning on using the drugs individually or in combination, to ensure stability of patients under anaesthesia but also guarantee smooth and uneventful induction, maintenance and recovery.

1.4 Study Objectives

1.4.1 General objective

Assessment of the physiological, hematological and biochemical effects of ketamine and propofol individually; and their combinations administered through TIVA in Africanis landrace mixed-breed dogs.

1.4.2 Specific objectives

i. To determine the physiological effects of ketamine, propofol and different ratios of the Ketamine-Propofol combination in Africanis landrace mixed breed dogs.
ii. To determine the hematological and biochemical effects of ketamine, propofol and different ratios of the Ketamine-Propofol combination in Africanis landrace mixed breed dogs.

1.5 Research Hypothesis

1.5.1 Null hypothesis

There is no effect on the physiological, hematological and biochemical parameters in mixed-breed dogs of the Africanis landrace caused by the anaesthetic agents individually or as a combination.

1.5.2 Alternative hypothesis

There is an alteration of the physiological, hematological and biochemical indices in the dogs after administration of the anaesthetic agents; and the parameters will be relatively more stable when the drugs are used in combination than when used individually.

1.6 Literature Review

The development of intravenous anaesthetic agents with rapid induction, distribution, and clearance such as propofol has contributed to increased interest towards TIVA among veterinary practitioners. These drugs have made it possible to induce and maintain anaesthesia through intravenous agents only (Waelbers *et al*., 2009). Propofol has often been used in TIVA, and studies have reported a volume of distribution of 4,863 ± 800 ml/kg and elimination half-life of 100 minutes after a single bolus. The elimination half-life however increases to 322 ± 27 minutes after 60 minutes of infusion (Joubert, 2014). The extension of half-life has been attributed to the slow return of propofol from poorly perfused body compartments.

Propofol however has little to no analgesic properties and significant cardiopulmonary depression. A decrease in arterial blood pressure following propofol use has been observed and is believed to be due to inhibition of the myocardial contractility and a decrease in systemic vascular resistance (Pagel & Warltier, 1993; Wouters *et al.*, 1995). Hypoventilation has also been reported when propofol was used and has been associated with depression of the central inspiratory drive and the animal's response to the partial pressure of carbon dioxide (Jonsson *et al*., 2005).

Patle *et al*. (2021) assessed the clinical efficacy of propofol and reported relatively longer periods for induction and shorter duration of anaesthesia when propofol was used alone, than when it was combined with fentanyl. In the combination, the induction was also smooth and there were less adverse cardiopulmonary effects. Other available drugs that could be used together with propofol include alpha -2- receptor agonists such as xylazine during premedication to counteract/counterbalance its side effects/deficiencies. Data on the effects of simultaneous use of the two drugs is however insufficient.

Studies have also assessed the effects of combining other anaesthetic agents like ketamine with propofol in a 1:1 ratio to form ‘ketofol’. Rastabi *et al.* (2021) evaluated the effect of ‘ketofol’ in mixed-breed male dogs and concluded that the induction and recovery scores were of higher quality in dogs when ‘ketofol’ was used for induction and maintenance. When comparing the cardiopulmonary effects of ‘ketofol’ in female Beagles, Kennedy and Smith (2015) reported that the combination resulted to higher heart rate and improved noninvasive mean blood pressure. However, there was a more significant respiratory depression than when propofol was used alone. In another study in mixed-breed dogs, Mannarino *et al.* (2012) concluded that there weren’t any significant hemodynamic effects of either propofol alone, or in combination with ketamine, but the heart rate and mean arterial blood pressure decreased significantly in both groups. Saikia *et al.* (2022) also studied dogs presented for elective ovariohysterectomy or castration, assessing the effect of ketamine and propofol individually and as a combination. They reported a significant decrease in hemoglobin concentration, packed cell volume and total erythrocyte count, with an increase in the blood glucose level. However, blood urea nitrogen and creatinine values increased more in the in the ketamine and propofol groups than in the ‘ketofol’ group.

All these studies highlight the need to carry out breed specific studies on the effects of anaesthetic agents, since it has been shown that different breeds respond differently to anaesthetic agents. There is also a necessity to assess various ratios of combination in the Ketamine-Propofol mixture, since most studies have combined the drugs at a 1:1 ratio; a combination which has been associated with exacerbated respiratory depression. The depression has partly been associated with a relatively high concentration of ketamine in the mixture, hence the need to adjust the ketamine ratio to determine if there could be an alternative optimum ketamine-propofol dose ratio that may perhaps be relatively safer for the animal.

1.7 Methodology

1.7.1 Ethical approval

The study was approved by the Research Ethical Committee of Sokoine University of Agriculture at the Directorate of Postgraduate Studies, Research, Technology Transfer and Consultancy.

1.7.2 Study area

The study was conducted at the College of Veterinary Medicine and Biomedical Sciences (CVMBS), Sokoine University of Agriculture in Morogoro Tanzania. The data collection, processing and analysis was performed at CVMBS, SUA.

1.7.3 Study design

The study was a repeated crossover experiment. Ten clinically healthy dogs were divided into groups to cover five treatments i.e. Ketamine (KK) and Propofol (PP) individually; and Ketofol at 1:1 (KP1), 1:2 (KP2) and 1:3 (KP3). Each treatment was administered twice with a one-week interval between treatments of the same protocol, and a two-week washout period when the animals were to receive a different treatment. The study was undertaken in two main phases, where the first phase used nine animals to assess three different ‘ketofol’ combinations; and the second phase used ten animals to compare effects of ketamine and propofol individually, together with the three ketofol combinations.

1.7.4 Study population

Ten clinically healthy mixed breed dogs of the Africanis landrace type with an average weight of 13 ± 2.7 kg were used. All the dogs included in the study were obtained from Morogoro municipality. The health status of the animals was ascertained through physical examination and assessment of the blood profile i.e. complete blood count (CBC) and serum biochemistry. The inclusion criteria were healthy male (n=5) and female (n=5) dogs in American Society of Anesthesiologists (ASA) category I or II, aged between 2-4 years. The exclusion criteria were very young or old age, pregnancy/lactation, aggressiveness, animals undergoing any form of therapy and those with signs/symptoms of disease. The dogs were kept for at least two weeks at the CVMBS kennels for acclimatization, health checkup, deworming, and vaccination prior to commencement of the study. They were fed on the same diet throughout the study period.

1.7.5 Data collection

The animals were fasted for 12 hours but provided with water ad libitum until 3 hours before anesthetic induction.

1.7.5.1 Premedication

Premedication comprising 0.04mg/kg atropine (Swiss Parenterals LTD®, India) and 2mg/kg Xylazine (Interchemie®, Netherlands) was administered via the intramuscular route on the semitendinosus muscles. Once fully sedated, the animals were moved to a surgical table and placed on sternal recumbency for catheterization and induction, then later maintained on left lateral recumbency. An 18 gauge cannula was inserted into the cephalic vein of one limb for administration of the anaesthetic agents.

The animals received five different treatments to cover Ketamine (KK) and Propofol (PP) individually; and Ketofol at 1:1 (KP1), 1:2 (KP2) and 1:3 (KP3). Each treatment was repeated twice, with a one-week period between treatments using the same protocol, and two-week interval when the animals were to receive a different drug or drug combination.

1.7.5.2 Induction and maintenance

Anaesthetic induction was through a single bolus slow intravenous administration; and maintenance through constant rate infusion using a burette infusion set (Neomedic®, United Kingdom) with a drop factor of 60 (60 drops per ml). In the KK group, induction was achieved by 5 mg/kg ketamine and maintenance was through 0.3 mg/kg/min for one hour. In the PP group, induction was achieved by 5 mg/kg propofol and maintenance was through 0.3 mg/kg/min for one hour. In KP1 group, induction was achieved by 4 mg/kg ketofol (i.e. 2 mg of ketamine + 2 mg of propofol per kg body weight) and maintenance was through 0.3 mg/kg/min for one hour (i.e. 0.15 mg/kg/min propofol and 0.15 mg/kg/min ketamine). In KP2, induction was achieved by 4 mg/kg ketofol (i.e. 1.3 mg of ketamine + 2.6 mg of propofol per kg body weight) and maintenance was through 0.3 mg/kg/min for an hour (0.1 mg/kg/min ketamine and 0.2 mg/kg/min propofol). In KP3, induction was achieved by 4 mg/kg ketofol (i.e. 1 mg of ketamine + 3 mg of propofol per kg body weight) and maintenance was through 0.3 mg/kg/min for an hour (i.e. 0.075 mg/kg/min ketamine and 0.225 mg/kg/min propofol). The ketofol mixture was reconstituted to make a total volume of 60 mls that was delivered for 60 minutes. The mixture was not used beyond one hour.

1.7.5.3 Monitoring

The parameters monitored were rectal temperature (degree Celsius, ^{0}C), respiration rate (breaths per minute, bpm), pulse rate (beats per minute, BPM) and oxygen saturation (percent, %). Temperature was measured using a digital thermometer whose probe was placed per rectum along the rectal mucosa. The respiration rate was obtained via auscultation using a stethoscope and observation of chest movements. The pulse rate and oxygen saturation were obtained from the pulse oximeter (Choice MMed®, China) attached to the animal's tongue. Rectal temperature and respiration rates were initially recorded before induction (0 min) to obtain baseline values. Once the animals were fully induced and had attained surgical anaesthesia, a pulse oximeter was placed on the tongue to record the pulse rate and oxygen saturation.

Anaesthetic maintenance and monitoring were also initiated, and parameters were recorded at 5, 10, 15, 20, 30, 40, 50 and 60 minutes while the animal was under maintenance. The infusion was discontinued at the 60th minute of maintenance, and the animal was allowed to recover from anaesthesia.

Other physiological factors assessed included the quality of induction and recovery, anaesthetic depth and side effects. Induction, surgical anaesthesia and recovery times were also determined. The quality of induction was scored on a 3-point scale, adopted from Mair *et al*. (2009) with minor alterations. Induction was ‘good’ if the animal was not excited and there were no signs of muscle twitching or paddling; ‘Fair’ if the animal showed mild signs of excitement and ‘poor’ if the animal appeared restless and spasmic.

The depth of anaesthesia was mainly determined by assessing pain perception through observing the animal’s response to the pedal reflex test. This was performed by fully clamping the toe web with a pair of hemostatic forceps for a minimum of 10 seconds while observing the animal’s reaction to the test, such as its ability to voluntarily withdraw the limb and any changes in the pulse rate. A negative test was an indication of loss of pain sensation. Other reflexes assessed were the anal and blink reflex. The degree of muscle relaxation was also observed.

Assessment of the quality of recovery was adopted from Kennedy and Smith (2015) and Rastabi *et al*. (2021) with minor changes. The quality was considered ‘good’ if there was no vocalization, paddling and the animal stood and walked without difficulty. A ‘fair’ recovery was characterized by mild vocalization, paddling and/or long lasting sternal recumbency; while a ‘poor’ recovery was assigned to animals who were unable to be in sternal recumbency, vocalized excessively and showed signs of delirium. A standing animal that responded well to auditory, visual and tactile stimuli was considered fully recovered.

Induction time was the time between administration of the anaesthetic agent to the time when the animal was considered to be fully under surgical anaesthesia. Time of anaesthesia was the time throughout which the animal was under surgical anaesthesia and with a negative pedal reflex. Recovery time was the time between discontinuation of the infusion of anaesthetic agent(s) and when the animal was able to support its own weight and walk freely.

Four milliliters of blood for biochemical and 1 ml for hematological assays were obtained from the cephalic vein in plain and Ethylene Diamine Tetracetic Acid (EDTA) tubes respectively. Blood was drawn at time 0 (before induction), 30 minutes and 60 minutes after maintenance was started i.e. while the animal was under constant rate infusion. Hematology was performed by an automatic hematology analyzer (Maccura F810®, China) while biochemistry by a UV spectrophotometer (Biochrom®, United Kingdom). Hematological assessment included absolute White Blood Cell count (WBC); relative Lymphocyte, Monocyte and Neutrophil count; together with the total Red Blood Cell (RBC), Hematocrit (HCT) and Hemoglobin concentration (HB). Biochemical parameters studied were Alanine aminotransferase (ALT), Aspartate transaminase (AST), Blood Urea Nitrogen (BUN) and Creatinine.

1.8 Data Analysis

Data analysis was done using Microsoft excel 15 (Microsoft corporation, USA) and the Statistical Product and Service Solutions (SPSS) version 20 (IBM Corporation, USA) for descriptive statistics and comparison of means. Linear plots were used to express the changes in parameters throughout the maintenance period. Means and standard deviations of numerical values were determined and analysis of variance (ANOVA) together with a Post Hoc Tukey's test and were used to compare variations between protocols. A dependent student's t-test was used to compare variations within each treatment protocol from the established baseline (pre-induction) values. Data was expressed in terms of means ± standard deviation, and the statistical level of significance was set at $p \leq 0.05$.

1.9 Study Limitations

Some of the limitations of this study include the fact that the maintenance period only lasted for 60 minutes. Short anesthetic periods were decided upon mainly for ethical reasons. Furthermore, mean arterial blood pressure and carbon dioxide concentration were not determined due to unavailability of the required equipment during the study. This would have provided a better understanding of the level of cardiopulmonary function of animals under anaesthesia. The study was also performed in healthy dogs, hence results obtained may vary when diseased or immune compromised animals are anaesthetized. This was done purposefully to form a uniform population.

1.10 Organization of the Dissertation

This dissertation has been prepared in accordance to SUA guidelines and layout of dissertation developed in a publishable manuscripts format. It has been divided into five main chapters. Chapter one comprises of a general introduction to the concept of TIVA, its main forms as well as the relative advantages of the technique in comparison to other anaesthetic modalities currently available. It also discusses some of the commonly used drugs and protocols with regards to their indications, mechanisms of actions and side effects. The chapter also states the main problem addressed in this study, its justification as well as the general and specific objectives. In addition, it has a brief review of literature to establish work that has already been done on the subject, and the knowledge gap that this research is expected to address. The chapter concludes with the methodology that was used to obtain the data together with the analytical tools used to describe the data and draw conclusions.

Chapter two is a paper-based chapter that comprises of a manuscript prepared following the format and guidelines of, and submitted to, the Applied Veterinary Research Journal (AVR); it mainly focuses on the physiological effects of different ketamine-propofol combination ratios in Africanis landrace mixed-breed dogs. It presents results of only three

treatment groups representing the three ‘ketofol’ combinations. The results of the remaining groups have been included in the appendix.

Chapter three is also a paper-based chapter containing a manuscript to be submitted to the Tanzania Veterinary Journal (TVJ). The manuscript presents the hematological and biochemical effects of ketamine, propofol and their combination ‘ketofol’ in Africanis landrace mixed breed dogs. It includes results of all the five treatment groups. Chapter four discusses the overall findings of this study. It compares the results obtained with studies that have already been conducted in mixed breed dogs or those belonging to officially recognized breeds. It also makes a comparison with studies performed in other species, using almost similar anaesthetic protocols, or assessing similar parameters.

Finally, chapter five draws general conclusions and recommendations from the findings of the study. It also states some of the limitations of the current study and suggestions for further research on this subject.

CHAPTER TWO

MANUSCRIPT I

2.0 Physiological effects of total intravenous anaesthesia (TIVA) using ketamine and propofol combinations in mixed-breed dogs

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The material contained in this chapter has been submitted to Applied Veterinary Research Journal (AVR).

Abstract

This study assessed the physiological effects of total intravenous anaesthesia (TIVA) using three different combination ratios of ketamine and propofol ("ketofol"). Nine adult mixed-breed dogs weighing 13.6 ± 2.7 kg received three treatments in a repeated crossover experiment. The treatments were Ketamine + Propofol in 1:1 (KP1), 1:2 (KP2) and 1:3 (KP3) ratios. A single bolus injection was used for induction while constant rate infusion (CRI) was utilized for maintenance of anaesthesia. All combinations lowered the respiration rates with no variations among the protocols (p=0.197). Within protocols, the rate was significantly low in comparison to pre induction values at the 30^{th} (p=0.019) and 40^{th} (p=0.041) in KP1, and 30^{th} minute (p=0.038) in KP2. The pulse rate was

within normal physiological range having no differences between protocols ($p=0.062$), however within protocols, the rate was significantly lower after the 30^{th} minute in KP1 and 15^{th} minute in KP2. The temperature was within the normal physiological range but relatively higher in KP1 ($p<0.001$). No differences were noted within protocols during maintenance ($p=0.925$). Oxygen saturation was generally low and did not differ significantly among the groups ($p=0.542$). The induction and recovery quality were good in all treatments. Surgical anaesthesia lasted for approximately 60 minutes under which the animals were maintained. Apneustic breathing was the most common side effect in all treatments, and recovery was relatively quicker in KP3. In conclusion, all ketofol ratios used were safe, however the 1:3 (KP3) combination had relatively more stable physiological parameters, less side effects and quicker recovery.

Keywords: ketofol, CRI, anaesthesia, dogs

2.1 Introduction

Total Intravenous Anaesthesia (TIVA) is a practice of administering anaesthetic drugs for induction and maintenance strictly through the intravenous route without the need for any inhalation agents (Campbell et al 2001). The development of intravenous anaesthetic agents with rapid induction, distribution, and clearance such as propofol has contributed to the increased interest towards TIVA among veterinary practitioners. This has also been further influenced by the high cost and unavailability of sophisticated anaesthetic equipment, especially in the field.

TIVA often involves anaesthetic induction by administering a bolus dose aimed at achieving the required blood concentration of the anaesthetic drug, then followed by maintenance which can be through Target-Controlled Infusion (TCI), intermittent bolus infusion or Constant Rate Infusion (CRI) (Waelbers et al 2009). TCI aims at delivering a predicted concentration of the anaesthetic agent in the specified body compartment. It uses a computer-controlled infusion pump which delivers a pre-determined drug concentration based on the patient's weight or age so as to achieve the required plasma or brain drug concentration. The availability of the equipment for this technique and consequently its application is, however, limited. In intermittent bolus infusion, an estimated amount of drug is administered within a short time. It is characterized by high peaks of plasma concentration and excessive depth

of anaesthesia. The approach is commonly used during induction of anaesthesia but may also be applied in maintenance. Recently, this technique has been replaced to a great extent by CRI (Seliskar et al 2007).

Constant Rate Infusion delivers a constant amount of the anaesthetic drug per unit time thus producing a more stable plane of anaesthesia. The agents commonly used are short acting drugs which are to be provided over extended periods to cover the amount of time required for the animal to be under general anaesthesia. The common delivery methods for CRI are calibrated syringe pumps, but intravenous infusion sets such as buretrols may also be used (Rastabi et al 2021). In addition to the relatively steadier plane of anaesthesia, CRI is also more likely to be adopted even in remote veterinary practices since the requirements are readily available. All it necessitates is placement of an intravenous line through which the anaesthetic drugs can be delivered to the patient. The technique has also been reported to result in less cardiopulmonary depression and other negative effects in comparison to intermittent bolus infusion, thus relatively safer for the animal (Njoku 2015).

One of the commonly used drugs in TIVA is propofol (Ambros et al 2008). Propofol is an alkyl phenol compound with rapid and smooth induction, short duration of action, non-cumulative properties, good muscle relaxation as well as quick, smooth, and excitement-free recovery

of animals (Hall et al 2001). It has been the drug of choice in short procedures in animals, either used alone or in combination with other drugs (Lerche et al 2000). Propofol has however been associated with dose-dependent respiratory depression (Aguiar et al 2001), apnea, little to no analgesia, decreased cardiac output and reduced arterial blood pressure (Smith et al 1993), but the effects are well tolerated by healthy dogs and cats (Berry 2015). It has also shown compatibility with drugs such as xylazine and ketamine; hence the drugs may be used to potentiate its action and counterbalance its side effects (Hall et al 2001; Celestine et al 2014).

Xylazine is an alpha-2-adrenergic receptor agonist which has good analgesic properties thus may be used during premedication (Hall et al 2001). The drug is thought to produce its effect by stimulating the alpha adrenoceptors in the spinal cord and brain; thus inhibiting the release of the neurotransmitters norepinephrine and substance P (Saha et al 2005; Kolahian 2014).

Ketamine is a dissociative anaesthetic agent that stimulates the sympathetic nervous system. It assists in counterbalancing the depressant effects of drugs such as xylazine or propofol when used simultaneously, thus producing stable hemodynamics during anaesthesia (Lerche et al 2000; Waelbers et al 2009). It also assists in producing analgesia through the inhibition of the N-methyl, D-aspartate (NMDA) receptors in the

thalamic and limbic systems (Hall et al 2001) and through its effect on opioid, mainly mu (μ) receptors. Owing to this property, ketamine administered at low doses has been used to produce analgesia in anaesthetized dogs (Slingsby and Waterman-Pearson 2000). On the other hand, ketamine is a poor muscle relaxant. At anesthetic dosages, it has been associated with muscle stiffness, convulsions, high incidences of agitation and violent recoveries (Kennedy and Smith 2015; Lin et al 2015); thus the use of such drugs as xylazine during premedication or propofol during induction and maintenance may improve its effect (Hall et al 2001).

Ketamine mixed with propofol at a 1:1 weight ratio has been suggested for induction and maintenance of anesthesia aimed at achieving more cardiovascular stability and less undesirable effects in comparison to the use of either drug alone (Rastabi et al 2021). When ketamine is combined with propofol, the combination assists in decreasing the dosage and cardiovascular depression caused by propofol. In the mixture, the hypnosis and cardiovascular depression produced by propofol counterbalances the psychomimetic and cardiostimulatory effects of ketamine (Intelisano et al 2008). However, the 1:1 combination ratio has been associated with aggravated respiratory depression in dogs (Mair et al 2009). Another study by Rastabi et al (2021) also concluded that ketamine may not be a suitable co-induction agent of anaesthesia with propofol in dogs after reporting significantly higher incidences of post-induction

apnea in the group of dogs that received a relatively higher dose of ketamine for co-induction with target-controlled infusion of propofol.

Mixed-breed dogs are generally domestic dogs that do not belong to any officially recognized breed. They are estimated to be over 150 million worldwide (Morris 2008), and appear to be most common breed category of domestic dogs in Tanzania. The Africanis landrace is one of the most popular ecotypes in the country. It is lightly built, with a long slender muzzle and short coat, whose colour is often fawn but may vary from white, brown, brindle to black (Maggs and Sealy 2008). They are relatively more popular due to their higher resilience and lower susceptibilities to disease. The dogs are mainly kept for security and occasionally hunting purposes.

There is limited information on the general effects of combining ketamine and propofol to form “ketofol” at different combination ratios in dogs, since most studies have combined the two drugs at a 1:1 weight ratio; which has also been reported to worsen respiratory depression in some animals (Rastabi et al 2021; Kennedy and Smith 2015). Few studies have also been undertaken in mixed breed dogs to determine their physiological responses to the anaesthetic agents. Studies on a number of officially recognized breeds have revealed breed specific metabolic and physiological peculiarities that could affect the pharmacokinetics and pharmacodynamics of drugs in different dog breeds (Fleischer et al 2008).

This study was conducted to determine some of the effects of different ratios of the ketamine-propofol combination on physiological parameters in mixed breed dogs. The information may be used by small animal practitioners when planning on using the drugs in combination, to ensure stability of patients under anaesthesia but also guarantee smooth and uneventful induction, maintenance, and recovery.

2.2 Materials and Methods

The study was approved by the Research Ethical Committee of Sokoine University of Agriculture at the Directorate of Postgraduate Studies, Research, Technology Transfer and Consultancy. Nine clinically healthy mixed breed dogs with an average weight of 13.6 ± 2.7 kg were used. The dogs were randomly purchased from wards within Morogoro region, Tanzania. The health status of the animals was determined through physical examination and assessment of the blood profile i.e complete blood count (CBC) and serum biochemistry. The inclusion criteria were healthy male and female dogs in American Society of Anesthesiologists (ASA) category I or II, aged between 2-4 years. The exclusion criteria were very young or old age, pregnancy/lactation, aggressiveness, animals undergoing any form of therapy and those with signs/symptoms of disease. The dogs were kept for at least two weeks in separate cages for acclimatization, health checkup, deworming and vaccination prior to commencement of the study. They were fed on the same diet throughout the study period. The animals were fasted for 12 hours but provided with water ad libitum until 3 hours before anesthetic induction.

Premedication comprising 0.04mg/kg atropine (Swiss Parenterals LTD®, India) and 2mg/kg Xylazine (Interchemie®, Netherlands) was administered via the intramuscular route on the semitendinosus muscles. Once fully sedated, the animals were moved to a surgical table and placed on sternal recumbency for catheterization and induction, then later maintained on left lateral recumbency. An 18 gauge cannula was inserted into the cephalic vein for administration of the anaesthetic agents.

All the animals received three different treatments to cover ketofol at 1:1 (KP1), 1:2 (KP2) and 1:3 (KP3). Each treatment was repeated twice, with a one-week period between treatments using the same protocol, and a two-week wash out period when the animals are to receive a different protocol. Anaesthetic induction was through a single bolus slow intravenous administration; and maintenance through constant rate infusion for one hour using a buretrol set (Neomedic®, United Kingdom) with a drop factor of 60. In the KP1 group, induction was achieved by 4 mg/kg ketofol (i.e. 2mg of ketamine + 2 mg of propofol per kg body weight) and maintenance was through 0.3mg/kg/min (0.15mg/kg/min propofol and 0.15mg/kg/min ketamine). In KP2, induction was achieved by 4 mg/kg ketofol (1.3mg of ketamine + 2.6 mg of propofol per kg body weight) and maintenance was through 0.3mg/kg/min (0.1mg/kg/min ketamine and 0.2mg/kg/min propofol). In KP3, induction was achieved by 4 mg/kg ketofol (1mg of ketamine + 3 mg of propofol per kg body weight) and maintenance was through 0.3mg/kg/min (0.075mg/kg/min

ketamine and 0.225mg/kg/min propofol). The ketofol mixture was reconstituted to make a total volume of 60 mls and the mixture was not used beyond one hour.

The parameters recorded were rectal temperature, respiration rate, pulse rate and oxygen saturation. Rectal temperature was measured using a digital thermometer whose probe was placed per rectum along the rectal mucosa. The respiration rate was obtained via auscultation using a stethoscope and observation of chest movements. The pulse rate and oxygen saturation were obtained from the pulse oximeter (Choice MMed®, China) attached to the animal's tongue throughout the procedure.

Parameters were initially recorded before induction (0 min) to obtain baseline values. Once the dogs were fully induced and have attained surgical anaesthesia, anaesthetic maintenance and monitoring was initiated. Physiological parameters were recorded at 5, 10, 15, 20, 30, 40, 50 and 60 minutes while the animals were under maintenance. The infusion was discontinued at the 60^{th} minute post induction, and the animal allowed to recover from anaesthesia. Other physiological factors assessed included the quality of induction and recovery, anaesthetic depth and side effects. Induction, surgical anaesthesia and recovery times were also determined.

The quality of induction was scored on a 3-point scale, adopted from Mair et al (2009) with minor changes. Induction was ‘good’ if the animal was not excited and there weren’t any signs of muscle twitching or paddling; ‘fair’ if the animal showed mild signs of excitement and ‘poor’ if the animal appeared restless and spasmic. Surgical anaesthesia was determined by assessing pain perception through observing the animal’s response to the pedal reflex. This was performed by fully clamping the toe web with a pair of hemostatic forceps for a minimum of 10 seconds while observing the animal’s reaction to the test, mainly the ability to voluntarily withdraw the limb and the change in pulse rate. A negative test was an indication of loss of pain sensation. The anal and blink reflex reflexes were also assessed and level of muscle relaxation observed.

Assessment of the quality of recovery was adopted from Kennedy and Smith (2015) and Rastabi et al (2021) with minor changes. The quality was considered ‘good’ if there was no vocalization, paddling and the animal stood and walked without difficulty. A ‘fair’ recovery was characterized by mild vocalization, paddling and/or long lasting sternal recumbency; while a ‘poor’ recovery was assigned to animals who were unable to be in sternal recumbency, vocalized excessively and showed signs of delirium. A standing animal that responded well to auditory, visual, and tactile stimuli i.e. able to walk on its own without hitting obstacles and respond to commands, was considered fully recovered.

Induction time was the time between administration of the anaesthetic agent to the time when the animal was considered to be fully under surgical anaesthesia. Time of surgical anaesthesia was the time throughout which the animal was under anaesthetic maintenance and with a negative pedal reflex. Recovery time was the time between discontinuation of the infusion of anaesthetic agents and when the animal was able to support its own weight and walk freely.

Data analysis was done using Microsoft excel 15 (Microsoft corporation, USA) and the Statistical Product and Service Solutions (SPSS) version 20 (IBM Corporation, USA) for descriptive statistics and comparison of means. Linear plots were used to express the changes in parameters throughout the maintenance period. Means and standard deviations of numerical values were determined and analysis of variance (ANOVA) together with a Post Hoc Tukey's test and were used to compare variations between protocols. A dependent student's t-test was used to compare variations within each treatment protocol from the established baseline (pre-induction) values. Data was expressed in terms of means ± standard deviation and the statistical level of significance was set at $p<0.05$.

2.3 Results

The overall quality of induction and recovery was good in all the three groups and the induction time was approximately 20 seconds in all

groups. The average recovery time was 50 ± 26, 55 ± 38 and 40 ± 29 minutes in the KP1, KP2 and KP3 groups respectively; with non-significantly quicker recoveries observed in KP3. Some of the side effects observed in different dogs in KP1 were apneustic breathing, characterized by lack of spontaneous breathing for 10-15 seconds; urination, tachypnea, tachycardia and vomiting. KP2 had tachypnea and irregular breathing in 1 dog and apneustic breathing in 3 dogs. In the KP3, there was urination in three dogs during recovery and apneustic breathing in one dog (Table 1). There were generally low respiration rates in all the protocols (Figure 1). The differences among the protocols were not significant (p=0.197). KP3 however had a relatively more stable rate (10 ± 1.1 cycles/minute) in comparison to KP1 ((10 ± 3.6 cycles/minute) and KP2 (10 ± 1.6 cycles/minute). Comparisons within protocols revealed significant reductions in the respiration rates at the 30^{th} (p=0.019) and 40^{th} (p=0.041) minutes in KP1; but also the 30^{th} (p=0.038) minute in KP2. No statistically significant differences were observed in KP3 (Figure 1). Comparison of the pulse rates among the protocols (Figure 2) did not show any significant differences (p=0.062). However, the pulse rate in KP3 (104 ± 5.3 beats/minute) appeared relatively more stable when compared with KP1 (122 ± 15.2 beats/minute) and KP2 (111 ± 27.9 beats/minute). Within protocols, statistically significant reductions were noted at the 30^{th}, 40^{th} and 50^{th} minutes in KP1 as well as 15^{th}, 20^{th}, 30^{th}, 40^{th} and 50^{th} minutes in KP2. No statistically significant differences were observed in KP3 (Figure 2).

The temperature remained within the normal physiological range in all the 3 groups (Figure 3) but there were significant differences in the temperatures among the treatment protocols ($p<0.001$). Higher and relatively more stable mean temperatures were observed in the KP1 (39.1 ± 0.05 ^{0}C) when compared with KP2 (38.8 ± 0.16 ^{0}C) and KP3 (38.7 ± 0.08 ^{0}C). There were no significant differences within protocols during maintenance ($p=0.925$) (Figure 3). The oxygen saturation in all groups remained relatively low in comparison to standard physiological values (Figure 4), and there weren't any significant differences among the groups ($p=0.542$). However, KP3 had slightly higher and more stable values (89 ± 2.2 %) in comparison to KP1 (86.1 ± 6.1 %) and KP2 (87 ± 3.2 %). Within group comparisons did not reveal any statistically significant differences (Figure 4).

2.4 Discussion

2.4.1 Anaesthetic drug considerations, induction time, duration of anaesthesia and side effects

The ketofol induction dose of 4mg/kg was selected following previous studies by Kennedy and Smith (2015) and Rastabi et al (2021). The maintenance dose also followed results from Kennedy and Smith (2015). Generally, combining ketamine and propofol reduced the dose required for induction and maintenance of propofol. A similar observation was made by Kennedy and Smith (2015) in female Beagles and Mannarino et al (2012) in mixed breed dogs. Further reductions in anaesthetic

requirements can also be achieved using premedication, due to the anaesthetic sparing effects of some premedication drugs such as alpha-2-adrenergic agonists (Dewangan et al 2010; Kinjavdekar et al 2010). In this study, xylazine was used as a premedication agent. The induction quality and time observed in this study coincides with other reports when propofol was used, where the induction time has ranged from 20-30 seconds, with a smooth transition to unconsciousness (Berry 2015). Slightly longer recovery periods have been observed when ketamine was used and may be a result of its slow clearance from body compartments due to redistribution.

Studies have reported post induction apnea (absence of spontaneous respiration for 15-30 seconds) in ketamine use (Lerche et al 2000; Mair et al 2009). Similar effects were also observed in this study where animals lacked spontaneous breathing for almost 15 seconds in all the groups. Differences in the incidences of post induction apnea may result from the higher dosages and rates of administration of the drug (Mair et al 2009) since a slightly lower incidence (18%) was reported after slow administration of propofol by Aguiar et al (2001). Slow titration of up to 90 seconds has been suggested by Berry (2015) to reduce the incidence of postinduction apnea.

Vomiting observed in some animals in this study has been associated with the alpha-2-adrenoceptor agonist, xylazine. The drug has been reported to

stimulate the chemoreceptor trigger zone of the brain in cats, resulting to nausea and thus vomiting (Colby et al 1981). The same mechanism may have resulted to vomiting observed in this study.

2.4.2 Monitoring of vital parameters

2.4.2.1 Respiration and oxygen saturation

The respiratory depressant effect of ketamine-propofol combinations observed in this study has also been reported in other studies (Kennedy and Smith 2015; Rastabi et al 2021). It may be due to the effect of ketamine and propofol in decreasing the response to carbon dioxide and arterial hypoxemia (Rastabi et al 2021). Clarke and Trim (2013) also documented respiratory depression as a classical complication of xylazine, which in this study was used during pre-medication. There is a possibility that the drug could have contributed to the decreased respiration rate observed in all animals. Furthermore, it has been reported that higher doses of ketamine may exacerbate respiratory depression (Kennedy and Smith 2015). Combining the three drugs; xylazine, propofol and ketamine may also likely have exacerbated respiration depression. The initial reduction in the rate in the early stages of the maintenance period as observed in the KP1 and KP2 groups may have been an effect of the induction dose that was administered as a single bolus. Bolus infusions are characterized by high peaks of plasma concentrations of the anaesthetic drugs and sometimes excessive depth of anaesthesia. Once the animals were on CRI, the rate gradually increased, but still remained low in comparison to standard physiological values.

The low oxygen saturation observed in all protocols corresponds with reduced respiration rates, hence may have resulted from the low supply of oxygen due to reduced respiration. It could also be associated with the apneustic breathing patterns that were observed in this study, where animals had shorter expirations in comparison to inspiration, resulting to reduced clearance of carbon dioxide. Kuusela et al (2000) also associated lower readings on a pulse oximeter with vasoconstriction as a result of xylazine and ketamine use, drugs which were also used in this study.

2.4.2.2 Pulse rate

Studies have reported significant increases in the heart rate when ketofol was used in comparison to when propofol was used alone (Kennedy and Smith 2015). This has been mainly attributed to the effect of ketamine in the mixture since ketamine has been reported to increase heart rate and systemic arterial pressure (Haskins 1985). In the current study, the reduction in pulse rate observed in the KP3 group might have been due to the lower ketamine dose used in the combination since protocols with a higher ketamine proportion had slightly higher pulse rates. It is also possible that the overall trend of reduced pulse rates in the animals was a result of the effects of xylazine that was administered during pre-medication. The drug results to a physiological sino-atrial and atrioventricular heart block, thus causing bradycardia (Hall et al 2001).

2.4.2.3 Temperature

The temperature remained relatively constant in KP1. A non-significant reduction in temperature was observed in KP2 and KP3. Adetunji et al (2002) and Seliska et al (2007) have also reported reduction in temperature of dogs under anaesthesia. It is usually attributed to the physiological effects of the anaesthetic agents used, that cause central nervous system depression and reduction in muscle activity (Seliska et al 2007). Adetunji et al (2002) also associated a reduction in environmental temperature to the overall reduction in an animal's body temperature.

2.5 Limitations

Some of the limitations of this study include the fact that only clinically healthy animals were used, and that the maintenance period only lasted for 60 minutes. More animals and longer maintenance and observation periods would have provided further insight in the general effects of the drug combinations. Furthermore, mean arterial blood pressure and carbon dioxide concentration were not determined and could have provided additional understanding on the effects in the cardiopulmonary system.

2.6 Conclusion and Recommendations

Generally, the 1:1, 1:2 and 1:3 Ketamine: Propofol combination ratios can safely be used in mixed breed dogs pre-medicated with xylazine and atropine in procedures expected to last for approximately one hour. However due to low respiration rates and oxygen saturation, it is

imperative to diligently monitor the animals while under anaesthesia, but also ensure a patent airway. Under the conditions of this study, the 1:3 Ketamine:Propofol combination (KP3) appeared to result in relatively more stable and predictable physiological effects with less side effects in comparison to the 1:1 and 1:2 Ketamine:Propofol ratios. Further studies with increased sample sizes and time for maintenance together with post-anaesthetic follow-up would provide more insight into the short- and long-term effects of the drug combinations in mixed breed dogs.

2.7 Acknowledgement

We wish to extend our utmost and sincere gratitude to the Sokoine University of Agriculture (SUA) through the Sokoine University of Agriculture's Higher Education for Economic Transformation (SUA-HEET) project for providing the financial support to conduct this study.

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Table and Figures

Table 1: Summary of Anaesthetic Indices in Ketofol at 1:1(KP1), 1:2(KP2) and 1:3 (KP3) Ratios

Parameter	KP1	KP2	KP3
Induction time (secs)	≈ 20	≈ 20	≈ 20
Induction quality	Good	Good	Good
Surgical anaesthesia time (mins)	60	60	60
Recovery time (mins)	50 ± 26	55 ± 38	40 ± 29
Recovery quality	Good	Good	Good
Side effects			
Apnoea	√	√	√
Tachypnea	√	√	
Urination	√		√
Tachycardia	√		
Vomiting	√		
Irregular breathing		√	

Figure 1: Mean Respiration Rate Following CRI of Ketofol at 1:1(KP1), 1:2(KP2) and 1:3 (KP3) Ratios

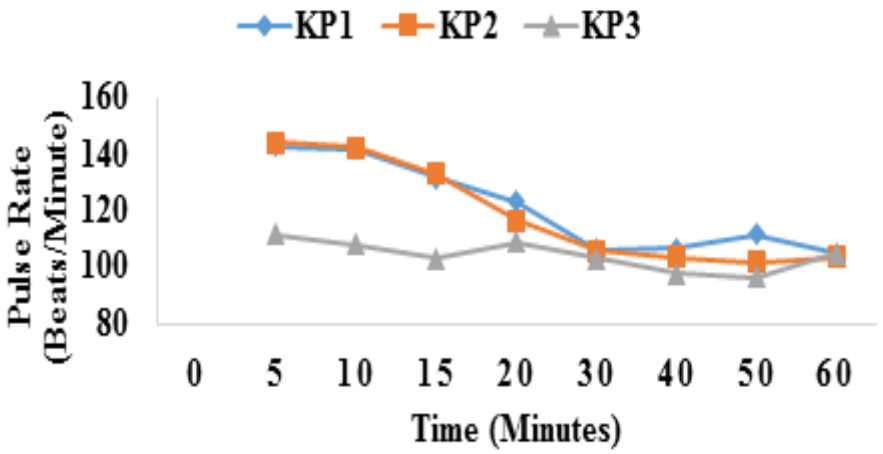

Figure 2: Mean pulse rate following CRI of ketofol at 1:1(KP1), 1:2(KP2) and 1:3 (KP3) ratios

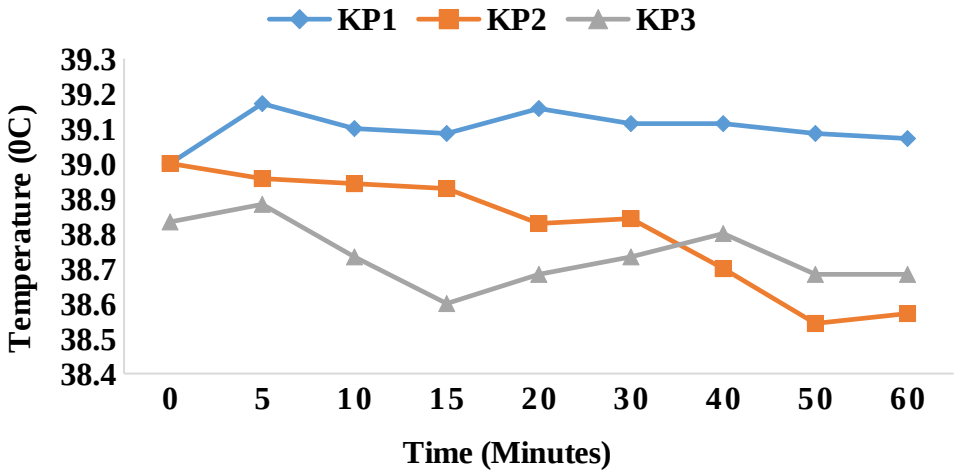

Figure 3: Mean Temperature Following CRI of Ketofol at 1:1(KP1), 1:2(KP2) and 1:3 (KP3)

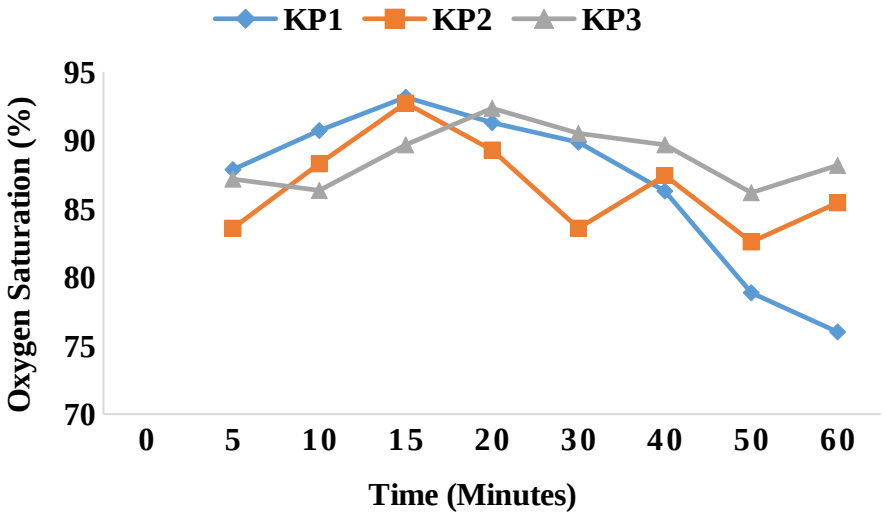

Figure 4: Mean Oxygen Saturation Following CRI of Ketofol at 1:1(KP1), 1:2(KP2) and 1:3 (KP3) Ratios

CHAPTER THREE
MANUSCRIPT II
3.0 Hematological and Biochemical Effects of Total Intravenous Administration of Ketamine, Propofol and their Combinations In Mixed-Breed Dogs

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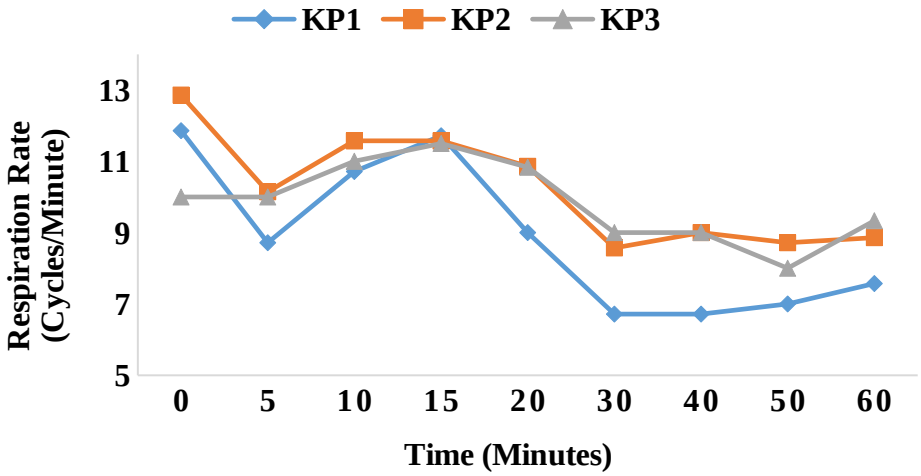
The Material contained in this chapter is in preparation for submission to the Tanzania Veterinary Journal (TVJ).

Summary

This study assessed effects of ketamine, propofol and their combinations administered through Total Intravenous Anaesthesia (TIVA) on hematological and biochemical indices in mixed breed dogs. Ten adult dogs were provided with five different treatments in a repeated crossover experiment. Treatments were Ketamine (KK) and Propofol (PP) individually; and as a combination in 1:1 (KP1), 1:2(KP2) and 1:3 (KP3) Ketamine + Propofol rations. KP2 had the highest mean absolute WBC



count while KP1 the lowest. Differences in the RBC, HCT and HB were not significant among the groups. KP3 group had a significant decrease in the RBC ($p=0.006$) and hemoglobin concentration ($p=0.049$) at the 60th minute. KP2 had the highest mean ALT while KP1 had the lowest. There was a significant decrease in ALT in the PP group at the 30th minute ($P<0.001$). PP has the least mean AST concentration, while KP2 had the highest. KP2 had a significant decrease in AST at the 60th minute ($p<0.001$). Non-significant differences were noted in the creatinine and BUN concentration among groups. There was a significant decrease in creatinine in KP2 from the 30th to 60th minute ($p<0.001$), and a significant increase in BUN in the PP and KP3 groups at the 60th minute ($p<0.001$).

Due to decrease in red blood cell counts, hematocrit and hemoglobin concentration noted in all the protocols; it is imperative to diligently

monitor the animals, but also ensure a patent airway when TIVA is to be used.

Keywords: TIVA, Ketofol, CRI, Hematology, Biochemistry

3.1 Introduction

Total Intravenous Anaesthesia (TIVA) is a practice of administering drugs for induction and maintenance of anaesthesia strictly through the intravenous route without the need for any inhalation agents (Campbell *et al*., 2001). This method often involves anaesthetic induction by administering a bolus dose aimed at achieving the required blood concentration of the anaesthetic drug, then followed by maintenance which can be through delivering intermittent boluses, Constant Rate Infusion (CRI) or Target-Controlled Infusion (TCI) (Waelbers *et al*., 2009).

Commonly used anaesthetic drugs in TIVA include propofol and ketamine individually or as a combination (Ambros *et al*., 2008; Waelbers *et al.,* 2009). Some anaesthetic drugs may affect the oxidant-antioxidant

state of cells responsible for the immunity of the animal such as lymphocytes. Oxidative stress may result to cellular damage that may predispose the animal to lymphocytopenia and immunological deficits (Delogu *et al.*,2004). Studies on a number of mixed as well as officially recognized breeds have also revealed breed specific metabolic and

physiological peculiarities that could affect the pharmacokinetics and pharmacodynamics of drugs in different dog breeds (Fleischer *et al*., 2008). Saikia *et al.* (2022) studied dogs presented for elective ovariohysterectomy or castration, assessing the effect of ketamine and propofol individually and as a combination. They reported a significant decrease in hemoglobin concentration, packed cell volume and total erythrocyte count, with an increase in the blood glucose level. However; blood urea nitrogen and creatinine values increased more in the ketamine and propofol groups than in the ‘ketofol’ groups.

There is limited information on the hematological and biochemical effects of ketamine and propofol in the mixed-breed dogs administered through TIVA. This study assessed some of the hematological and biochemical effects of ketamine and propofol individually, but also as a combination administered through TIVA in mixed-breed dogs.

3.2 Materials and Methods

A written approval of the study was obtained from the Research Ethical Committee of Sokoine University of Agriculture at the Directorate of Research, Technology Transfer and Consultancy (reference number SUA/DPRTC/R/186/VOLIII). Ten clinically healthy dogs with an average weight of 13 ± 2.7 kg were divided into groups to cover five treatments i.e. Ketamine (KK) and Propofol (PP) individually; and Ketofol at 1:1 (KP1), 1:2 (KP2) and 1:3 (KP3) in a repeated crossover experiment. Each treatment was administered twice with a one week interval between treatments of the same protocol, and a two week washout period when the animals are to receive a different treatment.

The inclusion criteria were healthy male and female dogs in American Society of Anesthesiologists (ASA) category I or II, aged between 2-4 years. The exclusion criteria were very young or old age, pregnancy/lactation, aggressiveness, animals undergoing any form of therapy and those with signs/symptoms of disease. The dogs were kept for at least two weeks at the CVMBS kennels for acclimatization, health

checkup, deworming and vaccination prior to commencement of the study. The animals were fasted for 12 hours but provided with water ad libitum until 3 hours before anesthetic induction. Premedication comprising 0.04mg/kg atropine (Swiss Parenterals LTD®, India) and 2mg/kg Xylazine (Interchemie®, Netherlands) was administered via the intramuscular route on the semitendinosus muscles. Once fully sedated, the animals were moved to a surgical table and placed on sternal recumbency for catheterization and induction, then later maintained on left lateral recumbency. An 18 gauge cannula was inserted into the cephalic vein of one limb for administration of the anaesthetic agents.

The animals received five different treatments to cover Ketamine (KK) and Propofol (PP) individually; and Ketofol at 1:1 (KP1), 1:2 (KP2) and 1:3 (KP3). Each treatment was repeated twice, with a one-week period between treatments using the same protocol, and two-week interval when the animals are to receive a different drug or drug combination.

Anaesthetic induction was through a single bolus slow intravenous administration; and maintenance through constant rate infusion using a burette infusion set (Neomedic®, United Kingdom) with a drop factor of 60.

In the KK group, induction was achieved by 5 mg/kg ketamine and maintenance was through 0.3 mg/kg/min for one hour. In the PP group, induction was achieved by 5 mg/kg propofol and maintenance was through 0.3 mg/kg/min for one hour. In KP1 group, induction was achieved by 4 mg/kg ketofol (i.e. 2 mg of ketamine + 2 mg of propofol per kg body weight) and maintenance was through 0.3 mg/kg/min for one hour (i.e. 0.15 mg/kg/min propofol and 0.15 mg/kg/min ketamine). In KP2, induction was achieved by 4 mg/kg ketofol (i.e. 1.3 mg of ketamine + 2.6 mg of propofol per kg body weight) and maintenance was through 0.3 mg/kg/min for an hour (0.1 mg/kg/min ketamine and 0.2 mg/kg/min propofol). In KP3, induction was achieved by 4 mg/kg ketofol (i.e. 1 mg of ketamine + 3 mg of propofol per kg body weight) and maintenance was through 0.3 mg/kg/min for an hour (i.e. 0.075 mg/kg/min ketamine and 0.225 mg/kg/min propofol).

The ketofol mixture was reconstituted to make a total volume of 60 mls that was delivered for 60 minutes. The mixture was not used beyond one hour. Once fully induced, 4 mls of blood for biochemical and 1 ml for hematological assays were obtained from the cephalic vein in plain and Ethylene Diamine Tetracetic Acid (EDTA) tubes respectively. Blood was drawn at time 0 (before induction) as well as 30 minutes and 60 minutes after induction and maintenance. Hematology was performed by an automatic hematology analyzer (Maccura F810®, China). Serum for biochemical analysis was extracted and stored at -20 ^{0}C prior to assay by a UV spectrophotometer (Biochrom®, United Kingdom).

Hematological assessment included absolute White Blood Cell count (WBC); relative Lymphocyte, Monocyte and Neutrophil count; together with the total Red Blood Cell (RBC), Hematocrit (HCT) and Hemoglobin concentration (HB). Biochemical parameters studied were Alanine aminotransferase (ALT), Aspartate transaminase (AST), Blood Urea Nitrogen (BUN) and Creatinine.

3.3 Data Analysis

Data analysis was done using Microsoft excel 15 (Microsoft corporation, USA) and the Statistical Product and Service Solutions (SPSS) version 20 (IBM Corporation, USA) for descriptive statistics and comparison of means. Linear plots were used to express the changes in parameters throughout the maintenance period. Means and standard deviations of numerical values were determined and analysis of variance (ANOVA) together with a Post Hoc Tukey's test and were used to compare variations between protocols. A dependent student's t-test was used to compare variations within each treatment protocol from the established baseline (pre-induction) values. Data was expressed in terms of means ± standard deviation, and the statistical level of significance was set at $p \leq 0.05$.

3.4 Results

3.4.1 Hematology

3.4.1.1 White Blood Cells (WBC)

There were statistically significant differences in the WBC count among the five protocols ($p=0.002$). The KP2 group had the highest mean count

($9.27 \pm 2.67 \times 10^3/\mu L$) while KP1 had the lowest ($4.78 \pm 0.92 \times 10^3/\mu L$). Comparisons of the count within particular protocols against pre-induction values revealed a significant decrease in the count in KP3 group at the 30th (p=0.009) and 60th (p=0.0330) minutes. There was also an insignificant gradual decrease in the count in KK, PP and KP1 groups at the 30th and later 60th minute. KP2 had an initial insignificant increase at the 30th minute that was followed by a decrease at the 60th minute. Generally, the mean WBC count at the 60th minute was less than the pre-induction count in all groups.

3.4.1.2 Lymphocytes

There were non-significant differences in the differential lymphocyte count among and within the protocols. However, a slight decrease in the count in KP2 and KP3 groups was observed in the first 30 minutes of maintenance. This was followed by a gradual increase towards the 60th minute. KK and PP groups had insignificant gradual increases through the 30th and 60th minutes, while and KP1 had a non-significant increase at the 30th minute which was followed by a decrease in the count at the 60th minute to a level below pre-induction values.

3.4.1.3 Neutrophils

The differences in the relative neutrophil count were statistically insignificant among protocols. Within particular protocols, KK and KP1 groups had an initial insignificant decrease at the 30th minute, which was followed by a gradual increase towards the 60th minute. The PP group had an initial increase in the count at the 30th minute, and later a decrease towards the 60th minute to levels beyond pre-induction values. KP2 however had a significant increase in the count throughout the maintenance period ($p=0.041$), while KP3 had an insignificant gradual decrease at the 30th and 60th minutes of maintenance.

3.4.1.4 Monocytes

There were statistically non-significant differences in the differential monocyte count between and within the protocols. The count however decreased in PP in the first 30 minutes, and later increased during the last 30 minutes of maintenance. A similar trend was also observed in KP1. In KK and KP3, the count initially increased at the 30th minute, then decreased at the 60th minute. KP2 had a steady count in the first 30 minutes, followed by a decrease towards the 60th minute. All the protocols

however recorded less counts at the end of the maintenance in comparison to pre-induction values.

3.4.1.5 Red Blood Cells (RBC)

The differences in the RBC count were not statistically significant among the protocols. Within particular protocols, there was a significant decrease in the count in the KP3 group at the 60th minute (p=0.006). KK, PP and KP1 groups also had a gradual decrease through the 30th and 60th minutes, however the differences were not significant. KP2 had an initial non-significant increase at the 30th minute, and later a decrease towards the 60th minute. There was a general decrease in the count in all groups when pre-induction values were compared to those at the end of the maintenance period.

3.4.1.6 Hematocrit (HCT)

Differences in the HCT were not statistically significant between and within particular groups. There was however an overall reduction in HCT when baseline values were compared to the values at the end of the maintenance period in all groups. PP group had the lowest mean HCT (26.92 ± 2.80 %) while KP2 had the highest (34.97 ± 5.16 %).

3.4.1.7 Hemoglobin Concentration (HB)

The differences in the HB among groups were not statistically significant. Within particular groups, there was a significant reduction in HB in the KP3 group at the 60th minute (p=0.049). Within the other groups, the differences were not statistically significant; however, PP had the lowest mean HB count (9.76 g/dL ± 0.89) while KP3 had the highest (11.67 g/dL ± 0.57). There was also a decreasing trend in the HB throughout the maintenance period in all the protocols.

3.4.2 Biochemistry

3.4.2.1 Alanine Aminotransferase (ALT)

The difference in ALT levels among the groups was significant (p=0.047). The KP1 group had the least mean concentration (5.71 ± 0.00 U/L) while KP2 had the highest (10.74 ± 0.24 U/L). KP2 and KP3 groups had an overall insignificant increase in the concentration at the 60th minute in comparison to pre induction values. The ALT level decreased non significantly in KK, but significantly in PP (p<0.001) at the 30th minute, which further decreased up to 60th minute. The concentration in KP1 was almost constant throughout the maintenance period.

3.4.2.2 Aspartate Transaminase (AST)

Differences in the AST concentration were statistically significant ($p=0.012$). PP has the least mean concentration (6.06 ± 1.98 U/L), while KP2 had the highest (12.22 ± 3.1 U/L). KK and KP1 had a non significant increase in the concentration at the 60^{th} minute, while KP2 had a significant decrease ($p<0.001$). Minor variations were observed in KP3 throughout the maintenance period. There was however an overall decrease in the mean AST level throughout the maintenance period in all groups.

3.4.2.3 Creatinine

Differences in the creatinine level were not significant ($p=0.346$). Comparisons at different times of the maintenance period revealed an insignificant increase in the KK group at the 30^{th} minute, which later decreased at the 60^{th} minute to a level below pre-induction values. KP3 had a non significant decrease from the 30^{th} to the 60^{th} minute. A significant increase was noted in KP2 from the 30^{th} to 60^{th} minute ($p<0.001$). The concentration in PP and KP1 also increased, however the differences were not statistically significant.

3.4.2.4 Blood Urea Nitrogen (BUN)

Differences in the BUN were not significant. Comparisons at different times of maintenance revealed a significant initial decrease in the PP group at the 30th minute, which later increased towards the 60th minute ($p<0.001$). A similar trend was also observed in KK and KP3; however, the differences were not statistically significant in KK, but significant in KP3 ($p<0.001$). KP1 had a non-significant increase from the 30th to 60th minute, while KP2 had a non-significant decrease at the 30th minute, which later gradually increased to the 60th minute. There was a general increase in the mean concentration of BUN in all groups at the 60th minute in comparison to pre-induction values.

3.5 Discussion

The decrease in WBC count observed in this study is consistent with previous studies that used either ketamine, propofol or their combination in dogs. Saikia *et al.* (2020) reported insignificant decreases in the WBC count at the 60th minute in dogs anaesthesitied with ketamine, propofol and ketofol (1:1). Costa *et al.* (2013) also reported a decrease in WBC count during maintenance in animals anaesthetized with propofol.

Similarly, Camkerten *et al*. (2013) reported non-significant decline in the WBC count during anaesthesia in Bozova greyhounds anaesthetized with a xylazine-ketamine combination. The reduction has mainly been associated with the anaesthetic plane and side effects of the drugs. The anaesthetic plane reduces the stress response of the animal, and side effects of anaesthetic agents affect the immune system of the animal (McBride *et al*., 1996). In addition to this, catecholamines and glucocorticoids that are released during anaesthesia may also impair the immune system (Elenkov and Chrousos, 2002). All these effects may result to a decrease in lymphocyte production and function.

The general relative lymphocyte count went down in the first 30 minutes under maintenance, then gradually increased in PP, KP2 and KP3 groups. KK had minor changes in the count while KP1 had a slight reduction in the count towards the 60^{th} minute. Costa *et al*. (2013) reported non-significant differences in lymphocytes in dogs anaesthetized with propofol, however there was a general trend of decrease in the lymphocyte count as the animals were under anaesthesia in comparison to basal values. The decrease has been associated with suppression of the

immune system cells by anaesthetic agents via indirect mechanisms operating through the central nervous system (Molina, 2006). The CNS regulates the concentration of humoral substances like cortisol or epinephrine that affect lymphocytic activity in lymphoid organs (Straub *et al*., 1998), hence an increase in concentration of norepinephrine may result in suppression of the lymphocyte production (Costa *et al.*, 2013).

In this study, there was a significant increase in the relative neutrophil count in the KP2 group, and a non-significant increase in the KP1 group as well. Saikia *et al.* (2020) reported insignificant neutrophilia up until the 60^{th} minute when ketamine, propofol and ketofol (1:1) were used in dogs. Non-significant neutrophilia was also reported by Jena *et al.* (2014) in dogs anaesthetized with propofol. Similar results were also reported by Sanker *et al.* (2011) who recorded neutrophilia in both ketamine or propofol anaesthetized dogs; Venugopa *et al.* (2014) when ketamine was used and Kumar *et al.* (2014) when ketofol was used. Neutrophilia has been associated with anaesthetic stress coupled sometimes with painful surgeries (Sanker *et al*., 2011; Saikia *et al.,* 2020). The stress stimulates the adrenal cortex, resulting to production of glucocorticoids. The

glucocorticoids act on the circulating neutrophils, resulting to transient neutrophilia. Anaesthetic stress may have resulted to the neutrophilia observed in KP1 and KP2 groups in this study, since the animals were not subjected to any pain while being monitored.

The mean relative monocyte count in all protocols at the 60th minute was less than the pre-induction values; suggesting a decrease in the overall monocyte count. The decrease was however not statistically significant. Saikia *et al.* (2020) also reported insignificant decreases in the monocyte count at the 15th and 30th minutes in ketamine, propofol and ketofol (1:1) groups. Costa *et al.* (2013) also reported insignificant differences in monocytes in dogs anaesthetized with propofol. Sanker *et al.* (2011) however reported a significant decrease in monocytes in both ketamine and propofol anaesthetized dogs. Similar trends were also reported by Jena *et al.* (2014), Venugopa *et al*. (2014), and Kumar *et al.* (2014) when propofol, ketamine and ketofol were used respectively.

All the protocols in this study had varying levels of decrease in the RBC count at the 60th minute in comparison to pre-induction values. A study by

Camkerten *et al*. (2013) reported a non-significant decrease in the RBC count in Bozova greyhounds anaesthetized with a xylazine-ketamine combination. There was also an overall reduction in the HCT in all groups in the study when pre-induction values were compared to values at the 60^{th} minute. Camkerten *et al*. (2013) also reported non-significant reductions in the HCT in Bozova greyhounds anaesthetized with a xylazine-ketamine combination.

The hemoglobin concentration decreased in all groups at the 60^{th} minute. The decrease was statistically significant in KP3 but not significant in PP, KK, KP1 and KP2 groups. Costa *et al.* (2013) also reported non-significant differences in HB in dogs anaesthetized with propofol.

Another study by Saikia *et al.* (2020) reported a significant decrease in the HB in all dogs treated with either ketamine, propofol or their combination (ketofol at 1:1) at the 30^{th} and 60^{th} minutes. Similar results were also reported by Sanker *et al.* (2011) who recorded a significant decrease in HB in both ketamine and propofol anaesthetized dogs. In general, decreases in RBC, HCT and HB have been associated with splenic pooling of erythrocytes as a result of a decrease in sympathetic

stimulation. This is a common side effect of most of the anaesthetic agents (Kinjavdekar *et al.,* 2010; Singh *et al.,* 2013; Saikia *et al.,* 2020). Propofol has however been reported not to cause significant splenic enlargement (O'brien *et al.,* 2004; Wilson *et al.,* 2004) thus the decrease, especially in propofol use may also result from sequestration of red blood cells into non-splenic sites (Costa *et al.,* 2013). The decline has also been associated with fluid shifts between body compartments in order to maintain normal cardiac output (Wagner *et al.,* 1991) or hemodilution as a result of fluid therapy (Kinjavdekar *et al.,* 2010; Singh *et al.,* 2013). Small volumes of fluids were administered in this study as well, this may have contributed to the decline in values of some parameters observed.

Alanine aminotransferase (ALT) is a specific enzyme produced by the liver (Nagode *et al*., 1966). Changes in the ALT level observed in this study were not uniform among the five treatment groups. KK and PP had an overall decrease in the concentration of the enzyme; while KP2 and KP3 had insignificant increases at the 60^{th} minute. Saikia *et al.* (2020) described a significant increase in the ALT towards the 60^{th} minute in dogs that received ketamine, propofol and their combination ketofol (1:1).

Similar findings were also reported by Celestine *et al.* (2014) in West African dwarf goats. Camkerten *et al.* (2013) also reported insignificant declines in the ALT level in Bozova greyhounds that were anaesthetized with a xylazine-ketamine combination. Increases in ALT have been associated with a combined effect of hypotension and hypoxemia, which may result to the release of the enzyme from the liver (Celestine *et al.*, 2014). The increase in ALT may also be an indication of hepatocellular damage. In this particular study, only two groups (KP2 and KP3) had increases in the ALT levels, one maintained a constant concentration (KP1) while the remaining two (KK and PP) had a decrease in the concentration of the enzyme. The reason for the decrease is still unclear.

The AST level serves as an indication of the status of muscle and liver damage. In this study, the KK group had a gradual increase in AST during the maintenance period up to the 60^{th} minute. An increase in AST following ketamine use has also been reported by Saikia *et al.* (2020). Saikia *et al.* (2020) suggested that the relatively higher increase in AST in the ketamine group when compared to propofol and ketofol groups may be indicative of the less adverse effects of the latter on body tissues when

compared with ketamine. The increase however is of less clinical significance when compared to ALT.

Creatinine levels may be used as an indication of renal function. There was a general combined increase in the mean creatinine concentration in all groups during maintenance. In particular; PP, KP2 and KP3 groups had increases throughout the maintenance period, while KK had an initial increase in the first 30 minutes followed by a decrease towards the 60th minute. Saikia *et al.* (2020) also recorded a significant increase creatinine in the ketamine, propofol and ketofol groups. The increase in creatinine level has been linked to reduced renal blood flow due to the inhibitory effects of the drugs; but also the production of the substance due to muscle damage and degeneration of amino acids (Restitutti *et al.,* 2012; Singh *et al*., 2013). Some of the decreases in creatinine levels may have resulted from sample deterioration since some samples were stored prior to analysis.

Urea assays are mainly an indication of renal function and to a lesser extent, liver function. The overall increase in BUN in KK, PP, KP1 and KP3 groups observed in this study was also reported by Saikia *et al.*

(2020). Manat *et al.* (2004) reported an increase in BUN after propofol use as well. The increase has been associated with temporary inhibition of renal blood flow by the anaesthetic drugs. The inhibition may haveresulted to a decrease in the glomerular filtration rate, thus an increase in the level of circulating BUN (Gonz'alez *et al*., 2003; Saikia *et al.*, 2020). Blood Urea Nitrogen (BUN) can also be altered by dehydration and to a greater extent by either protein catabolism, synthesis or both processes (Fox, 1989).

Generally, the protocols have shown to have minimal effects on hematological and biochemical indices studied. This is due to the fact that there were insignificant differences in most of the parameters when pre-induction values were compared to those at the end of the one hour infusion. However, Due to the varying levels of decrease in red blood cell counts, hematocrit and hemoglobin concentration noted in all the protocols; it is imperative to diligently monitor animals under anaesthesia, but also ensure a patent airway when TIVA is to be used in mixed breed dogs.

3.6 Acknowledgement

We extend our utmost and sincere gratitude to Sokoine University of Agriculture (SUA) through the Sokoine University of Agriculture's Higher Education for Economic Transformation (SUA-HEET) project for providing the financial support to conduct this study.

3.7 Conflict of Interest

Authors do not have any conflict of interest.

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Figures

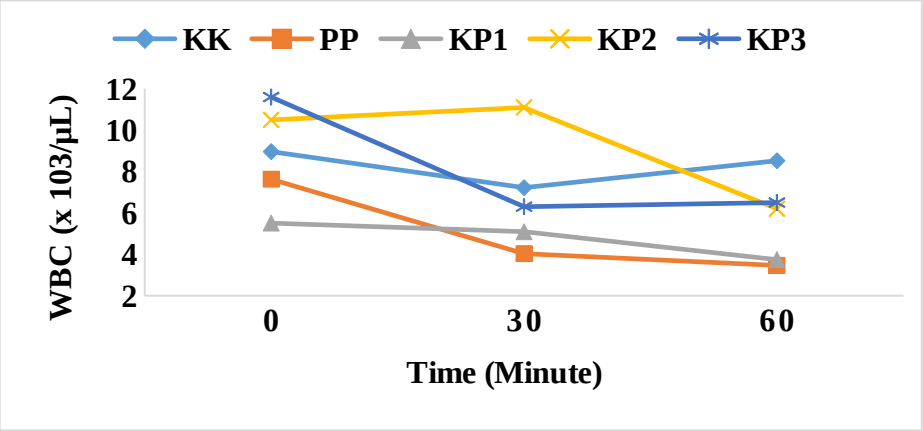

Figure 1: Mean absolute WBC count following CRI of Ketamine (KK), Propofol (PP); ketofol at 1:1 (KP1), ketofol at 1:2 (KP2) and Ketofol at 1:3 (KP3) ratios in mixed breed dogs

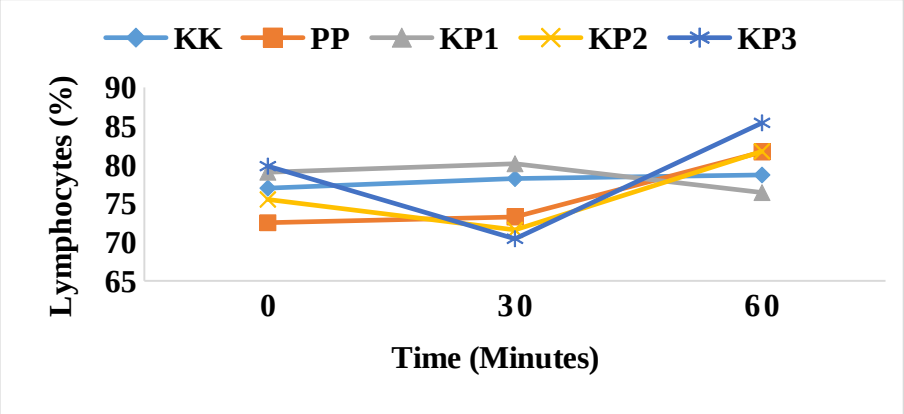

Figure 2: Mean differential lymphocyte count following CRI of Ketamine (KK), Propofol (PP); ketofol at 1:1 (KP1), ketofol at 1:2 (KP2) and Ketofol at 1:3 (KP3) ratios in mixed breed dogs

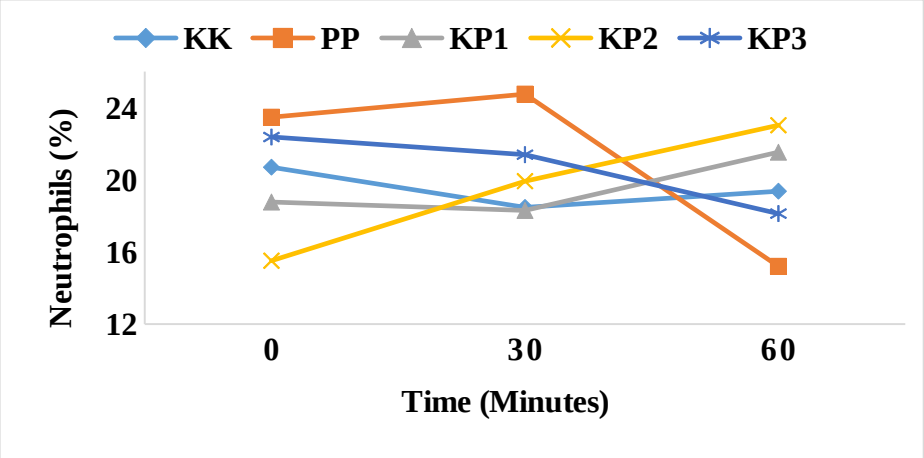

Figure 3: Mean differential neutrophil count following CRI of Ketamine (KK), Propofol (PP); ketofol at 1:1 (KP1), ketofol at 1:2 (KP2) and Ketofol at 1:3 (KP3) ratios in mixed breed dogs

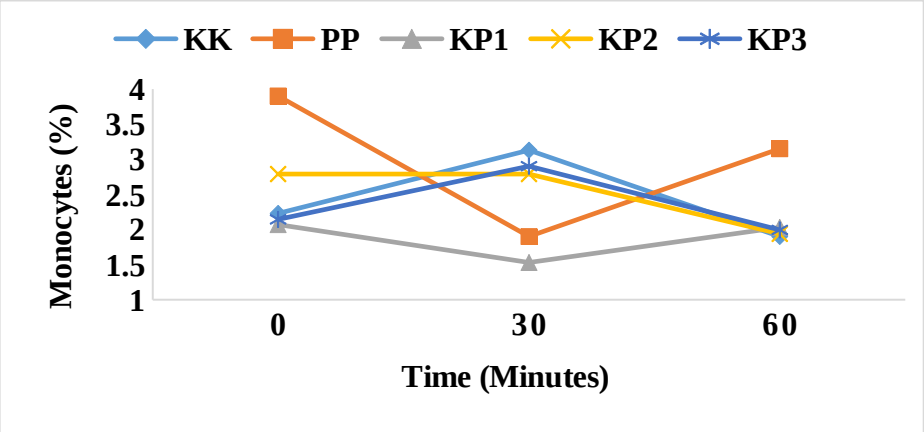

Figure 4: Mean differential monocyte count following CRI of Ketamine (KK), Propofol (PP); ketofol at 1:1 (KP1), ketofol at 1:2 (KP2) and Ketofol at 1:3 (KP3) ratios in mixed breed dogs

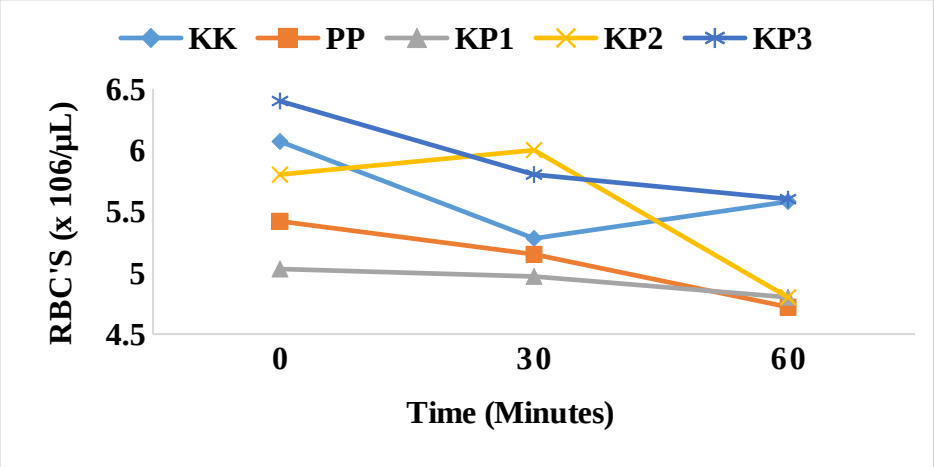

Figure 5: Mean RBC count following CRI of Ketamine (KK), Propofol (PP); ketofol at 1:1 (KP1), ketofol at 1:2 (KP2) and Ketofol at 1:3 (KP3) ratios in mixed breed dogs

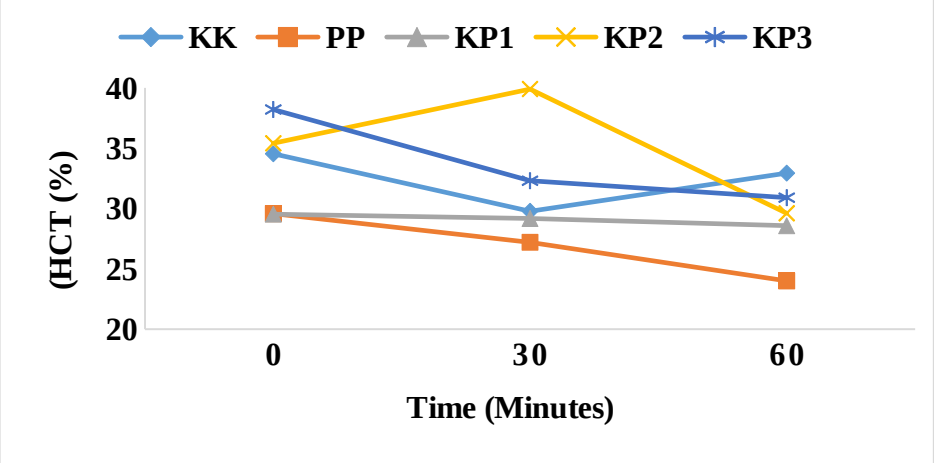

Figure 6*:* Mean HCT following CRI of Ketamine (KK), Propofol (PP); ketofol at 1:1 (KP1), ketofol at 1:2 (KP2) and Ketofol at 1:3 (KP3) ratios in mixed breed dogs

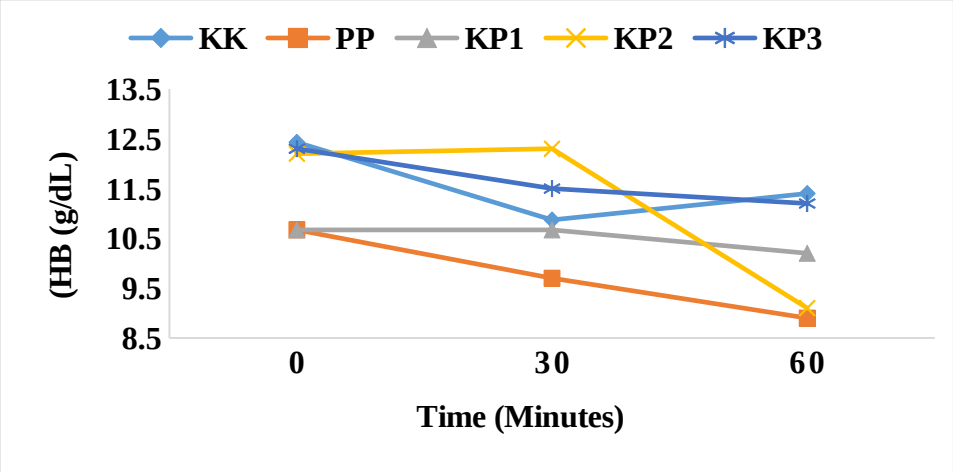

Figure 7: Mean HB following CRI of Ketamine (KK), Propofol (PP); ketofol at 1:1 (KP1), ketofol at 1:2 (KP2) and Ketofol at 1:3 (KP3) ratios in mixed breed dogs

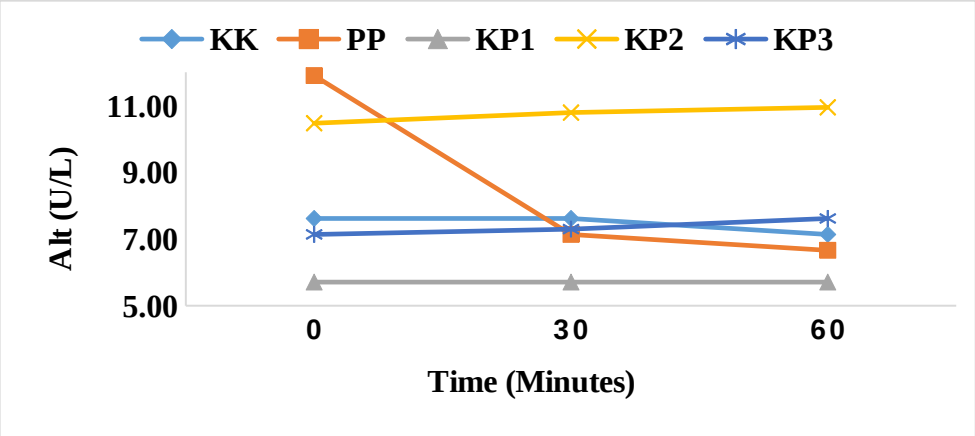

Figure 8: Mean ALT concentration following CRI of Ketamine (KK), Propofol (PP); ketofol at 1:1 (KP1), ketofol at 1:2 (KP2) and Ketofol at 1:3 (KP3) ratios in mixed breed dogs

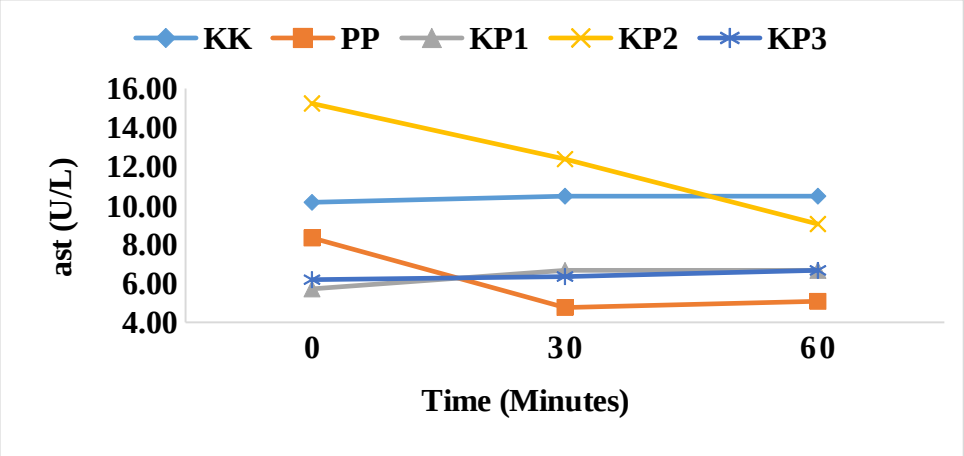

Figure 9: Mean AST concentration following CRI of Ketamine (KK), Propofol (PP); ketofol at 1:1 (KP1), ketofol at 1:2 (KP2) and Ketofol at 1:3 (KP3) ratios in mixed breed dogs

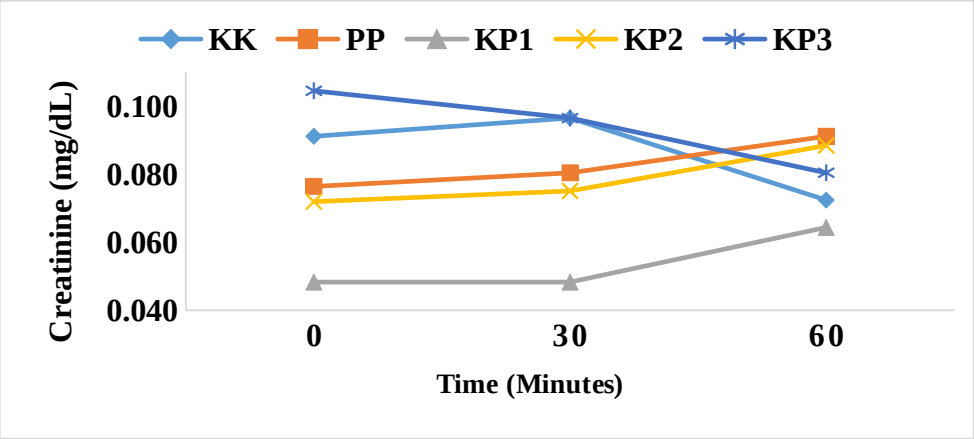

Figure 10: Mean creatinine concentration following CRI of Ketamine (KK), Propofol (PP); ketofol at 1:1 (KP1), ketofol at 1:2 (KP2) and Ketofol at 1:3 (KP3) ratios in mixed breed dogs

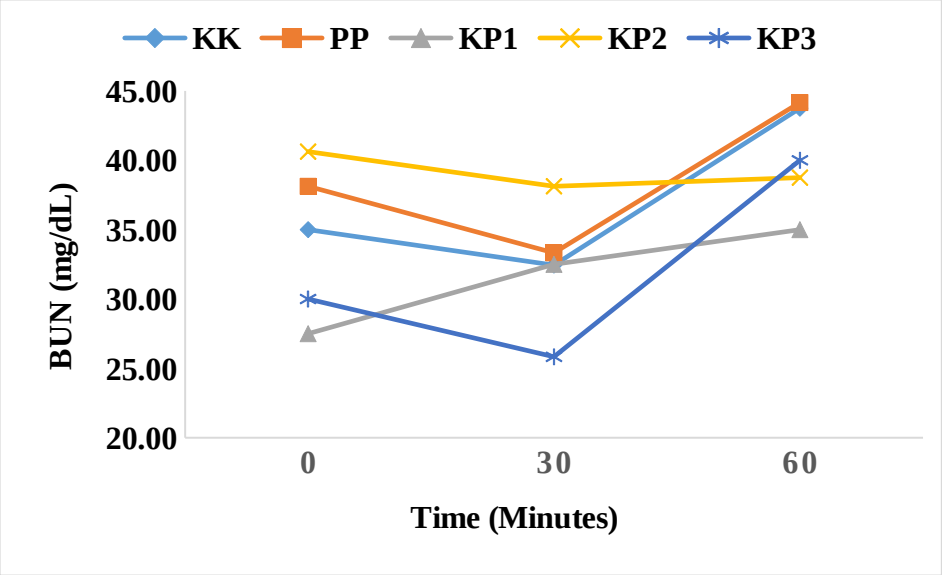

Figure 11: Mean BUN concentration following CRI of Ketamine (KK), Propofol (PP); ketofol at 1:1 (KP1), ketofol at 1:2 (KP2) and Ketofol at 1:3 (KP3) ratios in mixed breed dogs

CHAPTER FOUR

4.0 GENERAL DISCUSSION

4.1 Anaesthetic Drugs and Dosage

The drugs used in this study were mainly two pre anaesthetic agents, i.e. xylazine and atropine, and the injectable anaesthetics which were ketamine, propofol or their combinations 'ketofol' at different ratios. The use of xylazine as a premedication agent was mainly due to its dose dependent sedative, muscle relaxation and analgesic properties (Hall *et al.,* 2001). When combined with ketamine, the drug can also provide a short duration of surgical anaesthesia. Xylazine may also assist in reducing the dose of anaesthetic agents required for induction and maintenance of anaesthesia (Ramsey, 2008). Some of the side effects observed in this study that may partly be associated with the use of xylazine include respiratory depression as also documented by Clark and Trim (2013). The dogs in all treatments had relatively lower respiratory rates in comparison to the normal physiological values. The other side effect observed that may be associated with xylazine use was vomiting (Colby *et al.,* 1981). Studies have also linked xylazine to reduced oxygen saturation readings on the pulse oximeter due to its vasoconstrictive effects (Kuusela *et al*., 2000). The same effect may partly be associated with the reduced oxygen saturation observed in all treatments in the current study.

Atropine was also part of the premedication protocol as it assists in counterbalancing the bradycardia (Ramsey, 2008) that could have resulted from either xylazine or propofol use. The drug has however been associated with transient tachycardia, especially after intravenous administration. It was administered through the intramuscular route to prevent this effect.

Ketamine was mainly aimed at producing dissociative anaesthesia and partly analgesia through its blockade of NMDA receptors. However, it results to mild stimulation of the circulatory system and muscle

hypertonicity (Haskins, 1985), thus the need for co-administration of an alpha-2 adrenergic agonist, which in this study was xylazine in order to minimize the effects. The cardiostimulatory effect of ketamine was also noted in this study since the groups that received relatively higher proportions of ketamine (KK and KP1) had relatively higher mean pulse rates than those with less or no ketamine. Despite its cardiostimulatory effects, ketamine has been associated with exacerbated respiratory depression in animals (Kastner, 2007; Kennedy & Smith, 2015). A similar trend was observed in this study. Other side effects that were observed in this study and have been associated with ketamine use include apnoea (Kastner, 2007; Lerche *et al.,* 2000) and convulsions (Seliskar *et al.*, 2007). The drug when administered for an extended period of time may result to its accumulation and hence prolonged recovery in animals (Ramsey, 2008). This effect was also observed in this study since animals that received ketamine individually or higher proportions of ketamine recovered relatively slower than those that received less or no ketamine.

Propofol was included in the protocol as an anaesthetic agent due to its rapid action and non-cumulative properties. Due to the rapid clearance of the drug, animals that received propofol alone or in a relatively higher propofol proportion in the ketofol mixture recovered relatively quicker than those that received less or none. Its mechanism of action is not fully understood, but has been thought to modulate the inhibition of the GABA receptors (Ramsey, 2008). Some of the side effects reported in propofol use that were also observed in the study were respiratory depression, apnoea and bradycardia (Aguiar *et al*., 2001; Lerche *et al.,* 2000; Mair *et al*., 2009; Ramsey, 2008). It also has poor analgesic properties, hence the need to include xylazine and/or ketamine in the protocol.

Combining ketamine and propofol reduced the dose required for induction and maintenance of both drugs as it has been reported by Henao-Guerrero and Ricco (2014); Kennedy and Smith (2015); Lerche *et al*. (2000) and Mannarino *et al*. (2012). Further reductions in

anaesthetic requirements can also be achieved using premedication, due to the anaesthetic sparing effects of the premedication drugs such as alpha-2-adrenergic agonists (Dewangan *et al.*, 2010; Kinjavdekar *et al.,* 2010).

4.2 Physiological effects

4.2.1 Respiration and oxygen saturation

As it is with most anaesthetic agents, many of the protocols used had varying degrees of respiratory depression in animals. Propofol has been associated with dose dependent respiratory depression (Aguiar *et al*., 2001; Ramsey, 2008), ketamine combined with propofol at a 1:1 ratio has also been linked to aggravated depression of the respiratory system in dogs (Mair *et al.,* 2009; Rastabi *et al.,* 2021). This has been thought to be due to the higher proportions of ketamine in the mixture. The drugs tend to decrease the response of the animal to carbon dioxide and arterial hypoxemia (Rastabi *et al.,* 2021). Clarke and Trim (2013) however also associated respiratory depression with the use of xylazine. The reduced respiration in combination with vasoconstriction as a result of xylazine and ketamine use has also been linked with the low oxygen saturation recorded in the majority of protocols used in this study (Kuusela *et al.,* 2000).

4.2.2 Pulse and heart rate

The pulse rate was used as an indication of the heart rate and overall state of the circulatory system. Differences in the pulse among protocols were insignificant, and the rates remained within the normal physiological range. A significantly higher heart rate has been reported after ketamine use due to its cardiostimulatory effects. Ketamine increases the heart rate through stimulation of the sympathetic nervous system and release of catecholamine (Haskins, 1985). This has also been evident in this study since animals that received ketamine alone or in a higher proportion in the ketofol mixture had relatively higher pulse rates than those that received lower or no ketamine. A similar observation was also reported by Kennedy and Smith (2015) when ketofol was used.

Lower rates were observed in the propofol groups. This has also been reported by Lerche *et al.* (2000) and attributed to dose dependent vagal stimulation. Xylazine use has also been linked to reduced heart rates since the drug has been reported to result in bradycardia due to sino-atrial and atrioventrivular blockade (Hall *et al*., 2001).

4.2.3 Temperature

There was an overall mean reduction in temperature as the animals were under anaesthesia in this study. Similar trends have also been reported by Adetunji *et al. (*2002) and Seliska *et al.* (2007). It has mainly been associated with CNS depression and reduction in muscle activity as the animal is under anaesthesia (Seliska *et al.,* 2007). This reduces the animal's ability to respond to temperature changes. Decreases in environmental temperature and contact with cold surfaces have also been reported to result to reduced body temperatures (Adetunji *et al.,* 2002). In this study, the animals also received intravenous infusions which were maintained at room temperature, and not pre-warmed. This may have added to further reductions in the core body temperature.

4.3 Hematology

Changes in complete blood cell counts have mainly been associated with response of the animal to stress and side effects of some anaesthetic drugs. A number of mechanisms may account for some of the changes observed in this particular study. Some anaesthetic agents in combination with catecholamines and glucocorticoids released during anaesthesia impair the immune response of an animal (Elenkov & Chrousos, 2002; Molina, 2008). Immune cells can also be suppressed via indirect mechanisms operating through the CNS which assists in regulating the concentration of humoral substances like cortisol or epinephrine (Straub *et al*., 1998). Increases in the concentration of norepinephrine may end up suppressing lymphocyte production and hence a decrease in the lymphocytic count (Costa *et al.,* 2013). On the other hand, increased production of glucocorticoids has been associated with transient neutrophilia (Sanker *et al*., 2011; Saikia *et al.,* 2020). The

same mechanisms may have resulted to the changes in the cell counts observed in this study.

The decrease in RBC, HCT and HB observed in this study may have been a result of splenic pooling of erythrocytes as a result of a decrease in sympathetic stimulation. This is a common side effect of most anaesthetic agents (Kinjavdekar *et al.,* 2010; Singh *et al.,* 2013; Saikia *et al.,* 2020). Propofol has however been reported not to cause significant splenic enlargement (O'brien *et al.,* 2004; Wilson *et al.,* 2004) thus the decrease, especially in propofol use may also result from sequestration of red blood cells into non-splenic sites (Costa *et al.,* 2013). The decline has also been associated with fluid shifts between body compartments in order to maintain normal cardiac output (Wagner *et al.,* 1991) or hemodilution as a result of fluid therapy (Kinjavdekar *et al.,* 2010; Singh *et al.,* 2013). Small volumes of fluids were used in this study as well, this may have contributed to the decline in values of some parameters observed.

4.4 Biochemistry

Studies on the effect anaesthetic agents on the liver and kidney function have reported various changes that could directly be a result of the anaesthetic agents or other metabolic processes. In this study, changes in the level ALT and AST were used as indicators of the effect on the liver, while BUN and creatinine were used to indicate the effect on renal function. Both ALT and AST increased and decreased at different intervals among the protocols. The differences could not be directly linked to a single factor or causation, but rather a number of possible reasons. An increase in ALT has been associated with hypotension and hypoxemia, which may result to the release of the enzyme by the liver (Celestine *et al.*, 2014). The hypotension and hypoxemia may be a direct effect of the anaesthetic agents used. In this study, KK and PP had an overall decrease in the concentration of the enzyme; and a general increase in the oxygen saturation throughout the maintenance period, thus explaining the effect of hypoxemia on the release of the enzyme.

Increases in AST have been linked to damage of muscle and liver tissue (Saikia *et al.,* 2020); however, the concentration is of less clinical significance when compared to ALT. The reason for the changes observed in this study is not clear since the animals were not under any form of surgical procedure. Creatinine and BUN changes have been associated with changes in renal blood flow. Temporary inhibition of renal blood flow has been linked to increases in both creatinine and BUN (Gonz'alez *et al*., 2003; Saikia *et al.*, 2020). Increases in creatinine can also be due to increased production of the substance because of muscle damage or degeneration of amino acids (Restitutti *et al.,* 2012; Singh *et al*., 2013) while decreases may be due to sample deterioration that may result into a decrease. Since some of the samples were also stored prior to assays, it is possible that some decreases in concentration may be attributed to this factor. BUN can also be altered largely by the level of protein catabolism, synthesis or both processes (Fox, 1989).

CHAPTER FIVE

5.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1 General Conclusions

Generally; ketamine, propofol and the 1:1, 1:2 and 1:3 Ketamine: Propofol combination ratios can safely be used in mixed breed dogs of the Africanis landrace type pre-medicated with xylazine and atropine in procedures expected to last for approximately one hour. The protocols have shown to have minimal effects on hematological, biochemical and some physiological indices studied. This is due to the fact that there were insignificant differences in most of the parameters when pre-induction values were compared to those at the end of the one-hour infusion.

However due to low respiration rates and oxygen saturation, coupled with a decrease in red blood cell counts, hematocrit and hemoglobin concentration in all the protocols; it is imperative to diligently monitor the animals while under anaesthesia, but also ensure a patent airway. This would guarantee adequate tissue perfusion. Owing to the relatively more stable pulse, higher and more stable oxygen saturation, less severe side effects and shorter recovery time, the 1:3 ketamine: propofol combination ratio appears to be a slightly better alternative among the three ketofol combinations to be used in mixed breed dogs.

5.2 Recommendations

Diligent monitoring of the patients owing to the respiratory depressant effects of the anaesthetic drugs administered through TIVA.

i. Adjustment of the infusion rate is necessary to meet the animal's immediate requirements but also ensure stability of the patient under anaesthesia and a successful and uneventful recovery.
ii. Performing extensive studies on a wider population of animals and longer maintenance periods would provide further and more consistent insight on the general effects of the drugs or drug combinations.

iii. Assessment of more parameters such as arterial blood pressure, carbon dioxide concentration, glucose concentration, cortisol, more liver and kidney function tests and other stress related indices together with post anaesthetic follow-up would also create a better understanding of the short- and long-term effects of the drugs administered.

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APPENDICES

Appendix 1: TABLES

Table 1: Summary of anaesthetic indices in the treatment groups

Parameter	KK	PP	KP1	KP2	KP3	
Induction time (secs)	≈ 20	≈ 20	≈ 20	≈ 20	≈ 20	
Induction quality		Good	Good	Good	Good	Good
Anaesthesia time (mins)	58	55	60	60	60	
Recovery time (mins)	67 ± 35	29 ± 10	50 ± 26	55 ± 38	40 ± 29	
Recovery quality	Fair	Good	Good	Good	Good	
Side effects						
Apnoea	√	√	√	√	√	
Tachypnea	√	√	√			
Urination	√	√			√	
Tachycardia					√	
Bradycardia	√					
Vomiting	√					
Irregular breathing			√			
Open eyelids		√		√		
Vocalization		√				
Convulsions		√				

Table 2: Mean Respiration Rate (Cycles/Minute)

Time (Minutes)										
PROTOCOL	**0**	**5**	**10**	**15**	**20**	**30**	**40**	**50**	**60**	**AVG ± SD**
KK[a]	13	12	11	12	11	11	12	12	12	**12 ± 0.5**
PP[bc]	11	11*	12*	13*	12	13	13	13	14	**12 ± 0.9**
KP1[ab]	12	9	11	12	9	7	7*	7*	8	**9 ± 2.1**
KP2	13	10	12	12	11	9*	9	9	9	**10 ± 1.6**
KP3[c]	10	10	11	12	11	9	9	8	9	**10 ± 1.1**

***** Statistically significant differences in means in comparison to pre-induction values within particular groups ($p<0.05$)

[abcd] Protocols with the same superscript have statistically significant differences in means ($p<0.05$) following a Post Hoc Tukey's test

Table 3: Mean Pulse Rate (BPM)

Time (Minutes)									
PROTOCOL	**5**	**10**	**15**	**20**	**30**	**40**	**50**	**60**	**AVG ± SD**
KK	127	126	124	114*	117*	113*	111*	109*	**118 ± 7.1**
PP	119	116	106	105	106	92*	96	95	**104 ± 9.7**
KP1	143	142	131	123	106*	106*	111*	105*	**121 ± 16.0**
KP2	144	142	133*	116*	105*	104*	102*	103	**119 ± 18.1**
KP3	111	108	103	109	103	98	96	105	**10 4 ± 5.3**

Table 4: Mean Oxygen Saturation (%)

Time (Minutes)									
PROTOCOL	**5**	**10**	**15**	**20**	**30**	**40**	**50**	**60**	**AVG ± SD**
KK[abc]	73	77*	77	83*	83*	83*	84*	83	**80 ± 3.9**
PP	79	78	81	82	86	82	86	88*****	**84 ± 3.6**
KP1[a]	88	91	93	91	90	86	79	76	**86 ± 6.1**
KP2[b]	84	88	93	89	84	87	83	85	**87 ± 3.4**
KP3[c]	87	86	90	92	91	90	86	88	**89 ± 2.2**

Table 5: Mean Temperature (^{0}C)

Time (Minutes)										
PROTOCOL	**0**	**5**	**10**	**15**	**20**	**30**	**40**	**50**	**60**	**AVG ± SD**
KK[a]	38.8	38.7	38.9	39.0	38.9	38.8	38.7	38.6	38.4	**38.8± 0.18**
PP[b]	38.9	38.6	38.6	38.6	38.5	38.5	38.4	38.3	38.5	**38.5± 0.16**
KP1[abcd]	39.0	39.2	39.1	39.1	39.2	39.1	39.1	39.1	39.1	**39.1± 0.05**
KP2[c]	39.0	39.0	38.9	38.9	38.8	38.8	38.7	38.5	38.6	**38.8± 0.16**
KP3[d]	38.8	38.9	38.7	38.6	38.7	38.7	38.8	38.7	38.7	**38.7± 0.08**

Table 6: Mean Absolute WBC Count (x 10^3/µL)

Time (Minutes)				
PROTOCOL	**0**	**30**	**60**	**AVG ± SD**
KK	8.96	7.22	8.52	**8.23 ± 0.90**
PP	7.63	4.03	3.46	**5.04 ± 2.26**
KP1	5.51	5.09	3.74	**4.78 ± 0.92**
KP2	10.5	11.1	6.2	**9.27 ± 2.67**
KP3	11.6	6.3*	6.5*	**8.13 ± 3.00**

Table 7: Mean Differential Lymphocyte Count (%)

Time (Minutes)				
PROTOCOL	**0**	**30**	**60**	**AVG ± SD**
KK	76.97	78.20	78.67	**77.95 ± 0.88**
PP	72.50	73.25	81.65	**75.80 ± 5.08**
KP1	79.03	80.13	76.40	**78.52 ± 1.92**
KP2	59.5	56.4	64.4	**60.10 ± 4.03**
KP3	62.9	55.5	67.3	**61.90 ± 5.96**

Table 8: Mean Differential Neutrophil Count (%)

Time (Minutes)				
PROTOCOL	**0**	**30**	**60**	**AVG ± SD**
KK	20.70	18.47	19.37	**19.51 ± 1.12**
PP	23.47	24.75	15.20	**21.14 ± 5.18**
KP1	18.77	18.30	21.53	**19.53 ± 1.75**
KP2	15.51	19.92*	23.03*	**19.49 ± 3.77**
KP3	22.37	21.39	18.13	**20.63 ± 2.22**

Table 9: Mean Differential Monocyte Count (%)

Time (Minutes)				
PROTOCOL	**0**	**30**	**60**	**AVG ± SD**
KK	2.23	3.13	1.90	**2.42 ± 0.64**
PP	3.90	1.90	3.15	**2.98 ± 1.01**
KP1	2.07	1.53	2.03	**1.88 ± 0.30**
KP2	2.79	2.79	1.94	**2.51 ± 0.49**
KP3	2.14	2.90	1.99	**2.35 ± 0.49**

Table 10: Mean RBC Count (x 10^6/µL)

Time (Minutes)				
PROTOCOL	**0**	**30**	**60**	**AVG ± SD**
KK	6.07	5.28	5.58	**5.64 ± 0.40**
PP	5.42	5.15	4.72	**5.10 ± 0.35**
KP1	5.03	4.97	4.80	**4.93 ± 0.12**
KP2	5.8	6.0	4.8	**5.53 ± 0.64**
KP3	6.4	5.8	5.6*	**5.93 ± 0.42**

Table 11: Mean HCT (%)

Time (Minutes)				
PROTOCOL	**0**	**30**	**60**	**AVG ± SD**
KK	34.53	29.77	32.93	**32.41 ± 2.42**
PP	29.57	27.20	24.00	**26.92 ± 2.80**
KP1	29.53	29.17	28.57	**29.09 ± 0.48**
KP2	35.4	39.9	29.6	**34.97 ± 5.16**
KP3	38.2	32.3	30.9	**33.80 ± 3.87**

Table 12: Mean HB concentration (g/dL)

Time (Minutes)				
PROTOCOL	**0**	**30**	**60**	**AVG ± SD**
KK	12.43	10.87	11.40	**11.57 ± 0.79**
PP	10.67	9.70	8.90	**09.76 ± 0.89**
KP1	10.67	10.67	10.20	**10.51 ± 0.27**
KP2	12.2	12.3	9.1	**11.20 ± 1.82**
KP3	12.3	11.5	11.2*	**11.67 ± 0.57**

Table 13: Mean ALT Concentration (U/L)

Time (Minutes)				
PROTOCOL	**0**	**30**	**60**	**AVG ± SD**
KK	7.62	7.62	7.14	**07.46 ± 0.27**
PP	11.90	7.14*	6.66*	**08.57 ± 2.90**
KP1	5.71	5.71	5.71	**5.71 ± 0.00**
KP2	10.47	10.79	10.95	**10.74 ± 0.24**
KP3	7.14	7.30	7.62	**7.35 ± 0.24**

Table 14: Mean AST Concentration (U/L)

Time (Minutes)				
PROTOCOL	**0**	**30**	**60**	**AVG ± SD**
KK	10.15	10.47	10.47	**10.37 ± 0.18**
PP	8.33	4.76	5.08	**06.06 ± 1.98**
KP1	5.71	6.66	6.66	**6.35 ± 0.55**
KP2	15.23	12.38	9.04*	**12.22 ± 3.10**
KP3	6.19	6.35	6.66	**6.4 ± 0.24**

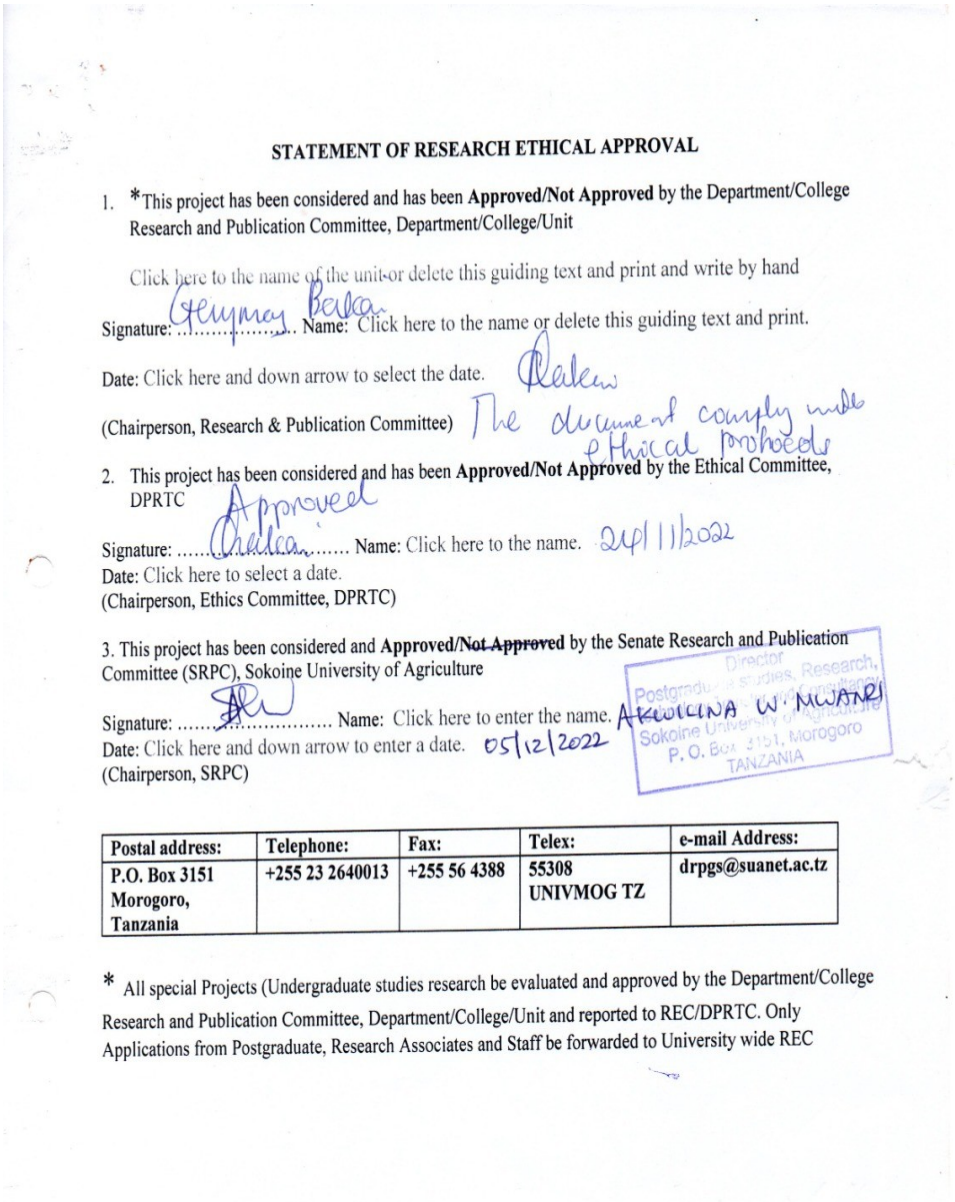
Table 15: Mean Creatinine Concentration (mg/dL)

Time (Minutes)				
PROTOCOL	**0**	**30**	**60**	**AVG ± SD**
KK	0.091	0.096	0.072	**0.087 ± 0.013**
PP	0.076	0.080	0.091	**0.083 ± 0.008**
KP1	0.048	0.048	0.064	**0.054 ± 0.009**
KP2	0.072	0.075*	0.088*	**0.078 ± 0.009**
KP3	0.105	0.096	0.080	**0.094 ± 0.012**

Table 16: Mean BUN Concentration (mg/dL)

Time (Minutes)				
PROTOCOL	**0**	**30**	**60**	**AVG ± SD**
KK	35.00	32.45*	43.75	**37.07 ± 5.93**
PP	38.13	33.33	44.17	**38.54 ± 5.43**
KP1	27.50	32.50	35.00	**31.67 ± 3.82**
KP2	40.63	38.13	38.75	**39.17 ± 1.30**
KP3	30.00	25.83*	40.00*	**31.94 ± 7.28**

Appendix 2: Ethical clearance form



STATEMENT OF RESEARCH ETHICAL APPROVAL

1. *This project has been considered and has been **Approved/Not Approved** by the Department/College Research and Publication Committee, Department/College/Unit

   Click here to the name of the unit or delete this guiding text and print and write by hand

Signature: ..... Name: Click here to the name or delete this guiding text and print.

Date: Click here and down arrow to select the date.

(Chairperson, Research & Publication Committee) The document comply made ethical protocols

2. This project has been considered and has been **Approved/Not Approved** by the Ethical Committee, DPRTC

Signature: ..... Name: Click here to the name.

Date: Click here to select a date.

(Chairperson, Ethics Committee, DPRTC)

3. This project has been considered and **Approved/~~Not Approved~~** by the Senate Research and Publication Committee (SRPC), Sokoine University of Agriculture

Signature: ..... Name: Click here to enter the name.

Date: Click here and down arrow to enter a date.

(Chairperson, SRPC)

Director
Postgraduate studies, Research, Consultancy
Sokoine University of Agriculture
P. O. Box 3151, Morogoro
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* All special Projects (Undergraduate studies research be evaluated and approved by the Department/College Research and Publication Committee, Department/College/Unit and reported to REC/DPRTC. Only Applications from Postgraduate, Research Associates and Staff be forwarded to University wide REC

SECTION P : FOR OFFICIAL USE

(i) APPROVAL

Date received : Click here and arrow to enter a date. 24/11/2022	Received by: Click here to type names. LUCIA MADALLA
Date of approval: Click here and arrow to enter a date. 05/12/2022 **Name**: AKWILINA MWANRI **Title**: COODINATOR POSTGRADUATE Approving authority in capital letters (example: SRPC, Departmental /College/Centre R&PC Click here to enter the name of approving authority.	Approval reference number: Click here to enter number. SUA/DPRTC/R/186/VOL III Approval is valid from Click here and arrow to enter a date. 05/12/2022 To: Click here to enter a date. .....Director..... Technology Transfer and Consultancy Sokoine University of Agriculture P. O. Box 3151, Morogoro TANZANIA
*All undergraduate studies shall be evaluated and/or approved by the College/Centre R&PC and Reports submitted to the chair Research Ethica Committee, DPRTC	
(ii) **NOT APPROVED** ☐ The applicant is required to revise the application by addressing reviewer's concerns (Reviwer's comments are provided to the applicant) ☐Other reasons (Describe briefly)	
Click here to enter text.	

Kuhusu Tasnifu Hii

Utafiti huu ulitathmini athari za kuingiza dawa za usingizi kwenye mishipa ya damu kwa mbwa. Dawa aina ya ketamine, propofol na mchanganyiko wake zilitumika. Utafiti ulibaini kupungua kwa kasi ya upumuaji na kiwango cha oksijeni. Mapigo ya moyo na joto vilisalia ndani ya wastani wa kawaida. Madhara yaliyoonekana yalikuwa kukojoa, kupiga kelele, kutetemeka, macho kutofumba na kutapika nyakati tofauti. Hesabu ya wastani wa seli nyeupe na nyekundu za damu ilikuwa chini baada ya dakika 60 za usingizi ikilinganishwa na kabla ya usingizi. Tofauti ya viashiria vya utendaji kazi wa ini na figo haikuwa kubwa. Kwa ujumla; dawa hizi zinaweza kutumika kwa mbwa mchanganyiko kwa mfumo wa kuingiza kwenye damu. Japo, kwa sababu ya kiwango cha chini cha upumuaji na oksijeni; pamoja na upungufu wa seli nyekundu za damu kilichoonekana, ni muhimu kuwafuatilia wanyama kwa ukaribu pindi wanapokuwa katika usingizi na kuhakikisha njia ya hewa iko wazi ili kurahisisha upumuaji.