MODULATORY EFFECTS OF ASCORBIC ACID ON NEUROBEHAVIOURAL AND PHYSIOLOGICAL RESPONSES INDUCED BY PROPOFOL ANAESTHESIA IN GOATS

BY

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BY

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DEPARTMENT OF VETERINARY PHYSIOLOGY,
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JULY, 2014

DECLARATION

I declare that the work in the thesis entitled, 'Modulatory Effects of Ascorbic acid on Neurobehavioural and Physiological Responses induced by Propofol Anaesthesia in Goats.' has been performed by me in the Department of Veterinary Physiology, Faculty of Veterinary Medicine, under the supervision of Prof. J. O. Ayo, Dr. S. F. Ambali, and Dr. T. Aluwong.

The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has previously been presented for the award of a degree or diploma at this or any other university.

Veronica Inyamu OCHIGBO

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Name of Student	Signature	Date

CERTIFICATION

This thesis entitled "MODULATORY EFFECTS OF ASCORBIC ACID ON NEUROBEHAVIOURAL AND PHYSIOLOGICAL RESPONSES INDUCED BY PROPOFOL ANAESTHESIA IN GOATS" by Veronica Inyamu OCHIGBO, meets the regulations governing the award of Master of Science degree in Veterinary Physiology of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to God Almighty, the breath of my existence and to my parents Mr. A. Ochigbo (of blessed memory) and Mrs. M. O. Ochigbo. I could not have been what I am today without you.

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ABSTRACT

The aim of the study was to evaluate the modulatory effect of ascorbic acid on neurobehavioural and physiological changes, induced by propofol anaesthesia in goats. Twenty-four Red Sokoto goats divided into four groups were used for the experiment. Goats in group I (VC) received only ascorbic acid (200 mg/kg). Goats in group II (PFOL) received only propofol (5 mg/kg), while those in group III (VC200 + PFOL) and group IV (VC300 + PFOL) were administered with ascorbic acid at the dose rates of 200 mg/kg and 300 mg/kg, respectively prior to propofol (5 mg/kg) induction. Pain response after the administration of ascorbic acid and propofol was evaluated in each goat by monitoring and scoring its behaviour. Onset and duration of anaesthesia in the goats were also recorded. Haematological parameters, taken before and after anaesthetic induction, were analyzed for leucocyte count, packed cell volume (PCV), haemoglobin concentration, erythrocyte count and erythrocyte osmotic fragility. The serum obtained from each blood sample was analyzed for electrolytes (Cl^-, Mg^{2+}, Na^+, K^+, and HCO_3^-), the activity of antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPx) and enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Goats (57.1%) in group IV had a pain score of 0, and none of the goats scored 3. Pain score was highest in goats administered with propofol alone, and scores of 3 and 2 were recorded in 40% and 60% of the treated goats, respectively. There was a significant ($P < 0.05$) increase in duration of anaesthesia in the VC300+PFol group (27.29 ± 4.11 min), when compared to the VC200+Pfol (15.29 ± 1.19 min) and PFol groups (10.6 ± 2.23 min). There was a significant ($P < 0.05$) decrease in onset of anaesthesia in the VC300+PFol group (2.0 ± 0.22 s) and VC200+Pfol (2.57 ± 0.29 s) when compared to the PFol groups (4.6 ± 0.68

s). There was a significant ($P < 0.05$) increase in Mg^{2+} concentration in only the groups administered with VC but no significant change occured in other electrolytes and serum enzymes. The activity of GPx and SOD rose in the Pfol group ($P < 0.05$) from 30.4 ± 1.33 IU/L to 36.8 ± 0.97 IU/L and 1.3 ± 0.08 IU/L to 1.7 ± 0.06 IU/L respectively indicating that propofol may be a potent antioxidant. In conclusion, the administration of ascorbic acid prior to propofol anaesthesia ameliorated pain caused by propofol and prior administration of ascorbic acid decreased the onset and increased the duration of propofol anaesthesia in goats.

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LIST OF ABBREVIATIONS

AA	Ascorbic acid
ACTH	Adrenocorticotropic hormone
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
Cl^-	Chloride ion
CNS	Central nervous system
COX-2	Cycloxygenase-2
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetraacetic acid
EOF	Erythrocyte osmotic fragility
GPx	Glutathione peroxidase
h	Hour
Hb	Haemoglobin concentration
HCO_3^-	Bicarbonate ion
HR	Heart rate
IV	Intravenous
K^+	Potassium ion
LD_{50}	Median lethal dose
Mg^{2+}	Magnesium ion
Na^+	Sodium ion
NaCl	Sodium chloride
$O_2^{\bullet-}$	Superoxide
PCV	Packed cell volume

PGE_2	Prostaglandin E 2
RBC	Red blood cell
ROS	Reactive oxygen species
RR	Respiratory rate
RT	Rectal temperature
SOD	Superoxide dismutase
TP	Total protein
UK	United Kingdom
WBC	White blood cell

CHAPTER ONE

INTRODUCTION

1.1 Background

Pain is a complex interaction involving sensory, emotional and behavioural factors (Serpell, 2006). Animal pain is an aversive sensory experience representing awareness by the animal of damage or threat to the integrity of its tissues. It changes the animal's physiology and behaviour to reduce or avoid the damage, to reduce the likelihood of its recurrence and to promote recovery (Molony, 1997). Pain typically involves a noxious stimulus or event that activates nociceptors in the body's tissues and conveys signals to the central nervous system, where they are processed and generate multiple responses (NRC, 2009). Painful stimuli evoke not only discrete sensory perceptions and somatic motor responses, but also marked changes in emotional and autonomic states (Gauriau and Benard, 2002). The ability to quantify the degree of pain experienced by animals is an important component in the assessment of animal welfare (Barnett, 1997), and may provide useful information on the outcome of intervention to ameliorate pain. Pain causes discomfort, impairs functions and immune responses in the body (Hellebrekers, 2000), resulting in negative consequences on livestock production. Inflammatory responses associated with injuries and pains have direct adverse impact on feed intake. Anorexia and lethargy are induced by cytokines, including interleukin-1, interleukin-6 and tumour necrosis factor-α, released by the inflammatory process, the stimulation of tissue catabolism and exerts their effects on the central nervous system (Johnson, 1997).

The identification and assessment of pain in animals may be achieved not by using a single tool but by multiple measures (Barnett, 1988; Molony and Kent, 1997). A combination of several indicators such as changes in behavioural pattern, physiological and biochemical parameters may be useful in making an informed judgement as to whether an animal is experiencing pain (Anil *et al.,* 2002).

Anaesthesia is an indispensable pre-requisite to most of the surgical interventions, both in humans and animals, so that the surgeon can perform surgical procedures with maximum precision (Muhammad *et al.,* 2009). In addition to routine surgical procedures such as disbudding and castration, goats are often presented to the veterinary surgeon for types of surgery which are associated more with companion animals; for example, fracture repair (Reid *et al.,* 1993). The development of new sedatives and analgesics in recent years has solved some of the problems in handling complicated surgical problems in these species (Khan *et al.,* 2004). Except for a few, patients like to remain sedated during the surgical procedure. The state of wakefulness can produce anxiety and reduce cooperation in animals (Tejinder and Ravishanka, 2005).

Propofol (2, 6- diisopropylphenol) is a unique non-steroid, short-acting general intravenous anaesthetic agent (Hofmeister *et al.,* 2008). It enhances γ-aminobutyric acid (GABA)-ergic neurotransmission, mediated by the $GABA_A$ receptor complex (Irifune *et al*., 2003). Propofol chemically resembles the chain-breaking antioxidant, α-tocopherol because it also has a phenolic hydroxyl group (Murphy *et al.,* 1992). It has been established that this phenolic hydroxyl group is responsible for the antioxidant properties of propofol (Murphy *et al.,* 1992, 1993; Adembri *et al.,* 2006). Thus, during anaesthesia for cardiac or vascular and transplantation surgery or during sedation of the

critically ill, propofol may be an effective inhibitor of lipid peroxidation (Aarts *et al*., 1995). Since its introduction, propofol has been rapidly displacing other anaesthetic agents (Dutta *et al.,* 1997) due to its rapid recovery rate.

Ascorbic acid (also known as vitamin C) is a water-soluble, chain-breaking antioxidant vitamin that is capable of scavenging and neutralizing reactive oxygen and reactive nitrogen species at low concentration (Sauberlich, 1994; Naidu, 2003). Ascorbic acid is probably the most effective, least toxic antioxidant identified in mammalian systems as it is more superior to other water-soluble plasma antioxidants (uric acid and bilirubin), and to the lipoprotein-associated antioxidants (α-tocopherol, β-carotene, and lycopene) (Sauberlich, 1994). Ascorbic acid is an antioxidant vitamin which accumulates in the brain from the blood supply, and is maintained at high concentrations in both neuronal and glial cells (Rebec and Pierce, 1994; Rice and Russo-Menna, 1998). It serves as a neuromodulator in normal brain functioning and as a neuroprotective agent in various conditions of oxidative stress (Grunewald, 1993). The neuroprotective action of ascorbic acid has been mainly attributed to its antioxidant capacity. It exerts a modulatory influence on the central nervous system (Elsa and Ubandawaki, 2005; Alireza and Goudarz, 2007). Impairment of central nervous system functions has been reported to occur after a deficiency of the vitamin (Ezenwanne and Anuka, 1991). Ascorbic acid is readily available, affordable and is almost non-toxic (Minka and Ayo, 2012).

1.2 Statement of the Problem

Pain is a core component of the stress response to injury, and therefore, should be managed appropriately to optimize recovery and minimize complications (Serpell,

2006). Despite considerable efforts to improve pain management, farm animals receive a lower priority than companion animal species or species in which the individual animal is of high economic value (Anil *et al.,* 2005). Extensive research focusing specifically on farm animals is needed to identify and quantify pain and to develop inexpensive, simple to use and safe pain medication before use of analgesia can be expected. Surgery and anaesthesia are events that pose considerable stress on the patient (Tejinder and Ravishanka, 2005). Pain a common stressor, inhibits a positive operating room and anaesthesia experience by patients undergoing surgical procedure and propofol has a substantial potential for causing pain and a burning sensation at the intravenous site (Singleton, 2000). The combined effect of stress and anaesthesia may result in detrimental consequences, such as cardio-respiratory arrests (Elsa and Ubandawaki, 2005).

1.3 Justification of the Study

Food animal production is an important economic enterprise. There is a need for a balance between economics and animal well-being, as pain management can result in economic loss (Anil *et al.,* 2005). Besides, the need for treatment of pain in farm animals is in line with the Animal Welfare Act (AWA, 1990). Propofol is gradually gaining popularity in both human and veterinary surgery worldwide because of its excellent recovery characteristics. However, its intravenous administration is associated with a high incidence of pain and discomfort (Andrzejow, 1995). Propofol is apparently, an ideal anaesthetic agent, yet it has no analgesic property and must be used in conjunction with an analgesic agent (Symington and Thakore, 2006).

Various methods have been used to reduce the pain on propofol injection, including prior injection of opoids (Nathanson *et al*., 1996), use of cold propofol (Barker *et al*., 1991), mixing of propofol with lignocaine (Barker *et al*., 1991) or prior treatment with lignocaine with tourniquet (Mangar and Holak, 1992). Although a number of drugs and methods have been described for the relief of pain on propofol injection, a satisfactory alternative has not yet been found (Ashwani and Radhika, 2009). Most pain research using animal models has been conducted to increase our understanding of the mechanisms underlying human pain, and the recent growth of veterinary clinical interest in the subject has opened doors to studies of pain for the benefit of livestock production (Hansen, 2003). Ascorbic acid has been recommended for administration before trapeziometacarpal arthroplasty (Zollinger *et al.*, 2008) and after a wrist fracture (Zollinger *et al.*, 2007) as it prevents complex regional pain. The impact of anaesthesia in the development of oxidative stress and consequent post-anaesthetic complications is poorly investigated in veterinary medicine (Simeonova *et al.*, 2004). Some studies have demonstrated the effects of ascorbic acid on ketamine anaesthesia in rabbits (Elsa and Ubandawaki, 2005) and dogs (Marjani *et al.*, 2008), indicating the ability of the vitamin to deepen and prolong the depth of anaesthesia. There is paucity of information in the available literature on the effect of ascorbic acid on propofol-induced anaesthesia in goats.

1.4 General Aim

The aim of this study was to evaluate the modulatory effect of ascorbic acid on neurobehavioural and physiological changes induced by propofol anaesthesia in goats.

1.5 Specific Objectives

1. To evaluate the neurobehavioural and physiological changes induced by propofol anaesthesia in goats.
2. To determine the effect of ascorbic acid on neurobehavioural and physiological changes due to intravenous administration of propofol in goats.

1.6 Research Hypotheses

H_o: 1. Propofol anaesthesia does not induce neurobehavioural and physiological changes in goats.

H_o: 2. Ascorbic acid does not exert any effect on neurobehavioural and physiological changes, induced by propofol administration in goats.

CHAPTER TWO

LITERATURE REVIEW

2.1 Pain

Pain as defined by the International Association for the Study of Pain is an unpleasant sensory and emotional experience, associated with actual or potential tissue damage, or described in terms of such damage (IASP, 1989). Price (1999) proposed that pain should be understood as a somatic perception, involving a bodily sensation with qualities similar to those reported during tissue-damaging stimulation. It is an experienced threat associated with this sensation, and a feeling of unpleasantness or other negative emotions, based on the experienced threat. Rajagopal (2006) summarised the definition of pain as a sensory and emotional experience. Pain, as a sub-modality of somatic sensation, has been defined as a complex constellation of unpleasant sensory, emotional and cognitive experiences, provoked by real or perceived tissue damage and manifested by certain autonomic, psychological, and behavioural reactions (Dubin and Patapoutian, 2010). The emotional component is variable from person to person and in the same person from time to time.

Animal pain has been confused with human pain, but experiences have been reported as similar, but are not the same (Molony, 1986, 1992). Animal pain is an aversive sensory emotional experience, representing awareness by the animal of damage or threat to the integrity of its tissues; it changes the animal's physiology and behaviour to reduce or avoid damage, to reduce the likelihood of recurrence and to promote recovery (Molony and Kent, 1997). Aversiveness is a consistent characteristic or component of pain. This

aversive quality can be rated and often separated from the sensory – discriminative component of the sensation, which is revealed in judgements about intensity, quality and location (Heinricher, 2004).

There are many similarities between animals and humans regarding pain perception (Morton and Griffiths, 1985); as many of the pain management strategies in humans were developed on the basis of animal models. According to the animal welfare act, a painful procedure is defined as any procedure that reasonably would be expected to cause more than slight or momentary pain or distress in a human being (Anonymous, 2000). Pain is argued to have sensory and emotional components (Anil *et al.,* 2005), as defined by the international association for the study of pain, but whether animals have emotions, an attribute defined as the ability to relate the present with the past and future, is a controversial subject. Almost all definitions of animal pain focus on physical pain, yet discussions on pain almost invariably include an emotional component. The lesser development of the neocortex has been cited as evidence that animals (except primates) cannot experience emotional pain (Bermond, 1997). The sensory component, thus, needs further attention, given that animals are limited in their ability to communicate the degree and location of pain (Anil *et al.,* 2005).

2.1.1 Classification of pain

Classification of pain is complicated and clear distinctions may not always be possible (Cole, 2002), the more simplistic the classification of pain, the greater the number of omissions and overlaps that occur (Pasero *et al.*, 1999). Several publications have classified pain into various categories using different criteria, which include its duration,

location, frequency, underlying cause, intensity and pathophysiologic mechanisms (Cole 2002; Vadivelu *et al.*, 2009). Pertusi (2004) classified pain typically as acute or chronic. Here acute is referred to as a response to injury, primarily nociceptive and designed to stimulate a withdrawal from the noxious source. Chronic pain, on the other hand, persists long after any appreciable tissue damage has resolved, and it may be due to sensitization of the central nervous system. Rajagopal (2006) simply classified pain in terms of therapeutic application into nociceptive and neuropathic. Woolf (2004) also stated that pain may be divided into two categories and five subcategories.

1) Nociceptive pain which may be subdivided into somatic, visceral and inflammatory pain.
2) Neurogenic pain, subdivided into causalgia and functional pain.

Nociceptive pain is transistory in response to nociceptive stimuli that could be mechanical, thermal or chemical. It plays an important protective role; however in certain situations, the pain will persist even after healing of the initial injury (Marchand, 2008). Inflammatory pain is associated with the healing process following a lesion, and inflammatory substances are released into the periphery by cells in an area of damaged tissue, but can also arise from hyperactivity of the nociceptive neurones in the central nervous system (CNS), a phenomenon known as neurogenic inflammation (Leis *et al.*, 2004). Neurogenic pain is pain arising as a direct consequence of diseases affecting the somatosensory system (Treede *et al.*, 2008). Neurogenic pain even of a peripheral origin is frequently associated with sensitisation of the CNS (Marchand, 2008). Functional neurogenic pain is a sub-category of neurogenic pain, occurring in the absence of a defined anatomic lesion within the nervous system, but rather representing a dysfunction of pain modulation mechanisms. This may occur as a result of central

activation of endogenous excitatory systems that will amplify the nociceptive signal or by a dysfunction of endogenous inhibitory mechanisms (Marchand, 2008).

2.1.2 Pain recognition and assessment in domestic animals.

Recognising pain and assessing its intensity are both essential for its effective management (NRC, 2008). The development of methods of recognition and assessment of animal pain has been delayed by the unwillingness of some people to accept that animals are capable of experiencing or suffering pain when subjected to injury, disease or other noxious events. This may be due to expediency, or confusion of animals to human pain (Anil *et al*., 2005). The assessment of pain in farm animals is often difficult (Stubsjoen *et al*., 2009) and the ability to quantify the degree of pain experienced by animals is an important component in the assessment of animal welfare (Barnett, 1997). The degree to which an animal exhibits signs of pain may be affected by environment, presence of others (humans and animals) species, medication, severity of pain and previous experience (Seksel, 2007). Direct measurement of subjective experiences is not possible; therefore, assessment of animal pain is of necessary value judgment, relying on physiological and behavioural indices to provide indirect evidence of this mental state (Molony and Kent, 1997).

Anil *et al* (2002) stated that there are intraspecies and interspecies variation in responses to painful stimuli and even the response of the same animal may not be the same in all cases (NRC, 2008). This variation may be attributed to stage of development, sex, environment, and prior pain experience. This makes identification and assessment of pain in animals difficult (Anil *et al.,* 2005). Biochemical indicators and indeed many

physiological indicators, although used in pain assessment under laboratory conditions, require specialized equipment and skills. Ethics, technology, economics, regulations and scientific knowledge all need to be amalgamated (Fraser and Leonard, 1993) to find a satisfactory solution. Subjective assessment remains a major tool for pain identification and assessment (Barnett, 1997). Even pain assessments involving objective measures such as heart rate, plasma cortisol and plasma beta-endorphins, conclusions on the meaning of changes with respect to pain are arrived at subjectively (Mellor *et al.,* 2000; Mellor and Stafford, 2000).

In humans, self report of pain is the standard by which assessment techniques may be judged; although there are limitations and biases, even when using this approach. For animals and very young humans who cannot self-report (or those with cognitive impairment), other assessment tools are necessary (Zwakhalen *et al.,* 2006; Ranger *et al.,* 2007). In assessing the degree of pain in animals, the common indicators are vocalisation, aggression, abnormal gait, reluctance to move, changes in temperature, respiratory rate and heart rate (Seksel, 2007). In goats, some behavioural indicators of pain include, vocalisation, inappetence, depression, increased respiratory rate and teeth grinding (Molony *et al*., 2002; NRC, 2008).

2.1.3 Pain scales

The human patient is able to provide a pain score which can be recorded, while the assessment of animal pain is more complex and there is a need to learn how to recognize the signs of pain, which involve both behavioural and physiological responses (Mathews, 2000; Holton *et al.,* 2001). Pain scales have been developed over the years

(Short, 1999; Bianchi *et al*., 2003), unfortunately they were developed in consideration to- and used in small animals.

1) Simple descriptive scale (SDS)
2) Visual analogue scale (VAS)
3) Numerical rating scale (NRS)
4) Composite scale (CS)

The SDS scale is said to be unadaptable to animals since it is not precise and specific, while the VAS is a more predictable scale to evaluate animal pain, compared to the SDS (Holton *et al.,* 2001). The VAS uses both behavioural and physiological parameters (Bufalari *et al.,* 2007). The minimum value is 0 (no pain) and the maximum value is 100 (the worst possible pain). The NRS uses behavioural parameters and the maximum pain score is 10. The behavioural categories used to assess the NRS pain score in dogs are vocalisation, movement and agitation (Short, 1999). Subjective pain scores are primarily based on behaviour, so it is also important to evaluate the behavioural responses to any drug administered in the absence of painful stimuli (Carroll *et al.,* 2007). The use of subjective pain scores in addition to biochemical markers advances the validity of pain scoring (Flechnell, 1997).

2.1.4 Pain and behaviour

Pain scoring can be influenced by behavioural parameters such as temperament, vocalisation, posture, locomotion and other behavioural changes (Bufalari *et al.,* 2007). To be considered is the fact that threshold and response to pain vary according to species, breed, health status and age. Behaviours reflective of pain include learned responses that may manifest differently in experienced or naive subjects (Bufalari *et al.,*

2007). The individual response to pain may contribute to make the evaluation even more difficult since previous behaviour or temperament of highly anxious animals may predispose them to a more intense pain state (Hardie, 2000). Behavioural and endocrine indicators of pain in lambs, cattle and pigs have been established by a number of research groups (Mellor and Stafford, 2000; Molony *et al*., 2002; Hay *et al*., 2003). These measures have been developed largely to aid in the evaluation of the welfare benefits of modifying standard agricultural practices (NRC, 2008).

2.1.5 Mechanism of pain

Pain results from diverse mechanisms, some of which are unique to one painful condition; others are present in multiple clinical syndromes, or may be expressed at different times during the natural history of a syndrome (Scholz and Woolf, 2002). Indeed, physiological mechanisms of pain are very complicated as many excitatory and inhibitory neuronal mechanisms are involved in the generation of pain (Devulder *et al.,* 2002).

The exact pain centre in the nervous system is not easy to determine, but there is a network containing a tightly connected multiple system of relations, overlapping each other elements, which participate in the procession of the different aspects of pain (Gyulahazi, 2010). Multiple mechanism that can produce pain have been identified; they include, nociception, peripheral sensitisation, phenotypic switches, central sensitisation, ectopic excitability, structural reorganisation, and decreased inhibition (Woolf and Salter, 2000; Julius and Basbaum, 2001; Scholz and Woolf, 2002).

The sensation of pain is mediated in the periphery by multiple sets of specialized afferents called nociceptors (Brazeau *et al*., 1998). The diameter of the peripheral process (1-15μm) and the presence or absence of a non-neuronal covering (myelin) determine the rate at which afferents conduct impulses. This forms the basis for anatomic criteria by which afferents are classified. An injury that causes a potential risk for the animal will activate free nerve endings that respond to nociceptive stimulation (Brazeau *et al*., 1998).

2.1.5.1 Nociception

Nociception represents the peripheral and CNS processing of information about the internal or external environment as generated by nociceptor activation. It could also be defined as the process, through which information about peripheral stimuli is transmitted by primary afferent nociceptors to the spinal cord, brainstem, thalamus, and subcortical structures (NRC, 2008). It is the sole mechanism that causes nociceptive pain and comprises the process of transduction, conduction, transmission and perception (Woolf, 2004). Transduction defines responses of peripheral nociceptors to traumatic or potentially damaging chemical, thermal or mechanical stimulation (Vadivelu *et al.*, 2009). Transduction is the conversion of noxious stimulus into electrical activity in the peripheral terminals of nociceptor sensory fibres (Woolf, 2004). This process is mediated by specific receptor ion channels expressed only by nociceptors. Nociceptors are distinguished from other afferent groups (those mediating touch, tickle, pressure, warmth and cold) by their transducing capacity (Brazeau *et al*., 1998). Conduction is the passage of action potentials from the peripheral terminal along axons to the central

terminal of nociceptors in the central nervous system (Woolf, 2004). Myelination and increasing size of a nerve fibre facilitate the speed of conduction of stimulus.

Transmission is the synaptic transfer and modulation of input from one neurone to another (Woolf, 2004). Nociceptors are generally electrically silent (Woolf and Ma, 2007) and transmit all-or-none action potentials only when stimulated and the speed of transmission is directly correlated to the diameter of axons of sensory neurones and whether or not they are myelinated (Dubin and Patapoutian, 2010).

Nociception, the perception of noxious stimuli, is initiated by stimuli that activate the peripheral terminals of nociceptors, a highly specialized subset of primary sensory neurones that respond only to intense stimuli (Dubin and Patapoutian, 2010). Afferent fibres originating in the periphery fall into three groups: Ab fibres, C fibres, and Aδ fibres (Brazeau *et al*., 1998; Scholz and Woolf, 2002; Marchand, 2008). Nociceptor messages are mainly transmitted by two classes of fibres, the larger myelinated Aδ fibres and the thin unmyelinated C fibres. The nociceptors are frequently referred to by the characteristics of their fibres (Marchand, 2008). The Ab (or Aβ) fibres are principally involved in the conduction of non-nociceptive input, such as vibration, movement, or light touch. The Ab fibres are large myelinated fibres with a rapid conduction velocity (35 m–75 m per second). Besides conducting the non-nociceptive signal, the stimulation of Ab fibres will recruit inhibitory interneurones in the *substantia gelatinosa* of the dorsal horn of the spinal cord, which will inhibit nociceptive input at the same spinal segment.

The Ad fibrers are relatively large myelinated fibres with slower conduction velocity than the Ab fibres, but faster (5 m–30 m per second) than the C fibres. They represent the majority of the myelinated fibres. Two types of Ad fibres exist, depending on the specificity of their responses to different stimulation (Treede *et al.,* 1998). The mechanonociceptors respond preferentially to intense and potentially harmful mechanical stimulation; the polymodal Ad fibers respond to mechanical, thermal, and chemical stimulation. However, the mechanonociceptor Ad fibres also increase their discharge after intense thermal stimulation, a phenomenon known as hyperalgesia. Due to the rapid conduction velocity, the Ad fibres are responsible for the first pain sensation, a rapid pinprick, sharp, and transient sensation (Marchand, 2008). Since their small calibre and lack of myelin, the conduction of the C fibres is relatively slow (0.5m–2 m per second). They represent three quarters of the sensory afferent input and are mostly recruited by nociceptive stimulation. However, they are also involved in non-nociceptive somatosensory information, such as the sensation of pruritus (Stander *et al*., 2003). Most of these fibres are polymodal and they respond to different modalities, including mechanical, thermal, and chemical stimulation (Meyer *et al*., 2006). Typically, nociceptor activity begins well before tissue damage is imminent, but reaches a peak as tissue failure forces (tissue destruction) are approached (Loughner *et al.,* 1997). This feature is important in understanding how injection volume can affect pain upon injection.

2.1.5.2 Peripheral sensitisation

Injury and inflammation of tissue result in profound changes to the chemical environment of the peripheral terminal of nociceptors (Woolf, 2004). Damaged cells

release intracellular contents, such as adenosine triphosphatase and K^+ ions; pH decreases; and cytokines, chemokines, and growth factors, are produced by inflammatory cells recruited to the site of damage (Vadivelu *et al.*, 2009). Some of these factors act directly on the nociceptor terminal to activate it and produce pain (nociceptor activators), and others sensitize the terminal so that it becomes hypersensitive to subsequent stimuli (nociceptor sensitizers). Activation by adenosine triphosphatase of the ligand-gated purinoreceptor enables immediate detection by nociceptors of tissue damage (McCleskey and Gold, 1999). The prostanoid prostaglandin E_2 and nerve growth factor bind to G-protein–coupled prostaglandin E and tyrosine kinase A receptors, respectively to alter the sensitivity of the terminal without producing direct nociceptor activation (Shu and Mendell, 1999). Bradykinin, a peptide produced by kallikrein-mediated cleavage of kininogens, activates and sensitizes the terminal (Walker *et al.*, 1995). Prostanoid production at the site of tissue injury, a major element of the inflammatory reaction, results from generation of arachidonic acid from membrane phospholipids by phospholipase A_2. Cyclooxygenase-2 (COX-2) converts arachidonic acid into prostaglandin H, which is converted into specific prostanoid species, such as prostaglandin E_2, by prostaglandin synthases (Vane *et al.*, 1998).

Activation of adenyl cyclase by prostaglandin E increases levels of cyclic adenosine monophosphate, which activates cyclic adenosine monophosphate–dependent protein kinase A. Release of calcium stores from microsomes in the terminal or calcium entry through channels in the membrane activates the calcium-activated protein kinase C (McCleskey and Gold, 1999). More action potentials than normal are generated in the nociceptor terminal (Gold *et al.*, 1998). Several sensitizers are present (prostaglandin

E2, nerve growth factor, and bradykinin), therefore blocking production of only one of these substances at any time will not eliminate peripheral sensitisation. Peripheral sensitisation decreases the threshold of high-threshold and silent nociceptors and plays a major role in increasing sensitivity to pain at the site of injury or inflammation (Woolf, 2004).

2.1.5.3 Central sensitization

Central and peripheral sensitisations are the major causes of hypersensitivity to pain after injury. Central sensitisation amplifies and facilitates the synaptic transfer from the nociceptor central terminal to dorsal horn neurones (Woolf, 2004). Initially, it is triggered in central neurones by nociceptor input into the spinal cord (Ji *et al.*, 2003). Central sensitisation requires brief but intense nociceptor activity to be initiated. It is produced by sensitized nociceptors during inflammation and by spontaneous ectopic activity, generated in sensory neurones after nerve injury (Vadivelu *et al.*, 2009). Central sensitisation begins with a cascade of events in the dorsal horn of the spinal cord that are triggered by release of transmitters from nociceptor central terminals, leading to alterations in synaptic receptor density, threshold, kinetics, and activation, and, thus, dramatically increasing transmission of pain (Woolf, 2004). One key receptor involved in these changes is the glutamate-activated NMDA receptor (Burke and Shorten, 2009).

During central sensitisation, this receptor is phosphorylated, which increases its distribution from intracellular stores to the synaptic membrane and its responsiveness to glutamate (Costigan *et al.*, 2009). Increased responsiveness to glutamate occurs by removal of the voltage-dependent Mg^{2+} ion block of the NMDA channel and increasing

the time, during which the channel is open (Ossipov *et al.*, 2010). The increase in excitability of the cell means that it can be activated by inputs that are normally sub-threshold and that its response to suprathreshold inputs increases (Costigan *et al*., 2009). The recruitment of subthreshold inputs manifests as a reduced threshold for eliciting pain (allodynia), an exaggerated or amplified response to noxious stimuli (hyperalgesia), and the spread of sensitivity to non-injured areas (secondary hyperalgesia) (Woolf, 2004).

2.1.6 Pain management

Pain management should be a priority in terms of ethics, to ensure animal welfare and, in terms of global medical management, to reduce morbidity and mortality associated with inadequate pain management (Roux, 2010). Management of pain involves a balance between inhibition of pain and minimisation of side-effects; therefore, in developing new analgesic compounds, the effects of treatment on both pain processing and behaviour must be considered (Neubert *et al.*, 2007).

2.2 Oxidative Stress

Oxidative stress refers to an imbalance between reactive species production and antioxidant defences (Halliwell, 2007). It is also a disturbance in the pro-oxidant antioxidant balance in favour of the former, leading to potential damage (Sies, 1991). Halliwell and Whiteman (2004) defined oxidative damage as the biomolecular damage caused by attack of reactive species upon the constituents of living organisms. Oxidative stress is an imbalance between the formation of free radicals and reactive oxygen species on one hand, and antioxidant defence systems on the other, which results in damage to biomolecules such as lipids, proteins and nucleic acids (Simeonova

et al., 2004). This damage may originate from an over-expression of reactive oxygen or nitric oxygen species or from a depletion of the antioxidant systems. Reactive oxygen species are generated during oxygen metabolism in mitochondrial respiratory chains, in addition of haemoglobin and in respiratory burst in granulocytes during phagocytosis (Marks *et al.*, 1996). The production of reactive oxygen species in low concentration is absolutely necessary for some physiological processes such as cell differentiation and proliferation, apoptosis and cell-mediated immunity (Lajarin *et al.*, 1999). However reactive oxygen species also play an important role in the pathogenesis of different diseases and cause cellular destruction by oxidative damage of DNA, proteins, lipids and carbohydrate. They alter the structure and function of key cellular constituents that result in mutation, cell damage and death. Metabolic activity is a primary source of free radicals, which are unavoidable by-products of ATP synthesis. Free radicals and non-free radical pro-oxidants are prone to react with any molecules, causing oxidative damage (Costantini and Verhulst, 2009). Every increase over normal concentrations of reactive oxygen species is connected with dangerous toxic reactions, which directly affect the cell framework and function (Gadjeva *et al.*, 2000). Increased levels of oxidative damage can result not only from oxidative stress, but also from failure of repair or replacement systems; for example, a rise in levels of biomarkers of oxidative damage. Many exogenous factors such as increased or decreased partial pressure of oxygen in blood, pain, chemicals and drugs could be sources of reactive oxygen species (Hug *et al.*, 1997).

Membrane peroxidation is regarded as a major mechanism whereby free radicals damage biological tissue (Halliwell and Gutteridge, 1990). Radical-mediated

peroxidation of erythrocyte membrane leads to haemolysis and can be induced by a variety of reactive oxygen species, such as hydrogen peroxide, superoxide anion and peroxyl radicals derived from thermal decomposition of water-soluble and lipid-soluble azo compounds (Niki, 1990).

2.2.1 Pain as a stress factor

Reactive oxygen species have been implicated in many degenerative neurological conditions such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (Hensley *et al.*, 1997; Lewen *et al.*, 2000) trauma (Rokyta *et al.*, 2003) and other brain dysfunctions, ranging from brain injury to ageing (Jenner, 1994). In addition, oxidative stress is an important determinant of degenerative and painful pathological conditions in peripheral nerve fibres (Wagner, 1998).

Wang *et al.* (2004) reported that superoxide, a type of reactive oxygen species, mediates pain that accompanies inflammation. Their experimental results indicate that an application of synthetic compound (M40403) that mimics the enzymatic function of superoxide dismutase (SOD) prevented the development of inflammation and hyperalgesia after injection of an inflammatory agent into the rat paw. Furthermore, the SOD mimetic given after induction of inflammation also reduces the developed hyperalgesia. These results suggest that superoxide participates in the nociceptive signalling cascade and in nociceptive sensitisation. Wang *et al.* (2004) suggest also that superoxide promotes cytokine production in the periphery and induces central sensitisation.

Other reactive oxygen-containing species may play important roles in pain, and there may be painful conditions other than inflammatory pain in which reactive oxygen

species participate (Chung, 2004). Nitric oxide, a form of reactive oxygen species, has been studied in pain mechanism. In the study, nitric oxide synthase, the enzyme that produces nitric oxide, reduced both the increased concentration of nitric oxide and some of the pain related behaviours that follow inflammation or neuropathic lesions (Haley *et al.,* 1992; Salter *et al.,* 1996). Painful stimulation increases lipoperoxidation (Rokyta *et al.,* 2003).

2.2.2 Anaesthesia as a stress factor

Anaesthesia is the elimination of sensation by controlled reversible suppression of nervous function (Egwu *et al*., 2011). It is a state of unconsciousness produced by a process of controlled reversible drug-induced intoxication of the central nervous system in which the patient neither perceives nor recalls noxious stimuli (Hall and Clarke, 1991).

The impact of anaesthesia in the development of oxidative stress and consequent post anaesthetic complications is poorly investigated in veterinary medicine (Simeonova *et al.,* 2004). Anaesthesia has been observed to produce stress response in goats (Eissa *et al*., 1990, Sanhouri *et al*., 1990).

It has been reported that oxidative activity occurs under sevoflurane anaesthesia and it was compared with the oxidative activity, produced under propofol anaesthesia (Allaouchiche *et al.,* 2001, Tsuchiya *et al.,* 2008). In these studies, sevoflurane produced oxidative activity in higher levels than propofol. Sevoflurane increases intracellular reactive oxygen species levels in neutrophils and phagocytic leucocytes (Wong *et al.,* 2006). Other studies have found increased radical generation during general anaesthesia with halothane (Neri *et al*., 1993) and isoflurane (Naziroglu and

Gunay, 1999). The study revealed that blood malonaldehyde concentration was high during standard halothane anaesthesia. Anaesthesia provokes a stress reaction accompanied by neurohumoural and metabolic responses with the purpose of maintaining homeostasis.

The mechanisms of lipid peroxidation in halothane anaesthesia are complex. On one hand the hypoventilation and the hypoxia usually accompanying halothane anaesthesia could be responsible for reactive oxygen species generation (El-Bassiouri *et al.,* 1998). The same authors stated that the tissue hypoxia depresses the activity of liver superoxide dismutase – an antioxidant enzyme at the frontline of defence. Hypoxia, however, was not a feature in another study (Simenova, 2004). Hypothermia accompanying anaesthesia can induce oxidative stress and aggravate hypoxia (Dede *et al*., 2002). Simeonova *et al.* (2004) recorded hypothermia in all anaesthesia groups at 120 and 140 minutes, but it only corresponded with the increased malonaldehyde concentrations during deep halothane anaesthesia.

Sub-anaesthetic doses of ketamine have been shown to increase oxidative stress in the brain of rats (Larissa *et al.,* 2009). The results in this study indicated that lipid peroxidation and tissue protein oxidation were affected by varying sub-anaesthetic doses of ketamine in multiple cerebral structures. Additionally, the activities of the antioxidant enzymes catalase and superoxide dismutase were measured and they were also found to alter in most of the structures tested.

2.2.3 Antioxidants

An antioxidant is any substance capable of preventing oxidation (Valko *et al*., 2005). Systemic enzyme and non-enzyme antioxidant defences maintain reactive oxygen

species concentrations within the physiological range (Simeonova *et al*., 2004). Antioxidants involve a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralise free radicals (Jacob, 1995). These components include:

- Nutrient–derived antioxidants like ascorbic acid, tocopherols, tocotrienols and carotenoids (Sies and Stahl, 1995).
- Antioxidant enzymes, including, superoxide dismutase, glutathione peroxidase and glutathione reductase, which catalyze free-radical quenching reactions (Percival, 1998).
- Metal-binding proteins, such as ferritin, lactoferrin, albumin, and ceruloplasmin that sequester free iron and copper ions that are capable of catalyzing oxidative reactions (Percival, 1999).

2.3 General Anaesthetics

General anaesthetics are some of the most widely used and important therapeutic agents **(**Yamakura *et al.,* 2001). General anaesthetic agents are used widely, both in clinical medicine and in neuroscience research. In neuroscience research, anaesthetic agents have been used to identify neuronal substrates that are involved in conscious perception, to explain neural mechanisms of sleep and arousal (Rudolph and Antkowiak, 2004). General anaesthesia is a behavioural state that requires critical degrees of immobility, amnesia, hypnosis/unconsciousness, analgesia, and muscle relaxation (Yamakura *et al.,* 2001). It is a state of unconsciousness and loss of protective reflexes, resulting from the administration of one or more general anaesthetic agents. The main synaptic actions which have been suggested to cause general anaesthesia are increases in the effects of

GABA (at $GABA_A$ receptors), decreases in excitatory amino acid transmission (Sawada and Yamamoto, 1985) and alterations in various ion channels, such as calcium (Twombly *et al*., 1990) and potassium (Nicoll and Madison, 1982). Potentiation of the effects of GABA occurs in many different types of compound, such as barbiturates, steroids (Little *et al*., 2000), inhalational agents (Longon *et al*., 1993) and propofol (Trapani *et al*., 2000).

Anaesthetic drugs used can be divided into intravenous and inhalation (volatile) agents (Digger and Viira, 2003). The term intravenous anaesthetic agents imply inducing anaesthesia by drugs administered intravenously (Lee[2], 2006). Intravenous anaesthesia is now well established in most areas of anaesthetic provision and is the preferred technique (Sneyd, 2004). The phrase "balanced anaesthesia" originated from the co-administration of drugs to produce an "ideal" anaesthetic state. Today, the ideal anaesthetic state implies adequate hypnosis, analgesia, muscle relaxation, and relief from stress (Muir and Yamashita, 2000). The aim of drugs used in sedation is to relieve both pain and anxiety while avoiding cardio-respiratory depression or loss of airway reflexes (Symington and Thakore, 2006). All drugs currently in use for sedation have potential for adverse effects, particularly impaired respiration or circulation. The ideal drug regimen would have a few side-effects, a short duration of action, and acceptable safety margins (Symington and Thakore, 2006).

2.3.1 General anaesthesia in goats

Goats are widely kept in farms and households in Nigeria for the production of meat, milk, skin and fibre (Udegbunam and Adetunji, 2007). Goat keepers often seek

veterinary services which may necessitate the use of anaesthetics, such as rumenotomy, caesarean section, mastectomy and relief of intestinal obstruction (Gray and McDonell, 1986). Generally the danger of regurgitation, bloating and inhalation of ingesta during general anaesthesia is much greater in ruminants compared to other common domestic species (Lee[1], 2006; Caulkett, 2003). However, some procedures, with economic justifications, are better performed under general anesthesia, and with certain precautions, general anesthesia can be carried out safely without complications (Lee[1], 2006). Starving the animals of food and water deprivation prior to anaesthesia for 12-24 hours can prevent or minimize these complications (Caulkett, 2003; Lee[1], 2006; Dzikiti *et a*l., 2011). Standing surgical operation in cattle under sedation plus local anaesthesia is not practicable in sheep and goats due to their small size, therefore these animals must be placed in recumbent positions for major surgeries. Thus, for humane reasons and for optimal technical efficiency, the use of a general anaesthetic agent for small ruminants appears rational (Adetunji and Ogunyemi, 1998). Unlike sheep, goats can be particularly sensitive to stress and pain, therefore it is important to perform procedures under adequate sedation and analgesia (Caulkett, 2003).

2.3.2 Nervous system monitoring during general anaesthesia

Nervous system monitoring during anaesthesia helps to assure that the patient is unconscious, amnesic and adequately analgesic. Central nervous function is evaluated on the basis of muscle reflexes (Kronen, 2007). The palpebral reflex and the tone of the jaw muscles are often used to evaluate a degree of muscle relaxation. The pedal or interdigital reflex test (absent during surgical planes of anaesthesia), is commonly applied to evaluate the adequacy of analgesia, as it tests the complex nociceptive system

reflexes (withdrawal). Many anaesthetists judge adequacy of depth of anaesthesia from a combination of nervous, cardiovascular and respiratory system signs.

2.4 Propofol

Propofol is a popular intravenous anaesthetic drug, used for induction and maintenance of anaesthesia owing to its rapid onset and short duration (Kang *et al*., 2010). Since its introduction in 1986, propofol has been rapidly displacing other anaesthetic techniques (Dutta *et al*., 1997), and propofol has become established for induction as well as maintenance of anaesthesia in veterinary practice (Joubert, 2000). Propofol marketed as Dipirivan® is a phenolic derivative formulated as an oil-in –water emulsion (McKeage and Perry, 2003).

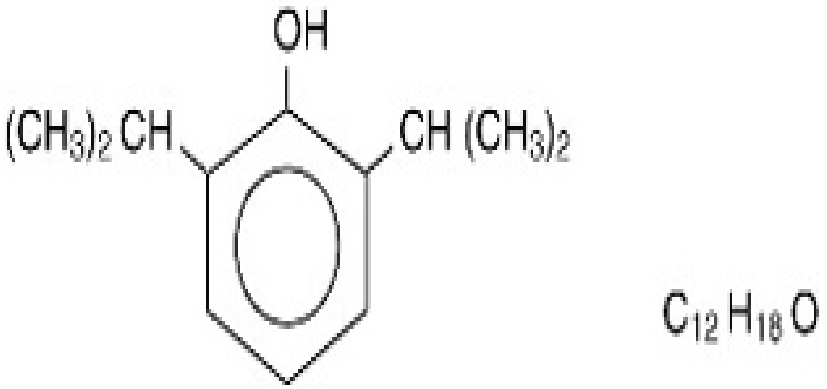


Figure 2.1: Structure of Propofol.

Propofol consists of active ingredient, 2,6-diisopropyl phenol, in addition the formulation also contains soyabean oil, glycerol, egg lecithin, sodium hydrochloride, water and disodium ethylene diaminetetraacetate to reduce the risk of infection (AstraZeneca, 2001) or disodium metabisulphite is added to retard the growth of microorganisms (De Cosmo *et al*., 2005).

Propofol was originally developed in the UK in 1973 using a form solubilised in cremophor. However due to anaphylactic reactions to Cremophor, this formulation was withdrawn from the market and subsequently reformulated as an emulsion of soya oil/propofol mixture in water (Thompson and Goodale, 2000). The general anaesthetic properties of propofol were initially discovered during a screen of 97 alkylphenols in mice and rabbits, following up on the initial observation that 2, 6-diethylphenol possessed potent anaesthetic effects (James and Glen, 1980). Clinical trials with the emulsion formulation of propofol began in 1983 in Europe, and the first commercial launching occurred in New Zealand and the UK in 1986 by Imperial Chemical Industries now ASTRA ZENECA under the brand name Dipirivan. The currently available preparation is 1 % propofol, 10 % soybean oil and 1.2 % purified egg phospholipid (emulsifier), with 2.25 % glycerol as a tonicity–adjusting agent and sodium hydroxide to adjust the pH (De Cosmo *et al.*, 2005). It also contains ethylenediaminetetraacetic acid as an antimicrobial agent. Due to its amnestic effects and appearance as a white liquid, propofol has been humorously dubbed 'milk of amnesia' by medical professionals (Euliano and Gravenstien, 2004). After its launch in 1986, propofol rapidly became the most commonly used intravenous anaesthetic agent, with thiopental, ketamine and etomidate reserved for specific indications (Sneyd, 2004).

2.4.1 Mechanism of action of propofol

There had been a number of efforts to understand the molecular mechanism of action of propofol (Trapani *et al*., 2000). Experimental evidence has shown the ability of propofol to positively modulate the function of $GABA_A$ receptors, a property common to many other general anaesthetics (Krasowski and Harrison, 1999; Trapani *et al*., 2000).

Propofol has been shown in biochemical, molecular and electrophysiological assays to allosterically enhance or potentiate the actions of GABA at the $GABA_A$ receptors (Hales and Lambert, 1992; Irifune *et al*., 2003) and prolong inhibitory postsynaptic currents mediated by $GABA_A$ receptors (Orser *et al*., 1994). Propofol can also open the $GABA_A$ receptor ion channel in the absence of GABA (direct activation), although this usually occurs at higher concentrations of propofol than necessary to potentiate submaximal receptor responses to GABA (Hales and Lambert, 1992; Hara *et al*., 1993; Jones *et al*., 1995). Propofol is thought to potentiate the binding of GABA to receptor sites in the central nervous system (Symington and Thakore, 2006).

2.4.2 Properties of propofol

2.4.2.1 Effects of propofol on blood pressure, heart rate and its heamodynamics

Induction of anaesthesia with propofol generally causes a decrease in mean arterial pressure (Oztekin *et al*., 2000). Propofol causes a decrease in blood pressure, which is generally dose-dependent (Karski *et al*., 1994) and thought to be due to a combination of decreased systemic vascular resistance and myocardial contractility (Angelini *et al*., 2001). In a study conducted by Roekaerts *et al.* (1993), propofol and midazolam was given to 30 patients following a coronary artery surgery, mean arterial pressure decreased by 15 % in the propofol group compared to 7 % in the midazolam group (Mckaege and Perry, 2003). Propofol also causes a decrease in heart rate (Mckaege and Perry, 2003). Carrasco *et al.* (1998) reported that patients sedated for approximately 14 hours showed a significant ($P < 0.05$) reduction in mean heart rate from base-line (from 110-93 beats/min), whereas patients receiving midazolam showed a slight increase in mean heart rate (from 106-112 beats/min). Tramer *et al.* (1997) suggested that the

decrease in heart rate is due to the inhibitory effect of propofol on baroreflexes and sympathetic activity. In other studies however, propofol was found to increase heart rate (Robinson *et al*., 1994; Shah *et al*., 2011).

Propofol does not alter haematological, fibrinolytic, or hepatic function (De Cosmo *et al.,* 2005), but it tightly binds to erythrocytes and serum albumin. As a result of this property, a prolongation of effect may be expected in case of hypoalbuminaemia, particularly during prolonged administration (Mazoit and Samii, 1999). However, Morey *et al.* (2006) reported that reductions in some haematological indices were observed after propofol induction in dogs. There were decrements in the erythrocyte count, haemoglobin concentration, haematocrit, absolute reticulocyte count and relative reticulocyte count. A decrease in the total concentration of leucocytes also occurred in the study.

2.4.2.2 Effects of propofol on respiratory rate

Propofol is a profound respiratiory depressant, even when used for conscious sedation in sub-anaesthetic doses (De Cosmo *et al.,* 2005). Respiratory depression is not of concern to patients undergoing mechanical ventilation, but it is an important consideration during weaning from ventilation (McMurray, 1991). The respiratory pattern is usually characterized by a decrease in tidal volume and an increase in respiratory rate. Propofol-induced depression of upper airway reflexes can prove helpful during intubation or laryngeal mask placement in the absence of paralysis (De Cosmo *et al.,* 2005). Since propofol is rapidly cleared from the blood, the respiratory depressant effect resolves quickly once the drug is stopped (Barr, 1995). A reduction in peak

inspiratory pressure has been associated with propofol infusions, which suggest a mild bronchodilator effect (Conti *et al.*, 1993).

2.4.2.3 Effects of propofol on the central nervous system

Propofol is a hypnotic agent that provides a dose-dependent suppression of awareness from mild depression of responsiveness to obtundation (De Cosmo *et al.*, 2005). It is a potent anxiolytic and a potent amnestic agent (Veselis *et al*., 2002). Propofol decreases cerebral blood flow and intracranial pressure, and has been used in neurosurgical patients to reduce elevated intracranial pressure. It is highly lipophilic, facilitating rapid penetration of the blood-brain barrier and a fast onset of action (usually within 40 seconds) (McKeage and Perry, 2003).

2.4.2.4 Anticonvulsant properties of propofol

Propofol is a unique, non-barbiturate anaesthetic agent with proven anticonvulsant properties (Dam *et al*., 2003), although the exact anticonvulsant mechanism is unknown (Brown and Levin, 1998). It probably acts by causing a uniform depression of the central nervous system, potentiating GABA-mediated pre- and post-synaptic transmitters, glutamate and aspartate (Dam *et al*., 2003).

Stecker *et al.* (1998) compared propofol with high dose barbiturates in the treatment of *refractory status epilepticus* and they concluded that propofol resulted in a rapid control of *refractory status epilepticus,* compared to high dose barbiturates. Also, recurrent seizures were common with sudden discontinuation of propofol, but not with gradual tapering.

2.4.2.5 Antiemetic properties

Propofol has been shown to have antiemetic effects (Gan *et al*., 1996) at subhypnotic doses after thyroidectomy. However, the mechanism by which propofol acts as an antiemetic is unclear (Ewalenko *et al.,* 1996). Thyroidectomy is a procedure accompanied by intense surgical stimulation of vagal afferents. Low doses of propofol may induce anxiolysis (Cavazzuti *et al.,* 1991), sufficient to modify cortical afferents to the vomiting centre. Propofol has also been shown to possess weak anti-serotonin properties (Barann *et al*., 1993), suggesting a possible effect on the chemoreceptor trigger zone, but not enough to fully explain the efficiency of the drug in emetic syndromes refractory to serotonin antagonist therapy (Scher *et al*., 1992).

2.4.2.6 Metabolism of propofol

Propofol is extensively metabolized via hepatic conjugation and excreted in the urine as sulphate and/or glucoronide conjugates of the parent compound or its hydroxylated metabolite (Propofol glucoronide, quinol glucoronides, and 4-quinol sulphate). The metabolites have a very short half-life (Timpe *et al*., 2006). However, the metabolites of propofol do not contribute to the sedating effect of propofol (Cussonneau *et al.,* 2007). Despite a half-life of 13-44 hours, duration of action is approximately 10 minutes owing to rapid redistribution from the central nervous system tissue to muscle and fat (Symington and Thakore, 2006).

2.4.3 Pain on injection of propofol

Since its introduction into clinical practice, pain on injection of propofol has been noted to occur in about 70-80 % of patients, who received it for induction of anaesthesia, in

the absence of other treatment regimens (Picard and Tramer, 2000). Akeson (2008) noted that there have been more than 400 publications regarding pain on injection of propofol, which has been considered to be the most bothersome memory of the anaesthetic induction experience (Aldrete *et al.,* 2009). Propofol belongs to the group of phenols that can directly irritate the skin, mucous membrane and venous intima. It could immediately stimulate nociceptors and free nerve endings (Ambesh *et al*., 1999).

The nature of pain has been described as extremely sharp, aching or burning (Kang *et al.,* 2010), and it has been ranked as the seventh most important drawback in practice of clinical anaesthesia by American anaesthesiologists (Macario *et al*., 1999). Efforts to avoid such pain have included a myriad of manoeuvres and a combination of medications, with only partial success (Picard and Tramer, 2000). This includes adding lignocaine, applying nitroglycerine ointment to the venopuncture site, diluting the propofol with 5 % dextrose or intralipid (Uzun *et al*., 2008), use of a larger vein (McCulloch and Lees, 1985). Intravenous lignocaine is the most commonly used pretreatment, but has a failure rate of 13 % to 32 % (Scott *et al*., 1988; King *et al*., 1992) and may even be up to 38.3 % (Woon *et al.,* 2005). Some patients do not respond well to the use of lignocaine with propofol and still a good number continue to complain (Massad *et al.,* 2006). Singleton (2000) conducted a study and reported that the incidence of pain in the propofol group (53 %) to that in the lidocaine group was not significantly different ($P > 0.05$).

The mechanism of pain relief by lignocaine has been speculated to be two fold; first by the reduction of propofol concentrations in the aqueous phase and second by lignocaine acting as a stabilizer of kinin cascade (Scott *et al*., 1988). Still the effects of adding

lignocaine are debatable. Eriksson *et al.* (1997) reported a reduction in anaesthetic potency with the dose required to induce hypnosis in rats increasing by 64-68 %. Lilley *et al.* (1996) found that addition of lignocaine to propofol causes a destabilisation of emulsion, although it was unlikely to have clinically significant effects.

Factors that affect pain on injection have been speculated to include:

a) The intrinsic drug property such as emulsion composition, pH of the formulation, temperature, injection volume and osmolarity (Kang *et al*., 2010).

b) The injection procedure itself which could contribute to injection pain (Brazeau *et al*., 1998) such as speed of pushing the medication during injection, propofol concentration in aqueous phase, speed of intravenous carrier fluid, use of local anaesthesia and buffering effect of blood (Scott *et al*., 1988; Klement and Arndt, 1991).

The mechanism of pain following propofol injection had been unclear until only recently (Kang *et al*., 2010). Nakane and Iwama (1999) found a potential mechanism for propofol induced pain, in which the lipid solvent for propofol activates the plasma kallikrein-kinin system and produces bradykinin which modifies the local vein by its vasodilatory and hyperpermeability actions. This modification in the local vein increases contacts between the aqueous phase propofol and free nerve endings, resulting in aggravation of pain on injection (Nicholas *et al*., 2005). Iwana *et al* (1998) also suggested kinin generation as a cause of pain since nafamostat mesilate, a synthetic serine protease inhibitor which inhibits kallikrein activity, was able to inhibit pain on injection.

Wong and Cheong (2001) postulated that pain could be either due to the direct irritant effect of propofol giving rise to an immediate sensation of pain, or an indirect effect via the release of mediators leading to a delayed onset. The latter theory involves the release of kininogens when propofol comes in contact with the vascular endothelium (Scott *et al*., 1988).

2.4.4 Propofol in goats

After induction of anaesthesia, a rapid recovery is desirable in ruminants because extended recumbency enhances the risk of tympany and hypoxaemia, and rapid recovery of swallowing reflex minimizes the risk of aspiration of regurgitated rumen contents (Prassinos *et al*., 2005). The rapid onset and short duration of action, with rapid recoveries make propofol useful in ruminants, in which these features are particularly desirable. Reports on the use of propofol for induction and maintenance of anaesthesia have indicated its suitability in goats (Nolan *et al*., 1991; Reid *et al*., 1993; Pablo *et al*., 1997; Carrol *et al*., 1998) and even sheep (Correia *et al.,* 1996), where it was used at 4 mg/kg but with halothane as maintenance and acepromazine as pre-medication in goats and sheep, respectively.

Induction time, quality of induction and recovery and the incidence of side-effects such as apnoea, regurgitation, hypersalivation, tympany, and the recovery time have been found to be best in goats using propofol, when compared to other anaesthetic agents such as ketamine and thiopentone (Prassinos *et al*., 2005). Ketamine has been shown to cause increased tonic-clonic muscle activity when used alone in ruminants (Hall *et al*., 2001). For this reason, it must be combined with sedative tranquillizers such as diazepam and or xylazine. Ruminants, however, are not always sedated before induction

of anaesthesia in order to avoid prolonged recoveries and, or an increased risk of regurgitation (Prassinos *et al*., 2005). Propofol can be used in small ruminants for the induction and maintenance of general anesthesia. It provides rapid induction and is very rapidly eliminated from the plasma, 5-6 mg/kg IV produces 4-9 minutes of anesthesia (Lee, 2006).

2.4.5 Propofol as an antioxidant

Free-radical scavengers or antioxidants represent an important component of the body's defence against oxygen-centred, free-radical-mediated injuries, thus boosting these defences by pharmacological means may emerge as an important therapeutic goal (Volti *et al*., 2006). Weir *et al* (1989) observed for the first time that propofol attenuated reperfusion injury in the cerebral cortex, thus pointing out the antioxidant properties of this compound. Propofol contains a phenolic O-H group (Aarts *et al.,* 1995) and many polyphenols have antioxidant properties (Volti *et al.,* 2006). Murphy *et al.* (1992) demonstrated that propofol reacts with peroxynitrite, leading to the formation of a more stable propofol-derived phenoxyl radical and had, therefore, been hypothesized to be a peroxynitrite scavenger. It has even been reported that propofol could replace α-tocopherol as an antioxidant (Aarts *et al*., 1995) as it chemically resembles α-tocopherol, which also has a phenolic hydroxyl group. It has been shown that introducing propofol into α-tocopherol–depleted microsomes restores the GSH-dependent protection. As such, propofol is a potent inhibitor of lipid peroxidation (Murphy *et al.,* 1992). Murphy *et al.* (1996) also carried out an *in vitro* study to determine the effect of propofol on free-radical-mediated oxidative stress of

erythrocytes and concluded that propofol is capable of protecting erythrocytes against oxidant stress.

The phenolic hydroxyl group is responsible for the antioxidant properties of propofol as found in various *in vitro* (Murphy *et al*., 1993, 1992) and *in vivo* experiments (Stratford and Murphy, 1998; Zhang *et al*., 2004; Ma *et al*., 2009). Due to its good lipid solubility, propofol is able to penetrate into membranes. Green *et al*. (1994) evaluated the specificity of propofol as a scavenger of oxygen and organic radical species, and showed that propofol preferentially scavenges organoradical species. This is because propofol was able to reduce significantly riboflavin radicals and the formation of malonaldehyde degradation products, generated from lipid hydroperoxides of arachidonic acid.

2.5 Ascorbic Acid

Ascorbic acid (vitamin C) is a water soluble micronutrient required for multiple biological functions (Duarte and Lunec, 2005). The structure of ascorbic acid is shown in Fig 2.2. It is one of the most important water soluble vitamins and is unique among vitamins for several reasons. It is present in various foods, particularly of plant origin, in quantities (typically 10-100 mg/100 g) higher than those of other vitamins (Padayatty *et al*., 2003). This is related to the fact that it is formed from sugars, which are abundant compounds. It is also one of the rare compounds containing a hydroxyl group that is so acidic as to be completely dissociated at neutral pH (carbon-3-hydroxyl pka=4.2) (Linster and Schaftingen, 2007). Ascorbic acid comprises two conjugated double bonds and a resonance form can be written for the deprotonated monoanionic form.

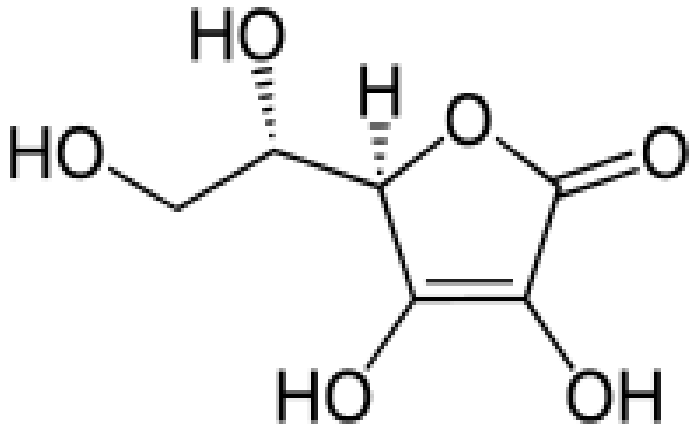


Figure 2.2: Structure of Ascorbic Acid

Most plants and animals synthesize ascorbic acid in the form of D-glucose or D-galactose and a majority of animals produce relatively high levels of ascorbic acid from glucose in the liver (Naidu, 2003). However, guinea pigs, fruit-eating bats, apes and humans cannot synthesize ascorbic acid due to the absence of the enzyme L-gulonolactone oxidase (Aguirre and May, 2008). Ascorbic acid is a labile molecule; it may be lost from foods during cooking/processing, even though it has the ability to preserve foods by virtue of its reducing property. The major metabolites of ascorbic acid in human are dehydroascorbic acid, 2,3-diketogulonic acid and oxalic acid. The main route of elimination of AA and its metabolites is through urine. It is excreted unchanged when high doses are consumed (Naidu, 2003).

2.5.1 Ascorbic acid as an antioxidant

All known physiological and biochemical actions of ascorbic acid are due to its action as an electron donor or a reducing agent (Sarkar *et al*., 2009; Schlueter and Johnston, 2011). As an antioxidant, its primary role is to neutralize free radicals (Iqbal *et al*., 2004; Aguirre and May 2008). Ascorbic acid is an antioxidant because of the shared

ability of the hydroxyl groups on carbons -2 and -3 to donate a hydrogen atom to a variety of oxidants, including nitrogen and oxygen-based free radicals, peroxides and superoxide (Buettner, 1993).

Ascorbic acid acts as a potent water-soluble antioxidant in biological fluids by scavenging physiologically relevant reactive oxygen and nitrogen species (Halliwell, 1996). These include free radicals such as hydroxyl radicals, aqueous peroxyl radicals, superoxide anion, nitrogen dioxide, and non-radical species such as hypochlorous acid, ozone, singlet oxygen, nitrosating species (N_2O_3/N_2O_4), nitroxide, and peroxynitrite (Carr and Frei, 1999). Since Ascorbic acid is water-soluble, it combats free-radical damages within and outside the cell (Iqbal *et al*., 2004). It protects the DNA of cells from damage caused by free radicals and mutagens to prevent harmful genetic alterations within cells, and it protects lymphocytes from mutation (Gaby and Singh, 1991). In addition to scavenging reactive oxygen and nitrogen species, ascorbic acid can regenerate other small molecule antioxidants, such as α-tocopherol, glutathione (GSH), urate, and β-carotene, from their respective radical species (Carr and Frei, 1999; Aguirre and May, 2008).

2.5.2 Physiological functions of ascorbic acid

2.5.2.1 Ascorbic acid and collagen production

One important function of ascorbic acid is in the formation and maintenance of collagen (Iqbal *et al*., 2004). Ascorbic acid acts as a co-factor during many steps of the process and without sufficient levels of ascorbic acid, collagen formation is disrupted. This disruption leads to a variety of disorders such as scurvy- a disease where the body cannot produce collagen and, as a result, essentially falls apart as these support

structures (collagen) deteriorate (Sharma *et al.,* 2008). Collagen is the major protein component of connective tissue and as much as 60 % of the total body protein in mammals is collagen (Oguntibeju, 2008).

Collagen protein requires ascorbic acid for hydroxylation, a process that allows the molecule to achieve the best configuration and prevents collagen from becoming weak and susceptible to damage (Harrison and May, 2009). There is also evidence that ascorbic acid increases the level of pro-collagen messenger RNA (Sharma *et al.*, 2008). Collagen subunits are formed within the fibroblasts as pro-collagen which are excreted into extracellular spaces, and ascorbic acid is required to export the pro-collagen molecules out of the cell (Iqbal *et al.*, 2004). Collagen synthesis occurs continuously throughout life to repair and replace damaged collagen tissue or build new cellular structures (Sharma *et al.,* 2008). Collagen contains large amounts of two amino acids that are not inserted directly by ribosomes - hydroxyproline and hydroxylysine. They are derived from proline and lysine in enzymatic processes of post-translational modification, for which ascorbic acid is required (Oguntibeju, 2008). Thus ascorbic acid deficiency may cause scurvy, a disease that can lead to loss of teeth and easy bruising caused by reduction in strength of connective tissue due to lack of collagen or defective collagen (Sharma *et al.,* 2008).

Ascorbic acid also plays a critical role in wound repair and healing/regeneration process as it stimulates collagen synthesis (Naidu, 2003). Adequate supply of ascorbic acid is necessary for normal healing process, especially for post-operative patients. This is

because rapid utilisation of ascorbic acid occurs during collagen synthesis at the site of wound (Sharma *et al.*, 2008) during the post-operative period (Naidu, 2003).

2.5.2.2 Ascorbic acid and neurotransmission

The non-antioxidant functions of ascorbate in the brain and neural-derived tissue centre on neurotransmitters as it is well established that ascorbate is essential for catecholamine biosynthesis in neural tissues, serving as a cofactor for dopamine β-hydroxylase in the conversion of dopamine to noradrenaline (Harrison and May, 2009). Ascorbate has been proposed to function as a neuromodulator of both dopamine and glutamate-mediated neurotransmission (Grunewald, 1993; Rebec and Pierce, 1994). The mechanisms by which ascorbate affects neuronal transmission has not been established, but may relate in part to redox changes in the N-methyl-D-aspartate (NMDA) receptor. This is because ascorbate has been shown to protect neurones from excitotoxicity, induced by activation of the NMDA receptor, and it prevented glutamate-induced cell damage and death in cultured cerebellar granule cells (Ciani *et al.*, 1996).

There is evidence that ascorbate is involved in the regulation of both acetylcholine and catecholamine release from synaptic vesicles (Harrison and May, 2009). In addition to the effects of ascorbate on neurotransmitters, ascorbate has been shown to be involved in neuropeptide amidation (Eipper *et al.*, 1983) and release in the brain (Glembotski, 1987).

Ascorbic acid acts as an endogenous agent, capable of potentiating GABAergic neurotransmission in the CNS (Calero *et al.*, 2011). Increased concentrations of extracellular ascorbic acid can potentiate $GABA_C$- and $GABA_A$- mediated currents,

enhancing local inhibition. Redox modulation of $GABA_A$ receptors has been demonstrated in heterologous systems and in different areas of the CNS, including the retina (Amato *et al*., 1999).

2.5.3 Intravenous use of ascorbic acid

Ascorbic acid plasma concentrations are tightly controlled when the vitamin is taken orally, even at the highest tolerated amounts (Padayatty *et al.*, 2004). By contrast, intravenous administration bypasses tight control and results in concentrations as much as 70-fold higher than those achieved by maximum oral consumption. Subjects consuming 200-300 mg per day of ascorbic acid in five or more daily servings of fruits and vegetables were reported to have a fasting steady state plasma concentrations of about 70-80 µmol/L (Levine *et al*., 2001). Even with maximally tolerated oral doses of 3 g every 4 hours, peak plasma concentrations are estimated not to exceed 220 µmol/L (Padayatty *et al*., 2004).

Intravenous administration of ascorbic acid bypasses tight control for several hours, until homeostasis is restored by renal excretion. Depending on the dose and infusion rate, peak plasma concentrations obtained intravenously are estimated to reach 14,000 µmol/L and concentrations above 2000 µmol/L may persist for several hours (Padayatty *et al.*, 2006). Intravenous ascorbic acid is widely used by complementary and alternative medicine practitioners for a variety of conditions, including infection and fatigue (Padayatty *et al.*, 2010). Apart from the known adverse effects of ascorbic acid (the potential for renal stone formation and for haemolysis in glucose-6-phosphate dehydrogenase deficiency), intravenous administration of ascorbic acid by these

practitioners, which averaged 28 g every 4 days for 22 treatments, was evidently safe and well tolerated (Schlueter and Johnston, 2011). Most of the positive effects of ascorbate follow parenteral administration of the vitamin because much higher effective doses can be given by this route compared to oral administration (Harrison and May, 2009).

2.5.4 Ascorbic acid as an ergogenic aid

Nutritional ergogenic aids are primarily aimed at enhancing performance by either directly affecting energy metabolism in the muscle or stimulating the CNS (Gonzalez *et al.,* 2005). Ascorbic acid has been found to be a safe, non-toxic and effective ergogenic alternative (Gonzalez *et al*., 2005). Ascorbic acid may have been neglected as an ergogenic aid; mainly because of the lack of knowledge of ascorbates' biochemical and physiological functions, when given in large doses especially by intravenous route. Ascorbic acid is distributed in varying concentrations throughout the body and is involved in a variety of metabolic reactions, related to exercise such as synthesis and activation of neuropeptides, formation and repair of collagen and synthesis of carnithine (Peak, 2003).

Ascorbic acid protects muscle cells from harmful effects of highly reactive free radicals, produced when the rate of oxygen consumption is substantially increased during strenuous exercise (Kanter, 1995). Exercise may result in damage to the muscle structure and post exercise soreness; this peaks one to three days after exercise and is referred to as delayed onset muscle soreness (DOMS). The pain associated with this condition has been reported to be significantly reduced by vitamin C at 200 mg

(Kaminski and Boal, 1992). Kanter (1995) also reported with preliminary evidence that ascorbic acid may benefit muscle function and reduce plasma levels of malondialdehyde, a secondary product of lipid peroxidation in addition to diminishing muscle soreness. In his work, it was suggested that reduction in inflammation may explain the decreased soreness present in the ascorbic acid supplement group.

Reduction in plasma concentration of pro-inflammatory cytokines such as interleukin-6, tumour necrosis factor-α and interleukin-8 have been reported following high-dose oral supplementation of 1500 mg ascorbic acid (Nieman *et al.,* 2000). It was suggested that high doses of vitamin C may also reduce cortisol (Peters *et al*., 2001; Brode *et al*., 2002) as athletes supplemented orally with 500-1500 mg of the vitamin in the week before and on the day of a race significantly attenuated post-exercise serum cortisol responses (Peters *et al*., 2001). It is well documented that cortisol production increases in response to acute stress (Van Uum *et al*., 2008). Of the many physiological changes that emerge in response to stressors, one of the most fundamental, and most frequently studied, is the increase of cortisol concentrations in blood, saliva or urine that accompanies stressful situations. Increased secretion of cortisol by the adrenal cortex is a fundamental feature of the stress response (Michaud *et al*., 2008). High doses of ascorbic acid may inhibit the synthesis of cortisol possibly through a pro-oxidant effect (Gonzalez *et al.,* 2002) under certain conditions. Peak (2003) stated that declines in plasma ascorbic acid interestingly corresponds to a rapid and dramatic increase in plasma cortisol and ACTH concentrations. Both cortisol and ascorbic acid have a regulatory influence on gluconeogenesis, an important energy mechanism (Gonzalez *et al.,* 2005).

2.5.5 Role of ascorbic acid in pain relief

Ascorbic acid has been reported to exert a substantial analgesic effect at pharmacological doses (Werbach, 2000). In a double-blind crossover study, supplementation of normal volunteers with 1 gram ascorbic acid three times daily reduced delayed-onset muscle soreness following strenuous exercise (Kaminski and Boal, 1992). Also under double blind conditions, severely ill cancer patients receiving 10 g of ascorbic acid daily experienced a significant reduction in pain (Creagen *et al*., 1979), while in an open trial 10 g of ascorbic acid daily reduced the sensitivity of teeth to air and water (Lytle, 1988).

Ascorbic acid has also been reported as effective for the treatment of neuralgia (Chen *et al.*, 2006). In this study, a 78-year-old male, who suffered from intractable T3-6 postherpetic neuralgia, with constant aching pain and intermittent, spontaneous, shooting pain over his right anterior chest and upper back, was treated with intravenous ascorbic acid at 2.5g daily for three days and his pain was resolved within a week.

Ascorbic acid scavenges reactive oxygen species, and has been shown to play a crucial role in neuropathic pain (Kim *et al.*, 2004). The mechanism by which ascorbic acid acts has been thought to be by enhancing adenyl cyclase–cAMP and also by augmenting the production and release of β-endorphins from the hypothalamic neurones in rats (Yang *et al*., 1996). β-endorphin appears to be effective in alleviating neuropathic pain (Wu *et al.*, 2004). Hanck and Weiser (1985) reported a dose-dependent pain reduction by oral administration of ascorbic acid in rats. Ascorbic acid is helpful in the relief of back pain and pain from inflamed vertebral discs (Iqbal *et al*., 2004). A plant called the rose hip,

high in ascorbic acid levels have been found to relieve pain in patients with osteoarthritis, and the relief obtained is comparable with that of non-steroidal anti-inflammatory drugs and acetylsalicylic acid (Winther *et al.,* 1999).

2.5.6 Toxicity and overdose of ascorbic acid

Ascorbic acid generally exhibits low toxicity (Robert, 1996). The LD_{50} is generally accepted to be 11,900 mg/kg in rat populations (Safety (MSDS), 2005). It is likely that high ascorbic acid intakes have low toxicity, except under certain conditions (Padayatty *et al*., 2006). Intravenous haemolysis was reported after massive ascorbic acid administration in people with glucose-6-phosphate dehydrogenase deficiency (Levine *et al.,* 1999). Ascorbic acid enhances iron absorption (Fleming *et al*., 2002). Iron poisoning can become an issue to people with rare iron overload disorders such as haemochromatosis, a genetic condition that results in inadequate levels of the enzyme glucose-6-phosphate dehydrogenase (G6PD). Thus deficiency causes sufferers to develop haemolytic anaemia after ingesting specific oxidizing substances such as very large doses of ascorbic acid (Cook and Reddy, 2001). Single doses of ascorbic acid greater than 1000 mg/day can cause gastro-intestinal distress, nausea, and osmotic diarrhoea. This is because the body attempts to rid itself of the high intraluminal concentration of ascorbic acid. The risk for kidney stones does increase also in individuals supplementing ascorbic acid (Schlueter and Johnston, 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Animals

Twenty four healthy Red Sokoto goats of both sexes, aged between 1-2 years and weighing about 10 - 20 kg were obtained from a livestock market in Sabon-Gari Local Government Area of Kaduna State. They were housed in the Research Goat Pen of the Department of Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The goats were pre-conditioned for a period of four weeks before the commencement of the experiment, during which prophylactic treatment with anthelmintic and ectoparasiticide was carried out. Albendazole and cypermetrin were administered orally and topically at a dose rate of 7.5 mg/kg and 1 ml/kg, respectively. The goats were fed on a commercial concentrate ration, and water was provided *ad libitum* throughout the study period.

The experiment was conducted at the Research Laboratory of the Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

3.2 Anaesthetic Protocol

Prior to anaesthetic induction, the goats were fasted from food and water for 24 and 12 h, respectively. They were weighed individually and divided into four groups. Group I (VC) was given ascorbic acid (Philo VC; Greenfield Pharmaceutical Jiang Su, China) intravenously at a dose rate of 200 mg/kg. The intravenous administration of drugs was done using the jugular vein with 21 gauge needles. Group II animals (PFol) were

administered with propofol (Dipirivan; AstraZeneca, Place Renault, Milano, Italy. 1 % Emulsion) intravenously at a dose rate of 5 mg/kg. Group III (VC200+Pfol) was given a combination of propofol (5 mg/kg) and ascorbic acid at a dose rate of 200 mg/kg. Group IV (VC300+PFol) was administered with ascorbic acid at a dose rate of 300 mg/kg and propofol (5 mg/kg). Ascorbic acid was administered 30 s prior to propofol injection. Time of anaesthetic administration was recorded. This was used as reference to describe the changes in various reflexes over time of induction.

The time taken for onset of induction, duration of anaesthesia and recovery time of the reflexes from anaesthetic condition to normal reflexes and complete recovery from anaesthesia were recorded (Khan *et al*., 2004)

3.3 Physiological Parameters

Physiological parameters of rectal temperature (RT), respiratory rate (RR) and heart rate (HR) were recorded using standard methods (Ghurashi *et al.,* 2009). Briefly, the RT which was recorded as an indicator of the body temperature was measured using a digital clinical thermometer (The Hartman's Company, PLC, England). The thermometer was inserted 5 cm deep into the rectum of each goat via the anus until an alarm sound was heard, indicating the end of the reading. The RR and HR were recorded using a hand-held stethoscope (3M Littmann, USA). The readings were taken prior to induction of anaesthesia, and continued throughout the induction period for every 5 minutes until full recovery was attained.

3.4 Neurobehavioural Study

All behavioural events were recorded for critical examination using a video recorder (Kodak C813, China). The reflex, behavioural and pain studies were carried out as indicated below:

3.4.1 Evaluation of reflexes

The effect of propofol on various body reflexes was studied as described by Khan *et al.* (2004), Kronen (2007) and Ghurashi *et al.* (2009). Reflexes evaluated were the ocular reflex (palpebral reflex), jaw tone, toe-pinch test (pedal reflex), and swallowing reflex (Prassinos *et al.*, 2005). Briefly, the ocular reflex was elicited by touching the cornea of the eye, while the jaw tone was monitored by opening the mouth of the goats. The pedal reflex was induced by applying pressure on the digits, and the swallowing reflex was elicited by applying pressure on the tongue of the goat. The reflexes were used to assess anaesthetic depth. The animals were monitored closely throughout the period of anaesthesia and observations were recorded. Time of abolishment and return of reflexes were also recorded.

3.4.2 Behavioural and pain study

The pain scoring system was carried out as described by Carroll *et al.* (2001), but with little modifications. The pain scores used were as follows: 3 = fractious (jumping and vocalisation); 2 = stamping of feet, fidgeting and teeth grinding; 1 = standing still or laying down, but eyes wide open; 0 = relaxed normal posture. Behaviour and all events observed from injection to complete recovery of the goats were noted and recorded using the video recorder. In addition to the pain score, other parameters studied were: a)

Time taken (onset) for induction of anaesthesia and b) Duration of anaesthesia (Egwu *et al.*, 2011)

3.5 Haematological Parameters

Blood samples (5 ml) were obtained from each goat just before and immediately after anaesthetic induction for haematological analyses. The haematological parameters analyzed were: leucocyte count, packed cell volume (PCV), haemoglobin concentration (Hb), erthrocyte count (RBC), and erythrocyte osmotic fragility (EOF). Each blood sample was collected and placed in a sample bottle containing anticoagulant EDTA at the rate of 1 mg/1 ml of blood.

3.5.1 Determination of blood cell counts and packed cell volume

The values of WBC, PCV, RBC, and Hb were analyzed using Abbott Haematological Analyzer Cell-Dyn 1700 (Abbott Laboratories, Abbott Park, Illinois, U.S.A.). Counting of blood cells is based on the volumetric impedance method that directly measures haematological values as described by Petani *et al.* (1997).

3.5.2 Erythrocyte osmotic fragility

Erythrocyte osmotic fragility was determined as described by Oyewale (1993). Briefly, sodium chloride stock solution was prepared in volumes of 500 ml for each test in concentrations, ranging from 0.0% to 0.9%. A set of 10 test tubes were used, and each tube contained 10 ml of the corresponding NaCl concentration from the stock solution. The test tubes were then labelled with the corresponding concentrations and arranged serially in a rack of 10 test tubes. One ml pipette was then used to transfer 0.02 ml of each blood sample into each of the 10 test tubes in a set, after which the contents of the

tubes were mixed by gentle inversion and allowed to stand at room temperature for 30 minutes. Thereafter, the contents of the test tubes were centrifuged at 1000 g for 10 minutes. The supernatant was transferred into a glass cuvette. The concentration of haemoglobin in the supernatant was then measured at a wavelength of 540 nm using a spectrophotometer (Jenway, 645, Japan) by reading the absorbance. The percent haemolysis was calculated based on the following formula (Faulkner and King, 1970):

$$\text{Percent Haemolysis} = \frac{\text{Optical density of test}}{\text{Optical density of standard (distilled water)}} \times 100$$

3.6 Biochemical Parameters

Blood samples for serum electrolytes and liver enzymes were collected in anticoagulant free sample bottles and allowed to clot. The clotted blood was centrifuged at 1000 g for 10 min, and the serum obtained was evaluated for the concentration of albumin, total protein, urea-nitrogen and globulin. The activities of the enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), and concentration of electrolytes (Cl^-, Mg^{2+}, Na^+, K^+, and HCO_3^-) were determined. All the parameters were assayed for using an auto-analyzer (Bayer Express Plus, Bayer, Germany, serial number: 15950).

3.7 Determination of Superoxide Dismutase, and Glutathione peroxidase.

3.7.1 Superoxide dismutase activity

Activity of SOD in the goat serum was determined using NWLSS SOD assay kit (Product

NWK-SOD02, Specificity: Cu/Zn, Mn and Fe superoxide dismutase, sensitivity: 5 U/mL).

The assay kit is based on the principle of superoxide inhibition of autooxidation of hematoxylin as described by Martin *et al*. (1987).

3.7.2 Glutathione peroxidase activity

The activity of GPx was assessed using NWLSSTM cGPx (GPx1) ELISA assay kit (Product NWKGPX02, Specificity: glutathione peroxidase, sensitivity: 12.5 pg/ml). The NWLSS™ cGPx assay is based on a sandwich enzyme-linked immunosorbent assay (ELISA), where sample GPx concentration is determined by comparing the 450 nm absorbance of sample wells to the absorbance of known standards (Takebe, 2002).

3.8 Data Analysis

Data obtained were analyzed using Paired *t*-test and one-way analysis of variance with Tukey's multiple comparison post-hoc test. The program used for the analysis was GraphPad prism version 4.0 (from GraphPad Software, San Diego, California, U.S.A.). Values were expressed as mean ± standard error of the mean (Mean ± SEM). The pain score was expressed as percentages. Values of $P < 0.05$ were considered significant.

CHAPTER FOUR

RESULTS

4.1 Effect of Ascorbic Acid and Propofol on Onset and Duration of Anaesthesia

4.1.1 Effect of ascorbic acid and propofol on onset of anaesthesia

The PFol group showed a longer onset (4.6 ± 0.68 s) of anaesthesia compared to the VC300+PFol (2.0 ± 0.22 s) and VC200+PFol (2.57 ± 0.29 s). There was no significant ($P > 0.05$) difference between VC200+PFol and VC300+PFol in the onset of anaesthesia. However, VC200+PFol had a longer onset (2.57 ± 0.29 s) of anaesthesia than the VC300+PFol group (2.0 ± 0.22 s) (Fig. 4.1).

4.1.2 Effect of ascorbic acid and propofol on duration of anaesthesia

The VC300+PFol group had the longest duration of 27.29 ± 4.11 min, and this value was significantly ($P < 0.05$) higher than that of the VC200+PFol (15.29 ± 1.19 min). The PFol group showed the lowest duration (10.6 ± 2.23 min) of anaesthetic induction. There was no significant ($P > 0.05$) difference between the duration of anaesthesia in the VC200+PFol and Pfol groups (Fig. 4.2).

4.2 Effect of Ascorbic acid and Propofol on Pain Score

Goats (57.1 %) in group IV had a pain score of 0, which was significantly ($P < 0.05$) greater when compared to the 0 % obtained in goats in group II, having a score of 0 (Table 4.1). However, 40 % of the goats in the propofol group had a score of 3, which was significantly greater than the 0 % recorded in goats in group IV. 60 % of the goats

in group II scored 2; while 14.3 % of goats administered with AA prior to propofol injection had a score of 2.

4.3 Effect of Ascorbic Acid and Propofol on Physiological Parameters

4.3.1 Effect of ascorbic acid and propofol anaesthesia on heart rate

There was no significant ($P > 0.05$) difference in heart rate within the groups. However, the VC group showed a 22.07 % decrease in heart rate after administration of ascorbic acid. The PFol group also had a decrease (1.85 %) in heart rate during induction, but it increased (16.23 %) again after induction. The VC200+PFol and VC300+Pfol groups both had non-significant increases in heart rate during induction. The heart rate in the VC200+PFol group rose further (9.76 %) after induction but that in the VC300+Pfol group decreased after anaesthetic induction (Fig. 4.3).

4.3.2 Effect of ascorbic acid and propofol anaesthesia on respiratory rate

There was a significant ($P < 0.05$) decrease in the respiratory rate in the VC300+Pfol group during (21.1 ± 1.68 breaths/min) induction and a significant ($P < 0.05$) increase in respiratory rate after (25.14 ± 0.73 breaths/min) induction. The VC group had a non-significant ($P > 0.05$) increase (3.03 %) in respiratory rate after administration of ascorbic acid. The PFol group had a steady decrease in respiratory rate during (21.4 %) and after (16.4 %) induction though not significant while the VC200+PFol group also had a steady decrease (25.5 %) during and after (2.1 %) anaesthetic induction (Fig 4.4).

4.3.3 Effect of ascorbic acid and propofol anaesthesia on rectal temperature

There was no significant ($P > 0.05$) difference in rectal temperature within the groups before, during and after anaesthetic induction. The VC group had a 0.79% increase in rectal temperature after administration of ascorbic acid when compared to the value obtained before the administration. Propofol when given alone did not change ($P > 0.05$) the rectal temperature during or after anaesthesia of the goats. Administration of ascorbic acid prior to propofol anaesthesia also did not alter the rectal temperature in the goats (Fig. 4.5).

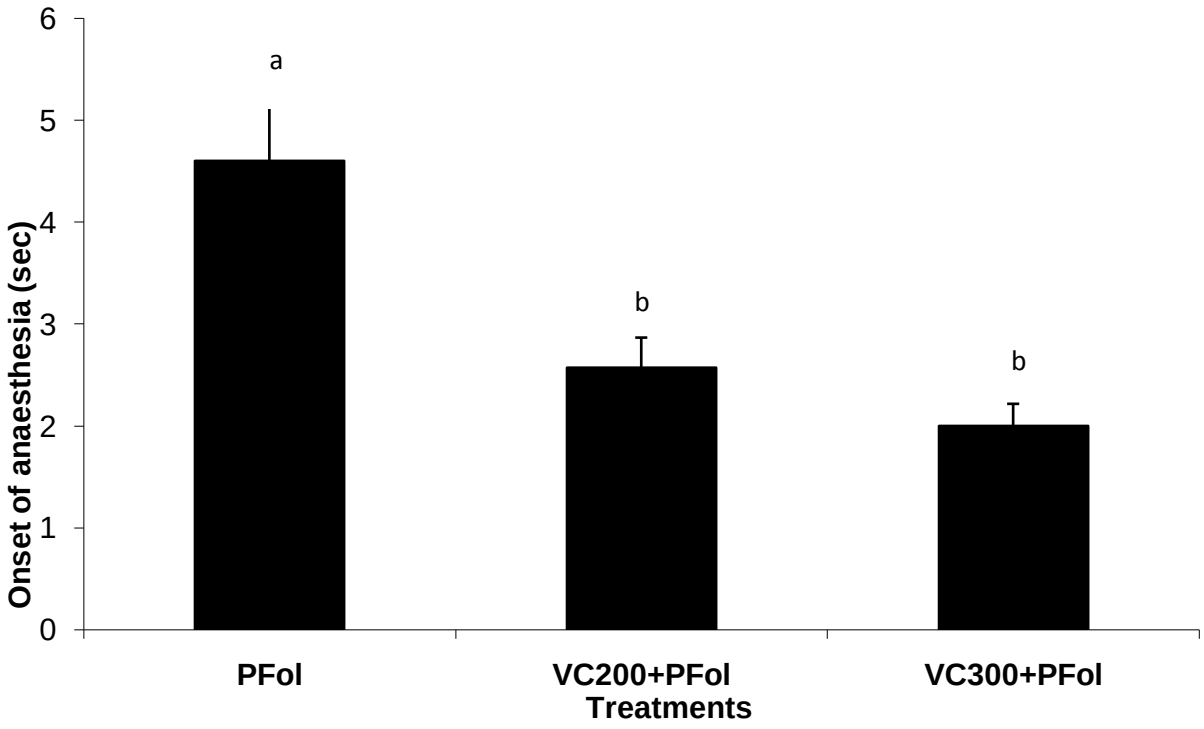


Figure 4.1: Effect of ascorbic acid and propofol on onset of anaesthesia in goats

PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

^{a,b} = Means with different superscript letters in the chart are significantly ($P < 0.05$) different

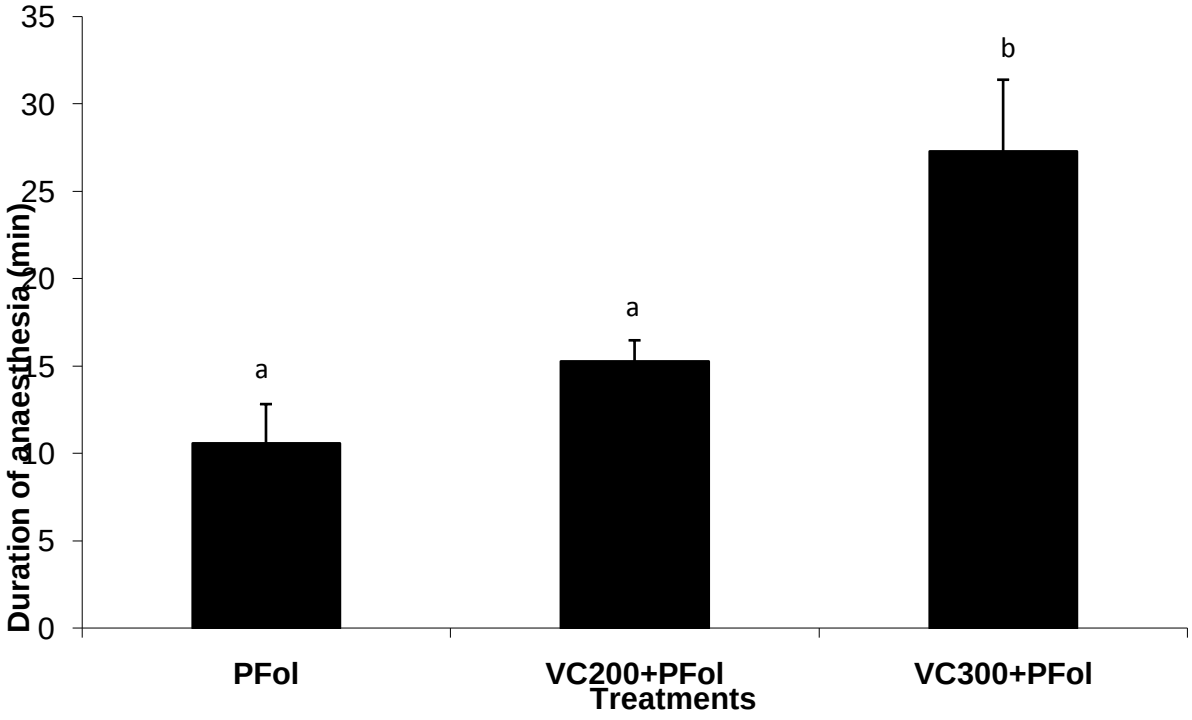


Figure 4.2: Effect of ascorbic acid and propofol on duration of anaesthesia in goats

PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

^{a,b} = Means with different superscript letters in the chart are significantly ($P < 0.05$) different.

Table 4.1: Effect of Ascorbic acid and Propofol on Pain Score in Goats

Pain score	VC (n=5)	Pfol (n=5)	V200+Pfol (n=7)	V300+Pfol (n=7)
0	20.0%[a]	0%[a]	42.9%[b]	57.1%[b]
1	40.0%[a]	0%[b]	28.5 %[b]	28.6% [b]
2	40.0%[a]	60%[a]	14.3%[b]	14.3%[b]
3	0.0 %[b]	40%[a]	14.3%[a]	0%[b]

VC= Ascorbic acid, PFOL = Propofol, VC200 mg/kg+PFOL = 200 mg/kg Ascorbic acid and propofol, VC300 mg/kg+PFOL = 300 mg/kg Ascorbic acid and propofol.

a,b = Means with different superscript letters in the same row are significantly ($P < 0.05$) different

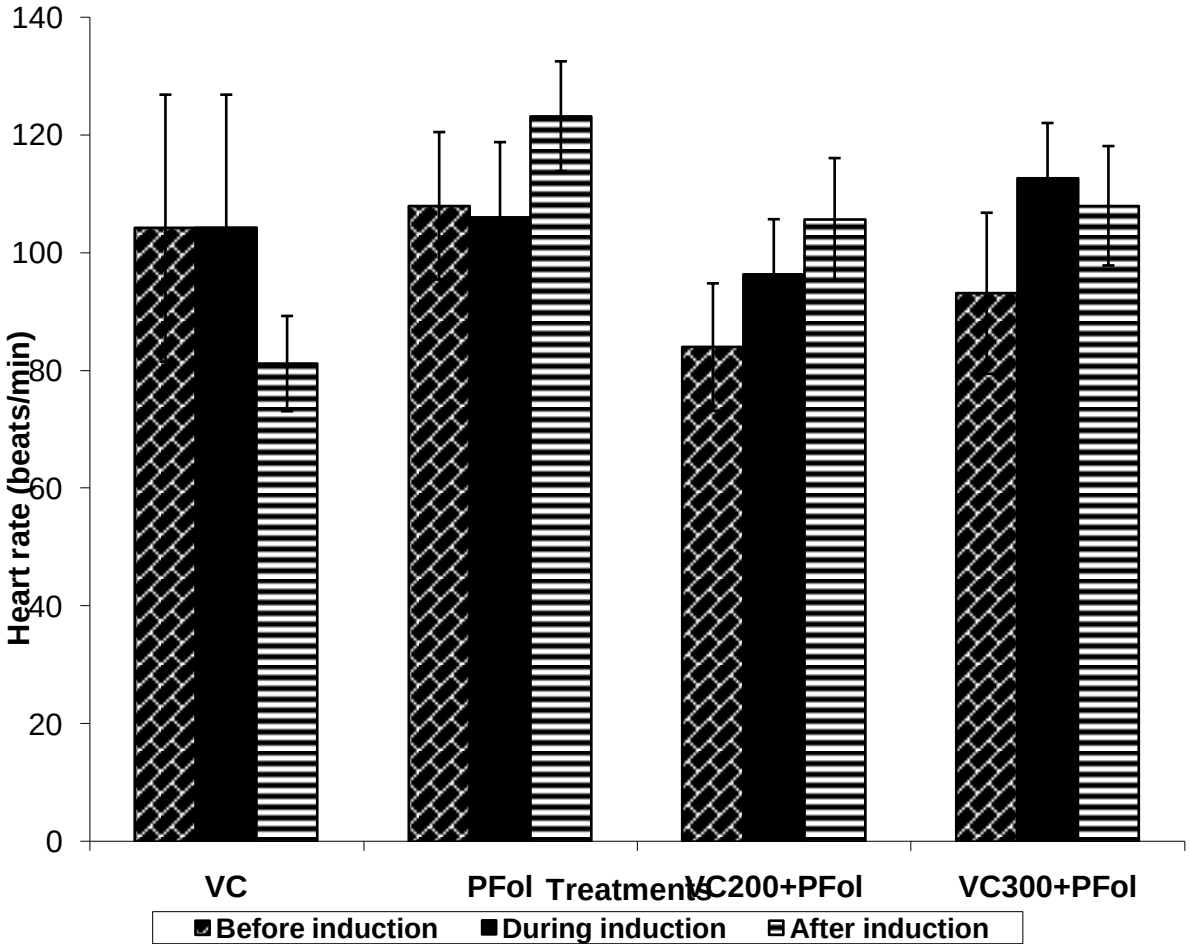


Figure 4.3: Effect of Ascorbic acid and Propofol anaesthesia on Heart rate in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

Values are not significantly ($P > 0.05$) different.

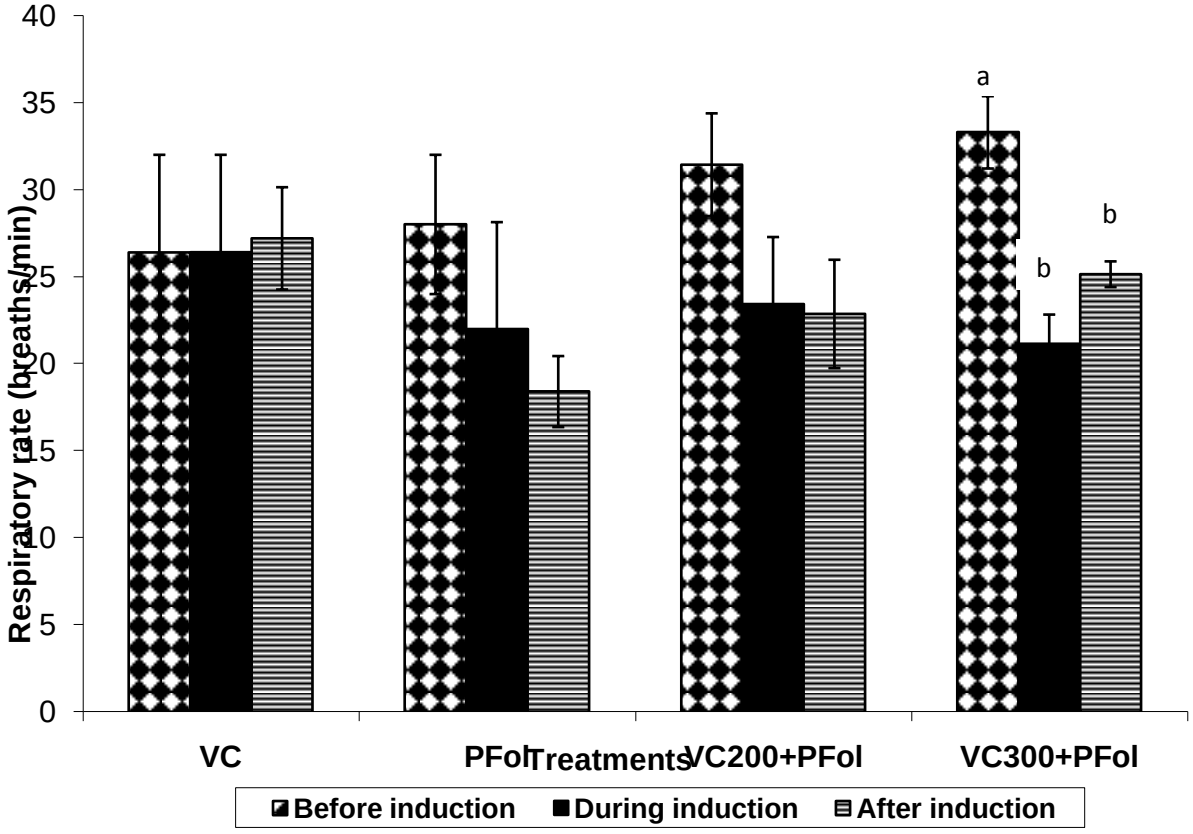


Figure 4.4: Effect of Ascorbic acid and Propofol anaesthesia on Respiratory Rate in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

^{a,b} = Means with different superscript letters in the chart are significantly ($P < 0.05$) different

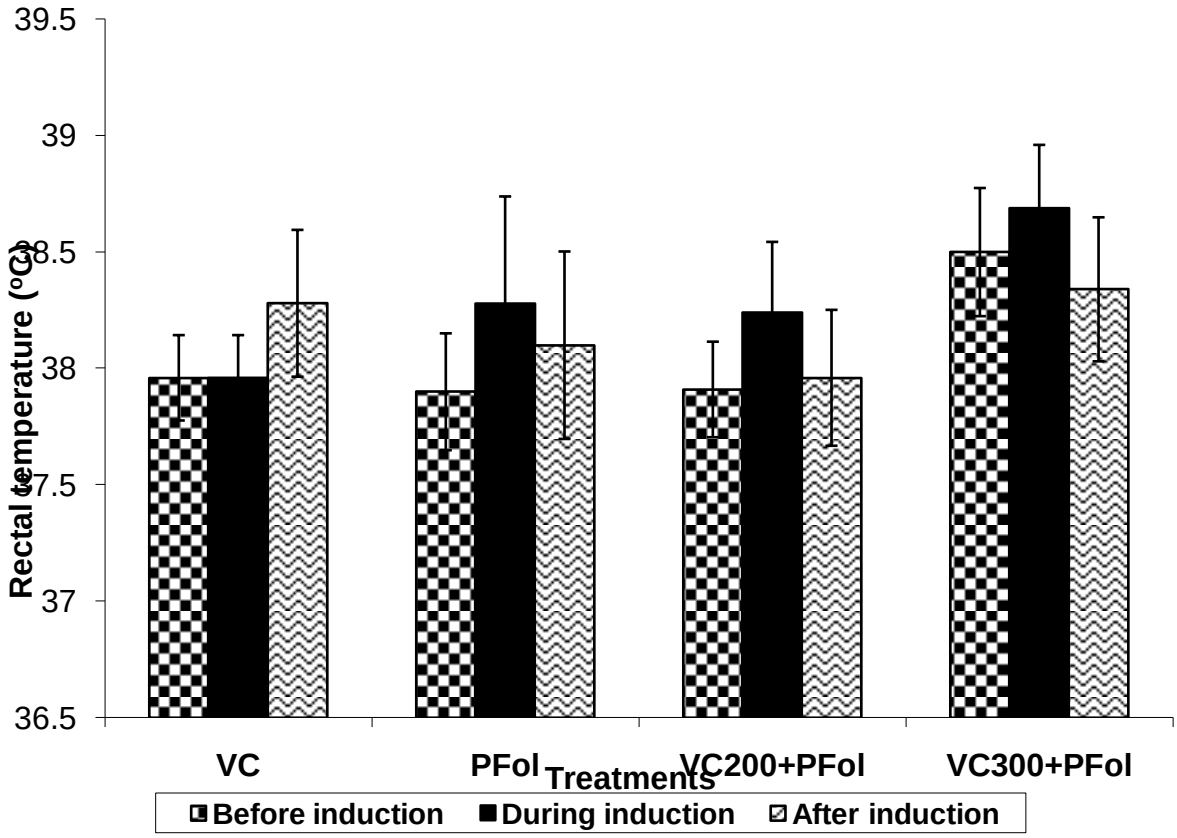


Figure 4.5: Effect of Ascorbic acid and Propofol anaesthesia on Rectal Temperature in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

4.4 Effect of Ascorbic acid and Propofol anaesthesia on Haematology

4.4.1 Effect of ascorbic acid and propofol anaesthesia on packed cell volume (PCV)

There was no significant ($P > 0.05$) difference in PCV within the groups; however there was a general decrease in PCV in all the groups after treatment. VC group had a decrease of 1.22 % in PCV after ascorbic acid administration. PFol group had a decrease of 14.02 % in PCV while the VC200+PFOL and VC300+PFol both had decreases of 19.52 % and 9.97 % respectively (Fig 4.6).

4.4.2 Effect of ascorbic acid and propofol anaesthesia on leucocyte count

There was no significant ($P > 0.05$) difference in leucocyte count within the groups; however PFol group had a non-significant ($P > 0.05$) increase of 2.89% in leucocyte count after anaesthesia, while the VC, VC200+PFOL and VC300+PFol all had non-significant ($P > 0.05$) decreases in leucocyte count (Fig. 4. 7).

4.4.3 Effect of ascorbic acid and propofol anaesthesia on red blood cell count

There was no significant ($P > 0.05$) difference within the groups. All the groups had non-significant ($P > 0.05$) decreases in red blood cell count. VC had a decrease of 7.79 % in red blood cell count after administration with ascorbic acid and the PFol had a decrease of 3.16 % after induction with anaesthesia. The VC200+PFOL and VC300+PFol both had decreases in red blood cell count of 6.59 % and 12.83 %, respectively (Fig 4.8).

4.4.4 Effect of ascorbic acid and propofol anaesthesia on heamoglobin concentration

There was no significant (P > 0.05) change in haemoglobin concentration within the groups. Administration of ascorbic acid and or propofol did not alter haemoglobin values (Fig. 4.9).

4.4.5 Effect of ascorbic acid and propofol anaesthesia on erythrocyte osmotic fragility

There was no significant (P > 0.05) difference in the percentage of erythrocyte osmotic fragility among the goats in the various groups at 0.1, 0.3, 0.5, 0.7 and 0.9 % of NaCl concentration. Generally, irrespective of the treatment groups, percent haemolysis decreased with increasing NaCl concentration. Haemolysis was complete (100%) in the standard solvent (distilled water). At 0.1 % of NaCl concentration, there was a general decrease in haemolysis in all the groups. At 0.3 % of NaCl concentration the VC and VC200+PFol group showed decreases in erythrocyte fragility while, PFol and VC300+PFol groups had increases in mean erythrocyte fragility. At 0.5 % of NaCl concentration, only the VC group showed a non-significant (P > 0.05) decrease in erythrocyte fragility from 100 ± 0.0 % to 97.2 ± 1.8 %. PFol, VC200+PFol and VC300+PFol all had increases in erythrocyte fragility. At 0.7 % of NaCl concentration VC and PFol groups showed decreases in erythrocyte fragility, while VC200+PFol and VC300+PFol groups showed increases in erythrocyte fragility. At 0.9 % of NaCl concentration only the PFol group showed a decrease in mean erythrocyte fragility from an initial value of 9.94 ± 3.16 % to 8.76 ± 2.39 %. The VC, V200+PFol and V300+PFol groups all had increases in erythrocyte fragility (Figs 4. 10 and 4.11).

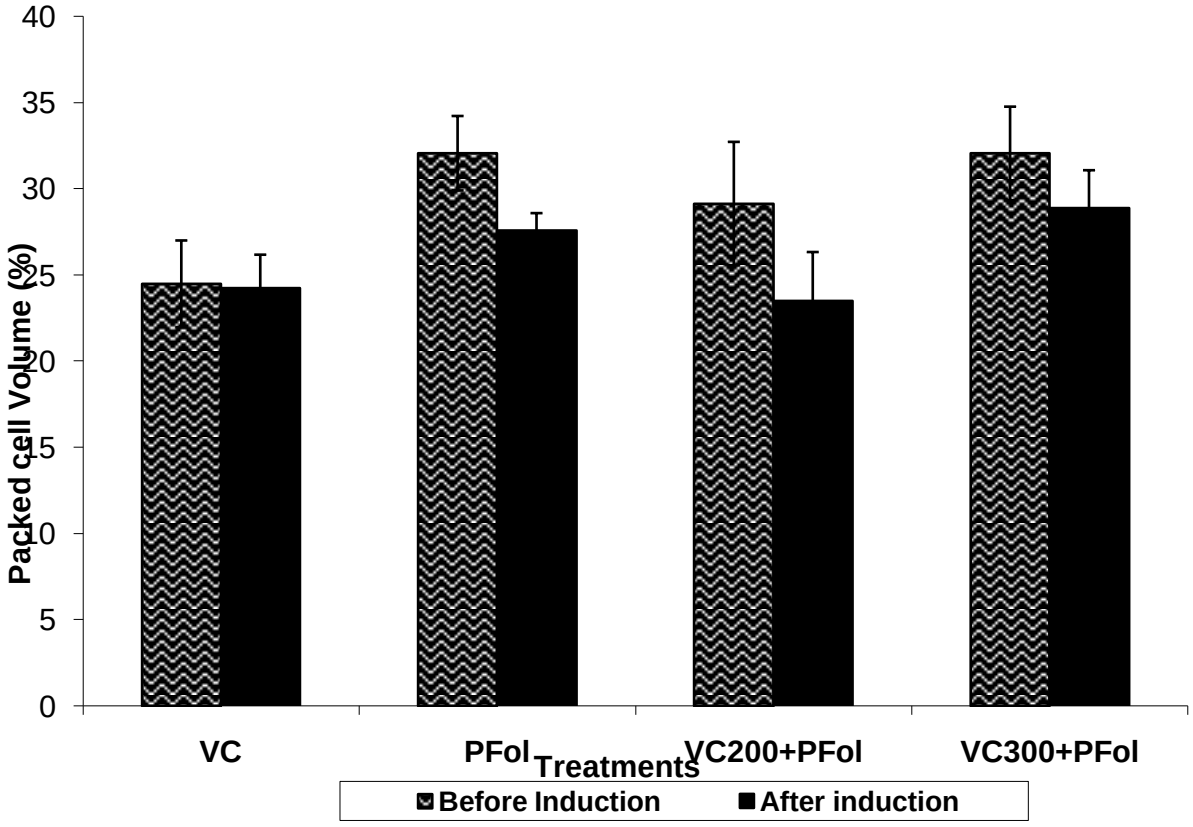


Figure 4.6: Effect of Ascorbic Acid and Propofol on Packed cell Volume in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

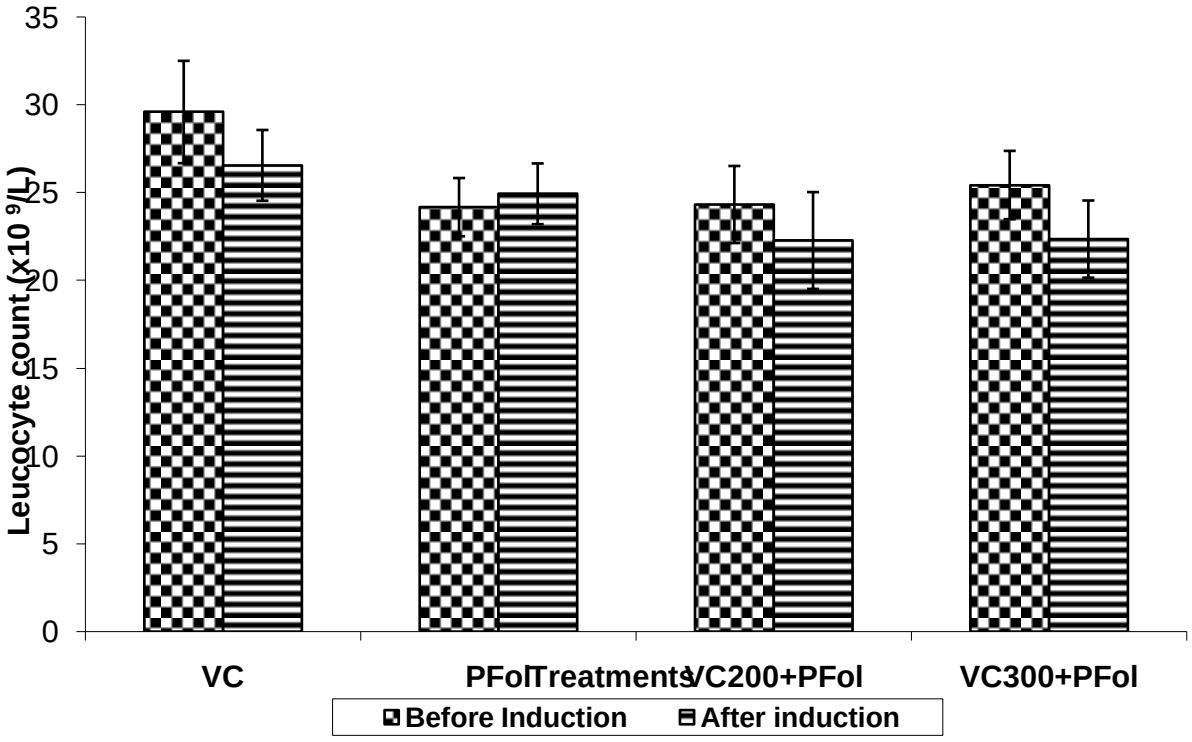


Figure 4.7: Effect of ascorbic acid and Propofol on Leucocyte Count in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

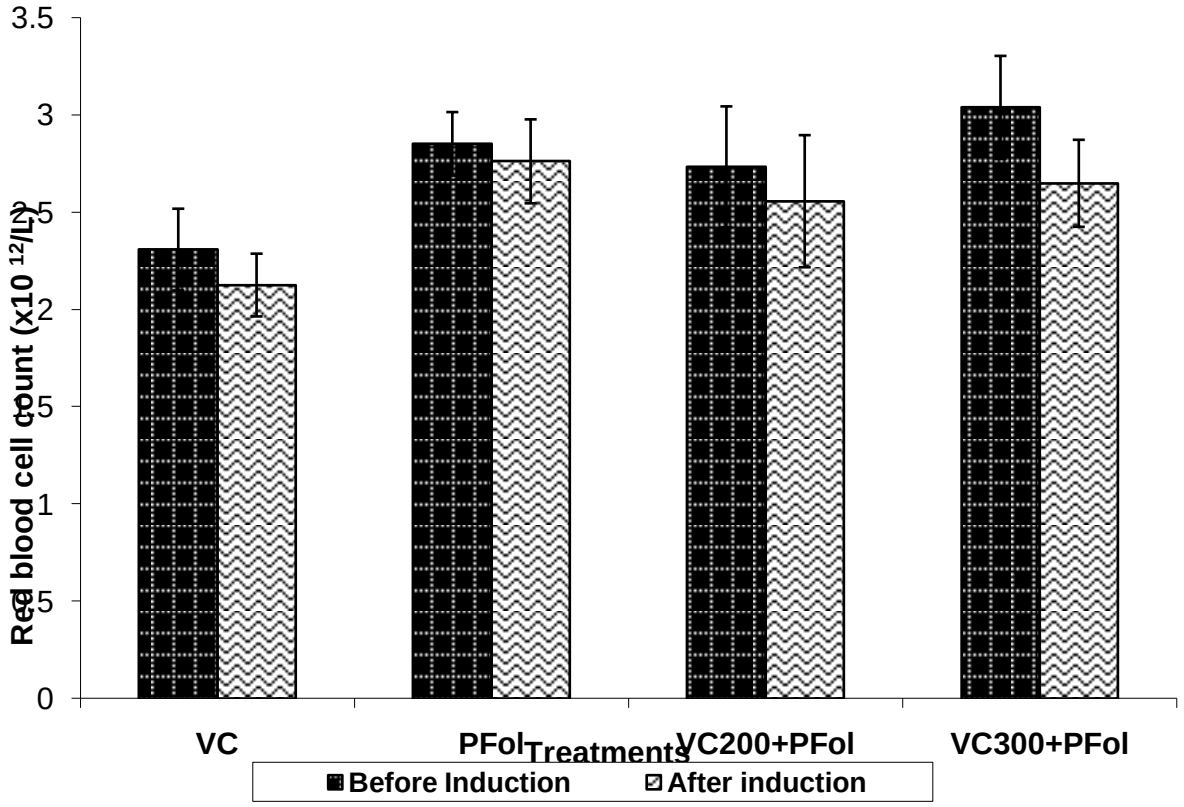


Figure 4.8: Effect of Ascorbic acid and Propofol on Red Blood cell count in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

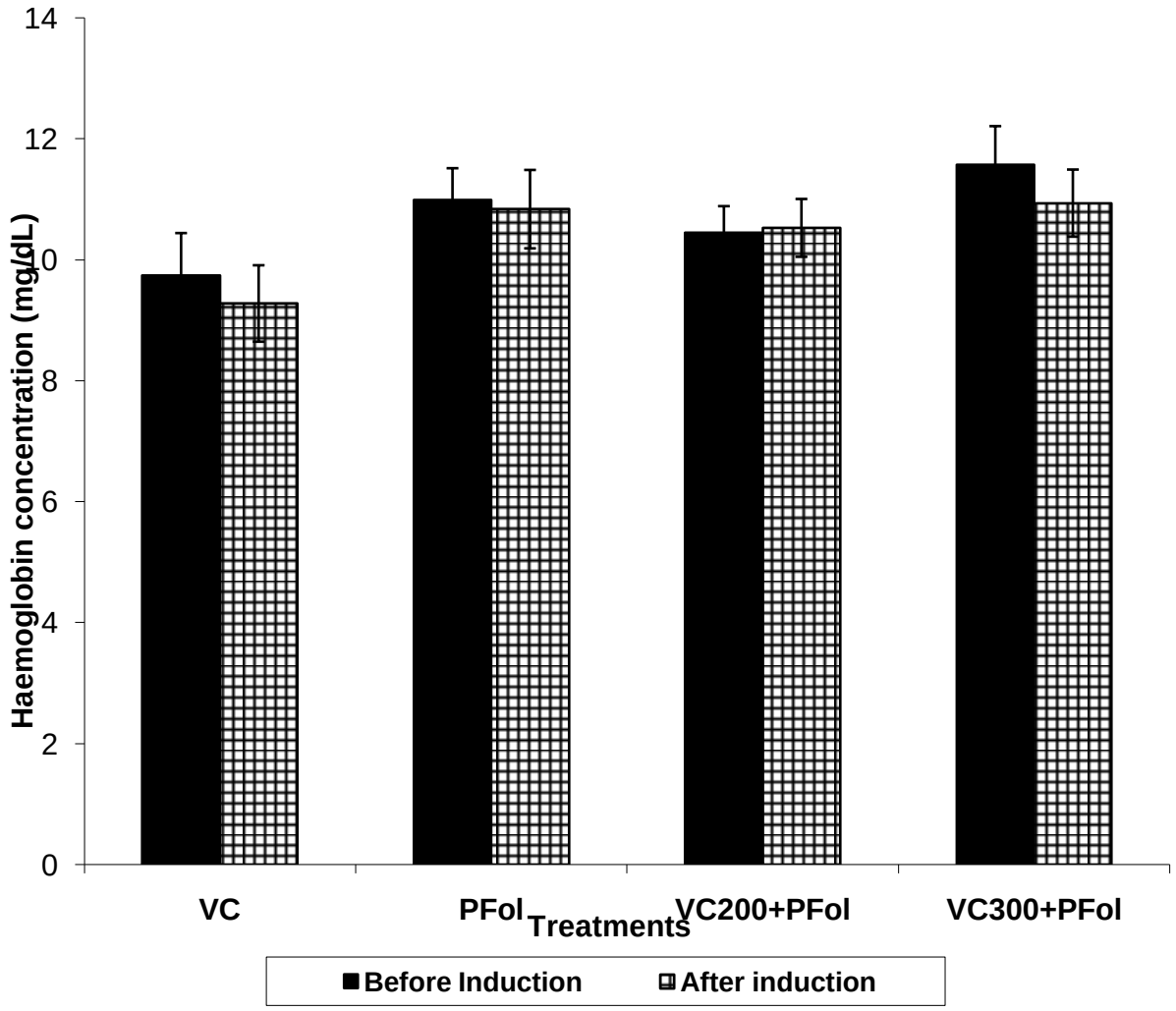


Figure 4.9: Effect of Ascorbic acid and propofol on Haemoglobin Concebtration in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

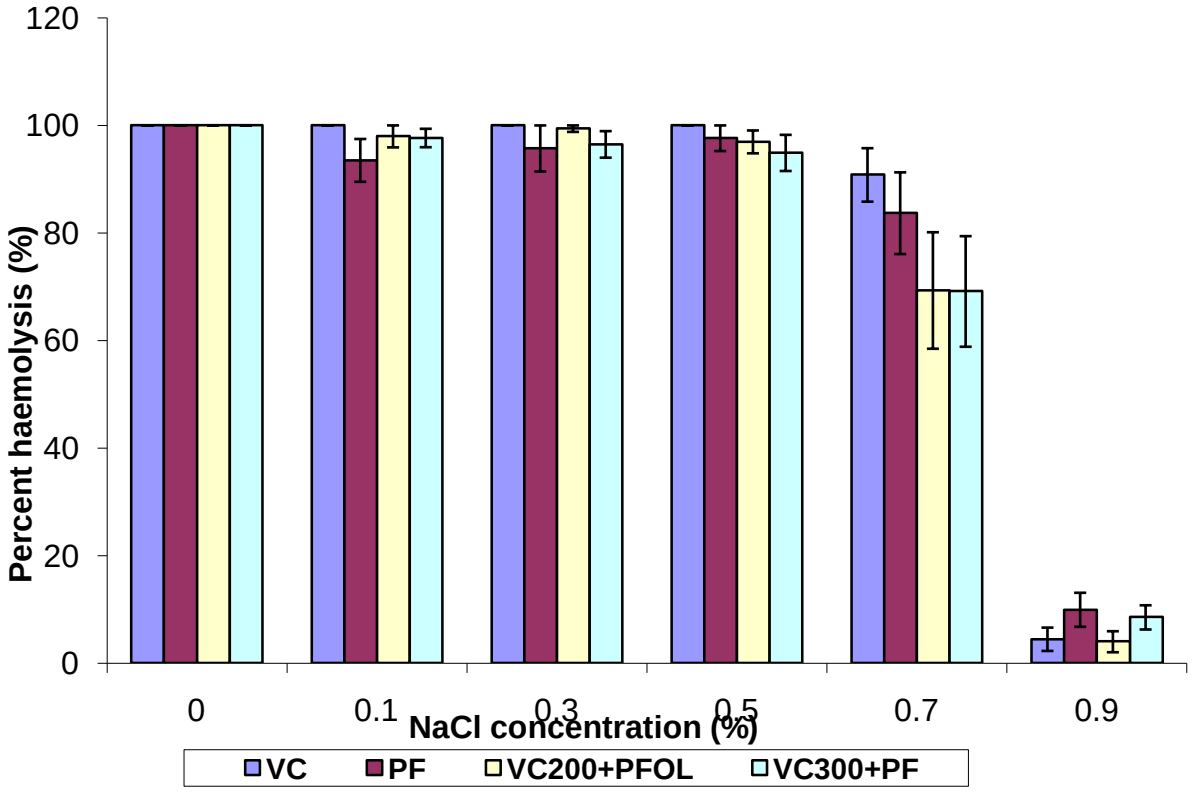


Figure 4.10: Erythrocyte osmotic fragility in the Red Sokoto goats before treatment with ascorbic acid and propofol.

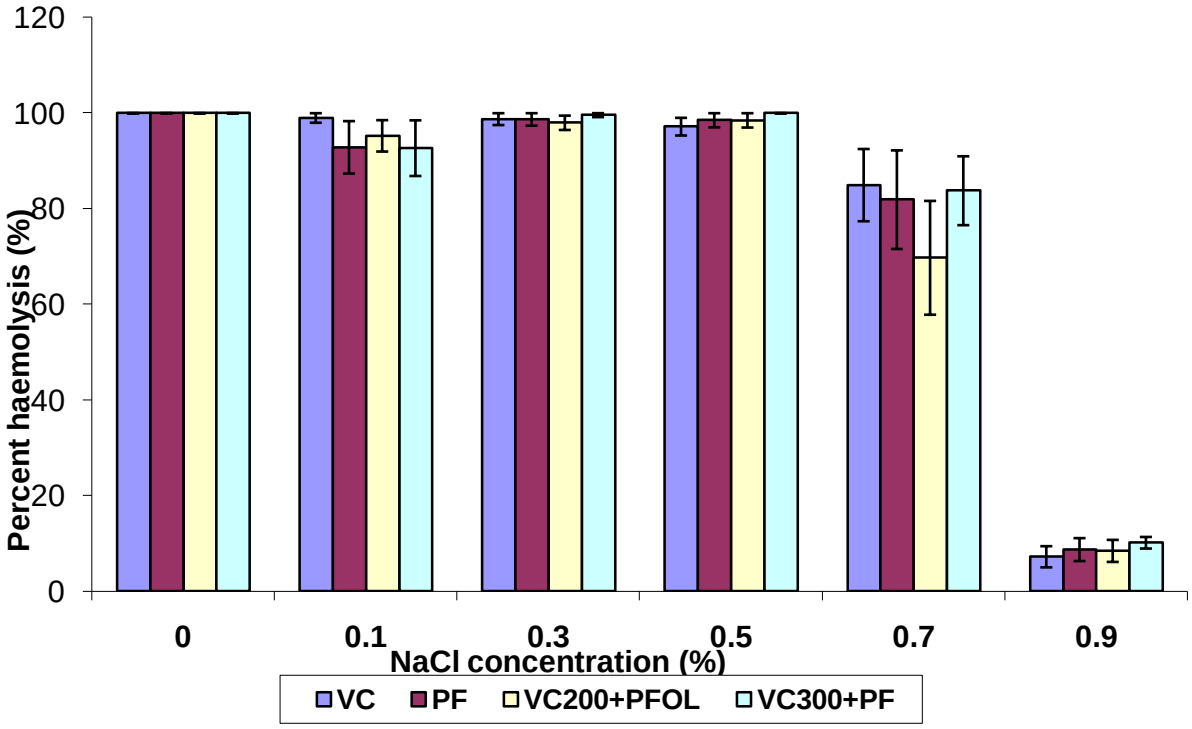


Figure 4.11: Erythrocyte osmotic fragility in the Red Sokoto goats after treatment with ascorbic acid and propofol.

4.5 Effect of Ascorbic acid and Propofol Anaesthesia on Serum Biochemistry

4.5.1 Effect of ascorbic acid and propofol anaesthesia on total protein, globulin and albumin concentration

4.5.1.1 Effect of ascorbic acid and propofol anaesthesia on total protein

The VC group had a non-significant ($P > 0.05$) decrease of 2.32 % in total protein after administration of ascorbic acid. The PFol also had a non-significant ($P > 0.05$) decrease of 0.57 % in total protein after induction with anaesthesia. The VC200+PFol had a non-significant ($P > 0.05$) increase in total protein after induction from a mean value of 66.57 ± 1.94 mg/dl to 70.14 ± 2.36 mg/dl. There was a significant ($P < 0.05$) increase in total protein values in the VC300+Pfol group after induction from a mean value of 67.29 ± 0.89 mg/dl to 70.43 ± 1.02 mg/dl (Fig. 4.12).

4.5.1.2 Effect of ascorbic acid and propofol anaesthesia on globulin

The VC and PFol showed no difference ($P > 0.05$) in globulin concentration before and after administration of ascorbic acid and propofol alone. There was a significant ($P < 0.05$) increase in globulin concentration in the VC200+Pfol group from a value of 28.71 ± 0.84 mg/dl to 31.43 ± 0.81 mg/dl after induction of anaesthesia. The increase (4.39 %) in globulin concentration recorded in VC300+Pfol group after anaesthesia did not differ from value recorded before induction of anaesthesia (Fig. 4. 13).

4.5.1.3 Effect of ascorbic acid and propofol anaesthesia on albumin concentration

The VC group had a non-significant ($P > 0.05$) decrease of 4.1% in albumin concentration after administration of ascorbic acid. The Pfol group showed a non-significant ($P > 0.05$) increase in albumin levels following anaesthesia. The VC200+PFol and VC300+Pfol groups both showed non-significant ($P > 0.05$) increases of 2.90 % and 4.80 % in albumin levels respectively after anaesthetic induction (Fig. 4.14).

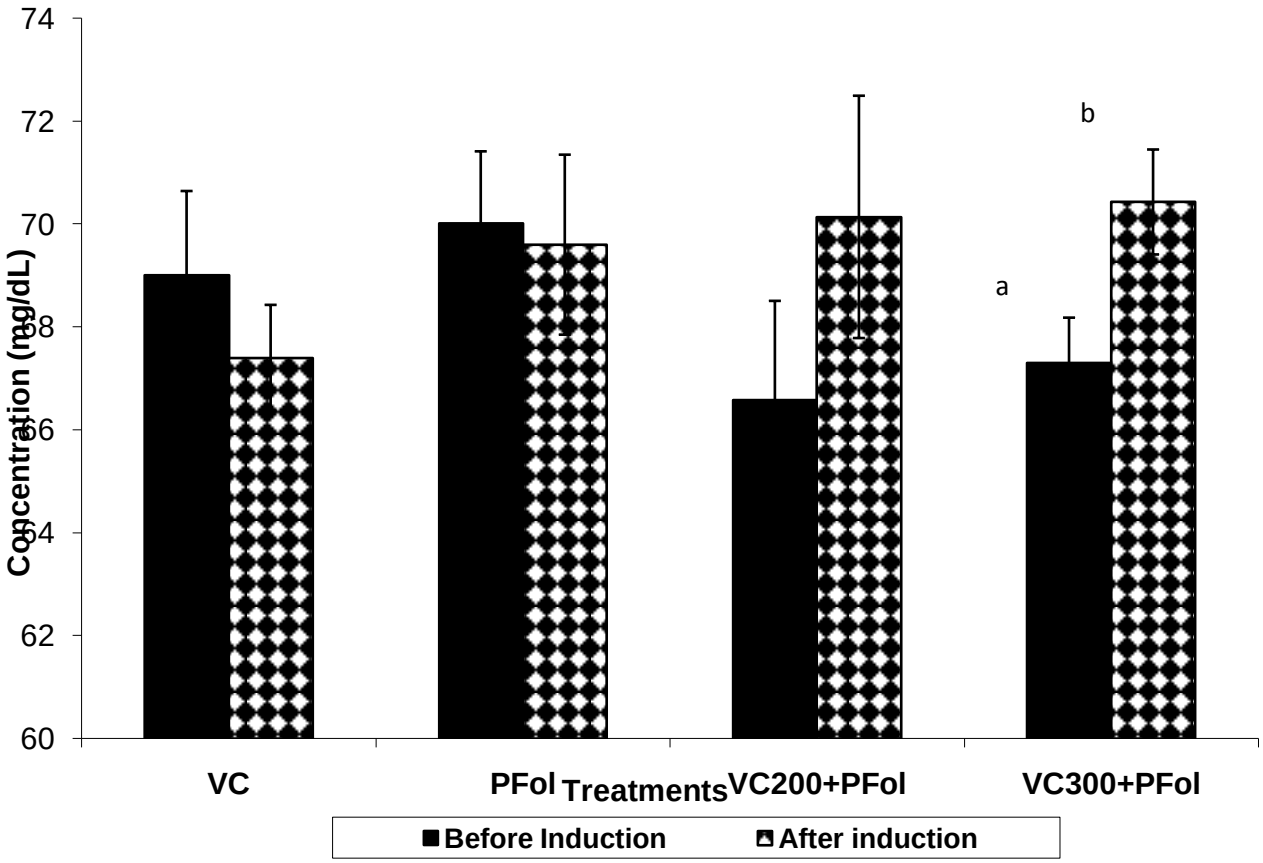


Figure 4.12: Effect of Ascorbic acid and Propofol on Total protein in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

^{a,b} = Means with different superscript letters in the chart are significantly ($P < 0.05$) different

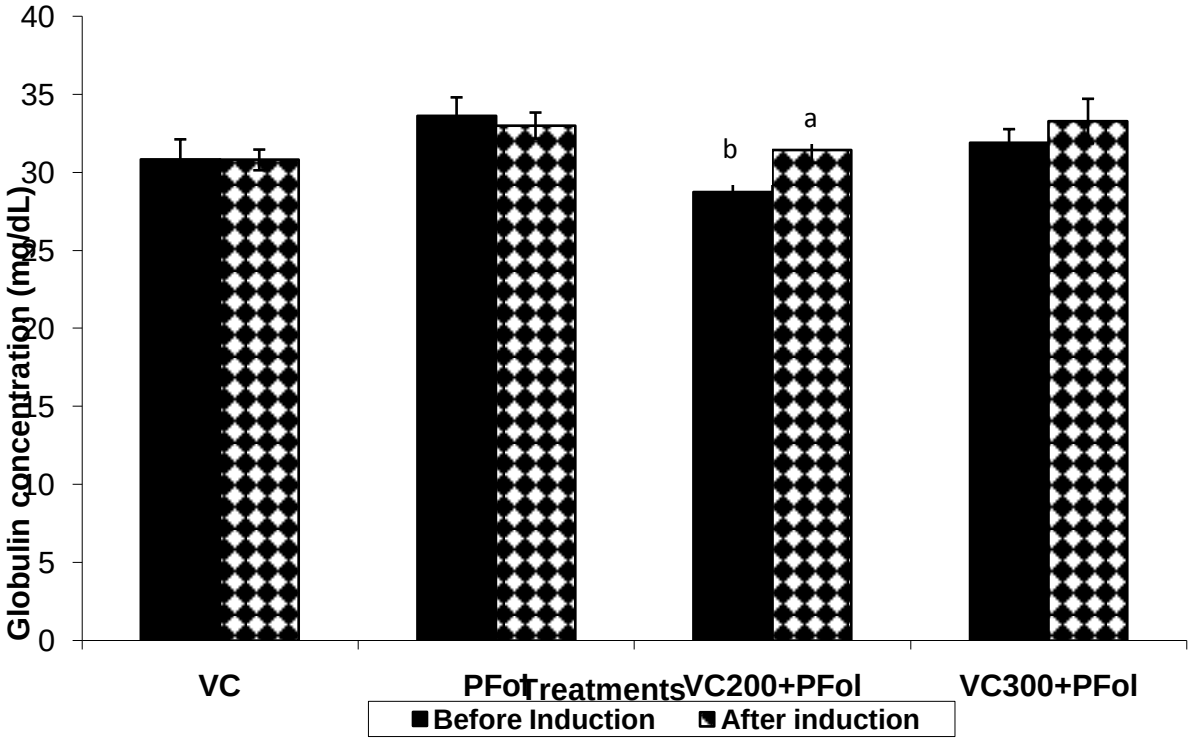


Figure 4.13: Effect of Ascorbic acid and Propofol on Globulin concentration in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

^{a,b} = Means with different superscript letters in the chart are significantly ($P < 0.05$) different

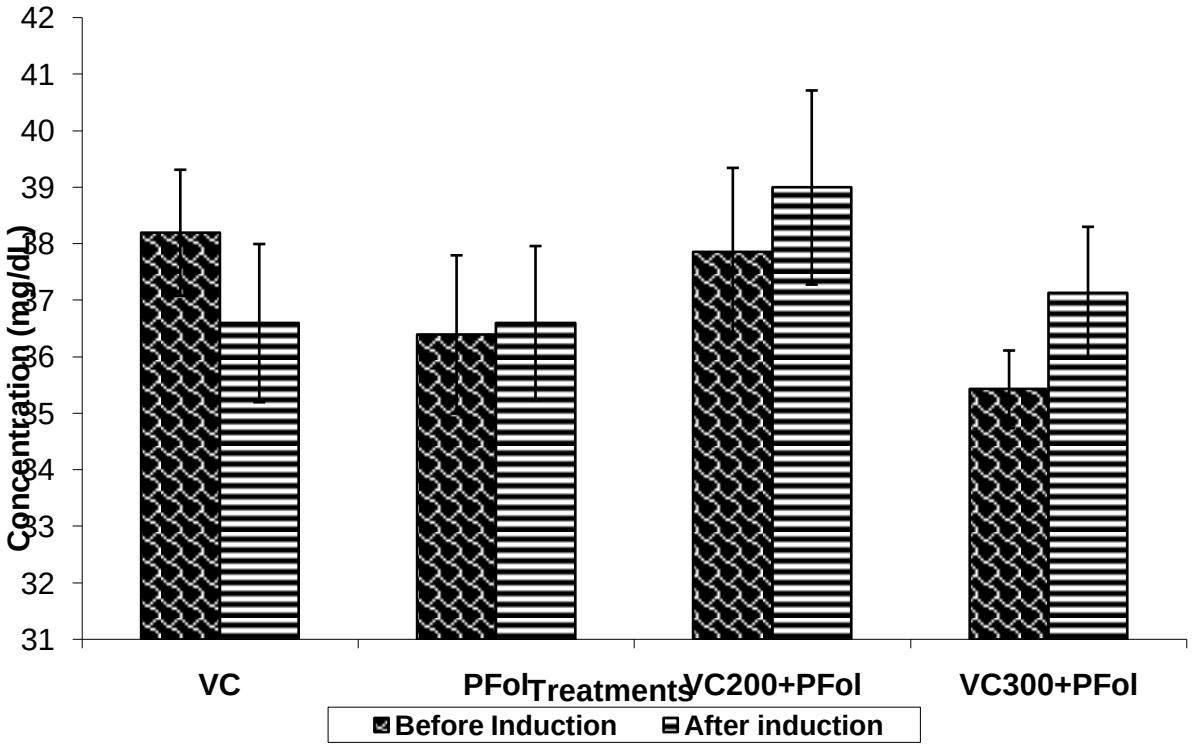


Figure 4.14: Effect of ascorbic acid and Propofol on Albumin in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

4.5.2 Effect of ascorbic acid and propofol anaesthesia on serum urea levels

There was no change in urea levels in all the groups after treatment; however the VC group had a non-significant ($P > 0.05$) decrease of 4.19 % in urea levels after administration of ascorbic acid. The Pfol and VC300+Pfol group also had non-significant ($P > 0.05$) decreases in urea levels after induction. The increase (2.73 %) in urea levels observed in VC200+Pfol group after anaesthetic induction was not significant (Fig. 4. 15).

4.5.3 Effect of ascorbic acid and propofol anaesthesia on serum antioxidant enzymes

4.5.3.1 Effect of ascorbic acid and propofol anaesthesia on superoxide dismutase activity

The VC, VC200+Pfol and VC300+Pfol groups all showed non-significant increases of 10.9 %, 22.0 % and 24.4 % in SOD activity after treatment. The Pfol group had a significant ($P < 0.05$) increase (30.77 %) in SOD activity from an initial value of 1.3 ± 0.08 IU/L to 1.7 ± 0.06 IU/L after induction (Fig. 4. 16).

4.5.3.2 Effect of ascorbic acid and propofol anaesthesia on glutathione peroxidase activity

The VC group showed a non-significant ($P > 0.05$) increase (5.67 %) in glutathione peroxidase activity after administration of ascorbic acid alone. The Pfol group showed a significant ($P < 0.05$) increase (21.1 %) in glutathione peroxidase activity after induction from a mean value of 30.4 ± 1.33 IU/L to 36.8 ± 0.97 IU/L. The VC200+Pfol group showed a non-significant ($P > 0.05$) decrease in glutathione peroxidase activity

after anaesthetic induction; while the VC300+Pfol group showed a non-significant (P > 0.05) increase in glutathione peroxidase activity after induction (Fig. 4. 17).

4.5.4 Effect of ascorbic acid and propofol anaesthesia on serum enzymes

4.5.4.1 Effect of ascorbic acid and propofol anaesthesia on Aspartate aminotransferase activity

Before treatment, V200+PFol group had the highest AST activity of 35.29 ± 1.67 IU/L and the Pfol group had the lowest value of 22.0 ± 1.41 IU/L. After treatment V200+Pfol group had the highest value of 38.29 ± 1.43 IU/L while VC300+Pfol group had the lowest AST values of 23.14 ± 0.94 IU/L. There was however, no significant (P > 0.05) difference within each group before and after treatments (Fig. 4. 18).

4.5.4.2 Effect of ascorbic acid and propofol anaesthesia on alanine aminotransferase activity

The PFol group had the highest ALT value (45.0 ± 2.0 IU/L), while the VC200+PFOL group had the least ALT (34.0 ± 1.18 IU/L) value before induction. After induction, the VC300+PFol had the highest mean ALT value (45.57 ± 2.93 IU/L) while the VC200+PFol group had the least mean ALT value (35.7 ± 1.15 IU/L). There was no significant (P > 0.05) difference however within each group in ALT levels before and after induction (Fig. 19).

4.5.4.3 Effect of ascorbic acid and propofol anaesthesia on alkaline phosphatase activity

The VC, PFol and VC200+PFol group all showed non-significant increases in ALP values after anaesthetic induction. The VC300+PFol group showed no change in ALP value (68.29 ± 1.87 IU/L) after anaesthetic induction (Fig. 4. 20).

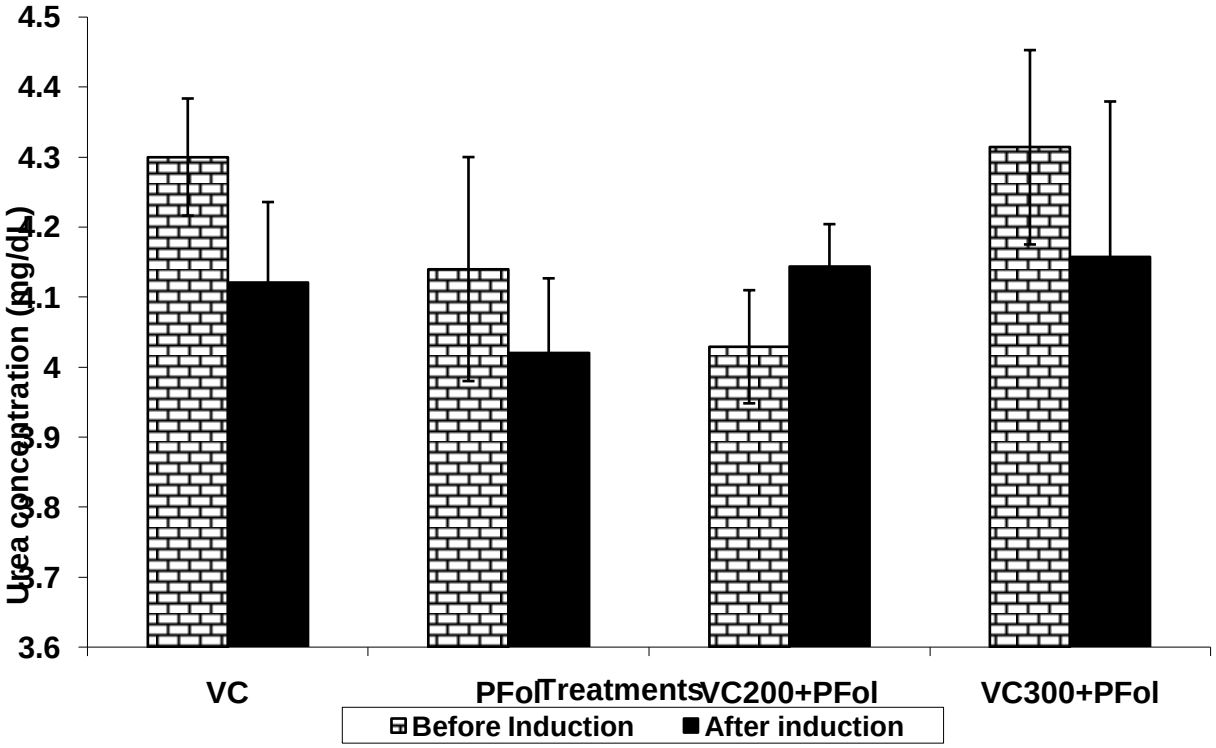


Figure 4.15: Effect of Ascorbic acid and Propofol on serum urea concentrations in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

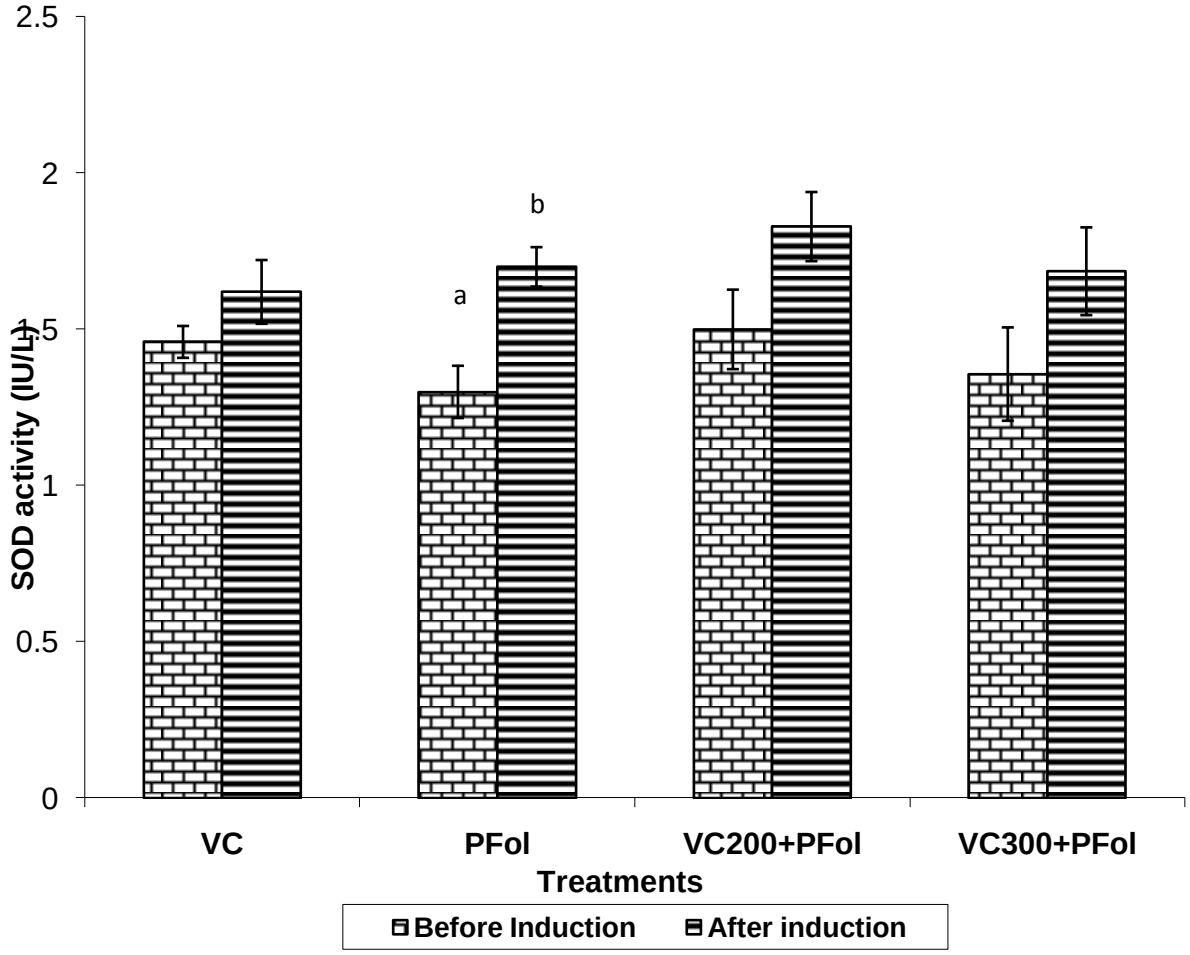


Figure 4.16: Effect of ascorbic Acid and Propofol on Superoxide dismutase in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

^{a,b} = Means with different superscript letters in the same column are significantly ($P < 0.05$) different

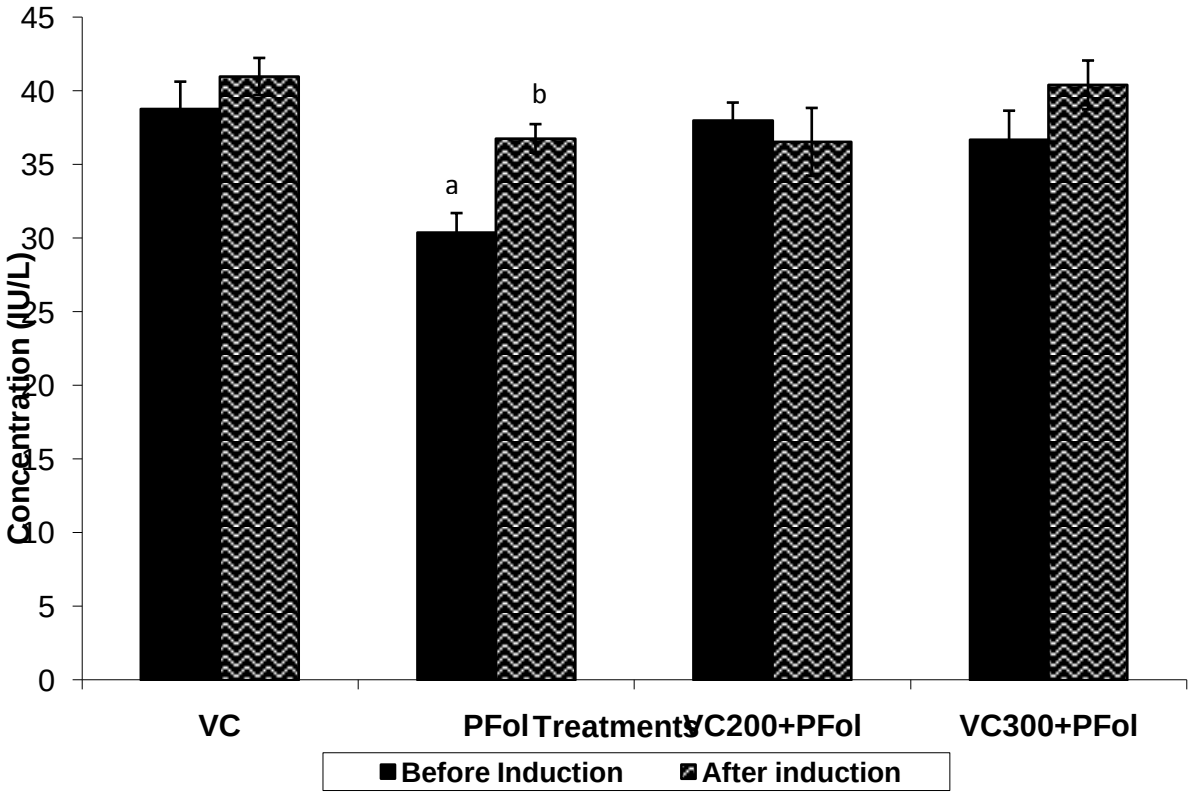


Figure 4.17: Effect of Ascorbic acid and propofol on Glutathione peroxidase in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

^{a,b} = Means with different superscript letters in the same column are significantly ($P < 0.05$) different

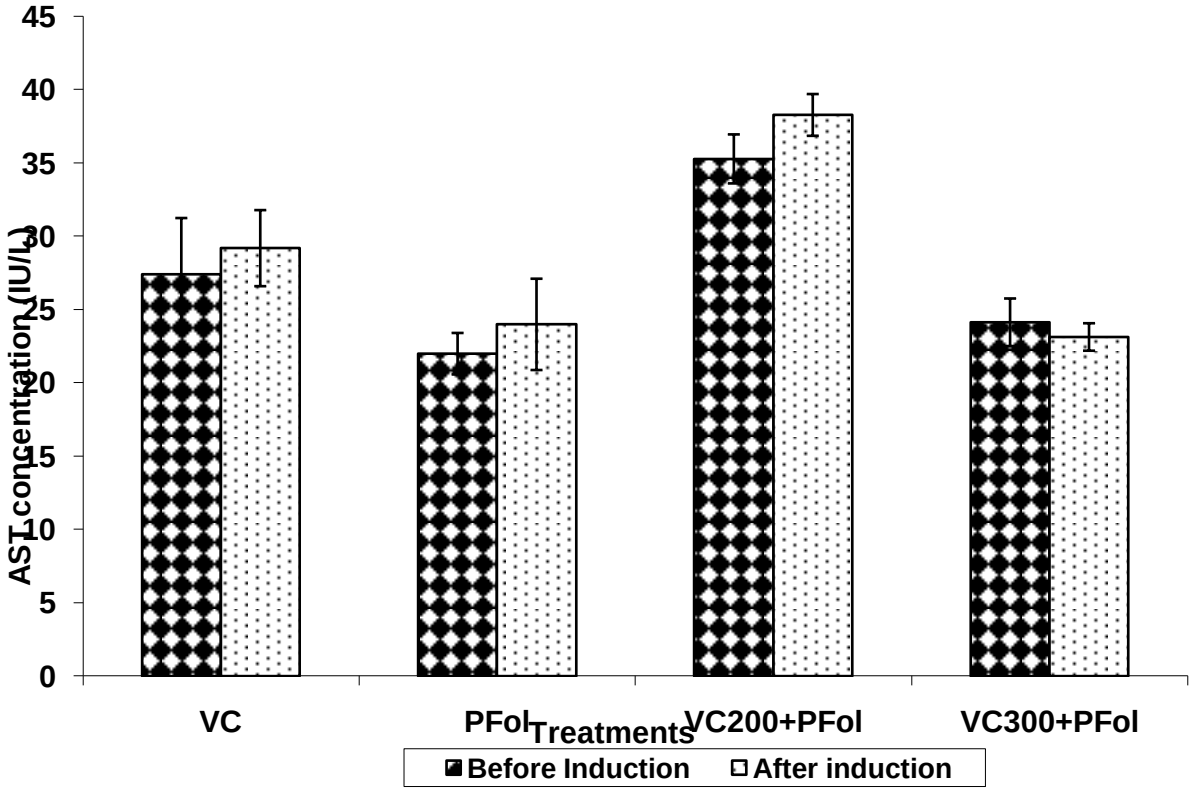


Figure 4.18: Effect of ascorbic acid and Propofol on AST in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

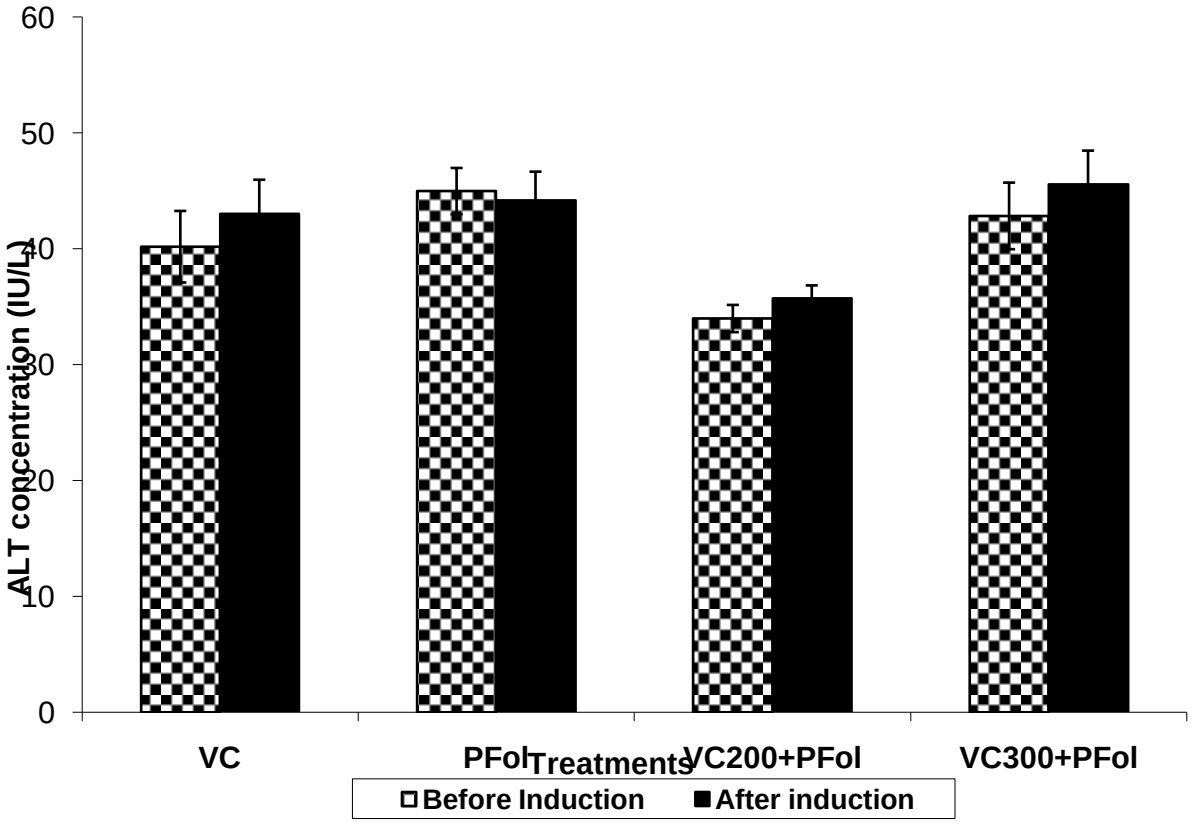


Figure 4.19: Effect of Ascorbic acid and Propofol on ALT in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

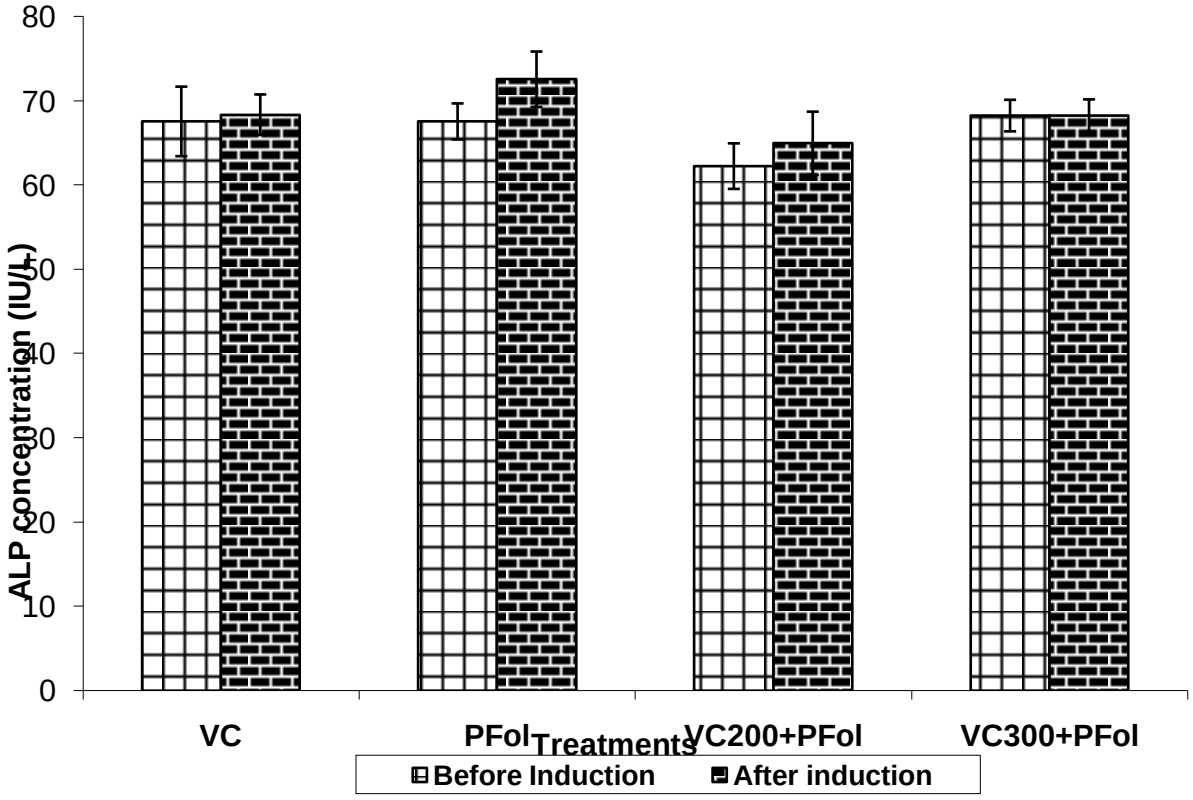


Figure 4.20: Effect of ascorbic acid and Propofol on ALP in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

4.5.5 Effect of ascorbic acid and propofol anaesthesia on serum electrolyte concentration

4.5.5.1 Effect of ascorbic acid and propofol anaesthesia on serum chloride ion concentration

There was no significant ($P > 0.05$) difference in chloride ion concentration within the groups before and after induction (Fig. 4. 21).

4.5.5.2 Effect of ascorbic acid and propofol anaesthesia on serum magnesium ion concentration

There was a significant ($P < 0.05$) increase in Mg^{2+} concentration in the VC group after administration of ascorbic acid from an initial value of 1.14 ± 0.05 mmol/dl to 1.38 ± 0.09 mmol/dl. The PFol group had a non significant increase in Mg^{2+} concentration from an initial value of 1.10 ± 0.07 mmol/dl to 1.26 ± 0.05 mmol/dl after anaesthetic induction. The VC200+PFol group showed a significant ($P < 0.05$) increase in Mg^{2+} concentration from an initial value of 1.23 ± 0.05 mmol/dl to 1.50 ± 0.06 mmol/dl after anaesthetic induction. The VC300+PFol group ha a non-significant increase in Mg^{2+} concentration after anaesthetic induction from a value of 1.27 ± 0.08 mmol/dl to 1.33 ± 0.09 mmol/dl (Fig 4. 22).

4.5.5.3 Effect of ascorbic acid and propofol anaesthesia on potassium ion concentration

There was no significant ($P > 0.05$) difference in K^+ concentration within the groups before and after treatment. The VC had a non-significant decrease in K^+ concentration of 2.36 % after ascorbic acid administration. The PFol had a non-significant decrease in K^+ concentration of 7.80 % after induction. The VC200+PFol group showed a non-

significant increase in K^+ concentration after anaesthetic induction. The VC300+PFol group showed an insignificant decrease in K^+ concentration after anaesthetic induction (Fig. 4.23).

4.5.5.4 Effect of ascorbic acid and propofol anaesthesia on sodium ion concentration

There was no significant ($P > 0.05$) difference in sodium ion concentration within the groups before and after treatment. The administration of ascorbic acid alone did not alter ($P > 0.05$) the sodium ion concentration in the goats. There was no change ($P > 0.05$) in sodium ion concentration in the PFol after induction of anaesthesia. Administration of ascorbic acid prior to propofol anaesthesia also did not alter the sodium ion concentration in the VC200+PFol and VC300+PFol (Fig. 4. 24).

4.5.5.5 Effect of ascorbic acid and propofol anaesthesia on bicarbonate ion concentration

There was no significant ($P > 0.05$) difference in bicarbonate ion concentration within the groups before and after anaesthetic induction. The administration of AA alone did not alter ($P > 0.05$) the bicarbonate ion concentration in the goats. Propofol when given alone did not change ($P > 0.05$) the bicarbonate ion concentration after anaesthesia of the goats. Administration of AA prior to propofol anaesthesia also did not alter the bicarbonate ion concentration in the goats (Fig. 4. 25).

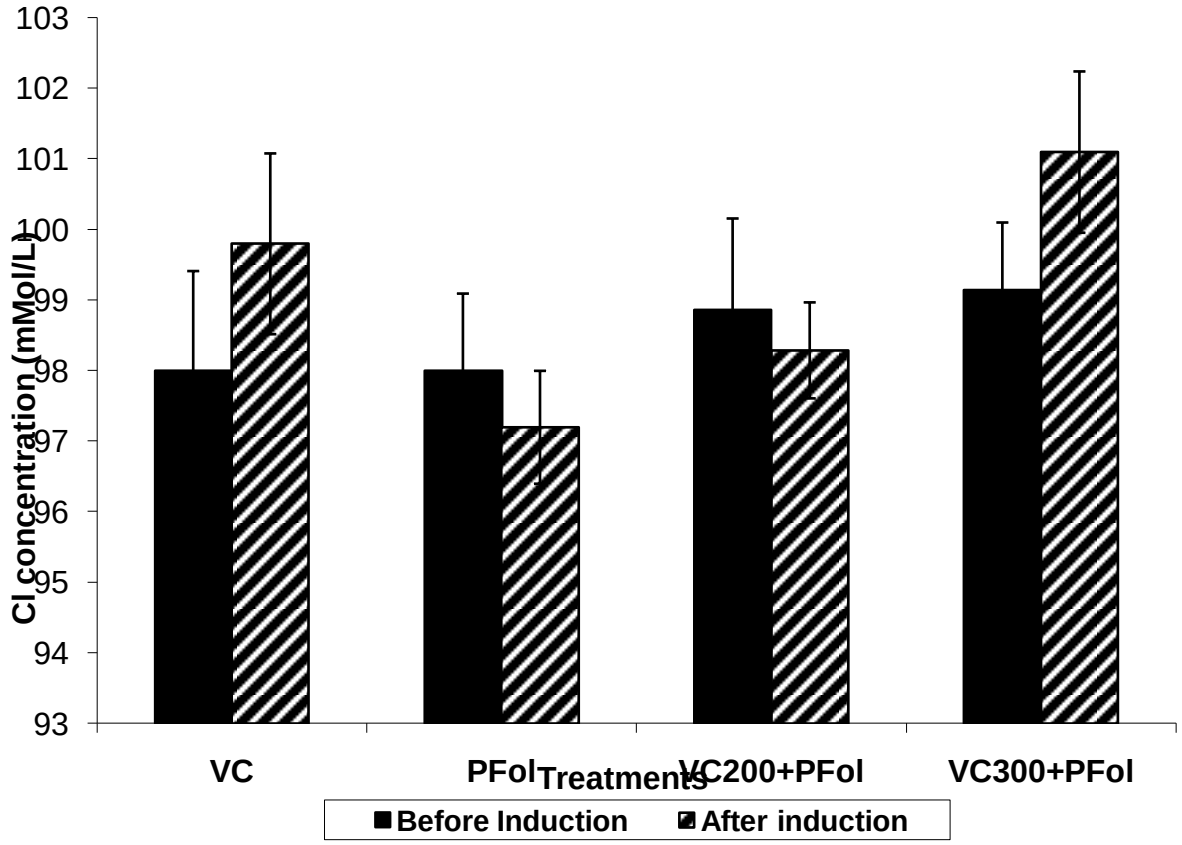


Figure 4.21: Effect of Ascorbic acid and Propofol on Chloride ion in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

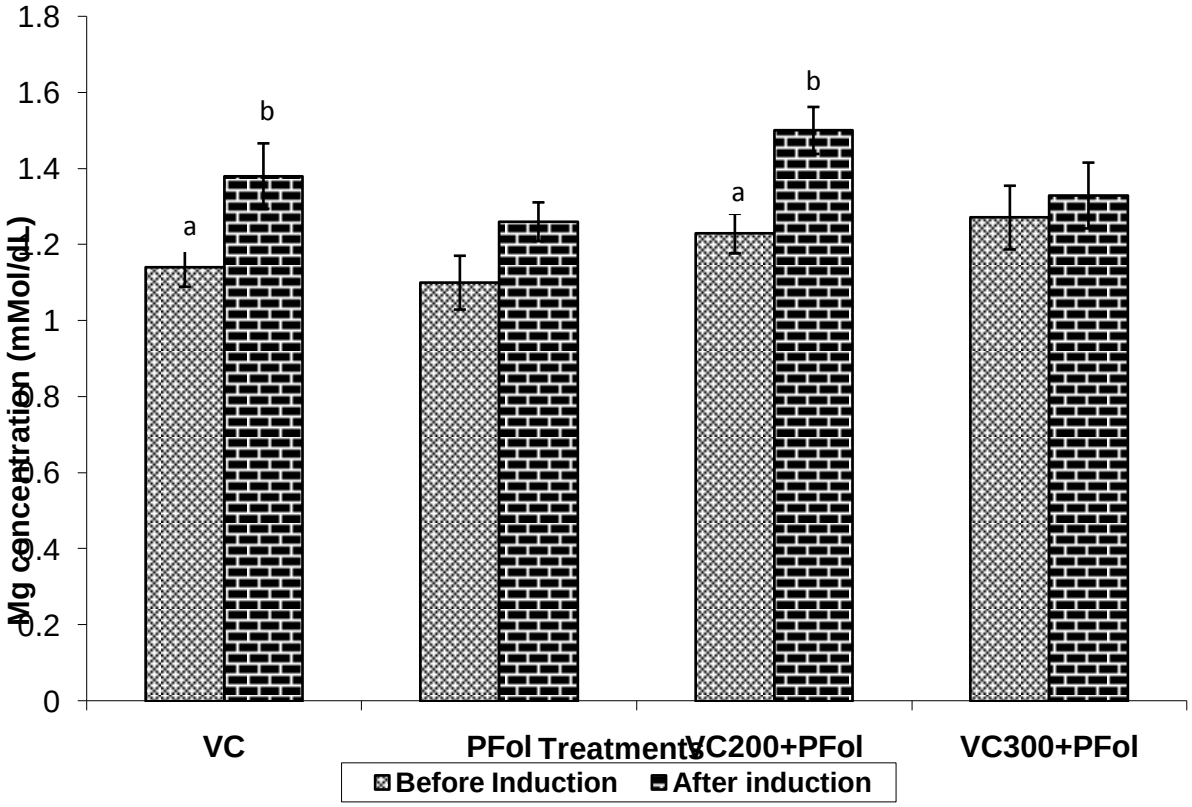


Figure 4.22: Effect of Ascorbic acid and Propofol on Magnesium ion in goats

^{a,b} = Means with different superscript letters in the same column are significantly ($P < 0.05$) different

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

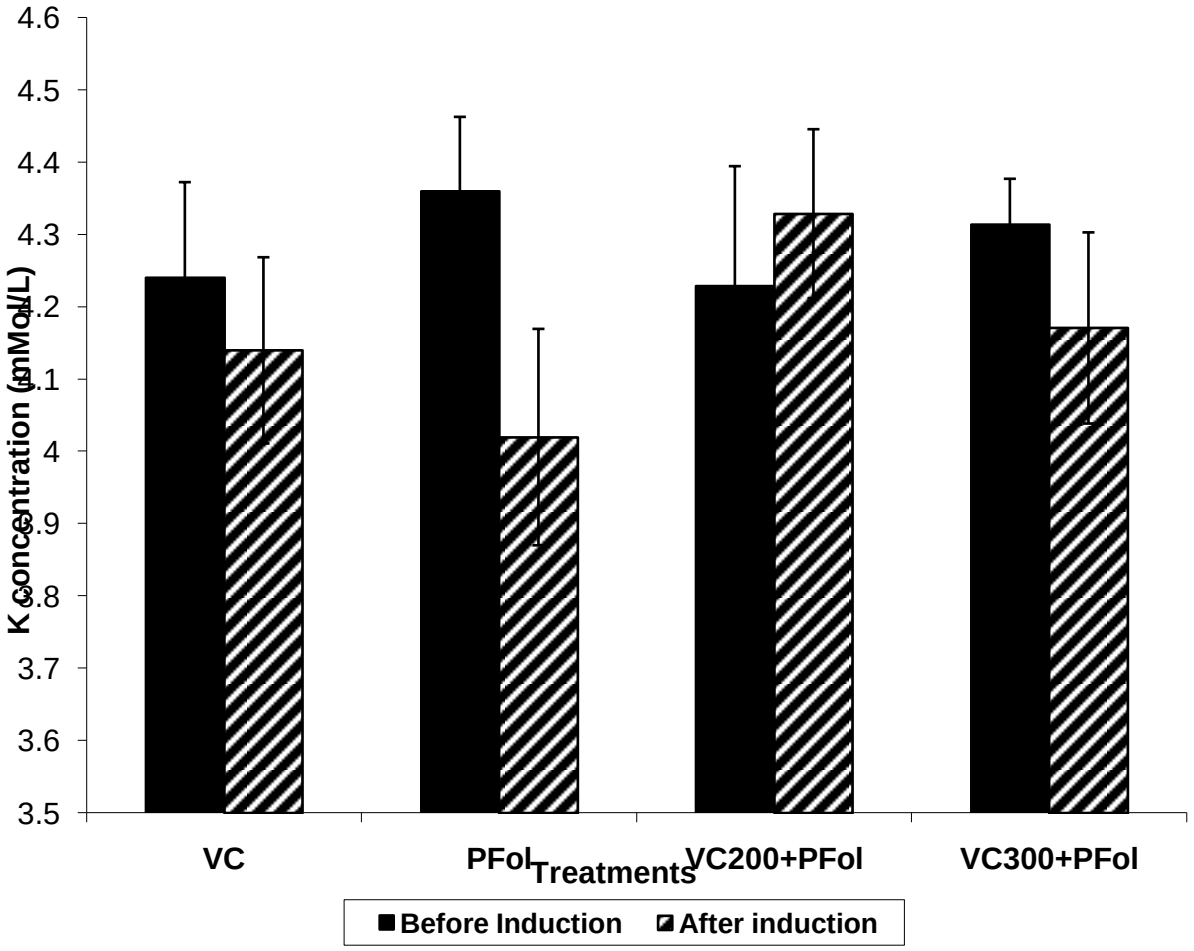


Figure 4.23: Effect of Ascorbic acid and Propofol on Potassium ion in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

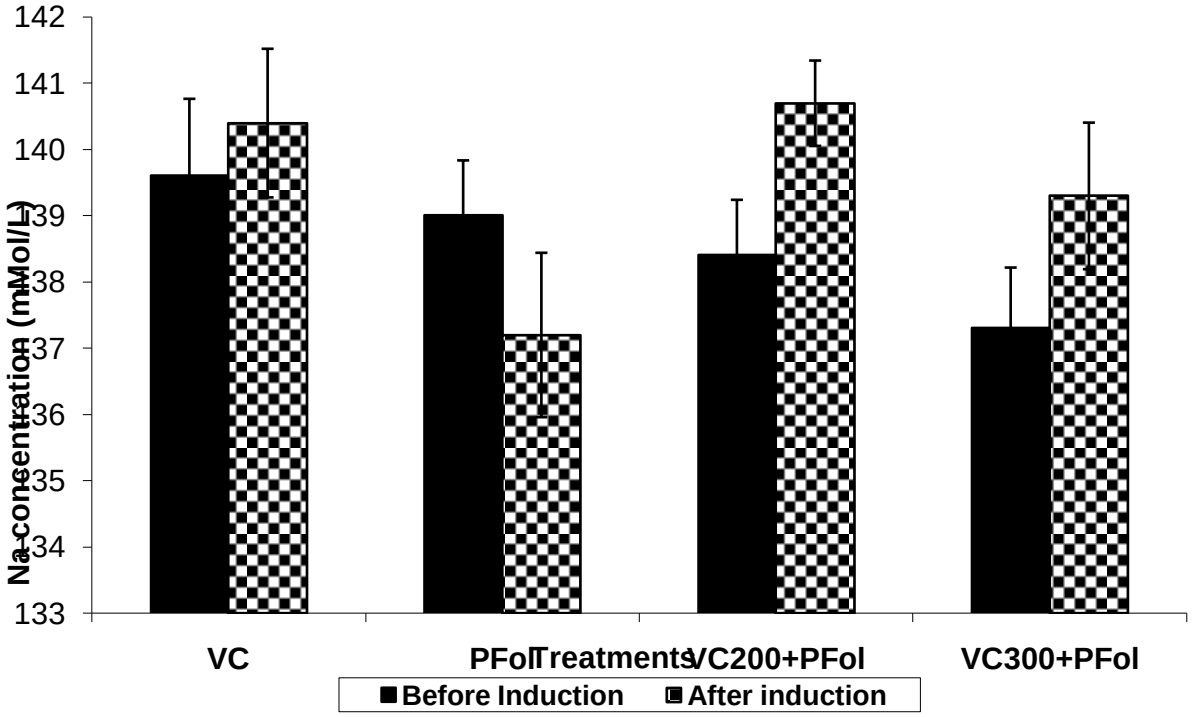


Figure 4.24: Effect of Ascorbic acid and Propofol on sodium ion in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

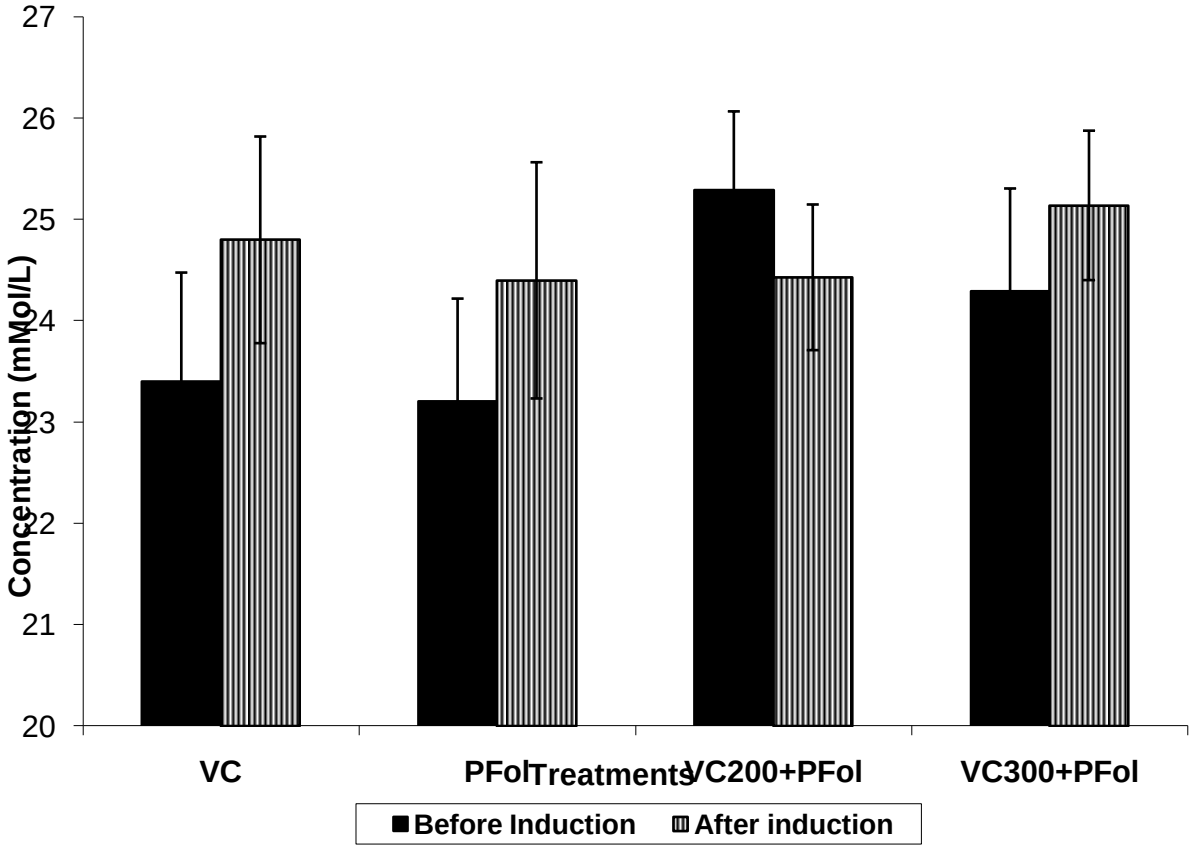


Figure 4.25: Effect of Ascorbic acid and Propofol on Bicarbonate ion in goats

VC = Ascorbic acid, PFol = Propofol, VC200+PFol = 200 mg/kg ascorbic acid and propofol, VC300+PFol = 300 mg/kg ascorbic acid and propofol.

4.6 Effect of Ascorbic Acid and Propofol Anaesthesia on Reflexes

4.6.1 Effect of ascorbic acid and propofol anaesthesia on pedal reflex

There was no significant ($P > 0.05$) difference in the onset of loss of pedal reflex between the groups. The PFol group had the longest time for onset of loss of pedal reflex (53.3 ± 33.8 s) and 60 % (Table 4.2) of the animals in this group lost the pedal reflex. The VC200+PFol had an onset time of 18.8 ± 5.4 s with 85.7 % losing the pedal reflex while the VC300+PFol showed a mean onset time of 21.0 ± 7.3 s and all animals in this group lost the pedal reflex. There was a significant ($P < 0.05$) increase, however, in the duration for loss of pedal reflex in the V300+Pfol group when compared with those of the PFol and VC200+Pfol groups. Pfol had duration of 5.33 ± 2.03 min, while VC200+Pfol and VC300+Pfol had mean durations of 7.5 ± 0.34 min and 18.1 ± 2.68 min for loss of pedal reflex, respectively.

4.6.2 Effect of ascorbic acid and propofol anaesthesia on swallowing reflex

There was no significant ($P > 0.05$) difference in the onset of loss of swallowing reflex between the groups. The PFol group showed an onset time of 30.0 ± 0.0 s for swallowing reflex and 60 % of animals in this group lost the swallowing reflex. The VC200+PFol and VC300+PFol showed an onset time of 17.14 ± 3.43 s and 18.43±7.18 s respectively, and both groups had 100% of the animals losing their swallowing reflex. The VC300+PFOL group showed a significant ($P < 0.05$) increase in the duration of loss of swallowing reflex when compared to the PFol and VC200+Pfol groups. The VC300+Pfol had the longest duration (18.14 ± 2.67 s), followed by the V200+PFol (6.0 ± 0.98 s) and the PFol group (5.67 ± 2.03 s) had the shortest duration of loss of swallowing reflex.

Table 4.2: Effect of Propofol and Ascorbic Acid on tested reflexes

Reflexes	Percentage of animals that lost the reflex		
	PFol	V200+Pfol	V300+PFol
Pedal	60%	85.7%	100%
Swallowing	60%	100%	100%
Jaw tone	60%	100%	100%
Palpebral	20%	85.7%	100%

Table 4.3: Effect of ascorbic acid and propofol on onset of loss of reflexes in goats, s. (Mean ± SEM).

Reflex	PFOL	VC200+PFOL	V300+PFOL
Pedal	53.33 ± 33.8	18.83 ± 5.4	21.00 ± 7.3
Swallowing	30.00 ± 0.0	17.14 ± 3.4	18.43 ± 7.2
Jaw tone	26.67 ± 3.3	17.57 ± 3.4	18.43 ± 7.2
Palpebral	40.00 ± 0.0	65.67 ± 36.2	37.00 ± 15.5

Table 4.4: Effect of ascorbic acid and propofol on duration of loss of reflexes in goats (Mean ± SEM), min.

Reflex	PFOL	VC200+PFOL	V300+PFOL
Pedal	5.333 ± 2.028^{a}	7.500 ± 0.3416^{a}	18.14 ± 2.676^{b}
Swallowing	5.667 ± 2.028^{a}	6.000 ± 0.9759^{a}	18.14 ± 2.668^{b}
Jaw tone	5.667 ± 2.028^{a}	6.857 ± 0.8845^{a}	18.14 ± 2.676^{b}
Palpebral	2.000 ± 0.0	6.000 ± 0.8165	7.14 ± 2.915

^{a,b} = Means with different superscript letters in each row are significantly ($P < 0.05$) different

4.6.3 Effect of ascorbic acid and propofol anaesthesia on jaw tone

There was no significant ($P > 0.05$) difference in the onset of loss of jaw tone reflex between the groups. The PFol group showed an onset time of 26.7 ± 3.33 s for jaw tone reflex and 60 % of animals in this group lost their jaw tone during anaesthetic induction. The VC200+PFol and VC300+PFol showed an onset time of 17.6 ± 3.43 s and 18.43 ± 7.18 s respectively. All the animals in both groups also lost the jaw tone reflex during anaesthetic induction. The VC300+PFOL group showed a significant ($P < 0.05$) increase in the duration of loss of jaw tone reflex when compared to those of the PFol and VC200+Pfol groups. The VC300+Pfol had the longest duration (18.14 ± 2.68 min), followed by the VC200+PFol (6.9 ± 0.89 min) and the PFol group (5.67 ± 2.03 min) showed the shortest duration of loss of jaw tone reflex.

4.6.4 Effect of ascorbic acid and propofol anaesthesia on palpebral reflex

Only 20 % of the animals in the PFol group lost the palpebral reflex, but in the VC200+PFol and VC300+PFol, 85.7 % and 100 % of animals, respectively lost the palpebral reflex.

CHAPTER FIVE

DISCUSSION

The present study showed a significant decrease in onset and significant increases in duration of anaesthesia in the groups administered with propofol and pre-medicated with ascorbic acid when compared to the group administered with propofol alone. This finding agrees with those of several workers that ascorbic acid decreases the onset of anaesthesia, but increases its duration in ketamine anaesthesia in rabbits (Elsa and Ubandawaki, 2005),a combination of xylazine and ketamine in rats (Alireza and Goudarz, 2007), and xylazine anaesthesia in rabbits (Egwu *et al.,* 2011) premedicated with ascorbic acid. The decrease in onset and increase in the duration of anaesthesia appear to be dose dependent, as the decrease in the time of onset as well as the increase in the duration of anaesthesia showed that at low doses the changes were insignificant but significant at high doses. Increasing the dose of ascorbic acid requires caution because ascorbic acid may act not only by exerting modulating influences on the central nervous system, inducing depression but also impairment of the functions of central nervous system when administered at high doses (Elsa and Ubandawaki, 2005). Although the proximate mechanism of action of propofol and ascorbic acid was not investigated in the present study, propofol has been shown to cause central nervous system depression by enhancing or potentiating the actions of GABA at the $GABA_A$ receptors (Hales and Lambert, 1992; Irifune *et al.*, 2003), while ascorbic acid has also been reported to potentiate GABAergic neurotransmission in the CNS (Calero *et al.*, 2011). The combined effect of both propofol and ascorbic acid on $GABA_A$ receptors may have lead to the increase in duration and decrease in the onset of anaesthesia.

The present study showed that intravenous ascorbic acid at large doses ameliorated the pain caused by injection with propofol. Werbach (2000) reported that ascorbic acid appears to exert a substantial analgesic effect at pharmacologic dosages. Ascorbic acid was found to inhibit cyclooxygenase (COX)-2-mediated PGE_2 production in neuronal cells. It is possible that ascorbic acid acts on the catalytic site of COX-2, which is the primary mechanism of action of aspirin-like drugs (Candelario-Jalil *et al*., 2006). This effect is probably the result of more complex redox-sensitive mechanisms, involved in specific interaction between propofol and ascorbic acid. Ascorbic acid was also reported to reduce interleukin-1β-mediated formation of ROS and PGE_2 in neuronal cells and this cytokine is one of the key agents involved in brain and peripheral inflammation (Candelario-Jalil *et al*., 2006). AA is a potent vitaminergic inhibitory neurotransmitter in the central nervous system (Karanth *et al.*, 2000), and this mechanism may be involved in its potentiation of analgesic effect in goats injected with propofol.

Another possible explanation for the analgesic action of ascorbic acid may be due to its indirect antagonistic effect on the *N*-methyl-D-aspartate (NMDA) receptor. NMDA, a glutamic acid receptor, is localized in the central nervous system and is responsible for the development of abnormal pain status, such as hyperalgesia and allodynia. It also plays a role in the transmission of noxious signals (Iwatsu *et al*., 2002). The result of the present study shows that ascorbic acid caused significant increases in magnesium levels, and magnesium is known to be a potent NMDA receptor antagonist, which explains its analgesic property (Memis *et al.*, 2002). Magnesium is also a calcium channel blocker, and calcium channel blockers have antinociceptive effects in animals (Memis *et al.*,

2002). This observation may further contribute to the decrease in pain score, observed in goats administered with ascorbic acid prior to propofol induction.

The decrease in heart rate caused by the administration of ascorbic acid alone disagrees with the result obtained by Egwu *et al.* (2011), who reported an increase in heart rate following the administration of ascorbic acid (120 mg/kg) alone to rabbits. This difference may be caused by the use of a higher dose (200 mg/kg) of ascorbic acid in this study. The observed decrease apparently resulted from the depressant activity of ascorbic acid on the central nervous system (Elsa and Ubandawaki, 2005).

In this study, propofol when administered alone was shown to cause an initial decrease in heart rate, and this finding is in agreement with the results obtained by some workers (Tramer *et al.*, 1997; Carrasco *et al.*, 1998). Propofol depresses the sympathetic nervous system directly, as do most other intravenous anaesthetic induction agents. The decrease in heart rate may be induced by direct depressant action of propofol on the sinus node (Mcintosh *et al.,* 2012). However, propofol administration in the present study was shown to cause an increase in heart rate after induction, which is in agreement with the findings of Robinson *et al.* (1994) and Shah *et al*. (2011), who reported an increase in heart rate after propofol administration. Other workers (Oztekin *et al.*, 2000, Kanaya *et al*., 2003) did not obtain any significant change in heart rate while Mcintosh *et al.* (2012) reported in rabbits, an initial increase in heart rate during propofol anaesthesia, later followed by a decrease.

In this study, ascorbic acid when administered alone non-significantly increased the respiratory rate of the animals. This disagrees with Egwu *et al*, (2011), who reported a decrease in respiratory rate after ascorbic acid administration in rabbits. Propofol when administered alone caused a decrease in respiratory rate during and after induction in the present study, and this is in agreement with the finding of Cleale *et al.* (2009), who reported a decrease in respiratory rate in cats after propofol administration. Propofol is known to have profound respiratory depressant properties. The groups administered with ascorbic acid prior to propofol induction showed a significant decrease in respiratory rate during induction, but there was also an increase after induction, especially in the group administered with 300 mg/kg of ascorbic acid. Since ascorbic acid induces central nervous system depressant activity at large doses, it is possible that the combined depressant effect of propofol and large doses of ascorbic acid caused the significant respiratory depression. However after induction there was an increase in respiratory rate in the VC300+PFol group. This finding may be due to the longer duration of propofol anaesthesia recorded in this group. The respiratory depressant effect of propofol resolves quickly once the drug is cleared from the blood, and propofol rapidly clears from the blood (Barr, 1995).

Ascorbic acid when given alone caused an insignificant increase in rectal temperature, which is in agreement with the result obtained by Egwu *et al.* (2011), who reported a slight increase in temperature of rabbits after administration of ascorbic acid. This result may be due to the modulating effect of ascorbic acid. Propofol when administered alone also caused an increase in rectal temperature during anaesthetic induction and a decrease in rectal temperature after the induction. This result is similar to the finding of

Cleale *et al.* (2009), who reported an increase in temperature of cats five minutes into anaesthesia and a decrease in temperature ten minutes afterwards.

In the present study, All the groups showed non-significant decreases in haematological values of PCV, leucocyte count, RBC count and Hb concentration, except the PFol group, which showed a slight increase in leucocyte count after propofol induction; and the V200+Pfol group which showed a slight increase in Hb concentration after anaesthetic induction. This finding is in agreement with that of Morey *et al.* (2006), who reported a decrease in RBC, leucocyte count, Hb, and PCV values after propofol induction over time in dogs. However the decrease in haematological values observed in this study was non-significant, which agrees with result of De Cosmo *et al.* (2005), who reported that propofol does not alter haematological, fibrinolytic, or hepatic function.

The erythrocyte membrane is rich in polyunsaturated fatty acids which is susceptible to lipid peroxidation and this result in the loss of membrane fluidity and cellular lysis (Alhassan *et al.*, 2010). The present study showed that propofol being a potent antioxidant (Tsuchiya *et al.*, 2002) protected erythrocyte membrane from lipid peroxidation. Ascorbic acid is known to have a protective effect on EOF, but in the present study, the combination of both ascorbic acid and propofol caused and increase in EOF. This is in disagreement with the results obtained by Tsuchiya *et al.* (2002) who reported that ascorbic acid enhances the antioxidant effect of propofol in oxidative hemolysis. The increase in EOF caused by the combination of ascorbic acid and propofol may be due to the overwhelming presence of antioxidants beyond the existing demand of erythrocytes as studies have shown that in large concentration, antioxidants

manifest some pro-oxidant effects. This results in their toxic build up, causing damages to the erythrocyte membrane.

In this study propofol caused a non-significant decrease in total protein and globulin concentration and a slight increase in albumin levels. Pascoe *et al.* (2006) reported a decrease in total protein during propofol anaesthesia in cats. The decrease in total protein observed after propofol anaesthesia in this study is, apparently, due to a decrease in globulin concentration. Propofol binds tightly to serum albumin, and because of this property a prolongation of effect may be expected in hypoalbuminaemia (Mazoit and Samii, 1999). In this study however, there was an increase in albumin concentration and a prolongation of the duration of anaesthesia was observed only in the groups pre-treated with ascorbic acid. Ascorbic acid, when administered alone, caused a decrease in total protein but not a change in globulin concentration. The albumin concentration however, decreased. In contrast, the VC200+PFol and V300+PFol groups showed elevated total protein, albumin and globulin concentration, with the V200+PFol group showing a significant increase in globulin concentration.

The study demonstrated that administration of ascorbic acid and propofol caused no significant alteration in the serum levels of Na^+, K^+, Cl and HCO_3.This result agrees with that of Eteng *et al.* (2006) that ascorbic acid administration does not have any adverse impact on plasma electrolytes. A significant increase was, however, observed in Mg levels in the VC and VC200+Pfol groups. Propofol, on the other hand, caused non-significant decreases in Cl, K^+, and Na^+. This report partly disagrees with the result of Lee *et al.* (2011) who reported hyperkalaemia in a young woman following induction with propofol.

In this study, glutathione peroxidase activity was significantly increased in the PFol group after anaesthesia. This result is consistent with those of Allam *et al.* (2008) and Farid and Ali (2000). The antioxidant effects of propofol have been attributed to its chemical similarity with other known antioxidants such as butylhydroxytoluene and tocopherol. These substances bind to cell membrane phospholipids and capture free radicals, thus disrupting the chain of transmission of lipoperoxidation in cell membrane fatty acid molecules. Eriksson *et al.* (1992) and De La cruz *et al.* (1998) concluded that propofol does not only inhibit lipid peroxide formation, but also increases the activity of the glutathione antioxidant system. At clinically relevant doses, apart from inhibiting lipid peroxidation, propofol may also act on enzyme systems (in particular on the glutathione system) which may lead to an increase in the activities of glutathione peroxidase, glutathione reductase and glutathione transferase. This effect increases cellular deposits of reduced glutathione, and hence, defensive cellular deposits of antioxidants, thereby protecting tissues from oxidative stress. The groups administered ascorbic acid also showed increases though not significant, indicating that propofol may be a stronger antioxidant than ascorbic acid.

In this study, there was also a significant increase in SOD activity in the group administered with propofol alone. This result agrees with that of Allam *et al*. (2008) who reported elevated activity of SOD in patients 10 minutes after anaesthesia with propofol. SOD is a metallo-enzyme which catalyses the dismutation of $O_2^{\bullet-}$ into O_2 and H_2O_2. It is important in the anti-oxidative defense mechanism and protects against lipid peroxidation (Nazifi, *et al.*, 2009).

In the present study, propofol caused an increase in ALP and AST activities, but a decrease in ALT activity. This finding partly disagrees with the result obtained by Mavadati *et al.* (2011), who reported non-significant decreases in AST, ALT and ALP activities in rabbit's plasma after anaesthesia with propofol. The result of the present study however agrees with that of Martin *et al*. (1997), who reported a non-significant increase in AST activity and a decrease in ALT activity after propofol infusion in nine children. AST and ALT activities are markers of liver injury (Annecke *et al.,* 2007) and researchers have demonstrated the toxic effect on liver cells of some anaesthetic agents such as diazepam (Mavadati *et al.,* 2011). However, longer post-anaesthetic patient evaluations are required to determine a possible hepatotoxicity effect of the anaesthetic agent used. Hypotension in combination with hypoxaemia (Baumgartner *et al.*, 2010) may be associated with the release of these enzymes from the heart muscle or liver during anaesthesia. However in the present study, plasma ALT and AST and ALP activities were within normal range for goats and the changes observed were non-significant. Overall, the results obtained in the present study agree with the finding of Mavadati *et al.* (2011) that propofol may be a safe anaesthetic with low adverse effect on biochemical parameters.

During anaesthesia, the central nervous function is evaluated on the basis of muscle reflexes (Kronen, 2007). The increase in duration of loss of reflexes observed in the groups administered with ascorbic acid prior to propofol anaesthesia may be as a result of the depressant effect of large doses of ascorbic acid. The loss of the tone of jaw muscles in this group indicated that at large doses AA adequately provided muscle relaxation during anaesthesia. The long duration of loss of pedal reflex indicated the ability of AA to adequately provide analgesia while the loss of the swallowing reflex

showed that AA provided a deeper and prolonged anaesthetic depth when compared to the other groups (Kronen, 2007).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, the study has shown that:

1. Administration of ascorbic acid prior to propofol decreases the onset and increases the duration of propofol anaesthesia.

2. At large doses, intravenous administration of ascorbic acid ameliorates pain caused by intravenous injection of propofol.

3. Propofol may be a more potent antioxidant than ascorbic acid.

4. Administration of propofol after premedication with ascorbic acid is safe and exerts low adverse effect on haematological and biochemical parameters.

6.2 Recommendations

1. Ascorbic acid should be administered prior to propofol anaesthesia in goats, especially when the animal has to remain anaesthesized for long periods.

2. Further work should be done to elucidate the mechanism by which ascorbic acid exhibits analgesic property.

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APPENDICES

APPENDIX I: Pain scores observed in goats following intravenous administration of ascorbic acid and propofol.

VC	PFOL	VC200+PFOL	VC300+PFOL
2	2	2	1
1	2	1	0
2	3	0	0
1	3	1	0
0	2	0	2
		3	0
		0	1

APPENDIX II: Heart rate (beats/min) observed following intravenous administration of ascorbic acid and Propofol in goats (Mean ± SEM).

Treatment	n	Before induction	During induction	After induction
VC	5	104.2 ± 22.7	Not applicable	81.2 ± 8.1
PFOL	5	108.0 ± 12.5	106.0 ±12.8	123.2 ± 9.3
VC200+PFOL	7	84.0 ± 10.8	96.3 ± 9.4	105.7 ± 10.4
VC300+PFOL	7	93.1 ± 13.7	112.6 ± 9.5	108.0 ± 10.1

APPENDIX III: Respiratory rate (breaths/min) observed following intravenous administration of ascorbic acid and Propofol in goats (Mean ± SEM).

Treatment	n	Before induction	During induction	After induction
VC	5	26.4 ± 5.60	Not applicable	27.2 ± 2.90
PFOL	5	28.0 ± 4.00	22.0 ± 6.10	18.4 ± 2.00
VC200+PFOL	7	31.4 ± 3.00	23.4 ± 3.80	22.9 ± 3.10
VC300+PFOL	7	33.3 ± 2.10*	21.1 ± 1.70	25.1 ± 0.70

APPENDIX IV: Rectal temperature (^{o}C) values observed following intravenous administration of ascorbic acid and Propofol in goats (Mean ± SEM).

Treatment	n	Before induction	During induction	After induction
VC	5	37.9 ± 0.18	Not applicable	38.2 ± 0.32
PFOL	5	37.9 ± 0.25	38.3 ± 0.46	38.1 ± 0.40
VC200+PFOL	7	37.9 ± 0.21	38.2 ± 0.30	38.0 ± 0.30
VC300+PFOL	7	38.5 ± 0.30	38.7 ± 0.30	38.3 ± 0.70

APPENDIX V: Haematological values observed following intravenous administration of ascorbic acid and Propofol in goats (Mean ± SEM).

		PCV (%)	WBC ($\times 10^9$/ L)	RBC ($\times 10^{12}$/L)	Hb (mg/dL)
VC	Before	24.50 ± 2.52	29.60 ± 2.91	2.31 ± 0.21	9.74 ± 0.70
	After	24.24 ± 1.95	26.56 ± 2.01	2.13 ± 0.16	9.28 ± 0.63
Pfol	Before	32.08 ± 2.16	24.18 ± 1.66	2.85 ± 0.16	10.98 ± 0.54
	After	27.60 ± 1.00	24.94 ± 1.73	2.76 ± 0.22	10.84 ± 0.65
V200	Before	29.16 ± 3.58	24.33 ± 2.20	2.73 ± 0.31	10.44 ± 0.45
+PFOL	After	23.50 ± 2.85	22.28 ± 2.76	2.56 ± 0.34	10.53 ± 0.48
V300	Before	32.07 ± 2.71	25.44 ± 1.95	3.04 ± 0.27	11.57 ± 0.64
+PFOL	After	28.90 ± 2.19	22.36 ± 2.20	2.65 ± 0.22	10.94 ± 0.56

APPENDIX VI: Changes in antioxidant enzyme activity observed following intravenous administration of ascorbic acid and Propofol in goats (Mean ± SEM).

		GSH (IU/L)	SOD (IU/L)
VC	Before	38.80 ± 1.86	1.46 ± 0.05
	After	41.00 ± 1.27	1.62 ± 0.10
Pfol	Before	30.40 ± 1.33	1.30 ± 0.08
	After	36.80 ± 0.97	1.70 ± 0.06
V200	Before	38.00 ± 1.23	1.50 ± 0.13
+PFOL	After	36.57 ± 2.30	1.83 ± 0.11
V300	Before	36.71 ± 1.97	1.36 ± 0.15
+PFOL	After	40.43 ± 1.66	1.69 ± 0.14