EVALUATION OF ROCURONIUM AND ITS REVERSAL BY NEOSTIGMINE AND GLYCOPYRROLATE COMBINATION IN PROPOFOL-ISOFLURANE ANESTHETIZED DOGS

THESIS

By

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CERTIFICATE I

This is to certify that the thesis entitled **"Evaluation of Rocuronium and its reversal by Neostigmine and Glycopyrrolate combination in Propofol-Isoflurane anesthetized dogs"**submitted in partial fulfilment of the requirements for the award of the degree of **Master of Veterinary Science** in the discipline of **Veterinary Surgery and Radiology** of CSK Himachal Pradesh Krishi Vishvavidyalya, Palampur is abonafideresearch work carried out by **DrYudhvir Rana (Admission No. V-2020-30-040)**, sonof Smt. Roshani Devi and Sh. Albar Rana, under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

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Date: 05.11.2022 Major Advisor

CERTIFICATE II

This is to certify that the thesis entitled **"Evaluation of Rocuronium and its reversal by Neostigmine and Glycopyrrolate combination in Propofol-Isoflurane anesthetized dogs"** submitted by **Dr Yudhvir Rana (Admission No. V-2020-30-040),**son of Smt. Roshani Devi and Sh. Albar Rana, to the CSK Himachal Pradesh Krishi Vishvavidyalya, Palampur in partial fulfilment of the requirements for the ward of the degree of **Master of Veterinary Science** in the subject of **Veterinary Surgery and Radiology** has been approved by the advisory committee after an oral examination of the student in collaboration with an External Examiner.

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CONTENTS

CHAPTER	TITLE	PAGE
1	**Introduction**	1-2
2	**Review of Literature**	3-13
3	**Materials and Methods**	14-27
4	**Results and Discussion**	28-63
5	**Summary and Conclusions**	64-67
	Literature cited	68-78
	Appendices	79-82
	Brief Resume of the Student	

ABBREVIATIONS

NMBA	Neuromuscular Blocking Agent
IPPV	Intermittent Positive Pressure Ventilation
NMB	Neuromuscular Block
$PaCO_2$	The partial pressure of carbon dioxide in the arterial blood
PaO_2	The partial pressure of oxygen in the arterial blood
I:E	Inspiratory to Expiratory ratio
TV	Tidal volume
T_0	Base value (before administration of the anaesthetic drugs)
T_1	Just after induction and the start of Isoflurane maintenance
T_5	5 minutes after Rocuronium was injected
T_{10}	10 minutes after Rocuronium was injected
T_{15}	15 minutes after Rocuronium was injected
T_{20}	20 minutes after Rocuronium was injected
T_{40}	40 minutes after Rocuronium was injected and post reversal
HR	Heart Rate
RT	Rectal Temperature
ECG	Electrocardiogram
RA	Right arm
LA	Left arm
RL	Right leg
LL	Left leg
NIBP	Non-invasive Blood Pressure
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
MAP	Mean Arterial Pressure
CVP	Central Venous Pressure
SpO_2	Oxygen saturation of Haemoglobin
EtO_2	End-tidal oxygen
FiO_2	Fractional inspired oxygen
$EtCO_2$	End-tidal carbon dioxide
$FiCO_2$	Fractional-inspired carbon dioxide

EtISO	End-tidal Isoflurane
FiISO	Fractional inspired Isoflurane
MAC	Minimum Alveolar Concentration
mA	Milliampere
ms	Millisecond
Amp.	Amplitude
min.	Minute
sec.	Second
mg%	Milligrams per cent
g%	Grams per cent
mg/dl	Milligrams per decilitre
mmol/l	Milli mole per litre
$p<0.05$	Statistically significant at a 5% level
$p<0.01$	Statistically significant at a 1% level
$p<0.001$	Statistically significant at a 0.1% level
SE	Standard error
conc.	concentration
ALT	Alanine transaminase
AST	Aspartate transaminase
PAL	Alkaline phosphatase
Inj.	Injection
@	At the rate of
ECG	Electrocardiogram/Electrocardiography
et al.	et alii (and others)
I/M	Intramuscular
I/V	Intravenous
Kg	Kilogram
b.wt.	Body weight
HR	Heart rate
RR	Respiration rate
RT	Rectal Temperature
Hb	Haemoglobin

PCV	Packed Cell Volume
TLC	Total leucocyte count
TEC	Total erythrocyte count
CRTN	Creatinine
BUN	Blood Urea Nitrogen
TP	Total Protein
Cl^-	Chloride ion
Na^+	Sodium ion
K^+	Potassium ion
IU/L	International unit per litre
IOP	Intra Ocular Pressure
ASA	American Society of Anesthesiologists
NIBP	Non-Invasive Blood Pressure
EDTA	Ethylene Diamine Tetra Acetic acid
^0F	Degree Fahrenheit
Temp.	Temperature
IBP	Invasive Blood Pressure
PLT	Platelet Count
PNS	Peripheral nerve stimulation

LIST OF PLATES

Plate no.	Title	Page no.
3.1	Peripheral nerve stimulation	17
3.2	Drugs used in the study	18
3.3	Anaesthesia monitoring equipment	25
3.4	Equipment used during the study for IPPV and IOP	26
3.5	Equipment for haemato-biochemistry and electrolyte analysis	26
3.6	Peripheral nerve stimulation equipment	27
4.1	Supracondylar fracture of femur managed by rush pinning technique	61
4.2	Femur fracture managed by ADMIT pinning technique	61
4.3	Pictures showing the centring of the ocular globe after administration of Rocuronium in the Propofol-Isoflurane anaesthetized dog	62
4.4	The pattern of ECG (Lead II) for Propofol-Isoflurane anaesthetized dogs at different time intervals, the animal was under NMBA between T_5– T_{20} and NMB was reversed between T_{20} and T_{40} (Paper speed= 25 mm/sec and 1 mV= 1 cm)	63

LIST OF FIGURES

FIGURENO.	TITLE	PAGE
4.1	Mean ±SE values of rectal temperature(^{0}F) at different time intervals. (n=21)	31
4.2	Mean ± SE of Intraocular pressure (mmHg)at various time intervals. (n=21)	32
4.3	Mean ± SE of Heart rate (beats/minute) at various time intervals. (n=21)	33
4.4	Mean ± SE of P amplitude (mV)at various time intervals. (n=21)	35
4.5	Mean ± SE of P interval(sec) at various time intervals. (n=21)	36
4.6	Mean ± SE of PR interval (sec) at various time intervals. (n=21)	36
4.7	Mean ± SE of R amplitude(mV)at various time intervals. (n=21)	37
4.8	Mean ± SE of QRS interval (sec) at various time intervals. (n=21)	37
4.9	Mean ± SE of QT interval(sec) at various time intervals. (n=21)	38
4.10	Mean ± SE of SBP (mmHg), DBP(mmHg) and MAP (mmHg) at various time intervals. (n=21)	40
4.11	Mean ± SE of CVP (cm of H_2O) at various time intervals. (n=21)	41
4.12	Mean ± SE of SpO2 (%) of at various time intervals. (n=21)	42
4.13	Mean ± SE of Isoflurane vaporizer concentration (%) at various time intervals. (n=21)	43
4.14	Mean ± SE of Respiration rate (breaths per minute) at various time intervals (n=21)	44
4.15	Mean ± SE of End Tidal Oxygen (vol %) and Fractional Inspired Oxygen (vol %) at various time intervals. (n=21)	45
4.16	Mean ± SE of EtCO2 (mmHg)at various time intervals. (n=21)	46

4.17	Mean ± SE of FiCO2(vol %) at various time intervals. (n=21)	47
4.18	Mean ± SE of End Tidal Isoflurane (vol %) and Fractional Inspired Isoflurane(vol %) at various time intervals. (n=21)	48
4.19	Mean ± SE of Minimum Alveolar Concentration (vol %) at various time intervals. (n=21)	49
4.20	Mean ± SE of Haemoglobin (g/dl) and Packed Cell Volume (%) at various time intervals. (n=21)	50
4.21	Mean ± SE of Total leukocytic count (10^9/µl) and Total erythrocytic count (10^{12}/ µl) at various time intervals. (n=21)	51
4.22	Mean ± SE of Platelet count(10^9/µl) at various time intervals. (n=21)	52
4.23	Mean ± SE of Plasma Glucose (mg/dl) at various time intervals. (n=21)	53
4.24	Mean ± SE of Alanine Transaminase (IU/l) and Aspartate Transaminase (IU/l) at various time intervals. (n=21)	54
4.25	Mean ± SE of Total Protein (g/dl), Blood Urea and Nitrogen (mg/dl) and Creatinine (mg/dl) at various time intervals. (n=21)	55
4.26	Mean ± SE of Sodium ion(mmol/l) and Chloride ion (mmol/l) concentration at various time intervals. (n=21)	56
4.27	Mean ± SE of Potassium ion concentration (mmol/l) at various time intervals. (n=21)	57
4.28	Mean ± SE of time elapsed(minutes) for recovery parameter to exhibit. (n=21)	58

LIST OF TABLES

Table No.	Title	Page
3.1	Study Design.	15
3.2	Ventilator settings.	16
3.3	Settings of Peripheral nerve stimulator.	16
3.4	Surgical procedures performed during the study and ASA classification.	19
3.5	Time intervals for taking and recording various observations.	19
3.6	Time intervals for recording Haemato-biochemical parameters and IOP (Intraocular pressure)	20
4.1	Patient signalment and ASA categorization. (n= 21)	28
4.2	Doses of different drugs, their time of onset and duration of action. (n= 21)	29
4.3	Mean ± SE of Rectal temperature (°F), Heart rate (beats/minute) Respiration rate (breaths per minute) at various time intervals. (n= 21)	30
4.4	Mean ± SE of Intraocular pressure (mmHg)at various time intervals. (n= 21)	32
4.5	Mean ± SE of Electrocardiogram Parameters at various time intervals. (n= 21)	34
4.6	Mean ± SE of Systolic Blood Pressure (mmHg), Diastolic Blood Pressure (mmHg) and Mean Arterial pressure (mmHg) at various time intervals. (n= 21)	39
4.7	Mean ± SE of Central venous pressure (cm of H_20) at various time intervals. (n= 21)	40
4.8	Mean ± SE of Pulmonary Parameters at various time intervals. (n= 21)	44
4.9	Mean ± SE of Haematological Parameters at various time intervals. (n= 21)	50
4.10	Mean ± SE of Biochemical Parameters at various time intervals. (n= 21)	52
4.11	Mean ± SE of electrolyte Parameters at various time intervals. (n= 21)	56
4.12	Mean ± SE of time elapsed(minutes) for recovery parameter to exhibit. (n= 21)	58

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ABSTRACT

This study was executed in the clinical setup to document the effects of Rocuronium and its reversal by Neostigmine and Glycopyrrolate combination in Propofol-Isoflurane anaesthetized dogs. Rocuronium-induced neuromuscular blockade was applied to 21 clinical cases. Out of which 19 cases of orthopaedic surgeries and 2 cases of ovariohysterectomy were performed. Every animal included in this study was premedicated using Inj. Butorphanol tartrate at the dose rate of 0.2mg/kg b.wt I/M, then after a gap of 30 minutes Inj. Atropine sulphate was administered at the dose rate of 0.02mg/kg b.wt I/M, again after a gap of 10 minutes animal was premedicated with Inj. Diazepam at the dose rate of 0.5mg /kg b.wt. I/V and followed immediately by Inj. Propofol I/V(to the effect), for the induction of general anaesthesia. Soon after induction, the animal was maintained on a mixture of Isoflurane and oxygen. After the animal had stabilized, the relaxation of muscle was induced using Inj. Rocuronium at the dose rate of 0.5 mg /kgb.wt. I/V. Immediately IPPV was provided in volume control mode, with settings, a respiration rate of 15 breaths per minute, tidal volume at 10ml/kg body weight and inspiratory expiratory ratio as 1:2. Neuromuscular blockade was reversed at the first sign of asynchrony on the bellow and the multipara monitor. A single syringe combination drug Inj.Myopyrolate I/V having both Neostigmine and Glycopyrrolate was administered at the dose rate of 0.05mg/kg b. wt. and 0.01mg/kg b.wt respectively. Rocuronium caused the centring of the ocular globe gradually within 30 seconds. The central position of the ocular globe facilitates many kinds of surgical interventions and examinations in the eyes. During orthopaedic surgeries, the reduction of fractured ends became effortless. Easy reduction of the fracture without tissue trauma was observed. In ovariohysterectomy surgeries, the appropriate level of abdominal muscle relaxation further helped in the easy exteriorization of the ovarian stump. The onset time for Rocuronium was 17.64±1.10 sec and it provided muscle relaxation for 27.82 ± 0.72 min. The use of Rocuronium along with IPPV caused minimal alteration of the physiological parameters with no consequences clinically and thus can be considered a complication-free anaesthetic protocol for interventions demanding muscle relaxation.

Student | Major Advisor
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CHAPTER 1 Introduction

General anaesthesia has three components: narcosis, analgesia, and muscle relaxation. All three components can be produced using a single general anaesthesia drug, but for achieving effective muscle relaxation deep level of general anaesthesia is required which in turn produces adverse effects such as respiratory depression and circulatory failure.

With the introduction of neuromuscular blocking agents, a light level of anaesthesia with good analgesia and adequate muscle relaxation became a possibility, thus any degree of relaxation could be achieved irrespective of the depth of anaesthesia.

The neuromuscular blocking drugs act specifically on the neuromuscular junction thereby causing paralysis or relaxation of voluntary striated muscle. These drugs have no other important effect on the body except on the neuromuscular junction and they produce fast and effective muscle relaxation with no or minimal effect on the central nervous system and circulatory system.

The main indication for use of muscle relaxants or more specifically neuromuscular blocking agents (NMBAs) are to relax skeletal muscles to make surgical access easier, prevent movement during ophthalmic, orthopaedic, aural and abdominal procedures, to allow the initiation of intermittent positive pressure ventilation and since NMBAs are part of balanced anaesthesia as they reduce the amount of general anaesthetic required.

There are two types of neuromuscular blocking agents namely depolarising and non-depolarising neuromuscular blocking agents. They are quite different according to the effects they produce. The depolarising neuromuscular blocking agents are characterized by relatively rapid onset, muscle fasciculations and non-reversal by anticholinesterases.

Whereas the non-depolarising neuromuscular blocking agents are mostly mono or biquaternary salts and are hydrophilic, they have a relatively slow time of onset and muscle fasciculations are not produced. Anticholinesterases are used for the reversal of muscle relaxation provided by non-depolarising neuromuscular blocking agents. Non-depolarising neuromuscular blocking agents are preferred in Veterinary clinical practice because of the lesser side effects and comparatively longer duration of action than depolarising neuromuscular blocking agents.

Rocuronium is an aminosteroidial competitive, non-depolarizing neuromuscular blocking agent with a rapid to intermediate onset depending on the dose and an intermediate duration of action. The Rocuronium is eliminated primarily by the liver in dogs and a small fraction is eliminated by the kidney. It is freely soluble in water and has a longer shelf-life and does not appear to induce malignant hyperthermia.

The animal under neuromuscular blockade should be monitored carefully so that the surgery can be performed on the animal in a relaxed state of musculature and residual blockade, if any, during recovery can be detected and treated. While calculating the dose of neuromuscular blocking agent its interaction with inhalant anaesthetic and other drugs of the anaesthetic protocol should be kept in mind.

The reversal agents in case of non-depolarising neuromuscular block, when used in the proper manner do not allow the residual neuromuscular block to set in and thus recurarization does not occur. The effects of neuromuscular block provided by Rocuronium can be effectively antagonized with acetyl cholinesterase inhibitors such as Neostigmine. The effects of the increase in the concentration of acetylcholine produced by acetyl cholinesterase inhibitors are not specifically limited to the neuromuscular junction, but also occur at other cholinergic sites of the patient's body. The muscarinic effects include emesis, enhanced intestinal tone, bronchoconstriction, salivation and bradycardia.

To prevent muscarinic effects, specifically bradycardia, Glycopyrrolate or Atropine may be given in small animals along with acetyl cholinesterase inhibitors. The Neostigmine and Glycopyrrolate combination is preferred as it has a slower onset and a longer duration of effect.

The clinical experience with Rocuronium in veterinary patients is limited. So, this research was formulated to evaluate the use of Rocuronium as a muscle relaxant and its reversal by Neostigmine and Glycopyrrolate combination (Myopyrolate) in Propofol-Isoflurane anaesthetized dogs with the following objectives:

1. To study the effects of Rocuronium in Propofol-Isoflurane anaesthetized dogs.
2. To evaluate the reversal of Rocuronium neuromuscular blockade by Neostigmine and Glycopyrrolate combination in dogs.

CHAPTER 2 Review of literature

To relax skeletal muscles, it is important to abolish voluntary muscle contractions and modify the slight tension i.e. tonus of muscle. Neuromuscular blocking agents are an important part of the anaesthetic protocol in human anaesthesia and gradually gaining importance in veterinary practice also. The neuromuscular blocking agents provide skeletal muscle relaxation thereby making surgical access easier, facilitate control of respiration, assist in the reduction of dislocated joints and cause centering of the ocular globe for various ophthalmic interventions. The reversal of neuromuscular block using acetylcholinesterase inhibitors is important in preventing residual blockade associated with the use of neuromuscular blocking agents. Therefore, keeping the objectives of the present study in mind, the available literature has been reviewed under the following headings:

2.1 Muscle relaxant

2.2 Neuromuscular blocking agent

2.3 Rocuronium

2.4 Neostigmine and Glycopyrrolate

2.5 Diazepam

2.6 Propofol

2.7 Isoflurane

2.8 Mechanical ventilation

2.9 Monitoring of the neuromuscular block (NMB)

2.1 Muscle relaxant

Rex (1968) reported that balanced anaesthesia should provide muscle relaxation, sleep and analgesia, further stating that the use of a specific drug for the purpose of muscle relaxation had a sparing effect on general anaesthetic, also surgical trauma was reduced when there was good access to the surgical site due to relaxation of skeletal muscles.

Both risk and surgical trauma were reduced very much by the use of muscle relaxants thereby increasing the probability of the successful outcome of surgery. The anaesthetist using the muscle relaxant must use them with utmost care and must understand that these were potent drugs, also their use should only be undertaken when a skilled anaesthetist and the required facilities were available (Stevenson1960).

The first use of muscle relaxant in a human subject in 1942 was a major achievement, a purified form of curare was injected intravenously into 25 patients under a light plane of anaesthesia, and in every subject complete relaxation of musculature was quickly produced for some period of time with apparently no side effects (Griffith and Johnson1942) and the first use of curare in the pure form was attempted in dogs (Pickett1951).

2.2 Neuromuscular blocking agent

Kopman (1989) suggested that the onset time of neuromuscular block was inversely proportional to the potency of the nondepolarizing neuromuscular blocking drug.

Martin-Flores et al (2018) surveyed that almost all veterinary practitioners were wary of the residual effect of neuromuscular block, but most of them took relatively few measures to decrease the chance of occurring of residual neuromuscular block, for example, regular use of quantitative neuromuscular block monitoring and reversal of neuromuscular block regularly.

The relaxation of musculature that was caused due to neuromuscular blocking agent helped a lot in carrying out surgical interventions in canine subjects by making the position of the eyeball favourable for surgery (Young et al. 1991)

For the majority of corneal and intraocular procedures, the use of neuromuscular blocking medications after general anaesthesia had been stabilised which allows for the best exposure of the ocular globe. The whole cornea was exposed because of the paralysis and lack of tone of extraocular musculature by these medications in dogs (Gelatt et al. 2011).

As neuromuscular blockade gives better surgical access to the abdominal cavity and promotes organ manipulation, neuromuscular blocking drugs (NMBA) were often used in human or animal laparoscopic surgeries (Rauser et al. 2015).

2.3 Rocuronium

Rocuronium bromide was a reliable nondepolarizing neuromuscular blocking agent having a good effect on the canine. Rocuronium has a monoquartenary structure and was an analogue of Pancuronium, also it was in close relation to vecuronium, it produces a neuromuscular block which has a quick onset time and an intermediate duration of action (Tranquili et al, 2007; Dugadale et al. 2002). As Rocuronium had low potency, it was required to be given in high dosages to achieve an adequate number of molecules at the neuromuscular junction causing its quick action (Hunter 1996).

Rocuronium bromide had appreciable chemical stability and very little hepatic biotransformation and in form of an infusion, it was reliable for a moderate duration in subjects having normal liver and kidney function. The lack of detectable metabolites shows minimal metabolism of Rocuronium in the body (McCoy et al. 1996). The inactive metabolite 17-deactyl-Rocuroniumwas detected in very fewer amounts in humans, mice and canines. The Rocuronium bromide was excreted through biliary excretions in greater concentration after its uptake by the liver. It was found that about 26% of the dose of Rocuronium given to human patients was excreted in urine and about 30% of the dose was excreted in faeces within 7days of time duration of giving Rocuronium to the human patient (Proost et al. 2000).

Neves et al. (2014) administered Rocuronium at the dose rate of 0.1 mg/ kg and reported that it caused a very small change in respiratory parameters and enhanced the relaxation of muscle thus making surgical access easy.

Dugdale et al. (2002) reported that neuromuscular block with Rocuronium had an onset time of 98±52 seconds and neuromuscular block has a duration of 32.3±8.2 minutes and top-up dose of 0.16mg/kg has a duration of 20.8±4.9 minutes and he further reported that the drug showed no tendency to accumulate so Rocuronium should be safe for use as continuous rate infusion. A mild increase in arterial blood pressure without a corresponding increase in heart rate was reported.

In humans, Rocuronium was slightly vagolytic in action so it causes a little transient tachycardia, particularly in children when a high dose is administered. (Khuenl-Brady and Sparr 1996).

Muir et al. (1989) reported that the absence of cardiovascular side effects makes Rocuronium a reliable neuromuscular blocking agent in the anaesthesia protocol of canines. The tachycardia after the neuromuscular block was due to the cardiac muscarinic receptor blockage, release of noradrenaline in increased amounts and blocking of the process of its reuptake, blocking of nicotinic choline receptor at ganglia or release of histamine. He further stated that the metabolite of Org 9426 (Rocuronium) contributing to neuromuscular block or having cardiovascular side effects or causing residual neuromuscular block was highly unlikely.

The use of Rocuronium has not shown any evidence of the release of histamine (Cason et al 1990). Auer (2007) stated that administration of Rocuronium at a dose rate of 0.3 and 0.6 mg/kg produces a good neuromuscular block ranging from 23 minutes to 32 minutes respectively. There was not much change in the pharmacodynamic parameters of the Rocuronium with changes in the age of the dog, nerve stimulation sites, weight and anaesthesia protocol being used to anaesthetize the dog.

In humans, the onset time of Rocuronium in older subjects was the same as that of their younger counterparts but the duration of neuromuscular block was for the longer time interval in older patients as compared to younger subjects, because of decreased plasma clearance along with the decrease in volume of distribution. Both volume distribution and plasma clearance contribute towards the elimination half-life of a drug (Matteo et al. 1993)

Sakata et al. (2019) stated that sevoflurane was able to potentiate the effect of Rocuronium-induced neuromuscular blockade in a dose-dependent manner in dogs. Auer et al. (2007) stated that Rocuronium injected intravenously in dogs at the dosage of 0.1 mg /kg b.wt causes the central position of the eyeball but had very less effect on ventilation.

In present veterinary practice as in human medical practice, all the major surgeries must be undertaken under balanced anaesthesia and it becomes more important in orthopaedic interventions. Popovici et al. (2014) found that with a balanced anaesthesia protocol using Rocuronium in canines with orthopaedic ailments, there was only a little change in HCT, RBC and Haemoglobin that was considered normal. The leukocytic count was within the normal range. Blood glucose decreased to a lower level after surgery than it was present before surgery. The normal level of urea and creatinine shows that renal perfusion was good. Increased levels of total protein, globulin and albumin signify a catabolic state post-surgery. The increment in ALT and AST levels was due to muscle injury and the increment in ALP (alkaline phosphatase) was because of injury to bone, the level of these enzymes was not affected by the anaesthetic protocol. Levels of sodium and potassium remained in the normal range. Rocuronium was advantageous for orthopaedic surgeries because it provided good muscle relaxation and it was free from any major haematological, biochemical or electrolyte change.

The residual neuromuscular block was defined as the partial recovery of the neuromuscular transmission during the postoperative period after the neuromuscular blocking drug had been used. In humans the cases of residual neuromuscular block vary from 2% and 64% according to the methods used, the kind of neuromuscular blocking agent used and whether the neuromuscular block has been reversed or not (Murphy and Brull 2010)

Murphy et al. (2008) in a study on human patients concluded that the residual effect of neuromuscular block could lead to respiratory complications in the postoperative period in general anaesthesia. Lack of expertise on the part of the clinicians for assessing recovery from the neuromuscular block and no timely monitoring of neuromuscular block in the peri-operative duration was reported. Proper management of neuromuscular block by optimising the dose of neuromuscular blocking agent, timely reversal of neuromuscular blockade and proper monitoring might decrease the chance of residual block and uneventful recovery can occur.

Haga et al. (2019) concluded that to maintain a good neuromuscular block in varied canine populations there was a significantly large variation in the amount of Rocuronium infused. Out of all the variables studied, diabetes mellitus was the only one which had a significant influence on the dose rate of Rocuronium. Thus, canines having diabetes mellitus needed a higher rate of infusion of Rocuronium bromide as compared to nondiabetic canines.

Sakai et al. (2017) concluded that when Rocuronium was injected at a rate of 0.6 mg/kg b.wt in a dog anaesthetized with inhalant Isoflurane and kept under continuous dexmedetomidine infusion, neuromuscular block at larynx recovers more slowly than neuromuscular blockade at the pelvic limb. They further reported that quantitative monitoring of the neuromuscular blockade being done at the hind limb using the peroneal nerve might miss the residual block present in the laryngeal musculature.

Alderson et al. (2007) in their research on dogs injected Rocuronium first as a bolus, at the dose rate of 0.5 mg/kg b.wt and then Rocuronium as the infusion was given immediately at the dose rate of 0.2 mg/kg/hr. They further found this protocol to be satisfactory in dogs and documented that CRI (constant rate infusion) of the Rocuronium can be effectively applied to clinical cases.

Sakai et al. (2018) in their work on cats, after giving an anaesthesia combination of Ketamine and Diazepam, injected the animals with Rocuronium at the dose rate of 0.3mg/kg b.wt., reported that the above protocol helped to improve the condition for intubation and decreases the length of time and number of times the intubation need to be attempted in the animal. Also, with this protocol animals showed apnoea for a very short time period.

Kuls et al. (2016) reported anaphylaxis in a single dog after a Rocuronium injection. The animal was injected with Rocuronium at the dose rate of 0.4mg/kg b. wt and the animal was anaesthetized with Midazolam, Propofol and Isoflurane combination. The animal showed signs of increased heart rate, along with increased blood pressure and bronchospasm.

2.4 Neostigmine and Glycopyrrolate

Mirakhur et al. (1977) made the comparison of the Glycopyrrolate and Neostigmine combination with the Atropine and Neostigmine combination for the reversal of neuromuscular block induced by Pancuronium in humans. The Glycopyrrolate and Neostigmine combination was concluded to be safe and effective. The heart rate was more stable with Glycopyrrolate and also anti sialagogue property of Glycopyrrolate was better than Atropine.

Increased concentration of acetylcholine produced due to acetylcholinesterase inhibitors was not specific in effect to the neuromuscular junction but also affects other cholinergic sites. Muscarinic effect acetylcholinesterase inhibitors include emesis, bradycardia, enhanced intestinal tone, bronchoconstriction, salivation and miosis (Plumb1999; Adam 2001).

It was promoted that Edrophonium should be used in combination with Atropine due to its rapid onset and short duration of action and Neostigmine should be used in combination with Glycopyrrolate as it has a slow onset and its effect is for a longer duration of time (Clutton and Glasby2008).

Jones et al. (2015) concluded that residual neuromuscular block had been associated with complications in human subjects and must be prevented by proper monitoring. The adequate use of reversal agents prevents the residual neuromuscular block from setting in and thus preventing recurarization. When required anticholinesterase inhibitors, Neostigmine and Edrophonium were used as reversal agents.

2.5 Diazepam

Diazepam frequently caused dogs to get too excited and did not have a calming effect. With the exception of a considerable increase in heart rate, it had little impact on the cardiopulmonary system (Haskins et al. 1986).

Regarding its impact on cardiovascular and haemodynamic parameters, Khurana (2013) found that the combination of Diazepam, Butorphanol, and Halothane was safer than that of Acepromazine, Butorphanol, Propofol and Halothane. Using Diazepam and Acepromazine as premedication drugs helped to reduce the dose of Propofol for the induction in both the anaesthetic combinations.

2.6 Propofol

According to Deutschman et al. (1994), Propofol induction and maintenance caused bradycardia because it decreases sympathetic tone more than the parasympathetic tone in human patients

After injecting a Propofol bolus in dogs, Bayan et al. (2002) noticed a temporary rise in respiratory rate followed by a drop, as well as transient apnoea lasting 15–35 seconds in dogs. After induction, the respiratory minute volume and respiratory tidal volume rapidly fell for the first 15 minutes, and subsequently steadily reduced.

As per Yoo et al. (2002), when Propofol was given alone, the animal's heart rate increased and then progressively declined. An insignificant decrease in the mean arterial pressure was also observed by them in their study.

According to Sano et al. (2003), premedication with Medetomidine, Acepromazine-Butorphanol, or Midazolam-Butorphanol was considered to be beneficial for the induction of anaesthesia with Propofol in dogs in a clinical context.

2.7 Isoflurane

Reid et al. (2001) in their study on humans reported that the continuous administration of inhalant anaesthesia Sevoflurane and to some extent Isoflurane resulted in delayed reversal of Rocuronium-based neuromuscular block when antagonised with Neostigmine.

Dragne et al. (2002) demonstrated that when Isoflurane was used as a general anaesthetic agent the dose rate of Rocuronium has to be reduced when a stable condition of anaesthesia was maintained whereas no change in dosage was required when dogs are maintained on Propofol anaesthesia. The potentiation of neuromuscular block set in by Rocuronium was caused by the Isoflurane and was pharmacodynamic in nature, which makes continuous monitoring essential. In surgical interventions of one hour or less, potentiation by Isoflurane of the neuromuscular block was not of clinical significance and if the incremental dose of Rocuronium was to be administered no change in dose was required. Whereas for the surgical interventions which were of longer duration, the dosage of Rocuronium was to be decreased to avoid potentiation of neuromuscular block by

Isoflurane. The dose of Rocuronium used for maintenance in Propofol anaesthesia is reduced to about fifty per cent when Isoflurane was used for maintenance of anaesthesia and surgery was about two hours or above in duration.

Kuusela et al. (2003) concluded that Propofol/Isoflurane anaesthesia was better than using Propofol infusion for inducing general anaesthesia in dogs. The respiratory system was undermined to a lesser extent and recovery from Propofol/Isoflurane anaesthesia was better and in lesser time.

Coskun and Saritas (2011) in their study on dogs equated the results of the inhalant anaesthetic agent Isoflurane with those of sevoflurane in relation to their effect on intraocular pressure. They concluded in this study that sevoflurane was better and safer as compared to Isoflurane because it alters intraocular pressure and haemodynamic measurements to a lesser extent.

2.8 Mechanical ventilation

Vesal (2012) utilized intermittent positive pressure ventilation for surgery of a diaphragmatic hernia in a dog. Intermittent positive pressure ventilation was started as soon as the animal was induced and the airway was secured with an endotracheal tube. It was recommended that to avoid barotrauma, a high setting for inspiratory pressure should not be used.

Senthilkumar (2013) stated that the use of a mechanical ventilator for providing intermittent positive pressure ventilation is essential when a neuromuscular blocking agent like Rocuronium bromide was administered to dogs under the anaesthetic protocol. Also, in Rocuronium-induced neuromuscular block, the oxygen requirement is calculated by taking the tidal volume of 20ml/kg body weight, which was sufficient to maintain optimum oxygenation and ventilation. The oxygen gas required in order to drive the bellows in the ventilator during the process of ventilation may differ according to the compliance of the lungs of the patients. It was further concluded that the total need for oxygen as driving gas should be calculated for the estimated duration of mechanical ventilation and it was made available to keep the patient safe during the process of neuromuscular block.

Kumar (2017) concluded that anaesthesia protocols with atracurium and vecuronium under intermittent positive pressure ventilation had very little effect on normal physiological parameters and thus both these anaesthesia protocols are without any complications and provide the required muscle relaxation. Neuromuscular blocking agent caused relaxation of muscle and centring of the eye which facilitated the surgical intervention in the eyeball or cornea. Muscle relaxation made surgical access to corrective osteotomies and also made an intervention in fracture having contracture simpler. He further concluded that a neuromuscular blocking agent under intermittent positive pressure ventilation decreased the overall demand for Isoflurane.

2.9 Monitoring of the neuromuscular block

It was important to ascertain proper recovery from the neuromuscular block and so it was of utmost importance to monitor the neuromuscular block. The technique that uses electrical stimulation of the peripheral nerve for monitoring neuromuscular block should be used. In companion animals, the force of contraction of the levator nasolabialis due to stimulation of the facial nerve or the force of contraction of the digital extensor muscle of the pelvic limb due to stimulation of the peroneal nerve can be monitored (Clutton2007).

Postoperative Recurarization was an enhanced level of neuromuscular block after a period of recovery. The electrically stimulated muscle could be judged for neuromuscular block subjectively through visual observation. Objective monitoring techniques like acceleromyography, electromyography and mechanomyography were used to prevent a neuromuscular block (Fuchs-Buder et al. 2009).

Tactile and visual monitoring of a neuromuscular block includes gauging the strength of twitch and fade was confirmed through touching or observing. Monitoring neuromuscular block through the tactile method was done by using fingertips as preload for the muscles to be stimulated and in the process gauging the strength of contraction of the muscle. The tactile method of monitoring neuromuscular block was more reliable than the visual method of monitoring, as per studies on human subjects (Klein 1981; Dorsch and Dorsch 2008).

Gaston *et al*. (2015) in their study on sheep concluded that the anaesthetic combination of Diazepam and Ketamine led to an instant fall in intraocular pressure whereas there was no change in intraocular pressure when Diazepam was used in combination with Propofol to induce anaesthesia. Also, Li et al. (2020) observed that neuromuscular blocking agents namely Rocuronium, Mivacurium and Cisatracurium when used in humans, did not cause any appreciable change in intraocular pressure.

CHAPTER 3 MATERIALS AND METHODS

3.1 Study Design

The study was undertaken in the Department of Veterinary Surgery and Radiology, Dr G.C.Negi College of Veterinary and Animal Sciences, C.S.K.H.P.K.V-Palampur, Himachal Pradesh. The study was undertaken in two phases, the standardization phase and the clinical trial phase.

3.2 Standardization phase

In this phase, pilot trials were conducted on unlinked canine subjects. A total of 8 dogs were divided into two groups, group 1(n=2) and group 2(n=6). These animals were separate from those subjects that were used in the clinical trial phase.

In group 1(n=2) animals were used for standardisation of doses of Rocuronium[5]with the clinically proven anaesthetic protocol of Butorphanol[1], Atropine[2], Diazepam[3] and Propofol[4] and maintenance was done with Isoflurane[7] and reversal of the neuromuscular block was not undertaken in these animals. In group 2(n=6) animals were used for the standardisation of doses of both Rocuronium and its reversal, a single syringe combination drug of Neostigmine and Glycopyrrolate (Myopyrolate[6]) with clinically proven anaesthetic protocol mentioned above.

The animals were administered the neuromuscular blocking agent Rocuronium and were connected to a mechanical ventilator[8], intermittent positive pressure ventilation (IPPV) was provided to the animals under volume control mode. The settings of the ventilator under volume control were kept as a respiration rate of 15 breaths per minute, tidal volume at 10ml/kg body weight and inspiratory expiratory ratio as 1:2. The dose rate of Rocuronium was finalized as 0.5mg/kg b.wt I/V and that of Neostigmine and Glycopyrrolate(Myopyrolate) were finalized as 0.05mg/kg b. wt I/V and 0.01mg/kg b.wt I/V respectively. The literature available and the preliminary observations made during pilot trials were used to arrive at the final doses.

3.3 Clinical trial phase

The study included client-owned dogs of either sex, various breeds, having a mean age of 19.70±3.94 months (range 3months to 5yrs) and mean body weight of18.82 ± 1.70 kgs (range 9kgs to 30 kgs) presented to the Department of veterinary surgery and radiology of the veterinary college for various surgical interventions.

Table 3.1: Study Design

Drugs	Group (n=21)
Pre anaesthetics	Inj. Butorphanol tartrate (0.2mg/kg b.wt. I/M) Inj.Atropine sulphate (0.02 mg /kg b.wt. I/M)
Premedication	Inj. Diazepam (0.5mg/kg b.wt. I/V)
Induction	Inj.Propofol (till the desired effect is obtained, I/V)
Maintenance	Isoflurane mixed in oxygen. (Coaxial circuit)
NMBA	Inj.Rocuronium (0.5 mg/kg b.wt I/V)
Antagonist for NMBA	Inj.Myopyrolate (Inj. Neostigmine 0.05mg/kg b.wt + Glycopyrrolate 0.01mg/kg) I/V

3.4 Technique of drug administration

The dogs were kept off feed for about twelve hours and kept without water for about four hours. A detailed physical examination of the animal was done. Every animal was restrained in lateral recumbency. Different sites for putting ECG electrodes were prepared and ECG was recorded after giving about 10minutes of stabilisation time to the animal, electrodes were applied at the standard positions to obtain base value (T_0). The area around the cephalic and saphenous veins on the limbs was aseptically prepared for collecting blood samples, injecting drugs and infusing ringer lactate. All parameters were recorded to form base values (T_0)

After obtaining the base values of all the parameters, the administration of drugs was done according to the doses mentioned in Table 3.1. After surgical anaesthesia had been induced with Propofol, the airway of the animal was secured by endotracheal intubation and the animal was quickly shifted to an inhalant anaesthetic machine. After the animal had stabilized physiologically, the neuromuscular blocking agent was administered at the dose rate mentioned in Table 3.1and the animal was immediately shifted onto a ventilator providing intermittent positive pressure ventilation according to settings mentioned in Table 3.2 Animal was kept on IPPV and was maintained throughout surgery on Isoflurane mixed with oxygen. Neuromuscular block induced by Inj.

Rocuronium was reversed at the first sign of asynchrony on the bellow of the ventilator and the multiparameter monitor[9]. A single-syringe combination drug having both Neostigmine and Glycopyrrolate (Myopyrolate) was administered at the dose rate mentioned in table 3.1.

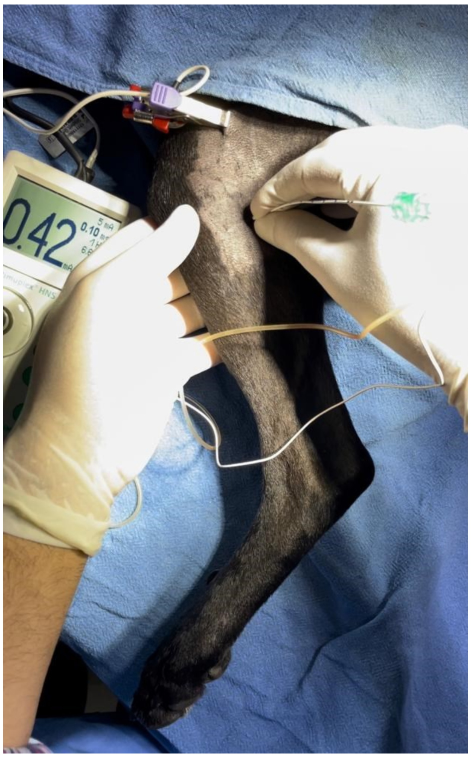
Table 3.2: Ventilator settings	
Ventilation mode	Volume control
I: E	1:2
Tidal Volume	10 ml/kg b.wt
Breaths per minute	15

3.5 Peripheral nerve stimulation

Peripheral nerve stimulation was used to ascertain that the animal was out of the neuromuscular blockade and could be safely weaned from the ventilator. It was helpful in preventing residual nerve block. Anatomical landmarks were identified on the limbs and the needle of the nerve stimulator was applied near the anatomical position of the nerve and the nerve was stimulated by the peripheral nerve stimulator[15]. The nerve stimulation was carried out with the help of a nerve stimulator as per the settings mentioned in Table 3.3.

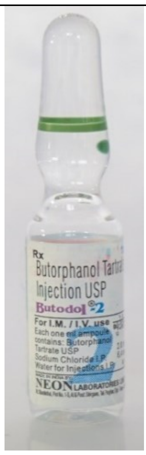
The peroneal nerve could be located caudal and distal to the head of the fibula and the ulnar nerve could be found on the medial aspect of the olecranon process as shown in plate 3.1.

Table 3.3: Settings of Peripheral nerve stimulator	
Voltage	2mA (gradually reduced to 0.3 to 0.4mA)
Frequency	1 hertz
Impedance	Calculated by the simulator itself
Duration of electrical impulse	0.10 ms

Plate 3.1: Peripheral nerve stimulation	
	
Ulnar nerve	**Peroneal nerve**

- 1. Inj. Butodol, Neon Laboratories Limited, Maharashtra
- 2. Inj. Tropine, Neon Laboratories Limited, Maharashtra
- 3. Inj. Lori, Neon Laboratories Limited, Maharashtra
- 4 Inj. MCT-ROF, Neon Laboratories Limited, Mumbai.
- 5 Inj..Rocunium , Neon Laboratories Limited ,
- 6 Inj.Myopyrolate, Neon LaboratoriesLimited,
- 7 Liquid Sosrane , Neon Laboratories Limited, Maharashtra
- 8 Athena skanray and Dispomed Ventilator
- 9 L & T Star 55 and Skanray multi-parameter monitor
- 10 Drager Isoflurane Vaporizer
- 11. Anaesthesia Gas Monitor: AGM 55, Larsen and Turbo Limited
- 12. Blood cell Counter: BC – 2800 vet, Auto Haematology Analyzer, Mindray
- 13. Blood chemistry Analyzer: Microlab 300, Merck
- 14. Blood Electrolyte Analyzer: CB-LYTE Automatic Electrolyte Analyze
- 15 Stimuplex HNS 12, nerve stimulator for Peripheral Regional anaesthesia, Braun
- 16 Blood Chemistry Analyzer: MISPA NANO, AGAPPE

Plate 3.2: Drugs used in the study



Butorphanol

Atropine

Diazepam

Propofol

Isoflurane

Rocuronium

Myopyrolate

3.6 Patient classification and procedures

All the animals were given Rocuronium and different surgical procedures listed in Table 3.4 were performed under intermittent positive pressure ventilation (IPPV).

Table 3.4: Surgical procedures performed during the study and ASA classification

Sr.No.	Surgical procedure	No. cases	ASA classification
1	Intra medullary pinning	**5**	ASA II
2	Bone plating	**4**	ASA II
3	Intra medullary pinning with cerclage wire	**3**	ASA II
4	Rush pinning	**5**	ASA II
5	Plate and rod combination	**2**	ASA II
6	ovariohysterectomy	**2**	ASA I

3.7 Parameters to be investigated

All the parameters were recorded at different time intervals as mentioned in Table 3.5 and Table 3.6.

Table 3.5: Time intervals for taking and recording various observation

Time interval	Time /stage
T_0(T_{BASE})	Base value
T_1	Just after induction
Rocuronium administered	
T_5	5 minutes after the Rocuronium was injected
T_{10}	10 minutes after Rocuronium was injected
T_{15}	15 minutes after Rocuronium was injected
T_{20}	20 minutes after Rocuronium was injected
Neuromuscular block was reversed (25-35 min)	
T_{40}	40 minutes after Rocuronium was injected and post reversal

Table 3.6: Time intervals for recording Haemato-biochemical parameters and IOP (Intraocular pressure)	
Time interval	**Time/stage**
Preoperative	Base value
Intraoperative	During surgery
Postoperative	After surgery

3.7.1 Clinical observations

The clinical parameters that were recorded in all the cases were as follows:

i. Body weight

The body weight of all animals was measured and then doses of various drugs to be used in the anaesthetic protocol were calculated according to the body weight of the animal.

ii. Rectal temperature

Recording of rectal temperature was done with help of a digital thermometer or multi parameter monitor through the rectal probe

3.7.2 Ophthalmic observation

Intraocular pressure of all the animals was recorded using a Schiotz tonometer at different time intervals as mentioned in Table 3.6.

3.7.3 Cardiovascular parameters:

i. Heart rate (beats per minute)

In the beginning heart rate of the animal was observed using a stethoscope and after the animal was anaesthetized the heart rate was monitored with help of a multi-parameter monitor.

ii. Electrocardiogram (ECG)

The animal was kept on an insulated surface to prevent electrical interference. The animal was positioned in such a manner that its limbs were perpendicular to the long axis of the body and were parallel to the floor.

An electrocardiogram (ECG) was recorded using the system of base apex bipolar lead. The ECG electrode marked as RA (right arm) and LA (left arm) were attached in the area proximal to the olecranon process of the ulna on the caudal aspect of the corresponding foreleg of the animal. Also, electrodes marked as RL (right leg) and LL (left leg) was attached in the area above the stifle fold of the corresponding hind leg. Lead II in the ECG was considered to evaluate the heartbeat rhythm, voltage and time parameters. The speed of the paper was fixed at 25mm/sec. The amplitude was calibrated at 1mV = 1cm on Y-axis. First of all, T_0 as a base reading of ECG was recorded, and then all the recordings as per the time interval mentioned in Table 3.5 were taken through a multiparameter monitor during the induction and maintenance phase. ECG gel was used to apply ECG electrodes to the skin of the animal. Different components of the electrocardiogram such as voltage and time function were recorded according to the following reading:

a. P interval, the distance between the origin and end of the P wave.
b. PR interval, the distance between the origin of the P wave and the beginning of QRS complex
c. QRS interval, the distance between the beginning of the QRS complex and the end of the QRS complex.
d. QT interval, the distance between the start of the QRS complex and the end of the T wave.
e. P amplitude, the amplitude of P wave (beginning of P wave to the maximum peak of P wave)
f. R amplitude, the amplitude of R wave (beginning of R wave to the maximum peak of R wave)

3.7.4 Observation pertaining to haemodynamic system

a. Non-Invasive blood pressure (NIBP)

Non-Invasive Blood Pressure (NIBP) cuff attached to a multi-parameter monitor was applied around the forelimb or hind limb as per suitability. The blood pressure parameters stated below were recorded by automated NIBP oscillometric technique at suitable time intervals mentioned in Table 3.5

i. **Mean Arterial Pressure (MAP): mmHg**
ii. **Systolic Blood Pressure (SBP): mmHg**

iii. Diastolic Blood Pressure (DBP): mmHg

b. Invasive blood pressure (IBP)

I. Central Venous Pressure (CVP)

A central venous catheterization set was used for the catheterizing jugular vein and central venous pressure (CVP) reading was taken with the help of a saline manometer. The jugular vein was catheterized and the catheter was advanced until its anterior portion was placed in the cranial vena cava. Then the catheter was connected to the manometer, position of the manometer was adjusted until the level of fluid in the manometer tube changed with the change in respiration of the dog. The CVP was recorded as per the time intervals mentioned in Table 3.5.

3.7.5Oxygen saturation of haemoglobin (SpO_2)

The SpO_2 probe was used to measure the oxygen saturation of haemoglobin and was applied to the tongue of the dog or other areas of the body like the web of digits and ear etc. The multi-parameter monitor was used for recording the oxygen saturation of haemoglobin. The SpO_2 was recorded at various time intervals as mentioned in Table 3.5

3.7.6 Anaesthetic Observations

i.Vaporizer Concentration (%)

After induction recording of vaporiser[10] concentration of Isoflurane in percent was recorded on the re-breathing co-axial circuit as per time intervals mentioned in Table 3.5

3.7.7 Pulmonary Observations

i. Respiration Rate (breaths/min)

The respiration rate before induction was recorded by counting the thoraco-abdominal excursion and after induction, the recording of the respiration rate was done by the movement of the rebreathing bag attached to the anaesthetic machine.

ii. Fractional Inspired Oxygen (FiO_2) and End Tidal Oxygen (EtO_2)

Anaesthesia Gas Monitor[11] was used to measure fractional inspired oxygen concentration (vol %) and end-tidal oxygen concentration (vol %) in an inhalation gas circuit and values were recorded at different time intervals as given in table 3.5

iii. Fractional Inspired Carbon dioxide ($FiCO_2$) and End Tidal Carbon dioxide ($EtCO_2$)

Anaesthesia Gas Monitor was used to measure fractional inspired carbon dioxide and end-tidal carbon dioxide in an inhalation gas circuit and values were recorded at different time intervals as given in Table 3.5

iv. Fractional Inspired Isoflurane (FiISO) and End Tidal Isoflurane (EtISO)

Anaesthesia Gas Monitor was used to measure fractional inspired Isoflurane concentration and end-tidal Isoflurane in an inhalation gas circuit and values were recorded at various time intervals as given in Table 3.5

v. Minimum Alveolar Concentration (MAC)

Anaesthesia Gas Monitor was utilised to estimate the Minimum Alveolar Concentration (Vol %) of Isoflurane with the help of an inbuilt algorithm and the values were noted down at various time intervals as mentioned in Table 3.5

3.7.8 Ventilator Settings

i. Respiration Rate

When an animal was shifted to a ventilator the respiration rate was fixed at fifteen breaths per minute.

ii. Tidal Volume (TV)

During one respiratory cycle, the amount of gas that moves inside and outside of the patient is known as tidal volume. The tidal volume of the patient for ventilator settings was calculated at the rate of 10ml/kg b.wt.

iii. Inspiratory Expiratory Ratio (I:E)

The ratio of the inspiratory time to the expiratory time is known as the inspiratory expiratory ratio. The value of I:E was fixed as 1:2 on the ventilator for every patient.

3.7.9 Recovery Parameters

i. Time to weaning from the ventilator

The period from putting the animal on the ventilator to the time of weaning the animal from the ventilator was recorded as the time to weaning from the ventilator.

ii. Palpebral reflex time

The period from the stoppage of Isoflurane in the anaesthetic circuit to the time when the animal starts showing palpebral reflex was recorded as palpebral reflex time

iii. Pedal reflex time

The period from the stoppage of Isoflurane in the anaesthetic circuit to the time when the animal started showing pedal reflex was recorded as pedal reflex time

iv. Time to first head raise

The period from the stoppage of Isoflurane in the anaesthetic circuit to the time when the animal tries to lift its head properly was recorded as the time to first head raise.

v. Time to the first standing

The period from the stoppage of Isoflurane in the anaesthetic circuit to the time when the animal acquired the standing posture was recorded as the time to first standing.

3.7.10Haematological Parameters

To estimate haematological parameters venous blood of about 1ml from each animal was collected using three percent ethylene-diamine-tetra-acetic acid (EDTA) coated vials. The blood sample for haematology was collected pre-operatively, intra-operatively and post-operatively at different time intervals according to Table 3.6. The following haematological parameters were estimated with the help of an automatic haemo-analyser[12]:

i) Haemoglobin (Hb)

ii) Packed cell volume (PCV).

iii) Total leukocyte count (TLC)

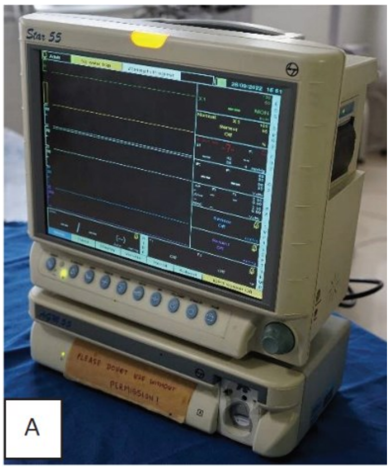
iv) Total erythrocyte count (TEC)

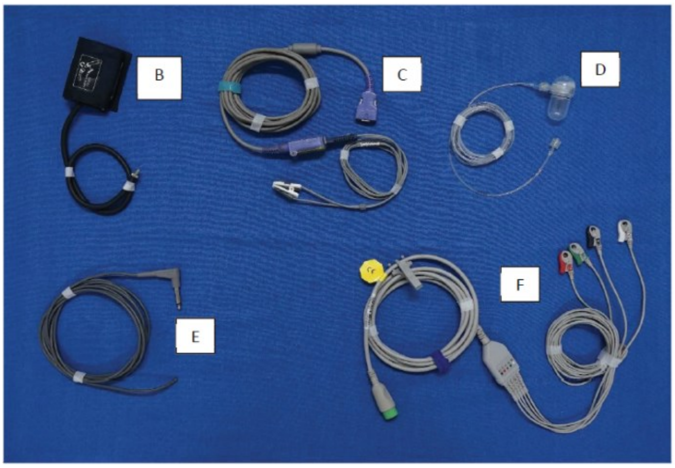
v) Platelet count (PLT)

3.7.11Biochemical Parameters:

Venous blood 5ml in volume was collected in heparinised (10 units/ml) syringes at various time intervals as mentioned in Table 3.6. Plasma was separated by centrifugation for 15minutes at 2500rpm. A fully automatic blood chemistry analyser[16] was used to estimate Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Total Protein (TP) and Creatinine (CRTN). Blood Urea Nitrogen (BUN) and glucose were estimated using a semi-automatic blood chemistry analyser[13]. Plasma Sodium, Chloride and Potassium were estimated using an automatic electrolyte analyser[14].

Plate 3.3: Anaesthesia monitoring equipment

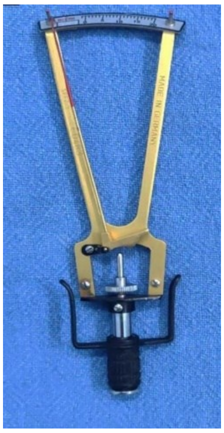




A- L&T Multipara monitor (Star 55) with anaesthesia gas monitor (AGM 55)
B- NIBP Cuff C- SpO2 probe D- Capnography sampling line with water trap
E- Temperature probe F- ECG leads

Plate 3.4: Equipment used during the study for IPPV and IOP

Anaesthesia machine with ventilator

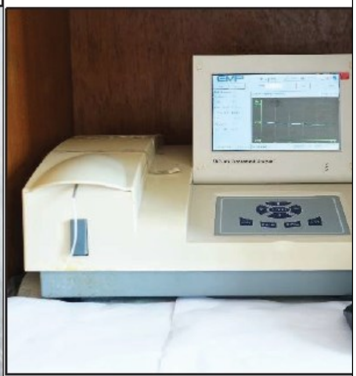


Schiotz tonometer

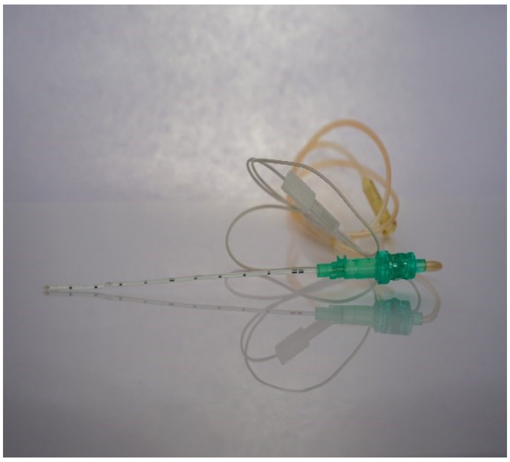
Plate3.5: Equipment for haemato-biochemistry and electrolyte analysis

A

B



A- BC-2800 Vet Auto Haematology Analyzer for Complete Blood Count. B- CB-LYTE Electrolyte analyser. C- Microlab-300 semi-automatic biochemistry analyser

Plate 3.6: Peripheral nerve stimulation equipment	
	
Peripheral nerve stimulator	**Echogenic needle**

3.8 Statistical Analysis

- Statistical analysis was done with GraphPad Instat® Software, Version 3.01, 32 bit
- Analysis of variance (ANOVA) method was done by Student-Newman at 5%, 1% and 0.1% levels of significance.
- The intra-group comparison was done using the student-Newman test

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Distribution of animal

A total of 21 client-owned dogs of either sex were tested for a neuromuscular blocking agent Rocuronium after induction of general anaesthesia. All the dogs were advised to be kept off feed for about twelve hours and off water for about four hours. Every animal included in this study was premedicated using Inj. Butorphanol tartrate at the dose rate of 0.2mg/kg b.wt. I/M, subsequently after a gap of 30 minutes Inj. Atropine sulphate was administered at the dose rate of 0.02mg/kg b.wt. I/M, again after a gap of 10 minutes animal was premedicated with Inj. Diazepam at the dose rate of 0.5mg /kg b.wt. I/V followed immediately by Inj. Propofol I/V (given till effect), for the induction of general anaesthesia.

After surgical anaesthesia had been induced with Propofol, the airway of the animal was secured by endotracheal intubation and the animal was quickly transferred on inhalant Isoflurane mixed in oxygen. Soon after physiological stabilization, Inj. Rocuronium at the dose rate of 0.5 mg /kg b.wt. I/V was administered and the animal was immediately shifted onto a ventilator providing intermittent positive pressure ventilation (IPPV) till the neuromuscular block wanes off. The neuromuscular block induced by Inj. Rocuronium was reversed at the first sign of asynchrony detected on the ventilator bellow and the multiparameter monitor. A single syringe combination drug, Inj.Myopyrolate I/V was administered at the dose rate of 0.05mg/kg b.wt.(Neostigmine) and 0.01mg/kg b.wt. (Glycopyrrolate).

Table 4.1: Patient signalment and ASA categorization (n= 21)

Signalment	Age		19.70±3.94 months (range 3months to 5yrs)
	Weight		18.82 ± 1.70 kgs (range 9kgs to 30 kgs)
	Gender	Male	10
		Female	11
General condition	ASA I		2
	ASA II		19

4.2 Doses of different drugs and their time of onset and duration of action

Mean ± SE of doses of Propofol, Rocuronium and Myopyrolate and time of onset and duration of action are given in Table 4.2

Table 4.2: Doses of different drugs and their time of onset and duration of action. (n=21)			
Drug(conc./ml)	**Mean±SE of doses used(ml)**	**Time of onset action**	**Duration of action (min)**
Propofol (10mg/ml)	4.64 ± 0.42 ml	Till effect to induction	Used for induction
Rocuronium(10mg/ml)	0.94 ± 0.08 ml	17.64 ± 1.10 seconds	27.82 ± 0.72 min.
Myopyrolate (Neostigmine 0.05 mg/ml + Glycopyrrolate0.01mg/ml)	1.87 ± 0.17 ml	Reversal 2.7±0.149 minutes	-

The Rocuronium and Myopyrolate were administered at the dose rate standardized during the standardisation phase as mentioned in Table 3.1. The mean time of onset and duration of action for Rocuronium was recorded as 17.64 ± 1.10 seconds and 27.82 ± 0.72 minutes respectively. Also, 2.7±0.149 minutes was recorded as the meantime for Myopyrolate to reverse the block. Dugadale et al. (2002) reported in their study with Rocuronium on dogs, a duration time of 32.3±8.2 minutes and onset time of 98±52 seconds with a dose rate of 0.4mg/kg b.wt. I/V of Rocuronium. Popovici et al. (2014) recorded the onset of action as 60-120 seconds and the duration as 40-90 minutes for 0.4mg/kg b.wt. I/V dose of Rocuronium. Jones et al. (2015) reported a dose of 0.04mg/kg b. wt. of the Neostigmine and that of Glycopyrrolate to be 0.01 mg/kg b. wt. to reverse a neuromuscular block produced by Rocuronium in dogs.

Marshall et al. (1994) documented that Rocuronium had the shortest onset time among all the available NMBAs but it had an intermediate duration of action, as was recorded for an equally potent dose of Vecuronium. Tranquilli et al. (2007) stated that Rocuronium was a Vecuronium derivative and its potency was one-eighth that of Vecuronium, due to lower potency high dose of rocuronium was used, that in turn places a larger number of molecules of Rocuronium close to the neuromuscular junction thus causing the faster onset of neuromuscular block. Being a non-depolarising NMBA Rocuronium competitively binds to the nicotinic receptors of the neuromuscular junction but did not activate them. Mechanism of action was characterised by the gradual weakening of muscle contraction which finally led to paralysis. Morris et al. (1983) documented that the hepatic system was the main route of elimination of the rocuronium and some fraction was also eliminated through the renal system.

4.3 Clinical Observations

4.3.1 Body weight

The range of body weight of the animal used in the clinical trials was between 9 kgs to 30 kgs with a Mean±SE value of 18.82 ±1.70 kgs.

4.3.2 Rectal Temperature (RT)

There was a steady decrease in the rectal temperature from the baseline value till T_{40}. The decrease in rectal temperature at T_5, T_{10}, T_{15}, T_{20} and T_{40} time intervals was statistically significant (P<0.001). The Mean ± SE of rectal temperature at various time intervals has been shown in Table 4.3 and Figure 4.1.

Table 4.3: Mean ± SE of Rectal temperature (°F), Heart rate (beats/minute) Respiration Rate (breath per minute) at various time intervals. (n=21)

Parameters	T_{BASE}	T_1	T_5	T_{10}	T_{15}	T_{20}	**25-35 MINREVERSAL**	T_{40}
	BASE	**GA**	**NMBA (IPPV)**					**POST REVERSAL**
Rectal Temp (ºF)	101.24 ± 0.19	100.88 ± 0.17	99.92 ± 0.25^{***}	99.25 ± 0.23^{***}	98.71 ± 0.23^{***}	98.03 ± 0.22^{***}		97.27 ± 0.26^{***}
Heart rate (beats/min)	106.76 ± 3.45	125.00 ± 3.97^{***}	131.82 ± 2.02^{***}	122.94 ± 1.86^{***}	120.41 ± 2.02^{**}	117.35 ± 2.05^{*}		116.05 ± 2.03^{*}
Respiration rate (per min)	25.23 ± 1.47	20.11 ± 0.66^{***}	15.0 ± 0.0^{***}	15.0 ± 0.0^{***}	15.0 ± 0.0^{***}	15.0 ± 0.0^{***}		11.23 ± 0.47^{***}

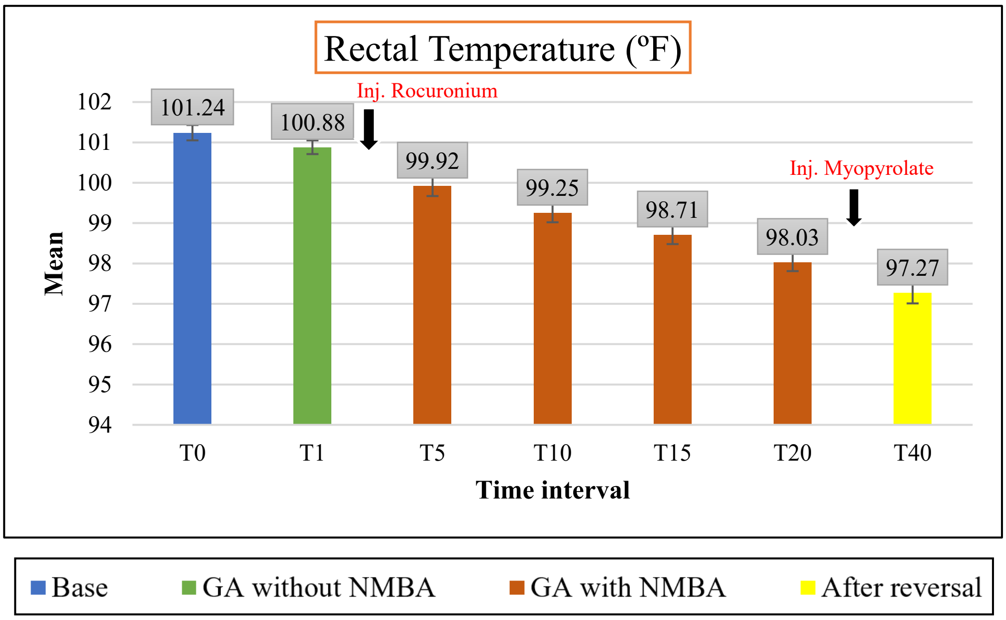
**** - The mean difference within the groups is significant when compared with base value (P < 0.05)***

***** - The mean difference within the groups is significant when compared with base value (P < 0.01)***

****** -The mean difference within the groups is significant when compared with base value (P< 0.001)***

The trend of gradual decline in the rectal temperature from the initial phase to the later phase of this study was also observed by Redondo et al. (2012), who in their study on dogs recorded a 2.0ºC fall in the body temperature of the animals in the first hour of the surgery, they further observed that in the premedication phase, the animal was under the influence of analgesics and sedatives, therefore, the basal metabolism and amount of heat produced in the body of the animal were both reduced and heat dissipation was increased. The surgical preparation of the animals and patent I/V line for administration fluid contributed to the decrease in the temperature of the animal. They further observed that post-induction, the fall in body temperature was gradual. During the phase of general anaesthesia animal's ability to regulate the temperature through behaviour was lost, leaving the animal on autonomic defences to manage the change in the temperature. There was inhibition of shivering and vasoconstriction in an anaesthetised animal. Inhibition of the process of thermoregulation by the anaesthetic was dose-dependent. Thomas and Lerche (2017) observed that normal body temperature in an anaesthetized dog range between 97ºF and 100ºF.

Figure 4.1: Mean±SE values of rectal temperature (ºF) at **different time intervals. (n=21)**



Hypothermia during anaesthesia was aggravated since extra heat loss occurred due to the inhalation of dry gases by the subject and also due to exposure of internal organs and cavities of the body to the external environment. (Matsukawa et al. 1995 and Doufas 2003). The depressed thermoregulatory centre, excess heat loss compared to metabolic heat production and the process of mixing core blood with peripheral blood as a result of vasodilation under anaesthesia might also cause hypothermia (Tranquilli et al.2007)

So, the gradual decline in the rectal temperature from the initial phase to the later phase of this study could be considered normal under anaesthesia. This decrease in the temperature did not have any deleterious effect on the animals under study as all the haemodynamic parameters and the tissue perfusion was normal at different time intervals under anaesthesia and no animal showed prolonged recovery due to hypothermia.

4.4 Ophthalmic observation

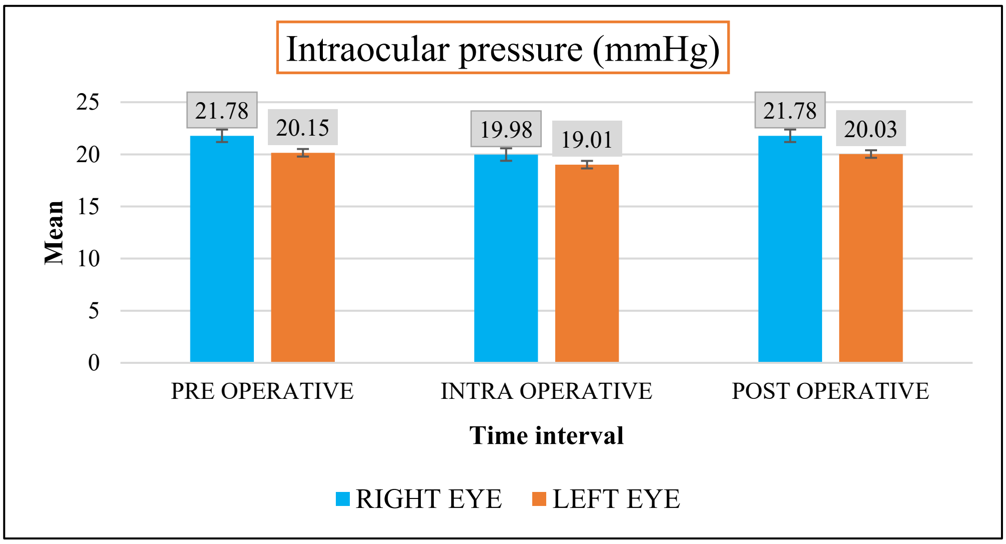
No major fluctuation in the Intraocular pressure (IOP) was observed during the study and it remained well within physiological limits. There was a statistically insignificant decrease in the intra-ocular pressure (IOP) during the intra-operative period compared to the pre-operative period, which again rose to match the baseline value in the postoperative period as shown in Table 4.4 and Figure 4.2.

Table 4.4: Mean ± SE of Intraocular pressure (mmHg) at various time intervals. (n=21)

IOP	Pre-op	Intra-op	Post-op
Right eye	21.78 ± 0.76	19.98 ± 0.74	21.78 ± 0.71
Left eye	20.15 ± 0.72	19.01 ± 0.49	20.03 ± 0.60

No significant difference

Figure 4.2: Mean ± SE of Intraocular pressure (mmHg)at various time intervals. (n=21)



The insignificant decrease in intraocular pressure (IOP) might be due to the fact that the Isoflurane causes a slight decrease in IOP and the neuromuscular blocking agent (NMBA) administered to the dog, which had already been maintained on Isoflurane did not further affect IOP (McMurphy et al.2004). It was reported that anticholinergic drugs did not have or have a very little incremental effect on IOP (Frischmeyer et al. 1993 and Kovalcuka et al. 2015).The benzodiazepines like Midazolam and Diazepam caused minimum or no effect on IOP but at the same time, these drugs could effectively negate the incremental effect of Propofol on IOP (Hofmeister et al.2006 and Kovalcuka et al.2013).Therefore it could be concluded that all the components of the anaesthesia protocol along with the NMBA(Rocuronium) used in the present study minimally altered the intraocular pressure of the ocular globe.

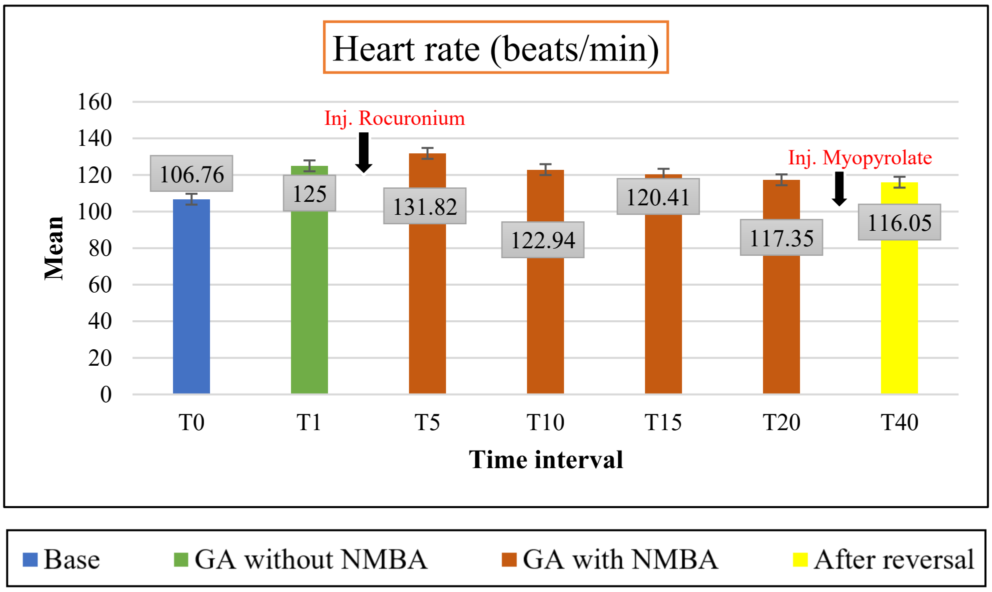
4.5 Cardiovascular Observations

4.5.1 Heart rate

The heart rate increased significantly($P<0.001$) from the base value to the T_1 and it further increased significantly ($P<0.001$) at T_5 just after Inj.Rocuronium was administered to the animal and thereafter gradually decreased significantly($P<0.05$) up to T_{40} as is evident from Figure 4.3 and Table 4.3. Although, at all the time intervals at which the heart rate was recorded, it was within the normal physiological limits.

The significant ($P<0.001$) increase in heart rate from the base value at T_1might be attributed to Diazepam because the cardiovascular sparing property was attributed to it. Increased heart rate in dogs might occur after they had been premedicated with Diazepam as observed by Kapil(2014) in her work on Ketamine-Propofol anaesthesia in dogs. A slight increase in heart rate just after administration of Rocuronium was in agreement with the findings of Khuenl-Brady and Spar (1996) who reported a slight transitory increase in the heart rate after administration of Inj. Rocuronium, which may be attributed to the mild vagolytic activity of Rocuronium in humans. After T_5 there was a gradual non-significant decrease in heart rate up to T_{20} and it continued after the reversal of the neuromuscular block, but the heart rate was always within normal physiological limits.

Figure 4.3: Mean ± SE of Heart rate (beats/minute) at various time intervals. (n=21)



Mama et al. (1996) and Muir and Gadawski (1998) opined that enhanced sympathetic activation due to consciousness loss in the subject or the compensation provided by the body to overcome the vasodilation of the arteries might be the reason for increased heart rate.

Xue et al. (1998) documented that Rocuronium bromide had very little effect on cardiovascular parameters and it did not cause any histamine surge in humans. Booij (1997) and Miranda et al (2008) stated that Rocuronium only in higher doses might be associated with a little increment in the heart rate and appreciable increment in blood pressure due to its vagolytic property.

Hence in the present study, the insignificant increment in the heart rate just after the animal was induced could be attributed to the agents of induction. This effect gradually decreased during the maintenance period under inhalant anaesthetic and consequently, the heart rate showed a gradually decreasing trend. The nonsignificant increase in the heart rate just after the administration of Rocuronium might be due to the mild vagolytic property of Rocuronium, but the heart rate was within the normal physiological range during all phases of this study so it was of no clinical significance.

4.5.2 Electrocardiogram Parameters (ECG) Parameters

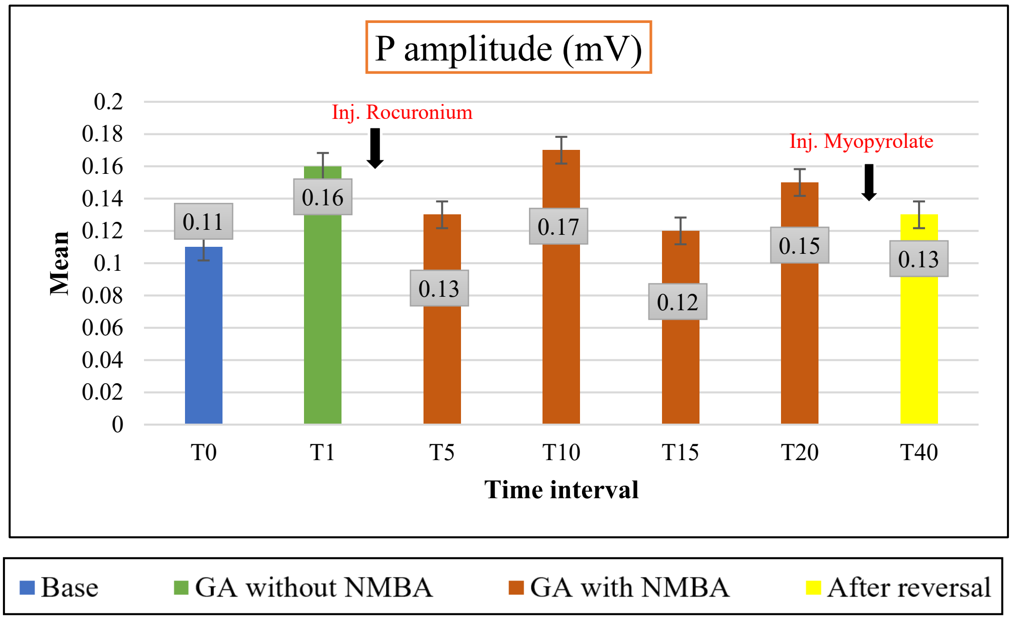
Table 4.5 presents recordings of various components of ECG at different time intervals. All the ECG parameters were within the normal physiological range. (Plate 4.4)

Table 4.5 Mean ± SE of Electrocardiogram Parameters at various time intervals. (n=21)

Parameters	T_{BASE}	T_1	T_5	T_{10}	T_{15}	T_{20}		T_{40}
	BASE	**GA**	**NMBA (IPPV)**				**25-35 MIN REVERSAL**	**POST REVERSAL**
P interval (sec)	0.04 ± 0.00	0.04 ± 0.002	0.04 ± 0.002	0.04 ± 0.0	0.04 ± 0.0	0.04 ± 0.002		0.04 ± 0.002
PR interval (sec)	0.08 ± 0.007	0.08 ± 0.005	0.08 ± 0.0007	0.07 ± 0.0009	0.09 ± 0.008	0.08 ± 0.006		0.08 ± 0.006
QRS interval (sec)	0.06 ± 0.007	0.06 ± 0.007	0.05 ± 0.002	0.04 ± 0.006	0.06 ± 0.0	0.05 ± 0.003		0.05 ± 0.003
QT interval (sec)	0.18 ± 0.01	0.18 ± 0.01	0.20 ± 0.01	0.18 ± 0.02	0.20 ± 0.00	0.21 ± 0.0		0.21 ± 0.0
P amplitude (mV)	0.11 ± 0.01	0.16 ± 0.01*	0.13 ± 0.01	0.17 ± 0.02*	0.12 ± 0.01	0.15 ± 0.01		0.13 ± 0.01
R amplitude (mV)	1.26 ± 0.08	1.30 ± 0.09	1.26 ± 0.08	1.35 ± 0.09	1.25 ± 0.08	1.32 ± 0.09		1.33 ± 0.10

** - The mean difference within the groups is significant when compared with the base value (P < 0.05)*

Figure 4.4: Mean ± SE of P amplitude (mV) at various time intervals. (n=21)



ECG is the recording of the electrical potential that is generated by the cardiac muscles during a cardiac cycle. The different voltages are generated by the process of depolarisation and repolarisation of the individual cardiac muscle cell. So, the different portions of the ECG could be attributed to the particular anatomical or physiological portion of the heart.

The P wave is the first wave to be recorded and it comes before the contraction of atria, P amplitude signifies the process of depolarisation of atria. As is clear from Figure 4.4 and Table 4.5, there was a significant($P<0.05$) increase in the P amplitude at T_1 and T_{10} compared to the base, although the value of P amplitude was well within the normal physiological range of 0.4mV. Thus, these changes in the P amplitude were of no clinical significance.

P interval or width of the P wave represents the time needed by the impulse to reach the atrioventricular (AV) node after starting from the sinoatrial (SA) node, as depicted in Table 4.5 and Figure 4.5 the P interval always remained within normal physiological limits at different time intervals as the width of P wave has a normal value of up to 0.04 sec.

The PR interval signifies activation of the AV (atrioventricular) junction of the heart. The normal physiological range for PR interval in dogs varies from .06 sec to

0.13sec. As shown in Figure 4.6 and Table 4.5 there was no significant change in the PR interval during the study and all values were within the normal physiological range

Figure 4.5: Mean ± SE of P interval(sec) at various time intervals. (n=21)

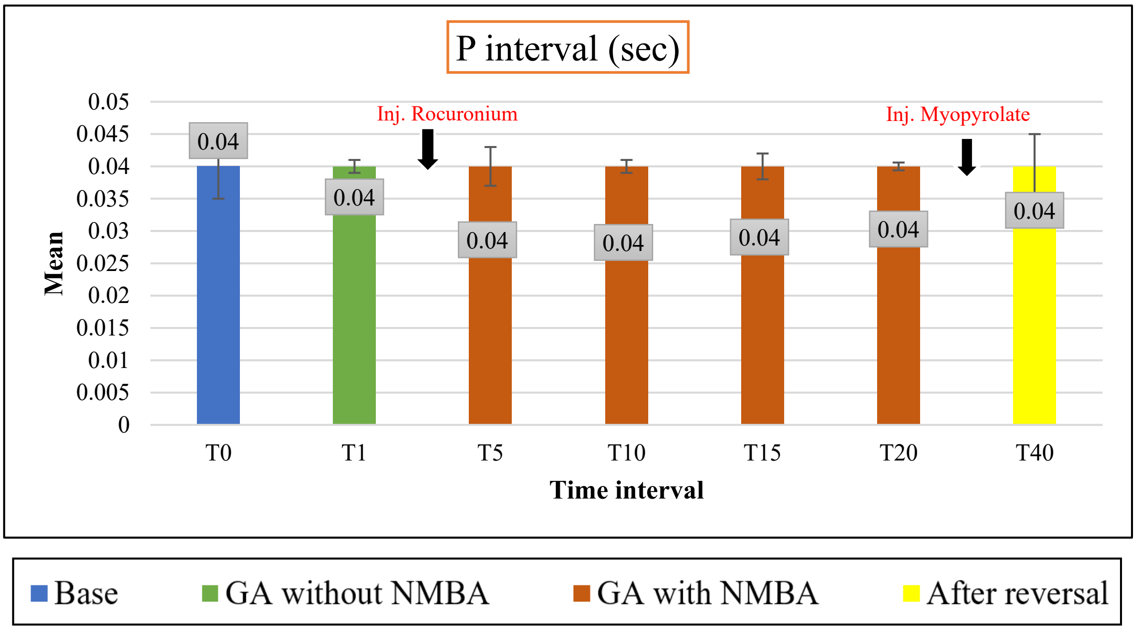
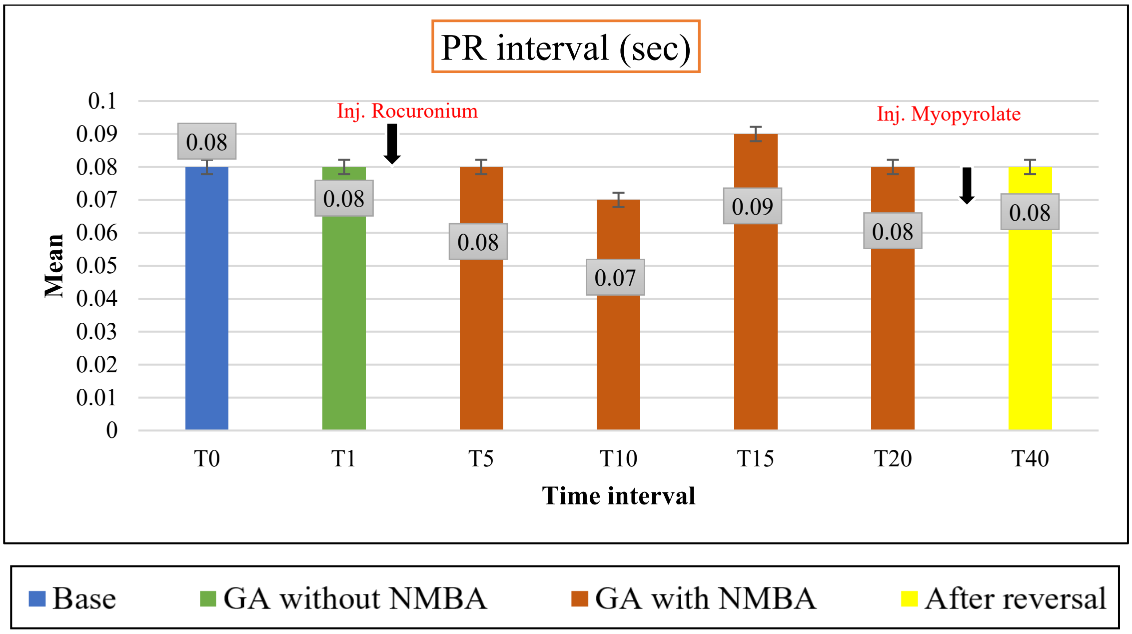


Figure 4.6: Mean ± SE of PR interval (sec) at various time intervals. (n=21)



The R wave amplitude and the QRS complex (Figure 4.7, Figure 4.8 and Table 4.5) did not show any statistically significant fluctuation beyond normal physiological limits for the heart of a dog. The depolarisation of the ventricles is represented by the

QRS complex (Tilley and Burtinick 2009). The normal value for the R wave and QRS complex is up to 30mV and 0.06 sec respectively.

Figure 4.7: Mean ± SE of R amplitude(mV)at various time intervals. (n=21)

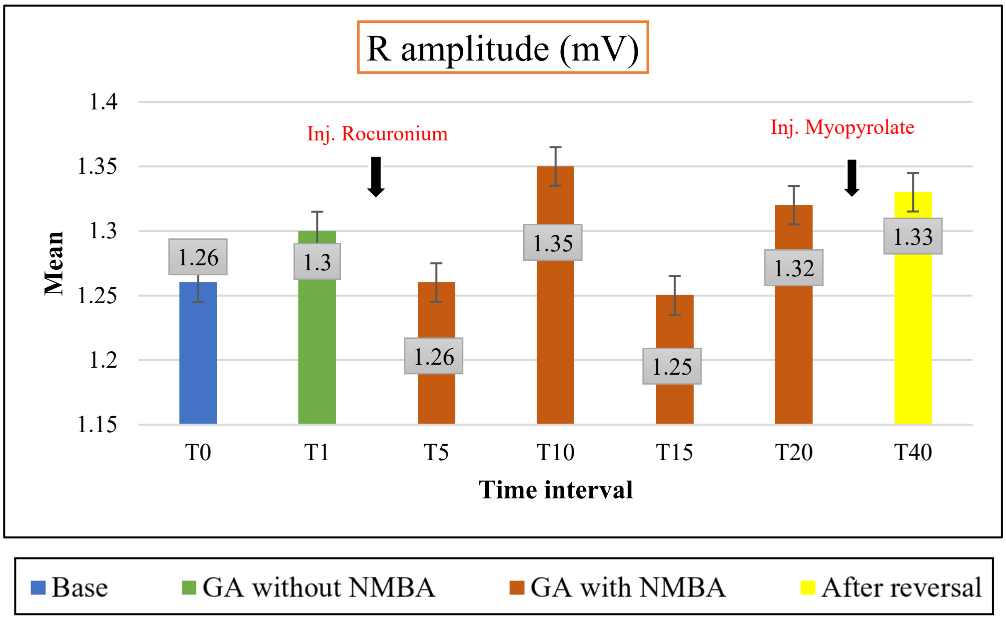
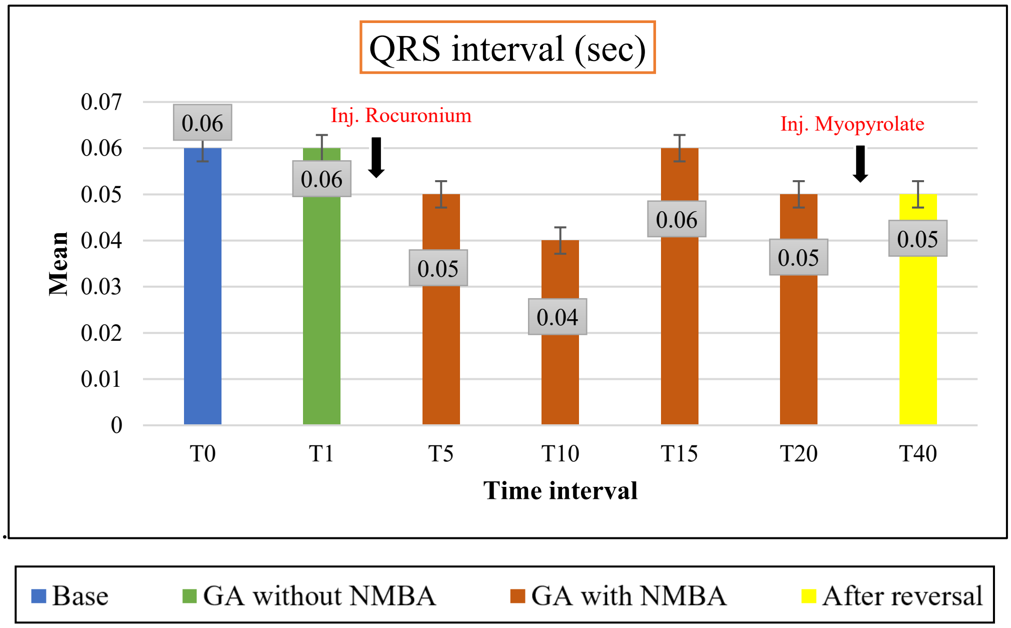


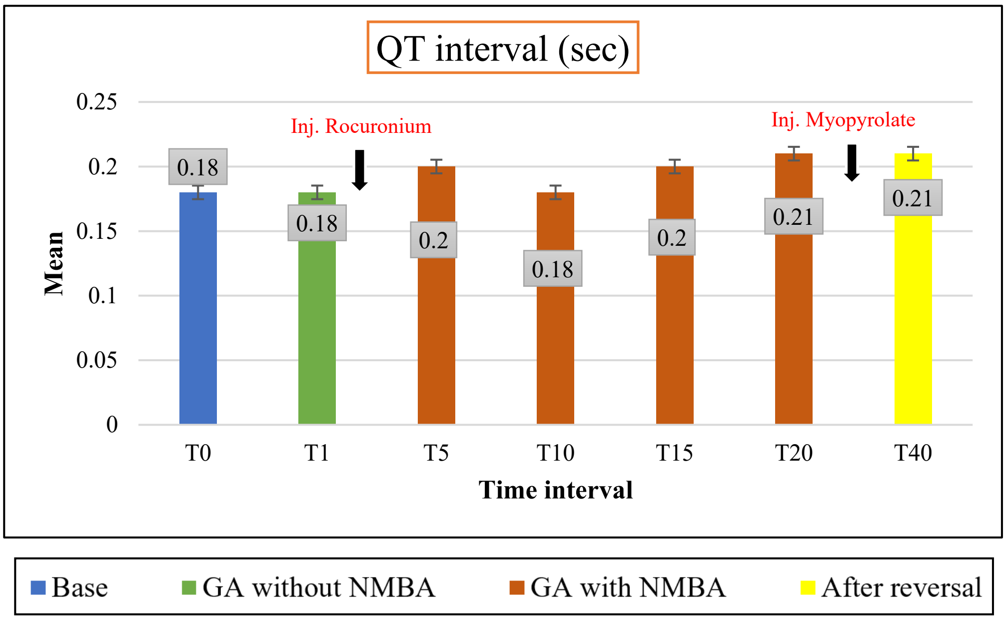
Figure 4.8: Mean ± SE of QRS interval (sec) at various time intervals. (n=21)



QT interval in this study remained within the normal physiological limits given for canines (Figure 4.9 and Table 4.5). QT interval is the summation of the depolarisation of ventricles and repolarisation of the ventricles and signifies the systole of the ventricles.

Although not statistically significant, the gradual increase in the QT value at T5, T15, T20 and T40 compared to the base was consistent with the findings of Riley et al. (1988) who in their study with dogs found that inhalant agents like Isoflurane cause prolongation of QT interval. Auer (2007) in his study observed that dogs premedicated with butorphanol and Diazepam, induced with Propofol, maintained on Isoflurane and injected with Rocuronium, for muscle relaxation showed no cardiovascular changes. Alderson et al. (2007) in their study on dogs also reported that no animal showed any kind of cardiovascular complication. In horses, it was reported that no cardiovascular side effects were observed when the neuromuscular blocking agent Rocuronium was used (Auer and Moens 2011).

Figure 4.9: Mean ± SE of QT interval(sec) at various time intervals. (n=21)



All the components of the ECG were well within the physiological limits. The slight elongation of the QT interval might be due to inhalant anaesthetic used for the maintenance of the animals. There was no elevation or depression of the ST segment during different phases of the study and no cardiac arrhythmias with any major clinical significance were recorded.

Thus, it could be inferred that the anaesthetic protocol along with the neuromuscular blocking agent, Rocuronium and its reversal did not affect the cardiac function in any adverse way.

4.6 Haemodynamic Observations

4.6.1 Non-invasive blood pressure

4.6.1.1 Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP) and Mean Arterial pressure (MAP)

The levels of systolic blood pressure (SBP), diastolic blood pressure (DBP) and Mean Arterial pressure (MAP) are shown in Figure 4.10 and Table 4.6.

Table 4.6: Mean ± SE of Systolic Blood Pressure (mmHg), Diastolic Blood Pressure (mmHg) and Mean Arterial pressure (mmHg)at various time intervals. (n=21)

Parameters	T_{BASE}	T_1	T_5	T_{10}	T_{15}	T_{20}		T_{40}
	BASE	**GA**	**NMBA (IPPV)**				**25-35 MIN REVERSAL**	**POST REVERSAL**
SBP (mmHg)	132.64 ± 3.85	123.88 ± 3.85	127.11 ± 3.90	116.23 ± 3.73*	122.29 ± 3.08	125.94 ± 2.91		126.64 ± 2.81
DBP (mmHg)	76.76 ± 2.62	71.88 ± 2.95	75.15 ± 2.31	75.23 ± 2.33	73.58 ± 2.06	74.17 ± 1.43		77.76 ± 1.37
MAP (mmHg)	95.41 ± 2.43	89.29 ± 3.04	92.47 ± 2.48	88.47 ± 2.12	89.64 ± 2.15	91.23 ± 1.44		94.35 ± 1.54

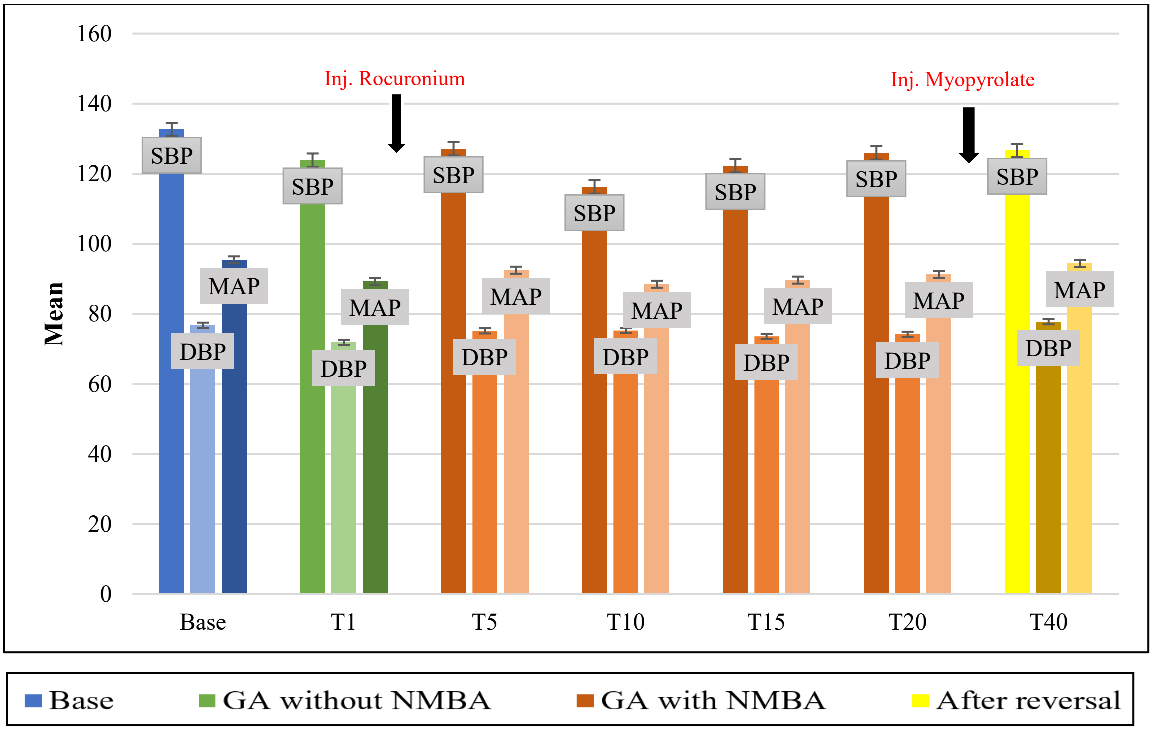
** - The mean difference within the groups is significant when compared with the base value (P < 0.05)*

The Systolic Blood Pressure (SBP) showed a statistically significant($P<0.05$) decrease at T_{10} compared to the base value, however it remained within the normal physiological limit at all time intervals during the study. The Diastolic Blood Pressure (DBP) showed no statistically significant change at different time intervals during the study. The decline in the Mean Arterial Pressure below 70 mm Hg or the decline in Systolic Blood Pressure below 90 mm Hg in dogs could be considered hypotension. (Johnson 1999)

The Mean Arterial Pressure (MAP)represented the most important parameter for monitoring blood pressure during anaesthesia because it was the mean driving pressure for tissue perfusion. (Tranquilli et al. 2007). No statistically significant change in the value of MAP was observed at any time interval during this study. The decrease in the value of MAP at the time interval T_1 which was just after induction may be due to the hypotensive property of Propofol.

A slight increase in the value of MAP at T_5, that was just after Rocuronium is injected, as compared to T_1 was there and a similar trend was observed by Dugadale et al. (2002) in their study on dogs. As MAP was within the normal physiological limits in this study, it could be inferred that no major haemodynamic alteration was caused due the anaesthetic protocol used.

Figure 4.10: Mean ± SE of SBP (mmHg), DBP (mmHg) and MAP(mmHg) at various time intervals.(n=21)



4.6.2 Invasive blood pressure

4.6.2.1Central Venous Pressure (CVP)

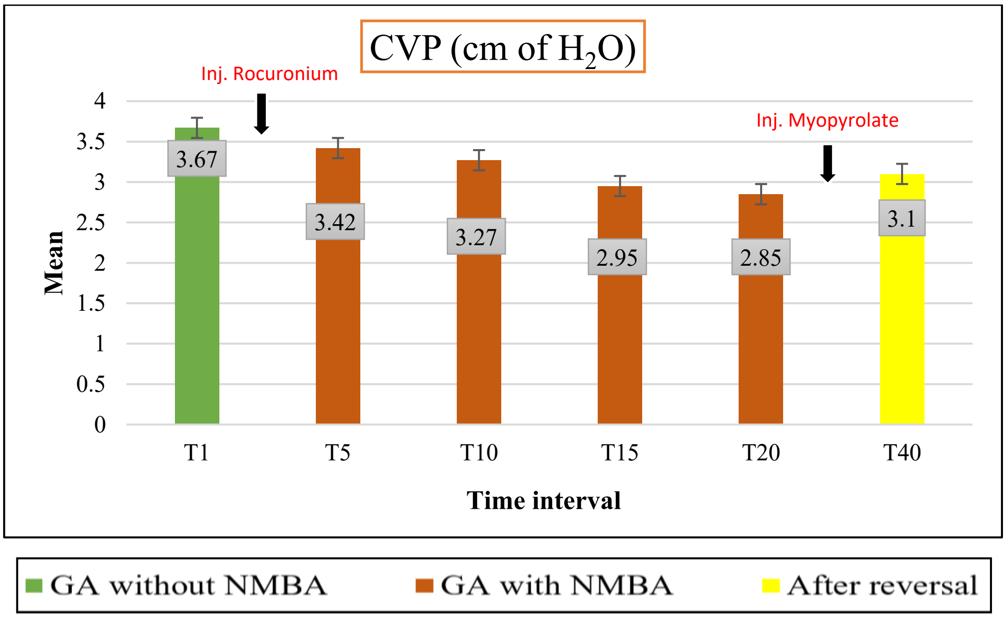
The trends in the central venous pressure (CVP) are highlighted in Table 4.7 and Figure 4.11

Table 4.7: Mean ± SE of Central venous pressure (cm of H_2O) (n=21)							
Parameters	T_1	T_5	T_{10}	T_{15}	T_{20}	25-35 MIN REVERSAL	T_{40}
	GA (BASE)	**NMBA (IPPV)**					**POST REVERSAL**
CVP (mmHg)	3.67 ± 0.11	3.42 ± 0.09	3.27 ± 0.06*	2.95 ± 0.12***	2.85 ± 0.16***		3.10 ±0.07**

** - The mean difference within the groups is significant when compared with base value (P < 0.05)*
*** - The mean difference within the groups is significant when compared with base value (P < 0.01)*
**** -The mean difference within the groups is significant when compared with base value (P< 0.001)*

The normal range of CVP was 0 to 10 cm of H_20 for small animals. The lower range or below range values were associated with hypovolemia and demanded rapid administration of fluid bolus. The higher range or above range values were indicative of hypervolemia and warranted fluid stoppage (Riebold 1990). The Central Venous Pressure (CVP) decreased significantly at T_{10}($P<0.005$) and T_{20}($P<0.001$) time intervals then again increased at T_{40} to lie just below the recordings made at the T_1 time interval. CVP remained within the normal physiological range at different time intervals. In this study, all the dogs were given continuous fluid therapy, which might be the reason for the maintenance of normal CVP.

Figure 4.11: Mean ± SE of CVP (cm of H_2O) at various time intervals(n=21)

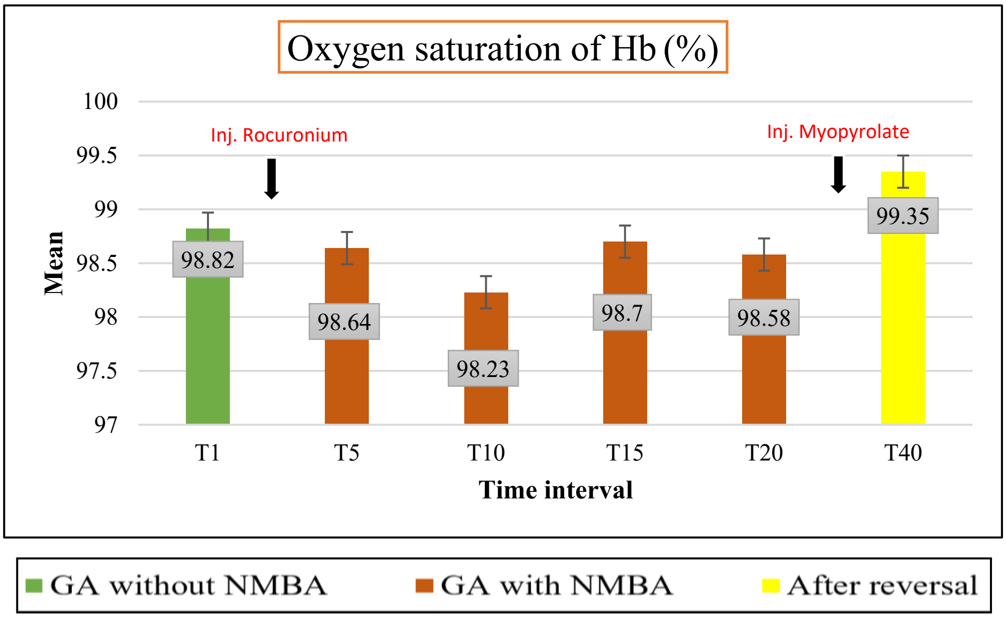


4.7 Oxygen saturation of haemoglobin (SpO_2)

The trend in the percentage of SpO_2during different time intervals is shown in Figure 4.12 and the Mean ± SE value is given in Table 4.8.

As per the oxygen haemoglobin dissociation curve, the levels of SpO_2 and PaO_2 (partial pressure of oxygen in the arterial blood) had a sigmoid relationship, SpO_2 of 100 % corresponds to PaO_2 level of 100 mm Hg or more and SpO_2 of 90% corresponds to PaO_2 level of 60 mm Hg, which could be considered as a critical level of hypoxemia and required intervention (Clark 2009). In his studies on humans, Madan (2017) documented that the PaO_2 value decreased by 4 mm Hg for every 1% reduction in SpO_2 value (for SpO_2 values between 100% to 90%).

Figure 4.12: Mean ± SE of SpO_2 (%) at various time intervals. (n=21)



The SpO_2showed a statistically nonsignificant decrease at the T_{10} time interval, although the oxygen saturation of haemoglobin always remained between 98 to 100 per cent. Foluso et al. (2018) found identical results, that there was no significant change in SpO_2 readings of the animals which were receiving oxygen supplementation. Also, Rauser et al. (2016) in their study concluded that neuromuscular blocking agents did not cause any significant effect on respiratory parameters including SpO_2.

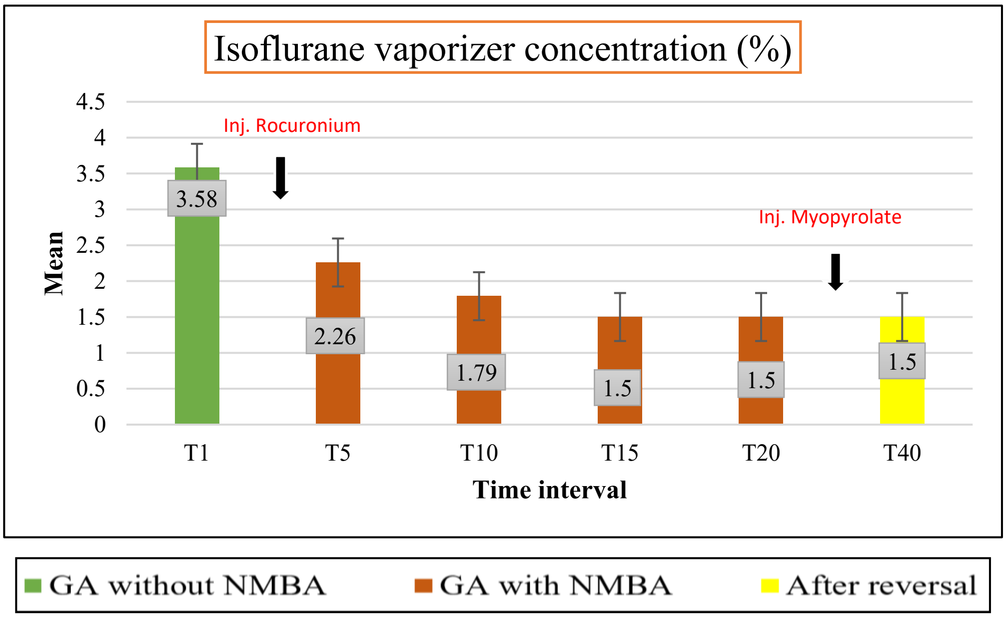
Thus, it could be stated that during different phases of the present study the oxygen saturation of available haemoglobin was adequate in the arterial blood of the animals.

4.8 Vaporizer Setting (%)

The vaporizer concentration (%) at different time intervals is shown in Figure 4.13 and Table 4.8. There was a significant (P<0.001) decrease in the average concentration of anaesthetic gas required to maintain the animal in the surgical plane of anaesthesia after the animal had been Injected with Rocuronium. Kumar (2017) who did his study on Vecuronium and Atracurium in dogs found a similar inhalant-sparing effect of the neuromuscular blocking agents. In addition, it had been documented that neuromuscular blockade was potentiated by inhalant anaesthetic agents (Nagahama et al.2006 and Sakata et al. 2019).

During this study it was recorded that the requirement of isoflurane to maintain the animal under anaesthesia was significantly reduced under neuromuscular blockade induced by rocuronium, substantiating the fact that neuromuscular blocking agents had an inhalant (Isoflurane) sparing effect.

Figure 4.13: Mean ± SE of Isoflurane vaporizer concentration (%) at various time intervals(n=21)



4.9 Pulmonary Parameters

4.9.1 Respiration Rate (breaths/min)

The respiration rate at different time intervals has been depicted in Figure 4.14 and Table 4.8

There was a significant ($P<0.001$) decrease in the respiration rate at the T_1 time interval, that was just after induction. A similar fall in the respiration rate following premedication with Diazepam and induction by Propofol was observed by Naryal (2020). The fall in respiration rate following induction with Propofol was also observed by Musk et al. (2005) and Hammond and England (1994) in their respective studies on dogs.

Figure 4.14: Mean ± SE of Respiration rate (breaths per minute) at various time intervals. (n=21)

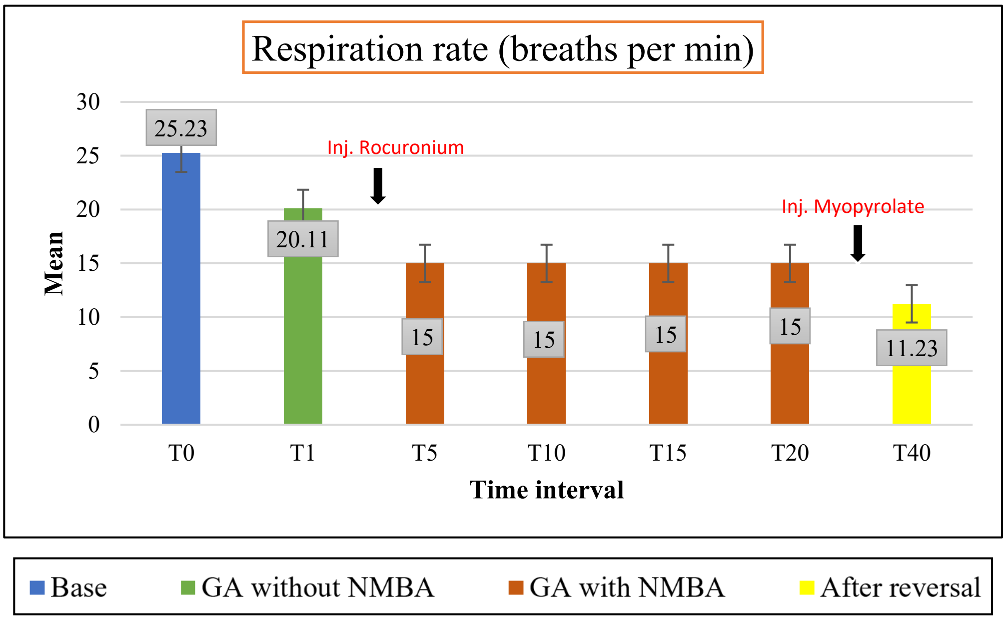


Table 4.8 Mean ± SE of Pulmonary Parameters at various time intervals. (n=21)

Parameters	T_1	T_5	T_{10}	T_{15}	T_{20}		T_{40}
	GA (BASE)	**NMBA (IPPV)**				**25-35 MIN REVERSAL**	**POST REVERSAL**
Isoflurane vaporizer concentration (%)	3.58 ± 0.11	2.26 ± 0.06^{***}	1.79 ± 0.07^{***}	1.5 ± 0.0^{***}	1.5 ± 0.0^{***}		1.5 ± 0.028^{***}
SpO_2 (%)	98.82 ± 0.29	98.64 ± 0.20	98.23 ± 0.30	98.70 ± 0.21	98.58 ± 0.23		99.35 ± 0.18
$EtCO_2$mmHg	38.29 ± 1.13	39.41 ± 1.03	40.41 ± 1.30	41.23 ± 1.36	41.11 ± 1.02		42.58 ± 1.17
$FiCO_2$ (Vol %)	0.05 ± 0.05	0.11 ± 0.07	0.11 ± 0.07	0.23 ± 0.10	0.17 ± 0.09		0.17 ± 0.09
EtO_2 (Vol %)	77.35 ± 1.26	79.23 ± 0.69	80.70 ± 0.85	80.47 ± 0.99	79.52 ± 1.03		80.52 ± 1.04
FiO_2 (Vol %)	83.94 ± 0.63	85.17 ± 0.25^{*}	86.41 ± 0.32^{**}	86.47 ± 0.49^{**}	85.58 ± 0.44^{*}		86.17 ± 0.38^{**}
Et_{iso}(Vol %)	2.70 ± 0.04	2.03 ± 0.03^{***}	1.85 ± 0.02^{***}	1.64 ± 0.02^{***}	1.16 ± 0.03^{***}		1.59 ± 0.03^{***}
Fi_{iso}(Vol %)	3.70 ± 0.10	2.54 ± 0.09^{***}	2.22 ± 0.02^{***}	2.06 ± 0.02^{***}	1.94 ± 0.03^{***}		1.84 ± 0.04^{***}
Mac (Vol %)	1.82 ± 0.02	1.44 ± 0.02^{***}	1.28 ± 0.01^{***}	1.15 ±0.01^{***}	1.10 ± 0.00^{***}		1.08 ± 0.00^{***}

* ***- The mean difference within the groups is significant when compared with the T_1 value ($P < 0.05$)***

** ***- The mean difference within the groups is significant when compared with the T_1 value ($P < 0.01$)***

*** ***- The mean difference within the groups is significant when compared with the T_1 value ($P< 0.001$)***

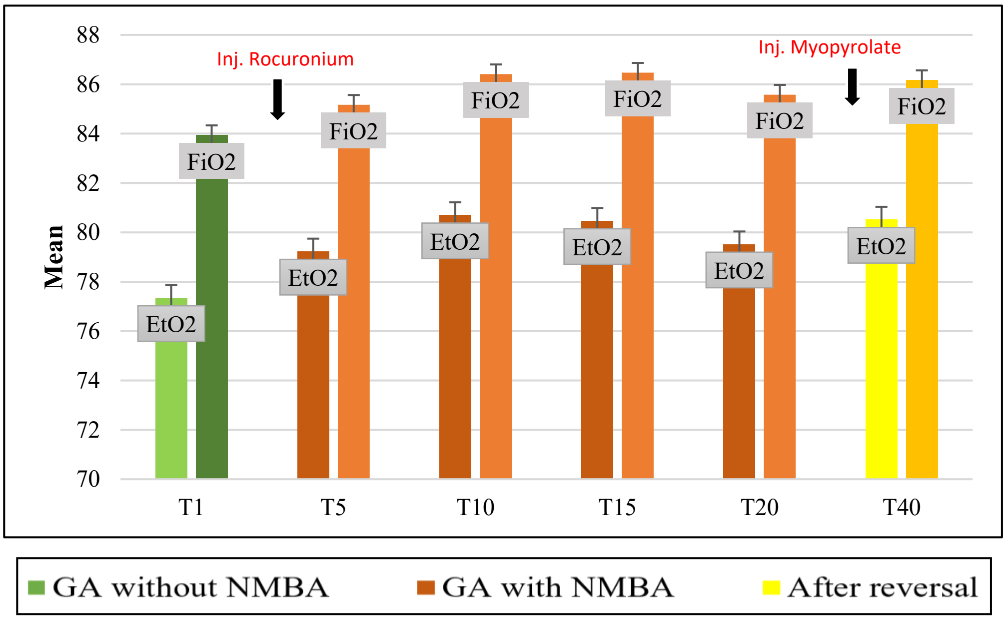
The respiration rate was constant at T_5, T_{10}, T_{15} and T_{20} as the animal was on a ventilator during these time intervals and the respiration rate was fixed at 15 breaths per minute. Further, there was a statistically significant($P<0.001$) decrease in the respiration rate after reversal at T_{40}, as the neuromuscular block had already been reversed between T_{20} and T_{40} and the animal had been weaned from the ventilator but it was still maintained on inhalant anaesthesia (Isoflurane). Thus, taking the above facts into account the decreased respiration rate at T_{40} was in the normal range for the animal under anaesthesia.

In this study, the dogs were kept on the ventilator for respiratory support during the neuromuscular blockade. The ventilator settings as mentioned in Table 3.2 were standardized by Kumar (2017) in his study on intermittent positive pressure ventilation (IPPV) in dogs during anaesthesia under Vecuronium and Atracurium.

4.9.2 End Tidal Oxygen (EtO_2) and Fractional Inspired Oxygen (FiO_2)

The concentrations (vol%) of end-tidal oxygen (EtO_2) and fractional-inspired oxygen (FiO_2) are highlighted in Figure 4.15 and Table 4.8

Figure 4.15: Mean ± SE of End Tidal Oxygen(vol%) and Fractional Inspired Oxygen (vol%) at various time intervals. (n=21)



There were statistically significant ($P < 0.01$) changes in the conc.(vol%) of fractional inspired oxygen (FiO_2) at different time intervals between T_5 and T_{40}, although FiO_2(vol%) always remained above 83%. The EtO_2(vol%) showed a statistically non-significant increase at T_5 from the base value and remained above 77% throughout the study

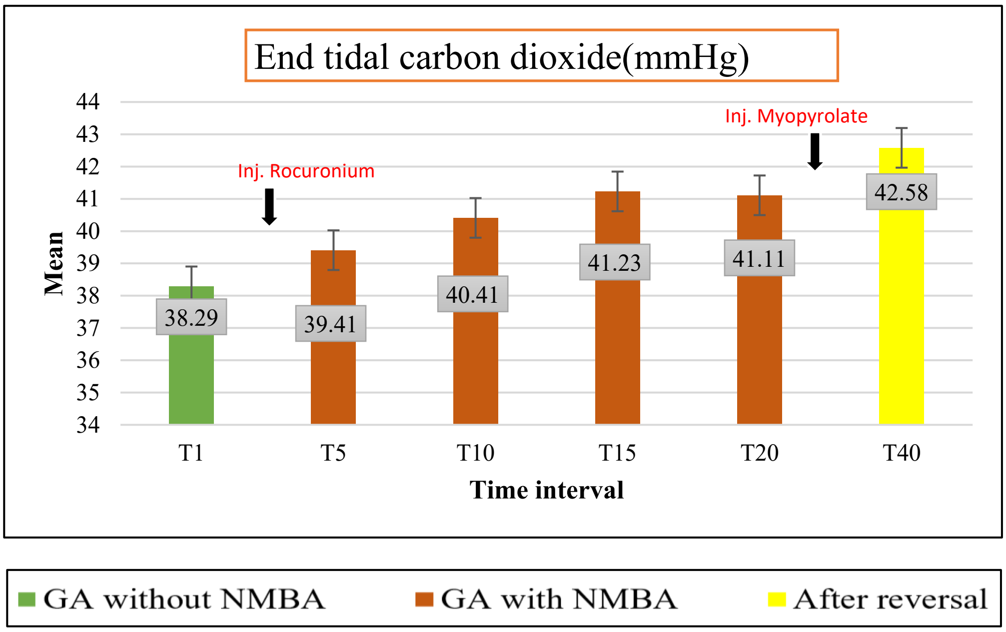
Linko and Paloheimo (1989) documented that the difference between FiO_2 and EtO_2increased in the periods of hypoventilation and decreased in the periods of hyperventilation.

In the present study, the difference betweenFiO_2 and EtO_2 always remained between 5 % to 7% indicating proper ventilation of all the animals during different phases of the study. Moreover, 100% oxygen was supplied to all the animals during the study.

4.9.3 End Tidal Carbon Dioxide ($EtCO_2$) and Fractional Inspired Carbon Dioxide ($FiCO_2$)

The levels of end-tidal carbon dioxide ($EtCO_2$) and fractional-inspired carbon dioxide ($FiCO_2$) are given in Figure 4.16 and Figure 4.17 and Table 4.8

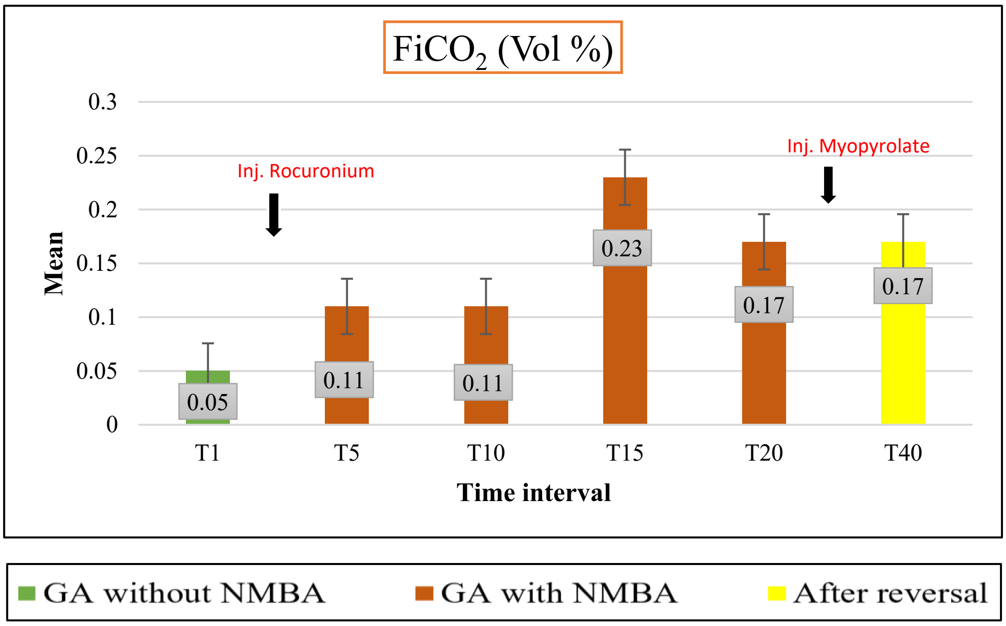
Figure 4.16: Mean ± SE of $EtCO_2$(mmHg)at various time intervals(n=21)



The end-tidal carbon dioxide ($EtCO_2$) showed a gradual statistically non-significant increase from T_5 to T_{40} time intervals.

The gradual rise in the $EtCO_2$ might be due to depression in the respiratory system produced by the anaesthetic agents thereby increasing the carbon dioxide retention in the body, normal value for end-tidal carbon dioxide ($EtCO_2$) for an anaesthetized animal was up to 55 mmHg (Thomas and Lerche 2017).

Figure 4.17: Mean ± SE of $FiCO_2$(vol%) at various time intervals. (n=21)



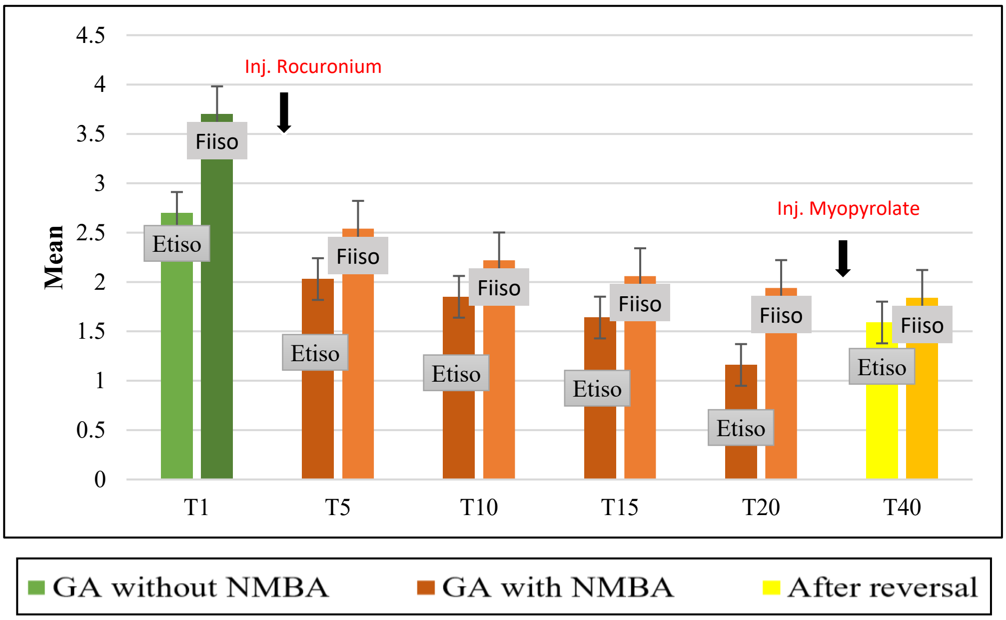
The $PaCO_2$ was usually used as an index of the respiratory system response to general anaesthetics. Inhalation anaesthetics caused depression in alveolar ventilation and as à consequence caused increment in $PaCO_2$ in a dose-related manner. The $PaCO_2$showed the ventilatory status of a subject and ranged between 35 mm Hg to 45mm Hg. $PaCO_2$might be slightly high in anaesthetized small animals and was much higher (60 to 80 mm Hg) in horses and cattle. $PaCO_2$above 60 mm Hg might be associated with excessive respiratory acidosis and caused sufficient hypoventilation to demand positive-pressure ventilation in small animals. The $PaCO_2$ below 20 mm Hg was associated with respiratory alkalosis and decreased cerebral blood flow that might hamper cerebral oxygenation. $PaCO_2$might also be estimated indirectly by measuring the carbon dioxide in a sample of gas drawn at the end of exhalation ($EtCO_2$). $EtCO_2$ is usually 2 to 4 mm Hg less than $PaCO_2$ in dogs and 10 to 15 mm Hg less in horses. In addition, $EtCO_2$ was more useful compared to SpO_2 in the early detection of hypoventilation because $EtCO_2$ would be elevated much before haemoglobin would desaturate, particularly in animals kept on 100% oxygen (Tranquilli et al. 2007)

The $FiCO_2$ at all the time intervals in this study did not exceed 0.3 %, which was well within the permissible limit. It showed that during the maintenance of anaesthesia almost no rebreathing of carbon dioxide occurred. The trend of a gradual rise in the $EtCO_2$value in the study could be because of the respiratory depression as a result of anaesthesia, however end-tidal carbon dioxide ($EtCO_2$) always remained within the normal physiological limit at different phases of the study.

4.9.4 End Tidal Isoflurane (EtISO) and Fractional Inspired Isoflurane (FiISO)

The concentrations (vol %) of end-tidal Isoflurane (EtISO) and fractional-inspired Isoflurane (FiISO) are depicted in Figure 4.18 and Table 4.8

Figure 4.18: Mean ± SE of End Tidal Isoflurane(vol%) and Fractional Inspired Isoflurane(vol%) at various time intervals. (n=21)



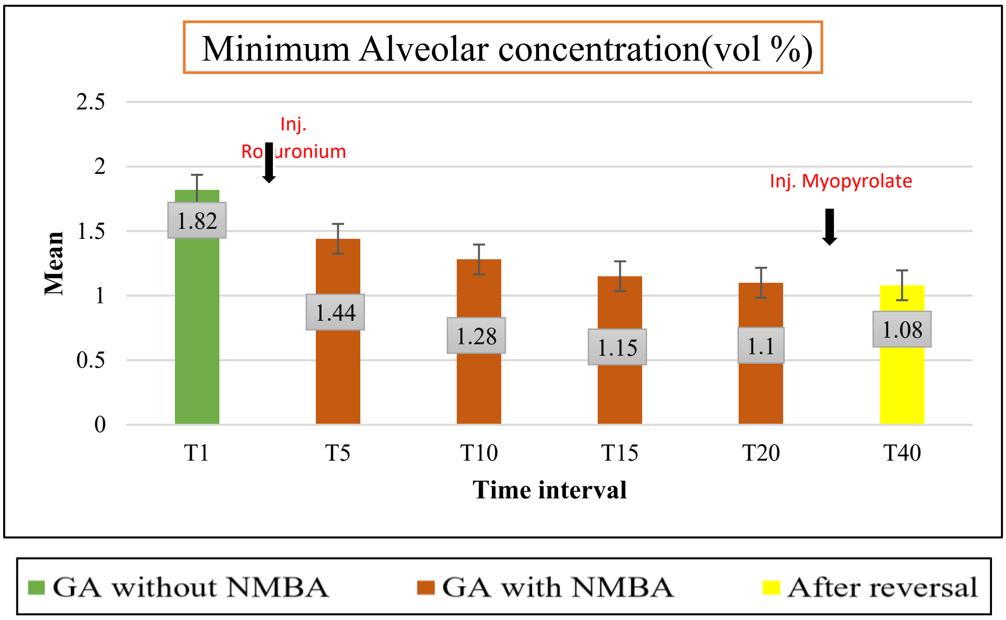
The fractional inspired Isoflurane (FiISO) showed a progressive significant($P<0.001$) decline from T_5 to T_{40} whereas the EtISO also showed a gradual statistically significant ($P<0.001$) decline from T_5 to T_{20} time interval and then a slight rise at T_{40}. Kumar (2017) stated in his study on IPPV in dogs that as the animal kept on inhalant anaesthesia got stabilized on Isoflurane, the absorption of the inhalant gas declined in the tissue thereby increasing the value of EtISO with increased duration of anaesthesia. In addition, the EtISO clinically showed the minimum alveolar concentration of the inhalant anaesthetic required that abolished all the movement in the animal including reflexive and non-purposeful movements (Seddighi et al 2011)

In the present study, the decline in the FiISO corresponds directly to the decreased vaporizer concentration (vol%) of the isoflurane. The EtISO in turn corresponds to the FiISO as both are directly related. The slight increment in the EtISO in the later phase of the study might be due to the fact that on saturation of body tissue of the animal with the isoflurane the absorption of isoflurane decreased in the later phase of the study.

4.9.5 Minimum Alveolar Concentration (MAC)

The level of minimum alveolar concentrations (MAC) (vol %) are highlighted in Figure 4.19 and Table 4.8

Figure 4.19: Mean ± SE of Minimum Alveolar Concentration (vol%) at various time intervals. (n=21)



The conc. (vol %) of MAC showed a gradual statistically significant ($P<0.001$) decline from T_5 to T_{40}.The MAC was calculated by an inbuilt algorithm of AGM and directly corresponds to the vaporizer concentration (vol %) at different time intervals. The trend for a decrease in MAC is in agreement with the study of Miyakazi et al. (2016) who reported a decrease in MAC of Isoflurane in rats after neuromuscular block with Pancuronium. Kumar (2017) found in his study on dogs, where he compared the anaesthesia protocol (Propofol-Isoflurane) under IPPV using Atracurium and Vecuronium that MAC of Isoflurane got increased in the initial phase of the neuromuscular block and then decreased gradually to later phases of the neuromuscular block.

It was observed in the present study that the requirement for isoflurane decreased significantly during the neuromuscular block induced by the Rocuronium thereby causing a decline in the MAC (vol %) of Isoflurane.

4.10 Haematological Parameters

Table 4.9 Mean ± SE of Haematological Parameters at various time intervals. (n=21)

Parameters	Pre-op	Intra-op	Post-op
Hb (g/dl)	12.95 ± 0.61	10.21 ± 0.52**	9.78 ± 0.56***
PCV (%)	37.00 ± 1.60	29.67 ± 1.40**	28.39 ± 1.53***
TLC ($x10^9/\mu l$)	16.58 ± 1.08	12.17 ± 1.02**	11.37 ± 1.06**
TEC ($x10^{12}/\mu l$)	6.42 ± 0.26	5.21 ± 0.26**	4.95 ± 0.29**
PLT($x10^9/\mu l$)	234.43 ± 26.82	196.59 ± 22.72	197.35 ± 19.78

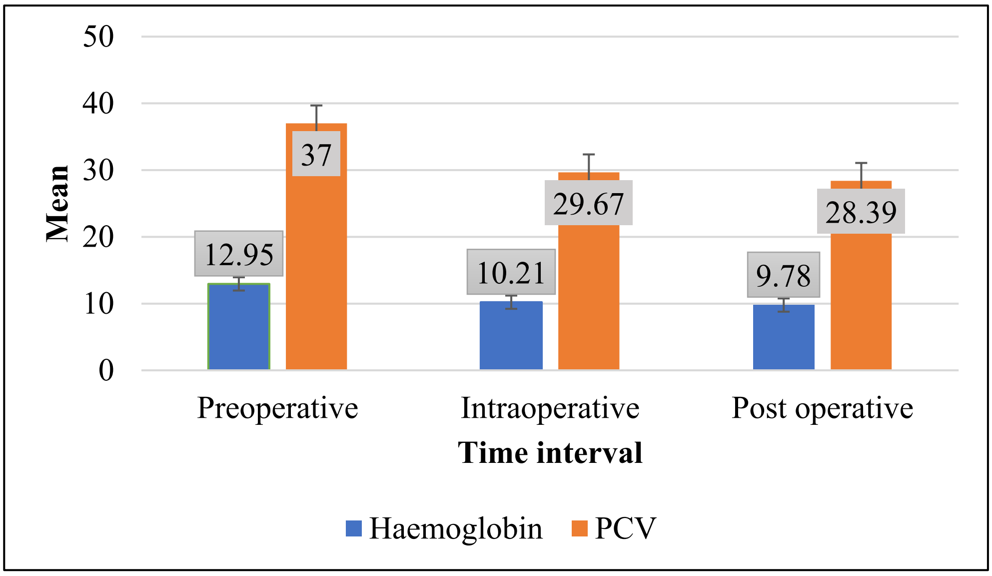
** *- The mean difference within the groups is significant when compared with the preoperative value (P < 0.01)*

*** *- The mean difference within the groups is significant when compared with the preoperative value (P< 0.001)*

4.10.1 Haemoglobin (Hb) and Packed Cell Volume (PCV)

The haemoglobin (Hb) and packed cell volume (PCV) showed a significant decrease at the intraoperative period(P<0.01) and then again during the postoperative period(P<0.001) compared to preoperative period as shown in Figure 4.20 and Table 4.9

Figure 4.20: Mean ± SE of Haemoglobin (g/dl) and Packed Cell Volume (%) at various time intervals(n=21)



The decrease in the levels of haemoglobin (Hb) and PCV during the general anaesthesia were attributed to the vasodilation at the micro capillary level and due to their dilatation, many RBCs entered these micro capillaries thereby decreasing the haemoglobin and PCV values measured in the blood sample from peripheral veins

(Naghibi et al. 2002). In addition, Tranquiliet et al.(2007) stated that Hb and PCV decreased during anaesthesia due to vasodilation induced by the anaesthetic agent, splenic dilatation, depression of compensatory reflexes, administration of fluid and blood loss.

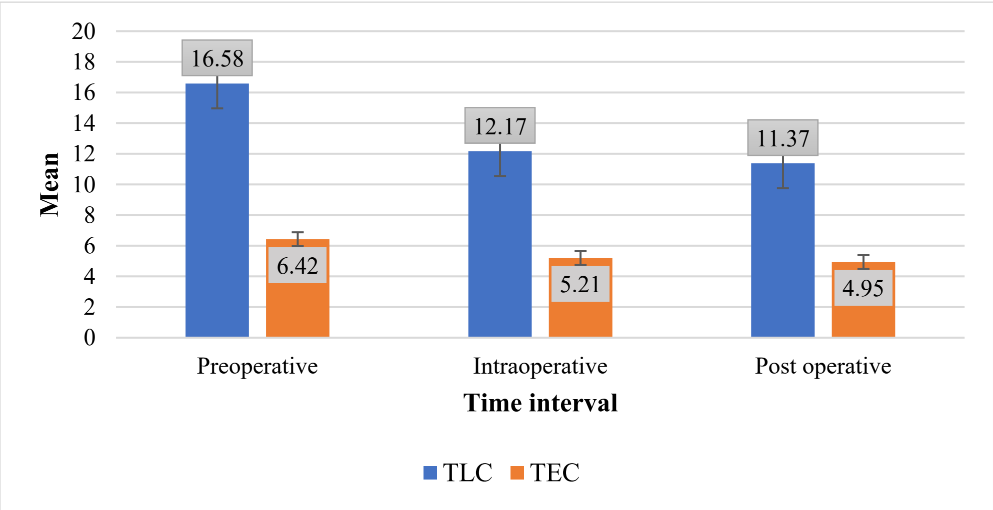
So, in the light of above findings, it could be stated that the gradual decrease in the level of haemoglobin and PCV during this study might be because of the general anaesthesia and to some extent due to the blood loss involved in the surgery.

4.10.2 Total leukocytic count (TLC), Total erythrocytic count (TEC)and Platelet count (PLT)

The total leukocytic count (TLC) and total erythrocytic count (TEC)showed a significant ($P<0.01$) decrease during the intraoperative period and postoperative period as compared to the preoperative period (Figure 4.21and Table 4.9). The decrease in the TLC during general anaesthesia could have been caused by the pooling of blood cells of the circulatory system into the spleen or other reservoirs as a result of sympathetic activity (Wagner et al 1991). The total leukocytic count might fall due to the migration of white blood cells from circulation to the site of surgery (Popovici et al. 2014).

Thus, the fall in TLC during this study could be attributed to splenic pooling due to anaesthesia and the response of the animal's immune system to the surgery. The reasons for the decrease in the TEC were the same as those mentioned for the decrease in Hb and PCV, as all three were closely related.

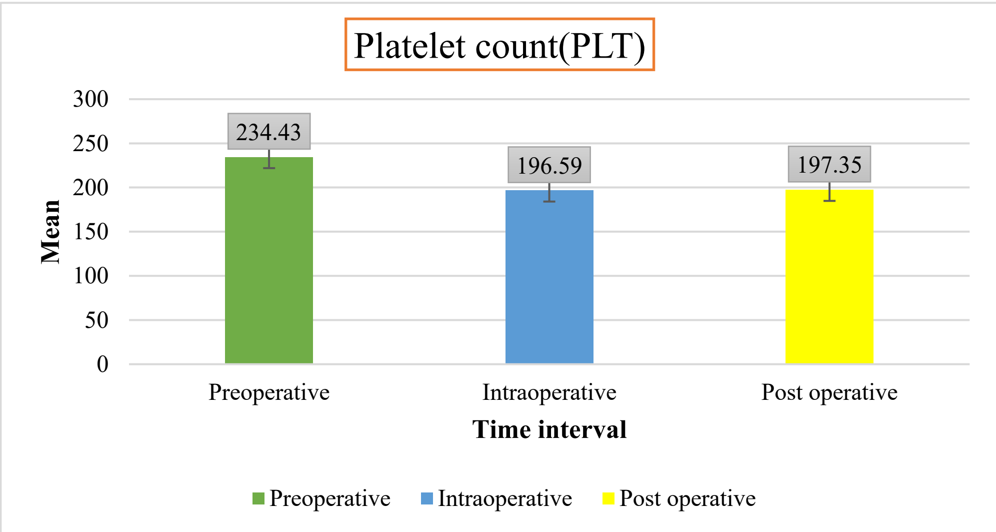
Figure 4.21: Mean ± SE of Total leukocytic count (10^9/µl) and Total erythrocytic count (10^{12}/µl) at various time intervals. (n=21)



The Platelet count (PLT) decreased non-significantly in the intra-operative and postoperative period compared to the baseline (Figure 4.22 and Table 4.9.)

In their study on dogs undergoing orthopaedic procedures maintained with isoflurane and which were instituted ringers lactate solution during the surgery Chohan et al. (2011) documented a significant decrease in the platelet count as a result of haemodilution one-hour post ringer lactate infusion. The trend of gradually decreasing platelet count (PLT) in the present study simulated with above-mentioned research findings. Therefore, the decreased platelet count in this study could be attributed to haemodilution due to the fluid infusion. However, the platelet count always remained within the normal physiological limit at different time intervals during the present study.

Figure 4.22: Mean ± SE of Platelet count($10^9/\mu l$) at various time intervals. (n=21)



4.11 Biochemical Parameters

The Mean± SE of different biochemical parameters are mentioned in Table 4.10

Table 4.10 Mean ± SE of Biochemical Parameters at various time intervals. (n=21)

Parameters	Pre-op	Intra-op	Post-op
Glucose (mg/dl)	82.47 ± 3.44	92.6 ± 3.40*	96.98 ± 3.15**
ALT (IU/l)	35.54 ± 3.10	30.94 ± 4.18	30.67 ± 4.81
AST (IU/l)	38.32 ± 7.95	20.5 ± 2.29*	20.23 ± 2.32*
BUN (mg/dl)	18.65 ± 1.15	20.82 ± 1.62	21.62 ± 1.54
Creatinine (mg/dl)	0.76 ± 0.07	0.84 ± 0.04	0.84 ± 0.03
Total Protein (g/dl)	5.47 ± 0.25	5.02 ± 0.18	5.34 ± 0.19

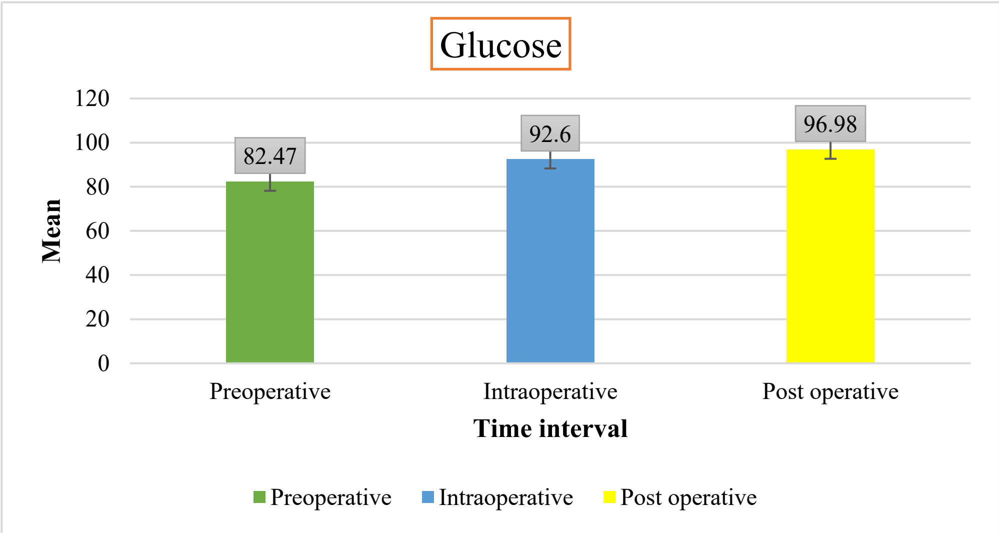
* - ***The mean difference within the groups is significant when compared with the preoperative value (P < 0.05)***

** - ***The mean difference within the groups is significant when compared with the preoperative value (P< 0.01)***

4.11.1 Plasma Glucose

The statistically significant increase($P<0.05$) in the value of glucose in the intraoperative and again significant($P<0.01$) increase in the postoperative period compared to the preoperative period is shown in Table 4.10 and Figure 4.23

Figure 4.23: Mean ± SE of Plasma Glucose (mg/dl) at various time intervals. (n=21)



The increase in the glucose level during and after surgery might occur due to a hypermetabolic response to the stress, in which the production of glucose was increased with simultaneous insulin resistance (Duncan 2012). In their study on dogs Maeda et al. (2018) opined that hormones related to stress namely cortisol, epinephrine and mediators of inflammation were responsible for raised levels of glucose during the perioperative period. Inhalant anaesthetic agents like Isoflurane and sevoflurane depress the glucose metabolism of the blood by decreasing the secretion of insulin hormone from the pancreas. (Diltoer and Camu1988; Vore et al.2001; Tanaka et al.2005). The agent used for intravenous anaesthesia like Propofol had also been associated with glucose metabolism suppression leading to an increased level of glucose (Kitamura et al.2009)

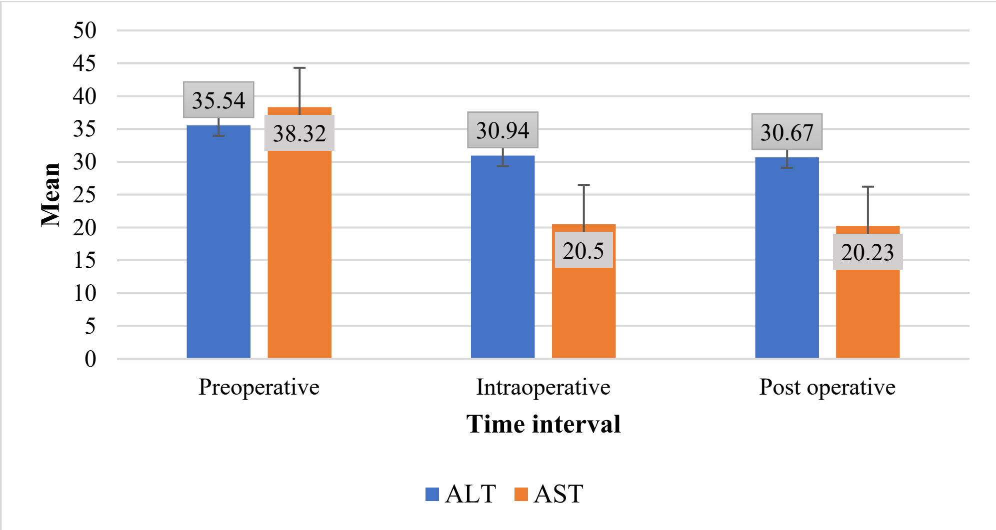
Dikshit and Prasad (1971) reported that due to the fall in basal metabolic rate and muscle activity during anaesthesia, the use of glucose by the muscle tissue was decreased thereby causing an increased concentration of blood glucose in the body.

Therefore, in the present study, the significant increase in the level of glucose from the initial phase to the later phase of the study might be caused due to anaesthesia, the stress due to surgery and the inactivity of the muscles during surgery.

4.11.2 Alanine aminotransaminase (ALT) and Aspartate aminotransaminase (AST)

The level of ALT showed a statistically non-significant decrease in the intraoperative and postoperative period compared to the base value. Similarly, there was a statistically significant ($P<0.05$) decrease in the level of AST in the intraoperative and postoperative periods compared to the preoperative level. (Figure 4.24 and Table 4.10.)

Figure 4.24: Mean ± SE of Alanine Transaminase (IU/l) Aspartate Transaminase (IU/l) at various time intervals. (n=21)



The preoperative high values of AST and ALT might be due to muscle Injury (Alvarez and Whittemore, 2009). A similar trend of decrease in the values of ALT and AST postoperatively was also recorded by Popovici et al. (2014) in their study on dogs undergoing orthopaedic surgery. Improved supply of blood to the liver as a result of administering Propofol might have caused a fall in the ALT and AST value in dogs (Khurana et al.2014). In addition, Frink Jr et al. (1992) in their work on dogs reported that isoflurane helped to maintain good hepatic blood flow whereas Gelman et al. (1984) documented that isoflurane improved the blood flow to the liver in dogs

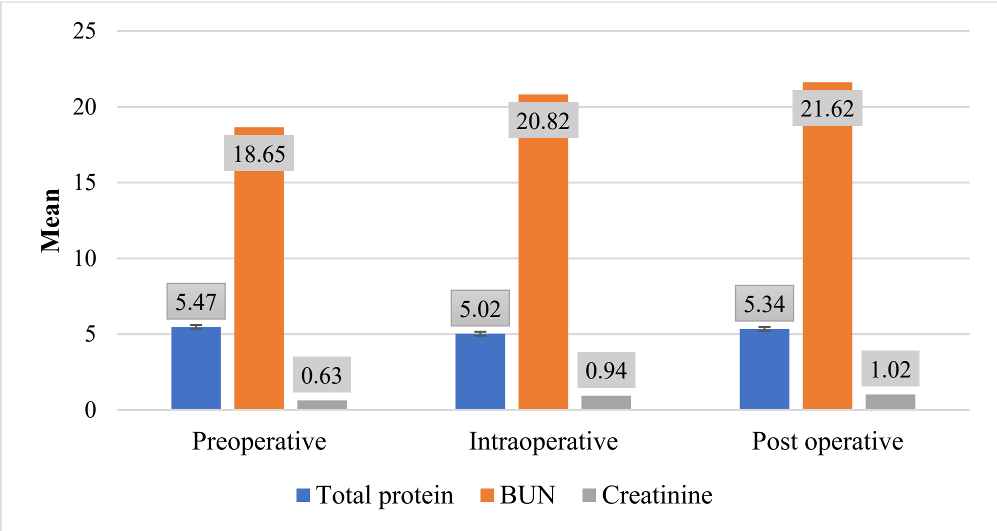
Thus, it could be inferred that in this study the initial higher levels of ALT and AST might be due to skeletal muscle trauma. The improved supply of blood to the liver due to anaesthesia might be responsible for the decreased intraoperative and post-operative levels of the above-mentioned enzymes. All the levels of ALT and AST measured at different time intervals were within the normal physiological limits.

4.11.3 Total Protein (TP), Blood Urea Nitrogen (BUN) and Creatinine (CRTN)

The trends in the plasma concentration of TP, BUN and CRTN are depicted in Figure 4.25 and Table 4.10. showed their Mean± SE values.

There was a statistically non-significant decrease in the value of total protein (TP) in the intraoperative period and postoperative period compared to the base value, the level of total protein was within the normal physiological range. The decrease in the value of total protein (TP) might be attributed to the haemodilution caused as a result of fluid administered during this study. Kapil (2014) and Kumar (2017) observed similar trends in their studies on dogs.

Figure 4.25: Mean ± SE of Total Protein (g/dl), Blood Urea and Nitrogen (mg/dl) and Creatinine (mg/dl) at various time intervals. (n=21)



The BUN and creatinine showed a non-significant increase in the intraoperative and postoperative periods as compared to the base level. The level of BUN and creatinine were within the normal physiological range during all phases of the study. The findings of normal values of BUN and creatinine are in the agreement with the studies of Popovici (2014) conducted on dogs anaesthetized with Propofol-sevoflurane combination and Rocuronium was used for neuromuscular blockade and Kumar (2017) on dogs anaesthetized with a Propofol-Isoflurane combination under vecuronium and atracurium as NMBAs. The normal levels of BUN and CRTN signified that renal function of the animals was normal with the anaesthetic protocol used during this study (along with the Rocuronium and its reversal).

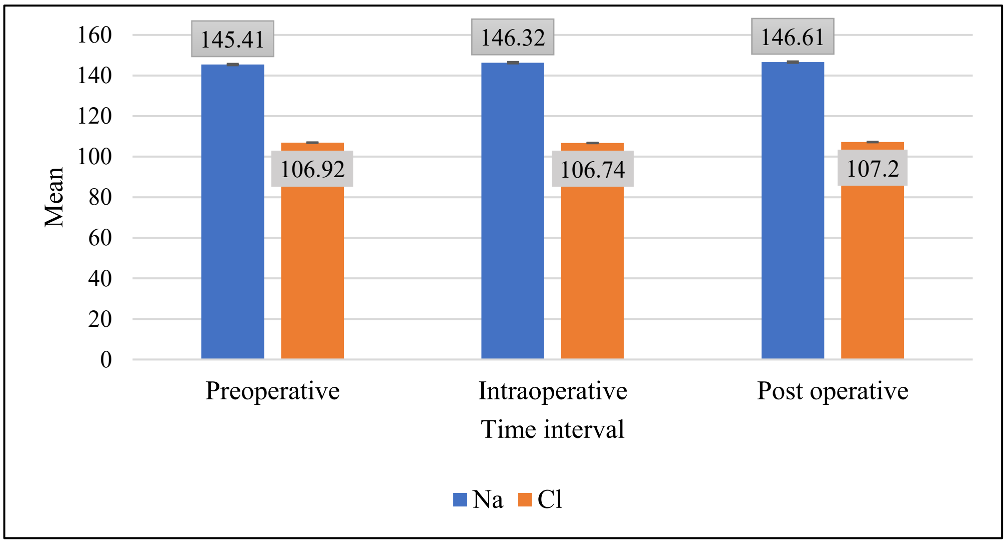
4.11.4 Sodium (Na), Potassium (K) and Chloride (Cl)

The concentration of electrolytes namely Sodium (Na), Potassium (K) and Chloride (Cl) as depicted in Table 4.11, Figure 4.26 and Figure 4.27were all within the physiological limits. There was no statistically significant change in the values of the above-mentioned electrolytes at different time intervals throughout the study. These findings were the same as those of Kumar (2017) whose study was conducted on dogs anaesthetized with a Propofol-Isoflurane combination under neuromuscular blocking agents, Vecuronium and Atracurium.

Table 4.11 Mean ± SE of electrolyte Parameters at various time intervals. (n=21)

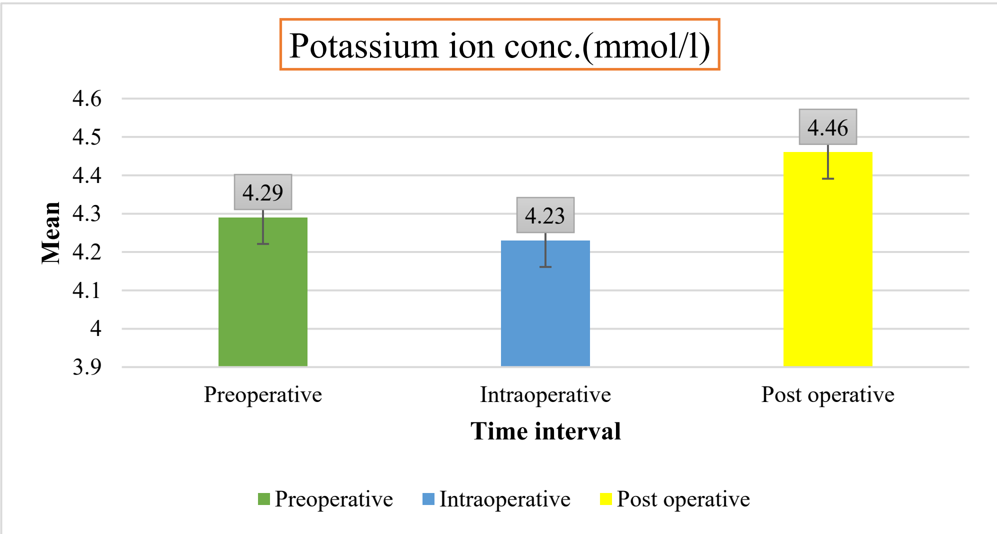
Parameters	Pre-op	Intra-op	Post-op
Na^+ (mmol/l)	145.41 ± 0.46	146.32 ± 0.64	146.61 ± 0.78
K^+ (mmol/l)	4.29 ± 0.06	4.23 ± 0.07	4.46 ± 0.10
Cl^- (mmol/l)	106.92 ± 0.39	106.74 ± 0.49	107.72 ± 0.76

Figure 4.26: Mean ± SE of Sodium ion(mmol/l) and Chloride ion (mmol/l) concentration at various time intervals.(n=21)



Tranquiliet al. (2007) highlighted the importance of electrolyte balance during anaesthesia and stated that hypokalaemia (serum level of potassium ion less than 3.0 mmol/L) led to hyperpolarisation of those cells that could be electrically stimulated ultimately leading to paralysis and ECG showed prolonged PR, QRS and QT interval, depression of ST segment and a flattened or inverted T wave. Whereas hyperkalaemia (serum level of potassium ion more than 6.5 mmol/L) leads to hypopolarisation and further causes myocardial arrhythmias. Decreased myocardial contractility and bradycardia might occur, ECG showed peaked T-waves and P-wave amplitude declined. Hyponatremia (serum sodium ion conc. of less than 136 mmol/L) ECG might show a widened QRS complex with an elevation of the ST segment. Hypernatremia (serum sodium ion conc. of more than 156 mmol/L) might cause non-respiratory alkalosis. Sudden changes in sodium concentration should be avoided because they could lead to unpredictable transcellular water migration and permanent brain damage. Chloride ions along with sodium, potassium and carbon dioxide keep the balance of body fluids and also maintains the acid-base balance.

Figure 4.27 Mean ± SE of Potassium ion concentration(mmol/l) at various time intervals. (n=21)



4.12 Recovery Parameters

All the animals recovered normally and uneventfully from the neuromuscular block and anaesthesia. There was no case of prolonged recovery in the present study. Different recovery parameters are shown in Figure 4.28 and Table 4.12

Figure 4.28: Mean ± SE of time elapsed(minutes) for recovery parameter to exhibit(n=21)

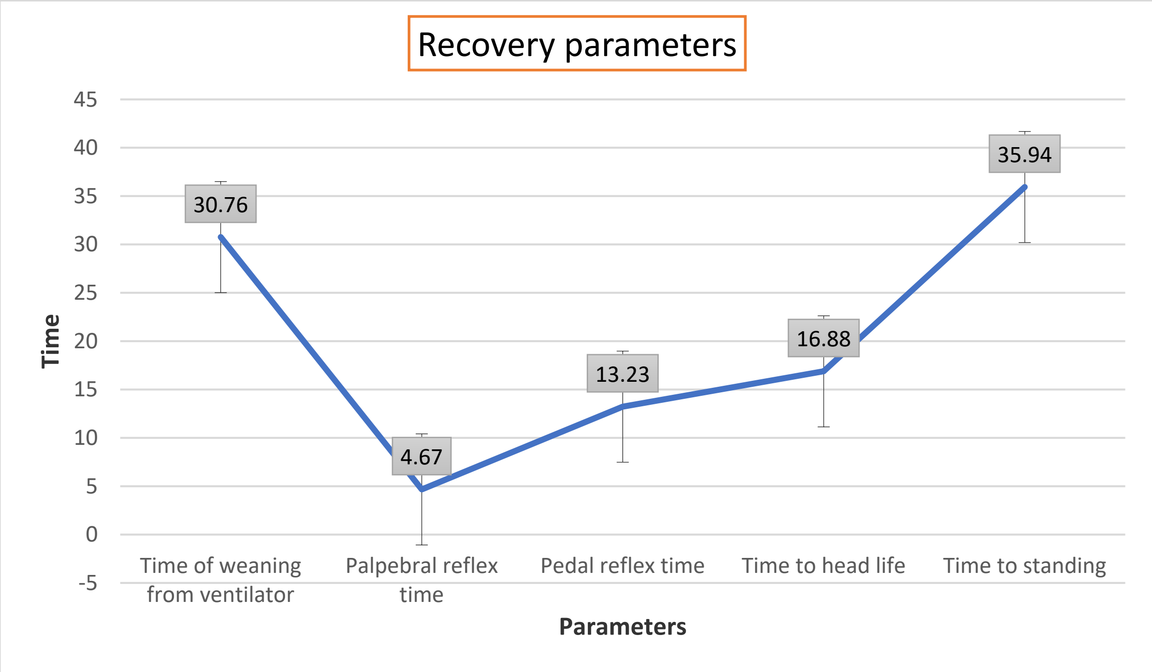


Table 4.12 Mean ± SE of time elapsed(minutes) for recovery parameter to exhibit. (n=21)	
Time of weaning from the ventilator	30.76 ± 0.65 min.
Duration of IPPV under NMBA	27.82 ± 0.72 min
Time of weaning from the ventilator after reversal of NMBA	2.7 ± 0.14 min.
Time-lapse till the end of surgery after reversal of NMBA	15.8±3.12 min
Isoflurane discontinued	
Palpebral reflex time	4.67 ± 0.44 min.
Pedal reflex time	13.23 ± 0.50 min.
Time to first head raise	16.88 ± 0.69 min.
Time to first standing	30.94 ± 1.29 min.

4.12.1 Time to weaning from the ventilator

The time to weaning from the ventilator was observed as the time that transpired between putting the animal on the ventilator and weaning the animal from the ventilator. The mean value of this parameter was recorded as 30.76 ± 0.65 minutes.

4.12.2 Palpebral reflex time

The period from the stoppage of Isoflurane in the anaesthetic circuit to the time when the animal started showing palpebral reflex was recorded as palpebral reflex time. The mean value for this parameter was recorded as 4.67 ± 0.44 min.

4.12.3 Pedal reflex time

The period from the stoppage of Isoflurane in the anaesthetic circuit to the time when the animal started showing pedal reflex was recorded as pedal reflex time. The mean value for this parameter was recorded as 13.23 ± 0.50 min.

4.12.4 Time to first head raise

The period from the stoppage of Isoflurane in the anaesthetic circuit to the time when the animal tries to lift its head properly was recorded as the time to first head raise. The mean value for this parameter was recorded as 16.88 ± 0.69 min.

4.12.5 Time to first standing

The period from the stoppage of Isoflurane in the anaesthetic circuit to the time when the animal acquired the standing posture (sternal recumbency in case of orthopaedic surgery) was recorded as a time to first standing. The mean value for this parameter was recorded as 30.94 ± 1.29 min.

Peripheral nerve stimulation

Additionally, peripheral nerve stimulation was used to ascertain the safety of neuromuscular blockade and the animal could be safely weaned from the ventilator. It was helpful in preventing residual nerve block.

The peripheral nerve stimulation (PNS) was carried out just before giving the neuromuscular block to ascertain the viability of the nerve being used to monitor the block and also to see the progression of the neuromuscular block after Rocuronium had been administered to the animal. The ulnar nerve and peroneal nerve were used for PNS.

Peripheral nerve stimulation was again carried out just after the neuromuscular block had been reversed by Myopyrolate to ascertain that the animal was out of the neuromuscular block. Monitoring of the neuromuscular block was done by subjective visual observation of muscle twitching by nervous stimulation. The settings of the nerve stimulator used for peripheral nerve stimulation were standardised by Sharma (2021) in his research on dogs (Voltage-2mA gradually reduced to 0.3 to 0.4mA, Frequency - 1 hertz, Impedance- calculated by nerve stimulator itself, Duration of electrical impulse - 0.10 ms)

Martin-Flores et.al (2014) in their study concluded that chances of residual curarization in the postoperative period were high if the assessment of recovery from the neuromuscular block was done just based on spontaneous respiration. Therefore, as a precautionary measure, peripheral nerve stimulation was instituted in the present study.

4.14 Centring of the ocular globe during neuromuscular block

Under inhalant anaesthesia, the ocular globe of the animal was in the ventromedial position. As Rocuronium was administered to the animal, the ocular globe gradually adopted a central position within 30 seconds. (Plate 4.3). The central position of the ocular globe facilitates many kinds of surgical interventions and examinations of the eye. Auer et al. (2007) in their study found similar results with the neuromuscular blocking agent, Rocuronium. Briganti et al. (2015) reported that Rocuronium facilitated centring of the eyeball by relaxing the extraocular musculature in dogs anaesthetized with Isoflurane and the duration of relaxation depended on the dose of the Rocuronium. Kumar et al. (2020) in their study on Atracurium and Vecuronium in dogs, found similar results pertaining to muscle relaxation for orthopaedics and ocular surgeries.

4.15 Muscle relaxation during neuromuscular block

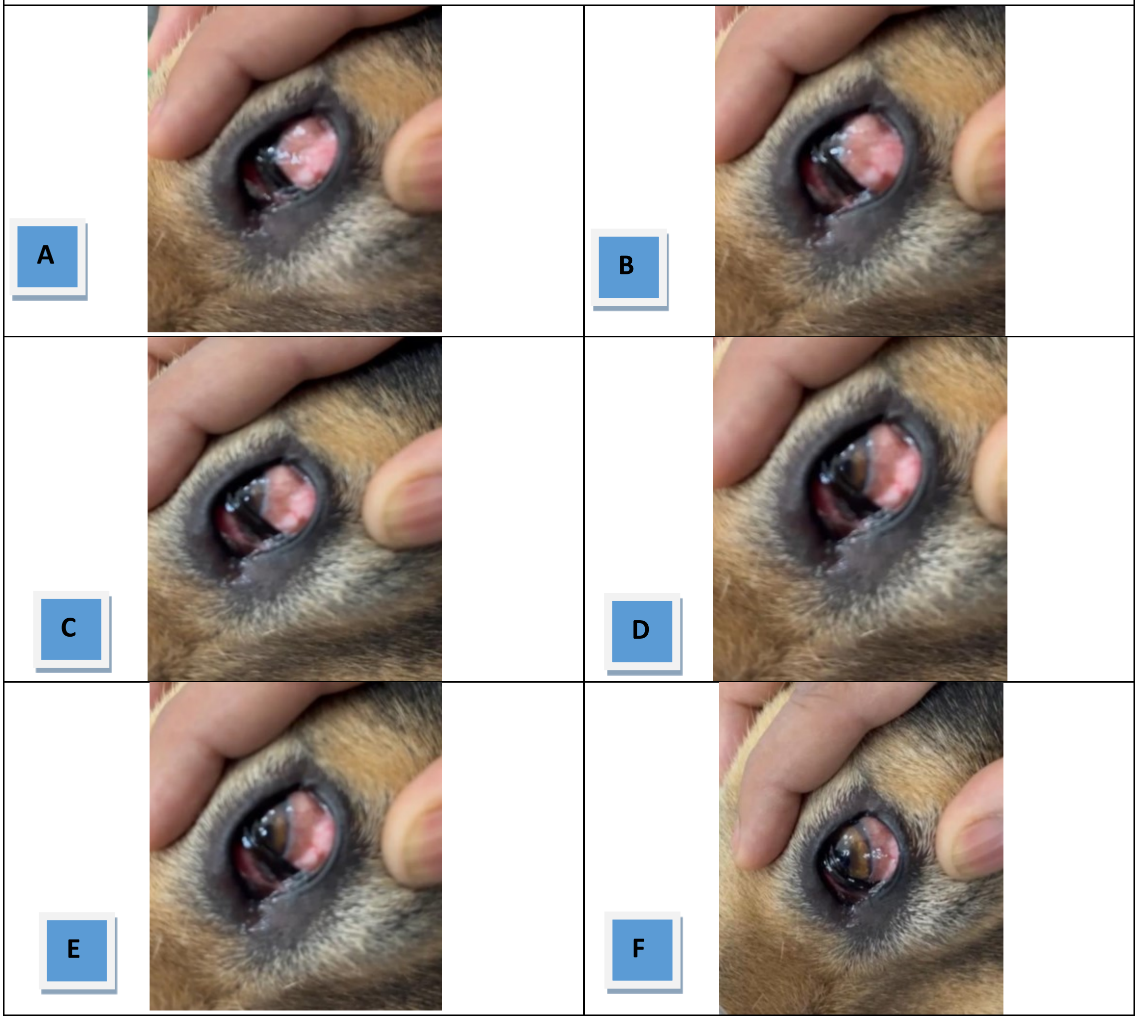
In the blind loop study, during orthopaedic surgeries, the reduction of fractured ends became effortless with minimal time thus reducing overall operative time. Easy reduction of the fracture without tissue trauma and also no bone nibbling was required for reduction of the fracture ends with Rocuronium (Plate 4.1 and Plate 4.2). Popovici et al. (2014) in their study used Rocuronium for orthopaedic surgeries in dogs and concluded that Rocuronium provided very good muscle relaxation.

In ovariohysterectomy surgeries, the appropriate level of abdominal muscle relaxation and overall easiness in surgical handling was observed which further helped in the easy exteriorization of the ovarian stump. Neves et al. (2014) reported a similar result in their study on female dogs using Rocuronium with acepromazine-Propofol-Isoflurane anaesthesia protocol.

Plate 4.1: Supracondylar fracture of femur managed by rush pinning technique			
			
Medio-lateral projection	Cranio-caudal projection	Medio-lateral projection	Cranio-caudal projection
Closed complete distal simple extraarticular fracture		Fracture repaired by Rush pinning technique	

Plate 4.2: Femur fracture managed by ADMIT pinning technique			
			
Medio-lateral projection	Cranio-caudal projection	Medio-lateral projection	Cranio-caudal projection
Closed complete diaphyseal simple transverse fracture		Fracture repaired by ADMIT pinning technique	

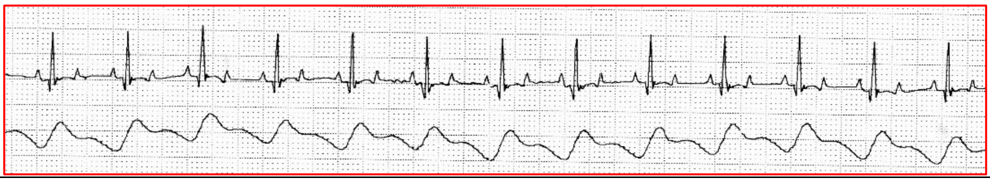
Plate 4.3: Pictures showing centring of the ocular globe after administration of Rocuronium in Propofol-Isoflurane anaesthetized dog.



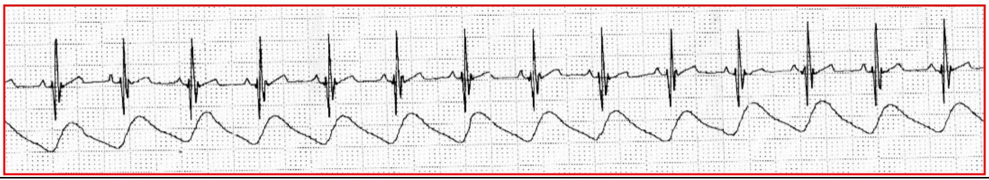
A: Ventromedial position of the ocular globe in Propofol-Isoflurane anaesthetized dogs

B-F: Series showing relaxation of the ocular globe and its gradual return to the central position

Plate 4.4: The pattern of ECG (Lead II) for Propofol-Isoflurane anaesthetized dogs at different time intervals, the animal is under NMBA between T_5 – T_{20} and NMB was reversed between T_{20} and T_{40} (Paper speed= 25 mm/sec and 1 mV= 1 cm)

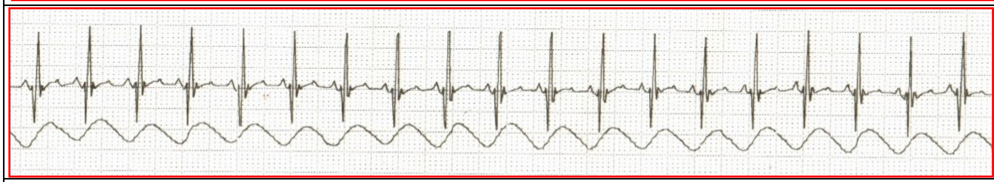


T_0



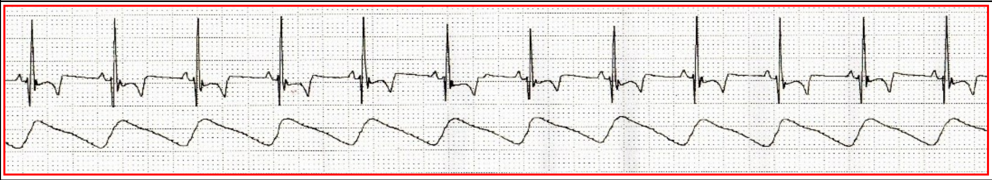
T_1

T_5

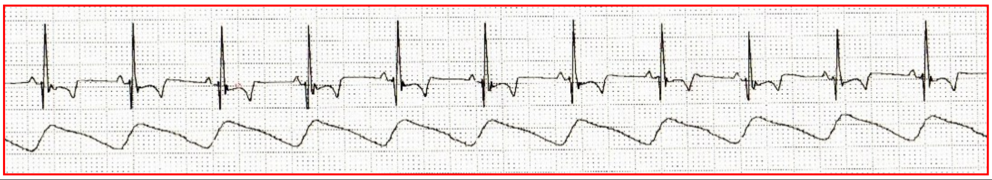


T_{10}

T_{15}



T_{20}



T_{40}

Chapter 5 SUMMARY AND CONCLUSIONS

This study was executed in the clinical setup to document the effects of Rocuronium and its reversal by Neostigmine and Glycopyrrolate combination (Myopyrolate) in Propofol-Isoflurane anaesthetized dogs. The study design had standardization and clinical trial phases. Rocuronium-induced neuromuscular blockade was performed in 21 clinical cases. Out of which 5 cases of intra-medullary pinning, 4 cases of bone plating, 3 cases of intramedullary pinning with cerclage wire, 5 cases of rush pinning, 2 cases of plate rod combination and 2 cases of ovariohysterectomy were performed.

Every animal included in this study was premedicated using Inj. Butorphanol tartrate at the dose rate of 0.2mg/kg b.wt. I/M, after a gap of 30 minutes Inj.Atropine sulphate was administered at the dose rate of 0.02mg/kg b.wt. I/M, subsequently after a gap of 10 minutes animal was premedicated with Inj. Diazepam at the dose rate of 0.5mg /kg b.wt I/V followed immediately by Inj. Propofol I/V(given till effect) for the induction of general anaesthesia.

Soon after induction, the animal was maintained using inhalant Isoflurane mixed in oxygen. After the animal had stabilized, neuromuscular block was induced using Inj. Rocuronium at the dose rate of 0.5 mg /kgb.wt. I/V. Immediate connecting of the animal to a mechanical ventilator was undertaken and intermittent positive pressure ventilation (IPPV) was provided in volume control mode, with settings i.e. a respiration rate of 15 breaths per minute, tidal volume at 10ml/kg body weight and inspiratory expiratory ratio as 1:2.

Neuromuscular block induced by Inj. Rocuronium was reversed at the first sign of asynchrony on the bellow of the ventilator and the multiparameter monitor. A single syringe combination drug Inj.Myopyrolate I/V was administered at the dose rate of 0.05mg/kgb.wt.(Neostigmine) and 0.01mg/kgb.wt. (Glycopyrrolate).

To assess the effect of the above protocol on the physiology of the animal various parameters like body temperature, respiratory rate, IOP, heart rate, electrocardiogram ,haemodynamic observations, vaporizer concentration and pulmonary parameters were critically monitored at different time intervals.T_0 (baseline value before giving any anaesthesia),T_1(immediately the following induction),T_5(5 minutes after Rocuronium was injected), T_{10}(10 min. after Rocuronium was injected),T_{15}(15 min. after Rocuronium was injected),T_{20}(20 min. after Rocuronium was injected).The reversal of the neuromuscular block was undertaken between T20 and T40 at about 25 to 35 minutes after the administration of Rocuronium. T_{40} (40 min after Rocuronium was injected and post-reversal). Haematological parameters, biochemical parameters and electrolytes were estimated preoperatively, intraoperatively and postoperatively. Statistical analysis was done using Graph Pad Instat Software, Version 3.01, 32 bits

The decrease in temperature was significant but within the normal range for the anaesthetized animal. The significant increase in the heart rate was transitory and the heart rate was within normal physiological range at all the time intervals. No statistically significant change in different components of ECG was observed. IOP was minimally altered and remained within the physiological limits

The mean arterial pressure (MAP) along with systolic blood pressure (SBP), diastolic blood pressure (DBP) and central venous pressure (CVP) were well within the normal physiological range. The requirement of inhalant anaesthetic decreased statistically significantly after the neuromuscular blockade was established in the animal. Thus, confirming the Isoflurane-sparing effect of the Rocuronium.

There was a statistically significant decrease in the respiration rate after the animal had been weaned from the ventilator as the animal was still under Isoflurane and thus the decreased value of respiration was normal under anaesthesia. All the remaining respiratory parameters like EtO_2, FiO_2, $EtCO_2$, $FiCO_2$, EtISO and FiISO were well within the normal range.

The value of oxygen saturation of haemoglobin (SpO_2) varied between 98 to 100 per cent during the study. All the haematological and biochemical parameters remained within the normal physiological range. An increase in the level of glucose from the intraoperative to postoperative period was observed. Also, there was a significant fall in the value of haemoglobin and packed cell volume from the intraoperative period to the postoperative period during the study. All the above alterations could be attributed to anaesthesia-related changes in the body of the animals.

Under inhalant anaesthesia, the ocular globe of the animal was in the ventromedial position. As Rocuronium was administered to the animal the ocular globe gradually changed to a central position within 30 seconds. The central position of the ocular globe facilitates many kinds of surgical interventions and examinations in the eyes. During blind loop orthopaedic surgeries, the reduction of fractured ends became effortless with minimal time thus reducing overall operative time. Easy reduction of the fracture with minimal tissue trauma was observed and no bone nibbling was required for reduction of the fracture. In ovariohysterectomy surgeries, the appropriate level of abdominal muscle relaxation and overall easiness in surgical handling was observed which further helped in the easy exteriorization of the ovarian stump.

Duration of IPPV under NMBA was 27.82± 0.72 min., time of weaning from the ventilator after reversal of NMBA was 2.7±0.14 min., time-lapse till the end of the surgery after reversal of NMBA was 15.8 ± 3.12 min. Then after the Isoflurane was discontinued the palpebral reflex time was 4.67 ± 0.44 min., the pedal reflex time was 13.23 ± 0.50 min., the time to first head raise was 16.88 ± 0.69 min and the time to first standing was 30.94 ± 1.29 min. The recovery of the animals in all the cases from the neuromuscular blockade and anaesthesia was smooth and uneventful.

CONCLUSIONS

- Inj. Butorphanol tartrate@ 0.2mg/kg b.wt I/M, Inj.Atropine sulphate@ 0.02mg/kg b.wt I/M,Inj. Diazepam @ 0.5 mg/kg b.wt I/V, Inj. Propofol @ 2.5-5 mg/kg b.wt I/V, Inj. Rocuronium @ 0.5 mg/kg b.wt. I/V and maintenance with Isoflurane under IPPV provided safe anaesthesia for a variety of surgeries.

- Rocuronium @ 0.5mg/kg b. wt IV in dogs produced neuromuscular blockade within 17.64±1.10 sec of administration and provided muscle relaxation for 27.82 ± 0.72 min.

- Neuromuscular blockade produced by Rocuronium was completely reversed by a single syringe combination drug of Neostigmine and Glycopyrrolate (Myopyrolate) I/V at the dose rate of 0.05 mg/kg b.wt and 0.01mg /kg b.wt respectively.

- Rocuronium-induced muscle relaxation provided the centring of the ocular globe within a few seconds without any significant effect on IOP, therefore its use in ophthalmic surgery can be explored.

- The use of Rocuronium along with IPPV caused minimal alteration of the physiological parameters with no consequences clinically and thus can be considered a complication-free anaesthetic protocol for interventions demanding muscle relaxation.

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Appendix – I

Proforma for Research

Case no: Name and Address: Date:

<table>
<tr>
<td>

Species: Breed:

Age: Sex: Body wt.:

Temperament

- ☐ Docile
- ☐ Aggressive
- ☐ Co-operative

Chief complaint:

Activity level

- ☐ Excited
- ☐ Normal
- ☐ Calm
- ☐ Depressed

Urination:

- ☐ Normal
- ☐ PU/PD
- ☐ Oliguria
- ☐ Anuria

Concurrent disease:

Any history of epilepsy:

Medication:

Previous anaesthetic risk:

</td>
<td>

General body condition:

- ☐ Normal ☐ Obese
- ☐ Weak ☐ Cachexic
- ☐ Pregnant

Hydration status:

Sclera:

- ☐ Normal ☐ Jaundiced
- ☐ Congested

CVS evaluation:

- ☐ HR ☐ PR ☐ CRT

Blood Pressure:

- ☐ DBP
- ☐ SBP
- ☐ MAP

Heart Auscultation:

Pulmonary Testing:

RR:

Mucus membrane:

Lung auscultation:

Temperature:

Integument:

- ☐ Neoplasia
- ☐ Emphysema
- ☐ Ectoparasites
- ☐ Trauma

Neurological deficits:

Muscular tone:

</td>
</tr>
</table>

Class of patient according to ASA: I II III IV V

IOP:

Pre-operative		**Intra-operative**		**Post-operative**	
OD-	OS-	OD-	OS-	OD-	OS

HAEMATOLOGICAL PARAMETERS:

PARAMETER	**PRE-OPERATIVE**	**INTRA-OPERATIVE**	**POST-OPERATIVE**
Hb			
PCV/HCT			
TEC			
TLC			
PLATELETS			

BIOCHEMICAL PARAMETERS:

Parameters			
Glucose			
ALT			
AST			
BUN			
CRTN			
TP			
Cl			
K			
Na			

PROCEDURE	**TIME**	**REMARKS**
Induction		
Start of inhalant		
Start of IPPV		
NMBA		
Start of Surgery		
End of surgery		

ANAETHESIA:

DRUG	DOSE RATE	ROUTE	TIME
Inj.Atropine			
Inj.Butrum			
Inj.Diazepam			
Inj.Propofol			

NMBA:

DRUG	DOSE & ROUTE	TIME (admin)	TIME FOR ONSET	TIME (effect wave off)/DURATION
Inj.Rocuronium				

REVERSAL OF NMBA:

DRUG	DOSE & ROUTE	TIME (admin)	TIME (reversal of NM block)
Inj Myo-Pyrolate			

RECOVERY PHASE

Time to weaning from ventilator	
Palpebral reflex time	
Pedal reflex time	
Time to first head raise	
Time to first standing	

MAINTENANCE:

Parameter	O min	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
Isoflurane vaporizer																					
SpO2																					
O2 flow																					
Eye ball (c/v)																					
HR																					
RR																					
SBP																					
DBP																					
MAP																					
CVP																					
RT																					
TV																					
I:E																					
EtCO2																					
FiCO2																					
EtO2																					
FiO2																					
EtISO																					
FiISO																					
MAC																					
Fluid rate																					

Brief Resume of the Student

Name	:	Dr Yudhvir Rana
Father's Name	:	Sh. Albar Rana
Mother's Name	:	Smt. Roshani Devi
Date of Birth	:	03.03.1979
Permanent Address	:	Vill. Aima, P.O. Bandla Tea Estate, Palampur, Distt. Kangra, H.P.

Academic Qualifications:

Qualification	**Month/ Years**	**School/Board/ University**	**Marks (%)**	**Division**	**Major subject**
10^{th}	1994	HP Board of Secondary Education, Dharamshala	72	1st	Mathematics, Science, Social Science, Hindi, English, Arts, Sanskrit
10+2	1996	CBSE, New Delhi	58.68	2nd	Mathematics,Biology, Chemistry, Physics and English
B.V.Sc. & A.H.	2003	CSKHPKV, Palampur	6.56	1st	All courses covered under VCI curriculum

Fellowships/Scholarships/Gold Medals/Awards/Any Other Distinction: nil

Publications:

Research papers: 1

Scientific Popular Articles: nil

Others: nil

Visits abroad along with duration and purpose of visit: nil