Received: 16 July 2024 Revised: 06 September 2024 Accepted: 24 September 2024
DOI: https://doi.org/10.29244/primatology.3.01.17-28 E-ISSN: 2964-5441

RESEARCH ARTICLE

INAJP INDONESIAN JOURNAL OF PRIMATOLOGY

Effect of Chlordiazepoxide or Haloperidol Oral Premedication during Midazolam-Ketamine Anaesthesia on Physiological and Blood Gas Parameters in Adult Male Bonnet Macaques (*Macaca radiata*)

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Abstract

Capturing wild animals with minimal stress is crucial to reduce morbidity and mortality. Oral premedicants can ease handling and subsequent anaesthetic administration. This study evaluated anaesthetic protocols and physiological changes during vasectomy in twelve adult male bonnet macaques. Group I received 10 mg/kg chlordiazepoxide, and Group II received 1 mg/kg haloperidol orally four hours before midazolam-ketamine anaesthesia. Sedation quality, induction, maintenance, and recovery were assessed. Physiological and blood gas changes were monitored at 0, 5, 10, 15, and 20 minutes. Both groups exhibited sedation post-premedication. Induction, maintenance, and anaesthesia depth were similar. Group I had better analgesia, while Group II had superior muscle relaxation. Blood gas analysis indicated mild stress and respiratory acidosis in both groups, with Group II showing more significant hypoxemia and respiratory acidosis. Haloperidol-premedicated animals were easier to handle but exhibited increased stress parameters compared to chlordiazepoxide-premedicated animals. Recovery was better in Group I. Both protocols effectively induced sedation and anaesthesia in bonnet macaques.

Keywords: blood gas analysis, injectable anaesthesia, monkey anaesthesia, stress.

1. Introduction

Non-human primates are most commonly premedicated and anaesthetised for various aspects of fundamental primatology and research purposes, such as physical examination, drug administration, surgical sterilisation, and diagnostic procedures. Monkeys are also chemically restrained for recreational purposes in zoos all over the world. In the domain of fundamental primatology, the strategic implementation of oral premedication is a critical component in research protocols, playing a pivotal role in alleviating stress during primate handling (Aenemo and Caulkett 2007; Lee *et al*. 2010). This stress reduction holds profound implications, enriching our understanding of primate behaviour, ecology, and morphology. By mitigating anxiety during handling, premedication allows for more accurate observations of natural behaviour, shedding light on non-human primate social structures (McCann *et al.* 2007). Capturing wild animals for various purposes is said to have started in the 1950's (Fahlman 2008). Since then, many capture techniques have been developed and standardised for wild animals. Uncontrolled stress and death due to capture myopathy have been recognised as the most severe concern during the handling and restraint of wild animals (Nielsen 1999; Paterson 2007).

The decreased stress positively influences ecological studies, unveiling more authentic interactions with the environment. Recognising the interconnected relationship between stress, behaviour, and physiology, premedication may potentially impact primate morphology, revealing more precise anatomical features. In the field of non-human primate research, oral premedication emerges as a valuable tool, significantly contributing to studies in behavioural, ecological, and morphological dimensions. Stress reduction during experimental manipulations facilitates a more naturalistic expression of primate behaviour, particularly in social interactions. In ecological studies, it aids in accurate observations by minimising stress-induced alterations in foraging and locomotor behaviours (Fahlman 2008; Rennie and Buchanan-Smith H.M 2006a, 2006b, 2006c).

Usually, free-ranging macaques are anaesthetised by darting or trapped in large cages and by injecting the anaesthetic agents after transferring them to squeeze cages or smaller cages. Captive bonnet macaques in

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zoos may also be darted in their enclosures or hand-injected after physical restraint. Premedication is not usually practised in such cases. Darting small-sized, unpremedicated monkeys can be dangerous for the animal and a difficult task because of their frantic fast movements. Unpremedicated macaques are challenging to handle for injecting drugs by hand because of their speed, dexterity, and intelligence, as well as their potential to cause serious physical injury to the handler. Moreover, physical and chemical restraint of monkeys is associated with stress, as in other wild animals (Kleiman *et al.* 2010). Ease of handling and drug administration can be improved, as well as reducing stress response in these animals by premedicating them with oral tranquillisers before handling or anaesthesia (Pulley *et al.* 2004). Several tranquillisers, sedatives, and anaesthetic drugs have established themselves as crucial agents for reducing stress and related issues during wild animal restraint. Improvement of the capture technique using appropriate drugs to minimise stress is a priority from the wildlife conservation and welfare point of view as well. Reduced stress in animals also increases the safety of the handler and the quality of the samples obtained from the study animals (Olberg 2007).

Chlordiazepoxide (a benzodiazepine derivative) and haloperidol (a butyrophenone derivative) have been defined as long-acting tranquillisers which can be administered orally as premedication. The potential to reduce stress during handling and ease the subsequent administration of anaesthetic drugs has been evaluated in the present study. The advantage of these long-acting drugs is that their effect would last for sufficient time to allow gastric emptying before the administration of anaesthetic drugs in spite of being administered orally. Anaesthetic drugs may be administered parenterally after the onset of action of the premedicant and after providing an appropriate period of time for gastric emptying. The combination of midazolam and ketamine has already been proven to produce satisfactory anaesthesia with minimal cardio-respiratory changes in animals and is routinely used for anaesthetising macaques (Fagundes *et al.* 2020).

Clinical effects of premedication and anaesthesia alter the physiological and blood gas parameters. Previous studies have reported the alterations in these parameters during anaesthesia (Galante *et al.* 2019; Gomes *et al.* 2021). Evidence has been reported for alterations like hypoxia, hypothermia and hypotension due to altered homeostasis during anaesthesia. The alterations due to deleterious effects of anaesthesia can also affect the result of research (Aguiar 2010; Wright 1982).

Under these circumstances, a study was conducted on adult male captive bonnet macaques undergoing vasectomy at the State Museum and Zoo, Thrissur, to address several research questions. Does oral premedication with chlordiazepoxide or haloperidol enhance sedation and ease of handling during midazolam-ketamine anaesthesia in adult male bonnet macaques? What are the effects of chlordiazepoxide or haloperidol premedication on physiological and blood gas parameters during and after midazolam-ketamine anaesthesia? How do chlordiazepoxide and haloperidol premedication compare in terms of the quality of anaesthesia induction, maintenance, and recovery times? Does premedication with chlordiazepoxide or haloperidol effectively reduce stress and anxiety in macaques compared to non-premedicated controls? The study also includes a specific focus on blood gas analysis under both protocols.

2. Materials and Methods

2.1 Humane Care Guidelines

The present study was approved by the Institutional Animal Ethics Committee, KVASU, Kerala, India. The study was conducted on twelve adult healthy bonnet macaques at the State Museum and Zoo, Thrissur, Kerala, India, as per the guidelines of the Central Zoo Authority of India (CZA). We followed the best practices for handling and surgery of the bonnet macaques as per the guidelines of CZA.

The bonnet macaques were provided with a meticulously planned diet comprising fruits, vegetables, nuts, and seeds to fulfil their nutritional requirements. Regular deworming protocols were followed to control parasites and maintain optimal gastrointestinal health. Additionally, vaccines are administered based on the recommendations of CZA, for preventing diseases to ensure the overall well-being of the bonnet macaques. Twelve apparently healthy adult male macaques which underwent routine vasectomy procedures to control their population in the zoo as per directions of CZA were selected for the present study. The animals were randomly selected from a group of 95 monkeys, which were held in three enclosures of 30×15×30 feet (length×breadth×height). All bonnet macaques were raised in accordance with the guidelines set by the CZA, taking into consideration their physical, social, and psychological needs.

The selected animals were randomly allotted into two groups, Group I and Group II of six each. The separated animals were conditioned for 15 days to take pineapple fruit juice prior to the procedure. The macaques were fasted for eight hours, and water was withheld for five hours prior to the administration of fruit juice containing premedication drugs.

2.2 Administration of Oral Premedicants

Based on the experience of the zoo veterinarian, the body weight of the animal was estimated (estimated body weight), and the dose of premedication and anaesthetic drugs were calculated and administered. Later, the body weight was evaluated after induction of anaesthesia, and these doses were corrected according to actual measured body weight. The actual body weights of the animals were 7.44±0.96 and 5.67±0.65 in Group I and Group II, respectively. Animals of Group I were premedicated with chlordiazepoxide at the rate of 10 mg/kg body weight orally in pineapple

fruit juice. Animals of Group II were premedicated with haloperidol at the rate of 1 mg/kg body weight orally in pineapple fruit juice. The tablets form of premedicants were powdered two minutes prior to oral administration, mixed with the fruit juice and administered one by one at the rate of 3 ml/kg.

$$\text{Corrected Dose Rate} = \frac{\text{Estimated Body Weight} \times \text{Dose Rate of the Agent}}{\text{Actual Measured Body Weight}}$$

Behavioural responses prior to and after the premedication were recorded by an observer who was unaware of which drug was administered.

2.3 Midazolam and Ketamine Hydrochloride Combination Anaesthesia

All the animals were physically restrained manually or by using a net for injecting anaesthetics. Animals of both groups were anaesthetised using a combination of midazolam and ketamine hydrochloride intramuscularly at the rate of 0.1 and 10 mg/kg body weight, respectively, into the gluteal muscles. The quality of induction and time of induction were recorded by the observer based on a graded scorecard modified from one described by Jianhua and Hongbin (2009).

2.4 Monitoring of Anaesthesia

All the animals were monitored by the same individual for quality of induction, quality of maintenance, depth of anaesthesia, and recovery from anaesthesia. The parameters were recorded as per the recommendations of Bakker *et al.* (2013) and Jianhua and Hongbin (2009). Time of induction, time of recovery, duration of anaesthesia, duration of immobilisation, and complications during anaesthesia were also monitored. The quality of maintenance of anaesthesia was monitored from the time of induction until the first signs of recovery.

Depth of anaesthesia was scored based on the level of physiologic reflexes noticed during the period of maintenance of anaesthesia. The scorecard was modified based on the sedation depth scoring system reported by Jianhua and Hongbin (2009) and Bertrand *et al.* (2016). Analgesia was assessed during the time of cephalic venepuncture immediately after the induction of anaesthesia and recorded as per recommendations of Sun *et al.* (2003). Duration of immobilisation, muscle relaxation, palpebral reflex, pedal withdrawal reflex, time of recovery and quality of recovery from anaesthesia were assessed and recorded in the scorecard based on the previous recommendations (Lee *et al*. 2010; Bakker *et al.* 2013). Duration of anaesthesia was calculated and recorded as the time from induction of anaesthesia to first signs of recovery. Any complications observed during the period of anaesthesia like regurgitation or vomiting, hypoxia, and arrhythmia were recorded. Tongue and jaw movements, extremity movements, vocalisation, struggling, and other signs of recovery from anaesthesia were recorded during the period of anaesthesia.

2.5 Evaluation of Physiological Parameters

Physiological parameters were monitored and recorded every five minutes from the time of induction of anaesthesia until the 30th minute after induction in both groups (i.e., at 0-minute mark, 5th, 10th, 15th, 20th, 25th, and 30th minute from lateral recumbency).

In addition to this, the degree of muscle relaxation, palpebral reflex, pedal withdrawal reflex, and analgesia were also monitored. The blood pressure was measured oscillometrically from the brachial artery using a neonatal cuff as per recommendations of Bertrand *et al.* (2016). The haemoglobin oxygen saturation was measured using a pulse oximeter connected to a multi-parameter monitor as suggested by Lee *et al.* (2010). All the observations and parameters were entered in an anaesthetic record for future reference and evaluation.

Lead II electrocardiogram was monitored throughout the surgical procedure. All the ECG tracings were recorded at 25 mm/sec paper speed and at 10 mm/mV using Cardiart 6108T8 (BPL Medical Technologies Pvt. Ltd., Palakkad, Kerala, India).

2.6 Blood Gas Analysis

Blood gas analysis was performed using the venous blood collected immediately after induction of anaesthesia and from arterial blood samples at the 20th minute after induction of anaesthesia. Blood samples were collected either from the femoral artery or by cephalic venipuncture. All blood samples were analysed immediately within 10 minutes of collection. Air bubbles, if any, were removed by keeping the syringe in an upright position and by squirting out some drops of blood along with the air bubbles. Arterial and venous blood samples were evaluated for blood pH, partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2), bicarbonate ion concentration (HCO_3^-), standard base excess (BE) and lactate values using a portable blood gas analyser (epoc® Blood Analysis System, and epoc BGEM Test Card, Epocal, INC., Ottawa, ON Canada) and the observations were recorded. The blood gas values were corrected to the rectal temperature of the animal using the provision available in the equipment.

2.7 Serum Cortisol, Creatine Kinase, Aspartate Aminotransferase, and Alanine Aminotransferase Estimation

Serum cortisol was estimated from the venous blood collected immediately after induction of anaesthesia and at the 30th minute of induction by electrochemiluminescent immunoassay (ECLIA) method using the commercially available kit in an automated analyser. Creatine Kinase, Aspartate Aminotransferase, and Alanine Aminotransferase were also estimated at the same time intervals.

2.8 Statistical Analysis

The data obtained was subjected to statistical analysis as described by Snedecor and Cochran (1994) using the statistical software SPSS version 16.0. Independent sample t-test was used to compare the Means±Standard Errors (SE) of physiological parameters, atmospheric temperature, relative humidity, time taken for premedication, onset time, induction time, recovery time, duration of anaesthesia, immobilisation time, and total procedure time between groups. A paired sample t-test was used for comparing pH, PCO_2, and HCO_3^- values of blood gas analysis.

Independent sample t-test and paired sample t-test were used for comparing biochemical and haematological parameters. Independent sample t-test was used to compare between the groups, and paired sample t-test was used for comparing observations before and after treatment, respectively.

2.9 Ethical statement

The present study was approved by the Institutional Animal Ethics Committee, KVASU, Kerala, India. The study was conducted in twelve adult healthy bonnet macaques at the State Museum and Zoo, Thrissur, Kerala, India, as per the guidelines of the Central Zoo Authority of India. We followed the best practices for handling and surgery of the bonnet macaques as per the guidelines of the Central Zoo Authority of India.

3. Results

3.1 Premedication Parameters

Mean±SE values of corrected oral dose against the actual measured body weight of animals were evaluated in both groups. The corrected doses of chlordiazepoxide and haloperidol were found to be 10.33±0.20 and 1.12±0.07 mg/kg body weight in Group I and Group II, respectively. No significant difference was noticed between Group I and Group II in these parameters. All the animals took a complete dose of fruit juice. The time taken to complete the premedication was 26.16±8.96 and 12.66±7.53 minutes in Group I and Group II, respectively.

3.2 Quality of Induction of Anaesthesia

Mean±SE of the dose rate of midazolam and ketamine used for anaesthetising animals of Group I were 0.103±0.005 and 10.33±0.20 mg/kg body weight, respectively. Mean±SE values of the dose rate of midazolam and ketamine used for anaesthetising animals of Group II were 0.112±0.007 and 11.28±0.77 mg/kg body weight, respectively. There was no significant difference in the dose rate, induction time, quality of induction, depth of anaesthesia, muscle relaxation, pedal withdrawal reflex, and quality of analgesia between the two groups of animals. A significant difference was noticed in the quality of recovery from anaesthesia between both the groups and the data of these parameters were recorded in Table 1.

3.3 Quality of Maintenance of Anaesthesia

The additional dose of the anaesthetic combination at the rate of one-third of the original induction dose had to be administered intravenously to three animals of each group to maintain anaesthesia to complete the vasectomy procedure. Two animals of Group I showed movements of the jaw and tongue, and two animals each of Group I and Group II showed movements of the extremities during the maintenance of anaesthesia. Mean±SE values of duration of anaesthesia after the initial induction dose were 44.33±6.04 minutes in Group I and 39.50±4.95 minutes in Group II. There was no significant difference in the duration of anaesthesia between Group I and Group II. Observations like the time taken from the onset of anaesthesia until recovery during the whole procedure were recorded (Table 2).

The depth of anaesthesia was scored, and the median values were found to be 3.5 in both groups. There was no significant difference in the depth of anaesthesia between Group I and Group II. Three animals of both Group I and Group II showed surgical anaesthesia (deeper level of anaesthesia). Three animals from both Group I and Group II showed lighter planes of anaesthesia.

The quality of analgesia was scored, and the median values for both groups were found to be 3.0 in Group I and 4.0 in Group II, respectively. The median values of scores for muscle relaxation were 2.0 in Group I and 1.0 in Group II, respectively. The median value score for pedal withdrawal reflex was 2.0 in both groups. The scores for the palpebral reflex were found to be zero at almost all times in both groups, indicating the presence of reflex throughout the period of anaesthesia.

Several complications like hypoxia, hypercarbia, apnoea, and regurgitation were noticed during the

Table 1. Scores obtained during anaesthesia

Parameter	Median		Z-value	p-value
	Group I	Group II		
Quality of induction	4.0	4.0	1.087	0.277
Depth of anaesthesia	3.5	3.5	0	1
Muscle relaxation	2.0	1.0	1.682	0.093
Pedal withdrawal reflex	2.0	2.0	0.123	0.902
Quality of analgesia	3.0	4.0	1.734	0.083
Quality of recovery from anaesthesia	2.0	3.5	2.682**	0.007

**Significant at 0.01 level

Table 2. Time taken from onset of anaesthesia until recovery during the procedure

Parameter	Mean±SE		t-value	p-value
	Group I	Group II		
Time of onset (nodding) (s)	66.33±22.41	80.0±13.60	0.521	0.614
Time of head sagging (s)	130.33±43.44	148.33±20.80	0.374	0.716
Time taken for recumbency (s)	164.66±49.64	152.50±20.48	0.227	0.825
Time taken for induction of anaesthesia (s)	246.33±41.04	291.50±58.12	0.635	0.540
Duration of anaesthesia (min)	44.33±6.04	39.50±4.95	0.618	0.550
Immobilisation time (min)	57.83±6.29	45.16±4.69	1.613	0.138
Time taken for recovery from anaesthesia (min)	61.50±6.80	38.33±5.94	2.564**	0.028

*Significant at 0.05 level; SE – Standard error

Table 3. Complications observed during anaesthesia

Observation	Present (% of animals affected)	
	Group I	Group II
Hypoxia	50	33.33
Hypercarbia	16.66	16.66
Apnoea	0	16.66
Regurgitation	50	33.33

Table 4. Blood gas analysis

Parameter	Time (min)	Mean±SE		t-value	p-value
		Group I	Group II		
pH	0	7.41±0.02	7.33±0.01	2.926*	0.015
	20	7.37±0.02	7.27±0.04	1.816	0.115
Partial pressure of carbon dioxide (PCO_2) (mmHg)	0	42.01±2.29	41.60±3.71	0.096	0.926
	20	42.75±2.65	41.03±3.38	0.399	0.698
Bicarbonate ion concentration (HCO_3^-)	0	24.56±2.03	22.16±1.27	0.998	0.342
	20	23.96±2.46	22.76±1.49	0.416	0.686
Base excess (BE) (mmol/L)	0	1.45±1.45	-3.90±0.82	3.189	0.10
	20	0.43±0.56	-1.88±1.29	1.636	0.147
Partial pressure of oxygen (PO_2) (mm Hg)	0	61.43±5.91	59.51±5.61	0.235	0.819
	20	78.38±7.09	91.80±8.21	1.236	0.245
Lactate values (mmol/L)	0	3.42±0.78	4.91±0.61	1.491	0.167
	20	3.13±0.87	3.77±0.82	0.533	0.606

t-value# for comparing before and after treatment
* Significant at 0.05 level; SE – Standard error

maintenance of anaesthesia, as shown in Table 3. The median values of quality of recovery from anaesthesia were 2.0 in Group I and 3.5 in Group II. Mean±SE values of time taken for recovery from anaesthesia were 61.50±6.80 and 38.33±5.94 minutes in Group I and Group II, respectively.

3.4 Blood Gas Analysis Parameters

The results of blood gas analysis at the 0-minute mark and 20th minute are given in Table 4. Mean±SE values of pH at the 0-minute mark were found to be 7.41±0.02 and 7.33±0.01 in Group I and Group II, respectively. A significant difference was observed at the 0-minute mark between Group I and Group II. Mean±SE values of BE levels were -3.90±0.82 and -1.88±1.29 mmol/L at the 0-minute mark and 20th minute, respectively, in Group II. A significant difference was also noticed in the BE levels of Group II between the 0-minute mark and 20th minute.

Mean±SE values of potassium concentration were found to be 3.21±0.27 and 3.45±0.23 mmol/L at the 0-minute mark and 20th minute, respectively, in Group II. A significant difference was noticed in the Group II between the 0-minute mark and 20th minute.

3.5 Plasma Glucose, Serum Cortisol, Creatine Kinase, Aspartate Aminotransferase, and Alanine Aminotransferase Estimation

Mean±SE values of glucose levels were found to be 98.16±13.11 and 155.0±8.94 mg/dL at the 0-minute mark and 20th minute, respectively, in Group II. A significant difference was noticed in Group II between the 0-minute mark and 30th minute. Mean±SE values of creatinine kinase levels were found to be 313.31±35.38 and 412.36±30.69 U/L at the 0-minute mark and 30th minute, respectively, in Group II. A significant difference was noticed in creatinine kinase levels of Group II between the 0-minute mark and 30th minute. Mean±SE values of cortisol levels were found to be 20.49±3.70 and 40.09±5.96 mg/dL at the

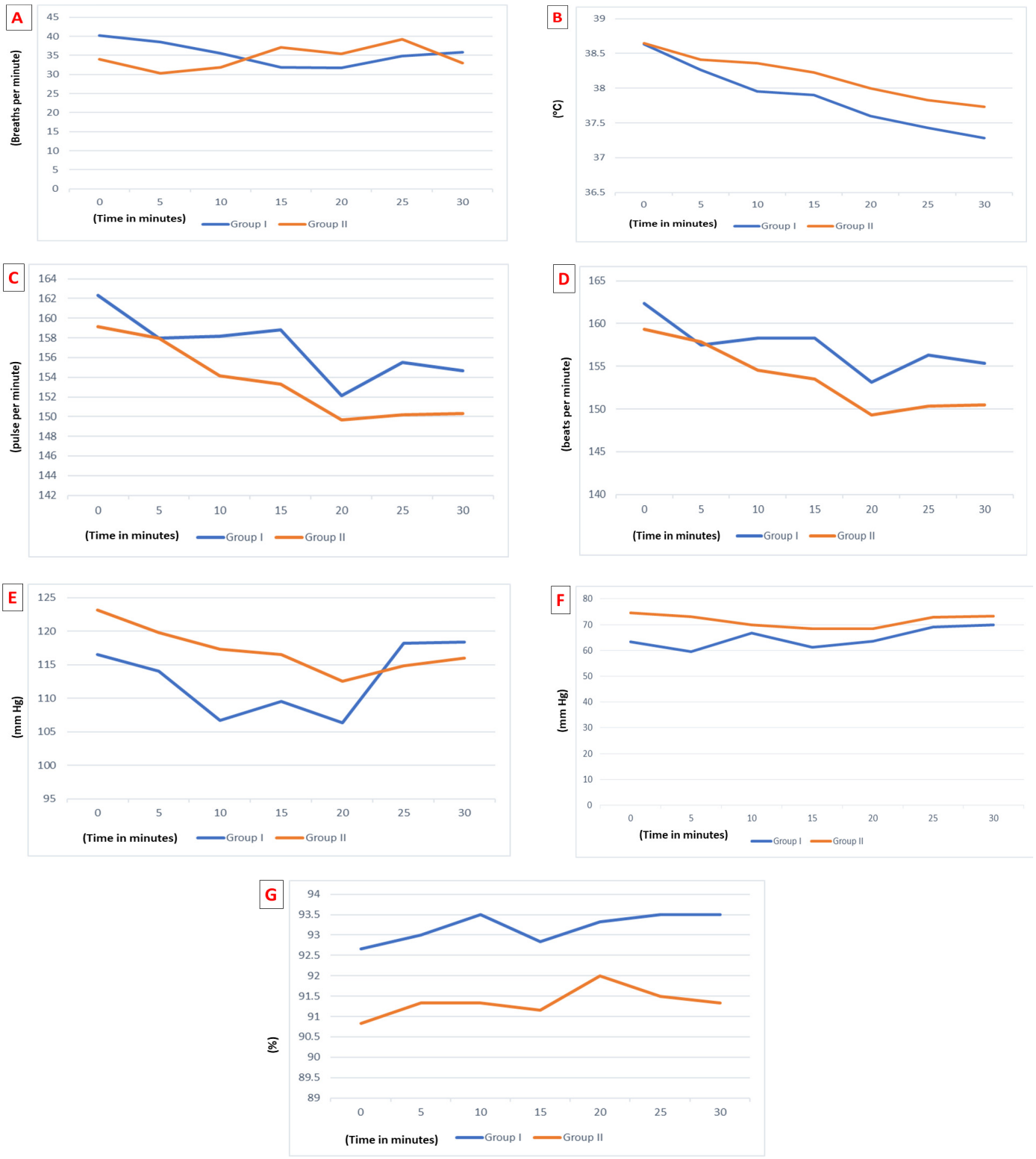

Figure 1. Comparison of physiological parameters recorded throughout the period of anaesthesia between groups and time periods. (A) Respiratory rate (breaths per minute), (B) rectal temperature (ºC), (C) pulse rate (pulse per minute), (D) heart rate (beats per minute), (E) systolic blood pressure (mm Hg), (F) diastolic blood pressure (mm Hg), (G) haemoglobin oxygen saturation (SpO_2) (%).

0-minute mark in Group I and Group II, respectively. A significant difference was also noticed during induction between Group I and Group II.

Values of aspartate aminotransferase and alanine aminotransferase were found to be not much altered in both groups. The values of all the parameters are given in Table 5.

3.6 Physiological Parameters

Physiological parameters were recorded throughout the period of anaesthesia at 5-minute intervals. Physiological parameters at the 0-minute mark and 20th minute are given in Table 5. Physiological parameters like respiratory rate (Graph A), rectal temperature

Table 5. Physiological parameters at the 0-minute mark and 20th minute

Physiological parameter	Time (min)	Mean±SE	
		Group I	Group II
Respiratory rate (breaths per minute)	0	40.16±3.60	34.00±2.78
	20	31.66±4.70	35.33±4.12
Rectal temperature (ºC)	0	38.63±0.33	38.65±0.24
	20	37.60±0.31	38.00±0.15
Pulse rate (pulse per minute)	0	162.33±6.92	159.16±4.34
	20	152.16±9.25	149.66±5.21
Heart rate (beats per minute)	0	162.33±6.92	159.33±4.30
	20	153.16±9.35	149.33±4.82
Non-invasive blood pressure (systolic/diastolic in mmHg)	0	116.50±11.25/ 63.33±9.63	106.33±8.51/ 63.50±8.50
	20	123.16±10.87/ 74.50±8.67	112.50±10.22/ 68.33±8.98
Haemoglobin oxygen saturation (%)	0	92.66±0.71	90.83±1.77
	20	93.33±0.66	92.00±1.15

SE – Standard error

(Graph B), pulse rate (Graph C), heart rate (Graph D), blood pressure (systolic - Graph E) (diastolic – Graph F), and haemoglobin oxygen saturation (Graph G) at 0-minute mark, 5th, 10th, 15th, 20th, 25th, and 30th minutes after the induction of anaesthesia are given as graphical representation (Figure 1).

The respiratory rate decreased gradually until the 20th and 10th minute after induction of anaesthesia in Group I and Group II, respectively. Later, it was stable until the 30th minute in both groups. No statistically significant difference was seen in this parameter between groups.

The rectal temperature was found to be within the normal range until the 30th minute after the induction of anaesthesia. The rectal temperature was 38.63±0.33 and 37.28±0.32 ºC at the 0-minute mark and 30th minute, respectively, in Group I and 38.65±0.24 and 37.73±0.13 ºC, respectively, in Group II. The rectal temperature was found to be gradually decreasing from 0 to 30 minutes after the induction of anaesthesia in both groups. There was no statistically significant difference in pulse rates between groups.

The pulse rate was found to be stable throughout the period of anaesthesia in both groups. The maximum and minimum pulse rate was noticed at the 0-minute mark and 20th minute in both groups. The pulse rate was found to be 162.33±6.92 and 152.16±9.25 per minute at the 0 and 20th minute, respectively, in Group I and 159.16±4.34 and 149.66±5.21 per minute, respectively, in Group II. A slight reduction was noticed gradually from the 0-minute mark to the 20th minute. There was no statistically significant difference in pulse rates between groups.

The heart rate was found to be stable throughout the period of anaesthesia in both groups. The minimum and maximum heart rates were noticed at the 0-minute mark and 20th minute, respectively, in both groups. The heart rate was found to be 162.33±6.92 and 153.16±9.35 beats per minute at the 0-minute mark and 20th minute, respectively, in Group I and 159.33±4.30 and 149.33±4.82 beats per minute, respectively, in Group II. A slight reduction was noticed gradually from the 0-minute mark to the 20th minute. Later, it was found to be approaching normal in both groups.

The blood pressure was found to be gradually decreasing until the 20th minute in both groups. Mean±SE values of blood pressure (systolic/diastolic) were found to be 116.50±11.25/63.33±9.639 and 106.33±8.51/63.50±8.50 mmHg at 0-minute mark and 20th minute, respectively, in Group I and 123.16±10.87/74.50±8.67 and 112.50±10.22/68.33±8.98 mmHg at 0-minute mark and 20th minute, respectively in Group II. Blood pressure was found to be approaching normal by the 30th minute in both groups. There was no statistically significant difference in blood pressure between groups.

Haemoglobin oxygen saturation was found to be stable throughout the period of anaesthesia in both groups. The minimum and maximum values of 92.66±0.71 and 93.50±0.71 per cent in Group I were observed at the 0-minute mark and 25th minute, respectively. The minimum and maximum values of 90.83±1.77 and 92.00±1.15 per cent in Group II were observed at the 0-minute mark and 20th minute, respectively. There was no statistically significant difference in haemoglobin oxygen saturation between and within groups.

3.7 Electrocardiography (ECG)

All the animals of Group I had normal ECG while one animal in Group II was found to have S-T segment-depression at 25th minute after induction of anaesthesia. No arrhythmia was noticed in animals of both groups during the study.

4. Discussion

4.1 Sedative Efficacy and Handling Ease

The administration of pre-anaesthetic and anaesthetic drugs in non-human primates serves a

dual purpose: reducing stress for the animals and enhancing safety for the handlers (Zausig 2009). Drugs inducing tranquillisation, sedation, analgesia, and muscle relaxation, or causing unconsciousness and hypnosis, are commonly used. Dissociative anaesthetics, often employed alone or in combination, achieve desired anaesthetic effects and extend the duration of anaesthesia (Kanaya 2003; Tranquilli and Grimm 2015). Undesirable effects like ataxia and injury during recovery from anaesthesia are managed using specific reversing agents in protocols consisting of opioids, α-2 agonists, and benzodiazepine (Williams *et al*. 2003). Common reversing agents include atipamezole for α-2 agonists, flumazenil for benzodiazepines, and naloxone for opioids (Fagundes *et al.* 2020). The dosages of drugs used in this study were consistent with those reported in existing literature (Authier *et al.* 2006; Longley 2008; Lemoy *et al.* 2012; Sun *et al.* 2013; Raposo *et al.* 2015).

Administering long-acting tranquillisers such as benzodiazepines or alpha-2 adrenergic agonists orally has been shown to facilitate the handling of non-human primates by inducing calmness and reducing stress, thereby improving cooperative behaviour during research activities (Wenger *et al.* 2013). This method improves the primates' well-being and streamlines procedures like physical examinations. The efficacy of long-acting neuroleptics in reducing stress among wild animals has also been documented (Fick *et al.* 2007). Sedation quality scores averaged 3.17±0.32 in Group I and 2.83±0.16 in Group II, reflecting moderate sedation and calmness induced by chlordiazepoxide and haloperidol. Haloperidol is reportedly more effective at inducing sedation than chlordiazepoxide, likely due to its stronger sedative properties (Weiss *et al.* 1977). Though all subjects in the study consumed the complete dose of fruit juice, the consumption time varied, with Group I taking longer due to the bitterness and higher dosage of chlordiazepoxide. Chlordiazepoxide has been recognised for its ability to reduce aggression and anxiety in monkeys (Crowel-Davis and Murray 2006; Reiser *et al.* 1962), although it has been associated with cerebellar ataxia (Gaalen *et al.* 2014). Meanwhile, haloperidol's sedative effects were observable within six hours in Spotted Deer (Johns 2014), with similar sedative signs in both groups of primates.

4.2 Impact on Physiological Parameters

Premedication with chlordiazepoxide or haloperidol led to noticeable calmness and decreased activity in the primates, with effects becoming evident approximately four hours after administration. These effects align with findings from previous studies that have highlighted the tranquillising properties of these drugs (Heuschele 1962; Weiss *et al.* 1977; Crowel-Davis and Murray 2006; Redrobe *et al.* 2008). Anaesthesia administered four hours post-premedication reached peak effectiveness, thus facilitating the handling as per the recommendations by Pulley *et al.* (2004). Both chlordiazepoxide and haloperidol are categorised as long-acting tranquillisers (Rang *et al.* 2005; Hofmeyr 1981), which was evident in the prolonged action observed in bonnet macaques. In contrast, non-premedicated howler monkeys, which were physically restrained before midazolam-(S+)-ketamine anaesthesia, exhibited stress behaviours such as defecation and urination (Chagas *et al*. 2010). In this study, no such side effects were noted, likely due to the sedative effects of premedication that reduced apprehension and stress during anaesthesia induction.

The intramuscular administration of midazolam and ketamine (Bertrand *et al.* 2016) resulted in a median induction quality score of 4.0 for both groups, indicating smooth and rapid induction without signs of excitement. Previous research has found that intravenous midazolam can enhance induction quality during ketamine anaesthesia in young vervet and rhesus monkeys (Jacobs *et al.* 1993). The combination of midazolam with ketamine alleviates the latter's side effects (Chagas *et al.* 2010; Fagundes *et al.* 2020; Gomes *et al.* 2021). The induction times in Groups I and II averaged 246.33±41.04 seconds and 291.50±58.12 seconds, respectively. These times are shorter than those reported for tiletamine-zolazepam anaesthesia in bonnet macaques (Bush *et al.* 1977) and butorphanol-midazolam-ketamine anaesthesia in howler monkeys (Fagundes *et al.* 2020). In a study by Furtado *et al.* (2010), induction times for marmosets using racemic ketamine and midazolam were recorded at 1.6±1.0 and 2.9±2.1 minutes. The shorter induction times observed in the present study could be attributed to the synergistic effects of the premedication drugs, which enhance the sedative and anaesthetic properties of the administered agents (Sobti *et al.* 1990).

4.3 Anaesthesia Quality and Recovery

The mean induction dose in this study was effective in maintaining dissociative anaesthesia, consistent with findings in other studies on non-human primates (Raposo *et al.* 2015; Fagundes *et al.* 2020). The anaesthetic protocols used were appropriate for minor procedures, and the observed anaesthetic quality and maintenance suggest a synergistic effect between the premedication and anaesthetic agents. The duration of anaesthesia was found to be 44.33±6.04 minutes for Group I and 39.50±4.95 minutes for Group II. This difference in duration could be due to the synergistic effects of midazolam and chlordiazepoxide metabolites, as noted in previous studies (Bourin and Renard 2001). Comparatively, midazolam at 0.5 mg/kg with dextroketamine at 10 mg/kg provided 44 minutes of restraint in capuchin monkeys, while ketamine at 8.0 mg/kg with midazolam at 0.2 mg/kg resulted in 41 minutes of anaesthesia in *Macaca mulatta* (Fowler *et al.* 2001). The shorter durations observed in this study might be attributed to lower drug doses. The median depth of anaesthesia was 3.5, indicating light to surgical anaesthesia in both groups. Studies in howler monkeys have reported similar excellent sedation scores during midazolam and (S+)-ketamine anaesthesia (Chagas *et al.* 2010).

Recovery times differed between the two groups,

with Group I averaging 61.50±6.80 minutes and Group II averaging 38.33±5.94 minutes. The longer recovery in Group I could be due to the prolonged action of chlordiazepoxide in the cerebellum (Adams 2001; Gaalen *et al.* 2014). Previous studies have documented recovery periods ranging from 44.17±0.51 minutes with dysphoria following ketamine anaesthesia in bonnet macaques (Allwin *et al.* 2016) to 63.3 minutes in howler monkeys (Fagundes *et al.* 2020). Midazolam is known to reduce recovery times (Langrehr *et al.* 1981), and the recovery times observed in Group II align with findings by Authier *et al.* (2006), indicating that haloperidol does not prolong recovery when combined with midazolam and ketamine. Flumazenil, a benzodiazepine antagonist, could potentially reduce recovery times even further (Fagundes *et al.* 2020).

4.4 Stress Reduction

Analgesia was assessed using a scoring method by Bakker *et al.* (2013) for common marmosets, with scores of 3.0 in Group I and 4.0 in Group II. Chlordiazepoxide's weak analgesic effects in monkeys (Crowel-Davis and Murray 2006) contrast with the effective analgesic properties of haloperidol for postoperative pain in humans (Daw *et al.* 1981; Judkins *et al.* 1982). The current study indicates that haloperidol offers superior analgesia compared to chlordiazepoxide. Muscle relaxation, as well as pedal and palpebral reflexes, were enhanced in Group I due to the combined effect of midazolam and chlordiazepoxide. Excellent muscle relaxation has been reported during midazolam-ketamine anaesthesia in non-human primates (Chagas *et al.* 2010; Fagundes *et al.* 2020; Furtado *et al.* 2010; Raposo *et al.* 2015). The absence of pedal reflex and the presence of palpebral reflex throughout the anaesthesia in both groups align with the observations by Bush *et al.* (1977) during ketamine or tiletamine-zolazepam anaesthesia.

Venous blood pH at 0-minute mark was 7.41±0.02 in Group I and 7.33±0.01 in Group II, indicating acidemia in Group II. Chagas *et al.* (2010) reported similar results during midazolam and (S+) ketamine anaesthesia in howler monkeys. Respiratory depression and slightly elevated venous pCO_2 in Group II may result from the combined effect of haloperidol and midazolam. The decrease in venous bicarbonate and increase in base excess from 10 to 20 minutes are indicative of compensated respiratory alkalosis in Group II. These findings suggest that the combination of haloperidol and midazolam may lead to hyperventilation, decreased bicarbonate levels, and respiratory acidosis in *M. radiata*. Blood pH in Group I decreased gradually, with pCO_2 levels comparable to those reported by Fowler *et al.* (2001). Respiratory acidosis was observed at 20 minutes, leading to hypoxia and hyperlactatemia in Group I. The higher lactate concentration in Group II suggests lactic acidosis, potentially due to rapid metabolism and compensatory hypoxia, a finding supported by studies in marmosets and rhesus macaques (Fowler *et al.* 2001; Furtado *et al.* 2010).

4.5 Blood Gas Analysis

The combination of chlordiazepoxide and haloperidol as premedication significantly influenced blood gas parameters, affecting the primates' physiological stability during anaesthesia. In Group I, premedication with chlordiazepoxide resulted in mild respiratory acidosis at the 20-minute mark, evidenced by decreased blood pH levels and elevated pCO_2 values. These findings are consistent with earlier studies that have reported similar respiratory changes in non-human primates under anaesthesia (Sobti *et al.* 1990). On the other hand, Group II, premedicated with haloperidol, exhibited a different blood gas profile. This group experienced a slight acidemia and hypoxia, accompanied by respiratory depression. The blood pH levels were lower in Group II, indicating a more pronounced acid-base imbalance compared to Group I. The hypoxic state in Group II, as indicated by decreased oxygen saturation levels, might be due to the combined sedative effects of haloperidol and midazolam, leading to reduced respiratory drive and oxygen uptake (Abbott *et al.* 2003). The lactate levels, which serve as an indicator of metabolic stress and anaerobic metabolism, were also different between the two groups. Group I showed lower serum lactate levels compared to Group II, suggesting that chlordiazepoxide premedication resulted in better physiological stability and lower metabolic stress. This aligns with findings from previous research, where lower lactate levels were associated with reduced stress and better physiological outcomes during anaesthesia (Adameova *et al.* 2009).

This study underscores the importance of selecting appropriate premedication to enhance sedation, maintain stable physiological parameters, and improve the overall quality of anaesthesia in non-human primates. The oral administration of chlordiazepoxide and haloperidol as premedication prior to midazolam-ketamine anaesthesia in bonnet macaques has proven to be an effective method for reducing stress and enhancing anaesthesia outcomes. While both chlordiazepoxide and haloperidol were effective in achieving the desired sedative effects, each drug exhibited unique benefits and limitations. Chlordiazepoxide was associated with better physiological stability and stress reduction, making it a preferable choice for procedures requiring longer recovery periods. Conversely, haloperidol provided superior analgesia and facilitated a quicker recovery, which could be advantageous for shorter procedures or when rapid post-anaesthesia recovery is desired. These findings highlight the potential of using tailored anaesthesia protocols to optimise outcomes in veterinary and research settings involving non-human primates. Further research is warranted to explore the long-term effects of these agents on animal well-being and their potential applications in other primate species. The study involved only six animals per premedicant, suggesting future studies with a control group and a large number of animals for better efficacy assessment.

Acknowledgements

The authors are thankful to the Kerala Veterinary and Animal Sciences University, Authorities of the State Museum and Zoo, Thrissur, Kerala, and Kerala Forest and Wildlife Department for providing the facility to perform the present study.

Conflict of interest: None of the authors has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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