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RESEARCH ARTICLE

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Effect of Oral Preanesthesic Sedation with Chlordiazepoxide and Haloperidol Before Anesthesia For Adult Male Bonnet Macaques (Macaca radiata)

Kuskur Sannappa Naik Kamalesh Kumar [a], George Chandy [b], Serendran Sooryadas [b], Parathazathayi Dinesh [b]

[a] Department of Animal Husbandry and Veterinary Services, Karnataka, India.
[b] Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, Pookode, Wayanad-673 576, Kerala, India.

ABSTRACT

It is important to capture wild animals with minimal stress to reduce morbidity and mortality. Oral premedicants have the potential to reduce stress during handling and ease the subsequent administration of anaesthetic agents. We evaluated the efficacy of premedication with chlordiazepoxide or haloperidol independently prior to midazolam-ketamine anaesthesia in 12 male Bonnet Macaques. Animals were randomly grouped into two groups of six (n=6). Animals of Group I were administered chlordiazepoxide (10 mg/kg) and animals of Group II were administered haloperidol (1 mg/kg) orally. The temperament of each animal was recorded prior to premedication. Behavioral responses after pre-medication were assessed for 4 h. Glucose and cortisol levels were measured from the venous blood sample collected after anaesthesia induction. Sedation was obtained in both groups, whereas the quality of sedation was comparatively better in Group II. Analgesia was better in Group I compared to Group II. Haloperidol-premedicated animals were easy to handle, but increased cortisol and glucose levels were recorded. According to our findings, pre-medication with chlordiazepoxide and haloperidol produced optimum sedation to handle the Bonnet Macaques for inducing anaesthesia.

Keywords

Preanesthetic Sedative, Chlordiazepoxide, Haloperidol, Non human primate, Stress reduction

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Abbreviations

NHP: Non human primate
VTA: Ventral tegmental area
PFC: Cerebral Blood Flow
NAc: Nucleus accumbens
ECLIA: Electrochemiluminescence Immunoassay
CZA: Central Zoo Authority
HPA: Hypothalamic-pituitary-Adrenal axis
CRH: Corticotropin-releasing hormone
ACTH: Adrenocorticotropic hormone

Corresponding author: *K.S Kamalesh Kumar*
Email: *kamalesh.kumar@ka.gov.in*
Tel: *+91(91)6428-2969*

Introduction

Uncontrolled stress and death due to capture myopathy have been recognized as the most serious concerns during the handling and restraint of wild animals [1]. It is important to capture wild animals with minimal stress to reduce morbidity and mortality. Improvement of the capture technique using appropriate medications to minimize stress is a priority from the welfare point of view in wildlife conservation, zoos, and laboratory-housed NHPs/macaques.

NHPs are routinely used for research in laboratories all over the world [2]. These animals are anaesthetized for several scientific studies in laboratories as well as for routine procedures, such as surgical sterilization and microchipping, in captivity. Capturing and handling NHPs requires experience, thorough knowledge of animal behavior, and technical skills to ensure the safety of animals and handlers. NHPs in captivity may also be darted in their enclosures or hand-injected after physical restraint. Free-ranging macaques are trapped in large cages and anaesthetized by darting or injecting the anaesthetic agents after transfer to squeeze cages or smaller cages. NHPs are not premedicated prior to darting in these conditions. However, darting small-sized unpremedicated monkeys can be dangerous for the animal and is a difficult task because of their frantic fast movements. In addition, NHPs are difficult to handle for drug injection by hand because of their speed, dexterity, intelligence, and 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. Premedicating these animals with oral tranquilizers before handling or anaesthesia improves the ease of handling and drug administration as well as reduces their stress response [3].

Tranquilizers, sedatives, and anaesthetic medicines have been established as crucial agents for reducing stress and related issues during wild animal restraint. Chlordiazepoxide [4] and haloperidol [5] have been defined as long-acting tranquilizers. Long-acting neuroleptics have been reported successful in reducing anxiety and producing sedation during treatment and translocation in wild animals [6-9]. Chlordiazepoxide has been observed to produce mild sedation, diminish spontaneous mobility, walking, grooming, and increase the lying period in Rhesus macaques [10]. Haloperidol also has been proven as a long-acting neuroleptic agent in NHPs, dogs, and other wild animals [5, 11, 12].

Both medications can be administered orally as premedicants and have the potential to reduce stress during handling and ease the subsequent administration of anaesthetic agents. The advantage of these long-acting premedicants is that their effect would last for sufficient time to allow gastric emptying before administrating anaesthetic drugs despite being administered orally. Anaesthetic agents may be administered parenterally following the onset of the premedical action and after providing an appropriate period for gastric emptying. Several anaesthetic combinations, such as midazolam and ketamine, have already been proven to produce satisfactory anaesthesia with minimal cardio-respiratory changes in animals and are routinely used for anaesthetising macaques. Therefore, a study was conducted in adult, male, captive Bonnet Macaques undergoing vasectomy at the State Museum and Zoo, Thrissur, Kerala to compare the efficacy of oral premedication with chlordiazepoxide or haloperidol before midazolam-ketamine anaesthesia.

Result

The temperament of each animal is presented in Table 1. Three animals in each group were stoic, two animals in each group were apprehensive, one animal in Group I was calm and relaxed, and one animal in Group II was aggressive. Mean ± SE of estimated body weight and actual measured body weight are presented in Table 2. There was no significant difference between estimated and actual measured body weights in both groups.

Ease of the Acceptance of Premedicants and Duration

The mean ± SE of the corrected oral dose of chlordiazepoxide against the actual measured body weight in the animals of Group I was 10.33 ± 0.20 mg/kg body weight. The mean ± SE of the corrected oral dose of haloperidol against the actual measured body weight in the animals of Group II was 1.12 ± 0.07 mg/kg body weight. The time taken to complete the premedication was 26.16 ± 8.96 and 12.66 ± 7.53 min in Group I and Group II, respectively. Ease of acceptance of fruit juice laced with premedicant, ease of netting or handling, and response to handling or injection were recorded and shown in Table 3. There was no significant difference between Group I and Group II in these parameters.

Behavioural observations

Behavioral responses were observed before premedication and for 4 h at intervals of 30 min after premedication. Various behavioural responses, including anxiety, aggression, and hyperactivity (Fig. 1 and Graph 1); active and playful (Fig. 2 and Graph 2); relaxed, calm, and reduced activity (Fig. 3 and Graph

Table 1.
Temperament of the animal

Parameter	Group I	Group II
Stoic	3 (50 %)	3 (50 %)
Aggressive	-	1 (16.66 %)
Apprehensive	2 (33.33 %)	2 (33.33 %)
Nervous	-	-
Calm and relaxed	1 (16.66 %)	-

Table 2.
Corrected body weight (kg)

Parameter	Mean ± SE		t-value	p-value
	Group I	Group II		
Estimated body weight	7.68 ± 1.07	6.16 ± 0.33	1.345 ns	0.208
Actual measured body weight	7.44 ± 0.96	5.67 ± 0.65	1.515 ns	0.161

Table 3.
Scores for ease of accepting premedicant, netting and resoponse to handling

Parameter	Median		Z-value	p-value
	Group I	Group II		
Ease of acceptance of fruit juice laced with premedicant	1.5	2.0	0.433 ns	0.665
Ease of netting or handling	2.0	2.0	0 ns	1
Response to handling or injection	2.0	2.0	0 ns	1

Ease of Handling after Premedication

The ease of handling and response to handling were recorded during netting and hand injecting of the drug. The median value for the ease of netting or injecting and response to handling or injection was 2 in both groups (Table 3). There was no significant difference in this parameter between the groups. Five (83.3%) of the premedicated animals were observed to be calm with reduced activity 4 h after premedication in both groups. One animal in each group remained active throughout 4 h.

Cortisol and Glucose Levels

Mean ± SE values of cortisol levels were 20.49 ± 3.70 and 40.09 ± 5.96 mg/dl after 4 h of oral premedication in Group I and Group II, respectively. Groups I and II were significantly different in terms of cortisol levels during induction. Mean ± SE of glucose levels was found to be 83.83 ± 7.66 and 98.16 ± 13.11 mg/dL after 4 h of oral premedication in Group I and Group II, respectively. No post-operative complication was observed in either group. All the animals were easily accepted back into their groups without any infighting.

3); stuperous look (Fig. 4 and Graph 4); half-open mouth (Fig. 5 and Graph 5); sitting with relaxed hind limbs (Fig. 6 and Graph 6); self-grooming (Graph 7); interaction with adjacent caged animal (Fig. 7 and Graph 8); ataxia (Fig. 8 and Graph 9); yawning (Fig. 9 and Graph 10); recumbency (Fig. 10 and Graph 11); leaning against wall or grill (Fig. 11 and Graph 12); drowsy (nodding/head down) (Graph 13); and sleeping in sitting posture (Fig. 12 and Graph 14) were recorded. All the observations are presented in Table 4. Graphical observation represents the changes of half an hour in each behavioral response.

Quality of Sedation after Oral Premedication

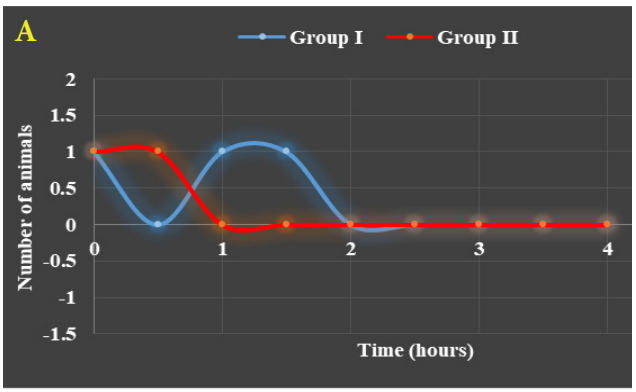
The median value for the quality of sedation after oral premedication was observed to be 1.5 in Group I and 2.5 in Group II. There was no significant difference between Group I and Group II in this parameter.

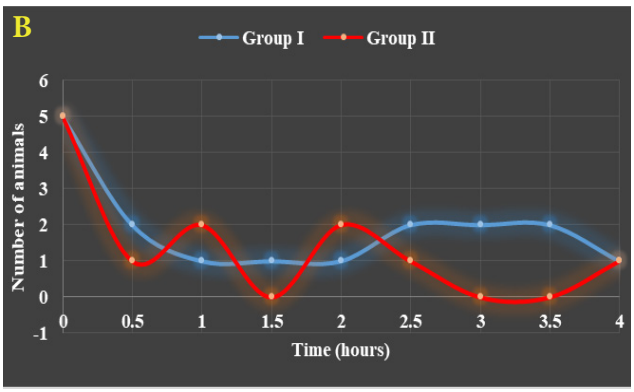
Discussion

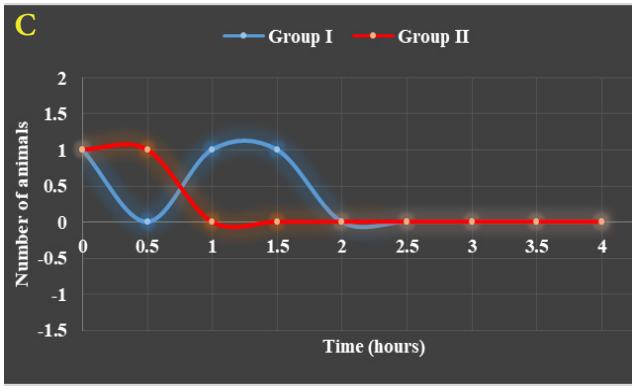
The current study aimed to evaluate the sedative effect of two premedicants separately and to assess their practical applications and feasibility for inducing general anesthesia with minimal stress to Bonnet Macacques.

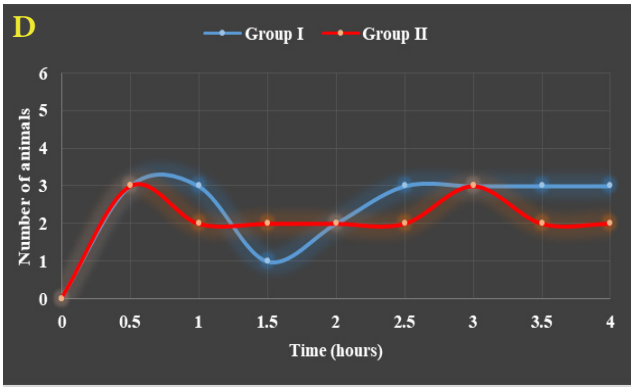
Prior observation of temperament helped assess the change in the behavior of the animals after oral premedication and during post-operative stress evaluation as opined by Fowler (2008) and Murphy (2008) [13, 14]. The corrected doses of drugs used in the present study were observed to be similar to the required dose as reported in many studies [15-19]. All the animals were conditioned to take pineapple juice for 15 days prior to the procedure as opined by CWINP-ILAR

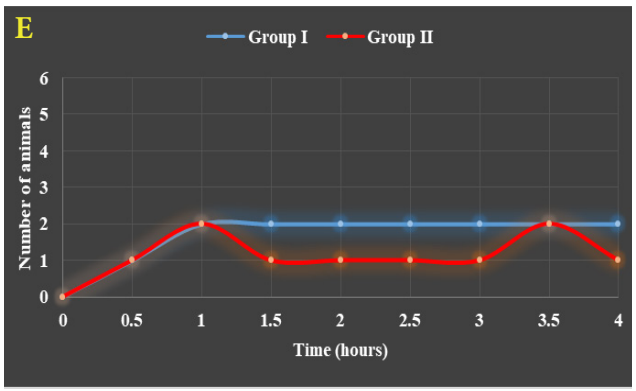
Figure 1.
A-L. Behavioral responses observed after premedication: (A) Anxiety, aggression, and hyperactivity; (B) Active and playful; (C) Relaxed, calm, and reduced activity; (D) Stuperous look; (E) Half-open mouth; (F) Sitting with relaxed limbs; (G) Social behavior; (H) Ataxia; (I) Yawning; (J) Recumbency; (K) Leaning against wall/grill; (L) Sleeping.

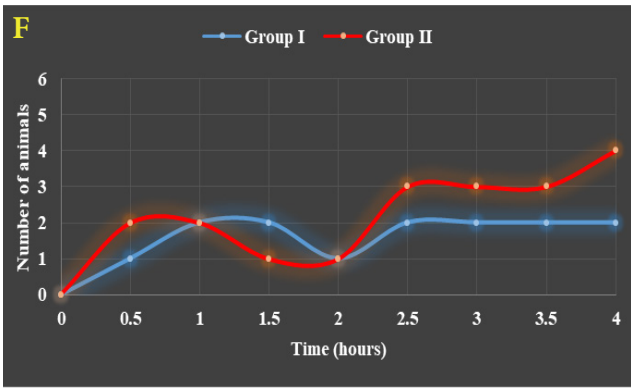

A
Group I
Group II
Number of animals
Time (hours)

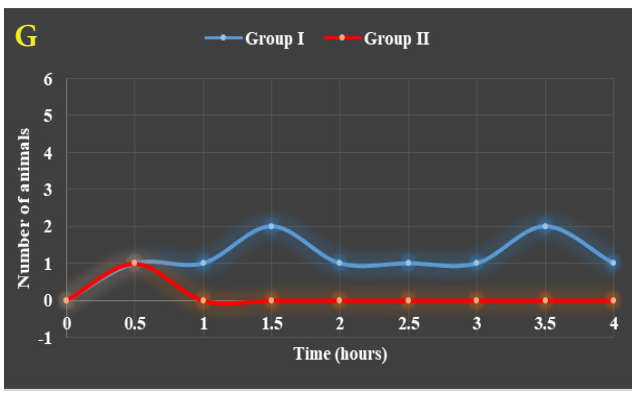

B
Group I
Group II
Number of animals
Time (hours)

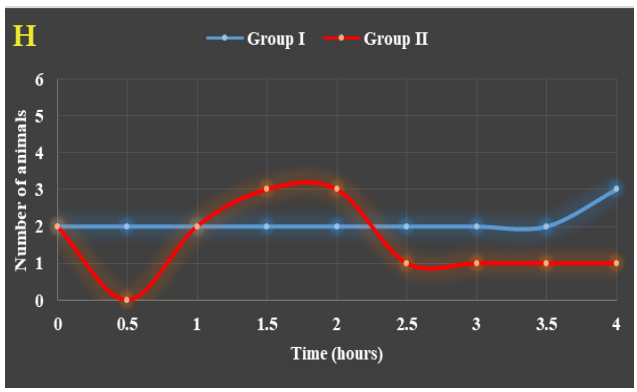

C
Group I
Group II
Number of animals
Time (hours)

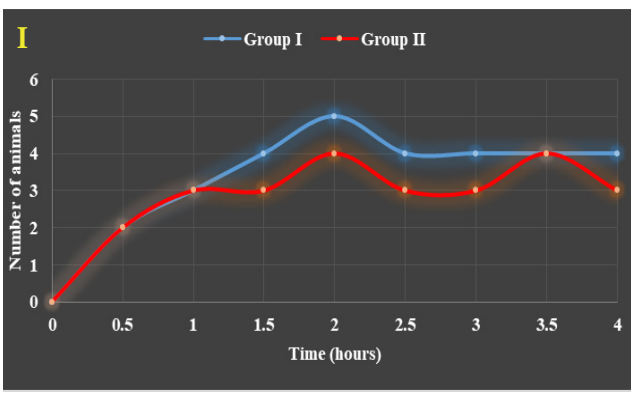

D
Group I
Group II
Number of animals
Time (hours)


E
Group I
Group II
Number of animals
Time (hours)


F
Group I
Group II
Number of animals
Time (hours)


G
Group I
Group II
Number of animals
Time (hours)


H
Group I
Group II
Number of animals
Time (hours)


I
Group I
Group II
Number of animals
Time (hours)

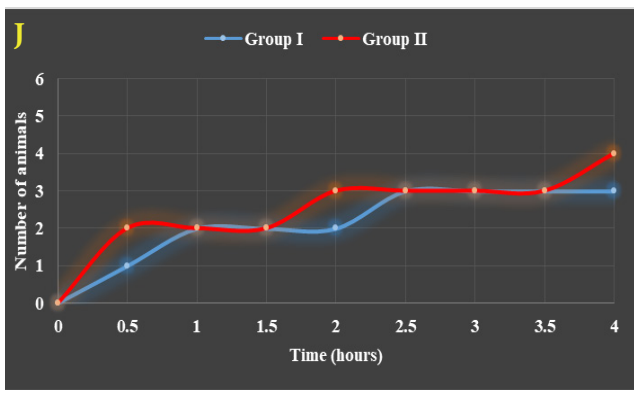

J
Group I
Group II
Number of animals
Time (hours)

Figure 2. Cont.

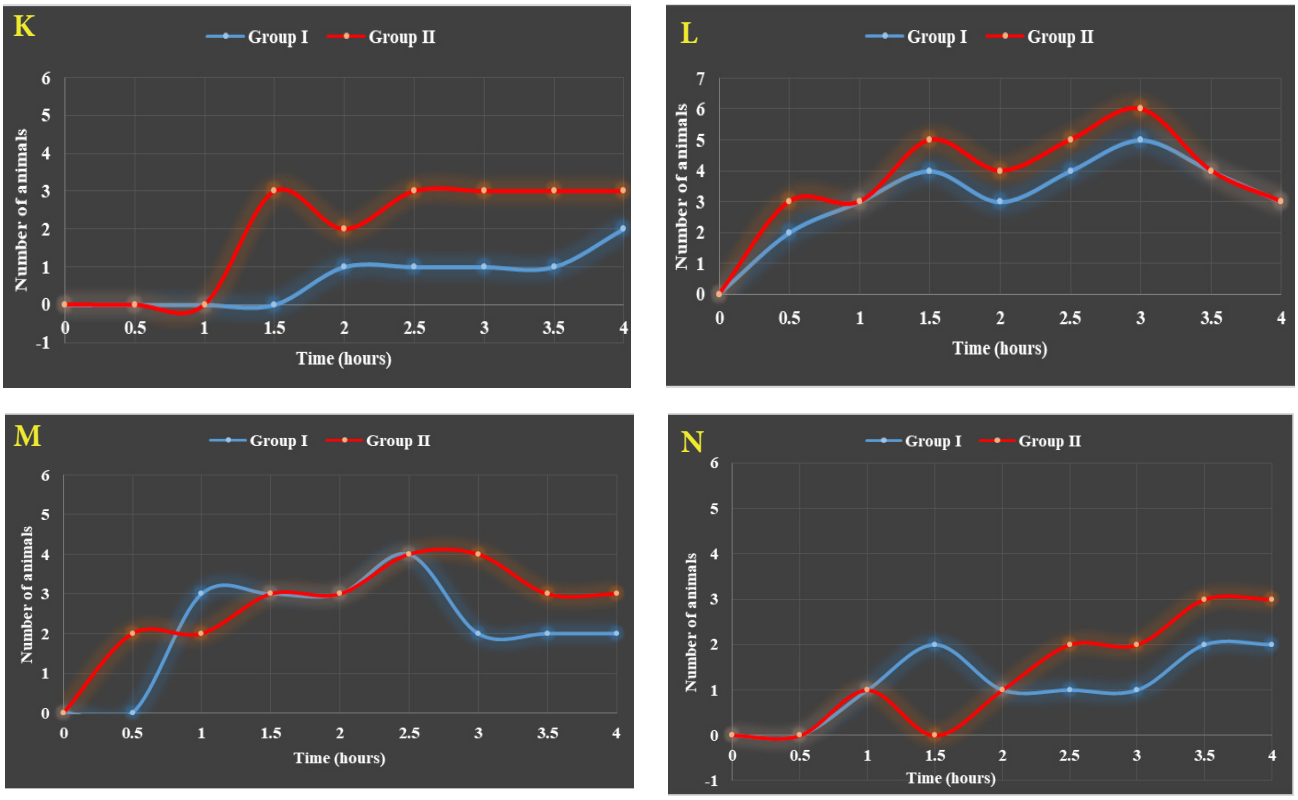


Figure 2.
Graph A-N. Graphical representation of behavioral responses observed after premedication: (A) Anxiety, aggression, and hyperactivity; (B) Active and playful; (C) Relaxed, calm, and reduced activity; (D) Stuperous look; (E) Half-open mouth; (F) Sitting with relaxed limbs; (G) Self-grooming; (H) Social behavior; (I) Ataxia; (J) Yawning; (K) Recumbency; (L) Leaning against wall/grill; (M) Drowsy; (N) Sleeping.

(1998) and Fowler (2008) [13, 20]. The slightly increased time taken to complete premedication and the lesser median value for the ease of administering fruit pulp in Group I could be due to the bitter taste and the larger dose of chlordiazepoxide.

Behavioural Observations

The effectiveness of the use of long-acting neuroleptics in wild animals to reduce stress was described by Fick et al. (2007) [21]. Oral administration of long-acting tranquilizers has been observed to facilitate the handling of NHP [22]. The administered dose of premedication was sufficient to produce the required effect as in earlier studies [10, 12, 23]. The scorecard used by Pulley et al. (2004) [3] in a study involving Rhesus Macaques was effective in monitoring the quality of sedation after oral premedication, which is similar to the present study. The median values for the quality of sedation were observed to be 1.5 (mild-moderate) in Group I and 2.5 (moderate-good) in Group II indicating increased sedation in Group II, but without any significant difference. Haloperidol was more effective in producing sedation than chlordiazepoxide. Increased sedative behavioral responses or tranquillization seen in haloperidol-premedicated animals may be due to its higher sedative effect [12]. The effects of haloperidol are not limited to dopamine D-2 receptor blockade. It also affects other neurotransmitter systems, including serotonin and norepinephrine [24, 25]. Disruptions in these systems have contributed to producing sedation by altering the balance of neurotransmitters causing CNS depression, reduced activity levels, decreased responsiveness to stimuli, and overall sedation.

One animal which had shown calm and relaxed behavior before premedication in Group I had a score of 3 indicating higher sedation. One animal which had shown aggressive behavior before premedication in Group II showed incoordination

Table 4.
Behavioural observations (per cent)

Observations	Prior to premedication		Four hours after premedication	
	Group I	Group II	Group I	Group II
Anxiety, aggression & hyperactive (Grinning and clicking)	16.66	16.66	0	0
Active & playful	83.33	83.33	16.66	16.66
Relaxed, calm & reduced activity	0	0	83.33	83.33
Stuperous look	0	0	50	33.33
Half open mouth	0	0	33.33	16.66
Sitting with relaxed hind limbs	0	0	33.33	66.66
Ataxia	0	0	66.66	50
Interaction with adjacent caged animal	33.33	33.33	50	0
Self-grooming	33.33	33.33	50	16.66
Yawning	0	0	50	66.66
Recumbency	0	0	33.33	50
Leaning against wall/grill	0	0	50	50
Drowsy (nodding/head down)	0	0	33.33	50
Sleeping in sitting posture	0	0	33.33	50

and ataxia (score 2). Two animals that were apprehensive in Group I showed minimal sedation (score 1). All the other animals showed mild to moderate sedation (score 2 - 3). Chlordiazepoxide primarily acts as a positive allosteric modulator of GABA-A receptors that contain alpha-1, alpha-2, alpha-3, or alpha-5 subunits. The alpha-1 subtype, in particular, is associated with sedative effects [24, 25]. By binding to these receptor subtypes, chlordiazepoxide promotes the opening of chloride channels, which further enhances the inhibitory effects of GABA. By enhancing GABAergic activity, chlordiazepoxide can lead to CNS depression. Minimal sedation in Group I could be due to the reduced intake of chlordiazepoxide or reduced dose of the drug leading to mild relaxation and anxiolysis.

None of the animals showed anxiety, aggression, or hyperactivity at the end of 4 h after premedication. Relaxation, calmness, and reduced activity were observed in all animals by the end of 4 h. Reduced aggression, anxiety-relieving, tranquilizing, and muscle relaxant properties of chlordiazepoxide have been also reported in monkeys by Reiser et al. (1962) [5]. Antianxiety, sedative, weak analgesic, and appetite-stimulating effects of the drug were reported in monkeys by Crowel-Davis and Murray (2006) [26]. The effectiveness of chlordiazepoxide in reducing aggressiveness and producing calmness has been already reported by Heuschele (1962) [23]. Hence both medications are proven to reduce anxiety, aggression, and hyperactivity in Bonnet Macaques.

A stuperous look and half-open mouth were noticed more in Group I than in Group II. By binding to GABA-A receptors, chlordiazepoxide increases the frequency of chloride ion channel opening when GABA is present. This hyperpolarizes the neurons, making them less likely to fire [24, 25]. In the context of muscle relaxation, this effect helps reduce the transmission of signals from nerves to muscles. The CNS-depressing effects of chlordiazepoxide affect the transmission of signals along the spinal cord and other pathways involved in muscle contractions. The anxiolytic (anti-anxiety) effects of chlordiazepoxide can indirectly contribute to muscle relaxation. Anxiety and stress can lead to muscle tension, so by reducing anxiety, chlordiazepoxide can help alleviate muscle stiffness and tightness. Muscle relaxation and anxiety-relieving properties of chlordiazepoxide could have increased the occurrence of these behavioral responses.

Long therapy using haloperidol influences dopamine receptors in the basal ganglia of NHP, producing extrapyramidal effects, such as muscle stiffness. The anticholinergic activity of haloperidol causes a reduction in the activity of the neurotransmitter acetylcholine, leading to some de-

gree of muscle relaxation due to the dampening of cholinergic transmission, which is involved in muscle contraction.

Ataxia was noticed in four and three animals of Group I and Group II, respectively. Benzodiazepines have been reported to have benzodiazepine type I (BZ I) receptor predominantly in cerebellum, causing cerebellar ataxia [27, 28]. Haloperidol causes ataxia within 6 h of oral premedication in Spotted Deer [4]. Haloperidol is a potent dopamine receptor antagonist, particularly targeting D2-type dopamine receptors [25]. By blocking these receptors, it decreases the effects of dopamine, a neurotransmitter involved in motor control and coordination. Dopamine pathways in the basal ganglia are important for regulating movement. When these pathways are disrupted due to dopamine receptor blockade, as seen with haloperidol use, it can lead to motor-related side effects, such as ataxia. Dopamine pathways also influence cerebellar function. The disruption of these pathways following dopamine receptor blockade by haloperidol can result in impaired communication between the basal ganglia and the cerebellum, contributing to ataxia. The degree of ataxia varied in each macaque. Factors, such as genetic predisposition, dose of haloperidol administered, and the specific neurochemical makeup of an individual's brain, can influence the severity of ataxic effects. Yawning, recumbency, leaning against wall/grill, drowsiness (nodding/head down), and sleeping in sitting posture could be due to the higher level of sedation shown by animals of both groups. One animal of Group I showed self-grooming which was in agreement with the findings of Kumar et al. (1999) in Rhesus Macaques [29].

Behavioral observations, including sleep-like state; calm and quiet state; and mild hypotension, have been recorded within 5 min of intravenous premedication of haloperidol in dogs [11]. The effectiveness of haloperidol along with zuclopenthixol to produce deep sedation during the transportation of an adult male gorilla from Germany to South Africa has been reported by Redrobe et al. (2008) [30]. Haloperidol produces deep sedation lasting about 2-4 h in Cebus Monkeys and Squirrel Monkeys when administered orally [12]. Haloperidol blocks postsynaptic dopamine (D2) receptors in the mesolimbic system of the brain reducing dopaminergic inputs from the ventral tegmental area (VTA) innervate brain regions involved in executive, affective, and motivational functions, including the prefrontal cortex (PFC), amygdala, and nucleus accumbens (NAc). The CNS-depressant effects of haloperidol can result in sedation and drowsiness. This sedative effect might contribute to improved sleep in NHP.

Five animals of each group could be easily handled after premedication and were observed to be calm with reduced activity by 4 h after premedication. The ease of handling and injecting the drug may have been due to the calming effect of premedication with either chlordiazepoxide or haloperidol. Calming and sedative effects of premedicants have been recognized by Heuschele (1962), Weiss et al. (1977), Crowel-Davis and Murray (2006), and Redrobe et al. (2008) [12, 23, 26, 30]. Administration of anaesthetics after 4 h of premedication helped to achieve peak effect and promoted handling as suggested by Pulley et al. (2004) [3]. Chlordiazepoxide (Rang et al., 2005) and haloperidol (Hofmeyr, 1981) have been defined as long-acting tranquilizers [7, 32]. Prolonged action of premedication agents was observed in Bonnet Macaques in the present study. The ease of handling and injecting drugs may have been due to the calming effect of premedication.

One animal in each group that had an aggressive and apprehensive temperament remained active throughout 4 h. Increased activity of these animals could be due to improper intake of bait or lower doses of premedication agents. The authors opine that pineapple juice could not mask the bitterness of chlordiazepoxide. Better replacement of oral bait can be used to mask the bitterness of the oral premedicants. The authors also suggested increasing the distance between cages to reduce the social behavior and activity of macaques.

Cortisol and Glucose Levels

Higher glucose levels observed in this study in Group II than Group I after 4 h of premedication

might be due to the excessive release of cortisol following psychological and physical stress related to handling. An increase in the cortisol levels may have led to gluconeogenesis in both groups. All the previous reports in Bonnet Macaques had reduced glucose levels in non-anaesthetized, ketamine-anaesthetized, and ketamine-xylazine-anaesthetized animals [32-36].

Circulating cortisol levels have already been reported as an important indicator of stress in wild animals [37, 38]. Normal cortisol levels during tiletamine-zolazepam anaesthesia in trained Rhesus Macaques between 0 and 60 min were between 27.9±1.7 and 21.2±2.0 µg/dL [39]. The significant increase in cortisol level in Group II- may have been due to its sensitivity to physical and psychological stress. HPA axis is a complex neuroendocrine system that controls the body's response to stress and regulates cortisol production. The hypothalamus releases CRH, which stimulates the pituitary gland to release ACTH, which in turn, signals the adrenal glands to produce cortisol [40]. Changes in neurotransmitter activity can influence the release of CRH and ACTH, which could subsequently affect cortisol production. By reducing anxiety and arousal, haloperidol might indirectly influence cortisol levels, especially during situations of acute stress.

Increased cortisol levels due to stress related to cage restraint and ketamine anaesthesia have been reported in Rhesus Monkeys [41]. The injection technique and blood sampling process have been observed to raise cortisol levels in untrained monkeys compared to trained ones [39]. Contradictory results maintaining stable endocrine responses have been reported by Fuller et al. (1984) [42] in Cynomolgus Monkeys. Long-term administration of haloperidol has been observed to produce movement disorders and tardive dyskinesia which resulted in delayed and potentially irreversible motor complications with typically stereotyped abnormal movements (peculiar postures, writhing, stretching, and oral movements) without acute dystonic reactions [43]. None of the animals in the present study showed any adverse effect associated with haloperidol, which may be because the drug was administered only once.

Haloperidol was observed to improve the ease of handling animals when evaluated subjectively by the handler. However, evaluation of parameters associated with stress showed that animals premedicated with haloperidol were more prone to stress than those premedicated with chlordiazepoxide. The quality of recovery in animals premedicated with haloperidol was better than that in animals premedicated with chlordiazepoxide, which showed ataxia during recovery. The present study involved a small group of six to evaluate each premedicant drug. A study involving more animals in the future would be beneficial for the better assessment of the efficacy of chlordiazepoxide and haloperidol as oral premedicants for the anaesthesia of NHP. The authors evaluated the effect of oral premedication using two medications without a control group. To determine the baseline data and to assess whether the premedication was useful in reducing handling stress or not, incorporating a control group would have been superior to the present study. The authors opined to include a control group in future studies.

Chlordiazepoxide and haloperidol at the doses of 10 and 1 mg/kg body weight, respectively, may be used as oral premedicants in Bonnet Macaques 4 h prior to the anaesthetic procedure to reduce stress and to ease the handling of animals during induction. However, bitter taste and a larger dose of chlordiazepoxide may reduce its acceptability. The long-acting nature of chlordiazepoxide and haloperidol ensures sufficient gastric emptying after their oral administration before the induction of anaesthesia and improves the quality of induction and maintenance of anaesthesia with minimal side effects. Pre-medication with chlordiazepoxide and haloperidol during midazolam-ketamine hydrochloride anaesthesia may be recommended for vasectomy and other procedures, such as physical examination, tuberculin testing, and wound dressing.

Materials and Methods

Ethical Considerations

The present study was approved by the Institutional Animal Ethics Committee, Kerala Veterinary and Animal Sciences University, Kerala, India. The study was conducted at the State Mu-

seum and Zoo, Thrissur, Kerala, India. Twelve healthy adult male macaques underwent routine vasectomy procedures to control their population in the zoo as per directions of the CZA of India and were selected for the current study. All the animals were cared for properly and were used humanely during capture, translocation, and study by following the best practices of veterinary care as per the guidelines of the CZA of India.

Animals, Husbandry, and Housing

The twelve animals used for the study were randomly selected from a group of 95 monkeys kept in three enclosures of 30 × 15 × 30 feet (l × b × h). Nutritionally fit animals which weighed more than 4 kg were considered for the study. The selected animals were randomly allotted into two groups of six each (website: random.org). All the twelve animals of the study were separated from their group and were kept in an enclosure sized 30 × 15 × 30 feet (l × b × h). The animals were fed the routine diet of the zoo in the evening. The segregated animals were conditioned in the morning hours to take pineapple fruit juice for 15 days before the procedure. The animals were fasted for 8 h and water was withheld for 5 h prior to the administration of premedication agents.

Assessment of Temperament

The temperaments of the animals were evaluated based on the response to the threatening human in individual cages before the study as suggested by Capitanio (1999) [44]. The temperament of each animal was recorded prior to premedication, which helped to assess the change in behavioral responses later.

Administration of Premedications

The dose of premedication and anaesthetic medications was calculated and administered based on the estimated body weight. These doses were corrected later according to actual measured body weight following anaesthesia. Animals of Group I and Group II were premedicated with chlordiazepoxide (Librium®, Abott Healthcare Pvt Ltd., Himachal Pradesh, India) and haloperidol (Serenace 10®, RPG Life Sciences Ltd., Ankleshwar) at the dose of 10 mg/kg body weight and 1 mg/kg body weight, respectively, orally in pineapple fruit juice. The premedica tablets were powdered, mixed with the fruit juice two minutes before oral administration, and were given in a steel bowl. Fruit juice was administered at a dose not exceeding 3 ml/kg body weight. The time taken to complete the premedication was measured from the moment it was placed in front of the animal until it had completely consumed it. The data relating to the dose of anaesthetics during induction and maintenance of midazolam-ketamine anaesthesia, including the hematological and biochemical parameters during haloperidol premedication have been provided by Kumar et al. (2017) [45].

Efficacy of Oral Premedicants

Ease of administering the premedication agents was scored based on a drug administration index used by Pulley et al. (2004) [3]. The ease of accepting fruit juice laced with premedicant was recorded based on the scorecard prepared (Score Card 1).

Corrected Dose Rate = Estimated Body Weight × Dose Rate of the Agent

Actual Measured Body Weight

Behavioral responses were recorded before and after premedication at intervals of 30 min for a period of 4 h by a blinded observer. All the animals were restrained inside the cages to induce anaesthesia either by netting or physical restraint. Ease of handling was assessed during the intramuscular injection of an anaesthetic mixture into gluteal muscles. Ease of handling before anaesthesia induction was recorded in a scorecard (Score Card 2). Quality of sedation throughout 4 h after premedication was recorded in a graded scorecard modified from one described by Pulley et al. (2004) (Score Card 3) [3].

Cortisol and Glucose Level Assessment

Serum cortisol was estimated from the venous blood collected 4 h after premedication immediately after the induction of anaesthesia by the ECLIA method using a commercially available kit (Cobas ECLIA Kit, Roche Diagnostics, Mannheim, Germany) and an automated analyzer. Plasma glucose was also estimated from the venous blood sample collected after the induction of anaesthesia.

Score card 1:
Ease of acceptance of fruit juice laced with premedicant

Score	Observation
2	Easily taken
1	Taken with apprehension
0	Taken with prolonged time

Score card 2:
Ease of acceptance of fruit juice laced with premedicant

Items of observations	Score	Comments
Ease of netting or handling	2	Easy
	1	Difficult
Response to handling or injection	2	Calm
	1	Excited

Statistical Analysis

The obtained data were analyzed as described by Snedecor and Cochran (1994) using the statistical software SPSS version 16.0. Independent samples t-test was used to compare the means ± SE of dose and time taken for premedication. The Mann-Whitney U-test was used for all the parameters in the study involving scor-

Score card 3:
Quality of sedation after oral premedication

Score	Quality of sedation after oral premedication
0	None: Normal behaviour
1	Mild: Upright, mild incoordination and ataxia (But able to move freely)
2	Moderate: Upright, severe incoordination and ataxia (Sitting with extended hind limbs)
3	Good: Recumbent, easily aroused
4	Heavy/Profound: Recumbent, very little response to stimulus (able to safely hand inject)

ing (ease of administering the premedication agents, ease of the acceptance of fruit juice laced with premedicant, ease of handling prior to the induction of anaesthesia, quality of sedation). Paired samples t-test was used for comparing observations before and after premedication and anaesthesia.

Authors' Contributions

K S Kamalesh Kumar and George Chandy have planned carried out the experiments. Both of them took the lead in writing the manuscript. S Sooryadas, P T Dinesh have provided critical feedback and helped shape the research, analysis and manuscript.

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Competing Interests

The authors declare no conflict of interest.

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