EFFECT OF ORAL PREANESTHESIC SEDATION WITH CHLORDIAZEPOXIDE AND HALOPERIDOL PRIOR TO ANESTHESIA FOR ADULT MALE BONNET MACAQUES (*Macaca radiata*)

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Abstract:

Background 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 drugs. **Aim** The efficacy of premedication with chlordiazepoxide or haloperidol independently prior to midazolam-ketamine anaesthesia was evaluated in 12 male Bonnet Macaques. **Method** Animals were randomly grouped into two groups of six animals each (n=6). Animals of Group I were administered chlordiazepoxide (10mg/kg) and animals of Group II were administered haloperidol (1mg/kg) orally. The temperament of each animal was recorded prior to premedication. Behavioural responses after pre-medication were assessed for 4 hours. Glucose and cortisol levels were assessed from the venous blood sample collected after induction of anaesthesia. **Result** Sedation was obtained in both the group of animals whereas quality of sedation was comparatively better in

Group II. Analgesia was better in Group I compared to Group II. Haloperidol-premedicated animals were observed to be easy to handle, but increased cortisol and glucose levels were recorded. **Conclusion** Pre-medication with chlordiazepoxide and haloperidol have produced optimum sedation to handle the Bonnet Macaques for inducing anaesthesia.

Keywords: Oral Premedication, Chlordiazepoxide, Haloperidol, Non-human primates, Bonnet Macaque.

Abbreviations: Non-human primates (NHP), Ventral tegmental area (VTA), prefrontal cortex (PFC), Nucleus accumbens (NAc), Electrochemiluminescent immunoassay (ECLIA), Central Zoo Authority (CZA), Hypothalamic-Pituitary-Adrenal (HPA) axis, Corticotropin-releasing hormone (CRH), Adrenocorticotropic hormone (ACTH).

1. Introduction:

Uncontrolled stress and death due to capture myopathy have been recognised as the most serious concern during 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 drugs to minimize stress is a priority from the welfare point of view in wildlife conservation, zoos and laboratory-housed nonhuman primates/macaques.

Non-human primates (NHP), are routinely used for research in laboratories all over the world [2]. NHP are anaesthetized for several scientific studies in laboratories as well as for routine procedures like surgical sterilization, microchipping etc. in captivity. Capturing and handling of NHP requires experience, thorough knowledge of animal behaviour and technical skills to ensure safety to the animal as well as the handler. NHP in captivity may also be darted in their enclosures or hand injected after physical restraint. Usually, free ranging macaques are trapped in large cages and anaesthetised by darting or injecting the anaesthetic agents after transferring them to squeeze cages or smaller cages. NHP are not premedicated prior to darting in these conditions. However, darting of small sized unpremedicated monkeys can be

dangerous for the animal and a difficult task because of their frantic fast movements. In addition, NHP are difficult to be handled 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. Ease of handling and drug administration can be improved as well as the stress response reduced in these animals by premedicating them with oral tranquilizers before handling or anaesthesia [3].

Tranquilizers, sedatives and anaesthetic drugs have established themselves 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, 7, 8, 9]. Chlordiazepoxide has been observed to produce mild sedation and reduce the spontaneous mobility, walking, grooming and increased lying period in Rhesus macaques [10]. Haloperidol also has been proven as a long-acting neuroleptic drug in non-human primates, dogs and other wild animals [5, 11, 12].

Both the drugs can be administered orally as premedicants and have the potential to reduce stress during handling and ease the subsequent administration of anaesthetic drugs. The advantage of these long acting premedicant drugs is that their effect would last for sufficient time to allow gastric emptying before 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. 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 for vasectomy in Bonnet Macaques.

3 Results:

The temperament of each animal was presented in Table 1. Three animals in each group were stoic, two animals in each group were apprehensive, one animal of Group I was calm and relaxed and one animal of Group II was aggressive. Mean±SE values of estimated body

weight and actual measured body weight is presented in Table 2. There was no significant difference between estimated and actual measured body weights in both groups.

3.1 Ease of Acceptance of Premedicant used in the Study and its Duration

Mean±SE value of corrected oral dose of chlordiazepoxide against the actual measured body weight in animals of Group I was 10.33±0.20 mg/kg body weight. Mean±SE value of corrected oral dose of haloperidol against the actual measured body weight in animals of Group II was 1.12±0.07 mg/kg body weight. Time taken to complete the premedication was 26.16±8.96 and 12.66±7.53 minutes 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 was recorded and shown in Table 3. There was no significant difference noticed between Group I and Group II in these parameters.

3.2 Behavioural observations

Behavioural responses were observed prior to premedication and for four hours at an interval of 30 minutes after premedication. Various behavioural responses like 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 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 were recorded in Table 4. Graphical observation represents the half an hourly changes in each behavioural responses.

3.3 Quality of Sedation after oral Premedication

Median value for 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.

3.4 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 ease of netting or injecting and response to handling or injection was observed to be 2.0 in both the groups (Table 3). There was no

significant difference in this parameter between the groups. Five (83.3 per cent) of the premedicated animals were observed to be calm with reduced activity by 4 hours after premedication in both groups. One animal in each group remained active throughout the period of four hours.

3.5 Cortisol and Glucose levels

Mean±SE values of cortisol levels were observed to be 20.49±3.70 and 40.09±5.96 mg/dl after 4 hours of oral premedication in Group I and Group II, respectively. Significant difference was noticed in cortisol levels during induction between Group I and Group II. Mean±SE values of glucose levels were observed to be 83.83±7.66 and 98.16±13.11 mg/dL after 4 hours of oral premedication in Group I and Group II, respectively.

No post-operative complications were observed in either group. All the animals were easily accepted back to their respective groups without any infighting.

4 Discussion and Conclusion:

The aim of the current study was to evaluate and examine the sedative effect of two premedicants separately and to assess its practical application and feasibility for induction of general anesthesia with minimal stress to Bonnet Macacques.

Prior observation of temperament was helpful in assessing the change in behaviour of the animals after oral premedication and during post-operative stress evaluation as opined by Fowler (2008) and Murphy (2008) [13,14]. The corrected dose rates of drugs used in the present study were observed to be similar to the required dose as reported in many studies [15, 16, 17, 18, 19]. All the animals were conditioned to take pineapple fruit juice for 15 days prior to the procedure as opined by CWINP-ILAR, (1998) and Fowler, (2008) [13,20]. Slightly increased time taken to complete premedication and lesser median value for ease of administering fruit pulp in Group I could be due to bitter taste and larger dose of chlordiazepoxide.

4.1 Behavioural observations

The effectiveness of use of long-acting neuroleptics in wild animals to reduce stress is described by Fick *et al.* (2007) [21]. Oral administration of long-acting tranquillizers has been observed to facilitate handling of NHP [22]. The administered dose rate 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 observed to be effective in monitoring quality of sedation after oral premedication in the present study also. The median values for quality of sedation was 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 observed to be 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]. Haloperidol's effects are not only limited to dopamine D-2 receptors blockade. It also affects other neurotransmitter systems, including serotonin and norepinephrine [24, 25]. Disruptions in these systems have contributed to produce sedation by altering the balance of neurotransmitters causing CNS depression leading to reduced activity levels, decreased responsiveness to stimuli, and overall sedation.

One animal which had shown calm and relaxed behaviour prior to premedication in Group I had a score of 3 indicating higher sedation. One animal which had shown aggressive behaviour prior to premedication in Group II showed incoordination and ataxia (score-2). Two animals which 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 central nervous system (CNS) depression. Minimal sedation in Group I could be due to reduced intake of chlordiazepoxide or reduced dose of the drug leading to mild relaxation and anxiolysis.

None of the animals were observed to have shown anxiety, aggression and hyperactivity at the end of the four hours after premedication. Relaxed, calm, and reduced activity was observed in all the animals by the end of 4 hours. 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 drugs 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 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. CNS depression effects of chlordiazepoxide affect the transmission of signals along the spinal cord and other pathways involved in muscle contractions. Chlordiazepoxide's anxiolytic (anti-anxiety) effects 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 behavioural responses.

Long therapy using haloperidol has been observed to influence on dopamine receptors in the basal ganglia of NHP producing extrapyramidal effects like muscle stiffness etc. The anticholinergic activity of haloperidol causes a reduction in the activity of the neurotransmitter acetylcholine leading to some degree of muscle relaxation due to the dampening of cholinergic transmission, which is involved in muscle contraction.

Ataxia has been noticed in four and three animals of Group I and Group II, respectively. Benzodiazepines have been reported to have benzodiazepine type I (BZI) receptor predominantly in cerebellum causing cerebellar ataxia [27, 28]. Haloperidol has been observed to cause ataxia within six hours 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 like ataxia. Dopamine pathways also influence cerebellar function. The disruption of these pathways by haloperidol's dopamine receptor blockade can result in impaired communication between the basal ganglia and the cerebellum, contributing to ataxia. The degree of ataxia experienced 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 how pronounced the ataxic effects are.

Yawning, recumbency, leaning against wall/grill, drowsiness (nodding/head down) and sleeping in sitting posture could be due to higher level of sedation shown by animals of both groups. One animal of Group I showed self-grooming during observation period which was in agreement with the findings of Kumar *et al.* (1999) in Rhesus Macaques [29].

Behavioral observations like sleep-like state; calm and quiet state; and mild hypotension have been recorded within five minutes of intravenous premedication of haloperidol in dogs [11]. The effectiveness of haloperidol along with zuclopenthixol to produce deep sedation during transportation of an adult male gorilla from Germany to South Africa has been reported by Redrobe *et al.* (2008) [30]. Haloperidol has been observed to produce deep sedation lasting about 2 to 4 hours 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). Haloperidol's central nervous system depressant effects 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 hours after premedication. The ease of handling and injecting drug may have been due to the calming effect of premedication with either chlordiazepoxide or haloperidol. Calming and sedative effect of premedicants has been recognised 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 four hours 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 tranquillizers [7, 32]. Prolonged action of the premedication drugs was observed in Bonnet Macaques in the present study also. The ease of handling and injecting drug may have been due to the calming effect of premedication.

One animal in each group which were having aggressive and apprehensive temperament remained active throughout the period of four hours. Increased activity of these animals could be due to improper intake of bait or lower doses of premedication drugs. The authors opine that pineapple juice couldn't 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 to increase the distance between cages to reduce the social behaviour and activity of macaques.

4.2 Cortisol and glucose level

Higher glucose levels observed in this study in Group II than Group I after 4 hours of premedication might be due to excessive release of cortisol due to psychological and physical stress related to handling. 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-anaesthetised, ketamine anaesthetised and ketamine-xylazine anaesthetised animals [32, 33, 34, 35, 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 0th and 60th minute was observed to be between 27.9±1.7 and 21.2±2.0 µg/dL [39]. Significant increase in cortisol level in Group II may have been due to its sensitivity for physical and psychological stress. Hypothalamic-Pituitary-Adrenal (HPA) axis is a complex neuroendocrine system that controls the body's response to stress and regulates cortisol production. The hypothalamus releases corticotropin-releasing hormone (CRH), which stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH). ACTH, 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 has been reported in Rhesus Monkeys [41]. Injection technique and blood sampling process have been observed to increase 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 ease of handling of 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. Quality of recovery in animals premedicated with haloperidol was observed to be better than that in animals premedicated with chlordiazepoxide, which showed ataxia during recovery. The present study involved a small group size of six for the evaluation of each premedicant drug. A study involving more animals in the future would be beneficial for better assessment of the efficacy of chlordiazepoxide and haloperidol as oral premedicants for anaesthesia of NHP. Authors evaluated the effect of oral premedication using two drugs without a control group. To determine the baseline data and to determine whether the premedication was useful in reducing handling stress or not, incorporating a control group would have been superior than present study. The authors opined to include control group in future studies.

Chlordiazepoxide and haloperidol at the dose of 10 and 1 mg/kg body weight, respectively may be used as oral premedicants in Bonnet Macaques four hours prior to the anaesthetic procedure to reduce stress and to ease handling of animals during induction. However, bitter taste and a larger dose of chlordiazepoxide may reduce its acceptability. Long-acting nature of chlordiazepoxide and haloperidol ensures sufficient gastric emptying after their oral administration before induction of anaesthesia and improves 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 like physical examination, tuberculin testing and wound dressing.

2. Materials and Methods:

2.1 Humane Care Guidelines:

The present study was approved by Institutional Animal Ethics Committee, Kerala Veterinary and Animal Sciences University, Kerala, India. The study was conducted at State Museum and Zoo, Thrissur, Kerala, India. Twelve apparently healthy adult male macaques underwent routine vasectomy procedures to control their population in the zoo as per directions of Central Zoo Authority (CZA) of India were selected for the present study. All the animals were cared properly and they were used humanely during capture, translocation and study by following best practice of veterinary care as per the guidelines of CZA of India.

Ethics approval statement: *'The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. Ethical approval was not required because no animals were used for research in this study.'*

2.2 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 x 15 x 30 feet (lxbxh). Animals that are nutritionally fit and are weighing more than 4 kg body weight 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 of size 30 x 15 x 30 feet (lxbxh). The animals were fed a 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 prior to the procedure. The animals were fasted for eight hours and water was withheld for 5 hours prior to administration of premedication drugs.

2.2 Assessment of Temperament

The temperaments of the animals were evaluated based on the response to the threatening human in individual cages prior to 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 behavioural responses later.

2.3 Administration of Premedications

The dose of premedication and anaesthetic drugs 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 prior to oral administration and were given in a steel bowl. Fruit juice was administered at a dose not exceeding more than 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 dose of anaesthetics during induction and maintenance of

midazolam-ketamine anaesthesia, haematological and biochemical parameters during haloperidol premedication has been provided by Kumar *et al*., (2017) [45].

2.3.1 Study of Efficacy of Oral Premedicants

Ease of administering the premedication drugs was scored based on a drug administration index used by Pulley *et al.* (2004) [3]. Ease of acceptance of fruit juice laced with premedicant was recorded based on the score card prepared (Score Card 1).

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

Behavioural responses were recorded prior to premedication and after premedication at 30 minutes interval for a period of four hours by a blinded observer. All the animals were restrained inside the cages for inducing anaesthesia either by netting or physical restraint. Ease of handling was assessed during the intramuscular injection of anaesthetic mixture into gluteal muscles. Ease of handling prior to induction of anaesthesia was recorded in a score card (Score Card 2). Quality of Sedation over a period of 4 hours after premedication was recorded in a graded score card modified from one described by Pulley *et al.* (2004) (Score Card 3) [3].

2.4 Cortisol and glucose level assessment

Serum cortisol was estimated from the venous blood collected at 4 hours after premedication immediately after the induction of anaesthesia by electrochemiluminescent immunoassay (ECLIA) method using commercially available kit (Cobas ECLIA Kit, Roche Diagnostics, Mannheim, Germany) in an automated analyzer. Plasma glucose (mg/dL) was also estimated from the venous blood sample collected after induction of anesthesia.

2.5 Statistical Analysis

The data obtained were analysed 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, time taken for premedication and to compare means±SE between the groups. Mann-Whitney U-Test was used for all the parameters in the study involving scoring (Ease of administering the premedication drugs, Ease of acceptance of fruit juice laced with premedicant Ease of handling prior to induction of anaesthesia, Quality of Sedation). Paired

samples t-test was used for comparing observations before and after premedication and anaesthesia, respectively.

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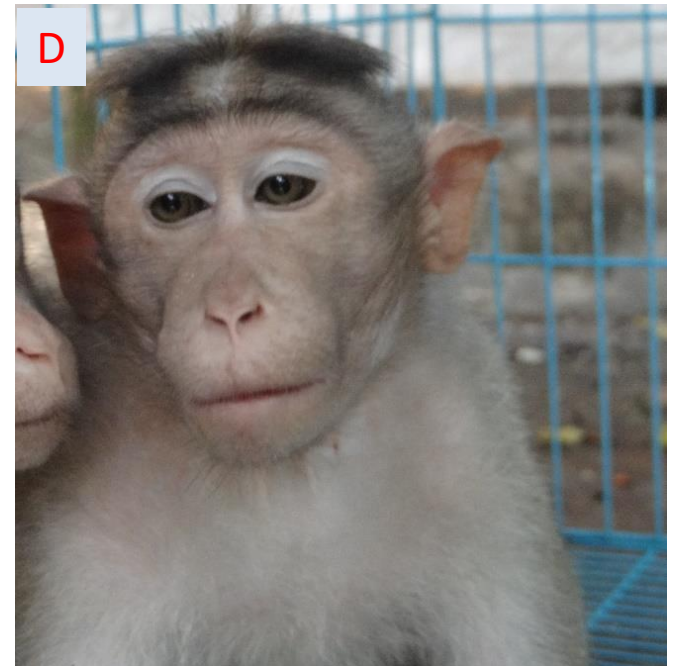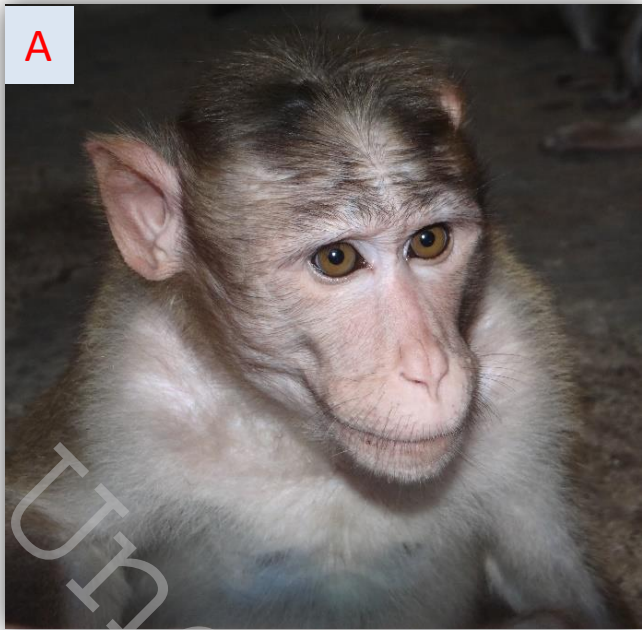
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Fig.1. A-L. Behavioral responses observed after premedication: (A) Anxiety, aggression, and hyperactivity; (B) Active and playful; (C) Relaxed, calm, and reduced activity; (D) Stuperous look; (E) Half-open mouth; (F) Sitting with relaxed limbs; (G)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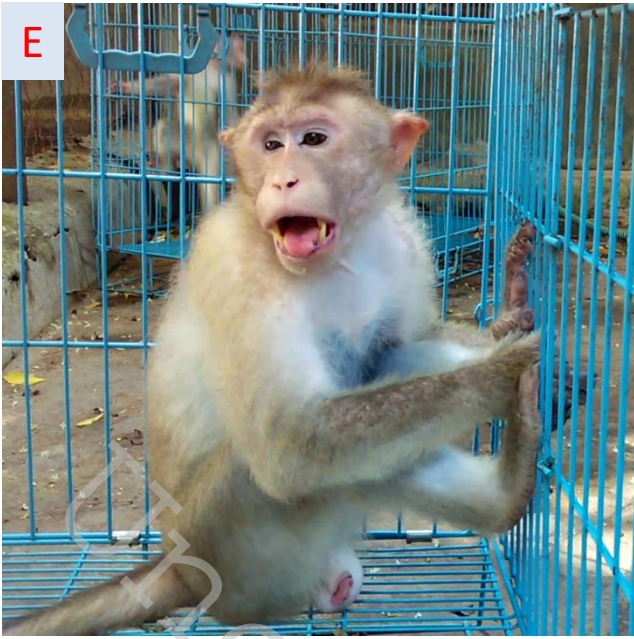
Graph A-N. Graphical representation of behavioral responses observed after premedication: (A) Anxiety, aggression, and hyperactivity; (B) Active and playful; (C) Relaxed, calm, and reduced activity; (D) Stuperous look; (E) Half-open mouth; (F) Sitting with relaxed limbs; (G) Self-grooming; (H) Social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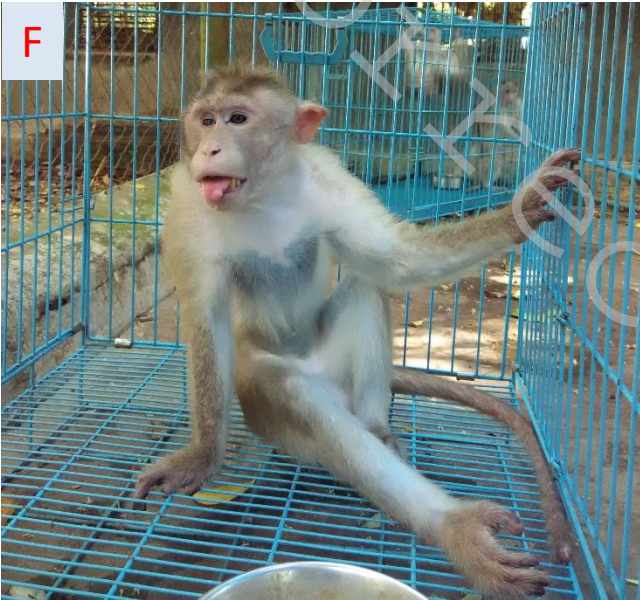 Leaning against wall/grill; (M) Drowsy; (N) Sleeping.