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Research Article

Effects of Midazolam-Ketamine Anesthesia on the Haematological and Biochemical Parameters Using Haloperidol or Chlordiazepoxide Premedication in Adult Male Bonnet Macaques *(Macaca radiata)*

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ABSTRACT

Introduction: 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 anesthetic drugs. This study was conducted to evaluate the hematological and serum biochemical changes associated with anesthesia in male Bonnet Macaques using haloperidol or chlordiazepoxide premedication.
Materials and methods: Twelve adult male Bonnet Macaques aged around 4 to 6 years were randomly allotted to two groups of six each. The duration of the study was five hours. Animals of Group I were administered chlordiazepoxide (10 mg/kg body weight) orally and animals of Group II were administered haloperidol (1 mg/kg body weight) orally four hours before anesthetizing with the intramuscular injection of midazolam (0.1 mg/kg body weight) and ketamine (10 mg/kg body weight). Hematological parameters such as hemoglobin concentration, erythrocyte, total leucocyte count, the volume of packed red cells, granulocyte, monocyte, and lymphocyte count were evaluated. Biochemical parameters such as creatine kinase, aspartate aminotransferase, alanine aminotransferase, cortisol, glucose, calcium, sodium, and potassium were evaluated from the venous blood sample collected at 0th minute and 30th minute after induction of anesthesia.
Results: The results of the current study indicate that in hematological parameters, the volume of packed cells was significantly different between 0th and 30th minute in both groups. The total leucocyte count was significantly different at 0th and 30th minute in Group I and Group II, and the monocyte count was significantly different at 0th and 30th minute in Group I. For biochemical parameters, a significant difference was observed in creatine kinase in group II between 0th and 30th minute and cortisol at time 0th between Group I and Group II.
Conclusion: These results highlight the impact of anesthesia protocols on stress responses in Bonnet Macaques. Haloperidol premedication was linked to a greater increase in cortisol and creatine kinase, indicating higher stress and muscle damage compared to chlordiazepoxide.

1. Introduction

Wild animals have fascinated humans since ancient times when coexistence in forests was common. Trapping and hunting for food were routine before the advent of agriculture over ten thousand years ago. Live capture of wild animals began in the 1950s[1], leading to the development of various capture techniques. Using

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poisoned blowpipe darts and arrows for hunting has a history of thousands of years[2]. Selective breeding has reduced fear and stress in these species, yet capture myopathy remains a significant concern[3]. Minimizing stress during capture is crucial to reduce morbidity and mortality. Tranquilizers, sedatives, and anesthetics play essential roles in stress reduction during wild animal restraint. Improving capture techniques with appropriate drugs is vital for wildlife conservation and welfare.

India's diverse wildlife population, including many species in state zoos, faces challenges such as overbreeding in Bonnet Macaques. India boasts one of the richest diversities of primates, with macaque species being particularly abundant. Among these, the Bonnet macaque (*Macaca radiata*) stands out as an old-world monkey that is densely populated and widely distributed among the eight macaque species found in India. This species is endemic to India, specifically inhabiting the southern regions of the Indian peninsula and the Western Ghats[4,5]. Monkeys, in particular, often come into conflict with humans in both rural and urban settings. The Central Zoo Authority of India (CZA) recommends surgical sterilization, such as vasectomy, as a humane and scientific population control method.

Non-human primates are commonly used for research worldwide. Animal models have become increasingly important for studying human physiology, anatomy, pathology, and pharmacology. With significant advancements in drug development, biomedicine, and pre-clinical trials, these models are crucial for evaluating therapeutic outcomes and drug safety for potential human use[6]. Non-human primates need anesthesia for various research purposes, requiring skilled handling to ensure safety for both animals and handlers. Typically, macaques are trapped, transferred to smaller cages, and anesthetized, though darting unpremedicated monkeys can be dangerous due to their quick movements. In these situations, bonnet macaques must be relocated for menace control and conservation purposes. However, trapping and translocation can cause injuries and stress, leading to increased morbidity and mortality[4,5]. Premedication with oral tranquilizers can improve handling and reduce stress. Long-acting tranquilizers like chlordiazepoxide and haloperidol, administered orally, can ease subsequent anesthetic administration. Their effects last long enough to allow gastric emptying before administering anesthetics. Drugs like midazolam and ketamine provide satisfactory anesthesia with minimal cardio-respiratory changes and are commonly used for macaques.

Identifying factors that influence blood parameters is vital for making accurate comparisons between populations or reference ranges[7]. Reference hematological and serum biochemistry data are available for many non-human primate species with extensive laboratory research histories, including chimpanzees, bonnet macaques, cynomolgus macaques, and rhesus macaques[8-16]. Hematological and serum biochemical tests are crucial for assessing animal health and diagnosing diseases[7,17]. Compared to other non-human primates like Rhesus Macaques (*Macaca mulatta*), Bonnet Macaques have fewer reported parameters. Due to the growing utilization of macaques in biomedical research, establishing their hematological and biochemical parameters is essential. Reference values are crucial for selecting healthy animals and interpreting laboratory data in non-human primate (NHP) models[18]. While there are published reports on hematological and serum biochemical indices in bonnet macaques, the data provides normal reference values specific to their laboratory or captive conditions[4,7,17,19]. Physical and chemical restraints, along with habitat, living conditions, and nutrition, can influence these values[20-22].

Under these circumstances, a study was conducted in adult, male, captive Bonnet Macaques undergoing vasectomy at the State Museum and Zoo, Thrissur, to study the hematological and serum biochemical changes associated with anesthesia for vasectomy in Bonnet Macaques using both protocols. The authors of the current study present the average values of bonnet macaques under captivity at the time of induction (0 minutes) and their variation after 30 minutes during the anesthesia protocol.

2. Materials and Methods

2.1. Ethical approval

The present study was approved by the Institutional Animal Ethics Committee, KVASU, Kerala, India (10.53°N 76.22°E).

2.2. Animals

The study was conducted in 12 adult healthy male Bonnet Macaques aged around 4 to 6 years were anesthetized for routine vasectomy procedures to control their population as directed by the CZA of India. The authors of the current study followed the best practices for handling and surgery of the Bonnet Macaques as per the guidelines of CZA of India[23]. The animals were randomly selected from a group of 95 Bonnet Macaques housed in three enclosures (9.1 x 4.5 x 9.1 meters). Selected macaques were separated and housed individually in cages (90 x 55 x 55 cm) for easy handling, with a mean body weight of 4.17±0.78 kg. All animals were dewormed 15 days before the procedure and fed a normal zoo diet.

2.3. Chemical restraint and blood collection

Health status was visually assessed the day before the procedure. The macaques fasted for eight hours and then given oral chlordiazepoxide (10 mg/kg, Librium®, Abott Healthcare Pvt Ltd., Himachal Pradesh, India) in Group I and haloperidol (1 mg/kg) in Group II. The administered dose rate of premedication was sufficient to produce the

required effect as in earlier studies[24, 25, 26, 27]. Pineapple juice was used as a vehicle for giving oral tablets. After four hours, they were physically restrained and anesthetized with intramuscular midazolam (0.1 mg/kg) and ketamine (10 mg/kg)[28, 29, 30]. Once anesthetized, the forearm was shaved and prepared using isopropyl alcohol and povidone-iodine. A 22G intravenous cannula was used to catheterize the cephalic vein, and 4 ml of blood was collected. Half was placed in K3 EDTA vacutainers for hematology, and the rest in clot activator tubes and blood gas analyzer strips for biochemical evaluation.

2.4. Haematology and biochemistry

Hematological parameters (TEC, TLC, hemoglobin, VPRC, DLC) were analyzed using a veterinary hematological analyzer. Plasma glucose, sodium, calcium, and potassium concentrations were measured with a portable blood gas analyzer. Serum creatine kinase, aspartate aminotransferase, and alanine amino-transferase levels were assessed using a semi-automatic analyzer with appropriate kits.

2.5. Evaluation of hematological and biochemical parameters

The veterinary hematological analyzer (Mythic 8 VET®, ORPHEE, Switzerland) was calibrated for Bonnet Macaque blood cells and reference ranges were pre-set for the analysis of the blood sample. Total erythrocyte count (106/µL), total leucocyte count (103/µL), hemoglobin concentration (g/dL), the volume of packed red cells (percent), and differential leucocyte count (percent) were evaluated immediately after induction of anesthesia and at 30th minute after induction. Plasma glucose (mg/dL), calcium (mmol/L), sodium (mg/dL), potassium (mmol/L), and glucose (mg/dL) were estimated using the portable blood gas analyzer (epoc® Blood Analysis System, and epoc BGEM Test Card, Epocal, INC., Ottawa, ON Canada) from the venous blood sample collected after induction of anaesthesia and from arterial blood at 30th minute after induction.

Serum cortisol was estimated from the venous blood collected from the cephalic venepuncture immediately after induction of anesthesia and at 30th minutes of induction by electrochemiluminescent immunoassay (ECLIA) method using commercially available kit (Cobas ECLIA Kit, Roche Diagnostics, Mannheim, Germany) in an automated analyzer (Elecsys® Cortisol II, Roche Diagnostics International AG, Rotkreuz, Switzerland).

Creatine Kinase, Aspartate Aminotransferase, and Alanine Aminotransferase were also estimated at same time intervals using commercially available kits (Liquick Cor-ASAT/ALAT, PZ CORMAY S.A., Poland, Liquick Cor-CK, PZ CORMAY S.A., Poland, Marketed by Inscon Medical Solutions Pvt. Ltd., Kochi) in a semi-automated analyzer (Master-T®, Hospitex Diagnostics, Italy).

2.7. Statistical analysis

The data obtained was analyzed statistically following the methods outlined by Snedecor and Cochran[31] using SPSS version 16.0 software. For comparing biochemical and hematological parameters, independent samples t-tests were used to compare between groups, while paired samples t-tests were employed to compare observations before and after treatment ($P<0.05$).

3. Results

3.1. Haematological Parameters

Results of hematological analysis at 0th and 30th minute are given in Table 1. Total Leucocyte Count (TLC)

Table 1. Hematological parameters in adult male Bonnet macaques using midazolam-ketamine anesthesia with haloperidol or chlordiazepoxide premedication

Parameter	Time (minutes)	Mean±Standard deviation	
		Group I	Group Ⅱ
Hemoglobin concentration	0	13.76±0.61	12.51±1.67
	30	13.33±1.15	11.95±1.84
Total erythrocyte count	0	5.14±0.59	5.18±1.05
	30	5.06±0.51	5.17±0.73
Total leucocyte count	0	15.43±1.45^{a}	12.84±3.18^{b}
	30	15.84±1.59^{a}	12.83±1.79^{b}
Volume of packed red cells	0	39.83±1.81^{aA}	38.00±3.16^{bA}
	30	38.50±1.49^{aB}	37.00±2.89^{bB}
Granulocyte count	0	45.63±16.19	41.68±17.73
	30	45.26±16.71	39.38±13.86
Monocyte count	0	8.53±4.07^{A}	8.58±4.46
	30	13.01±5.7^{B}	9.61±2.47
Lymphocyte count	0	45.65±12.88	49.73±14.40
	30	43.38±9.50	50.83±12.79

^{a,b} Values with different small superscript letters differ significantly ($p < 0.05$) between groups.
^{A,B} Values with different capital superscript letters differ significantly ($p < 0.05$) between the two times.

Table 2. Biochemical parameters in adult male Bonnet macaques using midazolam-ketamine anesthesia with haloperidol or chlordiazepoxide premedication

Parameter	Time (minutes)	Mean± Standard deviation	
		Group I	Group Ⅱ
Creatine kinase	0	303.21±161.42	313.31±86.66[A]
	30	310.75±109.20	412.36±75.17[B]
Aspartate amino transferase	0	38.54±13.35	41.53±13.64
	30	38.82±14.06	40.98±10.88
Alanine aminotransferase	0	20.54±9.09	26.31±8.38
	30	20.04±8.35	25.31±9.43
Cortisol	0	20.49±9.06[a]	40.09±14.60[b]
	30	25.01±7.84	33.10±15.46
Glucose	0	83.83±18.76	98.16±32.11
	30	129.33±61.46	155.00±21.90
Calcium	0	0.67±0.20	0.61±0.17
	30	0.70±0.15	0.64±0.17
Sodium	0	142.83±4.21	144.83±7.91
	30	144.66±3.26	145.50±6.79
Potassium	0	3.28±0.71	3.21±0.66
	30	3.46±0.66	3.45±0.56

[a,b] Values with different small superscript letters differ significantly ($p < 0.05$) between groups.
[A,B] Values with different capital superscript letters differ significantly ($p < 0.05$) between the two times.

was found to be 15.84±0.65 and 12.83±0.73 $\times 10^3/\mu L$ at 0th and 30th minute in Group I and Group Ⅱ. A significant difference between Group I and Group II was found in TLC at the 30th minute. VPRC levels were found to be 38.0±1.29 and 37.0±1.18 percent during 0th and 30th minute, respectively, in Group Ⅱ. A significant difference was noticed in VPRC levels between 0th and 30th minute in both groups. Monocyte count was found to be 8.53 ± 1.66 and 13.01±2.33 percent during 0th and 30th minute, respectively, in Group I. Significant difference was noticed in monocyte count between 0th and 30th minute in Group I.

3.2. Biochemical Parameters

Results of biochemical analysis are given in Table 2. Mean±SD values of creatinine kinase levels were found to be 313.31±35.38 and 412.36±30.69 U/L at 0th and 30th minute, respectively, in Group Ⅱ. Significant difference was noticed in creatinine kinase levels of Group Ⅱ between 0th and 30th minute. Mean±SD values of cortisol levels were found to be 20.49±3.70 and 40.09±5.96 mg/dL at 0th minute in Group I and Group II, respectively. Significant difference was noticed in cortisol levels during induction between Group I and Group Ⅱ.

4. Discussion

Primates are extensively utilized in research on human diseases due to their close resemblance to humans. Consequently, they play a significant role in advancing medical science and other related fields[3]. Hematological and biochemical parameters serve as crucial indicators in biology and medical research. These parameters are employed to assess the health status of animals, offering valuable references in pathology and toxicology studies. They also provide direct and indirect insights into organ functions[16].

Previous studies have examined hematology and biochemistry in various primate species, including chimpanzees[11], Cynomolgus macaques[8,32,33], Rhesus macaques[10,22,34] African green monkeys[35], Tibetan macaques[35-37,] and black howler monkeys[38]. However, there are limited reports on the hematological and serum biochemical parameters of Bonnet macaques.

Numerous studies on the hematological and biochemical parameters of non-human primates have been conducted under ketamine anesthesia[7,39-42]. Bolliger et al.[43] found that ketamine, followed by Telazol®, sevoflurane, or isoflurane, is the most commonly used anesthetic agent in these studies. Research on Bonnet macaques has been performed under physical restraint[15,44], ketamine anesthesia[7], ketamine and xylazine anesthesia[17,45], and ether anesthesia[46].

Physical restraint and anesthesia of untrained monkeys have been shown to be stressful, potentially altering hematological and serum biochemical parameters[14,20-22]. Both captive[7,15,45,47] and free-ranging animals[36,38] have been included in earlier studies.

4.1. Haematological parameters

There were some differences between the hematology parameters reported in previous studies and those observed in the current study. The acute stress caused by handling animals without anesthesia leads to an 'alarm reaction,' leading to hemoconcentration, neutrophilia, and lymphocytosis[48].

Total erythrocyte count (TEC), total leucocyte count (TLC), haemoglobin concentration, volume of packed red cell (VPRC), granulocyte percent, monocyte percent, and lymphocyte percent were recorded immediately after induction and at 30 minutes after it in the present study.

Results of TEC and TLC were found to be in agreement with the results of Ramachandra et al.[15] in non-anaesthetised Bonnet Macaques indicating normal counts. Pierre et al.[7] and Palanivelrajan et al.[45] reported slightly

higher TEC during ketamine hydrochloride and ketamine-xylazine anaesthesia, respectively, in Bonnet Macaques. Balasubramanyam et al.[47] reported lower TEC in Bonnet Macaques.

Findings of TLC of both groups were found to be in agreement with the results of available references in Bonnet Macaques[15, 45].

Haemoglobin levels during induction were found to be in agreement with the findings of Ramachandra et al.[15] and Mythili et al.[44] during studies in non-anesthetized Bonnet Macaques. A slight non-significant reduction was noticed in the hemoglobin levels of both groups by 30 minutes. Contradictory results have been reported in non-human primates during different anesthetic protocols[43].

Lymphocyte counts of both groups were found to be in agreement with the findings of Pierre et al.[7] in adult Bonnet Macaques during ketamine anesthesia. Balasubramanyam et al.[47] also reported similar results.

Significant decrease in the levels of VPRC was noticed at 30th minute in both groups compared to the level at induction. VPRC levels during induction were found to be in agreement with the results of Pierre et al.[7] in ketamine anaesthetized Bonnet Macaques. Ramachandra et al.[15] and Palanivelrajan et al. [45] reported slightly higher values during ketamine-xylazine anesthesia and physical restraint, respectively, while Balasubramanyam et al.[47] reported lower values in non-anesthetized Bonnet Macaques. A significant decrease in the level of VPRC may have been due to the administration of intravenous fluids during the procedure. The reduction in VPRC levels could also be attributed to the effects of ketamine, as indicated by Loomis et al.[20] and Yoshida et al.[21]. Studies have shown that ketamine anesthesia decreases VPRC levels in Rhesus monkeys[20] and Cynomolgus monkeys[21].

Monocyte count (13.01±2.33) was found higher in the present study in comparison with previous studies by Ramachandra et al.[15], Mythili et al.[44], Balasubramanyam et al.[45] and Palanivelrajan et al.[47]. The higher monocyte count may have been due to release of corticosteroids during physical restraint and induction of anesthesia as suggested by Benjamin[49]. But the results of monocyte count were contradictory to blood gas and biochemical results.

The present study involved a small group size of six for the evaluation of each premedical drug. A study involving more number of animals in future would be beneficial for better assessment of the effects on haematological parameters during chlordiazepoxide and haloperidol as oral premedicants for anaesthesia of non-human primates.

4.2. Biochemical parameters

Mean± SD values of cortisol levels were found to be 20.49±3.70 and 40.09±5.96 µg/dL during induction between Group I and Group II. Normal cortisol levels during tiletamine-zolazepam anaesthesia in trained Rhesus Macaques between 0th and 60th minute was found to be between 27.9± 1.7 and 21.2± 2.0 µg/dL[50]. The significant increase in cortisol level in Group Ⅱ may have been due to its sensitivity to physical and psychological stress. Circulating cortisol levels have already been reported as an important indicator of stress in wild animals[51,52]. Increased cortisol levels due to stress related to cage restraint and ketamine anaesthesia has been reported in Rhesus Monkeys[53]. Injection technique and blood sampling process have been found to increase cortisol levels in untrained monkeys compared to trained one[50]. Contradictory results maintaining stable endocrine responses have been reported by Fuller et al.[54] in Cynomolgus Monkeys.

The creatine kinase levels during induction and at 30th minute, creatine kinase levels increased significantly in Group Ⅱ (412.36±30.69 U/L) at 30th minute. Haloperidol has been found to increase the levels of creatine kinase enzyme during antipsychotic therapy[55,56]. Very mild variation in creatine kinase levels in Group Ⅰ may be due to trauma of skeletal muscles associated with intramuscular drug administration or vasectomy[57]. Creatine kinase isoenzyme tests could aid in confirmatory diagnosis of the observed alterations [58]. Creatine kinase values reported by Ramachandra et al.[7] and Pierre et al.[15] in non-anesthetized and ketamine anaesthetized Bonnet Macaques were higher than that of the present study indicating lower skeletal muscle damage in our study.

Aspartate aminotransferase enzyme levels were found to be same during induction and at 30th minute in Group I and Group II. AST Levels were found to be higher than that of the reports of Pierre et al.[7] and Ramachandra et al.[15] in non-anesthetized and ketamine anaesthetised Bonnet Macaques. Creatine kinase level was found to be higher at 30th minute in Group Ⅱ, without any significant change in AST levels. Increase in the levels of creatine kinase without significant increase in AST levels has been reported during skeletal muscle damage[50]. The increase in creatine kinase in the present study may have been because of skeletal muscle damage during physical restraint.

Glucose levels were found to be increasing with time in both groups. The level of glucose in both groups at the time of induction were higher than the normal fasting blood glucose levels (40-80 mg/dl) reported by Hall and Everds[59] in macaques but were found to be in agreement with the findings of Ramachandra et al.[15] in non-anaesthetized Bonnet Macaques.

The higher glucose levels observed in Group II compared to Group I during induction of anesthesia and 30 minutes after induction may be attributed to excessive cortisol release caused by psychological and physical stress from handling, which likely triggered 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[7,14,15,17,44].

Slight increase in the potassium levels was noticed in both groups compared to its levels at the time of induction. Woodward and Weld[58] reported significant

increase in the levels of potassium during tiletamine-zolazepam anaesthesia in Rhesus Macaques. A significant increase in potassium levels between induction and 30th minute in Group Ⅱ may be due to the excessive release of potassium ions from the myocytes due to damage that happened during physical restraint[59]. The potassium level (3.21±0.27) in this study is consistent with the results of Pierre et al.[7], Ramachandra et al.[15], and Mythiliet al.[44] Rahaman et al.[46] although observed slightly higher values. The serum calcium level (0.61± 0.07) found in this study was lower than those reported by Pierre et al.[7], Ramachandra et al.[15], Mythili et al.[44], and Rahaman et al.[46]. Ketamine anesthesia has also beenshown to reduce serum calcium levels in Cynomolgus monkeys[21]. The sodium level (144.83±3.23) observed in this study aligns with the findings of Pierre et al.[7], Ramachandra et al.[15], and Mythili et al. [44], but is higher than the value reported by Rahaman et al.[46]. Variations in electrolyte levels may be attributed to differences in nutrition and metabolism, as noted by Palanivelrajan et al. [17].

5. Conclusion

The estimated hematological and biochemical parameters showed minimal alterations, indicating that the premedication and anesthesia protocol used were physiologically stable for the macaques. However, significant variations were also noticed in certain haematological and biochemical parameters, such as total leucocyte count, volume of packed red cells, monocyte count, cortisol levels, and creatine kinase levels between the two groups. Notably, haloperidol premedication was associated with a more pronounced increase in cortisol and creatine kinase levels, indicating a higher stress response and muscle damage compared to chlordiazepoxide. These findings underscore the impact of anesthesia protocols on stress and physiological responses in Bonnet Macaques, providing valuable insights for optimizing anesthesia techniques in non-human primates.

Declarations

Competing interests

The authors declare that there is no competing of interest in this manuscript.

Authors' contributions

George Chandy was responsible for the conceptualization, methodology, supervision, and final correction of the draft. Kuskur Sannappa Naik Kamalesh Kumar handled data curation and the preparation of the original draft. Surendran Sooryadas, Parathazhathayil Dinesh, Kurishinkal Dominic John Martin, Padinhare Meleppatt Deepa, and Binoy Babu contributed to the methodology and editing. All authors have read and approved the final manuscript.

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Ethical considerations

The authors declare that this manuscript is original and is not being considered elsewhere for publication. Other ethical issues, including consent to publish, misconduct, fabrication of data, and redundancy, have been checked by the authors.

Availability of data and materials

The data in the present manuscript were collected by searching of literature as well as involving authors' own materials and are available in the present article.

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