Original Research Article

Haemato-biochemical profile assessment of immobilized wild rhesus macaques (*Macaca mulatta*) in Jabalpur

.

ABSTRACT

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| Indian rhesus macaque has been extensively used in biomedical research. Due to increased use of non-human primates in research studies, it is necessary to understand the effect of immobilizing drugs on haemato-biochemical values in wild rhesus macaques. The anesthetic drug combination used was Inj. Xylaxine at the rate of 2mg/kg body weightcombined with Inj.Ketamine hydrochloride at the rate of 5mg/kg body weight. This study has aimed to present an accurate hematological and biochemical values of anesthetized wild Indian rhesus macaques. The mean values of TEC, TLC, PCV and Hb observed in the present study were towards lower side of the normal reference range, however, the mean values of DLC, MCV, MCH and MCHC remained within the normal reference range. |

*Keywords: Wild rhesus macaque, immobilization, haematology, serum biochemistry.*

1. INTRODUCTION

Non-human primates are the crucial animal model used in pharmaceutical and biologic drug research. Numerous ape species including new world monkeys and old-world monkeys have been employed in various biomedical research to control various human physiological processes (Choi et al., 2016 and Zhu et al., 2023). The results of these studies were found more reliable and accurate when compared to other animal model (including rodents, canine, birds or pigs). *Macaca mulata* (rhesus monkey) and *Macaca fascicularis* (cynomologus monkey) are extensively used in preclinical testing in development of vaccines and the study of pathogenesis of HIV/AIDS, Parkinson’s disease, hepatitis, dentistry, orthopaedic surgical techniques, cardiovascular surgeries, psychological disorders and toxicological studies (Mukherjee et al., 2022).

Due to increased use of non-human primates in research studies, it is necessary to understand the effect of immobilizing drugs on haemato-biochemical values in wild rhesus macaques.

Numerous investigations have detailed the haematological values and blood chemistry in rhesus monkeys, taking into account the different elements that affect the viability of the findings, including age, sex, type of confinement, nutrition, health status, assessments of various medications used in chemical restraint, and, lastly, seasonal and geographic variations(Ibáñez‐Contreras et al., 2013 and Bakker et al., 2023).

According to earlier researches confinement, ambient circumstances, and chemical constraint can cause variations in normal haematological and biochemical parameters. Chemical immobilization through ketamine hydrochloride is a most prevalent practice in the restraining of nonhuman primates (Kumar et al., 2024). Various literatures have described the effects of ketamine anesthesia on the haemato-biochemical parameters of rhesus monkeys. Specific experimental setup including indoor living, typical environmental and husbandry circumstances, and acclimated physical restraint techniques without anaesthesia was used to collect clinical laboratory data in this experimental investigation (Shah et al., 2022). Nevertheless, very scarce information is available about hematological and biochemical changes due to immobilization in wild rhesus monkeys.

In the present study, we have aimed to present an accurate hematological and biochemical values of anesthetized wild Indian rhesus macaques.

2. material and methods

2.1 Location and place of work

The present study was conducted in territorial forest division, Jabalpur in its seven ranges namely Jabalpur (1147 km2), Shahpura (878 km2), Patan (607 km2), Sihora (475 km2), Panagar (477 km2), Majholi (643 km2) and Kundam (984 km2). Among these, Jabalpur range is having the largest area.

2.2 Selection of animals

The animals selected in the proposed study were sixteen wild adult male rhesus macaques in different areas of the Territorial Forest Division, Jabalpur. Macaques were first located in the site and visually assessed for potential immobilization. Such macaques were then either followed or provisioned food bait in a little quantity to attract them, until a good opportunity arose to safely dart them using DAN inject gun.

The anesthetic drug combination used was Inj. Xylaxine (Anased®, 100 mg/ml- Aspen Veterinary Resources®, Ltd. Liberty, Missouri, USA.) at the rate of 2mg/kg body weightcombined with Inj.Ketamine hydrochloride (Vetalar®, 100 mg/ml- Boehringer Ingelheim Vetmedica. Inc, St. Joseph, Missouri, USA.) at the rate of 5mg/kg body weight. CO2 powered variable pressure DAN-Inject riffle (Model No. 9361 MOD JM) using 3 ml light weight plastic darts and 1.5 X 30 mm needles with side port was used to dart the macaques.

The macaques were then followed in an attempt to reduce the post-darting travel distance. When the macaques gained lateral or sternal recumbency and did not show any positive response to touch, they were placed on sterilized cotton cloths. The hairs of one of the hind limbs were clipped and prepared for the blood collection from femoral vein. Then the anesthetized macaques were kept in a cage after recording various physiological parameters and observed for recovery from anesthesia thereafter in the cage itself. They were fed and watered at about 3 hours after complete recovery from anesthesia and later released in safe and natural habitat.

2.3 Blood collection

Following all aseptic procedures, 3 ml of blood was collected 30 minutes post immobilization from femoral vein of the rhesus macaques using 22-gauge scalp vein. Then, 1 ml aliquot from the blood samples was individually transferred into tripotassium ethylenediaminetetraacetic acid (EDTA-K3) coated vials for haematological analysis and remaining 2 ml aliquots were transferred into clot activator vials for the estimation of biochemical parameters. The serum was separated by centrifugation at 3000 rpm for 15 minutes. Hematological parameters were estimated using fully auto haematology analyzer (Shenzhen Procan Electronics Inc., Nanshan, Shenzhen, China) and the analysis of serum was conducted within 24 hours of blood extraction at room temperature using semi-auto chemistry analyzer (ARK Diagnostic System Pvt. Ltd., Mumbai, India) at School of Wildlife Forensic and Health, NDVSU, Jabalpur (M.P.). ERBA biochemical reagent kits manufactured by Transasia Bio-medical Ltd., Daman for total protein, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine and glucose were used after development of each metrics for each estimate. The methodology and set reagents used in respect to each parameter were as per the recommendations of manufacturer of analyzer system.

**2.4 Statistical Analysis**

Statistical analysis of data was carried out to find the mean± SE.

3. results and discussion

The blood samples were collected from the wild rhesus macaques 30 minutes post immobilization. The collected blood samples were analyzed for various haemato-biochemical parameters.

**3.1 Haematological parameters**

The mean values of total erythrocyte count (TEC), total leukocyte count (TLC), packed cell volume (PCV) and haemoglobin (Hb) observed in the present study were towards lower side of the normal reference range. However, the mean values of differential leukocyte count (DLC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) remained within the normal reference range (Table 1).

**Table 1: Haematology of rhesus macaques post immobilization**

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| **Variables** | **Mean ± SE** | **Normal reference values#** |
| **RBC (106/µl)** | 5.19 ± 0.04 | 5.62 ± 0.69 |
| **WBC (103/µL)** | 7.32 ± 0.16 | 7.84 ± 3.50 |
| **PCV (%)** | 37.82 ± 0.29 | 41.6 ± 4.7 |
| **Hb (g/dL)** | 11.52 ± 0.19 | 13.4 ± 1.6 |
| **MCV (fL)** | 72.86 ± 0.80 | 74.6 ± 4.9 |
| **MCH (pg)** | 22.19 ± 0.40 | 24.1 ± 1.9 |
| **MCHC (g/dL)** | 30.46 ± 0.49 | 32.4 ± 4.8 |
| **Neutrophil (%)** | 62.22 ± 1.08 | 51.5 ± 15.9 |
| **Lymphocyte (%)** | 33.15 ± 1.18 | 38.9 ± 14.6 |
| **Monocyte (%)** | 2.15 ± 0.13 | 3.5 ± 2.3 |
| **Eosinophil (%)** | 2.73 ± 0.14 | 3.9 ± 3.6 |
| **Basophil (%)** | 0.12 ± 0.03 |  |
| **Platelet (103/µL)** | 318.67 ± 14.55 |  |

# Bollinger et al., 2010

Total erythrocyte count measures the number of red blood cells i.e. erythrocytes per unit volume of blood. The function of erythrocyte is to carry oxygen from the lungs to the tissue and carbon dioxide from the tissue to lung. The mean value of TEC was 5.19 ± 0.04 million per cm3 post induction in the immobilized rhesus macaques (Table 1). The normal total erythrocyte count is 5.62 ± 0.69 million per cm3 as per Bollinger et al. (2010)and 5.90 ± 0.45 million per cm3 in wild sourced male macaques according to Shah et al.(2022). In consistence with our findings, Kanu et al. (2018) reported decrement in the value of total erythrocyte count. The mean value of TEC was 5.49 ± 0.89 million/cm3 immediately after induction which decreased to 5.15 ± 0.89 million/cm3 after 30 minutes of inductionin captive rhesus macaques who were anaesthetized by using Ketamine @ 5 mg/kg body weight and Xylazine @ 1 mg/kg body weight intramuscularly. The decrease in the value of total erythrocyte count is attributed due to relaxation of the spleen and consequent splenic sequestration of erythrocytes(Bennet et al.,1992 and Venkatesan et al., 2006).

Leukocytes are white-colored blood cells that defend animal’s body against infections and diseases. The mean value of TLC in rhesus macaque according to Bollinger et al. (2010)is 7.84 ± 3.50 thousand cells per cm3. The mean value of TLC in the present study 30 minutes after the administration of anesthesia was 7.32 ± 0.16 thousand per cm3 (Table 1) which was lower than the normal range. On contrary to our findings, Tewari (2012) reported an increase in mean value of TLC from 7.83 thousand per cm3 at early phase of anesthesia (5-10 minutes) to 9.59 thousand per cm3 at late phase of anesthesia (45-50 minutes) in rhesus macaques. Kim et al. (2005) and Venkatesan et al. (2006) stated that the reduction in leukocyte count occur due to redistribution of WBC from circulation into extravascular pool. Therefore, the reason behind reduction in leukocyte count in our study might be due to the redistribution of white blood cells from circulation to extravascular pool.

Packed cell volume is the proportion of blood occupied by RBCs expressed in percentage. The mean ± SE values of packed cell volume after 30 minutes of anesthesia in the present study was 37.82 ± 0.29 % which is towards lower side as compared with the normal range (Table 1). Our findings are partially in consistence with Tewari(2012) who reported the mean value of PCV was 42.39 % at early phase (5-10 minutes) and 38.55% at late phase of anesthesia (45-50 minutes) in rhesus macaques immobilized by single syringe combination of Ketamine @ 2 mg/kg and Xylazine @ 8 mg/kg bodyweight. The decrease in PCV after immobilization can be attributed to reversal of excitement or alarm reaction due to anesthesia induced by an adrenolytic catecholamine decrease leading to relaxation of spleen and redistribution of red blood cells from blood circulation into spleen(Tewari, 2012) or may be due to sequestration of erythrocytes in the capillary beds (Wall et al., 1985). There was a decrease in the PCV values after anesthesia in most of the studies. The variations observed in the degree of decrement might be due to the different anesthetic combinations and the doses used; and also due to the species and captive or wild status of the macaques.

Haemoglobin functions as good buffer. By this action, it regulates the hydrogen ion concentration and thereby play role in the maintenance of acid-base balance. The mean ± SE value of haemoglobin in the present study was 11.52 ± 0.19 g/dL which is lower than the normal reference range (Table 1). The decreases in hemoglobin concentration following Ketamine injection was attributed to a reversal of stress or "alarm reaction’ or due to relaxation of the spleen and consequent splenic sequestration of erythrocytes (Bennet et al., 1992 and Venkatesan et al., 2006).

**3.2 Biochemical parameters**

The observed mean value of total protein in the present study was 6.08 ± 0.17 g/dL which is slightly towards the lower range as compared to 7.8 ± 0.5 g/dL reported by Buchl and Howard (1997) (Table 2). Similar finding on the total protein was also reported by Bennet et al., (1992)who found a decrease in the total protein value from 8.1 ± 01 g/dL to 7.1 ± 0.1 g/dL before and after 15 minutes of Ketamine injection in rhesus macaques. The decrease in total protein values suggests osmotic haemodilution, perhaps related to hyperglycemia leading to influx of fluid into the vascular space (Hernandez‐Godinez et al., 2019).

**Table 2: Serum biochemistry of rhesus macaques post immobilization**

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| **Variables** | **Mean ± SE** | **Normal reference values\*** |
| **Total protein (g/dL)** | 6.08 ± 0.17 | 7.8 ± 0.5 |
| **Alanine transaminase (U/L)** | 35.43 ± 1.19 | 33 ± 8 |
| **Aspartate aminotransferase (U/L)** | 42.10 ± 1.34 | 38 ± 10 |
| **Blood urea nitrogen (mg/dL)** | 23.32 ± 0.91 | 20 ± 3 |
| **Creatinine (mg/dL)** | 1.02 ± 0.06 | 1.1 ± 0.1 |
| **Glucose (mg/dL)** | 87.77 ± 2.17 | 76 ± 16 |

\* Buchl and Howard (1997)

Alanine transaminase (ALT), also known as serum glutamic pyruvic transaminase (SGPT) is an enzyme found in a variety of tissues but is mainly found in liver and act as an important biomarker to determine the health status of liver. Aspartate aminotransferase (AST), also known as serum glutamic oxaloacetic transaminase (SGOT), is a protein made by liver cells. When liver cells are damaged, AST leaks out into the blood stream and the level of AST in the blood get elevated. The mean value of ALT and AST observed in the present study was 35.43 ± 1.19 U/L and 42.10 ± 1.34 U/L respectively which is higher than the normal reference value reported by Buchl and Howard (1997) (Table 2). These findings can be attributed to the fact that Ketamine and Xylazine affect the liver through metabolization of these drugs and Ketamine when combined with proteases can lead to hepatic inflammation. This leads to the elevation of liver enzymes (Lugo‐Roman et al., 2010).

The mean value of blood urea nitrogen (BUN) and Creatinine in our study was 23.32 ± 0.91 mg/dL and 1.02 ± 0.06 mg/dL respectively which is within the normal reference range as reported by Buchl and Howard (1997) (Table 2).These values remained within the normal reference range that might be attributed to the fact that the immobilizing drugs used in the present study were all readily metabolized by the liver before they are subjected to renal clearance(Lugo‐Roman et al., 2010).

The mean value of serum glucose in the present study was 87.77±2.17 mg/dL, which is higher than the normal reference value of 76±16 mg/dL reported by Buchl and Howard (1997) (Table 2). Xiao et al., 2013 also reported that the intramuscular injection of Xylazine (1-2 mg/kg) significantly increased blood glucose in normoglycemic cynomolgus monkeys. The increased level of serum glucose post anesthesia is due to the administration of Ketamine alongwith Xylazine. This can be attributed to the fact that α2-adrenoreceptor agonists have inhibitory effect on the β-cells and a stimulatory effect on the α-cells of pancreas. This leads to the suppression of release of insulin and stimulation of glucagon release, respectively (Sinclair, 2003 and Vaughan et al., 2014)

**4. CONCLUSIONS**

The study on hematological and serum biochemical values in wild adult male rhesus macaques in different areas of the Territorial Forest Division, Jabalpur immobilized by Inj. Xylazine and Inj. Ketamine combination is reported first time, which may serve as guidelines for future studies for the same species.

**DISCLAIMER**

Authors hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

**ETHICAL APPROVAL**

The research described in the study was conducted in compliance with the ethical standards and guidelines of the Institutional Animal Ethics Committee (IAEC) and due permission was received from the ethical committee of the University.

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