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# Haematological Profile of Various Inbred Strains of Mice Maintained at IMTech Centre for Animal Resources and Experimentation (iCARE)

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## Abstract

Animal models are frequently employed in scientific studies to collect data from an entire set of experiments. Haematological tests on these laboratory mice are essential for confirming scientific findings because diverse mouse strains have been used to examine various diseases and novel therapeutic methods. This work is aimed to establish haematological values in mouse strains used in biomedical research. Blood samples were taken from the retro-orbital sinus of mice from various strains and haematological parameters including total White Blood Cells (WBC), Red Blood Cells (RBC), Haemoglobin (HGB), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Red Blood Cell Dimension Width ( RDW), platelets, Mean Platelet Volume (MPV) and Platelet Dimension Width (PDW) were assessed. Compared to other strains (BALB/c, M:9.61, F:9.51; C57BL6/N, M:10.13, F:10.73; DBA/2, M:11.96, F:10.88), the mean values of WBCs (*$10^3$/µL) in DBA/1 mice were higher (M:14.02; F:14.65) and lower in NOD (M:7.14; F:6.54). The mean RBCs in different strains of mice used in this study ranged from 8.78-11.67*$10^6$/µL. Male mean haemoglobin valves in BALB/c, C57BL/6N, DBA/1, DBA/2 and NOD were 14.60, 13.32, 13.00, 14.35 and 13.48 (g/dL), respectively, while in females were 14.3, 14.09, 13.11, 12.65 and 13.24 (g/dL), respectively. The mean platelet counts in NOD mice (M:1826.60 and F:1557.80*$10^3$/µL) were significantly higher than other strains. Haematological profiles of the mice used in the study were consistent as reported previously for mice maintained in different animal facilities. The haematological values presented in this study can be used as reference values in biomedical research.

**Keywords:** BALB/c, C57BL/6N, DBA/1, DBA/2, Haematology, Mice

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# 1. Introduction

Mice and rats constitute nearly 95% of the experimental animals used in biomedical research. *Mus musculus* belongs to the order Rodentia and is the most commonly used species in clinical research. The advantages of using the mice as a test model are numerous, including cost-effectiveness, small size, easy housing, and maintenance, small reproductive cycle (21 days), and shorter lifecycle. Also, information regarding their anatomy, genetics, biology, and physiology are well documented[1]. The mechanisms behind many diseases have been better understood because of developments in transgenic mice, opening up new therapeutic avenues in both human and veterinary medicine[2]. BALB/c and C57BL/6 are inbred strains, genetically homogenous populations helping researchers use fewer mice. 99% of the alleles in BALB/c mice are homozygous, employed to generate monoclonal antibodies from plasmocytes[3] and immunological and infectious disease research. The C57BL/6 mice are frequently employed in investigations on cardiovascular disease, developmental biology and genetics, as well as immunological and neurological studies. Sub-lineages like C57BL/6J, are frequently employed in creating transgenic mice[4]. DBA/1 is a suitable model for immunology and inflammation research specifically for autoimmune diseases like rheumatoid arthritis and atherosclerosis[5]. DBA/2 is an inbred strain which has a low propensity for atherosclerotic aortic lesions, and a propensity for hearing loss in the high frequencies, audiogenic seizures, and the emergence of progressive eye abnormalities that closely resemble human hereditary glaucoma[6]. The Non-Obese Diabetic (NOD) mice are the model of Type I Diabetes that replicates several aspects of human diabetes[7].

The haematological and biochemical parameters of mice vary depending on age, nutrition, environment, routes of collection, and other aspects. The physiological profile of essential biological structures can be evaluated using haematological and biochemical markers, which can also be documented to generate reference values for clinical disease diagnosis. Although articles explaining laboratory parameters for healthy mice have been published in the literature, several research labs need more the capacity to develop their own set of reference values for use in their investigations[8,9]. It is crucial to appreciate variations and the distinctive characteristics of mouse haematology to conduct thorough and reliable haematologic studies of disease and response to medications for exploratory therapy. Increased knowledge of pathogenic pathways and cross-species experimental therapies has resulted from the discovery of disease-replicating mouse models, which has benefited biomedical research[10]. The normal ranges for several haematological evaluation measures must be understood since pathological processes can alter metabolism and change the outcomes of experimental methods. Haematologic values are important disease markers in both human and veterinary medicine. Established haematological reference standards in mice are necessary for assessing their health and well-being. Still, there isn't much data on these values in the literature because they primarily serve as a comparative base. In order to assist researchers in selecting the ideal experimental model, we checked numerous haematological parameters in different strains of mice namely, BALB/c, C57BL/6N, DBA/1, DBA/2, and NOD.

# 2. Materials and Methods

## 2.1 Animals

All the procedures described in this paper were sanctioned by the Institutional Animal Ethics Committee of CSIR-Institute of Microbial Technology (CSIR-IMTech), and the approval number is IAEC/QC/1. A barrier-maintained animal facility, namely, IMTech Centre for Animal Resources and Experimentation (iCARE) CSIR-IMTech, Chandigarh, keeps multiple inbred strains of mice in Individually Ventilated Cage systems (IVCs) in various rooms. Healthy, 4-8 week old mice, BALB/c (n = 60 Male and 60 Female), C57BL/6N (n = 60 Male and 60 Female), DBA/1 (n = 25 Male and 25 Female), DBA/2 (n = 25 Male and 25 Female) and NOD (n = 10 Male and 10 Female) from the breeding stock were used in this study and sample size was based on size of the breeding colonies of various inbred mice. All mice were kept in standard environmental conditions, including 24-25 °C temperature, 30-70 % relative humidity, and light/dark cycle of 12 hours, as well as free access to pelleted feed and water. The mice were closely monitored all times for any unusual behaviours. When the blood

samples from the mice were collected, no skin lesions or inflammations were visible upon examination.

## 2.2 Mice Handling and Blood Sampling Method

The Committee for Control and Supervision of Experiments on Animals (CCSEA), Ministry of Fisheries, Animal Husbandry, and Dairying, India, established guiding principles for conducting experiments on mice to minimise discomfort and suffering in animals by administering analgesics and/or anaesthetics were followed. A mouse was placed on a surface, such as the wire cage top or lid, after being gently yet firmly grabbed at the base of the tail with one hand. Using the opposite hand's thumb and first finger, while moving forward on the back to the neck, touching the ears gently pressed down to grab the skin and make a scruff. Lifted the mouse, secured the tail with ring finger and restrained the mouse with one hand. The local ophthalmic anaesthetic, Paracain (Proparacaine hydrochloride, 0.5%) was applied on to the eyelids and eyeball prior to blood collection. Blood was withdrawn from the retro-orbital sinus using a haematocrit capillary tube with the other hand's assistance. Immediately after collection, 20µl of fresh blood samples were added to 1.6ml diluent.

## 2.3 Blood Analysis

The diluted blood samples were processed on an automatic haematology analyser (Mindray BC-2800Vet, China).

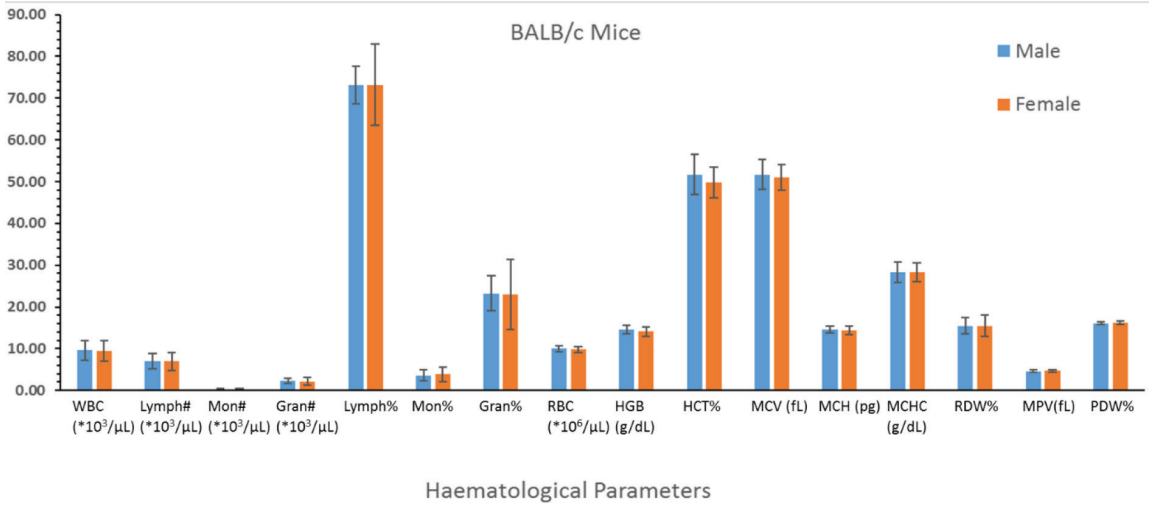



**Figure 1.** Haematological values of BALB/c mice (n = 60).

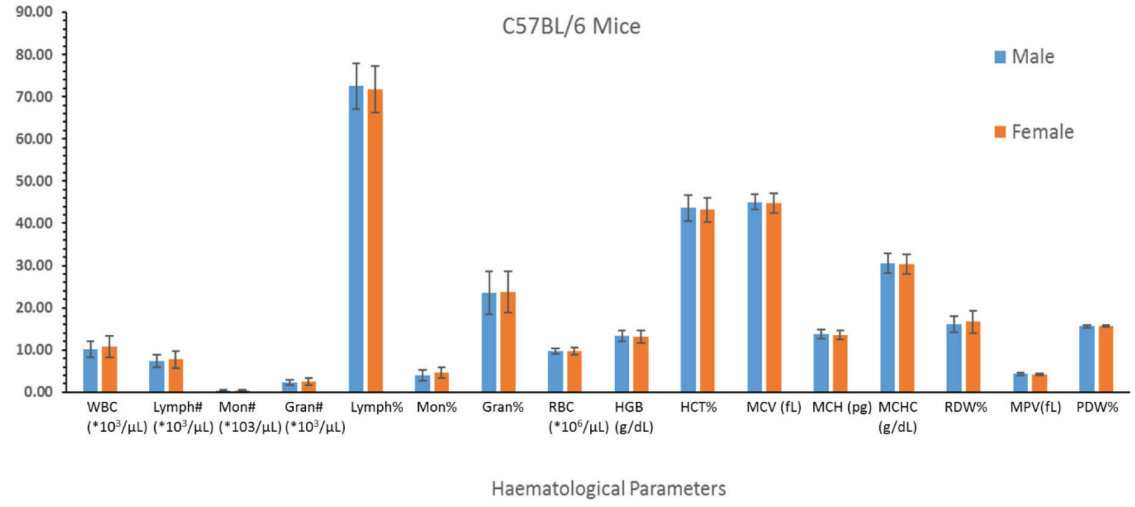



**Figure 2.** Haematological values of C57BL/6N mice (n = 60).

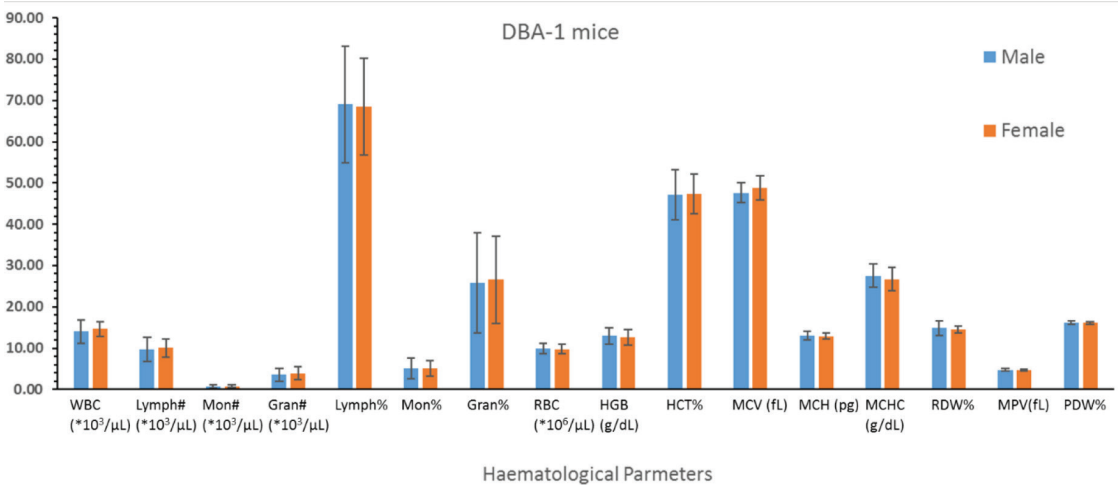



**Figure 3.** Haematological values of DBA/1 mice (n = 25).

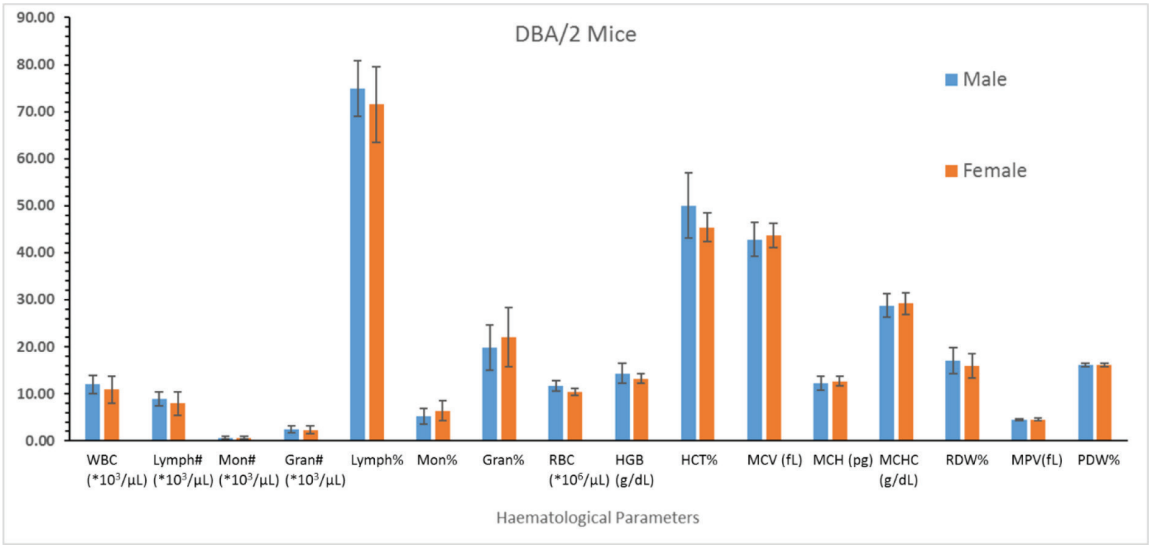



**Figure 4.** Haematological values of DBA/2 mice (n = 25).

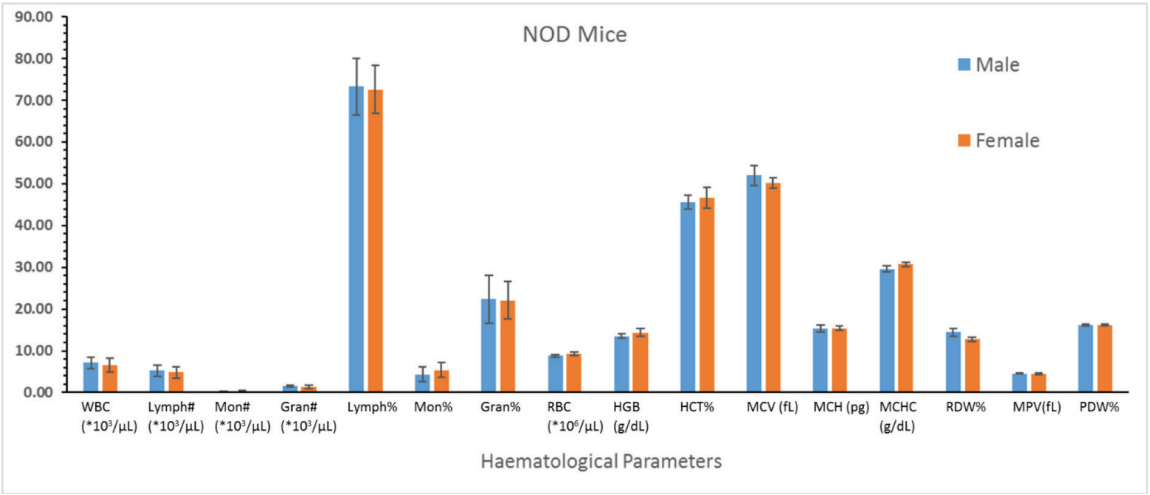



**Figure 5.** Haematological values of NOD mice (n=10).

**Table 1.** Haematological values of different strains of mice

| Strains of Mice | BALB/c | | C57BL/6N | | DBA/1 | | DBA/2 | | NOD | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Parameters** | **Male** (n = 60) | **Female** (n = 60) | **Male** (n = 60) | **Female** (n = 60) | **Male** (n = 25) | **Female** (n = 25) | **Male** (n = 25) | **Female** (n = 25) | **Male** (n = 10) | **Female** (n = 10) |
| WBC (*$10^3$/µL) | 9.61±2.36 | 9.51±2.40 | 10.13±1.86 | 10.73±2.54 | 14.02±2.78 | 14.65±1.78 | 11.96±2.01 | 10.88±2.91 | 7.14±1.36 | 6.54±1.73 |
| Lymphocyte (*$10^3$/µL) | 7.07±1.88 | 6.99±2.13 | 7.37±1.53 | 7.73±1.95 | 9.79±2.92 | 10.05±2.17 | 8.96±1.51 | 7.89±2.54 | 5.29±1.35 | 4.79±1.35 |
| Monocyte (*$10^3$/µL) | 0.33±0.15 | 0.36±0.20 | 0.39±0.15 | 0.47±0.15 | 0.68±0.33 | 0.73±0.29 | 0.62±0.25 | 0.65±0.27 | 0.28±0.09 | 0.34±0.16 |
| Granulocyte (*$10^3$/µL) | 2.21±0.61 | 2.16±0.91 | 2.37±0.62 | 2.54±0.79 | 3.55±1.50 | 3.87±1.55 | 2.38±0.78 | 2.34±0.77 | 1.57±0.29 | 1.41±0.44 |
| Lymphocyte % | 73.14±4.45 | 73.16±9.74 | 72.47±5.37 | 71.72±5.46 | 69.02±14.05 | 68.40±11.70 | 74.88±5.86 | 71.54±8.03 | 73.28±6.79 | 72.57±5.68 |
| Monocyte % | 3.62±1.40 | 3.86±1.79 | 4.04±1.21 | 4.60±1.24 | 5.12±2.44 | 5.01±1.87 | 5.26±1.68 | 6.36±2.16 | 4.34±1.71 | 5.41±1.86 |
| Granulocyte % | 23.25±4.16 | 22.99±8.41 | 23.50±5.11 | 23.72±4.86 | 25.86±12.14 | 26.59±10.60 | 19.86±4.77 | 22.09±6.28 | 22.38±5.75 | 22.02±4.50 |
| RBC (*$10^6$/µL) | 10.00±0.65 | 9.79±0.67 | 9.71±0.62 | 9.67±0.82 | 9.93±1.23 | 9.74±1.17 | 11.67±1.03 | 10.43±0.70 | 8.78±0.25 | 9.27±0.47 |
| HGB (g/dL) | 14.60±1.00 | 14.09±1.21 | 13.32±1.23 | 13.11±1.45 | 13.00±1.98 | 12.65±1.83 | 14.35±2.05 | 13.24±1.06 | 13.48±0.50 | 14.33±0.98 |
| HCT % | 51.69±4.84 | 49.74±3.66 | 43.66±3.11 | 43.17±2.93 | 47.16±6.14 | 47.31±4.82 | 50.02±6.88 | 45.42±3.11 | 45.53±1.65 | 46.57±2.55 |
| MCV (fL) | 51.73±3.55 | 51.02±3.02 | 45.04±1.80 | 44.80±2.29 | 47.58±2.41 | 48.84±2.98 | 42.83±3.54 | 43.65±2.58 | 51.98±2.36 | 50.27±1.25 |
| MCH (pg) | 14.56±0.82 | 14.38±0.97 | 13.68±1.07 | 13.51±1.07 | 13.02±1.01 | 12.93±0.69 | 12.25±1.44 | 12.66±0.96 | 15.33±0.76 | 15.40±0.54 |
| MCHC (g/dL) | 28.35±2.41 | 28.33±2.29 | 30.50±2.40 | 30.29±2.34 | 27.54±2.88 | 26.70±2.80 | 28.72±2.52 | 29.17±2.37 | 29.59±0.73 | 30.69±0.59 |
| RDW% | 15.47±1.96 | 15.45±2.57 | 16.06±1.93 | 16.64±2.65 | 14.84±1.73 | 14.46±0.80 | 16.99±2.81 | 15.89±2.55 | 14.39±0.97 | 12.73±0.53 |
| PLT (*$10^3$/µL) | 1210.95± 222.50 | 1218.52± 74.45 | 1371.02± 259.89 | 1291.62 ±196.59 | 1189.16± 207.97 | 1307.80± 245.57 | 1043.40± 166.22 | 1095.44± 159.45 | 1826.60± 184.06 | 1557.80± 136.28 |
| MPV(fL) | 4.65±0.34 | 4.67±0.28 | 4.35±0.35 | 4.27±0.19 | 4.70±0.30 | 4.66±0.21 | 4.44±0.18 | 4.54±0.21 | 4.52±0.14 | 4.48±0.15 |
| PDW % | 16.08±0.34 | 16.16±0.38 | 15.66±0.33 | 15.69±0.22 | 16.16±0.35 | 16.09±0.29 | 16.08±0.41 | 16.08±0.36 | 16.10±0.23 | 16.09±0.20 |

### 2.4 Statistical Analysis

In an Excel data sheet, the mean and standard deviation were determined. The data acquired was described as mean value and standard deviation (Mean±SD).

## 3. Results

The results are presented in the bar graph separated by strains viz. BALB/c (Figure 1), C57BL/6N (Figure 2), DBA/1 (Figure 3), DBA/2 (Figure 4) and NOD (Figure 5) mice, and Table 1. The data are provided, being reflected as a reference of haematology from the iCARE Facility. In order to determine the total and percent numbers of each distinct leukocyte type such as lymphocytes, monocytes, and granulocytes, we used a three-part differential cell counter haematology analyser. All male and female haematological values were similar in respective strains of mice. Interestingly, the mean platelet count in NOD mice was significantly higher than published literature, but otherwise, we did not find any differences between the haematological values of the mice and previous published data of these strains (Table 1).

## 4. Discussion

Making reference haematological values are crucial to interpret differences brought on by diseases. The evaluation of haematological parameters are relevant to figuring out the physiological profile of various stocks of mice[11]. Therefore, in this study, we evaluated the haematological parameters of different inbred strains *viz.* BALB/c, C57BL/6N, DBA/1, DBA/2, and NOD mice housed at the iCARE facility for last 10-15 years. Haematological indicators are biomarkers for detecting infections, parasites, and other pathologies, for diagnosing organ or tissue damage, animal reproduction, and other disorders[9]. Peripheral blood samples can reveal bodily anomalies that frequently lead to numerous health risks, such as autoimmune or metabolic illnesses. The circulatory system carefully planned and managed to ensure harmonious functioning, connects various body systems[12]. Numerous pre-analytical factors, such as strain, sex, age, circadian cycle, and nutrition, affect the haematological parameters of mice. Also, collecting reliable haematology data from mice requires minimizing the effects of animal handling and sample processing. The RBCs, WBCs, and platelet numbers reported for BALB/c and C57BL/6 in our study are consistent with the haematological profile published for male and female mice of the same age[13,14]. Fukuda *et al.,* used three mouse strains, C57BL/6, BALB/c, and DBA/2 in similar analyses of mouse haematological parameters with a haematology analyser. They recommended that the mouse stock be taken into account while conducting haematological assessments[15]. The haematological values similarly influence the experiment model and study plan[9].

The evaluation of the phenotypes and pathogenesis of mouse models of human and veterinary diseases, as well as the evaluation of responsiveness to novel treatment studies are all made possible and practicable by high-quality haematology tests[10]. The findings of this study are consistent with that data from Charles River, Jackson, and Taconic Lab[13,14,16-20] and other researchers[8,9,15]. The creation of reference values are crucial because, as previously mentioned, several variables, including age, gender, genetic variability, food, and environmental situations, could affect the findings of these investigations. Use of healthy mice also result in reproducible results which further will help the scientific community in reducing the number of mice as per the principles of 3 R's, i.e., reduction, replacement, and refinement.

## 5. Conclusion

The study results are presented as a range of haematological values that are regarded as reference values suitable for biomedical research.

## 6. Acknowledgement

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