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Reference values for selected hematological, biochemical and physiological parameters of Sprague-Dawley rats at the Animal House, Faculty of Medicine, University of Colombo, Sri Lanka

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Abstract

Background: Lack of available reference values in a research setting under local conditions can be a drawback for beginners, as the accuracy of data from control samples cannot be checked at the beginning of a research project. This affects comparisons with data from test samples. To avoid these complications in their research projects, beginners tend to have a greater number of animals in the control group compared to test groups in order to have control group measurements within 2 SDs of the mean.

Methods: As non-availability of reference values was a long-felt need, the described project was conducted in order to establish a reference database for selected haematological, biochemical and physiological parameters using apparently healthy Sprague-Dawley rats bred in the Animal House of Faculty of Medicine, University of Colombo (UCFM).

Results: Differences in mean values of packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), serum creatinine and blood glucose levels between the two genders were statistically significant. Lipid profile measurements did not differ significantly between genders, but mean and median values of triglycerides (TG) between male and female rats showed a difference of more than 10 mg/dL. The liver enzymes alkaline phosphatase (AP) and aspartate aminotransferase (AST) were also statistically significantly different between sexes. Despite wide variation in mean alanine aminotransferase (ALT) between sexes, the difference was not statistically significant.

Conclusion: The findings of this project should support to a certain extent the "Reduction" aspect of the 3Rs concept of Russell and Burch by reducing the number of Sprague-Dawley rats used in future research projects at UCFM.

KEYWORDS
3Rs concept, rat, reference values, Sprague-Dawley

1 | INTRODUCTION

About 95% of all lab animals in medical research are rats and mice bred specifically for research purposes. The reasons for their wide usage include their small size, ease of handling and housing, rapid reproduction, short life span and the ability to observe several generations in a short period of time. Rats also share about 95% of the human DNA and hence are more or less susceptible to similar diseases to humans and respond to treatments in a similar manner.[1-3]

Numerous strains of rats, both inbred and outbred, are used in experimental research. Out of the strains of rats available for research, Sprague-Dawley rats, an outbred albino strain of rats that shows calmness and ease in handling, are widely accepted and a general-purpose research model used in many studies. Their research applications include toxicology, safety and efficacy testing, reproduction and development, behavior, nutrition and pharmacology studies.[4,5] This strain of rats is widely used in Sri Lanka. They were introduced to the Animal House of the Faculty of Medicine, University of Colombo in 1977 and the Animal House has been supplying Sprague-Dawley rats for various research projects through continuous breeding for many generations.

When rats are obtained from an animal breeder, the breeder publishes a set of reference values for the different parameters measured under a given set of conditions. Those values can be used in research when it is necessary to confirm whether values obtained for the control group are within the reference range. However, such a set of reference values is currently unavailable for the rats bred under local conditions in the Animal House of UCFM. In addition, the present generation of Sprague-Dawley rats bred in the Animal House was produced through inbreeding initially and later through outbreeding for many generations and may have undergone significant genetic and physiological changes over the years. The factors that influence the reference data supplied by the animal breeder might now be different from those that were available for the first generation of rats obtained by the Animal House.

Data published in international studies carried out in the Animal houses, when compared with breeders' information, indicates that there are similarities and at the same time certain variations in the outcome.[6-9] In the past there have been instances in Sri Lanka when researchers faced problems of accepting the values of different measurements of rats in the control group and then comparing them with that of test groups of animals, due to the inconsistency of the values obtained.

Thus, establishing a reference database for different parameters of the Sprague-Dawley rats in the Animal House of UCFM will be of great importance for future researchers and the present study reports measurements of several selected hematological, biochemical and physiological parameters of Sprague-Dawley rats at the Animal House of UCFM.

2 | MATERIALS AND METHODS

2.1 | Animals

Apparently healthy 4- to 5-month-old male (n = 10) and female (n = 10) Sprague-Dawley rats bred in the Animal House, UCFM, Sri Lanka were used for the study (We did not adopt any statistical method to calculate sample size in this research project and kept the sample size to a minimum as indicated in the ethical application submitted for approval.) The rats were fed with a pelleted diet (Vet House Pvt. Ltd, Sri Lanka) and tap water ad libitum. All the animals were housed and treated in accordance with internationally accepted laboratory animal use and care guidelines and the guidelines for the ethics review of research proposals involving animals in Sri Lanka. During the study animals were housed for 6 days per week in standard rat cages based on sex (group housing, five animals per cage, 1344 cm^2 floor area per cage, with saw dust and wood peeling as the bedding material from a retail outlet for animal requirements, Colombo, Sri Lanka) and for 1 day per week they were kept in metabolic cages for data collection (water intake, food intake, urine output and body weight) under the necessary environmental conditions (temperature 23 ± 2°C, humidity 55%-60%, 12/12 hours light-dark cycles, ventilation set at 10-15 changes per hour). The bedding was changed and the cages were cleaned daily. The Ethics Review Committee of UCFM granted ethics approval for the project (EC-14-028).

2.2 | Sample collection

Before recruitment to the study, the body weight, and food and water consumption of all the rats were recorded.

Approximately 1 ml of blood was drawn from the tail vein of each rat once a week, between 8.30 and 10.00 AM. This was repeated for 15 consecutive weeks, in order to perform selected hematological and biochemical parameter measurements in alternate weeks. This arrangement permitted analysis of 50 samples for each hematological and biochemical parameter for each gender using 10 animals over the 15-week period. EDTA anti-coagulated blood was used in hematological investigations and serum was used in biochemical investigations.

Hematological investigations performed by manual methods included red blood cell count (RBC), white blood cell count (WBC), platelet count (PLT), differential count (DC), hemoglobin concentration ([Hb]), packed cell volume (PCV), or hematocrit. PCV was measured by the micro-hematocrit method using capillary tubes while RBC and WBC were measured manually using an improved Neubauer counting chamber. The differential count and the platelet count were measured manually using a thin blood film stained with Leishman stain and hemoglobin concentration was determined by Shali's method. Although the cyanomethemoglobin method is a more accurate measure of [Hb] than Shali's method, based on the facilities we have in the laboratory of the Department of Physiology, Shali's method was adopted for [Hb] measurement. [Hb] in all the blood samples was performed by one experienced senior technical officer to avoid any observer variations. Using RBC, PCV, and [Hb], mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated according to the standard formulae given in physiological and hematological textbooks.[10-12]

Biochemical investigations were performed using POINTE reagent kits on a semi-automated chemistry analyzer (POINTE-180, Scientific Inc., USA). These measurements included random blood glucose level (Glucose), lipid profile (total cholesterol, high density lipoprotein (HDL) and triglycerides (TG)), and serum creatinine level (Scr) and blood urea nitrogen level (BUN) for renal function and alkaline phosphatase (AP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme levels to determine liver function. Low density lipoprotein (LDL) was derived according to the formula given in the reagent kit using other measurements in the lipid profile.

Physiological parameters (measured 150 times for each gender from 10 rats during the 15-week period), such as urine output food and water intake, were determined for 24 hours per week during the study period when the animals were kept in individual metabolic cages. Measured amounts of food and water were provided at the beginning when animals were transferred to metabolic cages and at the end of 24 hours the remaining amounts were measured to determine the food and water consumption (FC and WC) over 24 hours. At the same time, urine was collected from the funnel of the metabolic cage and the total amount of urine collected was measured to determine urine output (UOP) per animal per day. Body weight (BW) was also measured once a week during the study, while behavior and the physical appearance of animals such as exploratory activity, cuddling and sleeping, consumption of water and food, appearance of fur, cleanliness of the coat and body, appearance of feces were observed throughout the study to see whether there were any deviations from the normal behavior of rats.

2.3 | Statistical presentation of data

The means ± SD (standard deviation), median and ranges of the measured parameters were computerized separately for male and female rats. Mean values of the selected hematological, biochemical and physiological parameters among males and females were compared separately by paired *t* test for statistical significance using the SPSS-2017 computer package.

3 | RESULTS

Measured hematological, biochemical and physiological parameters of rats are given in Tables 1, 2 and 3. The rats appeared normal and healthy till the end of the study and there were no major differences in the body weight, food and water consumption of animals before and at the end of the project. Although mean and median values of hematological parameters of males and female rats were closely similar to each other, based on the statistical comparisons, differences in mean values of PCV, MCV and MCHC between male and female rats were statistically significant ($P < 0.05$). Differences in renal function between male and female rats measured by serum creatinine levels were also statistically significant, as were differences in mean blood glucose level between two genders. Lipid profile measurements did not show a statistically significant difference between genders. However, mean and median values of TG differed between male and female rats by more than 10 mg/dL (mean TG difference between males and females, 11.14 mg/dL; median TG difference between males and females, 15.8 mg/dL). The liver enzymes AP and AST also showed a gender difference that was statistically significant. Despite wide variation in mean ALT between the genders, the differences were not statistically significant. Among the physiological parameters measured, differences in mean food and water consumption measurements were statistically significant. Thus, even though there were close similarities in the age, environmental conditions, water and food consumption of the rats, the results show individual variations resulting in a wide range of values for measured parameters.

TABLE 1 Mean, median and range of measured hematological parameters of UCFM Sprague-Dawley rats

	Mean ± SD		Median		Range	
Parameter	Male	Female	Male	Female	Male	Female
RBC ($\times 10^6$/μL)	5.26 ± 0.9	5.16 ± 1.0	5.36	5.05	3.8-6.68	2.9-6.8
[Hb] (g/dL)	13.63 ± 1.4	12.86 ± 1.9	13.8	13.0	10.4-16.5	8.6-15.38
PCV (%)	**39.38 ± 7.6**	**36.54 ± 8.5**	43.0	39.0	18-48	10-47
PLT ($\times 10^5$/μL)	3.44 ± 0.8	3.28 ± 1.0	3.24	3.17	1.7-5.57	1.48-6.15
WBC (per mm^3)	9142 ± 2773	7733 ± 2807	8850	8000	4400-14 800	3600-14 500
DC (%)						
Neutrophils	22.64 ± 6.4	24.79 ± 8.3	20.5	22.5	13-36	13-61
Lymphocytes	75.17 ± 6.6	72.87 ± 8.3	77.5	75.5	61-86	55-86
Eosinophils	2.17 ± 1.5	2.04 ± 1.7	2	2	0-6	0-8
Basophils	0.26 ± 0.5	0.22 ± 0.5	0	0	0-2	0-2
Monocytes	0.18 ± 0.4	0.16 ± 0.4	0	0	0-1	0-1
MCV (fL)	**77.04 ± 20.1**	**73.53 ± 22.9**	77.52	75.13	29.41-123.07	15.15-119.44
MCH (pg)	26.53 ± 4.8	25.7 ± 6.2	25.37	25.35	18.37-36.98	13.07-41.57
MCHC (g/dL)	**36.69 ± 12.5**	**37.61 ± 12.8**	31.82	33.79	25.41-80.55	21.16-95.0

Statistically significant data and parameters are bold.

TABLE 2 Mean, median and range of measured biochemical parameters of UCFM Sprague-Dawley rats

	Mean±SD		Median		Range	
Parameter	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Glucose (mg/dL)	**106.51 ± 34.6**	**112.15 ± 38.9**	101.8	106.35	62.4-201.8	56.1-197.2
Total cholesterol (mg/dL)	41.06 ± 14.9	45.20 ± 14.5	42.8	43.9	14.4-81.7	20.4-87.6
TG (mg/dL)	21.74 ± 12.1	32.88 ± 15.2	18.3	34.1	2.7-47.8	8.7-60.7
HDL (mg/dL)	22.79 ± 12.6	30.15 ± 12.1	22.4	29.7	9.7-42.1	0.2-63.3
LDL (mg/dL)	13.75 ± 16.5	8.03 ± 18.4	13.04	6.4	–20.66 to 49.82	–28.68 to 49.32
Scr (mg/dL)	**0.49 ± 0.30**	**0.52 ± 0.27**	0.4	0.4	0.2-1.2	0.2-1.2
BUN (mg/dL)	28.87 ± 6.7	26.0 ± 9.6	27.8	24.34	17.26-45.12	12.33-77.6
AP (U/L)	**545.27 ± 181.8**	**367.93 ± 120**	537.25	388.65	160.8-838.3	195-724.2
AST (U/L)	**215.83 ± 199**	**203.51 ± 106**	167.2	177.7	0.2-838.3	20.8-470.2
ALT (U/L)	93.7 ± 71.6	136.42 ± 64.7	106.2	134.95	1-223.3	2.1-426.9

Statistically significant data and parameters are bold.

TABLE 3 Mean, median and range of measured physiological parameters of UCFM Sprague-Dawley rats

	Mean ± SD		Median		Range	
Parameter	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
UOP (ml/rat/d)	8.9 ± 6.3	9.0 ± 4.3	7.0	8.5	1.5-24.5	1.0-20.5
WC (ml/rat/d)	**265 ± 35**	**223 ± 39**	275	225	200-350	150-325
FC (g/rat/d)	**108 ± 11**	**103 ± 12**	107	101	80-134	75-149
BW (g)	216.06 ± 24.5	198.35 ± 16.3	218	195	113-275	160-233

Statistically significant data and parameters are bold.

4 | DISCUSSION

In the long history of medical research, animals have always been important investigation tools. The use of animals enables the researcher to carry out experiments on development of new pharmaceuticals, vaccines, new surgical materials and procedures, investigation of diseases, safety and toxicity testing of different substances, etc. Since humans cannot be used for most of these experiments, animals make a good substitute. In this context, the hematological, biochemical and physiological parameters of rats are of significance to researchers as they are used to evaluate vital information about the response of the body to different diseases and treatment. These parameters depend on various factors including age, nutrition, environment and genetic factors, and changes in any of these conditions would affect the reference values of the above-mentioned parameters.

The current study was the first to be conducted with the objective of establishing a reference database for selected hematological, biochemical and physiological parameters of the Sprague-Dawley rats in the Animal house of UCFM for the benefit of future researchers. Although there were significant differences in several measurements between the sexes, this could not be related to the micro- and macro-living environments of the animals, the food and water source and supply, bedding in the cages and cleaning of the cages, as all these were common to all animals recruited for the study. Bleeding once a week for 15 weeks might have subjected the animals to a stressful situation. Repetitive bleeding over a long period could be a reason for the significant differences observed in the PCV, MCV and MCHC values between male and female rats. Other than this, the differences observed could only be attributed to individual differences, which may be related to genetics resulting from inbreeding and outbreeding.

We observed similarities and differences among our measured values compared with those of international breeders and researchers.[4,6,9,13-15] Mean RBC, [Hb], PCV, WBC and differential neutrophil and lymphocyte count parameters in the present study were low compared to the values for male and female SD rats (n = 5 for each gender, with a single blood sample per animal) determined by Ingle et al, in India,[13] while mean MCV, MCH and MCHC values were higher in the current study than those of Ingle et al. This is a good example of how, using the same species, researchers observe differences in measured parameters, based on the age of the animals used, site of blood collection, number of blood collection times, number of samples analyzed and the method of determination of the parameters. Furthermore, not all studies report the ranges for all hematological parameters based on gender differences. The measured hematological parameters such as [Hb], PCV and platelet count of SD rats in the control group in the study conducted by Gunatilake et al in 1996, although closely similar, are higher than the values obtained in the present study.[14] However, measured

data in control rats in a study by Perera et al, who conducted research at the Animal House of UCFM in recent years, are within our reference values.[15] Variation in the differential count of neutrophils and lymphocytes is a normal finding in rats compared to humans.[2]

The users of the "Interspecies Database (www.interspeciesinfo.com)" of the 3Rs-Centre Utrecht Life Sciences, which provides data on physiological, anatomical and biochemical parameters of different animal species and humans, report an average 20% reduction in the animal use annually. Similarly, we hope that the reference values determined for the UCFM Sprague-Dawley rat model will be helpful for future generations of researchers for comparison with their test group values and thereby contribute to a reduction in the number of animals used in future research projects.

5 | CONCLUSION

It is evident that measured hematological, biochemical and physiological parameters of SD rats can be affected by different factors/conditions. These reference values may help to verify results using Sprague-Dawley rats as a model and also to reduce to some extent the number of rats in the control groups of future research projects.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

SLD, MG, VB and MDS contributed to the development of the proposal, conducted statistical analysis and wrote the manuscript. MLBD, SLD and MDS conducted the project and collected samples. SSB, AHU and PBW analyzed the samples.

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