Establishment of Historical Control Data on Rats and Mice for Preclinical Studies

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ABSTRACT

Toxicology studies and other biomedical research can only be interpreted when adequate reference ranges established from healthy control animals are available. Each animal species being evaluated requires a unique set of reference values for various hematology and biochemical parameters. The stock animals were selected from Mass Biotech, Chengalpet, Tamil Nadu, India, during the years 2019 to 2023. The selected animals were found to be healthy under veterinary examination. Wistar rats (*Rattus norvegicus*) and Swiss albino mice (*Mus musculus)* were selected from the age group of 5-12 weeks. Heamatology, biochemistry, feed consumption and body weight were estimated and mean reference range was tabulated with standard deviation. The mean SGPT value of male rat was 70.16; the female rat was 41.14. The mean SGOT value of the male rat was 124.49, and that of the female rat was 95.3. The mean SGPT value of female mice was 31.14, and that of male mice was 46.72. The mean SGOT was 64.31 in female mice and 170.83 in male mice. The mean ALP value of female mice is 147.7. The mean haemoglobin value of male mice is 9.22, and the mean RBC and WBC count is 3.30 and 6.39, respectively. Other hematology and biochemical parameters of both sexes were estimated. The estimated variations in each parameter, with wider ranges, might be due to animal sources, geographical variations in terms of environment and genetic factors. So, the estimated values could be comparable with the data generated in similar geographical regions.

Keywords: Rodents, Haematology, Biochemistry, Reference range value

INTRODUCTION

Toxicology research can be interpreted only when adequate reference values are established from the healthy control animals. Each animal species being evaluated requires a unique set of reference intervals (Pessini *et al*., 2020). Numerous physiological factors, such as age, sex, housing, food and water intake, circadian rhythm, increased or decreased exercise, stress, or sexual cycle, all affect these parameters (de Kort *et al.*, 2020). Historical data from laboratory animals are used to interpret the toxicity and effectiveness of food additives, pharmaceuticals, and chemicals. However, despite their importance and value, these data are influenced by factors such as age, breeding condition, diet, measurement tool, and testing method (Keenan *et al.*, 2009; Lee *et al.*, 2012). Because it is useful to compare data from studies with historical control data to assess toxicity, the availability of historical control data is crucial (Geerlofs *et al.*, 2020). For proper interpretation of experimental results and assessment of reproductive and developmental toxicity, historical control data on studies of toxicity are helpful. To distinguish between changes caused by treatment and those that occur naturally in specific species or strains, historical control data are valuable (Ema *et al.*, 2014). The findings of toxicity and haematology studies are also explained by creating control groups. However, when the control data are inconsistent or the experimental values are at the extremes of the control data, such results are challenging to interpret. As a result, a random set of animals from a breeding facility is selected for historical data, which allows researchers to compare the test findings to the historical control group. Rats and mice are

the more widely used laboratory animal species for standardising developmental toxicity testing, but rats have been employed extensively in toxicological investigations, pharmaceuticals and many other biomedical research.

MATERIALS AND METHODS

Mass Biotech is a breeding facility and research organization recognized by the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India, since 2019 onwards. As the facility supplies lab animals for research purposes to CCSEA-registered institutions, contract research organisations, academics, biomedical research units and universities, it is inevitable to maintain reference control data to support any analysis of animal experiments. The healthy animals were selected from Mass Biotech, Chengalpet, Tamilnadu, India, during the years 2019 to 2023. As the breeding colony was maintained in-house for both Wistar rat and Swiss albino mice, it was convenient to select the different age groups of required animals. Wistar rats (*Rattus norvegicus*) and Swiss albino mice (*Mus musculus)* were selected from the age group of 5-12 weeks. The data of both species were collected from regular health monitoring programs from 2019 to 2023, as well as specifically allotted animals for reference data collection. The in-house institutional animal ethics committee approval (MB/IAEC/2021/02/03) was obtained.

All animals were kept in an animal room with the environmental conditions like 23±3°C temperature, relative humidity at 50±20%, a photo period of 12hr light/12hr dark, and the sound level of less than 65 db. Individual Polypropylene cages were used to house the animals with corn cob bedding (size:8mm) from Matha Agrotech, Haveri, Karnataka, India. and they had *ad libitum* access to RO water and a commercially available Rodent pellet diet from Krishna Valley Agrotech, Kupwad, Maharashtra, India, with 18% protein. These conditions were ensured as per CCSEA regulations.

Observation and Examination

Body weights were taken on a weekly basis, using an electronic balance. Blood samples for hematology were collected from the animals by retro-orbital sinus in mice and retro orbital plexus method using a heparinised capillary tube for bleeding, and EDTA vials were used for analysis. The parameters were measured using Exigo analyzer (Boule Medical AB, Swedan 5 part): red blood cell count (RBC), white blood cell counts (WBC) (neutrophils, basophils, eosinophils, lymphocyte, monocyte), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Mean Platelet Volume (MPV) (Brereton *et al.*, 2016).

Blood samples for biochemistry were collected from the animals by retro-orbital sinus method in the case of mice, and retro-orbital plexus in rats using capillary tubes. The serum was separated from collected blood samples by centrifugation at 3000 rpm for 15 min. The following parameters were measured with a Biosystem fully auto analyzer, Spain: Total bilirubin, direct bilirubin, aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT), alkaline phosphatase (ALP), total protein, albumin, A-G ratio (Petterino and Argentino-Storino, 2006).

RESULTS AND DISCUSSION

Table 1 lists the body weight and feed consumption of male and female rats, and Table 2 that of mice. Figures 1 and 2 show the body weights of rats and mice, respectively. The body weight of the rat gradually increased from 5 weeks to 12 weeks, where the female rat's body weight was less than the male rat's body weight, which correlated with Lee et al., 2012. The weight gains cited by Lee et al., 2012 was higher than the observed weight, which might be due to high-quality feed in

terms of a higher protein percentage. Both in rats and mice, the mean body weight of females was slightly greater than that of males in the last three weeks, due to more feed consumption in both rats and mice of the male sex.

Figure 1: Body Weights (g) of Rat

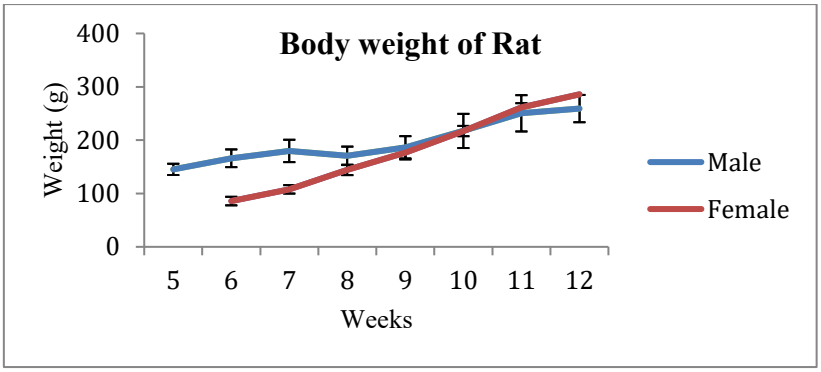

Figure 2: Body Weights (g) of Mice

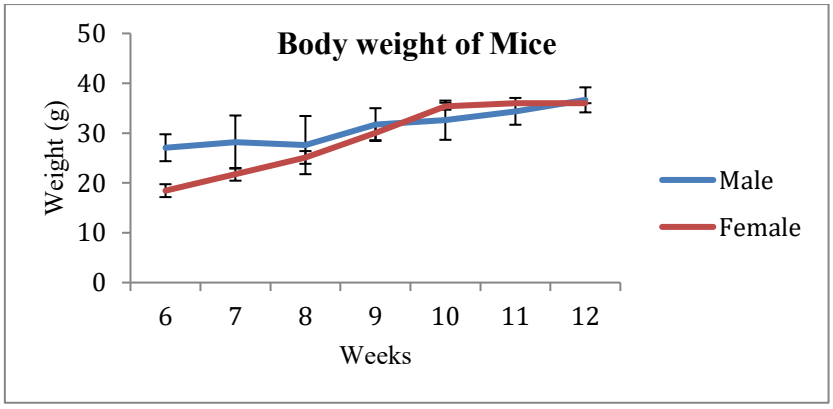

Table I: Body Weights and Feed Consumption of Rats

Male Rats				Female Rats			
Weeks	No.	Body Weights	Feed Consumption	Weeks	No.	Body Weights	Feed Consumption
		Mean±SD	Mean±SD			Mean±SD	Mean±SD
5	17	145.20±10.50	15.12±2.77	6	17	85.82±3.03	9.34±3.02
6	70	166.04±35.89	14.26±3.76	7	17	107.76±8.01	9.9±1.90
7	99	179.77±20.95	16.58±6.15	8	17	144.18±7.89	12.7±3.94
8	58	170.60±17.28	20.28±4.97	9	17	176.70±9.72	15.36±4.79
9	100	186.55±20.90	19.18±4.3	10	17	217.58±12.56	18.18±4.44
10	120	217.25±32.02	18.57±8.33	11	17	261.58±9.61	20.68±3.70
11	148	250.31±34.01	19.26±4.93	12	17	285.88±7.67	21.64±4.67
12	53	259.16±25.60	15.83±3.20				

Table II: Body Weights and Feed Consumption of Mice

Male Mice				Female Mice			
Weeks	No.	Body Weights	Feed Consumption	Weeks	No.	Body Weights	Feed Consumption
		Mean±SD	Mean±SD			Mean±SD	Mean±SD
6	85	27.05±2.7	2.4±1.98	6	18	18.44±1.27	1.21±0.159
7	85	28.20±5.32	2.8±1.99	7	18	21.72±1.27	1.40±0.21

8	85	27.59±5.8	2.62±1.99	8	18	25.11±1.27	2.35±0.21
9	41	31.70±3.28	3.2±2.89	9	18	30.05±1.47	2.91±0.46
10	41	32.52±3.90	3.86±2.88	10	18	35.38±0.69	3.36±0.32
11	33	34.36±2.69	3.1±0.37	11	18	35.27±0.82	3.60±0.44
12	33	36.66±2.50	3.7±0.31	12	18	35.61±0.69	3.77±0.29

The biochemical parameters of Wistar rats (male and female of age 6-8 weeks) and Mice (male and female of age 6-8 weeks) are presented in Tables 3 and 4, respectively. Blood samples were collected at 8 weeks in both rats and mice. The mean SGPT value of the male rat was 70.16, and that of the female rat was 41.14. The mean SGOT value of the male rats was 124.49, and that of the female rats was 95.3. When the mean values of observed data are similar to those of the control data of Lee *et al.*, 2012; Ochiai *et al.*, 2017; Okamura *et al.*, 2011). The mean SGPT value of female mice was 31.14, and the mean SGOT was 64.31. The mean ALP value of female mice was 147.7. The values obtained are relatively lower than those reported by Silva-Santana *et al.*, 2020, which might be due to the geographical variations and genetic variations of different mouse strains.

Table III: Biochemical Parameters of Rats (6-8 Weeks)

Parameters	Unit	Male		Female	
		No.	Mean±SD	No.	Mean±SD
Total Bilirubin	(mg/dl)	16	0.87±0.34	40	0.12±0.02
Direct Bilirubin	(mg/dl)	16	0.48±0.15	40	0.05±0.04
SGOT	(IU/L)	26	124.49±92.49	40	95.30±12.50
SGPT	(IU/L)	26	70.16±47.34	40	41.14±4.38
Alkaline Phosphatase	(IU/L)	26	250.86±123.28	40	65.57±19.53
Total protein	(g/dl)	26	7.01±0.65	40	8.34±0.17
Albumin	(g/dl)	26	3.45±0.73	40	3.15±0.18
Globulin	(g/dl)	26	3.56±1.20	40	4.49±0.41
A-G Ratio		26	1.15±0.61	40	0.70±0.06

Table IV: Biochemical Parameters of Mice (6-8 Weeks):

Parameters	Unit	Male		Female	
		No.	Mean±SD	No.	Mean±SD
Total Bilirubin	(mg/dl)	16	0.93±0.046	18	0.32±0.09
Direct Bilirubin	(mg/dl)	16	0.56±0.577	18	0.55±0.05
SGOT	(IU/L)	26	64.31±50.42	18	170.83±38.49
SGPT	(IU/L)	26	31.14±11.19	18	46.72±8.57
Alkaline Phosphatase	(IU/L)	26	147.7±65.64	18	297.27±55.23
Total protein	(g/dl)	26	6.48±0.562	18	5.78±0.16
Albumin	(g/dl)	26	3.42±1.00	18	3.03±0.14
Globulin	(g/dl)	26	3.36±1.08	18	2.75±0.14
A-G Ratio		26	1.37±0.79	18	1.1±0.08

The hematological parameters of Swiss mice (male and female of age 6-8 weeks) are listed in Table 5. The mean haemoglobin, RBC and WBC values of mice were 12.88, 9.0 and 7.5, respectively. The values are more or less similar to those of Pessini *et al.*, 2020; Restell *et al.*, 2014; Silva-Santana *et al.*, 2020). In Table hematological parameters of Wistar rats (male and female of age 6-8 weeks) were enlisted. The mean haemoglobin value of the male rat was 15.11, and that of the female rat was 15.6. The mean RBC and WBC counts of

male rats were 9.48 and 6.18, respectively. The mean RBC and WBC values of female rats are 8.4 and 12.3, respectively. The values are comparable with those of Hayakawa *et al.*, 2013; Lee *et al.*, 2012; Okamura *et al.*, 2011).

Table V: Hematological Parameters of Rats and Mice (6-8 Weeks)

Parameters	Unit	Rats		Mice	
		Male (N=40)	Female (N=40)	Male (N=20)	Female (N=27)
		Mean±SD	Mean±SD	Mean±SD	Mean±SD
Total WBC	$10^3/mm^3$	6.81±1.55	12.30±1.6	3.30±4.07	3.84±3.42
Neutrophils	%	17.9±28.17	13.40±4.4	39.35±13.46	32.9±15.5
Lymphocytes	%	84±6.38	84.3±04.87	57.2±14.38	64.78±15.20
Monocytes	%	1.52±1.48	1.67±1.46	2.55±2.41	1.74±2.01
Eosinophils	%	0.83±0.90	0.6±0.71	0.5±0.88	0.81±1.00
Basophils	%	0	0	0.05±0.22	0.11±0.32
RBC count	Millions/ mm^3	9.48±11.03	8.40±0.40	6.39±0.95	6.56±1.89
Haemoglobin	gm/dl	15.11±0.53	15.60±0.67	9.22±1.44	9.58±2.64
PCV	%	43.11±6.46	40.15±4.66	30.75±2.82	33.33±9.70
MCV	fl	51.81±1.43	52.40±1.9	48.91±6.81	51.12±4.39
MCH	g/ml	19.23±0.556	22.90±30.65	14.58±2.06	14.95±1.27
MCHC	g/dl	37.13±0.613	35.45±0.42	29.99±3.69	29.40±2.41
RDW	%	22.79±1.16	22.88±0.78	20.63±3.42	19.49±3.83
Platelet count	$10^3/mm^3$	658.02±95.26	699.97±52.10	1836.6±665.7	1859.48±1159.56
MPV	fl	6.18±0.10	6.19±0.09	5.28±0.48	5.31±0.64

CONCLUSION

In the Mass Biotech animal facility, the above reference values of rats and mice with parameters like body weight, feed consumption, hematology and biochemistry had thus been created, which could be used in toxicological and biomedical research. The body weight graphs of both rats and mice might be useful for in-house experiments and other commercial and academic institutes that use animals from Mass Biotech for the *in vivo* experiments. Further, parameters like hormones, organ weights, enzymes, biomarkers, antioxidants, etc. need to be compiled to help such type of studies.

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