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RESEARCH

Physiological working ranges of hematological and serum biochemical parameters in BALB/C, NMRI and C57/BL6 mice strains for preclinical experimentation in Cuba

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ABSTRACT

The availability of standardized ranges of values for physiological parameters (hematological and serum biochemical parameters) is essential for establishing the health status of animals in experimentation. They are required also to evaluate the effects of different natural and biotechnological products once applied, and, remarkably, they are influenced by a series of factors (genetics, housing, feeding and handling, among others). Unfortunately, reference values for these parameters adapted to working conditions in Cuba were unavailable. Therefore, this study was aimed to establish the physiological working range of hematological and serum biochemical parameters of BALB/C, NMRI and C57/BL6 mice lines. For this purpose, 360 animals were tested for hematological and serum biochemical parameters, 120 samples (60 male and 60 female animals) were taken for each mouse strain (BALB/C, NMRI and C57/BL6), and results were compared between both sexes in each strain, and among animals of the same sex in different strains. Relevant differences were found between males and females in BALB/C mice in 40 % of the studied parameters, while 63 % in C57/BL6 and 22 % in NMRI mice strains and all the parameters were influenced in both sexes. Therefore, our results bring support to researchers in assessing the health status of these mice strains in preclinical studies. More importantly, these are functional working ranges of the physiological parameters analyzed for animal experimentation in toxicology determinations in Cuba. They must be carefully considered, since the establishment of reference values could involve a higher number of animals as set for other strains as the Charles River.

Keywords: mice, hematology, serum biochemical, sex, BALB/C, NMRI, C57/BL6

RESUMEN

Rangos fisiológicos de trabajo para los parámetros hematológicos y de bioquímica sanguínea de las líneas de ratones BALB/C, NMRI and C57/BL6 para la experimentación preclínica en Cuba. La disponibilidad de rangos de valores estandarizados sobre parámetros fisiológicos, hematológicos y de bioquímica sanguínea, es esencial para el establecimiento del estado de salud de los animales de experimentación. Para ello se necesita evaluar los efectos de los diferentes productos naturales y biotecnológicos tras su aplicación, dichos efectos influenciados por diversos factores (genética, hospedaje, alimentación y manipulación, entre otros). Sin embargo, no existían valores de referencia para dichos parámetros en las condiciones de trabajo en Cuba. El propósito de este estudio fue establecer el rango de trabajo fisiológico de los parámetros hematológicos y de bioquímica sanguínea para las líneas de ratones BALB/C, NMRI y C57/BL6. Se evaluó a 360 animales, 120 para cada línea (60 machos y 60 hembras), y se comparó los resultados entre ambos sexos para las tres líneas, y entre animales del mismo sexo entre las líneas. Se encontró diferencias relevantes en el 40 % de los parámetros entre animales machos y hembras de la línea BALB/C, en el 63 % de C57/BL6 y el 22 % de NMRI, con influencia del sexo en todos los parámetros. Estos resultados soportan la evaluación del estado de salud de estas líneas de ratones en estudios preclínicos, con rangos de trabajo funcionales para los estudios toxicológicos en Cuba, en base a los parámetros fisiológicos analizados. Dichos valores son valores de trabajo, dado que para considerar valores de referencia se suele emplear un mayor número de animales, tal como se establece para otras líneas de ratones por Charles River (700 animales, 400 hembras y 300 machos).

Palabras clave: ratones, hematología, bioquímica serológica, sexo, BALB/C, NMRI, C57/BL6

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Introduction

Blood is a connective tissue with chemical and cellular elements, that can reach up until 7 % of the body weight [1] and it's usually employed to determine animal's health conditions. Hence, the proper evaluation and analysis of blood components is highly relevant for the adequate interpretation of experimental results and the toxicological effects of different substances in biomedical research.

In this setting, the mouse (*Mus Musculus*) is the most used animal model in the study of the pathogenesis and the treatment of human diseases [2]. The mouse is among the smallest mammals and have a short generation time, and its use reduces the costs of animal experimentation and several strains are available for its application in preclinical studies [3]. Moreover, the genomic similarities between humans and mice are the

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basis of modern biology, and the animal practice with these species provides valuable information on the biological function and etiology of many human diseases [4]. Different lines of mice with unique genetic and biological characteristics have been generated, further requiring the selection of the appropriate mouse model for the given experimental purposes [5].

Therefore, it is essential to know the physiological ranges of several hematological and serum biochemical parameters of this experimental species, since pathological processes can be influence on its metabolism and alter the experimental results. However, these parameters may change due to the environmental and handling conditions to which animals are exposed [6], even if physiological parameters are internationally established in most rodents used in experimentation. Furthermore, clinical pathology results may also change, depending on the methods used for sample collection and processing [7]. Hence, it is demanding to have reference data from each colony of laboratory animals, while requiring a high number of animals.

In Cuba, there have been few reports on standardized physiological values for hematological and serum biochemical parameters, specifically for BALB/C, NMRI and C57/BL6 mice strains [8, 9]. Therefore, this work was aimed at analyzing the physiological working ranges for 22 hematological and serum biochemical parameters for these three mice strains, under laboratory conditions for preclinical studies in Cuba. Consequently, the physiological working ranges were set for the hematological and serum biochemical parameters studied, and they were found significantly different according to the gender of animals and among the different strains. Our results support the correct evaluation of the health status of the animals and would facilitate the adequate assessment of the possible effects of several substances in mice, further contributing to guarantee the reliability of animal experimentation in Cuba.

Materials and methods

Animals

A total of 360 mice of both sexes of the BALB/C, NMRI and C57/BL6 strains were examined. All animals were 6-8 weeks-old with an average body weight of 22 g, never exceeding ± 20 %. In all cases, the females were nulliparous, and all animals were healthy and without clinical signs of any pathological disorders. Mice were qualified as Specific Pathogen Free and kept under germ-free conditions. They were specifically free of *Salmonella* sp., *Streptobacillus monitiformis*, *Streptococcus haemophilicus*, *Bordetella bronchiseptica*, *Corynebacterium kutscheri*, *Cytobacter freundi*, *Streptococcus pneumoniae*, *Shigella* spp., *Pasteurella* spp., *Bacillus piliformis*, *Leptospira* spp., Reovirus 3, Mouse polyoma virus, Minute virus, Mouse pneumonia virus, Mouse adenoma virus, Mouse hepatitis virus, Lymphocytic choriomeningitis, Hantan virus and Mycoplasma.

Mice were handled and maintained following the American Association for Accreditation of Laboratory Animal Care (AAALAC) guidelines [10]. All the procedures were performed under the approval of the CIGB's Institutional Animal Care and Use Committee (IACUC), according to the Resolution No. 11/2013, Regulation No. 64/2013, Guidelines for the Constitution and Functioning of the Institutional Committees for the Care and Use of Laboratory Animals (CICUAL) [11] of the National Regulatory Authority, the Center for State Control of Medicines, Equipment and Medical Devices (CECMED), Cuba.

Origin, environmental conditions and location

Mice were provided by the Division of Gnotobiotic Rodents from the National Center for the Laboratory Animals Breeding (CENPALAB), and housed at the Center for Genetic Engineering and Biotechnology (CIGB). The animals were kept in the barrier zone of the Direction of Preclinical Research and Animal Experimentation of the CIGB, under conventional laboratory conditions, 18-22 ºC temperature, 53-67 % relative humidity and 12 light/12 h dark cycle. Mice were housed in groups of five animals per cage, in Makrolon T-11 cages (Tecniplast, Italy), with 3 cm of poplar wood. Animals were fed daily with 10 g of commercial diet (certified granulated formula EMO-1002 (NP 165; ALY Co™, Cuba) provided by CENPALAB. The water consumed by the animals was sterilized by ozonization and supplied *ad libitum*. Also, animals were maintained under environmental enrichment regimen with toys (tunnels, mazes and walking wheels) and foraging enrichment.

Animal blood sample collection

Whole blood samples (100 µL) were collected from the saphenous veins at first time in the morning (8:00 AM), and split in two equal aliquots. One sample was transferred into a tube containing EDTA as anti-coagulant for hematological analyses. The second was stored in a plastic tube without anti-coagulants and kept at room temperature for 30-60 min until coagulation. Serum was obtained by centrifugation at 1600 *g* for 15 min. Individual serum samples were stored in polypropylene tubes at –20 °C until use.

Study design

Each mice line (BALB/C, NMRI and C57/BL6) was studied in 120 animals (60 females and 60 males). Every animal was tested for up to 22 hematological and serum biochemical parameters, as described below. All evaluations were performed in a Clinical Laboratory of the Direction of Preclinical Research and Animal Experimentation of the CIGB. The mean values reached and the standard deviation of the main hematological and serum biochemical parameters were calculated, assuming a mean ± 1 standard deviation as the physiological range of each sex, in three mouse strains. Likewise, the results were compared between males and females of each strain and also between animals of the same sex.

Assessment of hematological and serum biochemical parameters

Hematological parameters determinations included the analysis of Total white blood cell count (WBC; 10^3/µL), Total red blood cell count (RBC; 10^6/µL), Hemoglobin concentration (HGB; g/dL), Hematocrit percentage (HCT; %), Mean corpuscular volumen (MCV; fL), Mean corpuscular hemoglobin (MHC; Pg), Mean

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corpuscular hemoglobin concentration (MCHC; g/dL) and Platelet count (PLT; $10^3/\mu L$).

Evaluations were made using a hematological analyzer NIHON KOHDEN (Celltac, model MEK 6450J, Japan). A differential leukocyte count was performed as the percentage of White blood cells, including neutrophils (NEUTRO%), lymphocytes (LYMPHO%), and monocytes (MONO%). For this, peripheral blood films were stained with the Giemsa reagent, and cell counts obtained under an optical microscope equipped with immersion lenses (VistaVision, MO 000004; Zeiss, Goettingen, Germany).

Serum biochemical analysis included the evaluation of serum concentration of alanine aminotransferase (ALT; UI/L), aspartate aminotransferase (AST; UI/L), alkaline phosphatase (ALP; UI/L), creatinine (CREA; mg/dL), total protein (PT; g/dL), glucose (GLU; mmol/L), total bilirubin (TB; mmol/L), albumin (ALB; g/dL) and cholesterol (CHOL; mg/dL). These measurements were performed with a HESKA automatic biochemistry analyzer (DRI-CHEM 7000, FUJIFILM, Japan).

All results were recorded in compliance with Standard Operation Procedures and the Program for Management and Use of Animal Laboratory for Experimental and Control of Biotechnological Products of the CIGB.

Statistical analysis

Normality tests were performed by the D'Agostino & Pearson test, variance homogeneity was demonstrated by the Bartlett's test. Data complying with normality and variance homogeneity were then analyzed by an unpaired t test. Data that did not fulfill mathematical conditions, even after transformations, were analyzed by the Mann-Whitney U test. Either the case, all data were shown as mean ± standard deviation, or median and percentile intervals (2.5th and 97.5th percentiles) when following a nonparametric distribution. Significance level was set to 0.05. Statistical calculations were made by using the package GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA).

Results

The hematological parameters of BALB/C mice, in the case of WBC, MCV, MCH and LYMPHO%, presented higher values in females ($p < 0.05$). PLT and NEUTRO% had the highest values in males ($p < 0.05$). In the case of serum biochemistry, males had higher values ($p < 0.05$) in ALAT, ALP and CHOL. The other parameters showed no differences between males and females (Table).

C57/BL6 males had the highest values of hematological parameters in WBC, HCT, MCHC and NEUTRO%, while the results were also statistically significant differences ($p < 0.05$) in HGB, MCV, MCH and PLT, with the highest values in females. Serum biochemical parameters revealed significant differences between both sexes ($p < 0.05$) for ALAT, ASAT and CREA. Furthermore, ALAT and CREA highest values were found in males, and ASAT in females (Table 2).

In NMRI mice, significant differences ($p < 0.05$) were found between males and females for NEUTRO% and LYMPHO%, the highest NEUTRO% and LYMPHO% values in males and females, respectively. Significant differences were also shown for ALT, GLU and TB between males and females, the highest values observed in males.

Moreover, hematological and serum biochemical results showed statistically significant differences ($p < 0.05$) among females of the three strains tested in all the evaluated parameters (Table). In females, NMRI mice showed the higher values in 5 % of the parameters, while BALB/C and C57BL/6 showed higher values in 18 and 22 % of the parameters, respectively. In males, NMRI mice had the highest values in 18 % of the parameters, and there were similarities for BALB/C mice in 22 % of the parameters and in C567BL/6 for 27 %.

Significant differences ($p < 0.05$) were detected between both sexes in BALB/C mice for 40 % of the studied parameters, 63 % in C57/BL6 and 22 % in NMRI. Likewise, all hematological and serum biochemical parameters were influenced by the strain background in males and females.

Discussion

Knowledge on the hematological and serum biochemical values is essential to select experimental healthy animals in preclinical studies. In this sense, several authors have conducted multiple studies to establish the standardized parameters in different laboratory animal species. In fact, they have demonstrated that experimental results are directly influenced by factors such as sex, age, diet, stress, environmental conditions, variability of the analytical techniques, animal management, sedation method, pregnancy and diseases [12-14]. Furthermore, results' interpretation will always be limited to the animal population studied.

There has been generally accepted that, in adult animals, the mean concentrations of red blood cells and hematocrit are higher in males than in females [15-17], presumably due to males having greater muscle development, which requires a increased oxygen supply [18]. Nevertheless, Aleman et al. did not detect differences between males and females for HGB and HCT in NMRI mice [19]. Our results were similar NMRI and BALB/C mice strains, not in C57BL/6 mice, where females showed significantly higher values.

The BALB/C mice, both sexes, had lower WBC levels than those established by Charles River for 8-10 week-old mice of the same strain [20]. This could be caused by different handling and housing environmental conditions, as well as the age range used in both laboratories. On the other hand, WBC variations may be related to the method of containment and collection of samples. This trigger animal stress manifestations during the procedure, promoting the release of adrenaline, which ultimately mobilizes neutrophils from the bone marrow into the blood vessels and, subsequently, increase neutrophils and total leukocytes [21]. Coincidently, Kile *et al*. [22] reported the lowest values of WBC in the BALB/C strain. We showed similar findings, but only in males, since C57BL/6 females displayed the lowest levels.

Instead, sexual steroids, especially 17 B estradiol and progesterone, affect the circulation and activation of leukocytes [23], leading to significantly higher WBC values in males of the BALB/C and C57BL/6

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Table. Working values of hematological and serum biochemical parameters analyzed in BALB/C, NMRI and C57/BL6 mice strains in Cuba*

Parameters	BALB/C			NMRI			C57/BL6		
	Females	Males	Statistical analysis	Females	Males	Statistical analysis	Females	Males	Statistical analysis
WBC	6.83 ± 2.36 a	5.63 ± 2.08 a	p < 0.001	10.76 ± 4.94 c	11.54 ± 4.11 b	NS	4.65 ± 2.83 b	10.23 ± 3.59 b	p < 0.001
RBC	9.98 ± 0.56 a	10.14 ± 0.69 a	NS	9.94 ± 0.62 a	10.02 ± 0.70 a	NS	8.99 ± 0.92 b	8.91 ± 1.14 b	NS
HGB	15.21 ± 0.79 a	15.27 ± 0.97 a	NS	10.69 ± 4.86 c	10.20 ± 5.09 b	NS	14.11 ± 1.44 b	11.58 ± 2.21 b	p < 0.001
HCT	46.87 ± 2.60 a	47.25 ± 3.07 a	NS	46.29 ± 6.39 a	47.35 ± 7.34 a	NS	40.32 ± 5.71 b	44.88 ± 4.61 b	p < 0.001
MCV	47.00 ± 0.94 a	46.62 ± 0.60 a	p < 0.05	46.44 ± 5.27 c	47.31 ± 7.43 a	NS	45.17 ± 6.67 b	39.83 ± 1.73 b	p < 0.001
MCH	15.24 ± 0.28 a	15.07 ± 0.25 a	p < 0.001	10.68 ± 4.77 b	10.22 ± 5.13 b	NS	15.75 ± 1.38 a	13.47 ± 1.08 b	p < 0.001
MCHC	32.45 ± 0.46 a	32.34 ± 0.52 a	NS	22.53 ± 9.71 b	21.00 ± 10.01 c	NS	35.56 ± 5.42 a	45.10 ± 4.39 b	p < 0.001
PC	670.30 ± 126.30 a	717.48 ± 95.12 a	p < 0.05	904.03 ± 190.72 c	936.18 ± 203 c	NS	554.98 ± 145.01 b	183.90 ± 108.06 b	p < 0.001
NEUTRO%	0.15 ± 0.06 a	0.19 ± 0.10 a	p < 0.01	0.19 ± 0.07 b	0.27 ± 0.11 c	p < 0.001	0.18 ± 0.11 ab	0.21 ± 0.11 a	p < 0.05
LYMPHO%	0.84 ± 0.05 a	0.80 ± 0.10 a	p < 0.01	0.80 ± 0.08 b	0.72 ± 0.13 b	p < 0.001	0.78 ± 0.10 b	0.76 ± 0.11 b	NS
MONO%	0 ± 0.01 a	0 ± 0.01 a	NS	0 a	0 a	NS	0 a	0 ± 0.01 a	NS
EO%	0 ± 0.01 a	0.01 ± 0.01 a	NS	0 a	0 c	NS	0.03 ± 0.03 b	0.01 ± 0.02 b	p < 0.01
BASO%	0 a	0 ± 0.01 a	NS	0 a	0 a	NS	0.01 ± 0.02 b	0.01 ± 0.01 b	p < 0.05
ALT	10.92 ± 10.52 a	14.60 ± 10.10 a	p < 0.05	28.14 ± 13.38 b	40.34 ± 12.42 c	p < 0.001	22.33 ± 7.31 b	24.48 ± 8.04 b	p < 0.05
AST	28.80 ± 12.62 a	29.57 ± 13.44 a	NS	53.92 ± 15.03 c	50.85 ± 15.45 c	NS	38.55 ± 14.70 b	32.63 ± 14.40 a	p < 0.05
ALP	169.10 ± 98.11 a	216.52 ± 99.84 a	p < 0.001	226.73 ± 61.23 a	228.78 ± 45.43 a	NS	308.83 ± 19.75 b	280.09 ± 86.37 b	p < 0.001
CREA	0.57 ± 0.17 a	0.58 ± 0.19 a	NS	0.85 ± 0.40 c	0.89 ± 0.34 b	NS	0.55 ± 0.44 b	0.61 ± 0.49 a	p < 0.001
TP	5.78 ± 0.64 a	5.90 ± 0.64 a	NS	5.78 ± 0.44 ac	5.91 ± 0.56 ac	NS	5.58 ± 0.38 ab	5.19 ± 1.56 ab	NS
ALB	3.75 ± 0.47 a	3.91 ± 0.77 a	NS	4.21 ± 0.72 b	4.15 ± 0.34 b	NS	4.12 ± 0.68 b	3.92 ± 1.47 ab	NS
GLU	124.09 ± 23.17 a	126.47 ± 24.06 a	NS	116.32 ± 1.03 b	117.33 ± 1.19 c	p < 0.001	126.49 ± 10.86 a	115.08 ± 39.11 a	NS
CHOL	173.07 ± 21.29 a	183.59 ± 22.00 a	p < 0.05	153.89 ± 17.40 c	157.24 ± 16.40 b	NS	174.82 ± 17.72 a	162.53 ± 58.05 a	NS
TB	0.33 ± 0.47 a	0.30 ± 0.38 a	NS	0.69 ± 1.14 b	3.96 ± 1.86 c	p < 0.001	0.20 ± 0.08 b	13.30 ± 39.41 b	NS

*Parameter values are expressed as means ± 1 SD (n = 120 animals per group of mice strain, 60 males/60 females). WBC: White Blood Cells ($10^3/\mu L$); RBC: Red Blood Cells ($10^6/\mu L$); HGB: Hemoglobin (g/dL); HCT: Hematocrit (%); MCV: Mean Corpuscular Volume (fL); MCH: Mean Corpuscular Hemoglobin (Pg); MCHC: Mean Corpuscular Hemoglobin Concentration (g/dL); PC: Platelet Count ($10^3/\mu L$); NEUTRO%: Neutrophils percentage (%); LYMPHO%: Lymphocytes percentage (%); MONO%: Monocytes percentage (%); EO%: Eosinophiles percentage (%); BASO%: Basophilos percentage (%); ALT: Alanine aminotransferase (UI/L); AST: Aspartate aminotransferase (UI/L); ALP: Alkaline phosphatase (UI/L); CREA: Creatinine (mg/dL); TP: Total Protein (g/dL); ALB: Albumin (g/dL); GLU: Glucose(mmol/L); CHOL: Cholesterol (mg/dL); TB: Total Bilirubin (mmol/L). Statistical analysis was run between sexes. NS: means no statistical differences in the same age group.

strains, coincident with reports by Mazzaccara *et al*. [24]. NMRI mice behave as reported by Doeing [25], with no statistical differences between both sexes and the highest WBC values in females.

Regarding female animal physiology, estrogens exert a depressive action on lymphocytes [26]. Similar finding were reported by Hoffman-Goetz [27] where high levels of estradiol exposure in C57BL/6 female mice induced significantly reduced lymphocyte blastogenesis responses. Despite, such an effect was not visible none of the three mouse strains studied, where female NMRIs had higher values of LINFO% and no statistically significant differences were not found in BALB/C and C57/BL6 mice between sexes. This effect may be caused by the specific timing in the estrous cycle of the animals tested at which blood was collected, which may had coincided with low estrogen levels. In these parameters, Kile *et al.* [22], reported the highest values of LYMPHO% in C57BL/6 mice, while in our study BALB/C mice showed the highest values for both sexes, above those of C57/BL6 and NMRI mice.

The number and function of NEUTRO% shows a time trend during the estrous cycle in females [28], plausibly accounting for the highest values in males in the three strains of mice tested. Coincidently, male BALB/c mice had higher platelet counts values than female BALB/c mice [29], in agreement with our results. Despite, C57/BL6 showed different values mice while retaining its original characteristics, for reasons that we consider to be related to the rusticity of this strain. Otherwise, Barrios *et al*. [30] detected higher levels of platelets in C57/BL6 than in BALB/C mice.

Regarding CREA, Laurie *et al*. [31] detected lower values than ours in several mice strains, while other groups [32] have found values very similar to those for C57/BL6 animals, pretty similar to those detected at CIGB, including BALB/C mice.

The BALB/C and NMRI mice used by Coman *et al*. [33] generally display higher serum biochemical values in males than females. Accordingly, differences were only found between males and females were found for ALAT, GLU and TB in NMRI mice, and for ALAT, ALP and CHOL in BALB/C, with highest values always detected in males. It was also reported that females had the highest values of serum biochemical parameters [30], into contrast with the highest value we found only for ASAT in females, out of the nine parameters tested. ALAT has been also reported at higher levels in NMRI mice of similar genetic origin to ours [19], which would demonstrate the influence of environmental and animal handling conditions on this parameter, while our ALP values were significantly higher for both sexes.

Furthermore, Mazzaccara *et al*. [23] and Gordon *et al*. [34] reported significantly higher values of AST, ALP and ALB in C57/BL6 females, while males showed higher values for TP, CHOL and ALT. That contrast with differences found between both sexes in ALAT, ASAT and CREA, higher in females for ASAT and in males for ALAT and CREA.

BALB/C and C57/BL6 males were shown to have TP, ALB and CHOL values higher than those obtained by Santos *et al*. [35], but higher GLU levels, possibly caused by the genetic differences between animals, even for similar strains. Other contrasting results have been evidenced by Zhou *et al*. [36], with higher CHOL and GLU values in C57/BL6, while there were

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no differences for these parameters between males and females in our study. In fact, we detected TP, ALB and CHOL values in BALB/C and C57/BL6 males higher than those by Santos *et al*. [35]. On the contrary, they reported higher GLU levels than ours, maybe due to differences in the genetic background of the animals even for the same strains.

Regarding ALB, this parameter behaved in BALB/C and C57/BL6 mice at higher values than as reported by Silva *et al*. [37], who also detected higher values of this parameter in C57/BL6 mice. Instead, we detected similar results only in females. Ultimately, TP values were higher in CB57/BL6 mice [37], in agreement with our results with differences for both sexes between BALB/C and C57/BL6 strains.

In summary, the physiological working ranges were set for the hematological and serum biochemical parameters in BALB/C, NMRI and C57/BL6 mice strains in Cuba. They considered the gender's effect, contributing to evaluate the animal health status and as assessing tool for establishing the changes when testing any natural or biotechnological product. Noteworthy, these working ranges have to be carefully considered, since establishing reference values normally requires testing a larger set of animals. For instance, the reference values are established for strains as the Charles River mice strain using 400 female and 300 male animals [38, 39]. Moreover, they have to be periodically revised due to their potential changes caused by changes in animal genetics, breeding, housing, feeding and handling conditions, among other factors specific for the animal facility where animals are maintained and tested. Otherwise, and as far as we know, this is the first study aiming for establishing such useful working range for the animal strains tested in the Cuban context, with impact in natural and biotechnological preclinical experimentation.

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Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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