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Study of the Variation of Some Biochemical Parameters in *Rattus norgevicus*

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Authors' contributions

All the authors mentioned have each contributed at their own level to the realization of the work. Author OCO produced and interpreted the manuscript and performed the statistical analysis. Author DSTA read and corrected the manuscript. Author GMB created the conditions for the breeding of the animals. Author AFE took the blood samples from the animals, performed the assays for the different parameters and wrote the manuscript. Author ADPB defined the theme of the work, supervised and gave scientific support to the work.

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ABSTRACT

The biochemical assessment in animals is of capital interest to define the diagnosis of many diseases. The variation of biochemical parameters (urea, glycemia, creatinemia, transaminases: GPT and GOT, total cholesterol, HDL-c, LDL-c, triglycerides and total lipids) is an indicator of the state of health of certain vital organs such as the kidney, liver, pancreas, veins, heart, etc... . For this study, to be able to determine the normal values of the rats, a set of batches of male and female Wistar rats was formed and acclimatized for 2 months. Each batch set consisted of thirty-nine (39) rats. Each rat was weighed and then sampled by venipuncture at the caudal level to collect their blood in a dry tube (red). The red tube was used to determine the biochemical parameters. The

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results of this study showed that some animals already had certain pathologies before the use of any beneficial or harmful substance on them. However, most of the animals used have normal biochemical parameters revealing the good state of their various vital functions (kidney, liver, heart, veins, pancreas, etc.). These values of determining standard biochemical parameters can form a basis for future tests.

Keywords: Rats; biochemical parameters; normal values; pathologies.

1. INTRODUCTION

Developing countries face enormous public health problems. Infectious diseases and malnutrition are the most prevalent despite the development of diagnostic tools. Clinical examinations are accompanied by biological analyses to guide or confirm the clinical diagnosis. Hematological and biochemical parameters are a reliable means for health and nutritional status assessment [1]. However, the interpretation of the results of these analyses is done by comparison with reference values of european populations provided by the manufacturer. As such, the diagnostic kits used in our laboratories are developed according to european or north american standards that do not necessarily reflect those of africans. In fact, studies made on the biological standards of africans are rare if not almost non-existent. Already, studies conducted by [2] in Côte d'Ivoire, [3] in Cameroon, and [4] in Congo have shown that there are differences between the mean values of certain biological parameters in the african and the european [5].

These differences would be due to nutritional and environmental variations, among others [6,5]. If we add the notion of intra- and inter-individual biological variations, we understand that the reference values cannot be transposed indifferently from one country to another. For example, during an international cooperative study on the transferability of reference values, [7] concluded that it was necessary to establish reference values adapted to geographical origin and taking into account the ethnic factor in Africa [5]. So, the establishment of baseline values is of paramount importance for a given population in terms of scientific, diagnostic, and therapeutic aspects [7,5]. Therefore, before establishing reference values for africans, it is essential to determine the biochemical and hematological parameters of animal models including rats. Indeed, hematological and biochemical characterization of animals is of particular interest in the diagnosis of many diseases [8,9, 10], especially subclinical forms, as well as for prognosis [8,9,10]. To do this, most pharmacological and toxicological experiments are performed on this small rodent. Thus, it is used as a study model in many areas of biomedical research [11]. Nevertheless, the rare work carried out on rats, i.e. the determination of their biochemical and hematological parameters, is only a recourse to verify the effect of a substance on the rat organism, and even to establish standard reference values for comparative studies [12,13] with reduced sampling.

It is to remedy this situation that the present study was conducted. Its objective was to study the variation of some biochemical parameters in *Rattus norvegicus*.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Animal material

The rats used in this study were *Rattus norvegicus*. They are 78 in number including 39 males and 39 females and were acclimatized for two months at the Animal Physiology Department of the University Jean Lorougnon Guédé (UJLoG) (Daloa, Côte d'Ivoire) in plastic jars with stainless steel covers. In each jar, there are 4 or 5 animals per group according to their sex. During this period, they were fed with pellets and subjected to controlled temperature and lighting conditions. The litter used was wood chips. It was renewed every two days during the entire experiment to ensure the good hygienic condition of the animals. Their age varied between 3 and 6 months. The males rats weighed between 75 and 175 g while the females weighed between 70 and 150 g.

2.1.2 Blood samples

Blood samples are taken by caudal venipuncture in rats. The blood of each rat was collected immediately in the dry tube and then transported in a cooler to the laboratory for the determination of biochemical parameters.

2.2 Methods

2.2.1 Method of analysis of biochemical parameters

For the biochemical analysis, the samples were previously centrifuged using a centrifuge at 3000 rpm for 5 minutes in order to obtain their serum. These were then stored at -20°C until the determination of biochemical parameters such as: glutamate oxaloacétate transaminase (GOT), glutamate pyruvate transaminase (GPT), urea, glycemia, creatinine, total cholesterol, triglycerides, low density lipoproteins (LDL), high density lipoproteins (HDL), total lipids. The assays were performed with a semi-automated or spectrophotometer (Kenza max).

2.2.2 Method of glutamo-oxaloacetic transaminase (GOT) analysis

For its realization, a tablet of substrate R_2 was dissolved in 15 mL of buffer R_1 for 2 to 3 minutes and then homogenized in order to obtain the working solution. This solution can be stable for 21 days at 2 to 8°C or 3 days (72 H) at room temperature (15 to 25°C). Manipulation was carried out using the kinetic method. It consisted of taking a volume of 100 µL of serum to which 1 mL of the previous working solution was added. After this step, the solution obtained was read using a UV-visible spectrophotometer at a wavelength of 340 nm and a temperature of 37°C.

2.2.3 Method of glutamo- pyruvic transaminase (GPT) analysis

The measurement of this parameter was made according to the previous procedure.

2.2.4 Method of urea analysis

For the preparation of urea, an enzyme tablet R_3 was dissolved in 250 mL of buffer solution R_1 to obtain a solution. The resulting solution is stable for 4 weeks at 2 to 8°C or 7 days at room temperature (15 to 25°C). For the urea analysis, a volume of 1 mL of the initially prepared solution was taken and transferred to a hemolysis tube. In this tube, a volume of 10 µL of serum was added and incubated at 37°C for 5 minutes. After this incubation period, a 1 mL volume of the reagent R2 (ClONa) previously prepared and supplied by the manufacturer was added and incubated again at 37°C for 5 minutes. After this second incubation period, the determination of urea was carried out according to the enzymatic colorimetric method known as the Berthelot method. The reading was performed using a UV-visible spectrophotometer at 580 nm and 37°C.

2.2.5 Method of blood sugar analysis

For the determination of blood glucose by the Tinder method, the working solution was initially prepared. A volume of 500 mL distilled water was added to the enzyme buffer of bottle R_1 using a measuring cylinder and homogenized. Subsequently, the resulting solution was left to dissolve completely for about 2 minutes. After this step, the reagent R_2 provided by the manufacturer was added to obtain the working solution. This solution is stable for at least 2 years in the absence of contamination. As a practical matter, a volume of 1 mL of the solution obtained after the complete dissolution was removed and distributed in the hemolysis tube. To this volume, a volume of 10 µL of serum was added and incubated at 37°C for 10 minutes or 20 minutes at room temperature. Absorbance readings were taken at 500 nm against the reactive blank using a UV-visible spectrophotometer at 37°C. Staining is stable for 15 to 20 minutes at 37°C and then slowly decreases.

2.2.6 Method of creatinine analysis

For the creatinine assay, reagents R_1 and R_2 have already been prepared by the suppliers (ready-to-use solution). These reagents are stable up to the expiry date indicated on the bottle in the absence of contamination. The working solution was obtained using equal volumes, i.e. 500 µL of R_1, 500 µL of R_2 was added. However, the working solution is stable for 30 days at 2 to 8°C in the absence of contamination. The analysis of creatinine was carried out according to the kinetic colorimetric method of Jaffé. For this purpose, a volume of 100 µL of serum was added to 1 mL of the previously prepared solution (R_1 and R_2). Then the reading of absorbances was carried out using a UV-visible spectrophotometer at 492 nm and 37°C.

2.2.7 Method of total cholesterol analysis

The determination of total cholesterol was done according to the enzymatic method [14,15]. The reagents supplied by the manufacturer are stable up to the expiry date indicated on the box label. However, to make the working solution, reagent

R_2 (enzyme) was dissolved in reagent R_1 (buffer). Then the mixing was carried out gently until R_2 was completely dissolved for about 2 minutes. The solution is stable for at least 2 years. For the determination of total cholesterol a volume of 1 mL of the working reagent was taken and transferred into a hemolysis tube. Then a volume of 10 µL of serum was also taken and added to this tube. Mixing was subsequently carried out and incubated for 5 minutes at 37°C or 10 minutes at room temperature. The absorbance reading was taken at 500 nm against the reagent blank, the staining was stable for one hour.

2.2.8 Method of triglycerides analysis

The determination of triglyceride in the blood was carried out using the enzymatic method [16,15]. The procedure for the analysis of this parameter is consistent with the previous one.

2.2.9 Method of HDL analysis

The determination of HDL-cholesterol (HDL-C) was performed by adding 0.2 mL of serum or plasma to 0.3 mL of the manufacturer's reagent composition precipitant. The mixture was mixed gently and incubated for 5 minutes at 25°C. Afterward it was taken to the centrifuge for 3000 tr for 10 minutes. A volume of 100 µL of the resulting supernatant was removed and then added to 1 mL of the cholesterol reagent (ready-to-use reagent). Therefore, it was previously distributed in all hemolysis tubes except the white test tube. It was then mixed well and incubated for 5 minutes at 37°C or 10 minutes at 25°C. The mixture was then mixed well and incubated for 5 minutes at 37°C or 10 minutes at 25°C. The absorbance reading of the standard and HDL-cholesterol white test was taken at 505 nm.

2.3.10 Method of LDL analysis

The formula of [17] calculated the LDL-cholesterol (LDL-C) level if the triglyceride level < 3.4 g/1. Knowing the levels of total cholesterol, HDL-cholesterol and triglycerides, the LDL-cholesterol level is determined by the difference method according to the equation:

LDL (g/L) = Total Cholesterol - HDL - Triglycerides/ 5

2.3.11 Method of total lipid analysis

For the analysis of total lipids, the working reagent and the standard are ready for use and supplied by the manufacturer. However, for its realization, a volume of 2.5 mL of the working reagent has been dispensed into the hemolysis tubes. A volume of 100 µL of standard and 100 µL of serum was then added to the tubes. Mixing was done entirely with a stirrer and incubation of the prepared tubes was carried out for 10 minutes in a water bath at 100°C. A volume of 50 µL of the standard reaction medium and 50 µL of the serum was then separately collected and added to 1 mL of the previously shared phosphovainillin reagent in the hemolysis tubes. Subsequently, the whole was mixed thoroughly with a stirrer and incubated for 15 minutes at 37°C. During this reaction, unsaturated lipids react with sulfuric acid to form carbonium ions, then these ions react with phosphovainille to give a pink color. The intensity of the color formed is proportional to the concentration of total lipids in the sample [18]. Next, the reading of the absorbances of the samples and the standard against the reagent blank was done at 520 nm. The final coloration was stable for one hour.

2.3.12 Method of statistical analysis

The statistical treatments were carried out on the data collected after the characterization of biochemical parameters. These include a one-factor analysis of variance (ANOVA). When the difference is significant, a multiple comparison test (Turkey HSB) was conducted at the 5% threshold to separate the samples.

3. RESULTS

3.1 Results of the Analyses of the Biochemical Parameters of the Males

The results of the analyses of the biochemical parameters of males rats are reported in the following Tables 1, 2 and 3.

The urea values obtained for males M2, M6, M10, M13, M16, M19, M20, M33, M35 and M37 are out of the reference value for urea (0.39-0.65 g/L) in Table 3.

For blood glucose, the values for males M1, M3, M7, M9, M10, M11, M13, M18, M20, M21, M22, M31 and M39 are also not included in the reference value for blood glucose (0.47-1.19 g/L) in Table 3.

With creatinine, the parameters values for males M2, M3, M8, M9, M10, M13, M20, M21, M28, M29, M30, M31 and M32 are outside the

reference value for this parameter (4.18-6.98 g/L) in Table 3.

For GPT , the parameters values of males M12, M15, M20, M21, M22, M24, M27, M30, M35, M38 and M39 are not included in its reference value (67.20-159.72 IU/L) in Table 3.

The GOT values for males M10, M12, M20, M21 and M22 are not in the reference value (222.68-438.04 IU/L) in Table 3.

The TG values for males M1, M5, M6, M8, M9, M12, M15, M16, M17, M21, M22, M37, M38 and M39 are outside its reference values (0.42-1.10 g/L) in Table 3.

The results of T Chol parameters values for males M1, M4, M7, M8, M13, M19, M22, M29, M32, M33, M34, M35, M36 and M37 reveal that they are not included in the reference value (0.81-1.21 g/dL) in Table 3.

For HDLc, the values obtained for males M2, M7, M12, M17, M19, M33, M36, M37 and M38 are excluded from its reference value (0.45-0.75 g/dL) in Table 3.

The results of the LDLc show that the parameters values for males M1, M2, M3, M4, M6, M9, M12, M13, M27, M29, M30, M32 and M39 are not within the reference value (0.10-0.42 g/dL) of Table 3.

With T lip, the parameters values for males M4, M5, M6, M8, M9, M12, M15, M17, M21, M22, M31 and M38 do not form part of the reference value (3.55-5.35 g/dL) in Table 3.

3.2 Results of the Analyses of the Biochemical Parameters of the Females

Tables 4, 5 and 6 show the results of the analyses of biochemical parameters of females rats.

With urea, all female values are within the reference value (0.50±0.27 g/L or 0.23-0.77 g/L) of Table 6.

For blood glucose, the values of the females parameters F4, F5, F9, F13, F15, F25, F27, F30, F32, F36, F37, F38, and F39 have values that are outside the reference value (0.72±0.39 g/L or 0.33-1.11 g/L) of Table 6.

As for creatinine, the values for females F1, F9, F23, F24, F26, F27, F29, F30, F34, F36 and F38 are not part of the reference value (5.92±1.59 mg/L or 4.33-7.51 mg/L) in Table 6.

The GPT values for females F2, F11, F16, F17, F25, F30, F32 and F35 are not included in the reference value (118.85±47.76 IU/L or 71.09-166.61 IU/L) of Table 6.

At the GOT level, the results for females F4, F5, F8, F11, F13, F25, F28, F32 and F34 give values that are not included in the reference value (332.90±134.01 IU/L or 198.89-466.91 IU/L) in Table 6.

For TG, the results obtained for females F6, F13, F29 and F33 are outside the reference value (1.16±1.02 g/L or 0.14-2.18 g/L) of Table 6.

As far as T Chol is concerned, the values of the parameters of the females F11, F22, F24, F25, F30, F31, F32, F34, F35, F36, F37 and F39 show that they are below the reference value (0.92±0.21 g/L or 0.71-1.13 g/L) in Table 6.

For HDLc, the values obtained for females F4, F5, F6, F8, F12, F34, F35 and F39 are not included in the reference value (0.63±0.21 g/L or 0.42-0.84 g/L) of Table 6.

The LDLc results reveal that the parameters values for females F4, F5, F6, F8, F11, F16, F19, F20, F21, F22, F24, F30, F32, F34, F37 and F39 are within the reference value (0.22±0.16 g/L or 0.06-0.38 g/L) of Table 6.

For T lip, the values of the parameters of the females F6, F13 and F33 are not included in the reference value (5.15±2.65 g/dL or 2.50-7.80 g/dL) of Table 6.

3.3 Results of the Statistical Analysis

Statistical calculations are often useful to biologists for the determination of normal values or, more precisely, reference values, as well as for the evaluation of precision and analytical accuracy [19, 20,15].

The result of the statistical analysis of the biochemical parameters of males and females is given in Table 7.

Table 1. Biochemical parameters of the males of lot 1

Parameters \ Males	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18	M19	M2O	M21	M22
UREA	0.46	**0.29**	0.43	0.55	0.46	**0.91**	0.55	0.6	0.57	**0.68**	0.57	0.63	**0.67**	0.48	0.57	**0.68**	0.49	0,58	**0,69**	**0.69**	0.64	0,64
GLYC	**0.46**	0.7	**0.39**	0.53	0.51	0.91	**0.3**	0.47	**0.31**	**0.26**	**1.26**	0.95	**0.45**	0.55	0.83	0.77	0.93	**0.1**	1.01	**1.67**	**1.43**	**1.5**
CREAT	6.2	**3.8**	**4.1**	5.8	5.3	4.3	4.9	**3.6**	**3.9**	**2.8**	4.7	6.2	**7**	4.5	5.3	6.2	6.7	6.5	5.8	**7.4**	**8.2**	6.9
GPT	82	78	105	94	77	74	105	74	96	153	138	**63**	95	128	**58**	98	88	93	88	**241**	**192**	**185**
TG	**0.35**	0.75	0.47	0.61	**0.32**	**1.38**	0.65	**0.28**	**0.25**	0.65	1.09	**1.12**	0.43	0.61	**0.2**	**0.35**	**0.2**	0.65	0.48	0.95	**1.32**	**1.35**
GOT	314	375	388	235	218	296	296	358	284	**480**	355	**176**	258	321	261	258	279	224	302	**632**	**725**	**537**
T CHOL	**1.36**	1.01	1.01	**0.78**	0.89	0.98	**1.26**	**0.75**	1.12	0.9	0.92	**1.11**	**1.27**	1.09	0.95	1.19	1.17	1.2	**1.31**	1.17	1.18	**1.35**
HDLc	0.73	**0.87**	0.49	0.62	0.46	0.65	**0.79**	0.56	0.63	0.51	0.48	**0.8**	0.58	0.61	0.57	0.75	**0.86**	0.65	**0.87**	0.74	0.56	0.75
LDLc	**0.56**	**0.08**	**0.43**	**0.04**	0.37	**0.05**	0.34	0.13	**0.44**	0.26	0.22	**0.08**	**0.6**	0.36	0.34	0.37	0.27	0.42	0.34	0.24	0.36	0.33
T Lip	4.3	4.4	3.7	**3.5**	**3.03**	**5.9**	4.8	**2.58**	**3.4**	3.9	5.03	**5.6**	4.3	4.3	**2.9**	3.9	**3.43**	4.6	4.5	5.3	**6.3**	**6.8**

Table 2. Biochemical parameters of males in lot 2

Parameters \ Males	M23	M24	M25	M26	M27	M28	M29	M30	M31	M32	M33	M34	M35	M36	M37	M38	M39
UREA	0.48	0.42	0.49	0.53	0.53	0.51	0.45	0.46	0.62	0.39	**0.36**	0.39	**0.37**	0.41	**0.3**	0.45	0.42
GLYC	0.88	0.84	0.92	0.84	0.62	0.96	0.51	0.93	**1.22**	0.98	0.86	1.13	1.09	0.81	1.07	0.92	**1.46**
CREAT	4.7	4.3	5.5	5.2	5.8	**3.4**	**3.8**	**3.6**	**8.7**	**7.4**	6.8	6.4	6.4	6.1	6.2	6.7	6.7
GPT	94	**65**	108	79	**61**	89	88	**67**	142	141	132	125	**176**	124	108	**189**	**232**
TG	0.77	0.85	0.8	0.7	0.96	0.69	0.54	0.62	1.08	0.87	0.89	1.1	0.73	0.97	**1.32**	**1.35**	**1.11**
GOT	246	244	389	276	252	300	267	295	334	333	334	315	356	292	312	382	385
T CHOL	0.96	0.89	1.01	1.04	0.92	1.05	**1.37**	0.87	1.06	**0.68**	**0.77**	**0.79**	**0.76**	**0.7**	**0.67**	0.95	0.92
HDLc	0.7	0.59	0.52	0.58	0.75	0.7	0.62	0.68	0.45	0.55	**0.32**	0.45	0.46	**0.29**	**0.27**	**0.44**	0.63
LDLc	0.11	0.13	0.33	0.32	**0.02**	0.21	**0.64**	**0.07**	0.39	**0.04**	0.27	0.12	0.15	0.22	0.14	0.24	**0.07**
T Lip	4.3	4.4	4.5	4.4	4.7	4.4	4.8	3.7	**5.4**	3.9	4.2	4.7	3.7	4.2	5	**5.8**	5.08

** In bold, abnormal parameters*

Table 3. Summary of the biochemical parameters of the males of the two batches

Parameters	Maximum	Minimum	Average	Standard deviation	Reference values
UREA	0.91	0.29	0.52	0.13	0.39-0.65 g/L
GLYC	1.67	0.1	0.83	0.36	0.47-1.19 g/L
CREAT	8.7	2.8	5.58	1.4	4.18-6.98 g/L
GPT	241	58	113.46	46.26	67.20-159.72 UI/L
TG	1.38	0.2	0.76	0.34	0.42-1.10 g/L
GOT	725	176	330.36	107.68	222.68-438.04 UI/L
T CHOL	1.37	0.67	1.01	0.2	0.81-1.21 g/dL
HDLc	0.87	0.27	0.6	0.15	0.45-0.75 g/dL
LDLc	0.64	0.02	0.26	0.16	0.10-0.42 g/dL
T lip	6.8	2.9	4.45	0.9	3.55-5.35 g/dL

Urea (g/L), Glyc : glycemia (g/L), Creat : creatinine (mg/L), GPT (UI/L), TG, : triglycerides (g/L), GOT (UI/L), T Chol (Total cholesterol) (g/L), HDLc (HDL cholesterol) (g/L), LDLc (LDL cholesterol) (g/L), T Lip (Total lipids) (g/L)

4. DISCUSSION

Since for urea, the values for males M2, M33, M35 and M37 are over the reference value (0.39-0.65 g/L); while those for males M6, M10, M13, M16, M19 and M20 are over the same reference value in Table 3, it can be said that the low values for males M2, M33, M35 and M37 would indicate hypo-uremia. This hypo-uremia would reflect severe liver failure or a deficiency in urea cycle enzyme [21]. In contrast, high values for males M6, M10, M13, M16, M19 and M20 would indirectly reveal renal and liver dysfunction [22, 21]. Renal and liver dysfunction are more important in the M6 male. As for the values for females, they would show normal kidney and liver function as these values are all within their reference value (0.50±0.27 g/L or 0.23-0.77 g/L). Statistical analysis ($p>0.05$) reveals that sex has no influence on urea.

The creatinine values of males M2, M3, M8, M9, M10, M28, M29 and M30 are under the reference value (4.18-6.98 mg/L) for males as are those of females F1, F9, F23, F24 and F26 for their reference value (5.92±1.59 mg/L or 4.33-7.51 mg/L) ; whereas those of M13, M20, M21, M31 and M32 are over their reference value as are those of females F27, F29, F30, F34, F36 and F38. Low creatinine values in males (M2, M3, M8, M9, M10, M28, M29 and M30) and females (F1, F9, F23, F24 and F26) could be a sign of cachexia [23,24,25]. While the high creatinine values in males (M13, M20, M21, M31 and M32) and females (F27, F29, F30, F34, F36 and F38) could be explained by the decrease in creatinuria, i.e. the elimination of creatinine by the kidneys because this excretion by the kidneys is more specific to creatinine than urea [25]. Thus, kidney dysfunction would be more marked in males M13, M20, M32, M21 and M31 respectively; in females, it is more visible with F34, F38, F27, F30, F36 and F29. Statistical analysis ($p>0.05$) reveals that gender does not influence creatinine.

The increase in serum urea concentration associated with that of creatinine would indicate renal failure [26,27,25]. Therefore, it is possible to infer that M13 and M20 males would obey this assertion.

In terms of carbohydrate metabolism, the blood glucose values of males M1, M3, M7, M9, M10, M13 and M18 and females F4, F5, F9 and F27 are lower than the respective reference values by 0.83±0.36 g/L (0.47-1.19 g/L) for males and 0.72±0.39 g/L (0.33-1.11 g/L) for females. This observation would reflect hypoglycemia. Hypoglycemia is thought to be caused by malnutrition or prolonged fasting, excess insulin secretion: insulinoma, polyadenomatosis, endocrine insufficiency: adrenal, pituitary, and liver disorder: acute hepatitis [5]. The males M11, M20, M21, M22, M32 and M39 and the females F25, F30, F32, F36, F37, F38 and F39 have values higher than their respective reference. This would mean hyperglycemia. Hyperglycemia would be due to insulin-dependent or non-insulin-dependent diabetes, pancreatic diseases: acute or chronic pancreatitis, endocrine diseases: pheochromocytoma, hypercorticism, corticosteroid therapy, and hypothyroidism [5]. According to Isler C [28]; blood glucose levels are also low in cases of anorexia and high in cases of diabetes. Consequently, it is possible to say that anorexia would be observed in males M1, M3, M7, M9, M10, M13 and M18, and females F4, F5, F9 and F27; while diabetes would be observed in males M11, M20, M21, M22, M32 and M39, and females F25, F30, F32, F36, F37,

Table 4. Biochemical parameters of the females of lot 1

Females / Parameters	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20	F21	F22
UREA	0.45	0.49	0.6	0.37	0.65,	0.68	0.42	0.62	0.63	0.48	0.39	0.42	0.59	0.47	0.52	0.58	0.53	0.63	0.62	0.54	0.56	0.46
GLYC	0.44	0.65	0.57	**0.15**	**0.1**	0.8	0.34	0.47	**0.1**	0.36	0.9	0.89	0.67	0.93	**0.32**	0.53	0.73	0.42	0.7	0.66	0.63	0.63
CREAT	**3.7**	5.8	7	4.5	4.7	4.6	4	6	**3.3**	4.4	6.3	5.3	5.9	5.4	5.9	7.5	6.2	6.5	5.5	4.7	5.8	7.2
GPT	82	**58**	80	117	125	94	82	91	101	157	**262**	130	79	96	85	**55**	**57**	117	94	97	83	89
TG	0.53	0.74	0.37	1.07	1.04	**6.31**	0.6	1.14	0.63	1.17	1.26	1.01	**2.45**	1.04	0.52	1.3	0.56	1.06	1.15	0.71	0.34	0.65
GOT	329	283	233	**491**	**148**	256	290	**193**	362	212	**555**	450	**183**	294	275	201	203	188	268	317	205	255
T CHOL	1.13	1.05	1.02	0.74	0.98	1.13	1.1	1.06	1.04	1	**1.17**	0.95	0.95	0.92	0.99	0.86	0.76	1.04	0.81	1.06	1.12	**1.32**
HDL c	0.64	0.71	0.81	**1**	**1.21**	**0.37**	0.75	**1.18**	0.83	0.65	0.53	**0.41**	0.75	0.55	0.64	0.61	0.42	0.8	0.6	0.45	0.66	0.73
LDL c	0.38	0.2	0.14	**0.47**	**0.44**	**0.5**	0.23	**0.47**	0.08	0.12	**0.39**	0.34	0.29	0.16	0.25	**0.01**	0.23	0.2	**0.02**	**0.57**	**0.4**	**0.46**
T lip	4.2	4.5	3.5	4.5	5.05	**18.6**	4.3	5.5	4.2	3.2	6.08	4.9	**8.5**	4.9	3.8	5.4	3.3	5.3	4.9	4.4	3.7	4.9

Table 5. Biochemical parameters of females in lot 2

Females / Parameters	F23	F24	F25	F26	F27	F28	F29	F30	F31	F32	F33	F34	F35	F36	F37	F38	F39
UREA	0.41	0.46	0.57	0.55	0.76	0.47	0.45	0.44	0.36	0.53	0.49	0.38	0.42	0.5	0.27	0,34	0,51
GLY	0.71	0.42	**1.65**	0.38	**0.3**	0.43	0.4	**1.35**	1.1	**1.42**	0.86	0.95	1.08	**1.39**	**1.24**	**1.28**	**1.18**
CREAT	**4**	**2.1**	6.3	**3.5**	**7.9**	5.1	**9.9**	**8**	6.2	7.2	6.9	**7.6**	6.9	**8.1**	6.4	**7.6**	7
GPT	85	99	**244**	107	111	89	124	**206**	144	**179**	123	147	**191**	162	136	97	160
TG	1.08	0.5	1.53	2.12	0.6	0.79	**2.21**	1.17	0.96	0.88	**2.88**	1.46	0.56	0.41	0.84	0.64	1
GOT	427	257	**772**	442	290	**195**	252	441	414	**481**	364	**626**	351	392	308	317	463
T CHOL	1	**1.36**	**1.22**	0.96	1.05	0.79	0.83	**0.68**	**0.58**	**0.61**	0.74	**0.57**	**0.67**	**0.68**	**0.59**	0.73	**0.65**
HDLc	0.65	0.83	0.84	0.77	0.79	0.53	0.54	0.48	0.48	0.48	0.45	**0.26**	**0.36**	0.44	0.44	0.52	**0.41**
LDLc	0.13	**0.43**	0.07	0.23	0.14	0.10	0.15	**0.03**	0.09	**0.05**	0.29	**0.02**	0.2	0.16	**0.02**	0.08	**0.04**
T lip	5.2	4.7	6.9	7.7	4.1	4	7.6	4.6	3.8	3.7	**9.05**	5.06	3.07	2.7	3.6	3.4	4.02

** In bold, abnormal parameters*

Table 6. Summary of biochemical parameters of the females of the two batches

Parameters	Maximum	Minimum	Average	Standard deviation	Reference values
UREA	0.76	0.27	0.50	0.27	**0.23-0.77 g/L**
GLYC	1.65	0.1	0.72	0.39	**0.33-1.11 g/L**
CREAT	9.9	3.3	5.92	1.59	**4.33-7.51 mg/L**
GPT	262	55	118.85	47.76	**71.09-166.61 UI/L**
TG	6.31	0.34	1.16	1.02	**0.14-2.18 g/L**
GOT	772	148	332.90	134.01	**198.89-466.91 UI/L**
T CHOL	1.36	0.57	0.92	0.21	**0.71-1.13 g/L**
HDLc	1.21	0.26	0.63	0.21	**0.42-0.84 g/L**
LDLc	0.57	0.01	0.22	0.16	**0.06-0.38 g/L**
T lip	18.6	2.7	5.15	2.65	**2.50-7.80 g/dL**

Urea (g/L), Glyc : glycemia (g/L), Creat : creatinine (mg/L), GPT (UI/L), TG, : triglycerides (g/L), GOT (UI/L), T Chol (Total cholesterol) (g/L), HDLc (HDL cholesterol) (g/L), LDLc (LDL cholesterol) (g/L), T Lip (Total lipids) (g/L)

Table 7. Statistical analysis of biochemical parameters by gender (ANOVA)

Parameters	Urea	Glycemia	Creatinine	GPT	GOT	T Chol	TG	HDLc	LDLc	T lip
Males	0.52 ± 0.13[a]	0.83 ± 0.36[a]	5.58 ± 1.4[a]	113.46 ± 46.26[a]	330.36 ± 107.68[a]	1.01 ± 0.2[a]	0.76 ± 0.34[b]	0.6 ± 0.15[a]	0.26 ± 0.16[a]	4.45 ± 0.9[a]
Females	0.50± 0.27[a]	0.88± 1.03[a]	5.92± 1.59[a]	118.85± 47.76[a]	332.90± 134.01[a]	0.92± 0.21[a]	1.16± 1.02[b]	0.63± 0.21[a]	0.22± 0.16[a]	5.15± 2.65[a]
F	0.610112	0.070536	1.183922	0.255738	0.008503	3.796161	**5.294026**	0.406417	1.149781	2.423517
p	0.437172	0.791278	0,279998	0.614527	0.926771	0.055062	**0.024142**	0.525710	0.286988	0.123681

p<0.05, sex has an influence on the biochemical parameter being studied.
p>0.05, sex has no influence on the biochemical parameter being studied.

F38 and F39. Statistical analysis ($p>0.05$) shows that gender has no influence on blood glucose levels.

In general, hepatic exploration implies the observation of certain parameters such as glutamo-oxaloacetic transaminase (GOT) and glutamo-pyruvic transaminase (GPT). So, the observation of the GPT values of males M12, M15, M24, M27 and M30 and those of females F2, F16 and F17 gave respective reference values of 113.46±46.26 IU/L (67.20-159.72 IU/L) for males and 118.85±47.76 IU/L (71.09-166.61 IU/L) for females below; while those of males M20, M21, M22, M35, M38 and M39 and females F25, F30, F32 and F35 are above their respective reference values.

For GOT, its values in males M10, M20, M21 and M22 and females F4, F11, F25, F32 and F34 are greater than the respective reference values of 330.36±107.68 IU/L (222.68-438.04 IU/L) for males and 332.90±134.01 IU/L (198.89-466.91 IU/L) for females; although the value in M12 and those in females F5, F8, F13 and F28 are lower than their respective reference values. In addition, measurements of liver enzyme activities of GPT and GOT would be used in the diagnosis and evaluation of liver disease [29,21]. Elevated transaminases may reflect hepatocellular damage or disruption of bile flow [21]. Therefore, elevation of transaminases (GPT and GOT) would be observed in acute hepatitis. However, GPT is a cytosolic enzyme secreted in liver cells which it is released into the bloodstream in the event of hepatic cell necrosis [30,31,32]. Thus, it is a liver-specific enzyme, making it an important and very sensitive indicator of hepatotoxicity [33, 34,32]. So, the GPT values of males M12, M15, M24, M27 and M30 and those of females F2, F16 and F17 would obey this assertion. GOT is also an indicator of hepatocyte destruction, although in addition to the liver it is found in the heart, skeletal muscle, lungs and kidneys [31, 32]. Therefore, the values for males M10, M20, M21 and M22 and females F4, F11, F25, F32 and F34 would indicate hepatocyte destruction. Statistical analysis ($p>0.05$) shows that sex has no influence on GPT and GOT.

In lipid metabolism, triglyceridemia values in males M1, M5, M8, M9, M15, M16 and M17 are within 0.76±0.34 g/L (0.42-1.10 g/L) of the reference value; while those of males M6, M12, M21, M22, M37, M38 and M39 and those of females F6, F13, F29 and F33 are also in excess of the respective reference values of 0.76±0.34 g/L (0.42-1.10 g/L) for males and 1.16±1.02 g/L (0.14-2.18 g/L) for females. According to Al-Shinnawy M, Djeffal A, [35,18]; the increase in triglycerides corresponds to hypertriglyceridemia and would be a risk factor in predicting cardiovascular disease. Indeed, triglycerides are the main constituents of cell membranes and the increase in their concentration would probably be the result of apoptosis. The males values M6, M12, M21, M22, M37, M38 and M39 and the females values F6, F13, F29 and F33 would conform to this principle. Statistical analysis ($p<0.05$) indicates that sex has an influence on triglycerides.

For cholesterolemia in males M4, M8, M32, M33, M35, M36 and M37, and females F30, F31, F32, F34, F35, F36, F37 and F39, the values obtained are respectively below their reference 1.01±0.2 g/L (0.81-1.21 g/L) and 0.92±0.21 g/L (0.71-1.13 g/L). According to Eastham RD, Diaby V, [36, 21]; this decrease in values in these individuals would indicate hypocholesterolemia. It is observed in liver damage, serious infections, anemia, treatment with particular drugs (hormones such as clofibrate and androsterone), mental retardation and congenital acyl-transferase deficiency. M1, M7, M13, M19, M22 and M29 males and F11, F22 and F24 and F25 females all have values above their respective standards. The authors suggest that this would show hypercholesterolemia. It is found in liver damage, kidney damage, pancreatic damage and thyroid damage. According to Heuillet M [37], hypercholesterolemia is characterized by a chronic inflammation of the arterial wall that develops in response to damage to the vascular endothelium. Atherosclerotic lesions develop on large and medium arteries, which are approximately 3 to 0.5 cm in diameter. Statistical analysis ($p>0.05$) indicates that gender does not influence total cholesterol.

For HDL-c, the values for males M33, M36, M37 and M38 and females F6, F12, F34, F35 and F39 are 0.6±0.15 g/L (0.45-0.75 g/L) and 0.63±0.21 g/L (0.42-0.84 g/L) respectively below their reference; while those of males M2, M7, M12, M17 and M19 and females F4, F5 and F8 are over their respective reference values. According to [38]; a 1% increase in HDL-c is associated with a 3-4% decrease in coronary risk, and a level below 0.40 g/L in humans is considered a risk factor for cardiovascular disease. Similarly, the work of [39] showed that an increase in HDL-c levels would indicate a protective factor for the heart muscle, thus revealing its beneficial effect

against cardiovascular complications such as atherosclerosis. Consequently, males M2, M7,

M12, M17 and M19 and females F4, F5 and F8 would observe this principle unlike males M33, M36, M37 and M38 and females F6, F12, F34, F35 and F39. Statistical analysis ($p>0.05$) reveals that sex has no influence on HDL-c.

With LDL-c, the values of males M1, M3, M9, M13 and M29 and females F4, F5, F6, F8, F11, F20, F21, F22 and F24 are above their respective reference values 0.26±0.16 g/L (0.10-0.42 g/L) and 0.22±0.16 g/L (0.06-0.38 g/L). Males M2, M4, M6, M12, M27, M30, M32 and M39, and females F16, F19, F30, F32, F34, F37 and F39 have values below their respective reference. According to Lewington S et al. Heuillet M [40,37]; a 0.38 g/L reduction in LDL cholesterol would be associated with a one-third reduction in coronary mortality. Thus, males M2, M4, M6, M12, M27, M30, M32 and M39, and females F16, F30, F32, F34, F37 and F39 would respect this assertion, as opposed males M1, M3, M9, M13 and M29 and females F4, F5, F6, F8, F11, F20, F22 and F24. Statistical analysis ($p>0.05$) indicates that gender does not influence LDL-c.

For T lip, the values for males M4, M5, M8, M9, M15 and M17 and females F6, F13 and F33 are above their respective reference values by 4.45±0.9 g/L (3.55-5.35 g/L) and 5.15±2.65 g/L (2.50-7.80 g/L). Males M6, M12, M21, M22, M31 and M38 are below its reference value. According to Gervois P et al. Heuillet M [41,37]; dyslipidemia is one of the main cardiovascular risk factors. Their detection is mainly based on an analysis called "exploration of a lipid abnormality" or "ELA", which combines the assay of several lipid compounds. In practice, ELA is performed on an empty stomach and includes the determination of total cholesterol (TC), triglycerides (TG), HDL-cholesterol and LDL-cholesterol. Dyslipidemia is differentiated into pure hypercholesterolemia, pure hypertriglyceridemia and combined hyperlipidemia. Thus, to the extent that the M22 male has both high Total Cholesterol and triglyceride values; while the F6, F13 and F33 females also have high triglyceride and total lipid values compared to their respective standards, it can be said that these aforementioned individuals may be exposed to cardiovascular risk factors. Statistical analysis ($p>0.05$) shows that gender has no influence on T lip.

5. CONCLUSION

The results of this study showed that most of the animals had normal biochemical parameters. The values of these individuals allowed the determination of standards at the rat level with more representative sampling. Nevertheless, some individuals had fluctuating biochemical parameters. Variations in these parameters can be the cause of many pathologies. Thus, these pathologies are observed in a particular way at the level of:

- 2 males out of 39 with kidney problems;
- 6 out of 39 males and 7 out of 39 females for problems with their pancreas;
- 7 out of 39 males and 4 out of 39 females with anorexia;
- 4 out of 39 males and 5 out of 39 females with liver problems;
- 7 out of 39 males and 4 out of 39 females with high triglycerides;
- 6 out of 39 males and 4 out of 39 females were hypercholesterolemic;
- 4 out of 39 males and 5 out of 39 females who had a risk factor for cardiovascular disease;
- 5 out of 39 males and 9 out of 39 females who were exposed to coronary heart disease;
- 6 out of 39 males and 3 out of 39 females had hyperlipidemia;
- 1 out of 39 males and 3 out of 39 females had dyslipidemia and therefore the possibility of developing cardiovascular disease.

Statistical analysis of the various biochemical parameters revealed that sex has an influence on triglycerides only.

On the basis of the standard biochemical parameters obtained, it would be plausible to carry out tests of certain substances (food and/or medical) *in vivo* in order to determine their harmful or beneficial effects on vital organs and by ricochet extend it to man.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gupta AR, Patra RC, Saini M, Swarup D. Haematology and serum biochemistry of chital (axis axis) and barking deer (*Muntiacus muntjak*) reared in semi-captivity. Vet Res Comm. 2007;31:801-08.
2. Yapo AE., Assayi MJ, Aka B, Bonetto R, Lonsdorfer A, Comoé L et al. The reference values of 21 blood biochemical constituents of presumed healthy Ivorian adults. African pharmacy. 1989;44:13-21.
3. Boum B, Tantchou J. Biochemical standards of Cameroonian in the region of Yaoundé. Science and Technical Review. 1985;11(1):103-7.
4. Acker P, Maydat L, Trapet P. Quelques constantes biochimiques actuelles de l'africain congolais normal. Bull Soc Pathol. 1987;1:460-7.
5. Siby S. Study of the variation of biochemical and hematological parameters in the district of Bamako. State Thesis in Medicine. Faculty of Medicine, Pharmacy and Odonto-Stomatology, University of Bamako, Mali. 2008;77.
6. Sandé J, Coulibaly JL, Njikeutchi F, Bouabré A, Boukary A, Guissou IP. Establishment of reference values for 15 biochemical blood constituents in adults from Burkina Faso in Ouagadougou (Burkina-Faso). Annals of Clinical Biology. 2004;62:229-34.
7. Vincent-Viry M, Henny J, Clerc M, Siest G. Discussion of some "Reference limits" of European and African populations. (Practical conclusions. International cooperative study). Med Afri Noire. 1987;34(5):459-465.
8. Popoff M. Biochemical data in sheep: application to the differential diagnosis of some metabolic diseases. Bull Soc vét prat Fr. 1981;9:695-06.
9. Obi TU, Oduye OO. Haematological changes in natural and experimental peste des petits ruminants virus infection in goats. Rev high med vet country too. 1985;38:11-5.
   Available:https://doi.org/10.19182/remvt.8534
10. Ndoutamia G, Ganda K. Determination of haematological and biochemical parameters of small ruminants in Chad. Rev Med Vet. 2005;156(4):202-06.
11. Descat F. Rat haematology: Haemogram and myelogram. Exercise thesis, National Veterinary School of Toulouse – ENVT. 2002;110.
12. Sakandé J, Ahiboh H, Edjemé A, Yapo AE. Study of the biological tolerance of a plant with antiplasmodial activity Momordica charantia (Cucurbitaceae). Mali Medical. 2003;XVIII(1-2):1-4.
13. Farougou S, Kpodekon M, Loko F, Brahi OHD, Agniwo.B, Djago.Y. Usual values of the main serum biochemical parameters in rabbits *(Oryctolagus cuniculus*) reared in Benin. RASPA. 2007;5(1-2):17-22.
14. Fasce CF. Serum Cholesterol determined colorimetrically with enzyme. Clin Chem. 1982;18:901.
15. Bouiddouh FZ. Evolution of serum biochemical parameters in Wistar rats treated with an ethanolic extract of colocynth (*Citrullus colocynthis*) seeds. Master's thesis in Biology Option: Applied Biochemistry, Faculty of Nature and Life Sciences and Earth and Universe Sciences, Abou Bakr Belkaid-Tlemcen University, Algeria. 2012;63.
16. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem. 1982;28:2077-80.
    DOI: 10.1093 /clinchem / 28.10.2077
17. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of Low Density Lipoprotein Cholesterol in plasma, Without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499-02.
    Available:https://doi.org/10.1093/clinchem/18.6.499
18. Djeffal A. Evaluation of the toxicity of a carbamate insecticide "methomyl" in Wistar rats: Oxidative stress and exploration of the protective effects of selenium and / or vitamin C supplementation. 3rd Cycle Thesis, Option: Applied Biochemistry. Faculty of Sciences, Badji Mokhtar-Annaba University, Algeria. 2014;170.
19. Amotte M. Introduction to statistical methods in biology 2th ed. Paris: Masson et Cie; 1971.
20. Schwartz D. Statistical Methods for the Use of Physicians and Biologists. 3th ed. Paris: Flammarion medicine-Sciences; 1992;311.

21. Diaby V. Biochemical, hematological and histological studies of cadmium sulfate in Wistar rats. Doctoral Thesis, Specialty: Functional and Molecular Biology, UFR Biosciences, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire. 2017; 130.
22. Grenier-Michaud S, Lyne C, Pierre N. Understanding renal function is an essential component of paraclinical surveillance. Nurse perspective. 2011;30-5.
23. Pitel Ph, Moulin M, Valette J-P, Dumontier S, Petit L, Fortier G, et al. Approach to haematological and biochemical values in two asina breeds. Equine Veterinary Practice. 2006;38(149):19-25.
24. Pritchard JC, Burn CC, Barr ARS, Whay HR. Haematological and serum biochemical reference values for apparently healthy working horses in Pakistan. Res Vet Sci. 2009;87:389-95.
DOI: 10.1016 / j.rvsc.2009.05.003
25. Zabré ZM. Determination of the usual biochemical parameters in small ruminants in Burkina Faso and their variations in subjects naturally infected with trypanosomiasis. Veterinary Thesis, Faculty of Medicine, Pharmacy and Odonto-Stomatology, Cheikh Anta Diop University, Dakar, Senegal. 2013;71.
26. Abenga JN, Anosa VO. Serum total proteins and creatinine levels in experimental Gambian trypanosomosis of vervet monkeys. Afr J Biotechnol. 2005;4:187-90.
27. Hilali M, Abdel-Gawad A, Nassar A, Abdel-Wahab A. Hematological and biochemical changes in water buffalo calves (*Bubalus bubalis*) infected with Trypanosoma evansi. Vet Parasitol. 2006;139:237-43. DOI: 10.1016 / j.vetpar.2006.02.013
28. Isler C. Evolution of biochemical parameters during left displacement of the abomasum in dairy cows: Study of four cases. Veterinary Thesis, National Veterinary School, Claude-Bernard University, Lyon I, France. 2007;111.
29. Kim WR, Flamm SL, Di Bisceglie AM, Bodenheimer HC Jr. Serum activity of alanine aminotransferase (ALT) as an Indicator of health and disease. Hepatol. 2008;1363-70.
DOI: 10.1002/hep.22109
30. Kaneko JJ, Harvey JW, Bruss ML. Clinical biochemistry of domestic animals. 5th ed. Academic Press, San Diego, California. 1997;932.
31. Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. Diagnosis and monitoring of hepatic injury II. Recommendation for use of laboratory tests in screening, diagnosis and monitoring. Clin Chem. 2000;46:2050-68.
DOI: 10.1093 / clinchem / 46.12.2050
32. Gomé MB, Kouakou K, Touré A, Traoré F. Study of the acute and subchronic toxicity of aqueous extract of *Passiflora foetida* Linn. (Passifloraceae) in rats and mice. IJBCS. 2011;5(5):1777-89.
DOI: http://dx.doi.org/10.4314/ijbcs.v5i5.1
33. Pratt DS, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. N Engl J Med. 2000;342:1266-71.
34. Al-Habori M, Al-Aghbari A., Al-Mamary M., Baker M. Toxicological evaluation of Catha edulis leaves: a long-term feeding experiment in animals. J Ethnopharmacol. 2002;83:209-17.
DOI: 10.1016/S0378-8741(02)00223-4
35. Al-Shinnawy M. Vitamin C as ameliorative agent against thiodicarb toxicated male albino rats (Rattus norvegicus). Egypt Acad J Biol Sci. 2008;1(2):177-87.
36. Eastham RD. Abbreviated as biological constants. ed. Masson: Paris, France. 1978;248.
37. Heuillet M. Development of reference methods for biomarkers of lipid balance: application to quality control in clinical biology. Doctoral Thesis, Discipline: Life Sciences, University of Burgundy, Paris, France. 2013;199.
38. Lahoz C, Mostaza JM. Atherosclerosis as a systemic Disease. Rev Esp Cardiol. 2007;60(2):184-95.
DOI: 10.1016/S1885-5857(07)60131-5
39. Bidié ADP, Adeoti FM, Yapo FA, Tiekpa JW, N'guessan JD, Djaman JA. Effect of total aqueous extract of Chrysophyllum perpulchrum on hematological, biochemical parameters and weight growth in wistar healthy rats. REVIST. 2016;28:33-348.
40. Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, Halsey J et al. Blood cholesterol and vascular mortality by age, sex, and blood pressure: A meta-analysis of individual data from 61

prospective studies with 55,000 vascular deaths. Lancet. 2007;370:1829-39. DOI: 10.1016/S0140-6736(07)61778-4

41. Gervois P, Balduyck M, Brousseau T. Chapter 10: Cardiovascular disease: markers of atherosclerosis, coronary heart disease and stroke. In: Durand G, Beaudeux J-L. 2th ed. Medical biochemistry: current markers and perspectives. EM inter. 2008;99-114.

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