Some Blood Indexes of the Tortoise *Testudo graeca* Linn., 1758, From Benghazi Province, Libya

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***Abstract*- Hematological and chemical tests have been used as tools to report some blood indexes means and confidence intervals (95%) of the adult "Moorish" or "Greek" male and female tortoises, *Testudo graeca* Linnaeus, 1758. The area of study was limited to Benghazi province, Libya. Cardiocentesis was employed to obtain blood samples from 25 males and 25 females, which apparently looked healthy tortoises. The anticoagulant of choice, when it was needed, was lithium heparin to avoid destructive effects of the potassium ethylenediaminetetraacetate on cellular elements. Means of the erythrocytes counts as well as confidence intervals of both sexes were below the million per cubic millimeter. Low values were also recorded for packed cell volume and hemoglobin concentration. Males had significantly higher erythrocytes counts (0.817 ± 0.245 X 10^6/uL), packed cell volume (27.72 ± 05.31 %) and hemoglobin concentration (08.04 ± 01.06 g/dL) means as compared with the corresponding means of the females (0.668 ± 0.126 X 10^6/uL, 23.68 ± 03.40 % and 07.32 ± 0.71 g/dL, respectively). The calculated mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration of the males (354.28 ± 66.46 fL, 105.04 ± 24.72 pg and 29.76 ± 04.96 g/dL, respectively) and females (363.12 ± 63.99 fL, 113.04 ± 20.71 pg and 31.24 ± 03.53 g/dL, respectively) lacked significant sex influence. Means of the males' serum sodium (137.16 ± 6.56 mmol/L), potassium (06.00 ± 0.88 mmol/L), chloride (118.72 ± 10.47 mmol/L), magnesium (05.64 ± 01.30 mg/dL), calcium (11.31 ± 01.10 mg/dL) and phosphorus (03.17 ± 0.76 mg/dL) were comparable (P > 0.05) with the corresponding means of the females (138.80 ± 9.02 mmol/L, 06.02 ± 0.84 mmol/L, 116.96 ± 09.07 mmol/L, 05.33 ± 01.27 mg/dL, 11.78 ± 01.28 mg/dL and 03.32 ± 0.83 mg/dL, respectively). The proteinous and non-proteinous nitrogenous compounds of both sexes had close means (P > 0.05). Means of the male tortoises total proteins (3.99 ± 0.96 g/dL), albumin (1.46 ± 0.31 g/dL), globulin (2.30 ± 0.84 g/dL), urea (43.16 ± 11.30 mg/dL), uric acid (2.94 ± 1.13 mg/dL) and creatinine (0.24 ± 0.15 mg/dL). On the other hand, the corresponding means of these nitrogenous compounds in females serum samples were, respectively, 3.62 ± 0.96 g/dL, 1.45 ± 0.44 g/dL, 2.20 ± 0.72 g/dL, 38.56 ± 09.99 mg/dL, 3.22 ± 1.59 mg/dL and 0.28 ± 0.15 mg/dL. Low levels of plasma glucose were recorded in both males (53.84 ± 07.88 mg/dL) and females (56.88 ± 18.86 mg/dL). Statistical significance could not be detected when those means were compared with each other (P > 0.05). Such absence of significance was also observed when mean of males serum cholesterol (225.28 ± 28.90 mg/dL) was compared with mean of female serum cholesterol (248.56 ± 69.80 mg/dL). Triglycerides concentration in serum samples of the female tortoises (242.56 ± 92.91 mg/dL) reached more than twice that of the males (103.92 ± 22.75 mg/dL). Comparing these triglycerides means with each other pointed out to the existence of a high significant difference (P = 0.00).**

***Index Terms*— Testudo graeca, Hematology and Blood Chemistry**

I. INTRODUCTION

In 1831 Charles Darwin started, on the surveying ship: the Beagle, what has been described as the most consequential voyage in the history of Biology. The chelonians (turtles and tortoises) occupied a considerable space in his observations. [1]. The chelonians of the pond, river and sea are termed turtles, whereas the term tortoises is used for the land dwelling chelonians. The American Tortoise Rescue (http:// www.tortoise.com), in 2008, sponsored May 23rd of each year as the World Turtle Day. It aimed at encouraging protection of

these creatures and getting involved in scientific research on their biology (www.en.wikipedia.org/ World_Turtle_Day; www.days of the year. com /days/05).

Hematological Tests

On the basis that blood status reflects health condition of the organism, scientific literature contains voluminous information on hematological values in health and disease. Some of the chelonian normal hematological data are influenced by several factors such as geographic location [2; 3], season [4; 5] and sex [6; 7; 8]. Captivity as well as any other kind of stress, including deprivation of forage, could result in significant changes in some hematological indicators. Rainfall patterns and availability of the herbaceous plants influenced the assessed hematological parameters [9; 10]. Even annual differences were observed in some blood indices [11]. Mathes *et al*. [12] observed significant differences in some blood reference values depending on the species of the tortoise within the same *Testudo* genus. Post hibernational dehydration that was reported in *Testudo graeca* and *Testudo hermanni* [13] could be a reasonable source of variation in some blood parameters. These above mentioned main sources of variations in hematological values have to be considered in establishing means and confidence intervals for the physically examined normal tortoise.

Erythrocyte count, packed cell volume and hemoglobin concentration values in blood samples of male and female desert tortoises of California, U. S. A., were reported by Christopher *et al*. [14]. Packed cell volume was also determined in tortoises of the same desert [7] and in tortoises of Arizona, U. S. A. [11]. Tortoises packed cell volume and hemoglobin concentration values were estimated in Nigeria [15] with significant sex differences.

Mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration values of the clinically healthy tortoise *Gopherus agassizii* were calculated according to the standard formulas. Considerable variation in the calculated values was observed with wide confidence intervals [14]. Blood samples of healthy and viral infected *Testudo graeca* from Spain were analyzed for erythrocyte count, packed cell volume and hemoglobin concentration. Mean values of the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were calculated. All corresponding means of the healthy and viral infected tortoises did not show significant variations [16]. Significant variations were observed under stress and disease conditions [17]

Chemical Tests

A wide range of tests have been performed to identify the chemical components of the chelonian blood. Some of the tests were directed toward endorsement of presence or absence. Isolation of antifreeze glycoproteins from serum and their leakages from liposomes was achieved on blood samples from *Testudo graeca* [18]. Presence of specific immunogama globulins was confirmed by enzyme-linked immunosorbent assay (ELISA) and associated with various pathogens infection [19; 20].

Plasma concentration of 25-hydroxycalciferol in captive *Testudo graeca*, *Testudo hermani* and *Testudo marginata*. Seasonal variations were observed without statistical significance, but values of the females were always significantly lower than those of the males [21]. Sex steroid hormones and adrenal cortex corticosterone were radioimmunoassayed in male and female tortoises *Gopherus agassizii* to establish their blood concentration patterns according to their seasonal activity. High levels of corticosterone in males were associated with the peak in spermatogenesis and male-male fighting [22]. Olayemi and Adeshina [23] analyzed blood plasma of the apparently healthy West African tortoise *Kinixys erosa* for levels of sodium, potassium, chloride, calcium, total proteins, albumin, globulin, cholesterol, triglycerides, urea, creatinine and the activity of the enzymes alkaline phosphatase (ALP), glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and gama-glutamate transferase (GGT).

Plasma total proteins, albumin, phospholipids, cholesterol, triglycerides, testosterone, progesterone, calcium and phosphorus were determined in wild *Testudo horsfieldi* in Uzbekistan during the activity period covering the months of March and April. The concentration of total proteins, albumin, lipids and phosphorus showed slow increments with increased activity in males, whereas the increment was rapid in females [24]. Plasma lipid fractions including total lipids, fatty acids, triglycerides, phospholipids, cholesterol and cholesterol esters were estimated in blood of the tortoise *Gopherus agassizii* during the reproductive cycle of the males and females. Excluding free fatty acids, all lipid fractions were significantly higher in females plasma as compared with those of the males [25]. Plasma sodium, uric acid and lactic acid dehydrogenase activity of the male tortoise *Geochelone radiata* were higher during winter as compared with female. On the other hand, male urea and lactic acid dehydrogenase activity during summer were higher than those of the female tortoises [26]. Blood urea and glucose concentrations were determined in captive *Testudo graeca* and *Testudo hermanni*. Values of both sexes did not show significant differences [4]. Sick chelonians showed variations in blood chemistry values as compared with those of healthy specimens [27].

The present study has been designed with an objective of reporting some blood indexes of the adult *Testudo graeca* from Benghazi Province. The objective has been achieved by means of hematological and chemical tests on blood samples of apparently clinically healthy male and female tortoises.

II. MATERIALS AND METHODS

A. Animals

Male and female tortoises of the species *Testudo graeca* were brought to the laboratory where they were washed with water to remove dirt. They were examined for their characteristic thigh tubercles and four rows of enlarged scales on the ventral side of their forelimbs. The carapace length of each of the Greek tortoises was measured to confirm adulthood. In males the carapace length ranged between 17 – 25 cm with an average of 20.4 cm, whereas in females the range was 18 – 25 cm with an average of 21.1 cm. The dark to yellow carapace scutes with extensive dark coloration on the marginal areas, beside areas of dark coloration on the humeral, pectoral, abdominal and femoral plastron scutes, favored the subspecies *Testudo graeca graeca*. Furthermore, the spots of dark coloration were more prominent on the abdominal and femoral plastron scutes (Figures 1 and 2). Males were recognized by their wider anal plastron scutes and their longer

and thicker tails. Criteria for identification of the species, subspecies, male and female have been described by Schleich *et al.* [28].

The animals were externally examined to confirm absence of lacrimation, nostril discharge, ectoparasites, pathologic lesions on the head, neck and extremities as well as damage to the carapace and plastron. A few number of tortoises were employed to practice collection of blood samples and carrying out the hematological tests. Then after, 25 males and 25 females were the source of the data that are presented in this investigation.

B. Chemicals

With the exception of tests whose reagents were supplied as kits or prepared packages, all chemicals of other tests were of laboratory technical grade. Liquid chemicals were not subjected to further purification. Aqueous solutions were prepared with the use of glass distilled water.

C. Blood Sampling

With the use of an electric drill, a hole (one millimeter in diameter) was made through the plastron on the cardiac site. A needle (gauge 19) was inserted through the hole into the heart. Blood used to flow freely. About two ml of blood were placed into a plastic tube containing lithium heparin as an anticoagulant. Another blood sample, about 2 ml, was placed into a plain plastic container.

D. Hematological Tests

1. Erythrocytes Count

During the preliminary trials, an automated analyzer was tried to obtain results on some of the hematological indexes (Sysmex Hematology Systems, Model KS- 12N, Sysmex Corporation, Japan). The instrument is used to read data on human blood as well as blood of some mammalian laboratory animals such as rats and mice. Apparently, because of the large-in-size nucleated erythrocytes of the tortoise, the analyzer could not work on the collected blood samples. Consequently, erythrocytes count (10^6/uL), packed cell volume (%) and hemoglobin concentration (g/dL) were manually obtained.

Improved Neubauer chamber (Weber Scientific International Ltd., Sussex, England) was employed to count the erythrocytes. Non-coagulated blood, to which lithium heparin was added, was diluted 200 times in a diluting pipette using Hendrick's diluting solution. This diluting solution was recommended for counting reptilian blood erythrocytes [29]. Houston [30] has described preparation steps of this solution and suggested its use for fish erythrocytes count.

2. Packed Cell Volume

The hematocrit value was obtained by the use of the heparinized capillary tubes and the Microhematocrit Centrifuge (Hettick Hematokrit, Superior, Tuttingen, Germany). The specified capillary tubes were incompletely filled with the non-coagulated blood, sealed at one end with plasticized clay then centrifuged at 3000 rpm for five minutes. Percentage of the packed cell volume was estimated by means of the circular ruler that was supplied by the centrifuge manufacturer.

3. Hemoglobin Concentration

The cyanomethemoglobin colorimetric method was employed for the determination of hemoglobin (g/dL) in the non-coagulated blood samples. Steps of the estimation procedure were described by the supplier of the analysis kit depending on development of stable colored by Drabkin solution (Biomaghreb, Tunisia). Spectrophotometer (LP 700, Lange, Gmbh, USA) was used to read the optical density of the prepared solutions at 540 nm wavelength. Turbidity of the final solution was observed because of the erythrocytes nuclei resulting in false high estimated hemoglobin values. Therefore, centrifugation was carried out at 3000 rpm for five minutes to ensure packed accumulation of the nuclei at the bottom of the tube leaving clear diluted solution through which the light beam of the spectrophotometer passes.

4. Calculation of Erythrocytes Indices

Data on erythrocytes count (RBC count), packed cell volume (PCV) and hemoglobin concentration (Hb) have been plugged into the standard formulas for estimation of the mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg) and mean corpuscular hemoglobin concentration (MCHC, g / dL). The formulas were described by Hall [31].

E. Chemical Tests

1. Sodium, Potassium and Chloride

Serum aliquots were analyzed for these three major cations (mmol/L). Analysis was carried out on an automated electrolytes analyzer (Electrolyte Analyzer, model AVL- 9180, AVL Scientific Corporation, USA). The required reagents were obtained from the manufacturer of the instrument (AVL ISE Snap Pak Reagents Kit, AVL Scientific Corporation, USA). The basis on which the tests are established as well as flow sheet of the procedure steps have been outlined in the leaflet of the reagent kit.

2. Magnesium

Magnesium (mg/dL) content of the serum samples was determined by means of a photometric method. The spectrophotometer (LP 700, Lange, Gmbh, USA) was set at wavelength 520 nm. The method is based on a chemical reaction with xylidylblau in presence of ethanolamine (pH 11). The reagents kits were supplied by DIALAB, Austria (Kit Reference: DO1243, DIALAB Produktion und Vertrieb von Chemisch- Technischen, Austria). Beside the reagents, description of the procedure and possible interference factors have been included in the attached leaflet.

3. Calcium

A kit of reagents (kit reference: D99097) designed for quantitative determination of calcium in serum, plasma or urine was obtained from DIALAB, Austria (DIALAB Produktion und Vertrieb von Chemisch- Technischen, Austria). The reagents were used for estimation of calcium (mg / dL) in serum samples of the tortoise *Testudo graeca*. The method is based on a defined chemical reaction leading to development of specific color. Change in absorbance was read on a spectrophotometer (LP 700, Lange, Gmbh, USA) at wavelength 570 nm.

4. Phosphorus

Ammonium molybdate method for estimation of inorganic phosphorus in serum, plasma or urine was employed to determine phosphorus (mg / dL) in the harvested serum samples. The reagent was supplied in a kit (Kit reference: D00362) by DIALAB, Austria (DIALAB Produktion und Vertrieb von Chemisch- Technischen, Austria). Absorbance of the prepared solutions was recorded on a spectrophotometer (LP 700, Lange, Gmbh, USA) at wavelength 340 nm.

5. Total Proteins

Total proteins (g / dL) of the serum samples were determined by means of the Biuret colorimetric method. DIALAB, Austria (DIALAB Produktion und Vertrieb von Chemisch- Technischen, Austria) was the supplier of the reagent kit (Kit reference: D95680). The wavelength of the

employed spectrophotometer (LP 700, Lange, Gmbh, USA) was set at 540 nm.

6. Albumin

A single reagent of bromocresol green in citrate buffer (pH 4.2) was employed for colorimetric determination of serum albumin (g / dL). The reagent kit was purchased from DIALAB, Austria (DIALAB Produktion und Vertrieb von Chemisch- Technischen, Austria). Light absorbance, at wavelength 540 nm, was observed on a spectrophotometer (LP 700, Lange, Gmbh, USA).

7. Globulin

Globulin (g / dL) of each serum sample was calculated by subtracting its albumin from its total protein.

8. Urea

The enzymatic (urease) colorimetric method was the method of choice for determination of serum urea (mg / dL). The required three reagents were in a kit (Kit reference: 402999) whose supplier was DIALAB, Austria (DIALAB Produktion und Vertrieb von Chemisch- Technischen, Austria). A spectrophotometer (LP 700, Lange, Gmbh, USA) was employed to estimate absorbance at wavelength 580 nm.

9. Uric Acid

A kit (Kit reference: D95459) containing two reagents was obtained from DIALAB, Austria (DIALAB Produktion und Vertrieb von Chemisch- Technischen, Austria) for determination of serum uric acid (mg / dL). The wavelength 520 nm was set on a spectrophotometer (LP 700, Lange, Gmbh, USA) to measure absorbance.

10. Creatinine

Quantitative assay of creatinine (mg / dL) in serum samples was carried out with the aid of three reagents in a kit (Fluitest Crea Kinetic, Kit reference: # 448). The provider of the kit was Biocon Diagnostik, Germany. The wavelength 490 nm was adjusted to read light absorbance on a spectrophotometer (LP 700, Lange, Gmbh, USA).

11. Glucose

Plasma aliquots were subjected for glucose assay (mg / dL). An enzymatic colorimetric method was employed. The required kit, of three reagents (Kit reference: # 4611), was obtained from Biocon Diagnostik, Germany. Changes in light absorbance by the prepared mixtures were determined at wavelength 546 nm of a spectrophotometer (LP 700, Lange, Gmbh, USA).

12. Cholesterol

Serum cholesterol (mg / dL) was assayed colorimetrically on a spectrophotometer (LP 700, Lange, Gmbh, USA). The instrument was set up at wavelength 500 nm to measure changes in light absorbance of the mixtures. The components of the assay kit (Kit reference: D95116) were provided by DIALAB, Austria (DIALAB Produktion und Vertrieb von Chemisch- Technischen, Austria). Test principles, test procedure, calculation as well as precautions of interfering substances are included in the leaflet that was usually attached with the purchased kits. The test is efficient within the range of 03 – 800 mg/dL.

13. Triglycerides

A ready to use single liquid reagent was obtained from DIALAB, Ausria (DIALAB Produktion und Vertrieb von Chemisch- Technischen, Austria) for photometric determination of the triglycerides (mg / dL) in serum samples. The ordered kit carried reference code of D00389. The triglycerides values were calculated after measurement of the changes in light absorbance by the prepared mixtures at wavelength 500 nm on a spectrophotometer (LP 700, Lange, Gmbh, USA).

F. Statistical Analysis

The obtained numerical data were statistically analyzed to test the significance of differences between the calculated means. The means were compared by using the t-test [32] and the P values, beside means and standard deviations were included in the tables. The P value of less than 0.05 was considered an indication of significant difference between the compared means. Confidence intervals (95 %) were also computed and included in the results tables. Statistica 6.0 (Microsoft Corporation, USA) was the program employed for statistical analysis.

III. R E S U L T S

A. HEMATOLOGICAL TESTS

1. Erythrocyte Count

Blood samples of the male tortoises have had erythrocytes count mean of 0.817 ± 0.245 10^6/uL. On the other hand 0.668 ± 0.126 10^6/uL was the females red blood cells count mean. Statistical analysis detected a significant difference between the compared males and females means (P = 0.0096). The confidence intervals (95 %) of the males and females erythrocyte count were, respectively, 0.715 – 0.918 and 0.615 – 0.720 10^6/uL (Table 1).

2. Packed Cell Volume

Statistically significant (P = 0.0024) high values of packed cell volume, with a mean of 27.72 ± 05.31 %, were recorded for males as compared with the corresponding means of the females, 23.68 ± 03.40 %. Confidence intervals of the packed cell volume for males and females blood samples were, respectively, 25.52 – 29.91 and 22.27 – 25.08 % (Table 1).

3. Hemoglobin Concentration

Table 1 also shows means and confidence intervals of the hemoglobin concentration (g / dL). Males *Testudo graeca* blood contained more hemoglobin (08.04 ± 01.06) than females blood did (07.32 ± 00.71) with a P value of 0.0078. Values of the males had a confidence interval of 07.59 – 08.48, whereas the confidence interval of the females values was 07.03 – 07.62.

4. Mean Corpuscular Volume

Means of the corpuscular volume of the male (354.28 ± 66.46 fL) and female (363.12 ± 63.99 fL) tortoises were comparable (P = 0.6340). The confidence interval (95%) was 326.84 – 381.71 for the males and 336.70 – 389.53 for the females (Table 1).

5. Mean Corpuscular Hemoglobin

Mean corpuscular hemoglobin of the males (105.04 ± 24.72 pg) did not show significant decrease (P = 0.2209) when compared with that of the females (113.04 ± 20.71 pg). The confidence intervals, as presented in Table 1, of the mean corpuscular hemoglobin values were 094.83 – 115.24 and 104.48 – 121.59 for males and females tortoises, respectively.

6. Mean Corpuscular Hemoglobin Concentration

Male tortoises had an average of 29.76 ± 04.96 g/dL for their mean corpuscular hemoglobin concentration. Females average was 31.24 ± 03.53 g/dL. Comparison of the males mean with that of the females showed lack of significant difference (P = 0.2310). Confidence intervals of the males and females means were, respectively, 27.70 – 31.81 and 29.77 – 32.70 g/dL.

B. CHEMICAL TESTS

1. Ions

Table 2 shows means, standard deviations, confidence intervals of males and females *Testudo graeca* tortoises blood ions. Serum sodium, potassium and chloride of the males recorded respective means of 137.16 ± 6.56, 06.00 ± 0.88 and 118.72 ± 10.47 whose corresponding confidence intervals were 134.44 – 139.87, 05.63 – 06.36 and 114.39 – 123.04 mmol/L. Means of these electrolytes in serum samples of the female tortoises were 138.80 ± 9.02, 06.00 ± 0.88 and 113.21 – 120.70 mmol/L for sodium, potassium and chloride, respectively. The corresponding confidence intervals for the females serum electrolytes were, respectively, 135.07 – 142.52, 05.67 – 06.37 and 113.21 – 120.70 mmol/L. P values were more than 0.05 indicating that the corresponding means of the males and females were comparable.

Serum magnesium, calcium and phosphorus means are also presented in table 2. Means of these ions in males serum samples did not show significant differences as compared with those of the female tortoises (P values were more than 0.05). Average values were 05.64 ± 01.30, 11.31 ± 01.10 and 03.17 ± 0.76 for male tortoises and 05.33 ± 01.27, 11.31 ± 01.10 and 03.32 ± 0.83 mg/dL for females magnesium, calcium and phosphorus, respectively. The confidence intervals for these ions concentrations were, respectively, 05.10 – 06.17, 10.85 – 11.76 and 02.86 – 03.49 for males, whereas those of the female tortoises were 04.80 – 05.85, 11.25 – 12.31 and 02.98 – 03.67 mg/dL.

2. Nitrogenous Compounds

Serum total protein, albumin and globulin means were 3.99 ± 0.96, 1.46 ± 0.31 and 1.95 – 2.65 for the male *Testudo graeca* and were 1.95 – 2.65, 1.26 – 1.63 and 1.90 – 2.49 g/dL for the females, respectively. The computed P values showed non-significant differences between the compared corresponding means (Table 3). The confidence intervals (95%) of these proteinous nitrogenous compounds were, respectively, 3.59 – 4.39, 1.32 – 1.58 and 1.95 – 2.65 in male tortoises. The respective confidence intervals for the female tortoises were 3.22 – 4.01, 1.26 – 1.63 and 1.90 – 2.49 g/dL.

Table 3 shows, also, the estimated means and confidence intervals of three non-proteinous nitrogenous compounds in sera of the male and female tortoises. Urea, uric acid and creatinine of males serum aliquots averaged 43.16 ± 11.30, 2.47 – 3.41 and 0.18 – 0.30 mg/dL, respectively, while the respective confidence intervals were 38.49 – 47.82, 2.47 – 3.41 and 0.18 – 0.30 mg/dL. On the other hand, analysis of serum samples of the female tortoises came out with respective means of 38.56 ± 09.99, 3.22 ± 1.59 and 0.28 ± 0.15, whereas the corresponding confidence intervals were 34.43 – 42.68, 2.56 – 3.87 and 0.21 – 0.34 mg/dL. Means of these three nitrogenous compounds in males and females sera did not show significant differences. All of the P values of the t-test were more than 0.05.

3. Glucose, Cholesterol and Triglycerides

Plasma glucose in male tortoises had means that were close to those of the females (P = 0.4608). Glucose concentration mean and confidence interval were 53.84 ± 07.88 and 50.58 – 57.09 mg/dL, respectively, in males. In female *Testudo graeca* tortoises the corresponding values were 56.88 ± 18.86 and 49.09 – 64.66 mg/dL (Table 4).

Data in Table 4 show cholesterol and triglycerides concentrations in serum samples of the male and female tortoises. Cholesterol concentration of the males (mean: 225.28 ± 28.90; confidence interval: 213.34 – 237.21) did not differ significantly (P = 0.1299) from that of the female tortoises whose mean and confidence interval were, respectively, 248.56 ± 69.80 and 219.74 – 277.37 mg/dL. Triglycerides showed elevated values in serum samples of the female *Testudo graeca* tortoises as compared with the corresponding values of the male tortoises (P = 0.0000). Triglycerides mean of the males serum samples were 103.92 ± 22.75, whereas the significantly elevated mean of the female serum samples was 242.56 ± 92.91 mg/dL. The confidence intervals was 094.52 – 113.31 for the males and that of the females was 204.20 – 280.91 mg/dL.

IV. DISCUSSION

In all cases, blood samples were obtained within 24 hours after capture of the tortoises. This step was necessary to cancel interference of captivity with hematological and chemical values. It is worthwhile, to restate that cardioncentesis collection of blood was performed only once from each individual tortoise. By doing so, dilution of blood in case of repeated collection of blood samples and possible hematological and chemical changes due to infection were avoided.

HEMATOLOGICAL TESTS

Ugurtas *et al*. [33] cited the hypothesis presented by Ryerson [34] on the inverse correlation between the number of erythrocytes and their size. Low means of erythrocyte count of the studied tortoises (Table 1) go along with this hypothesis since erythrocyte dimensions of *Testudo graeca* and *Testudo hermanni* recorded high values [33]. The present investigation, also, supported a significant difference between the high mean values of the males as compared with those of the females. Low erythrocyte count and sex differences were observed in blood samples obtained from the jugular vein of the tortoise *Gopherus agassizii* in California, U. S. A.. The median during fall was 0.64 X 10^6 and 0.52 X 10^6 / uL for males and females, respectively, with the corresponding confidence intervals (95%) of 0.33 – 1.31 and 0.32 - 0.90 [14]. Red blood cell count of the captive male and female *Testudo graeca* in Spain had a mean value of 0.45 ± 0.1 X 10^6/uL [16].

The reduction in number of the erythrocytes was associated with low mean values of the packed cell volume (Table 1). Again sex effect was significant. The observed low packed cell volume means and sex difference in favor of males was also reported in *Gopherus agassizii* of a desert in California, U.S.A., during fall season. Medians of the hematocrit values were 25.0 % with a confidence interval (95%) of 16.2 – 37.5 for males and 21.0 % with a confidence interval (95%) of 15.0 – 28.2 for females [14]. However, Dickinson *et al.* [11] did not record sex effect on mean values of the *Gopherus agassizii* males (25 ± 3.6 %) and females (24.3 ± 3.2 %) of another desert, Arizona, U.S.A., over the period from 1990 to 1995. In Spain, the hematocrit values of the male and female *Testudo graeca* in captivity had an overall mean of 21.7 % [16].

Hemoglobin concentration (Table 1) have been characterized with low mean values in both males and females with a significant effect in favor of male tortoises. Similar low values and sex differences were estimated, during fall season, in the jugular vein blood of the tortoise *Gopherus agassizii* in California, U.S.A.. Median values (g/dL) were 7.0 for males and 5.8 for females. The 95% confidence intervals for males

and females were 4.7 – 10.3 and 4.3 – 8.4, respectively [14]. In Arizona, U.S.A., the overall mean during 1990 – 1994 was 10.2 ± 1.5 for males and 10.3 ± 1.3 for females of the tortoise *Gopherus agassizii*. The compared means lacked statistical significance [11]. The mean hemoglobin concentration in captive males and females *Testudo graeca*, in Spain, was 7.64 ± 3.16 g/dL [16].

Values of the calculated mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration (Table 1) did not show significant effect for sex. Christopher *et al*. [14] reported mean values of these indexes and were not in favor of looking for sex effect on these calculated values due to the wide variations in the elements of the calculation equations. The medians of the three means and their 95% confidence intervals in the *Gopherus agassizii* of California were, 404.0 fL, 218 – 655 for mean corpuscular volume, 112 pg, 68 – 189 for mean corpuscular hemoglobin and 28 g/dL, 23 – 35 for mean corpuscular hemoglobin concentration. In Spain, Muro *et al*. [16] presented the calculated data of *Testudo graeca*, 10 males and 19 females in captivity, as 471.9 ± 164.6 fL, 181.4 ± 87.5 pg and 44.75 ± 2.54 g/dL, respectively. In fresh water turtles, there were significant correlations between red blood cells counts, hematocrit values and hemoglobin concentration. However, the parameters were not influenced by sex [35]. Zago *et al*. [36] also observed lack of significant differences due to sex in chelonian hematological profile. Hematological variables showed significant differences in five groups of age of captured sea turtles [37].

CHEMICAL TESTS

Sodium, potassium, chloride, magnesium, calcium and phosphorus concentration means in cardiocentesis blood samples of the employed adult male and female *Testudo graeca* tortoises (Table 2) were in most of the cases close to the corresponding means reported by other investigators on the same species or others within the family Testodinidae. In the *Gopherus agassizii* of California the medians and 95% confidence intervals of 144 mmol/L, 127 – 176 for sodium, 4.9 mmol/L, 3.3 – 7.1 for potassium, 113 mmol/L, 100 – 141 mmol/L for chloride, and 5.0 mg/dL, 3.8 – 7.1 for magnesium in blood of the jugular vein during fall season. The values were reported jointly for males and females since differences due to sex could not be detected. Calcium values were reported separately as 11.0 mg/dL, 9.2 – 13.0 for males and 15. mg/dL, 10.8 – 18.2 for female with a significant effect for sex. Phosphorus mean values were also reported for males: 1.8 mg/dL, 0.8 – 2.6 and for females: 1.9 mg/dL, 0.9 – 3.2 and the investigators claimed presence of sex influence in favor of the females [14]. The same species of tortoises in Arizona showed significant sex differences in calcium: 10.3 ± 1.0 mg/dL for males and 13.2 ± 1.5 mg/dL for females, and in phosphorus: 1.6 ± 0.6 mg/dL for males and 4.4 ± 1.9 mg/dL for females [11]. *Testudo graeca*, under captivity conditions in Spain and without taking sex differences into account, showed mean values of 135.0 ± 15.6 mmol/L for sodium, 3.5 ± 0.9 mmol/L for potassium, 117.0 ± 15.2 mmol/L for chloride and 8.0 ± 5.9 mg/dL for phosphorus [16]. Concentrations of sodium, potassium, chloride and calcium were estimated in jugular vein blood samples. The mean values were 117.8 ± 6.1 mmol/L for sodium, 6.38 ± 1.11 mmol/L for potassium, 85.83 ± 7.19 mmol/L for chloride and 8.73 ± 1.2 mg/dL for calcium [23].

The nitrogenous compounds: total proteins, albumin, globulin, urea, uric acid and creatinine of the analyzed blood samples did not show sex differences (Table 3). Lower vertebrates such as reptilian (3.0 – 7.0 g/dL) and avian (2.5 – 4.5 g/dL) species, normally have lower blood total proteins than that of the mammals. An increase in proteins, primarily globulin, is needed for yolk production, then a decline occurs. Therefore, high values could be recorded in reptilian and avian females during active folliculogenesis [38]. Blood samples of the present study were collected during a period followed reproductive activity and not far away from hibernation. This point might explain the low recorded proteins concentration in addition to lack of significant differences between values of the males and females nitrogenous compounds. In Captive *Testudo graeca* in Spain, means of total proteins (3.7 ± 1.9 g/dL), albumin (1.1 ± 0.6 g/dL), globulin (1.8 ± 0.8 g/dL), urea (31.9 ± 22.2 mg/dL), uric acid (1.9 ± 1.1 mg/dL) and creatinine (0.5 ± 0.3 mg/dL) were recorded [16]. Christopher *et al*. [14] observed concentration medians and 95% confidence intervals of total proteins (3.7 g/dL, 2.3 – 5.1), albumin (1.2 g/dL, 0.8 – 1.8), globulin (2.3 g/dL, 1.3 – 3.8), urea (9.0 mg/dL, 1.0 – 53), uric acid (3.4 mg/dL, 1.6 – 7.4) and creatinine (0.3 mg/dL, 0.2 – 0.4) in *Gopherus agassizii* of California, U.S.A., during fall season. In Arizona, U.S.A., jugular vein blood samples of males and females of the same foregoing species of tortoises were processed for determination of the concentration means of these nitrogenous compounds over the period 1990 - 1995. In males the means were 3.4 ± 0.43 g/dL for total proteins, 1.7 ± 0.5 g/dL for albumin, 1.7 ± 0.5 g/dL for globulin, 1.6 ± 2.4 mg/dL for urea, 4.8 ± 2.1 mg/dL for uric acid and 0.24 ± 0.13 mg/dL for creatinine. The female values were 3.9 ± 0.69 g/dL for total proteins, 1.7 ± 0.5 for albumin, 2.1 ± 0.5 for globulin, 1.0 ± 2.0 mg/dL for urea, 5.4 ± 1.8 mg/dL for uric acid and 0.25 ± 0.10 mg/dL for creatinine [11]. Dickinson *et al*. [11] came to the conclusion that desert tortoises showed significant differences in hematologic and blood chemistry parameters due to sex, site and season. Heparinized jugular vein blood of the West African tortoise *Kinixys erosa* was analyzed by Olayemi and Adeshina [23] for mean values of total proteins (6.18 ± 0.98 g/dL), albumin (3.1 ± 0.22 g/dL), globulin (3.08 ± 0.13 g/dL), urea (1.87 ± 0.52 mg/dL) and creatinine (1.47 ± 0.1 mg/dL).

Blood glucose mean values of the *Testudo graeca* males and females were close to each other (Table 4). Tortoise blood glucose was reported as not the only source of energy during hibernation. It was suggested that during hibernation, glucose derived from hepatic glycogen beside lipids and degraded endogenous protein constitute the energy source [13]. Whether such suggested triplet source of energy could be applied on other physiological conditions is a questionable point. However part of the answer came out from another study in which cholesterol and lipids recorded higher concentration values during period of oviposition in *Testudo graeca* and *Testudo hermanni* females as compared with values of the males [4]. Possibly, cholesterol and lipids are available for steroid hormone biosynthesis, for vitellogenesis as well as for participation in energy supply. *Testudo graeca* in captivity, where forage availability ensured, showed blood glucose of 113.58 ± 75.5 mg/dL [16]. Blood glucose value for *Gopher agassizii* in Arizona desert, U.S.A., averaged 132.6 ± 32.2 mg/dL in males and 127.1 ± 34.9 mg/dL in females [11] whereas the median value for both sexes was 73 mg/dL, with

45 – 130 as the 95 % confidence interval, was recorded for the same species of tortoises in California desert, U.S.A. [14]. The low glucose values of the present investigation and the variations in the reported values of other studies might be attributed to the effect of geographic site.

Concentration mean, as well as the 95 % confidence interval, of blood cholesterol in *Testudo graeca* females was not high enough to reach statistical significance as compared with mean value of the males (Table 4). Cholesterol of captive *Testudo graeca* in Spain had a mean value of 124.8 ± 90.1 mg/dL [16]. On the other hand, the West African tortoise *Kinixys erosa* had blood cholesterol average of 117.60 ± 10.75 mg/dL [23]. Male (77.1 ± 20.8 mg/dL) and female (175.8 ± 56.9 mg/dL) blood cholesterol mean values were recorded in *Gopher agassizii* tortoises of a desert in Arizona, U.S.A. and sex effect was significant [11]. A desert tortoise in California, U.S.A., had a blood cholesterol median value of 243 with a 95% confidence interval of 110 – 496 mg/dL at one site and a median value of 68 with a 95 % confidence interval of 32 – 146 mg/dL at another site during fall season [14]. The role of the geographic site, again, could not be overlooked.

Table 4, also, shows significant elevation of the female triglycerides concentration in comparison with that of the male. Such sex influence was encountered in mean values of the *Gopher agassizi* females (237.4 ± 187.7 mg/dL) and males (18.7 ± 25.1 mg/dL) in a desert area, Arizona, U.S.A. [11]. A triglyceride median of 329 with a 95 % confidence interval of 208 – 491 mg/dL at a site, and a median of 216 with a 95 % confidence interval of 27 – 299 mg/dL were recorded in *Gopher agassizii* at another site of a desert area, California, U.S.A. [14]. Lance *et al.* [25] documented the sex influence on cholesterol and triglycerides in favor of female tortoises. Such influence has also been supported by data of another trial [26]. Successive changes in triglycerides and cholesterol were noticed in captive juvenile, 1 month to three years, loggerhead turtles [39].

V. CONCLUSION

The present investigation has presented hematological and chemical values of blood samples that were collected from adult male and female Greek tortoise *Testudo graeca*. The wildlife animals were obtained from an area within Benghazi province, Libya. Further studies are needed to establish normal values of important hematological and chemical parameters during different seasons of the year. Annual variations due to changes in temperatures and rainfalls have to be included. Such studies should also be carried out in other geographic sites within the Libyan State covering chelonian species as well as other useful wildlife animals.

TABLE I. MEANS AND CONFIDENCE INTERVALS (95%) OF CLOTTING TIME, ERYTHROCYTES COUNT, PACKED CELL VOLUME, BLOOD HEMOGLOBIN CONCENTRATION, MEAN CORPUSCULAR VOLUME, MEAN CORPUSCULAR HEMOGLOBIN AND MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION OF MALES AND FEMALES *TETSTUDO GRAECA.*

Parameter	Sex	No.	Mean ± SD	Confidence Interval (95%)	P value*
Erythrocytes Count ($X10^6$/uL)	M	25	0.817 ± .245	0.715 – 0.918	0.0096
	F	25	0.668 ± .126	0.615 – 0.720	
Packed Cell Volume (%)	M	25	27.72 ± 5.31	25.52 – 29.91	0.0024
	F	25	23.68 ± 3.40	22.27 – 25.08	
Hemoglobin Concentration (g/dL)	M	25	08.04 ± 1.06	07.59 – 08.48	0.0078
	F	25	07.32 ± 0.71	07.03 – 07.62	
Mean Corpuscular Volume (fL)	M	25	354.28 ± 6.46	326.84 – 381.71	0.6340
	F	25	363.12 ± 3.99	336.70 – 389.53	
Mean Corpuscular Hemoglobin (pg)	M	25	105.04 ± 4.72	094.83 – 115.24	0.2209
	F	25	113.04 ±20.71	104.48 – 121.59	
Mean Corpuscular Hemoglobin Concentration (g/dL)	M	25	29.76 ± 04.96	27.70 – 31.81	0.2310
	F	25	31.24 ± 03.53	29.77 – 32.70	

* P value of less than 0.05 indicates significant difference between the compared means.

TABLE II. MEANS OF BLOOD SODIUM, POTASSIUM, CHLORIDE, MAGNESIUM, CALCIUM AND PHOSPHORUS OF MALE AND FEMALE *TESTUDO GRAECA*..

Parameter	Sex	No.	Mean ± SD	Confidence Interval (95%)	P value*
Sodium (mmol/L)	M	25	137.16 ± 6.56	134.44 – 139.87	0.4662
	F	25	138.80 ± 9.02	135.07 – 142.52	
Potassium (mmol/L)	M	25	06.00 ± 0.88	05.63 – 06.36	0.9094
	F	25	06.02 ± 0.84	05.67 – 06.37	
Chloride (mmol/L)	M	25	118.72 ±10.47	114.39 – 123.04	0.5284
	F	25	116.96 ±09.07	113.21 – 120.70	
Magnesium (mg/dL)	M	25	05.64 ±01.30	05.10 – 06.17	0.4029
	F	25	05.33 ±01.27	04.80 – 05.85	
Calcium (mg/dL)	M	25	11.31 ±01.10	10.85 – 11.76	0.1664

	F	25	11.78 ±01.28	11.25 – 12.31	
Phosphorus (mg/dL)	M	25	03.17 ± 0.76	02.86 – 03.49	0.4924
	F	25	03.32 ± 0.83	02.98 – 03.67	

* P value of less than 0.05 indicates significant difference between the compared means.

TABLE III. MEANS AND CONFIDENCE INTERVALS (95%) OF BLOOD TOTAL PROTEINS, ALBUMIN, GLOBULIN, UREA, URIC ACID AND CREATININE OF MALE AND FEMALE *TESTUDO GRAECA*.

Parameter	Sex	No.	Mean ± SD	Confidence Interval (95%)	P value*
Total Proteins (g/dL)	M	25	3.99 ±0.96	3.59 – 4.39	0.1742
	F	25	3.62 ±0.96	3.22 – 4.01	
Albumin (g/dL)	M	25	1.46 ±0.31	1.32 – 1.58	0.9707
	F	25	1.45 ±0.44	1.26 – 1.63	
Globulin (g/dL)	M	25	2.30 ±0.84	1.95 – 2.65	0.6302
	F	25	2.20 ± 0.72	1.90 – 2.49	
Urea (mg/dL)	M	25	43.16 ±11.30	38.49 – 47.82	0.1340
	F	25	38.56 ±9.99	34.43 – 42.68	
Uric Acid (mg/dL)	M	25	2.94 ± 1.13	2.47 – 3.41	0.4898
	F	25	3.22 ± 1.59	2.56 – 3.87	
Creatinine (mg/dL)	M	25	0.24 ± 0.15	0.18 – 0.30	0.4007
	F	25	0.28 ± 0.15	0.21 – 0.34	

* P value of less than 0.05 indicates significant difference between the compared means.

TABLE IV. MEANS OF BLOOD GLUCOSE, CHOLESTEROL AND TRIGLYCERIDES OF MALE AND FEMALE *TESTUDO GRAECA*.

Parameter	Sex	No.	Mean ± SD	Confidence Interval (95%)	P value*
Glucose (mg/dL)	M	25	53.84± 07.88	50.58 – 57.09	0.4608
	F	25	56.88 ±18.86	49.09 – 64.66	
Cholesterol (mg/dL)	M	25	225.28 ±28.90	213.34 – 237.21	0.1299
	F	25	248.56 ±69.80	219.74 – 277.37	
Triglycerides (mg/dL)	M	25	103.92 ±22.75	094.52 – 113.31	0.0000
	F	25	242.56 ±92.91	204.20 – 280.91	

* P value of less than 0.05 indicates significant difference between the compared means.

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