Serum biochemical parameters of Asian tortoise (*Agrionemys horsfieldi*)

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Key Words:
Asian tortoise; serum; biochemistry.

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Received 03 December 2009,
Accepted 18 May 2010

Abstract

At present, a great deal of attention is being focused on the tortoise as a domestic pet. Knowledge of the blood biochemical parameters in captivity of this animal would be helpful for evaluations of their health. In this regard, the serum biochemical values were measured in 12 Asian tortoises (6 males and 6 females) before hibernation. Serum values of total protein (TOP) 63.19 ± 7.57 g/L, Albumin (Alb) 47.24 ± 10.66 g/L, creatinine (Crea) 57.4 ± 4.68 µmmol/L, glucose (Glc) 81.46 ± 21.88 mmol/L, urea 7.52 ± 2.74 mmol/L, uric acid (UA) 0.11 ± 0.028 mmol/L, aspartate transaminase (AST) 0.46 ± 0.017 µkal/L, alanine transaminase (ALT) 0.44 ± 0.053 µkal/L, amylase 1,157 ± 33.96 µkal/L, calcium (Ca) 2.74 ± 0.65 mmol/L, magnesium (Mg) 1.98 ± 0.24 mmol/L, and inorganic phosphorus (P) 1.26 ± 0.101 mmol/L were determined respectively. There were no significant differences in TOP, Alb, Glc, Crea, urea, UA, AST, ALT, amylase, Ca and P, and also Mg levels between males and females.

Introduction

The Asian tortoise (*Agrionemys horsfieldi;* formerly classified as *Testudo horssieldii*) is one of the species of tortoise that lives in Iran, Pakistan, West China, Kazakstan, Turkmenistan and Russia (Asadi *et al.*, 2007). The Asian tortoise is not a large tortoise; the carapace is rounded and almost as broad as it is long and the supra-caudal region is undivided. Females of this species tend to be larger than the males (Bergman, 2001). The most notable aspect of the behavior of the Asian tortoise is their overall lack of activity. These tortoises are inactive most of the year, and are one of the only reptiles known to hibernate both during the winter and aestivate during the summer (Bergman, 2001). Olvera *et al.* (2003) have discussed the fact that hematological and biochemical parameters are essential for assessments of the health and clinical status of reptiles, and tortoises in particular.

Hypophosphatemia, hypocalcemia and increased AST activity have been observed in prolonged desert entrapped tortoises, compared with the results obtained from wildlife tortoises (Christopher, 1999). Seasonal variations in blood and plasma urea values and cyclic variations in blood glucose concentrations of *Testudo hermanni* tortoises have been shown previously (Lawrence *et al.*, 1987). Among the diseases of imported *Agrionemys horsfieldi* tortoises, metabolic disorders and alterations of blood parameters have been reported because they are kept in unsuitable conditions, such as over crowding, low temperatures, improper care and inadequate nutrition (Knotkova *et al.*, 2002). In this respect, THE physiological indicators for health should be established based on physical examination along with the evaluation of hematological and blood biochemical values (Goble and Spörle, 1992; Knotkova *et al.*, 2000). In the previous study, we have delineated the sera lipid and lipoprotein parameters of *Agrionemys horsfieldi* (Asadi *et al.*, 2007). The aim of the present study was to determine the serum values of total protein (TOP), albumin (Alb), globulin (Glb), magnesium (Mg), inorganic phosphorus (Pi), glucose (Glc), uric acid (UA), creatinine (Crea), alanine transaminase (ALT), aspartate transaminase (AST), alpha amylase (amylase), calcium (Ca), and urea in the Asian tortoise.

Materials and Methods

Twelve apparently healthy adult Asian tortoises (6 males and 6 females), mature but not pregnant, were kept in a normal environment in the Eram Park (Tehran, Iran) during the late October, 2004. The tortoises were fed with green leaves, grass, dandelions, strawberries, potatoes, tomatoes, carrots, and cheese ad libitum.

Blood was collected from the jugular vein of the animals. In all sera samples, the values of TOP, Alb, Glb, Mg, Pi, Glc, Urea, UA, Crea, AST, ALT, amylase and Ca were measured. TOP concentration was measured based on the biuret method, a formation of a violet complexing between cupric ions and protein (Silverman and Christenson, 1995). Alb concentration was determined using a dye-binding technique between Alb and bromocresol green, which results in a colored complex (Doumas *et al.*, 1976). The Alb**:**Glb ratio was determined by dividing the Alb concentration by the value for Glb. The Mg concentration was measured by the titan yellow method (Muller *et al.*, 1994; Wills *et al.*, 1986). Glc concentration was measured using glucose oxidize *p*-aminophenazone method. Pi values were measured with para aminophenol sulfate, which utilizes a colorimetric method. Urea concentration was measured by means of the diacetylmonoxime reagent and converted thereafter to BUN by timing at 0.467 (Dawson 1993). Crea concentrations were measured based on the Joffe reaction, which is a colorimetric reaction between Crea and alkaline picrate (Palm and Lundblad, 2005). AST and ALT activities were measured by the direct combination of oxalacetic acid with dinitrophenyl hydrazine and measurement of the color in an alkaline solution (Mazola *et al.*, 1985). Amylase activities were measured by the method of Tietz (Tietz, 1999). Ca values were measured by *O-cresolphetaleincomplexone* (Caudill and Boon, 1986; Annino *et al.,* 1967). Uric acid levels were determined by uricase method (Christopher 1999). All reagents were prepared by the Zist Chimie Biochemical Company (Tehran, Iran). Data were analyzes by Student's *t*-tests between sexes using the Sigma Stat 2 statistical package (Cystal Soft ware. Inc. Point, Richmond, CA, USA).

Results

Values of the serum biochemical parameters are expressed as mean ± standard deviation (SD) in Table 1. There were no significant differences in terms of TOP, Alb, Glc, Crea, UA, AST, ALT, amylase, Ca, Pi, Mg, and urea concentrations between sexes of the Asian tortoise.

Discussion

The values of different routine serum biochemical parameters in different tortoises have been shown in Table 2. The TOP values in the Asian tortoise were similar to those reported previously in the Mediterranean tortoise (Lawrence *et al.*, 1987), Desert tortoise (Christopher, 1999) and hinge-backed tortoise (Olayemi and Adeshina, 2002) However, its value was higher than those reported in the Russian tortoise, Mojave desert tortoise (Christopher *et al.,* 1999) and Marginated tortoise (Christopher *et al.,* 1999). In this respect, Christopher, discussed whether these variations could be due to seasonal variations in conjunction with the reproductive cycle and hibernation.

Table 1: Serum biochemical values of the Asian tortoise. Values were expressed as mean ± SD in both male and female tortoises. Statistical analysis showed no significant differences between sexes.

Analytes	Female	Male	Normal range
TOP (gl/L)	63.19 ± 7.57	62.12 ± 8.14	63.19 ± 7.57
Alb (g/L)	47.24 ± 10.66	21.69 ± 2.34	47.2 ± 10.66
Glc (mmol/L)	4.74 ± 1.603	3.43 ± 0.59	4.52 ± 1.21
Urea (mmol/L)	15.98 ± 5.86	1 6.26 ± 2.83	7.52 ± 2.74
UA (mmol/L)	0.12 ± 0.038	0.106 ± 0.014	0.11 ± 0.028
Crea (mmol/L)	44.46 ± 40.66	71.07 ± 66.3	57.4 ± 4.68
AST(ukal/L)	0.48 ± 0.21	0.44 ± 0.14	0.46 ± 0.17
ALT (ukal/L)	0.45 ± 0.04	0.42 ± 0.062	0.44 ± 0.053
Amylase (ukal/L)	1157 ± 29.25	1157 ± 41.017	1157 ± 33.96
Ca (mmol/L)	2.67 ± 0.73	2.8 ± 0.61	2.74 ± 0.65
Mg(mmol/L)	2.015 ± 0.27	1.921 ± 0.37	1.98 ± 0.316
P (mmol/L)	1.28 ± 0.11	1.98 ± 0.094	1.269 ± 0.101

Total protein (TOP), Albumin (Alb), glucose (Glc), uric acid (UA), creatinine (Crea), aspartate transaminase (AST), alanine transaminase (ALT), calcium (Ca) , magnesium (Mg) and inorganic phosphorus (P).

The current study showed higher values for serum Alb in the Asian tortoise than the Desert and hinge-backed tortoises (Olayemi and Adeshina, 2002). This was probably due to species differences (Christopher, 1999). Our findings with regards to serum Crea levels in the Asian tortoise were that they had lower values than those reported in the Marginated and West African hinge0backed tortoises (Olvera *et al.*, 2003).

The values of serum Glc concentration observed in the present study were similar to those reported in the Marginated tortoise (Olvera *et al.,* 2003) and Desert tortoise (Christopher *et al.,* 1999). On the other hand, serum Glc values in the Asian tortoise were higher than those of the Mediterranean tortoise (Lawrence, 1987). Peaks in the concentration of glucose may act as a trigger to its arousal, and it also appears to be associated with a rise in environmental temperature (Lawrence, 1987). Serum Glc levels in the Asian tortoise were lower than the Russian tortoise (Knotkova, 2002).

Our findings on the serum urea levels in Asian tortoise were similar to those reported in the *Mediterranian hemmini* tortoise (Lawrence *et al.,* 1987). On the other hand, serum urea values in Asian tortoise were lower than the *Mediterranean graeca* tortoise (Lawrence, 1987) and higher than the Marginated (Olvera *et al.,* 2003) and hinged-back tortoises (Olayemi *et al.,* 2002). This difference may be due to a shift from urea to uric acid excretion between the species, and can be triggered by increased environmental temperatures (Olayemi *et al.,* 2002). The rise in the blood urea concentration during hibernation can only result from increased protein degradation (Lawrence *et al.,* 1987).

Serum uric acid levels of the Asian tortoise were similar to those reported in the Western Mojave, Mojave and Desert tortoises (Christopher *et al.,* 1999; Christopher, 1999). Serum uric acid levels in the Asian

Table 2: Previous reports on normal serum biochemical parameters in different species of tortoise.

Tortoise species	TOP (g/L)	Alb (g/L)	Glc (mmol/L)	Urea (mmol/L)	UA (mmol/L)	Crea (μmol/L)	SGOT (μkal/L)	SGPT (μkal/L)	Ca (mmol/L)	P (mmol/L)	Cited literature
Mediterranean graeca	62-109	_	1.46 ± 0.41	8.2 ± 3.5	_	_	_	_	_	_	Lawrence (1987)
Mediterranean hermmani	67-106	_	1.6 ± 0.54	6.3 ±2	_	_	_		_	_	Lawrence (1987)
Marginate tortoise	32-39	_	0.72-4.66	0.54± 0.31	1.15 ±0.29	20,600 ± 18,390	1.15± 0.29	0.32±0.085	2.27-5.54	1.32-3.13	Lópes-Olvera (2003)
Hinge backed tortoise	61.83 ± 0.98	31±2.19	_	_	0.607± 0.109	1,303.9 ± 84.86	0.607± 0.109	0.43±0.14	2.18 ± 0.03	_	Olayemi (2002)
Mojave tortoise (D)	19-37	_	_	0.07-0.27	_	_	_	_	_	0.27-0.93*	Christopher *et al.*, (1999)
Mojave tortoise (GI)	23-51	_	_	0.09-0.44	_	_	_	_	_	_**	Christopher(1999)
Russian tortoise	45.1 ± 6.8	_	11.4 ± 1.3	0.095 ± 0.02	1.1± 0.3	_	1.1± 0.3	0.7±0.4	2.5± 0.9	1.4 ± 0.4	Knotkova(20020
Desert tortoise	1.5-52	6-23	1.94-5.38	0.04-0.32	10-73	_	10-73	1-5	_	0.64-1.45*	Christopher (1999)

Total protein (TOP), Albumin (Alb), glucose (Glc), uric acid (UA), creatinine (Crea), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), calcium (Ca) and inorganic phosphorus (P).

tortoise were higher than the Russian tortoise (Knotkova *et al.*, 2002) and lower than the Marginated tortoise (Olvera *et al.*, 2003).

Our findings with regards to the values of serum AST activity in the Asian tortoise were lower than those reported in the Marginated tortoise (Olvera *et al.*, 2003), hinge-backed Desert tortoise (Olayemi *et al.*, 2002), Russian tortoise (Knotkova *et al.*, 2002) and Desert tortoise (Christopher, 1999). Serum ALT activity levels in the Asian tortoise were similar to those reported in the hinge-backed Desert tortoise (Olayemi *et al.*, 2002). In this respect, serum values of ALT activities in the Asian tortoise were higher than the Marginated tortoise (Olvera *et al.*, 2003). Their values in the Asian tortoise were lower than the Russian tortoise and Desert tortoise (Knotkova *et al.*, 2002). Lower AST activities and Crea concentrations in the Asian tortoise compare to the hinge-backed ones may be due to the normal temperature of the animal's environment. Furthermore, plasma ALT and AST activities can rise in the presence of liver disease (Knotkova *et al.*, 2002). Serum Ca levels in the Asian tortoise were similar to those reported in the Marginated tortoise (Olvera *et al.*, 2003), hinge-backed tortoise (Olayemi and Adeshina, 2002) and Russian tortoise (Knotkova *et al.*, 2002). Furthermore, our findings on serum Pi levels in the Asian tortoise were similar to those reported in the Marginated tortoise (Olvera, 2003) and Russian tortoise (Knotkova *et al.*, 2002). However, their values were higher than the Mojave tortoise (Christopher *et al.*, 1999).

The different blood biochemical values among the different species of tortoise may be due to variations in age, sex, geographic locations, seasons, and other environmental factors (Olvera *et al.*, 2003; Christopher *et al.*, 1999). Furthermore, the site of venepuncture has an important influence on the hematological and biochemical values of tortoise, which should be considered in the interpretation of result (Christopher *et al.*, 1999).

In conclusion, the present findings on the serum biochemical parameters in Asian tortoise can be considered as the normal values for this type of tortoise when kept in captivity.

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