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RESEARCH ARTICLE

Liver enzyme activities of captive Asian elephants in Nay Pyi Taw area, Myanmar

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Abstract

Myanmar manages the largest captive population of Asian elephants in the world. For conservation, routine monitoring on the health status of elephants is important. As the liver is an important vital organ for regulation of the physiological status, liver enzymes are necessary tools for the health monitoring of the individual elephant. The liver enzyme activities of apparently healthy 31 captive adult Asian elephants (22 females and 9 males) aged 5-54 years, with body condition scores (BCS) 5-7, from camps of Kintha, Ngalite and Zoo, from Nay Pyi Taw area were examined. Feed, feeding practice and activities of the elephants from 3 camps were slightly different. Blood samples were obtained once during the study period from the elephants without sedation. After serum samples were collected, the liver enzyme activities such as alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and total bilirubin (BIL) were analyzed. The ranges of enzyme activities of ALP, AST, GGT and total BIL of all elephants were 37.0-244.0, 0.0-77.0, 0.0-6.0, 0.1-0.7, respectively. The liver enzyme activities of all elephants obtained from this study were within the normal range of Asian elephants. The activities of GGT and total BIL were significantly higher ($P < 0.05$) in male elephants than those of females. The mean AST activity of the elephants from Kintha camp was significantly higher ($P < 0.05$) than that of the elephants from Zoo but not significantly different ($P > 0.05$) with the elephants from Ngalite camp. Some liver enzyme activities were high in bull with musth and in lactating females. Individual baseline values obtained from elephants with healthy conditions in this study would be very useful for comparative clinical cases. Further study is still needed to investigate the serum enzymes of both captive and wild elephants depending on the management system, physiological status and locations in Myanmar.

Keywords:

Asian elephants, liver enzymes, Myanmar

1. Introduction

The Asian elephant (*Elephas maximus*) is an endangered species that plays an important role in the country history, culture and economy of Myanmar. In the country's logging industry, the elephants have been a significant workforce and also a symbol of power and great fortune (Leimgruber et al., 2011). Elephant protection was initiated through the Elephant Preservation Act of 1879, and expanded by subsequent laws including the Burma Wildlife Protection Act of 1936 (revised 1956), and the Protection of Wildlife and Wild Plants and Conservation of Natural Areas Law in 1994 (Aung, 1997; Uga, 2000).

Myanmar is home to the second largest total population of Asian elephants remaining worldwide (after India), including a captive population of approximately 5,000, the largest captive population in the world. Most captive elephants live in government-owned timber camps where elephant draught power has been utilized extensively for more than a century. Today, the captive elephants are also important economically as most captive elephants are used primarily for tourism. According to Myanma Timber Enterprise (MTE) record, most of elephant had been died because of old age, some infectious diseases such as bacterial and viral diseases. The other causes were parasitic diseases, accidents, malnutrition and diarrhea especially in baby elephants and orphans (Myanmar Timber Elephant Project, http://www.elephant-project.science/timber-elephants/).

Health is a state of physical and psychological wellbeing. Numerous biological, physiological, and environmental variables determine whether health or disease will prevail. Proper nutrition and housing, an appropriate social environment, sound disease control measures, and other positive husbandry practices support health. Accurate hematologic and biochemical reference intervals are useful for evaluating the health status of animals and for proper diagnosis of disease and evaluation of treatment efficacy (Janyamethakul et al., 2017). As the health monitoring program, most of the veterinarians are relying on evaluation of blood test to assist in diagnosis and treatment of disease. Serum biochemical tests are important for routine monitoring of the health status of elephants (Mikota, 2006). The common liver function tests used for elephants include Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), Alanine aminotransferase (ALT) and bilirubin (Kerr, 2002).

The study of liver enzyme activity will not only be useful in the complication of global information on elephant but also be important in proper diagnosis of disease and disorder, treatment, breeding, general well-being of both captive and wild Asian elephant. Some researchers have documented serum biochemical values of the African as well as Asian elephants (Allen et al., 1985; Pandit et al., 2015; Janyamethakul et al., 2017). Since the management system, environmental conditions and geographic locations differ in one place and another, base line liver enzyme activities are necessary to observe when the elephants are in healthy status in order to compare when they are sick. The aims of this study were to evaluate liver enzyme activities and to examine baseline data in elephants in three different elephant camps (Kintha and Ngalite elephant camps and Nay Pyi Taw zoo) from Nay Pyi Taw area.

2. Materials and Methods

2.1 Experimental Animals

Thirty one captive Asian elephants (22 females and 9 males) aged 5-54 years, from three different elephant camps (Kintha and Ngalite elephant camps and Nay Pyi Taw zoo) from Nay Pyi Taw area were examined in this study. Based on a physical examination (Mikota, 2006) performed by Myanmar veterinarians who have experienced with elephants, all of the ele-

-phants in this study were classified as healthy. All the elephants were in good body condition scoring (BCS) 5-7 accessed according to Fernando et al. (2009). One male elephant from Ngalite camp was in late musth but the physical examination of that elephant showed healthy. Two females, each from Ngalite and zoo and 3 females from Kintha camps were in lactation periods of 1-4 years. The elephants from Ngalite camp and Zoo in Nay Pyi Taw participated in elephant shows and visitor rides, however the elephants from Kintha camp did not participate in any activities. The elephants from Kintha camp were fed naturally with bamboo leaves, barks, roots and local plants and with no supplements. The elephants from Ngalite camp were fed roughage and supplement with palm sugar, flours and broken rice in blocks. The elephants from zoo were fed grass and bananas, palm sugar, wheat, rice bran and broken rice routinely. All the elephants were vaccinated against anthrax (AA) and Hemorrhagic Septicemia (HS) and dewormed regularly.

2. 2 Sample Collection

Blood samples were obtained from all the elephants once during the study period without sedation. About 10 ml of blood was withdrawn from the auricular vein of each elephant mostly in the morning by using 18 gauge needle and collected into vacuum tube. After standing at room temperature for one hour, sera were separated and collected into serum tubes. The tubes were then brought in cold storage to the University of Veterinary Science, Yezin on the same day of collection. When arrived at the University, the samples were centrifuged for 3 minutes at a 14,000 rpm for getting the clear sera. The sera were then transferred to another tubes and stored in -20°C until analyzing.

2.3 Analysis of Serum Samples

The liver enzyme activities such as alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and total bilirubin (BIL) were analyzed from all serum samples of elephants with Vet Test chemistry analyzer (INDEXX Vet test 8008, USA). The test was performed according to the manufacturer's instructions.

2.4 Statistical Analysis

The liver enzyme activities of all elephants were expressed as minimum and maximum values. The significant differences of mean liver enzyme activity between two groups of elephants were analyzed by student *t* test. The mean liver enzyme activity of the elephants among the different camps was compared by one way ANOVA and the significant differences were analyzed with DMRT. The value of $P < 0.05$ was taken as significant by using SPSS 19.

3. Results and Discussion

Serum chemistries, important diagnostic tool for elephants, may be altered by environmental and physiological factors, restraint techniques, sample taking procedure, laboratory methodologies and quality control, and numerous other variables. When clinical signs are nonspecific and other diagnostic techniques may not be available, most of the veterinarians rely on the serum chemistry for proper diagnosis of the disease status. Mikota (2006) stated that age or physiological status (e.g., lactation) may affect certain parameters, but gender differences are few.

The values of AST, ALP, GGT and total BIL (Table 1) obtained from the present study were found to be in normal range of Asian elephants, indicating the absence of any hepatic abnormalities and intravascular hemolysis of the examined elephants. The enzymes ALP and AST activities examined in this study were within the range of the previous report by Santos et al. (2020). Although the mean activities of ALP and AST were numerically higher in males than in females in this study, there was not significantly different, whereas

Santos et al. (2020) and Janyamethakul et al. (2017) found a significant difference in ALP levels between males and females in. In the present study, one male elephant from Ngalite camp, in late musth, showed higher AST, GGT and total BIL levels of 71, 6 and 0.7, respectively. This finding was agreed with that of Niemuller et al. (1990), in their study, musth bulls expressed high ALP and GGT levels than the other non-musth elephants parallel with high testosterone concentration. The significant difference ($P < 0.05$) between male and female GGT and total BIL activities in this study could be due to the higher enzyme activities of musth bull. GGT values obtained from this study were lower than those reported by Silva and Kuruwita (1993) in Sri Lanka and ALP and total BIL values were also lower than the findings of Pandit et al. (2015) in Nepal. The mean values of total BIL in male and female elephants of this study were in agreement with the findings of Allwin et al. (2015) from India and Silva and Kuruwita (1993) from Sri Lanka.

The enzyme ALP levels were found to be significantly higher ($P < 0.05$) in young elephants than the older in this study (Table 2) but the range was within the normal. This enzyme is widely distributed in the body, particularly in the bone, liver and intestinal wall and higher levels are found in the young animals due to high osteoblastic activity (Kerr, 2002). The normal range of ALP in elephants is 20-249 U/L in the study of Santos et al. (2020) and 60-450 U/L in the study of Mikota (2006). Pandit et al. (2015) observed higher ALP levels in young elephants under 6 years of age, up to 461-716 U/L in their study.

Table 1. Liver enzyme activities of all elephants

Liver enzymes	Unit	Elephants (n=31) (min-max)	Male, n=9 Mean±SE	Female, n=22 Mean±SE	P-value
ALP	U/L	37.0-244.0	142.11±17.70	113.82±13.66	0.384
AST	U/L	0.0-77.0	34.33±8.34	30.10±4.71	0.662
GGT	U/L	0.0-6.0	0.67±0.67	0.05±0.05	0.002**
Total BIL	mg/dl	0.1-0.7	0.2±0.07	0.13±0.02	0.028*

*significantly different between male and female ($P < 0.05$)
** highly significantly different ($P < 0.01$)

Table 2. The mean liver enzymes of elephants with respective age

Liver enzymes	Unit	Young elephants 5-30 years (n=19) (mean±SE)	Old elephants 31-55 years (n=12) (mean±SE)	P value
ALP	U/L	156.78±13.54	73.92±6.74	0.003**
AST	U/L	29.42±5.53	36.42±7.35	0.148
GGT	U/L	0.06±0.05	0.46±0.46	0.034*
Total BIL	mg/dl	0.12±0.01	0.19±0.05	0.006**

*significantly different between young and old
** highly significantly different ($P < 0.01$)

Table 3. The mean liver enzymes of female elephants in lactation and non-lactation

Liver enzymes	Unit	Lactating females (n=7) (Mean±SE)	Non lactating females (n=15) (Mean±SE)	P value
ALP	U/L	77.86±9.59	130.60±18.13	0.001**
AST	U/L	47.29±9.03	22.07±4.25	0.396
GGT	U/L	0.0±0.0	0.70±0.07	0.162
Total BIL	mg/dl	0.11±0.01	0.14±0.02	0.127

*statistically significant difference between lactating and non-lactating females

Table 4. The mean liver enzymes of elephants among the different camps

Liver enzymes	Unit	Ngalite, n=17 (Mean±SE)	Zoo, n=5 (Mean±SE)	Kintha, n=9 (Mean±SE)
ALP	U/L	142.42±16.67	96.00±12.97	98.00±16.35
AST	U/L	29.94±6.03^{ab}	16.40±6.40^{b}	42.22±5.83^{a}
GGT	U/L	0.41±0.35	0.00±0.00	0.00±0.00
Total BIL	mg/dl	0.19±0.04	0.12±0.02	0.10±0.00

^{a,b}the mean values with different superscripts within the same row were significantly different at P < 0.05 level

GGT is an enzyme involved in glutathione metabolism; found in most cells and has the highest activity in liver and kidney. GGT is a fairly liver specific indicator of cholestasis commonly used in horses, pigs, and domestic ruminants. The normal value of GGT for Asian elephant is 4-35IU/L (Mikota, 2006). As mentioned above, GGT may increase in musth bulls; non-musth and musth values for GGT were 1.74 ± 1.6 U/L and 7.89 ± 4.59 U/L, respectively, in an Asian bull monitored through two musths (Niemuller et al., 1990). In this study, except the higher GGT and total BIL values in musth bull of the older age group, the values of other elephants presented normal, and the differences between two age groups showed not statistically significant (P > 0.05).

Although ALP in lactating female elephants found in this study were significantly higher (p<0.05) than those of non-lactating females, the values were within the normal range of Asian elephants (Table 3). Since nine non-lactating elephants from this study were under 20 years of age and thus higher ALP levels of them might be age related and not with the physiological status because the other three enzymes were within the normal range. But the physiological status affecting the serum enzymes of elephants should be further examined with large sample size.

In this study, the mean ALP, AST, GGT and total BIL activities of the elephants based on the three different elephant camps were compared (Table 4) and found that all the mean values were within the normal range expressed by Santos et al. (2020) and Janyamathakul et al. (2017). The feeding management of the elephants was slightly different among the three camps, but the weather and geographical condition were not different in the present study. The elephants from Kintha camp were fed naturally on bamboo leaves, barks, roots and local plants and had no activity of show, trekking and riding. The elephants from Nga-

-lite camp, having activity of visitor rides, were fed on roughage naturally like Kintha camp and supplement with palm sugar, flours and broken rice in blocks. The elephants from zoo, having activity of elephant show and visitor rides, were fed grass and bananas, palm sugar, wheat, rice bran and broken rice in rotation. The mean AST activity of the elephants from Kintha camp (42.22±5.83) were significantly higher ($P < 0.05$) than that of the elephants from Zoo (16.40±6.40) but not with the elephants from Ngalite camp (29.94±6.03). The elephants in different countries or feeding management like captive elephant in zoos and private camps may have differences in hematologic and biochemistry values (International Species Information System, 2016).

Because, factors such as age, sex, management, exercise, as well as geographical location can affect values (e.g. horse, human), enzymes value may not be relevant across all populations (Yaqub, 2013). Therefore, the elephant in different country or feeding management may be different in haematologic and biochemical values. The most blood parameter data in Asian elephants are with wide range and interpretation may be difficult in clinical cases. Also the factors such as sample collected areas, stress due to physical exercise and work, management and nutrition are different, which can lead to different blood parameters (Janyamethakul et al., 2017). So it is important to examine the base line blood and/or enzyme values when the elephants are in healthy conditions which may help as comparative values whenever necessary. The liver enzyme activities of captive Asian elephants obtained from the present study were almost the same with the normal values in previous reports of captive Asian elephants from Sri Lankar (De Alwis et al., 2005), India (Allwin et al., 2015), Nepal (Pandit et al., 2015), Thailand (Janyamethakul et al., 2017) and Myanmar (Santos et al., 2020). But there are significant differences in some enzyme activities depending on the management and nutrition. As the liver is the most important organ which plays a pivotal role in regulating various physiological processes, the liver enzyme values observed in this study indicate proper health status of the sampled elephants and it can be clinically useful as base line data or comparative values for captive Asia elephants of Nay Pyi Taw area. Further study should be done to investigate the serum enzymes of both captive and wild elephants depending on the management system, physiological status and locations in Myanmar.

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Conflict of interest

The authors declare that there are no competing interests.

References

Allen JL, Jacobsn AER, Harvey JW, Boyce W. Hematologic and serum chemistry values for young African elephants (*Lxodonta Africana*) with variations for sex and age. J Zoo Wildl Med. 1985; 16:98-100.

Allwin B, G Senthil Kumar G, Kalaignan Pa, Senthil NR, Vairamuthu S, Stalin Vetamanickam, Kalyaan US, Dattatray Kisanrao Ingole. Serum biochemistry of the Asian elephant. IJAMR 2015;2:62-66.

Aung M. On the distribution, status and conservation of wild elephants in Myanmar. Gajah, Asian Elephant Specialist Group Newsletter. 1997;18:47-55.

De Alwis GKH, Wijesekera RD, Vithana D, Neththasinghe N, Ratnasooriya WD. Serum levels of some biochemical constituents of captive Sri Lankan elephants (*Elephas maximus maximus*). J Sci Univ Kelaniya. 2005;2:105-114.

Fernando P, Janaka PHK, Sampath KK, Ekanayaka HG, Nishantha, Pastorini J. A Simple Method for Assessing Elephant Body Condition. Gajah. 2009;31:29-31.

Myanmar Timber Elephant Project. http://www.elephant-project.science/timber-elephants/

International Species Information System. Physiological reference values for elephant. http://www.elephantcare.org/isismain.htm, *Accessed*: 25.02.2016.

Janyamethakul T, Sripiboon S, Somgird C, Pongsopawijit P, Panya-pornwithaya V, Klinhom S, Loythong J, Thitaram C. Hematologic and Biochemical Reference Intervals for Captive Asian Elephants (*Elephas maximus*) in Thailand. Kafkas Univ. Vet. Fak Derg. 2017; 23(4): 665-669.

Kerr MG. Laboratory Medicine Clinical Biochemistry and Haematology. Blackwell Scientific publications Ltd., Great Britain. 2nd edition. 2002;pp. 127-135.

Leimgruber P, Zaw Min O, Aung M, Kelly DS, Wemmer C, Senior B, Songer M. Current status of Asian elephants in Myanmar. Gajah. 2011;35:76–86.

Mikota SK. Hemolymphatic system. In: Fowler, ME, Mikota S K (eds.). Biology, Medicine, and Surgery of the Elephants. Blackwell Publishing, Ames, USA. 2006;pp. 325-345.

Niemuller C, Gentry PA, Liptrap RM. Longitudinal study of haematological and biochemical constituents in blood of the Asian elephant (*Elephas maximus*). Comp Biochem Physiol. 1990;96(1):131-134.

Pandit A, IDhakal IP, Girhe KP, Rana HB. Serum Biochemical Profile of The Elephants in Chitwan District, Nepal. Int J Recent Sci Res. 2015;6:3130-3135.

Santos DJF, Jackson J, Htoo Htoo Aung, Kyaw Nyein, Win Htut, Lummaa V. Sex differences in the reference intervals of health parameters in semicaptive Asian elephants from Myanmar. J Zoo Wildl Med. 2020;51(1):25-38.

Silva ID, Kuruwita VY. Hematology, plasma, and serum biochemistry values in free-ranging elephants (*Elephas maximus ceylonicus*) in Sri Lanka. J Zoo Wildl Med. 1993;24(4):434-439.

Uga. Conservation and Use of Wild Asian Elephants *(Elephas maximus)*. Forestry Department, Ministry of Forestry, Government of the Union of Myanmar. 2000; pp 1-15.

Yaqub LS, Kawu MU, Ayo JO. Influence of reproductive cycle, sex, age and season on haematologic parameters in domestic animals: a review. J Cell Anim Biol. 2013;7:37-43.