Serum Hepatobiliary Enzyme Activities Of Ruminants And Dogs Of Humid Tropics - A Comparison

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Abstract- *Activities of hepatobiliary enzymes such as, Alanine aminotransferase(ALT), Aspartate aminotransferase(AST), Alkaline phosphatase (ALP) and Gamma glutamyltransferase (GGT) in the serum samples of cattle, buffaloes, goats and dogs of mixed breeds were assessed. All the animals were apparently healthy, free of external parasites, dewormed regularly and vaccinated routinely against infectious diseases. Blood samples were collected by jugular venipuncture in ruminants and by cephalic/saphenous venipuncture in dogs. In the obtained sera samples, concentrations of ALT, AST, ALP and GGT were determined using an automated blood analyzer. The minimum and maximum levels, mean serum hepatobiliary enzyme activities, number of observations (n), standard error (SE), 95 % confidence interval for the lower and upper bound values of enzyme activities (reference range) were calculated. The current study revealed significant differences in the activities of ALT, AST, ALP and GGT in cattle, buffalo, goat and dogs of hot humid tropics.*

Keywords- hepatobiliary enzymes, reference range, hot humid climatic conditions

I. INTRODUCTION

The measurement of serum enzymes is an important tool for disease diagnosis in veterinary and human clinical practice. Most of the enzymes with diagnostic applications, functions within the cells in which they are synthesised and are present in high concentration in specific tissues. The routinely used enzymes to evaluate hepatic damage includes ALT, AST, ALP, GGT, Sorbitol dehydrogenase (SDH), Lactate dehydrogenase (LDH), Ornithine carbamoyl transferase (OCT) and 5' Nucleotidase (NTP) (Kaneko *et al.*, 2008). Among these clinically important enzymes, ALT, AST, ALP and GGT plays significant role in the diagnosis of liver disorders in animals. The enzymes routinely used in human beings for disease diagnosis may not give true indications of hepatic injury in veterinary practice. There is also lack of standard reference values for some species. Each animal species have their own specific hepatobiliary enzyme levels which vary from one species to another (Kaneko *et al.*, 2008). The available data on hepatobiliary enzyme levels from literature shows widely divergent values among different species and these data are mainly procured from the animals reared in temperate climate. Even though considerable information is available on normal serum hepatobiliary enzyme levels of domestic animals of exotic breeds, kept under different environmental and managemental conditions, use of these serum enzyme levels for monitoring health status of indigenous breeds may mislead the diagnosis. So for more accurate clinical interpretation of hepatic diseases, it is a prerequisite to reexamine the normal range for these enzymes.

II. MATERIALS AND METHODS

Adult healthy cattle, buffalo and goats maintained at university farms and dogs maintained at nearby kennels were selected randomly for the study. A minimum of thirty animals were selected from each species for the study. All the animals were fed on a balanced diet and were apparently healthy, free of external parasites, dewormed regularly and vaccinated routinely against infectious diseases.

Blood samples were collected by jugular venipuncture in ruminants and by cephalic/saphenous venipuncture in dogs using sterile needles (18 gauge) directly into clean dry sterile glass tubes without anticoagulants. Serum was harvested after 30 to 45 minutes following clot formation and by centrifugation for 10 minutes at 2000 g. The clear serum was immediately assayed for the following hepatobiliary enzymes ALT, AST, ALP and GGT within an hour of serum separation to serve as base line values. The enzyme assay was performed using Ecoline – Merck diagnostic kits (Merck Specialities Pvt. Ltd, Mumbai) on an automated blood analyzer (Microlab 200). To test the significant differences in enzyme activities between different species the experimental results obtained were analysed statistically by using analysis of variance (ANOVA) technique followed by Duncan Multiple Range Test as described by Snedecor & Cochran (1994) using computerised software programme, Statistical package for social sciences (SPSS).

III. RESULTS

Significant differences (P≤ 0.05) were noticed in the activity of hepatobiliary enzymes between the four species studied, although a few showed similarities. The highest ALT activity was observed in buffaloes with a mean of 50.00 ± 3.53 IU/L and the lowest value in goat (15.94 ± 0.84 IU/L) (Table 1). Serum ALT levels were significantly higher (P≤ 0.05) in the serum of buffalo and dog when compared to cattle and goat. Serum ALT levels of cattle and dog were 19.46 ± 1.54 and 33.56 ± 3.38 IU/L, respectively. The results revealed statistically significant difference (P≤ 0.05) in mean serum ALT levels between buffalo and dog, whereas no significant difference was found in mean serum ALT level of goat and cattle, but significantly different from that of buffalo and dog.

Comparison of AST activities between different species are presented in Table 1. The activity of AST was also highest in the serum of buffaloes (130.00 ± 7.29 IU/L) but lowest value was observed in dogs (35.83 ± 2.49 IU/L). Serum AST levels observed for cattle and goat were 68.67 ± 2.29 IU/L and 80.87 ± 3.71 IU/L, respectively. The results showed statistically significant difference (P≤ 0.05) between the mean serum AST levels of cattle, buffalo, goat and dog.

Serum ALP activities of cattle, buffalo, goat and dog are shown in Table 2. The highest ALP activity was observed in buffaloes with a mean of 323.6 ± 32.09 IU/L. Moreover, the results showed wide variation in the enzyme activity in buffaloes with a baseline value of 251.00 IU/L and an upper value of 396.19 IU/L. The lowest ALP activity was observed in dogs (92.90 ± 7.53 IU/L) as compared to buffalo, cattle (113.70 ± 7.59 IU/L) and goat (175.92 ±20.09 IU/L). Significant differences were not found between the mean serum ALP values of cattle and dog, whereas the ALP activities of goat and buffalo were significantly different (P≤ 0.05), which were also significantly different from that of cattle and dog.

The results showed comparatively lower GGT activities (Table 2.) and the values obtained were in a narrow range in all the four species. The lowest GGT activity was observed in dog serum with a mean of 4.0 ± 0.15 IU/L whereas, goat showed the highest GGT activity (35.27 ± 1.73 IU/L). The mean serum GGT values of cattle and buffalo were under homogenous groups and were not statistically significant different. But significant differences were observed (P≤ 0.05) between the mean GGT values of goat and dog which were also significantly different from that of cattle (13.15 ± 0.78 IU/L) and buffalo (10.11 ±1.28 IU/L). Figures 1 and 2 illustrates the normal serum ALT, AST, ALP and GGT activities in cattle, buffalo, goat and dogs and its species wise comparison respectively.

IV. DISCUSSION

In the present study, highest serum ALT activity was observed in buffaloes followed by dogs. The ALT activity obtained in cattle was comparable to that in goats and significant differences were not found in the serum ALT activity between the two species. Similar results were reported by Kaneko *et al.* (2008) who suggested a reference value of 3 to 23 IU/L and 6 to 19 IU/L for cattle and goat ALT activity which were also comparable to each other. The serum ALT activity depends upon its concentration in liver, so high serum ALT activity in dogs and buffaloes obtained in the present study indicate increased liver ALT concentration in these species. Lower ALT activity in cattle and goat serum indicates decreased liver ALT concentration in these species. The findings are in agreement with those of earlier workers (Kaneko *et al.,* 2008; Ettinger and Feldman, 2000 and Latimer *et al.,* 2003) who reported ALT as a liver specific enzyme in dogs and increased serum ALT activity was observed in acute hepatocellular necrosis and inflammation in these species whereas, low liver ALT activity was reported for horses, pigs, ruminants and birds. The present results are also supported by low ALT activity reported by Bartholomew *et al.* (1987) in cattle. However, in contrast to the low liver ALT activity reported for ruminants (Kaneko *et al.,* 2008 and Latimer *et al.,* 2003), the study showed a significantly higher ALT activity in buffaloes as compared to those observed for cattle and goat. Similar findings in buffaloes were also reported by earlier workers (Terzano *et al.,* 2005 and Grasso *et al.,* 2004). Therefore, ALT estimation can be recommended for diagnosis of hepatic damage in buffaloes also.

Significant differences were noticed in the mean serum AST values between cattle, buffalo, goat and dog. Between the species highest AST activity was observed in buffaloes and the lowest level in dogs. Serum AST reference range observed for adult healthy buffaloes in the present study is in close agreement the reports of Randhawa *et al.* (1997) and Grasso *et al.* (2004) and they reported a mean AST value of 134.6 ± 4.36 IU/L for adult healthy buffaloes and 146.84 IU/L for buffalo cows maintained under intensive system of management, respectively. The lowest AST activity observed for dog was supported by the findings of Caisey and King (1980) who reported AST activity of 32 IU/L in dogs. Compared to cattle, the serum enzyme activity was found to be higher in goats. The results of the present study supported the findings of Kahn (2005), who reported an AST activity of 66 to 220 IU/L for goats, even though the values of the present study is towards the lower margin. Increased serum AST

concentration is suggestive of the use of this enzyme to diagnose hepatic disorders in all the four species studied. The findings of the present investigation are supported by the studies of Kaneko *et al.* (2008), Ettinger and Feldman (2000) and Radostits *et al.* (2000). They suggested AST as an effective marker for assessing hepatic disorders in large and small animals. Since, increased AST level in serum is also associated with muscular damage its measurement in serum alone cannot be used as a specific hepatocellular damage marker (Benjamin, 2001).

Observations made in the present study showed highly variable values for mean serum ALP activity in all the four species studied. The presence of many isoenzymes may be the probable cause of variability. Serum ALP level was found to be within a wider range as compared to other hepatobiliary enzymes studied in the present experiment. Between species, highest ALP activity is observed in buffaloes and lowest in dogs. This is in close agreement with the studies of Grasso *et al.* (2004) who reported 370.11 IU/L of ALP activity in buffaloes maintained under intensive system of management, whereas a higher ALP values was observed for those maintained under traditional system (443.12 IU/L) and the lower limits of dog serum ALP activity was supported by the findings of Ariyibi *et al.*, (2002) who reported a mean serum ALP activity of 57.6 to 76.8 IU/L for Alsatian dogs. The results also showed no significant difference between the ALP values of dogs and cattle and they fall in a similar range. But the values observed in goats were significantly higher than that of cattle and dog. The ALP values obtained for female crossbred goats in the present study was within the range of 61 to 283 IU/L established by Kahn (2005). Laker (1996) reported physiological increase in ALP activity during active bone growth and pregnancy period and pathological increase in hepatobiliary and bone diseases. Importance of ALP in assessing cholestasis supports the findings of Schwartz (1973), Kaneko *et al.* (2008) and Radostits *et al.* (2000). Since, the animals selected for the present study were adult, non pregnant and apparently healthy, the values obtained serve as the normal reference level for future use in clinical veterinary practice for the assessment of hepatobiliary diseases in all the four species studied.

Normal serum level for GGT was found to be almost similar for cattle and buffalo serum. No significant difference was found between the two species. These findings were in accordance with the reports of Kaneko *et al.* (2008) and Hilali *et al.* (2006) who reported almost similar GGT reference ranges for adult cattle and buffaloes. Highest level of GGT was observed in goats whereas, lowest activity in dogs. The relatively higher GGT activity observed in cattle and goats and the lower values in dogs was supported by the findings of Braun *et al.* (1983) and Kaneko *et al.* (2008). However, the values obtained in the present investigation were under a narrow range in all the four species as compared to other hepatobiliary enzymes.

V. CONCLUSION

The obtained results could serve for a better understanding of serum activities of ALT, AST, ALP and GGT in cattle, buffalo, goat and dogs of hot humid tropics for estimating their physiological status as well as for diagnostic purposes in these species.

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Table 1. Serum ALT and AST activities (IU/L) in cattle, buffalo, goat and dog

Species	ALT				AST			
	Minimum	Maximum	Mean ± SE	95 % confidence interval	Minimum	Maximum	Mean ± SE	95 % confidence interval
Cattle	11	28	19.46 ± 1.54^{a}	16.11 - 22.81	58	84	68.67± 2.29^{b}	63.61 - 73.72
Buffalo	30	64	50.00 ± 3.53^{b}	42.02 - 57.98	105	172	130.0 ± 7.29^{c}	113.51 -146.49
Goat	11	24	15.94 ± 0.84^{a}	14.17 - 17.72	61	114	80.87 ± 3.71^{a}	72.89 - 88.84
Dog	15	48	33.56 ± 3.38^{a}	25.15 - 41.36	24	50	35.83 ± 2.49^{d}	30.35 - 41.31

* Means with different superscripts differ significantly (P≤ 0.05) at each column

Table 2. Serum ALP and GGT activities (IU/L) in cattle, buffalo, goat and dog

Species	ALP				GGT			
	Minimum	Maximum	Mean ± SE	95 % confidence interval	Minimum	Maximum	Mean ± SE	95 % confidence interval
Cattle	82	147	113.70± 7.59^{b}	96.53 – 130.87	9	19	13.15± 0.78^{b}	11.45 - 14.86
Buffalo	175	479	323.60± 32.09^{c}	251.00 - 396.19	4	15	10.11 ± 1.28^{a}	7.15 - 13.07
Goat	71	294	175.92± 20.09^{a}	131.71 - 220.13	27	45	35.27 ± 1.73^{a}	31.41 - 39.13
Dog	45	128	92.90 ± 7.53^{b}	75.87 – 109.93	3	5	4.00 ± 0.15^{c}	3.66 - 4.34

* Means with different superscripts differ significantly (P≤ 0.05) at each column

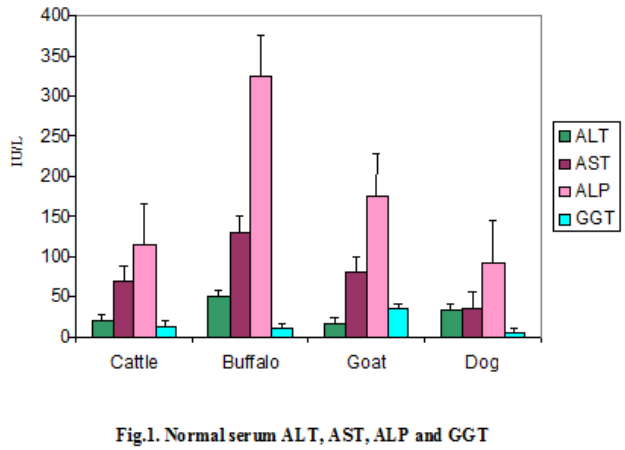

Fig.1. Normal serum ALT, AST, ALP and GGT

Fig.1. Normal serum ALT, AST, ALP and GGT activities of adult cattle, buffalo, goat and dog

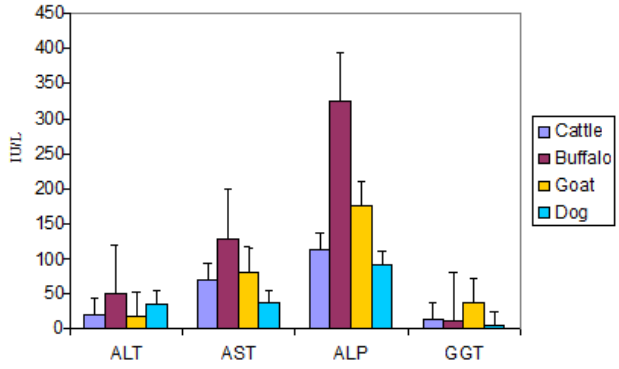

Fig.2. Species wise comparison of normal serum

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ALT, AST, ALP and GGT levels