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Effect of Storage Time and Temperature on the Stability of some Hepatobiliary Enzymes from Camel Serum

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ABSTRACT

Camels can survive in the desert without food and water for a few days because of their unique biology. The current study was performed to determine the effect of storage time and temperature on serum enzyme activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in young female camel. The sera were stored at 4°C, 25°C and 40°C for 0, 1, 2, 3,7,11, and 15 days, then the activities of these enzymes were determined. Our study revealed that the activity of AST, ALT, and ALP did not change over 15 days when stored at 4°C or at room temperature. Interestingly, at 40°C deceased stability of AST was apparent after 2 days, whereas, ALT and ALP show no loss of activity over 11 days. From these results it is therefore advisable to consider stability of each serum hepatobiliary enzymes for different animals separately before preserving sera samples to get more valid and reliable results.

Keywords - *Serum enzyme activity; Storage time; Storage temperature.*

INTRODUCTION

Each animal species has its own specific hepatobiliary enzyme levels which vary from one species to another. The capacity of camels to thrive well and reproduce in semi-arid and arid areas, where other livestock hardly survive, makes them the most important domestic animal in this areas.[1]

The measurement of serum enzymes is an essential tool for disease diagnosis in veterinary and human medicine. The routinely used enzymes to evaluate hepatic damage in animals include alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP). ALT and AST are made in the liver where they participate in the metabolism of amino acids and the production of proteins. ALT rises dramatically in acute liver damage, such as viral hepatitis.[2] AST, however, is not actually specific to the liver because it also present in red blood cells, cardiac and skeletal muscle.[3] ALP is responsible for the production of energy and the metabolism of phosphorus. ALP is an enzyme produced in the cells lining the biliary ducts of the liver. It is used extensively as a tumor marker and is also present in bone injury, pregnancy, or skeletal growth with elevated readings.[4] To detect real pathological changes in the body, the variations in sample collection and handling must be reduced to acceptable levels at which they cause no adverse effect on the results.[5] Standard guidelines for blood sample handling state that plasma or serum should be separated (within 20-30 min) from cells as soon as possible after clot formation is complete to avoid clot-induced changes in the concentration of serum composition.[6] Many studies have been done on the stability of human enzymes during storage.[7,8]

However, in studies about camels, it is not always practical to analyze the sera immediately because of the location of the camel, time of collection, and the availability of the laboratory facilities.

At present, as there are conflicting data regarding the effect of different temperatures and durations of storage on the stability of the activities of hepatobiliary enzymes of camels that are routinely analyzed for clinical diagnostic use, it is of primary importance to reexamine the storage stability of these enzymes.

MATERIALS AND METHODS

Blood samples were collected from the Jugular vein of nine starved healthy adult female camels (age 3-6 years) using sterile needles (18 gauge) directly into clean dry sterile glass tubes without anticoagulants. Serum was separated after 30-35min following clot formation by centrifugation for 10 min at 2000 g; the serum was dispensed into 3 tubes and stored at 4°C, 25°C and 40°C. At intervals, serum from each tube was taken and analyzed. The measurements were done immediately after serum separation (zero day), and on days 1, 2, 3, 7, 11, 15 days. The clear serum was assayed for the following hepatobiliary enzymes ALT, AST, and ALP. The serum enzyme kinetic was determining using commercial reagent kits (from Biomaghreb by JENWAY 6305 Spectrophotometer).

Statistical analysis: The stability of an enzyme activity under each temperature condition and time was determined by calculating the percentage change in activity from the

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mean fresh value (day zero) at each time point for each animal. To test the significant differences in enzyme activity over time at each temperature point, the data was analyzed statistically using *paired student t test* by Sigma Plot 2 programm.[9]

RESULTS

The serum enzyme activities of AST, ALT, and ALP in camel serum during storage at 4°C (Table 1), 25°C (Table 2), and 40°C (Table 3) are shown. AST activity was significantly decreased on day 15 in serum stored at 4°C. A negligible increase in all of the enzyme activities being studied here was observed at 4°C during the first week of storage, however, at 25°C ALP activity was significantly decreased after 7 days of storage. ALT activities were stable for 15 days in serum stored at 25°C without any significant variation (Table 2). Serum ALT was found to be the most stable enzyme in camel serum being stable at 40°C for 15 days without significant decrease in activity. In contrast, the most unstable enzyme was found to be AST. Its activity decreased significantly on 3rd day of storage at 40°C. ALP showed significant decrease in activity in serum preserved at 40°C for longer than 3 days (Table 3).

DISCUSSION

In this study, the effect of storage at room temperature (25°C), refrigeration (4°C) and 40°C for 0, 1, 2, 3, 7,

Table 1: Activity of ALT, AST and ALP in camel sera samples preserved at 4ºC for 15 days

Days of storage	AST		ALT		ALP	
	U/L	% change in activity	U/L	%change in activity	U/L	% change in activity
zero	56.7 ± 2	100	16.4 ± 2	100	181.5 ± 24	100
1	63.1 ± 2	111	18.3 ± 2	110	243 ± 47	130
2	72.0 ± 7	127	17.4 ± 5	100	243.3 ± 53	130
3	52.2 ± 1	92	16.4 ± 1	100	252.7±28	130
7	65.0 ± 11	115	23.6 ± 2	140	189.3 ± 13	100
11	59.1 ± 1	104	24 ± 5	140	154 ± 5	85
15	48.6 ± 0.4	86*	18.6 ± 8	110	137.5 ± 11	76*

Data represent the mean ± SD. (* indicates a statistically significant difference where $P < 0.05$, ** indicates a statistically significant difference where $P < 0.01$) to find out if the difference seen is statistically significant, the data of each time point in the table was compared to the corresponding zero time control sample. When no star is there it means no significant difference is observed.

Table 2: Activity of ALT, AST and ALP in camel sera samples preserved at 25°C for 15 days

Days of storage	AST		ALT		ALP	
	U/L	% change in activity	U/L	% change in activity	U/L	% change in activity
zero	56.7 ± 2	100	16.4 ± 2.8	100	181.5 ± 24	100
1	59.0 ± 1	104	16.9 ± 1.6	103	243 ± 17	134
2	58.4 ± 8	103	14.9 ± 1.6	91	250.7 ± 24	138
3	56.4 ± 19	99	16.6 ± 2	101	271.7 ± 4	150
7	54.4 ± 8	96	16.7 ± 0.8	102	150 ± 8	83*
11	56.1 ± 3	99	16.3 ± 0.8	99	150 ± 1.4	83*
15	47.9 ± 24	84*	14.1 ± 2	86	144 ± 14	79*

Data represent the mean ± SD. (* indicates a statistically significant difference where $P < 0.05$, ** indicates a statistically significant difference where $P < 0.01$) to find out if the difference seen is statistically significant, the data of each time point in the table was compared to the corresponding zero time control sample. When no star is there it means no significant difference is observed.

Table 3: Activity of ALT, AST and ALP in camel sera samples preserved at 40 for 15 days

Days of storage	AST		ALT		ALP	
	U/L	% change in activity	U/L	% change in activity	U/L	% change in activity
zero	56.7 ± 2	100	16.4 ± 2.8	100	181.5 ± 24	100
1	63.1 ± 2	111	24.2 ± 0.73	148	261 ± 24	144
2	58.1 ± 9	103	25 ± 7	152	221 ± 5	122
3	47.8 ± 1	84*	22.8 ± 8	139	174 ± 44	96
7	37.1 ± 5	65*	16.6 ± 0.8	102	139.6 ± 24	77*
11	28.6 ± 1.6	51**	14.8 ± 2.5	90	141.3 ± 8	78*
15	27.5 ± 1	49**	13.1 ± 2.7	80*	142 ± 0.7	78*

Data represent the mean ± SD. (* indicates a statistically significant difference where $P < 0.05$, ** indicates a statistically significant difference where $P < 0.01$) to find out if the difference seen is statistically significant, the data of each time point in the table was compared to the corresponding zero time control sample. When no star is there it means no significant difference is observed.

11, 15 days of ALT, ALP, and AST were investigated. Serum AST, ALT, and ALP activities found in this study are in reasonable agreement with values reported from previous studies.[10,11] Data on hepatobiliary enzymes of camels are gained under hot humid tropical conditions. Temperature and- duration of storage are two important factors in the instability of enzyme activity.[12, 13] The rate of activity of each enzyme shows a maximum value at a particular temperature then as the temperature increases the enzyme protein undergoes increasingly rapid heat denaturation and loss of its activity. Intracellularly, enzymes are protected from degradation when bound to their substrates and cofactors. In serum, enzymes, substrates and cofactors are dispersed and binding is uncommon, leaving the enzymes more susceptible to degradation.[12]

Our results showed that the activity of ALT of camel serum is stable over 15 days even when the serum is stored at 40°C. AST activity, however, was significantly decreased after 3 days when the serum is stored at 40°C. In contrast to the results obtained by Saeed *et al.* on camels.[14] ALP was also stable over the study period at the three temperature degrees selected in this study.

According to our results the activity of AST, ALT, and ALP in camel serum is more stable than that reported in goat serum. The effect of temperature and storage time on the hepatobiliary enzyme activities from goat serum was studied by Divya PD,[15] which showed that ALT was unstable at room temperature while AST was found to be stable for 8 days at room temperature. ALP showed great variation upon storage as compared to the other hepatobiliary enzymes and the authors suggested that its estimation should be performed in fresh serum samples.

CONCLUSION

One important aspect of this study is that it demonstrated the stability of camel serum when stored at 40°C, a temperature frequently reached during the summer. Further studies will have to address the question of what is the effect of this temperature point on other serum constituents of camel.

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