Use of serum gamma glutamyl transferase as a biomarker of stress and metabolic dysfunctions in *Rathi* cattle of arid tract in India

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The investigation was carried out to determine serum gamma glutamyl transferase enzyme as a biomarker of stress and metabolic dysfunctions in *Rathi* cattle of arid tract in India. Blood samples were collected to harvest serum from healthy male and female, drought affected, ketotic cows, recently aborted cows, cows with diarrhoea, cows with traumatic pericarditis, calves with urinary calculi, cows affected with urea poisoning and cows affected with acidosis. The mean values of \( \gamma \) glutamyl transferase showed significant variations \((p \leq 0.05)\) according to sex and age in the healthy group of animals. The normal range in healthy animals was from 12 to 34 UL\(^{-1}\). In affected group an average 23.69 times rise in the value was observed from that of healthy group. Cows affected with urea poisoning and acidosis were having highest mean values whereas drought affected animals were having least value. It was concluded that present study attempted to provide a new insight about an old enzyme. As the number of animals in the present study was statistically sufficient therefore the mean value of healthy group can be used as reference value for \( \gamma \) GT in *Rathi* cattle and other cattle breeds which can help to interpret the variations of serum \( \gamma \) GT in various metabolic diseases of cattle.

**Key words:** Gamma glutamyl transferase, metabolic dysfunction, *Rathi* cattle, serum
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Use of gamma glutamyl transferase (GGT or γ GT) as diagnostic marker in medicine is significant. However, its use as a stress marker in veterinary science is still in juvenile stage. It is a carboxypeptidase which cleaves C-terminal glutamyl groups and transfers them to peptides and other suitable acceptors. It occurs as a membrane associated aggregate and is also involved in glutathione metabolism by transferring the glutamyl moiety to a variety of acceptor
molecules including water, certain L-amino acids and peptides (Schulman et al., 1975). Glutathione breakdown results in the formation of cysteine, a thiol compound exerting antioxidant effects and helps to preserve intracellular homeostasis of oxidative stress. Increase in environmental oxidative stress may induce γGT via nuclear factor kappa-light-chain-enhancer of activated B cells or NFkB (Yokoyama, 2007). Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a protein complex that acts as a transcription factor and it is found in almost all animal cell types and is involved in cellular responses to stimuli such as stress, cytokines, free radicals, ultraviolet irradiation, oxidized LDL, and bacterial or viral antigens (Gilmore, 1999; Brasier, 2006; Gilmore, 2006; Perkins, 2007).

Gamma glutamyl transferase is also involved in leukotriene metabolism (Raulf et al., 1985). The activity of γ GT in the bovine is present in kidney (Szewczuk and Baranowski, 1963), bile ductules (Naftalin et al., 1969), brain capillaries (Orlowski et al., 1974), milk (Sobiech et al., 1974) and leukocytes (Sobiech and Sobiech, 1975). As serum γ GT is almost all derived from the liver, its determination can serve as a valuable test for metabolic dysfunction and hepatocellular damage in acute fasciolosis, ketosis and angiomatosis (Rico et al., 1977). An elevated serum γ GT appears to be a sensitive specific indicator of liver damage, making it a useful diagnostic aid even over serum aspartate aminotransferase (Blackshaw, 1978). It is also used to determine bile duct epithelial proliferation, cholestatic disorders, chronic and toxic hepatopathies. The visual hepatic damage due to parasitic load and serum levels of γ GT has been significantly positively related in the cattle (Molina et al., 2006).

Modern milk production often puts the production capabilities of cows at risk, which can result in metabolic disorders. In order to predict such disorders and eventual subclinical diseases it is necessary to determine physiological ranges of biochemical parameters in a clinically healthy herd. Gamma glutamyl transferase is one such parameter and its determination is important for systemic monitoring of health (Payne and Payne, 1987; Stojević et al., 2002 and Kida, 2003). Drought is also an important factor affecting milk production in the arid tract. There is a paucity of literature regarding the serum γ GT levels in the Rathi animals. The present investigation was carried out to find out the normal values of this enzyme along with its possible role as a biomarker of stress and metabolic dysfunctions in the cattle of Rathi breed.

MATERIALS AND METHODS

To carry out the investigation blood samples (300) were collected to harvest serum from the Rathi cattle belonging to farmers’ stock of arid tract in India. To obtain normal values, samples were collected from 150 healthy Rathi animals (healthy group) categorised as male (60) and female (90), which were further divided as calves and adults. To assess the use of γ GT as a biomarker of stress and metabolic dysfunctions, samples were collected from affected animals (150) due to stress and metabolic dysfunctions. This group comprised of drought affected adult Rathi animals (30), ketotic cows (20), recently aborted cows (20), cows with diarrhoea (20), cows with traumatic pericarditis (20), calves with urinary calculi (20), cows affected with urea poisoning (10) and cows affected with acidosis (10).

The blood samples were collected in sterile
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tubes without anticoagulants to harvest sera and analyses were made immediately after sera collection. The spectrophotometric method (Wolf and Williams, 1973) was used to determine serum γ glutamyl transferase with slight modification. Tris buffer was prepared by dissolving 14.54 g tris, 2.44 g magnesium chloride and 11.89 g glycylglycine in about 800 ml distilled water. The pH was adjusted to 7.8 at 37°C and final volume was made to 1 litre with distilled water. It was stored at 4°C. Substrate was prepared by dissolving 1.28 g L-γ-glutamyl-4-nitroanilide in 0.15 mol/l hydrochloric acid and final volume was made to 100 ml with the acid. It was stored at -20°C. Then in a test tube 100 µl serum and 1.0 ml of buffer were taken and warmed to 37°C. Then immediately the contents were transferred to a 1 cm cuvette and 0.1 ml of substrate was added. The optical densities were recorded at 405 mµ. To determine the change (ΔOD/minute) per minute, three ODs were recorded at one minute interval. As molar absorption coefficient of 4-nitroaniline at 405 mµ is 9900 1 mol⁻¹cm⁻¹, therefore the activity of enzyme in UL⁻¹ was determined by the formula:

\[
\text{U/l activity} = \frac{1000 \times \Delta \text{OD}_{405} \times 1.2}{\text{minute} \times 0.1 \times 9.9}
\]

Where 1.2 is the final volume in the cuvette and 0.1 ml the volume of serum.

Mean changes in serum γ GT levels of affected animals were compared from those of healthy animals by using statistical significance (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The mean ± SEM values are presented in table 1. The mean values of γ glutamyl transferase showed significant variations (p≤ 0.05) according to sex and age in the healthy group of animals. The values were higher in male animals than female. The serum γ GT activity decreased with the advancement in the age. It was higher in calves than adults of each sex. The normal range in healthy animals was from 6 to 34 UL⁻¹. In affected group an average 23.69 times rise in the value was observed from that of healthy group. Cows affected with urea poisoning and acidosis were having highest mean values whereas drought affected animals were having least value.

The mean value of serum γ GT in healthy group of Rathi animals was in the normal range reported for cattle (Starý and Rolencová, 1981), however, the range in present study was higher than the earlier reports (Blackshaw, 1978). Higher serum γ GT in male animals corroborated the earlier findings in cattle (Boonprong et al. 2007). Age wise variations were also noted in serum γ GT. Earlier work has also shown the influence of age on γ GT values (Stojevic et al., 2005). Calves had higher serum γ GT levels which can be used to predict serum IgG1 concentration (Parish et al., 2008).

Earlier reports suggested that calves suckling colostrum had 26 times greater serum activity of γ GT compared with concentrations at birth (Perino et al., 1993). Higher serum γ GT value in drought affected animals indicated the stress on the liver due to adverse conditions. Activity can be determined for suspicion of acute and chronic liver disease (Cebra et al., 1997). Higher levels during drought could also be due to low appetite because of low quality feed. Increased γ GT value in ketogenic cows (Steen, 2001) pointed towards a stressed liver as its activity is relatively high in liver (Tenant, 1997). It could be related to negative energy status of high yielders particularly in late pregnancy, in the
first weeks of lactation, and during disease (Cebra et al., 1997) as energy status have an influence on γ GT values (Stojevic et al., 2005).

Table 1. Serum levels of γ Glutamyl transferase (γ GT) in Rathi cattle

<table>
<thead>
<tr>
<th>Category</th>
<th>γ GT (UL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Healthy group (150)</td>
<td>23.31 ± 0.13 a</td>
</tr>
<tr>
<td>1. Male (60)</td>
<td>24.75 ± 0.21 c</td>
</tr>
<tr>
<td>(i) Calves (45)</td>
<td>28.0 ± 0.12 b</td>
</tr>
<tr>
<td>(ii) Adults (15)</td>
<td>21.5 ± 0.24 b</td>
</tr>
<tr>
<td>2. Female (90)</td>
<td>21.85 ± 0.22 c</td>
</tr>
<tr>
<td>(i) Calves (40)</td>
<td>25.2 ± 0.12 a</td>
</tr>
<tr>
<td>(ii) Adults (50)</td>
<td>19.5 ± 0.14 e</td>
</tr>
<tr>
<td>II. Affected group (150)</td>
<td>552.38 ± 11.16 d</td>
</tr>
<tr>
<td>1. Drought affected (30)</td>
<td>167.33 ± 7.2</td>
</tr>
<tr>
<td>2. Ketotic cows (20)</td>
<td>645.14 ± 10.2</td>
</tr>
<tr>
<td>3. Recently aborted cows (20)</td>
<td>503.26 ± 12.6</td>
</tr>
<tr>
<td>4. Cows with diarrhoea (20)</td>
<td>312.15 ± 9.4</td>
</tr>
<tr>
<td>5. Cows with traumatic pericarditis (20)</td>
<td>387.41 ± 16.7</td>
</tr>
<tr>
<td>6. Calves with urinary calculi (20)</td>
<td>435.25 ± 11.6</td>
</tr>
<tr>
<td>7. Cows affected with urea poisoning (10)</td>
<td>983.31 ± 20.2</td>
</tr>
<tr>
<td>8. Cows affected with acidosis (10)</td>
<td>985.25 ± 15.2</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate the number of animals.

b and e = Significant (p≤ 0.05) age effect

c = Significant (p≤ 0.05) sex effect

d = Significant (p≤ 0.05) difference from healthy group

Enhanced levels of serum γ GT value in diarrhoea cases can be related to its activity in intestine (Braun et al., 1983). Mechanism of increased serum γ GT in the cases of recently aborted cows and cows with traumatic pericarditis could be explained as stress response because γ GT is a consistent cellular and biochemical marker of stress responsiveness and stress-induced immunomodulation (Koner et al., 1997). It plays a central role in the homeostasis of the antioxidant glutathione. The expression of γ GT has been shown to be upregulated after oxidative stress.

Increased serum activity of γ GT in calves affected with urinary calculi signifies the presence of this enzyme in the kidneys (Goldberg, 1980) as urinary γ GT is considered as a good test for kidney damage (Braun et al., 1983). In various metabolic dysfunctions, like acidosis and urea poisoning the increased serum γ GT could be suggestive of restoration of intracellular homeostasis (Schulman, 1975). Increased level also indicates the liver stimulation thereby resulting in increased production of γ GT and its more leakage into plasma (Barouki, 1983).

It was concluded that present study attempted to provide a new insight about an old enzyme. As
the number of animals in the present study was statistically sufficient therefore the mean value of healthy group can be used as reference value for γ GT in Rathi cattle and other cattle breeds which can help to interpret the variations of serum γ GT in various metabolic diseases of cattle. From the practical point of view γ GT activity was easy to measure, it can be effectively used as a valuable diagnostic test for hepatic and metabolic disorders in the Rathi breed of cattle.

REFERENCES


