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HISTOENZYMIC DISTRIBUTION PATTERN IN GALL BLADDER OF SHEEP

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ABSTRACT

The present study was conducted on gall bladder of < 1 year-old sheep (n = 9) to elucidate the distribution of enzymes *viz*., alcoholic dehydrogenase, glucose-6-phosphate dehydrogenase, glutamic dehydrogenase, lactic dehydrogenase, malic dehydrogenase, reduced nicotinamide adenine dinucleotide diaphorase, reduced nicotinamide adenine dinucleotide phosphate-diaphorase and succinic dehydrogenase. The histoenzymic distribution of monoamine oxidase, non-specific esterase, alkaline phosphatase and glucose-6-phosphatase was also recorded. The study revealed moderate to intense activity in glandular areas of gall bladder for majority of enzymes reflecting higher metabolic activity. The enzyme activity was weak to moderate in different tunics of gall bladder and mild to moderate in vascular areas. The differential enzyme activity in different tunics of gall bladder was correlated with metabolic activity of cells.

Key words: Enzyme, gall bladder, histochemistry, postnatal, sheep

INTRODUCTION

The gall bladder is a storage reservoir that allows the supply of bile acids in high concentration and controlled manner to the duodenum for solubilization of dietary lipids (Suchy, 2016). All these functions are strongly influenced by various histomorphological and histochemical factors. The presence of many enzymes including phosphatases and dehydrogenases participate in major physiological roles in gall bladder. Alkaline phosphatase (AKPase) is associated with the ionic exchange across the membrane as well as for endocytosis and pinocytosis in cells (Singh and Singh, 2015). Diaphorase activity represents mitochondrial activity along with the cytoplasmic electron transport. Succinic dehydrogenase (SDH) is an essential part of Kreb's cycle and also found in all aerobic cells. Moreover, glucose-6-phosphatase (G-6-Pase) is a key enzyme which regulates glucose mechanism in intestine. G-6-Pase is a hydrophobic protein which was observed to be embedded within endoplasmic reticulum of the luminal membrane (Mithieux *et al.,* 2004).

Adequate reports have been made on histomorphology of gall bladder in domestic animals (Poddar, 1977; Bacha and Wood, 1990; Gheri *et al.,* 1990; Mokhtar *et al.,* 2013). However, very few reports are available on the distribution of enzymes in gall bladder of domestic animals. More-over, there is no report available on histoenzymology of gall bladder of sheep. Therefore, present study was aimed to explicate the tissue localization of various enzymes in gall bladder of sheep.

MATERIALS AND METHODS

The present study was conducted on gall bladder of <1 year-old sheep (n = 9) to assess the histo-enzymic distribution of different enzymes. The samples were collected from the slaughter houses present in and around Srinagar (Kashmir) and the approximate age of sheep (< 1 year) was determined on the basis of dentition (Sastry *et al*., 1982; Singh, 1988). After observing the dentition of post-natal sheep, the fresh and unfixed gall bladders were dissected out and fixed in liquid nitrogen. Cryosections of 10 µm at -20ºC were obtained on grease-free glass slides with the help of cryotome. These sections were incubated with various substrates to study histoenzymic distribution of alcoholic dehydrogenase (ALC), glucose-6-phosphate dehydrogenase (G-6-PD), lactic dehydrogenase (LDH), g1utamic dehydrogenase (GLD), malic dehydrogenase (MDH), reduced nicotinamide adenine dinucleotide diaphorase (NADH-D), reduced nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-D) and succinic dehydrogenase (SDH) and monoamine oxidase (MAO) as per the methods of Pearse (1972). The intensity of staining gave qualitative indication of the amount of enzyme activity. In addition, control sections were incubated in media devoid of substrate to check the specificity of the reaction. The non-specific esterases (Gomori, 1952) and phosphatases were studied by using simultaneous coupling azo dye method (Barka and Anderson, 1963) and lead nitrate method (Barka and Anderson, 1963), respectively.

RESULTS AND DISCUSSION

Dehydrogenases

A uniform weak alcoholic dehydrogenase (ALC) activity was observed in lamina epithelialis mucosae; whereas mild activity was observed in propria-submucosa of sheep gall bladder (Fig. 1). ALC is a group of dehydrogenase enzymes which is required for interconversion between alcohols and aldehyde or ketones, located in the cytoplasm of cell. Saleem *et al.* (1984) also detected ALC of gall bladder in human. The weak to mild activity of ALC in different tunics of gall bladder was correlated with differential metabolic activity of various tunics.

A weak glucose-6-phosphate dehydrogenase (G-6-PD) activity was observed in lamina epithelialis mucosae whereas weak to moderate activity was observed in glands and vascular areas of propria-submucosa (Fig. 2). The G-6-PD activity is associated with pentose phosphate shunt (Fennel and Pearse, 1961), which might be utilized for nucleic acid synthesis during development. The weak G-6-PD activity indicated developing pentose phosphate shunt in the cells of different tunics of gall bladder of sheep of < 1year age.

A weak glutamic dehydrogenase (GLD) activity was observed in lamina epithelialis mucosae, weak to moderate in vascular areas and glands of propria submucosa. Tunica serosa of sheep gall bladder was weakly positive. The GLD produces α-ketoglutaric acid by deamination of glutamic acid which is an intermediate in Krebs cycle (Shrader and Zeman, 1972). The differential activity might be associated with the development of glutamic dehydrogenase pathway in Krebs cycle.

Weak to moderate lactic dehydrogenase (LDH) activity was observed in lamina epithelialis mucosae. Moderate to strong LDH activity was noticed around venous channels, connective tissue and glandular epithelium of propria submucosa. Tunica muscularis of gall bladder during post-natal period was moderately positive (Fig. 3). LDH is a NAD-dependent enzyme found in the cells involved in glycolytic pathway. Moderate to strong LDH activity in present study might be due to the presence of glycolytic pathway in different tunics of gall bladder.

A uniform weak malic dehydrogenase (MDH) activity was observed in different tunics and vascular endothelium of lamina propria submucosa of postnatal gall bladder. Lamina epithelialis mucosae were moderately positive for succinic dehydrogenase (SDH) activity. Thevascular

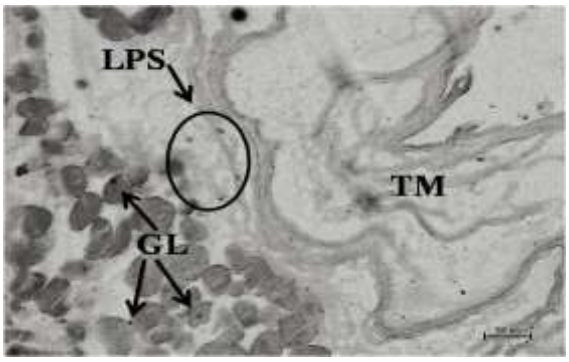

Fig. 1: Cryosection of gall bladder of postnatal sheep showing weak in the epithelium (TM), mild alcoholic dehydrogenase activity in the lamina propria (LPS) and glands (GL) of lamina propria-submucosa. Nitro BT method X100

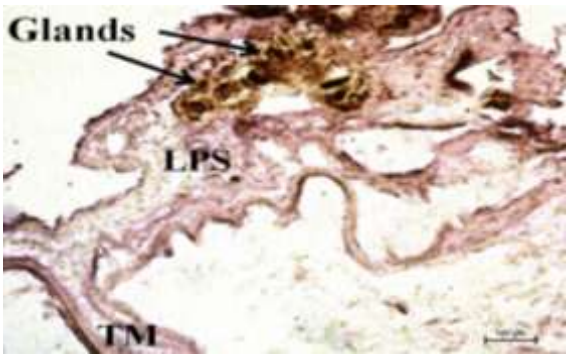

Fig. 2: Cryosection of gall bladder of postnatal sheep showing mild in the tunica mucosa (TM) and mild to moderate Glucose-6-phosphate dehydrogenase activity in in the lamina propria-submucosa (LPS). Nitro BT method X 100

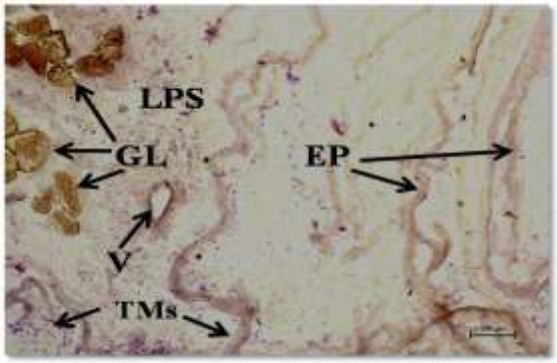

Fig. 3: Cryosection of gall bladder of postnatal sheep showing mild to moderate in the tunica mucosa (EP), moderate to strong lactic dehydrogenase activity in the muscles of tunica muscularis (TMs), blood vessel (V) and glands of propria-submucosa (LPS). Nitro BT method X100

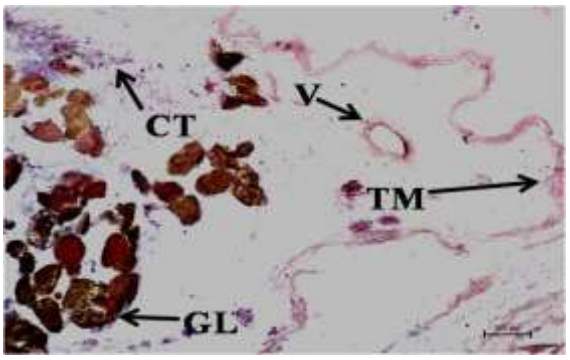

Fig. 4: Cryosection of gall bladder of postnatal sheep showing mild to moderate in tunica mucosa (TM), moderate to strong nicotinamide adenine dinucleotide diaphorase activity in glandular epithelium (GL) and vascular endothelium (V) and mild in connective tissue fibres (CT) of propria-submucosa. Nitro BT method X100

endothelium and glands of propria submucosa showed moderate to intense activity. The tunica muscularis showed moderate activity for SDH. The presence of SDH activity indicated mitochondrial localization and development of Krebs cycle activity in the cells (Lauwers, 1973).

Diaphorases

A weak to moderate activity of reduced nicotinamide adenine dinucleotide diaphorase (NADH-D) was observed in tunica mucosa. Moderate to strong activity was observed in vascular endothelium and glandular of propria submucosa of gall bladder of sheep (Fig. 4). The NADH-D is a coenzyme dehydrogenase and acts in the cell as a part of hydrogen transport chain (Raekallio, 1970). The distribution of reduced nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-D) was similar to that of NADH-D in gall bladder. NADPH-D and NADH-D being coenzyme dehydrogenases, their activity reflected the metabolic status of the cell (Shrader and Zeman, 1972).

Monoamine oxidase (MAO)

A moderate MAO activity was observed in the lamina epithelialis mucosae of tunica mucosa, moderate to intense activity in the glands and blood vessels of propria-submucosa and tunica muscularis of gall bladder of postnatal sheep (Fig. 5).

Non-specific esterases (NSE)

NSE activity was present in the form of granular deposits in the tunica mucosa. Non-glandular areas and blood vessels of the propria submucosa and tunica serosa showed weak activity whereas the glands were moderately positive for NSE (Fig. 6). Tunica muscularis was moderately positive for non-specific esterases in the gall bladder of sheep. Non-specific esterases are a group of enzymes associated with lipid metabolism. The presence of NSE activity suggested that these might be playing a role in synthesis of lipids and phospholipids which are used for formation of cell membranes (Monahan-Earley *et al.*, 1987).

Phosphatases

Tunica mucosa was weakly positive for adenosine triphosphatase (ATPase) while glandular epithelium in propria-submucosa showed weak activity. Vascular endothelium of blood vessels in propria-submucosa and tunica muscularis was moderately positive for ATPase. ATPase is a trans-mebrane/integral membrane protein that converts ATP to ADP and inorganic phosphate (Pi) and its activity was correlated with high energy requirement for transport of metabolites in the cells of blood vessels. A weak alkaline phosphatase (AKPase) activity was observed in the lamina epithelialis

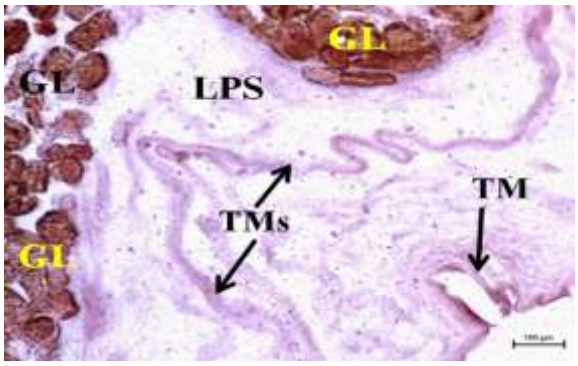

Fig. 5: Cryosection of gall bladder of postnatal sheep showing moderate monoamine oxidase activity in the tunica mucosa (TM), moderate to intense activity in the glands (GL) of propria-submucosa (LPS) and moderate in the tunica muscularis (TMs). Nitro BT Method X 100

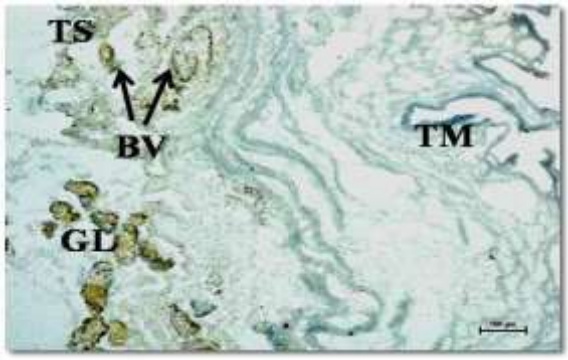

Fig. 6: Cryosection of gall bladder of postnatal sheep showing weak to mild in the tunica mucosa (TM) and tunica serosa (TS), moderate non-specific esterase activity in in the glands (GL) and blood vessels (BV) of propria-submucosa. Naptholacetate method X 100

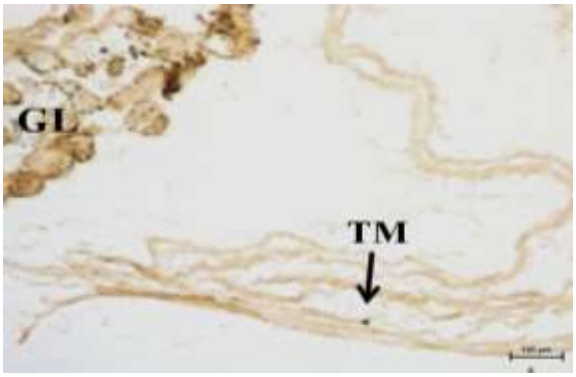

Fig. 7: Cryosection of gall bladder of postnatal sheep showing weak alkaline phosphatase activity in tunica mucosa (TM) and mild to moderate in the glands (GL) of propria-submucosa. Azo dye method X100

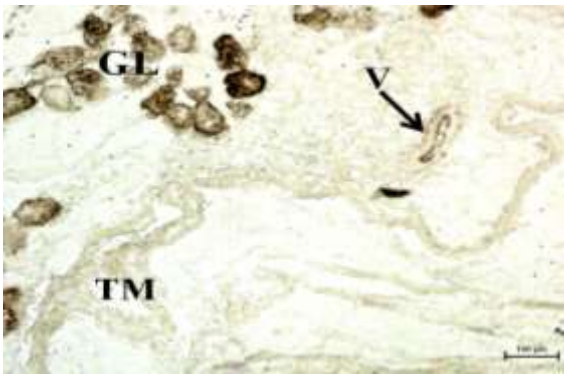

Fig. 8: Cryosection of gall bladder of postnatal sheep showing mild in tunica mucosa (TM) and moderate glucose-6-phosphatase activity in glands (GL) and blood vessel (V) of propria-submucosa. Lead nitrate method X100

mucosae, whereas weak to moderate activity was present in the glands of propria-submucosa of gall bladder of sheep (Fig. 7). The presence of AKPase activity around glands and capillaries may be correlated with ionic movement across epithelium and wall of capillaries. Al-Allaf *et al*. (2013) localized AKPase activity in human gall bladder containing cholesterol stones. The AKPase activity varied considerably in different parts of gall bladder and was correlated with morphological damage to gall bladder due to cholesterol stones.

A weak glucose-6-phosphatase (G-6-Pase) activity was observed in lamina epithelialis mucosae of gall bladder of sheep. Endothelial cells of the blood vessels and glands were moderately positive for G-6-Pase (Fig. 8). Glucose-6-phosphatase consists of amino acids, and is anchored to the endoplasmic reticulum (ER). The main function of this enzyme is to regulate glucose release into the circulation. Loss of this enzyme leads to a condition known as hepatic glycogen storage disease (Chayen *et al.,* 1969). Hill *et al*. (2004*)* also reported the presence of G-6-Pase at a lower concentration in the human gall bladder.

Ethical statement: The present study was conducted in compliance with the institutional ethical guidelines and after obtaining approval from the concerned bodies.

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