HISTOCHEMICAL STUDIES OF LIVER IN BUFFALO (BUBALUS BUBALIS)

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ABSTRACT

The gross histochemical studies of the 24 buffaloes livers (12 male and 12 female) were carried out. PAS reaction was intense in hepatocytes in all regions of liver in male and female except apex of caudate lobe in female where it was weak and below the portal vein in male which was intense. The other components showed moderate to weak reaction in both male and female buffaloes. Alkaline phosphatase reaction was weak in hepatocytes in all the regions of liver of male and female buffalo except upper third of parietal surface and below the portal vein in female, while other components showed weak to negative reaction in both male female buffaloes. Acid phosphatase reaction was moderate in hepatocytes in neck of gall bladder in both the sex and apex of caudate lobe in male, while moderate to negative reaction in other components of male and female. Ferric iron granules were present in the cytoplasm of hepatocytes of both male and female, which were pale blue in colouration.

Keywords: *Bubalus bubalis*, buffaloes, histochemistry, liver

INTRODUCTION

Very scanty literature is available on the histochemistry of the liver in buffaloes. Hence the present study is made.

MATERIALS AND METHODS

The present study was carried out on normal livers 12 male and 12 female adult buffaloes (*Bubalus bubalis*) of Murrah breed. The liver was examined insitu and all attachment and other particulars were recorded. The specimens were collected immediately after slaughter at Deonar Abattoir, Mumbai. The livers were separated from the pluck and cleared by remobing the fascia, blood vessels, nerves etc. in order to facilitate the observations. These separated livers were washed under running tap water to remove all blood clotm exudateand tissue debris. The organs were brought to the laboratory in ice-cooled box for further study.

For the histochemical study of the liver, five tissue pieces of 4 to 5 mm size in all dimension were collected from upper third of parietal surface, middle of the liver at center, just below the portal vein, from the neck of gall bladder and apex of

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caudate lobe. These tissue pieces were fixed in 10% neutral buffered formaline solution for 24 h. The tissue pieces were treated with routine method of dehydrationin in ascending grades of alcohol, cleared in cedar wood oil and embedded in paraffin wax as per the method of Drury et al. (1967) each prepared paraffin block was sectioned at 3 to 5 µm. Histochemical study was carried out for following cytological contents. Periodic acid Schiff's technique (PAS) for carbohydrates (mucopolysaccharides) as per the method of Culling (1969). Gomori's modified alkaline phosphatase cobalt method for alkaline phosphatase activity (Singh and Sulochana, 1997) and Gomori's modified lead nitrate method for acid phosphatase activity (Culling, 1969). Turnbull blue method for ferric iron as per the method of Pearse (1968).

RESULTS AND DISCUSSION

The histochemical activities were observed in various components of liver like hepatocyte, Von-Kupffer cells, portal vein, hepatic artery and central vein from the different regions of the liver like upper third of parietal surface, middle of liver at the center, below the portal vein, neck of the gall bladder and apex of the caudate lobe.

Periodic acid schiff (PAS) reaction

Hepatocytes showed intense reaction in all regions except apex of caudate lobe in female, which was weak and below the portal vein in male, which was intense, the other components showed moderate to weak reaction in both male and female buffaloes. (Figure 1).

Gomori's modified alkaline phosphatase cobalt

Hepatocytes showed weak reaction in all the regions of the liver, except upper third of parietal surface and below the portal vein in female, while other components showed weak to negative reaction in both male and female buffaloes. Wadhava *et al.* (1991) observed that loss of alkaline phosphatase activity in liver collected form downer's cow syndrome. Saigal *et al.* (1992) recorded moderate alkaline phosphatase activity in central vein and portal vein. Nanda *et al.* (1979) also recorded the similar observations in adult male buffaloes. (Figure 4).

Lead nitrate method for acid phosphatase

Hepatocytes showed moderate reaction in neck of gall bladder in both the sex and apex of caudate lobe in male, while moderate to negative reaction in other components. Saigal et al. (1992) recorded that very weak or negative reaction in central vein and portal vein, but moderate reaction in hepatocyte of the periportal region. The acid phosphatase activity in the hepatocytes had been recognized in other species like rat, mouse, dog and human being (Wachstein, 1959; Wachstein 1963; Novikoff, 1959; Novikoff, 1963; Novikoff and Essner, 1960). Similarly Manns and Mortimer (1969); Rtzlaff and Tyler (1973); Nanda et al. (1979) recognized it's distribution in calf, sheep, rat, aves and goat, respectively. Wadhawa et al. (1991) observed that increased in acid phosphatase activity in Von-Kupffer cells of liver collected from in downer's cow syndrome animals. (Figure 2).

Turnbull blue method for ferric iron

In present work, the presence of ferric iron granules were identified by pale blue colouration in the cytoplasm of hepatocytes of both male and female buffaloes. Khan *et al.* (1995) observed

Sr. No.	Parameters	Male			Female		
		PAS	ALk	АСр	PAS	ALk	АСр
A) UPPER THIRD OF PARIETAL SURFACE							
1	Hepatocyte	+++	+	+	+++		+
2	Von-Kupffer cell	++		+	++	+	+
3	Portal vein	++			++	+	
4	Hepatic artery	++	+	+	++		+
5	Central vein	++			+	+	+
	B)	MIDDLE C	F THE LIV	ER AT CH	ENTER		
1	Hepatocyte	+++	+	+	+++	+	
2	Von-Kupffer cell	++	+	+	++	+	+
3	Portal vein	++	+		++	+	+
4	Hepatic artery	+	+		+	+	+
5	Central vein	+			+		
		C) BELO	W THE PC	RTAL VE	ÍN		
1	Hepatocyte	+++		+	+++		
2	Von-Kupffer cell	+++	+		+++	+	+
3	Portal vein	+			+	+	
4	Hepatic artery	++			++		
5	Central vein	++	+		++	+	
		D) NECK	K OF GALI	BLADDE	R		
1	Hepatocyte	+++	+	++	+++	+	++
2	Von-Kupffer cell	++		++	+		++
3	Portal vein	++	+	+	++		+
4	Hepatic artery	++		++	++		++
5	Central vein	+	+	++	+	+	++
		E) APEX	CAUD	ATE LOB	E		
1	Hepatocyte	+++	+	++	++	+	+
2	Von-Kupffer cell	++	+	+	++	+	+
3	Portal vein	+	+	++	++	+	++
4	Hepatic artery	++		+	+	+	++
5	Central vein	++		+	++		++

Table 1. Histochemical activity in various components of different regions of liver in male and female buffaloes.

ALk = Alkaline phosphatase, ACp = Acid Phosphatase, +++ = Inense, ++ = Moderate,

+ = Weak and - = Negative.

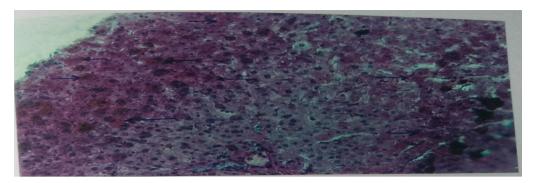


Figure 1. Microphotograph of the cross section of the liver: Arrow showing red coloured PAS activity. (PAS 66x)



Figure 2. Microphotograph of the cross section of the liver: Arrow showing the brownish-black coloured acid phosphatase activity. (Acid phosphatase 66 x)

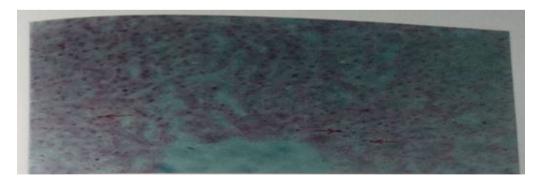


Figure 3. Microphotograph of the cross section of the liver: Arrow showing the pale-blue coloured ferric iron content. (Turnbull blue method for ferric iron 66 x)



Figure 4. Microphotograph of the cross section of the liver: Arrow showing the red coloured PAS activity.

the concentration of copper and iron, which were significantly different in male and female goats, while, Bires *et al.* (1995) observed the distribution of copper and iron in the liver of sheep and reported the significant levels between the young and old goat. (Figure 3).

RFFERENCES

- Bires, J., I. Maracek, P. Barko, M. Biresova and T. Weissova. 1995. Accumulation of trace element in sheep and the effects upon qualitative and quantitative ovarian changes. *Vet. Hum. Toxicol.*, **37**(4): 349-356.
- Culling, C.F.A. 1969. Handbook of Histopathological Technique (Including Museum Technique), 2nd ed. Butterworth and Co., Philadelphia, USA. p. 228-238 and 477-479.
- Drury, R.A.B., E.A. Wallington and R. Cameron. 1967. Carleton''s Histological Technique, 4th ed. Oxford University Press, London, England. p. 23-98.
- Khan, A.T., B.C. Diffay, D.M. Forester, S.J. Thompson and H.W. Mielke. 1995. Trace

element concentration in tissues of goats from Alabama. *Vet. Hum. Toxicol.*, **37**(4): 327-329.

- Manns, E. and P.H. Mortimer. 1969. Liver enzyme histochemistry - A comparative study of sheep, calf and rat. J. Com. Pathol., 79(2): 277-284. DOI: 10.1016/0021-9975(69)90017-6
- Nanda, B.S., A.J.J. A1-Sgaukgktm and A. Najada. 1979. Histoenzymic studies on the liver of goat. *Iraqi Biology J.*, 7: 56-71.
- Novikoff, A.B. 1959. Cell heterogenicity within the hepatic lobule of the rat: Staining reactions. *J. Histochem. Cytochem.*, 7(4): 240-244. DOI: 10.1177/7.4.240
- Novikoff, A.B. 1963. Lysosomes in physiology and pathology of cells - contribution of staining methods. Ciba Foundation Symposium on Lysosomes. Indiana, USA.
- Novikoff, A.B. and E. Essner. 1960. The liver cell. Some new approaches to its study. *A. J. Med.*, **29**: 102-131.
- Ratzlaff, M.H. and W.S. Tyler. 1973. A histochemical study of avian liver. *Poultry Sci.*, **52**: 1419-1426.

Saigal, R.P., S.K. Nagpal and B.S. Nanda. 1992.

Hydrolytic enzymes in buffalo liver a histoenzymic study. *Indian J. Vet. Anat.*, **4**(2): 58-63.

- Wachstein, M. 1959. Enzyme histochemistry of liver. *Gastroenterology*, **37**: 525-537.
- Wachstein, M. 1963. Cytochemistry of the liver. p. 137-194. In The Liver Morphology, Biochemistry and Physiology, 1st ed. Ch. Rouiller Academic Press, London, England.
- Wadhwa, D.R., B. Prasad and K.S. Roy. 1991. Histoenzymic studies on downer cow syndrome. *Indian J. Anim. Sci.*, **61**(3): 257-260.