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Gross Morphological, Histological and Histochemical Studies on Liver of Pati Ducks (*Anas platyrhynchos domesticus*) of Assam

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ABSTRACT

Background: *Pati* ducks have been playing a vital role in Assam's rural economy. It is an established fact that the liver is an important organ in the body. Since literature on a detailed anatomical study of the liver in *Pati* ducks of Assam is scare, hence the present study was designed to establish the gross, histological and histochemical details on liver with advancement of age.

Methods: The present study was conducted on 24 numbers of *Pati* ducks of Assam. They were divided into four (4) groups, each group containing six birds and kept for different ages *i.e*. 1 week, 8 weeks, 16 weeks and 24 weeks. The gross morphometry *viz*. length, breadth, thickness and weight of liver were observed. For histological study, tissue samples were fixed in 10% neutral buffered formalin. The processing and various methods staining were used according to Luna (1968). For histochemical study, samples were stored at -80°C in a deep freeze immediately after collection and they were treated according to Singh and Sulochana's method (1978).

Result: Morphometrical parameters showed an increasing trend with age. The lobules of liver were not distinct. Strong Alkaline phosphatase and Acid phosphatase activity were observed in the capsular area and bile canaliculi respectively.

Key words: Age, Gross, Histological, Histochemical, Liver, Pati duck.

INTRODUCTION

Ducks are one of the great converters of inferior quality waste products into excellent animal protein as egg and meat. The '*Pati*' duck of Assam was recognized by the ICAR Breed Registration Committee as a registered breed of duck with accession number INDIA_DUCK_0200_PATI_11001 in the year 2017 [National Bureau of Animal Genetic Resources, Karnal (Haryana), India. 2010] with an estimated population of about 18.21 lakh. *Pati* ducks have been playing a vital role in Assam's rural economy. It is an established fact that the liver is an important organ in the body. The liver performs a variety of functions necessary to sustain life, including the synthesis of blood proteins, the production and secretion of bile, detoxification, the absorption of nutrients, the metabolism of several substances and the storage of metabolites as reported by (Saez *et al*., 2012; Odokuma and Omokaro, 2015). However, a detailed anatomical study on the postnatal development of the liver in *Pati* ducks of Assam is scare and therefore, this study was designed on pati ducks aiming at understanding the gross morphological, histological and histochemical details on liver of *Pati* ducks with advancement of age.

MATERIALS AND METHODS

The present study was conducted on 24 numbers of apparently healthy *Pati* ducks of Assam of different age groups, irrespective of sex. It was decided to divide the ducks into four (4) groups as per their age so that the post natal development could be recorded along with the advancement of age. Each group contained six birds and each group was kept for a certain period of time, *i.e*. 1 week, 8 weeks, 16 weeks and 24 weeks. The experimental birds were sacrificed at the Department of Anatomy and Histology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati according to the method of Gracy (1986). Immediately after sacrifice, gross anatomical characteristics, location and relative topographic position of the liver were recorded. By using an electronic pan balance, the liver weights were measured after being removed from the body. A Vernier callipers was used to measure the length, breadth and thickness of each organ (McCance, 1974). For histological study, tissue samples from the liver from all age groups were fixed in 10% neutral buffered formalin solution and processed to prepare paraffin blocks according to the method described by Luna (1968). The paraffin blocks were sectioned in 5 µm thickness using a microtome and the sections were stained with Mayer's

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Haematoxylin and Eosin staining technique for histomorphological study. For study of collagen fibers, Van Gieson's method was used, for reticular fibers, Gomori's method was used, for elastic fibers, Hart's method was used and Bielschowsky's method was used for axis cylinder and dendrite as described by Luna (1968). Histological characteristics of liver were recorded after staining. On hematoxylin and eosin stained sections, different micrometrical parameters were recorded according to standard micrometrical methods using a camera mounted microscope and Image Pro Express Ver-2.0 software. For histochemical studies, birds from each group were utilized and the tissue samples were collected and preserved in liquid nitrogen at -196°C immediately after sacrifice. Samples of preserved tissue were transferred directly to cryostat microtome maintained at -22°C. The sections were cut at 7µm thickness and were treated for histochemical staining with Gomori's alkaline phosphatase cobalt method (Singh and Sulochana, 1978) for alkaline phosphatase, Gomori's method for acid phosphatase (Singh and Sulochana, 1978), Lead method for ATPase (Bancroft, 2008), Gomori's method for non-specific esterase (Bancroft, 2008) .

RESULTS AND DISCUSSION

Gross morphology

The liver of *Pati* ducks was located in the thoracic cavity above the sternum. It was found coudoventral to the lung and ventral to the proventriculus, gizzard, spleen, intestines and testis or ovary (Fig 1). Similar findings were reported by Abdulla (2015) in Iraqi ducks and Moslem (2015) in ostrich. The liver was reddish yellow in colour at 1 week of age and subsequently it was reddish brown colour in later stages of age. Similar findings were observed by Lakshmi *et al.* (1999) in duck, Sivagnanam *et al.* (2004) in guinea fowl and Kadhim *et al. (*2019) in Homing pigeon. The liver of *Pati* ducks consisted of two asymmetric lobes, the right and left lobes. It had two surfaces, the parietal surface and visceral surface. The gall bladder was located on the visceral surface of the right lobe of the liver (Fig 2). Similar observations were made by Lakshmi *et al.* (1999) in duck.

The average length of the right lobe of liver was 34.52±0.98 mm and 85.59±0.83 mm at 1 week and 24 weeks of Pati ducks, respectively. The average length of the left lobe was 26.51±0.52 mm and 57.18±0.89 mm at 1 week and 24 weeks, respectively. The average breadth of the right lobe was 18.26±0.99 mm at 1 week and 35.69±0.95 mm at 24 weeks of Pati duck of Assam. The average breadth of the left lobe was measured as 16.40±0.98 mm at 1 week and 27.23±0.99 mm at 24 weeks of Pati duck. The average thickness of the right lobe at 1 week and 24 weeks were 10.41±0.31 mm and 19.10±0.83 mm, respectively. The average thickness of the left lobe at 1 week and 24 weeks were 10.94±0.32 mm and 15.39±0.78 mm, respectively. The average weight of the liver was 3.92±0.24 gm at 1 week and 39.65±0.99 gm at 24 weeks of Pati duck of Assam as reported by Kadhim *et al. (*2019) in Homing pigeon. Aaraji (2015) reported that the weight of the liver of male turkey was 1.89±0.112% in relation with the body weight. The mean value of length, breadth, thickness and weight of liver of Pati ducks was significantly higher with the advancement of age (Table 1). The size and weight of the liver might be dependent on the breed, age and nutritional status of the individual bird.

Histology

The liver of *Pati* duck covered by a thin capsule of connective tissue (Fig 3). The connective tissue of the capsule contained collagen, reticular and elastic fibers as reported by Abdulla

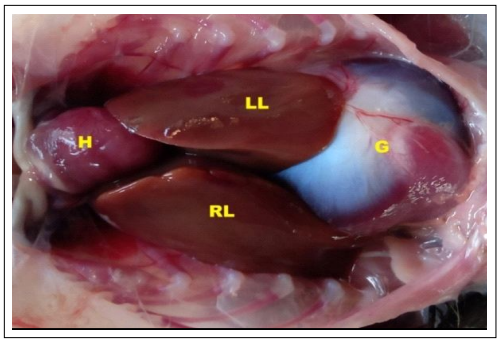

Fig 1: Photograph of Pati duck showing the in-situ position of liver consisting of left lobe (LL) and right lobe (RL), heart (H) and gizzard (G).

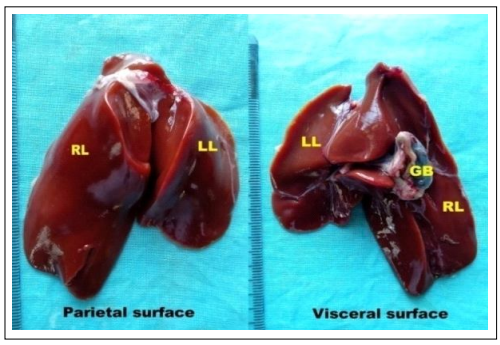

Fig 2: Photograph showing surfaces of the liver of Pati duck, gall bladder (GB), right lobe (RL) and left lobe (LL) of the liver.

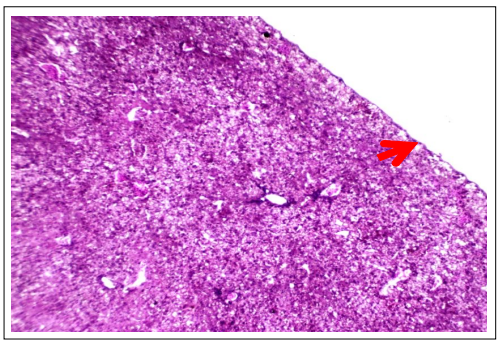

Fig 3: Photomicrograph of liver showing well differentiated capsule (arrow) in pati duck. H and E. ×10.

(2015) in ducks and Shehan *et al.* (2018) in flacon (Fig 6 and 7). Collagen and reticular fibers showed an increasing trend with the age (Fig 7 and 8). The thickness of the capsule of the liver increased along with the advancement of the age which was 0.71±0.03 µm in 1 week and 1.17±0.05 µm in 24 weeks (Table 2). Similar findings were observed by Kadhim *et al.* (2019) in pigeon. The capsular connective tissue penetrated into the parenchyma of the liver in the form of thin septa or trabeculae (Fig 7, 8 and 9). These septa or trabeculae entered the parenchyma dividing that organ into a number of indistinct lobules (Fig 4). These findings were similar with the observations of Sivagnanam *et al.* (2004) in Guinea fowl, Rajendranath *et al.* (2009) in emu, Moslem (2015) in ostrich and Khazraji (2017) in gull. Reticular fibres were also present in the septa or trabeculae. Presence of fine nerve fiber was observed in the capsule penetrating into the parenchyma through trabeculae (Fig 10). The lobules of Pati ducks were not distinct because of very thin interlobular connective tissue septa except the portal triads. So, the liver lobules were not separated from each other distinctly as reported by Faraj (2018) in marsh harrier. The average diameter of the hepatic lobule and distance between two central veins showed increasing trend with advancement of age (Table 2). However, at 24 weeks both measurements were decreased (Table 2). It might be due to formation of new hepatic lobule as reported by Johnson (2005) in pig. The hepatic lobule was centered on the central vein and the hepatocytes in cords were radiating from the central vein towards the periphery. The portal triad consisted of the portal vein, hepatic artery, bile duct and lymphatics in the periphery (Fig 5). Similar observations were made by Sivagnanam *et al.* (2004) in Guinea fowl and Kadhim *et al.* (2019) in pigeon. The central vein was lined by endothelium and its wall was supported by collagen and reticular fibers. Nerves fibres were observed along the course of blood vessels. Hepatocytes were arranged as branching and anastomosing cords and each hepatic cord consisted of double layers of cells. The spherical to ovoid vesicular nucleus of hepatocytes was observed in the basal part of the cells. The cytoplasm of hepatocytes was granular and eosinophilic. The hepatocytes were surrounded by a network of sinusoids. The kupffer cells were observed along with the sinusoidal endothelial cells (Fig 11). Similar finding were reported by Sivagnanam *et al.* (2004) in Guinea fowl, Rajendranath *et al.* (2009) in emu, Selman (2013) in coot bird, Abdulla (2015) ducks and Kadhim *et al.* (2019) in pigeon.

Histochemistry

The Alkaline phosphatase activity was strong in 1 week to 8 weeks, moderate in 16 weeks age and weak in 24 weeks age group (Table 3). The strong activity observed in the capsular region of the liver as presence of connective tissue fiber (collagen) and mucopolysaccharides (Fig 12). The alkaline phosphatase activity was located in the hepatic cells in the regions of the terminal hepatic venules. Ratzlafe and Tyler (1973) in avian liver reported the same. The activity of the acid phosphatase was weak in all age groups of pati

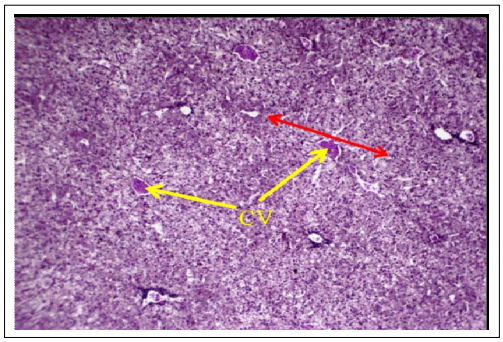

Fig 4: Photomicrograph of liver showing central vein (CV) and hepatic lobule (red arrow) in 16 weeks old duck, H and E, ×10.

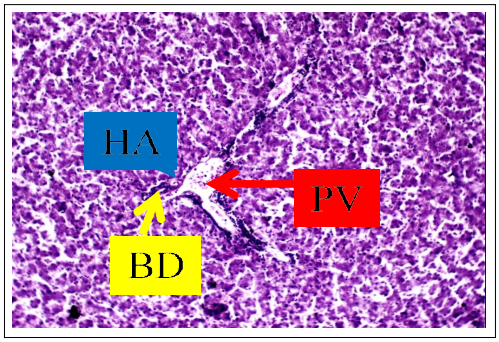

Fig 5: Photomicrograph of portal triad of liver showing portal vein (PV), hepatic artery (HA) and bile duct (BD) in pati duck, H and E, ×40.

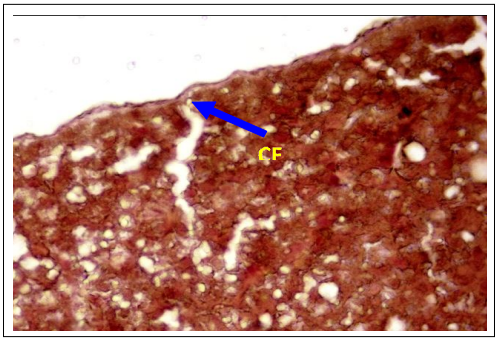

Fig 6: Photomicrograph of liver showing collagen fibers (CF) in the capsule of pati duck, Van Geison's, ×40.

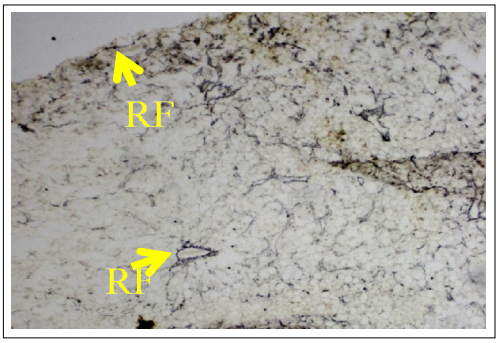

Fig 7: Photomicrograph of liver showing reticular fibers (RF) in 1 week old pati duckling, Gomori's, ×10.

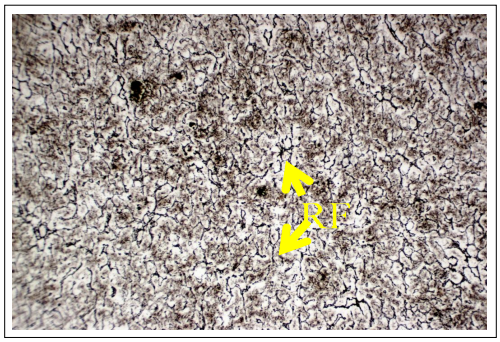

Fig 8: Photomicrograph of liver showing reticular fibers (RF) in 24 weeks old pati duck, Gomori's, ×10.

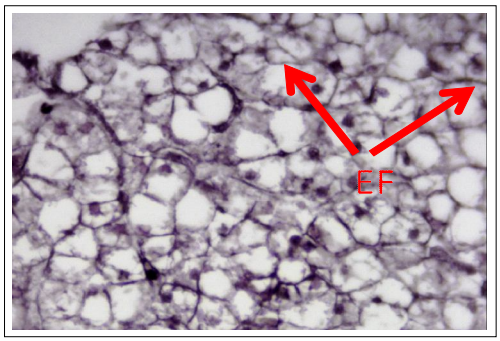

Fig 9: Photomicrograph of liver showing the elastic fibers (EF) in pati duck, Hart's, ×40.

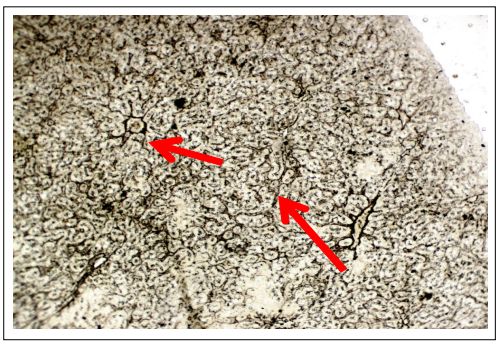

Fig 10: Photomicrograph of the liver showing nerve fibers (arrows), Bielschowsky's, ×10.

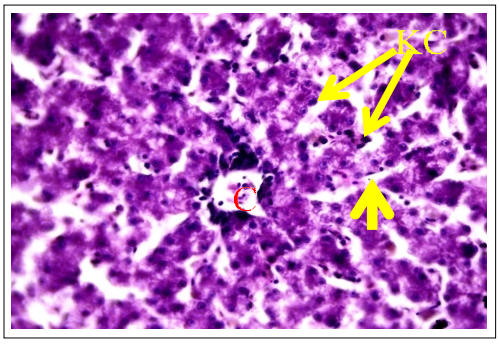

Fig 11: Photomicrograph of liver showing central vein (C), endothelial cell (arrow) and Kupffer cell (KC). H and E. ×40.

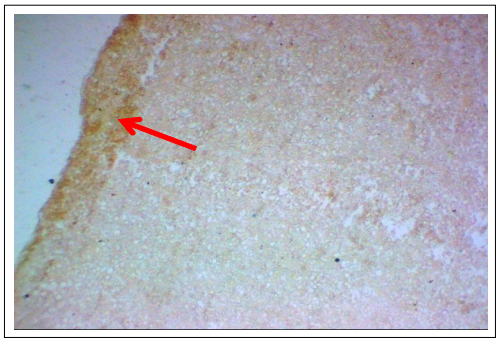

Fig 12: Photomicrograph of liver showing alkaline phosphatase activity (arrow) in Pati duck, ×10.

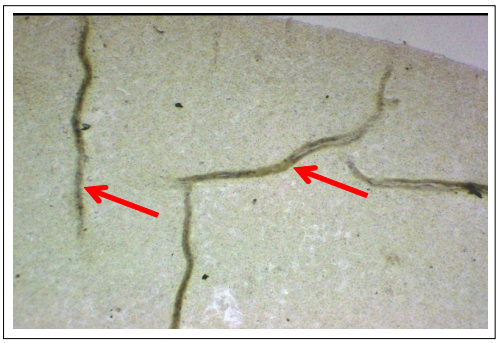

Fig 13: Photomicrograph of liver showing acid phosphatase activity (arrows) in Pati duck, ×10.

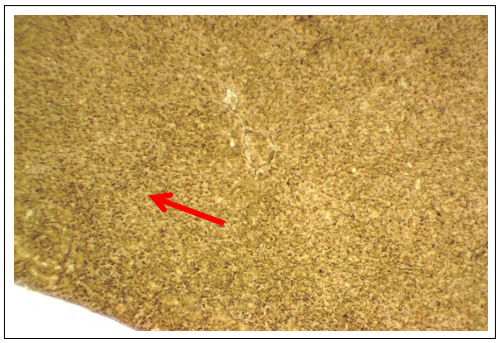

Fig 14: Photomicrograph of liver showing ATPase activity (arrow) in Pati duck, ×10.

Fig 15: Photomicrograph of liver showing non-specific esterase activity (arrow) in pati duck, ×10.

Table 1: Average (Mean±SE) length, breadth, thickness and weight of liver of pati duck during postnatal development.

Age group	Length (mm)		Breadth (mm)		Thickness (mm)		Weight (gm)
	Right lobe	Left lobe	Right lobe	Left lobe	Right lobe	Left lobe	
1 week	34.52±0.98^{d}	26.51±0.52^{d}	18.26±0.99^{c}	16.40±0.98^{c}	10.41±0.31^{c}	10.94±0.32^{b}	3.92±0.24^{d}
8 weeks	47.19±0.97^{c}	35.51±0.61^{c}	23.37±0.89^{b}	22.27±0.95^{b}	14.35±0.59^{b}	13.34±0.69^{ab}	13.86±0.90^{c}
16 weeks	76.87±0.77^{b}	54.25± 0.80^{b}	32.31±0.76^{a}	25.22±0.47^{ab}	17.88±0.55^{a}	13.99±1.30^{ab}	34.37±1.24^{b}
24 weeks	85.59±0.83^{a}	57.18±0.89^{a}	35.69±0.95^{a}	27.23±0.99^{a}	19.10±0.83^{a}	15.39±0.78^{a}	39.65±0.99^{a}

Sample size=06.

*Means with different superscript in column were significantly different.

Table 2: Average (Mean±SE) capsule thickness, diameter of the lobule and distance between two central veins of the liver of pati duck during postnatal development.

Age group	Capsule thickness (µm)	Diameter of the lobule (µm)	Distance between two central veins (µm)
1 week	0.71±0.03^{b}	18.18±0.42^{c}	20.53±0.87^{b}
8 weeks	1.05±0.02^{a}	24.55±0.52^{b}	20.91±0.78^{b}
16 weeks	1.12±0.04^{a}	27.39±0.83^{a}	26.24±1.76^{a}
24 weeks	1.17±0.05^{a}	13.35±0.23^{d}	15.57±1.05^{c}

Sample size=06.

*Means with different superscript in column were significantly different.

Table 3: Showing the activity of histoenzymic reaction in the liver of pati duck in different age groups.

HistoenzymicReaction	1 week	8 weeks	16 weeks	24 weeks
Alkaline phosphatase	+++	+++	++	+
Acid phosphatase	+	+	+	+
ATPase	+++	+++	+++	+++
Non specific esterase	++	++	++	++

Gradation for intensity.

Weak: +

Moderate: ++

Strong: +++

Intense: ++++

ducks. However, strong acid phosphatase activity was observed in the bile canaliculi as the cuboidal epithelium of bile canaliculi contains well developed nuclei (Fig 13). This was in accordance to Lakshmi (1998) in duck. The adenosine triphosphatase (ATPase) activity was strong in all the age groups of birds (Fig 14). Adenosine triphosphatase activity was located on the canalicular boundaries of the hepatic cells as recorded by Ratzlafe and Tyler (1973) in avian liver. The activity of non specific esterase was moderate in the liver of Pati duck (Fig 15). Similar observations were reported by Ratzlafe and Tyler (1973) in avian liver.

CONCLUSION

In the current investigation, most of the parameters which were recorded shown an increasing trend along with the advancement of age of the *Pati* ducks. This might be the result of the various anatomical, biochemical and physiological changes that occur in the body as they grow older. The present study also try to establish the different anatomical, histological and histochemical changes along with the advancement of age of the *Pati* ducks. The investigations revealed that the size, shape and conformation of the Liver and also changes of cellular activity along with age.

Conflict of interest: None.

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