Indian Journal of Veterinary Anatomy 38(01): 70-74, June 2026

Histochemical Studies on the Adrenal gland of Large White Yorkshire Crossbred Adult Pigs

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Received: 21.05.2026; Accepted: 29.06.2026

ABSTRACT

Histochemical study on the adrenal gland was conducted on twelve Large White Yorkshire pigs irrespective of their sex. The activity for neutral mucopolysaccharides was strong in capsule, zona glomerulosa, zona fasciculata and epinephrine zone, whereas, it was moderate in zona reticularis and weak in nor epinephrine zone. The activity for alkaline phosphatase was strong in capsule, zona fasciculata and nor epinephrine zone and weak in the epinephrine zone. The acid phosphatase activity was strong in the outer layer of capsule, zona reticularis and in the epinephrine zone. Strong deposits of lipids were found in epinephrine zone. Chromogranin 'A' immune positive reaction was strong in the epinephrine zone and weak in the nor epinephrine zone.

Key words: Adrenal gland, histochemistry, pig.

INTRODUCTION

The adrenal gland plays an important role in maintenance of electrolyte concentration in extracellular fluid, regulate carbohydrate metabolism and has a masculinizing effect as testosterone (Aughey and Frye, 2001). The adrenal glands are located adjacent to the cranial medial border of the kidneys and they represent two distinct endocrine tissues. The outer cortex secretes steroid hormones while the medulla produces catecholamines. Hormone production in each of these two regions of the gland is regulated independently (Eurell and Frappier, 2006). The present study was conducted as the literature pertaining to histochemical study on the adrenal gland was very scanty in pig.

MATERIALS AND METHODS

The present study was conducted on 12 Large White Yorkshire cross bred adult pigs (Above 1year age) irrespective of their sex. Fresh adrenal glands were collected from pigs immediately after slaughter from AICRP on pigs, College of Veterinary Science, Tirupati. After collection the specimens were washed thoroughly and they were subjected for histochemical studies, fresh tissue pieces were collected from the adrenal glands of pig and they were fixed at 4^0C in chilled 10% Neutral Buffered Formalin. Frozen sections of 15-20µm thickness were obtained by cryostat and they were subjected to following staining methods. Sudan Black B method for lipids, Gomori's method for Acid phosphatase activity and Gomori's method for Alkaline phosphatase activity (Singh and Sulochana, 1997). Some paraffin sections of 5-6µm thick were also used for histochemical study i.e. for neutral mucopolysaccharides by Periodic Acid Schiff (PAS) method (Singh and Sulochana, 1997).

For immunohistochemical study, paraffin sections of adrenal gland tissue samples were cut at 5µm thickness, mounted on APES (Amino Propyl Ethoxy Sialine) coated slides and incubated overnight at 37^0C (Lillie, 1999; Luna,1968; Lynch *et al*., 1969).

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RESULTS AND DISCUSSION

Capsule

In the present study, the capsule showed strong positive reaction for neutral mucopoly saccharides in outer fibrous and inner cellular layers and also around the blood vessels (Fig.1). In contrast to this, Hakeem (1990) stated that only the fibrous content of the capsule was intensely positive for neutral mucopolysaccharides in goat, which is in partial agreement with the present findings.

The capsule showed strong positive activity for alkaline phosphatase. The activity was moderately positive in the outer fibrous layer and strongly positive in the cellular layer (Fig.2). However, Pathak *et al.* (2015) stated that the capsule showed moderate activity of alkaline phosphatase in goat.

In the present study, the acid phosphatase activity was intensely positive in the outer fibrous layer and weak in the inner cellular layer of the capsule (Fig.3). However, moderate acid phosphatase activity was observed in capsule by Pathak *et al.* (2015) in goat. In the present study, the capsule showed mild deposition of lipids (Fig.4). In concurrence with this, Kumar *et al*. (2011) reported a negative to mild reaction of fine lipid droplets in the capsule of adrenal gland in buffalo.

Cortex

In the present study, the zona glomerulosa was intensely positive for neutral muco polysaccharides in the cells forming the cell cords and clusters and in the surrounding connective tissue (Fig.1). Moderate activity for alkaline phosphatase was observed in the cell cords of zona glomerulosa and weak activity in the connective tissue surrounding the cell cords (Fig.2). Less intense activity in cell cords and clusters and weak activity in sinusoids for acid phosphatase were noticed (Fig.3). In the present study, mild quantities of lipids were observed in cortex (Fig.4). Similar observations were observed in the adrenal cortex of heifer **(**Weber *et al.*, 1950), cow (Yamauchi, 1965), goat (Hakeem, 1990) and buffalo (Kumar *et al*., 2011). In the present study, the cell cords of zona fasciculata were strongly positive for neutral mucopolysaccharides (Fig.5). Similarly, Hakeem (1990) also noticed neutral mucopolysaccharides positive activity in the cortical cells in goat. In the present study, the zona fasciculata was strongly positive for alkaline phosphatase (Fig.6). The activity of acid phosphatase was moderate in cell cords and weak in the sinusoids of zona fasciculata and moderate quantity of lipids was present. Similar findings were also observed by Yamauchi (1965) in cow. However, Robinson *et al*. (1977) reported that the zona fasciculata cells did not store any lipids in sheep.

The cell cords of zona reticularis was moderately positive for neutral muco polysaccharides. Similar findings were reported in goat by Hakeem (1990). The moderate activity of alkaline phosphatase was noticed in the cell cords of zona reticularis at cortico-medullary junction. The cell cords were strongly positive for acid phosphatase, while weak activity was seen in cell cords and sinusoids (Fig.7). In the present study, only few lipids were noticed in this zone. In contrast to this, lipids were noticed intercellularly or intracellularly and were more abundant in the reticularis by Meyers and Charipper (1956) in golden hamster.

Medulla

The cell cords, connective tissue and the blood vessels of epinephrine zone showed strong activity for neutral mucopolysaccharides (Fig.8).

However, Hakeem (1990) observed neutral mucopolysaccharides only in the fibers of adrenal medulla in goat. In the present study, strong positive activity for alkaline phosphatase was observed in the sinusoids and the basement membrane of cell cords. However, the cells of this zone showed weak activity of alkaline phosphatase (Fig.9). The connective tissue around the cell cords showed strong positive reaction for acid phosphatase (Fig.7). In the present study, the cells of this zone were showed abundant deposition of lipids (Fig.10). It is in accordance with the findings of Raju and Rao (1982) in mongoose. However, Kour *et al*. (2017) observed that outer medullary zone of adrenal medulla in goat showed mild deposition of lipids.

In the present study, neutral muco polysaccharide activity was moderate in the connective tissue between cell cords and acini and weak in the cells. The wall of the central vein also showed moderate activity for neutral mucopolysaccharides (Fig.8). However, Hakeem (1990) observed neutral mucopolysaccharide activity only in the connective tissue fibers of medulla in goat. Alkaline phosphatase activity was strongly positive in the cell cords of nor epinephrine zone of adrenal medulla of pig. Moderate activity in the cell cords and mild activity for acid phosphatase in the connective tissue around the cell cords was observed in this zone. In the present study, this zone showed few lipids (Fig.10). In contrast to this, absence of lipids was noticed in the medulla of golden hamster (Meyers and Charipper, 1956), goat (Hakeem, 1990) and buffalo (Kumar *et al.*, 2011).

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In the present study, Chromogranin A reaction was not seen in the capsule, zona glomerulosa and zona fasciculata. But strong to moderate reaction of Chromogranin A was noticed in the cells of zona reticularis of adrenal cortex, while negative activity was seen in sinusoids. Lassmann *et al.* (1986) stated that the entire adrenal cortex was negative for chromogranin A in buffalo, whereas, Jelinek and konecny (2010) stated that in bovines, the reaction for chromogranin A was weak in majority of the cortical cells.

In the present study, strong immune positive reaction was observed for chromogranin A in epinephrine cells cords,while negative activity was seen in the connective tissue around the cell cords, blood vessels, nerve plexus, cortical cells and wall of the central vein of medulla. However, nor epinephrine cells showed moderate chromogranin 'A' reaction (Fig.11). Similarly, epinephrine cells of medulla in bovines showed strong positive reaction for chromogranin 'A' than norepinephrine cells as noted in the present study by Jelinek and konecny (2010).

CONCLUSION

The adrenal gland of Large White Yorkshire crossbred adult pigs exhibited distinct histo chemical and immunohistochemical characteristics reflecting its functional specialization. Strong positivity for neutral muco polysaccharides in the capsule, cortex, and medulla suggests their role in structural support and cellular organization. Alkaline phosphatase activity indicated active metabolic and secretory functions, while acid phosphatase activity reflected lysosomal and cellular turnover processes. Lipid deposition was prominent in the zona fasciculata and epine phrine zone, supporting their involvement in steroido genesis and endocrine activity. Chromogranin A immunoreactivity was strongest in epinephrine cells and moderate in norepinephrine cells, confirming their neuroendocrine secretory nature.

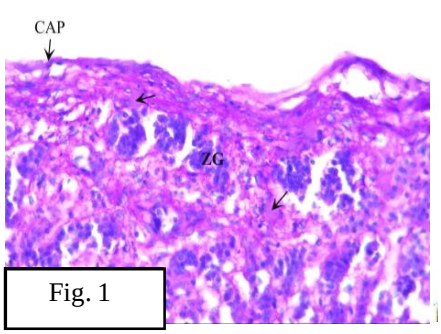

Fig. 1

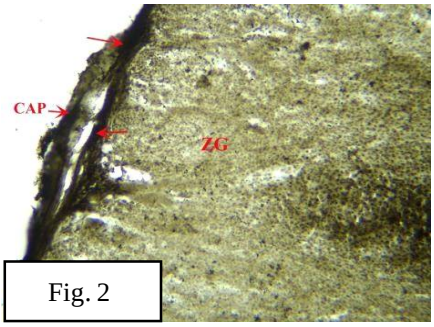

Fig. 2

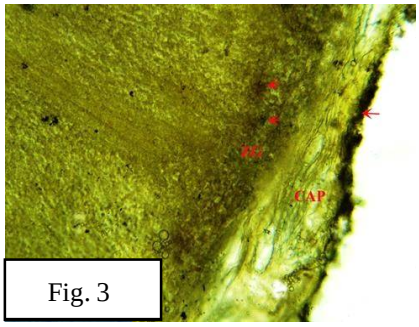

Fig. 3

Fig.1 Photomicrograph showing intense activity (arrows) for neutral mucopolysaccharides in the capsule (CP) and zona glomerulosa (ZG) of adrenal gland of pig. PAS×100.**Fig.2** Photomicrograph showing strong activity (arrow) for alkaline phosphatase in the capsule (CAP) of adrenal gland of pig. ZG-Zona glomerulosa. Gomori's×100.**Fig.3** Photomicrograph showing strong acid phosphatase activity (arrow) in capsule (CAP), zona glomerulosa (ZG) of adrenal gland of pig. Gomori's×100.

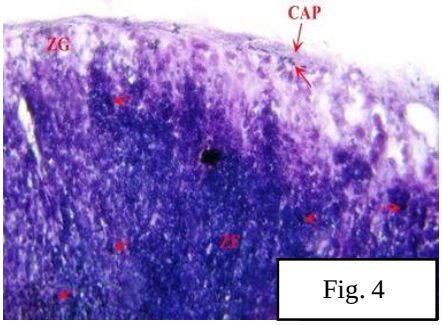

Fig. 4

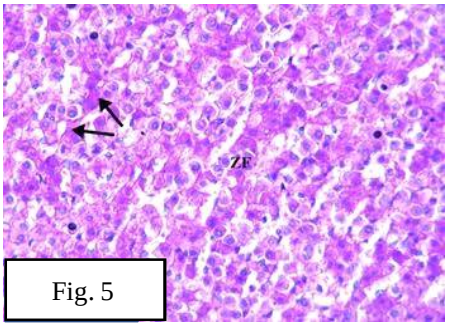

Fig. 5

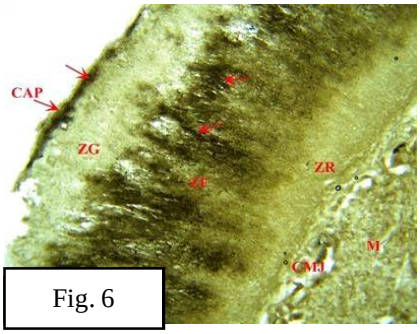

Fig. 6

Fig.4 Photomicrograph showing lipids (arrow) in zona fasciculata (ZF) of adrenal gland of pig. CAP-Capsule, ZG-Zona glomerulosa. Sudan black B × 100.**Fig.5** Photomicrograph showing intense neutral mucopolysaccharide activity (arrows) in zona fasciculata (ZF) of adrenal gland of pig. PAS × 400.**Fig.6** Photomicrograph showing strong alkaline phosphatase activity (arrow) in capsule (CAP) and zona fasciculata (ZF) adrenal gland of pig. ZG-Zona glomerulosa, ZR-Zona reticularis, M-Medulla, CMJ-Cortico-medullary junction. Gomori's×100.

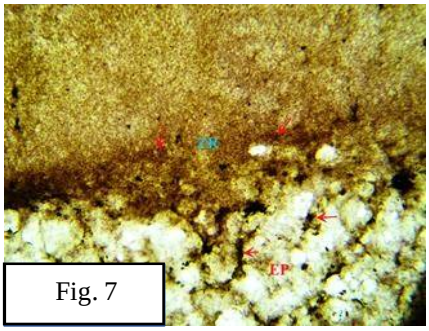

Fig. 7

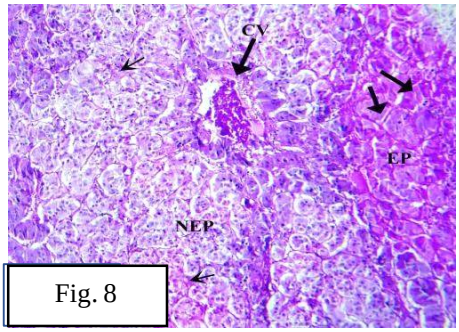

Fig. 8

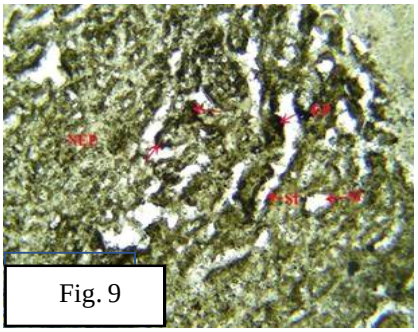

Fig. 9

Fig.7 Photomicrograph showing acid phosphatase activity (arrows) in zona reticularis (ZR) and epinephrine zone (EP) of adrenal gland of pig. Gomori's×400**. Fig.8** Photomicrograph showing neutral mucopolysaccharides (arrow) in epinephrine (EP) and nor epinephrine zones (NEP) of pig. CV-Central vein. PAS × 400. **Fig.9** Photomicrograph showing strong alkaline phosphatase activity (arrow) in epinephrine zone (EP) of adrenal gland of pig. EP-Epinephrine zone, SI-Sinusoids. Gomori's × 100.

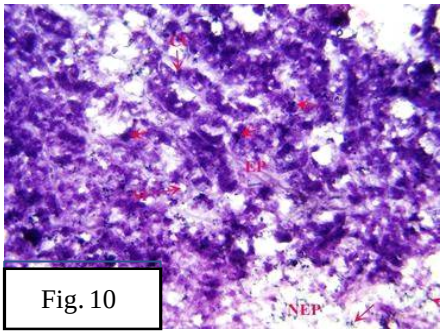

Fig. 10

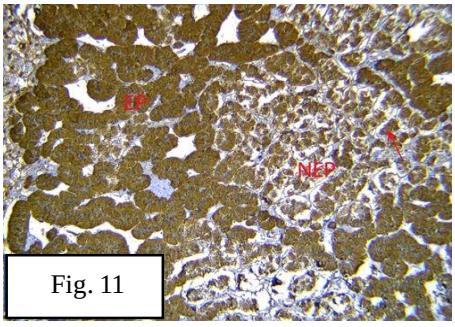

Fig. 11

Fig.10 Photomicrograph showing strong activity (arrows) for lipids in epinephrine zone (EP) of adrenal gland of pig. CC-Cell cords, NEP-Nor epinephrine zone, SI-Sinusoid. Sudan black B × 100. **Fig.11** Photomicrograph showing moderate activity (arrow) of chromogranin 'A' in nor epinephrine zone (NEP) of adrenal medulla of pig. EP-Epinephrine zone. Chromogranin A×400.

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