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Histochemical studies on cerebral and cerebellar cortices of pig (*Sus scrofa*)

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Abstract
The histochemical aspects of cerebral and cerebellar cortices were studied in Large White Yorkshire cross-bred pigs at different age groups. The neuronal cells and neuropil of cerebral neocortex, the neuropil and Purkinje cell layer of cerebellar cortex showed positive for PAS, Alkaline phosphatase and Acid phosphatase activities in all age groups of pigs. However the PAS, Alkaline phosphatase and Acid phosphatase activity was stronger in finisher age group. Negative Alkaline phosphatase activity was observed in the cerebral and cerebellar cortices of preweaner age group. The amount of fat deposition in cerebral and cerebellar cortices was more in the finisher age group when compared to preweaner pigs.

Keywords: Histochemistry, cerebral neocortex, cerebellar cortex, pig

Introduction
Pig husbandry is one of the best and profitable enterprises for small and marginal farmers as they possess most important economic traits such as high prolificacy, faster growth rate etc. Cerebral cortex is the site at which voluntary movements are initiated, sensations were brought to consciousness and higher functions such as reasoning and planning takes place (Frandson *et al*., 2009) [1]. The cerebellar cortex has become the focus of intense research because it is presumed to be responsible for planning movement and adapting to special conditions and involved in storing memories over various time-periods (Attwell *et al*., 2002) [2]. The literature available on histochemical aspects of cerebral neocortex and cerebellar cortex was very little in pig. Hence the present study was undertaken to provide the information pertaining to histochemical changes in cerebral and cerebellar cortices at different age groups of apparently normal pigs.

Materials and Methods
Total 18 pigs, of apparently normal health were obtained from the slaughter house of AICRP on pigs, College of Veterinary Science, Tirupati and were categorized into three groups based on their age, i.e., Preweaners (1 to 42 days), Growers (43 days to 8 months) and Finishers (9 months and above) with 6 animals in each group. Fresh tissue pieces were collected from cerebral neocortex and cerebellar cortex and they were fixed at 4 ^{0}C in chilled 10% Neutral Buffered Formalin and frozen sections of 10 - 15µm thickness were obtained from cryostat and they were subjected to various histochemical staining methods *viz*., Gomori's method for Acid phosphatase activity, Gomori's method for Alkaline phosphatase activity, Sudan Black B method for fats (Singh and Sulochana, 1997) [3]. Further, fresh tissue samples were processed for paraffin sections and 5-6 µm thickness sections were cut with the help of microtome (Singh and Sulochana, 1997) [3] and these sections were subjected to Periodic Acid Schiff (PAS) method for neutral mucopolysaccharides (Singh and Sulochana, 1997) [3].

Results and Discussion
In cerebral neocortex, PAS positive activity was observed in the cytoplasm of neuronal cells, neuropil and blood vessels. The PAS positive activity was mild in preweaner age group and moderate to high in grower age group (Fig.1). Whereas in finisher age group, intense PAS

positive activity was observed in the neuropil and moderate activity was seen in the blood vessels and few neuronal cells of the cerebral neocortex.

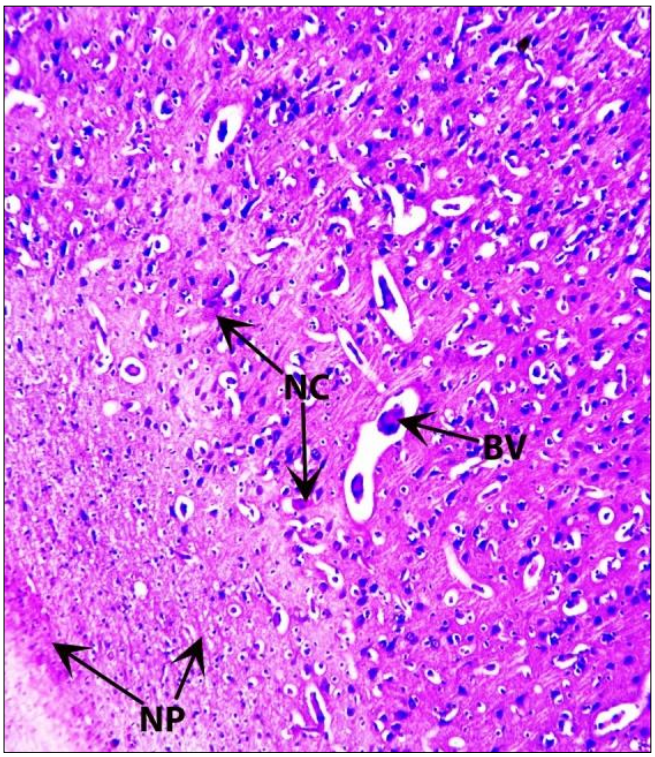

Fig 1: Photomicrograph of the cerebral neocortex of grower pig showing moderate PAS positive activity in cytoplasm of some neuronal cells (NC), neuropil (NP) and blood vessel (BV). (PAS X 100)

The cerebellar cortex of the preweaner and grower age groups showed mild PAS positive activity in the molecular layer and neuropil of the granular layer. However, the Purkinje cell layer showed moderate PAS positive activity in the preweaner age group and moderate to high PAS positive activity in the grower age group. In finisher age group, moderate to high PAS positive activity was noticed in the molecular layer and neuropil of the granular layer. However, PAS positive activity was intense in the Purkinje cell layer (Fig.2). Negative PAS activity was observed in the granule cells of granular layer of all age groups.

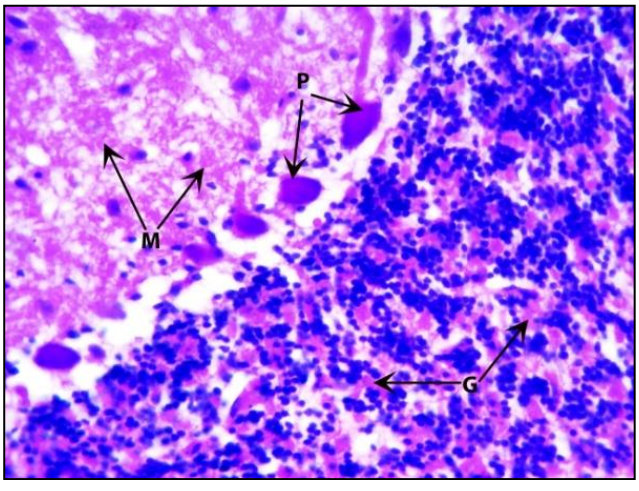

Fig 2: Photomicrograph of the cerebellar cortex showing high PAS positive activity in molecular layer (M), Purkinje cells (P) and neuropil of granular layer (G) in finisher pig. (PAS X 400)

The PAS positive activity indicates the presence of neutral mucopolysachaarides in cerebral and cerebellar cortices. There was a gradual increase in the PAS positive activity in cerebral neocortex and cerebellar cortex from preweaner to finisher age group as stated by Salankar (2017) [4] in goat. In contrary to the above findings, Sharma and Singh (1994) [5] reported decreased activity of PAS positive neurons with age in the parietal cortex of rat.

Negative Alkaline phosphatase activity was observed in the cerebral neocortex and cerebellar cortex of preweaner age group. The activity was moderate to high in the neuropil and mild in neuronal cells of cerebral neocortex of grower age group, while in finisher age group, the activity was strong in the neuropil and moderate to high in neuronal cells (Fig.3). In contrary to the above findings, Manocha (1970) [6] reported that the Alkaline phosphatase activity was absent in most of the neurons and neuropil of squirrel monkey. In the cerebellar cortex, the Alkaline phosphatase activity was mild in the molecular layer and moderate to high in the Purkinje cell and granular layers of grower and finisher age groups (Fig.4). Increase in alkaline phosphatase activity was observed with advancement of age in cerebral neocortex and cerebellar cortex, which agreed with the findings of Salankar (2017) [4] in goat.

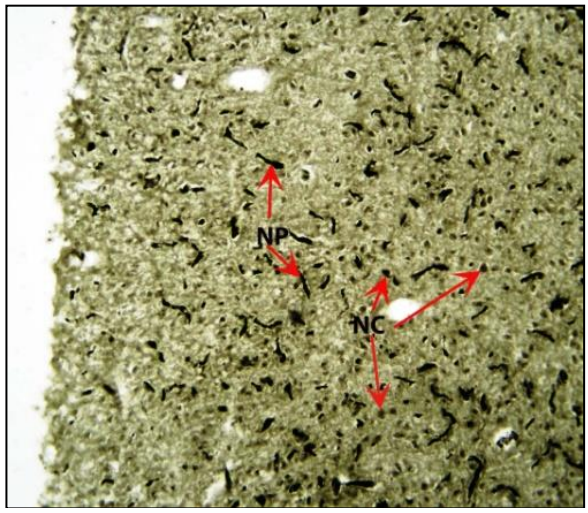

Fig 3: Photomicrograph of the cerebral neocortex showing strong alkaline phosphatase activity in neuropil (NP) and moderate activity in neuronal cells (NC) of finisher pig. (Gomori's method X 100)

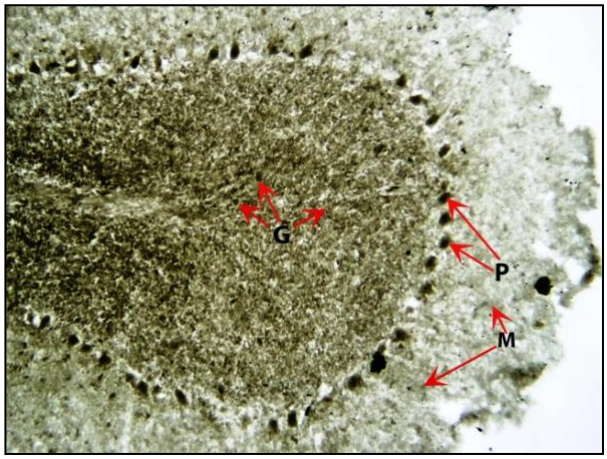

Fig 4: Photomicrograph of the cerebellar cortex of finisher pig showing mild alkaline phosphatase activity in molecular layer (M), high activity in the Purkinje cells (P) and granular layer (G). (Gomori's method X 100)

The Acid phosphatase activity was noticed in neuronal cells and neuropil of cerebral neocortex. It was found mild in preweaner and grower age groups and strong in finisher age group (Fig.5). An increase in acid phosphatase activity was observed from preweaner to finisher age groups, which concurs with the statement of Salankar (2017) [4] in goat and Ng and Tam (1986) [7] in mouse. In contrary to this, no significant change in acid phosphatase activity was reported with aging in the cerebrum of rat by Nakamura *et al.* (1989) [8].

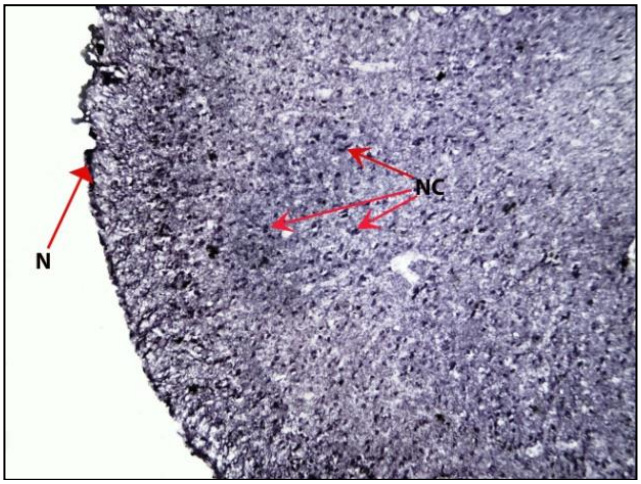

Fig 5: Photomicrograph of the cerebral neocortex of finisher pig showing strong acid phosphatase activity in neuropil (N) and neuronal cells (NC). (Gomori's method X 100)

The Acid phosphatase activity in the cerebellar cortex was mild in the neuropil and Purkinje cell layer of preweaner and grower age groups. In finisher age group, moderate to high activity was observed in the Purkinje cell layer and neuropil of granular layer and mild activity was noted in molecular layer (Fig.6**).** These findings concurs partially with the findings of Manocha (1970) [6] in squirrel monkey, Becker *et al*. (1960) [9] and Tewari and Bourne (1963) [10] in rat. In the present study, an increase in acid phosphatase activity was observed with the advancement of age from preweaner to finisher stage. This increased activity assumed to be an indicative of release of lysosomal enzymes associated with degeneration of neurons occurring during ageing**.**

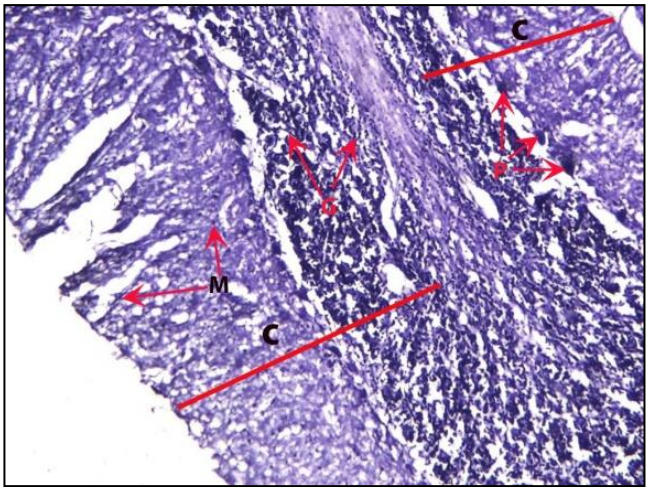

Fig 6: Photomicrograph of the cerebellar cortex(C) of finisher pig showing mild acid phosphatase activity in molecular layer (M), high activity in the Purkinje cells (P) and neuropil of granular layer (G). (Gomori's method X 100)

The deposition of fat was not observed in the cerebral and cerebellar cortices of preweaner age group. Whereas in grower (Fig.7) and finisher age groups, mild to moderate quantities of fat deposition was seen in cerebral neocortex. In the cerebellar cortex, mild deposition of fat was observed in granular layer of grower age group, whereas in finisher age group, mild to moderate deposition of fat was seen in the molecular layer, Purkinje cells and granular layer.

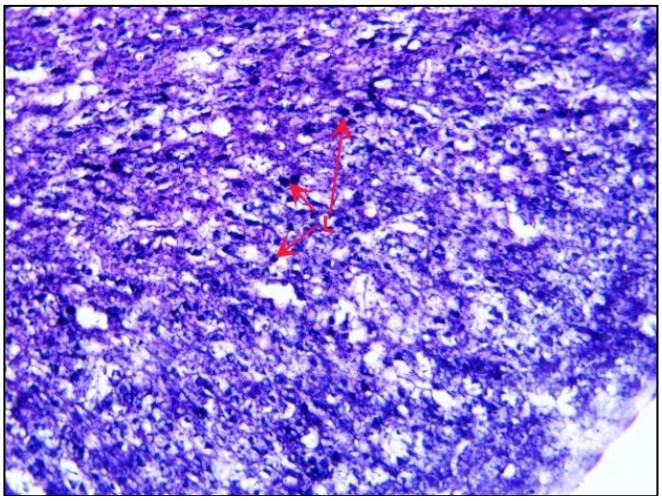

Fig 7: Photomicrograph of the cerebral neocortex showing moderate *Lipid deposition* (L) in grower pig. (Sudan black B X 100)

Conclusion

The PAS, Alkaline and Acid phosphatase activities were stronger in finisher age group when compared to preweaner and grower age groups in cerebral and cerebellar cortices. Mild to moderate deposition of fat was observed in finisher age group in cerebral and cerebellar cortices. Increase in PAS positive activity in finisher age group may indicate more energy requirement for carrying out the functions of the brain during advanced age.

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