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Histology

Postnatal Development of the Rabbit Cerebellum

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ABSTRACT

The purpose of the current study was to provide an overview of the rabbit cerebellum's postnatal developmental stages. Forty rabbit cerebella of postnatal ages (PN1, PN 5, PN8, PN15, PN22, 1M, 2M, 3M, 4M, 5M and 6M) were obtained from the Balady rabbits. In, PN1 the cerebellar cortex consisted of four layers: an external granular layer (EGL), a molecular layer, a Purkinje cell layer, and an internal granular layer. The thickness of the EGL reached its maximum, consisted of five to six layers of oval or spherical cells. The Purkinje cell layer retained immature. Purkinje cells had vesicular nuclei, prominent nucleoli, and an amphiphilic cytoplasm were arranged in two or three different layers. At PN 5, EGL decreased in thickness while Purkinje neurons were arranged in a well-developed single row. Myelination of the white matter appeared more evident. At later postnatal ages, the EGL was decreased in thickness until disappeared, while internal granular layer and molecular layer increased in thickness until the anatomical characteristics of the mature cerebellum were reached from two months onward. Cerebellum was important for motor coordination; muscle tones and maintaining the equilibrium of the body. An extended process that takes place during postnatal life gives the mature cerebellum its ultimate structure.

Key wards: Histology, Cerebellum, Postnatal Development, Rabbits, Histogenesis.

INTRODUCTION

The female rabbits are regarded as stimulated to ovulate because after mating the ovulation occurred (Spies et al, 1997 and Harkness et al, 2010) and rabbit have no regular cycle like other domestic species like cattle (Harkness et al, 2010). The female rabbits are regarded as stimulated to ovulate because after mating the ovulation occurred (Spies et al, 1997 and Harkness et al, 2010) and rabbit have no regular cycle like other domestic species like cattle (Harkness et al, 2010). The female rabbits are regarded as stimulated to ovulate because after mating the ovulation occurred (Spies et al, 1997 and Harkness et al, 2010) and rabbit have no regular cycle like other domestic species like cattle (Harkness et al, 2010).

Rabbit is considered as a small animal and suitable experimental animal model for studying a variety of morphological abnormalities and diseases in both human and animal toxicology, pharmacology antibody production and development of new surgical techniques (Abidu-Figueiredo et al., 2009). The female rabbits are considered as stimulated to ovulate due to ovulation has occurred after mating (Spies et al., 1997; Harkness et al., 2010; Tortereau et al., 2013; Gad,

2016) and rabbit does not have regular cycle like other domestic species like cattle (Harkness et al., 2010). According to the FAO (FAO, 2001), rabbit keeping provides additional income and supplies more protein for poor urban households. Rabbits are utilized for producing meat and fur, possess a little body size, fast growth rate, short intergenerational period and great capacity for reproduction that make rabbit suitable for raising tiny animals in underdeveloped countries (Cheeke, 1986; Hristov et al., 2006; Calasans-Maia et al., 2009; Amorim et al., 2011; Alves et al., 2011).

The cerebellum is important organ responsible for timing, coordination of movements and balance (Rivers, 2000; Sultan and Glickstein, 2007; Susanet al., 2008; Buckner, 2013; Butts et al., 2014; Sokolov et al., 2017; Mariën and Borgatti, 2018; Schmahmann, 2019; Hatten, 2020). It is recorded hat its measurements and structure are connected to extremity movements and species posture (Mial and Reckess, 2002). Increasing cerebellar foliation improving motor control and increasingly complex behaviors (Iwaniuk et al., 2006; Zachary et al., 2013).

Cerebellar cortex become the subject of extensive investigation. because it is involved in movement planning and in storing memories over various time periods (Attwell *et al*., 2002; Pal et al., 2003; Irimescu et al., 2015; Treuting and Dintzis, 2012; Danmai-goro et al., 2016; Sur et al., 2011; Musa et al., 2016; Beheiry, 2015). The literatures about rabbit's nervous system are still relatively limited. There are little researchs on rabbit's cerebellum development compared to another animals (Beaudoin et al., 2003).

MATERIALS AND METHODS

1 | Examined animals

The present study was carried out on forty females of Balady rabbits. They were collected from different popular markets in Menoufia governorate, Egypt, where all rabbits were kept and managed under the same condition of feeding, water supply and temperature. The ovulation in the house rabbits was induced; it happened only after the mating by ten to twelve hours (Sirotkin et al., 2010). Forty rabbit cerebella of postnatal ages (PN1, PN 5, PN8, PN15, PN22, 1M, 2M, 3M, 4M, 5M and 6M) were obtained from the Balady rabbits. All animal-handling procedures as well as samples' collection and disposal will be according to the regulations of Institutional Animal Care and Use Committee (IACUC) with oversight of the faculty of Veterinary Medicine, University of Sadat City. Ethical approval number: VUSC-041-1-20.

2 | Histological study

The cerebellum of rabbits was obtained and fixed in 10% formalin for at least 48 hours. All specimens were dehydrated using increasing concentrations of ethyl alcohol, then cleared in methyl benzoate and embedded in paraffin wax. Sections of 5-7µm in thickness were obtained using rotary microtome (Luna, 1968) and stained with: Harri′s hematoxylin and eosin (H&E), Crossmon′s trichrome stain (CT), Weigert′s elastic (WE) tissues stain, Gomori′s reticulin (GR) stain, Alcian blue (AB) at pH2.5, Periodic acid Schiff method (PAS), alcian blue at pH2.5 – periodic acid Schiff combination (AB+ PAS) and Phosphotungstic acid Haematoxylin stain.

2.2 | For Transmission Electron Microscope Study: -

Cerebellar samples were preserved in fresh 2.5% buffered

glutaraldehyde in 0.1 M a solution of phosphate buffer pH 7.4 at 4 °C, dehydrated and embedded in Araldite 502 resin. Leica ultra-microtome was used to cut the plastic molds, stained with 1% toluidine blue then examined and photographed. After semi-thin sections examined, sections of ultra-thin were cut and stained with uranyl acetate. Ultra-thin sections were photographed by using JEOL- JEM-100 SX transmission electron microscope, Japan, electron microscope unit, Tanta University.

All the aforementioned stains and techniques were outlined of Bancroft and Gamble (2008). The Olympus CHS microscope (Olympus Optical CO, LTD, Tokyo, Japan; Leica DM 2500, Leica Germany) was used to examine each stained section.

RESULTS

1 | Macroscopic features of the cerebellum

Cerebellum was cylindrical in shape and milky in color. The cerebellum formed of well-developed centrally located vermis and the bilateral cerebellar hemispheres. The cerebellar surface consisted of numerous convolutions (Folia cerebelli) and fissures (Fig. 1).

2 | Microscopic features of the cerebellum

At the age of one day post parturition, the cerebellar cortex formed of four layers: an EGL that located beneath the pia matter, a molecular layer, a Purkinje cell layer and the internal granular layer (Fig. 2 a, b, c). Thickness of the EGL reached its maximum, consisted of five to six layers of spherical or oval shaped cells. Molecular layer appeared as a pale area contained Purkinje cells that had vesicular nuclei with an amphiphilic cytoplasm and prominent nucleoli, were arranged in multiple layers (Fig. 2c). Purkinje cells had apical cone directed towards the surface and retained some characteristics of immaturity (Fig. 2d). Internal granular layer (IGL) consisted of darkly stained cells. The white matter was situated below the IGL and composed of less densely packed oval or fusiform cells. (Fig. 2a). The neutral mucopolysaccharide appeared PAS positive reaction in molecular layer and between granular cells (Fig. 2 e, f, g). The acid mucopolysaccharide showed weak positive reaction to alcian blue stain (Fig. 2h). The collagen fibers had appeared as thin fibers in dura matter and between granular cells (Fig. 2i). The elastic fibers also, had appeared as thin fibers (Fig. 2 j, k). The reticular fibers had appeared in dura matter (Fig. 2l).

At the age of five days post parturition, as the previous age but well-developed single layer of Purkinje cell was arranged. Myelination of the white matter appeared more evident (Fig. 3 a, b, c, d). The neutral mucopolysaccharide appeared PAS positive reaction in Purkinje cell layer and between granular cells (Fig. 3 e, f). The acid mucopolysaccharide showed weak positive reaction to alcian blue stain (Fig. 3g). The collagen fibers had appeared as thin fibers between granular cells (mossy fibers) (Fig. 3h). The elastic fibers also, had appeared as thin fibers (Fig. 3 I, j). The reticular fibers had appeared in dura matter (Fig. 3k).

At the age of eight days post parturition, the cortex was formed of four layers: the internal granular layer, the Purkinje cell layer, the molecular layer and the outer thick external granular layer (EGL) (Fig. 4 a, b, c). EGL layer decreased in thickness and formed of spherical darkly stained cells. Molecular layer was increased in thickness and consisted of few small cells (basket cells and stellate cells), dendritic processes of Purkinje cell

nerve fibers (climbing fiber) (Fig. 4 c, d). Purkinje cell layer was organized in one layer and appeared as pear-shaped cells and less stained nuclei with apparent nucleoli (Fig. 4 c, d). Populated granular cells of the internal granular layer appeared as densely stained small spherical cells (Fig. 4 c, d, e). Myelination of the white matter appeared more evident (Fig. 4e). The neutral mucopolysaccharide appeared PAS positive reaction in Purkinje cell layer, molecular layer and between granular cells (Fig. 4 f, g). The acid mucopolysaccharide showed weak positive reaction to alcian blue stain (Fig. 4h). The collagen fibers had appeared in dura matter and in blood capillary (Fig. 4 i, j). The elastic fibers also, had appeared in climbing fibers in molecular layer and mossy fibers in internal granular layer (Fig. 4 k, L). The reticular fibers had appeared in dura matter (Fig. 4m). The processes of Purkinje cells (axons) extended into the internal granular layer while, dendrites extended into molecular layer. The dendritic processes of Purkinje cells were short, less branched and did not reach the peripheral surface of molecular layer (Fig. 4 n, o, p).

At the age of 15 days post parturition, EGL decreased in thickness, composed of three or four layers of granule cells (Fig. 5a). Molecular layer thickness increased and formed from two cells, basket cells and stellate cells. Upper portion of the molecular layer contained the stellate cells that had spherical or oval cell bodies and darkly stained basophilic nuclei with distinct nucleoli, while the basket cells located in the bottom part of the molecular layer, close to Purkinje cell, had spherical or triangular shaped cells and contained vesicular nuclei with distinct nucleoli. Purkinje cell layer organized in one layer between ML and IGL. IGL was formed from densely packed cells. It consisted of two types of cells: granule cells and Golgi Type II cells. The granule cells were small, numerous and tightly packed. They were spherical with large spherical nuclei and minimal quantity of cytoplasm and gathered to create cluster of cells. Golgi cells were larger than the granule cells had an oval or triangular cell body with vesicular nucleus and more cytoplasm (Fig. 5b). Myelination of the white matter appeared more evident (Fig. 5c). The neutral mucopolysaccharide appeared PAS positive reaction in Purkinje cell layer, molecular layer and between granular cells (Fig. 5 d, e). The acid mucopolysaccharide showed weak positive reaction to alcian blue stain (Fig. 5f). The collagen fibers had appeared in blood capillary in white matter (Fig. 5g). The elastic fibers also, had appeared mossy fibers in internal granular layer and white matter (Fig. 5 h, i). The reticular fibers had appeared in dura matter (Fig. 5j).

At the age of 22 days post parturition, as the previous age but the external granular layer decreased in thickness. Thickness of molecular layer increased than preceding age (Fig. 6a, b, c). Internal granular layer became thicker and more distinct than the preceding age. There were small spaces between the granule cells called cerebellar glomeruli or islands appearing as irregular light areas (Fig. 6b). The neutral mucopolysaccharide appeared PAS positive reaction in Purkinje cell layer, molecular layer and between granular cells (Fig. 6 d, e). The acid mucopolysaccharide showed positive reaction to alcian blue stain (Fig. 6f). The collagen fibers had appeared in dura matter, blood capillary in white matter and mossy fiber (Fig. 6 g, h). Filamentous nerve fibers presented in molecular layer called climbing fibers (Fig. 6i) and mossy fibers presented

between granular cells (Fig. 6j). The reticular fibers had appeared in dura matter (Fig. 6 k, L). The dendritic arborization of Purkinje cells was long, branched (Fig. 6 m & n). Semithin section demonstrated that the Purkinje cells had ovoid shape, spherical vesicular nuclei with prominent nucleolus and basophilic cytoplasm. Internal granular layer had clusters of spherical cells. They had spherical euchromatic nuclei contained distinct nucleolus and little pale cytoplasm (Fig. 6o). Ultrastructurally, molecular layer contained basket cell that had large euchromatic (mainly extended chromatin) nucleus and scanty granulated cytoplasm and multiple myelinated nerve fibers, filled with mitochondria and surrounded by normal myelin sheath (Fig. 6p). The Purkinje cells had large spherical euchromatic nuclei with well-developed nucleoli and nuclear envelope. The cytoplasm contained multiple mitochondria, Golgi apparatus, free ribosomes and numerous well-developed RER (Fig. 6q). Granular cells had spherical nuclei contained clusters of condensed chromatin. They had few cytoplasm contained little mitochondria, ribosomes and RER (Fig. 6r).

At the age of one month, as the previous age but the external granular layer had disappeared in some areas (Fig. 7 a, b). At the age of two-month, cortex formed of granular layer, Purkinje cell layer, and molecular layer. EGL was completely disappeared. Thickness of molecular layer was increased compared to the preceding age. Molecular layer formed of two cells; basket cells and stellate. Stellate cells situated in uppermost portion of the molecular layer, had oval or spherical cell and had densely stained basophilic nuclei and distinct nucleoli, while were located in the bottom part of the molecular layer, close to the Purkinje cell, had spherical or triangular shaped cells and had lightly stained basophilic nuclei with prominent nucleoli. Purkinje cell layer was organized in one layer appeared as flask -shaped cells and lightly stained nuclei with distinct nucleoli (Fig. 8 a, b). The granular layer was formed of densely packed cells. It consisted of two types of cells: granule cells and Golgi Type II cells. The granule cells were small, numerous and tightly packed. They were spherical with spherical nuclei and little quantity of cytoplasm and. gathered to create a cluster of cells Golgi cells were larger than the granule cells had an oval or triangular cell body with vesicular nucleus and more cytoplasm (Fig. 8 b, c). Each cerebellar hemisphere's white matter contained large clusters of nerve cells called deep cerebellar nuclei. The nuclei were grouped from lateral to medial as the dentate, emboliform, globose, and fastigial (Fig. 8 d, e). The neutral mucopolysaccharide appeared PAS positive reaction in Purkinje cell layer, molecular layer and between granular cells (Fig. 8 f, g). The acid mucopolysaccharide showed positive reaction to alcian blue stain (Fig. 8 h, i). The collagen fibers were present in blood capillary (Fig. 8j). Climbing nerve fibers presented in molecular layer (Fig. 8k) and mossy fibers presented between granular cells (Fig. 8L). The reticular fibers had appeared in dura matter (Fig. 8m). The dendritic arborization of Purkinje cells was long, branched (Fig. 8 n, o). Semithin section that revealed that the Purkinje cells had oval shaped cells, spherical vesicular nuclei with prominent nucleolus and basophilic cytoplasm. Internal granular layer had cluster of spherical cells. These cells had spherical euchromatic nuclei contained apparent nucleolus and little pale cytoplasm (Fig. 8p). Ultrastructurally,

molecular layer contained basket cell that had large euchromatic nucleus and scanty granulated cytoplasm and multiple myelinated nerve fibers, filled with mitochondria and surrounded by normal myelin sheath (Fig. 8q). The Purkinje cells had large spherical euchromatic nuclei with well-developed nucleoli and nuclear envelope. The cytoplasm consisted of numerous mitochondria, numerous well-developed rough endoplasmic reticulum, free ribosomes and Golgi apparatus (Fig. 8r). Bergmann astrocyte ensheathed a Purkinje cell with their processes. It had euchromatic nucleus and pale cytoplasm contains ribosomes, mitochondria and surrounded by blood vessel (Fig. 8s). The granule cells had spherical heterochromatic nuclei. They contained few cytoplasm with little mitochondria, ribosomes and rough endoplasmic reticulum (Fig. 8t).

The morphological and histological features of the mature cerebellum were reached from two months onward. The cerebellum at 3M, 4M, 5M and 6M had the same histological structure of cerebellum at 2M.

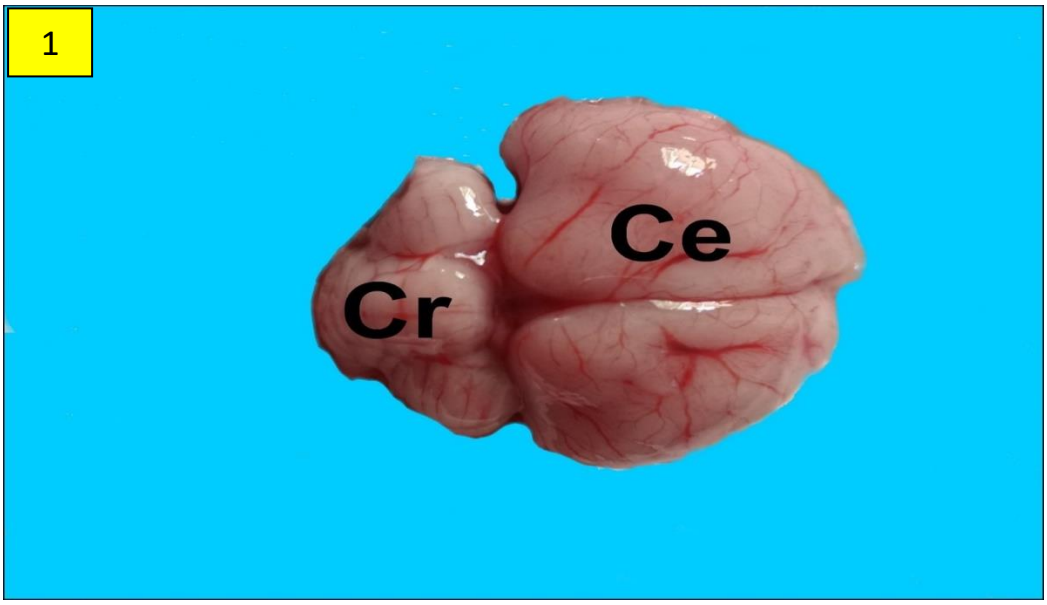


Fig. 1. Dorsal view of rabbit cerebellum. Cr, Cerebellum; Ce, cerebral hemisphere.

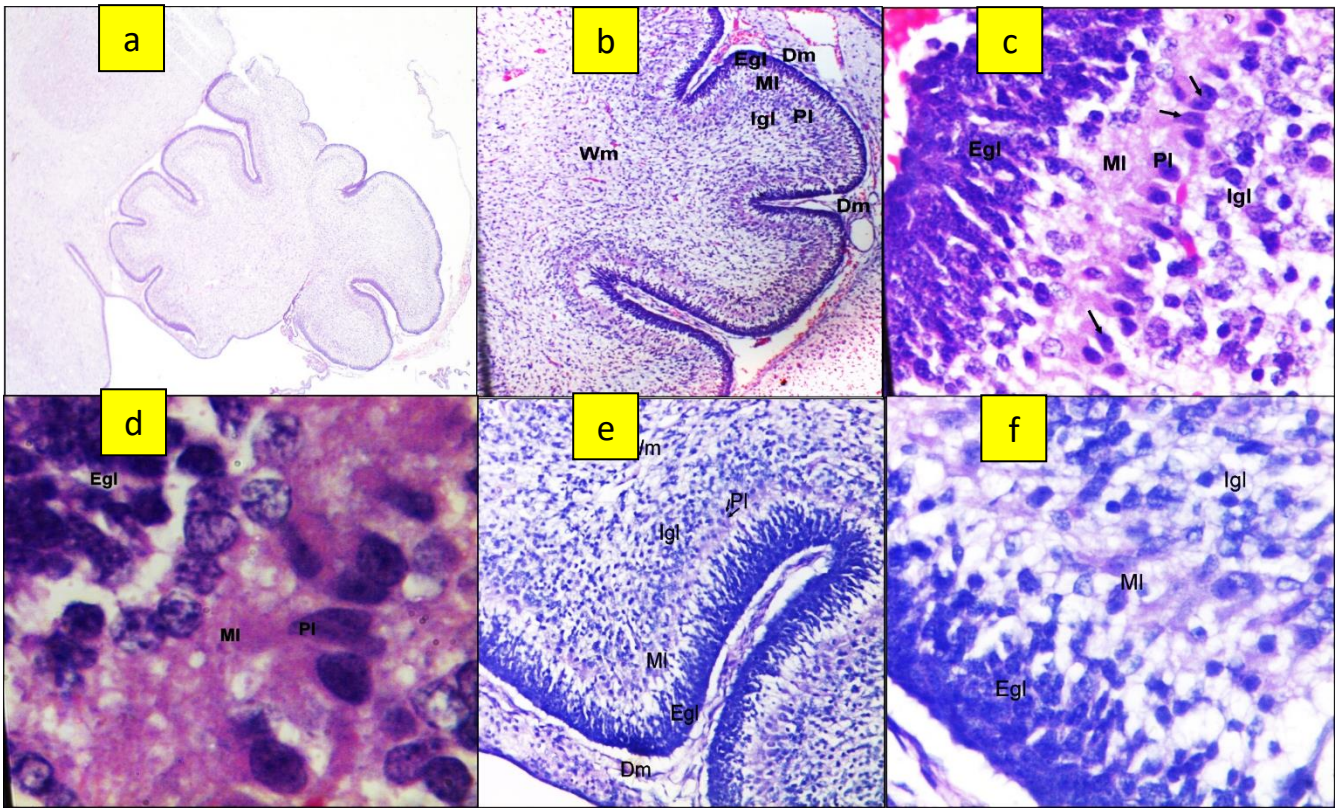
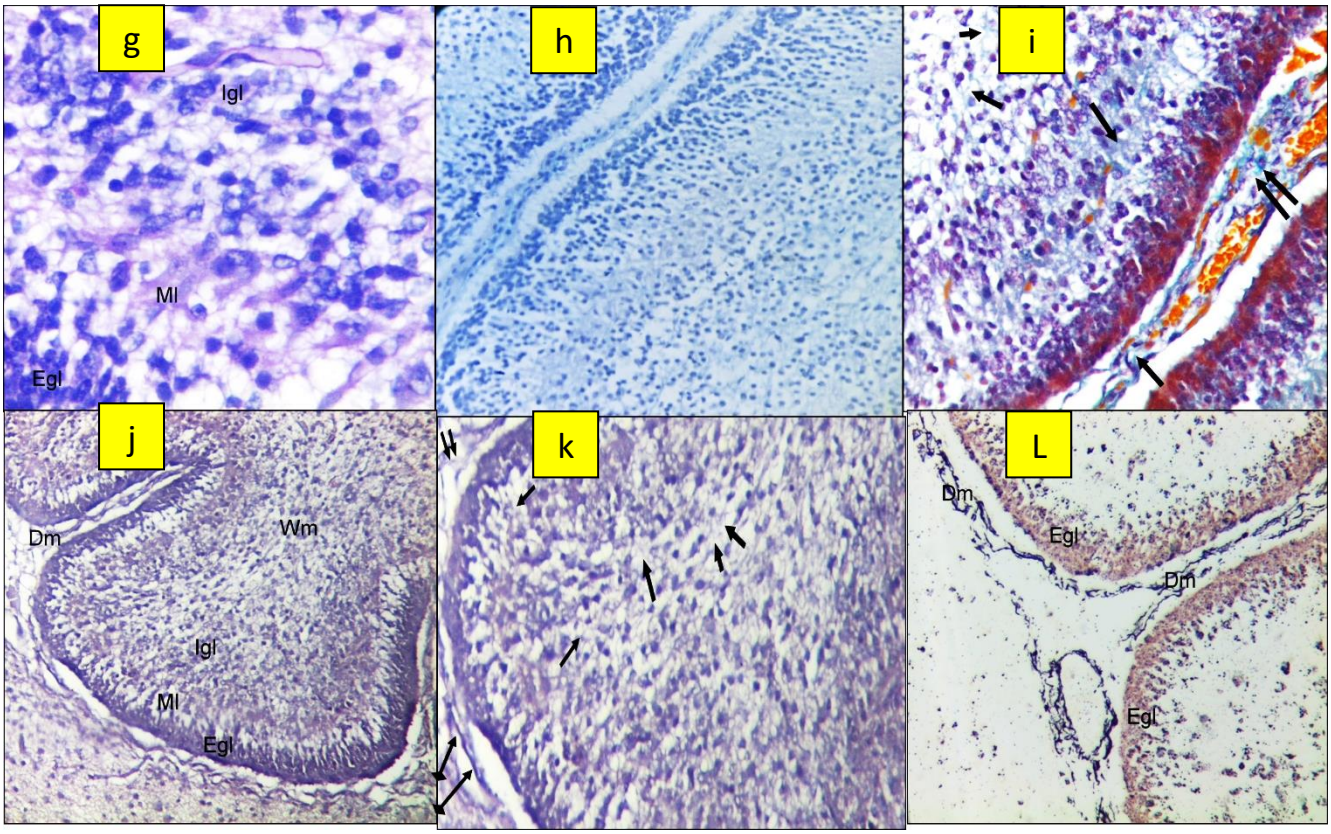


Fig. 2. Photomicrograph of rabbit cerebellum at one day post parturition. (a): H&E stain stereomicroscope×30. (b): Showing the external granular (Egl), Purkinje layer (Pl), internal granular layer (Igl), molecular layer (Ml), white matter (Wm) and dura matter (Dm). (H&E X 40). (c): Showing molecular layer (Ml), white matter (Wm), internal granular layers (Igl), and external granular layers (Egl). (H&E X 400). (d): Higher magnification of (c). (H&E X 1000). (e): Showing positive reaction to PAS (black arrow). (PAS stain X 400). (f): A higher magnification of (e). (PAS stain X 400).



(g): A higher magnification of (e)**.** (PAS stain X 400). (h): Showing weak positive acid mucin reaction. (AB pH 2.5 X 200). (i): Showing distribution of collagen fibers in dura matter (black arrow) (CT X 400). (j): Showing distribution of elastic fibers between granular cells (mossy fibers). (Weigert′s elastic tissues stain X 100).). (k): Showing distribution of elastic fibers (black arrow). (Weigert′s elastic tissues stain X 400). (L): Showing distribution of reticular fibers in dura matter (Dm). (GR stain X 200)**.**

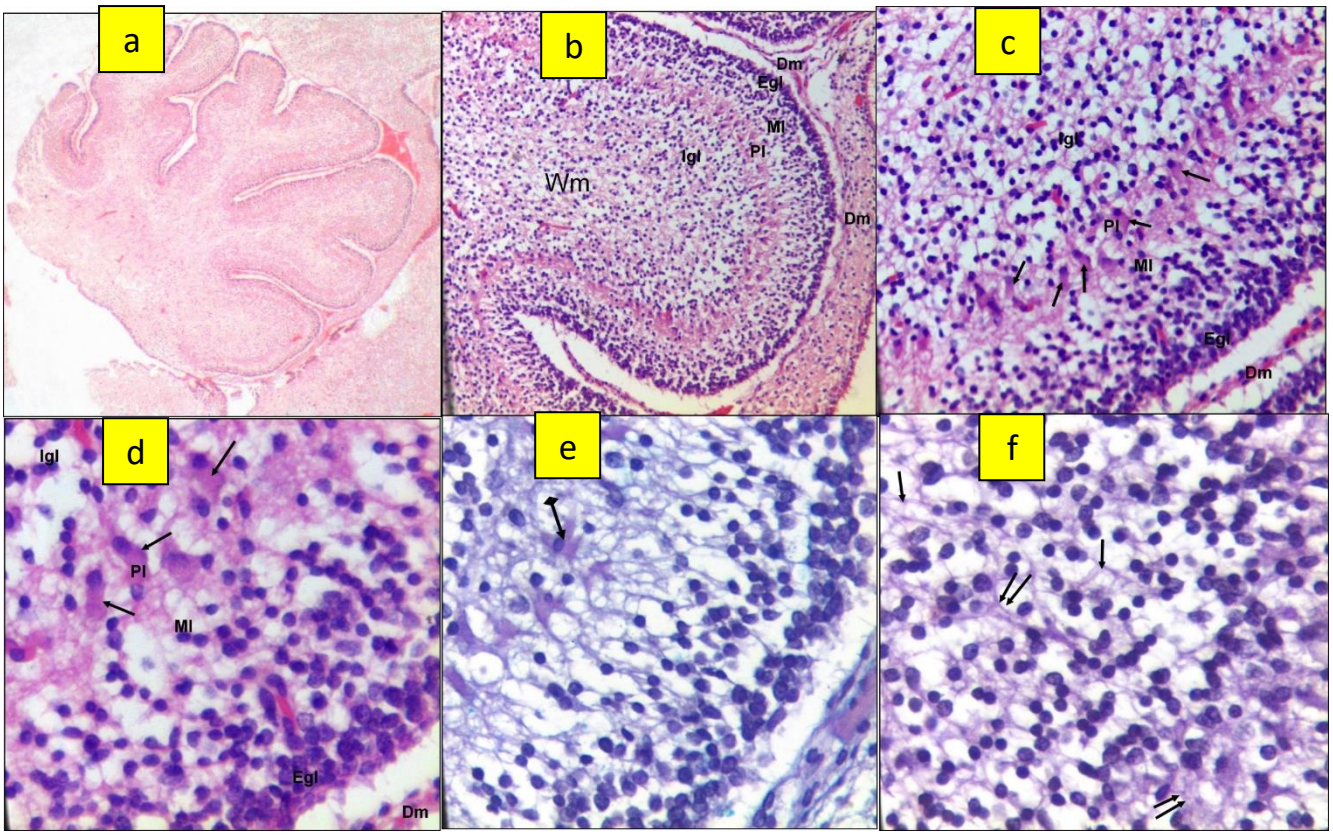
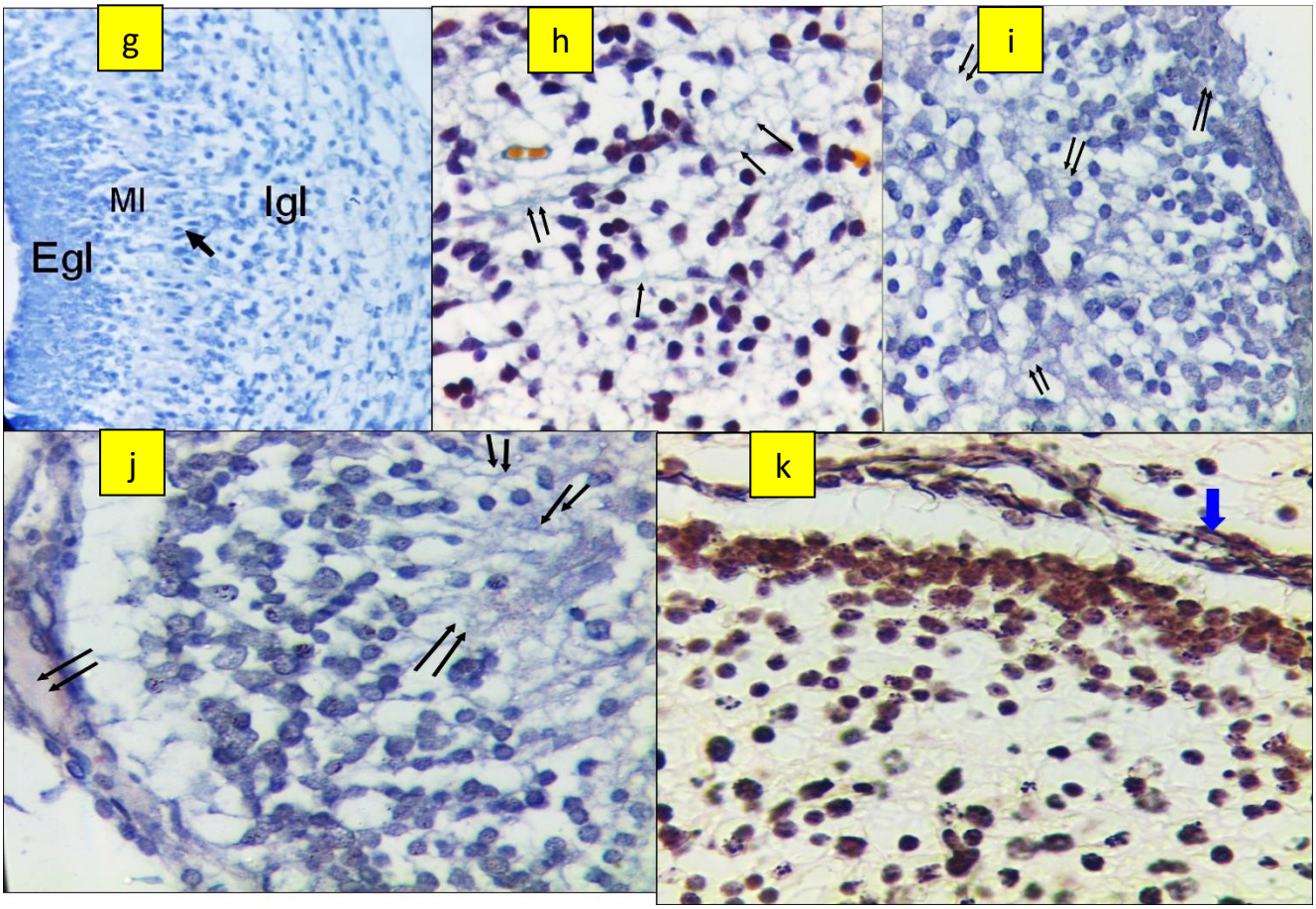


Fig. 3. Photomicrograph of rabbit cerebellum at five days post parturition. (a): H&E stain stereomicroscope×30. (b): showing the external granular (Egl), Purkinje layer (Pl), internal granular layer (Igl), molecular layer (Ml), white matter (Wm) and dura matter (Dm). (H&E X 40). (c): showing the external granular (Egl), molecular layer (Ml), internal granular layers (Igl) and white matter (Wm). (H&E X 200). (d): A higher magnification of (c)**.** (H&E X 400). (e): showing positive reaction to PAS in Purkinje cells. (PAS stain X 400). (f): showing positive reaction to PAS (black arrow)**.** (PAS stain X 400).



(g): showing weak positive acid mucin reaction. (AB pH 2.5 X 200). (h): showing distribution of collagen fibers (black arrow). (CT X 400). (i&j): showing distribution of elastic fibers in dura matter and between granular cells. (Weigert′s elastic tissues stain X 400). (k): showing distribution of reticular fibers in dura matter (blue arrows). (GR stain X 400)**.**

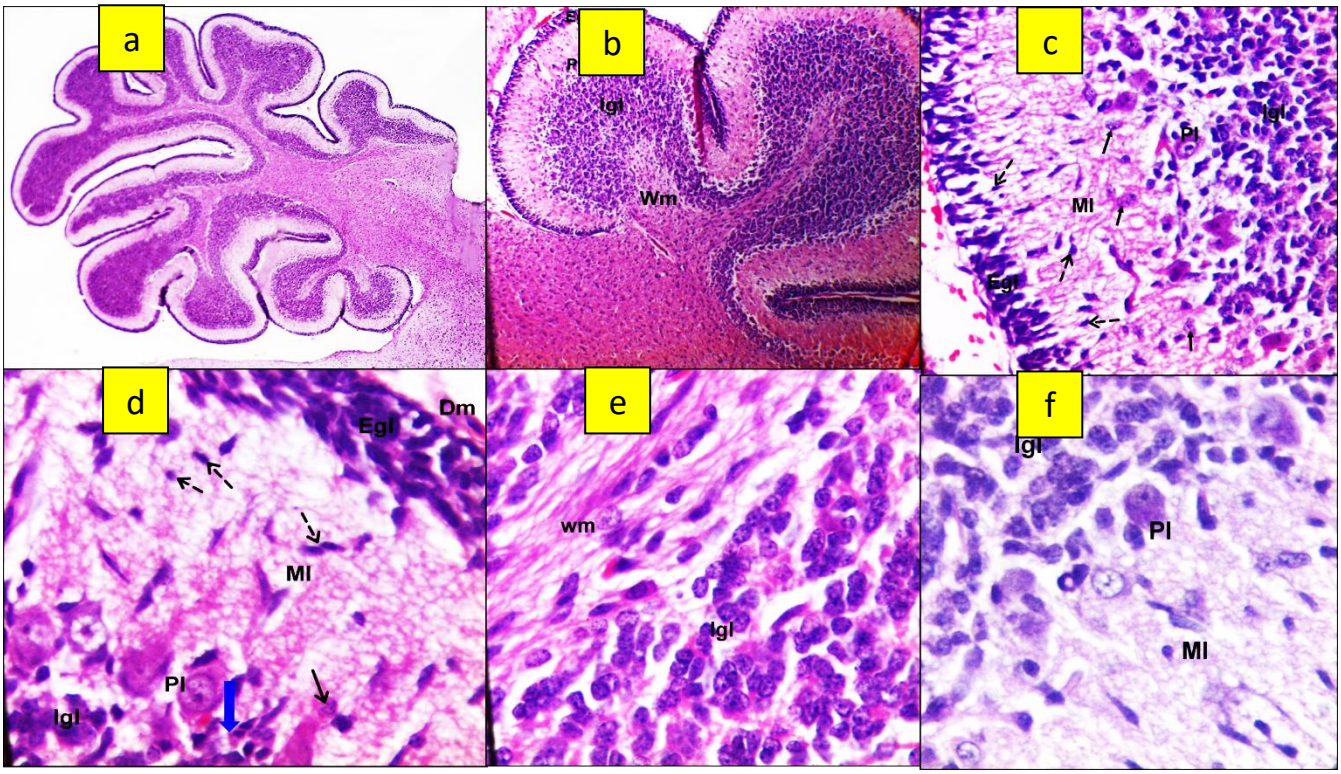
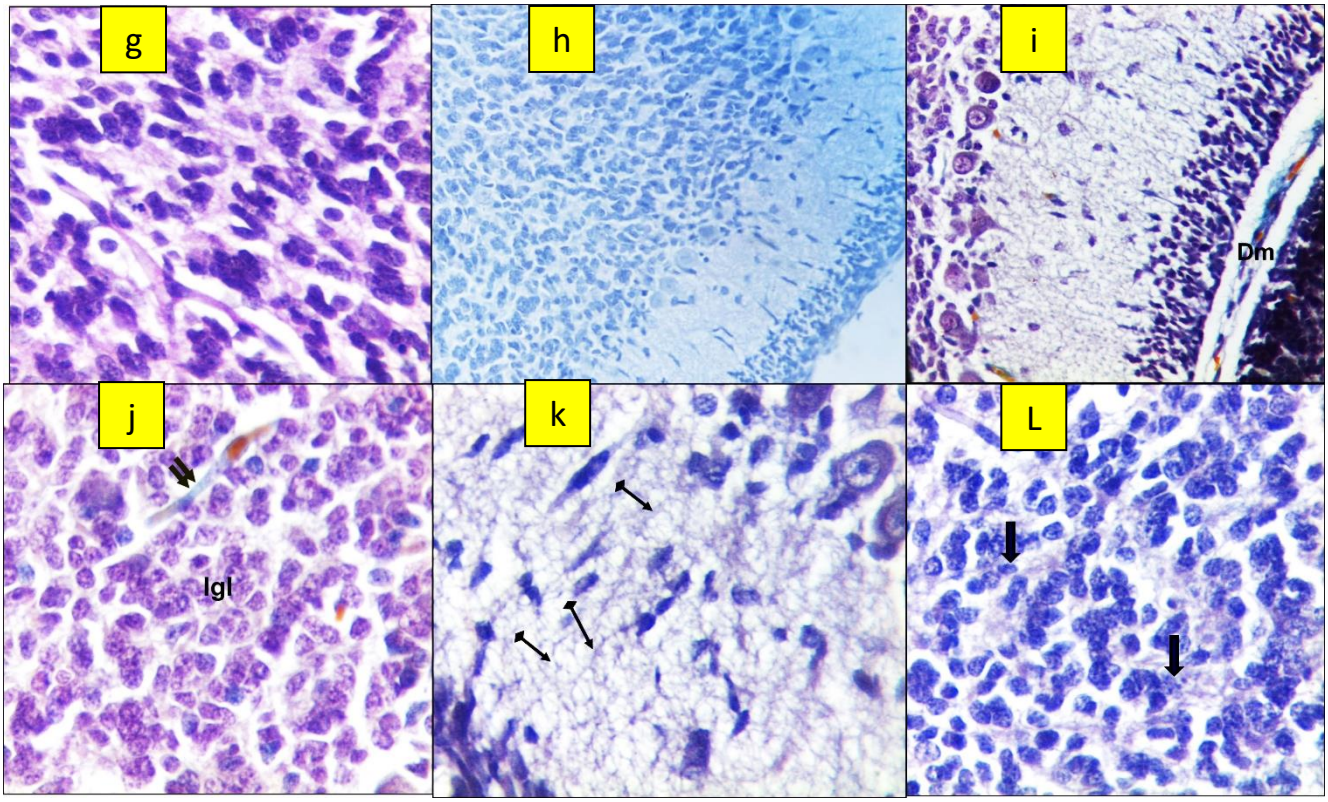
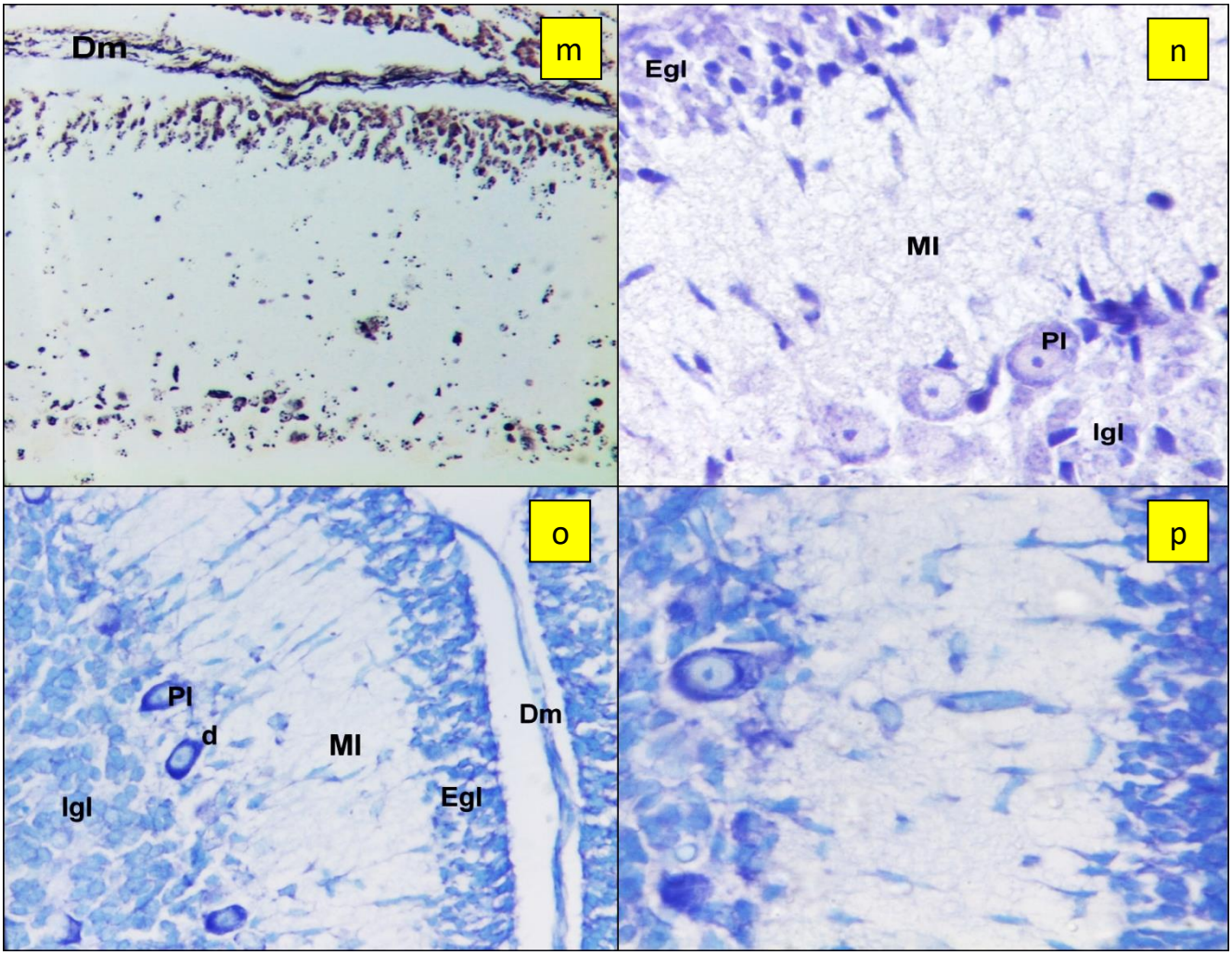


Fig. 4. Photomicrograph of rabbit cerebellum at eight days post parturition. (a): H&E stain stereomicroscope×30. (b): showing the external granular layer (Egl), molecular layer (Ml), Purkinje cell layer (Pl), internal granular layers (Igl) and white matter (Wm). (H&E X 40). (c): showing the external granular layer (Egl), molecular layer (Ml), internal granular layers (Igl), Purkinje cell layer (Pl), stellate cells (broken arrow), basket cells (black arrow) and Bergman astrocyte (blue arrow). (H&E X 200). (d &e): Higher magnification of (c)**.** (H&E X 400). (f): showing positive reaction to PAS in molecular layer and Purkinje cells. (PAS stain X 400).



(g): showing positive reaction to PAS**.** (PAS stain X 400). (h): showing weak positive acid mucin reaction. (AB pH 2.5 X 400). (i): showing distribution of collagen fibers in dura matter (Dm). (CT X 400). (j): showing distribution of collagen fibers in blood capillary (black arrow). (CT X 400). (k): showing distribution of elastic fibers in climbing fibers in molecular layer (black arrow). (Weigert′s elastic tissues stain X 400). (L): showing distribution of elastic fibers in mossy fibers (black arrow). (Weigert′s elastic tissues stain X 400).



(m): showing positive reaction to Gomori′s reticulin stain in dura matter (Dm). (GR stain X 400)**.** (n): showing short, dendritic branches of Purkinje cell (Pl) in the molecular layer (Ml). Phosphotungstic Acid Haematoxylin X 400)**.** (o&p):A semithin section showing short, dendritic branches (d) of Purkinje cell (Pl) extend in the molecular layer (Ml) the external granular (Egl) and internal granular layers (Igl). (TB stain X 400)**.**

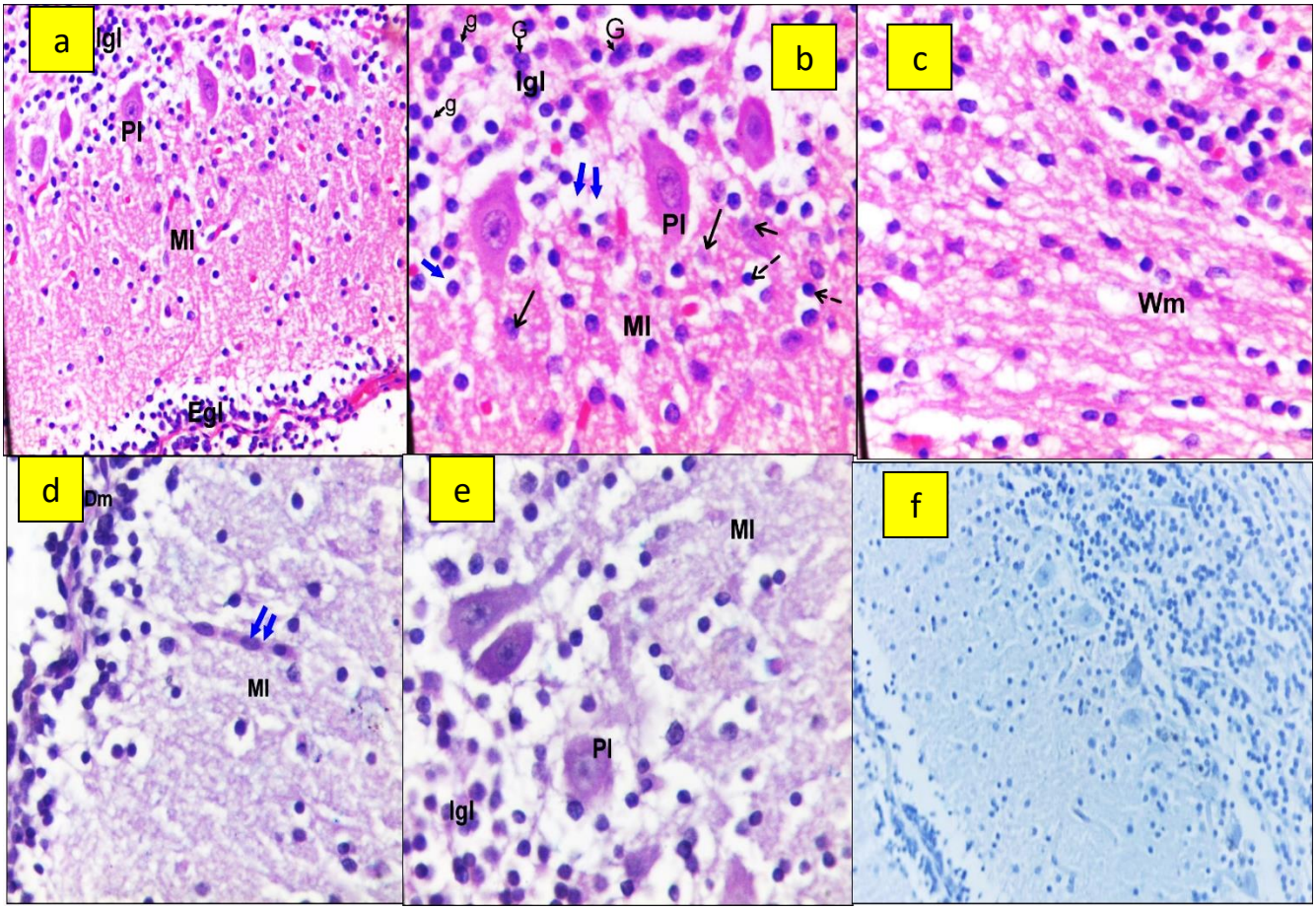
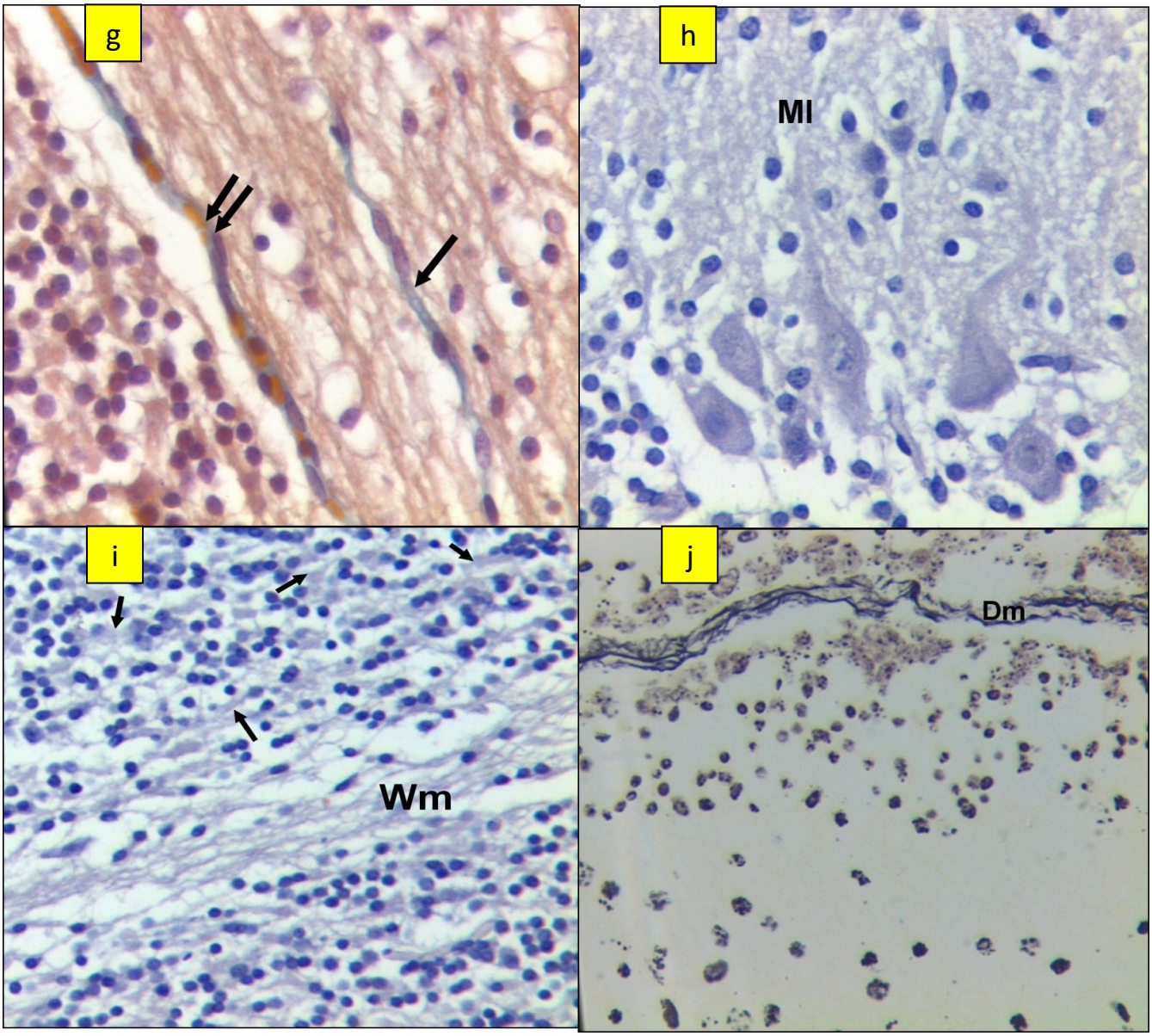


Fig. 5. Photomicrograph of rabbit cerebellum at 15 days post parturition. (a): showing Purkinje cell layer (Pl), internal granular layers (Igl), molecular layer (Ml) and the external granular (Egl). (H&E X 200). (b): A higher magnification of (a) denoting molecular layer (Ml), internal granular layers (Igl), Purkinje cell layer (Pl), stellate cells (broken arrow), basket cells (black arrow), Bregman astrocyte (blue arrow) granule cells (g), Golgi cells (G). (H&E X 400). (c): showing white matter (Wm). (H&E X 400). (d&e): showing positive reaction to PAS in dura matter (Dm), molecular layer (Ml), blood capillary (blue arrow) and Purkinje cells (Pl). (PAS stain X 400). (f): showing weak positive acid mucin reaction. (AB pH 2.5 X 400). (View g): showing distribution of collagen fibers in blood capillary in white matter (black arrow). (CT X 400).



(h): showing distribution of elastic fibers. (Weigert′s elastic tissues stain X 400). (i): showing distribution of elastic fibers in mossy fibers (black arrow) and in white matter. (Weigert′s elastic tissues stain X 400). (j): showing distribution of reticular fibers in dura matter (Dm). (GR stain X 400).

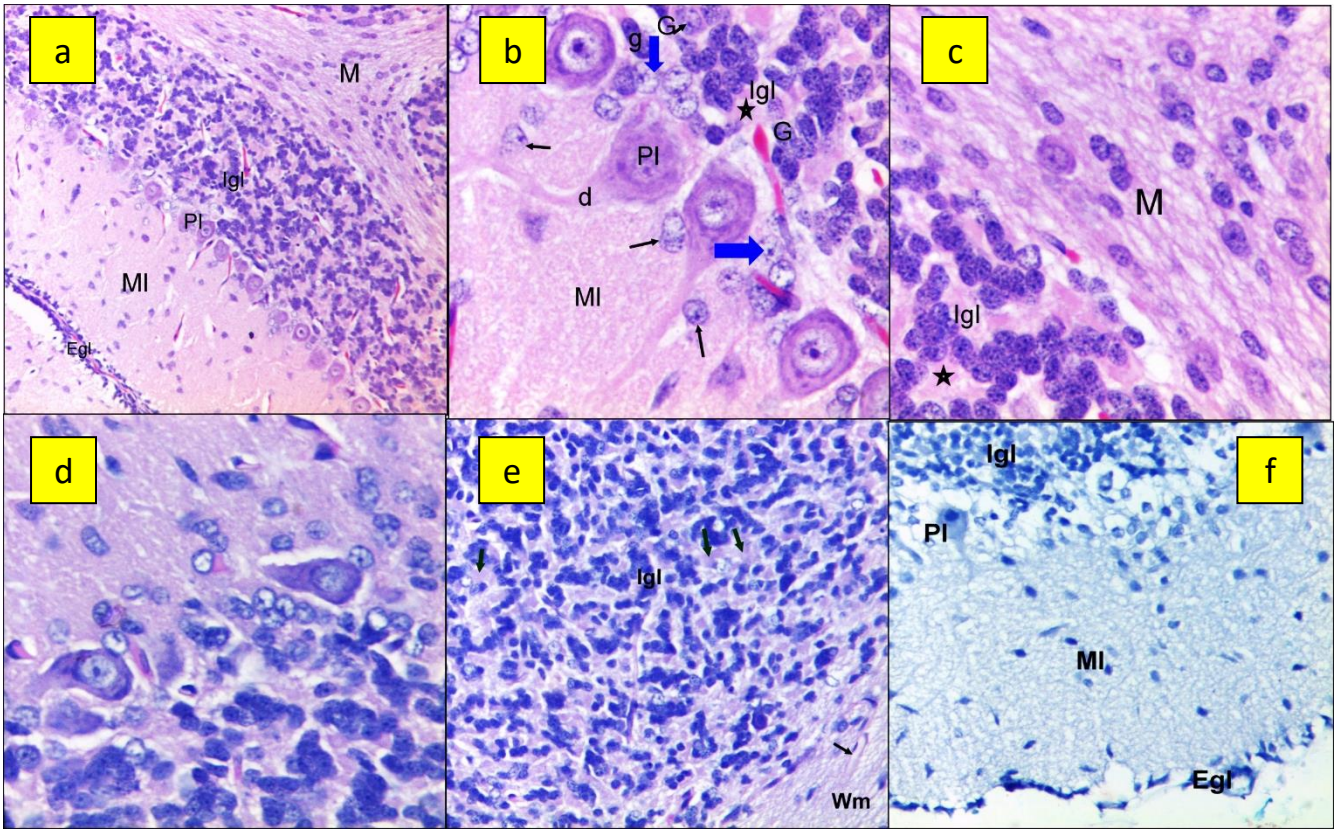
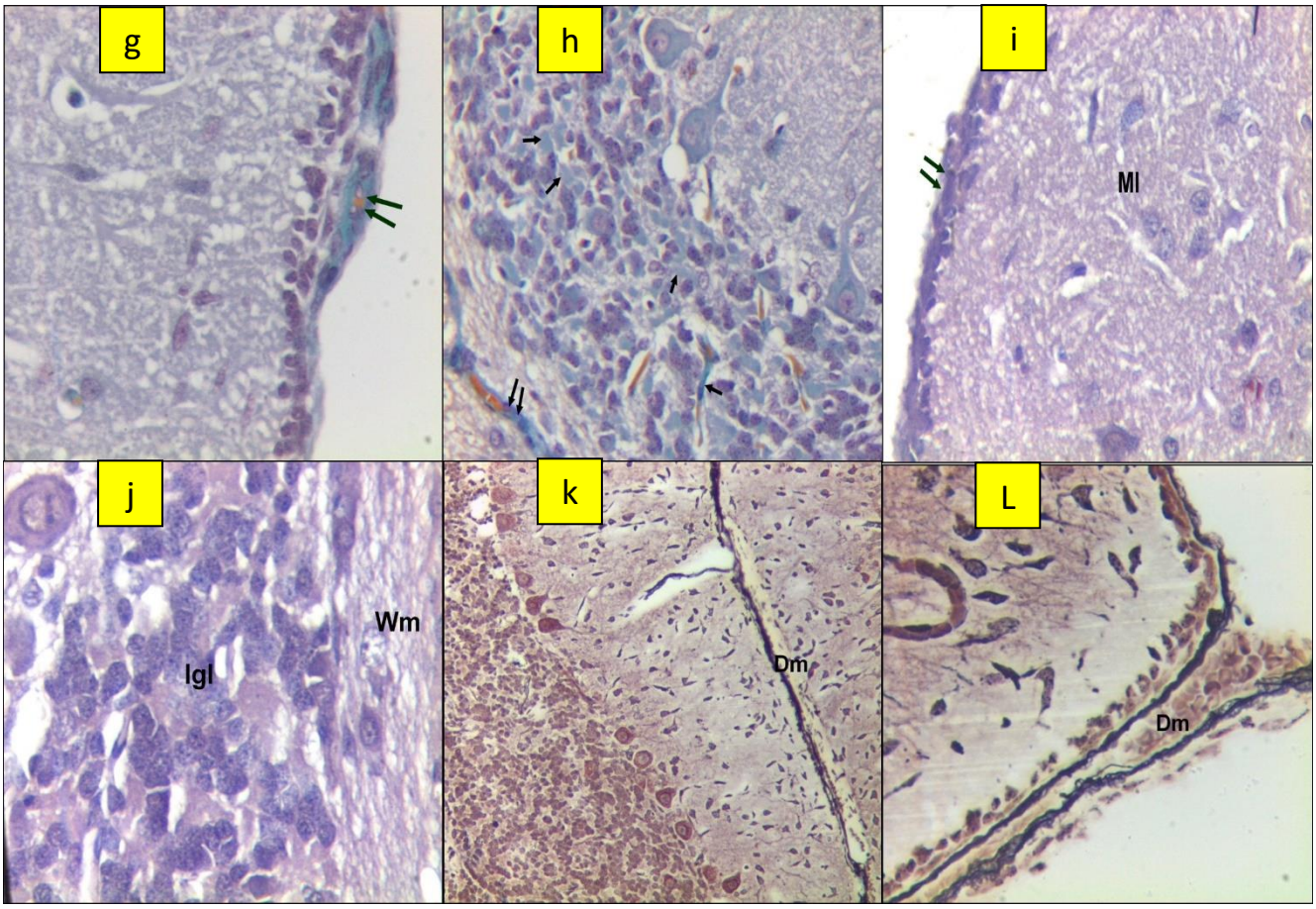
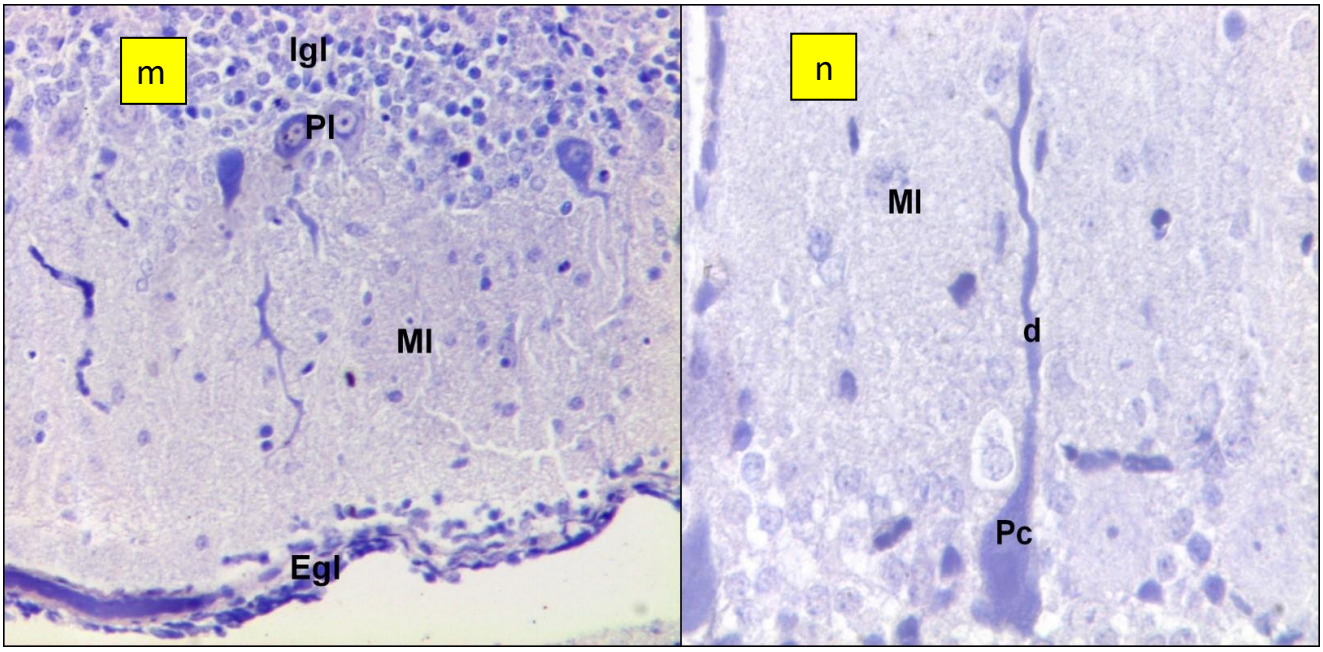


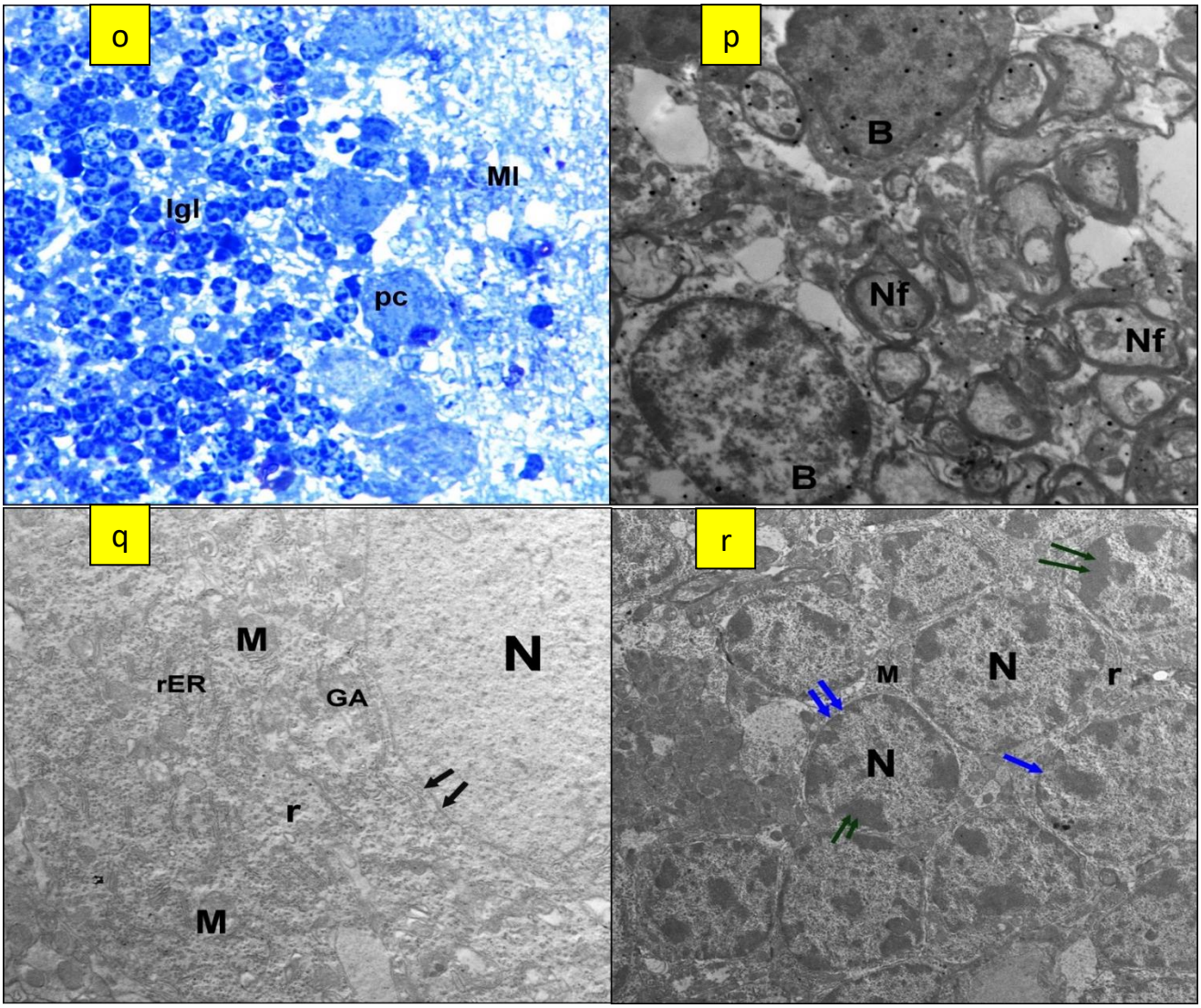
Fig. 6. Photomicrograph of rabbit cerebellum at 22 days post parturition. (a): showing the external granular layer (Egl), molecular layer (Ml), Purkinje cell layer (Pl), internal granular layers (Igl) and medulla (M). (H&E X 100). (b): showing molecular layer (Ml), internal granular layers (Igl), Purkinje cell layer (Pl), basket cells (black arrow), Bergman astrocyte (blue arrow), granule cells (g), Golgi cells (G), granular island (star) and dendrites (d). (H&E X 400). (c): showing medulla (M) and granular island (star). (H&E X 400). (d): showing positive reaction to PAS in molecular layer and Purkinje cells. (PAS stain X 400). (e): showing positive reaction to PAS (black arrows) in internal granular layer (Igl) and white matter (Wm). (PAS stain X 400). (f): showing positive acid mucin reaction. (AB pH 2.5 X 400).



(g): showing distribution of collagen fibers in dura matter (black arrow). (CT X 400). (h): showing distribution of collagen fibers in mossy fibers and blood capillary (black arrow). (CT X 400). (i&j): showing distribution of elastic fibers in dura matter (black arrow). (Weigert′s elastic tissues stain X 400). (k): showing distribution of reticular fibers in dura matter in dura matter (Dm). (GR stain X 200)**.** (L): A higher magnification of (k)**.** (GR stain X 400)**.**



(m &n): showing long, dendritic branches (d) of Purkinje cell (Pc) was extending in the molecular layer (Ml). Phosphotungstic Acid Haematoxylin X 400).



(o): A semithin section showing Purkinje cell (Pc), molecular layer (Ml) and internal granular layers (Igl). (TB stain X 400)**.** (p): A transmission electron micrograph showing molecular layer which containing a basket cell (B) with large nucleus and little granular cytoplasm and multiple myelinated nerve fibers (NF). (TEM× 2000). (q): showing the Purkinje cell with nucleus (N), inner layer of nuclear envelope (black arrows), mitochondria (M), Golgi bodies (GA), ribosome(r) and rough endoplasmic reticulum (rER). (TEM × 2000). (r): showing granular cells with roughly spherical nuclei (N) with cluster of condensed chromatin (black arrows), outer layer of the nuclear envelope (blue arrow), mitochondria (M), and ribosomes (r). (TEM × 2000).

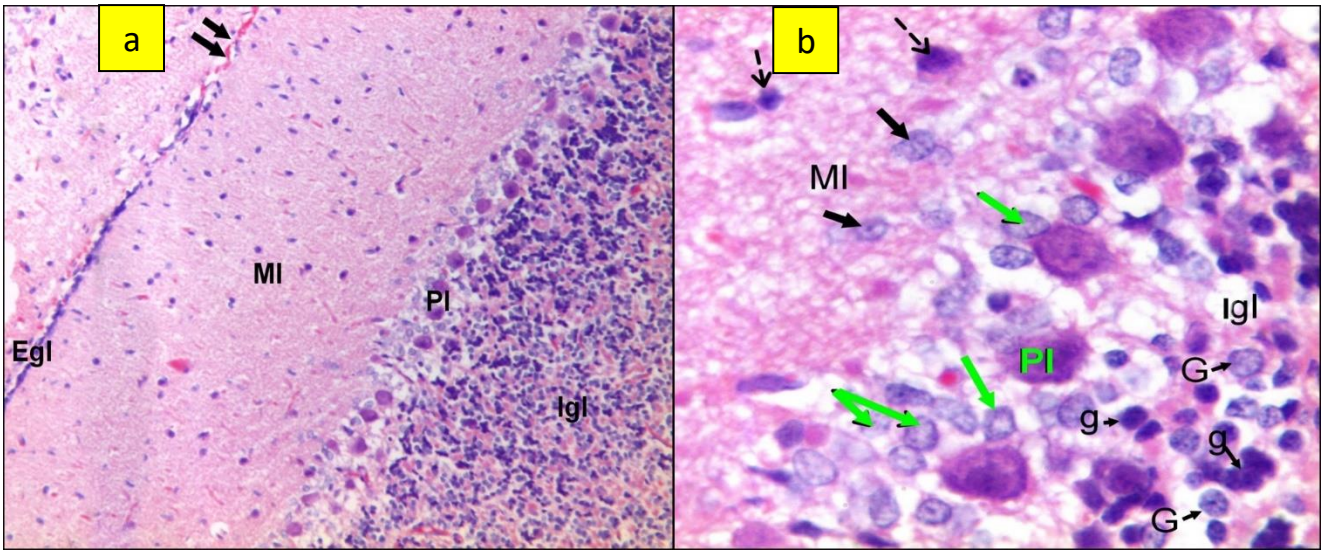


Fig. 7. Photomicrograph of rabbit cerebellum at one month age. (a): showing the external granular (Egl) that disappeared in some areas (black arrows), internal granular layers (Igl), Purkinje cell layer (Pl), molecular layer (Ml)and medulla (M). (H&E X 100). (b): showing molecular layer (Ml), internal granular layers (Igl), Purkinje cell layer (Pl), basket cells (black arrow), stellate cell (broken arrow), Bergman astrocyte (green arrow), granule cells (g) and Golgi cells (G). (H&E X 400).

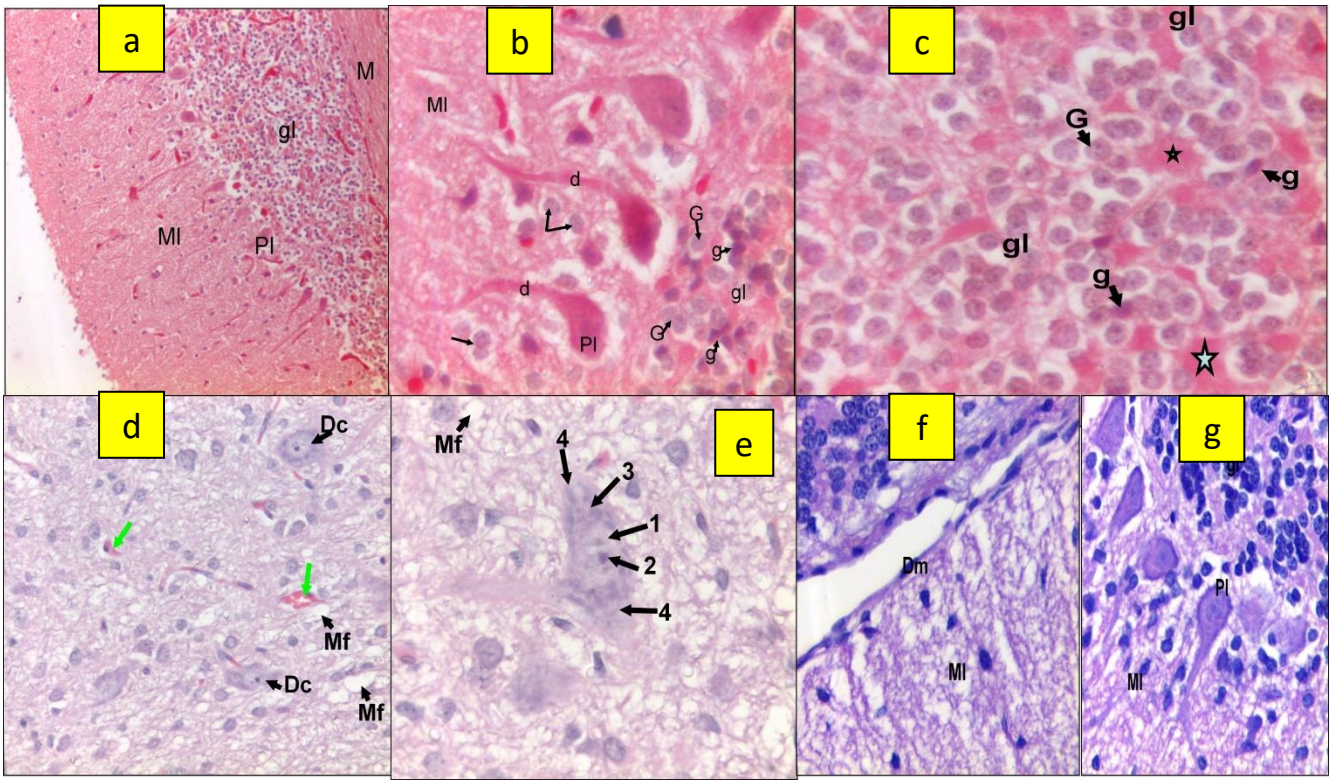
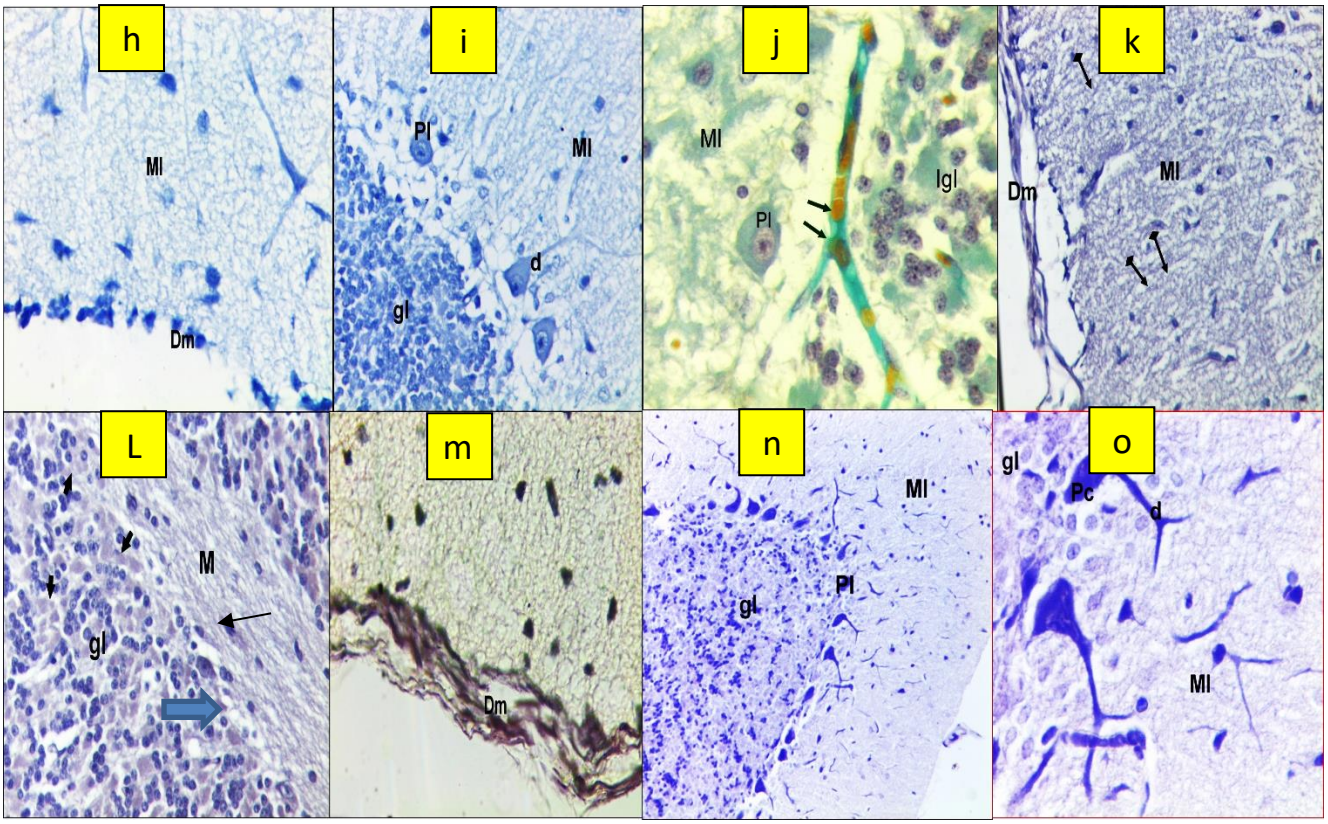
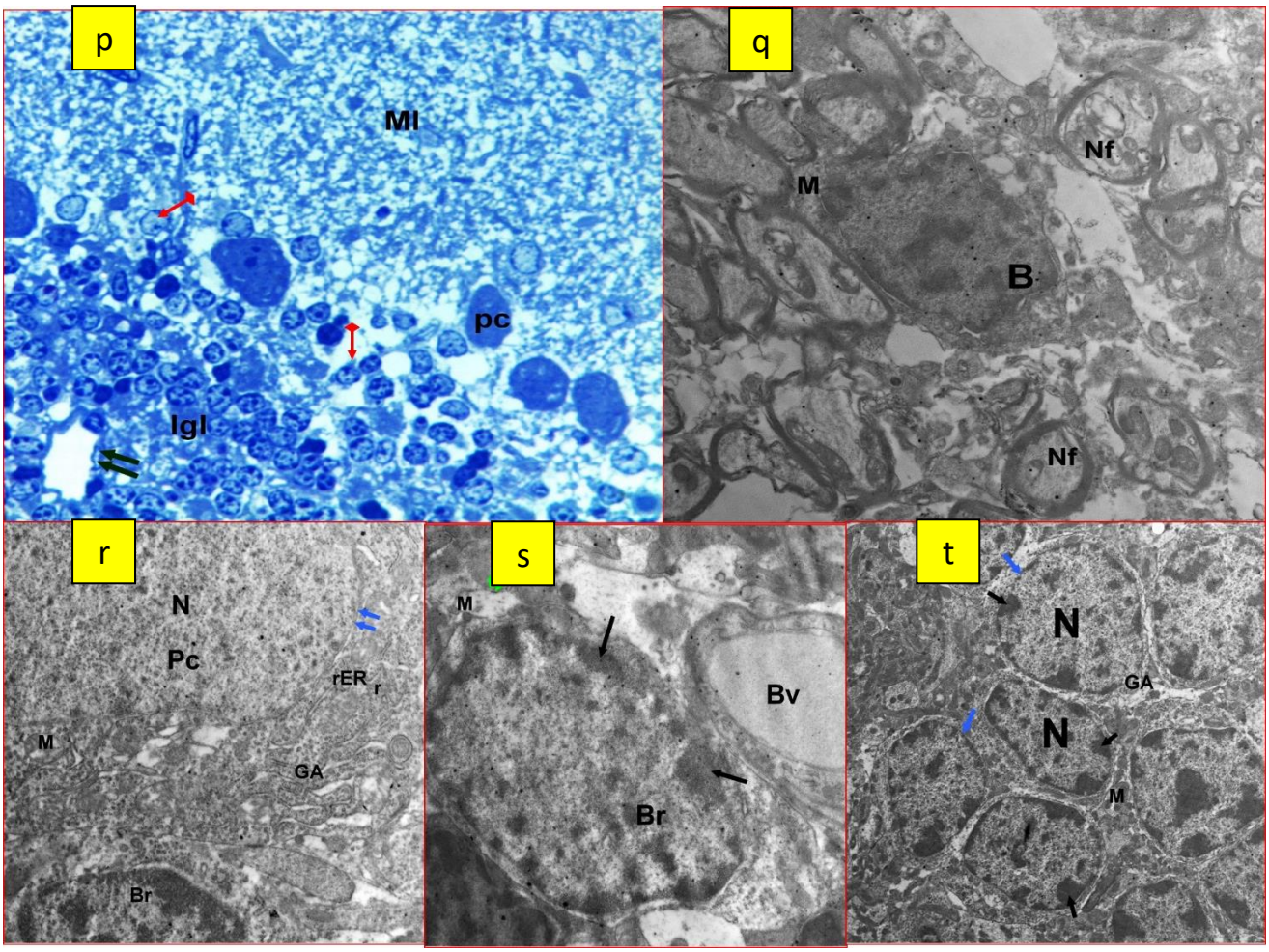


Fig. 8. Photomicrograph of rabbit cerebellum at two months age. (a): showing molecular layer (Ml), Purkinje cell layer (Pl), granular layer (gl) and medulla (M). (H&E X 100). (b): A higher magnification photomicrograph depcting molecular layer (Ml), Purkinje cell layer (Pl), dendrites (d), basket cells (black arrows), granule cells (g) and Golgi cells (G). (H&E X 400). (c): showing granular layer (gl), granular island (star), granule cells (g) and Golgi cells (G). (H&E X 400). (d): showing inner medulla, deep cerebellar nuclei (Dc) and myelinated fibers (Mf). (H&E X 100). (e): showing deep cerebellar nuclei, 1, nucleus; 2, nucleolus; 3, cytoplasm; 4, dendrite, and myelinated fibers (Mf). (H&E X 400). (f): showing positive reaction to PAS in molecular layer (Ml) and dura matter (Dm). (PAS stain X 400). (g): showing positive reaction to PAS in molecular layer (Ml), Purkinje cell layer (Pl), granular layer (gl). (PAS stain X 400).



(h): showing positive acid mucin reaction (AB pH 2.5 X 400). (i): showing positive acid mucin reaction molecular layer (Ml), Purkinje cell layer (Pl), granular layer (gl) and dendrite (d): (AB pH 2.5 X 400). (j): showing distribution of collagen fibers in blood capillary (black arrows). (CT X 400). (K): showing distribution of elastic fibers in dura matter (Dm) and in climbing fibers in molecular layer (black arrows). (Weigert′s elastic tissues stain X 400). (L): showing distribution of elastic fibers in mossy fibers (arrow**?**) and in medulla (M). (Weigert′s elastic tissues stain X 400). (m): showing distribution of reticular fibers in dura matter (Dm). (GR stain X 400)**.** (n): showing molecular layer (Ml), Purkinje cell layer (Pl) and granular layer (gl) Phosphotungstic Acid Haematoxylin X 200)**.** (o): Showing long, dendritic branches (d) of Purkinje cell (Pc) in the molecular layer (Ml). Phosphotungstic Acid Haematoxylin X 400)**.**



(p): A semithin section showing Purkinje cell (Pc), molecular layer (Ml), internal granular layers (Igl), Bergman astrocyte (red arrows) and blood capillary (black arrows). (TB stain X 400)**.** (q): An electron micrograph showing molecular layer containing a basket cell (B) with large nucleus, little granular cytoplasm, an aggregation of mitochondria at the axon hillock(M) and multiple myelinated nerve fibers (NF), filled with mitochondria (TEM× 2000). (r): showing the Purkinje cell (Pc)**,** with large euchromatic nucleus (N), outer layer of nuclear envelope (blue arrows), Bergman astrocyte (Br). The cytoplasm contains Golgi bodies (GA), rough endoplasmic reticulum (rER), ribosome(r) and mitochondria (M). (TEM × 2000). (s): showing Bergman astrocyte (Br) with clumps of heterochromatin (black arrows), mitochondria (M), processes (green arrows) and blood vessel. (TEM × 3000). (t): showing granule cells with nuclei (N), cluster of condensed chromatin (black arrows), outer layer of nuclear (blue arrows), mitochondria (M) and Golgi bodies (GA). (TEM × 2000).

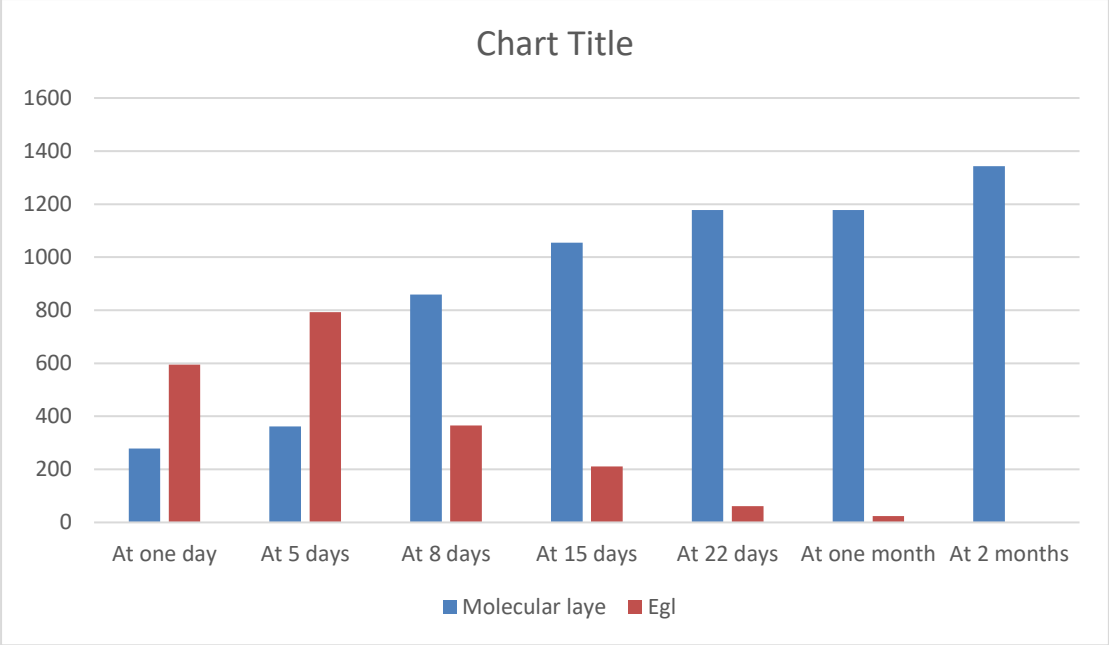


Fig. 9. Statistical analysis chart of molecular layer and external granular layer thickness using image j and measured by Mm.

Table (1): Showing the means of the thickness of the external granular layer and the thickness of the molecular layer of the cerebellar cortex from age 1 dPN to age 2 mPN:

Postnatal day	**Thickness of EGL**	**Thickness of molecular layer.**
PD 1	594.348	278.704
PD 5	792.134	361.655
PD 8	365.617	859.087
PD 15	211.398	1055.048
PD 22	61.88	1178.034
1M	23.557	1178.202
2M	0	1342.928

DISCUSSION

In the present study, at the age of one day post parturition, the cerebellar cortex formed of four layers: an EGL, a molecular layer, a Purkinje cell layer and the internal granular layer. Thickness of the EGL reached its maximum, consisted of five to six layers of spherical or oval shaped cells. Molecular layer appeared as a pale area contained Purkinje cells that had vesicular nuclei with an amphiphilic cytoplasm and prominent nucleoli, were arranged in multiple layers. Purkinje cells had apical cone directed towards the surface and retained some characteristics of immaturity. Internal granular layer consisted of darkly stained cells. The white matter was situated below the IGL and composed of less densely packed oval or fusiform cells as reported by (Elsawy et al., 2013; Fakhry et al., 2022). But Mohamed and Mohamed (2018) reported that, molecular layer not easily

detected at 2dPN. The EGL consisted of proliferating cells that, after migrating from the rhombic lip (Ryder & Cepko, 1994), covered the entire cerebellar surface and given rise to granule, stellate and basket cells of the adult cerebellum as reported by (Jacobson, 1991; Lossi et al.,1995) in rabbit.

At the age of five days post parturition, the current study illustrated that Purkinje cell layer was arranged in a well-developed single layer. Myelination of the white matter appeared more evident. This result agreed with (Lossi et al., 1995; Vastaghet al., 2005).

At the age of eight days post parturition, the current study illustrated that cortex was formed from outer thick external granular layer, molecular layer, Purkinje cell layer and internal granular layer. The thickness of EGL layer decreased and formed of spherical deeply stained cells. The molecular layer thickness was increased and formed of few small cells (basket cells and stellate cells), dendritic processes of Purkinje cell and nerve fibers (climbing fiber). Purkinje cell layer was organized in one layer and appeared as pear-shaped cells and less stained nuclei with apparent nucleoli. Populated granular cells of the internal granular layer appeared as densely stained small spherical cells. Myelination of the white matter appeared more evident. This result agreed with (Lossi et al., 1995; Ahmed and Eid. 2015; Fakhry et al., 2022).

At the age of 15 days post parturition, the current study illustrated that EGL decreased in thickness, composed of three or four layers of granule cells. The thickness of molecular layer increased and consisted of two cells; basket and stellate cells. Stellate cells situated in uppermost portion of molecular layer, had oval or round cell, darkly stained basophilic nuclei and distinct nucleoli, while the basket cells located in the bottom portion of the molecular layer, close to Purkinje cell, had spherical or triangular shaped cells and contained vesicular basophilic nuclei with prominent nucleoli. The Purkinje cell layer organized in one layer between ML and IGL. IGL was formed from densely packed cells. It consisted of two types of cells: granule cells and Golgi Type II cells. The granule cells were small, numerous and tightly packed. They were spherical with huge spherical nuclei and minimal quantity of cytoplasm and gathered to create a cluster of cells. Golgi cells were larger than the granule cells had an oval or triangular cell body with vesicular nucleus and more cytoplasm as reported by (Lossi et al., 1995; Marcelo and Fahad, 2002; Bahgat et al., 2006; Allam et al., 2011; Galas et al., 2017; Amin et al., 2019).

In the current investigation, at the age of 22 days post parturition, as the previous age but the external granular layer decreased in thickness. The thickness of molecular layer increased than previous age. There were small spaces between the granular cells called cerebellar glomeruli appeared as random light region as reported by (Lossi et al., 1995; Allam et al., 2011). Xu et al. (2013) reported that, a single raw of EGL was still present covering some areas, was absent completely in certain areas and had disappeared totally at 28 days postnatally in rat. Dalia (2002) reported that. EGL was disappeared completely at 21 days after parturition in guinea pig. Ultrastructurally, the molecular layer contained basket cell had large nucleus, minimal granulated cytoplasm and multiple myelinated nerve fibers, filled with mitochondria and surrounded by normal myelin sheath.

The Purkinje cells had large rounded euchromatic nuclei with well-developed nucleoli and nuclear envelope. The cytoplasm contained numerous mitochondria, Golgi apparatus, free ribosomes and rough endoplasmic reticulum. Granular cells had spherical nuclei with clumps of heterochromatin. They had few cytoplasm with little mitochondria, rough endoplasmic reticulum and ribosomes. This result agreed with Fakhry et al. (2022).

At the age of one month, as the previous age but the external granular layer was disappeared in some areas. These results agreed with (Lossi et al., 1995; Hayam et al., 2019).

At the age of two month, the current study illustrated that cortex formed of granular layer, Purkinje cell layer and molecular layer. External granular layer was completely disappeared. The molecular layer increased in thickness and consisted of two cells, basket cells and stellate cells. The stellate cells located in uppermost portion of the molecular layer, had oval or round darkly stained basophilic nuclei and prominent nucleoli, while the basket cells located in the bottom region of the molecular layer, close to the Purkinje cell, had spherical or triangular shaped cells, and lightly stained basophilic nuclei and prominent nucleoli. Purkinje cell layer was organized in one layer and appeared as flask-shaped cells contained vesicular nuclei and apparent nucleoli. The granular layer consisted of closely packed granular cells. It consisted of two cells: granule cells and Golgi Type II cells. The granule cells were small, numerous and tightly packed. They were spherical with spherical nuclei and minimal quantity of cytoplasm. Golgi cells were greater than the granule cells had an oval or triangular cell body with vesicular nucleus and more cytoplasm. This agrees with results of Lossi et al. (1995); Pal et al. (2003); Eurell and Frappier (2006); Zhang et al. (2006); El-Ghazali (2008); Salankar (2017); Hayam M. Farhoud et al. (2019); Fakhry et al. (2022). The white matter of each cerebellar hemisphere had large concentrations of nerve cells called deep cerebellar nuclei. These were grouped from lateral to medial; they were the dentate, emboliform, globose, and fastigial nuclei. This agrees with results of Eroschenko (2005).

Ultrastructurally, the molecular layer contained basket cell that had large nucleus and little granular cytoplasm and multiple myelinated nerve fibers, filled with mitochondria and surrounded by normal myelin sheath. The Purkinje cells had large spherical nuclei with well- developed nucleoli and nuclear envelope. The cytoplasm contained multiple mitochondria, numerous well-developed rough endoplasmic reticulum, free ribosomes and Golgi apparatus. Bergmann astrocyte ensheathed a Purkinje cell with their processes. It had euchromatic nucleus and pale cytoplasm contains ribosomes, mitochondria and surrounded by blood vessel. The granule cells had spherical heterochromatic nuclei. They had few cytoplasm with little mitochondria, ribosomes and rough endoplasmic reticulum. This result agreed with Fakhry et al. (2022).

Lossi et al. (1995) suggested that the EGL was decreased in thickness until disappeared, while IGL and molecular layer increased in thickness until the anatomical characteristics of the mature cerebellum were reached from two months onward.

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