Histological, immunohistochemical and Scanning Electron Microscopic Studies on the Cerebellum of Dromedary Camel (*Camelus dromedarius*)

Rasha Ragab Beheiry
Department of Histology and Cytology. Faculty of Veterinary Medicine, Zagazig University

With 8 figures **Received October, accepted for publication Novenber 2015**

Abstract

Little researches are known about the cerebellum of camel. This work aimed to study the morphology and histology of the cerebellum in ten camels by using histological paraffin sections and scanning electron microscope. Also, avidin-biotin complex peroxidase (ABC) method for the demonstration of glial fibrillary acidic protein (GFAP), and S-100 protein.

The results revealed that the cerebellum was formed from cortex and medulla. The cerebellar cortex consists of three layers, outer molecular layer, middle Purkinje cell layer and inner granular layer. The immunohistochemical findings show that S100 and GFAP immunoreactivity was found in the three layers of cerebellar cortex and in the medulla. The cells of the molecular layer, Purkinje cells and the granule cells were immunostained with anti-S-100 protein, in addition the glia cells and their processes, which were detected in form of network were also strongly immunostained. GFAP immunoreactivity in the molecular layer was detected but less dense than S100-immunereactivity. The Purkinje cells were surrounded by brown network of GFAP immunoreactive glial cells. In the granular layer, the GFAP immunoreactivity showed similar reaction to those of S100 - immunoreactivity. Scanning electron microscopic findings rvealed that the dorsal surface of the cerebellum showed two elevated ridges (folia) in between groove (sulci). The dorsal surface of cerebellar cortex showed a corrugation, which resembles the irregular bleb like protrusions.

Conclusions: the results obtained from this study revealed that the cerebellum of camel contains immunoreactive S100 protein and GFAP.

Keywords: histology, immunohistochemistry, scanning microscopy, GFAP, S100, cerebellum, camel.

Introduction

The camel is an important species adapted to hot and arid environments (Schwartz, 1992). In spite of its economic value, camel has for a long

time remained the most neglected animal in the field of scientific research. One of the important reasons for this neglect that it is mostly found in arid and tropical areas of Africa and Asia where poor nutrition and husbandry are the major shortcomings (Sohail, 1983). The literatures about their nervous system remain still relatively limited. The cerebellum is an important organ responsible for timing, balance, and coordination of movements. It was mentioned that its shape and size are related to extremity movements, gravity centre, and species posture (Mondal, 1997, Mial and Reckess, 2002). Two morphological features of the cerebellum are the variation in both its volume and foliation (amount of surface folding) between species: amphibians and reptiles have unfolded cerebella while birds and mammals have variably convoluted cerebella (Larsell, 1967 and Iwaniuk et al., 2006). Zachary et al. (2013) suggest that increasing cerebellar foliation may be allowing improved motor control and increasingly complex behaviours. In many studies, species differences in cerebellar size and morphology, including the relative size of individual folia and the overall degree of folding of the cerebellum have been documented (Iwaniuk and Hurd, 2005 and Iwaniuk et al., 2007) but less in camel. The intermediate filaments are cytoskeleton proteins present in most vertebrate cell types. Astrocyte precursors of the central nervous system usually express vimentin as the major intermediate filaments. Astrocyte maturation is followed by a switch between vimentin and glial fibrillary acidic protein (GFAP) expression, the latter being recognized as an astrocyte maturation marker (Eng, 1971). S100 protein is a 20 kDa calcium-binding protein that accumulates during central nervous system maturation in mammals. There is an increase in S100β mRNA and protein levels during infancy indicative of postnatal astrocytic maturation (Marks, 1996). The purpose of this study was to provide an overview about the histomorphology of camel cerebellum through the histological sections and study the presence of the glial marker proteins, the S-100 and the glial fibrillary acidic (GFAP) protein, and to describe the morphology of dorsal surface of cerebellum using scanning electron microscopy. These results provide reference for future studies in related research.

Materials and Methods

In this study camel's brain were collected from ten camel heads, 3 to 6 years old without sex differentiation, directly after slaughter from Zagazig abbatoir. After collection of the whole brain by careful dissection, pieces of cerebellum were made by a sharp knife on desired plane (the nodulus lobe) and fixed in 10% buffered neutral formalin for histological observation, the tissue samples were routine

histological and immunohistochemical processing. The slides stained by Harris's haematoxylin and Eosin for studying the general structures and Periodic acid Schiff technique for neutral and some acidic mucopolysaccharides (Bancroft and Gamble, 2001).

Tissue preparation for immunohisto-chemistry (Hsu et al., 1981).

For the immunohistochemistry, the streptavidin-biotin peroxidase complex (ABC) method was utilised for 5 µm thick sections. All staining procedure was carried out according to manufacturer protocol of Thermo Scientific. Sections were deparaffinized in xylene and rehydrated through a graded series of ethanol. To reduce nonspecific back ground staining due to endogenous peroxidase, incubate slides in H2O2 for 10 minutes then wash two times in phosphate-buffered saline (PBS; pH 7.4). Incubate slides in microwave EDTA (pH 8) for 15-20 minutes at 99 c then wash in tap water. Wash 4 times in buffer optional apply (Ultra V BlockVR (normal serum) and incubate for 5 minutes at room temperature to block nonspecific back ground stain. Wash four times in PBS. Apply the primary antibody (GFAP Cataloge. No. monoclonal antibody AB1-Ms 280 R & S100 Cat. No. monoclonal antibody AB1-Ms 296 R7 Thermo Fisher Scientific, LabVision Corporation) and incubate according to manufacture protocol. Wash four times in PBS. Apply in biotinylated goat antipoly valent and incubate for 10 minutes at room temperature then wash four times in PBS. Apply strept avidin peroxidase and incubate for 10 minutes at room temperature then rinse four times in PBS. Finally add drop of DAB (diaminobenzidin)+ chromogen to 2 ml of DAB+ substrate, mix good and apply to tissue then incubate for 5-15 minutes depending on the desired stain intensity. Wash in tap water. Sections were counterstained with hematoxylin for 3 min, dehydrated through an alcohol series, cleared in xylene, and mounted by using DPX**.**

Tissue preparation for scanning electron microscopy (Bozzola and Russel, 1999).

The specimen of the cerebellum of adult camel were fixed in 2.5% glutaraldehyde in 0.1 m sodium phosphate buffer, PH 7.3, then the specimens were washed 3 times in in 0.1 m sodium phosphate buffer, PH 7.3 for 10 minutes each and post fixed in 1% buffered osmium tetroxide for 2 hours at 4°C, the specimens were again washed in0.1 m sodium phosphate buffer, PH 7.3 (three times for 5 min each) and then in distilled water (2 times for 5 min each), dehydrated in ascending grades of alcohol and dried to the critical point. The specimens were mounted in metal base, sputtered with gold in an Emitech K550 sputter apparatus analyzed and pho-

tographed under LEO 435 VP and JEOL JSM 5200 scanning electron microscope in Faculty of Medicine Tanta University.

Results

I. Light microscopic findings

Histomorphology

The dorsal surface of the cerebellum in camel has many folia (elevated ridges) separated by sulci (deep grooves). The thickness of granular layer was greater than molecular layer on the folial summit (top of folia) and thinnest than the molecular layer at the bottom (fissure) of the folia. The cerebellum of camel was formed from outer cortex (grey matter) and inner medulla (white matter). The cerebellar cortex consists of three layers, outer molecular layer, middle Purkinje cell layer and inner granular layer (Fig. 1 a, b).

1) Cerebellar cortex (Grey matter)

A) Outer molecular layer

The outer molecular layer was formed from few numbers of nerve cell bodies. Two types of cells have been observed, stellate and basket cells. The stellate cells were found in the superficial region of the molecular layer and it was darkly stained with H&E stain (Fig. 2 a) while the basket cells were deeply situated and stained lightly with H&E stain (Fig. 2 b). Also it contained nerve fibers.

B) Purkinje cell layer

The Purkinje cells present in one row at the junction between molecular and granular layers. The cells were oval or round or flask in shape with round central nuclei. The nucleus was vesicular with a deeply stained nucleolus. The dendrites of the Purkinje cells extend to the molecular layer. At some places aggregations of granule cells were found between Purkinje cells. In PAS stained sections, the Purkinje cell cytoplasm has showed strong positive reaction (Fig. 3a,b).

C) Granular cell layer

The granular layer was between the white matter and the Purkinje cell layer and consisted of large number of small cells with nerve fibres. The granule cells represent the predominant cells in this layer; these cells were round with large round nuclei and small amount of cytoplasm. These cells were closely packed together in H & E stain, resembling lymphocytes. Between the granule cells there were small spaces called cerebellar islands or glomeruli appearing as irregular light areas. In addition, few glial cells and stellate neurons were present among them. Large Golgi cells were also found; these cells were round in shape and have irregular excentric nuclei. The granular layers showed positive reaction to PAS stain (Fig. 4a,b).

2) Cerebellar medulla (White matter)

The white matter formed from nerve fibers, few glia cells and blood ves-

sels which gave positive reaction to PAS stain (Fig. 5 a, b).

II. Immunohistochemical findings

S100: S100 immunoreactivity was found in the three layers of cerebellar cortex: molecular, Purkinje and granular cell layers (Fig. 6a). The basket cells and stellate cells in molecular layer were immunostained with anti-S-100 protein. The Purkin-je cells were, positive for S100 immunostaining, which detected beside the dendrites and around nerve cell body (Fig. 6b). In granular cell layer, the reaction with anti-S-100 protein was strongly positive which present in between the clusters of granular cells, in addition the glia cells and their processes which were detected in form of network were also strongly immunostained (Fig. 6c). S-100 immunoreactivity was also detected in the white matter (Fig. 6d).

GFAP: GFAP immunoreactivity was found in both cerebellar cortex and the medulla. In the molecular layer the GFAP immunoreactivity was detected with few or moderate reaction (less dense than S100-immuno-reactivity). The purkinje cells were surrounded by brown network of GFAP immunoreactive glial cell. In the granular layer, the GFAP immunoreactivity showed similar reaction to those of S100-immunereactivity. In the white matter, GFAP reactivity was seen surrounding the blood vessel and the glia cells and forming network around the nerve fibers (Fig. 7a,b, c).

III. Scanning electron microscopic findings

In low magnification the dorsal surface of the cerebellum of camel showed two elevated ridges (folia) in between groove (sulci) (Fig 8. a and b). In high magnification the dorsal surface of cerebellar cortex showed the corrugation, which resembles the irregular bleb like protrusions (Fig 8 c). The molecular layer showed different shapes of cells, the identification of these cells needs different technique (Fig. 8 d).

Discussion

The cerebellum was known to involve in cognitive processes, including learning, memory and language in humans (Ito, 1993). (Carpenter, 1995 and Pal et al., 2003) stated that the function of the cerebellum is modified by experience.

Differences in volume, morphology, and histology of the cerebellum appear to relate to the performance of other types of tasks, and some differences are known to exist according to species, general anatomic structure and behavioural features (Mondal, 1997, Mial and Reckess, 2002 and Iwaniuk et al., 2006). The present study revealed that the dorsal surface of the cerebellum in camel has many

folia separated by sulci grooves. The thickness of granular layer was greater than molecular layer on the folial summit and thinnest than the molecular layer at the bottom of the folia. These results were proved by some authors (Sur et al., 2001).

The observation revealed that in camel cerebellum the cortex consists of three layers, outer molecular layer, middle Purkinje cell layer and inner granular layer. These results are similar to the findings of some authors (Williams et al., 1995) and (Pal et al., 2003) in human cerebellum and in goat cerebellum (El-Ghazali, 2008).

In the current study, the outer molecular layer was formed from two types of cells, stellate and basket cells. The stellate cells were found in the superficial region of the molecular layer and it was darkly stained while the basket cells were deeply situated and stained lightly with H&E stain. The same results were obtained by some authors (Helmut, 1977), (Carpenter, 1995) and (Pal et al., 2003) in human cerebellum and (El-Ghazali, 2008) in goat.

The present work revealed that the Purkinje cells present in one row at the junction between molecular and granular layers. The cells were oval or round or flask in shape with round central nuclei. The nucleus was vesicular with a deeply stained nucleolus. The dendrites of the Purkinje cells extend to the molecular layer. At some places aggregations of granule cells were found between Purkinje cells. These observations were similar to the findings of (Kuhlenbeck, 1975). The present work revealed that in PAS stained sections, the Purkinje cell cytoplasm showed strong positive reaction. It also was observed around the blood vessels. Similar observations were made by (El-Ghazali, 2008) in goat.

This study revealed that the granular layer was between the white matter and the Purkinje cell layer and consisted of a large number of small cells with nerve fibers. The granule cells represent the predominant cells in this layer; these cells were round with large round nuclei and small amount of cytoplasm. Similar observations were made by some authors (Robert, 1964) and (El-Ghazali, 2008).

In the present work granule cells were closely packed together in H&E stain, resembling lymphocytes, this was in agreement with (El-Ghazali, 2008) in goat cerebellum. Between the granule cells there were small spaces called cerebellar islands or glomeruli, which appeared as irregular light areas. This was in agreement with (El-Ghazali, 2008 and Eroschenko, 2005). The observations showed that the cerebellar medulla form the white matter which formed from nerve fibers, few glia cells and blood vessels which gave positive reaction to PAS stain. This is in accordance to (Williams et al., 1995) and (El-Ghazali, 2008).

GFAP are regulated under developmental and pathological conditions. GFAP expression is one of the main characteristics of the astrocytic reaction, which was observed after central nervous system lesion. So the studies on GFAP regulation have been shown to be useful in both brain physiology and also neurological disease (Gomes et al., 1999, Eng et al., 2000 and Notle et al., 2001).

In the CNS of higher vertebrates, following injury, as trauma, disease or genetic disorders, astrocytes become reactive and respond in a typical manner, termed astrogliosis. Astrogliosis is characterized by rapid synthesis of GFAP and is demonstrated by increase in protein content or by immunostaining with GFAP antibody (Eng et al., 2000). Increase of GFAP in astrocytes occurs gradually throughout the adult lifespan of mice, rat and humans (Eng and Lee, 1998 and Nichols et al., 1993).

GFAP immunoreactivity in the mature CNS is restricted to glial filaments within protoplasmic and fibrous astrocytes, and radial glia in the cerebellum “Bergmann glia” and subependymal astrocytes adjacent to the cerebral ventricles. GFAP-like immunoreactivity was found in regenerating Telecast spinal cord, in Schwann cells, glia-like cells in the myenteric plexus, Kupffer cells and cells of the pineal gland. GFAP immunoreactivity also has been demonstrated in immature oligoden-drocytes, in pituicytes and pituitary adenomas, epiglottic cartilage, papillary meningiomas, and metastasizing renal carcinomas (Mc Lendon and Bigner, 1994 and Eng and Lee, 1995).

S100 protein is consists of two subunits S100α and S100 β that have different tissue distribution (Heizmann, 1999). S100 β localized to microtubules, centrioles, centrosomes and intermediary filament, while S100α is localized on intracellular membranes including triads, skeletal muscle cells and close to Z-discs in sarcomeres. S100α is most abundant in slow-twitch skeletal muscle cells, epithelial cells, olfactory receptor cells and some specific neuronal populations such as specific regions of the limbic system, whereas S100 β is most abundant in glia cells, melanocytes, chondrocytes, adipocytes and other neuronal subpopulations (Garbuglia, 1999 and Heizmann, 1999).

The present study showed that GFAP and S100 immunoreactivity was found in both cerebellar cortex and the medulla. Similar observations were made by some authors (Touimi et al., 1985) who revealed that astrocytes can be detected in the cerebellum of normal mice during the first 4 weeks of life. These cells are visualized with anti-GFAP (glial fibrillary acidic protein), anti-S100 and anti-vimentin immune sera. Their perikaryons, situated in the white matter or in the granu-

lar layer, extend long processes which are inserted onto the pial surface.

The current study showed that S100 immunoreactivity was found in both cerebellar cortex and the medulla. These results are similar to the results of some authors (Ghan-dour et al., 1981) in rat cerebellum, in rat vermis and flocculus few Purkinje cells showing S100 immunoreactivity (Rickmann and Wolff, 1995), also in chicken brain where a stronger immunoreactivity is observed (Goto et al., 1988).

The results of scanning electron micrograph were not recorded in available literature and it may be helpful for future researches.

References

Bancroft, J. D, Gamble, M. (2001): Theory practice of histological techniques,5th Ed. Churchill living stone.

Bozzola, J., Russel, L. (1999): Electron microscopy. Principles and techniques for biologists. 2nd ed. Boston, Toronto, London, Singapore: Jones & artlett Publishers; 2 Sub edition (October 1998); p. 148–201.

Carpenter's. (1995): Human Neuroanatomy 9th Ed. Williams and Wilkins, Baltimore USA: pp 538-622

El-Ghazali, H.M. (2008): Some morphological studies on the cerebellum of balady goat. M. V. Sc. Thesis, Faculty of Veterinary Medicine. Zagazig University.

Eng, L. F., Vanderhaeghen, J. J., Bignami, Gerste, B. (1971): An acidic protein isolated from fibrous astrocytes. Brain Research; 28: 351-354

Eng, L. F., Lee, Y. L. (1995): Intermediate filaments in astrocytes. In Neuroglia, pp. 650 –667. H. Kettermann and B. R. Ransom, eds. Oxford University Press.

Eng, L. F., Lee, Y. L. (1998): Glial response to injury, disease, and aging. In astrocytes in brain aging and neuro degeneratio , pp. 71– 89. H. M. Schipper, ed. R. G. Landes Co., Georgetown, TX.

Eng, L. F., Ghirnikar, R. S., Lee, Y. L. (2000): Glial Fibrillary Acidic Protein: GFAP-Thirty-One Years (1969–2000), Neurochemical Research; Vol. 25, Nos. 9/10, pp. 1439–1451

Eroschenko, V. P. (2005): Difor's atlas of histology with functional correlation, 10th Edition, W.B. Lippincott Williams and Wilkins. Pp 134-137

Garbuglia, M., et al. (1999): The calcium-modulated proteins, S100 A1 and S100 B, as potential regulators of the dynamics of type III intermediate filaments. Braz. J Med Biol- Res. 32:1117-1185

Ghandour, M. S., Labourdette, G., Vincendon, G. Gombos, G. A. (1981): Biochemical and Immunohistological Study of S100 Protein in

Developing Rat Cerebellum. Dev Neurosci; 4: 98–109.

Gomes, F. C. A, Paulin, D., Neto, V. M. (1999): Glial fibrillary acidic protein (GFAP): modulation by growth factors and its implication in astrocyte differentiation. BEfrfaezcitlsia onf Jgoruorwntahl ofaf cMtoerds iocna l GanFAd PB eioxlporgeicssailo Rnesearch; 32: 619-631

Goto, S. et al. (1988): A comparative immunohistochemical study of calcineurin and S100 protein in mammalian and avian brain. Exp. Brain. Res.; 69:645-650 (cited by Bastianelli E. Distribution of calcium binding protein in the cerebellum. Cerebellum 2003. 2:242-262)

Heizmann, C. W. (1999): Ca2+ binding S100 proteins in the central nervous system. Neurochem. Res; 24:1097-1100

Helmut, L. (1977): Translated by Winstaley, D. P.: In : human Histology, Cytology and microanatomy. George Thieme publishers' stuttgart. Germany: pp231-234

Hsu, S. M.; Raine, L.; Fanger, H. (1981): Use of Avidin-Biotin-Peroxidase Complex (ABC) in Immunoperoxidase Techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. Jour. Histochem. Cytochem ; 29 (4), S. 577–580.

Ito, M. (1993): Movement and thought: identical control mechanisms by the cerebellum. Trends Neurosci;16: 453–454.

Iwaniuk, A. N., Hard, P. L. (2006): Wylie DRW. The comparative morphology of the cerebellum in caprimulgiform birds: Evolutionary and functional implications. Brain Behav Evol; 67: 53–68.

Iwaniuk, A. N., Hurd, P. L. (2005): Brain Behav Evol, , 65, 15–230.

Iwaniuk, A. N., Peter, L. H. (2007): Douglas, RWW. Brain Behav Evol ; 69: 196–219

Kuhlenbeck, H. (1975): The Central Nervous System of vertebrates, Vol 4. Spinal cord and Deuterencephalon, S karger. Basel: pp 625-768

Larsell, O. (1967): The comparative anatomy and histology of the cerebellum from myxinoids through birds. Minneapolis, MN: University of Minnesota Press.

Marks, A., O'Hanlona, D., Leia, M., Percyb, M. E., Laurence, E., Beckerc, L. E. (1996): Accumulation of S100β mRNA and protein in cerebellum during infancy in Down syndrome and control subjects. Molecular Brain Research. Volume 36, Issue 2, March: 343–348

Mc Lendon, R, E., Bigner, D. D. (1994): Immunohistochemistry of the glial fibrillary acidic protein: Basic and applied considerations. Brain Pathol; 4: 221–228.

Mial, R. C., Reckess, G. Z. (2002): The cerebellum and the timing of coordinated eye and hand tracking. Brain Cognition;48: 212–226.

Mondal, R. K. (1997): Comparative gross anatomical and histomorphological studies on cerebellum of fish, amphibia, reptilia and mammalia. PhD Thesis. West Bengal University of Animal and Fisheries Science, Koklata, West Bengal, India.

Nichols, N. R., Day, J. R., Laping, N. J., Johnson, S.A., Finch, C. E. (1993): GFAP mRNA increases with age in rat and human brain. Neurobiol. Aging;14:421– 429.

Notle, C., Matyash, M., Pivneva, T., Schipke, C. G., Ohlemeyer, C., Hanisch, U., Kirchhoff, F., Kettenmann, H. (2001): GFAP Promoter-Controlled EGFP Expressing Transgenic Mice: A Tool to Visualize Astrocytes and Astrogliosis in Living Brain Tissue. GLIA; 33:72–86

Pal, B., Chowdhury, S., Ghosh, R. K. (2003): Comparative Anatomical Study of the Cerebellum of Man and Fowl, J Anat. Soc. India; 52(1): 32-37

Rickmann, M., Wolff, J.R. (1995): S100 protein expression in subpopulations of neurons of rat brains. Neuroscience; 67:977-991

Robert, M. Herndon. (1964): The fine structure of the rat cerebellum. II. The Stellate Neurons, Granule Cells and Glia. The journal of cell biology • VOLUME ~3,

Schwartz, H. J. (1992): Productive performance and productivity of dromedaries (*Camelus dromedarius*). Anim Res Dev; 35: 86–89.

Sohail, M. A. (1983): The role of the Arabian camel (Camelus dromedarius) in animal production. World Rev Anim. Prod; 19(3):38-40.

Sur, E., Oznurlu, Y., Ozaydin, T., Colakoglu, F., Unsal , S., Yener, Yno. (2001): Comparative histometrical study of the cerebellum and the dtermination of some Ag nor parameters in different avian species. Bull Vet Inst Pulawy; 55:261-265

Touimi, S. B., Jacque, C. M., Derer, P., De Vitry, F., Maunoury, R., Dupoueym, P. (1985): Evidence that mouse astrocytes may be derived from the radial glia: An immunohistochemical study of the cerebellum in the normal and reeler mouse. Neuroimmunology,; 9 : 87–97

Williams, P. L., et al: (1995): 38th Edn. Gray's Anatomy In: Nervous System. Churchill Livingstone, London, 1995 pp 1028-1065

Zachary, J, H., Sally, E. S.T., Susan, D. H. (2013): The evolution of cerebellum structure correlates with nest complexity. Biol Lett; 9. 20130687.

Corresponding author:
E-mail: rasharagab2006@yahoo.com

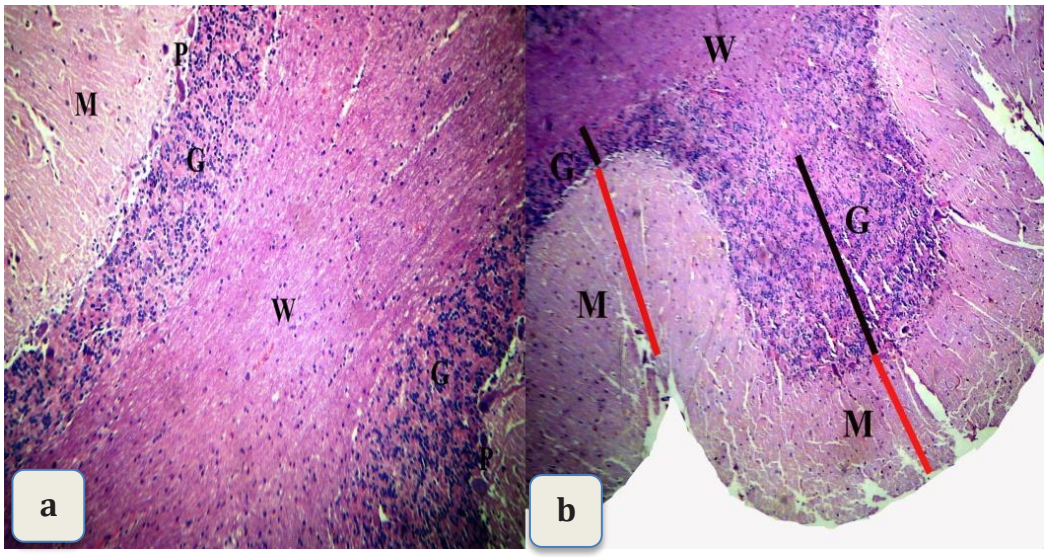

Fig (1): Photomicrograph of cerebellar cortex and medulla of camel. Molecular layer (M), Purkinje layer (P), granular layer (G) and white matter (W) in {a}. The thickness of granular layer was greater than molecular layer on the folial summit in {b}. Stain: H&E. x 100.

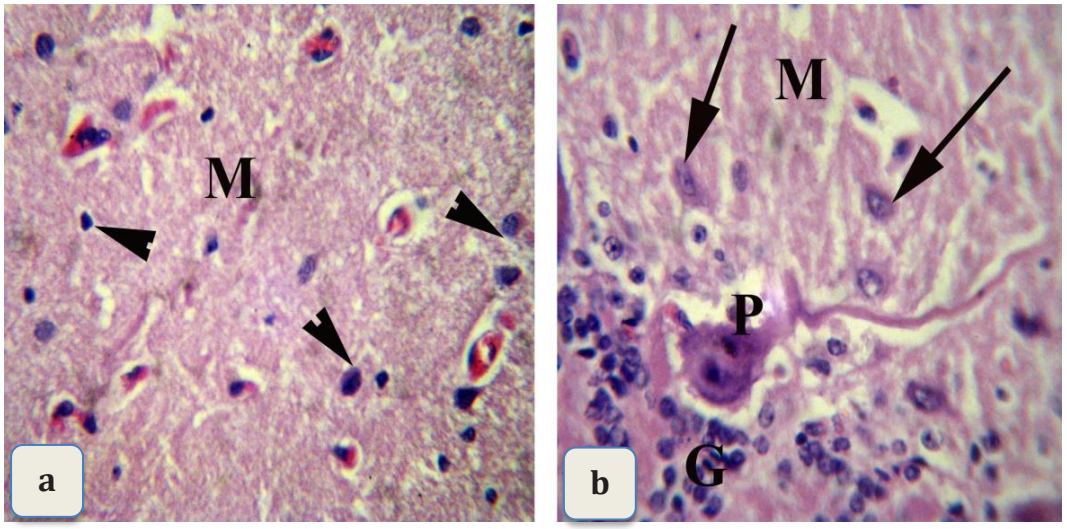

Fig (2): Photomicrograph of cerebellar cortex of camel. {a} showing the darkly stained stellate cells (arrow heads) in the superficial region of the molecular layer while in {b} the basket cells (arrows) were deeply situated and stained lightly with H&E stain. Molecular layer (M), Purkinje layer (P), granular layer (G). Stain: H&E.x 400.

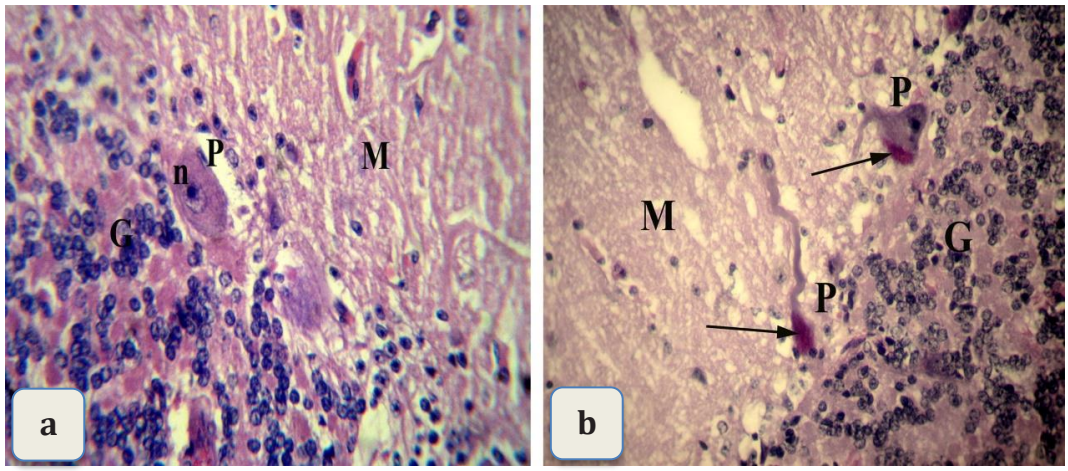

Fig (3): Photomicrograph showing the three layers of camel cerebellar cortex, molecular (M), Purkinje cell (P) and granular (G) cell layer. The Purkinje nucleus was vesicular with a deeply stained nucleolus (n) {a}. The dendrites of the purkinje cells extend to the molecular layer and the cytoplasm (arrows) showed strong PAS positive reaction {b}. Stain: H&E in {a} and PAS in {b}. x 400

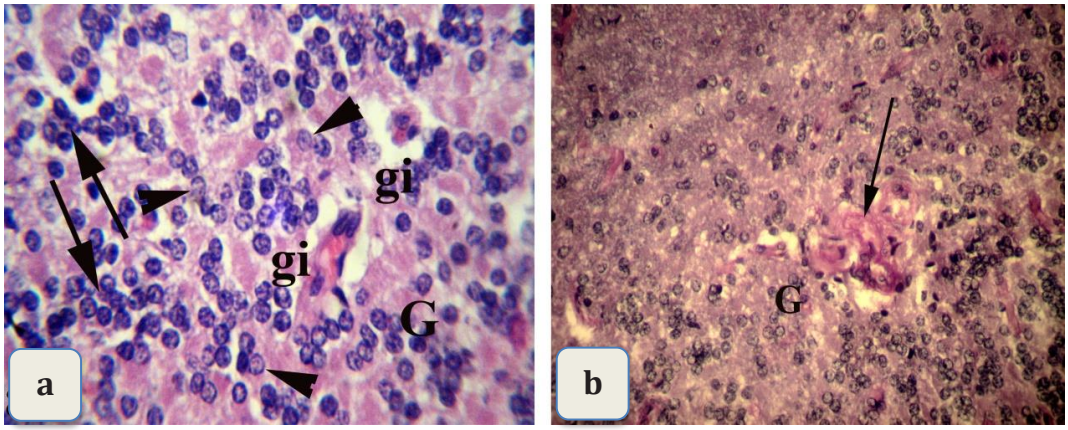

Fig (4): Photomicrograph showing the granular layer formed from granule cells (arrows), Golgi cells (arrow heads) and granular island (gi) {a}. The granular layers showed positive reaction to PAS stain (arrows) {b}. Stain: H&E in {a} and PAS in {b}. x 400

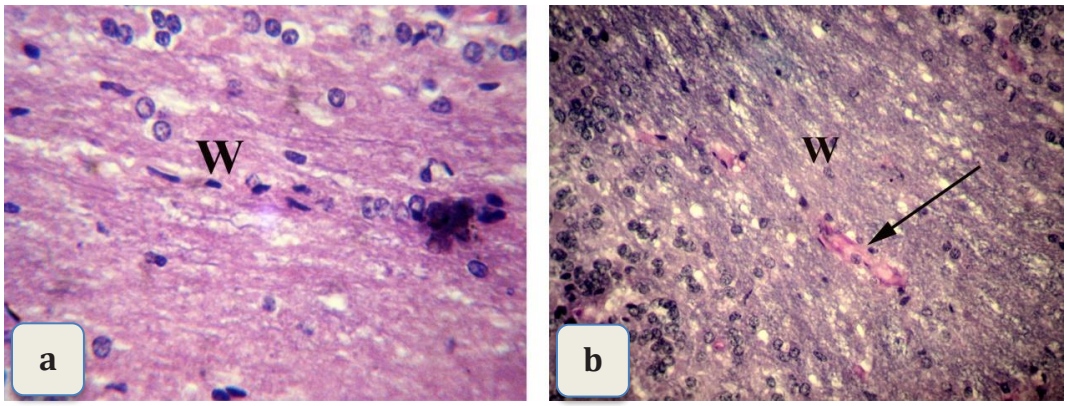

Fig (5): Photomicrograph showing the white matter (W) formed from nerve fibers, few glia cells and blood vessels which gave positive reaction to PAS stain (arrow). Stain: H&E in {a} and PAS in {b}.x 400

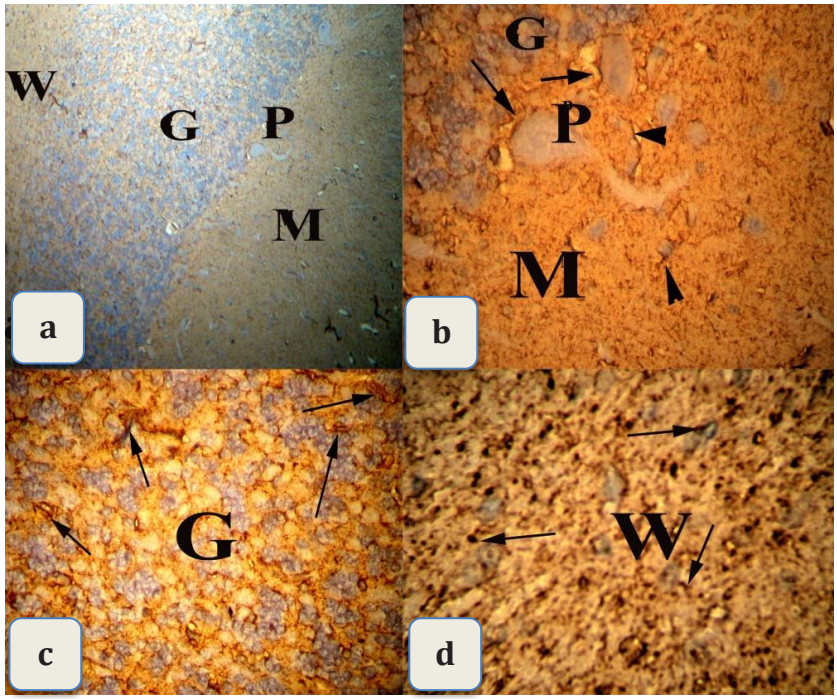

Fig (6): Immunostaining of S100 in cerebellar cortex and medulla of camel in {a}. Cerebellum was immunostained with anti-S100. Antibody antigen complexes were detected and using a peroxidase- ABC kit and DAB. Sections were counterstained with hematoxylin. Molecular layer (M), Purkinje layer (P), granular layer (G) and white matter (W). The reaction in the molecular layer (arrow heads) and around cell body of Purkinje cell (arrow heads) in {b}. The reaction in the granular layer in form of network (arrows) in {c}. The reaction in the cerebellar medulla (arrows) in {d}. Immunoperoxidase staining. x100 in a&400 in b,c,d

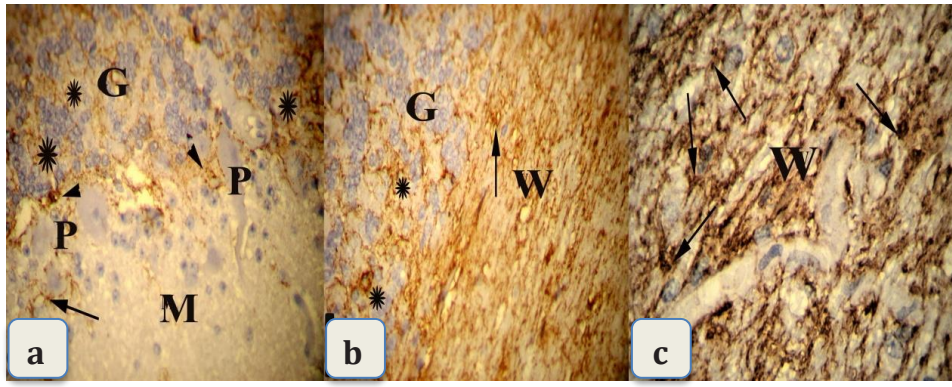

Fig (7): Immunostaining of GFAP in the cerebellar cortex of camel. Cerebellum was immunostained with anti-GFAP. Antibody antigen complexes were detected and using a peroxidase- ABC kit and DAB. Sections were counterstained with hematoxylin. Molecular layer (M), Purkinje layer (P), granular layer (G). In {a}, the reaction in molecular layer is few, the Purkinje cells were surrounded by brown network of GFAP immunoreactive glial cell (arrow heads) and (arrow) and the reaction in granular layer (stars). In {b}, the reaction in granular layer (stars) and white matter (arrow). In {c}, GFAP reactivity was observed surrounding in form of network around the nerve fibers (arrows). Immunoperoxidase staining. x 400.

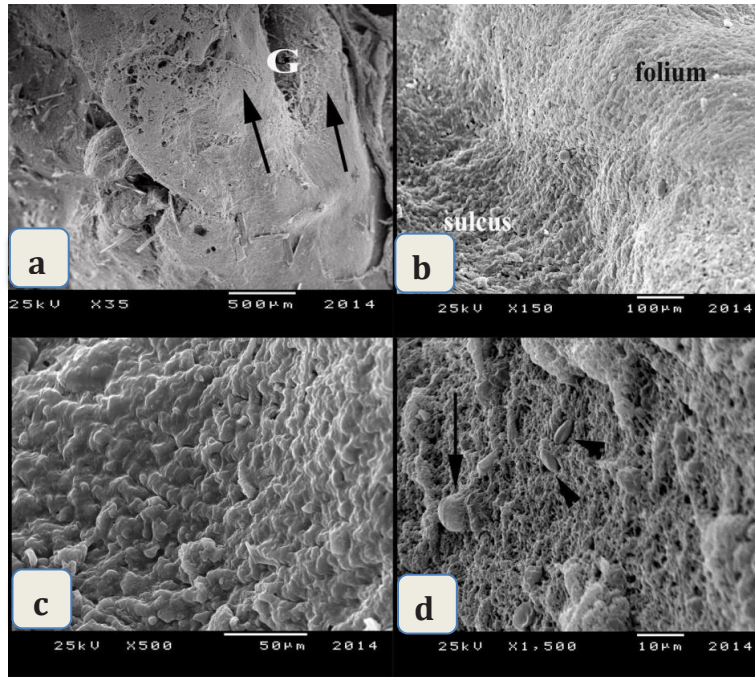

Fig (8): Scanning electron micrograph showing the dorsal surface of camel cerebellum at nodulus lobe resembling two elevated ridges (arrows) in between groove (G) in{a}. Micrograph {b} showing the folium and the sulcus. Micrograph {c} was magnification to the image {b} showing the corrugation of dorsal surface resembling the irregular bleb like protrusions. Micrograph {d} showing different types of cells (arrow heads and arrow) on the molecular layer of cerebellar cortex.