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Histological and Histochemical Studies on the Choroid Plexus in *Sciurus Linnaeus*

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Abstract

The present study aimed to investigate the histological and histochemical characteristics of the choroid plexus in adult male and female *Sciurus Linnaeus*. The current study included 30 adult Sciurus Linnaeus of both genders. The animals were divided into two groups: the first was used for histological studies, while the second was used for histochemical investigation. The animals were euthanized by intravenous injection of sodium phenobarbital (80 mg/kg). The sections were stained using hematoxylin-eosin, (PAS), combined PAS-AB (pH-2.5), Masson's trichrom, and Gomori trichrom stains. At magnification, the choroid plexuses of the lateral ventricle are characterized by parallel folds of different dimensions, while those of the third and fourth ventricles are composed of a great number of processes with a leaf-like appearance connected by a stem to the stromal tissue's more basal core. The stromal tissue expanded into the leaves, along with capillaries that broke up into capillary plexuses in the leaf body. The stromal tissue choroid plexus developed bigger, with a single layer of cuboidal epithelium that is nearly ciliated covering its highly vascular structure. A significant volume of connective tissue separates the epithelium from the blood vessels. The histochemical results showed that the choroid plexus and ependymal cells in the villi showed a positive reaction for PAS, Gomori trichrom, while the same parts gave a negative reaction AB. In the combined PAS-AB (PH-2.5) stains, the connective tissue revealed the presence of collagen fibers by using Masson's Trichrom.

Key words: Histological, Histochemical, Choroid Plexus, Sciurus Linnaeus.

Introduction

The choroid plexus was made up by a vascular folding from pia mater that was coated on the outside with an epithelium resulting from the ependymal coat of the ventricles [1]. The ventricles are four ducts that connected and fully occupied by the cerebrospinal fluid (CSF) inside the major

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regions of brain. Within these ventricles the choroid plexuses are positioned and provide the CSF and serve as a unique boundary between CSF and peripheral blood [2, 3]. Two essential roles of the choroid plexus: are inhibition of exogenous material entry to the brain as well as generation and regulation of components of the CSF [4, 5]. According to [6-9] the choroid plexus has two basic functions: it produces and regulates CSF components, it prevents foreign substances from entering the brain, and it produces numerous proteins with nutritional and neuroprotective properties to the brain. There is a dearth of knowledge regarding the choroid plexus in rodent animals. Therefore, the goal of the current study was to compare the choroid plexuses of the Sciurus Linnaeus to those of other species in order to better characterize them [10, 11].

Materials and Methods

A total number of thirty heads of *Sciurus Linnaeus* (males and females) were used. The samples were gathered from local suppliers in market animal. The specimens of Choroid plexus were separated from the heads and taken for the histological and histochemical study. Each was washed thoroughly with 0.9 % normal saline solution and kept in clean plastic containers. A part of specimens was fixing in 10% neutral buffered formaldehyde and other part was fixed in Bouin's solution, where they kept for two days and dehydrated by graded alcohol sequences as (70%, 80%, 90%, 95%, and 100%) for two hours for each concentration. The specimens were cleared in xylene for one hour in two changes and Infiltrated in molten wax of paraffin. The sections of 5μm thickness prepared from the blocked specimens by the use of the rotary microtome (Series MRS3500, Histo-Line laboratories Ltd, Italy), then were mount on glassy slides and staining using Mayer's Hematoxylin and Eosin to demonstrate the general histological components of the tissues [12, 13].

Also the specimens were stained with PAS stain and combined AB-PAS (pH-2.5) was used for determination acidic and neutral polysaccharide [12]. The collagenous and smooth muscle fibers were identifying by Masson’s trichrome stain and Gomori’s method for reticulum. All stained sections with routine and special stains were examined by a microscope and (LCD Digital Microscope (micros) with embedded Android based computer system, AUSTRIA.

Results

At small magnification the choroid plexuses of the lateral ventricle are characterized by parallel folds of different dimensions (Figure 1); while, the third and fourth ventricles were composed of a great number of processes with leaf-like appearance connected by a stem to the stromal tissue's more basal core. Secondary elevations with a leaf-like appearance might rise from the primary leaves (Figure 1). Inside the leaves the stromal tissue extends together with vessels that splitting up into capillary plexuses at the bulk of leaves (Figure 2).

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Cuboidal or epithelium of a single layer of covers the choroid plexus's highly vascular stroma (Figure 3). However, the epithelial covering of the third and fourth ventricle choroid plexuses appeared to be bigger than that of the lateral ventricle plexus due to their tree-like branching. The cytoplasm of cuboidal epithelium was mostly stained by eosin stain with granular appearance, particularly in the supranuclear area. Some cells have microvilli and cilia on their apical borders, whereas others appear bulged or have secretory protrusions (Figure 4). The cytoplasm is stained with PAS and has a negative reaction to AB (Figures 5, 6).

A connective tissue of big quantity separates the blood vessels from the choroidal epithelium. The collagen bundles are the most important stromal component (Figure 3). The stroma of the connective tissue appeared very thin that the endothelium of arteries can join to the basement membrane of choroid epithelia (Figures 4-6). The presence of many blood vessels distinguishes the connective tissue stroma. The lateral ventricle's blood channels are long, wide, slightly convoluted, and moderately parallel (Figures 3, 6). Choroid plexus of the lateral ventricle has a high vascular density (Figure 2).

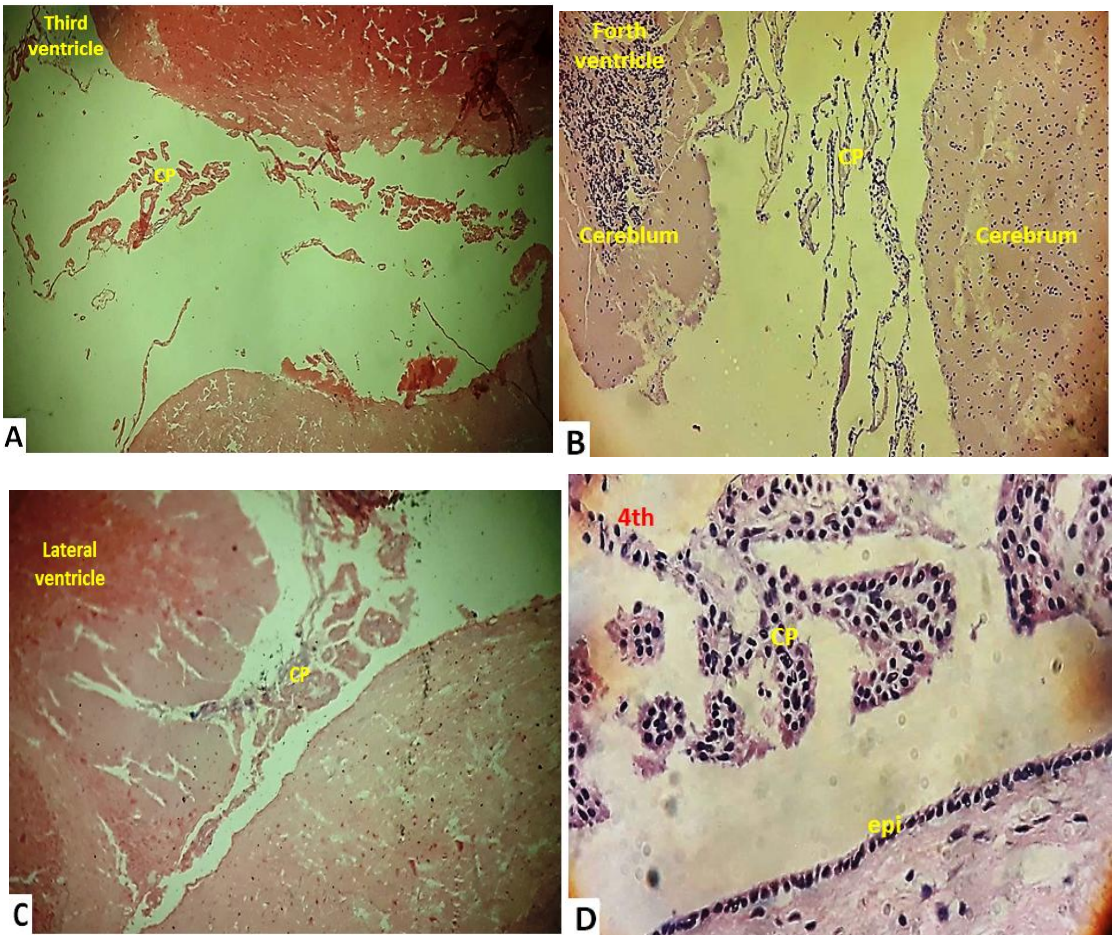

Figure (1, A-D): Masson trichrom and H&E stains sections: choroid plexuses (cp) of the: third, fourth and lateral ventricle showing, covering cuboidal epithelium (epi), the lumen of the ventricles (X200),

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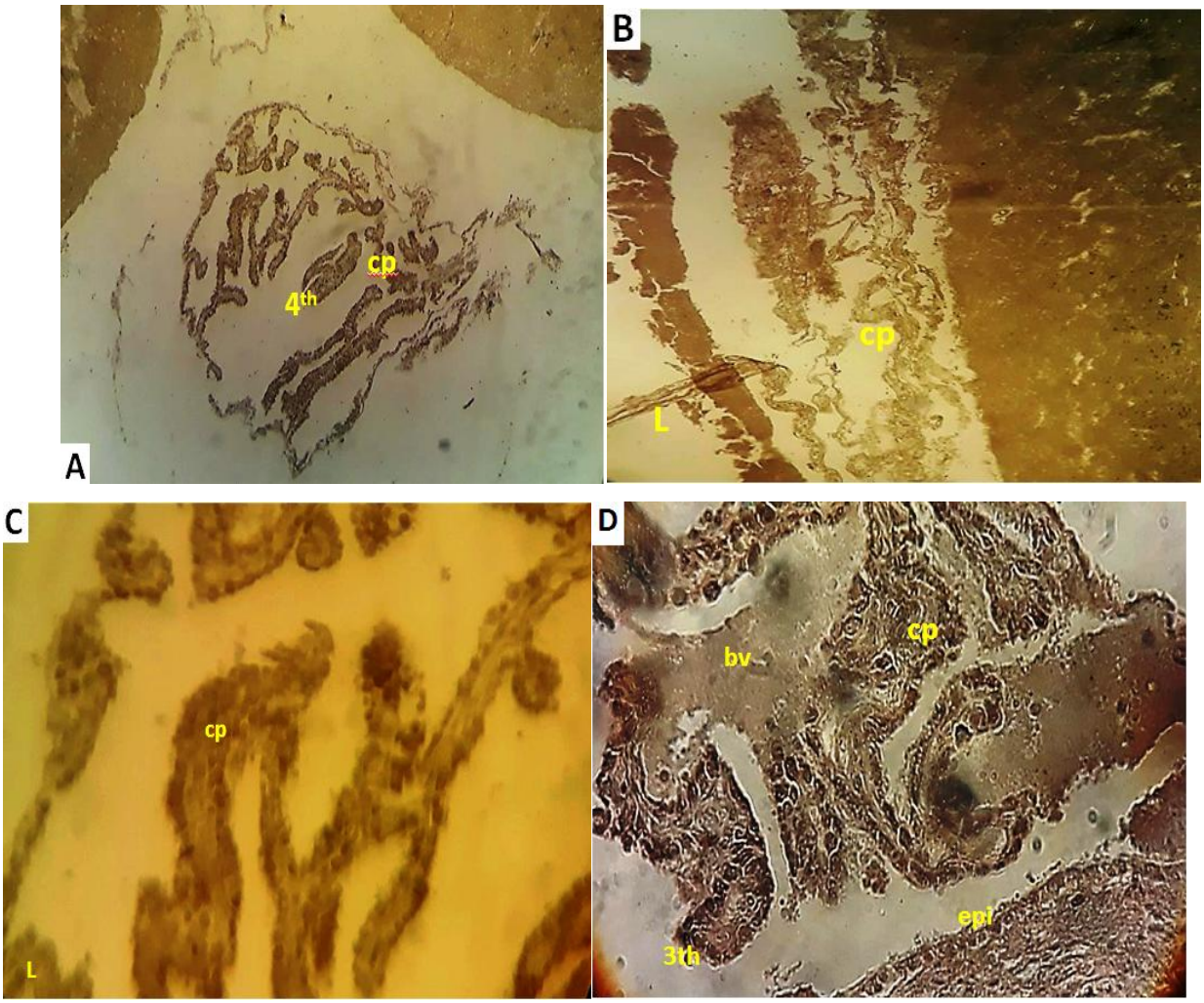
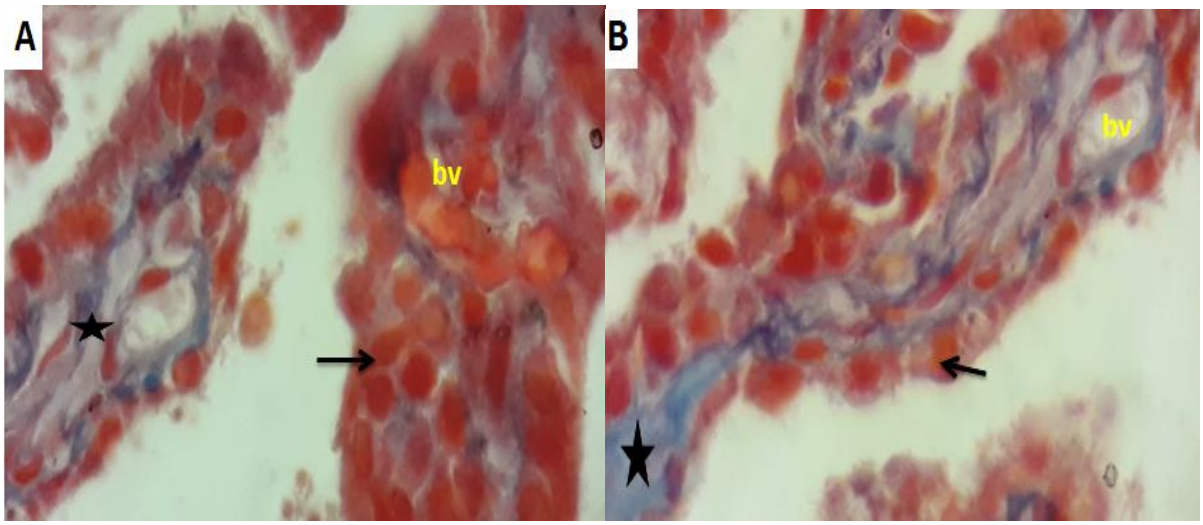


Figure (2, A-D): Gomori's stain shows high magnification of choroid plexus (cp) of 4th; lateral and third ventricle viewing epithelium is high cuboidal-low columnar (epi), lumen of the ventricles (L) and Blood vessels; A & B (100X), C&D (400X)



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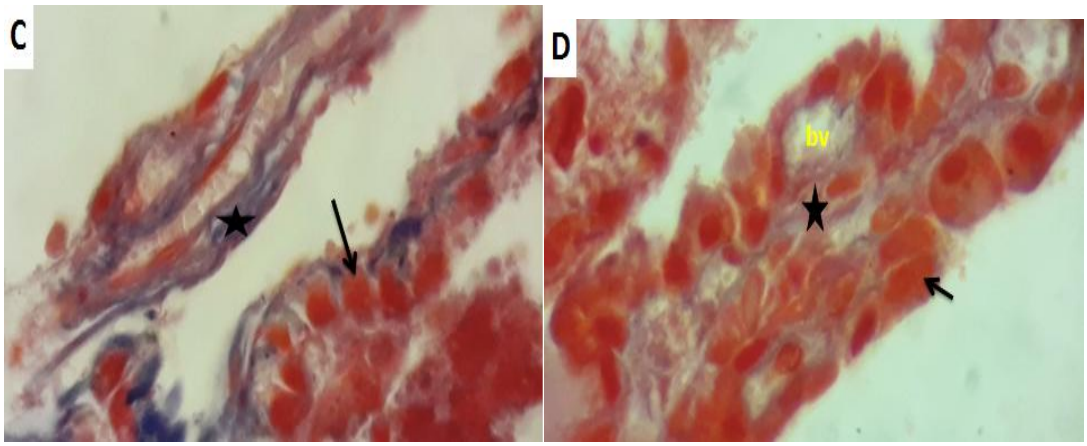

Figure (3, A-D): Masson trichrom stain sections; choroid plexus of ventricles screening cuboidal epithelia (black arrow), with cilia and swollen luminal border as well as the lumen of ventricles connective tissue stroma with collagen fiber (black star), 400X

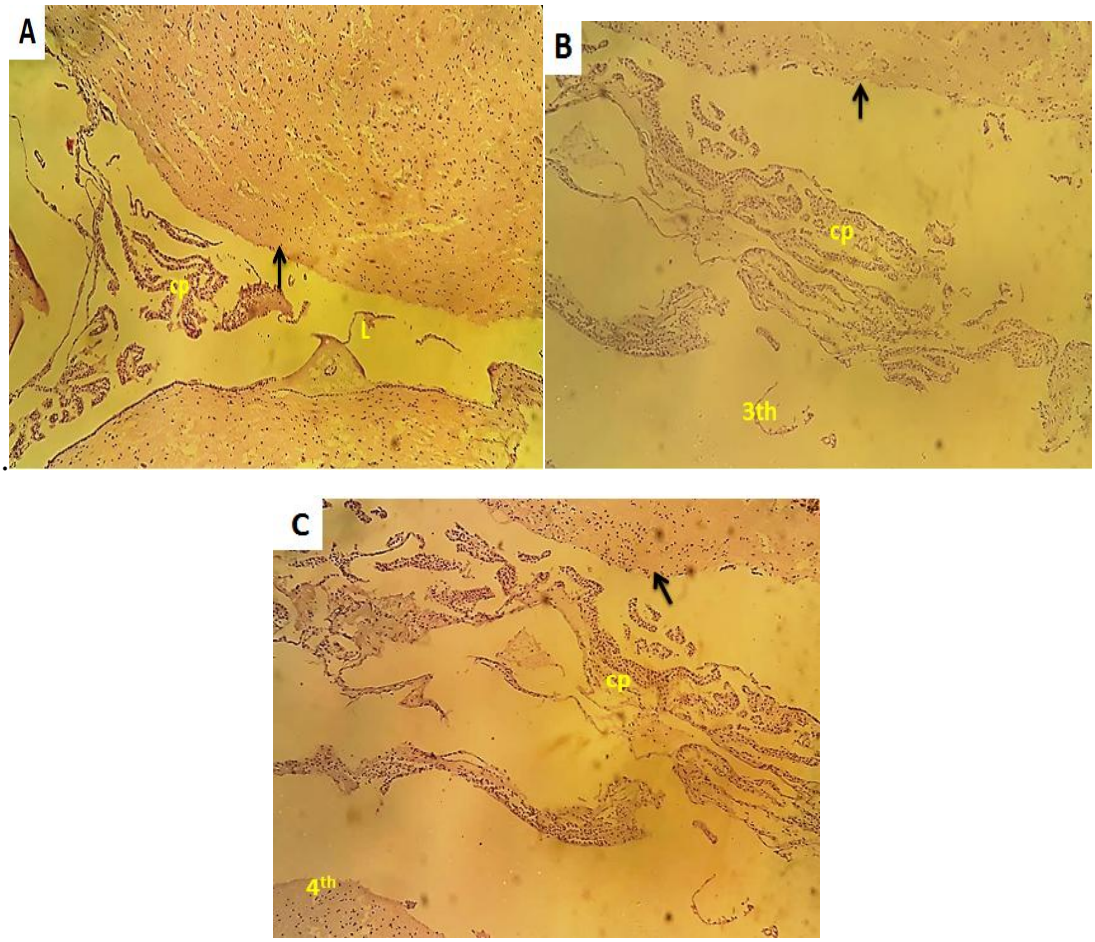

Figure (4: A-C): Combined PAS-stained sections at choroid plexus of ventricles viewing the covering epithelia (cp) and ependymal cells were faintly stained for PAS stain except for its apical border and negative to alcian blue stain 200X

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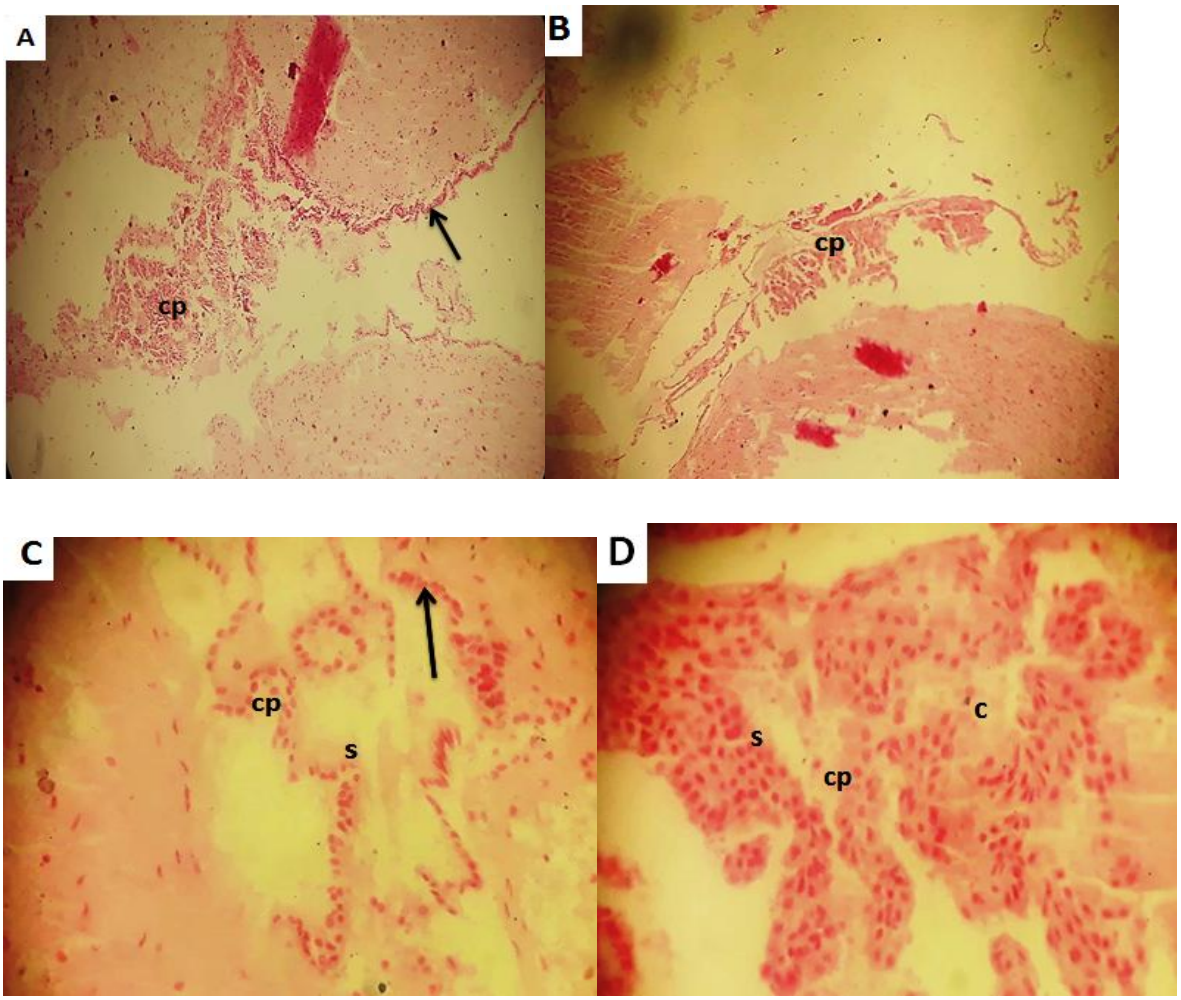
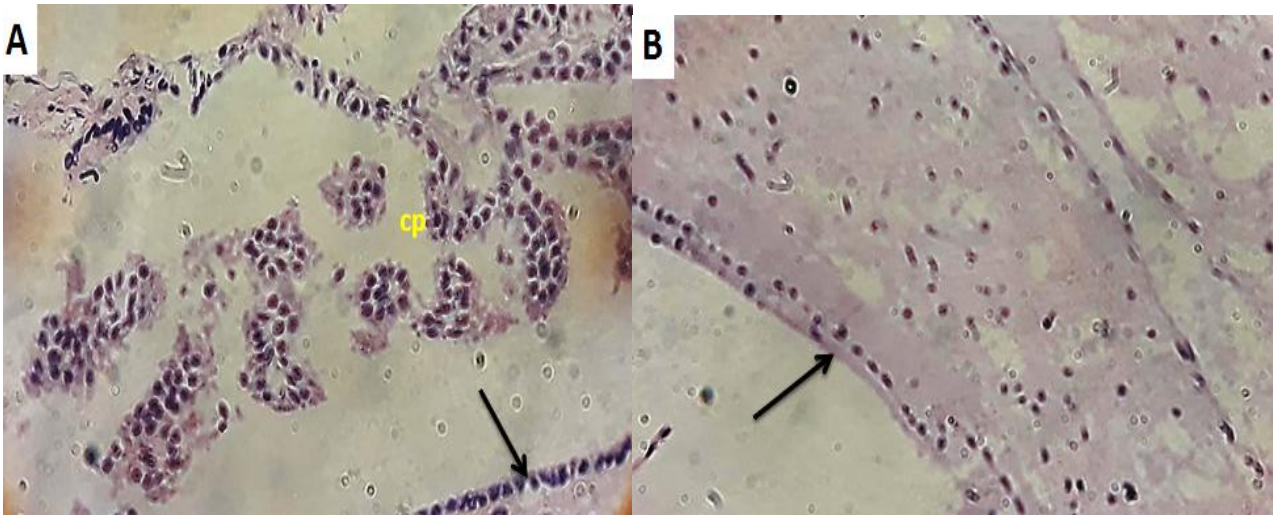


Figure (5, A-D): PAS-stain histological section in choroid plexus (cp) of 3rd, lateral and 4th ventricles display the epithelium (black arrow) that weakly stained excluding the apical edges; A&B (100X), C&D (400X)



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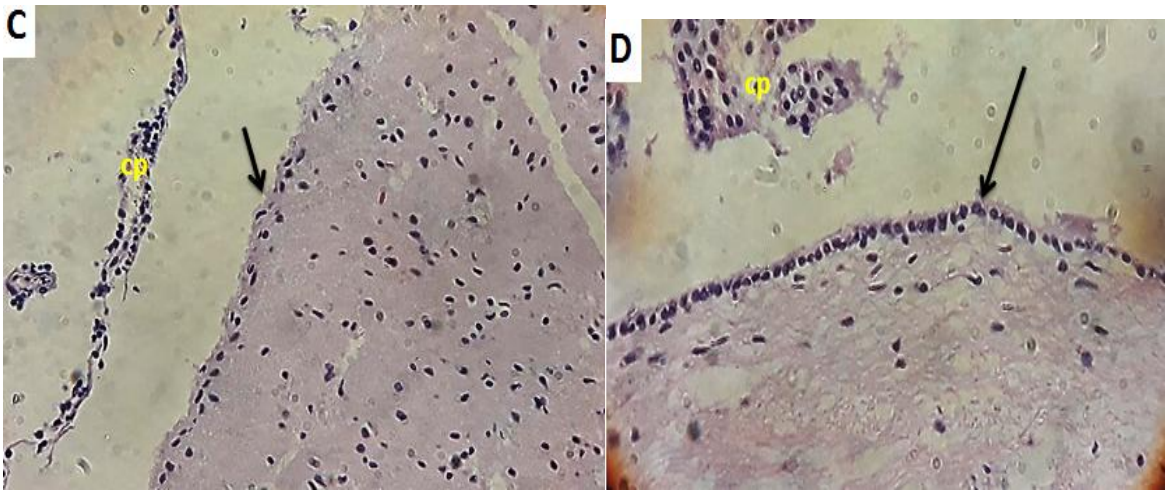

Figure 6, A-D): H&E-stained sections at choroid plexus (cp) of ventricles displaying the epithelia (cp) and ependymal cells (black arrow); 200X

Discussion

Sympathetic and parasympathetic nerves supply innervation to the choroid plexus. The superior cervical ganglion's sympathetic fibers govern blood stream into the choroid plexus, whereas the para-sympathetic fibers inhibit the creation of CSF. The ependyma is single layer of cuboidal epithelia that makes up the deepest part of the choroidal plexus. The ependymal cells make up ependymal layer, which has hair-like projections. They're dedicated glial cells which resulting of the neural stem cells that capable of producing cerebrospinal fluid and releasing it into the ventricles. Microvilli are tiny processes that are found on the apical layer of the ependymal cells. The ependyma's surface area is increased by microvilli. Humans [14, 15], cattle, horses, and dogs are all mammals that are similar to each other. These findings revealed that, the constitution of the choroidal plexuses of lateral ventricle below little magnification of the light microscope showed comparable folds of flexible dimensions; similar findings were previously mentioned by [15, 16] in sheep.

The apical edges of certain cells were appeared possess microvilli and cilia whereas other cells appeared swelled or showed secretory projections. These findings were comparable to those recorded by [5, 17] in horse and also [18, 19] in sheep.

Our findings reveal that the lateral ventricle has more vascularization than the third and fourth ventricles, which we explain to the choroid plexus's increased secretory activity in the lateral ventricle [15, 20].

Acknowledgement

The present study indicated that the apical edges of certain cells appeared possess microvilli and cilia whereas other cells appeared swelled or showed secretory projection.Our findings reveal that

the lateral ventricle has more vascularization than the third and fourth ventricles, which we explain to the choroid plexus's increased secretory activity in the lateral ventricle.

Conflict of interest

No

Ethical approval

All of the experimental procedures involving animals were conducted in accordance with institutional animal care guidelines of university of Diyala, Iraq.

Fund: No fund

References

[1] S. A. Liddelow, S. Temple, K. Møllgård, R. Gehwolf, A. Wagner, H. Bauer, *et al.*, "Molecular characterisation of transport mechanisms at the developing mouse blood–CSF interface: a transcriptome approach," *PloS one*, vol. 7, p. e33554, 2012.

[2] J. H. Jeon, S. W. Lee, J. K. Ko, B. G. Choi, S. H. Cha, G. S. Song, *et al.*, "Neuroendoscopic removal of large choroid plexus cyst: a case report," *Journal of Korean Medical Science*, vol. 20, pp. 335-339, 2005.

[3] C. G. Thanos and D. F. Emerich, "Choroid Plexus Epithelial Cell Transplants for Repair of the Brain," *Cellular Transplantation: From Laboratory to Clinic*, p. 399, 2007.

[4] B. R. Bill, D. Balciunas, J. A. McCarra, E. D. Young, T. Xiong, A. M. Spahn, *et al.*, "Development and Notch signaling requirements of the zebrafish choroid plexus," *PloS one*, vol. 3, p. e3114, 2008.

[5] Ebada, A. Helal, and M. AlKafafy. Morphological Studies on the Choroid Plexus in Balady Goat (Capra hircus), *J Vet Anatomy*, 4, pp. 79-92, 2011.

[6] Kaiser, K., Bryja, V.,. Choroid plexus: the orchestrator of long-range signalling within the CNS. Int. J. Mol. Sci. 21. 2020.

[7] Castro Dias, M., Mapunda, J.A., Vladymyrov, M., Engelhardt, B. Structure and junctional complexes of endothelial, epithelial and glial brain barriers. Int. J. Mol. Sci. 20. 2019

[8] Bill, BR., Balciunas, D., McCarra, JA., Young, ED., Xiong, T., Spahn, AM., Korzh, V. and Schimmenti, LA. Development and notch signaling requirements of the zebra fish choroid plexus. PLoS One. 2008, 3 (9): e3114.

[9] Damkier, H. H., Brown, P. D., and Praetorius, J. Cerebrospinal fluid secretion by the choroid plexus. Physiological reviews, 93(4), 1847-1892. (2013)

[10] Serot, JM.; Bene, MC. and Faure,GC.: Choroid plexus, aging of the brain, and Alzheimer's disease. Frontiers in Bioscience. 8 (Suppl.): s515- s521.

[11]Smith, D. M., Oliker, A., Carter, C. R., Kirov, M., and McCarthy, J. G. A virtual reality atlas of craniofacial anatomy.Plastic and reconstructive surgery, 120(6), 1641-1646. (2007).

[12] S. Suvarna, C. Layton, and J. D. Bancroft, "Theory and practice of histological techniques," *Pbl. London, Churchill Livingstone, Elsiver,* pp. 173-187, 2013.

[13] Al-Shaeli, S. J., Ethaeb, A. M., and Gharban, H. A. (2020). Molecular and histopathological dentification of ovine neosporosis (Neospora caninum) in aborted ewes in Iraq. *Veterinary world*, *13*(3), 597-603

[14] Snell, R. S. *Clinical neuroanatomy*: Lippincott Williams & Wilkins, 2010.

[15] J. L. Wilkinson, *Neuroanatomy for medical students*: Butterworth-Heinemann, 2014.

[16] Rajtová, V.,(2002) "Scanning electron microscopy of Plexus choroideus in adult sheep," *ActaVeterinaria Brno,* vol. 71, pp. 279-282,.

[17] O. J. Tamega, L. F. Tirapelli, and S. Petroni,(2000) "Scanning electron microscopy study of the choroid plexus in the monkey (Cebusapellaapella)," *Arquivos de neuro-psiquiatria*, vol. 58, pp. 820-825,.

[18] M. Alsulaiman, G. Muhammad, Y. Alotaibi, A. Mahmood, and M. Abdelkader, (*2009)*"Building a Speaker Recognition System with one Sample," in *The 2009 International Symposium Computer Science and Computational Technology (ISCSCT 2009)*, 2009, p. 330.

[19] M. Eggermont, P. Cornillie, M. Dierick, D. Adriaens, N. Nevejan, P. Bossier, *et al.*,(2020) "The blue mussel inside: 3D visualization and description of the vascular-related anatomy of Mytilusedulis to unravel hemolymph extraction," *Scientific reports,* vol. 10, pp. 1-16,.

[20] V. Rajtová, "The plexus chorioideus in the adult goat: A scanning electron microscope study," *Acta Vet Brno,* 69, pp. 167-171, 2000.