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Some Anatomical and Histological Features of the Brain in African Catfish (*Clarias Gariepinus*)

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Abstract

The purpose of the present study is to investigate the catfish brain morphology by gross anatomy, morphometric analysis in addition to light microscopy. A total of twenty African catfish (*Clarias Gariepinus*), 30-80 cm long, with body mass 300-900gm, were used in this study. Anatomically, the brain of catfish is divided into five parts: telencephalon, mesencephalon, diencephalon, myelencephalon and metencephalon. The most prominent parts of catfish brain are optic tectum, telencephalon, and cerebellum. The morphometric analysis of the brain of catfish revealed a large optic tectum in the mesencephalon, whilst telencephalon and the cerebellum are of medium size. The brain occupies the caudal part of the cranial cavity. The brain is slightly elongated and narrow, slightly wider in the middle portion near the mesencephalon (optic tectum), eminentia granularis and the diencephalon. The telencephalon (cerebral hemispheres) single layer formed from various neuirons which are supported by neuroglia. The optic tectum consisted of five layers stratum marginale; stratum opticum; stratum album centrale; stratum griseum centrale and stratum periventriculare. The cerebellum histologically, formed from three different layers molecular, purkinje cell and inner granular layers. The optic nerve in catfish appeared large and thick.

KEYWORDS
Anatomy, Histology, Brain, Catfish

INTRODUCTION

Catfish (*Clarias Gariepinus*) is one of the teleost species which is widely distributed in Africa and the Middle East due to the economic value as an inexpensive source of animal protein for various populations (Elsheikh, 2013). It is categorized as an omnivorous, fast growing, bottom feeder, sharp tooth and can feed on agriculture byproducts, live and dead animals (Appelbaum and Kamler, 2000). It has resistant mechanisms for habitat ecological changes, naturally inhabits rivers, lakes, swamp bottoms, floodplains, and intensively farmed aquaculture species in the Middle East long times ago (Bishai and Khalil, 1997).

The African catfish are freshwater fish. It deemed as one of the extremely valued species in Egypt that are preferable to other species in order that they can overcome a wide range of habitant conditions; including hypoxia, extreme temperatures, adaptation to poor quality water, and coexistence with bad food, while achieving high growth rates (Karkit *et al*., 2019).

The brain of vertebrates is basically divided into regions that control specific functions related to sense organs, motor coordination, behavior, and metabolic regulation (Butler and Hodos, 2005). The brain morphology in teleost fishes is varied and shows a degree of differentiation, hardly comparable to that of other vertebrate groups (Wagner, 2003).

Several studies have clarified that brain morphology has adaptive significance and affects physiology, behavior, and habits of fish. For example, the large cerebral hemisphere is linked with a higher degree of learning, sensory integration, and spatial navigation (Park and Bell, 2010), while a large optic tectum is associated with good vision and orientation response (Pollen *et al*., 2007). This study aimed to provide the baseline information on the anatomy and histology of the brain of the catfish.

MATERIALS AND METHODS

Sample collection

A total of twenty African catfish (*Clarias Gariepinus*), 30-80 cm long, with body mass weight 300-900g, were used in this study. They were caught live from the river Nile, at Delta region, Egypt, and transported in a plastic aquaria of 100-liter capacity, to the Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.

The handling of fish in this study was accompanied the guidelines of the Institutional Animal Care and the Research Ethics Committee of the Zagazig University, with an ethical approval number of (ZU-IACUC/2/F /164/2023).

Gross anatomy (Macroscopical) examination

All fish was anesthetized with 0.01% benzocaine and killed with cervical dislocation then used for anatomical and histological examination (Close *et al*., 1997).

Heads were detached from the body and held in an upright

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position for brain extraction. The soft tissues surrounded skull were removed. The skull was carefully cut with the help of a sharp cutter. The skull bone was carefully removed with the help of forceps to avoid damage to the dorsal part of the brain. The dorsal part of the cranium was removed, and the brain was exposed for a further incision. The lateral part of the cranium was removed with the help of small dissecting scissors. The optic and olfactory nerves were carefully exposed with the help of scissors to avoid damage to the brain. The brain from the bottom was gently lifted out. Few samples were missed out (04) during the extraction of the brain from the lateral part of the cranium. The photographs were captured through Sony digital camera (DSC-W690)16.1 mega pixels then edited and labeled on Adobe Photoshop version 8 and investigated using a dissection microscope (Nikon SMZ-2T; Tokyo, Japan).

Morphometric analysis

Morphometric analysis of different parts of the brain, the most prominent parts of catfish brain are the optic tectum, telencephalon, and cerebellum. The major anatomical parts of the brain were subjected to a morphometric measurement (cerebellum length, width, thickness and weight telencephalon length, width, thickness and weight tectum opticum length, width, thickness and weight,) following the standard guideline from Abrahão and Shibatta (2015).

The brain was weighed with the help of digital weight balance and the other measurements by Vernier Caliper.

Microscopical examination

Fixation and tissue processing

Six fish were used for the histological examination, specimens that were collected from brain parts, immediately fixed in Bouin's solution for 48 hours. All samples were processed for paraffin embedding technique and tissue segments paraffin blocks were sectioned in 5-micron thickness and stained with hematoxylin and eosin Stain.

The formalin preserved catfish's brain; specimens were processed in an automated tissue processor. The processing consisted of an initial two steps fixation and dehydration. Fixation comprising tissue immersion in 10% buffered formalin for 48 hours, followed by removal of fixative in distilled water for 30 minutes. Dehydration was then carried out by running the tissues through a graded series of alcohol (70%, 90% and 100%). The tissue was initially exposed to 70% alcohol for 120 minutes followed by 90% alcohol for 90 minutes and then two cycles of absolute alcohol, each for one hour. Dehydration was then followed by clearing the samples in several changes of xylene. It consisted of tissue immersion for an hour in a mixture comprising 50% alcohol and 50% xylene, followed by pure xylene for one and a half hour. Samples were then impregnated with molten paraffin wax, then embedded and blocked out. Paraffin sections (4–5 µm) were stained with hematoxylin and eosin (Suvarna *et al.*, 2018).

Finally, 4-5 µm paraffin sections were obtained and stained with hematoxylin and eosin (H and E) stain which were photographed via a digital Dsc-W 800 super steady cyper shot camera connected to an Olympus BX 21 light microscope at Histology and Cytology Department, Faculty of Veterinary Medicine, Zagazig University, Egypt.

The nomenclatures in this study were adopted according to Nomina Anatomica Veterinaria (2017).

Statistical analysis

The morphometric analysis was accomplished by one-way analysis of variance (ANOVA), followed by multiple ranges post hoc test for pairwise comparisons using SPSS 25.0 for Windows, Version 21, Statistical Analysis System package SPSS (2012). The statistical data were expressed as mean ± SE, and the data were considered statistically significant at p values <0.05.

RESULTS

Anatomically, the brain of catfish can be divided into five regions: telencephalon (fore brain), mesencephalon (optic tectum), diencephalon (epithalamus, thalamus and hypothalamus), myelencephalon (medulla oblongata and vagal lobes) and metencephalon (cerebellum).

The brain occupid the caudal part the cranial cavity. It was slightly elongated and narrow, slightly wider in the middle portion near the mesencephalon (optic tectum), eminentia granularis and the diencephalon (Fig. 1).

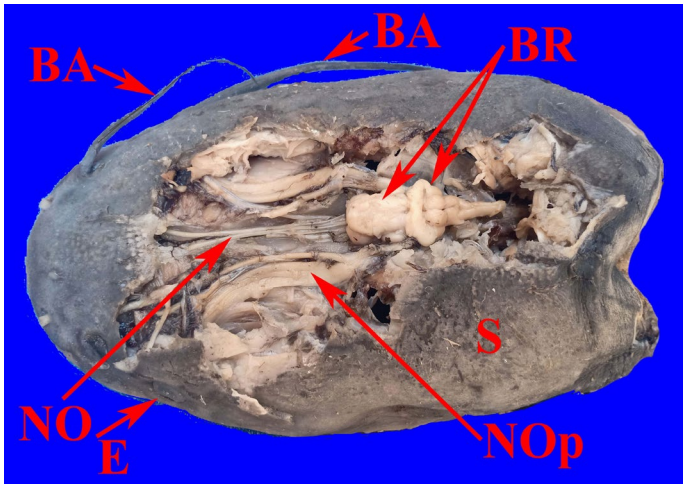

Fig. 1. A photomicrograph of catfish brain inside the head dorsal view showing (S) Skull (BR) Brain (BA) Barbel (NO) Nervus Olfactorii (NOp) nervus opticus (E) Eye.

The telencephalon was situated rostral to the optic tectum and the hypothalamus, in dorsal view; the distal portion of the telencephalon was positioned dorsal to the hypothalamus, Hypophysis and inferior lobe of hypothalamus. The telencephalon was elongated oval, its anterior and posterior margins were blunt (Figs. 2 and 3).

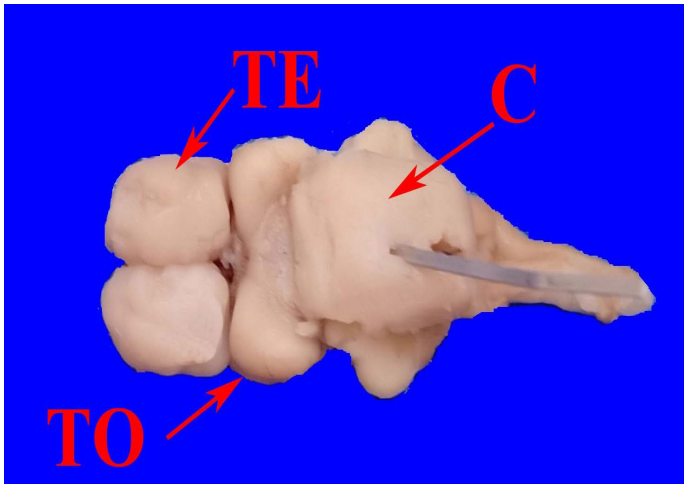

Fig. 2. A photomicrograph of catfish brain dorsal view showing after reflection of cerebellum, (C) cerebellum, (TE) Telencephalon, (TO) Tectum opticum.

The optic tectum was located at the dorsal portion of the mesencephalon, and ventral to the cerebellumin the dorsal view, dorsal to the lobus inferior hypothalami and the hypothalamic cerebri and caudal to the telencephalon in the dorsal view. The optic tectum was formed of two bilaterally compressed ovoid structures which was related dorsally to corpus cerebelli and ventrally to lobus inferior hypothalamic (Figs. 2 and 3).

The hypothalamus was elongated oval in shape in the ventral aspect of the brain; its anterior and posterior margins were rounded, with notched appearance dorsal surface. The hypothalamus was positioned at the ventral portion of the diencephalon, at the ventral view of the brain and caudal to the telencephalon and the chiasma opticum, medial to the lobus inferior hypothalami and rostral to the hypophysis.

The lobus inferior hypothalamic was situated at the outer part of the diencephalon, lateral to the hypophysis and hypothalamus it was paired crescentic in shape. The lobus inferior hypothalamus appeared larger than the hypothalamus, but smaller than the telencephalon and the optic tectum. The hypophysis was located caudal and ventral to the hypothalamus, on its mid axis, and it was rounded in shape (Fig. 3).

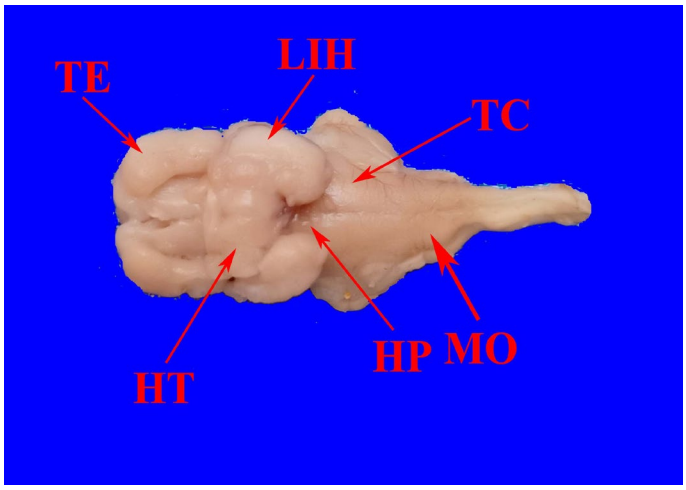

Fig. 3. A photomicrograph of catfish brain ventral view showing (TE) Telencephalon, (HP) Hypophysis, (HT) Hypothalamus, (LIH) Lobus inferior hypothalamus, (TC) Truncus cerebri (MO) Medulla oblongata.

The cerebellum was situated in the dorsal portion of the rhombencephalon, dorsal to the optic tectum and telencephalon and rostral to the eminentia granularis and lobus vagus. It was elongated oval in shape, with a wide end and rounded rostral end. The eminentia granularis was crescentic in shape and was located at the caudal aspect of the cerebellum (Fig. 4).

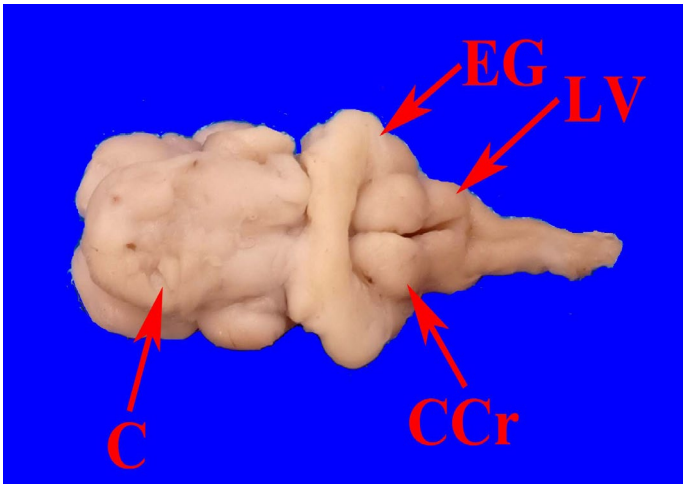

Fig. 4. A photomicrograph of catfish brain dorsal view showing (C) cerebellum, (EG) Eminentia granularis, (CCr) Cerebellar crest, (LV) Lobus vagus.

The truncus cerebri was located at the ventral aspect of the brain and caudal to the hypothalamic cerebri. The narrow medulla oblongata connected the brain and the spinal cord (Fig. 3).

The olfactory nerve (Nn. Olfactorii), which extended ventrocaudal part of telencephalon to the rostral part of the head. The optic nerve (N. Opticus) originated from the lateral aspect from the optic tectum and extended in rostral direction. The optic nerve in catfish was large and thick (Figs. 5 and 6). The narrow medulla oblongata joined the brain and spinal cord.

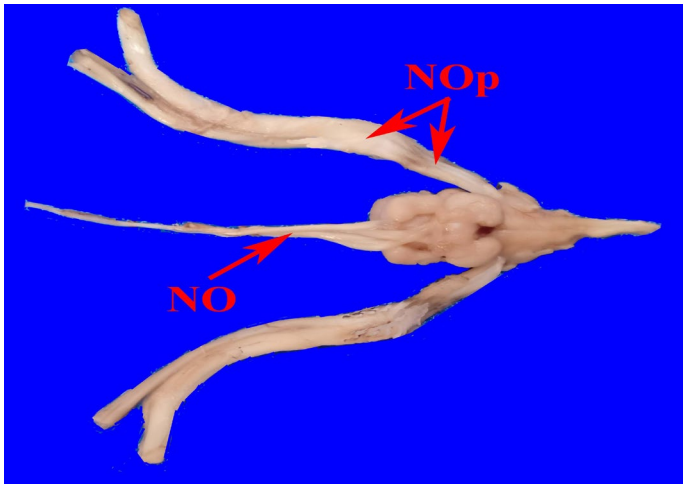

Fig. 5. A photomicrograph of catfish brain connecting with olfactory and optic nerve ventral view showing (NO) Nervus Olfactorii (NOp) nervus opticus.

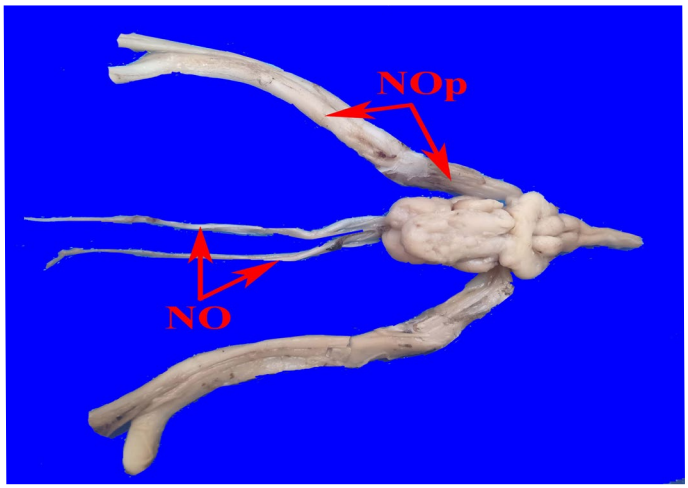

Fig. 6. A photomicrograph of catfish brain connecting with olfactory and optic nerve dorsal view showing (NO) Nervus Olfactorii (NOp) nervus opticus.

Morphometric analysis results

The optic tectum was obviously the largest part of the catfish brain, followed by the cerebellum and telencephalon (Table 1, Fig. 7).

Histological structure of the brain

On the base of histological structure and position the brain of catfish could be divided into five parts: telencephalon, mesencephalon, diencephalon, myelencephalon and metencephalon.

The telencephalon (cerebral hemispheres) as a single layer formed from various neurons which were supported by neuroglia (supporting cell) which were differentiated from neurons by their small nuclei surrounded by a thin acidophilic cytoplasm. The wall of the brain was covered by a meninx. Telencephalon was contained several blood capillaries (Fig. 8).

From external to internal, the optic tectum consisted of five-

Table 1. Morphometric analysis of the brain of catfish.

		No.	Mean	Std. Deviation	Std. Error
Length	1	20	0.81	0.29	0.09
	2	20	0.54	0.05	0.02
	3	20	0.55	0.06	0.02
	Total	60	0.63	0.21	0.04
Width	1	20	0.55	0.05	0.02
	2	20	0.79	0.04	0.01
	3	20	0.83	0.46	0.14
	Total	60	0.72	0.29	0.05
Thickness	1	20	0.26	0.03	0.01
	2	20	0.38	0.03	0.01
	3	20	0.50	0.03	0.01
	Total	60	0.38	0.11	0.02
Weight	1	20	0.13	0.01	0.00
	2	20	0.09	0.031	0.01
	3	20	0.21	0.01	0.00
	Total	60	0.14	0.05	0.01

1: Cerebellum; 2: Telencephalon; 3= 0ptic tectum

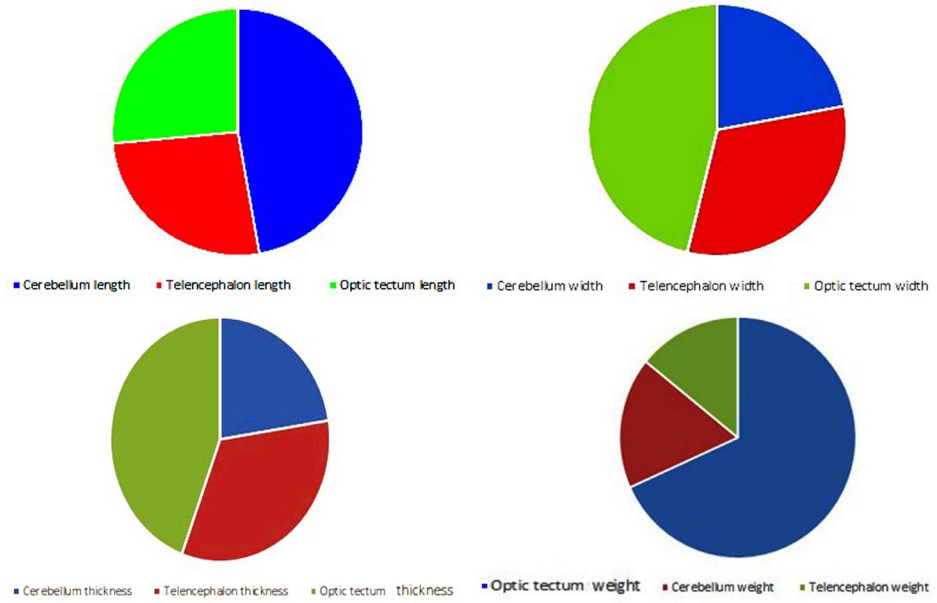

Fig. 7. Charts of catfish brain measurements.

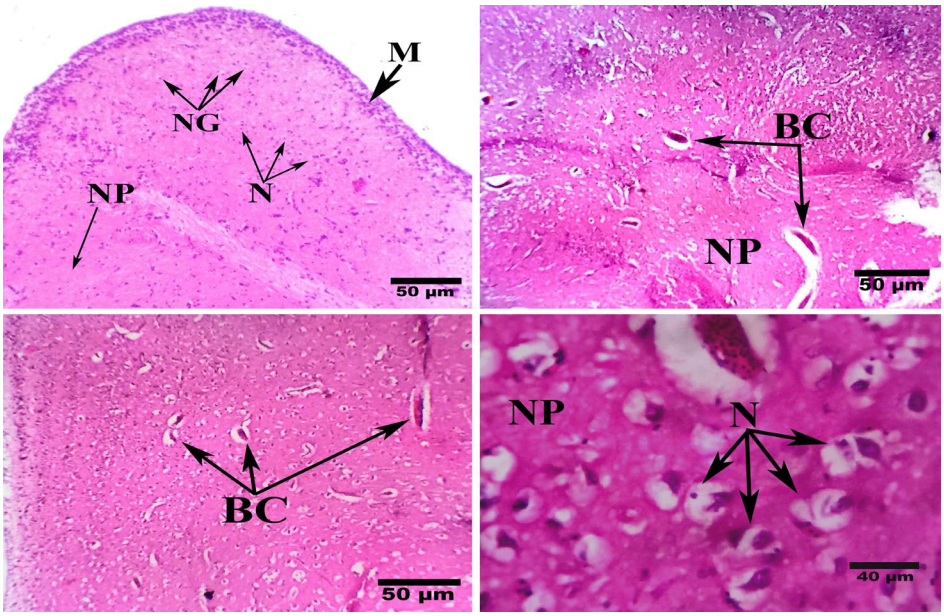

Fig. 8. A photomicrograph of catfish telencephalon showing (M) meninx (N) Neurons (nerve cells) (NP) Neuropil (NG) Neuroglia (BC) Blood capillary.

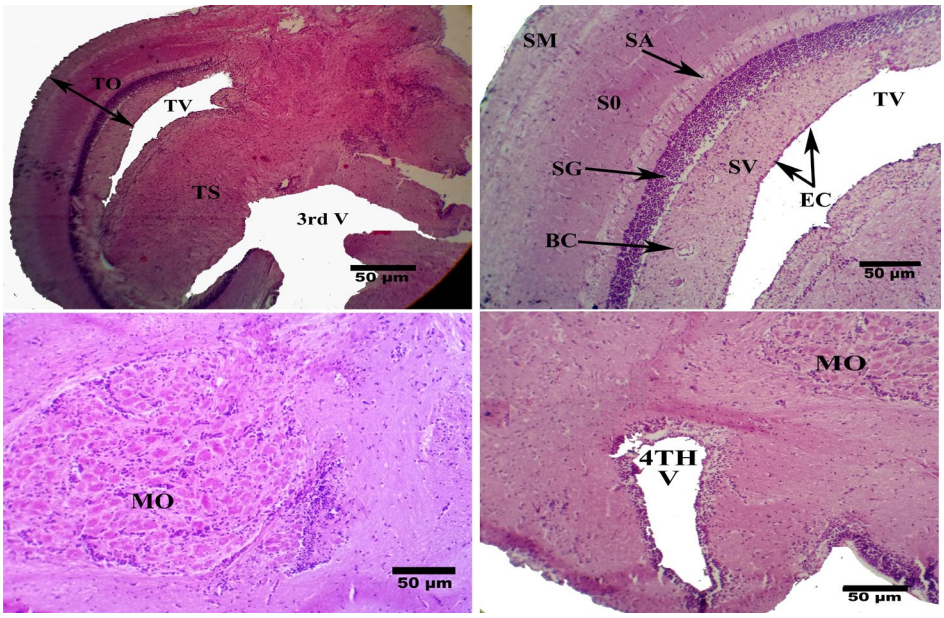

Fig. 9. A photomicrograph of catfish optic tectum and medulla oblongata shows (TO) Tectum opticum (TV) Tectal ventricle (3RD V) Third ventricle (4TH V) Fourth ventricle (TS) Torus semicircularis (SM) Stratum marginale (SO) Stratum opticum (SA) Stratum album central (SG) Stratum griseum central (SV) Stratum periventriculae (MO) Medulla oblongata (EC) Ependymal cell.

layers; stratum marginale, stratum opticum, stratum album central, stratum griseum centrale and stratum periventriculare. These layers were distinguished from their others by cellular compositions. The optic tectum ventrally separated from torus semicircularis by tectal ventricle. The optic tectum was separated from the diencephalon by the third ventricle. The ventricular wall was lined with the neuronal cell called ependymal cell. The medulla oblongata was located at the level of the fourth ventricle (Fig. 9).

The cerebellum histologically, formed from three different layers molecular, purkinje cell and inner granular layers. The optic nerve appeared in the form of bundles of nerve fibers (Fig. 10).

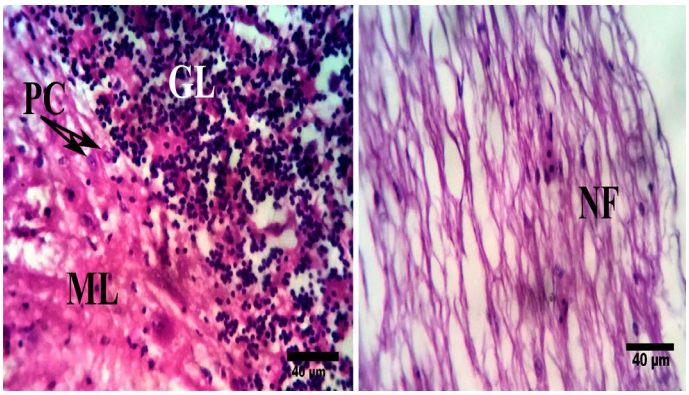

Fig. 10. A photomicrograph of catfish cerebellum and optic nerve shows (ML) Molecular layer (GL) Granula layer (PC) Purkinje cell layer (NF) Nerve fibers.

DISCUSSION

The optic tectum was apparently the largest part of the brain of catfish in all examined specimens followed by the cerebellum and telencephalon, this result was in accordance with Senarat *et al.* (2015).

The brain of Gray Mullet (Mugil cephalus) characterized by sizeable optic tectum (Hussein and Cao, 2019), the optic tectum was very large in this kind of fish. In contrast, Abrahão and Shibatta (2015), stated that in all examined species of Pseudopimelodus, the corpus cerebelli is the largest subdivision of brain. Senarat *et al.* (2016) showed that the largest part of the brain, optic tectum, was well-developed under the mesencephalon. The cerebellum of the metencephalon was moderately sized, located between the optic tectum and medulla oblongata. Angulo and Langeani (2017) added that the corpus cerebelli was the more voluminous brain subdivision in most specimens, followed by the optic tectum.

In agreement with Ito and Yamamoto (2009) the cerebrum consisted of a single layer in all teleosts; but it was lobulated in Epinephelus coioides (Savari *et al.*, 2013).

The cerebellum is histologically formed from three layers, molecular, Purkinje cell, and granular layer (Hussein and Cao, 2019; Senarat *et al.*, 2020). Except in Eleginops maclovinus fish the cerebellum is characterized by unordinary structure; as it consisted of of central molecular layer and lateral granular layer (Eastman and Lannoo, 2008). Senarat *et al.* (2015) stated that large Purkinje cells were developed in the cerebellum of Indo-Pacific seahorse fish.

The optic tectum ventrally separated from torus semicircularis and optic tegmentum by tectal ventricle this result following Eastman and Lannoo (2004) and Hussein and Cao (2019).

The current work revealed that, the optic tectum consisted of five layers, similar results were recorded by Senarat *et al.* (2020) in the brain of brain of the Indo-Pacific seahorse. This result was not in line with previous observations (Senarat *et al.*, 2016; Hussein and Cao, 2019; Northcutt, 2002), it consisted of six layers which were from cranial to caudal; stratum marginal, stratum opticum, stratum fibrosum et griseum superficial, stratum griseum central, stratum album central and stratum periventriculare.

CONCLUSION

The study on brain structure and morphology increases the data for systematics, and assist in understanding the behavior and habits of fishes. In addition to that, it is noticeable that there is a great scope for studies on brain anatomy regarding the large divergence of teleosts. Particularly, the largest optic tectum supposed a large ability for learning, good vision, orientation response and the tendency to devour fast-moving prey. Unless this hypothesis should be assured by further studies on the feeding behavior of catfish.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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