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Physiology

Histological and Histochemical Structure of the Pituitary Gland in the Mullet Fish *(Mugil cephalus)*

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ABSTRACT

This study aimed to elucidate the histological and histochemical structure of the pituitary gland of the mullet fish (*Mugil cephalus*). The results indicated that the pituitary gland of mullet fish (*Mugil cephalus)* was composed of a defined adenohypophyseal part interrupted by strands of neurohypophyseal arborization. Three regions were observed in the adenohypophysis, rostral pars distalis (RPD), proximal pars distalis (PPD), pars intermedia (PI). By certain histochemical staining methods, seven cell types were detected in the pituitary gland of *Mugil cephalus,* lactotrophs, adrenocorticotrophs in RPD, somatotrophs, gonadotrophs and thyrotrophs in PPD, melanotrophs and somatolactotrophs in PI. The neurohypophysis consisted of nerve fibers, pituicytes were dispersed within thin connective tissue rich in blood capillaries.

Key words: Histological, Histochemical, Pituitary gland, *Mugil cephalus.*

INTRODUCTION

The pituitary gland is an important endocrine gland in vertebrates. It regulates fundamental physiological processes such growth, environmental adaptability, immunological response, gonadal development, sexual differentiation, and reproduction. The pituitary gland in teleost fish was composed of adenohypophysis and neurohypophysis. The adenohypophysis was separated into three regions: the pars intermedia, rostral pars distalis, and proximal pars distalis (Oğuz and Ünal, 2022) while the neurohypophysis was made up of blood vessels, glial cells, and non-myelinated hypothalamic neurosecretory fibers (Schreibman *et al.*, 1973; Genten *et al.*, 2009). The fish species used in this study is *Mugil cephalus*, which is found in the coastal waters of the tropics and subtropics of all oceans (Robins and Ray, 1986). Too, it is exceedingly euryhaline, survive in a wide run of salinities from 0% in freshwater to hypersaline waters (Collins, 1985). Due to its high market value and easily cultivated by fish farmers, *Mugil cephalus* is a fish with an important economic impact (Bahnasawy *et al.*, 2009). This study aimed to clarify histological and histochemical structure

of the pituitary gland of the mullet fish, *Mugil cephalus.*

MATERIALS AND METHODS

1. Fish

Total 40 adult *Mugil cephalus* fish of both sexes (0.9-1kg B.Ws.) were collected in a good condition from freshwater and marine water. Freshwater fish were obtained from fish farm in Toulumpat Barseq, Abu Hummus center, El-Beheira Gover-norate, While marine fish were obtained from Deeba hatchery, Damietta Governorate. Pituitary gland with part of the base of the brain were dissected and fixed in neutral buffered formalin for light microscope study.

2. Histological procedures

The specimens were fixed in 10% neutral buffered formalin for 48 hrs, then dehydrated in a graded series of ethanol solutions, and embedded in paraffin. Sections 5 μm thickness were cut and mounted on slides. The selected sections were stained with the following stains and techniques: Harris hematoxylin and eosin (H&E), for general histological study. Peracetic acid-Alcian blue- Periodic acid Schiff- orange G (PAA-AB-PAS-OG) stain for differentiation of gonadotrophs, somatotrophs and lactotrophs. Lead hematoxylin for demonstration of adrenocorticotrophs and melan-otrophs. Periodic acid Schiff (PAS) for demonstration neutral mucopoly-saccharides. Masson's trichrome (MT) stain, for demonstration of collagen fibers and muscle cells. All the aforementioned stains and techniques were used outlined by Heath (1965) and Bancroft and Gamble (2002). Then photomicro-graphs were taken by microscope with digital camera (Leica EC3). (Ethical approval number: VUSC-022-1-18).

RESULTS

Morphologically, the brain of mullet fish (*Mugil cephalus)* was composed of forebrain (olfactory lobe & cerebrum), diencephalon (thalamus & hypothalamus), midbrain (optic lobes) and hindbrain (cerebellum & medulla oblongata). The pituitary gland of the mullet appeared bean-shaped organ, attached by a short and thin stalk to the floor of the hypothalamus, just behind the optic chiasm and anterior to saccus vasculosus, it lodged in small depression of the parasphenoid bone (*Sella turcica*). The gland consisted of the adenohypophysis and the neurohypophysis (Fig. 1&2).

A-*Adenohypophysis*

The adenohypophysis was divided into three regions anterior-dorsal rostral pars distalis (RPD), middle proximal pars distalis (PPD), and posterior-ventral pars intermedia (PI). The RPD occupied a large area about half of the adenohypophysis. PPD was the middle region between RPD and PI. It appeared as a triangular-shaped area with a broad dorsal surface penetrated by the neurohypophysis. The PI was clusters of secretory cells surrounded everywhere by large areas of the neurohypophysis (Fig. 3a & b). The connective tissue elements of the adenohypophysis was formed of thin capsule of collagenous fibrous and very delicate trabeculae and reticular meshwork that arranged the adenohypophyseal cells into an anastomosis cords or clusters of cells, it also carried blood and nerve supply (Fig. 4a & b).

1- Rostral pars distalis (RPD)

RPD was anterior-dorsal in position, composed of acidophils (lactotrophs) and basophils (adrenocorticotrophs). Acidophils occupied the largest area in RPD. They were polymorphic in shape with round nucleus**.** Basophils were arranged as cords of two or three layers of polyhedral cells of different sizes in the interphase between RPD and neurohypophysis (Fig. 5a & b).

1.a-Lactotrophs (PRL cells)

They were acidophilic cells that occupied a major area of RPD. Their cytoplasm had high affinity to orange G stain and appeared orange in color with PAA-AB-PAS-OG stain (Fig. 6a). PRL cells were arranged in cords besides blood sinusoidal capillaries. They were polymorphic in shape with round eccentric dark stained nuclei. The cytoplasm appeared dense orange color in most of PRL cells, other cells showed clear cytoplasm around the nucleus (Fig. 6b).

2.b-Adrenocorticotrophs (ACTH cells)

They were basophilic cells arranged as cords of two or three layers of polyhedral cells with large oval nuclei. They were mainly in contact with blood capillaries, occupied the area between the RPD and neurohypophysis. Some of ACTH cells were scattered as isolated cells between the PRL cells (Fig. 7a). They appeared blue in color with lead hematoxylin (Fig. 7b).

2-proximal pars distalis (PPD)

The PPD was the smallest area of the adenohypophyseal regions of the pituitary of *Mugil cephalus.* It was a narrow triangular area in the center of the gland between RPD and PI (Fig. 3). The dorsal broad part of the PPD was composed of somatotrophs, while the thyrotrophs occupied the area near the RPD and gonadotrophs covered the narrow ventral portion of PPD (Fig. 8). The gonadotrophs of the PPD showed vacuolated, basophilic cytoplasm in some cells, while the somatotrophs were arranged in cords around the neurohypophyseal branches (Fig. 9).

2.a-Gonadotrophs (FSH cells)

The histochemical methods revealed that these cells were PAS-positive, basophilic and strongly reacted with Masson's trichome. They occupied the small, narrow angular area at the ventral portion of PPD. The gonadotrophs were polygonal in shape with small, round dark stained nuclei. The cytoplasm in some of gonadotrophs cells was vacuolated and others showed positive reactions with PAS (Fig. 10a). They appeared dark red in color with PAA-AB-PAS-OG stain (Fig. 10b) and dark green in color with Masson's trichrome stain (Fig. 11a).

2.c-Somatotrophs (GH cells)

They were acidophilic cells that occupied the broad dorsal area of PPD surrounded by the neurohypophyseal branches and blood capillaries. They were arranged in discontinuous cords or follicles (Fig. 8). These cells were oval in shape containing an eccentric large oval nucleus. They were negative PAS reaction (Fig. 10a & 11b), however they had high affinity to orange G stain and appeared yellow in color with PAA-AB-PAS-OG stain (Fig. 10b & 11c).

2.c-Thyrotrophs (TSH cells)

They were arranged in anastomosing cords, located between somatotrophs and adrenocorticotrophs. They were surrounded by neurohypophysis (Fig. 8). They were irregular or elongated in shape with round or oval nuclei. They appeared light green in color with Masson's trichrome stain (Fig. 11d).

3.Pars intermedia (PI)

It was located in the posterior-ventral region of the pituitary gland of *Mugil cephalus*. The PI was an islets of cells surrounded everywhere by large areas of neurohypophysis. It consisted of two cell types, somatolactotrophs and melano-trophs (Fig. 12).

3.a-Somatolactotrophs (SL cells)

They were PAS-positive, weakly acido-philic cells. SL cells were organized as an interrupted cell cords laying against the neurohypophysis. They showed an oval or elongated shape, with an oval eccentric nuclei. They gave positive PAS reaction (Fig. 13a). The cytoplasm of SL cells showed high affinity to the Orange G and appeared dark orange color with PAA-AB-PAS-OG stain (Fig. 13b).

3.b-Melanotrophs (MSH cells)

They were polygonal, basophilic cells with round, large nuclei. These cells had no affinity to PAS or (PAA-AB-PAS-OG stain (Fig. 13).

B-Neurohypophysis (NH): -

The neurohypophysis was the neural part of the pituitary gland of *Mugil cephalus* originated from the base of the brain, the diencephalon. NH gave off ramifications to the different parts of the adeno-hypophysis but arborized and interdigitated extensively in the PI. It consisted of nerve fibers, pituicytes were dispersed within thin connective tissue rich in blood capillaries (Fig. 14).

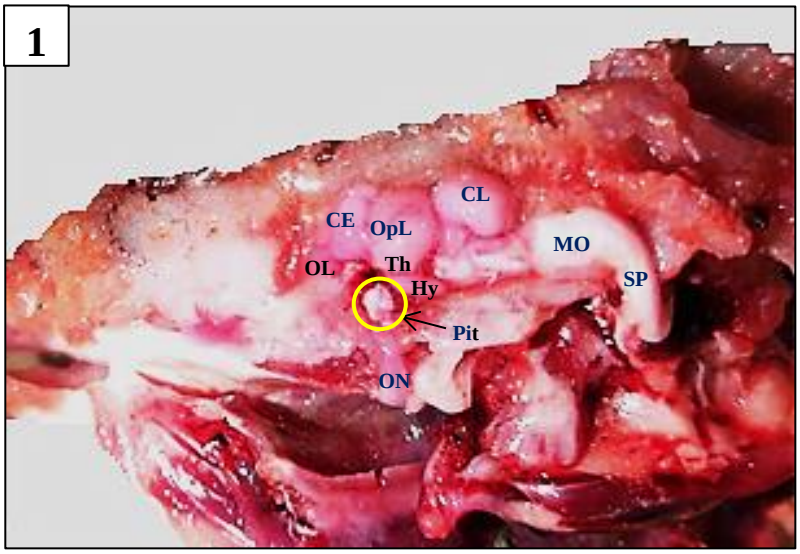

Fig. 1: Gross photograph of the brain of *Mugil cephalus* showing pitutary gland (Pit), thalamus (Th), hypothalamus (Hy), optic nerve (ON), olfactory lobe (OL), cerebrum (CE), optic lobe (OpL) cerebellum (CL), medulla oblongata (MO) and spinal cord (SP).

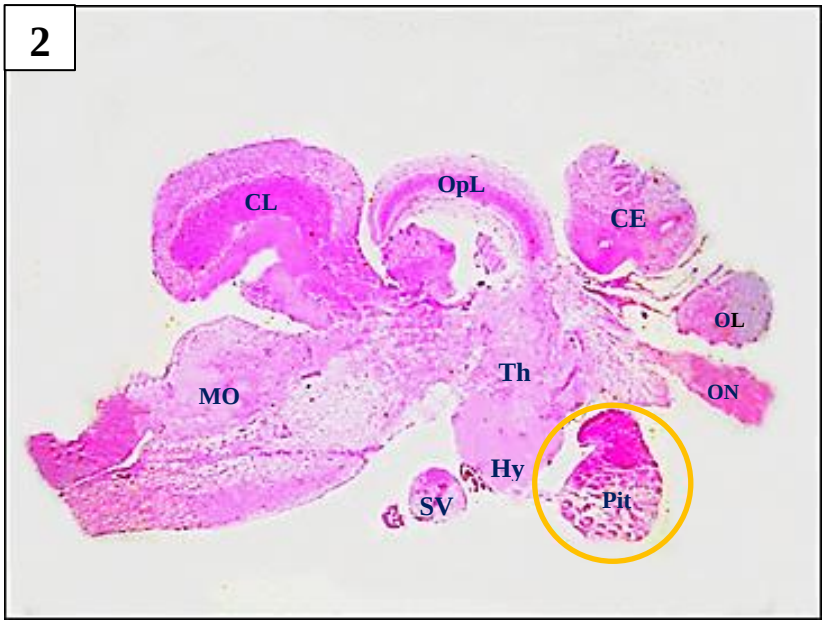

Fig. 2: Saggital section of the brain of *Mugil cephalus* showing pitutary gland (Pit), hypothalamus (Hy),saccus vasculosus (SV), optic nerve (ON), olfactory lobe (OL), thalamus (Th), cerebrum (CE), optic lobe (OpL) cerebellum (CL) and medulla oblongata (MO). (steriomicrscope, H&E).

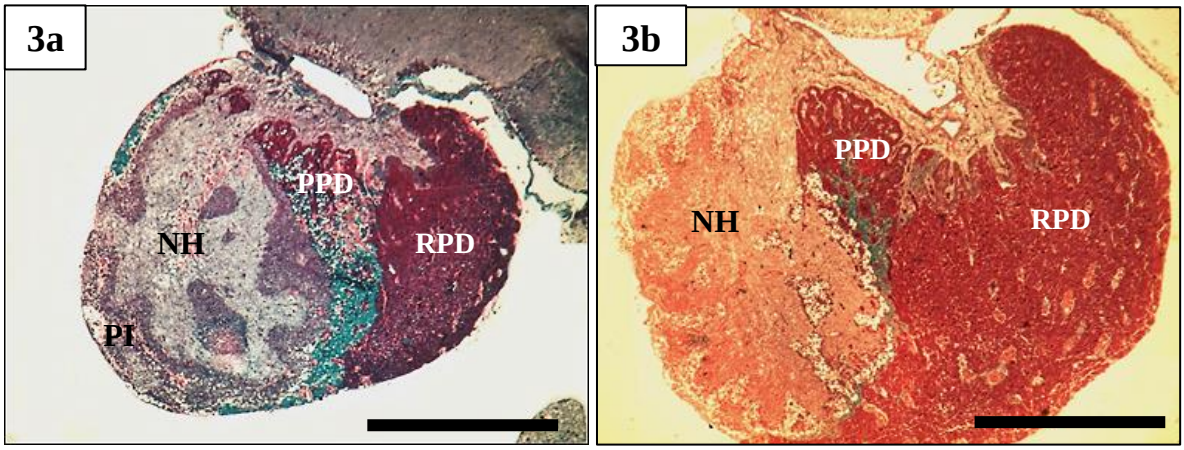

Fig. 3: Photomicrograph of pituitary gland of *Mugil cephalus* showing rostral distalis (RPD), proximal pars distalis (PPD), neurohypophysis (NH) and pars intermedia (PI), (**Fig.3a**): marine fish; (**Fig. 3b**): in freshwater fish. (MT, bar 500 µm).

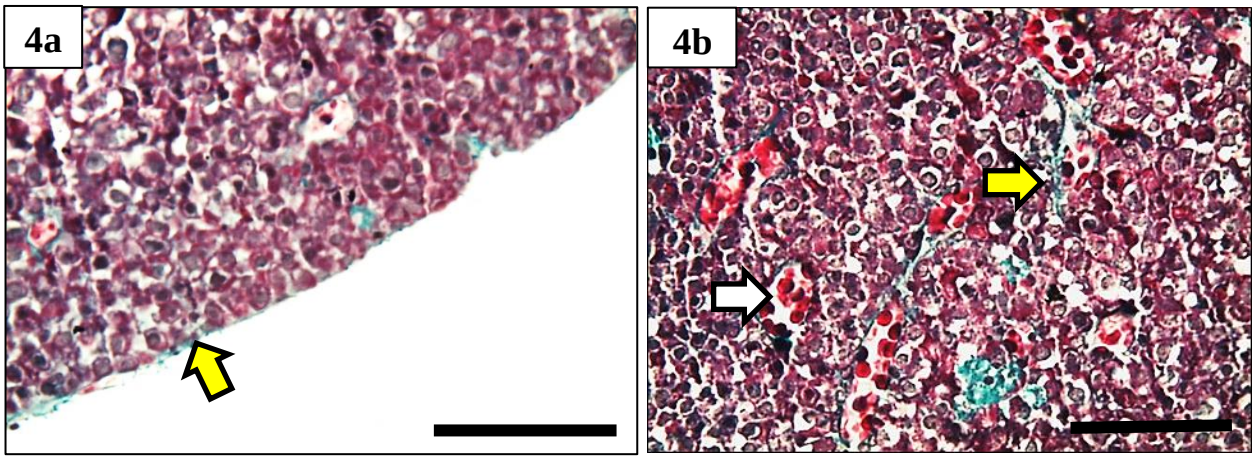

Fig. 4: Photomicrograph of adenohypophsis of *Mugil cephalus* showing thin capsule (yellow arrow) (**Fig. 4a,** Masson's trichrome (MT), bar 50 µm). Trabeculate (yellow arrow) carreid sinsudal capillaies (white arrow) (**Fig. 4b**, MT, bar 50 µm).

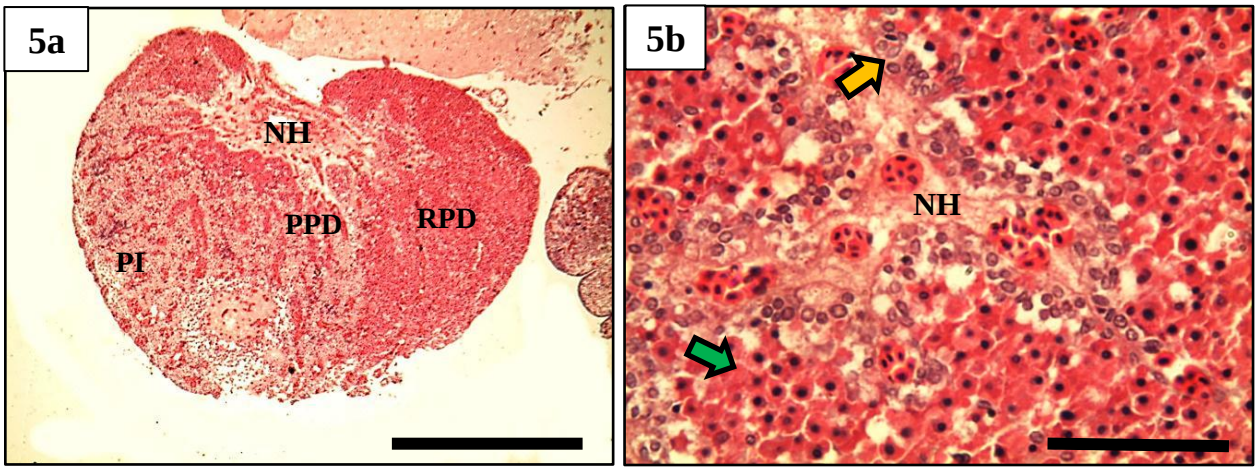

Fig. 5: Photomicrograph of pituitary gland of marine *Mugil cephalus* showing the area of the RPD in relation to the PPD and PI (**Fig. 5a**; H&E, bar 500 µm). A higher magnification of RPD showing acidophils (green arrow), basophils (yellow arrow) and NH. (**Fig. 5b**; H&E, bar 50 µm).

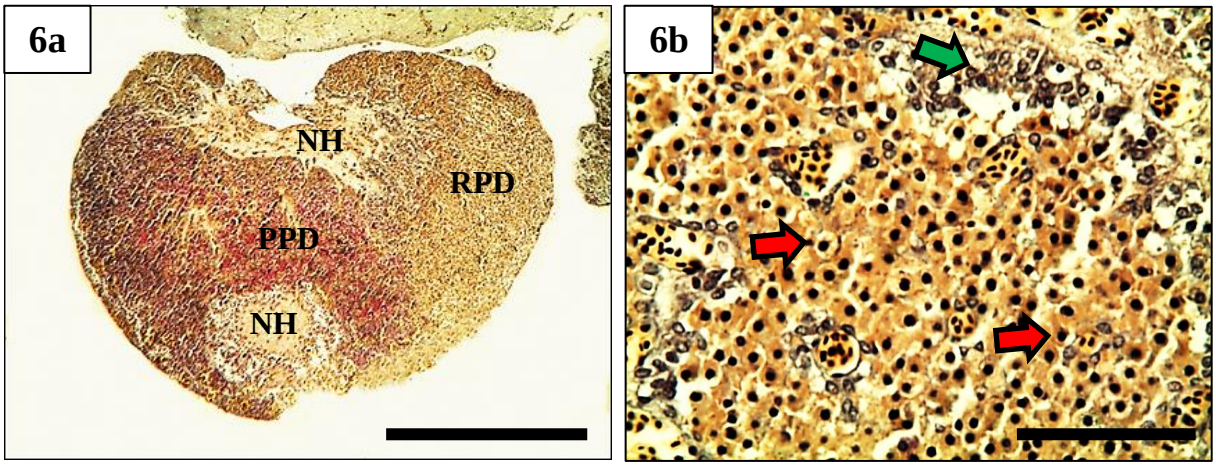

Fig. 6: Photomicrograph of pituitary gland of *Mugil cephalus* showing orange colored PRL cells in RPD, PPD and NH (**Fig. 6a**; PAA-AB-PAS-OG, bar 500 µm). A higher magnification showing orange colored polymorphic PRL cells with round nuclei (red arrows) and ACTH cells (green arrows) (**Fig. 6b**; PAA-AB-PAS-OG, bar 50 µm).

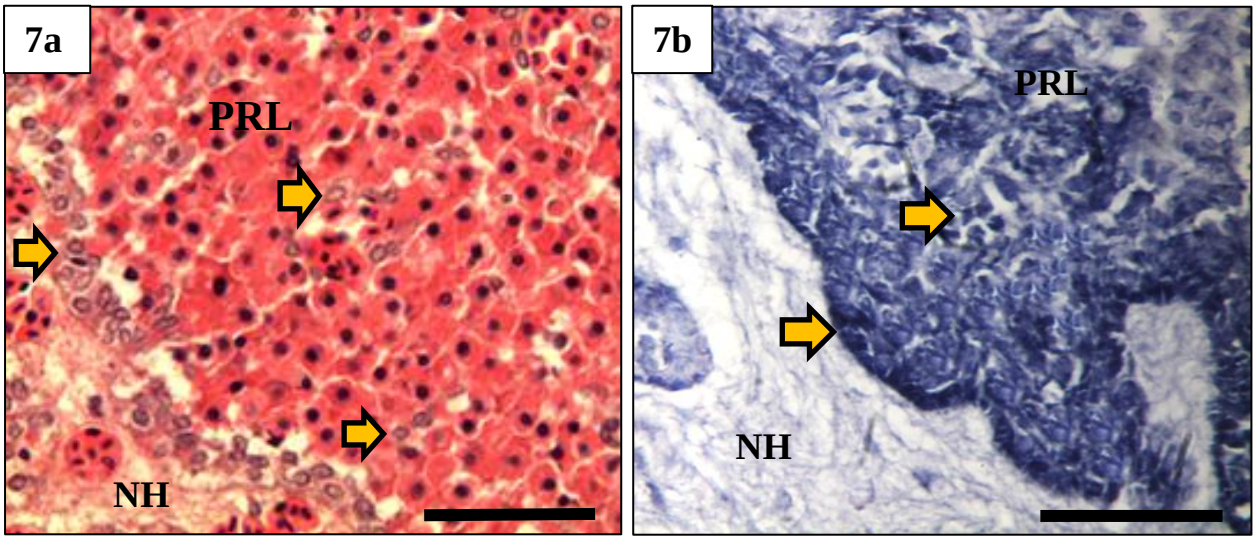

Fig. 7: Photomicrograph of RPD of *Mugil cephalus* showing the ACTH cells (yellow arrow) between RPD and NH, and some cells within the area of PRL cells. (**Fig. 7a**, H&E, bar 50 µm) and (**Fig. 7b**, Lead hematoxylin, bar 50 µm).

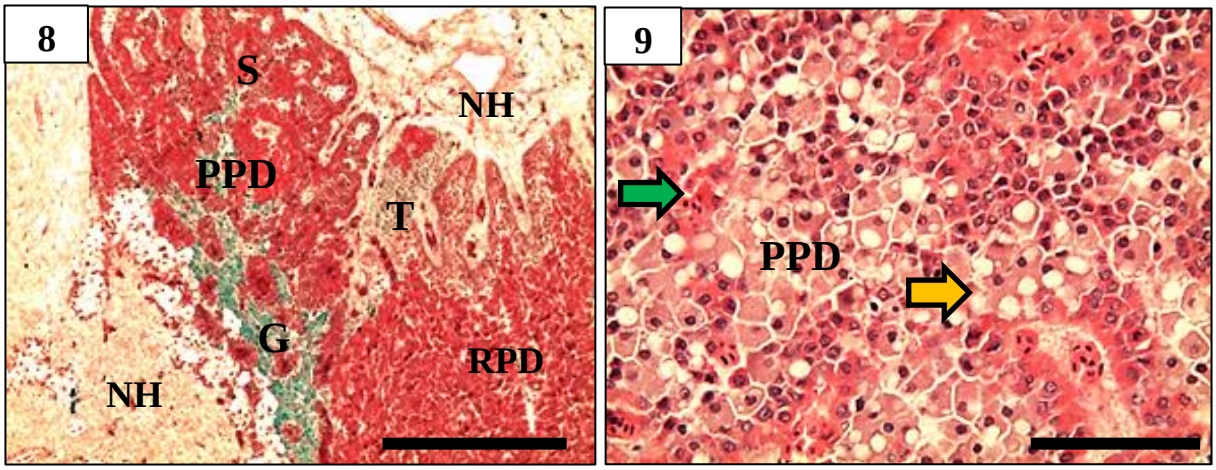

Fig. 8: Photomicrograph of pituitary gland of *Mugil cephalus* showing PPD, RPD, NH, somatotrophs (S), gonadotrophs (G) and thyrotrophs (T). ((MT), bar 200µm). **Fig. 9**: Photomicrograph of PPD of *Mugil cephalus* showing basophilic vaculated gonadotrophs (yellow arrow) and acidophilic somatotrophs (green arrow). (H&E, bar 50 µm).

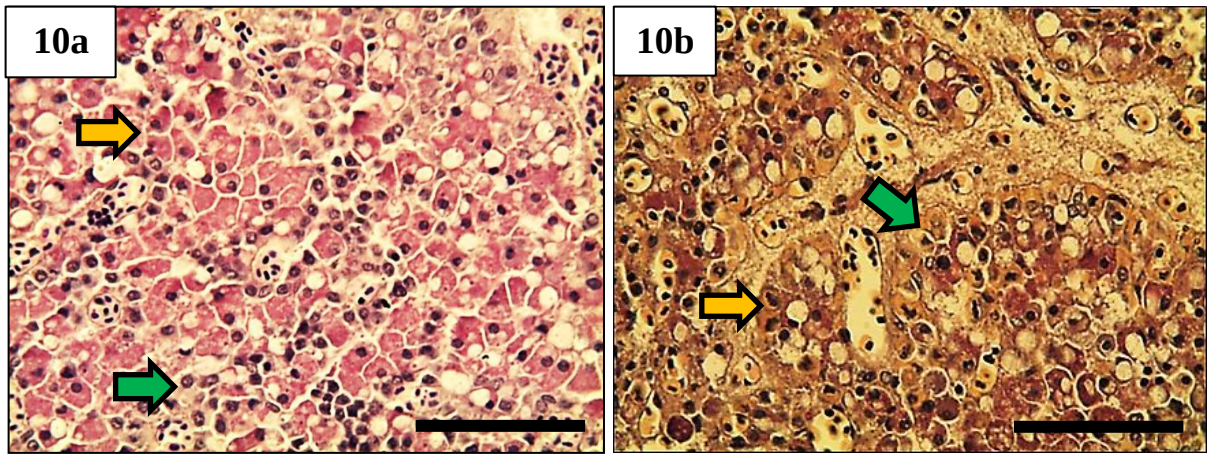

Fig.10: Photomicrograph of the PPD of *Mugil cephalus* showing gonadotrophs (yellow arrow) and somototrophs (green arrow), (**Fig. 10a**, PAS, bar 50µm; **Fig. 10b**, (PAA-AB-PAS-OG, bar 50 µm).

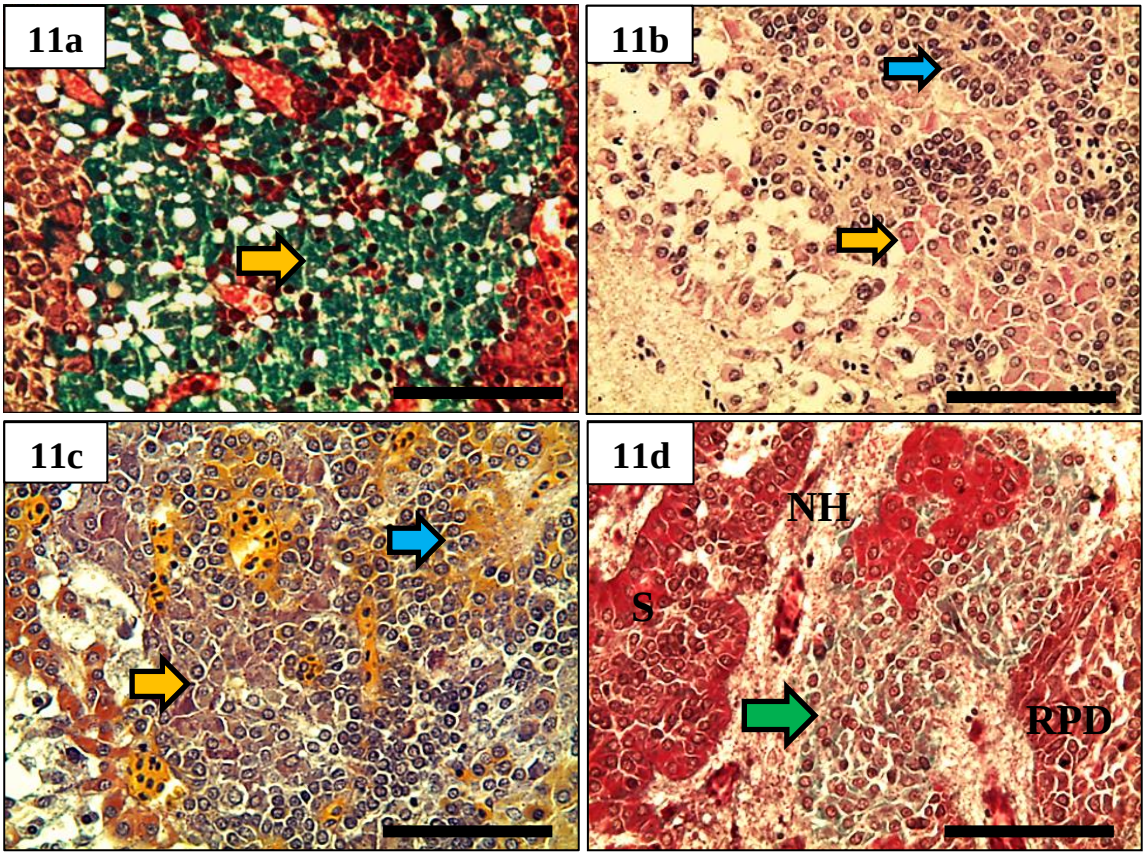

Fig.11: Photomicrograph of PPD of *Mugil cephalus* showing dark green gonadotrophs (yellow arrow). (**Fig. 11a**, MT). PAS positive reaction of gonadotrophs (yellow arrow) and negative reaction of somatotrophs (blue arrow) (**Fig.11b**, PAS). Somatotrophs showed a highly reactivity (blue arrow) (**Fig. 11c,** PAA-AB-PAS-OG). light green colored thyrotrophs (green arrow) located between somatotrophs (S) and RPD. **(Fig. 11d,** MT). bar 50 µm.

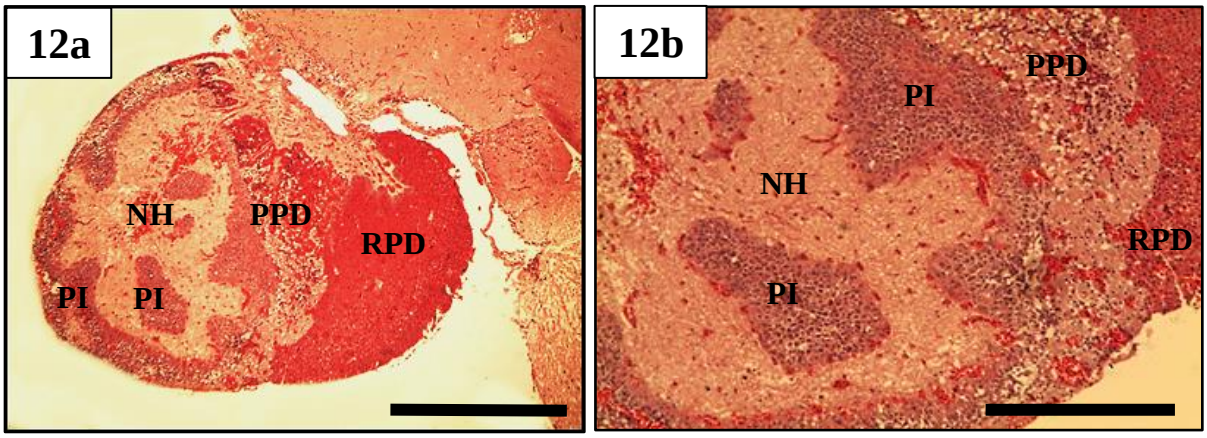

Fig. 12: Photomicrograph of pituitary gland of *Mugil cephalus* showing the pars intermedia (PI) in relation to PPD and NH (**Fig. 12a**; H&E, bar 500 µm). A higher maginfication showing the PI surronded by NH. (**Fig. 12b**; H&E, bar 200 µm).

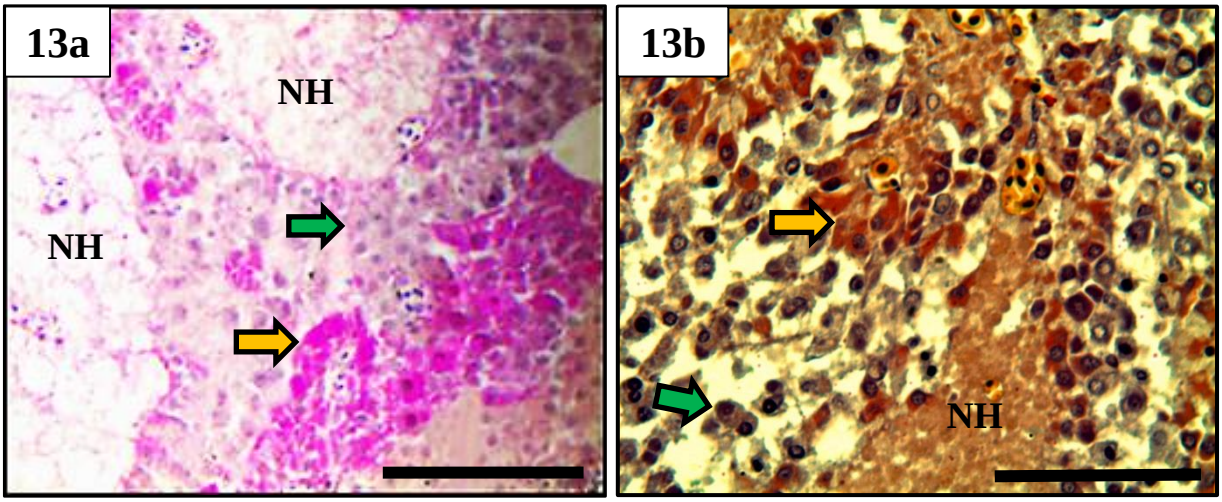

Fig. 13: Photomicrograph of the PI of *Mugil cephalus* showing somatolactotrophs (yellow arrow) and melanotrophs (green arrow) surrounded by NH (**Fig. 13a**, PAS, bar 50 µm; **Fig. 13b**, (PAA-AB-PAS-OG, bar 50 µm).

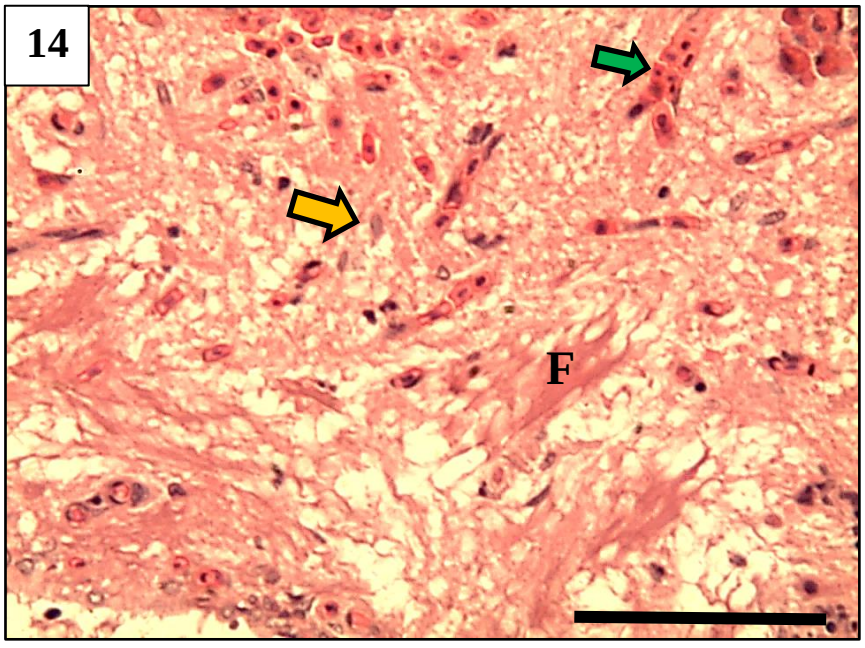

Fig. 14: Photomicrograph of pituitary gland of *Mugil cephalus* showing NH contaning unmylinated nerve fibers (F), pituicytes (yellow arrow) and sinusoial capillaries (green arrow). (H&E, bar 50 µm).

DISCUSSION

This study showed that the pituitary gland of mullet fish (*Mugil cephalus)* consists of two parts, adenohypophysis (glandular part) and neurohypophysis (nervous part). The adenohypophysis revealed three regions, RPD, PPD and PI, this result is similar to that obtained by Narayan *et al.,* (1985) in *Mugil cephalus*, Jafri and Ensor, (1980) in *Aequidens pulcher*, Groman, (1982) in striped bass, Narayan *et al.,* (1984) in *Valamugil cunnesius*, Peute *et al.,* (1984), EL-Zoghby *et al.,* (2008) in *Clarias lazera*, Munro, (1985) in *Aequidens pulcher*, Quesada *et al.,* (1988) in *Sparus aurata*, Toubeau *et al.,* (1991) in *Barbus barbus*, Mousa, (1998), Kasper *et al.,* (2006), El-Sakhawy *et al.,* (2011) and Hassanin and El Asely, (2015) in *Oreochromis niloticus*, Gaber, (2000) in *Bagrus docmac and Bagrus bayad*, Segura-Noguera *et al.,* (2000) in the white seabream, Rodríguez-Gómez *et al.,* (2001) in Bluefin tuna, Kharat and Khillare, (2013) in *Nemacheilus mooreh*, Hassnin and Shoeib, (2014) in *Cyprinus carpio L*; Ye *et al.,* (2020) in grass carp and Oğuz1 and Ünal, (2022) in the Lake Van fish but differed from others in the nomenclature of these three regions as Bachan L et al., (1963) in *Cirrhina mrigala*, Abd El-Rahman *et al.,* (2015) in *Liza carinata* where the pituitary gland was divided into pro-adenohypophysis, meso-adenohypophysis and meta-adeno-hypophysis.

By using certain histochemical staining methods, seven adenohypophyseal cell types were detectable in the pituitary gland of *Mugil cephalus*. Lactotrophs, adrenocorticotrophs in RPD, gonad-otrophs, somatotrophs and thyrotrophs in PPD, melanotrophs and somatolactin cells in PI. While, Narayan *et al.,* (1985) in *Mugil cephalus*, Jafri and Ensor, (1980) in *Aequidens pulcher*, Narayan *et al.,* (1984) in *Valamugil cunnesius*, (Munro, 1985) in *Aequidens pulcher*, Quesada *et al.,* (1988) in *Sparus aurata* and Toubeau *et al.,* (1991) in *Barbus barbus* reported that six adeno-hypophyseal cell types only are detect-able.

In *Mugil cephalus*, lactotrophs and adrenocorticotrophs located in RPD, gonadotrophs, thyrotrophs and somatotrophs located in PPD, the same results were obtained by Jafri and Ensor, (1980) in the roach, Munro, (1985) in *Aequidens pulcher*, Quesada *et al.,* (1988) in *Sparus aurata* and Toubeau *et al.,* (1991) in *Barbus barbus*, but these results differed from those obtained by Narayan *et al.,* (1984) in *Valamugil cunnesius* and Narayan *et al.,* (1985) in *Mugil cephalus* as they mentioned that lactotrophs and thyrotrophs in RPD, adrenocorticotrophs in the interphase between the neurohypophysis and RPD, somatotrophs and gonadotrophs in PPD.

The present study revealed that lactotrophs occupy the major part of RPD, on the other hand Dharmamba and Nishioka, (1968) in *Tilapia mossambica*; Massoud *et al.*, (1985) in *Malapterurus electricus*; Gaber (2000) in *Bagrus docmac* and *Bagrus bayad*; El-Zoghby *et al.,* (2008) in *Clarias lazera* and El-Sakhawy *et al.,* (2011) in Nile tilapia mentioned that the lactotrophs form the major component in RPD, also, islets of lactotrophs are detected in the PPD and individual cells appear subcapsular in PI.

Lactotrophs in *Mugil cephalus* were arranged in cords and not grouped into follicles, the same result was found by Ball and Baker, (1969) in *striped bass*; Benjamin, (1979) in *Crenilabrus melops;* Cinquetti and Dramis, (2006) in *Padogobius martensi* and El-Sakhawy *et al.,* (2011) in Nile tilapia, While Takashima and Hibiya, (1995) in rainbow trout; Gaber, (2000) in *Bagrus docmac and Bagrus bayad* and El Zoghby *et al.,* (2008) in *Clarias lazera* observed that

lactotrophs are arranged in definite follicles with ovoid or spherical lumina.

The current study recorded that adrenocorticotrophs are arranged in cords between RPD and neurohypophysis and no adrenocorticotrophs found in PPD while Gaber, (2000) in *Bagrus docmac and Bagrus bayad*; Weltzien *et al.*, (2003) in Atlantic halibut; El-Zoghby *et al.*, (2008) in *Clarias lazera;* Borella *et al.*, (2009) in *Arapaima gigas* and El-Sakhawy *et al.*, (2011) in Nile tilapia found that isolated adrenocorticotrophs are dispersed deep in PPD.

In *Mugil cephalus*, somatotrophs were located only in PPD and dispersed among gonadotrophs, on the other hand Takashima and Hibiya, (1995) in salmonids; Weltzien *et al.*, (2003) in Atlantic halibut, Kasper *et al.*, (2006) and El-Sakhawy *et al.*, (2011) in Nile tilapia observed that isolated somatotrophs present in PI between the melanotrophs and somatolactin cells bordering the neurohypophysis.

Our study revealed that gonadotrophs are polygonal cells with eccentric nuclei giving positive PAS reaction and located in PPD and scattered in the region between PPD and PI while, Yoakim, (1971) in *Synodontus schall*; Gaber, (2000) in *Bagrus docmac and Bagrus bayad*; El-Gohary, (2001) and El-Sakhawy *et al.*, (2011) in Nile tilapia found that small clusters of gonadotrophs are located along the periphery of PI and isolated cells are also observed intermingled with other cells of RPD.

Zohar *et al.*, (2010) stated that the gonadotrophic cells of teleost fishes play critical roles in the regulation of reproductive processes, so the optimal rates of synthesis and secretion of gonadotrophic hormones are necessary for successful gonadal maturation.

In this study, thyrotrophs were located in PPD, the same result was obtained by Jafri and Ensor, (1980) in the roach, Munro, (1985) in *Aequidens pulcher*, Quesada *et al.*, (1988) in *Sparus aurata* and Toubeau *et al.*, (1991) in *Barbus barbus*. Thyrotrophs showed the same histochemical staining affinities (as gonadotrophs), but they were weaker or lighter which support the result of Takashima and Hibiya (1995) in some species, such as chum salmon, rainbow trout, carp, tuna and red sea bream.

Thyrotrophs regulate thyroidal hormones secretion. The thyroidal hormones have been implicated in almost every aspect of teleost physiology including growth, differentiation, metamorphosis, maturation, reproduction, respiration, various aspects of metabolism, behavior, the central nervous system, seasonal adaptation, temperature tolerance, osmoregulation, and several others (Dodd and Matty, 1964)

In the current study two cell types were detected in PI, the melanotrophs and PAS-positive somatolactin cells, the same results were recorded in *Monopterus albus* (Wai-Sum and Chan, 1974); *Crenilabrus melops* (Benjamin, 1979); *Padogobius martensi* (Cinquetti and Dramis, 2006) and Nile tilapia (El-Sakhawy *et al.*, 2011).

In teleost fish, melanotrophs were the source of melanocyte stimulating hormone (MSH) (Ball *et al.*, 1972). Somatolactin cells may play a role in the control of calcium metabolism, background color adaptation, reproduction, and other processes (Olivereau *et al.*, 1981; Ball and Batten, 1981; Schreibman *et al.*, 1982).

Somatolactin role in the regulation of gonadal functions or sexual maturation, and response to environmental changes have all been suggested (Planas *et al.*, 1992; Rand-Weaver and Swanson, 1993; Olivereau and Rand-Weaver, 1994; Rand-Weaver *et al.*, 1995). However, the

exact function of somatolactin has not been fully understood.

Canepa *et al.*, (2006) concluded that somatolactin was a pituitary hormone present exclusively in fish and was involved in different physiological effects. They added that there was clear evidence towards a possible involvement of somatolactin in the adaptation to background colors in teleost together with MSH.

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