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Histological, Ultrastructural and Immunohistochemical Studies on the Skin of Catfish

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Abstract

This study was done on the skin of head, trunk and tail of 10 mature catfish collected from local markets to investigate the histological, ultrastructural and Immunohistochemical structure of the skin. The epidermis was consisted of stratified squamous epithelium containing Malpighian, mucous cells and club cells (alarm cells). The Malpighian cells were the most numerous cells of the epidermis of the skin. The mucous cells varied from rounded to goblet shape with basal basophilic nucleus and acidophilic cytoplasm. The club cells had different shapes; rounded, oval and elongated with spherical central basophilic nucleus. The dermis contained stratum spongiosum or adiposum depending upon the body region beside stratum compactum. Hypodermis comprised of loose connective tissue containing adipose cells. TEM investigation showed mucous cells contained mucous globules of different electron staining filling apical part of the cell with numerous mitochondria and abundant RER. Club cells had one or two nuclei with prominent nucleolus. The epidermal and club cells showed positive immunostaining with BAX, while the mucous cells showed a negative reaction.

Keywords: BAX, Catfish, Histology, Skin, TEM.

1. Introduction

The skin of a fish has several functions such as sensation, secretion, maintenance of osmotic pressure and acts as first line of defense against microorganisms in some teleost and family Sciaenidae [10]. Secretory function is one of the important functions of the skin owing to the presence of mucous cells and club cells in brown bull head, eel and catfish and carp [43,16]

The skin of fish composed of three main layers; epidermis, dermis and hypodermis. The epidermis consists of three basic layers are basal cuboidal or columnar, intermediate polyhedral and superficial flat cells [13] in Cyprinus carpio, [35] in Korean spined loach, Iksookimia koreensis and [15] in turbot fish. In contrast, the epidermis of some marine fish is composed of five layers; stratum basal, spinosum, granulosum, lucidum and corneum[4]. The intermediate layer of the epidermis is composed of polyhedral cells, mucous and club cells in palembang puffer fish and torrent catfish [21] and [36].

Malpighian cells are the most numerous cells of the epidermis of the skin in fish. In the basal layer of epidermis, Malpighian cells appear as columnar cells, while in the superficial layer, the cells become flattened as in marine teleost fish and eel fish [4,16].

Mucous cells have spherical or elongated shape with basal nucleus and pale acidophilic cytoplasm in catfish [However, they have saccular shape in [12]almon salar [38] . TEM revealed that the mucous cell contains basal nucleus and mucous granules filling the apical part of the cell in the arctic lamprey [39].

Club cells are differed from spherical to flask-shaped with slightly acidophilic cytoplasm and rounded basophilic centrally placed nucleus [37] in Indian major carp, while they are not found in Tilapia nilotica [14] Ultra-structurally, the club cells contain numerous organelles and large vacuoles. The plasma membrane characterized by presence of several invaginations in Neotropical catfish, Pimelodella lateristriga[5].

The dermis is divided into two well-differentiated layers, the superficial loose connective tissue layer (stratum spongiosum) and the deeper layer of compactly arranged bundles of collagen fibers (stratum compactum) as mentioned [28] , [6] in Mystus [15] in turbot fish and.

The hypodermis is composed of loose connective tissue. This layer is binding the dermis with underlying muscle bundles [24] [15] in trout and turbot fish respectively.

Therefore, the aim of this work is to throw more light on the histological structure of catfish skin with special reference to ultrastructural and immunohistochemical changes of the skin.

2. Materials and methods

2.1 Light microscopy

Skin specimens of 10 mature catfish of both sexes were taken from head, trunk and tail during period from September 2014 to September 2015.The used fish were collected from El-Kalyobia Governorate, Egypt. The specimens were fixed in 10% neutral buffered formalin, dehydrated in alcohol, cleared in xylene and embedded in paraffin wax. Sections of 5-7µm thick were cut then stained with Harri's haematoxylin and eosin (H&E), Periodic acid Schiff (PAS) techinque, Alcian blue method (PH 2.5) and Gomori's reticulin according to[2].

2.2 Transmission electron microscopy (TEM)

The specimens (nearly 1 mm3, each) were fixed by immersion in 5% glutaraldehyde prepared in 0.1M sodium cacodylate buffer. Specimens were secondary fixed by immersion in 1% osmium tetroxide prepared in 0.1M sodium cacodylate

buffer. The specimens were dehydrated in ascending grades of ethanol (60, 70, 80, 90 and 100%), each for 15 minutes in each grade. Dehydrated specimens were embedded in an epoxy resin for 1 hour at 40°C. Semithin sections (1 µm thick) were cut for light microscopy and stained with 1% toluidine blue . Ultra-thin sections of 60 nm thick were stained with uranyl acetate followed by lead citrate. Stained sections were examined with a JEOL 1010 Transmission Electron Microscope at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University. Sample preparations were done according to [3].

2.3 Immunohistochemical examination

Paraffin sections of 5 micrometers were prepared on positive charged microscope slides. Sections were deparaffinezed in xylene, rehydrated in descending grades of ethanol then distilled water and rinsed in phosphate buffered saline. Antigen retrieval was done by heating the tissue section in 10mM citrate buffer, PH 6.0 for 10minutes followed by cooling at room temperature for 20 minutes. Rabbit anti-human BAX polyclonal antibodies (catalog number E17990, spring bioscience, Pleasanton, California, USA). BAX at dilution 1:200 was incubated at room temperature for 30 minutes. Visualization was done as outlined [27].

3. Results

3.1 Light microscopy

The skin of catfish consisted of three layers; epidermis, dermis and hypodermis Figs (1,2) Head epidermis was more folded than trunk and tail epidermis Fig (3) Tail epidermis was the thinnest part of the skin Fig (4) The main three layers forming the stratified squamous epithelium were basal columnar cells with oval basal basophilic nucleus and acidophilic cytoplasm, intermediate polyhedral cells with spherical basophilic central nucleus and superficial flat cells with flat nucleus Fig (5).

The epidermis contained also mucous and club cells. Mucous cells were varied from rounded to goblet shape with basal oval basophilic nucleus and pale acidophilic cytoplasm. The mucous cells were mainly located at the superficial layer of epidermis Fig (6). Club cells (alarm cells) appeared; rounded, oval and elongated with centrally located and acidophilic cytoplasm. Some cells were binucleated. The club cells were located mainly at the middle layer of epidermis and sometimes presented at the superficial layer of the epidermis Fig (7). Both club and mucous cells showed weak positive reaction with PAS Fig (8). The mucous cells Fig (9) showed positive reaction with alcian blue (pH = 2.5).

The dermis of head and ventral trunk was formed of stratum adiposum and stratum compactum Fig (2), while in tail and dorsal trunk, dermis was composed of stratum spongiosum and stratum compactum Fig (1). The dermis of dorsal trunk contained numerous melanocytes than that of the ventral trunk Fig (10). The stratum spongiosum consisted of layer of loose connective tissue containing collagen fibers, melanocytes and blood vessels, while the stratum adiposum was differentiated into two layers. The upper thin layer consisted of loose connective tissue containing melanocytes, fibroblast cells and blood vessels. The lower thick layer was composed of adipose cells that arranged into clusters, each was surrounded by connective tissue capsule. The stratum compactum consisted of regular bundles of collagen fibers Fig (2).

Hypodermis was formed from layer of loose connective tissue containing white adipose cells Figs (1,2) .

3.2 Transmission electron microscopy

Mucous cells had goblet shape with basal, oval nucleus and mucous globules which had different shapes and electron density and filling the apical part of the cell Fig (11), numerous mitochondria and abundant RER. Club cells had elongated shape with spherical, central nucleus and sometimes contain two nuclei with prominent nucleolus Fig (12).

3.3 Immunohisto chemistry

Malpighian or epidermal cells especially middle one and club cells showed a positive cytoplasmic reaction and negative nuclear reaction with BAX, while the mucous cells showed a negative reaction Fig (13).

4. Discussion

The present study showed that the epidermis of the catfish is formed of basal, intermediate and superficial layer. The same result was mentioned [13] in Cyprinus carpio, [4] described five layers of the epidermis in some marine fish are stratum basal, spinosum, granulosum, lucidum and corneum. Unicellular gland as mucous and club cells are appeared in the epidermis of catfish which is similar to those illustrated in other teleost [4].

In our study the mucous cells appeared in different shapes; spherical, elongated and goblet shape. They located in the middle and superficial layer of epidermis similar to the corresponding ones of carp [12] Despite mucous cells comprised one of the epidermis components in catfish and serve in secretion of a gel-like viscous substance named surface mucus which has a role in lubrication, and protection against infection [44,45] .they are not found in the epidermis of Paddle fish [30] and Pimelodella lateristriga [5] . In the present study, the mucous cells showed positive reaction with both alcian blue (pH = 2.5) and PAS respectively. We suggested that this is due to the presence of acidic and neutral mucopolysaccharides. The TEM investigation of the present study revealed that the

mucous cells of the epidermis contained numerous mitochondria, abundant RER and many secretory vesicles of different electron density occupied apical and supranuclear region of the cell. This result in accordance with [20] in [39] in Arctic lamprey and [8] in Antarctic notothenioid Fish, Gymnodraco acuticeps.

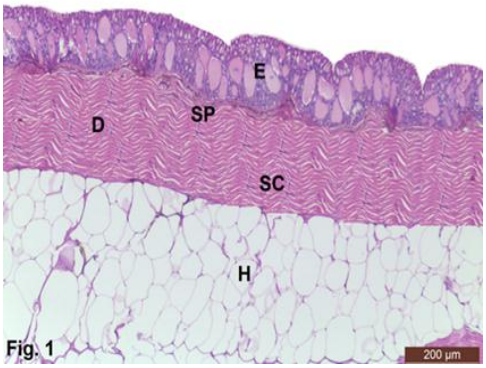

Fig (1) Photomicrograph of the skin from catfish dorsal trunk region showing epidermis (E), dermis (D) which contains stratum spongiosum (SP) and stratum compactum (SC) and hypodermis (H), H&E (200 µm)

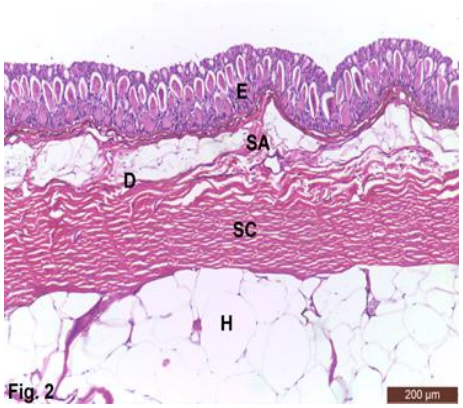

Fig (2) Photomicrograph of the skin from catfish ventral trunk region showing epidermis (E), dermis (D) which contains stratum adiposum (SA) and stratum compactum (SC) and hypodermis (H), H&E (200 µm)

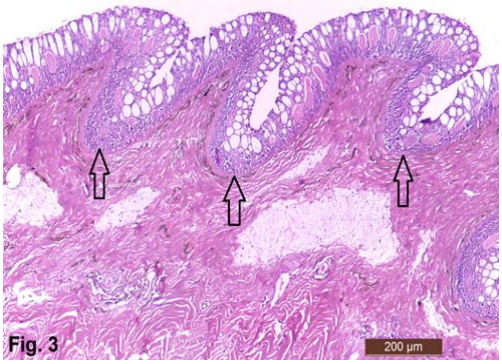

Fig (3) Photomicrograph of the skin from catfish head region showing highly folded epidermis (arrows), H&E (200 µm)

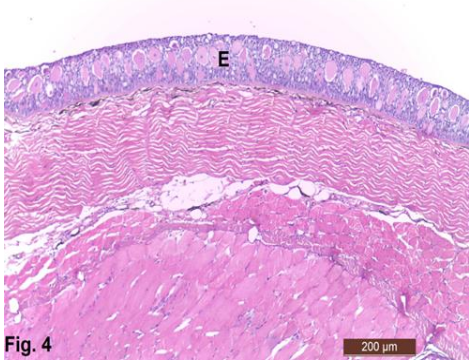

Fig (4) Photomicrograph of the skin from catfish tail region showing thin epidermis (E), H&E (200 µm)

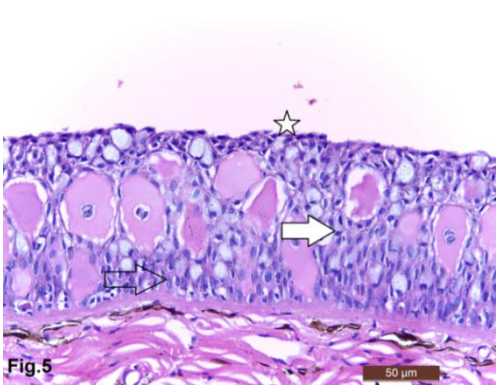

Fig (5) Photomicrograph of the skin from catfish epidermis showing basal columnar cells (arrow), middle polyhedral cells (white arrow) and superficial flat cells (star), H&E (50 µm)

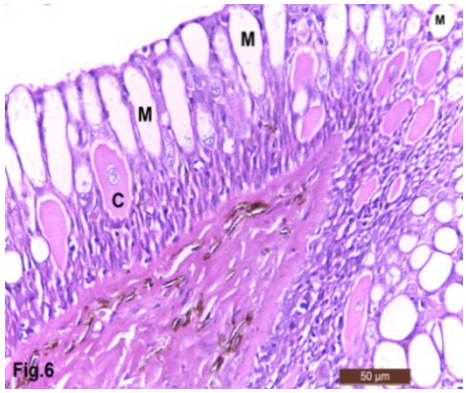

Fig (6) Photomicrograph of the skin from catfish showing mucous (M) and club cells (C), H&E (50 µm)

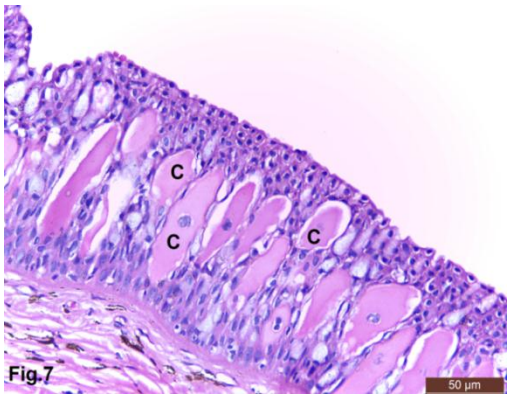

Fig (7) Photomicrograph of the skin from catfish showing club cells (C), H&E (50 µm)

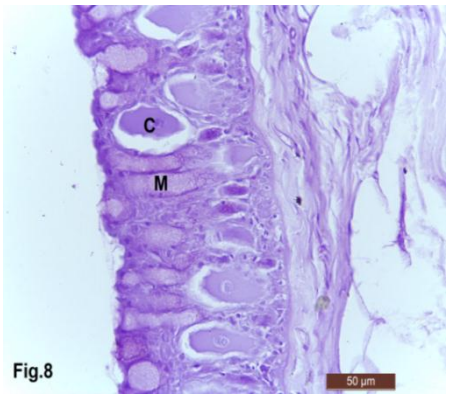

Fig (8) Photomicrograph of the skin from catfish epidermis showing positive reaction of club (C) and mucous (M) cells to PAS technique (50 µm)

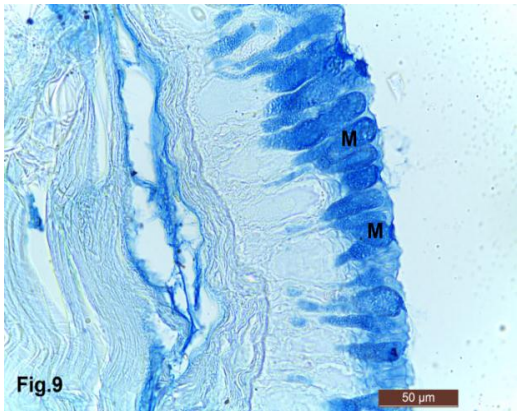

Fig (9) Photomicrograph of the skin from catfish epidermis showing the mucous cells (M) contain alcianophilic granules, AB (pH = 2.5) (50 µm)

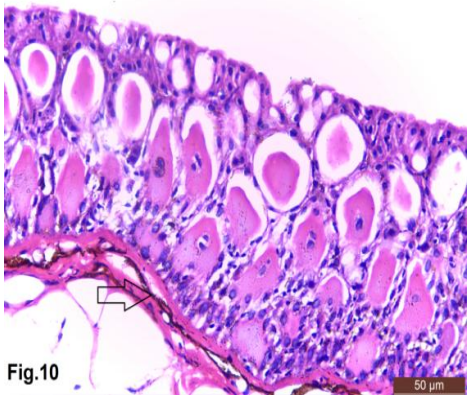

Fig (10) Photomicrograph of the skin from catfish dorsal trunk dermis showing numerous melanocytes (arrow), H&E (50 µm)

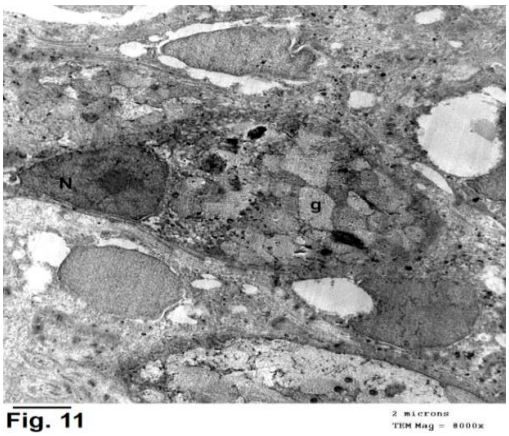

Fig (11) TEM micrograph showing mucous cell contains basal nucleus (N) and mucous globules (g), (8000X)

Fig (12) TEM micrograph showing club cell contain two neclei (N), (6000X)

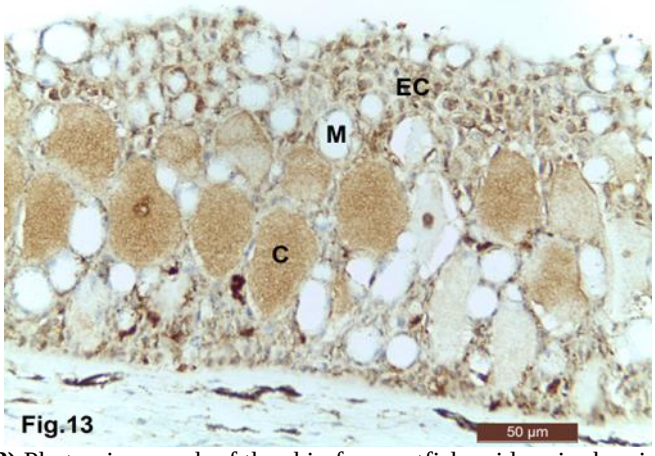

Fig (13) Photomicrograph of the skin from catfish epidermis showing positive reaction of club cells (C), BAX (50 µm).

The present study identified club cells in the epidermis of catfish that have a role in production of protein that initiates alarm reactions when perceived by olfactory organs of fish and may act as a warning of danger to the other fishes, meanwhile they are not found in Tilapia nilotica [14]. Club cells of catfish have large rounded or oval shape with central one or two nuclei that is similar to the siluroids[1] , Cyprinids [40] and neotropical catfish [5] Club cells of catfish give weak positive reaction to PAS due to presence of acidic mucopolysaccharides. This result is disagreed [42] and Park et al. (2003) in Siluriforms and [19] in Cyprinids. Ultrastucturally, the club cells contain one or two irregular, heterochromatic nuclei with prominent nucleolus. This is disagreed with [5] in Pimelodella lateristriga.

The present investigation revealed that the dermis of head, dorsal trunk and tail is composed of stratum spongiosum and stratum compactum. The same result was mentioned by [22] in Anguilla Anguilla and Cyprinus carpio and [14] in Anguilla vulgaris and Tilapia nilotica. However, the dermis of ventral trunk was similar to the structure of African catfish [18] which comprised of stratum adiposum and stratum compactum. The body dorsum is appeared darker than the ventral one due to high concentration of melanophores. This result is similar to that [30] in [24] in Trout.

The hypodermis is well developed layer of loose connective tissue mainly contained adipose cells. This is similar to hypodermis of Heteropneustes fossilis [33] and Tetradon fluviatilis [31]. However, the hypodermis of Bagrus bayad [33] is ill developed or absent.

The epidermis of fish contains different mucopolysaccharides, proteoglycans and carbohydrate-binding lectin proteins which have a role in immunoprotection [29] and [34] . For instance, carbohydrate-specific proteins, mucus and mucopolysaccharides are covering eel skin and responsible for innate immune reaction [7].

Distinguishing the tissue and organs during development as well as removing of terminally damaged cells are the main functions of apoptosis [23] which is regulated by many cellular signaling pathways [9] and biochemical events, which result in cellular changes including bleeding, shrinkage, nuclear fragmentation, chromatin condensation and chromosomal DNA fragmentation, and finally lead to cell death [9] and [11]. Bcl-2 family proteins, including BAX regulate apoptosis intrinsic pathway and responsible for an increase in the mitochondrial membrane permeability to cytochrome C. The positive reaction of BAX in the epidermal and club cells referred to continuous changes and turnover of these cells due to the process of apoptosis [17] and [26].

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