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Histomorphometric and Histochemistry of Mucous Secreting Cells in Different Parts of Skin in Shabut (*Barbusgrypus*, Heckel 1843)

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ABSTRACT

The role of skin in fish is providing a physical barrier, first line of defense against pathogens, osmotic pressure, physical injury and acts as a semi-permeable osmotic barrier to maintain ionic and fluid balance in marine and fresh water fish. This study was done since, there is some reports about skin of freshwater histology information but the histological structure and chemical composition of *Barbusgrypus* has not been reported. Skin samples obtained from 9 different zones of the body of ten healthy male and female *Barbusgrypus*. The routine procedures of preparation of tissues were followed and the paraffin blocks were cut at 6 microns, stained with H&E, PAS and AB and studied under light microscope. Results showed that, epidermis formed of non keratinized stratified squamous epithelium with mucous goblet like cells. Mucous cells were along the superficial cells layers and their distributions varied greatly. The highest number of mucous cells by using of histomorphometric and histochemistry was in the head and back regions, and the lowest was in the ventral region. Female had no higher number of mucous cells as compared to male. Ultrastructural finding revealed that mucus cells, with numerous aggregation and lacked of folding but there were numerous interphalangial joints between them.

KEYWORDS: Histomorphometric, mucous secreting cells, Shabut, *Barbusgrypus*

1.INTRODUCTION

Based on studies and according to the records of FAO Shabut, also known as *Barbusgrypus.* It is one of the most significant fish species listed in the fresh waters of the rivers along South and SouthwestIran, the Karoon river, in some rivers of Iraq, and also in the Euphrates River and Tigris Rivers in Turkey (Selki*et al.,* 2005; Zivotofskya& Amar, 2006; Dorostghoal *et al*., 2009 and Fuczyska*et al*., 2008).Skin as primary defence system in fish is the interface between the external and internal environment of the animal for example osmotic pressure and physical force(Al-Banaw *et al*., 2010). Forming the body's first line of defense, it comes into direct contact with all water toxic chemicals, parasites, communication, sensory perception, locomotion, respiration, excretion and thermal regulation (Hausen., 2005; Hiroi., 2004 and Murray *et al*., 2012). Skin is one of the largest organs in a fish (Mckim and Lien., 2001), making up approximately 10 percent of the body weight(Park, 2002; Wisenden *et al*., 2009). Morphologic and functional differences can be great as there are many species of fish occupying a multitude of habitats in both fresh and saltwater (Pinky *et al*.,2008; Sharifpour., 2004). Epidermis of skin in teleost's, for example *Barbusgrypus,* contain several types of secretory cells including mucous cells (Ghattas and Yani., 2010; Sire and Akimenko., 2004). Mucous secreted from these cells acts as natural defense against parasites and pathogenic organisms and helps marine in locomotion, osmoregulation and lubrication (Palaksha *et al*., 2008). Mucous glands, which aid in maintaining the water balance and offer protection from bacteria, are extremely numerous in fish skin(Scillitani *et al*., 2012).Distribution of mucous cells changes in their number and thickness of the epidermis, have been studied in salmonids by (Stocklosowa, 1966). Although there is some reports about skin of freshwater histology information but the histological structure and chemical composition of *Barbusgrypus* has not been reported. Therefore, the aims of this study were to study the structure of mucous secreting cells in *Barbusgrypus* skin by using light and scanning electron microscopic techniques and also to investigate the chemical composition of *Barbusgrypus* skin.

2.MATERIALS AND METHODS

All chemicals for microscopic observation were purchased from Merck (Darmstadt, Germany). Samples,2-3 years-old, for this research were obtained from warm water fish hatchery in then on-reproductive season from fresh water fish reproduction and development center, Iran. For this reason the skins of 10 adult *Barbusgrypus*, after biometry were removed. Both mature male (Av.

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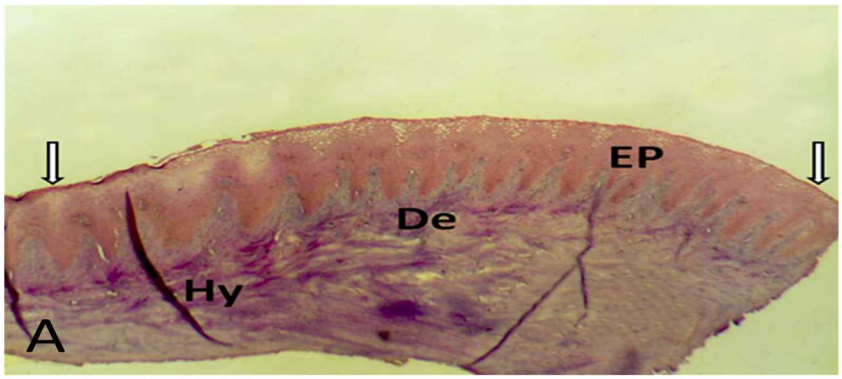
Wt: 1630.75±2.15 gr, Av. L: 62.8±1.58cm) and female(Av. Wt:2400.15±1.66 gr, Av. L: 67.50±1.51cm) were used in the experiment. The fishes were anaesthetized by gillyflower extract before taking out the skin samples. The skin of fish was removed manually. The cleaned samples were washed with tap water and drained. The skin was then cut into small pieces (0.5 cm) and placed in fixative. Samples were obtained from 9 zones of the body. The selected zones from different regions of the body were taken from the head region, upper and lower lips, maxillary and mandible barbell, dorsal, middle and ventral body near dorsal fin and caudal stalk region. Then for fixation, for light microscope and transmission electron microscopy (TEM) samples were located in neutral buffer formal in and glutaral dehydesolution. Routine procedures of preparation of tissues were followed and the paraffin blocks were cut at 5 to 6 microns, stained with H&E(hematoxylinandeosin), PAS(Periodic Acid Schiff)and AB (Alcian blue)(PH 2.5) studied under light microscope(Bancroft and Gamble., 2002;Deminique*et al*., 2004).For the measurement of the number of mucous secreting cells special eye piece micrometer was prepared as microscope was equipped with a Dinolitelens connected to PC. For TEM, the prepared skins with a thickness of 2–3 mm were rapidly fixed with 2.5% glutaraldehydein 0.2 M phosphate buffer (pH 7.2)and post fixed in 1% osmium tetroxide in the same buffer (Demeestere and Mast 2009). Routine preparation of tissues for TEM examination followed and ethanol dehydrated tissues were embedded in LX 112. Then blocks were cut at 5onm by ultra microtome, stained with Lead citrate and Uranyl acetate. They were observed and investigated by TEM (CM110, Philips, Holland).

3.RESULTS

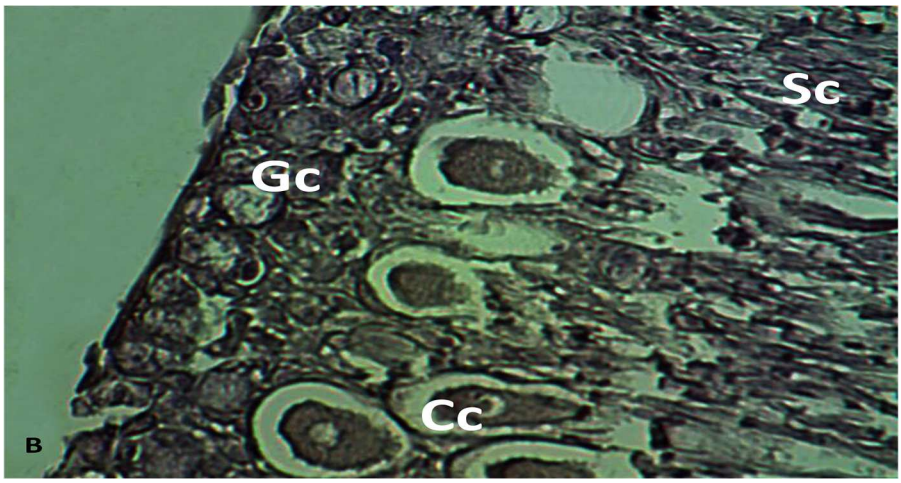
Histomorphology and Histochemistry

In almost all the zones, results showed that fish skin of *Barbusgrypus* consisted of epidermis which non-keratinized stratified squamous epithelium and underlined dermis which formed of dense regular connective tissue followed by hypodermis of loose connected tissue. Under normal skin histology examination of Shabut the sequence of cell types from the outer extreme towards the interior was pavement cells, the outermost cellular layer of the epidermis, followed the interior by randomly distributed mucous cells, filament cells and club cells. Rodlet cells and chloride cells were absent from the skin of freshwater Shabut. By histological examination, in the middle to the outer of epiderm, there were large spherical and dilated cells that were placed in several rows.

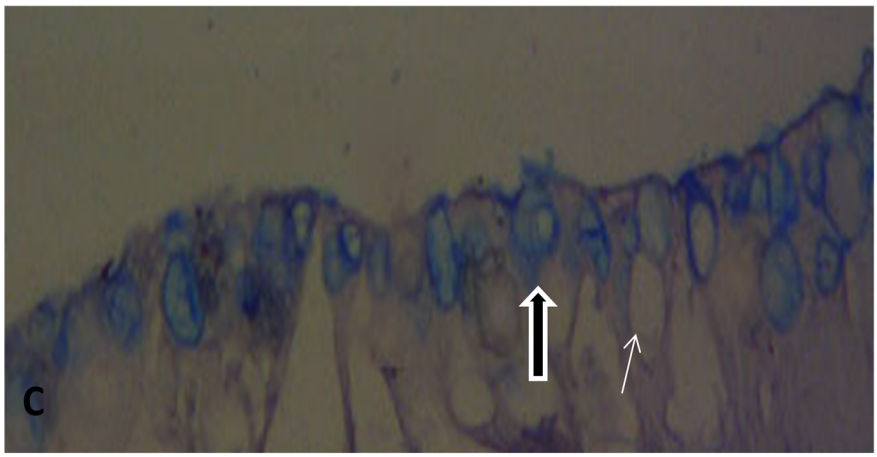
ThecellswerestainedwithH&Equiteclearcytoplasmandcytoplasmicstainingpurple to bright red with PAS that are called mucous secreting or goblet cells. These cells had a condensed heterochromatin appearance nucleus as in the surface area they were larger elongated but more spherical shapes were observed in midrace. With AB staining, two aspects of mucus cell activity in the Shabutskin have been registered in the course of our experiments. Based on this finding, mucuscell scontainingacid nature that consists mainly of sulfatedmucin and N –acetyl syalomucin were blue and the others containing Neutral nature were not taken color. The former were most and near the surface but the latter were the lowest number. Inultra structural finding, in contrast to epidermal epithelial cells that contain cells with folded nuclei, mucus cells lacked of folding but there were numerous interphalangeal joints between them. Mucous cells were numerous and were in various stages of development throughout the epidermis. They begin their differentiation in the stratum germinativum and migrate upward through the epidermis at a rate required to replace mature cells lost at the surface. As they move upward through the middle layer of the epidermis, they actively synthesize muslin packets and store them within the cell(Fig1, A-E).



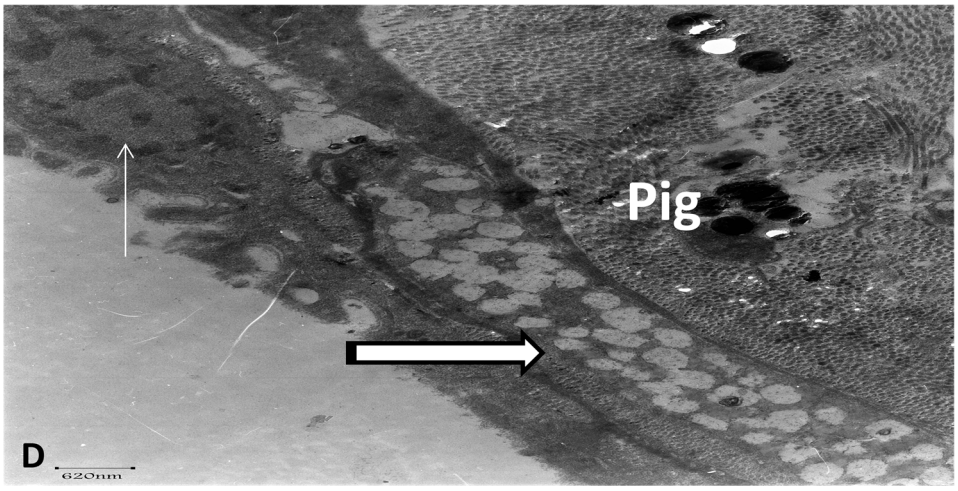
A: A photomicrograph of skin of lip of Shabut (*Barbusgrypus*) showing thick epidermis of stratified squamous epithelium (EP), with dermis (De), hypodermis (Hy), external region of lip (left arrow) and internal of lip (right arrow) (H&E ×100).



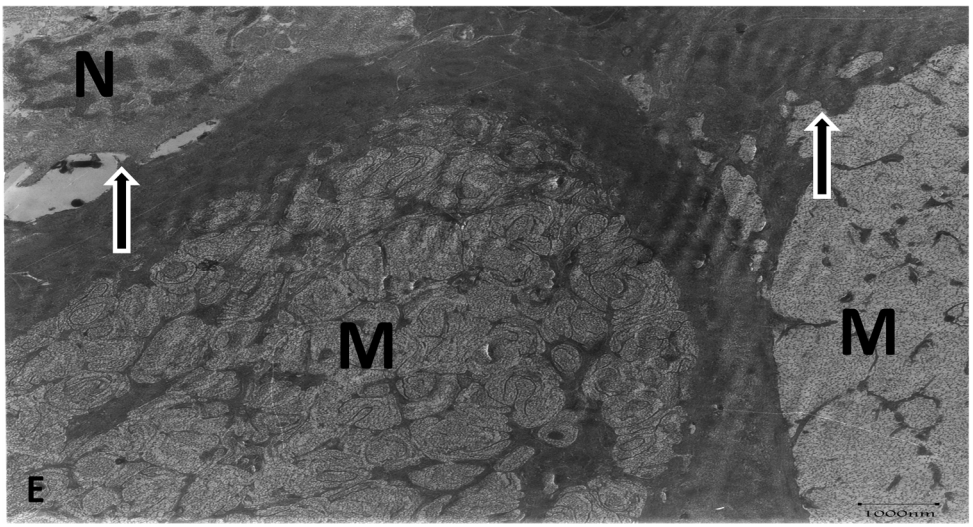
B: A photomicrograph of skin of barble of Shabut (*Barbusgrypus*) showing PAS positive mucus cells (Gc) against PAS negative club cells (Cc) and squamous cells (Sc) (PAS ×400).



C: A photomicrograph of skin of barble of Shabut (*Barbusgrypus*) showing acid mucous secreting cells (left arrow) with neutral mucous secreting cells (right arrow) (AB ×400).



D: A photomicrograph of skin of head of Shabut (*Barbusgrypus*) showing a mucus cell with numerous aggregation mucus (thick arrow), nucleus (arrow) and aggregation of pigment (pig) (×620nm).



E: A photomicrograph of skin of head of Shabut (*Barbusgrypus*) showing a mucus cell with cytoplasmic contents (M), finger joints with membrane of epidermal cells (arrows) and nucleus of epidermal cells (N) (×620nm).

4.Histometric

Having determined the mean and standard deviation, indifferent are as of the skin epidermis of Shabut fish showed mucus cells were various in number ($p<0.05$) (See Table I). Then using Tukey test, these different areas were compared pair wise (Fig2).Based on this fig, the skin of studied fish was divided into fourzones. So, skin of the head with the greatest number (351.8±4.92) while the ventral part of the body with the minimum number (15.20±1. 30), having significant with other are as formed an area (a) and(d).In comparison, outer surface of the lips and barbless with dorsal and middle regions of body together were in one area(b) and (c).As shown in this fig, the number of mucuscellsinthedorsal, ventral, middle and coudal stalk regions of body marked no significant difference (cd) ($p<0.05$).For neutral and acid mucous secreting cells results showed skin of studied fish was divided in to fourzones. As skin of the head with the greatest number (43.90±1.26, 132.70±3.76) while the ventral part of the body with the minimum number(1.94±0/65, 5.71±1.95) having significant with other are as (a) and (d) formed an area. In comparison, outer surface of the lips and barbles with dorsal and middle regions of body together (b) and (c) were in one area. According to this fig, the number of neutral and acid mucous secreting cells in the dorsal, ventral, middle and caudal stalk regions of body indicated no significant difference (cd) ($p<0.05$).

Table I. Number of different regions and factors studied in Shabut (*Barbusgrypus*)

	Head	Lips	Barbles	Dorsal body	Middle body	Ventral body	Caudal stalk
Number of mocous secreting cells	351.8±4.92	147.6±1.95	117.8±1.74	26.40±2.30	44.60±1.14	15.20±1. 30	38.20±0.83
Acid mocous secreting cells	132.70±3.76	56.70±1.46	44.15±1.30	9.90±0.84	16.32±0.42	5.71±1.95	14.32±0.62
Neutralmocous secreting cells	43.90±1.26	18.90±0.48	14.70±0.43	3.30±0.28	5.60±0.14	1.94±0.65	4.77±0.23

5.DISCUSSION

The function of fish skin is very important because it is the interface between the external and internal environment of the animal. Base on studies many styles, variations in structure, and adaptations in the fish skin are observed across the numerous marine and freshwater fish species that make up this highly diverse group of aquatic vertebrates (Fontenot and Neiffer, 2004).

The microscopy results from different areas of the skin of *Barbusgrypus* indicated that the basic structure of these regions differ in different parts of the body. Generally, the histological structure of the skin was the other aquatic, and forms the tree main categories in the outer epidermis, dermis and hypoderm is in the middle and the bottom as findings of this study consistent with other researchers on several species (Mastrodonato *et al*., 2005; Clayton *et al*., 2009; Evans and Claiborne., 2006).The outer skin layer or epidermis in *Barbusgrypus* like other species consists of squamous or cuboidal cells that contain filamentals by TEM results called to no fibrils that tie the epithelial cells together and give rigidity to the epidermis(Al-Banaw*et al*., 2010). Through the study, a unique feature of the epidermis of teleost is its richness of unicellular glands, the most abundant being the mucous cell. Mucus cells are numerous and in various stages of development throughout the epidermis in both freshwater and marine fish(Clayton et al., 2009). In micrographs of *Barbusgrypus* epidermis they begin their differentiation in the stratum germinativum

and migrate upward through the epidermis at a rate required to replace mature cells lost at the surface. As they move toward the surface through the middle layer of the epidermis, they actively synthesize mucin packets and store them within the cell (Demeestere and Mast 2009). On reaching the skin surface the biggest mucus cells were seen, and this area they forced together between epithelial cells, to release their contents. According to using ABon different parts of the skin of *Barbusgrypus*, two types of secreting mucus cells; all cells with acid secreting mucosa were mostly near or on the surface of the skin but cells with natural secreting mucosa were mostly below the surface layer and slightly lower than it had been. This finding was reported on cyprinidae skin by (Pinky *et al*, 2008) and cat fish skin by (Al-Banaw *et al*., 2010).The researchers also reported that the number, placement increase or decrease of mucosal cells exposed several factors may as temperature, season and reproductive stressors. The main function of mucus, reducingfrictioneasyswimduetothelargenumberofcellsintheheadandupperbody of the fish justifies (Mittal *et al*., 1994). This Corresponded with our study that the large number of mucus cells was on the head and upper part of the body and the lowest was on the ventral part of body in *Barbusgrypus*.

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