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Histomorphology of oesophagus and histochemical characterization of oesophageal mucin of the catfish *Heteropneustes fossilis*

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Abstract
The structural organization of catfish GIT consist of a short, thick-walled oesophagus connected with J-shaped stomach which terminates into three segmented intestine. The histology of gut wall, particularly oesophagus, reveals four typical layers, namely, serosa, muscularis, submucosa and mucosa. The innermost mucosal epithelium is stratified and is made up of basal cuboidal cells, intermediate columnar mucous cells and superficial flattened cells. The middle layer constitutes the major thickness of mucosal epithelium and the taste buds are absent. The mucopolysaccharides present in oesophageal mucosa stain positive with Alcian Blue (pH 2.5) and Periodic Acid Schiff reagent suggesting the presence of acidic and neutral glycoproteins (GPs) where the latter seems to be predominant. There is complete absence of sulphated GP in the oesophagus which is confirmed by the Methylation and Saponification. The neutral GP may aid in lubrication whereas carboxylated glycoproteins may protect the mucosal lining from the invasion of various pathogen. The absence of sulphated moiety may indicate that the functional necessity of this GP may either be diminished or not required at all.

Keywords: Catfish, esophagus, Histochemistry, mucin, glycoproteins

1. Introduction

The gastrointestinal tract (GIT) in all vertebrates, with mouth at one end and anus at the other, is essential for obtaining food from external source and passing it on to the internal environment for assimilation. In different vertebrate groups, there are large differences in the morphology and physiology of the gastrointestinal regions which may be attributed either to the diet or feeding strategy or to the type of the habitat which the animal occupies.

The oesophagus in fishes is very short, thick-walled and straight tube connected dorsally to pharynx and ventrally to stomach or directly to intestine in agastric fishes. The distensible nature of oesophagus is evident from the fact that most of the predaceous fishes have been found to engulf fishes as big as their own size and never suffer from blockage. The structural variations in the oesophagus, like GIT, may relate to functional attributes of the teleosts. In freshwater fishes, the oesophagus, in general, bears longitudinal folds which are lined with multilayered squmaous epithelium and contain large number of mucous cells. However, in the marine teleosts, it is more complex and is provided with highly vascularized columnar mucosal folds with few mucous cells. This structural feature has been associated with the osmoregulatory function in marine fishes. The structural variations in GIT, particularly oesophagus, necessitates that a large number of species should be studied to take the measure of such variations.

The catfish *Heteropneustes fossilis* is a commercially important food fish of Indian subcontinent. While various facets of its physiology have been extensively studied [1, 2, 3], no detailed report is available on the histomorphology and qualitative nature of the mucin in the oesophagus of this fish. Thus, the aim of the present investigation is to study (1) the GIT and histological organization of oesophagus and (2) to characterize the mucosubstances present on the oesophagus of the catfish *H. fossilis.* This study will give an insight into the functional aspects of the oesophagus particularly related to its dietary habits of this catfish which may serve as an important input to fishery scientists in aquaculture management.

2. Material and Methods

2.1 Experiment protocol

The experiments on GIT were performed on well acclimatized catfish *H. fossilis* (35 - 40 g, approximately 15 cm Length) obtained from local fish market of Aligarh and maintained under controlled laboratory conditions. Ten fish were anaesthetized and sacrificed by decapitation and the entire GIT from oesophagus to rectum was removed. The oesophagus was cut from the rest of the GIT and fixed in Carnoy's fluid for 2 h and processed for histological and histochemical studies as detailed below.

2.2 Histology of Tissues

The fixed tissues of oesophagus were processed following the standard methodology of paraffin block making and 5 micron thin sections were obtained using Leica microtome (Leica RM 2125 RTS).

2.3 Staining details

The oesophagus sections of the catfish were stained with Hematoxylin & Eosin (H/E) and Massons Trichrome for routine histology and mucopolysubstances in the oesophageal mucosa were characterized using the staining techniques given in the table below.

Table 1: Details of the stains used for characterization of mucopolysaccharides in the oesophagus of catfish *H. fossilis*

S.N.	Staining Techniques	Interpretation of staining reactions	References
1.	Alcian Blue (AB) (pH 2.5)	Glycoconjugates with acidic groups (carboxylated and sulphated)	Mowry (1963) [4]
2.	AB (pH 1.0)	Glycoconjugates with O-sulphate esters	Lev and Spicer (1964)[5]
3.	Active Methylation (AM)/AB (pH 2.5)	Glycoconjugates with O-sulphate esters	Spicer and Warren (1960) [6]
4.	AM/Saponification (KOH)/AB (pH 2.5)	Glycoconjugates with carboxylated moiety	Spicer and Lillie (1960) [7]
5.	Periodic Acid Schiff reagent (PAS)	Glycoconjugates with neutral moiety	McManus (1948) [8]
6.	PAS/AB (pH 2.5)	Glycoconjugates with neutral and acidic moiety	Mowry (1963) [4]
7.	PAS/AB (pH 1.0)	Glycoconjugates with neutral and sulphated moiety	Spicer *et al.* (1967) [9]

2.4 Visualization of Sections

The sections were visualized under Zeiss microscope (Carl Zeiss model Axioskop 40 FL) at the suitable magnification and images were captured with Axiocam ICc3 camera and five sections each from ten different fishes were quantified with Auto measure software (Rel. 4.8). For statistical analysis, the data were expressed as mean ± S.E. Analysis of data was carried out using GraphPadInStat software and one-way analysis of variance (ANOVA) followed by Tukey's test to calculate the statistical significance of the data.

3. Observations

3.1 Gross morphology of GIT

The *H. fossilis* is a bottom living omnivorous fish and the overall appearance of the GIT is that of a convoluted tubular structure. The GIT in this catfish, like other typical teleost, is divided into short oesophagus which is attached to J-shaped stomach and followed by intestine (Fig. 1 and 2). The stomach consisting of three regions, anterior (cardiac), mid (fundic) and posterior (pyloric) (Fig. 2) is connected to the intestine which is divided into anterior (duodenum), mid region (mid-intestine) and posterior (rectum) (Fig. 1).

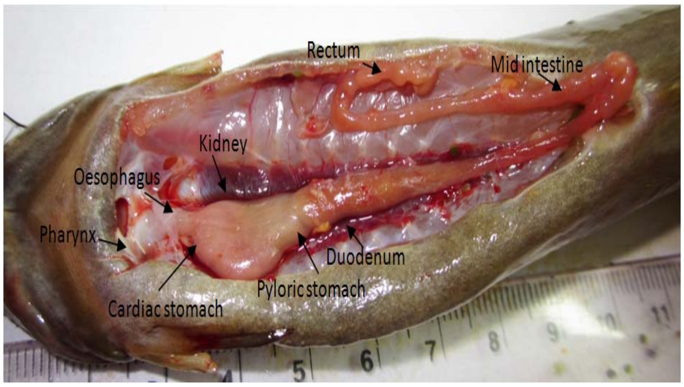


Fig 1: *In situ* gastrointestinal tract (GIT) of *H. fossilis*

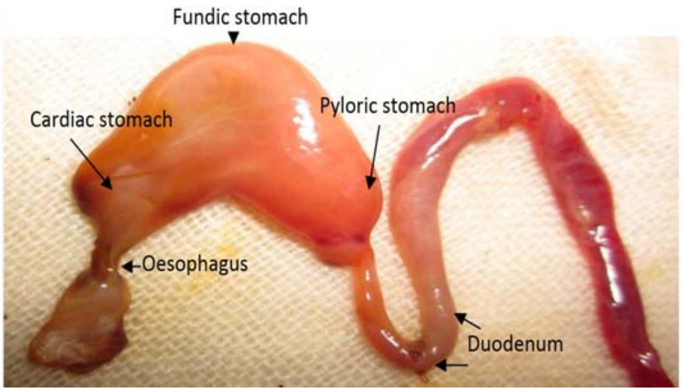


Fig 2: Magnified view of anterior GIT of *H. fossilis*

3.2 Histology of oesophagus

Like other vertebrates, the gut wall of catfish is divided in four layers i.e. tunica serosa (adventitia), tunica muscularis, tunica submucosa and tunica mucosa. In longitudinal and cross sections of catfish oesophagus, these four layers can be differentiated when stained with Hematoxylin & Eosin (H/E) and Massons Trichrome (Fig. 3 A, B). The serosa is well developed and tunica muscularis is composed of two types of smooth muscles i.e. outer circular and inner longitudinal muscles which are arranged in isolated bundles and scattered in the submucosa. The mean width of the outer circular muscles is 259.35 µm SD ± 64.518 ranging from 167.86 - 328.78 µm whereas the longitudinal muscles, being irregular in shape, their width cannot be measured (Fig. 3 A, B). The thin submucosa is composed of intermingled loose collagen fibers and large number of granulated cells. The total width of submucosa ranges between 196.75 - 496.17 µm (mean 319.63 µm SD ± 112.29). The mucosa shows closely arranged deep longitudinal folds which extend the entire length of oesophagus (Fig. 3 A). The distinct feature of mucosal epithelium is that it is made up of stratified epithelium consisting of basal cuboidal cells, intermediate columnar mucous cells and superficial layer of flattened cells (Fig. 3 C and D). The numerous columnar mucous cells which are present in entire mucosal epithelium constitute its major thickness which stain positive with Alcian Blue/Periodic Acid Schiff (pH 2.5) (Fig. 3 D). The mucosa epithelium rests on thin but prominent basement membrane which is visible with AB (pH 2.5)/PAS stains (Fig. 3 D). The mean width of mucosa is 253.99 µm SD ± 93.44 (range 181.74 – 349.28 µm) whereas the mean width of mucosal epithelium is 78.144 µm SD ± 25.863 (range 110.43 - 45.09 µm). The lamina propia is composed of connective tissue mostly containing collagen fibers, nerve plexes and blood vessels which are present beneath the mucosal epithelium (Fig. 3 C and D). No taste buds are seen in the mucosal epithelium in this catfish.

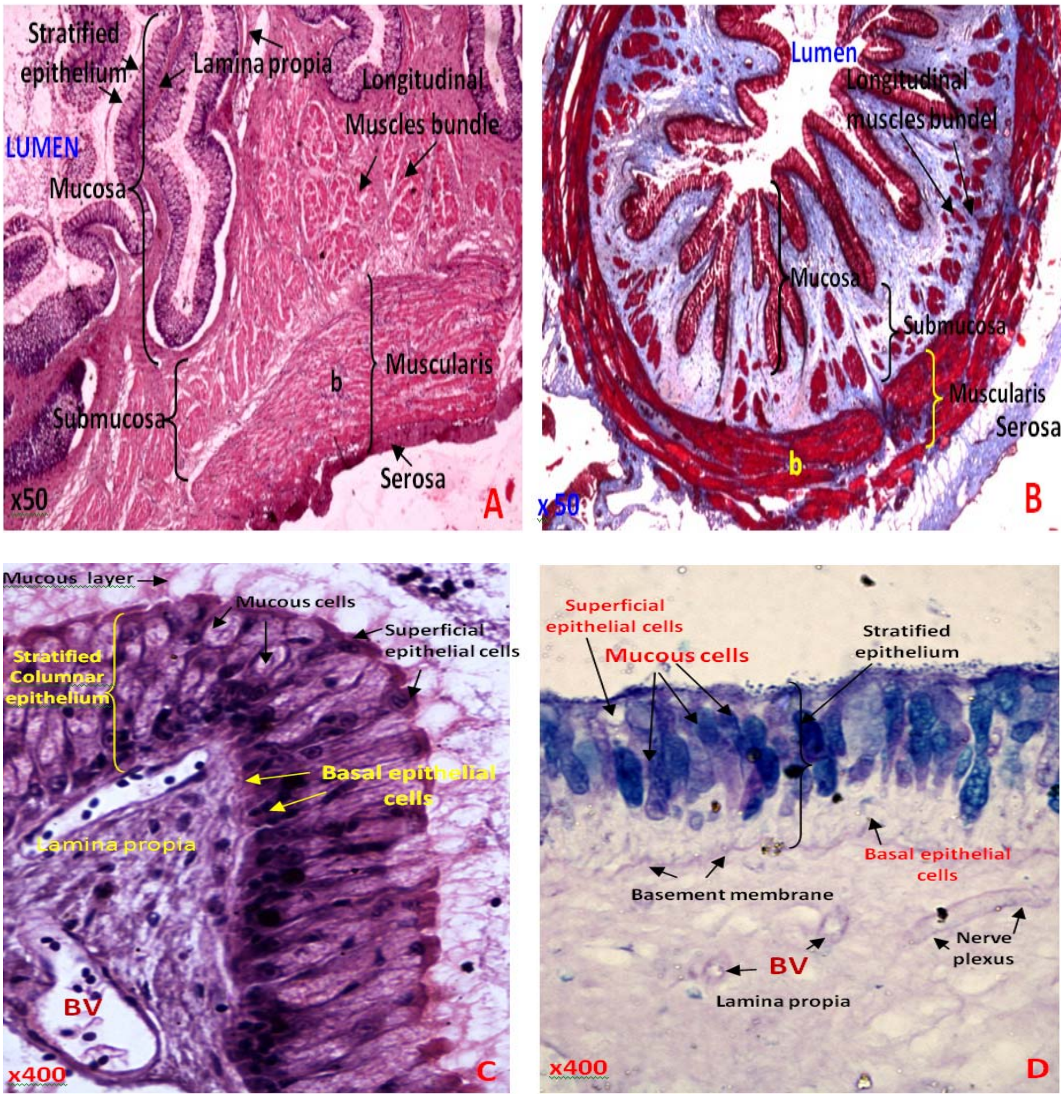
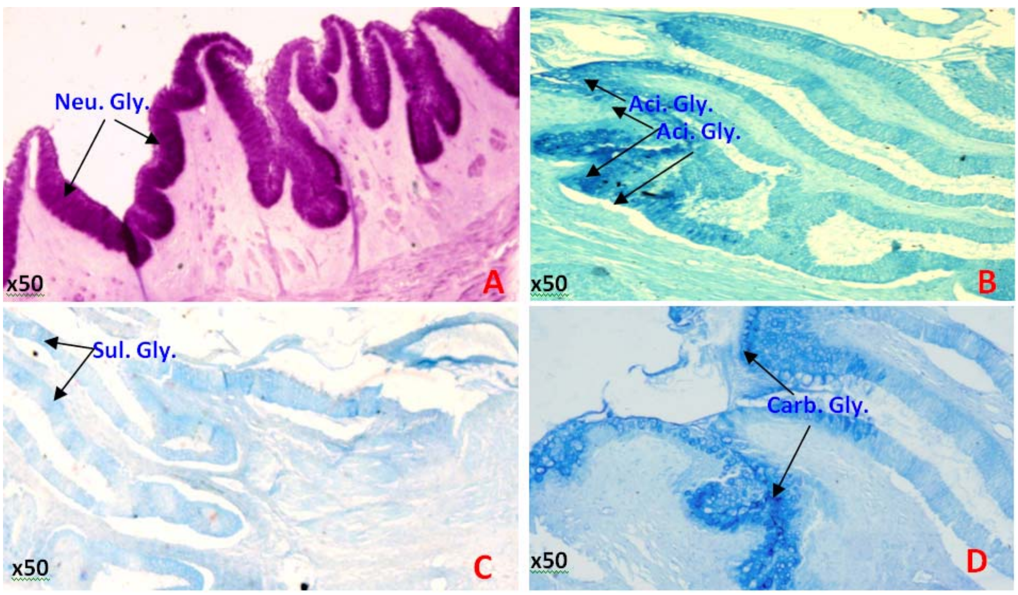


Fig 3: (A) Longitudinal and (B) cross section of oesophagus of *H. fossilis* showing four layers i.e. serosa, muscularis, submucosa and mucosa, (H&E and Massons Trichrome, x50). (C) & (D) are the magnified view of mucosa epithelial layer showing mucous cells, superficial and basal epithelial cells and basement membrane. (H&E, PAS-AB (pH 2.5), x400). (b, circular smooth muscles; BV, blood vessels).



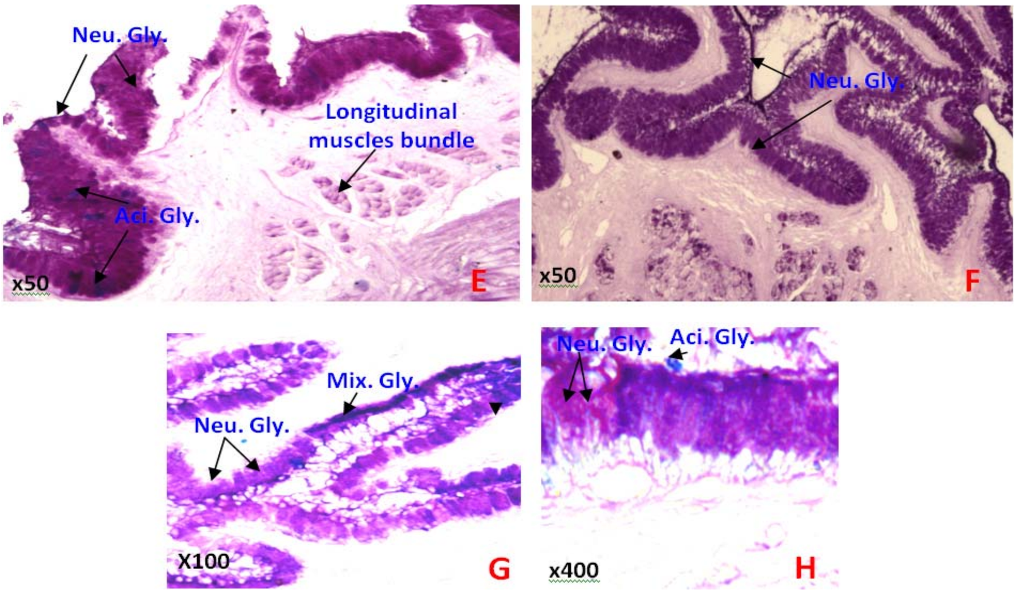


Fig 4: Longitudinal sections of oesophagus of catfish *H. fossilis* maintained in Tap Water (A) stained with PAS showing a great predominance of neutral glycoprotein, x50. (B) AB (pH 2.5) showing acidic glycoprotein only in some restricted region, x50. (C) Mucosa following active methylation (AM/AB (pH 2.5) for sulphated glycoprotein moiety x50. (D) Active methylation followed by saponification (KOH) and then stained with AB (pH 2.5) showing the carboxylated glycoprotein x50. (E) Mucosa stained with combination of PAS - AB (pH 2.5) showing abundance of neutral and limited number of darkly stained cells of acidic glycoprotein, x50. (F) Mucosa stained with AB (pH 1.0) - PAS showing magenta colour cells only neutral glycoprotein, x50. (G) magnified view of mucosa layer of oesophagus, x100. Note a well-demarcated predominant neutral glycoprotein rich oesophagus, (H) magnified view of mucosa layer showing mostly neutral and few acid glycoprotein, x400. (Neu. Gly, Neutral glycoprotein; Aci. Gly, Acidic glycoprotein; Sul. Gly, Sulphated glycoprotein; Carb. Gly; Carboxylated glycoprotein; Mix. Gly, Mixed glycoprotein).

3.3 Characterization of mucopolysaccharides

One of the distinguishing features of the distribution of mucopolysaccharides in the mucous cells (MCs) of GIT of *H. fossilis* is the conspicuous absence of sulphated moiety in the acidic glycoconjugates from the entire oesophagus.

The mucous cells in oesophagus stain positive both with PAS and AB (pH 2.5) indicating the presence of both neutral as well as acidic glycoproteins. However, the content and distribution of neutral glycoprotein seems to be much more predominant as compared to acidic glycoproteins (c.f. Fig. 4 A and B). The absence of sulphated moiety is confirmed by the fact that Methylation of the sections yield negative result due to the fact that methylation suppresses carboxylated component and specifically stains the sulphated moiety (Fig 4. C) which is also reaffirmed by using the combination of AB (pH 1.0) and PAS which only yield neutral moiety (Fig- 4. D). This observation is further corroborated by the reappearance of carboxylated moiety following saponification processes (Fig. 4 D). Further, while the mucous cells with neutral glycoproteins are distributed all over the mucosal lining of oesophagus, the acidic mucus seems to be confined only in the restricted region of oesophageal mucosa. Sections of oesophagus stained with the combination of PAS-AB (pH 2.5) show the purplish appearance with a greater tinge towards the magenta colour showing larger proportion of neutral glycoprotein (Fig. 4 E). These observations are further borne out by viewing these sections at high magnification (Fig. 4 G, H).

4. Discussion

4.1 Gross Histology

The gross morphological structure of digestive tract of *H. fossilis* essentially shows the same structural features which are found in other fish species such as *Leporinus friderici, L. taeniofasciatus, Clarias batrachus* and *Serrasalmus nattereri* [10, 11]. In some herbivorous fishes, oesophagus is directly connected with intestine due to the absence of stomach and consequently the anterior portion of intestine becomes bulky or swelled and is known as intestinal bulb which is presumably used as a storage organ of the ingested food. This feature has also been seen in some other fishes like *Lepidocephalichthys guntea* [12], *Cirrihinus mrigala, Labeo rohita* [13] and *Labeo niloticus* [14].

The internal histology of gastrointestinal tract of catfish *H. fossilis* is composed of four typical layers i.e. mucosa, submucosa, muscularis and serosa which is mostly similar in other fish species. Interestingly, the same architectural plan has been evident even in the fishes with different feeding habits such as in carnivores, omnivores and herbivores. There are many structural features in this freshwater catfish which bear similarities with many other freshwater teleosts. For instance, the oesophageal mucosal layer in *H. fossilis* shows deep longitudinally arranged folds which have also been reported in the fishes inhabiting freshwater environment such as Silverside *Odontesthes bonariensis* [15]. Mishra *et al*. (1999) [16] observed in two Indian freshwater teleost fishes, *Puntius sophore* and *Ompok bimaculatus,* that the longitudinal folds, unlike other fishes, are wide and sharp. The three layered mucosal epithelium in *H. fossilis* i.e. basal cuboidal cells, intermediate columnar mucous cells and superficial layer of flattened cells have also been observed in other freshwater fish species, *viz. A. japonica, A. anguilla* and *S. senegalensis* [17, 18, 19]. In catfish, like in other omnivorous fishes, the stratified and pseudo-stratified epithelium in oesophagus may protect this organ against abrasion and enable the rough items to pass along the tract more easily [10]. Girgis (1952) [20] described different adaptations in the oesophagus epithelium of *Labeo horie* (Cuvier), an herbivore bottom feeding cyprinoid where the epithelium contains goblet cells at the side and base and stratified epithelial cells at the top. Expectedly, a large number of mucous cells are found in the epithelium of different teleost species [21] which secrete mucus sheet over the epithelial surface. The continuous sheet connected with stratified epithelium generates lubrication for food particles during swallowing and protects the epithelial surface against mechanical damage and bacterial invasion and may also participate in ionic absorption [22, 10] suggesting thereby its role in osmoregulation [23]. The large number of columnar mucous cells secretes abundant quantity of mucus in this catfish which clearly suggests that the mucus in this fish, as also in others, is performing the function of protection and facilitating the process of swallowing of food during ingestion. In the catfish, the mucosal epithelium rests on the basement membrane which has also been observed in many other species but the muscularis mucosa consisting of stratified muscular fibers, which usually help in the movement of folds, has been found absent in the catfish *H. fossilis.* Hence, the functional role of muscularis mucosa should be performed by some other alternative segment which is difficult to speculate at this moment. Similarly, the occurrence of taste buds may vary in teleosts. While they may be absent in some teleost, like in this catfish, these are reportedly present in many other species such as *Neogobius gymnotrachelus* and *Rhamdia quelen* [24, 25]. The occurrence or the reported absence of taste bud may possibility relate to the gustatory abilities of the teleosts which may dependent on their feeding habit as well as the ecological niches occupied by them.

4.2 Mucopolysccharides in oesophagus
In the present study, the oesophagus mucosa lining shows the predominant presence of neutral GP even though acid GPs is also present in small measures. Similar qualitative distribution of GPs pattern in oesophagus has also been reported in other teleosts such as Northen pike *Esox lucius,* European catfish *Silurus glanis,* Atlantic bluefin tuna *Thunnus thynnus* [26, 27]. In addition, there are number of other studies where the presence of neutral and acidic GP in the oesophagus have been reported but the relative predominance of either of the two GPs in such studies have not been commented upon.

Interestingly, the sulphated glycoconjugates have not been observed in the gut mucosal lining of the catfish *H. fossilis* which corroborates the observation of Scocco *et al*. [28] in oesophagus of the Tilapia spp. However, most of the studies have shown the presence of sulphated glycoconjugates in oesophagus such as in *Umbrina cirrosa*, *Anguilla Anguilla* and *Odontesthes bonariensis* [29, 30, 15]. It has been shown that the sulphomucin confers high viscosity to mucus which aids in trapping small particles [31]. The absence of sulphomucin in the esophagus of catfish suggests that such a functional necessity in catfish may either be diminished or not required at all. In herbivore teleosts, acidic glycoconjugates predominate over the neutral glycoproteins in oesophagus as observed in *Arrhamphus sclerolepis*, *Tilapia spirurus* and *Odontesthes bonariensis* [31, 32, 15] but in carnivore fishes, there seems to be somewhat inconsistent pattern. Another curious fact is that most of the above fishes, unlike catfish, are euryhaline and endemic to sea water even though any habitat related functional significance is not presently evident.

5. Conclusion
The present study has described the structural organization of oesophagus of the catfish which show some common and other unique features which compare well with other omnivorous teleosts. Further, it has been clearly established that glycoprotein (GPs) present in the oesophagus are predominantly neutral with certain proportion of the acidic GP. However, the sulphated GP has been found to be altogether absent. The qualitative differences in the glycoconjugate moiety present in the mucus of oesophagus may be associated with the different functions. The absence of salivary glands in fishes may be duly compensated by the lubrication function provided by the mucus secreted by the oesophagus mucous cells. However, the predominance of neutral GP as observed in catfish may further aid in lubrication function whereas the acidic glycoprotein may protect the mucosal lining from the invasion of various pathogens which may enter with the swallowed food.

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