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Histochemical Analysis of DNA and RNA Identified by Methyl Green - Pyronin 'Y' Method and Feulgen's Reaction Staining in Liver of *Channa Punctatus*

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Abstract
The light microscopy was to identify cells, storage products, tissue deposits, and microbial pathogens. The Assessment of surrounding tissue with special stains may reveal aspects of interest for the tissue. We illustrate the expected staining characteristics of control *Channa punctatus* liver tissue with Methyl Green -Pyronin 'Y' and Feulgen's Reaction stains. The aim of this study was to characterize the normal structure of the liver of *Channa punctatus*, a carnivorous freshwater fish found in Hasanparthy Lake, using gross anatomy, histology and histochemistry. Anatomically, the liver presents C-shaped and only two lobes smaller right and bigger left. The gallbladder is located in right lobe and shows elongated shaped. Histological analysis demonstrated that the hepatic parenchyma is made of two hepatocytes plates surrounded by sinusoids. The fish moreover pancreatic tissue was observed in visceral portion of liver, formed by exocrine pancreatic tissue and islet organ, constituting an extrahepatic pancreas. The nucleic acids content in different tissues in the freshwater fish of *Channa punctatus.* The fish were scarified and the tissues such as liver and Pancreas removed and processed for determination of nucleic acids. The results obtained in the present study may provide a contribution to the knowledge of the characteristics of nucleic acid as parameters of estimation of DNA and RNA levels in Liver of the fish.

Keywords: *Channa punctatus,* Feulgen's Reaction, Liver, DNA

1. Introduction

Histochemistry has contributed a great deal not only to the understanding of biological phenomenon but also to clinical medicine. Histochemical techniques help to analyze not only the localization of protein, lipid and glycogen etc, but also molecular changes at cellular level. The main advantage of histochemistry lies in the analysis of biological phenomena in the "particular cells". The altered state of cell constituent can be studied by using histochemical techniques. The histochemical tests reveal the localization of chemical product of cellular activity. The present investigation deals with the histochemical nature of the skin, gills, liver and pancreas of *Channa punctatus* in order to discuss, the contrast among control and the effect of bacterial and fungal infected skin, gills, liver and pancreas.

The fish liver appears as does the liver of other vertebrates. It as a key organ which controls many life function and plays a prominent role in fish physiology, both in anabolism (proteins, lipids and carbohydrates) and catabolism (nitrogen, glycogenolysis, detoxification) and it acts as storage center for many substances, mainly glycogen (Takashima and Hibiya *et al*., 1995) [35], (Ostaszewska and Sysa *et al*., 2004) [25].

The liver is one of the digestive glands that composed of parenchyma cells and lattice fibers. It plays a prominent role in metabolism and acts as storage center for many substances. Fish liver with a higher phylogenic status had structures identical to the mammalian arrangement, which possessed higher metabolic functions. The fish liver is a relatively large organ. In wild fish, it is usually reddish brown in carnivores and lighter brown in herbivores, but at certain times of year it may be yellow or even off white. In farmed fish, it can be lighter in color than in an equivalent wild specimen but this is diet dependent. The liver may be a localized organ in the anterior abdomen. In some species, it may extend the length of the abdomen or are closely applied to the other viscera. In some species it is a compound organ in the form of a hepatopancreas. The fish liver does contain drug metabolizing enzymes and is one of the most frequently damaged organs, but it has been shown (in mammals)

that only 10% of hepatic parenchyma is required to maintain normal liver function. Fatty change (fatty metamorphosis; lipidosis) is an abnormal and excessive accumulation of intracellular fat. In an injury which, though reversible, can be severe and can cause severe disruption of cell function. Distinctn on staining vacuoles of fat lie in the cell cytoplasm, displacing and compressing the nucleus. It is most commonly seen in the liver, but also seen in kidney and heart.

The bacterial and fungal diseases create metabolic crisis and impairment in fish liver. The depletion in level of protein, lipid and glycogen, points towards exhaustion of cell energy to meet high demand of fish in stressful condition. Hence, in present study histochemical tests were used for localization of chemical products of cellular activities by the intensity of staining reactions. It is used for comparing the protein; glycogen and lipid contents present in the liver cells of the control and infected fish *Channa punctatus.*

Utmost earlier studies carried out in the field of histochemical studies on liver of fishes. The pancreas has been investigated (Macallum 1884, 1886) [17, 18] (Kristal *et al*., 1946) [14] in a large number of fishes. (Ogura 1959) [23] and (Miyawaki *et al*., 1961) [20] reported histological and histochemical investigations on the digestive gland of the cray fish, *Procambarus clarldi.* (Subhash 1966) [34] studied the histochemistry of liver and pancreas in a carp fingerling *Catla catla.* (Raymond 1969) [29] examined histology and histochemistry of the capsule of *proteocephalus* sp (cestoda) in *lepomis macrochirus.* (David *et al*., 1972) [8] investigated morphology and enzyme histochemistry in the liver of largemouth bass *(Micropterus salmotdes).* (Nakazawa *et al*., 1985) [21] investigated histochemistry of liver tumors induced by diethylnitrosamine and differential sex susceptibility to carcinogenesis in Oryzias latipc. (Hampton *et al*., 1985) examined functional units in rainbow trout *(Salrno gairdneri)* liver in arrangement and histochemical properties of hepatocytes. (Krapagaganapthy *et al*., 1988) describedhistochemical studies on the effects of lindane in the anabantid fish, *Colisa Mia.* (Ribelles *et al*., 1995c) [31] investigated the morphological and histochemical changes on the liver arid pancreas of giltheads, *Sparus Auratus* induced by acute action of the anionic detergent, sodium dodecyl sulphate observedbiochemical and histochemical properties of hepatic tumors of rainbow trout, *Oncorhynchus mykiss* (Lynn *et al*., 1993) [16].

According to (Rosety *et al*., 2001) examined morphohistochemical changes in the liver and intestine of young giltheads (fish nursery), *Sparus aurata,* induced by acute action of the anionic tension active alkylbenzene sulphonate. (Sakr *et al*., 2001) studied on histopathological, histochemical and physiological studies on the effect of the insecticide, hostathion, on the liver of the cat fish *Glorias gariepinus.* (Sakr and Jamal 2005) [32] have made observations on fenvalerate induced histopathological and histochemical changes in the liver of the catfish *Clarias Gariepinus,* (Anandhi and Murthy 2006) [1] made examined the chemical stress on the liver of *Glossogobius giuris.* (Rejane and Vildes 2008) [30] observed ultra structural and histochemical analysis of channel catfish *(Ictalurus punctatus)* liver treated with fumonisin. (Pathan *et al*., 2009) [27] described the histochemical changes in the liver of freshwater fish *Rasbora daniconius,* exposed to paper mill effluent. Reported the comparative histological, histochemical and ultra structural studies on the liver of flathead grey mullet *(Mugil cephalus)* and sea bream *(Sparus aurata)* (Naveen and Hekmat, 2010) [22].

2. Material and Methods

The freshwater fish's material for the present study was collected from Lake of Hasanparthy, resources in Warangal district, Telangana, India. The *Channa punctatus* EUS infected fish samples were collected and maintains was already described. The specimens were obtained alive by using fishing net, and they were brought immediately to the laboratory, in plastic containers with oxygen filled water. The specimens were deeply anaesthetized by immersion into 5 ml/L aqueous solution of ethylene glycol monophonylether. To study the gross anatomy of different regions of the dissected tissue part of liver were carefully removed and small pieces were taken to obtain proper fixations. For the histopathological and histochemical studies the required tissues were removed and the blood vessels and mucous attached to them were scrapped off smoothly without damaging the original stricture. The tissues collected for the present study included skin, gills, liver and pancreas. The procedure followed for preparation of histological sections and for histochemical analysis of different substances is similar to all the tissues. The tissues collected from control and infected fishes were immediately fixed and processed. The fixatives used in the present study were Alcoholic Bouin's, Susa, Formal clcium, Carnoy and Zenker. Specific fixatives were used for lipids, mucopolysaccharides and nucleic acids. For lipids formal calcium for nucleic acids Susa, Carnoy and for mucopolysaccharides modified Bouin's (Harris *et al*., 1973) [11] and cetyl pyridinium chloride (1% cetylpyridinium chloride in formalin) was used specially. Bouin's and Susa comparatively gave good results. After fixation in Bouin's for 18-24 hours material was washed in scots tap water. Susa fixed material has been treated with iodine alcohol. Formal calcium fixed material subjected to post chromatin has been used for testing lipids in tissue sections. Experience proved that Susa formed the choicest fixative for most histological and histochemical reactions with the exception of lipids, nucleic acids and mucopolysaccharides. After fixation and post treatment the material was washed and then dehydrated in graded series of alcohols. Cleared in xylene and embedded in paraffin wax. Depending upon the parts of the systems the infiltration time varied from (1- 4) hours was minimized and then serial transverse or sagittal sections were cut 3-6mμ thickness for all the tissues. Excellent staining results for histological studies could be obtained by using Dalafields haematoxylin counterstained with eosin (Gurr 1962 [10] and Heidenhain's Azan Gurr 1962).

For the EUS infection studies the fishes collected from the lake of Hasanparthy of fish like *Channa punctatus*. The procedures for determination of infected and control fishes. The tissues from control and infected groups were collected separately and processed individually, for histopathological and histochemical investigations.The following various histoichemical methods have been employed to elucidating the chemical nature of viz., the presence of nucleic acids in tissue liver of *Channa punctatus*. The procedure as outlined in (Pearse 1968) [28] for the different histochemical tests were adopted in the present study. However, the techniques mentioned in the following books were also referred (Gomori 1952) [9] (McManus and Mowry 1960) [19] (Lillie

1965) [15] (Humason 1967) [12] (Bancroft 1975) [3] (Carleton and Drury 1957) [6] (Barka and Anderson 1963) [4] (Chayen *et al*., 1973) [7] (Culling 1974) (Bancroft and Steyans1977) [2] (Kiernan 1999) [13] (Shyamasundari and Hanumantha Rao 2007) [33].

3. Identification of Nucleic Acids

To study the distrubitional pattern of nucleic acids, tissues were fixed in Carnoy fixative was used. The presence of DNA was demonsrated by Feulgen's reaction (Hydrolysis in 1 N HCL for 15 minutes at 60ºc) of (Pearse 1968) [28]. The Methyl green/ Pyronnin 'Y' method of (Pearse 1968) [28] was used to detect the DNA and RNA in the same section, while only RNA was demonstrated with Pyronin 'Y' alone.

4. Methyl Green – Pyronin 'Y'method

The use of mixture of basic dyes methyl green and pyronin – Y, to stain nuclei and cytoplasm differentially, is of long standing process. However, the mechanism by which this mixture causes the differentiation has been understood relatively (Chayen 1973) [7] and (Barnett 1954) [5]. It seems to be that the mechanism of this reaction is the competition between the slow staining of doubly charged methyl green and more rapidly singly charged pyronin-y. Consequently the methyl green stains DNA and pyronin- y stains RNA more frequently.

5. Feulgen's Reaction

The precise chemical reaction for this method is not clear. This may be related to the ability of the phosphate to migrate to different sites in sugar molecule. Mild hydrolysis (1 N Hcl at 60^0 C for few minutes) unmasks the aldehyde groups. During this process, this hydrolysis removes the purines from DNA and gives more aldehyde groups for staining. However, the prolonged hydrolysis renders the DNA soluble. The aldehyde groups are then exposed to Schiff's reagent and produce a colour. The microphotographs of various tissue sections have been taken under low and high magnification to visualize the variations both the structural and histochemical illustrations.

6. Results

6.1 Comparative Histochemical Analysis of Control and Infected Liver

A battery of histochemical techniques has been applied for elucidating the chemical nature of the substances of control liver and infected liver. The pancreatic tissue consists of both exocrine acinar and endocrine islet cells. Both the cell types usually respond in a similar way to all the histochemical reactions.

6.2 Nucleic Acids

The control liver of fish is generally made up of hepatocytes with centrally placed nucleus and densely stained nucleolus. In addition to acinar cells of hepatopancreas are polyhedral and compactly arranged. Moreover, acinar cells are arranged in two or three rows encircling blood capillaries and apical part contains zymogen granules (Palas Samanta *et al*., 2018) [26]. (Figure 1&3). In the EUS infected fish notable lesions observed in the liver of *C. Punctatus* under light microscopic study were severe degeneration in hepatocytes, excessive fat deposition, pyknotic nuclei, acentric nuclei in DNA and RNA and degenerative hepatopancreas under laboratory conditions (Figure 2&4). The extreme of damage in field condition was comparatively less than laboratory study which was included enlarged acentric nuclei and dilated hepatocytes.

6.3 Pyronin Y

The liver tissue reacts the Pyronin 'Y' react, strongly showing the presence of extracellular RNA in the control tissue sections. When the bacterial and fungal infected liver tissues were subjected to Pyronin 'Y'the tissue reacted moderately, suggesting the depletion of nucleic acids of RNA. This represents complete devoid of nucleic acids and their remnants were mainly located at the peripheries of the cells which showed the hepatic parenchymal cells. RNA is mainly stained by using basic dyes such as Toluidine Blue and methylene blue. Dyes that are used to stain both DNA and RNA include, Methyl green Pyronin'Y' stain which is used to observe the presence of nucleic acid; and Acridine orange which stains DNA in yellow-green and RNA in red-orange color. (Fig. 1 & 2).

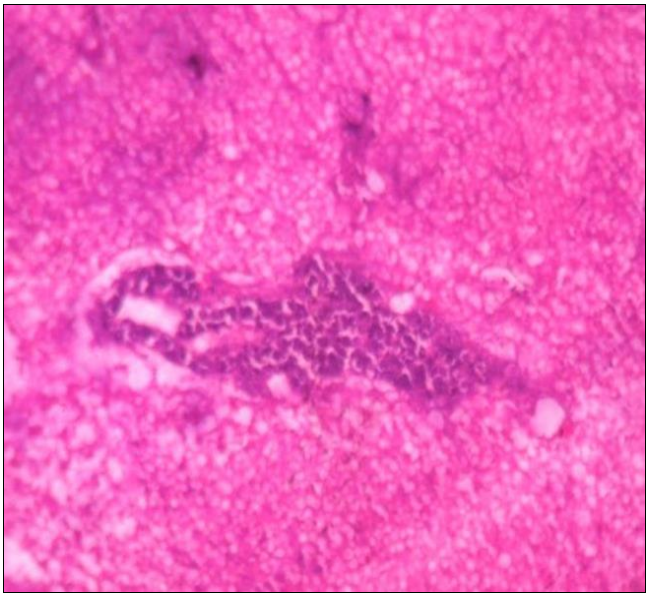

Fig 1: T.S of *Channa punctatus* Control liver (Pyronin 'Y')

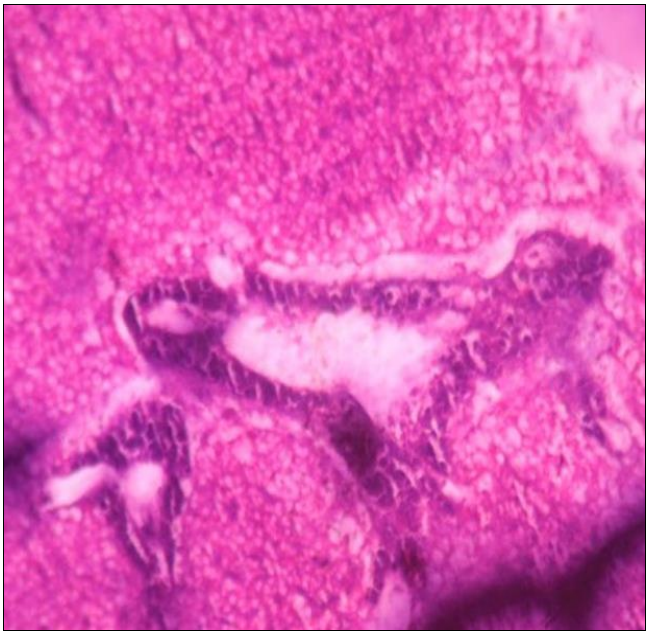

Fig 2: T.S of *Channa punctatus* Infected liver (Pyronin 'Y')

6.4 Feulgens Reaction

The control liver of fishes exhibited normal content of DNA in the cytoplasmic hepatocytes. Whereas the infected fishes with bacteria and fungi levels have a moderate rise of DNA content in the liver comparing to the control fish. On the other side, there was no incredible difference between liver DNA content of fishes exposed to different pathogenic contamination levels. From the histochemical reactions it was concluded that the control liver tissue of this fish. It is the most widely used method which is involved the principle of hydrolysis of DNA by Hydrocholoric Acid (HclS) which was exposes the deoxyriboses. Then Fuchsine reacts with the aldehyde group which colors the DNA in red. (Fig. 3 & 4).

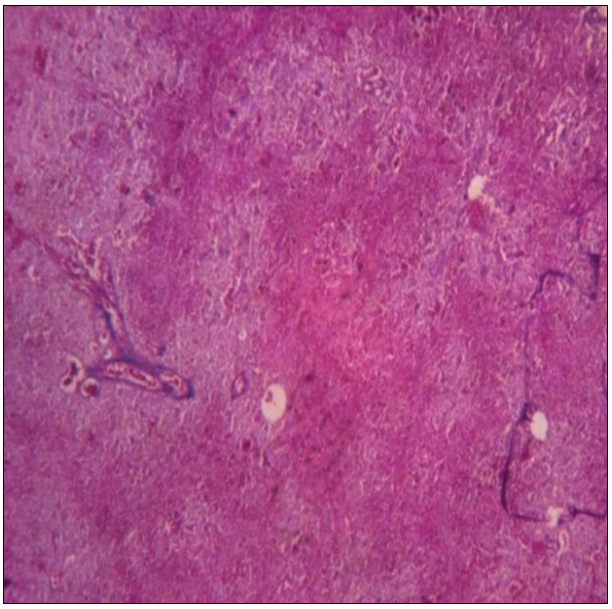

Fig 3: T.S of *Channa punctatus* Control liver (Feulegen Reaction)

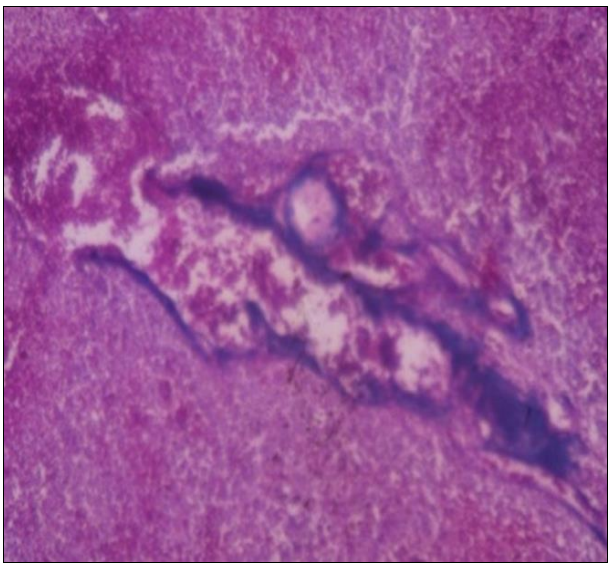

Fig 4: T.S of *Channa punctatus* Infected liver (Feulegen Reaction)

7. Discussion

The freshwater teleosts liver is the important digestive gland consisting of two main lobes. Each lobe is composed of polyhedral cells arranged radially around the hepatic vein in the form of ducts. Nemours blood vessels called sinusoids and intracellular bile caniliuli are scattered in the hepatocytes. The microscopic investigations of the liver parenchyma revealed that the histochemical alterations were not only restricted to the site of infections but also to various parts of organ tissues due to parasite replication, invasion and secretion of some toxins which may produce disruption, tissue injury and subsequently reduce the immune response and increase the histochemical alterations of the infected animals. In the present study a series of histochemical tests have been employed and elucidated the chemical nature of the liver cells. From the range of histochemical tests this could be established that the liver cells contain fairly good amounts of carbohydrates, proteins, lipids and nucleic acids. Pyronin 'Y' and Feulgen reaction demonstrated the presence of RNA and DNA.

In this present study the bacterial and fungal infections has produced rapid degeneration, hypertrophy, necrosis and vacuolation of hepatocytes with splitting off the tissue. Severe effects were observed when in the seasonal infection. This includes loss of characteristic hexongonal shape of hepatic cells, rupture of nuclear and cell membranes, formation of binucleate hepatocytes, higher degree of atrophy at the centre and disorganization of hepatic chords. The extent of damage to the liver was depends on the seasonal variations leads to EUS condition.

8. Conclusion

Histological studies of control fish gave knowledge of the tissue pattern of normal histology of liver of *Channa punctatus.* The histological study of control fish differed from the EUS infected fishes. The histochemical reactions were determined that the control liver. Extensive tissue damage has been observed in liver tissue. Sinusoids were distended and partially filled with granular, eosinophilic material. The tissue damages like degeneration of cytoplasm in hepatocytes, atrophy, formation of vacuoles, ruptures in blood vessels and disposition of hepatic cords are the histopathological changes observed in the liver.The findings from this study clearly showed that *Aeromonas hydropila, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella salmonicida* and fungi were *Aspergillus flavus, Fusarium solani, Rhizophus stolenifer, Aspergillus fumigatus, Penicillium chrysogenum, Aspergillus niger* and *Trichoderma viridae* are the major bacterial and fungal species causing Epizootic Ulcerative Syndrome in this fish. Disease prevention should be carried out by means of good culture practices and health management to ensure the optimum yield and best quality of the products.

9. Declaration of competing interest

The authors declare that they have no conflict of interest.

10. Ackonowledgements

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11. References

1. Anandhi UD, Murthy PS. Chemical stress on the liver of *Glossogobius giuris* (Ham.) An overview. Adv. Env. Technol, 2006, 79-96.

2. Bancroft JD, Stevenson JM. Theory and practical histochemical technique. Edinburg Churchill, 1977.
3. Bancroft JD. Histochemical Techniques. Butterworths, London and Boston, 1975.
4. Barka T, Anderson PJ. Histochemistry theory, practical and biblioFig.y.Hoeber Medical Division. Harper and Row Publishers Inc., New York, Boston and London, 1963.
5. Barnett RJ, Siligman AM. Histochemical demonstration of bound sulfahydrin and disulphide group of proteins. J. NAD: Cancer Ins. 1954; 14:769-803.
6. Carleton M, Drury RAB. Histochemical technique for normal and pathological tissues and the identification ofparasites.Oxford University Press, London, 1957.
7. Chayen T, Bitensky L, Butches RG. Practical Histochemistry. John Wiley and Sons, London, 1973.
8. David E Hinton, Robert L Snipes, Michael W Kendall. Morphology and Enzyme Histochemistry in the Liver of Largemouth Bass *(Micropterus sdmoides)* Fisheries Research Board of Canada. 1972; 29(5):531-534.
9. Gomori G. Microscopic histochemistry: Principles and Practice. Univ. of Chicago Press, Chicago, III, 1952.
10. Gurr E. Staining animal tissues: Practical and therotical. Leonard Hill (Books) Ltd., London, 1962, 631.
11. Harris JE, Watson A, Hunt S. Histochemical analysis of mucous cells in the epidermis of brown trout Salmo trutta L.J. Fish Biol. 1973; 5:345-351.
12. Humason GL. Animal tissue techniques.W.H. Freeman and Co. San Fransisco an Lundon, 1967.
13. Kiernan JA. Histological and histochemical methods: Theory and practice. Butterworth Heinenamannlinacre House, Jordan Hill, Oxford Ox28DP, 1999.
14. Kristal J. The exocrine pancreatic tissue of seven teleosts. S. Afr. J. Med. Sci. (Biol. Suppl). 1946; 11:79-81.
15. Lillie RD. Histopathological technique and practical histo- chemistry, 3rd Edition, Mc Graw Hill, New York, 1965.
16. Lynn M Parker, Darrel J Laure'n, Bruce D Hammock, Bruce Winder, David E Hinton. Biochemical and histochemical properties of hepatic tumors of rainbow trout, 1993.
17. Macallum BA. Alimatentary canal, liver, pancreas and air bladder of *Amiurus catus.* Proc. Can. Inst. 1884; 2:387-417.
18. Macallum BA. Alimatentary canal and pancreas of Acipenser, Amia and Lepidosteus. J. Anat. Physiol. 1886; 20:603-636.
19. McManus JFA, Mowry RW. Staining methods, Histologic and Histochemical. Harper and Row, New York, 1960.
20. Miyawaki M, Matsuzaki M, Sasaki N. Histochemical studies on the hepatopancreas of the cray fish, *Procambarus clarkii*. Kumamoto, J. Sci., Ser. B, Sec. 2. 1961; 5:161-169.
21. Nakazawa T, Hamaguchi S, KyonoHamaguchi Y. Histochemistry of liver tumors induced by diethylnitrosamine and differential sex susceptibility to carcinogenesis in Oryzias latipes. J Natl Cancer Inst. 1985; 75(3):567-73.
22. Naveen El-Said, Reda El-Bakary, Hekmat Lotfi ElGammal. Comparative histological, histochemical and ultrastructural studies on the liver of Flathead Grey Mullet *(Mugil cephalus)* and Sea Bream *(Sparus aurata)* Global Vererinaria. 2010; 4(6):548-553.
23. Ogura K. Midgut gland cells accumulating iron or copper in the cray fish, *Procambarus clarkii.* Annot. Zool. Jap. 1959; 32:133-142.
24. Rajamanickam N, Prakasam A, Rengarajan KV. Histochemical studies on the effects of lindane in the anabantid fish, *Colisa lalia* HamiltonBuchanan). Proc.Second National Symp.Ecotoxicol, 1988, 188-190.
25. Ostaszewska T, Sysa P. Development of hepatocytes in Nase *(Chondrostoma nasus L.)* larvae following hatching. Archive of Polish Fisheries. 2004; 12(2):151-161.
26. Palas Samanta, Pragya Kumari, Sandipan Pal, Aloke Kumar Mukherjee, Apurba Ratan Ghosh. Histopathological and Ultrastructural Alterations in Some Organs of Oreochromis niloticus. Exposed to Glyphosate-based Herbicide, Excel Mera 71. J Microsc Ultrastruct. 2018; 6(1):35-43.
27. Pathan TS, Thete PB, Shinde SE, Sonawane DL, Khillare YK. Histochemical changes in the liver of freshwater fish, *Rasbora daniconius*, exposed to paper mill effluent. Emir. J. Food Agric. 2009; 21(2):71-78.
28. Pearse AGE. Histochemistry: Theoretical and Applied. 2nd Edition, Little, Brown and Company, Boston, Mass, 1968.
29. Raymond C Bowen. Histology and rdstochernistry of the capsule of *proteocephalus* sp. (cestoda) in *lepomis macrochirus.* The Ohio Journal of Science. 1969; 69(4):243.
30. Rejane Maria, Ciira Scaff, Vildes Maria Scussel. Ultra Structural and Histochemical Analysis of Channel Catfish *(Ictdums punctatus)* Liver Treated with Fumonisin Bl. Braz. arch, biol. technol. 2008; 51(2):333-344.
31. Ribelles A, Carrasco *C*, Rosety M, Aldana M. Morphological and histochemical changes on the liver and pancreas of giltheads, *Spams Auratus* L., induced by acute action of the anionic detergent, sodium dodecyl sulphate. Histol. Histopathol. 1995c; 10:781-787.
32. Sakr SA, Jamal Al Laial SM. Fenvalerate induced histopathological and histochemical changes in the liver of the catfish, *Clarias gariepinus.* J. App. Sci. 2005; 1(3):263-267.
33. Shyamasundari K, Hanumantha Rao K. Histochemistry in focus. M.J.P Publishers, Chennai, India, 2007.
34. Subhash Chandra Saxena. Histochemistry of liver and pancreas in a carp fingerling *Catla catla* (Hamilton) Department of Zoology, Agra College, Agra, 1966.
35. Takashima F, Hibya T. An atlas of fish histology: Normal and pathological features, 2nd edi. Tokyo, Kodansha, 1995.