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Effect of curcumin supplementation on hepatic, renal and intestinal organization of *Anabas testudineus* (Bloch): Light and electron microscopic studies

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Summary

The ultrastructure of hepatic, renal and intestinal tissues of *Anabas testudineus* and the influence of dietary curcumin (0.5 and 1% for 6 months) on the organization of these tissues were studied. The results revealed an increased vascularity in curcumin-treated fish liver. The size of hepatopancreas and its nuclei and melanomacrophage centres increased in the treated liver while size of hepatocytes and their nuclei remained unchanged. The hypertrophy and hyper-activity of hepatopancreas might provide for proper digestion and absorption of food. In the intestine, the number of goblet's cells in the villi decreased in the curcumin treated group which might help the retention of food in the intestine. Therefore, based on the above results it can be concluded that curcumin is beneficial to fish and, so, could be of application in aquaculture practices.

Key words: *Anabas*; liver; melanomacrophage centre; hepatopancreas; ultrastructure.

Introduction

Anabas testudineus (commonly known as 'climbing perch') is a euryhaline teleost, inhabiting both freshwater and brackish water ecosystems of India and other South East Asian countries. It thrives well in water deficient in dissolved oxygen and is capable of migrating between ponds taking advantage of the accessory respiratory organs, the labyrinthine organs. The market demand for this fish is very high in view its high iron and copper content and prolonged freshness out of water. It is a valuable diet for sick and convalescent people since it contains easily digestible poly-unsaturated fatty acids and many essential **amino acids** (Kohinoor et al., 1991; Sarkar et al., 2005). This species of fish has an advantage due to its ability to tolerate adverse environmental conditions (Ponniah and Sarkar, 2000). In addition to its importance as a cultivable fish, *Anabas* is an excellent model organism for biological studies as it can be easily maintained under laboratory conditions and also due to the fact that it possesses well developed organ systems comparable to higher vertebrates. The physiological adaptations and effects of various hormones and chemicals on metabolism of *Anabas* have been investigated extensively (Johnson and Oommen, 1993; Nair and Oommen, 1995; Varghese and Oommen, 1999; Leena et al., 2000; Varghese et al., 2001; Sreejith and Oommen, 2006).

Curcumin, derived from the tuber of turmeric plant (*Curcuma longa*), is one of the best studied natural compounds. *In vitro* and *in vivo* experiments by various researchers have suggested a wide range of potential therapeutic or preventive effects associated with curcumin including anti-oxidant, anti-cancer, anti-microbial, hepatoprotective and gastro-protective effects (Soudamini and Kuttan, 1989; Elizabeth and Rao, 1990; Ruby et al., 1995; Allen et al., 1998; Ramý´rez-Tortosa et al., 1999; Kelloff et al., 2000; Park et al., 2000). Most of these effects were observed in studies conducted either in mammalian models or cell-lines. It would be advantageous if these beneficial effects of curcumin could be utilized

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for the growth of fish in aquaculture. As a preliminary step, *in vitro* and short term *in vivo* anti-oxidant effects of curcumin in *Anabas testudineus* were investigated in our laboratory. The results proved that curcumin prevented lipid peroxidation in fish too and did not produce any toxic side effects up to a period of 8 weeks (Manju et al., 2008, 2009, 2012). Since curcumin is entirely a new compound to fish body, it is essential that its prolonged effects on various tissues are investigated to recommend the compound for application in aquaculture of fish. Therefore, the present study was conducted to evaluate the effect of curcumin *in vivo* in a long-term study in *Anabas testudineus,* by examining the histology of liver, intestine and kidney adopting light and transmission electron microscopic techniques.

Materials and methods

Adult fish, weighing 40 ± 5 g were collected from the rivers of Thiruvananthapuram district, Kerala (Lat: 8.48333, Long: 76.91667) during June-July 2008. The fish were reared in large stock tanks with aerated well water (28-30ºC) and natural photoperiod (12L: 12 D) for a month. The fish were fed *ad libitum* with 40% protein feed (basal feed) once daily, prepared in the laboratory (Hardy, 1980). Its components (Table 1) and proximate composition (Table 2) were determined earlier in our laboratory (Johnson 2004). Curcumin was obtained from Synthite Industrial Chemicals Limited, Kochi, India. Two doses of curcumin, 0.5% and 1%, were selected for the study based on the results of previous dose-determination studies (unpublished data). The feed for the experimental fish was prepared by supplementing 0.5% and 1% curcumin (by mass) to the basal feed, made into pellets and air-dried under shade.

Table 1 Proportion of feed ingredients in the diet

Diet	Proportion ($g\ kg^{-1}$)	Protein content ($g\ kg^{-1}$)
Rice bran	0.01324	0.0011
Groundnut oil cake	0.03676	0.0084
Tapioca flour	0.01324	0.0029
Fish meal	0.03676	0.0201

(Johnson, 2004)

After acclimatization to laboratory conditions, as above, the fish weighing 40 ± 5 g were transferred to aquarium tanks (0.61×0.30×0.30 m) in which the conditions identical to the stock tanks were maintained. The tanks were labeled A1, A2, A3, B1 B2, B3, C1, C2, and C3, with eight fish in each. Tank A series fish were fed with basal feed (control), tank B series with 0.5%, and tank C series with 1% curcumin – supplemented feed in each set. Each tank received accurately weighed feed at 10% of the body weight, once daily during the morning hours. The fish were fed with curcumin-supplemented feed for six months, and the water was changed every day.

Table 2 Proximate composition of the diet used in the study

Properties (%)	Composition ± SD
Moisture	12.30 ± 0.71
Protein	40.02 ± 0.33
Lipid	9.14 ± 0.20
Carbohydrate	13.27 ± 1.01
Fiber	4.81 ± 0.14
Ash	2.11 ± 1.04

n = 6

(Johnson, 2004)

Histological analyses

Light microscopic analysis

At the end of six months, fish were fasted overnight and eight fish were selected at random from each triplicate group. They were anaesthetized in MS 222 and sacrificed by spinal concussion. Liver, kidney and intestine were dissected out and fixed in suitable fixatives depending upon the staining technique. Later, the tissues were washed in running water and dehydrated by passing through ethanol series (30-100%). The sections were taken on slides, rehydrated once again using the same alcohol series from 100 to 30% ethanol after which the sections were stained. The staining techniques employed include hematoxylin and eosin (H & E) (Clark, 1981), Mercuric Bromophenol Blue (Chapman, 1975), Periodic Acid-Schiff's (PAS) (Bedi and Horobin, 1976), Perls' Prussian Blue, Sudan Black B counter-stained with Neutral Red (Chiffelle and Putt, 1951) and Nile Blue Sulphate (Lillie, 1956). The stained sections were mounted in DPX mountant. The preparations were observed in a Leica research microscope and the images were captured in a Pentium IV computer using Qwin software (Leica, Jena, Germany). Ten microscopic fields (sorted randomly) of five slides with sections taken from five different fish in

each group were examined and scored in a blinded fashion by a single pathologist. Histometric measurements including surface areas of hepatopancreas (μm^2), hepatopancreas cell nucleus (μm^2), melanomacrophage centres (MMC) (μm^2), hepatocyte (μm^2) and hepatocyte nucleus (μm^2) were made using a Pentium IV computer and Qwin software.

Electron microscopic analysis

For transmission electron microscopy (TEM), the tissues were washed in wash buffer and fixed in 2.5% gluteraldehyde, prepared in cacodylate buffer, post-fixed in osmium tetroxide and embedded in thin viscosity resin. Semi-thin (1μm thick) sections were stained with toluidine blue O (TBO). Observations were made on these sections and the images were captured in a Pentium IV computer using Qwin software. Ultrathin sections obtained with a Leica ultra-microtome (Jena, Germany) were stained with uranyl acetate and lead citrate and subjected to transmission electron microscopic analysis using a Philips 201C transmission electron microscope (Amsterdam, Holland). The images were edited using Adobe Photoshop version 7.0.

Statistical analysis

The histometric data were statistically analyzed adopting one-way analysis of variance (ANOVA) using SPSS software. The results were expressed as mean ± SD. The significance of the difference between means was determined by Duncan's multiple range test at the level, $p<0.05$.

Results

Light microscopic histology and ultrastructure of liver of control fish

The liver is brown-colored with a prominent gall bladder. It is enclosed in a thin connective tissue capsule and composed of mainly the hepatic parenchyma. The parenchyma consists mainly of hepatocytes, arranged as tubular units, running irregularly. The hepatocytes are polyhedral in shape and nucleated. The nucleus possesses a prominent dark nucleolus. In section, four to seven hepatocytes are radially arranged around a central sinusoid. The sinusoids are lined by endothelial cells with elongated nuclei, and Kupffer cells with bean-shaped nuclei and stellate cytoplasmic extensions. The capillaries of hepatic artery and portal veins are scattered throughout the liver parenchyma, without a definite pattern. The lateral borders of hepatocytes form the bile canaliculi which further unite forming the bile duct (Figs. 1a, b).

Numerous islands of the exocrine pancreatic tissue or the hepatopancreas are seen throughout the parenchyma, easily distinguishable from the hepatocytes by their acinar arrangement. They are separated from the hepatocytes by thin septa of connective tissue. They are often found in the vicinity of branches of hepatic arteries or portal veins. They are covered by a thin layer of connective tissue containing reticular fibres. Each hepatopancreas consists of two tiers of cells. The cells are columnar and have a basally located nucleus with a dark nucleolus. Two types of zymogen granules are clearly visible at the apical portion of the cells. The hepatopancreatic ducts are also seen associated with them (Figs. 1c, d). Another important feature of the liver parenchyma is the presence of melanocytes and scattered melanomacrophage centres (MMCs). A MMC is an aggregate of melanomacrophages (MMs), a special category of macrophages, which accumulate melanin, lipofuscin and haemosiderin pigments, as indicated by positive staining with Perls' Prussian blue, Nile blue sulfate and Sudan black B. They are also PAS-positive. The MMCs are always found associated with the hepatopancreas. Occasionally, they are seen lying free among the hepatocytes, within the hepatopancreatic tissue, around the hepatopancreatic ducts, and even inside the portal vein, which is surrounded by the pancreatic tissue. These pigmented cells possess small darkly stained nuclei (Fig. 2). TEM analysis further clarified the liver histology. The have a single nucleus either centrally or basally located with a condensed heterochromatin located at the periphery of the nucleus. The nucleolus is highly electron dense. A well organized rough endoplasmic reticulum (RER) is present. Both round and elongated mitochondria are seen associated with the nucleus and RER (Fig. 3).

Histology and ultrastructure of liver of curcumin-treated fish

In the liver of curcumin-treated fish, there was no indication of any pathological change in both light and electron microscopy perspectives, independent of the dose (0.5 and 1.0%) of curcumin. However, the vascularity increased abundantly as evident in the distribution of blood capillaries (Fig. 4). The area of hepatopancreas, hepatopancreatic cell nucleus and MMC increased in the liver of treated fish while the area of hepatocytes and

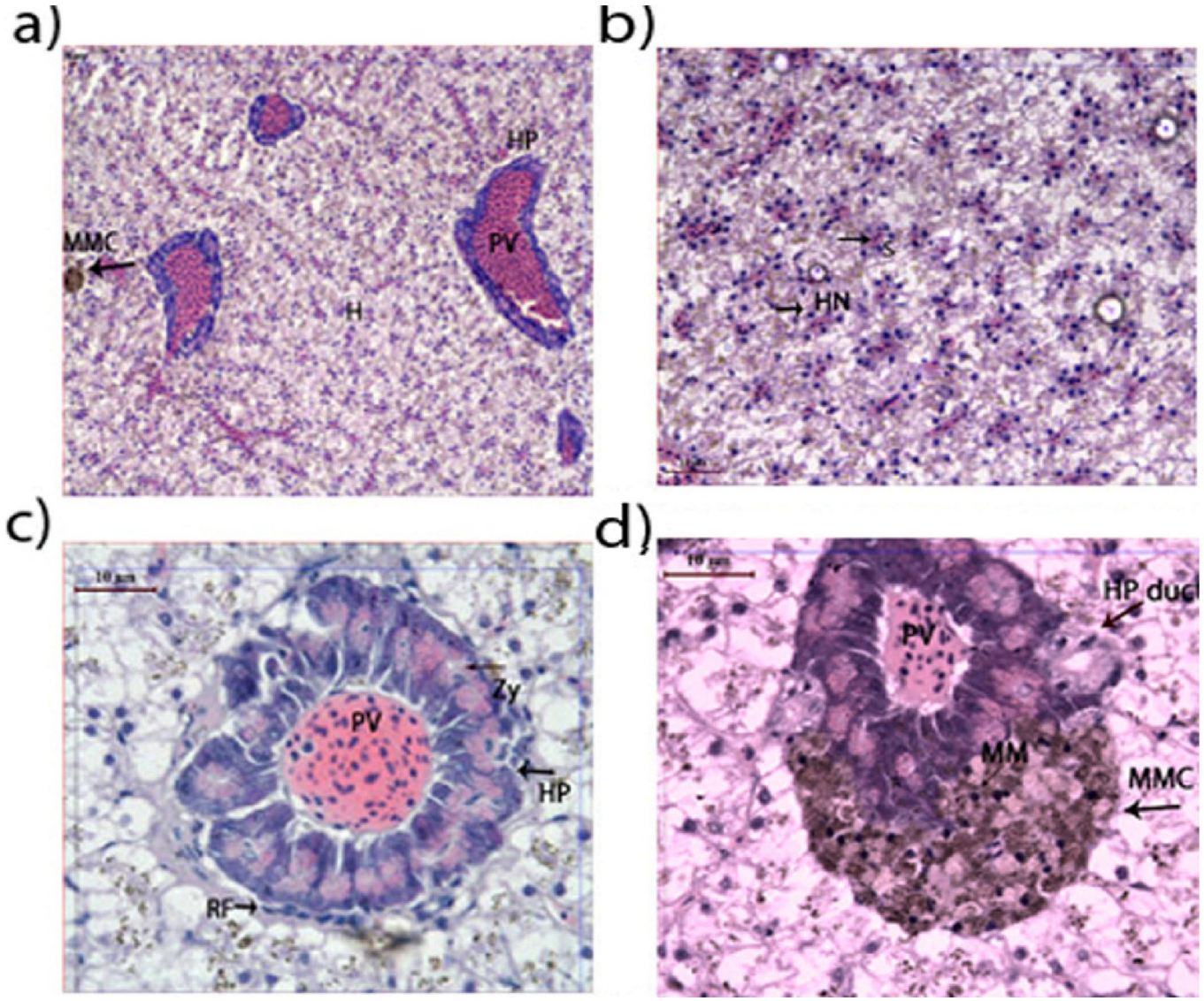

Fig. 1. Structure of liver of a control *A. testudineus.*

(a) Hepatocytes, hepatopancreas, portal vein and MMC.

(b) A section of liver showing the arrangement of hepatocytes and their nuclei.

(c) Structure of hepatopancreas around a hepatic portal vein showing two tiers of cells, with zymogen granules at their apical portions. Note the reticular fibers surrounding the hepatopancreas.

(d) Association of hepatopancreas with MMC. Note the darkly stained nuclei of MMC cells and the hepatopancreatic ducts on either side of the hepatopancreas. (Hematoxylin-Eosin). H, hepatocytes; HP, hepatopancreas; HN, hepatocyte nucleus; HP duct, hepatopancreatic duct; PV, portal vein; MM, Melanomacrophage, MMC, melanomacrophage centre, RF, reticular fibers; S, sinus. Scale bar =10µm.

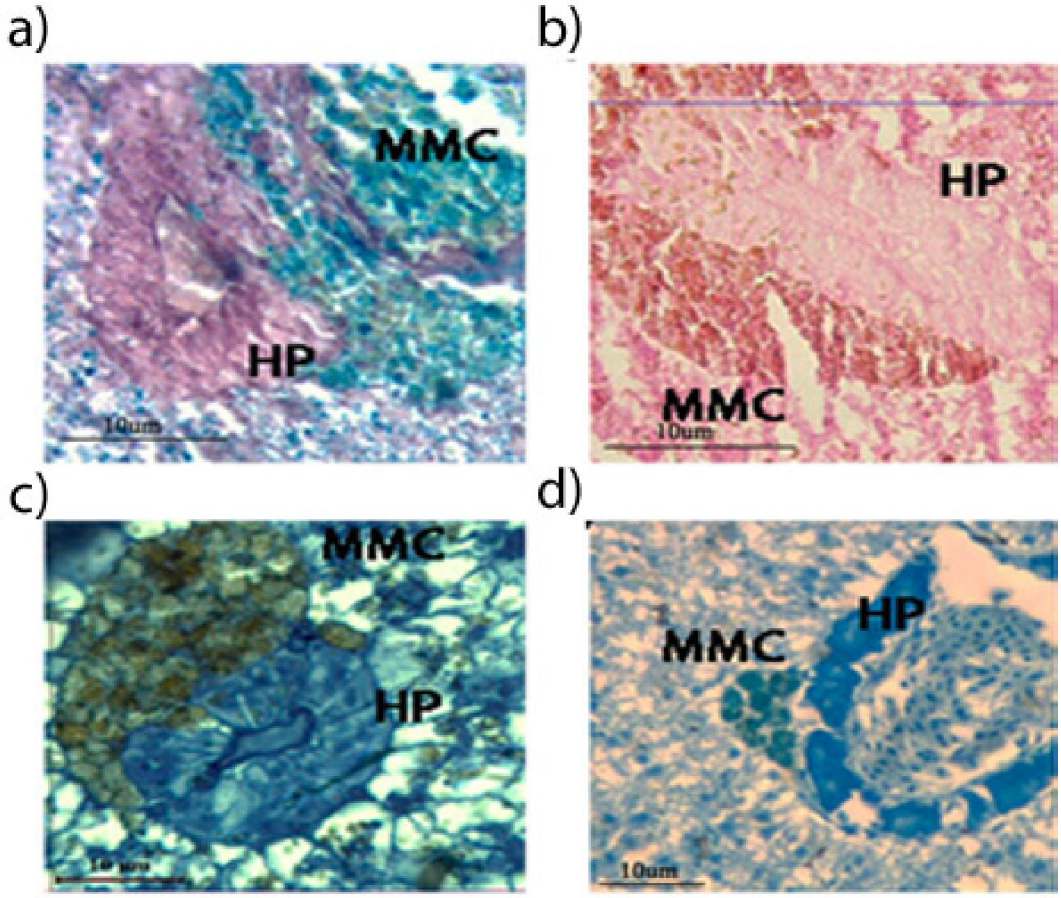

Fig.2. MMC and hepatopancreas of control fish.
(a) Perl's Prussian blue stained.
(b) PAS-stained.
(c) Bromophenpl blue-stained.
(d) Nile blue sulphate-stained. Note that MMCs stain positive with Perl's Prussian blue, Nile blue sulphate and PAS and negative with bromophenol blue. HP, hepatopancreas; MMC, melanocmacrophage centre. Scale bar = 10µm.

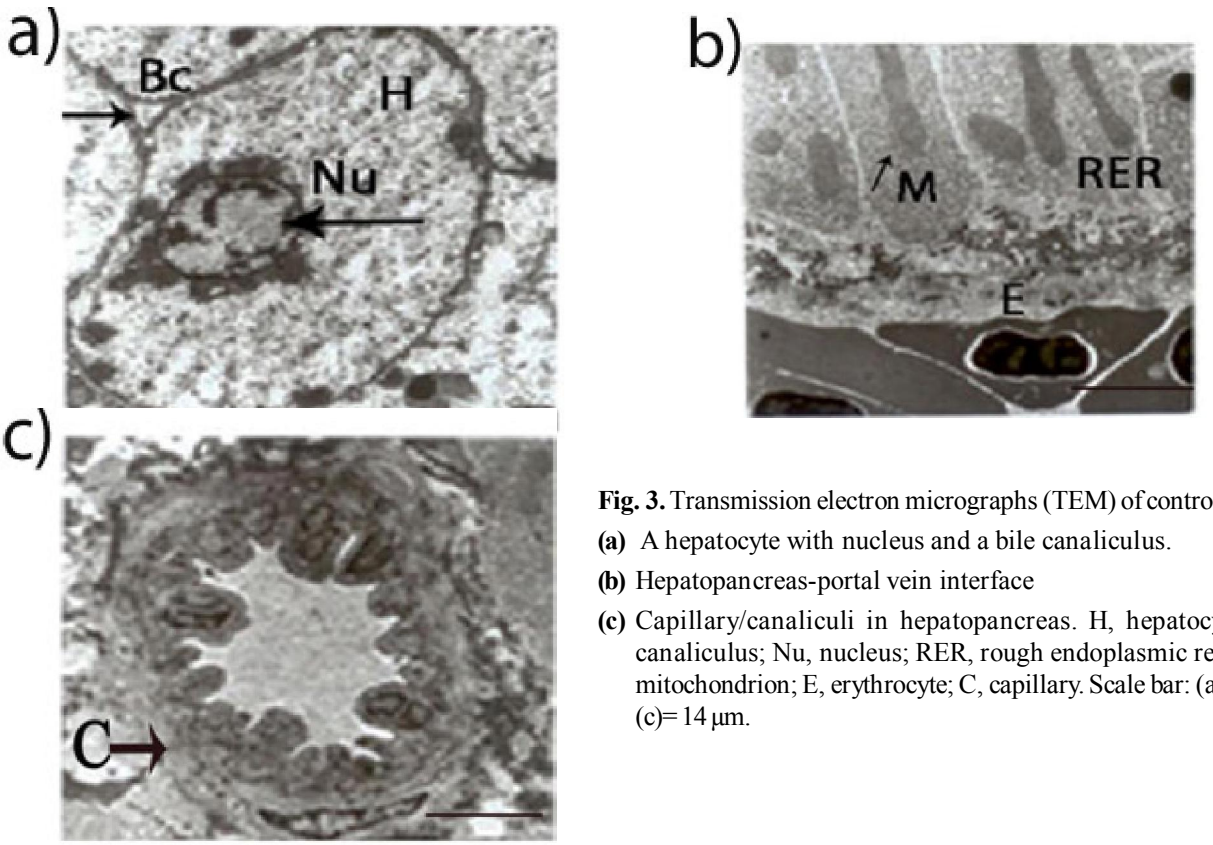

Fig. 3. Transmission electron micrographs (TEM) of control *Anabas* liver.
(a) A hepatocyte with nucleus and a bile canaliculus.
(b) Hepatopancreas-portal vein interface
(c) Capillary/canaliculi in hepatopancreas. H, hepatocytes; Bc, bile canaliculus; Nu, nucleus; RER, rough endoplasmic reticulum; MC, mitochondrion; E, erythrocyte; C, capillary. Scale bar: (a)=7µm; (b) & (c)= 14 µm.

their nuclei remained unchanged (Table 3). Ultrastructurally, there was hypertrophy of cell organelles, especially RER and mitochondria, in the hepatopancreatic cells of curcumin-treated fish**.** More abundant MMCs were present in the hepatopancreas of treated fish. There were melanocyte macrophages (MMs) within the pancreatic acini and also inside the portal blood vessels, indicating that the MMs are capable of migrating to the blood, showing the passage of MMs through the spaces of hepatopancreatic tissue. The hepatopancreas of treated fish indicated hyper-activity as seen by the abundance of zymogen granules and RER (Figs. 5, 6).

Histology and ultrastructure of intestine of control fish

Transverse section of intestine revealed the following structures from outside: connective tissue (serosa), longitudinal muscle, circular muscle, lamina propria and mucosa. The mucosa is in villous forms. The mucosal lining is tall columnar and the nuclei of cells are located basally. There are abundant goblet cells, secreting mucous, many of which were in direct continuity with the lumen, implying discharge of mucous in to the lumen (Fig. 7). TEM analysis showed several dense bodies, RER and mitochondria in the epithelium of the villi. The epithelial cells possess plenty of mitochondria located closer to the microvilli. The basal portion also possesses numerous mitochondria (Fig. 8).

Histology and ultrastructure of intestine of treated fish

In the curcumin-treated fish, there was no indication of any pathological change in the intestine. The only microscopically discernable change was fewer goblet cells (Fig. 9). Ultrastructural analysis revealed some dense aggregates, several dark cells and migratory cells (Fig. 10).

Histology and ultrastructure of kidney of control fish

The head kidney of *Anabas testudineus* is glomerular and composed of fused bilateral lobes. It is covered by a capsule of dense fibrous connective tissue. The kidney has two regions, the parenchyma and the kidney tubules (nephrons). The parenchyma is composed of the inter-renal gland, chromaffin tissue and groups of MMCs, in addition to lymphoid and haematopoietic tissues. Lipofuscin is the major pigment in *Anabas* kidney MMCs as revealed in Perls' reaction, in addition to haemosiderin and melanin. The MMCs are of two types viz., encapsulated and non-encapsulated. The inter-renal cells are cylindrical, polygonal or ovoid. The chromaffin cells are mostly round or elongated and larger than the inter-renal cells (Figs. 11, 12). The kidney tubule has glomerulus, surrounded by Bowman's capsule, and proximal, distal and collecting tubules. Glomeruli are less numerous.

Histology and ultrastructure of kidney of treated fish

In the kidney of treated fish, the glomeruli and proximal and distal tubules retained normal architecture. The glomeruli were lesser in abundance in both control and treated fish. Similar to those in the kidney of control fish, in the treated fish also MMCs contained lipofuscin as the major pigment. The kidney of treated fish had more encapsulated MMCs (Fig. 13).

Discussion

Accumulation of toxic chemicals is known to adversely affect the liver, kidney, muscles and other tissues of fish (Mohanta et al., 2010). The present study demonstrates that the liver, kidney and intestine of both the control and the curcumin-treated fish exhibited normal structure and there were no pathological changes indicating that curcumin, at the doses treated, is not at all toxic to fish but only produces some positive and favorable effects.

Microscopic examination of the liver, intestine and kidney of *Anabas testudineus* showed the typical structural organization comparable to other teleosts. As far as teleostean hepatic organization is concerned, several microscopic, anatomical and histological studies have been done on marine species such as hake *Merlucius hubbsi*, the white croaker *Micropogonias furnieri,* the stripped weak fish *Cynoscion gautuapa* and Atlantic croaker, *Micropogon undulatus* (Eurell and Haensly, 1982; Diaz et al., 1999). Among freshwater fishes, histology and ultrastructure of the liver of *Ictalurus punctatus*, *Hydrocynus forskahlii, Oncorhyncus mykiss*, *Oligosarcus jenynsii*, *Oreochromis niloticus*, *Hoplias malabaricus* and *Carassius auratus* have been described (Hinton and Pool, 1976; Geyer et al., 1996; Petoff et al., 2006; Rios et al., 2007; Vicentini et al., 2005; Bertolucci et al., 2008). The histological structure of *Anabas* liver closely resembles that described for *Oligosarcus jenynsii* (Petoff et al., 2006) and *Micropogon undulates* (Eurell and Haensly, 1982). The absence of hepatic lobules and lack of portal triads in *Anabas* are comparable with many other teleosts (Hampton et al., 1985; Gonzalez et

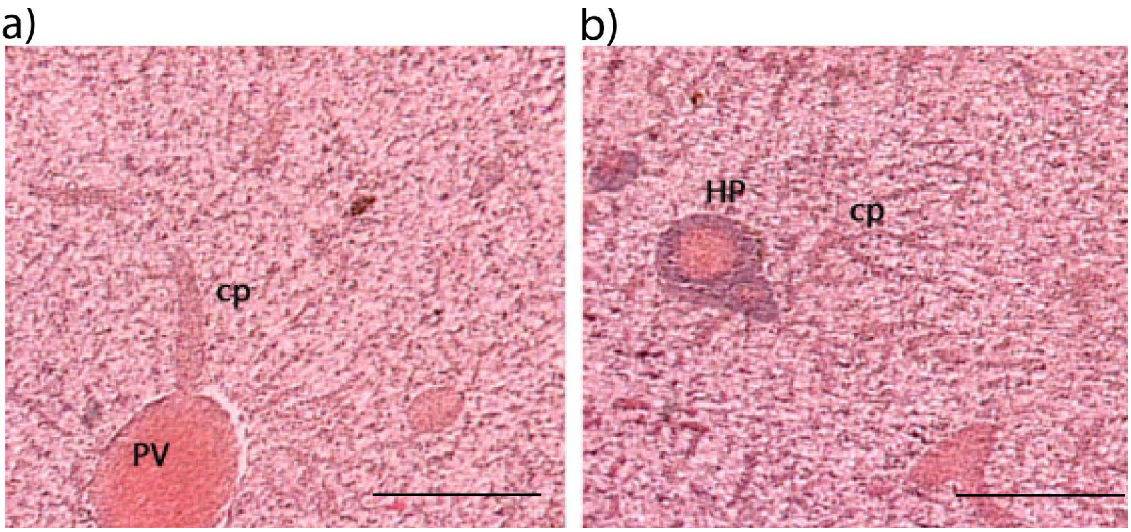

Fig. 4. Light micrographs of liver of control and treated fish. **(a)** Control fish liver showing portal vein and a few capillaries. **(b)** Treated fish liver showing numerous capillaries. (Hematoxylin-Eosin). cp, capillary; PV, portal vein; HP, hepatopancreas. Scale bar = 10 μm.

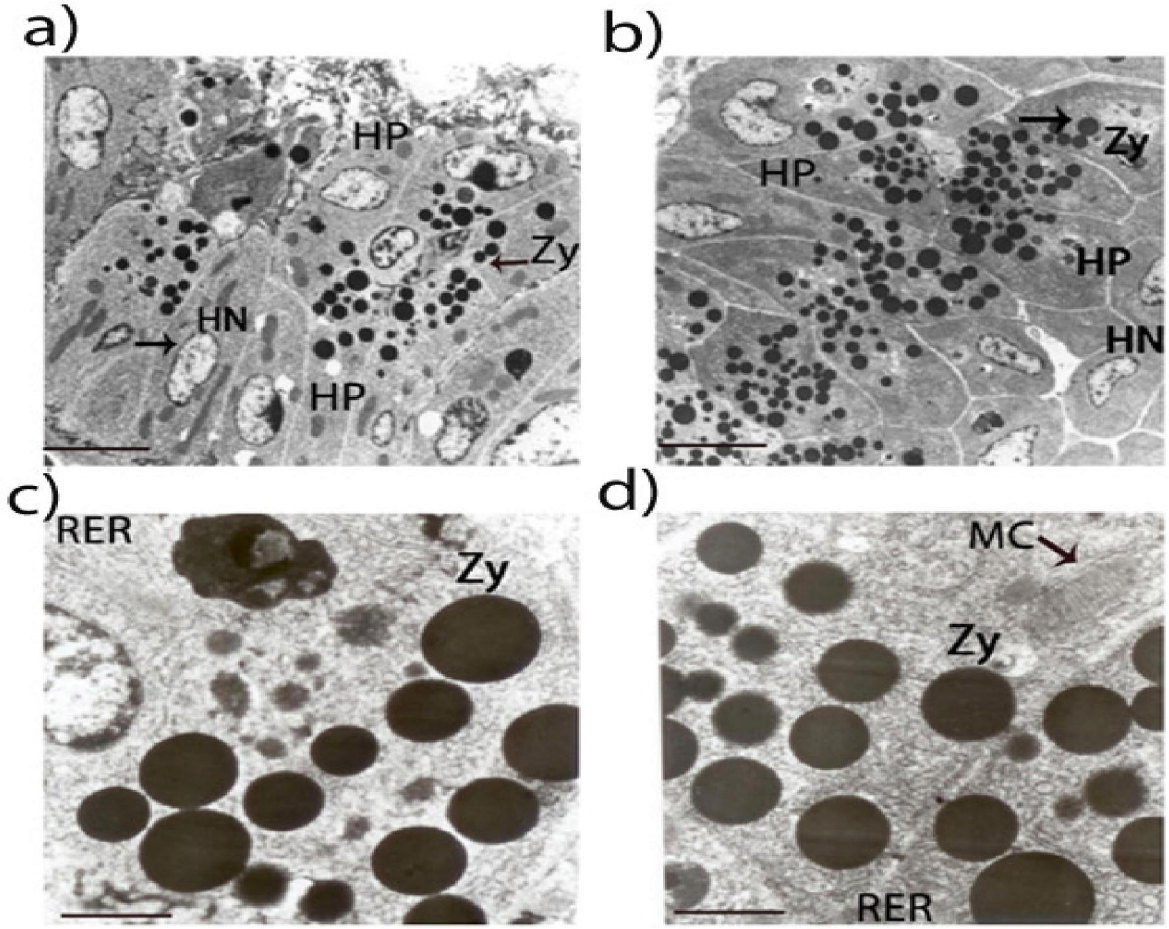

Fig. 5. TEM of control and treated hepatopancreas.
(a) A low power image showing the hepatopancreas. **(b)** Two tiers of HP cells showing greater amount of zymogen granules (light and dark granules) in the treated fish. **(c)** Enlarged view of control fish zymogen granules, the darker version.
(d) Enlarged view of control fish zymogen granules, the lighter version. HP, hepatopancreas, HN, hepatopancreas nucleus, Zy, zymogen granules, RER, rough endoplasmic reticulum; MC, mitochondrion. Scale bar: (a) = 22.5 μm; (b)= 20 μm; (c)= 4.5 μm; (d)= 4.4 μm.

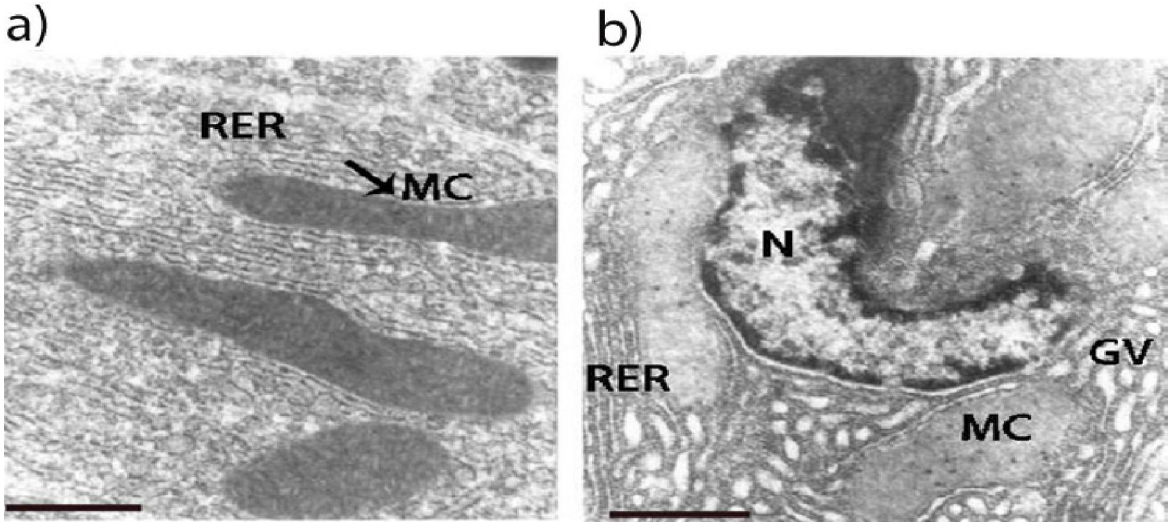

Fig. 6. TEM of a hepatopancreatic cell of control **(a)** and treated fish **(b).** Note that the cell of treated fish has larger mitochondria, prominent RER and Golgi vesicles indicating hyperactivity. RER, rough endoplasmic reticulum; MC, mitochondrion; GV, Golgi vesicle; N, nucleus. Scale bar: (a)=18 µm; (b)= 20 µm.

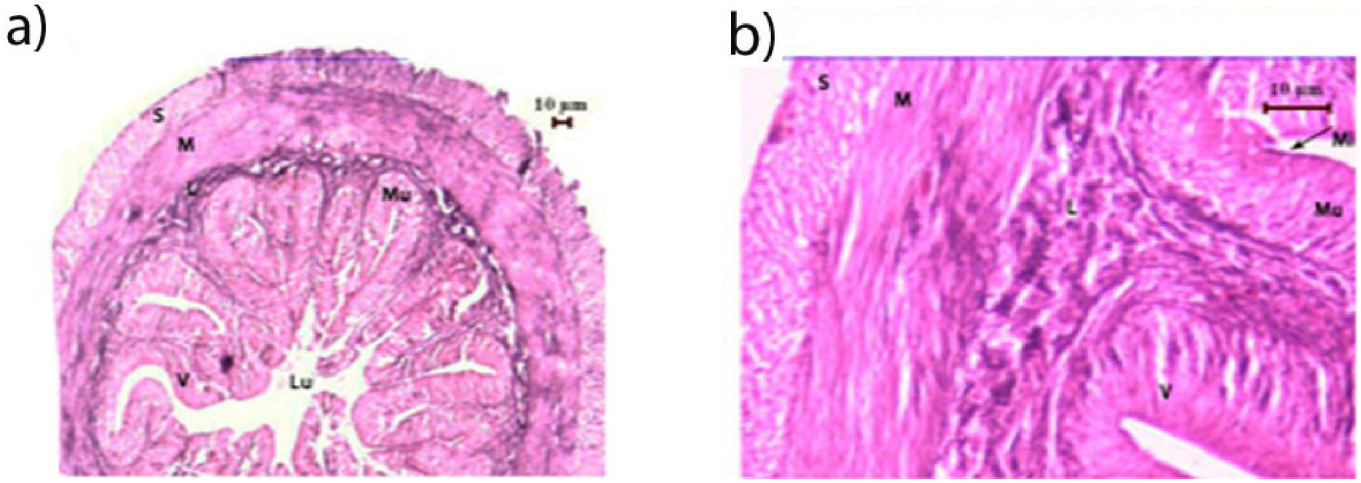

Fig. 7. Light micrographs of sections of intestine of control fish**.** (a) Transverse section showing the different layers in the intestinal wall. **(b)** A portion enlarged (Hematoxylin-Eosin). S, serosa; M, muscularis; L, lamina propria; Mu, mucosa; V, villus; Mi, microvilli. Scale bar: (a) = 20 µm; (b) = 10 µm.

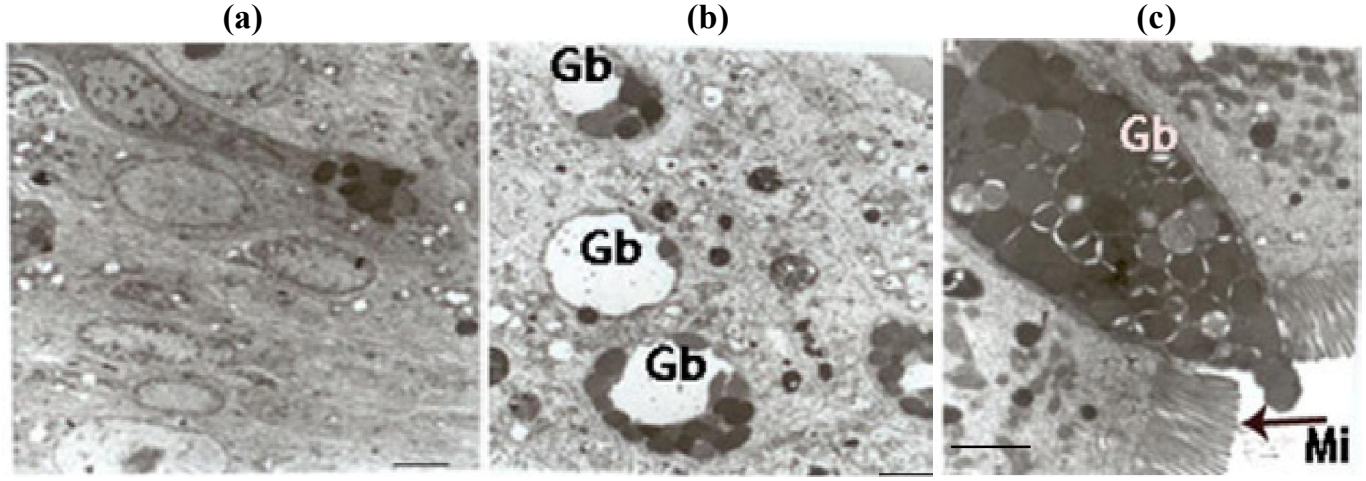

Fig. 8. TEM of intestinal epithelium of control fish. **(a)** Epithelium of villi. **(b)** Profiles of mucus secretion in goblet cell. **(c)** A goblet cell discharging mucus into the lumen. Gb, goblet cell; Mi, microvilli. Scale bar: (a).= 7.5 µm; (b) = 13.3 µm; (c) = 22.5 µm.

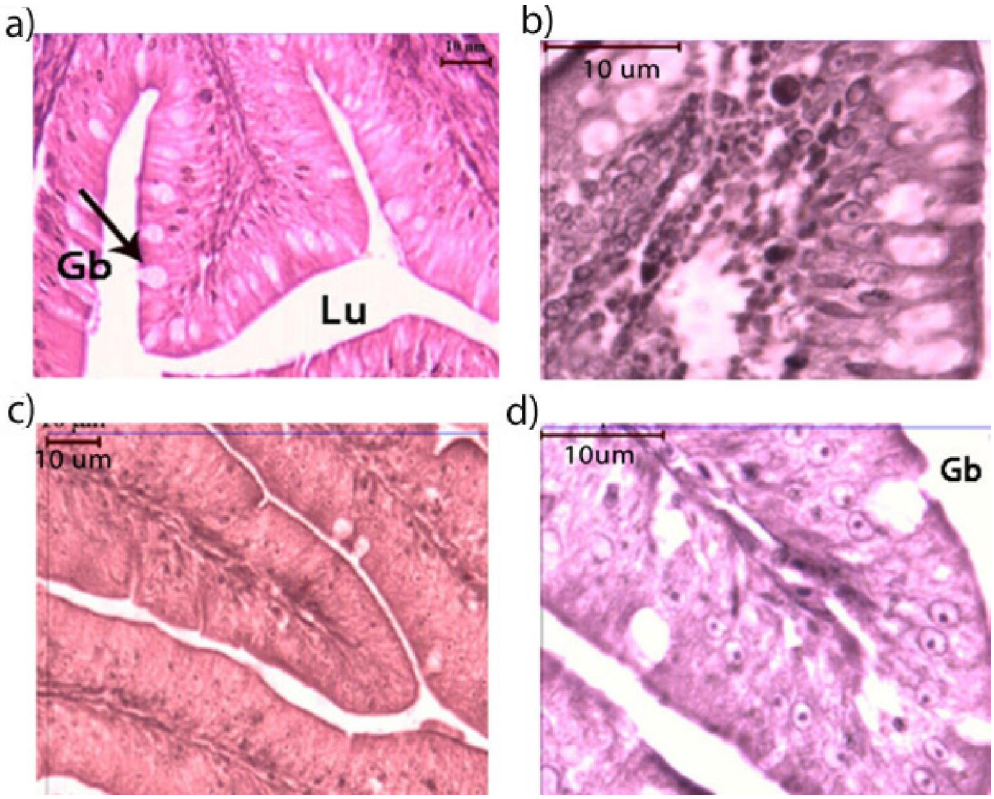

Fig. 9. Light micrographs of epithelium of intestinal villi of treated fish**. (a)** Low power photomicrograph of intestine of a control fish. **(b)** A magnified view. **(c)** Low power photomicrograph of epithelium of a treated fish showing fewer goblet cells. **(d)** A magnified view. (Hematoxylin-Eosin). Gb, Goblet cell; Lu, lumen of intestine. Scale bar: 10 µm.

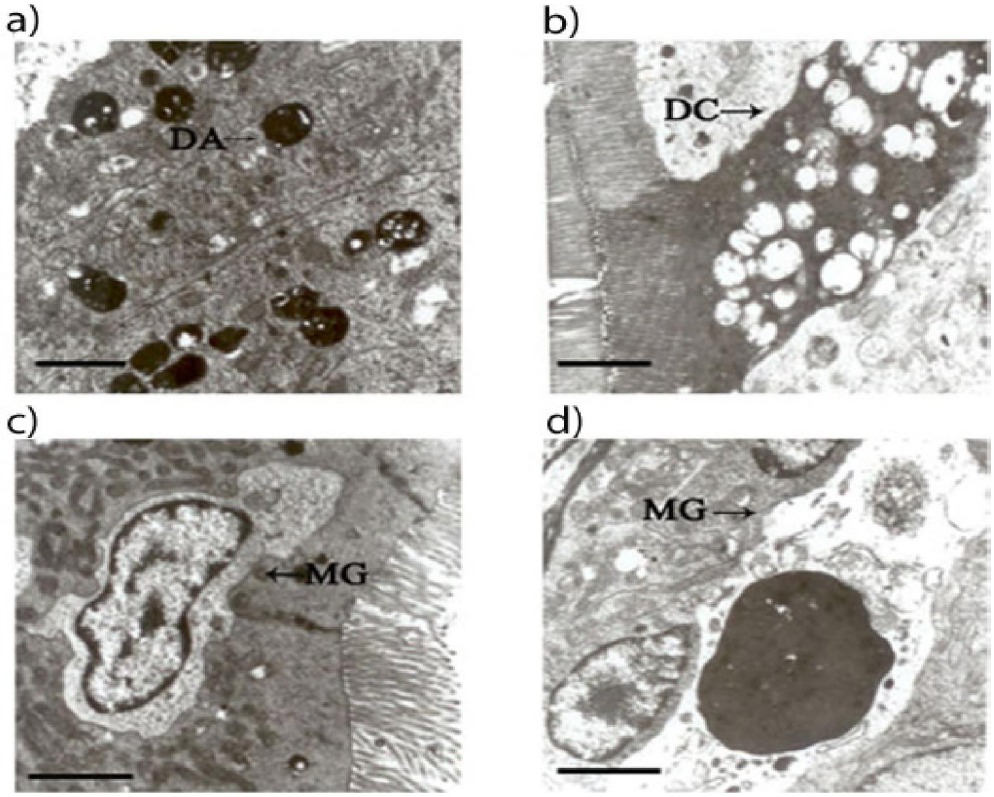

Fig. 10. TEM of epithelium of intestinal villi of treated fish. **(a)** Dense aggregates in the epithelium**. (b)** Dark cells**. (c)** A migratory cell in the epithelium. **(d)** A migratory cell in the intercellular space. DC, dark cell; MG, migratory cell; DA, dense aggregate. Scale bar: (a).= 25 µm; (b)= 17 µm; (c)= 16.7 µm; (d)= 13.3 µm.

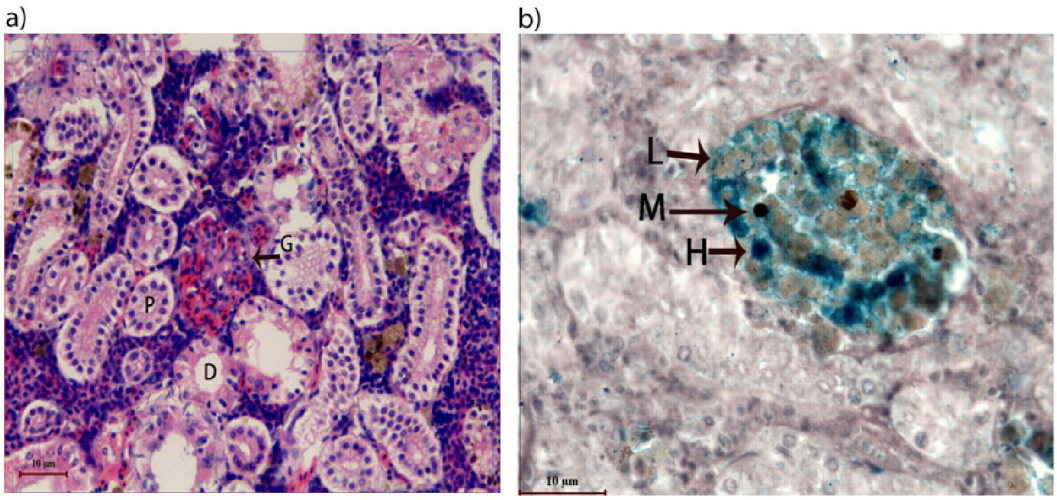

Fig. 11. Light micrographs of sections of kidney of control fish. **(a)** Shows kidney tubules, Bowman's capsule and MMCs (Hematoxylin-Eosin). **(b)**Pigment cells in MMC (Perl's prussian blue. L, lipofuscin; M, melanin; H, hemosiderin. Scale bar = 10 µm.

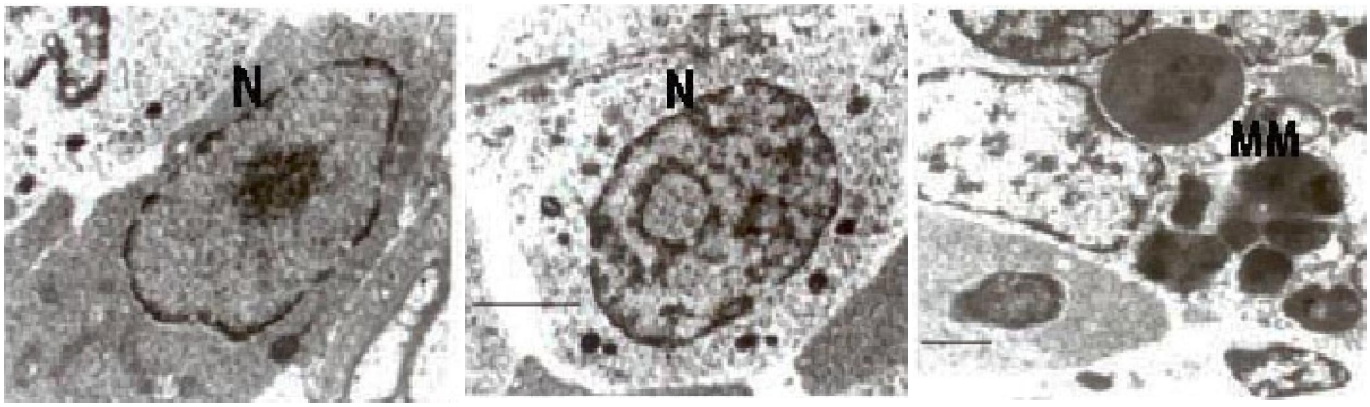

Fig. 12. TEM of kidney of control fish. **(a)** An inter-renal cell. **(b)** A chromaffin cell. **(c)** A melanomacrophage. N, nucleus; MM, melanocmacrophage. Scale bar: (a)= 14 µm; (b) = 15 µm; (c) = 12.5 µm.

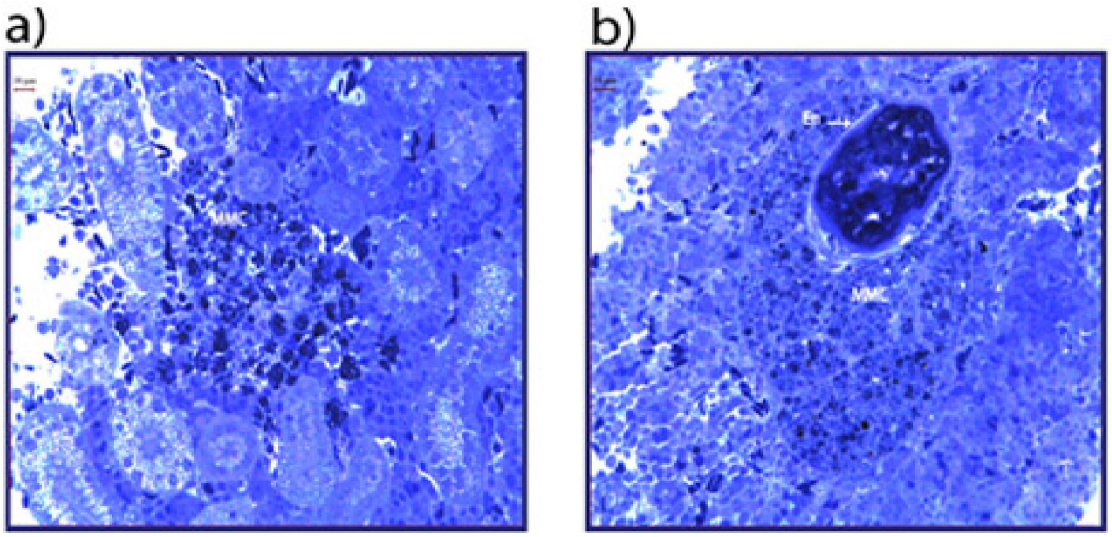

Fig. 13. Light micrographs of TBO-stained sections of kidney. **(a)** From a control fish, showing non-encapsulated MMC. **(b)** From a treated fish showing encapsulated MMC and a dense structure. MMC, melanomacrophage centre; En, an encapsulated dense structure. Scale bar = 10 µm.

Table 3. The effect of curcumin on area of hepatopancreas and melanomacrophage centres (MMC) in *Anabas*.

Area (μm^2)	Ctrl	0.5% Cur	1% Cur
Hepatopancreas	440.5 ± 6.8^a	583.2 ± 15.9^b	1033.6 ± 7.6^b
Hepatopancreas nucleus	2.2 ± 0.5^a	3.4 ± 0.3^b	3.9 ± 0.5^b
MMC	143 ± 3.1^a	364 ± 3.8^b	490 ± 5.9^b
Hepatocyte	8.3 ± 1.1^a	10.4 ± 1.5^a	8.9 ± 0.7^a
Hepatocyte nucleus	1.8 ± 0.1^a	1.9 ± 0.1^a	2.3 ± 0.1^a

Values are expressed as mean ± S.D. The significant difference among groups was analyzed by One-Way ANOVA. Mean values of different superscript letters (a & b) are significantly different ($p<0.05$) as determined by Duncan's Multiple range test.

al., 1993; Vicentini et al., 2005). Kupffer cells have not been described in many fishes (Eurell and Haensly, 1982; Datta, 1996). PAS staining of *Anabas* hepatocytes revealed lesser amount of glycogen in them. Unlike those of mammals, fish hepatocytes do not metabolize much glycogen (Hampton et al., 1985).

The increased vascularity in the liver of treated fish is a positive change indicating that curcumin induces vascularization in the liver parenchyma. This would facilitate increased blood supply, providing for nutrient enrichment to the hepatocytes and quick removal of the toxic metabolites, if any. It also suggests that curcumin at low doses has a positive effect in the liver and should be stimulating the factors of vasculogenesis (neovascularization). Curcumin and its analogs restored the normal histology of CCl_4-treated rat liver and caused mild sinusoidal dilation (Kamalakkannan et al., 2005). Administration of turmeric extract induced hepatotoxic effects in mouse and rat (Deshpande et al., 1998; Kandarkar et al., 1998) whereas toxic effects were not observed in rat, guinea pig, monkey, and pig (Wahlstrom and Blennow, 1978; Shanker et al., 1980; Bille et al., 1985). Feeding of turmeric to chicken through diet induced hepatic changes and these changes were not time- and dose-dependent (Al-Sultan and Gameel, 2004). In the present study also, the observed changes in the histology of *Anabas* were not dose-dependent.

In *Anabas* the exocrine pancreatic acini were observed in plenty, which often surrounded the portal areas. They were more basophilic than hepatocytes. The zymogen granules were clearly seen at the apical portion of the cell which was eosinophilic as evident from H and E staining and the positive staining with bromophenol blue. The hepatopancreatic duct which collects the digestive enzymes was clearly seen. This is the first report of pancreatic duct of a fish originating from within the liver. The association of hepatopancreas with the portal blood could be attributed to the supply of amino acids and other raw materials for the synthesis of digestive enzymes.

Melanocyte macrophage centers were abundant in the liver of *Anabas*, and in most of the cases they were associated with the hepatopancreas which in turn surrounds the portal veins. The MMCs can be considered as an integral part of the reticulo-endothelial system of teleosts acting as repository for effete materials which cannot be metabolized further or that are required for recycling (Robertis, 1975). The association of MMs with the pancreatic tissue leads us to raise two questions/ hypotheses: i) does this association of MMCs with hepatopancreas indicate the migration of MMs loaded with pigment or lipofuscin into the portal veins; and ii) does the secretion of hepatopancreas have anything to do with the management of pigment in MMCs? The Perls' Prussian blue staining indicated that the MMs contain hemosiderin (greenish blue), lipofuscin/ceroid (yellowish brown) and melanin (black). This was further confirmed by Nile blue staining which stained lipofuscins in blue green and melanin in pale green. The MMCs were PAS-positive, indicating the presence of glycoproteins. Liver MMCs are involved in the regular storage, relocation and recycling of iron compounds of effete or damaged RBCs from the portal blood system (Leknes, 2004). Agius and Robertis (1981) reported that MMC enlargement during starvation is sometimes associated with damage to tissues including

kidney and spleen. The central role of MMs is to phagocytose foreign particles and products from cell degradation (Vogelbein et al., 1987). It has also been suggested that macrophages loaded with cell debris or foreign materials are segregated as MMCs, which are destined for involution (Tsuji and Seno, 1990).

Although the light microscopic appearance of fish MMCs has been described many a times (Agius, 1980, 1985; Herraez and Zapata, 1986), less is known about their fine structure (Agius and Agbede, 1984; Fulop and McMillan, 1984; Wolke, 1992). Ultrastructurally, melano-macrophages are very complex. They have indented nuclei and a large number of membrane-bound vacuoles containing a variety of materials. The pigment granules often appear to be contained in groups in such vacuoles, suggesting phagocytosis. Within the macrophages, lipofuscin generally appears to be the most abundant pigment; melanin is often, but not always, the other major component (Robertis, 1975). Hemosiderin is a brown, granular, relatively insoluble pigment containing a protein and an iron (ferric) component. In higher animals, iron is normally stored in the body in the form of ferritin. When the body as a whole, or a particular organ or tissue, becomes saturated with ferritin, iron continues to be stored intracellularly, but in the form of hemosiderin rather than ferritin (Agius, 1979). Hemosiderin is composed of ferric iron and protein and is derived from the catabolism of hemoglobin from effete erythrocytes and is, therefore, an intermediate metabolic product that occurs during recycling of components for erythropoiesis (Kranz, 1989). There are two possible mechanisms by which the augmented hemosiderin-iron content may have come about: (i) the increased catabolism of damaged erythrocytes, and (ii) the increased retention of iron within MMCs as a protective mechanism. Hemosiderin can be present in considerable quantities under certain conditions such as hemolytic anemia. Functions ascribed to MMCs are many, including storage of cell-derived phospholipids and iron following erythrophagocytosis (Agius, 1979; Anguis and Robertis, 1981; Agius and Agbede, 1984) and deposition of resistant pathogens such as bacterial and parasitic spores (Robertis, 1975) and antigen processing in immune responses (Agius, 1985).

The intestinal mucosa is vulnerable to oxidative stress on account of the constant exposure to ROS generated by the luminal content and may result in conditions like ischemia (Halliwell and Gutteridge, 1999). Goblet cells of intestine secrete mucous which is a lubricant and can facilitate faster passage of luminal content. Curcumin treatment caused a decrease of goblet cells and this would reduce the mucous discharge. This itself can be a positive effect of curcumin at low doses in that lesser the mucous, slower will be the transit of luminal content which is food in the process of digestion and absorption. A faster transit of the luminal content would suggest lesser time for digestion and absorption. Slower transit under the influence of curcumin activity would provide for more duration of retention in the intestine, allowing for better digestion and absorption. Ukil et al. (2003) showed that luminal curcumin had a topical beneficial activity on colonic epithelial cells independent of systemic absorption.

Kidneys are the natural filtration system, performing many vital functions including removal of waste products from the blood stream, regulation of water balance and maintenance of *p*H of body fluids. Kidneys possess most of the common xenobiotic metabolizing enzymes thereby contributing to the metabolism of drugs and foreign compounds. Morphology and function of kidneys have been modified through evolution to fulfill different physiological requirements and the widest range of kidney types is found in fish (Charmi et al., 2009). Studies have shown that there is a relationship between structure of nephron and the external environment. In marine teleosts, nephrons are poorly developed. Here, the less abundant Bowman's capsule in *Anabas* may be an adaptation to tolerate wide range of aquatic habitats. The encapsulated structure in the kidney may be the degradation product of pigment moiety of curcumin. The MMCs may play a role in the elimination of toxic products. In the present study, curcumin treatment did not cause any pathological change of concern in the kidney. The kidney exhibited normal architecture.

In conclusion, the present study suggests that curcumin can increase the vascularity in liver which would help in the quick removal of toxic wastes resulting from metabolism of xenobiotics. Decrease in number of goblet cells would help in the retention of food in the intestine

providing longer time for better digestion and absorption. The hypertrophy and hyper-activity of hepatopancreas, as evidenced by the histometry, may help in proper digestion and absorption of food. Increase in MMCs may help in removing toxic materials and their elimination through the portal system. Therefore, it can be concluded that curcumin is beneficial to fish from the histological point of view as well, and curcumin supplementation would produce positive effects in aquaculture of fish.

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References

Agius C. (1979) The role of melano-macrophage centres in iron storage in normal and diseased fish. *J Fish Dis*. **2**:337-343.

Agius C. (1980) Phylogenetic development of melano-macrophage centres in fish. *J Zool*. **191**:11-31.

Agius C. (1985) The melano-macrophage centres in fish: A review. In: Manning MJ, Tatner MF (Eds) *Fish Immunology*. pp 85-105. London, UK: Academic Press.

Agius C, Robertis J. (1981) Effect of starvation on the melano-macrophages centres of fish. *J Fish Biol*. **19**:161-169.

Agius C, Agbede SA. (1984) An electron microscopic study on the genesis of lipofuscin, melanin and haemosiderin in the haematopoietic tissues of fish. *J Fish Bio*l. **24**:471-488.

Allen PC, Danforth HD, Augustine PC. (1998) Dietary modulation of avian coccidiosis. *Int J Parasitol*. **28**:1131–1140.

AL-Sultan SI, Gameel AA. (2004) Histopathological changes in the livers of broiler chicken supplemented with turmeric (*Curcuma longa*). Int J Poult Sci. **3**:333-336.

Bedi KS, Horobin RW. (1976) An alcohol soluble Schiff's reagent: A histochemical application of the complex between Schiff's reagent and phosphotungstic acid. *Histochemistry* **48**:153-159.

Bertolucci B, Vicentini CA, Vicentini IBF, Bombonato MTS. (2008) Light microscopy and ultrastructure of the liver of *Astyanax altiparanae* Garutti and Britski 2000 (Teleosti, Characidae). *Acta Sci: Biol Sci*. 30:73-76.

Bille N, Larsen JC, Hansen EV, Wurtzen G. (1985) Sub-chronic oral toxicity of turmeric oleoresin in pigs. *Food Chem Toxicol*. **23**:967-973.

Chapman DM. (1975) Dichromatism of bromophenol blue with an improvement in the mercuric bromophenol technique for protein. *Stain Technol*. **50**:25-30.

Charmi A, Bahmani M, Sajjadi MM, Kazemi R. (2009) Morphohistological study of kidney in farmed juvenile Beluga *Huso huso* (Linneaus, 1758). *Pak J Biol Sci*. **12**:11-18.

Chiffelle TL, Putt FA .(1951) Propylene and ethylene glycol as solvents for Sudan IV and Sudan black. *Stain Technol*. **26**:51-56.

Clark G. (1981) Staining procedures used by the Biological Stains Commission. In: *Staining Procedures*. pp 412-414. Baltimore: Williams and Wilkins.

Deshpande UR, Gadre SG, Raste AS, Pillai D, Bhide SV, Samuel AM. (1998) Protective effect of turmeric (*Curcuma longa L.)* extract on carbon tetrachloride induced liver damage in rats. *Indian J Exp Biol*. **36**:573–577.

Diaz AO, Gonzales CM, Garcia AM, Devincenti CV, Goldemberg AL. (1999) Morphological and histochemical characterisation of liver from stripped weak fish *Cynoscion guatucupa* (Cuvier, 1830). *Biociencia* **7**:67-78.

Dutta HM. (1996) A composite approach for evaluation of the effect of pesticides on fish. In: Munshi JSD, Dutta HM (Eds) *Fish Morphology: Horizon of New Research.* pp 249-277. USA: Science Publisher Inc.

Elizabeth K, Rao MNA. (1990) Oxygen radical scavenging activity of curcumin. *Int J Pharmacol*. **58**:237-240.

Eurell JA, Haensly WE. (1982) The histology and ultrastructure of liver of Atlantic croaker *Micropogon undulates* L. *J Fish Biol*. **21**:113-125.

Fulop GMI, McMillan D. (1984) Phagocytosis in the spleen of sun fish *Lepomis* spp. *J Morphol.* **179**:175-195.

Geyer HJ, Nel MN, Swanepoel JH. (1996) Histology and ultrastructure of hepatopancreas of tiger fish *Hydrocynus forskahlii*. *J Morphol*. **227**:93-100.

Gonzalez G, Crespo S, Brusle J. (1993) Histocytological study of the liver of the cabrilla sea bass *Serranus cabrilla* (Teleosti, Serranidae) an available model for marine fish experimental studies. *J Fish Biol*. **43**:363-373.

Halliwell B, Gutteridge JMC. (1999) Oxidative stress and antioxidant protection: Some special cases. In: Free radicals in biology and medicine. pp 530-533. Oxford: Clarendon Press.

Hampton JA, Mc Cuskey PA, Mc Cuskey RS, Hinton DE. (1985) Functional unit in rainbow trout (*Salmo gairdneri)* liver: Arrangement and histochemical properties of hepatocytes. *Anat Rec*. **213**:166-175.

Hardy R. (1980) Fish feed formulation. In: *Fish Feed Technology. Lectures Presented at the FAO/UNDP Training Course in Fish Feed Technology*. pp 233-239. Seattle: University of Washington.

Herraez MP, Zapata AG. (1986) Structure and function of the melanomacrophage centres of the gold *fish Carrassius auratus*. *Vet Immunol Immunopathol*. **12**:117-126.

Hinton DE, Pool CR. (1976) Ultra structure of the liver in channel catfish *Ictalurus punctatus* (Rafinesque). *J Fish Biol*. **8**:209-219.

Johnson C. (2004) Influence of certain plant extracts on growth and metabolism of teleosts (*Anabas testudineus* and *Labeo rohita*) and a mammal (*Rattus norvegicus*). Dissertation, University of Kerala, India.

Johnson B, Oommen OV. (1993) Cortisol and hepatic intermediary metabolism in lower vertebrates: A comparative study in a teleost, *Anabas testudineus* and an apodan amphibian *Gegeneophis carnosus*. *J Reprod Biol and Comp Endocrinol.* **5:**44-50

Kamalakkannan N, Rajagopalan R, Suresh PV, Viswanathan P, Rajasekharan KN, Menon VP. (2005) Comparative effects of curcumin and an analogue of curcumin in carbon tetrachloride-induced hepatotoxicity in rats. *Basic Clin Pharmacol Toxicol*. **97:15-21.**

Kandarkar SV, Sharda SS, Ingle AD, Deshpande SS, Maru GB. (1998) Subchronic oral hepatotoxicity of turmeric in mice- histopathological and ultrastructural studies. *Indian J Exp Biol*. **36**:675-679.

Kelloff GJ, Crowell JA, Steele VE, Lubet RA, Malone WA, Boone CW, Kopelovich L, Hawk *ET*, Lieberman R, Lawrence JA, Ali I, Viner JL, Sigman CC. (2000) Progress in cancer chemoprevention: development of diet-derived chemopreventive agents. *J Nutr*. **130**:467S–471S.

Kohinoor AHM, Akhteruzzaman M, Hussain MG Shah MS. (1991) Observation on the induced breeding of *Mystus cavasius* (Hamilton) in Bangladesh. *Bangladesh J Fish.* 14:73-77.

Kranz H. (1989) Changes in splenic melanomacrophage centres of dab *Limanda limanda* during and after infection with ulcer disease. *Dis Aquat Org*. **6**:167-173.

Leena S, Lakshmy PS, Oommen OV. (2000) Effect of carp growth hormone on lipid metabolism in a teleost, *Anabas testudineus* (Bloch). *J Endocrinol Reprod*. **4**:26-34

Leknes I. (2004) Melanomacrophage centres in the liver of platy fish *Xiphophorus maculates* Poecilidae: Teleostei. *Zoology* **107**:201-204.

Lillie RD. (1956) Nile-blue staining technique of the differentiation of melanin and lipofuscins. *Stain Technol*. **31**:151-153.

Manju M, Sherin TG, Rajeesha KN, Sreejith P, Rajasekharan KN, Oommen OV. (2008) Curcumin and its derivatives prevent hepatocyte lipid peroxidation in *Anabas testudineus*. *J Fish Biol*. **73**:1-13.

Manju M, Sherin TG, Rajasekharan KN, Oommen OV. (2009) Curcumin analogue inhibits lipid peroxidation in a freshwater teleost, *Anabas testudineus* (Bloch)—an *in vitro* and *in vivo* study. *Fish Physiol Biochem*. **35**:413–420.

Manju M, Akbarsha MA, Oommen OV. (2012) *In vivo* protective effect of dietary curcumin in fish *Anabas testudineus* (Bloch). *Fish Physiol Biochem*. **38**:309-318.

Mohanta MK, Salam MA, Saha AK, Hasan A, Roy AK. (2010) Effects of tannery effluents on survival and histopathological changes in different organs of *Channa punctatus*. *Asian J Exp Sci*. **1**:294-302.

Nair P, Oommen OV. (1995) Thyroid stimulating hormone stimulates the intermediary metabolism in *Anabas testudineus*. *Am Zool*. 35: 47A.

Park EJ, Jeon CH, Ko G, Kim J, Sohn DH. (2000) Protective effect of curcumin in rat liver injury induced by carbon tetrachloride. *J Pharm Pharmacol*. **52**:437–440.

Petoff GM, Diaz AO, Escalante AH, Goldenberg AI. (2006) Comparative histology of the kidney of *Oligosarcus jenynsi* and *Rhamdia sapo* from Los Pardres lake (Buenos Aires Province Argentina). *Rev R Acad Galega Cienc*. **21**:19-30.

Ponniah AG, Sarkar UK. (2000) *Fish Biodiversity of North East India*. Lucknow, India: NBFGR – NATP Publications.

Ramý´rez-Tortosa MC, Mesa MD, Aguilera MC, Quiles JL, Baro´ L, Ramirez-Tortosa CL, Martinez-Victoria E, Gil A. (1999) Oral administration of a turmeric extract inhibits LDL oxidation and has hypocholesterolemic effects in rabbits with experimental atherosclerosis. *Atherosclerosis* **147**:371–378.

Rios FS, Donatti LO, Fernandes MN, Kalinin AL, Rantin FT. (2007) Liver histopathology and accumulation of melanomacrophage centres in *Hoplias malabaricus* after long term food deprivation and re-feeding. *J Fish Biol*. **71**:1393-1406.

Robertis RJ. (1975) Melanin containing cells of teleost fish and their relation to disease. In: Ribelin WE, Migaki G (Eds) The Pathology of Fishes. pp 399-428. Madison: WI University of Wisconsin Press.

Ruby JA, Kuttan G, Dinesh Babu KV, Rajasekharan KN, Kuttan R. (1995) Anti-tumour and free radical scavenging activity of synthetic curcuminoids. *Int J Pharmacol*. **131**:1-7.

Sarkar UK, Deepak PK, Kapoor D, Negi RS, Paul SK, Singh S. (2005) Captive breeding of climbing perch *Anabas testudineus* (Bloch, 1792) with WOVA FH for conservation and aquaculture. *Aquacult Res*. **36**:941-945.

Shankar TN, Shanta NV, Ramesh HP, Murthy IA, Murthy VS. (1980) Toxicity studies on turmeric (*Curcuma longa*): Acute toxicity studies in rats, guinea pigs and monkeys. *Indian J Exp Biol*. **18**:73-75.

Soudamini KK, Kuttan R. (1989) Inhibition of chemical carcinogenesis by curcumin. *J Ethnopharmacol.* **27**:227–233.

Sreejith P, Oommen OV. (2006) Rapid regulatory effect of tri-iodothyronine (T_3) on antioxidant enzyme activities in a fish *Anabas testudineus* (Bloch): Short – term *in vivo* and *in vitro* study. *Indian J Biochem Biophys*. **43**:119-122

Tsuji T, Seno S. (1990) Melano-macrophage centres in the aglomerular kidney of the sea-horse: Morphological studies on its formation and possible function. *Anat Rec*. **226**:460-470.

Ukil A, Maity S, Karmakar S, Datta N, Vedasiromoni JR, Pijush KD. (2003) Curcumin, the major component of food flavour turmeric reduces mucosal injury in trinitrobenzene sulphonic acid induced colitis. *Brit J Pharmacol*. **139**:209-218.

Varghese S, Oommen OV. (1999) Thyroid hormone regulate lipid metabolism in a teleost, *Anabas testudineus* (Bloch). *Comp Biochem Physiol*. **124**:445-450.

Varghese S, Shameena B, Oommen OV (2001) Thyroid hormones regulate lipid peroxidation and antioxidant enzyme activities in *Anabas testudineus* (Bloch). *Comp Biochem Physiol B Biochem Mol Biol*. **128**:165-171.

Vicentini CA, Franceschini-Vicentini IB, Bombonato MTS, Bertolucci B, Lima SG, Santos AS. (2005) Morphological study of the liver in the teleost *Oreochromis niloticus*. *Int J Morphol.* **23**:211-216.

Vogelbein WK, Fournie JW, Overstreet RM. (1987) Sequential development and morphology of experimentally induced hepatic melanomacrophage centres in *Rivulinus marmoratus*. *J Fish Biol*. **31**:145-153.

Wahlstrom B, Blennow G. (1978) A study on the fate of curcumin in the rat. *Acta Pharmacol Toxicol.* **43**:86-92

Wolke RE. (1992) Piscine macrophage aggregate: A review. *Ann Rev Fish Dis*. **2**:91-108.