Chapter 4

Effect of curcumin on liver, intestine and kidney of *Anabas testudineus*:

Light and electron microscopic study

Introduction

Histopathological investigations have proved to be a sensitive tool to detect direct effects of chemical compounds or xenobiotics in target organs of animals in laboratory experiments and field studies. Tissues which are highly sensitive to xenobiotics are liver, intestine and kidney. Liver plays a key role in the metabolism and biochemical transformations of xenobiotics, which inevitably reflects on its integrity by creating lesions and other histopathological alterations of the parenchyma or the bile duct (Robertis, 1978). Intestine is a vulnerable site of oxidative stress on account of the constant exposure to ROS, generated by the luminal contents (Halliwell *et al.*, 2000). Kidney is one of the most vulnerable organs to various toxic compounds because many xenobiotic substances or their metabolites are excreted through urine from the blood. In the previous chapters, it was explained that curcumin plays a favorable role in the fish. Since curcumin is entirely a new compound to fish body, it is essential that its effects on normal tissue histology are investigated. Therefore, the present study was taken up to
evaluate the efficacy and safety of curcumin *in vivo* in an edible fresh water fish, *Anabas testudineus*, by examining the histology of liver, intestine and kidney.

**Experimental design**

**Histological analysis**

The experimental fish and the treatment modality were as provided in the chapter 3, except that the fish were fed for 6 months. The histology sampling, stains, and light and electron microscopy techniques used are given in the materials and methods section. The slides were randomized and scored in a blinded fashion by a single unbiased observer. Six slides from each group were analyzed. The structures were quantified in 15 randomly selected fields in each slide. Histometric measurements including surface areas of hepatopancreas (µm²), hepatopancreas cell nucleus (µm²), macrophage-melanocytes centre (MMC) (µm²), hepatocyte (µm²) and hepatocyte nucleus (µm²) were taken in a Pentium IV computer using Qwin software (Leica, Jena, Germany).

**Statistical analyses**

The histometric data were statistically analyzed by one-way analysis of variance (ANOVA) using SPSS set up. The results were expressed as mean ± S.E. The significance of the difference between means was determined by Duncan’s multiple range test (Duncan, 1955) at the level, p<0.05.
Results

Histology of control liver

The liver is a brown colored bi-lobed structure. The left lobe is slightly larger than the right one. The liver of *A. testudineus* is composed of a homogenous hepatic parenchyma covered by a thin capsule of connective tissue. The gall bladder is prominent and has an oval or round shape. Liver is divisible into parenchyma (hepatocytes) and stroma (non-hepatocytic tissue). Parenchyma is homogenous and composed of hepatocytes (the predominant cell type), occupying more than 70% of the total volume of the liver, arranged as tubular units. Tubular units ran irregularly through the parenchyma so that they are sometimes seen both in transverse and longitudinal sections in the same field of observation. Four to seven hepatocytes are radially arranged around a central sinusoid. Sinusoids are lined with endothelial cells and stellate reticulo-endothelial cells (Kupffer cells) and often contained erythrocytes. Kupffer cells have a bean-shaped nucleus and plump cytoplasm with star-shaped extensions. The sinusoidal surfaces of the hepatocytes are provided with microvilli which occupy the Space of Disse, the space between the sinusoid and hepatocytes. Endothelial cells are clearly visible. The hepatocytes are polyhedral in shape with their bases facing the sinusoids. They have large nuclei located centrally or basally and each possesses a prominent, dark nucleolus. Those facing the sinusoids have a basally located nucleus. Veins
are scattered throughout the liver parenchyma, without a definite pattern. Lateral borders of hepatocytes formed the bile canaliculi (Fig. 4.1).

Numerous patches of the exocrine pancreatic tissue or the hepatopancreas are seen throughout the parenchyma, easily distinguishable from the acinar arrangement of the hepatocytes. They are separated from the hepatocytes by a thin septa of connective tissue. They are often found in the vicinity of branches of hepatic arteries or portal veins and their size greatly varies. They are covered by a thin layer of connective tissue containing reticular fibres. Each hepatopancreas consisted of two rows of cells, the basal region of the inner row of cells is in contact with the vein while the basal region of the outer row contacts the outer layer of connective tissue. The cells are columnar and have a basally located nucleus with 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 (Fig. 4.2).

Another important feature of the liver parenchyma is the presence of scattered melanocyte macrophage centres (MMCs), an aggregate of melanocyte macrophages (MMs), a special category of macrophages, which accumulate melanin, lipofuscin and hemosiderin, as indicated by positive staining with Perls prussian blue, nile blue sulfate and sudan black B and melanocytes. They are also PAS-positive. The MMCs are always 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 and these pigmented cells have a small darkly stained nucleus (Fig. 4.2, 4.3 & 4.4).

The non-hepatocytic cells include the following: 1. Endothelial cells which lines the sinusoids are in plenty. They have indistinct cytoplasm and an elongated, darkly stained nucleus without nucleoli. 2. Biliary epithelium which consist of simple cuboidal cells (cholangiocytes) that line the bile duct lumen. They have a PAS-positive brush border. 3. A special category of macrophages (melanomacrophages) were observed in the parenchyma.

Transmission electron microscopic analysis further clarified the liver histology. The ultra-structure of hepatocytes show a single nucleus either centrally or basally located with more condensed heterochromatin located at the periphery of the nucleus. The nucleolus has a high electron dense visibility. A well organized rough endoplasmic reticulum (RER) is present. Both round and elongated mitochondria are seen associated with the nucleus and RER (Fig. 4.5).

Histology of treated liver

In the curcumin-treated liver, there was no indication of any pathological change in both light and electron microscopy irrespective of independent of the dose of curcumin used. However, the vascularity increased abundantly as evident in the distribution of blood capillaries (Fig. 4.6). The area of hepatopancreas, hepatopancreatic cell nucleus and MMC increased in the treated liver while area of
hepatocytes and their nuclei remained unchanged (Table 4.1). Ultrastructurally, there was hypertrophy of cell organelles, especially RER and mitochondria in the treated hepatopancreatic cells. More number of MMCs was associated with hepatopancreas of treated fish. There were melanocyte macrophages (MMs) within the pancreatic acini and also inside the portal blood vessel, indicating that the MMs are capable of migrating into the blood, showing the passage of MMs through the spaces of hepatopancreatic tissue. The treated hepatopancreas showed hyper-activity as seen in the abundance of zymogen granules. There were some cells or nuclei associated with MMC of treated fish. A peculiar densely nucleated structure was seen (Fig. 4.7, 4.8 & 4.9).

Histology of control intestine

The transverse section of intestine has the following structures from outside- connective tissue (serosa), longitudinal muscle, circular muscle, lamina propria and the mucosa. The mucosa is in villous forms. The mucosal lining was tall, columnar and nuclei of cells are located basally. There are abundant goblet cells, secreting mucous, many of which are in direct continuity with the lumen, implying discharge of mucous in to the lumen. (Fig. 4.10). TEM analysis showed several dense bodies, RER and mitochondria in the villi epithelium. The epithelial cells had plenty of mitochondria located closer to the microvilli. Basal portion also possessed numerous mitochondria. Goblet cells and their secretory vesicles were clearly visible (Fig. 4.11).
Histology of treated fish intestine

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. 4.12). Ultrastructural analysis revealed some dense aggregates, several dark cells and migratory cells (Fig. 4.13).

Histology of control kidney

The head kidney of *Anabas testudineus* is glomerular and composed of fused bilateral lobes. The right lobe is slightly larger than the left lobe. This organ is covered by a capsule of dense fibrous connective tissue. 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 cells of MMCs, in addition to lymphoid and haematopoietic tissues. Lipofuscin is the major pigment in *Anabas*, MMCs as revealed from Perl’s reaction, in addition to hemosiderin and melanin. The MMCs are of two types viz., encapsulated and non-encapsulated. The inter-renal cells are found to be cylindrical, polygonal or ovoid. The chromaffin cells are mostly round or elongated and larger than the inter-renal cells (Fig. 4.14, 4.15).

The kidney tubule has glomeruli, enclosed by Bowman’s capsules, proximal, distal and collecting tubules. Glomerulii are less in number. Kidney also possesses patches of MMCs along with free MMs in the hematopoietic tissue (Fig. 4.14).
Histology of treated kidney

In the treated kidney, the glomeruli, proximal and distal tubules retained normal architecture. The glomerulii were fewer in number in both control and treated groups. Similar to those in control kidney, treated MMCs contained lipofuscin as the major pigment. Treated kidney had more encapsulated MMCs (Fig. 4.16).

Discussion

Accumulation of the 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 experimental fish exhibit a normal architecture and there were no pathological changes.

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 *Merlucius hubbsi*, the white croaker *Micropogonias furnieri*, the stripped weak fish *Cynoscion gautuapa* and Atlantic croaker, *Micropogon undulatus* (Diaz et al., 1999; Eurell & Haensly, 1982). Among freshwater fishes, the histology and ultrastructure of the liver of Channel cat fish *Ictalurus punctatus*, the tiger fish *Hydrocynus forskahlii*, the trout *Oncorhyncus mykiss*, *Oligosarcus Jenynsi*,

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Oreochromis niloticus, Hoplias malabaricus and gold fish Carassius auratus have been described (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 & Haensly, 1982). The absence of hepatic lobules and lack of portal triads in Anabas are comparable with many other teleosts (Vicentini et al., 2005; Hampton et al., 1985; Gonzalez et al., 1993). Many of the authors have not described Kupffer cells in fish (Eurell & Haensly, 1982; Munshi & Datta, 1996). Hepatocytes lack much glycogen as evidenced by PAS staining. In contrast to those of mammals, fish hepatocytes do not metabolize much glycogen (Moon et al., 1985; Hampton et al., 1985).

Increased vascularity in liver 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 either stimulating the factors of vasculogenesis (neovascularization). Increased vascularity in the present study is consistent with a report by Kiran et al. (2007). Curcumin and its analogs restored the normal histology of CCl₄ treated rat liver and caused mild sinusoidal dilation (Kamalakkannan et al., 2005). Administration of turmeric extract induced hepatotoxic effects in mice and rats (Deshpande et al., 1998; Kandarkar et al.,
1998) whereas toxic effects were not observed in rats, guinea pigs, monkeys and pigs (Wahlstrom & Blennow, 1978; Bhavanishanker 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 and the effect of turmeric appears to depend on dose, duration of treatment and animal species (AL-Sultan & Gameel, 2004).

The exocrine pancreas acini were observed in plenty 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 is eosinophilic as evident from H & E staining and showed positive staining with bromophenol blue and also in H & E stain. The hepatopancreatic duct which collects the digestive enzymes was clearly seen. This is the first report of pancreatic duct originating from the liver. The association of hepatopancreas with the portal blood can be attributed to the supply of amino acids and other raw materials for the synthesis of digestive enzymes.

Melanocyte macrophage centres were abundant in the liver of *A. testudineus*, and most of the time they were associated with the hepatopancreas which in turn surrounds the portal veins. MMCs can be considered as an integral part of the reticulo-endothelial system of teleosts acting as repository centres for effete materials which cannot be metabolized further or that are required for recycling (Robertis, 1975). The association of MMs with the pancreatic tissue lead
us to raise two questions/hypotheses; Does this association of MMCs with hepatopancreas indicate the migration of MMs loaded with pigment or lipofuscin into the portal veins or does the secretion of hepatopancreas has 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). Some authors suggest that macrophages loaded with cell debris or foreign materials are segregated as MMC, which are destined for involution (Tsuji & Seno, 1990).

Although the light microscopic appearance of fish melano-macrophage centres has repeatedly been described in the literature (Agius, 1980, 1985; Herraez & Zapata, 1986), less is known about their fine structure (Agius & Agbede, 1984; Fulop & McMillan, 1984; Wolke, 1992). Ultrastructurally, melano-macrophages are very complex (Robertis, 1975). They have indented nuclei and large numbers
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. Haemosiderin 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 haemosiderin rather than ferritin (Agius, 1979). Haemosiderin is composed of ferric iron and protein and is derived from the catabolism of haemoglobin 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 haemosiderin-iron content may have come about: (i) the increased catabolism of damaged erythrocytes, and (ii) the increased retention of iron within melano-macrophage centres as a protective mechanism. Haemosiderin can be present in considerable quantities under certain conditions such as haemolytic anaemia. Functions ascribed to melano-macrophage centres are many, including storage of cell-derived phospholipids and iron following erythrophagocytosis (Agius, 1979, 1981; Agius & 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 & Gutteridge, 1999). Lipid peroxidation may bring about protein damage inactivation of membrane bound enzymes and decrease the fluidity of intestinal brush border membrane, either through direct attack by free radicals or their reactive derivatives (Ohyashiki et al., 1986).

Goblet cells of intestine secrete mucous which is a lubricant and can facilitate faster passage of luminal content. On the contrary, curcumin treatment brings about decrease of goblet cells and this reduces mucous discharge. This itself can be a positive effect of curcumin at low doses, that is, 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 time of retention in the intestine, allowing 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 pH of body fluids. Kidneys possess most of the common xenobiotic metabolizing enzymes and thus are able to contribute to the body's 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 fishes (Charmi et al., 2009). Studies have shown that there is a relation between structure of nephron and the external environment. In marine teleosts, nephrons are poorly developed. Here, the lesser number of 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 changes of concern. The kidneys exhibited the normal architecture.

In conclusion, the present study suggests that curcumin can increase the vascularity in liver which helps the quick removal of toxic waste resulted from metabolism of xenobiotics. Decrease in the number of goblets cells help 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, based on the above results it can be stated that curcumin is beneficial to fish from the histological point of view, as well.
Fig. 4.1

A)

B)
Fig. 4.2

A)

B)
Fig. 4.3

A) 

B)
Fig. 4.4
Fig. 4.7

A) 

B) 

C) 

D)
Fig. 4.9

A)

B)

10 μm
Fig. 4.10

A)

B)
Fig. 4.14

A)

B)
Fig. 4.1 Structure of liver of a control fish *A. testudineus* showing hepatocytes, hepatopancreas, portal vein and sinus. (H & E). H, hepatocytes; HP, hepatopancreas; PV, portal vein; HN, hepatocyte nucleus; S, sinus; MMC, melanocyte macrophage centre. Scale bar = 10 \( \mu \)m.

Fig. 4.2 Structure of hepatopancreas. A, hepatopancreas around a hepatic portal vein showing two tiers of cells, with zymogen granules at their apical portions (stained pink). Note the reticular fibers surrounding the hepatopancreas; B, association of hepatopancreas with MMC; note the darkly stained nuclei of MMC cells and the hepatopancreatic ducts on either side of the hepatopancreas. (H & E). Scale bar = 10 \( \mu \)m.

Fig. 4.3 A) Association of macrophage melanocytes with the hepatopancreas. Note a macrophage cell inside a pocket of hepatopancreas facing the portal vein. (H & E). B) Two types of zymogen granules (light- and dark-stained) in the hepatopancreas. (TBO). HP, hepatopancreas; PV, portal vein; MM, macrophage melanocyte; MMC, melanocyte macrophage centre; Zy, zymogen granule. Scale bar = 10 \( \mu \)m.

Fig. 4.4 MMC and hepatopancreas. A) Perl’s Prussian blue stained. B) PAS-stained. C) Bromophenpl blue-stained. D) Nile blue sulphate-stained. Note,
MMCs stain positive with Perl's prussian blue, nile blue sulphate and PAS and negatively with bromophenol blue. HP, hepatopancreas; MMC, melanocyte macrophage centre. Scale bar = 10 µm

Fig. 4.5 Transmission electron microscopic (TEM) images of control *Anabas* liver. A) A hepatocyte with nucleus and a bile canaliculus. Scale bar = 7 µm B) Hepatopancreas-portal vein interface. Scale bar = 14 µm C) capillary/canaliculi in hepatopancreas. Scale bar = 4.5 µm. H, hepatocytes; Bc, bile canaliculus; Nu, nucleus; RER, rough endoplasmic reticulum; MC, mitochondrion; E, erythrocyte; C, capillary.

Fig. 4.6 A) control liver showing portal vein and a few capillaries. B) Treated liver showing numerous capillaries. (H & E). Cp, capillary; PV, portal vein; HP, hepatopancreas. Scale bar = 10 µm

Fig. 4.7 TEM images of control and treated hepatopancreas. A) A low power image showing the hepatopancreas. Scale bar = 22.5 µm B) Two tiers of HP cells showing greater amount of zymogen granules (light and dark granules) in the treated fish. Scale bar = 20 µm C) Enlarged view of control fish zymogen granules, the darker version. Scale bar = 4.5 µm D) Enlarged view of control fish zymogen granules, the lighter version. Scale bar = 4.4 µm HP, hepatopancreas; HN,
hepatopancreas nucleus; Zy, zymogen granules; RER, rough endoplasmic reticulum; MC, mitochondrion.

Fig. 4.8 TEM images of a hepatopancreatic cell of control (A) and treated fish. Scale bar = 18µm (B). Note that the treated hepatopancreas has larger mitochondria, prominent RER and Golgi vesicles indicating hyperactivity. Scale bar = 20µm RER, rough endoplasmic reticulum; MC, mitochondrion; GV, Golgi vesicle; N, nucleus.

Fig. 4.9 A) A densely nucleated structure in the treated liver, shown by an arrow (TBO). B) L.S. of a duct arising from the hepatopancreas, shown by an arrow (H & E). Scale bar = 10µm

Fig. 4.10 Structure of control intestine. A) Transverse section showing the different layers in the intestine wall. B) A portion enlarged. (H & E). S, serosa; M, muscularis; L, lamina propria; Mu, mucosa; V, villus; Mi, microvilli. Scale bar = 10µm

Fig. 4.11 Ultrastructure of control villi epithelium A) Villi epithelial cells Scale bar = 7.5µm B) Profiles of mucous secretion in goblet cell. Scale bar = 13.3µm C)
A goblet cell secreting mucous into the lumen. Scale bar = 22.5µm Gb, goblet cell; Mi, microvilli.

**Fig. 4.12** Effect of curcumin on villi epithelium. A) Control villi epithelium with many goblet's cells. (H & E). B) A control villus in high power (H & E). C) Treated fish villi epithelium with fewer goblet cells. D) A treated fish villus in high power (H & E). Gb, goblet's cell; Lu, lumen of intestine. Scale bar = 10µm

**Fig. 4.13** Intestinal villi epithelium of treated fish. A) Dense aggregates in the epithelium. Scale bar = 25µm B) Dark cells. Scale bar = 17µm C) A migratory cell in the epithelium. Scale bar = 16.7µm D) A migratory cell in the intercellular space. Scale bar = 13.3µm. dense body; DC, dark cell; MG, migratory cell; DA, dense aggregate.

**Fig. 4.14** A) Structure of *Anabas* kidney showing kidney tubules, Bowman's capsule and MMCs (H & E). B) Pigment cells in MMC (Perl's prussian blue). L, lipofuscin; M, melanin; H, hemosiderin. Scale bar = 10µm

**Fig. 4.15** A) Ultrastructure of an inter-renal cell. Scale bar = 14µm B) A chromaffin cell. Scale bar = 15µm C) Melanocyte macrophage. Scale bar = 12.5µm. N, nucleus; MM, melanocyte macrophage.
Fig. 4.16 A) Control kidney showing non-encapsulated MMC. (TBO). B) Treated kidney showing encapsulated MMC and an encapsulated structure. (TBO) MMC, melanocyte macrophage centre; En, encapsulated structure. Scale bar = 10µm
Table 4.1 Effect of curcumin on surface area of hepatopancreas and MMC in *Anabas*

<table>
<thead>
<tr>
<th>Area (μm²)</th>
<th>Ctrl</th>
<th>0.5%</th>
<th>1%</th>
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<tr>
<td>Hepatopancreas</td>
<td>440.5 ± 6.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>583.2 ± 15.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1033.6 ± 7.6&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Hepatopancreas nucleus</td>
<td>2.2 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.9 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MMC</td>
<td>143.3 ± 3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>364.4 ± 3.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>490.5 ± 5.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hepatocyte</td>
<td>8.3 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.4 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.9 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hepatocyte nucleus</td>
<td>1.8 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.9 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
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Values are expressed as mean ± S.E. The significant difference between 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.