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# Histopathological effects of toxic alga *Nostoc muscurum* on juvenile grass carp fish (*Ctenopharyngodon idella* Val. 1844 )

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## Abstract

In the present study, the grass carp fish (*Ctenopharngodon idella*) was divided into three groups, each of them was force feeding ; group (A)on toxic alga *Nostoc muscurum* only , group (B) on mixture of toxic alga *N. muscurum* and clover and group (C) served as the control group on clover only. The tissue sections which were made from fish organs that have been investigated a long two periods (after 24 h and after 15 days) , and they proved that the alga *N. muscurum* have toxic effect on the organs of grass carp fishes (gills , intestine , liver and kidney) in A and B groups and the degree of damage depend on the long time the experiment tacks.

At first , the causal relationship of tissue damage with the presence of microcystin-containing (*N. muscurum* ) were investigated in grass carp fish .

In gills filament the histopathological changes are represented by aneurism and congestion of capillaries , the adhesion and change in the shape of the secondary gill lamellae , increase in the number of epithelial cells and swelling of epithelial cells .These changes increased after 15 days , they were represented by detachment of epithelial layer of the secondary gill lamellae . The chondrodysplasia in the supporting cartilage of gill filament and atrophy and disappearance of the secondary gills lamellae were observed in mixture group only as changes.

The histopathological changes of intestine were very severe after 15 days and were represented by the increase in the proliferation of columnar epithelial cells , necrosis of lining epithelial tissue and the vascular degeneration of smooth muscles cells in circular layer of tunica muscularis.

after 24 hours, the histopathological changes in liver were confined by the congesting of sinusoid with the appearance of yellow patches through the liver cords. The hepatocytes in the liver of group A were seen as normal , but in the mixture group B the location of nucleolus of these cells appeared abnormal .While after 15 days the latter changes appeared in hepatocytes of both groups (A and B) and represented the beginning of necrosis which were described .

In kidney , the histopathological changes occurred in all structures of the kidney of fish in the both groups (A and B) . In addition , after 24 hours , in renal tubules , the changes were represented by metaplasia in lining epithelial tissue and their hollow disappeared, the cells of that tubules are lost the polarization in tissue and a change in their nucleus . In glomerules , was noted the proliferative glomerionephritis then a disappearance of bowman's space . Also a necrosis occurred in hematopoietic tissue .However , after 15 days , all of that changes are also observe in two groups (A and B) , as well as the necrosis of some parts of the epithelial lining of renal tubules.

Keywords: Toxic effects , cyanobacterial algae , *Nostoc muscurum* , grass carp fish

## 1- Introduction

Cyanopbacteria were recorded to represent up to 17% of the phytoplankton investigated in fresh water fish ponds (Shaaban *et al*., 1999). In spite of great importance of cyanobacteria for aquatic life nutrition , specially for fish growth and productivity many of them were recorded to be highly toxic affecting the growth of aquatic organisms (Turell and Middlelbrook , 1988) . Cyanobacteria have been found flourishing during mid to lake summer in fresh water ponds and lakes in U.S.A , Canada , Russia , Europe ,S. Africa , S. America , India ,Japan , Midlle east and Australia (Charmichael *et al*., 1985) .

Cyanobacterial blooms have been detected in fresh water ponds and lakes all over the world some of these cyanobacterial were found to be the source of some potent toxins (Charmichael , 1988 ). Fish productivity in fresh water is correlated to flourishment of phytoplanktonic components , bacteria and algae ( Kund – Hansen and Batterson , 1994 ; Garg and Bahatragar , 1996 ).

The alga *Nostoc* is a cosmopolitan cyanobacterial genus occurring in both terrestrial and aquatic ecosystems . Strains of genus *Nostosc* are the most common cyanobacteria in symbiosis ( Dodds *et al*., 1995) . In Iraq AL-sultan , 2010 showed

one species from genus *Nostoc* ( *N. muscurum*) have the ability to produced hepatotoxins exactly Microcystin-LR with highly concentration 27.57 µg/ml with appeared toxic effect on some economic fish larvae and zooplankton .

Herbivorous fish are among the most abundant fish groups . The phytoplankifvorous fish are especially important to humans because of their role in aquatic ecocystems consumers of phytoplankton primary production , their importance as food fish , and their potential for biological management of algal blooms . ( Opuszynski and Shiremon , 1995 ; Xie and Liu , 2001 ) . In aquatic systems , fish stand at the top of the aquatic food chain , and are possibly affected by exposure to toxic cyanobacteria . ( Xie *et al*., 2004 ) .

Hepatotoxic microcystins ( MCs) are natural toxins produced by fresh water cyanobacteria such as *Microcystis* primarily *M. aeruginosa* , *Anabaena* , *Oscillatoria* , *Nostoc* ( Charmichael , 2001 ) . More than 80 chemical forms have been reported ( Feurstein *et al*., 2009 ) . MCs inhibit eukaryotic protein phosphatase type 1 and 2A , resulting in excessive phosphorylation of cytoskeletal filaments , ultimately leading to liver failure , and have been implicated in the death of birds , wild animals and livestock , and fish ( Charmichael , 1994 ; Kaebernick and Neilon , 2001 ) . The World Health Organization established 0.04 µg /kg body weight (BW) $day^{-1}$ as a tolerable daily intake (TDI) of MC-LR , one of the most potent MCs ( at least in acute terms ) , and provided a guideline value of 1 µg / L as a maximum allowable concentration of MC-LR in drinking water ( Falconer *et al*., 1999 ; Kuiper-Goodman *et al*., 1999 ) .

Until now ,more attention has been paid to human uptake of MCs through the drinking water than bioaccumulation of MCs in aquatic animals in natural water . Fish standing at the top of the aquatic food chain , are likely to be most affect by exposure to toxic cyanobacteria , and so their consumption may pose great risk to humans ( Chorus and Bartram , 1999 ; Xie *et al*., 2005 ) .

In Iraq a little studies about toxic effects of cyanotoxins on animal tissues such as fish , mice unless study of AL – Hilfi ( 2007 ) and AL- Sultan and Al-Ali ( 2010 ) , so this study aimed to visualize histopathological effect force feeding of toxic alga *N. muscurum* on herbivorous fish juvenile *C. idella* and mixture feeding ( Toxic alga *N. muscurum* and Clover plant ) compared with fed on normal food ( Clover plant ) only .

## Material and Methods

Isolation and purification of Toxic alga *N. muscorum*

The isolate of toxic cyanobacterial alga *N. muscurum* was obtained from Dr. Emad Yousif A. Al-Sultan / algal laboratory ( College of Education / Basrah university which isolated from sewage water for Al-Kandaq river / Basrah city southern of Iraq as Unialgal culture . This cyanobacterial species which has ability to produce toxins namely Microcystins especially MC-LR at concentration 27.570 µg / ml ( Al-Sultan , 2010 ) . Axienic culture were made according ( Weidman *et al*., 1984 ) .

Toxic alga was cultivated in modified Chu-10 liquid medium (Al-Aaragy-1996) with (-nitrogen) content . Conical flasks 3 L in volume filling with 2L of liquid medium ( chu-10) were used for culturing toxic alga *N. muscurum* as batch cultures , cultures were incubated in growth chamber with temperature 23 C° ±2 and continuous illumination .

Algal growth was made by measuring chlorophyll (a) concentration according (Vollenwieder, 1974). *N. muscurum* was classified according to ( Desikachary , 1959 and Prescott , 1975 ).

Source of *C. idella* juvenile

Grass carp fish were obtained from vertebrate department / marine science center / basrah university with mean weight 4.61 g . Fish juvenile were get on cork container 50L in volume until reaching to the laboratory . Fish were acclimated in plastic cages volume 30 L with using clover plant as food during acclimation period . Each cage contain 5 fish Juvenile . Quarter volume of each water cages were removed daily to save the quality of water .

Feeding Experiments

*C. idella* juvenile was force feeding on three types of food ( three groups) , group A- toxic alga was feed on *N. muscurum* only in portion (1m/L from axenic culture ) group B feed on mixture food 1ml /L ( Toxic alga and clover in portion 1:1 v/v) and group C feed on clover plant only as control group . Toxic alga was used as food after cultures reached to early stationary phase ( after 14 days ) ( Al-sultan , 2010 ) . Fish juvenile were fed daily on those three types of food separately until reach to saturated status . Feeding experiments were extended to 15 days . Three replicate were done for each treatments (each replicate contain 5 fishes juvenile). Two time periods were selective to get fishes sample for histopathological studies after 24 hours and 15 days of feeding . The temperature of water range between 22-24 C° and dissolved oxygen between 7.5-8.3 mg /L .

Preparation of histopathological sectioning

At each feeding two periods three fish juvenile were get from each groups and sectioning under dissecting microscope to obtain gills ,liver , intestine and kidney to study histopathological changes by using paraffin method according to Humason (1972) as following : each sample was fixated by using bouins fixative solution for 24 hours , then washed with ethanol 50% many time. For dehydration each samples transfer to increasing concentration of ethanol (70% , 90% and 100% ) for two changes each one .Then transfer to xylen for clearing . Finally , embedded by using paraffin wax in ( 58 ˚C ) , the paraffin block cut for sections thickness ( 6-7$\mu m$ ) in diameter. All sections were Stained with haematoxylin and eosin stains. sections were mounted with kanada palsam then were covered by cover slid and investigated under light microscope type ( Olympus ) .

**3-Results**

Feeding of grass carp fish on toxic alga *Nostoc muscurum* only and on mixture of *N. muscurum* and clover give rise to histopathological changes in the gills , intestine , liver and kidney. These changes differ according to types of tissues of these organs .

**1- Histopathological changes in Gills**

Examination of gills sections revealed similarity in histopathological changes between the first two groups (A and B) of fish where fed group A on toxic alga only and group B on mixture , but these changes became very sever with time .The histological examination show the normal structure of gills filaments which composed from secondary gills lamellae which involved epithelial tissue and capillary blood vessels , supporting cartilage and blood vessels (Fig 1).

**1-1 After 24 hours**

The histopathological changes in gills filament are represented by aneurism and congestion of capillaries of secondary gill lamellae. Generally, this congestion begins with the free end of secondary gill lamellae . These lamellae are close to each other .Frequently, the number of these lamellae were between 1-4 in each gill filament (Fig.2,3,4). These changes simultaneity with the alteration of the straight shape of the secondary gill lamellae so that some of these lamellae appeared as loop, zigzag and (L) shaped (Fig.4,5).

The adhesion of secondary gills lamellae was observed in other of gill filament. This adhesion occurs in the free end of these lamellae , and already outspread in regions of the gill filament. Frequently , the number of secondary gills

lamellae in which adhesion occurs among them was about 3-5 (Fig. 4,5).

The increase in the number of epithelial cells represents the beginning of hyperplasia , whereas the proliferative epithelial cells are confined in the one third basal of secondary gill lamella .Usually , hyperplasia occurs in lamellae which were suffering from that alteration in shape. (Fig. 4,5).

The other changes which occurred in epithelial cells are represent by swelling , therefore , they appeared as large cells in size with irregular external boundaries (Fig.6).

**1-2 After 15 days**

The histopathological changes which occurred after 24 hour , were also observed after 15 days (Fig.7,8,9) , but hyperplasia grew in some secondary gills lamellae . The epithelial cells hyperplasia begins with the proliferation from the one basal third toward the free end of that lamellae, in this case the hyperplasia comprises each lamella .

In this period , the secondary gills lamellae which became different in their shapes in previous stage came back to their straight shape (Fig.9).

Other histopathological changes are represented by the detachment of epithelial layer of secondary gill lamellae (Fig.10,11) . Also, the chondrodysplasia (hyperplasia) were observed in the supporting cartilage of gill filament at free end region , chondrodysplasia were observed in mixture group fish only , and it forms large cartilaginous mass on the blood vessel which seems congest . In that region , atrophy and disappearance of the secondary gills lamellae were observed in gill filament itself and the secondary gills lamellae of the close neighbor gills filaments (Fig. 12).

**2- Histopathological changes in Intestine**

The normal intestine of fish group C involved , mucosa layer consist of three layers the columnar epithelial tissue which included goblet cells , lamina propria and muscularis mucosa ; this show in (Fig. 13).

2-1 After 24 hour

The changes occurres in the both groups (toxic alga and mixture). They included ,the adhesion between villi in that part of intestine which is characterized by crowding and tallness of villi , this adhesion occurred between two or more of villi by their opposite sides. The longitudinal sections that passed through villi showed that the adhesion diffuses on the vertical axis of villus (Fig. 14).

In addition ,the histological examination of intestine showed that abnormal cells exist among the columnar epithelial cells of lining epithelial layer .

These cells differ from other cells in epithelial layer by their irregular shape , large size and loss of the polarization in epithelial tissue layer and they appear in different levels of the epithelial lining i.e they do not sit on the basement membrane . Cytoplasm of abnormal cells are contains either one mass or small masses of unknown substance , as for their nuclei - if it showed - dark color and location in side position (Fig. 15,16). Frequently , many cells of the lymphocytes type were observed as infiltration in both of the mucosa and submucosa layers . Also, the congestion of blood vessels were observed in the submucosa layer as another change at the intestine (Fig.17).

**1-2 After 15 days**

There are very severe changes at the wall of intestine. These changes are represented by the increasing proliferation of columnar epithelial cells in epithelial lining .Usually, this occurs in limited small areas in epithelial lining tissue of intestine. However , these cells seem very crowded and dark . Therefore , for all these reasons , the lining epithelial tissue of intestine seem stratified epithelial tissue (Fig.18) .

The part of intestine which has short villi was affected mostly at this period. Particularly, the effect was focused in the epithelial tissue. Whereas , the epithelial cells were suffering from necrosis; the process of necrosis is initiated by the lysis of the plasma membrane ,this is following by lysis the parts of cytoplasm , these were synchronized with the colorlessness (achromatism) of nucleus gradually. Next ,the lysis become comprised at all parts of the cell , thus , the process of cellular death was finished with the disappearance of nucleus leaving the parts residue of cell which is close to the connective tissue in villi .However, all the remains of the dead cell disappear from the tissue sections. Therefore , a gap is observed in the lining epithelial tissue of intestine opposite to the gut cavity directly (Fig.19 , 20) .

In some parts of intestine ,the histopathological changes extend to circular muscle layer of tunica muscularis ;therefore, the vascular degeneration was observed in cytoplasm of smooth muscles cells. (Fig. 21).

**3- Histopathological changes in Liver**

**3-1 After 24 hour**

The histopathological examination of the liver of group C show as normal tissue which included the center vein , hepatocytes arranged as cords and between them sinusoids ; this show in (Fig. 22). But the liver of both groups (A and B) of fish confines by the congesting of sinusoid in small areas of the liver , especially those which are adjacent to the capsule of liver. An simultaneity an appearance of yellow patches which have interspersed through

the liver cords is observed (Fig. 23 ,24) .While , the hepatocytes seem normal . But in some region of liver in the mixture group the nucleus of these cells appeared abnormal their nucleoli appear peripheral position (Fig. 25).

**3-2 After 15 days**

Hepatocytes suffer an alteration in the place of the nucleolus before appeared in the liver of fish of both groups (toxic alga and mixture) in this time. This change represents the beginning of more violent change in hepatocytes , whereas , most of these cells were suffering from necrosis . The area of necrotic cells in the liver was larger in fish of the mixture group than fish of toxic alga group.

The histological examination shows that the necrosis is beginning with the displacement of the nucleolus from its center , following the compression of this nucleolus at one side of nucleus , this synchronizes was accompanied by the largeness of nucleus size .Therefore , all these reasons make the nucleus like the adipocytes in its appearance which seem at it in the routine preparations of histological methods. Then , the necrotic cell , the process usually progressed in an orderly fashion , the nucleolus disappeared gradually , this concomitance with disappearance of the nucleus followed by the cytoplasm and the plasma membrane (Fig.25, 26,27,28).

**4- Histopathological changes in Kidney**

**4-1 After 24 hour**

Normal structure of kidney was clarified in group C and the tissue sections showed hematopoietic tissue between the renal tubules and renal corpuscles which consist of glomerules surrounded with narrow space (bowman's space) and bowman's capsule (Fig.29).

The histopathological changes occurred in all structures of kidney of fish in the both groups (A and B) . In renal tubules , the changes were represented by the addition new cells toward its hollow . So the simple epithelial tissue lining of these tubules seems as an stratified epithelial tissue , mostly a component of two layers , this change called metaplasia . The renal tubules are appears like closely-packed cellular mass without hollow. The cells of these tubules are lost the polarization in tissue , it is small in size and their nucleus seems pyknosis and have dark color.(Fig.30,31).

The proliferative glomerionephritis have occurred In some glomerules. This give rise to the stricture of the bowman's space then its disappearance (Fig. 30,31).

Necrosis of hematopoietic tissue was observed in some parts of kidney .The

necrosis illustrated by karyopyknosis and shrink of nucleus , following the lysis parts of cytoplasm and plasma membrane and disappearance gradually , in consequence. So the remnants of these cells observed in histological sections as pale stain of rounded or irregular structures (Fig. 30,31).

**4-2 After 15 days**

The histopathological changes which observed in previous time (after 24 hour) in kidney are observe also in both groups (A and B) of fish in this time . As well as that, the renal tubules suffered from very violent changes, These changes are represented by necrosis of some parts of the epithelial lining ,especially these tubules which lost their cells polarization and their hollow (Fig.32,33) .

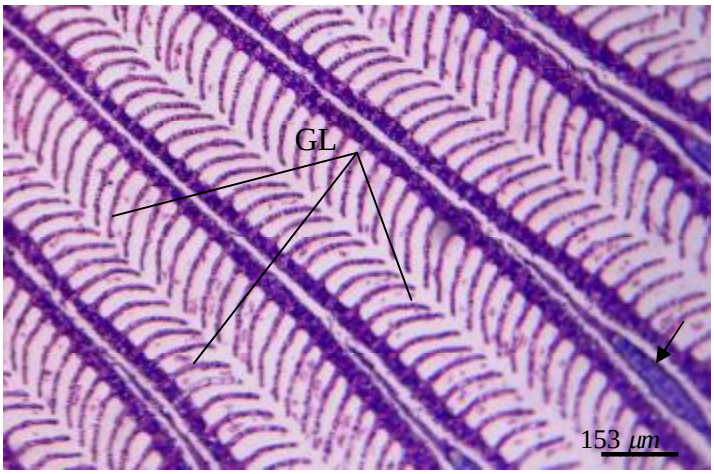


Fig. 1.Gills tissue of grass carp control fishes feeding on the clover only ,cartilage of gill filament (arrow) , secondary gills lamellae (GL), H&E.

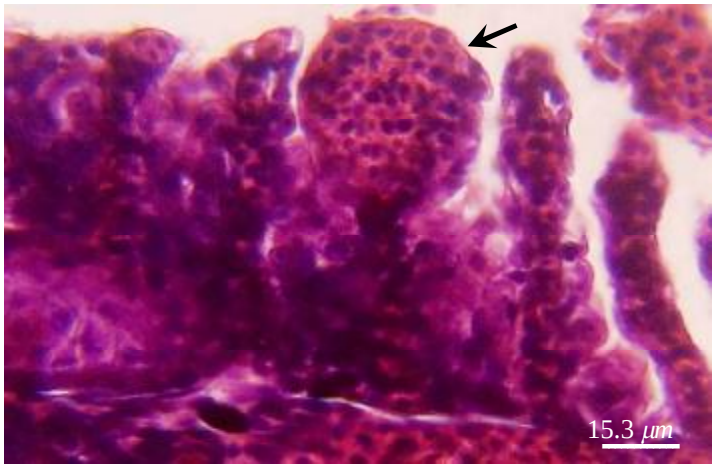


Fig.2. Gills filaments of grass carp fishes feeding on mixture of toxic alga *N. muscorum* and the clover post 24 h. Aneurism and Congestion of capillaries of secondary gill lamellae(arrows). H&E.

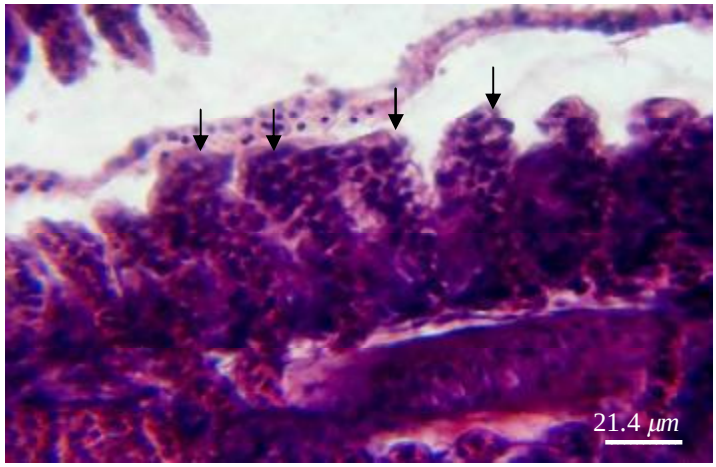


Fig.3. Gills filaments of grass carp fishes feeding on toxic alga *N. muscorum* only post 24 h. Aneurism and Congestion of capillaries of four secondary gill lamellae(arrows). H&E.

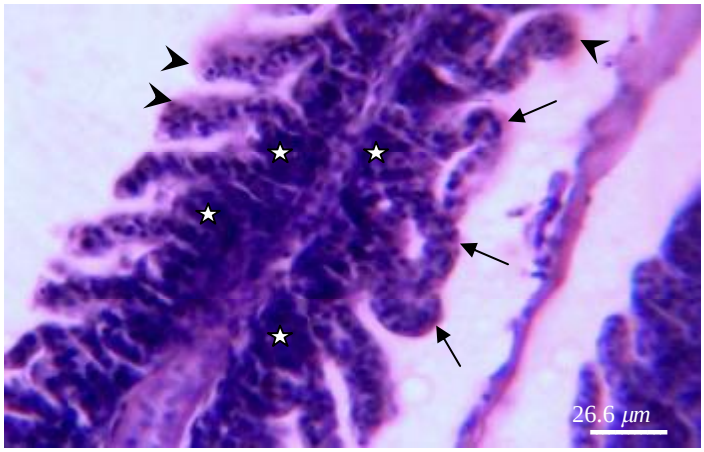


Fig.4. Gill filament of grass carp fishes feeding on toxic alga *N. muscorum* only post 24 h. The adhesion of secondary gills lamellae and lamellae appeared as zigzag (arrows), the beginning of hyperplasia (stars) , Congestion of capillaries (head arrow)H&E.

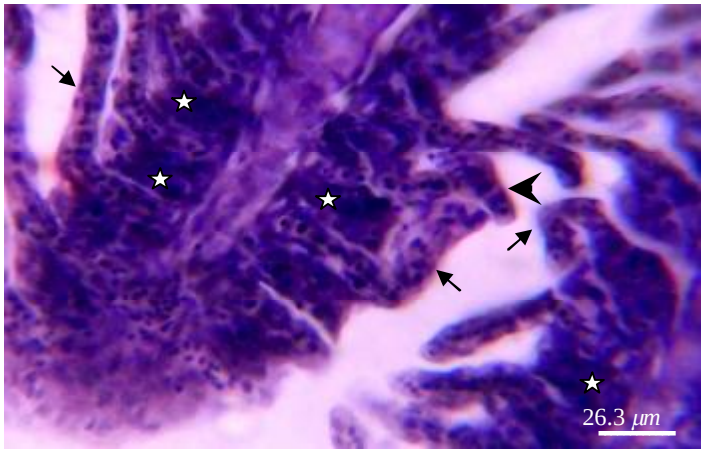


Fig.5. Gill filament of grass carp fishes feeding on mixture of toxic alga *N. muscorum* and the clover post 24 h. lamellae appeared as loop(arrows) and zigzag (head arrow), the beginning of hyperplasia (stars) , H&E.

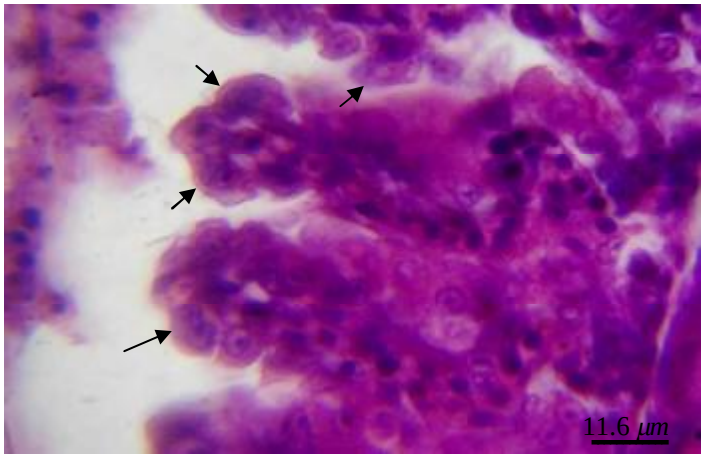


Fig.6. Secondary gills lamellae of grass carp fishes feeding on of toxic alga *N. muscorum* only post 24 h. epithelial cells appear swelling with irregular boundaries (arrows), look to the alteration of the straight shape of the secondary gill lamellae , H&E.

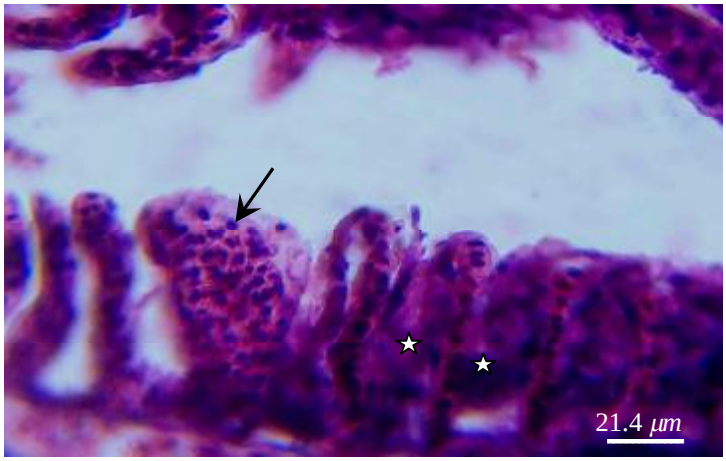


Fig.7. Secondary gill lamellae of grass carp fishes feeding on toxic alga *N. muscorum* alone post 15 day . Aneurism and Congestion of capillaries of secondary gill lamellae (arrows),beginning of hyperplasia of secondary gill lamellae (stars). H&E.

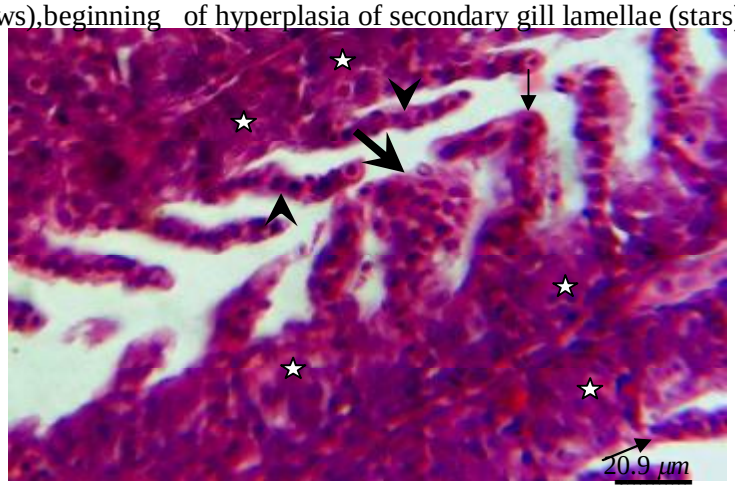


Fig.8. Gill filament of grass carp fishes feeding on mixture of toxic alga *N. muscorum* and the clover post 15 day . Aneurism and Congestion of capillaries ( thick arrow ), lamellae appeared as loop (thin arrows) and zigzag (head arrow) , the beginning of hyperplasia in the one basal third of secondary gill lamellae (stars) . H&E.

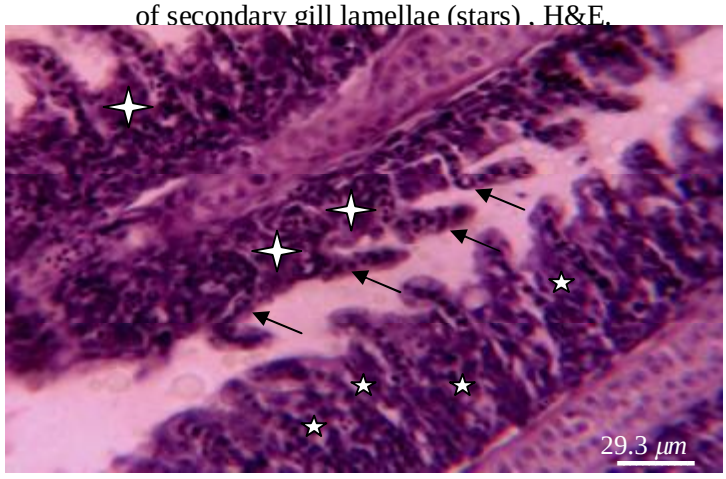


Fig.9. Gills filaments of grass carp fishes feeding on toxic alga *N. muscorum* only post 15 day .in some lamellae occur the beginning of hyperplasia in the one basal third , lamellae appeared as zigzag (arrows), in other lamellae the hyperplasia grow and it comprises each lamella which given back their straight shape (stars), H&E.

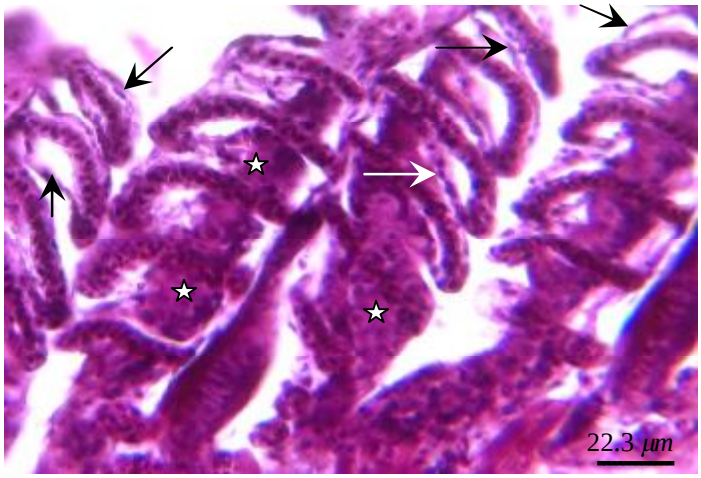


Fig.10. Gill filaments of grass carp fishes feeding on toxic alga *N. muscorum* only post 15 day . the detachment of epithelial layer of secondary gill lamellae (arrows) , the hyperplasia in the secondary gill lamellae (stars) , H&E.

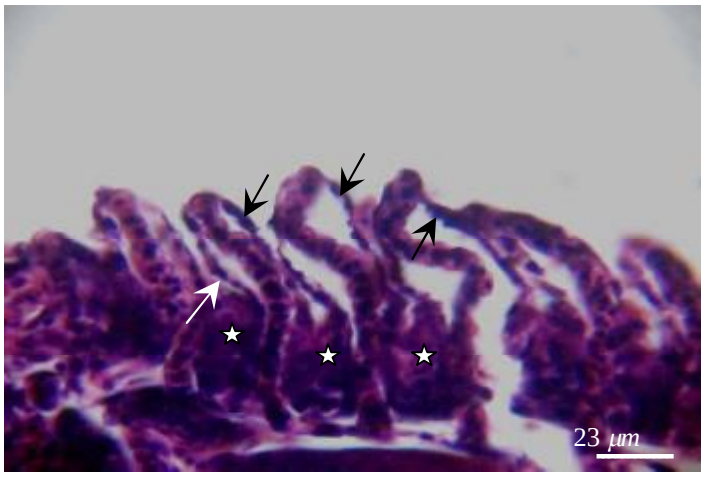


Fig.11. Gill filament of grass carp fishes feeding on mixture of toxic alga *N. muscorum* and the clover post 15 day . the detachment of epithelial layer of secondary gill lamellae (arrow) , the hyperplasia in the secondary gill lamellae (stars) , H&E.

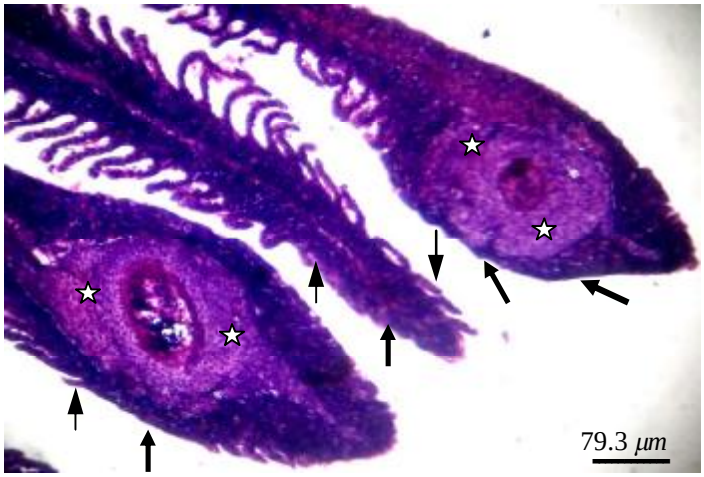


Fig.12. Gill filaments of grass carp fishes feeding on mixture of toxic alga *N. muscorum* and the clover post 15 day . Atrophy (thin arrow) and disappearance (thick arrow) of the secondary gills lamellae , the chondrodysplasia (hyperplasia) in the supporting cartilages of gill filaments (stars) , H&E.

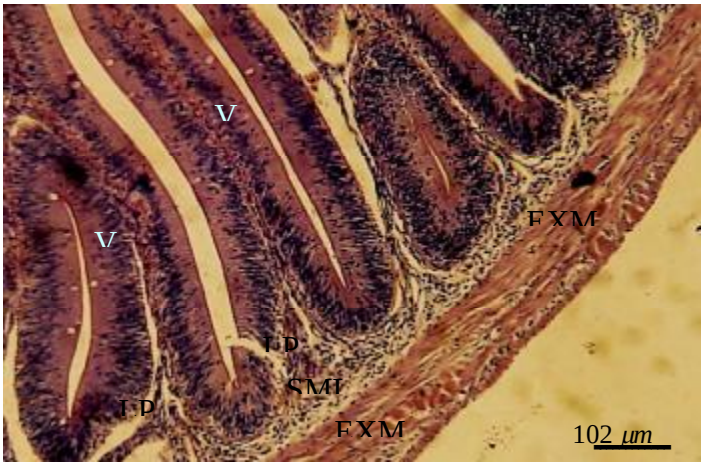


Fig.13.Intestine tissue of grass carp control ,Villi of small intestine (V) , External muscle layer (EX Submucosa layer (SML) , Lamina propriae (LP) ,H&E.

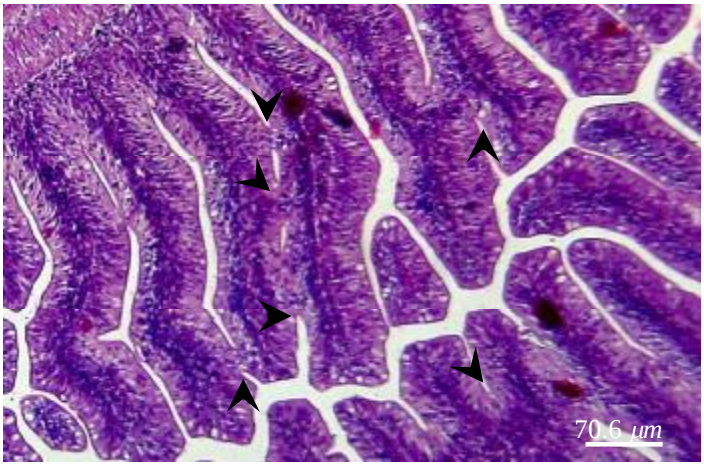


Fig.14.Cross section in Intestine of grass carp fishes feeding on mixture of toxic alga *N. muscorum* and the clover after 24 h . The adhesion between Villi ( head arrow) , H&E.

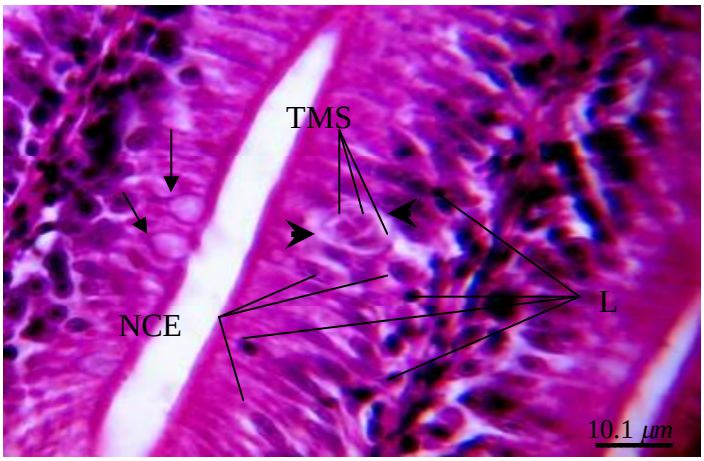


Fig.15. Villi of small intestine of grass carp feeding on mixture of toxic alga *N. muscorum* and the clover after 24 h . Show abnormal cells (head arrow) existed among the normal columnar epithelial cells (NCE) ,Three masses of unknown substance(TMS) , Lymphocyte (L) goblet cells (arrow) H&E.

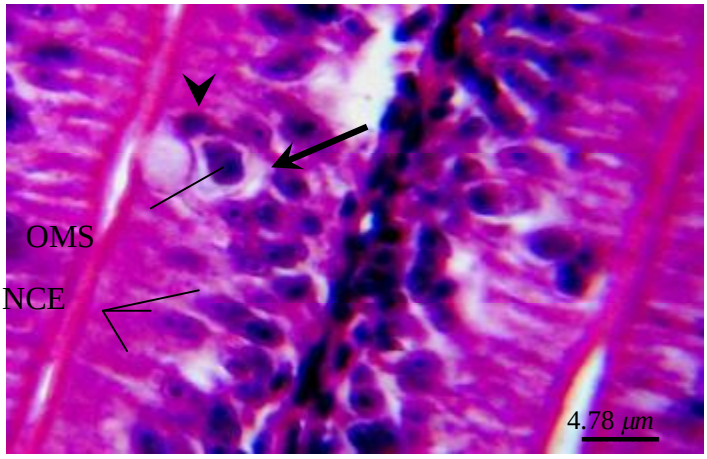


Fig.16. Villi of small intestine of grass carp feeding on toxic alga *N. muscorum* only after 24 h . Show abnormal cells (arrow) existed among the normal columnar epithelial cells (NCE) , one mass of unknown substance (OMS) , nucleus of abnormal cell(head arrow) H&E.

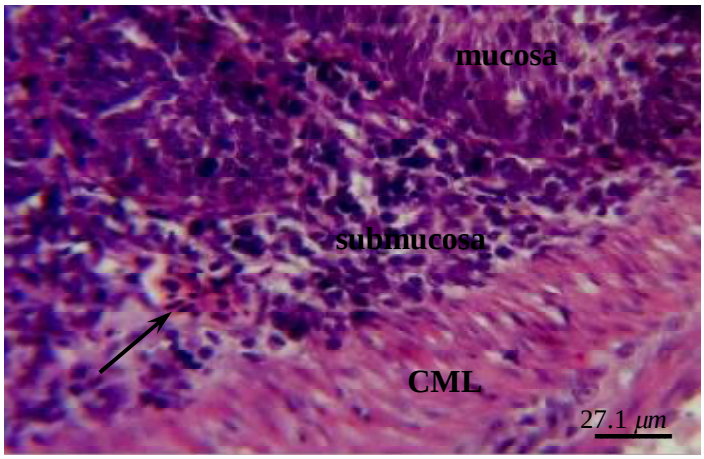


Fig.17.Cross section in small intestine of grass carp feeding on toxic alga *N. muscorum* only after 24 h . Show infiltration lymphocytes in both of the mucosa and submucosa layers, the congestion of blood vessels (arrow),circular muscle layer (CML), H&E.

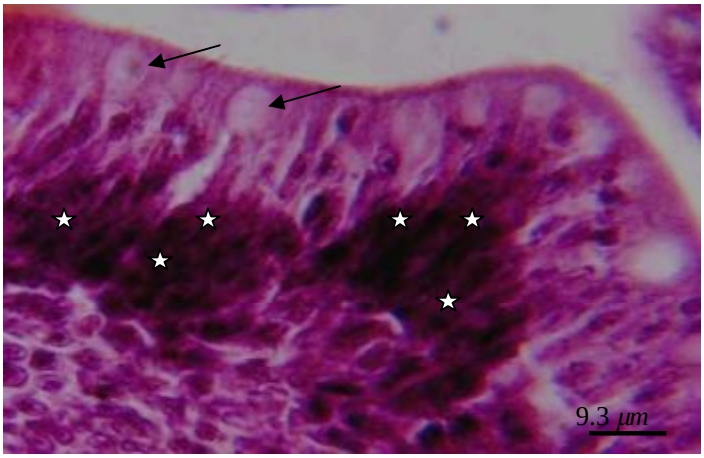


Fig.18. Villi of small intestine of grass carp feeding on toxic alga *N. muscorum* only after 15 days . Show increasing proliferation of columnar epithelial cells (stars) seemed very crowded and dark stain ,goblet cells (arrows) , H&E.

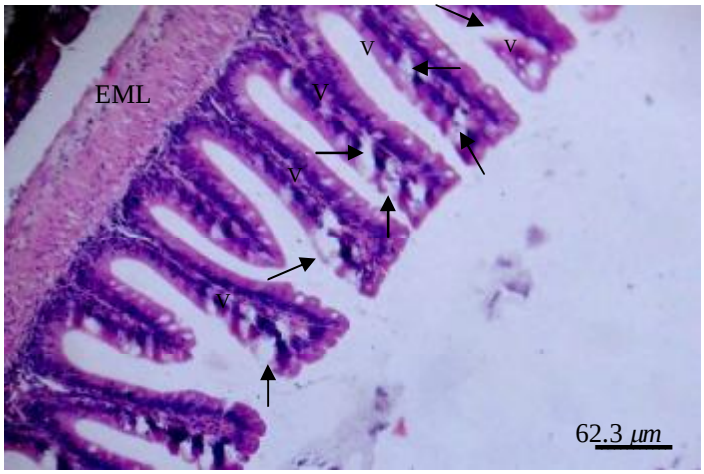


Fig.19. Cross section in the intestine of grass carp feeding on toxic alga *N. muscorum* and the clover post 15 days . Show gaps in columnar epithelial tissue (arrows), villi (V) , external muscle layer(EML), H&E.

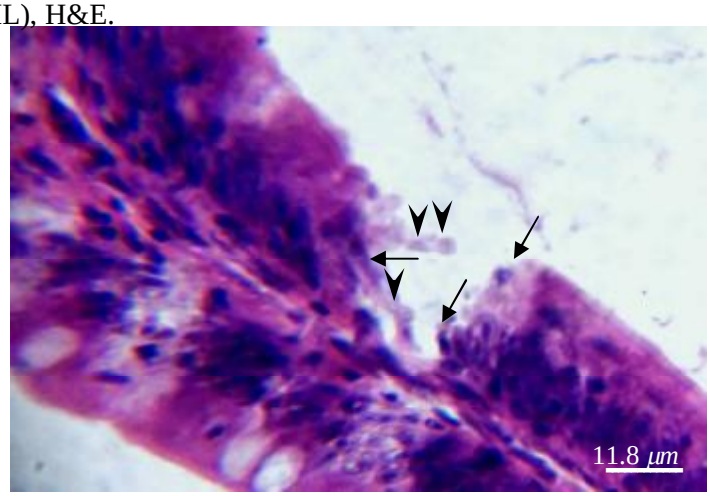


Fig.20. Villi of the intestine of grass carp feeding on toxic alga *N. muscorum* only post 15 days . Show pattern of necrosis in columnar epithelial cells (arrows), the parts residue of cells (head arrows), H&E.

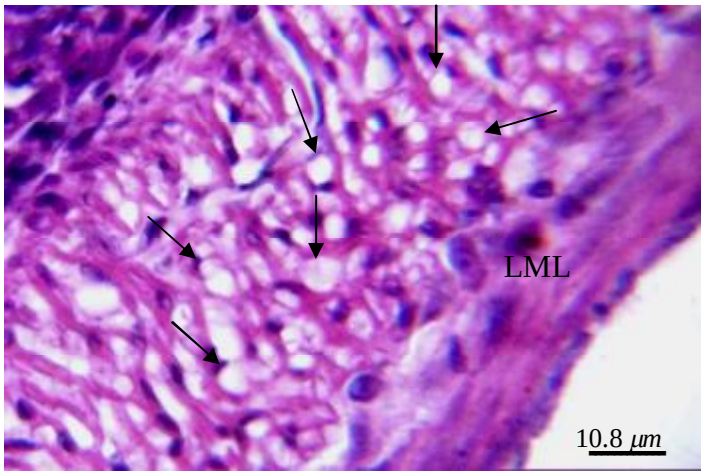


Fig.21. Cross section in the tunica muscularis of intestine of grass carp feeding on toxic alga *N. muscorum* and the clover post 15 days . Show the vascular degeneration in cytoplasm of smooth muscles cells of circular layer (arrows), longitudinal muscle layer(LML), H&E.

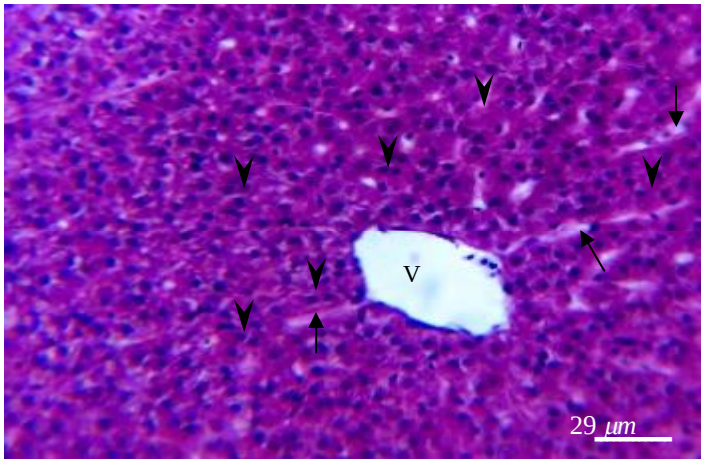


Fig. 22.Liver tissue of grass carp fish control , central vein (V) , hepatocytes (head arrows) , sinusoids (arrows) , H&E.

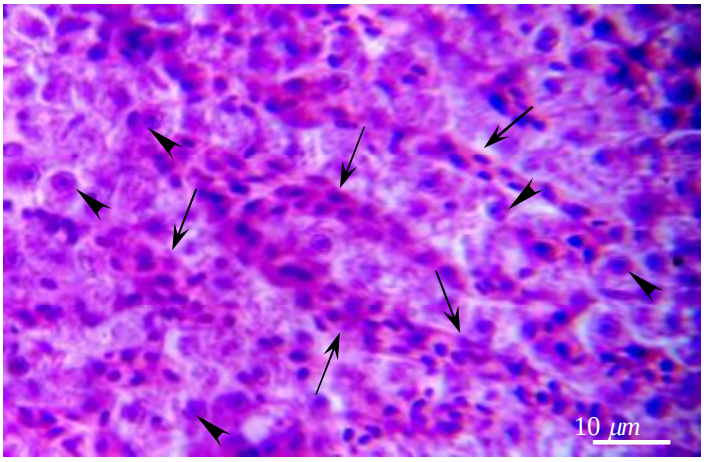


Fig.23. Liver of grass carp feeding on toxic alga *N. muscorum* only post 24 h , show the congestion of sinusoids (arrow) , normal nucleus of hepatocytes (head arrows) , H&E.

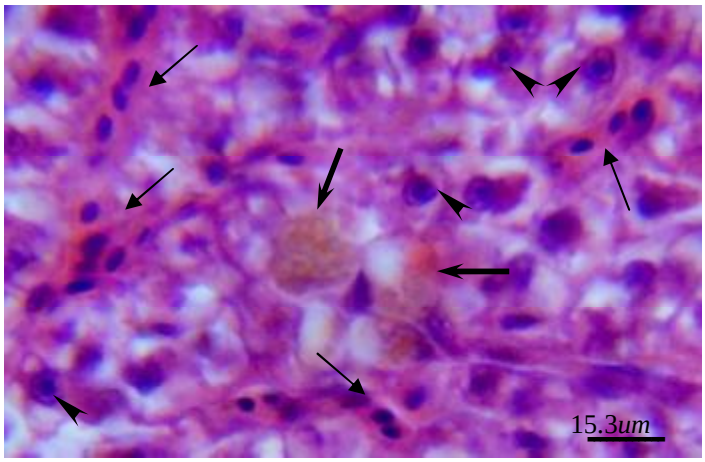


Fig.24. Liver of grass carp feeding on toxic alga *N. muscorum* and the clover post 24h , show the congestion of sinusoids (thin arrow) , yellow patches (thick arrow ) , nucleus of hepatocytes (head arrows) , H&E

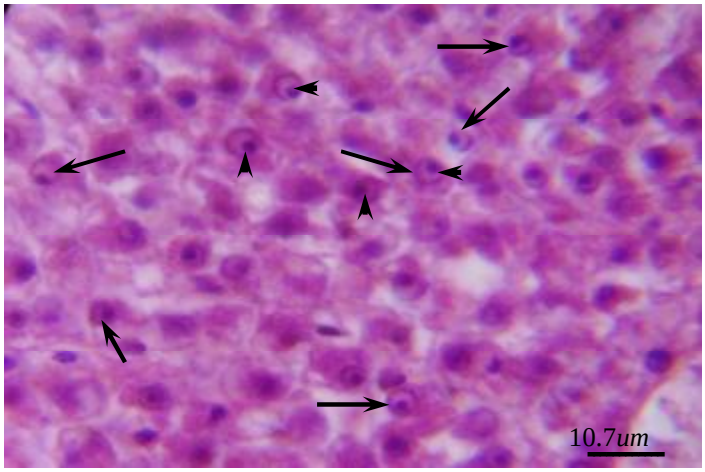


Fig.25. Liver of grass carp feeding on mixture of toxic alga *N. muscorum* and the clover after 24 h, abnormal nucleus of hepatocytes (arrows), nucleolus placed adhering to the nuclear envelop (head arrows) , H&E.

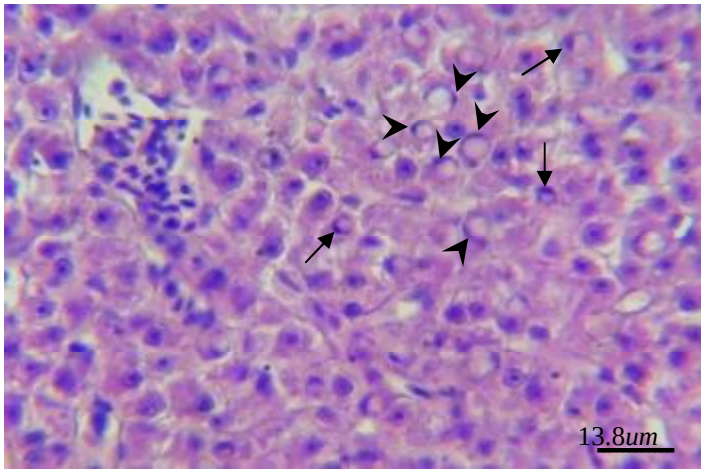


Fig.26. Liver of grass carp feeding on toxic alga *N. muscorum* only after 15 day, necrosis of hepatocytes , beginning of necrosis (arrows),then the compression of nucleolus at one side of nuclear envelope (head arrows) , H&E.

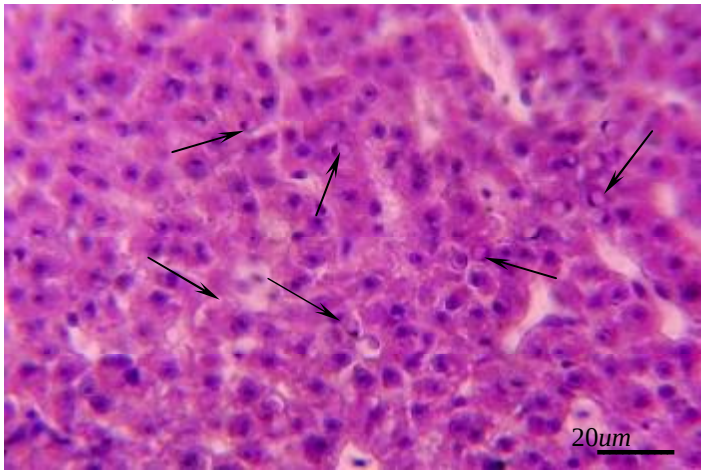


Fig.27. Liver of grass carp feeding on mixture of toxic alga *N. muscorum* and the clover post 15 day, necrosis of hepatocytes (arrows), H&E.

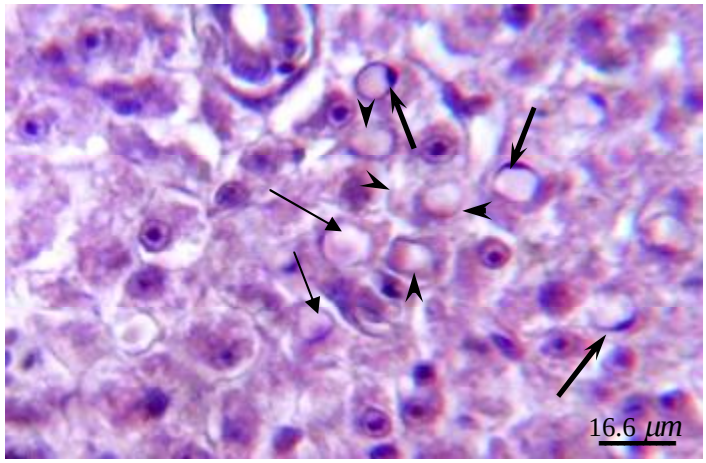


Fig.28. Liver of grass carp feeding on mixture of toxic alga *N. muscorum* and the clover after 15 day, show the pattern of hepatocytes necrosis , the compression of nucleolus at one side of nuclear envelope(thick arrows), nucleolus disappeared (thin arrows), lyses of all components of cell(head arrows) , H&E.

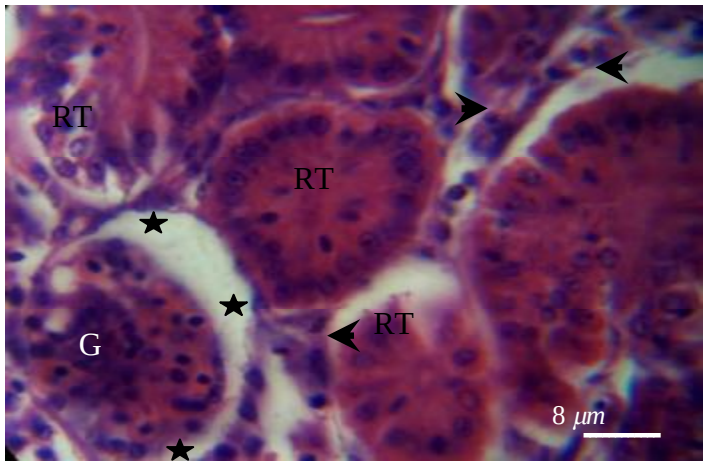


Fig.29.Kidney tissue of grass carp control, hematopoietic tissue ( head arrows) , bowmen space (stars) , glomerule (G) , renal tubule (RT) , H&E.

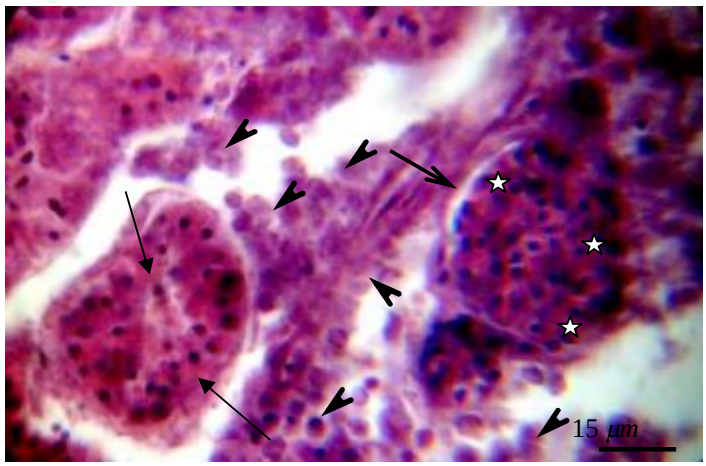


Fig.30.Kidney of grass carp feeding on toxic alga *N. muscorum* only after 24 h, metaplasia of renal tubules (arrows) , the proliferative glomerionephritis in glomerule (stars) and stricture of bowman's space (thick arrow) , necrosis of hematopoietic tissue (head arrows), H&E.

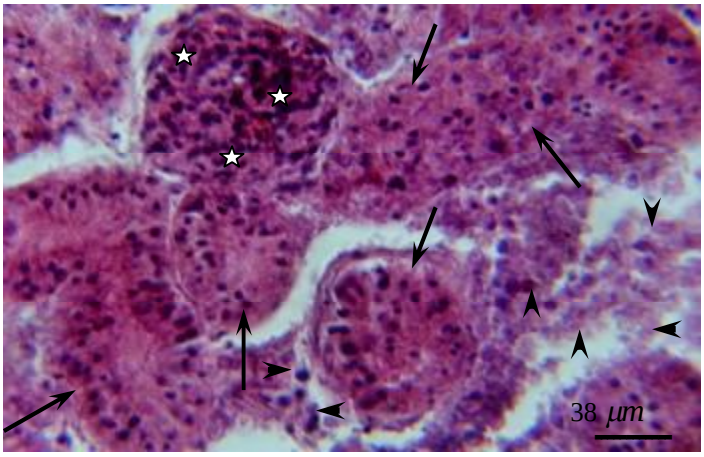


Fig.31.Kidney of grass carp feeding on toxic alga *N. muscorum* and the clover after 24 h, metaplasia of renal tubules (arrows) , the proliferative glomerionephritis in glomerule (stars) and disappear of bowman's space , necrosis of hematopoietic tissue (head arrows) ,H&E.

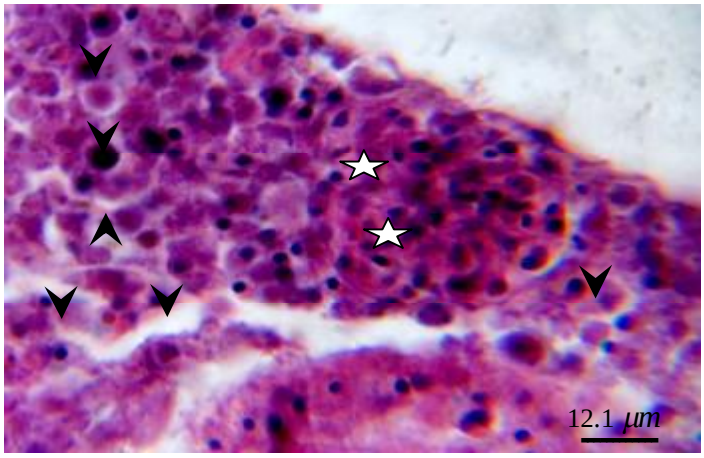


Fig.32.Kidney of grass carp feeding on toxic alga *N. muscorum* only after 15 day, the proliferative glomerionephritis in glomerule (stars) and disappear of bowman's space, necrosis of hematopoietic tissue (head arrows) ,H&E.

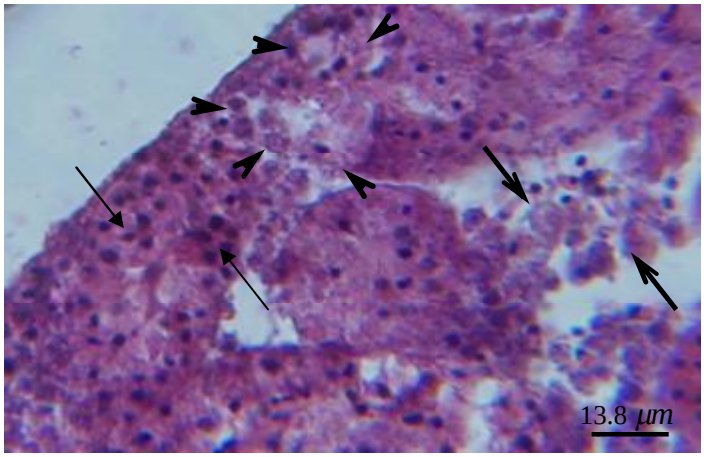


Fig.33.Kidney of grass carp feeding on toxic alga *N. muscorum* and the clover after 15 day, metaplasia of renal tubules (thin arrows) , necrosis of epithelial lining of renal tubules (head arrows), necrosis of hematopoietic tissue (thick arrows) ,H&E.

4- **Discussion**

The toxic alga *Nostoc muscurum* have toxic effect on the organs of grass carp fish (*Ctenopharngodon idella*) . Memorable, At first , the causal relationship of tissue damage with the presence of microcystin-containing (*N. muscurum* ) were investigated in grass carp fish .

Toxins of microcystins enter the organism by using bile acid transport system of hepatic and intestinal cells and transported by blood circulation to other organs as liver for transformation and/or storage, and if transformed in the liver it may be excreted trough the bile or pass back into blood for possible excretion by kidney or gill (Lindstoma-seppa *et al*., 1981 ; Zaccaroni and Scaravelli ,2007) . Therefore , when the fish take alga *N. muscurum* will be result of histopathological changes in gills , intestine , liver and kidney. Other histopathologist they mentioned the ingestion of small amounts of microcystin can be caused damages in liver , kidney and intestine (Turner *et al*.,1990 ; Carmichael ,1992 ; Carmichael ,1994 ; Carmichael ,1997 ; Carmichael ,2001 ) .The gross observations to the fish in the aquariums and tissue sections have proved the fish were taking the alga greedily in the two groups A and B , but the fish of group B have been taking it more greedily than the fish of group A . So in sometime the histopathological changes were more severe in group B than group A.

The present study has shown that changes intensity are increase according to long time of experiment and with continuously the fish have take their food in the two groups A and B , this agree with other histopathologist they mentioned the severity of tissue damage depends on the concentration of the toxicant and the period of exposure ( Karlson - Norrgen *et al*., 1985 ; Mallat 1985 ; Franchini *et al*., 1994 and Oliveira *et al*., 1996).

The gills, which participate in many important functions in fish, such as respiration, osmoregulation and excretion ,while the intestine ,act as way of absorption ,so that they remains in close contact with the external environment, and particularly sensitive to changes in the quality of the water, are considered the primary target of the contaminants (Poleksic & Mitrovic-Tutundzic, 1994; Mazon *et al*., 2002 and Fernandes & Mazon, 2003) include ichtyotoxins particular when their cells will be lyses (Falconer and Hampage , 2006 ) .

Aneurysm and congestion of blood vessels in gills ,intestine and sinusoids of liver occurred as inflammatory response (Weissman ,1992), because of toxic effect of *N. muscurum* .This is the same reason

which caused the adhesion of secondary lamellae in gills and intestinal villi , also alteration secondary gills lamellae shape and lymphocytes infiltration in intestine.

The alterations like epithelial lifting, hyperplasia of the epithelial cells, fusion of secondary gills lamellae, fusion of villi, detachment of epithelial layer of secondary gills lamellae and the alteration of some secondary gills lamellae are part of defense mechanisms for lessening the harm of toxicant. In general , all alterations result in the increase of the distance between the waterborne toxicants in external environment and the blood in gills and intestine and thus serve as barrier to entrance of contaminants (Mallatt, 1985; Poleksic & Mitrovic-Tutundzic, 1994 and Fernandes & Mazon, 2003) .

The abnormal cells which have appeared in lining epithelial tissue of intestine may be suggested as goblet cells which unable to liberation their mucus substance because of cytotoxic effect of *N. muscurum* .That phenomenon is called mucoid degeneration (Curran and Crocker, 2000)

During absorption, distribution, and elimination processes the toxicant will encounter various the cellular and organelle membranes before interacting with the target. These membranes act as barriers . Through that , toxicant may be caused changes in structure of membranes which lead to dysfunction particularly in transport processes (Hodgson , 2004) , this may be explain swelling of some epithelial cells of secondary gills lamellae and unable goblet cells liberate their mucoid substances ,thus accumulation these substances in cells. Carbis *et al* . (1996) have reported that 37% of the fish *Cyprinus carpio* which gavage by microcystin had gills with pinpoint necrosis , epithelial ballooning , folded lamellar tips and exfoliation . Also ,Gupta and Guha (2006) have reported swollen of epithelial lining and prominent of mucous cells and chloride cells after one hour of treatment fresh water fish *Heteropneustes fossilis* with *Microcystis aeruginosa* ,then ,in the ten - days stage, they reported necrotic and apoptotic cells of epithelial lining of gills .

Atrophy and disappearance of secondary gills lamellae and congestion of blood vessel which were observed - after 15 day - may result by the pressure which caused by mass of chondrocytes of hyperplasia (Cotran *et al.* , 1999) .

In spite of , the enterocytes (intestinal epithelium) possess an extensive capacity for metabolism of xenobiotics but that may be unable to tolerance toxicity of *N. muscurum* (Klaassen and Watkins 1999). Thereby ,the changes post 15 days

were very severe at the wall of intestine , these changes are represented by crowded and necrosis of columnar epithelial cells and vascular degeneration of smooth muscles .

The degeneration is reversible histopathological change, but when the tissue continue to incur to pathogenetic this may be result of transforms this change to irreversible change (necrosis ) ( Cotran *et al.* , 1999).

The proliferation of columnar epithelial cells in intestine, the proliferation glomerionephritis in glomeruli and genitive construction of new cells in renal tubules are examples of dysplasia due to toxic effect of toxic alga *N. muscurum* which may act as tumor promoters .Therefor , the alga *N. muscurum* may regulate cells proliferation as other microcystin by protein kinases which promoting cell division cycle and by protein phosphatases which inhibiting cell division (Zaccaroni and Scaravelli ,2007). Consequently, the development of hyperplasia in gills occurred as a irritant response with epithelial and cartilaginous tissues.

The liver is the primary organ for detoxification of organic xenobiotics, thereby ,it have suffering from many histopathological changes when the toxins were accumulation ( Cotran *et al.* , 1999). After 24 hour the hepatocytes seemed normal beyond their nucleolus. The yellow patches which have interspersed through the liver cords may represented a bile substance which leakage from the wall of bile canaliculi in the liver because of damage in functions of membrane (Hodgson , 2004).

The other changes of liver in our study were similar to that described in different fishes species by other authors, e.g. Garcia (1989), Rabergh *et al.* (1991) , Tencalla *et al.* (1994) and Carbis *et al* . (1996) .

In present study, the pattern of necrosis process of hepatocytes that have been described , is began with alter location of nucleolus that effect lead to death cell , thereby , this proved the cytotoxic effect of toxic alga *N. muscurum* on the nuclear component of hepatocytes. Palikova *et al*. (2004) and Al-Sultan and Al-Ali (2010) have reported alter location of nucleus of hepatocytes in *C. carpio* which exposure to cyanobacteria extract and *Poecilia sphenops* which feeding on *Hapalosiphon welwitschii* respectively.

Other changes in liver has also been described in various papers in other fishes gavage or injected by other microcystin. Tencalla and Dietrich (1997) have reported the effect of microcystin gavage in rainbow trout *Oncorhynchus mykiss* ,between 3-12hours the microhaemorraging was observed. The

hepatocytes nuclei began to condense and cytoplasm of these cells was highly vacuolated .While after 24 hours from intraperitoneal injection of microcystin in fish *H. fossilis* the changes in liver represented by swollen of hepatocyte , the cytoplasm of these cells appear granular and haemorrhage in sinusoid. But after 15 days of treatment of the liver ,apoptotic cells appear (Guputa and Guha 2006). Fischer *et al.(*2000) indicate the hepatocytes necrosis represents primary events in microcystin in the rainbow trout and the apoptotic cell death seems to be only secondary nature.

Interestingly, in kidney, most of the alterations in our cases were seen in the tubular cells rather than in the glomeruli, which were spared. This agree with Kotak *et al.* (1996) and Guputa and Guha (2006) , but later they show degeneration of glomeruli and dilation of bowman's space of *H.fossilis* in late stages. Kotak *et al.* (1996) stated the injection of microcystin in *O. mykiss* caused hepatic renal pathology .kidney lesions in fish consisted of coagulative tubular necrosis with a dilation of bowman's space . While Palikova *et al*. (2004) mentioned no changes were found in the kidney of fish *C. carpio* which exposure to cyanobacteria extract. All these changes in kidney may explain that different types of microcystin have variance mechanisms effect on different fishes .

The hematopoietic tissue disappeared gradually , the pattern of necrosis process of hematopoietic cells that have been described. This agree with Guputa and Guha (2006) have reported necrosis in the kidney tissue cells (lymphoid tissue) of *H.fossilis* , but many of these tissue cells show apoptosis.

In kidney the damage may causes renal failure as well as hyponeocytosis consequently hypocytosis , pancytopenia and hypovolemia (Davis and Berndt ,1994 ).

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# التأثيرات المرضية النسيجية للطحلب السام *Nostoc muscurum* على يافعات اسماك الكارب العشبي ( *Ctenopharyngodon idella* Val. 1844)

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**الخلاصة**

تم في الدراسة الحالية تغذية اصبعيات الكارب العشبي قسريا بعد تقسيمها إلى ثلاث مجاميع ، المجموعة الأولى(A) غذت على الطحلب السام *Nostoc muscurum* لوحده والمجموعة الثانية(B) غذت على خليط مكون من الطحلب نفسه ونبات البرسيم أما المجموعة الثالثة غذت على نبات البرسيم لوحده واعتبرت كمجموعة سيطرة. ودرست المقاطع النسيجية المأخوذة من أنسجة أعضاء الأسماك على فترتين (بعد 24 ساعة وبعد 15 يوم ) وبينت أن للطحلب السام *N. muscurum* تأثير سمي على أعضاء الأسماك (الغلاصم و الأمعاء والكبد والكلية)في المجموعتين A و B ، وكان مقدار الضرر يعتمد على طول فترة التجربة .

ومن الجدير ذكره انه لأول مرة تبحث العلاقة السببية لتضرر نسيج اسماك الكارب العشبي بفعل الطحلب السام *N. muscurum* .

ففي الخيوط الغلصمية تمثلت التغيرات المرضية النسيجية بتوسع واحتقان الأوعية الشعرية الدموية والتصاق الصفائح الغلصمية الثانوية مع بعضها البعض إضافة إلى تغير شكلها المستقيم كما لوحظ زيادة في اعدد الخلايا الطلائية وانتفاخها ، هذه التغيرات ازددت شدة بعد 15 يوم من بدا التجربة إذ تمثلت بانفصال الطبقة الطلائية للصفائح الغلصمية الثانوية وظهر في اسماك مجموعة التغذية المختلطة فرط تنسج في الغضروف الساند للخيط الغلصمي مع ضمور واختفاء لبعض الصفائح الغلصمية .

تبدو التغيرات المرضية النسيجية في الأمعاء أكثر شدة بعد 15 يوم وتمثلت بتوالد الخلايا الطلائية العمودية وتنخر بطانة النسيج ألطلائي وتنكس فجوي في سايتوبلازم الخلايا العضلية الملساء في الطبقة الدائرية من الغلاف العضلي لجدار الأمعاء .

اقتصرت التغيرات المرضية النسيجية في الكبد بعد 24 ساعة على احتقان أشباه الجيوب الكبدية مع ظهور بقع صفراء تخللت الحبال الكبدية . تبدو الخلايا الكبدية في كبد اسماك المجموعة A طبيعية لكن موقع نويات الخلايا في المجموعة المختلطة B يبدو غير طبيعي ، وهذا التغير ظهر بعد 15 يوم في كبد اسماك المجموعتين A و B ومثل بداية لتنخر الخلايا الكبدية وقد وصف نمط حدوث التنخر في الخلايا الكبدية بشكل تفصيلي .

وفي الكلية حدثت التغيرات المرضية النسيجية في كل تراكيب الكلية لأسماك المجموعتين A و B ، فتمثلت التغيرات في النبيبات الكلوية بعد 24 ساعة بحؤول بطانة النسيج ألطلائي لتلك النبيبات وفقدان خلايا تلك النبيبات قطبيتها في النسيج وتغير انويتها . أما في الكبيبة فلوحظ زيادة توالد خلايا الكبيبة ثم اختفاء مساحة بوما ن. كما لوحظ أن خلايا النسيج المكون للدم تعاني من التنخر . كل هذه التغيرات ظهرت بعد 15 يوم أيضا في تراكيب الكلية لأسماك المجموعتين Aو B ، إضافة لتنخر بعض أجزاء البطانة الطلائية للنبيبات البولية .

الكلمة المفتاح: التأثير السمي و طحالب السيانوبكتيرية و *Nostoc muscurum* و اسماك الكارب العشبي