Original Article

# Histopathological and Ultrastructural Alterations in Some Organs of *Oreochromis niloticus* Exposed to Glyphosate-based Herbicide, Excel Mera 71

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## Abstract

*Oreochromis niloticus* was exposed to glyphosate-based herbicide Excel Mera 71 for 30 days under field and laboratory conditions to investigate the histopathological and ultrastructural responses in gill, liver, and kidney. Gill displayed degenerative changes in the pillar cells of gill epithelium, curling of secondary lamella, and appearance of globular structure in laboratory condition under light microscopy. Scanning electron microscopic (SEM) observations revealed loss of microridges, disappearance of normal array of microridges, and damage in stratified epithelial cells under both the conditions, while severe vacuolation and necrosis were prominent under transmission electron microscopic (TEM) study in the laboratory condition. In liver, excess fat deposition and acentric nuclei in the laboratory condition were prominent under light microscopic and SEM study. TEM study showed necrosis in mitochondria, cytoplasmic vacuolation, degeneration in endoplasmic reticulum (ER), and reduced amount of glycogen droplets, but under field condition, lesions were less. Kidney showed fragmented glomerulus, excessive fat deposition, and hypertrophied nuclei under light microscope, while topological study showed shrinkage of glomerulus and degenerative changes under laboratory condition. TEM study also confirmed necrosis in mitochondria, dilation and fragmentation of ER, and appearance of severe vacuolation in the laboratory study, but no significant alterations were observed in field under SEM and TEM study. Therefore, the present study depicts that Excel Mera 71 caused comparatively less pathological lesions under field than laboratory condition, and finally, these responses could be considered as bioindicators for toxicity study in aquatic ecosystem.

**Keywords:** Excel Mera 71, glyphosate, histopathology, *Oreochromis niloticus*, ultrastructure

## Introduction

Glyphosate, N-(phosphonomethyl) glycine, a nonselective, postemergence herbicide, is extensively used worldwide to control broad-leaved annual and perennial weeds in agricultural fields, forestry, and aquatic systems.[1,2] Glyphosate is soluble in water (12 g/L at 25°C) but insoluble in most organic solvents. In addition, it binds tightly to organic matter and sediment/soil within six-inch depth and therefore becomes unavailable to plants or other aquatic organisms, and finally, its activity reduced significantly.[3] Moreover, the half-life of glyphosate in soil ranged between 2 and 197 days, in water ranged from 4 to 91 days, while in vegetation was <24 days.[4] Glyphosate is readily degraded both in water and soil by soil microbes to aminomethylphosphonic acid and carbon dioxide ($CO_2$).[5] Due to strong adsorptive characteristics, glyphosate are not likely to move to groundwater but have the potency to contaminate surface water.[4] In addition, it is under the toxicity class of III (on I–IV scale, where IV is least dangerous) for oral and inhalation exposure.[6] Its high efficacy and cost-effectiveness favor its repeated applications in commercial formulations.[7] In addition, commercial glyphosate formulations are more toxic because it contains nonionic polyethoxylene amine surfactant, which is toxic to aquatic organisms, especially

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fish species.[8] Moreover, indiscriminate use of this herbicide, careless handling, and runoff from agricultural fields caused adverse impacts on inhabitant fish populations and other aquatic life forms as they finally reach to the aquatic environment.

Ecotoxicological risks caused by pesticides/herbicides to nontarget aquatic organisms are well established;[9] however, at higher concentrations, these herbicides are known to reduce the survival, growth, and reproduction of fish. Therefore, toxicity study of these chemicals is very much essential to understand the extent of damage at cellular and subcellular level. In this regard, histopathological alterations are well documented in specific target organs, including gills, liver, and kidney,[10] responsible for respiration, biotransformation, and xenobiotic excretion, respectively. Although our previous studies have reported adverse effects of Excel Mera 71 on oxidative stress responses[11-17] and histological alliterations[18-20] in different fish species, presently available information to correlate the pathological alterations between light microscopic observations and ultrastructural observations is not sufficient to evaluate the responses in different fish tissues after this commercial glyphosate herbicide Excel Mera 71 in field contamination.

In the present study, *Oreochromis niloticus* (Linnaeus) was considered as model test organism for toxicity study because they grow fast, mature quickly, breed easily without inducement, and finally have good potentiality for cultivation.[21] In addition, it is surface-feeding omnivorous fish, belongs to the family *Cichlidae*, and is extensively used as protein source.[22] Therefore, the present study was aimed to investigate the toxic effects of Excel Mera 71 on *O. niloticus* both under the laboratory and field conditions on comparative basis through histological and ultrastructural observations in the gill, liver, and kidney.

## Materials and Methods

### Fish collection and maintenance

*O. niloticus* of both the sexes with an average weight of 38.57 ± 2.47 g and total length of 13.59 ± 0.496 cm, respectively, were procured from local fish farm market. After that, fishes were brought to the laboratory and were acclimatized for at least 15 days in aquarium (250 L). Fishes were continuously aerated and maintained at natural photoperiod (12-h light/12-h dark). During acclimatization period, the average value of water parameters were as follows: temperature, 26.49 ± 0.13°C; pH, 7.94 ± 0.04; electrical conductivity, 392.22 ± 0.62 µS/cm; total dissolved solids, 279.33 ± 0.69 mg/L; dissolved oxygen, 6.44 ± 0.05 mg/L; total alkalinity, 204 ± 7.30 mg/L as $CaCO_3$; total hardness, 180.44 ± 3.74 mg/L as $CaCO_3$; orthophosphate, 0.03 ± 0.001 mg/L; ammoniacal nitrogen, 1.66 ± 0.21 mg/L; and nitrate nitrogen, 0.21 ± 0.03 mg/L. After acclimatization, fishes were divided into two groups: one group was transferred to field ponds situated at Crop Research and Seed Multiplication Farm in the premises of the University of Burdwan and other group was transferred to the laboratory aquarium. During acclimatization and experimentation periods, fishes were fed commercial fish pellets (32% crude protein, Tokyu) once a day.

### Field experimental design

For field experiments, fish specimens of field group were again divided into two sets: one set of fish specimens was transferred to treatment pond and another set was transferred to control pond. Both ponds are free of contamination. A total of six cages (three for treatment pond and three for control pond) were prepared and installed at experimental ponds. Each cage contains 10 fish species. The dose (750 g/acre) recommended for rice cultivation was dissolved in water and applied to the treatment pond.[23,24] It was sprayed on the 1st day of the experiment. Duration of the experiment was 30 days. For field experiments, a special type of cage was prepared. Cages were prepared based on Chattopadhyay *et al*.[25] with some modifications. All cages were square in shape having an area of 2.5 m × 1.22 m and cage height was 1.83 m (submerged height was 0.83 m). Cages were framed by light strong bamboo. Four-sided wall, cage floor, and top of the cage cover were fabricated with nylon net and were embraced by two PVC nets: the inner and outer net-bearing mesh sizes of 1.0 mm × 1.0 mm and 3.0 mm × 3.0 mm, respectively. During the experimentation period, pond water showed the following average values: temperature, 24.03°C ± 0.20°C; pH, 6.56 ± 0.09; electrical conductivity, 347.00 ± 1.15 µS/cm; total dissolved solids, 247.67 ± 1.45 mg/L; dissolved oxygen, 7.00 ± 0.157 mg/L; total alkalinity, 221.33 ± 3.53 mg/L as $CaCO_3$, total hardness, 140 ± 2.31 mg/L as $CaCO_3$; orthophosphate, 0.24 ± 0.03 mg/L; ammoniacal nitrogen 0.74 ± 0.11 mg/L; and nitrate nitrogen, 1.66 ± 0.04 mg/L.

### Laboratory experimental design

Fishes under laboratory condition were again divided into two groups (control and glyphosate treated) and maintained in six aquaria (three for control and three for treatment) at Ecotoxicology Laboratory, Department of Environmental Science, the University of Burdwan. Each aquarium contains 10 fishes. Fishes were exposed to sublethal dose of glyphosate, i.e., 17.20 mg/L, for 30 days.[16,17] Dose was applied on every alternate day following dewatering and renewal on a regular basis. Experiments were carried out according to the guidelines prescribed by the University of Burdwan and were approved by Ethical Committee. During exposure period, control and glyphosate-treated aquaria were subjected to the same environmental conditions. During experimentation period, water parameters showed the following average values: temperature, 26.63°C ± 0.12°C; pH 7.93 ± 0.06; electrical conductivity, 426.00 ± 5.93 µS/cm; total dissolved solids, 302.89 ± 4.69 mg/L; dissolved oxygen, 5.06 ± 0.43 mg/L; total alkalinity, 209.80 ± 10.50 mg/L as $CaCO_3$; total hardness, 163.11 ± 3.04 mg/L as $CaCO_3$; orthophosphate, 0.04 ± 0.002 mg/L, ammoniacal nitrogen, 7.09 ± 2.15 mg/L; and nitrate nitrogen, 1.78 ± 0.26 mg/L.

### Sampling

During experimentation period, water-quality parameters were measured as per the APHA.[26] At the end of the experiment, after 30 days, fishes were collected both from aquariums and field ponds. After collection, fishes were anesthetized with tricaine methanesulfonate (MS 222), then desired organs namely gill, liver, and kidney, were dissected out, and tissues were fixed in respective fixatives accordingly and finally proceeded for histological, scanning, and transmission electron microscopic (TEM) observations.

### Histological analysis

For histological observation, fish tissues were fixed in aqueous Bouin's fluid solution overnight. After fixation, tissues were dehydrated through graded series of ethanol and finally embedded in paraffin. Paraffin sections were then cut at 3–4 μ using microtome (Leica RM2125). Finally, sections were stained with hematoxylin-eosin (H and E) solution and examined under a light microscope (Leica DM2000).

### Ultrastructural analysis

For scanning electron microscopic (SEM) study, tissues were fixed in 2.5% glutaraldehyde solution (prepared in phosphate buffer, 0.2 M and pH 7.4) for 24 h at 4°C and then postfixed with 1% osmium tetroxide solution (prepared in phosphate buffer, 0.2 M and pH 7.4) for 2 h at 4°C. After fixation, tissues were dehydrated through graded series of acetone, subsequently followed by amyl acetate. After that, tissues were dried using liquid $CO_2$ at critical point drier. Tissues were then mounted on metal stubs and sputter-coated with gold (thickness approximately 20 nm). Finally, tissues were examined under SEM (Hitachi S-530) at University Science Instrumentation Centre, the University of Burdwan, West Bengal, India.

For TEM study, fish tissues were fixed in Karnovsky fixative (mixture of 2% paraformaldehyde and 2.5% glutaraldehyde prepared in 0.1 M phosphate buffer, pH 7.4) for 12 h at 4°C and then postfixed with 1% osmium tetroxide solution (prepared in phosphate buffer, 0.2 M and pH 7.4) for 2 h at 4°C. After fixation, tissues were dehydrated through graded series of acetone, infiltrated, and finally embedded in epoxy resin (Araldite CY212). Ultrathin sections were then cut (thickness 70 nm) and collected on naked copper-meshed grids. Grids were then stained with uranyl acetate and lead citrate. Finally, grids were examined under TECNAI G2 high-resolution TEM at Electron Microscope Facility, Department of Anatomy, AIIMS, New Delhi, India.

## Results

### Gill

Gill of the control fish consists of primary gill lamellae which are composed of cartilaginous skeletal structure, multilayered epithelium, and vascular system. Between secondary epithelium, primary lamella is lined by stratified epithelium and numerous chloride cells in the basement. Secondary gill lamella consists of epithelial cells supported by pillar cells [Figure 1a]. Gills of fishes under glyphosate-treated laboratory condition showed degenerative changes in pillar cells, curling of secondary lamellae, blood congestion, lamellar disarrangement, and appearance of globular structure under light microscopic observations [Figure 1b], but lesions were not so much prominent in field condition [Figure 1c].

Topographical study observed under SEM depicted that each control gill filament is composed of primary and secondary gill lamellae and is embraced by stratified epithelial cells and horizontal flat filaments [Figure 1d]. Ultrastructural alterations in the gill were also supporting the light microscopic lesions as excessive mucus secretion over gill epithelium, loss of microridges structure, disappearance of normal microridges array, and damage in stratified epithelial cells [Figure 1e], while under field condition, stratified epithelial cells and microridges structures showed almost normal appearance [Figure 1f].

TEM observations of the primary epithelium of gills showed general appearance of chloride cells supported by tightly packed pavement cells under control condition [Figure 1g]. Gills of fishes under treated laboratory condition showed severe cytoplasmic vacuolation, degeneration in tubular vascular structures, necrosis, dilation in tight junction, abnormal-shaped nucleus [Figure 1 h], while comparatively less pathological lesions were observed under field condition which included dilated mitochondria and rough endoplasmic reticulum (ER) and vacuolation in some places [Figure 1i].

### Liver

Histologically, liver of the control fish is generally made up of hepatocytes with centrally placed nucleus and densely stained nucleolus. In addition, acinar cells of hepatopancreas are polyhedral and compactly arranged. Moreover, acinar cells are arranged in two/three rows encircling blood capillaries and apical part contains zymogen granules [Figure 2a]. Most notable lesions observed in the liver of *O. niloticus* under light microscopic study were severe degeneration in hepatocytes, excessive fat deposition, pyknotic nuclei, acentric nuclei, and degenerative hepatopancreas under laboratory condition [Figure 2b]. The extent of damage in field condition was comparatively less than laboratory study which included enlarged acentric nuclei and dilated hepatocytes [Figure 2c].

Under SEM observation, liver of the control fish showed normal palisade arrangement of hepatocytes, hepatic cords, and mucus mass over hepatocytes [Figure 2d]. Topological observation of the liver from laboratory condition under SEM study confirmed the pathological lesions of light microscopy showing severe damage in hepatocytes and hepatic cords and excessive mucus secretion [Figure 2e], but in field condition, lesions were comparatively less [Figure 2f].

TEM study of the control liver showed normal appearance of hepatocytes with centrally placed prominent nucleus and nucleolus [Figure 2g]. In addition, cytoplasm contains large amount of mitochondria, rough ER, and glycogen [Figure 2g]. Degenerative changes, such as necrosis in mitochondria,

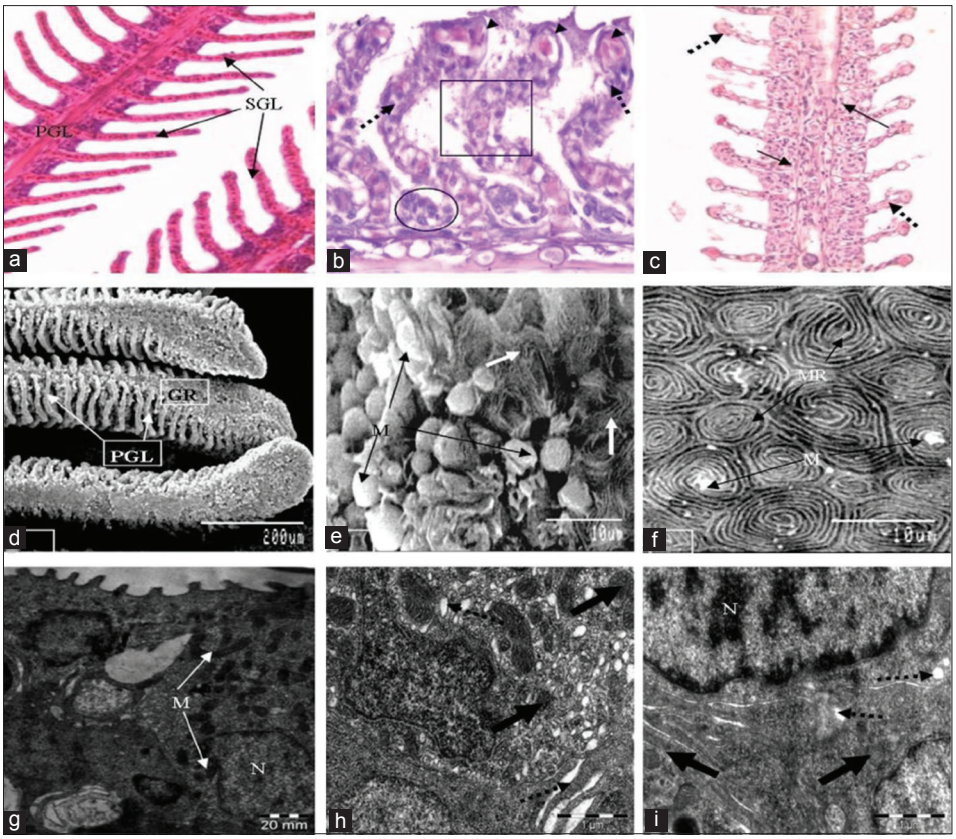


**Figure 1:** Histopathological photomicrographs of gill of *Oreochromis niloticus* under control condition (C), glyphosate-treated laboratory condition, glyphosate-treated field condition. (a) Normal structure of primary gill lamellae and secondary lamella under light microscopy (C, ×400). (b) Curling (square), congestion of blood vessel (arrowhead), distortion of chloride (oval) and pillar cells (broken arrow (GL, ×1000). (c) Atrophy and hypertrophy in interlamellar space between secondary gill lamellae (arrow) and damage in pillar cells (broken arrow) under light microscopy (GF, ×400). (d) Scanning electron microscopy showing normal arrangement of gill rakers with primary gill lamellae and stratified epithelial cells on the primary gill lamellae (C, ×200). (e) Gill epithelium showing loss of microridges over stratified epithelial cells (arrow) and mucin droplets (M) under scanning electron microscopy (GL, ×3000). (f) Almost normal appearance of microridge in stratified epithelial cells and excess mucins (M) droplets under scanning electron microscopic (GF, ×3000). (g) Gill epithelial cell under transmission electron microscopy showing normal chloride cell, pavement cells with prominent mitochondria (M) with apical pore (square) (C, ×1000). (h) Degenerative chloride cells (bold arrow) and severe vacuolation (broken arrow) under transmission electron microscopic (GL, ×6300). (i) Vacuolation (broken arrow) and dilated mitochondria (bold arrow) under transmission electron microscopy (GF, ×6300)

cytoplasmic vacuolation, dilation in ER, and reduced number of glycogen droplets, were prominent under laboratory study [Figure 2h]. On the other hand, in the field condition, hepatocytes showed almost normal appearance of mitochondria and dilation in some places and fragmented and vesiculated ER [Figure 2i]. Moreover, the lesions were comparatively higher under laboratory condition than the field.

## Kidney

Histologically, normal kidney is generally made up of large number of nephrons and hematopoietic tissues. In addition, nephron comprises Bowman's capsule and renal tubules. Moreover, Bowman's capsule contains glomeruli or Malpighian body and renal tubule consists of proximal convoluted tubule (PCT), distal convoluted tubule (DCT), and collecting duct. Renal tubules are mainly consisted of columnar and cuboidal epithelial cells [Figure 3a]. The most conspicuous alterations observed in the kidney of *O. niloticus* were fragmented glomerulus, severe degenerative changes in PCT and DCT such as swelling, excessive fat deposition, and hypertrophied nuclei [Figure 3b], while under field condition, comparatively less damage was observed in the kidney of *O. niloticus* [Figure 3c].

Under SEM observation, normal kidney showed glomerulus as cell mass, rounded PCT, oval-shaped DCT [Figure 3d]. SEM observations also showed degenerative changes in the kidney, i.e., shrinkage of glomerulus and distortion of PCT and DCT after glyphosate exposure under laboratory condition [Figure 3e], but in field condition, it was insignificant [Figure 3f].

TEM observation revealed that normal kidney contains electron dense mitochondria and nucleus and abundant vesicular structures in capillary epithelial cell cytoplasm [Figure 3g]. TEM study confirmed the necrosis in mitochondria, dilated and fragmented ER, and severe vacuolation in laboratory

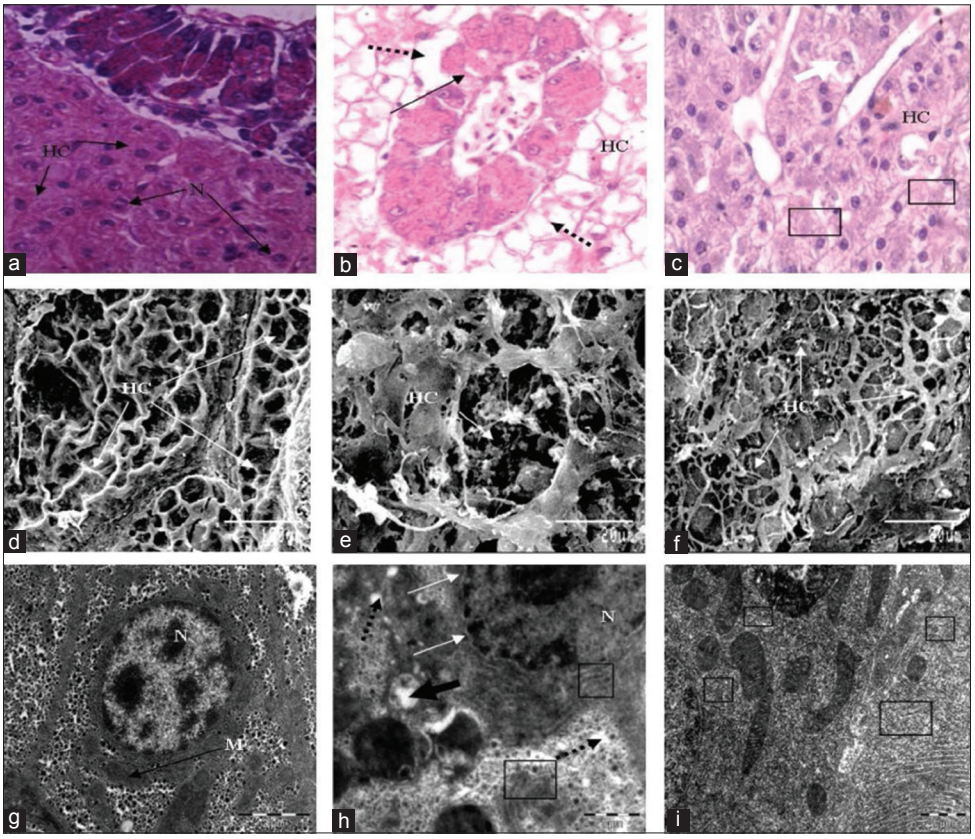


**Figure 2:** Histopathological photomicrographs of the liver of *Oreochromis niloticus* under control condition (C), glyphosate-treated laboratory condition, glyphosate-treated field condition. (a) Normal appearance of hepatocytes and compact arrangement around central vein with distinct nucleus (N) under light microscopy (C, ×1000). (b) Degenerative hepatopancreas (arrow), severe vacuolation in cytoplasm of hepatocytes (broken arrow) under light microscopy (GL, ×1000). (c) Light microscopy showing hypertrophied acentric nuclei (white arrow) and dilated hepatocytes (square) (GF, ×1000). (d) Normal hepatocytes under scanning electron microscopic observation (C, ×500). (e) Degeneration of cords under scanning electron microscopic observation (GL, ×600). (f) Scanning electron microscopic observation showing damage in hepatic cords (GF, ×500). (g) Normal appearance of hepatocytes with large number of mitochondria (M), rough endoplasmic reticulum, and glycogen droplets under transmission electron microscopy (C, ×3200). (h) Hepatocytes showing degenerated and necrosed mitochondria (bold arrow), vacuolation in nuclear membrane (white arrow), dilated and degenerated rough endoplasmic reticulum (square), and severe vacuolation (broken arrow) under transmission electron microscopy (GL, ×5000). (i) Under transmission electron microscopy, hepatocytes showing dilated, fragmented, and vesiculated rough endoplasmic reticulum (square) (GF, ×4000)

condition endorsed by light microscope [Figure 3h], but no significant alterations were observed under field condition, except dilation of ER [Figure 3i].

## Discussion

The present study is the maiden attempt to evaluate the comparative toxicity of Excel Mera 71 herbicide under two conditions, i.e., field and laboratory conditions with regard to histopathological alterations through light microscopic, SEM, and TEM observations in Indian freshwater fish, *O. niloticus*. However, Senapati *et al*.[27,28] recorded some histopathological alterations in the stomach and intestine of *Anabas testudineus* after Almix exposure under laboratory condition only.

Histopathological changes in the gill induced by glyphosate exposure were more prominent under laboratory condition than field observation. Hypertrophy and hyperplasia in the gill epithelium were common responses observed under both the conditions and were demonstrated by Hued *et al*.[29] and Ramírez-Duarte *et al*.[30] in the gill of *Jenynsia multidentata* and *Piaractus brachypomus* after Roundup exposure, respectively. In addition, mucus secretion and lamellar disarrangement indicated protective mechanism of gill epithelium to glyphosate exposure. The results were similar to the findings of Kossakowski and Ostaszewska[31] and Biagini *et al*.[32] Moreover, curling of secondary gill lamellae as observed in the present study under both the conditions was also reported in *Cyprinus carpio* after chlorpyrifos exposure by Pal *et al*.[33] In addition, appearance of globate structure at gill lamellae tip was also reported by Sorour and Harbey[34] in *Oreochromis* sp. collected from polluted and unpolluted Wadi Hanifah stream, Riyadh.

The most notable ultrastructural alterations (SEM) in gill stratified epithelium such as thinning and degeneration of microridges, upliftment of epithelial cells, and reduced number of mucous and chloride cells were also described by Johal *et al*.[35] in *C. carpio* after monocrotophos exposure. In addition, loss of microridge structure from pavement cells of gill epithelium

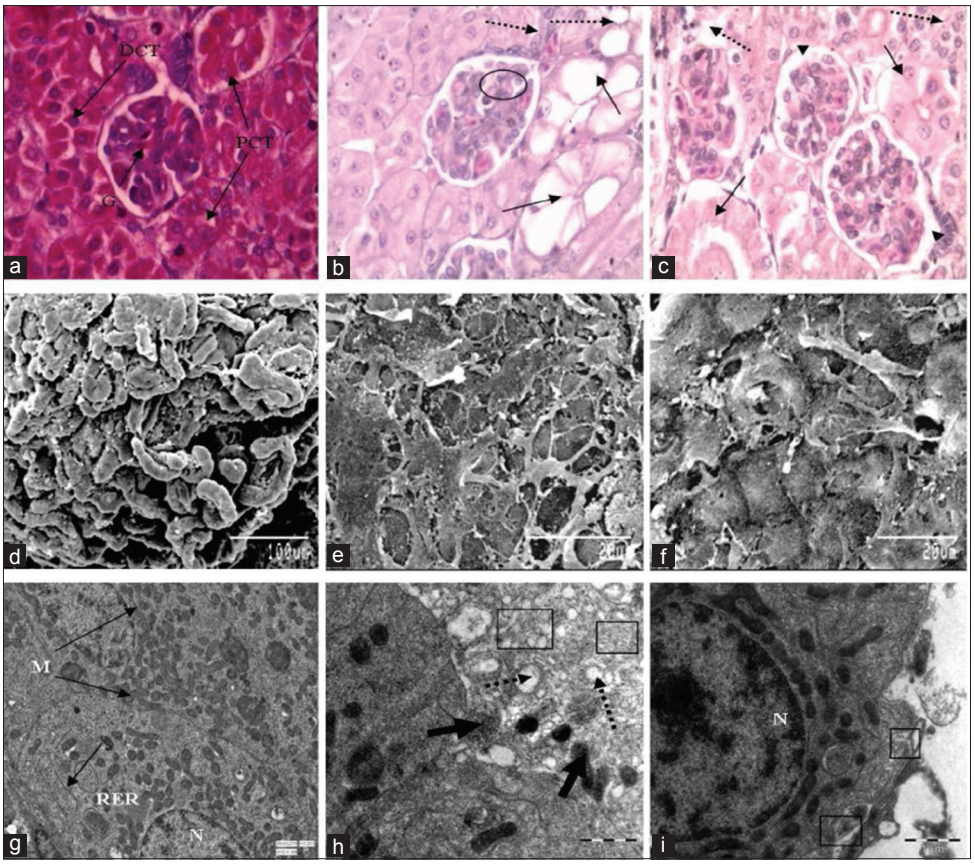


**Figure 3:** Histopathological photomicrographs of the kidney of *Oreochromis niloticus* under control condition (C), glyphosate-treated laboratory condition, glyphosate-treated field condition. (a) Normal proximal convoluted tubule, distal convoluted tubule, Bowman's capsule, and glomerulus under light microscopy (C, ×1000). (b) Fragmented glomerulus (oval), degenerative and hypertrophied proximal convoluted tubule and distal convoluted tubule (arrow), and vacuolation in the hematopoietic tissues (broken arrow) under light microscopy (GL, ×1000). (c) Light microscopy showing degenerative and hypertrophied proximal convoluted tubule and distal convoluted tubule (arrow), vacuolation in the hematopoietic tissues (broken arrow) (GF, ×1000). (d) Normal kidney with prominent proximal convoluted tubule and distal convoluted tubule under scanning electron microscopic observation (C, ×600). (e) Degenerative changes and shrinkage of glomerulus under scanning electron microscopic observation (GL, ×600). (f) Degeneration under scanning electron microscopic observation (GF, ×800). (g) Normal appearance of the kidney with electron dense mitochondria (M) and nucleus (N) under transmission electron microscopy (C, ×2550). (h) Necrosis in mitochondria (bold arrow), dilation, vesiculation, and fragmentation of rough endoplasmic reticulum (square) and severe vacuolation (broken arrow) under transmission electron microscopy (GL, ×5000). (i) Dilated rough endoplasmic reticulum (square) under transmission electron microscopy (GF, ×500)

was also reported by several authors.[32,34,36] Moreover, Mallatt[37] demonstrated that microridges are playing vital role in cellular protection against environmental contaminants by retention of mucus on gill epithelium. On the other hand, transmission electron micrographic observations such as hypersecretion of mucus and necrosis in gill epithelium indicated impaired gas exchange capacity by gill epithelium.[37,38] Therefore, these pathological lesions in gill morphology could lead to functional abnormalities and interference of normal fundamental processes such as maintenance of osmoregulation and antioxidant defense mechanism of gill epithelium.[39]

In liver, severe necrosis in hepatocytes indicated negative impact of herbicide (Excel Mera 71), which caused functional and structural impairments.[40] Similar observations along with cytoplasmic vacuolation and pyknotic nuclei were also reported by Rahman *et al*.[41] in *Corydoras punctatus* and *A. testudineus* after Diazinon 60 EC exposure. Cytoplasmic vacuolization in hepatocytes observed in the present study was also demonstrated by Biagianti-Risbourg and Bastide[42] in *Liza ramada* exposed to atrazine. In addition, vacuolization of hepatocytes indicates imbalance between rate of synthesis of substances in parenchymal cells and their release into systemic circulation, which ultimately suggest the stress condition of the fish.[43] In another study, Jiraungkoorskul *et al*.[19] noticed swelling of hepatocytes, pyknotic nuclei, severe cytoplasmic vacuolation, degenerated cell membrane, and severe leukocytes infiltration in the liver of *O. niloticus* after Roundup exposure. Degenerative changes in hepatopancreas and distortion in acinar cells seen under laboratory study indicated tissue damage particularly in columnar epithelial cells and this might be an adaptive compensatory response by organism itself to neutralize the glyphosate-induced stress.[43]

SEM observation showed severe damage in hepatocytes and hepatic cords and excessive mucus secretion under laboratory

study. The results were corroborated with the findings of Uguz *et al*.,[44] who reported similar findings and indicated that this might be due to increase in DNA/RNA ratio. Due to higher sensitivity of the hepatocytic ultrastructure, higher glyphosate concentration in the aquatic environment resulted in more extensive degenerative responses under laboratory condition. Most conspicuous alterations in hepatocytes such as necrotic mitochondria, vacuolated nuclear membrane, dilated and degenerative ER, severe cytoplasmic vacuolations, and reduced number of glycogen droplets were seen under laboratory study. Necrotic mitochondria observed in the present study was also reported by Bozzola and Russell[45] and indicated that this might be due to inhibition of large number of respiratory enzymes which oxidizes substrates to form ATP during phospholipid metabolism and fatty acid synthesis in mitochondria. In addition, dilation and swelling of rough ER was another most important lesion observed in the present study could be interpreted as morphological counterpart of ethoxycoumarin-O-deethylase and ethoxyresorufin-O-deethylase induction.[46] Moreover, the cytoplasmic vacuolation in hepatocytes indicated decreased protein synthesis due to reduced utilization of lipid-protein conjugation that accompanied hepatocyte injury.[47] However, reduced glycogen content in hepatocytes of *A. testudineus* under laboratory condition might be due to increased glycolytic activity to meet enhanced energy demand as compensatory mechanism.[48,49] Higher pathological lesions in hepatocytes under laboratory condition compared with field observation might be attributed to availability of natural food under field condition from natural water body.[50] Therefore, less cytopathological alterations were observed in field than laboratory fish.

In the present study, kidney showed fragmentation of glomerulus, severe degenerative changes in PCT such as swelling, excess fat deposition, and hypertrophied nuclei under laboratory condition. However, comparatively less pathological lesions in PCT, DCT, and glomerulus were observed in field condition. Similar results of excessive fat deposition and hypertrophied and pyknotic nuclei were also revealed by Jiraungkoorskul *et al*.[19] in the kidney of *O. niloticus* after Roundup exposure under laboratory study. In another study, Oulmi *et al*.[51] reported small cytoplasmic vacuoles and nuclear deformation in *Oncorhynchus mykiss* to linuron exposure and explained these nephro-histopathological alterations due to herbicidal stress as compensatory response. The nephro-histopathological alterations in the present investigation indicated disruption of several biochemical and physiological pathways including endocrine disruption and these are correlated with other findings.[52,53]

Ultrastructural alterations such as shrinkage of glomerulus and damage in PCT and DCT observed under SEM study were also reported by Fischer-Scherl *et al*.[54] On the other hand, TEM observation showed cytopathological alterations such as necrosis in mitochondria, dilation and fragmentation of ER, and appearance of severe vacuolation under laboratory condition in the kidney of *Carassius auratus* exposed to hexachlorobutadiene reported by Reimschüssel *et al*.[55] and Bravo *et al*.[56] in two Venezuelan cultured fishes, *Caquetaia kraussii* and *Colossoma macropomum* after triazine exposure. Fischer-Scherl *et al*.[54] also reported considerable ultrastructural changes in the kidney of rainbow trout to atrazine exposure and these responses resembled the symptoms of the present study under laboratory condition. However, the presence of hyaline droplets in renal tubules indicated occurrence of renal toxicity after herbicide exposure.[57]

## Conclusions

Histopathological and ultrastructural responses of Excel Mera 71 in gill, liver, and kidney of *O. nilotius* were studied and represented as an integrated cumulative effect of physiological and biochemical contaminants. Generally, these responses were more pronounced in laboratory-exposed fish than field exposed and indicating higher disturbances of cellular metabolism as well as stronger structural and functional alterations under laboratory condition. Therefore, these histopathological and ultrastructural responses could be considered as bioindicators to analyze the fish health status under contaminated aquatic environment.

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### Conflicts of interest

There are no conflicts of interest.

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