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Original Article

Toxic effects of glyphosate-based herbicide, Excel Mera 71 on gill, liver, and kidney of *Heteropneustes fossilis* under laboratory and field conditions

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ABSTRACT

The effects of glyphosate-based herbicide Excel Mera 71 under field and laboratory conditions were investigated to evaluate the pathological symptoms through light and electron microscopic study in the gill, liver, and kidney of *Heteropneustes fossilis* (Bloch) for a period of 30 days. Histological alterations like hypertrophy and fusion in secondary lamellae, damage in chloride cells were more prominent in laboratory conditions under light microscopy. Topological changes such as complete loss of microridges, swelling, and irregular arrangement of microridges in the gills were prominent under scanning electron microscopic study under laboratory conditions. Transmission electron microscopy (TEM) study depicted vacuolation and degeneration in chloride cells, dilation in rough endoplasmic reticulum (RER), and mitochondria in gill epithelium. The liver showed enlarged and pyknotic hepatocytes, vacuolation, excess fat deposition, and necrosis under laboratory conditions, while enlarged acentric nuclei, increased sinusoidal space, and less vacuolation in cytoplasm were observed under field conditions. TEM displayed cytoplasmic vacuolation and a reduced number of endoplasmic reticulum and glycogen droplets in the laboratory, but this was less pronounced under field conditions. In the kidneys, loss of hematopoietic tissue, degenerative changes in glomeruli, proximal and distal convoluted tubule, and epithelial cell lining of the renal tubules were comparatively less prominent under field conditions. Under TEM, epithelial cell necrosis, endoplasmic reticulum fragmentation, and mitochondrial degeneration were more prominent under laboratory conditions. The present study evaluated the comparative toxicity under field and laboratory conditions under long-term exposure to glyphosate herbicide and identified pathological responses as indicators in monitoring the herbicidal contamination in aquatic ecosystems.

1. Introduction

Histopathological study has been widely used for toxicity testing of the effects of xenobiotic compounds at the suborganismal or organismal level, as well as

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evaluation of overall health of the entire population in the ecosystem. The advantage of using histopathological symptoms in specific target organs like the gills, liver, and kidneys in environmental monitoring is that they are most effective to study the vital functions, such as respiration, accumulation and biotransformation, and excretion of xenobiotics in fish [1,2]. Histological changes appear as prime responses to sublethal stressors, while topological characterization of cell surface and subcellular organelles can be best analyzed under scanning electron microscopy (SEM) and transmission electron microscopy (TEM), respectively. Furthermore, the alterations in cells and tissues in vertebrates, especially fish, are recurrently used as biomarkers in many studies, but such changes also occur in all invertebrates inhabiting aquatic basins, and are normally easier to identify than functional ones [3], which ultimately serve as warning signs of damage to animal health [4,5]. Fish after exposure to these xenobiotic substances show several lesions in different tissue systems [6,7]. Gills [8,9], liver [4,10], and kidneys [11] are the most suitable organs for histological analysis in order to determine the effects of contamination. During xenobiotic exposure, the toxicants break down the adhesion between the epithelial branchial cells and the underlying pillar cells, accompanied by collapse of the structural integrity of the secondary lamellae and subsequent failure of the respiratory functions of the gills [12]. The liver is the central metabolic organ and plays a key role in biochemical transformations of the xenobiotic substances, which inevitably reflects on its integrity by creating lesions and other histopathological alterations in the liver parenchyma [13]. The kidneys perform an important function in maintenance of a stable internal environment and partially xenobiotic metabolism.

Aquatic bodies are contaminated by several pesticides, especially herbicides such as glyphosate, through irrigation water and surface run-off. Among the non-target aquatic organisms, fish represent the largest and most diverse group of vertebrates that are chronically exposed to these substances continuously. Therefore, the present study aimed to investigate the toxic effects of commercial formulations of the glyphosate herbicide, Excel Mera 71 (Excel Crop Care Limited, Mumbai, Maharashtra, India), at histopathological and ultrastructural levels through changes in the gills, liver, and kidneys of the fish *Heteropneustes fossilis* (Bloch) under laboratory and field conditions.

2. Materials and methods

2.1. Chemicals

Commercial formulation of the glyphosate herbicide (Excel Mera 71, Excel Crop Care Limited) was used in both the experiments. Excel Mera 71 is the trade name of glyphosate herbicide in the Indian Market. Delafield's hematoxylin stain, eosin yellow, xylene, Distyrene Plasticizer Xylene, amyl acetate, acetone, glutaraldehyde solution, sodium hydroxide, tricaine methonesulfonate, uranyl acetate (EM grade), ethanol, disodium hydrogen phosphate, dihydrogen sodium phosphate, lead citrate (EM grade), epoxy resin (EM grade), paraformaldehyde (EM grade), and araldite CY212 (EM grade) of analytical grade were purchased from Merck Specialities Private Limited (Mumbai, India). Osmium tetroxide was purchased from Spectrochem (Mumbai, India).

2.2. Fish

Freshwater teleostean fish *H. fossilis* (Bloch) of both the sexes with an average weight of 31.77 ± 3.440 g and total length of 16.58 ± 0.388 cm were procured from local markets and were acclimatized under congenial laboratory conditions for 15 days separately in aquaria of 250-L capacity. Fish were kept in continuously aerated water with a static system and experiments were conducted with a natural photoperiod (12-hour light/12-hour dark) and at an ambient water temperature. During acclimatization, the average values of water parameters were analyzed: temperature $26.49 \pm 0.127\,^{\circ}C$, pH 7.94 ± 0.04, electrical conductivity $392.22 \pm 0.62\ \mu S/cm$, total dissolved solids 279.33 ± 0.69 mg/L, dissolved oxygen 6.44 ± 0.05 mg/L, total alkalinity 204.0 ± 7.30 mg/L as $CaCO_3$, total hardness 180.44 ± 3.74 mg/L as $CaCO_3$, sodium 24.45 ± 0.56 mg/L, potassium 5.33 ± 1.02 mg/L, orthophosphate 0.03 ± 0.001 mg/L, ammoniacal nitrogen 1.66 ± 0.21 mg/L, and nitrate nitrogen 0.21 ± 0.030 mg/L. After acclimatization, fish were divided into two groups: one group was maintained in field ponds situated at Crop Research Farm premises of The University of Burdwan, West Bengal, India and the other group in laboratory aquarium. The fish were fed once a day with commercial fish pellets (32% crude protein, Tokyu® fish food, Thailand) during both acclimation and exposure periods. Therefore, the study was carried out under two different experimental conditions: field pond and laboratory, for a duration of 30 days.

2.3. Experimental design

2.3.1. Field experiment

Fish were maintained in two groups in two separate adjacent fields: three control groups containing 10 fish species in a cage in one field, and three glyphosate exposure groups containing 10 fish species in separate field and cages for 30 days. The desired dose of 750 g/acre, corresponding to the concentration recommended for use in rice culture, was dissolved in water and applied once. It was sprayed on Day 1 of the experiment on the surface of each glyphosate-treated cage. During experimentation, glyphosate-treated and control fish were subjected to the same environmental conditions. The cages were prepared for the culture of the experimental fish species as per Chattopadhyay et al. [14], with some modifications. All the cages were square in shape with an area of 2.5 m × 1.22 m and height of 1.83 m (submerged height was 0.83 m). The cages were framed by light strong bamboo. The four-sided wall, floor of the cage, and top of the cage cover was fabricated with nylon net and was embraced by two polyvinyl chloride nets: the inner and outer bearing mesh sizes of 1.0 mm × 1.0 mm and 3.0 mm × 3.0 mm, respectively. During the experimentation of 30 days, the field pond