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Histological Effects of Mancozeb on Intestinal Tissue of Zebrafish (*Danio rerio*)

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ABSTRACT

Mancozeb is a synthetic pesticide which has been used for decades. It is used to hinder the growth of fungi and plants against damage. Pesticides access aquatic ecosystems via ground waters and affect water quality and aquatic life. In our study, investigation the histopathological effects of mancozeb on intestine tissue of zebrafish were aimed. Degeneration and expansion at villi structure and increase in the number of goblet cells, mucus accumulation and expansion in muscular layer were detected at exposure groups. Depending on the increased number of leukocytes enteritis was monitored.

Introduction

Fungicides are pesticides that have potential toxic effects on animals. They have a wide range of usage area and are used for protecting tubers, fruits and vegetables during storage or are applied directly to ornamental plants, trees, field crops, cereals and turf grasses in agriculture (1). In industry, numerous fungicides are used to protect products during shipment, suppress mildews that attack painted surfaces, preserve wood, control fungal growth in paper pulps, and protect household carpet and fabric (2). In veterinary medicine, fungicides are commonly an antibacterial/antiseptic treatment for foot rot disease but, some fungicides, are also used a molluscicide to repel and kill slugs and snails (3).

Both alkyl and ethylene dithiocarbamates form salts with metals and both can be oxidized to the corresponding disulfides. Dithiocarbamates may decompose under certain circumstances into a number of compounds, such as sulfur, 5, 6-dihydro-3 H-imidazol [2,1-C]-1, 2, 4 dithiazole -3-thione, ethylenethiourea (ETU), andethylenediamine (EDA). ETU stable, has high watersolubility, and is of particular importance because of its specific toxicity (4,5).

Ethylenebisdithiocarbamates (EBDCs) are widely used in agriculture as fungicides, mainly on fruit, vineyard, and potato crops. Their extensive world-wide consumption can be ascribed to their low acute toxicity and their short environmental persistence (6). Mancozeb is one member of ethylenebisdiothiocarbamate (EBDC) fungicides. EBDC pesticides because of health concerns caused by ETU, including potential carcinogenic, developmental and thyroid effects (7). It uses to protect many fruit, nut, and field crops from a wide spectrum of fungal diseases (4). Mancozeb is effective in soils with ETU 5-10 days. (4).

In our study, investigation the histopathological effects of mancozeb on intestine tissue of zebrafish (*Danio rerio*) were aimed. The zebrafish is a small fish about 6cm in length and characterized by a series of five pigmented stripes running the entire length of each side of its body. The zebrafish are used as stress test model because they can survive fairly severe environmental changes without succumbing, surviving long enough to show developmental defects. Finally, easy and inexpensive raising making them an ideal animal model for researches.

Materials and Methods

Zebrafish were raised in a incubation chamber, and received 14 hours of day light and 10 hours of darkness every day. After one week adaptation period, zebrafish divided into four groups (n=10) as one control and 2 experimental groups (5 ppm, 7.5 ppm). For investigating the effects of mancozeb, intestine tissues were dissected after 5 days of the exposure. Tissues were fixed with 10% neutral buffered formalin and dehydration were carried out in an ascending series of ethanol. After tissues were cleared in xylene, embedded in paraffin wax and cut into 5 µm sections on a microtome. The sections were stained with hematoxylin (H&E) and results were evaluated with light microscope.

Results

In control and experimental groups any death and behavioral changes were not observed. In control group, normal intestine histology was monitored. Mucosa, submucosa and serosa layers and lamina propria were observed clearly (Figure 1a). Villi structures and goblet cells were easily monitored. Cell layers of mucosa epithelium and goblet cells between enterocytes which have columnar nucleus were photographed (Figure 1b).

In 5 ppm mancozeb exposure group, degeneration, disjoined and enteritis formation at villi structure were detected (Figure 2a). Expansion and increase in the number of goblet cells were monitored (Figure 2b). Contraction at lamina propria and expansion at villi structure were observed (Figure 2c). In addition, dysplasia and increase in the number of lymphocytes at villi were found (Figure 2d).

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In 7.5 ppm mancozeb exposure group, severe dysplasia were observed. Degeneration at villi structure (Figure 3a), mucus accumulation and expansion at muscularis layer were detected (Figure 3b). Enteritis formation, integration of villi (Figure 3c) and increase in the number of blood cells (Figure 3d) were monitored. Increase in the number of goblet cells were observed at exposure groups, compared with control group.

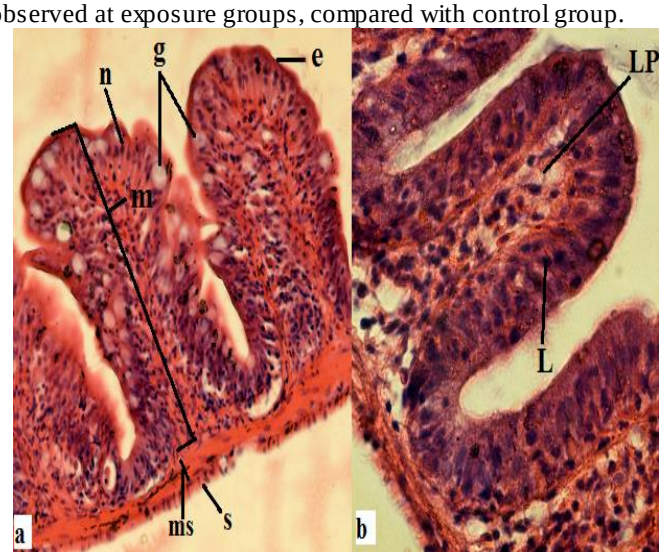

Figure 1. Intestine histology of control group,, a) mucosa (m), submucosa (ms), serosa(s), goblet cells (g), enterocyte (e), nucleus (n), b) lamina propria (LP), lymphocyte (L), Hematoxylin & Eosin a-x40 b-x100

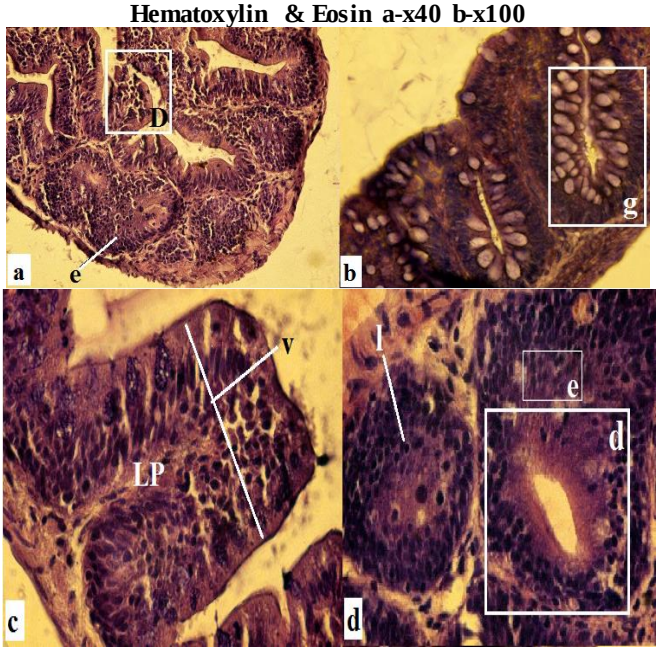

Figure 2. Intestine histology of 5 ppm mancozeb exposure group, a) disjoned at villi structures, enteritis (e), degeneration(D), b) increase in the number of goblet cells (g), c) Contraction at lamina propria (LP), expansion at villi structure (v), increase in the number of lymphocyte (l), dysplasia (d) and enteritis (e) Hematoxylin & Eosin a-x20 b,c-x40, d-x100

Discussion

Pesticides are especially used to satisfy food need all over the world. However, their use in an exaggerated manner leads to uncontrolled environmental pollution. In this study degenerative effects of mancozeb on zebrafish intestine was proved. Pesticides and xenobiotics have a great impact on the environmental quality as they result in a toxicity risk to non-target organisms especially fish and enter the food chain when they become accumulated in aquatic organisms (8-10).

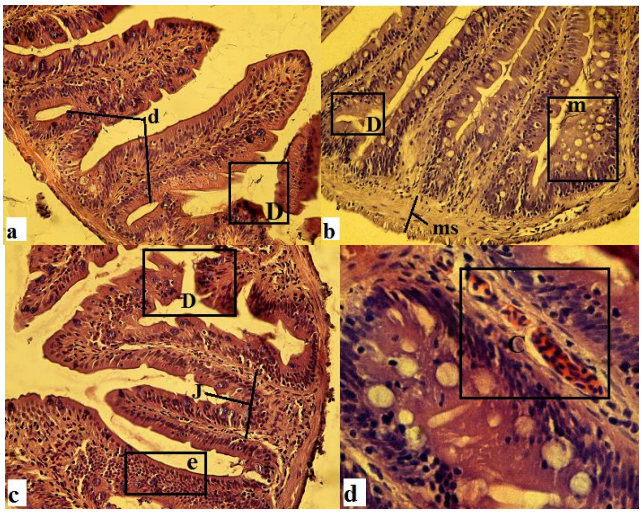

Figure 3. Intestine histology of 7.5 ppm mancozeb exposure group, a) dysplasia (d), degeneration (D), mucus accumulation (m), expansion at muscularis layer (ms), enteritis (e), integration of villi, (J), increase in the number of blood cells (C), Hematoxylin & Eosin a,b,c-x40, d-x100

Limited data are available on the toxicity of ETU to aquatic organisms; however, the available data show that ETU has low acute toxicity to aquatic organisms. ETU is practically nontoxic to cold water fish and slightly toxic to freshwater invertebrates (11). Our results are consistent with this study. In Mohan's study (12) about effects of malathion on *Glossogobius giuris* intestine, malathion was found to induce alterations both in the foregut and hindgut after exposing to each of 0.05, 0.25 and 0.5 ppm for 24, 48, 72 and 96-h of intervals. Malathion treatment resulted in breakdown and fragmentation of the serosal layer. In muscularis, the longitudinal muscle layer was greatly reduced and the circular muscle got thickened with an increase in the number of blood vessels. These findings aren't different from our study. Distinctively, in our study, increase in the number of goblet cells, dysplasia, mucus accumulation and integration at villi structure were found.

Karthigayani et al. (13) studied histological effects of cypermethrin on intestine and liver tissues of tilapia fish *Oreochromis mossambicus*. They exposed to sublethal concentration of 0.008ppm at different exposure time from 24 to 192 hrs. The histological observations of the intestine showed deleterious effect of cypermethrin at 96 hrs exposure time, the epithelial cells and the cells of the outer wall disintegrate which would eventually result in the breakdown of the intestinal functions. Cengiz and Unlu (14) investigated sublethal effects of commercial deltamethrin on the structure of the gill, liver and gut tissues of mosquitofish, *Gambusia affinis*. *They* exposed two sublethal concentrations of deltamethrin (0.25–0.50 µg/l) for periods of 10, 20 and 30 days. They detected infiltration of mononuclear leucocyte and eosinophils towards lamina propria. They also observed necrosis in the gut tissues of fish after 30 days of exposure to 0.50 µg/l deltamethrin.

Ganeswade et al. (15) found that dimethoate induced histological changes in the intestine of fresh water fish P*untius ticto*. They exposed to lethal (5.012ppm) and sublethal concentrations (2.506 and 1.253ppm) of dimethoate for 96 hrs and 60 days respectively. Acute exposure resulted into cloudy swelling and granular cytoplasm in mucosal cells, broken serosa, bulging and hypertrophic condition was noticed in columnar epithelial cells which secretes excess amount of mucus. Necrotic and bulging conditions were observed at the tip of villi which lead to rupture of villi. Chronic exposure results in broken serosa, vacuolated submucosal layer, vacuolated longitudinal

and circular layer, mucosal layer completely damaged, vacuolated villi and columnar cells were completely collapsed to higher concentration.

Kakuta et al., (16) reported hyperemia, degenerative changes in the tips of villi, loss of structural integrity of mucosal folds, hypertrophy vacuolation and necrosis in the intestine of *Cyprinus carpio* exposed to the pesticide atrazine. Similar histopathological effects of mancozeb were observed like atrazinein our study.

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