Biochemical and histopathological response of Oreochromis niloticus to malathion hepatotoxicity

Ahmed Th. A Ibrahim

Abstract

Malathion as pesticides had wide range uses for the control of insects on fruits and vegetables, mosquitoes, household insects, animal parasites. Its wide range of uses caused toxicity for non-targeted aquatic organisms including fish. So, the present work aimed to evaluate the biochemical and histopathological changes associated with chronic exposures of malathion (0.1 and 0.3 ppm) to liver of Oreochromis niloticus for 30 days of exposure. The negative effects of malathion on O. niloticus liver was revealed in terms of glucose, total protein (Tp), albumin, serum aspartic aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in blood serum. Also, Catalase (CAT), Superoxide dismutase (SOD), total antioxidant (TAO), glutathione (GSH) and glutathione-S-Transferase (GST) as antioxidant biomarkers. Protein carbonyl (PC), lipid peroxidation (LPO) and DNA damage as oxidative stress biomarkers in liver that could be used as environmental contamination biomarker for fish. The histopathological alteration of liver was reported.

Liver enzymes showed a significant increase in malathion exposed groups. However, TP, albumin and globulin showed a significant decrease in malathion exposed group. The activity of SOD, CAT, GSH and GST showed a significant increase (p<0.05) when compared to the control groups. However, TAO showed significantly decreased (p<0.05) than control group. A significant increase in DNA fragmentation, LPO and CP were detected with malathion doses increases. Liver showed many histopathological alterations in malathion exposed groups.

Introduction

As a result of agricultural and local activities; chemical pollutants caused continuously contamination in aquatic ecosystems. One of the most toxicants category is the pesticides, that have poisonous influences on aquatic life including fish that are economically important; a widely risk of these pesticides due to their toxicity on non-target organisms [1].

Malathion (0,0- dimethyl -S-1,2-bisethoxy carbonyl ethyl- phosphorodithioate) is organophosphate pesticide with wide range expansion. [2], which is mostly preferred for its high selectivity to target pests [3]. Malathion, even at low concentrations are harmful for fish. And, caused a reduction of glycose, proteins and lipids, which reported by Huculeci, Dinu [4] after fish exposure to Malathion. Malathion exposure on different animals showed that its ability to produce reactive oxygen species (ROS) which caused damage to various membranous cell components. When these ROS are formed; Oxidative stress happened. The enzymatic defense system, including catalase (CAT) and superoxide dismutase (SOD) could detoxified The ROS, while the activity of glutathione-S transferase (GST) could detoxified organic peroxides [5]. Changes in antioxidant enzyme activities levels could use as biomarkers in different aquatic organisms [6-8].

Liver as a vital organ, performing an extensive variety of body functions like detoxification, enzyme production, metabolism and homeostasis we selected the liver of Oreochromis niloticus as target organ for the present study. The antioxidant enzymes like CAT, SOD, GST, GSH and TAO along with DNA fragmentation, carbonyl protein (CP) and lipid peroxidation are well established tool for assessment of oxidative stress caused by toxicants [9, 10]. Thus, the present investigation aimed to determine biochemical change, antioxidant, oxidative stress biomarkers, and histopathological alteration as a response to malathion exposure for 30 days at two different doses in liver of Oreochromis niloticus.

Materials and methods

Eighteen specimens of adult Oreochromis niloticus were caught from the fish market, New Valley governorate. Fish were transported to fish biology lab in Zoology Department, Science faculty, New Valley University. Fish (110–135 g) (17.2±19.4 cm) were fed on a marketable pellet diet (2% of body weight/day) and kept in 110 L rectangular tanks containing tap water (conductivity 2000 ls/cm; pH 7.6; oxygen 85-95% saturation; temperature 25 °C; photoperiod 12:12 light: dark). After 2 weeks acclimation, fish were divided into three groups; control and two groups.

Chemicals

Malathion, O,O-dimethyl-S-1, 2-bisethoxy carbonyl ethyl-phosphorodithioate (50% purity), was obtained from Green River International Trade Co., Shang Hai, China.

Experimental setup

Two chronic doses of malathion pesticide were used (0.1 and 0.3 ppm). These chronic concentration were selected according to Al-Ghani [11], who reported the 96-h LC50 for malathion which was 10.06 ppm. The water and aquarium were completely replenished day after day for 30 days of the experiment. In clean glass bottles, the stock solution of malathion (1,000 mg/L) was prepared and stored then diluted to concentrations of 0.1 and 0.3 ppm, 1/10 and 3/10 of 96h LC50 respectively, (6 fish/tank): control and two malathion groups.
Biochemical parameters

After 30 days of malathion exposure, blood samples were collected from the caudal vein, left to coagulate. Then, centrifuged at 5000 rpm for 10 min to separate serum into Eppendorf tubes and stored at -20°C for biochemical analysis. Colorimetric and kinetic determinations of biochemical parameters were performed using spectrophotometer (Jasco-V530).

Glucose, total protein, albumin, serum aspartic aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were estimated in serum using assay kits supplied by Diamond Diagnostic, Egypt.

Liver preparations

Liver was wisely removed, and dried using filter paper, then washed with 7.4 pH phosphate buffer and homogenized in phosphate buffer containing of 1mMEDTA, 1mM DTT, 0.15M KG, 0.01% PMSF at about 4°C. Homogenization was carried out then centrifuged at 10,000 rpm for 20 min at 4°C and the obtained supernatants were used for determination of antioxidants and oxidative stress biomarkers.

Total carbohydrate determination

Tissues analyzed for carbohydrate content were first wet weighed (1 gm of liver) and then placed into centrifuge tubes containing 3 ml of KOH solution (30%). Carbohydrate content in liver was determined by Anthron method [12].

Antioxidants and oxidative stress biomarkers

Total protein contents was determined by Lowry method [13]. SOD activity was measured as described by McCord and Fridovich [14]. Catalase activity was measured as described by Aebi [15]. Glutathione S-transferase (GST) was measured using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate as described by Habig, Pabst [16] and adapted to a microplate reader by Stephensen, Sturve [17]. The GSH level was measured following the methods described by Cohn and Lyle [18]. The TAO was measured using a colorimetric assay kit (Randox Laboratories, Crumlin, U.K.) and values are expressed as mmol/L. Lipid peroxidation was measured according to the method of Levine, Garland [21]. Carbonyl protein was determined by the method of Kurita-Ochiai, Fukushima [20].

Histopathological Preparation

After 30 days of malathion exposure, fish were sacrificed, then dissected and liver were collected for histopathological analysis. Liver were fixed into 4% neutral formalin solution for 48 h and then dehydrated using alcohol series gradually (70 to 100%), cleared in xylene and embedded in paraffin. 5 to 7 μm thick paraffin sections were cut and stained with haematoxylin and eosin (H&E) and analyzed under light microscope (Ray Wild Opti 3 lab model, Germany) equipped with a camera (Tucsen, ISH1000, Korea).

Statistical analyses

Data are expressed as mean ± Std. Err. Statistical significance was evaluated by ANOVA. Differences were considered significant at P<0.05 and P<0.001 using the statistical software SPSS version 16.

Results

The changes in liver enzymes (AST, ALT and ALP) after 30 days exposure period of Oreochromis niloticus are presented in Table 1. Exposure of O. niloticus with malathion showed a significant increase (P<0.05) in ALT (182.5, 37.44 and 73.54 U/l) for the control, 0.1 and 0.3 ppm malathion, respectively) and AST (22.16, 49.26 and 86.56 U/l for the control, 0.1 and 0.3 ppm malathion, respectively) and a significant increase (P<0.05) in ALP (12.74, 28.17 and 66.51 U/l for the control, 0.1 and 0.3 ppm malathion, respectively) was reported with the increase of malathion dose. Current results showed a significant decrease (P<0.05) in TP (total proteins) from 6.01 ppm in the control to 4.21 mg/dl in the group exposed to 0.1 mg/L malathion and 2.87 mg/dl in fish serum that exposed to 0.3 mg/L malathion (Table 1). Also, albumin and globulin (Alb and Glo) levels showed a significant decrease with the increase of malathion dose (Table 1). Glucose (Glu) concentration of O. niloticus serum was significantly increased (P<0.05) from 72.54 mg/dl in the control to 95.78 and 138.54 mg/dl in fish serum that exposed to 0.1 and 0.3 ppm malathion, respectively (Table 1).

Our study reported significant variations (P<0.001) and (P<0.05) of oxidative stress and antioxidant biomarkers of treated groups when compared with control. To evaluate the oxidative stress and antioxidant status of O. niloticus liver that exposed to (0.1 and 0.3 ppm as 1/10 and 3/10 of LC50) of malathion for 30 days, the levels of CAT, SOD, TAO, GST, GSH and GPx activities and DNA fragmentation, CP, and LPO (assessed by MDA content), were measured (Table 2).

The enzymatic and non-enzymatic antioxidant (CAT, SOD, GSH, GST, and TAO) in liver of Oreochromis niloticus showed different behavior in increase or decrease. CAT, SOD, GSH and GST showed a significant increase in different malathion exposed group dependent manner with an upward trend. However, TAO level significantly decreased (P<0.05) when compared with the control group (Table 2).

The present study showed a significant increase in oxidative stress parameters: LPO, DNA fragmentation and CP content after the exposure to sublethal doses (0.1 and 0.3 ppm) of malathion on liver of O. niloticus as shown in Table 2. A highly significant increase (P<0.001) of DNA fragmentation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>22.16±0.68</td>
<td>49.26±3.01***</td>
<td>86.56±6.48***</td>
</tr>
<tr>
<td>ALT</td>
<td>182.5±0.93</td>
<td>37.44±2.48*</td>
<td>73.54±5.35***</td>
</tr>
<tr>
<td>ALP</td>
<td>12.74±0.41</td>
<td>28.17±3.08**</td>
<td>66.51±4.18**</td>
</tr>
<tr>
<td>TP</td>
<td>6.01±0.45</td>
<td>4.21±0.51***</td>
<td>2.87±0.08***</td>
</tr>
<tr>
<td>Albumin</td>
<td>2.81±0.18</td>
<td>2.11±0.13***</td>
<td>1.67±0.22***</td>
</tr>
<tr>
<td>Globulin</td>
<td>4.73±0.34</td>
<td>1.97±0.21*</td>
<td>1.18±1.572**</td>
</tr>
<tr>
<td>Glucose</td>
<td>72.54±6.52</td>
<td>95.78±7.76*</td>
<td>138.54±11.38**</td>
</tr>
</tbody>
</table>

* showed significant at (p<0.05). **, *** showed significant at (p<0.001).

The present study showed a significant decrease in liver protein content after exposed to different doses of malathion. Remia, Logaswamy [30] attributed that decrease to increase of proteolysis and metabolism under pollutants stress, or due to its using to alleviate the energy demand under stress [38]. That decrease in protein contents confirmed the intoxication induced by Malathion, which disturbed cell function [39]. Similar results were obtained for Clarias gariepinus [22] and Labeo rohita [40, 41] after pesticides exposure.

Antioxidant enzymes protect cells from increased Reactive Oxygen Species (ROS) level [42, 43]. High production of ROS was reported in our study due to increase in ROS level and that’s caused simultaneous increase in antioxidant enzymes activities. The antioxidant defense system of Oreochromis niloticus that exposed to malathion showed alteration in terms of elevation of CAT, SOD, GST and GH activities and declining in TAO. However, oxidative stress showed a significant increase of LPO, CP and fragmented DNA.

Increase of CAT activity in liver of Oreochromis niloticus as its response to scavenge H2O2 primarily as a result of increase in the production of free radicals due to malathion exposure. Catalase was used to catalyze H2O2 conversion to molecular oxygen and water in biological system protection against ROS [8, 44]. Also, SOD activity elevated as its association of superoxide anions production. [45] report the mechanism of superoxide anion radical’s conversion to H2O2 and molecular oxygen by SOD catalyzes to protect cells against oxidative damage induced by superoxides.

The central role of GSH in modifying oxidative stress induced malondialdehyde production, as it works as a reducing substrate in oxidative reactions [46]. So, GSH provide the premier protection against cellular damage that cause by ROS [37]. Malathion exposure elevated GSH content in rohu hepatocytes [46]. These results similar to that of Ibrahim and Harabawy [8], Srivastava and Reddy [49], Ullah, Zuberi [50].

The present study showed a significant increase of GST level. Li [51] attributed this increase to detoxify and remove ROS. Similar results were observed by Ibrahim and Harabawy [8] in Clarias gariepinus, Ullah, Zuberi [40] in Labeo rohita and Ullah, Husan [41] in Labeo rohita after exposed to different types of pesticide.

On the other hand, TAO showed a significant decrease in treated Oreochromis niloticus with different doses of malathion. That decrease of hepatic total antioxidant capacity due to the overproduction of free radicals during pesticide detoxification [50, 52].

Shen and Liu [53] suggested that apoptosis activation cause by Oxidative stress and ROS production. These oxidative stress attack the integrity of DNA in nucleus [54]. In the present study, DNA fragmentation percentage showed a significant increase in liver of treated groups comparing with the control fish. That damage of DNA due to increases in ROS production [55]. DNA fragmentation reflects the breaks of DNA strand that induced by oxidative stress due to malathion exposure. Similarly, Moradi, Mozdarani [56] observed a significant elevation in DNA damage in Cyprinus carpio that exposed to malathion. Ibrahim and Harabawy [8] also reported that increase of DNA damage in Clarias gariepinus that exposed to carbophuran.

The protein oxidation caused formation of carbonyl protein, as a result of direct attack of ROS to protein [57]. Carbonyl proteins formation is nonreversible, causing conformational changes and ultimately resulting.
owing to elevate susceptibility to protease action, in breakdown of proteins by proteases [58]. Our results showed a significant elevation in CP after exposed of *O. niloticus* to malathion. Similar result was obtained by Vineetkumar and Muniswamy [59], who found that protein carbonyl levels increased as an indicator for malathion intoxication that induced disruption in cellular protein metabolism.

As malathion is lipophilic in nature. Its accumulation in membranes generating free radicals production causing increase in LPO and hepatocytes damage. LPO is the highly resultant harmful attacks by free hydroxide radical through Fenton reaction [40], resulting of oxidative damage to different tissues [60]. Lipid per oxidation increases in current study suggests that the antioxidant enzymes cannot scavenged ROS totally, and reflected that malathion caused LPO due to free radicals production, which induced molecular and clastogenic damage [61]. Our results similar to that of Ullah, Zuberi [40] who exposed different fish species cypermethrin, and Ibrahim and Harabawy [8] to carbofuran.

Liver is very important organ for detoxification of xenobiotics. Therefore, the alterations in liver are nothing but reflections of toxic effects on contaminants. However, toxicant (malathion) elsewhere a certain concentration can disturb liver normal mechanisms, which accordingly caused severe histopathological changes as in this study. The importance histological studies in ecotoxicology due to rapid response of tissues to contamination or pollutants. Normal structure of hepatocytes control group with central nuclei. The present study of expose of *O. niloticus* to malathion caused many morphological changes in the liver, which considered as pollutant biomarker [62], Pugazhvendan, Narendiran [63], in their studies, observed normal histological structure of liver in control fish but the histopathology of experimental fish liver degenerative and scattered with necrosis of hepatic cells.

The detoxifying function of liver against pesticides during metabolism, alerted its histopathological form. Many authors have reported pesticides induced histopathological changes in liver of different fish species (Benli and Özkul [64] on *Oreochromis niloticus*, Tabassum, Ashfaq [65] on *Channa punctata* and Ullah, Zuberi [50] on *Labeo rohita*).

**Conclusion**

Generally, the present study showed that exposure of *O. niloticus* to two different doses of malathion as a pesticide induced changes in biochemical and histopathological parameters and caused many disturbances in defense system as well as induction of oxidative stress parameters in fish of polluted ecosystems. So, the present results give a clear evidence about *O. niloticus* response to malathion exposure and reflect the ability of *O. niloticus* to be a good bioindicator for aquatic systems.

**Ethical statement**

All experiments were carried out in accordance with the Egyptian laws and University guidelines for the care of experimental animals. All procedures of the current experiment have been approved by the Committee of the Faculty of Science of New Valley University, Egypt.

---

**References**


34. Tendulkar M, Kulkarni A (2012) Cypermethrin-Induced Toxic Effect on GlycogenMetabolism in Estuarine Clam, Marcia Optima (Gmelin, 1791) of Ratnagiri Coast.Maharashtra, J. Toxicol. 3.


42. Lushchak VI (2011) Environmentally induced oxidative stress in aquatic animals. Aquatic Toxicology. 101: 13-30.


47. Regoli F, Principato G (1995) Glutathione, glutathione dependent and antioxidant enzymes in mussel, mytilus galloprovincialis, exposed to metals under field and laboratory conditions: Implications for the use of biochemical biomarkers. Aquatic Toxicology. 31: 143-164.


Author information

Ahmed Th. A Ibrahim (Ibrahim AT)
Zoology Department, Faculty of Science, New Valley University, Egypt

Corresponding Author:
Ahmed Th. A Ibrahim, Zoology Department, Faculty of Science, New Valley University, Egypt.
Email: Ahmed1983(at)yahoo.com; Ahmed1983(at)scinv.au.edu.eg; Tel: +2 0100 7221152