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EVALUATION OF MALATHION INDUCED OXIDATIVE STRESS IN *TILAPIA MOSSAMBICA*

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ABSTRACT
Malathion is a universal organophosphate pesticide and well known to cause severe disorders in metabolism, nerve poisoning, histology and biochemical aspects in non-targeted species including fish. The present study is aimed to find out the toxic effects of malathion on histopathological and oxidative damage in liver of a fresh water fish, *Tilapia mossambica*. The LC_{50} values of malathion for *Tilapia mossambica* (96 hours) were determined by probit regression analysis and was found to be 3.609 mg/L. Fishes were exposed for 21 days to three different sub lethal concentrations i.e. 1/10% (360µg/L), 1/20% (720µg/L) and 1/30% (1080µg/L) using ten specimens in each aquarium. To detect the hepatotoxic effects, changes in Lipid Peroxidation (LPO), antioxidant enzymes like Catalase (CAT), Glutathione peroxidase (GPx) Superoxide Dismutase (SOD), Glutathione-s-transferase (GST) and histopathological modifications were assayed. The activities of antioxidant enzymes like Catalase (CAT), Glutathione peroxidase (GPx) Superoxide Dismutase (SOD), Glutathione-s-transferase (GST) significantly increased in all experimental fish compared to control ($P<0.01$). The histological studies revealed significant degenerative changes like cellular swelling, hypertrophy, pyknosis aggregation of melano macrophages and necrosis in liver. Lipid peroxidation significantly increased in liver tissue of all experimental fishes in dose dependent manner. This study clearly revealed malathion as an effective hepatotoxic pesticide. High dose of malathion exposure was associated with histopathological damage and oxidative stress in liver. The study also underscores the significance of using histopathological and oxidative stress biomarker and recommends incorporating these parameters in Water Quality Monitoring Programs to evaluate the health status of the fish and ecosystem. Our results may provide valuable information for future studies on antioxidant system in aquatic environment.

KEYWORDS: Histopathology, Malathion, Oxidative stress, *Tilapia mossambica*

INTRODUCTION

Malathion is one of the most notorious toxic pesticides and is responsible for many severe pesticide-poisoning incidents around the world. Aquatic bodies are a major sink for municipal, industrial and other anthropogenic compounds (Reddy, P.B. and Baghel, B.S., 2010, Reddy, P.B. and Singh, R.K., 2011, Reddy, P.B., 2012[a]. Reddy, P.B., 2013). Of many chemicals released into the aquatic environment, pesticides have gained much consideration. Malathion is an organophosphate that blocks with the normal function of the nervous system which consequently can affect many additional organs and functions by generating reactive oxygen species (ROS) to induce oxidative stress (Reddy, 2016[a]; Ullah *et al*, 2016, 2018; Akbel *et al*, 2018). A number of physiological, biochemical, histopathological and molecular biomarkers are usually used to evaluate the toxic effects of pollutants as an early warning signal of negative biological effects on different animal species (Reddy, 2012[b], Reddy and Rawat, 2013; Reddy and Kusum, 2013; Reddy, 2016b).

However, many authors usually use antioxidant enzymes, lipid peroxidation (LPO) and histopathological biomarkers to evaluate the toxic effects in ecotoxicological research (Reddy, 2012b; Reddy, 2016b; Patil and Reddy, 2017; Awasthi *et al*, 2018; Sharma *et al*, 2018).

Being a vital organ, performing an extensive variety of body functions like detoxification, enzyme production, metabolism and homeostasis we selected the liver of *Tilapia mossambica* as target organ for the present study. The antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST) and glutathione peroxidase (GPx) along with lipid peroxidation are well established tool for assessment of oxidative stress caused by toxicants (Patil, A. and Reddy, P.B., 2017, Srivastava, B and Reddy, P.B, 2017, Sharma *et al*, 2018). We therefore conducted an experiment to study the toxic effects of Malathion on the liver *Tilapia mossambica* using histopathological, antioxidant enzyme system and malondialdehyde formation as substitute biomarkers.

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MATERIALS AND METHODS

Fish: The healthy and uniform sized juvenile fish, *Tilapia mossambica* (*Oreochromis mossambica*) with an average length of 11.82cm (±0.38) and weight of 36.27g (±1.84) were obtained from the local commercial aqua culture ponds irrespective of the sex and were acclimatized to the laboratory conditions for 15 days prior to the experiment. Physico-chemical characters of water were examined by following the APHA (1985). Commercial grade Malathion (50% EC, manufactured by Coromandal fertilizer limited, Coromandal house, pesticide division, Ranipet, Vellore (TN), India) was procured from the local market and stock solution was prepared in acetone and mixed in water to obtain required dilutions.

Experimental design: A group of 10 healthy and acclimatized fishes were exposed to diverse concentrations of pesticide Malathion to calculate the medium lethal concentration LC50 value. Dose-response curves and LC50 values were calculated by probit analysis (Finney, 1971) and all graphs were plotted by using xlstat in windows10. The LC50 values corresponding to 12, 24, 48, 72 and 96 h of exposure of Malathion in the present study were 7.681, 5.929, 4.767, 4.066, and 3.609 mg/L, respectively. Fishes were exposed for 21 days to three different sub lethal concentrations i.e. 1/10% (360µg/L), 1/20% (720µg/L) and 1/30% (1080µg/L) using ten specimens in each aquarium. The aquarium water was changed for every 24 hours with freshwater in order to avoid the accumulation of excretory materials of animals and probable biodegradation products of pesticides. Animals were kept in well-aerated aquaria in a quiet and well-ventilated room, crowding was avoided, sufficient amount of nutritious food was provided, fishes were hold softly and only when necessary.

Histopathological biomarkers: After termination of experiment, few fish from each group were sacrificed and liver tissue was taken out. It was fixed in 4% calcium formol fixative at 4^0C for one week before being dehydrated in graded ethanol series, cleared in xylene series and finally embedded in paraffin wax (melting point 65^oC). The tissues were sectioned at 8u on a rotary microtome and stained with haematoxylin and eosin (H&E). Histopathological analysis was performed under a light microscope (NIKON ECLIPSE E 400, USA) and photographed by using digital camera attached to the microscope.

Oxidative stress markers: The liver tissue was homogenized using homogenizing buffer (50mM Tris-HCl mixed with 1.15 KCL and pH adjusted to 7.4) using a motor-driven Teflon Potter– Elvejhem homogenizer. The resulting homogenate was centrifuged at 10,000 g for 15 min. The residual supernatant was used for enzymatic assays by using a UV-VIS spectrophotometer (Systronics, Type, 119). Catalase activity (CAT, µmol/ min/ mg protein) was assayed by following Chance and Maehly (1955). The activity of Glutathione Transferase (GST) was determined according to Habig et al. (1974). Superoxide Dismutase (SOD, µmol/ min/ mg protein) was assayed by following Kakkar et al. (1984) with some modifications. Glutathione Peroxidase was estimated by following Mohandas et al. (1984). Lipid peroxidation was assayed by measuring malondialdehyde (MDA) formation by Thiobarbituric Acid Reactive Substances (TBARS) method (Armstrong, D. and Browne, R. 1994).

Data was analyzed by using computer software http://www.xlstat.com and have been presented as mean values ± standard error (S.E.).

RESULTS

Water parameters: There was no mortality in any experimental group during experimental period. The physico-chemical characteristics of the water for all the experimental periods remained constant and found to be within the limits of WHO standards. No significant differences were noticed between aquaria (Table 1).

Table 1. Physio chemical parameters of water used in the experiments. The values are represented as mean ± SD (n = 5).

Water parameters	Group I(control)	Group II	Group III	Group IV
Temperature (^{0}C)	22.66 ± 1.27	22.88 ± 2.01	23.01 ± 1.85	22.91 ± 2.08
pH	7.33 ± 0.12	7.12 ± 0.14	7.26 ± 0.21	7.42 ± 0.32
Dissolved oxygen (mgO2/ L)	7.10 ± 0.1	7.24 ± 0.02	7.3 ± 0.21	7.25 ± 0.11
Conductivity (µS cm^{-1})	104.1 ± 8.92	102.6 ± 9.93	109.6 ± 7.96	108.8 ± 8.77
Total hardness	250.1 ±11.1	243.3± 12.4**	239.8 ±12.7	247.6 ±9.67

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LC50 value: Twenty healthy individuals of Tilapia mossambica were tested against Malathion concentration for the determination of 96-hr LC50 value at constant temperature (27 ^{o}C) and pH (7.3) of water. The calculated 96 h LC50 value (95% confidence limits) of Malathion using a static bioassay to fingerlings of *Tilapia mossambica* was 3.609 mg/L.

Table.1. Effects of Malathion exposure on the activities of antioxidant enzymes GSH-Px (nmol of GSH oxidized/min/mg protein),), SOD (µmol/min/mg protein), CAT (µmol/min/mg protein), GST (µmol of chloro-2, 4-dinitrobenzyne conjugated formed/min/ mg protein)) in the liver of *Tilapia mossambica*. Data are represented as Mean ± SE (n = 8).

Oxidative markers	Group I (Control)	Group II	Group III	Group IV
LOP	11.96 ± 1.04	24.1 ± 2.31	42.69 ± 2.78	71.54 ± 4.14
CAT	211.18 ± 5.13	277.44 ± 4.96	309.65 ± 5.66	349.89 ± 6.42
SOD	144.22 ± 4.74	177.41 ± 5.16	219.29 ± 5.53	264.72 ± 6.14
GST	1387.0 ± 19.9	2364.0 ± 27.8	3565.21 ± 28.32	4907.29 ± 29.17
GSH-Px	1.98 ± 0.14	2.28 ± 0.18	2.86 ± 0.23	3.48 ± 0.42

Histopathological studies: In the present study, the control fish liver exhibited normal architecture of histology.

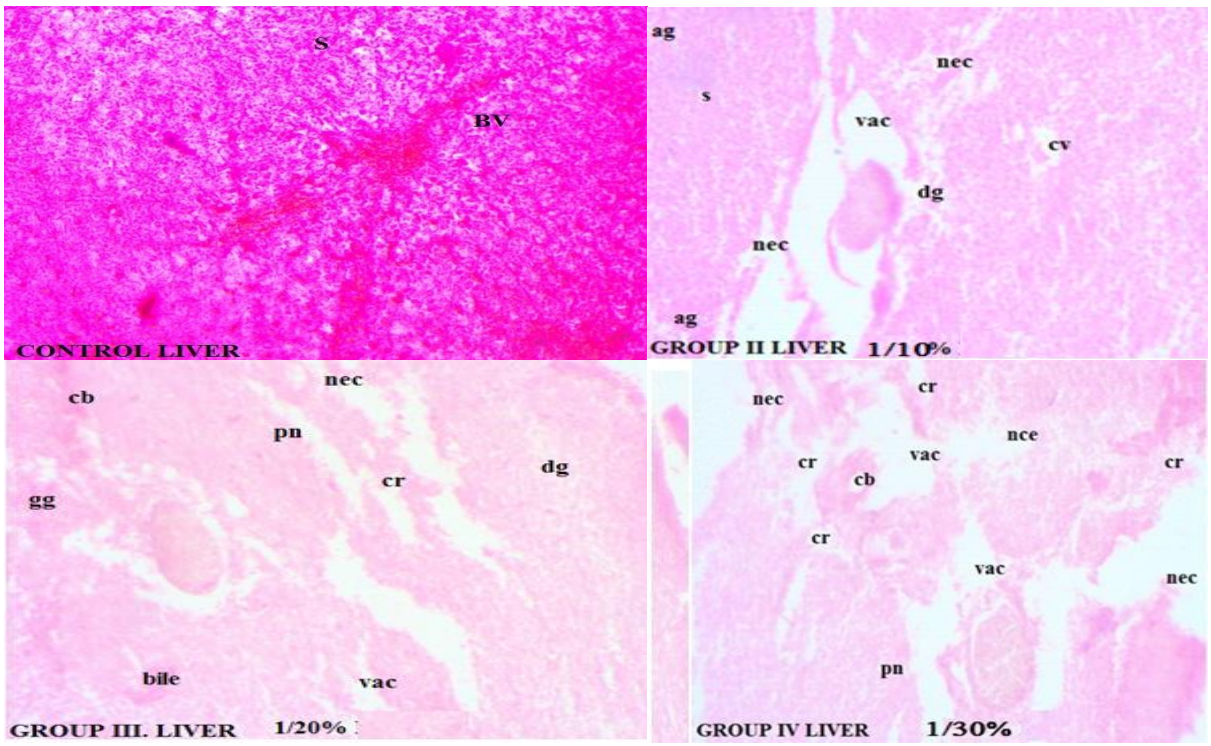

Figure 1. Effects of sub lethal concentration of Malathion on histopathology of liver of *tilapia mossambica*.
A. Control fish show normal structure.
B. Liver from Group II showing swelling. (x 1000)
C. Histology of liver of fish from Group III. Showing acute cellular swelling, hypertrophy and pyknosis
D. Showing acute cellular swelling, hypertrophy and pyknosis). (All sections are H&E Stained x400). (ag) aggregation of melanomacrophages, (S) sinusoid, (BV) blood vessel (V) central vein, (nec) necrosis, (vac) vacuolation, (dg) patchy degeneration, (cb) blood congestion, (pn) pyknotic nucleus, (cr) cellular rupture, (gg) glycogen granules.

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Oxidative enzymes and lipid peroxidation (LPO): The results of oxidative stress markers in liver of *Tilapia* are presented in Table 2. Results confirm that all four oxidative enzyme activities (CAT, GST and SOD) were significantly (P> 0.01) elevated in fish treated with 1/10%, 1/20% and 1/30% of Malathion in comparison with control. The results of LPO level of liver was also significantly increased ($P < 0.01$) in all experimental groups in with increasing the dose (P<0.001). The CAT activity significantly increased ($P < 0.01$) in the liver of all experimental fishes. Comparing the control value (211.18 ± 5.13 µmol/min/mg protein) the highest increase in CAT activity was observed (349.89 ± 6.42) in the liver of fish from Group IV. The GSH, GSH-Px and SOD activities were also followed similar pattern. The activities of all enzymes reached the maximum value in the liver of Group IV animals treated with 1/30% sub lethal concentration of Malathion. TBARS levels determined in the liver of fish from control group were approximately 11.96 ± 1.04 (µmol/ min/ mg protein MDA/g tissue) while it was 24.1 ± 2.31, 42.69 ± 2.78 and 71.54 ± 4.14 (µmol/ min/ mg protein MDA/g tissue) in treated group II, III and IV respectively.

Hepatocytes were large with centrally located nuclei, polygonal in shape and with uniform eosinophilic cytoplasm. However, the fish exposed to various sub lethal concentrations of malathion exhibited many alterations like, irregular-shaped nuclei, nuclear hypertrophy, vacuolation and the presence of eosinophilic granules in the cytoplasm. Blood sinusoids were also observed which detached hepatic cords one from another (Fig.1). Hepatic tissue was damaged from slightly to moderately in different groups according to the dose concentration. The damage symptoms are severe in the liver Group IV animals.

DISCUSSION

LC50 value: The calculated 96 h LC50 value (95% confidence limits) of malathion to fingerlings of *Tilapia mossambica* was 3.609 mg/L which is very much higher than 0.5925 µg/L in *Oreochromis mossambicus* (Tilapia) (Subburaj, A., et al, 2018) much less than 5.495 mg/L (*Tilapia mossambica*) (Sailatha *et al.*, 1981) and 5.6 mg/L (*Tilapia mossambica*) (Sahib and Rao, 1980). The variation in LC50 value of the same substance against same or different species, or the LC50 value of different chemicals against same or different fish species is maybe due to the design, formulation and stereochemistry of the pesticides or their active molecules (Al-Ghanim, K.A., 2012, Ullah, et al 2018). The intensity of toxicity also depends on water quality parameters, health, size, weight, and age of the fish (Reddy, .2012[a], 2016[b]). Besides, it is well known fact that an inverse relationship exists between body weight and pesticide toxicity (WHO, 1992).

Oxidative stress: The organophosphate pesticide malathion, is known to affect the normal function of organism by inducing oxidative stress (Ullah, M., et al, 2016, 2018, Akbel, E., et al, 2018). Histopathological and oxidative stress enzymes are sufficient for biomarkers studies and this has been confirmed tools for biomonitoring programmes as early warning signals of possible damage in aquatic ecosystems (van der Oost et al., 2003, Kumar, N et al, 2017, Kumari, K. and Khare, A., 2018). The outcome of the current study clearly reflects oxidative damage and decline in antioxidant defense due to malathion-induced oxidative stress. Lipid peroxidation (LPO) is considered as the extremely consequent harmful attacks by reactive oxygen species which result oxidative damage to tissues or organs (Ullah *et al*, 2016; Patil and Reddy, 2017; Srivastava and Reddy, 2017). The elevated LPO in the present study clearly revealed that malathion produced oxidative stress by producing excessive amount of free radicals which consequently resulted tissue damage (Valavanidis *et al*, 2006, Srivastava and Reddy, 2017, Awasthi *et al*, 2018). The results of the present study are in agreement with the result of the earlier studies on different fish species exposed to different pesticides including atrazine in *Channa punctatus* (Nwani *et al*, 2010), diazinon in *Oreochromis niloticus* (Üner *et al*, 2006), cypermethrin in *Oreochromis niloticus* and *Cyprinus carpio* (Üner *et al*, 2001), endosulfan in *Channa punctatus* (Pandey *et al*, 2001) and endosulfan in *Tilapia mossambica* (Patil and Reddy, 2017). The elevated levels of antioxidant enzymes in the present study clearly reflected the defence mechanism of fish, which it tried to resist, remove, or neutralize the toxic effects of reactive oxygen species (ROS) and to protect the system from oxidative stress. Living organisms have antioxidant enzymes to neutralize or to resist ROS.

The antioxidant enzyme system consists of different enzymes such as CAT, SOD, and GSH-Px, GST etc., which acts on antioxidants to maintain a stable ROS state/level. Nevertheless, under stress, antioxidant enzymes increased to help the cell to remove elevated ROS level. In the present study SOD activity increased significantly in the fishes of malathion treated groups may be to catalyze the conversion of superoxide anion radicals (induced by malathion) to H2O2 and molecular oxygen to protect the cell against oxidative damage. Catalase (CAT) is a key and sensitive biomarker enzyme in antioxidant protection system defending animals from oxidative stress (Srivastava and Reddy,

2017; Awasthi *et al,* 2018). The higher CAT activity in the present study indicates an increase in the scavenging capability of the fish against free radicals. GSH has a vital role in adjusting oxidative stress induced by LPO. It also provides secondary protection against induced oxidative stress by supporting a reduced state of the cell (Tort *et al,* 1996). The sub lethal concentration of malathion exposure increased the GSH content in the liver of *Tilapia*. The dose dependent increase in the GSH in the present study might be as a protective response of *Tilapia* against malathion induced oxidative stress. GSH-Px is considered as complementary to CAT that protects the cells by scavenging free radicals induced by malathion. The results of antioxidant enzymes of the present study are in agreement to the previous studies (Kavitha and Rao, 2007, Srivastava and Reddy, 2017: Narra *et al,* 2017: Ullah *et al,* 2018: Wang *et al,* 2018).

Histopathological studies: Histopathological symptoms are directly connected to the oxidative stress biomarkers, as many toxins have to undergo metabolic activation to stimulate cellular modifications in the affected organism. Histopathological examinations gained importance and has been established as a reliable biomarker in toxicological studies (Reddy and Rawat, 2013; Reddy and Kusum, 2013; Kumar *et al,* 2017; Ramesh *et al,* 2018). Liver is the chief organ for detoxification of xenobiotics. Therefore, the alterations in liver are nothing but reflections of toxic effect of contaminants. In the present study the liver from the control group did not show any alteration in structure (Figure 1). The liver sections of fish exposed to different sublethal concentration of malathion showed remarkable histopathological changes like necrosis, ruptured central vein, vacuolization, macrophage and lymphocytes infiltration, ruptured and degenerated hepatocytes. These antagonistic alterations in the liver can lead to severe physiological harms and in the end may cause death of the fish. The liver plays an important role in detoxification of harmful substances including pesticides. However, toxicant (malathion) beyond a certain concentration can interrupt the normal regulating mechanisms in the liver, which accordingly leads to severe histopathological alterations as in this study.

The current results are in analogous with earlier research studies revealing different histopathological changes induced by pesticides in the liver of different fish species including *Brachydanio rerio* (Rodrigues, E.D.L. and Fanta, E., 1998), *Heteropneustes fossilis* (Joshi et al., 2007), *Clarias gariepinus* (Velmurugan et al., 2009), zebra fish Danio Rerio (Bhuvaneshwari *et al,* 2015) *Channa punctata (*Tabassum *et al,* 2016), *Labeo rohita* (Ullah *et al,* 2018).

CONCLUSIONS

The results of the current investigation indeed point out that malathion causes oxidative stress in fishes. The elevated levels of CAT, SOD, GST and GPx in liver of exposed fish suggest the decisive role of these enzymes in cell protection against the toxic effects of pesticide and development of adaptive response to malathion toxicity. From the present results, it is concluded that oxidative stress may be credited to malathion induced hepatic toxicity. The present results also contribute to improve our knowledge about possible development of oxidative stress induced by malathion exposure in aquatic organisms. The biomarkers assayed in this study could provide useful information for evaluating the toxicological effects of malathion and other toxicants on the fish. Therefore, it is expected to include these biological parameters in biomonitoring program in areas polluted to assess the health of both aquatic organisms and ecosystem.

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