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Sublethal Malathion Exposure Disrupts Neurotransmitter Homeostasis and Redox Status in the Freshwater Fish, *Oreochromis mossambicus* (Peters, 1852)

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Abstract: The extensive use of malathion, an organophosphate insecticide, has raised concern as it is known to cause adverse health effects in humans and non-target organisms including fish. The lack of information regarding alterations in neurotransmitters by disrupting redox status in fish brain lured to investigate the sublethal effects of malathion in the freshwater fish *Oreochromis mossambicus*. The present study investigated the hypothesis by exposing sublethal concentration (50 ng L^{-1}) of malathion to the fish for 1, 4, 7, and 15 d along with the control group. Malathion exposure resulted in a negative allometric growth of fish as indicated by a decline in the condition factor. Exposure to malathion caused a significant ($P<0.05$) reduction in the activities of acetylcholinesterase, monoamine oxidase, and the level of 5-hydroxy indole acetic acid whereas the levels of other monoamine neurotransmitters like serotonin, noradrenaline, adrenaline, and dopamine increased than the respective control groups suggesting that the altered neurochemistry affects normal neurotransmission and brain functions. The current study showed a significant ($P<0.05$) reduction in the activities of antioxidant enzymes and total antioxidant capacity while elevating the levels of hydrogen peroxide, lipid peroxidation, nitrite, and peroxynitrite due to the overproduction of oxygen and nitrogen free radicals thereby leading to redox stress in fish brain. The profound effect on behavior and histological alterations like necrosis and vacuolization reflected neurotoxicity under malathion intoxication. Hence, the study recommends restricting the use of pesticide applications, and to prevent the release of untreated effluents in the vicinity of water bodies by creating buffer zones.

Keywords: Malathion, Brain, Neurotransmitters, Redox stress, Antioxidant enzymes, *Oreochromis mossambicus*, Histology, Behavior

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Introduction

Pesticide contamination in aquatic ecosystems has gained increasing attention in recent decades as chronic exposure and accumulation of such toxicants in aquatic biota adversely affect both target and non-target organisms. Malathion, an organophosphate insecticide, is widely used in residential landscaping and agriculture for the control of mosquitoes, therefore, becomes a part of the Malaria Eradication Programme in India (Dash *et al*., 2008). The Government of India in 2020 has banned the manufacture, import, and use of about 40 pesticides across the country, which do not include malathion. In India, the installed annual production capacity of malathion in the fiscal year 2021 has been reported as 4,000 metric tonnes. Malathion is used for the treatment of lice, where the application of 0.5% solution twice a week intervals kills human head lice, scabies mite, and crab lice (Idriss and Levitt, 2009).

Malathion has been known to enter the water bodies either by direct spray to control mosquitoes or indirectly through surface runoff from agricultural sites. The groundwater samples collected from the various hand pumps located in agricultural and industrial areas of Kanpur, Uttar Pradesh, India contained malathion at a maximum concentration of 29.835 µg L^{-1} (Sankararamakrishnan *et al*., 2005). The presence of malathion in the Sheonath river in the Rajnandgaon city of Chhattisgarh state, India occurred in a range between 1.7 and 2 ppm concentration (Thetwar *et al*., 2007). A recent meta-analysis and probabilistic risk assessment study has stated that malathion exists at a high level in drinking water followed by surface waters and ground waters (Vasseghian *et al*., 2022). The water solubility of malathion has been known to be 143 mg L^{-1} at 20ºC (Bowman and Sans, 1983). In addition, the high lipophilicity of malathion tends to accumulate in lipid-rich tissues like the brain causing neurotoxicity (Basarslan *et al*., 2014).

It has been reported that the half-life of malathion is 2 to 18 days in water, 17 days in soil, and 5 days in the air depending on the conditions like temperature and pH, and also readily gets absorbed through the skin, lungs and gastrointestinal tract based on the concentration and site of exposure (Menczel *et al*., 1983). According to one of the reviews on malathion toxicity in fish, the LC_{50} values of various fish species ranged between microgram to milligram level, and the United States Environmental Protection Agency (US-EPA 2016) has reported that the LC_{50} value of malathion in *Tilapia mossambicus* was 2000 ppb, which denotes 2 mg L^{-1} concentration (Deka and Mahanta, 2016). However, in another study 50% rate of mortality occurred in the fish, *Oreochromis mossambicus* after 0.5 µg L^{-1} concentration of malathion was exposed for 96 h (Shincy *et al*., 2020). Malathion exposure has been shown to cause serious adverse health effects on humans, and other non-target organisms including fish, amphibians, birds, reptiles, and mammals. Malathion exerts neurotoxicity through oxidative desulfuration that gets converted to malaoxon, which inhibits acetylcholinesterase in nervous tissues.

Some notable deficits like cholinesterase inhibition, alterations in the levels of brain neurotransmitters, sensory deprivation, and changes in the thyroid or gonadal hormone levels have been found associated with behavioral instability in fish (Pessoa *et al*., 2011). The behavioral change in an organism is an integrated output of the nervous system and is also considered an ideal endpoint reflecting a series of toxic effects and compensatory responses at the organism level. It also leads to biochemical, morphological, or physiological disturbances as an ultimate impact of neurobehavioural dysfunctions. The toxicity of most pesticides including organophosphates is associated with the production of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), which results in redox stress. Currently, there is a scarcity of scientific data regarding the effects of malathion on the levels of neurotransmitters and its

associated free radical generation that influence the overall health and well-being of the fish. Hence, the present study was designed to focus on the gap area in the brain of the freshwater fish, *Oreochromis mossambicus*.

Materials and Methods

Acclimatization of test animal:

Oreochromis mossambicus (8 ± 1.5 g; 7 ± 1 cm), were collected from the Angelfarm, Kunnamangalam, Kozhikode district, Kerala, India. They were transported to the laboratory and kept in glass tanks for 30 min - 1 h to allow the temperature to equalize. Then fish were acclimatized to the laboratory conditions for at least two weeks before the experiment, and those found unhealthy were removed from the study.

Glass tanks (40 L capacity) holding well-aerated, dechlorinated water having a constant photoperiod (12 h: 12 h light:dark) was maintained throughout the study. During acclimatization, fish were fed with commercial fish pellets twice a day (10:00 am and 5:00 pm). Fish were maintained in well-aerated, dechlorinated, and semi-static water, which was changed every alternate day to reduce the accumulation of toxic ammonia. Besides, probable mortality was recorded daily, and dead ones were removed from the tanks. The care and use of the fish complied with the Animal Welfare Board of India and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) under the Ministry of Environment, Forest, and Climate Change, Government of India.

Preliminary tests:

All physico-chemical parameters of the tap water were maintained according to the standard protocols as prescribed in APHA guidelines (APHA, 1998) to ensure that the dilution in the test medium never influences the test results. Water temperature (28 ± 3 °C), pH (7.5 ± 0.4), dissolved oxygen (8.64 ± 0.6 mg L^{-1}), and salinity (<100 ppm) were maintained during the entire study period. The chemical analysis ensured the water quality for the survival and growth of fish without any sign of stress, and care was taken not to exceed the limits specified in OECD guideline 203 throughout the experimental duration (OECD, 1992).

Chemicals:

Malathion 50% EC was purchased from National Pesticides and Chemicals, Amravati, Maharashtra, India. Pyrogallol, nicotinamide adenine dinucleotide phosphate (NADPH), ethylene-diaminetetraacetic acid (EDTA), glutathione oxidized, sodium azide, glutathione reductase, glutathione reduced, horseradish peroxidase, trichloroacetic acid, malondialdehyde, gallic acid, Ellman's reagent, O-phthaldialdehyde (OPT), sodium sulphite, and benzylamine were obtained from Himedia Research Laboratories, Mumbai, India. The ELISA kit of 5-hydroxy-3-indoleacetic acid (5-HIAA) was purchased from Immuno Biological Laboratories Inc, Minneapolis, USA. All other chemicals were of analytical grade and obtained from local commercial sources.

Grouping and design of experiment:

The median lethal concentration (LC_{50}-96 h) of malathion determined using probit analysis was 500 ng L^{-1} concentration, which coincided with the previous studies (Karmakar *et al*., 2016; Shincy *et al*., 2020). In the present study, one-tenth of LC_{50} value i.e., 50 ng L^{-1} was chosen as the sublethal concentration. After acclimatization, fish (N=100) were randomly categorized into separate aquarium glass tanks (40 L; 30 cm width × 60 cm length × 30 cm depth) with a density of 20 fish per tank (10 fish, in replicates, for each treatment). The fish were not fed during the treatment period in all experimental groups. Fish were grouped into five, a control and four malathion-treated groups. Fish were exposed to malathion at 50 ng L^{-1} concentration for 1, 4, 7, and 15 d, along with a control group without toxicant.

Behavioral analysis:

Behavioral changes in the fish after malathion exposure were monitored continuously for 2 to 3 h immediately after the toxicant exposure, and for 1 h continuously at every 24 h interval (Eissa *et*

al., 2010), and the scoring pattern was followed as described by Sahu *et al*. (2018).

Collection, preparation, and analysis of tissue samples:

Fish were sacrificed by decapitation and weighed with and without mucous deposition to find the percentage of mucous production. The whole brain tissue was dissected out, cleaned in cold normal saline, weighed, and divided into three portions. One portion of brain tissue homogenate (1% w/v) was prepared in ice-cold isolation buffers (0.1 M phosphate buffer, 0.3 M sodium acetate, 0.1 M hydrochloric acid-butanol, 0.1 M tris buffer) separately using a motor-driven tissue homogenizer on crushed ice for 1 min. All homogenization and centrifugation steps were performed in the prescribed isolation medium of the assays. The supernatants were separated and were used after 30 min of recovery and within 4 h of preparation for the analyses of neuro-transmitters such as acetylcholinesterase activity (Ellman *et al*., 1961), serotonin, dopamine, noradrenaline, and adrenaline concentrations (Schlumpf *et al*., 1974), and activity of monoamine oxidase (Christ *et al*., 1973). The quantitative determination of 5-hydroxy-3-indoleacetic acid (5-HIAA) was assayed using a commercial ELISA kit (Immuno Biological Laboratories Inc, Minneapolis, USA) strictly according to the manufacturer's protocol.

Another portion of brain tissue homogenate (1% w/v) was prepared in ice-cold 0.9% normal saline using a motor-driven tissue homogenizer on crushed ice for 1 min. The brain homogenate of all experimental groups was centrifuged at 800 *g* for 15 min. The supernatants were then used for biochemical analyses such as superoxide dismutase (Marklund and Marklund, 1974), catalase (Claiborne, 1985), glutathione reductase (Carlberg and Mannervik, 1985), glutathione peroxidase (Mohandas *et al*., 1984), hydrogen peroxide generation assay (Pick and Keisari, 1981), lipid peroxidation (Ohkawa *et al*., 1979), and total antioxidant capacity (Prieto *et al*., 1999). The total protein contents were determined according to the method of Lowry *et al*. (1951). The third portion of brain tissue was processed for histological analysis (Roberts and Smail, 2001).

Statistical analyses:

Statistical analyses were performed using the International Business Machine Corporation-Statistical Package for the Social Sciences (IBM-SPSS) software, SPSS v24.0. The statistically significant difference between the means of different groups was compared using a one-way analysis of variance (one way-ANOVA) followed by Duncan's Multiple Range test as the post-hoc test. Data were represented as a box plot for ten animals per group in replicates. In the box plots, the boundary of the box closest to zero indicates the first quartile, a black line within the box marks the mean, and the boundary of the box farthest from zero indicates the third quartile. Whiskers above and below the box indicate the tenth and ninetieth percentiles. The point above and below the whisker indicate outliers outside the tenth and ninetieth percentile. Asterisks (*) in Figures denote significant ($P<0.05$) against the control group.

Results

Length-weight relationship and condition factor of the fish:

The body weight of the fish *Oreochromis mossambicus* showed a significant reduction after 7 and 15 d of malathion exposure when compared with the corresponding control group (Table 1). However, a slight reduction in body weight was observed after 1 and 4 d of malathion exposure to the control group (Table 1). The length-weight relationship and condition factor (K values) were found less than two, and the reduction occurred in a time-dependent manner (Table 1).

The relative weight of brain tissue:

The relative weight of brain tissue showed a significant ($P<0.05$) reduction after exposure to malathion in a time-dependent manner than that of the control group (Fig. 1).

Table 1: Length-weight relationship and condition factor of the fish, *Oreochromis mossambicus* after malathion exposure (n = 10/ group, in replicates)

Experiment groups		Final body weight (g)	Logarithmic equation (Log W = log a + b log L)	Correlation coefficient 'r'	Coefficient of determination 'r²'	Condition factor 'K'
Control		9.85	Log W = log 12.67 + 0.39 log L	0.952	0.9076	1.430
Malathion (50 ng L^{-1})	1 d	9.24	Log W = log 10.29 + 0.15 log L	0.836	0.6996	1.342
	4 d	9.21	Log W = log 9.60 + 0.084 log L	0.843	0.7109	1.338
	7 d	9.12	Log W = log 10.15 + 0.144 log L	0.891	0.7940	1.325
	15 d	9.11	Log W = log 7.92 + 0.099 log L	0.981	0.9628	1.323

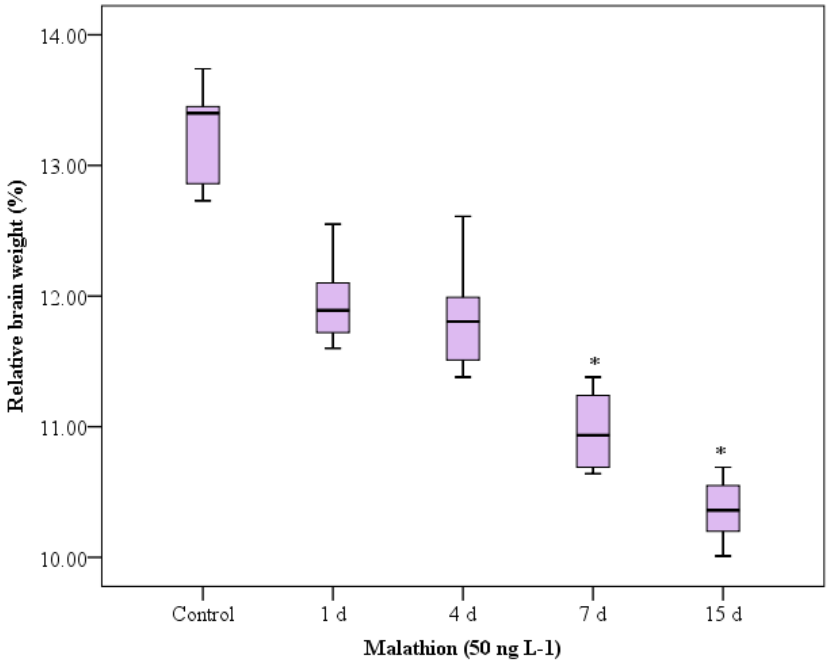

Fig. 1: Effect of malathion on the relative brain weight of the fish, *Oreochromis mossambicus* (n = 20, i.e., 10/ group, in replicates).

Table 2: Scoring of behavioral alterations in the fish *Oreochromis mossambicus* after malathion exposure

Behavioral parameters	Control group	Malathion-exposed groups			
		1 d	4 d	7 d	15 d
Surfacing and air engulping	-	++++	+++	++	+
Avoidance behavior	-	++++	++++	++++	++++
Cornering	-	-	++	+++	++++
Wall hitting	-	-	+++	+++	+++
Physical encounter	-	+	++	+++	++++
Mucus deposition	-	+	++	++++	++++
Loss of equilibrium	-	+	++	++	+++
Bottom settlement	-	-	+	+++	++++
Skin hemorrhage	-	-	-	+	++++
Swirling movement	-	-	-	-	++++
Exophthalmic eyes	-	-	-	+++	+++
Reduced opercular movement	-	++	++	++++	++++

+ denotes presence of behavioral modifications, and an increase in + sign indicates severity of the response

Behavioral changes:

Malathion exposure at 50 ng L^{-1} concentration for 1, 4, 7, and 15 d showed remarkable changes in the behavior of the fish. *O. mossambicus* when compared with the control fish. The severity of changes in the behavioral responses was more prominent on an increase in the exposure duration, which is shown in Table 2. Fish on initial exposure to malathion showed an immediate response that includes aggressive swimming, frequent surfacing and engulping of air, jumping or escaping tendency to avoid the toxicant (Fig. 2).

The avoidance response continued for a day, later in the day 4 exposure group, fish was observed with an increase in the gill opercular movement, erratic swimming, and knocking at the walls of tanks. Fish then changed the swimming pattern from surfacing to bottom settlement, however, a few fish showed aggressive behavior by hitting each other. On day 7 of malathion exposure, most of the fish were lethargic and settled towards the corner of the tanks with reduced movement. Fish exposed to malathion for 15 d showed skin hemorrhage, descaling, exophthalmic eyes, fading of colors, and reduced opercular movement. Fish maintained in the control group were active and healthy with normal swimming patterns and showed no abnormal behavior (Fig. 2).

Brain neurochemicals:

The activity of acetylcholinesterase enzyme in the brain tissue of the fish *Oreochromis mossambicus* decreased significantly ($P<0.05$) in all exposure duration of malathion (Fig. 3A). However, the levels of serotonin, noradrenaline, adrenaline, and dopamine increased significantly in all treatment groups in a time-dependent manner (Figs. 3B-E). Malathion exposure resulted in a significant ($P<0.05$) reduction in the activity of monoamine oxidase enzymes, which was duration-dependent (Fig. 3F). Similarly, the metabolite of serotonin, 5-hydroxy-3-indoleacetic acid (5-HIAA) also reduced significantly ($P<0.05$) after malathion exposure in a time-dependent manner than the control group (Fig. 3G).

Brain redox status:

The activities of the antioxidant enzymes such as superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase decreased significantly ($P<0.05$) after malathion exposure in a time-dependent manner when compared with

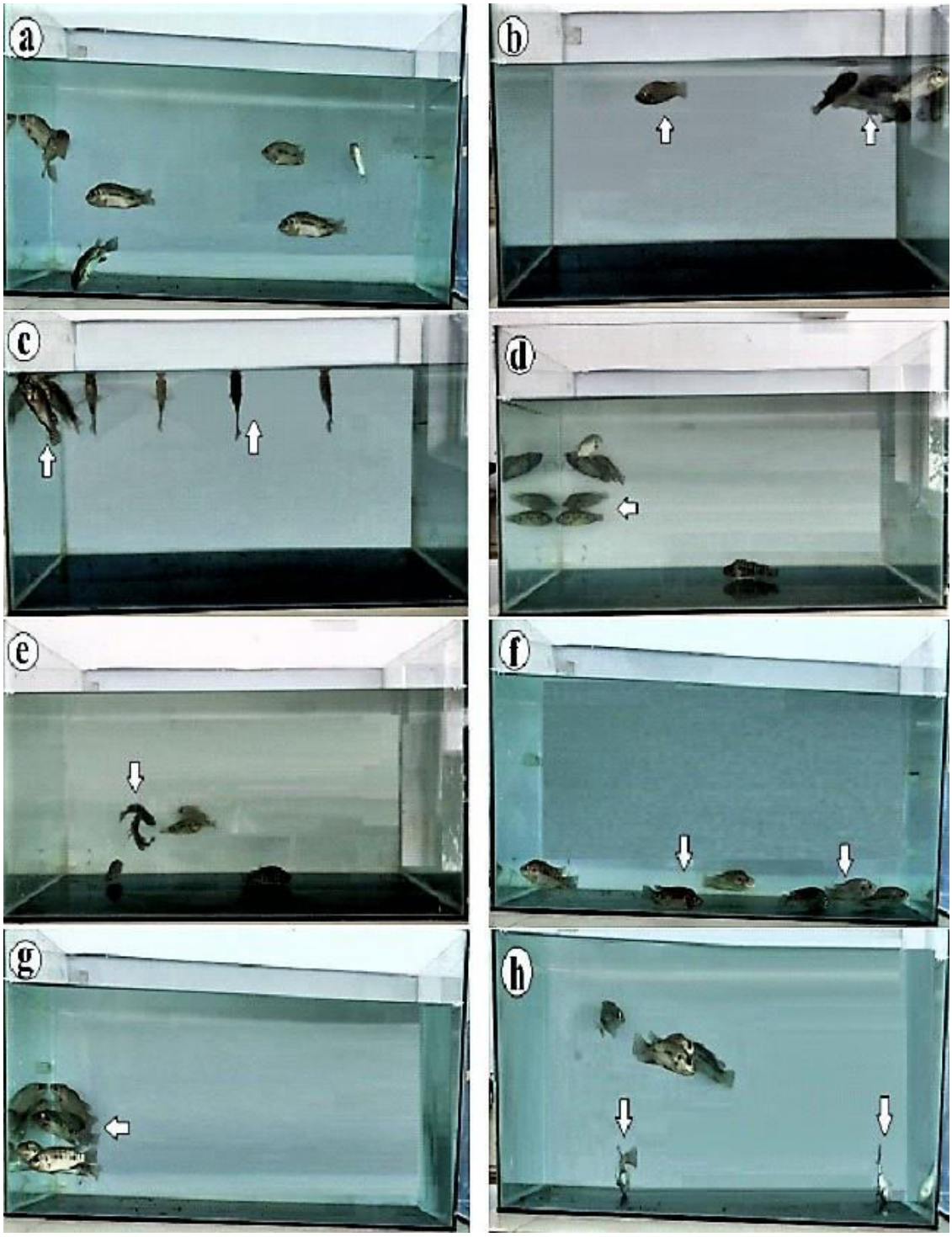

Fig. 2: Behavioural modifications in the fish, *Oreochromis mossambicus* after malathion exposure. (a) Control fish group; (b-h) Malathion-expoed groups; (b) Surfacing; (c) Air engulping; (d) Hitting on the walls of the experimental tank; (e) Hitting on each other; (f) Bottom settlement; (g) Cornering on the edge of the tank; (h) Swirling movement (Modifications are pointed out using white arrows).

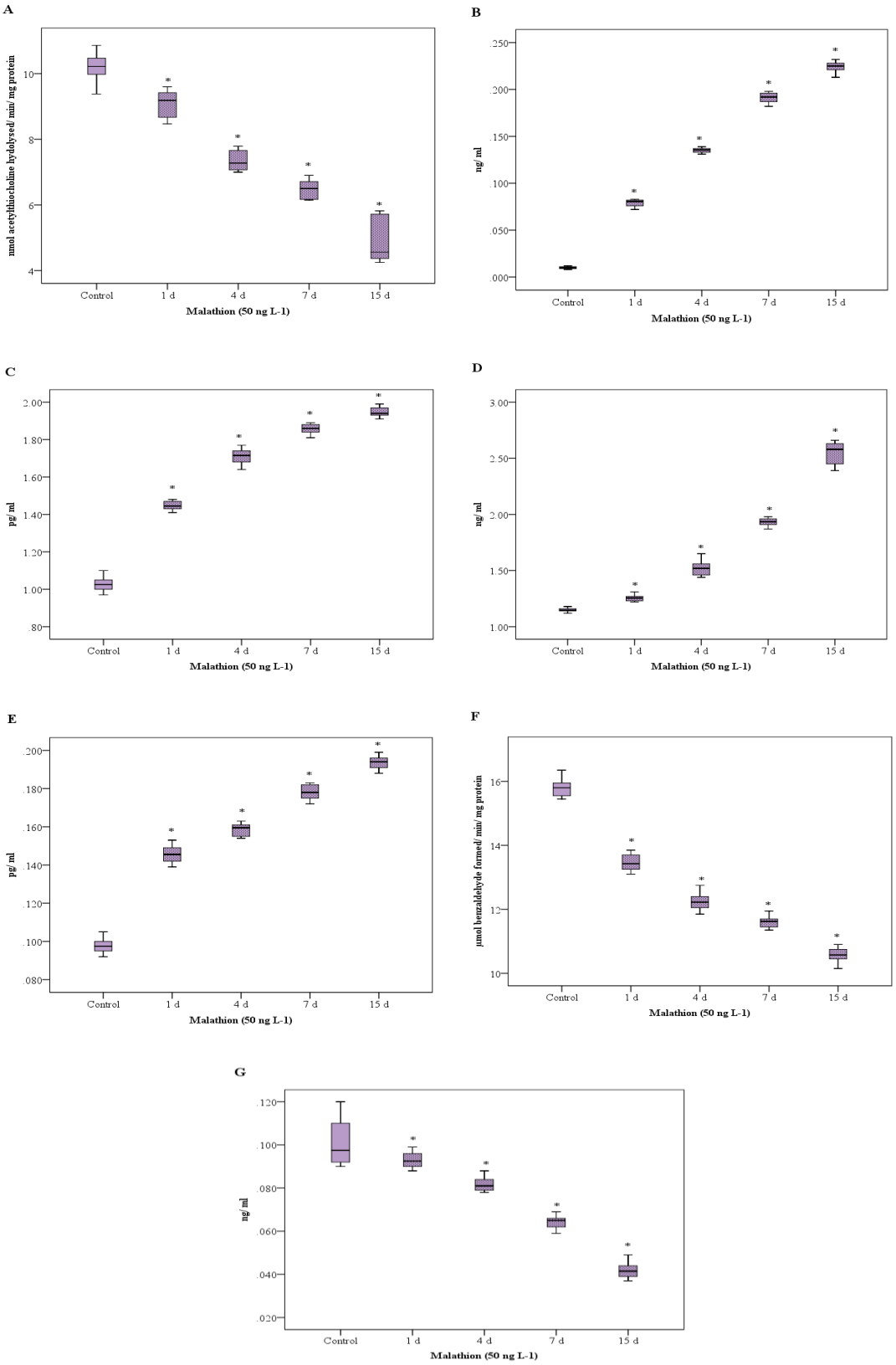

Fig. 3: Effect of malathion on brain neurotransmitters in the fish, *Oreochromis mossambicus* (n = 20, i.e., 10/ group, in replicates). (A) Acetylcholinesterase; (B) Serotonin; (C) Noradrenaline; (D) Adrenaline; (E) Dopamine; (F) Monoamine oxidase; (G) 5-hydroxy indole acetic acid.

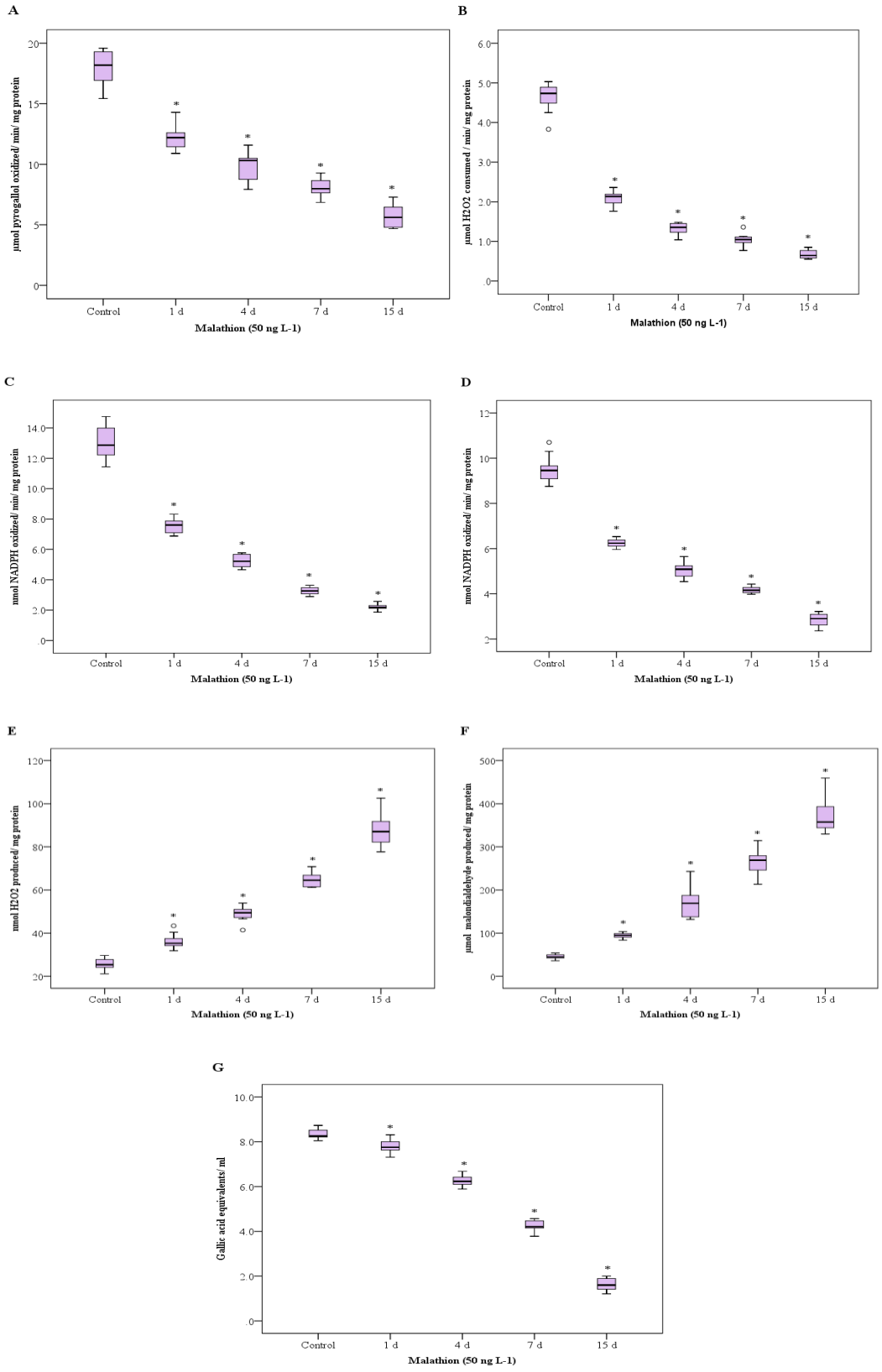

Fig. 4: Effect of malathion on brain redox status in the fish, *Oreochromis mossambicus* (n = 20, ie., 10/ group, in replicates). (A) Superoxide dismutase; (B) Catalase; (C) Glutathione reductase; (D) Glutathione peroxidase; (E) Hydrogen peroxide; (F) Lipid peroxidation; (G) Total antioxidant capacity.

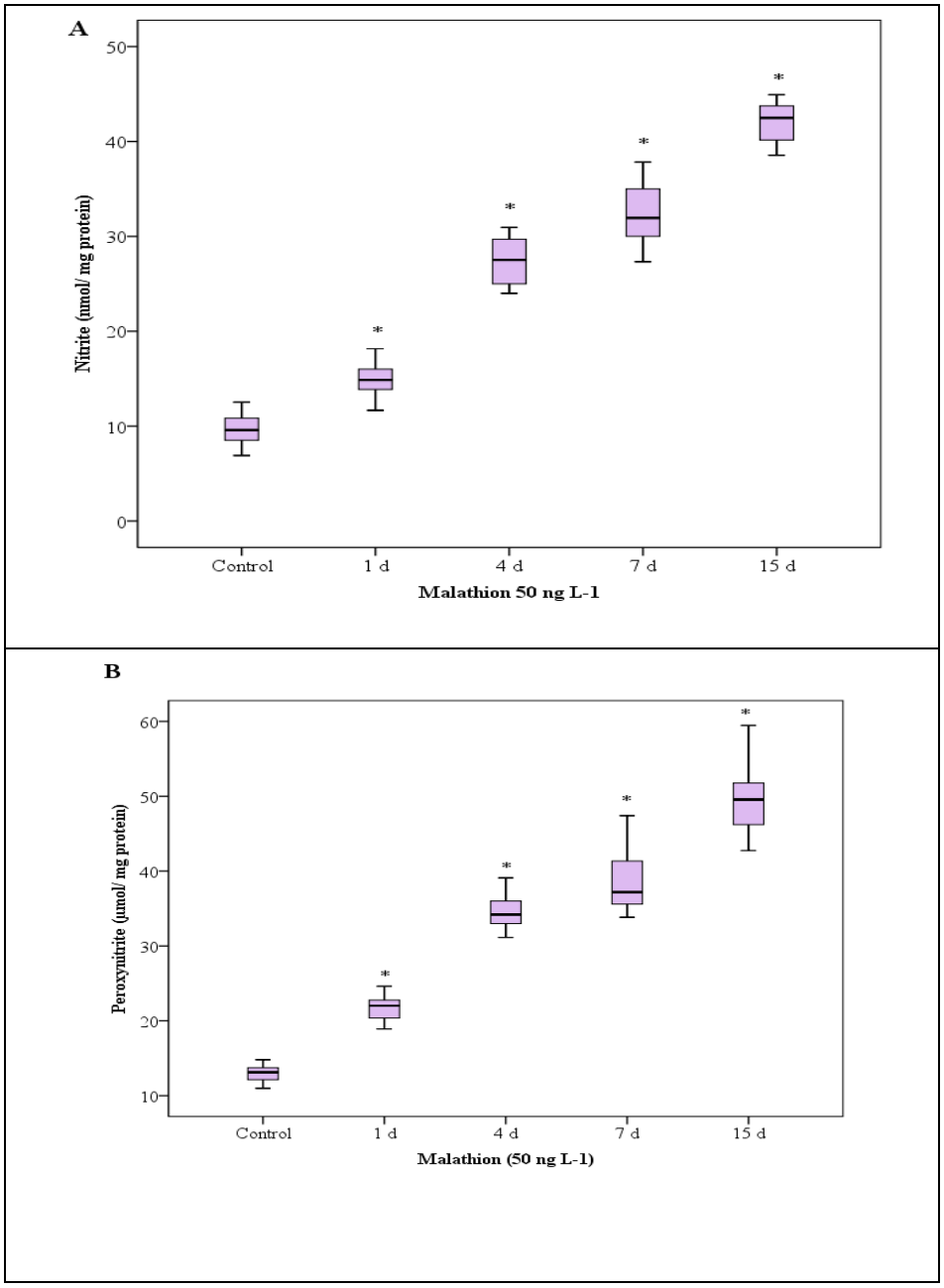

Fig. 5: Effect of malathion on the levels of (A) Nitrite; (B) Peroxynitrite in the brain of the fish, *Oreochromis mossambicus* (n = 20, i.e., 10/ group, in replicates).

the corresponding control groups (Figs. 4A-D). However, the levels of hydrogen peroxide, lipid peroxidation, nitrite, and peroxynitrite increased significantly ($P<0.05$) on an increase in the exposure duration (Figs. 4E-F; 5A-B). However, the total antioxidant capacity in the brain of the fish decreased significantly ($P<0.05$) after malathion exposure in a duration-dependent manner (Fig. 4G).

Histology of brain tissue:

The histological study examined both the optic tectum region of the mid brain and the cerebellar cortex regions of the hind brain. The optic tectum of control brain tissue showed distinct regions such as stratum marginale, stratum opticum, stratum fibrosum, stratum album, stratum griseum, and stratum periventriculae. Similarly,

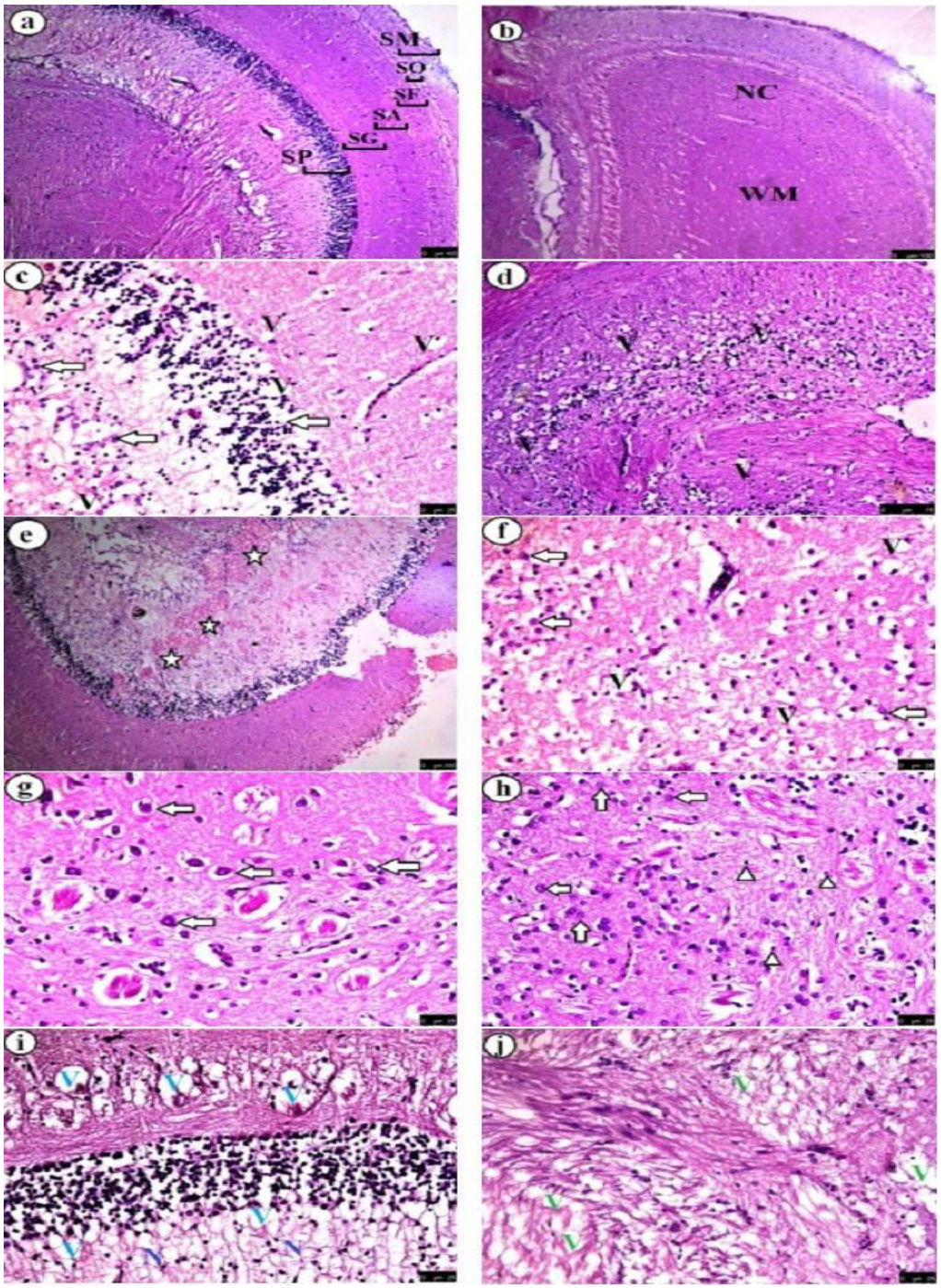

Fig. 6: Histology of brain tissues in the fish, *O. mossambicus.* (a) Control optic tectum, SM-Stratum marginale, SO-Stratum opticum, SF-Stratum fibrosum, SA-Stratum album, SG-Stratum griseum, SP-Stratum periventriculae; (b) Control cerebellar region, NC-Neuroglial cells, WM-White matter; (c and d) Malathion-exposed tissue for 1 d, Sparsed and disorganized nuclei (←); V-Vacuolization; (e and f) Malathion-exposed tissue for 4 d, Inflammatory cell infiltration (*), Karyorrhexic nucleus (←), Vacuolization (V); (g) Malathion-exposed tissue for 7 d, Oligodendroglioma (←); (h) Malathion-exposed tissue for 7 d, Pyknotic (Δ), karyorrhexic (←), karyolytic (↑) nucleus; (i and j) Malathion-exposed tissue for 15 d, Necrosis (N), Vacuolization (V).

the cerebellar cortex region of brain tissue showed compact neuroglial cells and white matter (Figs. 6a-b). Exposure to malathion for 1 d showed sparsed and disorganized nuclei in the optic tectum, and vacuolization in the cerebellar cortex (Figs. 6c-d). Malathion exposure for 4 d resulted in inflammatory cell infiltration in the optic tectum, while karyorrhexis nucleus and vacuolization

were prominent in the cerebellar cortex region (Figs. 6e-f). Fish exposed to malathion for 7 d, caused oligodendroglioma in the optic tectum whereas the cerebellar cortex was prominent with the formation of pyknotic, karyorrhexis, and karyolytic nuclei (Figs. 6g-h). Exposure to malathion for 15 d showed severe necrosis, vacuolization, and degeneration of neuronal cells in both regions (Figs. 6i-j).

Discussion

Pesticides used in the control of pests are known to reach non-targeted sites like air, soil, and water mainly from the agricultural fields as runoff, and alter the physico-chemical properties of water causing negative impacts on aquatic organisms. Many pesticides are resistant to environmental degradation so they persist in treated areas ultimately enhancing their effectiveness (Mojiri *et al*., 2020). The main route of pesticides to enter the fish occurs through skin epithelial absorption, gill operculum, or oral engulping thereby reaching gut and systemic circulation (Schlenk, 2005; Banaee, 2012). Pesticide intoxication causes damage to vital organs leading to several physiological and biochemical alterations (Banaee *et al*., 2011). The present study aimed to investigate the hypothesis that sublethal exposure to malathion could alter neurotransmitters and its possible role in the brain redox status of the freshwater fish *Oreochromis mossambicus*.

In toxicology studies, regular monitoring of body weight has been routinely performed to interpret compound-related toxic effects (Sreedevi and Chitra, 2014). The present study observed a progressive decline in the body weight of the fish *Oreochromis mossambicus* in the treatment groups, and this could be due to the stress attained after sublethal malathion exposure. Similar results have been observed in the fish *Oreochromis niloticus* after malathion exposure suggesting the loss of some body constituents as evidenced by histological analysis (Al-Ghanim, 2012). The health status of the fish was quantitatively evaluated by assessing the length-weight relationship and condition factor, which showed negative allometric growth in malathion-exposed groups.

The structural complexity and functional diversity of the fish brain allow it to control all functions in the effector organs of the body, which include memory, vision, hearing, hunger, thirst, emotion, etc. Evaluation of organ-to-body weight relationship or relative organ weight is recommended in toxicological studies to assess the tissue-specific treatment-related toxic effects. Fish exposed to malathion resulted in a reduction of relative brain weight, and this might be due to brain tissue damage such as necrosis, or atrophy as suspected in histological analysis (Chitra and Mohan, 2014). The structural or functional alterations in the nervous system hamper fish behavior that is mediated through biochemical, morphological, and physiological variations. Many factors are responsible for the changes in the normal behavioral pattern of fish, which includes exposure to predators, environmental cues, water quality, and toxicant exposure. Daily behavioral variations in the fish were monitored to examine the impact of malathion, and the changes were found related to the neurotoxic effects of the toxicant. *Oreochromis mossambicus* exposed to malathion exhibited an initial alteration in the swimming pattern as evident by aggressive swimming and frequent surfacing to gulp air and jumping or escaping tendency to avoid the toxicant. Air engulping and surfacing phenomena are the avoidance behavior that indicates a need for more oxygen to avoid the stress induced by the toxicant. Likewise, *Catla catla* exposed to an organophosphate insecticide, dimethoate resulted in jerky moments and frequent gulping of air (Hussain *et al*., 2015).

An increase in the treatment duration for 4 d showed an increase in gill opercular movement, erratic swimming, knocking at the walls of tanks, and this unsteady swimming pattern of fish fails to maintain posture and equilibrium. Later, fish changed their swimming pattern from surfacing to bottom settlement while a few fish still showed aggressive behavior by hitting each other. *Labeo*

rohita exposed to a biopesticide sank to the bottom of the tank and became motionless (Bhat *et al*.**,** 2012). The bottom settlement, lethargy, and moving towards the corner of tanks were more prominent when the exposure duration was increased to 7 d illustrating the adverse toxic effects of malathion.

In the 15 d malathion exposure group, fish showed hemorrhage and descaling of skin, exophthalmic eyes, fading of colors, and reduced opercular movement as detrimental behavioral alterations caused by the neurotoxic effects of the exposed compound. The abnormal behavior observed in malathion-exposed fish perhaps indicates alterations in the level of brain neurotransmitters (Ramesh and Muniswamy, 2009; Sumi and Chitra, 2019). Similar abnormal behavioral responses like erratic swimming, surfacing, rapid gill opercular movement, air gulping, and bottom settlement have been observed in the fingerlings of European catfish, *Silurus glanis* exposed to the organophosphate pesticide dichlorvos (Ural and Koprucu 2006). Malathion exposure also increased the deposition of mucus, and this is probably due to irritation of the skin in direct contact with the toxicant. It is a defensive mechanism of the fish to prevent the entry of the toxicant. Similarly, sublethal exposure of chlordecone to *Etroplus maculatus* showed an increase in mucus deposition stating the formation of physical and chemical barriers on the epidermal surface to defend against the toxicant (Asifa *et al*.**,** 2014).

Neurotransmitters are endogenous chemical messengers that perform several functions including initiation, amplification, and modulation of nerve signals between neurons and other cells in the body through the chemical synaptic transmission process (Olesti *et al*.**,** 2019). Acetylcholine (ACh) is a signaling molecule that performs neurotransmission at neuromuscular junctions and in the brain (Panula *et al*.**,** 2010). The signaling molecule ACh has been known to activate two classes of receptors namely ionotropic nicotinic ACh receptors (nAChRs) and G-protein-coupled muscarinic AChRs (mAChRs). The role of nAChRs is to modulate glutamate release (Kabbani *et al*.**,** 2013), and memory formation (Kenney *et al*.**,** 2010) whereas the mAChRs are involved in neurotransmission, neuromodulation (Brown 2010), and olfactory mechanisms (Durand *et al*.**,** 1998). The ACh-mediated signaling is regulated by the activity of the acetylcholinesterase enzyme, which is a serine hydrolase related to the type B carboxylesterase family that cleaves acetylcholine into choline and acetate (Dobransky and Rylett, 2003). The present study observed that malathion exposure inhibited acetylcholinesterase activity in brain tissues of the fish *Oreochromis mossambicus* indicating organophosphate toxicity, and also associated with behavioral changes.

The aminergic neurotransmitters include dopamine and serotonin, among which dopamine mediates several important brain functions like locomotion, cognition, emotion, and reward mediated through G-protein coupled receptors (Schultz, 2002). Serotonin modulates brain physiology and behavior and plays an important role in regulating perception, aggressiveness, anxiety, sexual behavior, appetite, vascular function, and pain (Lucki, 1998). Despite neural communication, serotonin also plays a vital role in the influence of plasticity in the fish brain (Li *et al*.**,** 2011). In the present study, malathion exposure increased the levels of dopamine and serotonin in a time-dependent manner suggesting general behavioral inhibition, including suppression of aggressive behavior, feeding, and locomotion (Winberg and Nilsson, 1993). During the study, fish exhibited bottom settlement, lethargy, and cornering in the fish tank on an increase in exposure duration, and this could be due to elevated levels of aminergic neurotransmitters.

The catecholamine hormones, adrenaline, and noradrenaline are routinely measured in the brain of fish as indicators of the adrenergic stress response (von Euler, 1961). The current study documented an increase in the levels of adrenaline and noradrenaline in the brain tissues of fish

exposed to malathion, which denotes the stress response. Exposure to stressors augments the sympathetic and adrenomedullary synthesis, and the concentration of tyrosine hydroxylase enzyme catalyzes catecholamine biosynthesis in the presence of pyridoxal phosphate as a cofactor (Goldstein, 2010). Thus the elevated levels of catecholamines after malathion exposure can be considered an important component of the stress response. Besides, an increase in serotonin has been shown to stimulate the levels of adrenaline and noradrenaline in the rainbow trout indicating serotonin-mediated catecholamine release (Fritsche *et al*.**,** 1993).

Monoamine oxidases (MAO) catalyze the oxidative deamination of several endogenous and dietary amines, including dopamine, serotonin, norepinephrine, and the first histamine metabolite, tele-N-methylhistamine that plays a major role in neuronal and neurocognitive abnormalities in fish (Brunner *et al*.**,** 1993). Malathion exposure inhibited the activity of monoamine oxidase in the brain of the fish *Oreochromis mossambicus*. There is evidence that neurotoxicity caused by an excess serotonin concentration has been linked with decreased MAO activity, and more likely developed antisocial behavior in zebrafish (Sallinen *et al*.**,** 2009). In the present study, malathion exposure also showed a similar pattern of MAO inhibition by hyperserotonemia in the brain along with behavioral impairment indicating neurotoxicity in the fish. Malathion exposure decreased the level of 5-hydroxy-3-indoleacetic acid (5-HIAA), a metabolite of serotonin in the brain of the fish, and this is due to the inhibition of the monoamine oxidase enzyme, which failed to convert serotonin into its metabolite. The decline in brain 5-HIAA has been found associated with inhibition of aggressive behavior (Miczek *et al*.**,** 1989), which is in agreement with the present findings after malathion exposure.

The toxicity of most pesticides including organophosphates is associated with the production of free radicals, which alters redox status and thereby damages all components of cells including carbohydrates, proteins, lipids, and nucleic acids with consequent loss of their biological functions (Livingstone, 2003). Fish have a well-developed antioxidant defense system that is highly sensitive to variations in environmental conditions. The alterations in the antioxidant system are used as a biomarker to detect environmental pollution, which disturbs the balance between pro-oxidants and antioxidants with the production of free radicals. The toxic effects of free radicals were neutralized by the action of several antioxidant enzymes thus maintaining normal cellular oxidative homeostasis (Halliwell and Gutteridge, 1999; Yang and Lee, 2015).

In the present study, malathion exposure at sublethal concentration decreased the activities of superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase enzymes in the brain tissue of the fish in a time-dependent manner than the respective control groups. Superoxide dismutase and catalase are the first-line defensive enzymes against the production of oxygen free radicals (Pandey *et al*.**,** 2003). Malathion exposure resulted in uncontrolled production of free radicals that exceed the antioxidant capacity in the brain tissue of exposed fish, and this is mainly because of highly rich polyunsaturated fatty acids rendering brain tissue highly vulnerable to oxidative damage, and hence lipid peroxidation occurs (Aitken *et al*.**,** 2010).

The excessive production of hydrogen peroxide after malathion exposure contributed to the decline in superoxide dismutase enzyme activity, while the superoxide anion produced due to the failure of the enzyme has declined the activity of the catalase enzyme (Bagnyukova *et al*.**,** 2006). The present findings demonstrated that brain tissue was unable to neutralize the ROS produced by malathion exposure, which in turn could have reacted with membrane lipids for the production of lipid peroxidation thereby leading to oxidative stress (Hermes-Lima, 2004). The present study observed that malathion decreased

total antioxidant capacity in brain tissue as a consequence of oxidative damage. Another study has demonstrated that exposure to heavy metals decreased antioxidant capacity in the zebrafish brain thereby mediating impairment of cholinergic signal transmission by the inhibition of the acetylcholinesterase enzyme (Richetti *et al*., 2011). Exposure to malathion elevated the level of nitrite, one of the stable metabolites of nitric oxide demonstrating nitrative stress whereas an increase in peroxynitrite contributes to damage brain tissues (Radi, 2013).

The structural modifications in brain tissue after malathion exposure was assessed by histological analysis. The study examined both the optic tectum region of the midbrain, and the cerebellar cortex region of the hindbrain after malathion exposure, and compared the structural alterations with control tissues. The midbrain or mesencephalon is relatively large consisting of the optic tectum and ventral tegmentum. The optic tectum is composed of six distinct layers such as stratum marginale, stratum opticum, stratum fibrosum, stratum album, stratum griseum, and stratum periventriculae (Mishra and Devi, 2014). The optic tectum is the visual center of the fish brain mainly involved in the sense of feeding, locomotion, decision making, and maintenance of equilibrium in swimming (Springer *et al*., 1977). Malathion exhibited neurotoxicity as evidenced by sparse and disorganized nuclei, inflammatory cell infiltration, oligodendroglia, severe necrosis, vacuolization, and degeneration of neuronal cells in the optic tectum, and this could be the reason for behavioral modification in the fish during the exposure period. The common carp *Cyprinus carpio* exposed to atrazine and chlorpyrifos induced brain tissue damage as evidenced by severe degeneration, loss of granule cells, and reduction of Nissl bodies along with the induction of oxidative stress suggesting peroxidative tissue damage (Xing *et al*., 2010).

The cerebellar cortex region of the fish hindbrain consists of a molecular layer formed of Purkinje cell dendrites and glia cells, and a granular cell layer that receives the nerve impulse, which functions to control swimming equilibrium, orientation, and coordination of muscular tone (Finger, 1983). Unlike higher vertebrates, there are no distinct layers of the cerebellum in teleost fish where the neuroglial cells and granule cells form a continuous zone (Miyamura and Nakayasu, 2001). In the present study, malathion exposure caused neurodegeneration in the cerebellar cortex region as identified with vacuolization, pyknotic, karyorrhexis, and karyolytic nuclei, necrosis, and degeneration of neuroglial cells. All these damages were remarkably severe with an increase in exposure duration of malathion. In a study, glyphosate herbicide exposed to catfish, *Clarias gariepinus* has been characterized by severe degeneration of Purkinje cells, edema, vacuolization, necrosis, and degenerated neurons (Erhunmwunse *et al*., 2014). The histological changes observed in the brain tissue after malathion exposure suggested severe neurological and behavioral disturbances in the freshwater fish *Oreochromis mossambicus*.

Conclusion

Sublethal exposure to malathion altered neurotransmitters in brain tissues and the neurotoxicity was mediated through the induction of redox stress in the fish *Oreochromis mossambicus*. The failure of the antioxidant defense system by the elevation of lipid peroxidation, and nitrative stress in the brain tissue have contributed to the alteration in brain neurochemistry. Besides, behavioral modifications and histological damage in the brain tissue of the fish suggest the neurotoxicity of the compound. Pesticide poisoning in the aquatic environment also affects human health by consuming the exposed fish, hence it is necessary to improve safety profiles for the formulation, use, handling, and discharge of pesticide residues into the natural environment. The present study predicted the potential hazards of malathion and thus contribute to the environmental risk assessment and monitoring.

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