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Effects of Diisononyl phthalate on the Antioxidant Status in Gill, Liver and Muscle Tissues of the Fish, *Oreochromis mossambicus*

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ABSTRACT: Phthalates, commonly used as plasticizers in PVC products, are frequently released into the aquatic ecosystem causing adverse toxic impacts on the inhabitants. The hypothesis of the present study was to investigate the toxic effects of one of the phthalates, diisononyl phthalate (DINP) on the antioxidant status in gill, liver and muscle tissues of the fish, *Oreochromis mossambicus*. DINP at 300ppm concentration was exposed to fish for short-term (24, 48, 72 and 96 h) and long-term (7, 14, 30 and 60 days) durations maintaining vehicle control (propylene glycol as solvent) and negative control (without solvent) groups. In gill tissue, the activities of antioxidant enzymes such as superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase showed significant (P<0.05) increase initially during short-term exposure followed by significant (P<0.05) reduction in all treatment groups of long-term exposure. The level of lipid peroxidation and the activities of acid and alkaline phosphatase increased significantly (P<0.05) after DINP exposure in all treatment groups. Liver tissue showed significant (P<0.05) reduction in the activities of all antioxidant enzymes except glutathione reductase in all treatment groups with concomitant increase in the level of lipid peroxidation and the activities of alanine and aspartate aminotransferase. Similarly, reduction in the activities of antioxidant enzymes and induction of lipid peroxidation along with significant (P<0.05) increase in the marker enzymes were observed in the muscle tissues. The study revealed that DINP induced toxicity by the alteration of antioxidant status in gill, liver and muscle tissue. The patterns of variations observed in different tissues are duration dependent and also indicate that all tissues are equally susceptible to oxidative stress by the exposure to the toxicant.

Keywords: DINP; gill; liver; muscle; antioxidant enzymes and *Oreochromis mossambicus*.

INTRODUCTION: Phthalates are the esters of phthalic acids used as plasticizers to increase the flexibility, durability, longevity and transparency in polyvinyls. Approximately 8.4 million tones of plasticizers are produced globally where it accounts for 10-60% of the total weight of plasticized products (ECHA, 2010). DINP, a colourless and odourless oily liquid of high molecular weight phthalates is composed of more than six carbons in their backbone which give more durability and permanency. DINP is the major component to make vinyl flexible, without which the existence of electric cables and synthetic leather is impractical. Thus the use of DINP extends over in many products including automobiles, cables and wire, buildings and constructions, roofing materials, toys, flooring industries, coated fabrics etc (CPSC, 2010). Some of the non-polyvinyl chloride products such as rubbers, paints, inks, pigments, lacquers, adhesives, sealants etc also contain DINP as a softener (EC JRC, 2003). The Consumer Product Safety Improvement Act (CPSIA) in 2008 enacted an interim ban for using more than 0.1% of DINP in toys that was placed in the mouth of children. DINP has also been detected in water, household products, food and in other sources of human consumption (US-EPA, 2009). The occurrence of DINP and its metabolites have been found in urine, cord serum, saliva, and in breast milk of human (Lin et al., 2011). In human, the elimination of DINP and its metabolites occur by multiphase elimination pattern showing elimination half-life of 3 h in first phase followed by 5 h for oxidized metabolites. The elimination half-life for the second phase initiates after 24 h of post-dosing (Koch and Angerer, 2007). The half-life of DINP is 0.7 days in air, 50 days in surface water, 300 days in soil and 3000 days in sediments (Oehlmann et al., 2008).

Some studies reported DINP as safe are now regarded as toxic and have wide concern since they are shown to cause cancer, developmental abnormalities and reproductive effects (Su et al., 2012). DINP has been shown to have endocrine disrupting properties thereby known to mimic or antagonize the action of endogenous hormones, which consequently result in adverse effects on reproduction, growth and development (Fisher, 2004). There is an increasing concern that DINP is detected regularly in aquatic ecosystem as

they are released continuously into the environment and pose a potential environmental threat to aquatic organisms (Liu et al., 2014). As soon as DINP reaches the aquatic environment it could find the way to higher organisms, including human through the food chain. Mixture of phthalates has been shown to exhibit enhanced estrogenic activity in medaka, *Oryzias melastigma* (Chen et al., 2014). Recently, it has been reported that exposure to DINP affected growth and maturation of oocytes leading to abnormal gonadal development and reproduction in zebrafish (Santangeli et al., 2017). The adverse toxic effects of DINP in fish could be due to slow metabolism and elimination than mammals or birds (Greulich and Pflugmacher, 2003). DINP has been shown to alter the lipid metabolism and endocannabinoid system in the liver of the female zebrafish (Forner-Piquer et al., 2017).

There are several test procedures adopted in ecotoxicology to detect the toxic effects of any compounds in aquatic animals. Antioxidant defense system play a crucial role in maintaining cellular homeostasis and have received much attention in ecotoxicology because induction of oxidative stress by environmental pollutants was considered as one of the primary mechanisms of toxicity in aquatic organisms (Santos et al., 2004). The environmental contaminants have been shown to induce reactive oxygen species (ROS) production in fish as a result of the failure of antioxidant defence mechanism (Monteiro et al., 2010). In stable condition, cells or tissues of fish are equipped with well-defined antioxidant defense system to scavenge the ROS formed. Initial increase in ROS reset the original balance by the scavenging activities of antioxidant enzymes. However, continuous production of ROS as a result of toxicant exposure could imbalance the pro-oxidant/antioxidant status in the tissue which could lead to oxidative stress. It is necessary to prove the direct consequence of DINP in the rate of ROS production and also its cause and effect on various tissues remains scarce. Thus the present study focused on the antioxidant status in gill, liver and muscle tissues of the fish, *Oreochromis mossambicus* as exposure to any toxicant could influence the production of free radicals.

MATERIAL AND METHODS:

Acclimatization in animal model: Freshwater fish, *Oreochromis mossambicus* weighing 3.5±0.75g and length 5.5±1.5cm were collected from a fish farm, Safa Aquarium, Kozhikode, Kerala. Fish were acclimatized to the laboratory conditions before the experiment in well-aerated dechlorinated water (40 L capacity). The physico-chemical features of the tap water were estimated as per APHA guidelines (1998) maintaining water temperature (28 ± 2°C), oxygen saturation of water (70 to 100 %), pH (7.4 to 7.6) in both control and treatment groups throughout the study.

Chemicals: Diisononyl phthalate (DINP)- CAS No. 28553120 of 99% purity was obtained from Sigma Aldrich Chemical Co., USA. Malondialdehyde, NADPH, glutathione oxidized, thiobarbituric acid, pyrogallol, sodium pyruvate, 2,4-dinitrophenyl hydrazine, L-aspartate, 2-oxoglutarate, DL-α-alanine were obtained from Himedia Laboratories, Mumbai, India. All other chemicals were of analytical grade and obtained from local commercial sources.

Preparation of test chemical: The organic solvent that do not produce toxic effects at certain dose levels, and are not suspected to undergo chemical reaction with the test substance is usually used as vehicle control. In the present study, propylene glycol (16 µl; 1 M) was used as a solvent to dissolve DINP by sonicating at 50 Hz for 5 min with 30sec pulse interval. The concentration of DINP at 300ppm was chosen in the present study according to the maximum solubility as described earlier and the concentration was maintained throughout the experiment (Revathy and Chitra, 2015). Negative control, without solvent or test chemical was also maintained along with other treatment groups.

Experimental design: Fish were exposed to 300ppm concentration of DINP for short-term (24, 48, 72 and 96 h) and long-term (7, 14, 30 and 60 days) durations maintaining vehicle control group (propylene glycol as solvent) and negative control (solvent-free and toxicant-free) group. In each treatment groups ten fishes were maintained and the health conditions were continuously monitored during the experiment. All experimental tubs were properly aerated using tubed motorized pumps. Monofilament netting was used to cover the tanks to avoid the jumping of fish from the test solutions.

Tissue preparation: At the end of every exposure period, fish were captured gently using small dip net with least disturbances in order to avoid stress, weighed and killed by decapitation. Gill, liver and muscle were carefully excised and cleaned from mucous and debris, weighed and 1% (w/v) crude tissue homogenates were prepared in ice-cold saline using a motor driven tissue homogenizer. The homogenates were centrifuged at 3000 rpm for 15min at 4^{o}C and the supernatants collected were then used for the biochemical analysis.

Biochemical analysis: Activities of antioxidant enzymes such as superoxide dismutase (Marklund and

Marklund, 1974), catalase (Claiborne, 1985), glutathione reductase (Carlberg and Mannervik, 1985) and glutathione peroxidase (Mohandas et al., 1984) and the level of lipid peroxidation (Ohkawa et al., 1979) were assayed in the supernatants of gill, liver and muscle tissues. Total protein concentration in the tissues was determined by the method of Lowry et al. (1951). The activities of acid phosphatase (Torriani, 1960) and alkaline phosphatase (Bessey et al., 1946) were assayed in gill and liver tissues. The activities of alanine and aspartate aminotransferase were measured by the method of Bergmeyer (1974) in muscle tissue.

Statistical analysis: Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test using statistical package SPSS 17.0. Differences were considered to be significant at $p<0.05$ against the control groups. Data are presented as mean± SD for ten animals per group and all biochemical estimations were carried out in triplicate.

RESULTS AND DISCUSSION:

Effect of DINP on body weights and tissue weights: The body weight of the animal remained without any significant change throughout the experiment period when compared to the control groups (Table 1). The weights of gill and liver tissues showed significant ($P<0.05$) decrease only after 30 and 60 days of DINP exposure (Table 1).

Effect of DINP on the antioxidant status in gill tissue: The activity of superoxide dismutase (SOD) increased significantly ($P<0.05$) after 96h of DINP exposure whereas significant ($P<0.05$) reduction in the enzyme activity was observed in all treatment groups of long-term exposure (Fig. 1A). Catalase (CAT) activity increased significantly ($P<0.05$) in time-dependent manner up to 96 h duration and thereafter a significant reduction was observed in gill tissue (Fig. 1B). Activity of glutathione reductase showed 6-8 fold increase during the short-term exposure whereas no significant changes were noted in the long-term exposure group (Fig. 1C). The activity of glutathione peroxidase decreased significantly ($P<0.05$) from 72 h duration onwards in time-dependent manner in both short and long-term exposure groups (Fig. 1D). The level of lipid peroxidation increased significantly ($P<0.05$) after short and long-term exposure of DINP (Fig. 1E). The activities of gill marker enzymes, acid and alkaline phosphatases, increased significantly ($P<0.05$) in all treatment groups while compared with control groups (Figs. 4A and 4B). Exposure to vehicle-control showed no significant changes in the activities of all enzymes and in the level of lipid peroxidation when compared to solvent and toxicant-free control groups.

Table 1: Effect of DINP on the body weight and tissue weights of the fish, *Oreochromis mossambicus*.

Parameters	Control	Solvent control	DINP (300ppm)							
			Short-term exposure					Long-term exposure		
			24 h	48 h	72 h	96 h	7 days	14 days	30 days	60 days
Body weight (g)	3.55±0.71	3.52±0.47	3.47±0.90	3.48±0.5	3.49±0.33	3.49±0.42	3.45±0.52	3.44±0.16	3.43±0.59	3.51±0.19
Gill weight (mg)	123±0.28	122±1.5	121.5±1.37	122±1.03	123±1.09	122.5±0.54	120.8±0.98	119.8±1.32	117.6±1.96*	113±0.21*
Liver weight (mg)	53±0.83	51±1.86	52±0.75	51±0.13	51.3±0.16	50.6±0.81	49.8±0.14	49.6±0.16	48.8±0.17*	48±0.17*

*Values expressed in Mean±SD; n=10/group; *P<0.05 against the control groups.*

Effect of DINP on the antioxidant status in liver tissue: Liver tissue showed significant ($P<0.05$) reduction in the activities of superoxide dismutase and catalase after short-term and long-term exposure groups when compared to the control groups (Figs. 2A and 2B). The activity of glutathione reductase showed significant ($P<0.05$) increase after short-term exposure and a significant ($P<0.05$) decrease was noted only after 60 days of DINP treatment (Fig. 2C). Glutathione peroxidase activity decreased significantly ($P<0.05$) from 96 h of the toxicant exposure showing a time-dependent reduction up to 60 days (Fig. 2D). The level of lipid peroxidation increased significantly ($P<0.05$) in both short-term and long-term exposure groups when compared with the corresponding control groups (Fig. 2E). The activity of alanine aminotransferase in liver tissues showed significant ($P<0.05$) increase from 72 h of exposure showing a time-dependent increase up to 60 days of exposure (Fig. 4C) whereas the activity of aspartate aminotransferase showed significant ($P<0.05$) increase only after 30 and 60 days of DINP exposure (Fig. 4D).

Figure 1: Effect of DINP on the antioxidant status in the gill tissue of the fish, *Oreochromis mossambicus.*

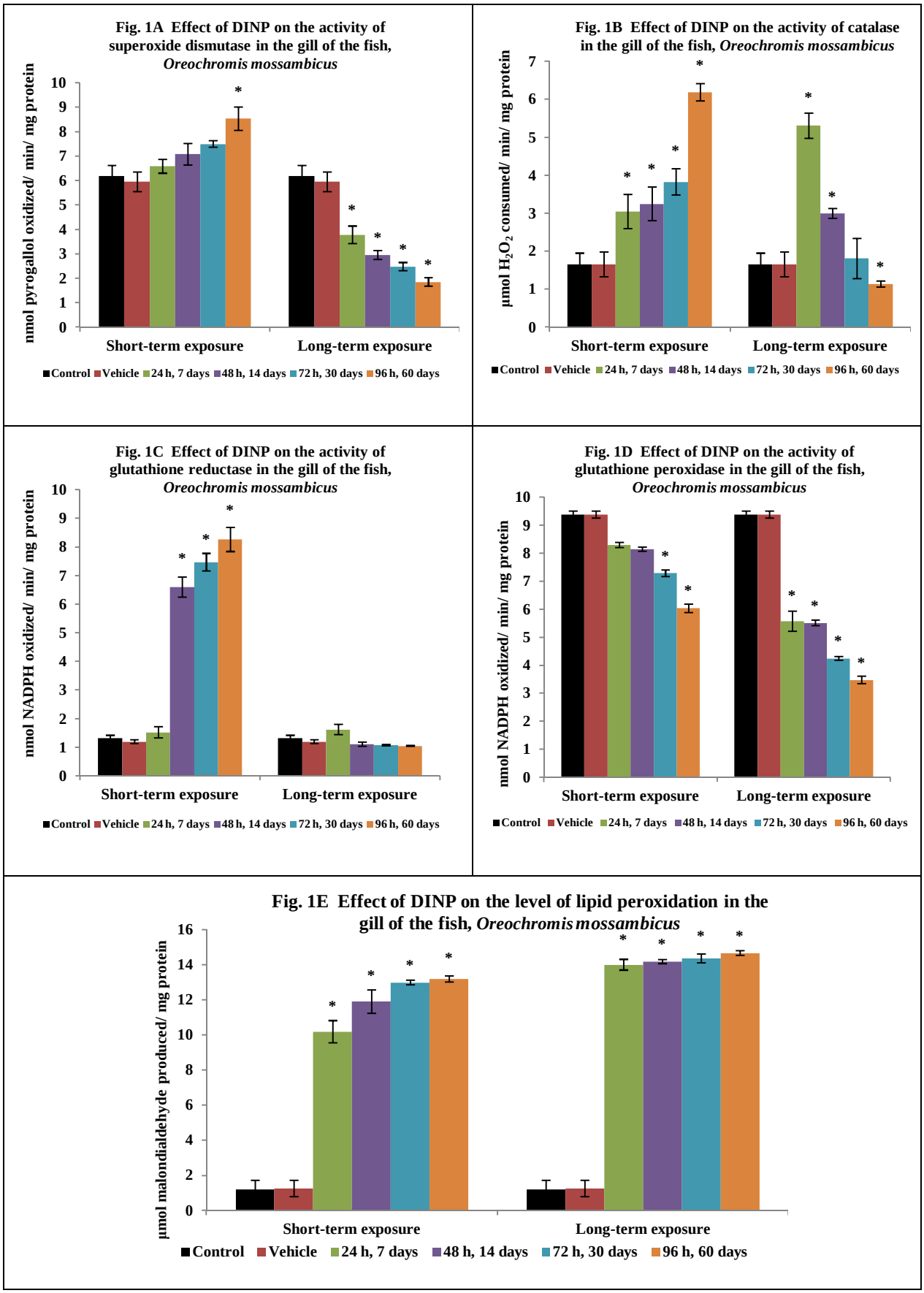


Figure 2: Effect of DINP on the antioxidant status in the liver tissue of the fish, *Oreochromis mossambicus.*

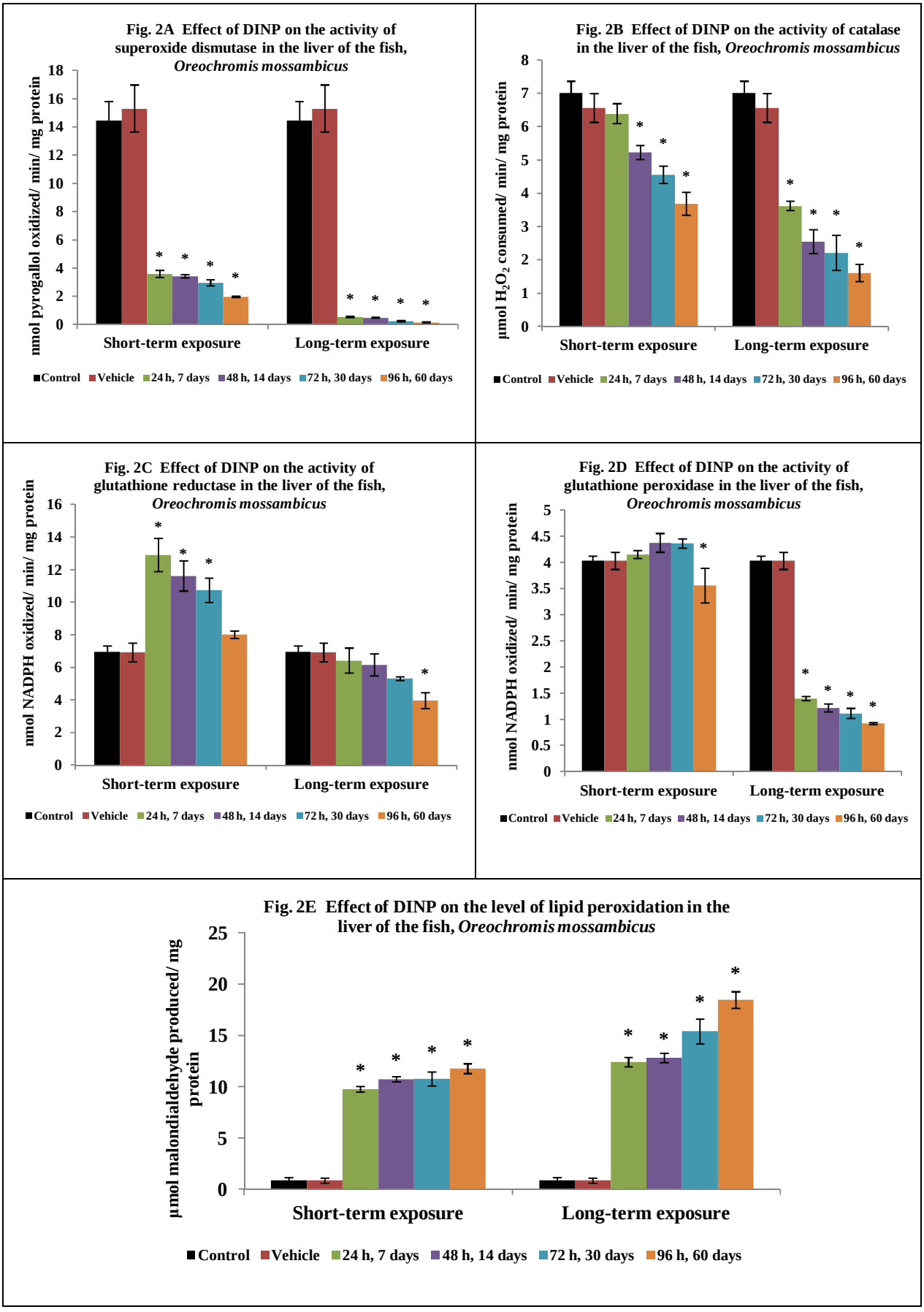


Figure 3: Effect of DINP on the antioxidant status in the muscle tissue of the fish, *Oreochromis mossambicus.*

Fig. 3A Effect of DINP on the activity of superoxide dismutase in the muscle of the fish, *Oreochromis mossambicus*

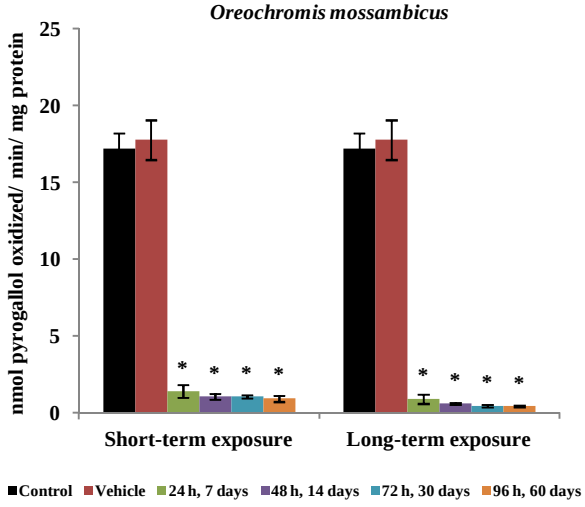


Fig. 3B Effect of DINP on the activity of catalase in the muscle of the fish, *Oreochromis mossambicus*

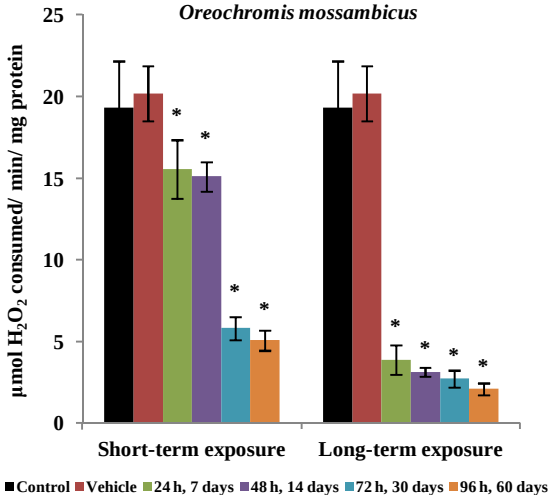


Fig. 3C Effect of DINP on the activity of glutathione reductase in the muscle of the fish, *Oreochromis mossambicus*

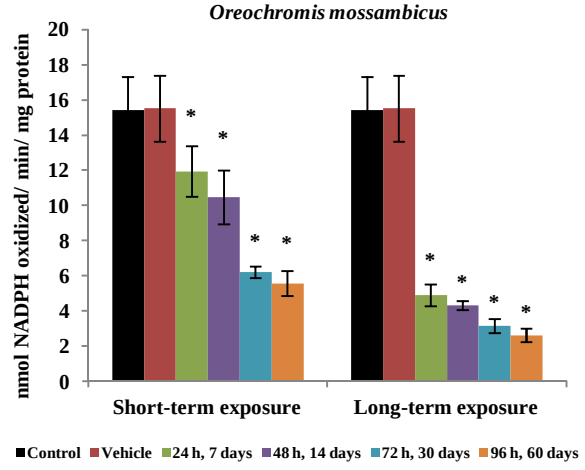


Fig. 3D Effect of DINP on the activity of glutathione peroxidase in the muscle of the fish, *Oreochromis mossambicus*

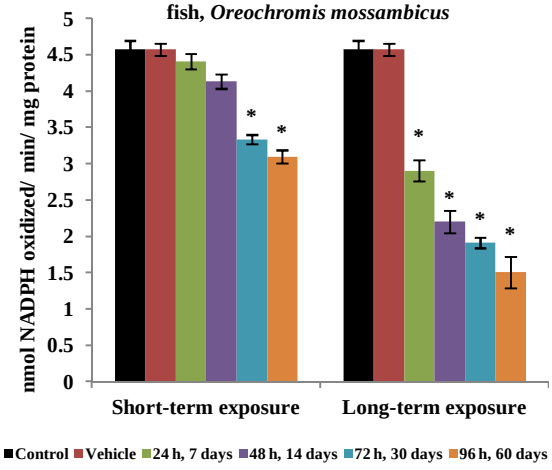


Fig. 3E Effect of DINP on the level of lipid peroxidation in the muscle of the fish, *Oreochromis mossambicus*

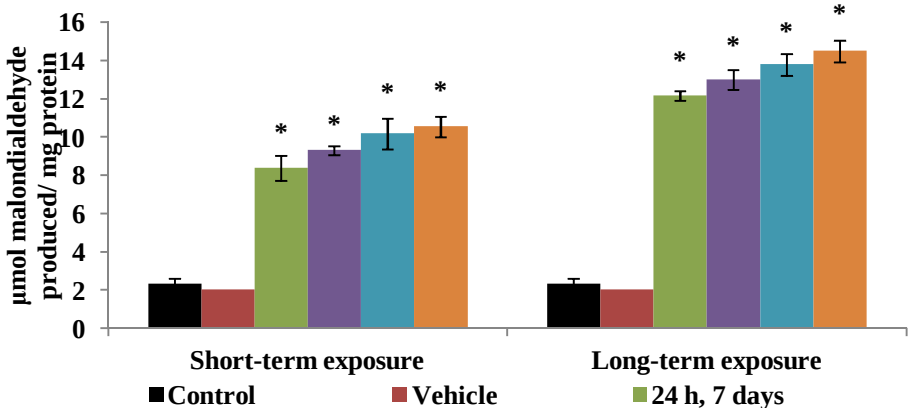
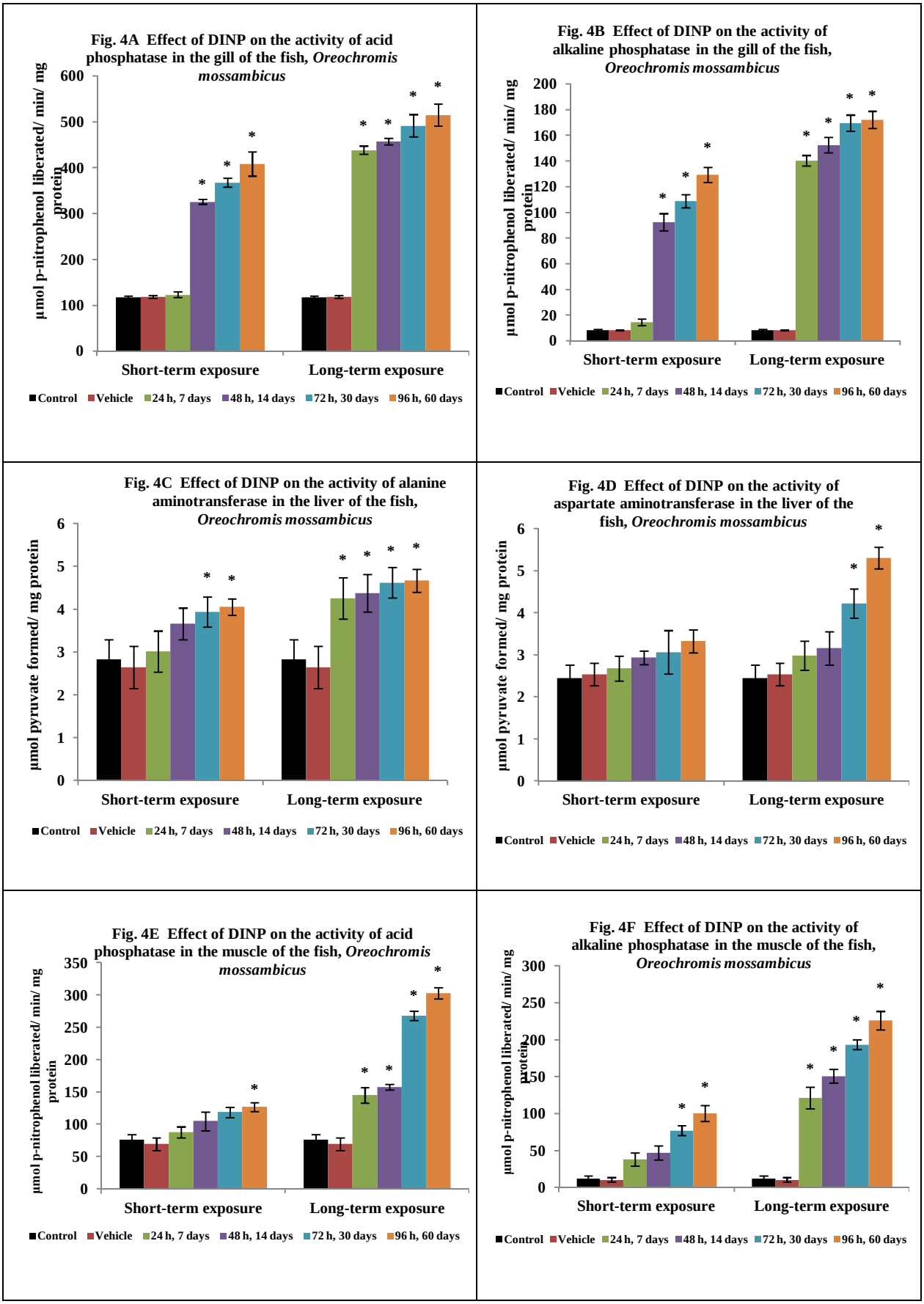


Figure 4: Effect of DINP on the activities of tissue marker enzymes in the fish, *Oreochromis mossambicus.*



Effect of DINP on the antioxidant status in muscle tissue: Exposure to DINP showed significant ($P<0.05$) reduction in the activity of superoxide dismutase in both short and long-term exposure groups than that of the control groups (Fig. 3A). The activities of catalase and glutathione reductase showed significant ($P<0.05$) and time-dependent decrease in both exposure groups (Figs. 3B and 3C). Similarly, a time-dependent reduction in the activity of glutathione peroxidase was observed from 72 h of exposure onwards (Fig. 3D). The level of lipid peroxidation significantly ($P<0.05$) increased in both exposure groups (Fig. 3E) along with significant increase in the activities of acid and alkaline phosphatases in time-dependent manner (Figs. 4E and 4F).

Discussion: Di-isononyl phthalate (DINP), widely used monomeric plasticizers of polyvinyl chloride (PVC), is commonly found in many plastic products including toys. DINP is considered as toxic and the use was strictly regulated by the European Union. However, in India there are no checks on its usage in the toys of children thereby raising much alarm to the health of infants. The concentrations of DINP found in aquatic environment varies from 1.8 to 9.7 μg/L based on the locations using DINP in paints, adhesives, sealants and inks, close to the production and processing sites and from the non-PVC processing centers (NRC, 2008). The occurrence of DINP in fish has been documented in various literatures with the levels ranging from undetectable to 11,576μg/Kg (Munshi et al., 2013). The water solubility of DINP is less than 0.001mg/ L (Staples et al., 1996) and the highest soluble concentration of DINP dissolved in propylene glycol as organic solvent was found to be 300 ppm, where no mortality was noticed for 96 h (Revathy and Chitra, 2015). According to the maximum solubility limit 300ppm was selected as the test concentration in the present study. Prediction of toxicity of any chemicals on single species provides basic information to improve the quality of life and protect the environment from the adverse effects of the toxicant. Thus the present study adopted short-term exposure of DINP for 96 h using single test species, *Oreochromis mossambicus*. However, short-term tests have some limitations because it provides only hypothetical incisive information on the toxic compounds and it lack adequate knowledge on the behavior of chemical. In order to estimate the adverse toxic effects of the chemical it is always necessary to set extensive limit on the range of exposure and thus long-term exposure for 60 days was also conducted to anticipate the outcome of toxicant on the chronic exposure. The test solution was maintained in static with renewal system at every 96h regular interval in long-term exposure groups by transferring fish to another tank of freshly prepared toxicant. The replacement of test solution improves the constancy of DINP concentration throughout the experiment.

In aquatic toxicology, fish is commonly selected as test species for many reasons as it is highly sensitive to pollutants in the environment, requires self protection from contaminants, source of wealth in aquatic ecosystem, availability of diverse species and a direct consumer to human through the food chain. In the present study, *Oreochromis mossambicus* was selected as the test species owing to the ecological tolerance such as high resistance to toxicants and rate of survival at high salinity, low oxygen saturation, altered pH, and extreme high or low temperature. However, the physicochemical properties of water were maintained in normal standards as per APHA guidelines throughout the test period. Alterations in the body weight and tissue weights are considered as sensitive index to estimate the systemic toxic effects of pollutants in the environment. There was no treatment related changes in the body weight of the fish after DINP exposure, however, the weights of gill and liver tissues showed reduction after 30 and 60 days of DINP exposure and this could be due to treatment related necrosis or tissue damage. There are several biomarkers in ecotoxicology assessment, among which the role of antioxidant defense system in exposure to environmental toxicants is the most significant current topic of interests. It is well documented in many literatures that exposure and bioaccumulation of toxicants activate the intracellular redox reactions which lead to the generation of free radicals, such as superoxide anion, hydrogen peroxide, hydroxyl radicals and their by-products (Lushchak, 2011). Generally, free radicals are very unstable and react with biomolecules in the body, leading to cell injury, which is compensated by the action of various antioxidant enzymes (Velisek et al., 2011). The antioxidant defense system includes both enzymatic and non-enzymatic antioxidants. The major enzymatic antioxidants are superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase whereas non-enzymatic antioxidants include vitamin C and E, glutathione, β-carotene etc. The generation of free radicals severely damage membrane lipids, which could result in lipid peroxidation. Thus monitoring the level of antioxidant enzymes and lipid peroxidation provides valuable evidences regarding the biochemical changes on cells or tissues, which could serve as sensitive biomarkers of oxidative stress (Paskerova et al., 2012).

In gill tissue, exposure to DINP showed increase in the activity of superoxide dismutase following 96 h duration whereas after 7 days of treatment showed reduction in the enzyme activity. Superoxide dis-

mutase is a metal-containing primary defensive antioxidant enzyme that functions to eliminate the free radicals of oxygen formed during cell metabolic processes thereby converting into oxygen and hydrogen peroxide (McCord and Fridovich, 1969). DINP exposure increased the activity of catalase in time-dependent manner up to 96 h duration and thereafter a significant reduction was observed. Catalase detoxifies the oxygen reactive radicals by the decomposition of hydrogen peroxide into water and oxygen (Cheng et al., 1981). The initial increase in the activities of superoxide dismutase and catalase after DINP exposure denotes the primary defensive mechanism of gill tissue to remove the free radicals formed. However, the reduction in the activities of antioxidant enzymes during long-term exposure indicates the failure of defensive mechanism to cope up with the production of free radicals. Activity of glutathione reductase showed 6-8 fold increase during the short-term exposure whereas no significant changes were noted in the long-term exposure group. Exposure to DINP decreased the activity of glutathione peroxidase from 72 h duration onwards in time-dependent manner in both short and long-term exposure groups and this could be the failure of the enzymes to protect against the radical damage. The failure of antioxidant enzymes to remove the reactive oxygen species is reflected by increase in the level of lipid peroxidation in both short and long-term exposure of DINP. The present results suggest the failure of pro-oxidant/ antioxidant balances in the gill tissue as a result of DINP exposure which resulted in oxidative stress. The results were found in agreement when one of the environmental contaminants, chlordecone exposed to gill tissues of the cichlid fish, *Pseudetroplus maculatus* (Asifa and Chitra, 2017). In addition, the activities of gill marker enzymes, acid and alkaline phosphatases increased significantly in all treatment groups. Phosphatases are the enzymes involved in wide variety of metabolic activities such as cell permeability, growth and differentiation, protein synthesis, absorption and transport of nutrients and also used as a reliable marker tool for the assessment of environmental pollutants. The higher activity of acid and alkaline phosphatases indicates severe tissue damage due to DINP exposure and disturbance of membrane transport across the gill tissue and signifies the intensity of tissue damage during the prolonged exposure.

Liver plays an important role in protecting the animal from potential toxicants through detoxification mechanism by converting lipophilic compounds into more water-soluble metabolites. Liver is also well-equipped with antioxidant defense system to protect the hepatocytes from oxidative injury as a result of toxicant exposure. In the present study, liver tissue showed reduction in the activities of superoxide dismutase and catalase after short-term and long-term DINP exposure groups. The activity of glutathione reductase increased after short-term exposure and decreased only after 60 days of DINP treatment. Glutathione peroxidase activity decreased from 96 h onwards in time-dependent manner. The alterations in the antioxidant enzymes led to the induction of lipid peroxidation in both short-term and long-term exposure groups. Lipid peroxidation is oxidative destruction of membrane lipids initiated by the attack of free radicals on phospholipids or polyunsaturated fattyacids. Lipid hydroperoxides are the primary products of lipid peroxidation and among the secondary products, malondialdehyde and 4-hydroxynonenal are extensively studied. The most mutagenic product of lipid peroxidation is malondialdehyde whereas 4-hydroxynonenal has been considered as the most toxic (Esterbauer et al., 1990). Once formed, malondialdehyde undergo metabolism to react on the cellular or tissue proteins or DNA to form adducts resulting in the alteration in the biochemical properties of biomolecules. The present findings suggest that DINP induced lipid peroxidation which may facilitate in the development of liver tissue damage in the fish, *Oreochromis mossambicus*. Besides, the activities of alanine and aspartate aminotransferase in liver tissues were found increased after DINP treatment. These are enzymes present primarily in liver and used as specific biomarker enzymes to detect liver damage induced by toxicants and the increased activities of the enzymes indicate inactive transamination and oxidative deamination processes in liver tissues. The results are in agreement with another study when different levels of paraquat were exposed to the fish, *Clarias gariepinus* (Chimela et al., 2014).

In fish muscle, the pro-oxidant is balanced by the presence of natural antioxidant defense system. Muscle tissues possess the ability to maintain the redox state by eliminating reactive oxygen species by removing catalytic metal ions, decreasing local oxygen concentration, scavenging singlet oxygen etc. (Symons and Gutteridge, 1998). Thus the antioxidant system directly or indirectly inhibits the initiation and propagation of lipid peroxidation. In the present study, DINP exposure showed reduction in the activities of antioxidant enzymes as superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase in time-dependent manner throughout the treatment period. Therefore, it was clearly demonstrated that DINP exposure caused inability of muscle tissue to eliminate the free radicals. Similarly, the exposure to acrylamide has been shown to decrease the activities of antioxidant enzymes and induction of lipid peroxidation in the muscle tissue of *Oreochromis niloticus*

(Gopika et al., 2018). Generation of free radicals activate lipid peroxidation chain reactions where the lipid radical reacts with oxygen to produce peroxyl radical. Peroxyl radical initiates a chain reaction and transforms polyunsaturated fatty acids into unstable lipid hydroperoxides, which decompose readily to secondary products such as aldehydes including malondialdehyde. The present study confirms 4 to 7 fold increase in the production of malondialdehyde in the muscle tissues after DINP exposure. Induction of lipid peroxidation disturbs the integrity of cell membranes that eventually leads to muscle tissue damage and also used as biomarker of oxidative stress. Acid and alkaline phosphatases are generally used as stress marker enzymes that serve as a diagnostic tool to detect toxicity stress of chemicals. In the present study, DINP exposure increased the activities of acid and alkaline phosphatase in time-dependent manner. Acid and alkaline phosphatases are involved in the ester linked hydrolysis and act under acidic and alkaline conditions, respectively. Sublethal concentration of benzyl butyl phthalate (BBP) has been shown to increase the activities of acid and alkaline phosphatases in gill, liver and muscle tissues of zebrafish, *Danio rerio* (Sepperumal and Saminathan, 2014). Thus the alteration in the enzyme activities could be due to defensive mechanism of muscle tissue to overcome the toxic stress caused by DINP.

CONCLUSION: The present investigation indicates that one of the high molecular weight phthalates, DINP induced oxidative stress in gill, liver and muscle tissues of the fish, *Oreochromis mossambicus*. Fish, the most diverse among the group of non-target aquatic organisms are highly susceptible to oxidative stress mediated by DINP toxicity. Furthermore, the results suggested that antioxidant status may be used as sensitive tool in ecotoxicology to detect plasticizer pollution in the aquatic ecosystem.

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