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Research Paper

Toxicity Study of Diethyl Phthalate on Nile Tilapia *Oreochromis Niloticus*

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Abstract
Diethyl phthalate (DEP) is a plasticizer reported to be a pollutant of aquatic ecosystems. Therefore, short term definitive test by static renewal bioassay method was conducted to determine the acute toxicity (LC50) of DEP in the Nile tilapia Oreochromisniloticus. Fingerlings were exposed to different concentrations (0, 30, 35, 40, 45, 50 and 75 mg/L) of DEP for 96 h. The acute toxicity value was found to be 48.1 mg/L in O.niloticus. One tenth of LC50 (48.1mg/L) value was selected as the sub-lethal concentration for sub-acute studies. Fish were exposed to sub-lethal concentration for 7 days and allowed to recover in DEP-free medium for 7 days. Behavioural responses and morphological deformities were studied during experimental tenures. Fish in toxic water displayed avoidance behaviour and hyper excitability, irregular, darting and erratic swimming movements within 1h compared to controls. At 24 h, DEP exposed fish showed sluggishness with some loss of equilibrium than controls. After 48h onwards, all exposed fish to DEP were noticed less active compared to controls and, shortly thereafter, displayed a more severe loss of equilibrium further more fish exposed to DEP resulted in dark pigmentations on body, caudal fin bending and abdomen bulging, while no morphological alterations in control fish. Interestingly all morphological and behavioural alterations of fingerlings have been recovered when introduced to toxic free water within 7 days in recovery group except lesion in gills structure, caudal fin bending and bulged abdomen. In conclusion results of the present investigation evidence that DEP exposure is primarily toxic to O. niloticusand induced high morphological and behavioural alterations on fingerlings at sub lethal concentrations.

Keywords: *DIETHYL PHTHALATE, NILE TILAPIA, MORPHOLOGICAL DEFORMITIES, PLASTICIZER.*

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I. Introduction

Phthalates are industrial chemicals used as lubricants, solvents, additives, softeners etc. They are present in number of day to day used products such as PVC products, building materials (paint, adhesive, wall covering), personal-care products (perfume, eye shadow, moisturizer, nail polish, deodorizer etc.), detergents and surfactants, packaging, children's toys, pharmaceuticals and food products, textiles, household applications such as shower curtains, floor tiles, food containers and wrappers etc. They can be easily released into the water environment via various routes and are identified as one of the most prevalent organic pollutants [15]. However, once entering the environment, they pose remarkable toxicological threats to the myriad of non-target organisms, discover its way to the food chain, and threaten ecological balance and biodiversity of nature [18].

Diethyl phthalate (DEP) is one of the most extensively used industrial chemical and employed in products such as insecticides, mosquito repellents, camphor substitute, plasticizer for cellulose, bathing soaps, cosmetics, pharmaceutical coatings, after shave-lotion, detergent, ester plastic film and sheets etc.. [14]. It has also been estimated that approximately 1% the diethyl phthalate ester content of plastic materials in direct contact with water or other liquids are released into the aquatic environment [24] and DEP may be discharged from industries as effluent [21]. DEP has been detected in water [30], sediments [6] and agricultural soil [28] throughout the globe. DEP is known to be a contaminant of freshwater ecosystems. Fish can absorb phthalates from contaminated water through gills during respiration, dermal exposure and intake of contaminated food particles and sediments [3]. Several *in-vivo* studies showed that DEP exposure caused endocrine disruption [2],

oxidative stress [15] neurotoxic [12] embryo toxic [16], hepatotoxic [29], hematotoxic [25], behavioural [12] and histopathlogical effects [11] in different culturable fish species.

Although several studies have been demonstrated the toxic effects of DEP on freshwater fishes, for example the acute toxicity (measured as 96-h lethal concentration LC50s) of DEP to bluegill *Lepomismacrochirus* was found to be 110 mg/L [4], to rainbow trout was 12 mg/L [1] to fathead minnows *Pimephalespromelas*was 31.8 mg/L [10], and 72-h LC_{50} of DEP to *Cirrhinamrigala*was 50 ppm [12] Despite these studies, till date there were no studies have been focused on acute toxic effects of DEP on nile tilapia *Oreochromisniloticus*.

In the present investigation the nile tilapia *O. niloticus fingerlings* are used as the experimental fish because of their high growth rates, efficiency in adapting to diverse diets, great resistance to diseases and handling practices, easy reproduction in captivity at prolific rate, good tolerance to a wide range of environmental conditions [7] and it is also a good biological model for toxicological and immune toxicity studies [24]. Therefore, a study was designed to determine the toxic effects of DEP on a nile tilapia *O. niloticus* fingerlings.

II. Materials and methods

Fish collection and maintenance:

Healthy *O. niloticus*embryowerecollected from private fisheries farm (14.515400N; 75.810799E) Gangnarasi village, HarihartalukDavanagere District, and embryos were treated with 0.05% $KMnO_4$ reared in cement fish tank (5 X 3 X 5 feet) Department of Studies in Zoology, Davangere University, Shivagangothri Campus, Davanagere, Karnataka, India. After 40 days fingerlings were then transferred to 12L capacity glass aquaria (size, 30 X 26 X 14 cm; L X W X H) filled with aged tap water two weeks prior to the experiment for acclimatization to laboratory conditions at a stocking density of 07 fingerlings/aquaria. The mean water temperature was 21.56±0.16°C, whereas the mean dissolved oxygen was 6.85 ± 0.56 mg/L during the acclimation and experimentation. Each aquarium were provided with continuous aeration to ensure the supply of oxygen, maintained under normal day - night light duration and fed with commercially available small food pellets (Optimum, Perfect companion (M) Sdn. Bhd. Malasiya) during the rearing and acclimation.

Toxicant used:

Diethyl phthalate (GRM4655, 99.99% purity) obtained from Himedia, was used in present study. Various working concentrations of DEP were prepared by dissolving appropriate amount of DEP in distilled deionized water because DEP is soluble in water.

Determination of LC50 of DEP:

Fingerlings (3.50±0.22 g, 4.1±0.45 cm) were randomly divided into 07 groups (7 fish / group / aquarium) and definitive test was conducted using concentrations (0, 30, 35, 40, 45, 50 and 75 mg/L) of DEP earlier determined based on pilot studies. The control and DEP exposed fish were aerated continuously to prevent hypoxic condition of medium and were kept under continuous observation during the experimental period 24, 48, 72 and 96 hours (h) for LC. During this period water was replaced daily and fresh toxicant (DEP) was added for 96 h. Fish were monitored at regular intervals and mortality data was recorded. The LC_{50} of DEP for 96 h was calculated using probit analysis [7]. The experiment was conducted following OECD standardized guidelines for semi-static bioassays (OECD guidelines @2019).

Mortality:

Mortality was recorded at an interval of 24 h over a period of 4 days (96 h). Fingerlings were taken dead when they turned upside down and sank to the bottom of the tank or when their tail showed no form of movement even prodded with a glass rod [18].

Sub-lethal studies:

21 fingerlings were divided into three groups namely, Group I- Experimental control maintained without toxicant, Group II- exposed 4.81 mg/L of DEP ($1/10^{th}$ of LC_{50}) and recovery group III, were also exposed to DEP ($1/10^{th}$ of LC_{50}) for period of seven days. Water was changed daily in order to avoid the accumulation of fish waste and any leftover feed and renewed by adding the fresh toxicant. After 7days Group III fingerlings were used for recovery studies.

III. Results and Discussion

Phthalates are emerging environmental pollutant. Low-molecular weight phthalates such as Dimethyl phthalate (DMP), Dibutyl phthalate (DBP), Diisobutyl phthalate (DIBP), Butylbenzyl phthalate (BBP) and including DEP were found to be acutely toxic to aquatic ecosystem because they are not covalently bounded to their substrate are therefore easily leached out ending up in the environment. DEP exposure has been reported to

cause endocrine disruption [2], oxidative stress [15], neurotoxic [12], embryo toxic [16] hepatotoxic [29], hematotoxic [25], behavioural [12], histopathlogical effects [11] and mortality [27] in different culturable fish species.

The acute toxicity (LC*50*) of diethyl phthalate calculated over 96 h periods for *O. niloticus* fingerlings was found to be 48.1 mg/L. Similar to our results, several other studies reported acute toxicity of DEP to bluegill *L. macrochirus* was found to be 110 mg/L [4], to rainbow trout was 12 mg/L [1], and to fathead minnows *P. promelas*was 31.8 mg/L [10]. Furthermore, the results of present study indicated that percentage mortality of *O. niloticus* fingerlings increased with increase in concentration of DEP. The observed percentage of mortality was dose dependent increased from 14.28%, 28.50%, 42.80%, and 57.14% for the fingerlings exposed to 35, 40, 45 and 50 mg/L DEP respectively, whereas 100% mortalities were recorded in fish exposed to concentrations of 75mg/L. However, no mortality was recorded in the control and 30 mg/L exposed groups.

Some of the common behavioural and morphological abnormalities of DEP exposure on *O. niloticus* fingerlings includes fish attempted to leap out of test medium, dense schooling behavior, irregular, darting and erratic swimming movements, hyperexcitability, loss of equilibrium followed by muscular incoordination, convulsions, tremors sinking to the bottom and hiding and abnormal surface behaviors. The fingerlings showed abdomen bulged, anal casts and body colour turned to dark pigmentation, which pronounced on dorsal surface and enhanced with exposure. Finally, death with widely extended gill covers. Similar observations were recorded in freshwater fish, *Cirrhinamrigala*[12], *Clariasgariepinus*fingerlings [20].

Gills are most susceptible to the toxicant exposure due to their direct interaction with the environment therefore; studies of gills are frequently used as biomarkers [22]. The results of present study showed severe lesion and blood clotting in gills structure of DEP-exposed *O. niloticus* fingerlings. Similar to our results, *C. gariepinus* fingerlings exposed to different sub-lethal concentrations of DEP showed histopathlogical alternations (fusion, rising of the filaments, oedema, uplifting and loss of epithelium) in the gills structure [20]. Nevertheless, the functional significance of DEP- induced alterations in gill histology requires further study. Fish mortality could be due to rapid inhibition of critical enzymes of vital organs due to passage of free DEP through critical sites, resulting in respiratory arrest and death. Critical sites for DEP cytotoxic in freshwater organisms include central nervous system, myocardium, the gills, and other sites where gaseous exchange and osmoregulatory processes occur. Although we could not confirm these.

In present study we also investigated the influence of a sublethal ($1/10^{th}$ of LC_{50} 4.81mg/L) concentration of DEP-induced toxicity on morphological and behavioural changes of *O. niloticus* fingerlings for a period of seven days. Many studies have been reported that exposure of fish to sub lethal concentration/or dose of DEP for short terms exhibited more severe behavioural and morphological [27]. Fish exposed to 4.81mg/L DEP displayed avoidance behaviour and hyperexcitability, irregular, darting and erratic swimming movements within 1h compared to controls. At 24 h, DEP exposed fish showed sluggishness with some loss of equilibrium than controls. After 48h onwards, all exposed fish to DEP were noticed less active compared to controls and, shortly thereafter, displayed a more severe loss of equilibrium and dense schooling behavior. Animal behaviour is a neurotropically regulated phenomenon, which is mediated by neurotransmitters [5]. Loss of equilibrium follows erratic and darting swimming movements with muscular incoordination and imbalanced body activity, which might be due to inhibition enzymes and causes cytotoxic hypoxia in brain, which results changes in electrical activity of brain thus causing damage to the region of the brain associated with the maintenance of equilibrium [26]. Morphological alteration in body colour to dark pigmentations, caudal fin bending and abdomen bulging are common in fish exposed sub lethal concentrations of phathalates [27]. Previous studies have reported similar morphological alterations in *Clariasgariepinus* fingerlings [20] and *Cirrhinamrigala*fingerlings [12], exposed to sub lethal DEP. In the present study, similar exposure to DEP for a period of seven days resulted in dark pigmentations on body, caudal fin bending and abdomen bulging, while no morphological alteration in control fish (Fig. 2). Interestingly all morphological and behavioural alterations of *O. niloticus* fingerlings have been recovered when introduced to toxic free water within seven days in recovery group except lesion in gills structure, caudal fin bending and bulged abdomen.

IV. Conclusions

Indiscriminate release of pollutants into the environment may disturb the delicate ecological balance of the earth. DEP is one such chemical reported to influence the aquatic biota. Results of the present investigation evidenced that DEP exposure is primarily toxic to *O. niloticus* fingerlings and induced high morphological and behavioural alterations on fingerlings at sub lethal concentrations. Further studies should attempt to study the underlying molecular mechanism of DEP toxicity in aquatic organisms.

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