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Acute toxicity effect of dimethyl phthalate (DMP) on *Oreochromis mossambicus* juveniles

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Abstract
Dimethyl phthalate (DMP), a widely used plasticizer, is a subchronic toxicant and endocrine-disrupting chemical that impairs reproductive health. This study determined the 96-h LC_{50} of DMP and evaluated its sub-acute toxicity in the Mozambique tilapia *Oreochromis mossambicus* using a static-renewal bioassay. Fingerlings were exposed to different concentrations of DMP (0, 20, 40, 60, 80, and 100 mg/L) for 96 h. The 96-h LC_{50} value for the fish was determined to be 42.8 mg/L. Subsequently, one-tenth of the LC_{50} value (4.2 mg/L) was selected as the sub-lethal concentration for the sub-acute study. Fish were exposed to this sub-lethal concentration for 7 days and then allowed to recover in DMP-free water for an additional 7 days. Behavioural responses and morphological abnormalities were evaluated during both the exposure and recovery periods. DMP exposure induced significant behavioural, morphological, and physiological changes in fish, including abnormal swimming patterns, loss of equilibrium, dark pigmentation, caudal fin bending, abdominal bulging, gill lesions, and reduced liver and gonad mass. Following a 7-day recovery period in toxicant-free water, most behavioural alterations were reversed; however, persistent morphological abnormalities indicated incomplete recovery from DMP-induced toxicity. In conclusion, the present study demonstrates that DMP is toxic to *O. mossambicus* fingerlings, inducing significant behavioural and morphological alterations at sublethal concentrations. This study also provides the first estimate of the 96-h LC_{50} of DMP for *O. mossambicus*, contributing baseline toxicity data for this ecologically and commercially important species.

Keywords: Dimethyl phthalate, *oreochromis mossambicus*, acute toxicity, plasticizer, phthalate esters

Introduction
Dimethyl phthalate (DMP), a phthalic acid ester (PAE), is an anthropogenic industrial chemical widely used in cellulose acetate production and as a component of paints, adhesives, printing inks, and coatings (Prasad & Suresh, 2015). [11] Due to its extensive industrial applications, DMP has been recognized as an important environmental contaminant, with a reported half-life of 3.2 years under natural ambient conditions (Staples *et al.*, 1997) [14].Continuous release of DMP into aquatic ecosystems can impair the reproductive health and sexual behaviour of aquatic organisms. Phthalates can enter fish through multiple routes, including uptake via gill respiration, dermal absorption, ingestion of suspended particles, and consumption of other organisms containing phthalate residues (Barnhorn *et al.*, 2004). [2] Several investigations have demonstrated that DMP exposure induces behavioural alterations, reduced growth performance (Rana *et al.*, 2017) [12], endocrine disruption (Wang *et al.*, 2019) [16], and oxidative stress (Cong *et al.*, 2020) [6] in different fish species. In addition, the toxic effects and acute toxicity thresholds of DMP have been reported in several fish species. The 96-h lethal concentration (LC_{50}) of DMP was reported to be 55.8 mg/L in black molly *Poecilia sphenops* (Rana *et al.*, 2017) [12] 45.8 mg/L in zebrafish *Danio rerio* (Cong *et al.*, 2020) [6], 50 mg/L in bluegill *Lepomis macrochirus* (Buccafusco *et al.*, 1991) [3], and 29 mg/L in the marine fish sheepshead minnow *Cyprinodon variegatus* (Adams *et al.*, 1995). [1] However, the acute toxicity of DMP in the freshwater fish *Oreochromis mossambicus* has not yet been investigated. Therefore, the present study aimed to determine the median lethal concentration (LC_{50}) of DMP in *O. mossambicus*.

In the present investigation, Mozambique tilapia *O. mossambicus* fingerlings were selected as the experimental model due to their rapid growth, adaptability to various diets, resistance to diseases and handling stress, successful reproduction under captive conditions, and tolerance to a wide range of environmental conditions (Faheem and Lone, 2017) [7]. Furthermore, *O. mossambicus* is considered a suitable biological model for toxicological and immune toxicological studies (Giron-Perez *et al.*, 2009) [8].

Materials and Methods
Experimental animals
Mozambique tilapia *Oreochromis mossambicus* fingerlings were obtained from the Shanti Sagar lake (14.1495168º N, 75.8935031º E) Sulekere, Channagiri, Davanagere, weigh about (4.5±0.5 g). The fish were acclimated for 20 days in 10-L glass aquaria (30 × 26 × 14 cm; L × W × H) at a stocking density of 12 fish per aquarium containing aged tap water. During the acclimation and experimental periods, the mean water temperature was maintained at 22.92 ± 0.10°C, total dissolved solids (TDS) at 533 ± 0.62 mg/L (ppm), and dissolved oxygen at 7.08 ± 0.25 mg/L. Continuous aeration was provided to maintain adequate dissolved oxygen levels, and the fish were maintained under a natural photoperiod. During the acclimation period, the fish were fed commercial pelleted fish feed (Tokiyo Special Fish Food Pvt. Ltd., Thanakulam, Tamil Nadu, India).

Determination of LC_{50} of DMP
O. mossambicus fingerlings were randomly divided into six groups (12 fish / group / aquarium with 10 L water). A definitive acute toxicity test was conducted using dimethyl phthalate at minimum concentrations 0 (controls), 20, 40,

60, 80 and 100 mg/L. DMP (≥99% purity; CAS No. 84-66-2; HiMedia Chemicals Pvt. Ltd., Maharashtra, India) was used as the test chemical. Fish mortality was monitored and recorded at 24, 48, 72, and 96 h of exposure. Under semi-static exposure conditions, the test medium was renewed every 24 h, and freshly prepared DMP solutions were added daily to maintain the nominal exposure concentrations. The 96-h median lethal concentration (LC_{50}) was determined using probit regression analysis following the method described by Faheem and Lone (2017). [7] The experiment was conducted in accordance with the Organisation for Economic Co-operation and Development (OECD) guidelines for semi-static acute toxicity bioassays (OECD, 2019) [10].

Mortality

Mortality was recorded at 24-h intervals throughout the 96-h exposure period. Fingerlings were considered dead when they lost equilibrium, settled at the bottom of the aquarium, and failed to respond to gentle stimulation with a glass rod, following the criteria described by Mgbaeruhu (2002) and Viddyanand *et al.,* (2023) [9, 15]

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Results and Discussion

Dimethyl phthalate (DMP) is considered a toxicant, although its toxicity is generally lower than that of several other phthalates. It is widely used worldwide in the manufacture of plastics. Due to its extensive use and continuous release from industrial sources, DMP has become a significant environmental contaminant, posing potential health risks to both terrestrial and aquatic organisms (Chi *et al.,* 2021; Cao *et al.,* 2024) [5, 4].

In the present study, *O. mossambicus* were exposed to varying concentrations of DMP (0, 20, 40, 60, 80, and 100 mg/L), and the mortality of the fish at each concentration was recorded (Table 1). The mortality observed during the experiment increased with increasing concentrations of DMP, indicating a dose-dependent response. Probit analysis revealed that the median lethal concentration (LC_{50}) of DMP for *O. mossambicus* was 42.8 mg/L (Fig. 1). The acute toxicity (LC_{50}) of DMP in fish has been reported in only a limited number of studies. Previously reported 96-h LC_{50} values include 55.8 mg/L for black molly *Poecilia sphenops* (Rana *et al.,* 2017) [12], 45.8 mg/L for zebrafish *Danio rerio* (Cong *et al.,* 2020) [6], 50 mg/L for bluegill *Lepomis macrochirus* (Buccafusco *et al.,* 1991) [3], and 29 mg/L for the marine fish *Cyprinodon variegatus* (Adams *et al.,* 1995). [1] The 96-h LC_{50} value obtained in the present study (42.8 mg/L) falls within the range reported for other fish species, suggesting that *O. mossambicus* exhibits a moderate level of sensitivity to DMP exposure. The observed interspecific variation in LC_{50} values may be attributed to differences in species-specific tolerance, physiological and metabolic characteristics, life stage, water quality parameters, and experimental conditions employed across studies.

Complete mortality (100%) was observed in fish exposed to 80 and 100 mg/L DMP, with mortality occurring within 24 h of exposure. In contrast, mortality rates of 33.33%, 41.66%, and 75% were recorded in the 20, 40, and 60 mg/L treatment groups, respectively, while the surviving fish remained alive until the completion of the exposure period. No mortality was observed in the control group (0 mg/L), confirming that mortality was associated with DMP exposure. To the best of our knowledge, this study provides the first estimation of the median lethal concentration (96-h LC_{50}) of DMP in *O. mossambicus.* The LC_{50} value determined in the present study was 42.8 mg/L, indicating that DMP exhibits considerable acute toxicity toward juvenile *O. mossambicus*. The higher sensitivity of juveniles may be attributed to their greater vulnerability to environmental contaminants compared with adult fish. Juvenile fish possess immature physiological defense mechanisms, including underdeveloped detoxification systems such as hepatic biotransformation pathways, which may reduce their ability to metabolize and eliminate toxic compounds. Furthermore, the external fertilization process in fish may increase the likelihood of early-life-stage exposure to contaminants present in the surrounding aquatic environment. These factors may contribute to the enhanced susceptibility of juvenile *O. mossambicus* to DMP toxicity (Zhu *et al.*, 2022) [17].

The behavioural and morphological abnormalities observed in *O. mossambicus* fingerlings following DMP exposure indicate severe physiological stress and toxic effects. Behavioural changes such as erratic swimming, hyperexcitability, loss of equilibrium, tremors, and abnormal surface activity suggest disruption of the nervous system. In addition, morphological abnormalities including abdominal bulging, darkened body pigmentation, and extended gill covers observed at the time of death indicate respiratory impairment and metabolic disturbances. Similar behavioural and morphological alterations have been reported in *Clarias batrachus* (Satpathi *et al.,* 2016) [13] supporting the present findings that DMP exposure adversely affects the normal physiological and behavioural functions of fish.

In the present study, the influence of a sublethal concentration of DMP (1/10th of the 96-h LC_{50}; 4.28 mg/L) on the behavioural and morphological responses of *O. mossambicus* fingerlings was investigated over a seven-day exposure period. The results demonstrated that exposure to this sublethal concentration induced noticeable behavioural and morphological alterations, indicating the onset of toxic stress even at non-lethal levels. Fish exposed to 4.28 mg/L DMP exhibited attempts to leap out of the test medium, dense schooling behaviour, irregular and erratic swimming, hyperexcitability, loss of equilibrium, muscular incoordination, tremors, bottom settling, hiding behaviour, and abnormal surface activity. These behavioural responses are widely recognized as indicators of stress and neurotoxicity caused by toxicant exposure. Morphological alterations, including abdominal bulging, anal casts, progressive dark pigmentation of the body, particularly on the dorsal surface, and widely extended gill covers at death, further suggest severe systemic toxicity affecting osmoregulatory, respiratory, and metabolic functions. Similar behavioural and morphological abnormalities following sublethal exposure to phthalate compounds, including DMP, have been reported in several fish species, indicating that these responses are reliable biomarkers of toxic stress. Comparable observations have also been documented in *Clarias batrachus* (Satpathi *et al.*, 2016) [13] supporting the present findings. Collectively, these results demonstrate that sublethal DMP exposure disrupts normal physiological and behavioural functions in *O. mossambicus*, potentially compromising survival, growth, predator avoidance, and overall ecological fitness under prolonged environmental exposure.

Interestingly, most of the behavioural and morphological alterations observed in *O. mossambicus* fingerlings were reversible following transfer to toxicant-free water, with substantial recovery occurring within seven days. However, certain abnormalities, including gill lesions, caudal fin bending, and a bulged abdomen, persisted even after the recovery period, suggesting that these alterations may represent more severe or irreversible effects of DMP toxicity.

Conclusions

The present study demonstrates that DMP is toxic to *O. mossambicus* fingerlings, inducing significant behavioural and morphological alterations at sublethal concentrations. This study also provides the first estimate of the 96-h LC_{50} of DMP for *O. mossambicus*, contributing baseline toxicity data for this ecologically and commercially important species. Although most alterations were reversible after seven days in toxicant-free water, persistent gill lesions, caudal fin bending, and abdominal bulging indicate the potential for lasting toxic effects. Further studies are needed to elucidate the underlying molecular mechanisms of DMP toxicity and its long-term ecological impacts.

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