Toxicity of formalin on behaviour and respiration in *Danio rerio*

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ABSTRACT

Formalin is an effective chemotherapeutic agent for combating several infectious conditions affecting fish of aquacultural importance and ornamental fish. It is applied as bath, flush or flowing treatment method and has proved to be effective against a variety of fish parasites and microbes. The toxicity of formalin on the behavior and respiration of the popular aquarium Zebra fish *Danio rerio* was evaluated 96 hour LC50 value was found to be 30ppm. Abnormal behavior and swimming movements, reduced opercular beats, increased mucus secretion and mortality were recorded due to formalin toxicity on zebrafish. Oxygen consumption of zebrafish exposed to sub-lethal concentration of formalin showed a time dependent decrease with 62.4% reduction at the end of 96 hours. The need for judicious use of fish chemotherapeutants like formalin and their impact on fish life are discussed.

Key words: Formalin, Toxicity, Behaviour, Oxygen consumption, Zebrafish.

1. Introduction

Fishes are a rich source of protein available in a digestible and convertible form. Besides being a nutritional delicacy, fishes have been valued for many years as excellent bio-indicators of water quality bio-control agents of mosquito larvae (Jhingran, 1977) and have also been attracting attention for their ornamental nature and as game fishes (angling).

In aquaculture, factors such as unfavorable water conditions, overcrowding, frequent administration of medicines, handling and transportation stress (Schaperclaus, 1991) have resulted in defective growth in fishes, mass attack by parasites and episodes of mass mortality. For the prophylaxis and treatment of fish diseases, a large number of disinfectants, anaesthetics, antibiotics and other drugs are in use for a long time (Schaperclaus, 1991). According to Roberts (1978), therapy can be applied to fishes in three ways external treatment involving baths, dips, etc., systemic treatment via the diet and parenteral treatment involving injection of drugs.

Despite the fact that effects of several chemicals employed in fish disease therapy have been analysed by various workers, there seems to be little attention given to formalin (Razia & Radhakrishnan, 1987). Formalin (37-40% formaldehyde) is an effective chemotherapeutic agent successfully used against several infectious conditions, particularly those affecting the body surface and skin of fishes (Roberts & Shepherd, 1979).

Formalin can be applied by bath, flush or flowing treatment methods and has proved effective against a variety of parasites and micro-organisms (Bauer et. al 1969; Schaperclaus, 1991; Shuzo Egusa, 1991). There has been very little work on the toxicity of formalin on fishes.
Smith & Piper (1972), Williams & Wootten (1981) and Razia & Radhakrishnan (1987) had cautioned on the need for close monitoring of oxygen levels following formalin treatment as it is a reducing agent.

The Zebrafish, *Danio rerio*, is a tropical freshwater fish belonging to the family Cyprinidae. It is a popular aquarium fish, frequently sold under the trade name Zebra Danio, and is an important model organism in scientific research.

*Danio rerio*, is a common and useful model organism for studies on vertebrate development and gene function (Lieschke & Currie, 2007). Its greatest advantages for use as a model system include:

- Fully sequenced genetic code;
- Well understood, easily observable and testable developmental behaviors;
- Availability of well-characterized mutants.
- Rapid embryonic development. Large, robust and transparent embryos that develop outside the mother and ability to regenerate fins, skin, heart and the brain (in larval stages).

2. Materials and methods

Fish response to formalin was monitored and recorded daily throughout the 96 hours bioassay. Dead fish were counted & removed. Fish were regarded as dead when all opercular movements ceased. Regular visual observations of the fish (maintained in different concentration of formalin) was made to record the general behaviour, opercular movements, swimming orientation etc. during the entire exposure period.

Using the mortality record, LC$_{50}$ for 96 hours for formalin was determined graphically following the method of Varma & Srivastava (1984)).

Oxygen consumption of the control and treated fish was determined following Strickland and Parsons (1968).

3. Results

In the control fish, no apparent abnormalities in the behaviour pattern were observed. In the case of formalin exposed fish, there was not much change in the behavioral patterns at concentrations of 10ppm and 20ppm. At 40ppm, the fingerlings showed increased swimming movements and decreased rate of opercular movements in comparison to the control fingerlings. At 80ppm, no fingerlings survived at the end of 96 hours exposure to formalin. The fingerlings showed initial restlessness followed by abnormal movements such a leaping to the surface, decreased respiratory frequency (opercular movement) and secretion of mucus. At 40 ppm concentration of formalin, the fingerlings showed an avoidance response by exhibiting increased swimming and opercular movements immediately after exposure. Later within a few hours of exposure, the fingerlings showed loss of equilibrium, secretion of mucus, swimming at upside down position, followed by death usually within 24 hours.

The mortality record for 96 hours exposure at various concentration of formalin is given in Table 1. It is evident that at concentrations of 10 ppm and 20 ppm the % mortality is less than 50%, at 40 ppm it is 70% and shows a marked increase at 80 ppm (100%). Graphical determination of 96 hours LC$_{50}$ value was found to be 30 ppm by the straight line graphical method.
A change in the respiration rate is one of the common physiological responses to toxicants and is easily detectable through changes in oxygen consumption rate, which is frequently used to evaluate the changes in metabolism under environmental deterioration. It is evident from the study that formalin affected the oxygen consumption of Zebrafish *Danio rerio* sub-lethal concentration of the rate of O$_2$ consumption in treated fish showed a progressive decrement compared to the control. At 24 hours, oxygen consumption in the treated fish was 1.48 ml/g/hr, followed by 1.01, 0.08 and 0.65 ml/g/hr at 48, 72 and 96 hours respectively. The % decrease in O$_2$ consumption was 10.6, 19.8, 37.5 and 62.4 during the study period and this was statistically significant at P< 0.01.

4. Discussion

Chemotherapy is widely practiced for treating various infectious disease of fishes. According to Schaperclaus (1991), there are a large number of drugs, antiparasitics, disinfectants, vitamins, hormones etc. at our disposal whose scientific application could give satisfactory results. Among the various chemotherapeutic agents in use, formalin and KMnO$_4$ are commonly used as antiparasitics against a variety of pathogenic organisms. According to Strelkow (1983), the usage of these agents depends essentially on the working spectrum, toxicity, metabolism and residue formation, technological and economical aspect of applicability.

Formalin is one of the most effective and widely used compounds in fish culture for therapeutic and prophylactic treatment of fungal infection and external parasites of fish and fish eggs (Roberts, 1978). Shuzo Egusa (1991) recommended the use of formalin at 25 ppm for the white spot disease caused by *Ichthyophthirius multifilis* and at 15 – 25 ppm in ponds for alternate days for 5 – 7 days to control trematodes. According to Kabata (1970) treatment for 1 hour with 250 ppm formalin destroyed the larvae of the copepod *Salmincola* sp.

The mortality rate and behavior of Zebra fish during experimentation was found to be dependent on duration of exposure and concentration of formalin which is in accordance with similar results obtained for other toxicants (Vutukuru, 2005). Behavioural toxicology is an important tool for hazardous assessment of water pollution and helps in understanding the manifestations of environmental stress on fish (Rao and Rao, 1987). The abnormal behavioural changes exhibited by zebra fish on exposure to formalin such as erratic swimming behavior, increased secretion of mucous, reduced opercular movements have also been reported in fishes exposed to various toxicants like lead, cadmium, chromium and fertilizers (Konar and Sarkar, 1983; Olaifa et al., 2003; Tawari-Fufeyin et al., 2008). The behavioural changes seen in zebra fish in the present study may be an indicative of internal disturbances of body functions such as inhibition of enzymes, impairment of neural transmission, disturbances in metabolic pathways and respiratory distress (Shah and Altindag, 2005).

The rate of O$_2$ consumption of a fish is considered a reflection of total metabolism and hence the metabolic state of the fish. The present study clearly indicates that fingerlings of Zebra fish exposed to various concentrations of formalin showed toxicity & mortality. This could partly be attributed to the role of formalin as a reducing agent resulting in reduction of oxygen levels in the water (Roberts, 1978). Reduced oxygen tension along with other environmental stressors like high ammonia levels, suspended solids will impose stress and predispose the fishes to various secondary microbial infections (Shepherd and Poupard, 1975). Another reason for the reduction in the rate of oxygen consumption by the fingerlings...
may be due to damage caused to the gill epithelia by exposure to formalin (Smith and Piper, 1972).

Hence, judicious use of the chemotherapeutant with proper monitoring is essential to understand its toxicity on fish and thereby help to achieve the aim of preventing the spread of fish infections and infestations.

**Table 1:** Mortality of Zebra fish fingerlings at different concentration of formalin

<table>
<thead>
<tr>
<th>S. NO</th>
<th>FORMALIN CONCENTRATION (ppm)</th>
<th>% MORTALITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CONTROL</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>70</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 2:** O$_2$ consumption in *Danio rerio* exposed to sub lethal concentration of formalin

<table>
<thead>
<tr>
<th>S. NO</th>
<th>TIME (HOUR)</th>
<th>OXYGEN CONSUMPTION IN CONTROL (ml/g/hr)</th>
<th>OXYGEN CONSUMPTION IN TREATED FISH (ml/g/hr)</th>
<th>% INCREASE OR DECREASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>1.4</td>
<td>1.48</td>
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<tr>
<td>2</td>
<td>48</td>
<td>-</td>
<td>1.01</td>
<td>-19.8</td>
</tr>
<tr>
<td>3</td>
<td>72</td>
<td>-</td>
<td>0.080</td>
<td>-37.5</td>
</tr>
<tr>
<td>4</td>
<td>96</td>
<td>-</td>
<td>0.065</td>
<td>-62.4</td>
</tr>
</tbody>
</table>

(Significant at P< 0.01)

5. **References**


