Effect of sublethal levels of nitrite on some blood parameters of juvenile *Labeo rohita* (Hamilton-Buchanan)

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Juveniles of *L. rohita* were exposed to sublethal levels of nitrite (0.02, 0.1 and 0.4 mg/l) for 2, 24, 48 and 96 hr. The time of exposure at individual concentrations of nitrite did not show any significant difference in haemoglobin, cortisol, chloride and lactic acid. Haematocrit showed significant reduction with increasing concentration of nitrite irrespective of duration of exposure. Fishes exposed to 0.4 mg/l nitrite showed significantly high levels of glucose beyond 2 hr. The mean erythrocytic fragility of fishes exposed to the 3 concentrations of nitrite for 3 exposure periods showed significant higher sensitivity to osmotic stress. The results suggest decrease in haematocrit and cell wall strength of erythrocytes creating stress to fish.

**Keywords:** Blood, Erythrocytes, *L. rohita*, Nitrite, Stress

The accumulation of nitrite in the environment is one of the principal problems in freshwater fish culture. Nitrite is highly toxic to freshwater fishes, but its toxic action is poorly understood. Formation of methemoglobin, a substance that interferes with the ability of blood cells to carry oxygen when concentration reach 30-40% of total haemoglobin concentration is the most recognizable sign of nitrite toxicity in humans. Fish exposed to nitrite in the water are able to accumulate the anion in the blood plasma and tissues, with nitrite freely crossing the red cell membrane and oxidizing haemoglobin to methemoglobin. This gives a reddish brown colouration to blood, thereby reducing the functional haemoglobin.

Sublethal effects of nitrite have been studied in salmonids, *Ictalurus punctatus*, *Salmo gairdneri*, fat head minnows, *Catla catla*, *Ctenopharyngodon idella* and in *Mugil platanus*. The present study has been undertaken to evaluate the changes in various blood parameters of the fish *Labeo rohita* (Hamilton-Buchanan) exposed to sublethal levels of nitrite (0.02, 0.1 and 0.4 mg/l) for 96 hr.

**Materials and Methods**

Hatchery bred fingerings of *L. rohita* of average length and weight (115 mm and 15.0 g) were acclimatized in the laboratory in fibre glass tank containing chlorine free tap water at a stocking density of 0.4 g/l with continuous aeration at 12:12 L:D cycle. The water quality conditions were pH (7.7-7.8), water temperature (27°C-28°C), alkalinity (136-160 mg/l) and hardness (170-190 mg/l). All the fishes were fed tubifex worms *ad libitum*. Water was exchanged daily and excretory products were siphoned out. Water quality parameters were measured as per APHA.

After acclimatization for 1 month, 240 healthy fishes without any parasitic infestation were exposed to three sublethal levels of nitrite. The 96 hr LC_{50} of nitrite for the fishes was found to be 2.5 mg/l. The fishes were divided into four groups. The fish in group 1 were not exposed to any treatments and served as control. Fishes in groups 2-4 were exposed to 0.02, 0.1 and 0.4 mg/l nitrite respectively for 96 hr.

All the experimental fishes were kept at the rate of 30 fish in 450 l of tap water in duplicate tanks with continuous aeration. The pH of the all tanks was maintained at 8±0.02 units with the temperature being at 28°C. Nitrite was introduced as reagent grade sodium nitrite and concentrations were confirmed by an azo-dye method.

To measure red cell fragility, 50 μl of freshly drawn heparinised blood was added to each 10 tubes containing 3 ml unbuffered saline solution of 0.9, 0.7, 0.6, 0.5, 0.45, 0.4, 0.35, 0.3, 0.2, 0.1 g/dl NaCl. After 5 min the tubes were vortexed and centrifuged to
sediment the whole red cells, cell fragments and blood clots. The absorbance of the decanted supernatant was determined at 460 nm using a 0.9% saline blank. Maximum haemolysis occurred at 0.1 g/dl NaCl. A plot of haemolysis versus saline concentration produced a sigmoid curve and the concentration at which 50% haemolysis occurred was the median corpuscular fragility in g/dl NaCl.

Blood sampling and analysis—Ten fishes from each group were sacrificed after 2, 24, 48 and 96 hr from the start of the experiment. To obtain blood, L. rohita fingerlings were netted gently and rapidly anaesthetized using MS 222 (ethyl m-amino-benzoate methane sulphonate) at the dose of 60 mg/l and the fishes were immobilized within 1 min of application. Blood was collected by severing the caudal peduncle. Heparin was used as an anticoagulant. Immediately after collection blood was centrifuged at 3000 rpm for 5 min in cold and plasma separated out was either used for analysis immediately or stored at −20°C for analysis later. Sampling procedure of netting, anaesthesia and plasma storing was completed within 10 min to avoid influence of netting, combined with anaesthesia on the basal cortisol levels. The methods employed for estimation of various blood parameters are glucose, plasma chloride, lactic acid, haematocrit, cortisol, chloride and lactic acid. In haematocrit nitrite application showed significant reduction in the value irrespective of duration of exposure. Exposure of nitrite @ 0.4 mg/l to fishes showed significantly high levels of glucose over control beyond 2 hr.

Osmotic fragility—Statistically significant higher sensitivity to osmotic stress, compared to control (0.375 mg/l) was observed (Table 1). However, after 2 hr of exposure, the 0.02 mg/l nitrite exposed fishes were less sensitive to osmotic stress than control with mean erythrocytic fragility (MEF) being 0.365 g/dL of NaCl.

Discussion
It is evident that exposure to nitrite in water reduced the levels of haemoglobin in the fishes. The nitrite ions in water enter fish through the chloride cells. From the blood plasma, nitrite diffuses into red blood cells, where it oxidizes the iron in haemoglobin to the +3 oxidation state, which lacks the capacity to bind oxygen reversibly. Degeneration of red cell structural and functional properties may cause an increased removal of red cells from the circulation and contribute to the

<table>
<thead>
<tr>
<th>Nitrite concentration (mg/l)</th>
<th>2 hr</th>
<th>24 hr</th>
<th>96 hr</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.375 ±</td>
<td>0.375 ±</td>
<td>0.375 ±</td>
</tr>
<tr>
<td>0.02</td>
<td>0.0058</td>
<td>0.0058</td>
<td>0.0058</td>
</tr>
<tr>
<td>0.1</td>
<td>0.365 ±</td>
<td>0.425 ±</td>
<td>0.435 ±</td>
</tr>
<tr>
<td>0.0033</td>
<td>0.0029</td>
<td>0.0029</td>
<td>0.0029</td>
</tr>
<tr>
<td>0.1</td>
<td>0.420 ±</td>
<td>0.435 ±</td>
<td>0.452 ±</td>
</tr>
<tr>
<td>0.0029</td>
<td>0.0039</td>
<td>0.0039</td>
<td>0.0039</td>
</tr>
<tr>
<td>0.4</td>
<td>0.435 ±</td>
<td>0.430 ±</td>
<td>0.450 ±</td>
</tr>
<tr>
<td>0.0025</td>
<td>0.0087</td>
<td>0.0087</td>
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<td>0.0029</td>
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decrease in total haemoglobin seen in nitrite-exposed fish \textsuperscript{26}. Shrinkage in red blood cells was evident in blood films of nitrite-exposed fishes. This may have reduced the haematocrit levels in various exposed fishes. This is in agreement to the observation Jensen \textit{et al.}\textsuperscript{26}.

The elevations in cortisol and glucose at 0.4 mg/l nitrite exposed fishes were a result of general stress related physiological changes. Elevation of hypothalamus pituitary interrenal (HPI) axis causes the rise in blood cortisol\textsuperscript{27}, which in turn enhances the blood glucose level\textsuperscript{28}. Elevation of plasma cortisol and glucose have been well documented as primary and secondary stress responses respectively, in fish\textsuperscript{29}. Carmichel \textit{et al.}\textsuperscript{30} reported an increase in plasma cortisol and glucose in stressed large mouth bass.

Reduction of plasma chloride observed is a characteristic effect in nitrite-exposed fish\textsuperscript{26} where

![Graphs showing changes in physiological parameters](image)

Fig.1—Changes in the physiological parameters of \textit{L. rohita} fingerlings of control and subjected to sublethal nitrite (0, 0.02, 0.01 and 0.4 mg/l) for 2, 24, 48 and 96 hr (a: haemoglobin, b: haematocrit, c: plasma cortisol, d: plasma glucose, e: plasma chloride, f: plasma lactic acid). **Significant at 1\% level.
passive efflux of CI ions takes place and active branchial CI uptake is partially converted to NO3 uptake. Perrone and Meade proposed that nitrite present in the environment is actively pumped across the gills at the chloride uptake sites (chloride cells) of fishes. Nitrite enters the fish via the same route as uptake. This uptake mechanism rationalizes why an elevation of environmental chloride levels protects against nitrite accumulation and toxicity. Nitrite increases chloride cell activity to maintain ion homeostasis. Cortisol is known to cause proliferation of chloride cells on the gills. The increased cortisol levels as observed in known to cause proliferation of chloride cells on the increased chloride cells density may enhance the chloride uptake capacity in response to the chloride depletion inside the system, which at the same time may be maladaptive by increasing nitrite uptake from the environment.

Except in fishes exposed for 2 hr in 0.02 mg/l concentration of nitrite, all fishes at different exposure period and at different concentrations exhibited decrease in cell wall strength. But the haemoglobin levels recovered possibly by recruitment of new RBC as a result of stress induced adrenaline stimulation of spleen.

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