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Responses of *Labeo rohita* fingerlings to N-acetyl-p-aminophenol toxicity

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ABSTRACT

The short term (96 h) toxicity of N-acetyl-p-aminophenol (0.58 mg/L – Treatment I and 0.29 mg/L – Treatment II) on certain health indicators (haematology, biochemical, and enzymology) of an Indian major carp *Labeo rohita* was studied. When compared to control, N-acetyl-p-aminophenol treated fish showed a significant ($P < 0.05$) decrease in haemoglobin (Hb), haematocrit (Hct), and erythrocyte (RBC) levels throughout the study period. Whereas, a significant ($P < 0.05$) increase were noted in leucocyte (WBC) counts (except 48 h in Treatment-I), mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) values (except 24 h in Treatment-I). Mean corpuscular haemoglobin concentration (MCHC) values were found to be decreased significantly ($P < 0.05$) in fish exposed to 0.58 mg/L of N-acetyl-p-aminophenol, whereas in 0.29 mg/L exposed fish the values were found to be increased significantly ($P < 0.05$) (except 72 h). A significant ($P < 0.05$) increase in plasma glucose levels was noticed in fish exposed to 0.58 mg/L of N-acetyl-p-aminophenol (except 96 h). However, a biphasic trend in plasma glucose level was observed at 0.29 mg/L of N-acetyl-p-aminophenol exposed fish. Protein levels were found to be increased in both the treatments except at the end of 48 and 96 h in 0.58 and 0.29 mg/L, respectively. In both the treatments fluctuations of enzyme (GOT, GPT, and LDH) activities in gill and liver were also noted. However, these enzyme activities found to be significantly ($P < 0.05$) decreased in kidney and plasma of fish. From the result we conclude that the drug N-acetyl-p-aminophenol upon short term exposure could pose a risk to fish and the alteration of these parameters can be used to ecological risk assessment of pharmaceuticals in aquatic organisms.

1. Introduction

Continuous release of xenobiotics and their metabolites into the aquatic environment is a matter of serious concern all over the world. Pharmaceuticals a diverse group of substances can enter the aquatic environment from wastewater treatment plants in the form of non-metabolized parent compounds or their metabolites (Fent et al., 2006; Ramesh et al., 2018). The occurrence of pharmaceuticals has been detected in aquatic recipients such as sewage treatment plants, municipal wastewater, agricultural run-off, natural surface water, ground water, drinking water (Corcoran et al., 2010; Liao et al., 2015), and also in aquatic organisms (Mottaleb et al., 2016). Studies have reported that the concentrations of these pharmaceutical medications in various aquatic matrices are in µg or mg levels (Phillips et al., 2010; Ramaswamy et al., 2011; Shanmugam et al., 2014). In contrast to reports available on the occurrence of pharmaceutical and their residues in aquatic biota, toxicity and effects on aquatic organisms were poorly understood (Gilroy et al., 2014).

Among various pharmaceuticals, non-steroidal anti-inflammatory drugs (NSAIDs) are highly prescribed medications for many diseases. Their role is to act against enzymes involved in prostaglandin synthesis (cyclo-oxygenase-1 and 2) (Day and Graham, 2013; Nakatsu et al., 2018). N-acetyl-p-aminophenol is a type of NSAIDs and predominantly used as a first line remedy against pain, inflammation, and fever (Ramos et al., 2014). Moreover, N-acetyl-p-aminophenol is also used in > 100 products either alone or in combination with other medications (Shankar and Mehendale, 2014). It is a weak acid (pKa: 9.5), while in digestive track, it is neutral, thus rapidly absorbed (Abdel-Daim et al., 2017). As an active substance in NSAIDs, it is found up to an average of 500 mg (one-component) and 325 mg (one of the components). More researches needed to be focused on the toxicology profile of this component because; it has been believed “safe at the applied doses in medications”. It is a prescribed medication for breastfeeding and pregnant women, and also an alternative for deadly ibuprofen or acetylsalicylic acid (used as pain reliever by pregnant women). It has a vital role against diseases in animals (poultry and

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Table 1
Changes in the haematological parameters in a freshwater fish *L. rohita* treated (Treatment-I: 0.58 mg/L; Treatment-II: 0.29 mg/L) with N-acetyl-p-aminophenol.

Parameters	Exposure	Haematology		
	period (h)	Control	Treatment- I	Treatment- II
Hb (g/dl)	24	13.08 ± 0.27^{a}	8.18 ± 0.05^{c} (− 37.46)	11.77 ± 0.11^{b} (− 10.01)
	48	13.22 ± 0.19^{a}	10.33 ± 0.18^{c} (− 21.86)	11.31 ± 0.06^{b} (− 14.44)
	72	13.20 ± 0.09^{a}	9.43 ± 0.46^{b} (− 28.61)	8.56 ± 0.24^{b} (− 35.20)
	96	13.15 ± 0.03^{a}	7.36 ± 0.26^{c} (− 44.03)	10.46 ± 0.27^{b} (− 20.45)
Hct (%)	24	38.70 ± 0.76^{a}	24.04 ± 0.17^{c} (− 37.88)	34.56 ± 0.29^{b} (− 10.69)
	48	38.78 ± 0.72^{a}	30.56 ± 0.55^{c} (− 21.19)	33.04 ± 0.32^{b} (− 14.80)
	72	38.98 ± 0.31^{a}	27.78 ± 1.34^{b}(− 28.73)	25.28 ± 0.77^{b} (− 35.14)
	96	38.44 ± 0.07^{a}	21.78 ± 0.79^{c} (− 43.34)	30.90 ± 0.67^{b} (− 19.61)
RBC (millions/cu mm)	24	6.78 ± 0.11^{a}	4.28 ± 0.11^{b} (− 36.87)	3.78 ± 0.14^{c} (− 44.24)
	48	6.78 ± 0.11^{a}	3.90 ± 0.19^{b} (− 42.47)	4.00 ± 0.14^{b} (− 41.00)
	72	6.74 ± 0.05^{a}	3.34 ± 0.17^{b} (− 50.44)	3.14 ± 0.28^{b} (− 53.41)
	96	6.76 ± 0.18^{a}	3.38 ± 0.30^{b} (− 50.00)	3.10 ± 0.26^{b} (− 54.14)
WBC (1000/cu mm)	24	36.70 ± 0.03^{c}	45.80 ± 0.01^{a} (+ 24.79)	42.66 ± 0.02^{b} (+ 16.23)
	48	36.83 ± 0.03^{b}	35.64 ± 0.01^{c} (− 03.23)	37.37 ± 0.01^{a} (+ 01.46)
	72	36.66 ± 0.01^{c}	44.70 ± 0.01^{a} (+ 21.93)	39.37 ± 0.01^{b} (+ 07.39)
	96	36.21 ± 0.01^{c}	39.44 ± 0.01^{b} (+ 08.92)	40.23 ± 0.01^{a} (+ 11.10)
MCV (fl)	24	57.08 ± 0.81^{b}	56.29 ± 1.38^{ab} (− 01.38)	92.00 ± 3.96^{a} (+ 61.17)
	48	57.29 ± 1.72^{b}	79.12 ± 4.24^{a} (+ 38.10)	83.01 ± 3.07^{a} (+ 44.89)
	72	57.84 ± 0.67^{b}	83.76 ± 4.83^{a} (+ 44.81)	83.05 ± 7.40^{a} (+ 43.58)
	96	57.02 ± 1.54^{b}	66.02 ± 5.04^{b} (+ 15.78)	84.97 ± 3.49^{a} (+ 49.01)
MCH (pg)	24	19.26 ± 0.25^{b}	19.05 ± 0.45^{b} (− 01.09)	31.19 ± 1.33^{a} (+ 61.94)
	48	19.46 ± 0.51^{b}	26.67 ± 1.50^{a} (+ 37.05)	28.30 ± 1.11^{a} (+ 45.42)
	72	19.57 ± 0.20^{b}	28.33 ± 1.64^{a} (+ 44.76)	28.01 ± 2.55^{a} (+ 43.12)
	96	19.43 ± 0.55^{b}	22.17 ± 1.62^{b} (+ 14.10)	34.73 ± 3.05^{a} (+ 78.74)
MCHC (g/dl)	24	33.87 ± 0.04^{a}	33.85 ± 0.06^{a} (− 0.05)	33.90 ± 0.07^{a} (+ 0.08)
	48	33.85 ± 0.05^{ab}	33.70 ± 0.09^{b} (− 0.04)	34.07 ± 0.17^{a} (+ 0.64)
	72	33.86 ± 0.07^{a}	33.82 ± 0.10^{a} (− 0.11)	33.70 ± 0.10^{a} (− 0.47)
	96	33.80 ± 0.20^{a}	33.61 ± 0.19^{a} (− 0.56)	34.03 ± 0.23^{a} (+ 0.68)

Values are expressed as the Mean ± S.E of five individual observations. Values in the parentheses represent % change over control. (+) Denotes percent increase over control. (−) Denotes percent decrease over control. Means within a row bearing same letters are not significantly different ($P < 0.05$) according to DMRT.

cattle) (Smith et al., 2017). In contradiction, Dierkes et al. (2014) mentioned that N-acetyl-p-aminophenol has could elicit reproductive disorders in male, and respiratory defect in children. Recommended dose of N-acetyl-p-aminophenol to adults and children are 4 g/day and < 75 mg/kg/day, respectively, over dose as a one-component is toxic to adults (7 g), and children (150 mg/kg) (Mazaleuskaya et al., 2015) which may cause hepatic necrosis and renal damage in humans and other experimental animals (Mazer and Perrone, 2008). As per the U.S. Food and Drug Administration (FDA), N-acetyl-p-aminophenol is considered as “black box warning” (Krenzelok, 2009). Prescribed medication and aniline precursors are the potential sources of N-acetyl-p-aminophenol in humans, and half lives in biological samples (plasma, urine, and breast milk) is 2.7 h (Bitzén et al., 1981). Metabolism of N-acetyl-p-aminophenol mostly occurs in liver followed by kidney, and intestine and converted to inactive glucuronide and sulfate conjugates (Mazaleuskaya et al., 2015). Few percentages undergo oxidization by the enzymes (cytochrome P450) to form N-acetyl-p-benzoquinoneimine, a relative metabolite and excrete as cysteine and mercapturicate conjugates. But rest of N-acetyl-p-aminophenol is excreted as in its original form through urine (Dierkes et al., 2014).

The concentrations of N-acetyl-p-aminophenol and residues have been detected in sewage treatment plant effluents (Ternes, 1998), natural water (Kolpin et al., 2002), river (Roberts and Thomas, 2006), surface waters (Grujic et al., 2009), and in drinking water (Fram and Belitz, 2011) of many countries. Moreover, the stress from these biologically active compounds on aquatic organisms including fish is known to be a serious environmental problem throughout the world. In this line, fish are commonly used for monitoring of the impact of pharmaceuticals due to their relatively big body size, long life cycle, easy to raise and also can be considered as a reliable indicator of environmental stressors (Ramesh et al., 2018).

The impact of pharmaceuticals on aquatic organisms can be assessed by several physiological processes. A combination of several physiological measurements may provide better understanding of the effects of environmental contaminants (Fleeger et al., 2003; Poopal et al., 2017). For example the analysis of haematological parameters in fish may be used for identifying the health condition of the fish (El-Sayed et al., 2007; Ramesh et al., 2014). Likewise evaluation of the biochemical parameters of fish could be used as possible biomarker to assess the toxicity of chemicals (Polakof et al., 2011; Parolini et al., 2018). Abdel-Daim et al. (2017) mentioned that transaminases are ideal biomarker to assess the toxicity of N-acetyl-p-aminophenol at organ level. Consequently, we attempted to study the impacts of N-acetyl-p-aminophenol on certain haematological, biochemical, and enzymological parameters in an Indian major carp *Labeo rohita*.

To our best knowledge, the reports on the human pharmaceutical drug N-acetyl-p-aminophenol on Indian major carps are scanty. Moreover, the selected fish species *Labeo rohita* is widely distributed in Indian freshwater and also form a suitable fish for aquaculture due to its fast growing nature and taste.

2. Materials and methods

2.1. Chemicals

N-acetyl-p-aminophenol (CAS no. 103-90-2, purity > 99%) was purchased from Sigma–Aldrich Corporation, USA. N-acetyl-p-aminophenol (1.0 g) was diluted with double-deionized water to make up to 1000 mL to obtain a stock solution. RBC and WBC diluting fluids, haemoglobin reagent (Drabkin solution), O-Toluidine reagent, Folin phenol reagent, and other chemicals with highest analytical grade were purchased from Fischer Scientific India Pvt. Ltd., India. GOT, GPT, and