



DNA alterations in blood cells of *Channa punctatus* after acute exposure to 4-Nonylphenol

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Received: 17 February 2019; Accepted: 26 September 2019

Keywords: Blood cells, Comet assay, Genotoxicity, 4-Nonylphenol

Biodiversity of freshwater is a valuable resource in terms of economic, aesthetic, cultural, educational and scientific values. But in developing countries which are densely populated almost all the rivers receive huge amount of waste from agricultural, industrial and urban discharge, due to which almost all the water bodies are contaminated with countless number of ill defining mixture of chemicals. There are a number of studies which show that in a country like India there is mismanagement of waste water as a number of rivers receive waste water directly emerging from the industries and household waste (Jadhav and Singare, 2015).

Among various organic and inorganic pollutants released into aquatic ecosystem Nonylphenol ethoxylates (NPEOs) have attained a considerable attention due to its endocrine disrupting effect. NPEOs break down to Nonylphenol (NP) in the environment due to microbial degradation (Nakada *et al.* 2006). But the concern grows as NP is lipophilic, hydrophilic, bio-accumulative, non-biodegradable and due to its pervasiveness in the environment. NP is widely distributed in sediments, air, water, soil and waste water (Sharma and Chadha, 2018). NP has been found above permissible levels in a number of rivers in all over the world (Gautam *et al.* 2015; Selviraj *et al.* 2014). Till date main work done on 4-NP with reference to its toxicity are mainly on its endocrine effect associated with the reproductive system (Duan *et al.* 2019; Cheng *et al.* 2019). But only few studies are related to genotoxicity using fish as a model (Sharma and Chadha, 2016).

Fish are in intimate contact with polluted water and are more susceptible to the changes, as it inhabit all zones of aquatic habitat and bio-accumulate the environmental pollutants. Furthermore, fish genotoxicity biomarkers are valuable parameters for environmental risk assessment. Comet assay is most promising and accepted method and extensively applied due to proven suitability to fish. Comet assay having sensitivity for detecting minimum intensity of DNA fragmentation and require a small amount of blood sample (Delmond *et al.* 2019). Blood cells in fish are a major and reliable indicator of stress.

Channa punctatus has certain favourable characteristics like easy maintenance in the aquarium, wide distribution, available throughout the year, and commercial importance which make it an excellent experimental model (Sharma and Chadha, 2017). So present study has been undertaken in the Department of Zoology, Guru Nanak Dev University, Amritsar, in the year 2015, to assess the genotoxic effect of 4-NP in blood cells of fish *C. punctatus* in order to assess the damage and an insight into functional consequences.

4-nonylphenol used in the present study was obtained from Himedia (India). A stock solution was prepared in ethanol. The 96 hours LC₅₀ value of 4-nonylphenol was determined as 1.27 mg/l for *C. punctatus* (Sharma *et al.* 2014). Three sublethal concentrations were decided, which were 1/8th (0.15 mg/l), 1/4th (0.31 mg/l) and 1/2th (0.63 mg/l) of LC₅₀. Glass aquaria of 200 liters capacity were used for the experiment. The physicochemical properties of test water, namely temperature -23.9±0.19, pH -7.4±0.20, dissolved oxygen -3.2±0.30 mg/l, total alkalinity -16.65±0.30, free CO₂ 8±0.23 mg/l, TDS- 0.4±0.01 g/l, TSS- 0.5±0.01, TS-0.9±0.02 g/l were analyzed by standard methods (AWWA, APHA and WPCF 2005). Exposure was given for 96 hours and blood sampling was done after 24, 48, 72 and 96 hours of exposure and analysed for single gel electrophoresis assay. The specimens maintained in tap water were considered as negative control while in ethanol as positive control.

For comet assay methodology of Singh *et al.* 1998 was carried out with slight modifications to suit the present study. Two slides were prepared for each group and analyzed further under fluorescent microscope. Slides were stained with Ethidium bromide. The captured digital images were scored through CASP Lab. Hundred randomly chosen cells were scored. DNA damage was quantified by studying different parameters including Tail length (TL), Tail moment (TM), per cent tail DNA (% DNA) and Olive tail moment (OTM).

The results were expressed as mean±SE and to study the significance of difference among treated and control group, one-way analysis of variance (ANOVA) followed by Tukey-HSD test was applied with the help of SPSS.

Different parameters used in this study are TL, TI, TM

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Table 1. Effect of different concentrations of 4-NP on different parameters of comet assay after 24, 48, 72 and 96 h of exposure in blood cells.

Concentration	Time duration	TL (μm)	TI (%)	TM	OTM
0.15 mg/litre	Control	3.28 \pm 0.09 ^a	0.11 \pm 0.69 ^a	1.12 \pm 0.26 ^a	0.02 \pm 0.013 ^a
	Ethanol	5.5 \pm 0.86 ^a	0.37 \pm 0.16 ^a	0.02 \pm 0.01 ^a	0.1 \pm 0.04 ^a
	24 h	25.34 \pm 9.67 ^b	12.67 \pm 5.87 ^b	4.55 \pm 2.27 ^a	4.31 \pm 2.06 ^b
	48 h	10.36 \pm 1.6 ^{ab}	6.21 \pm 1.13 ^{ab}	1.06 \pm 0.39 ^a	1.64 \pm 0.52 ^{ab}
	72 h	11 \pm 0.67 ^{ab}	7.3 \pm 1.03 ^{ab}	1.44 \pm 0.29 ^a	1.52 \pm 0.23 ^{ab}
0.31 mg/litre	96 h	14.73 \pm 0.25 ^{ab}	9.22 \pm 0.28 ^{ab}	2.14 \pm 0.02 ^a	1.79 \pm 0.29 ^{ab}
	Control	4.5 \pm 0.86 ^a	1.5 \pm 0.41 ^a	0.07 \pm 0.03 ^a	0.36 \pm 0.08 ^a
	Ethanol	12.5 \pm 0.86 ^c	6.39 \pm 2.54 ^{abc}	0.86 \pm 0.37 ^a	1.45 \pm 0.59 ^{ab}
	24 h	28.1 \pm 0.37 ^b	11.3 \pm 0.80 ^{bc}	6.64 \pm 1.35 ^b	4.22 \pm 0.69 ^c
	48 h	18.9 \pm 1.00 ^d	7.51 \pm 0.21 ^{bc}	1.82 \pm 0.11 ^a	2.86 \pm 0.19 ^{bc}
0.63 mg/litre	72 h	9.54 \pm 0.97 ^c	4.49 \pm 0.18 ^{ab}	0.76 \pm 0.09 ^a	1.13 \pm 0.12 ^a
	96 h	43.91 \pm 0.37 ^c	25.5 \pm 0.70 ^d	13.44 \pm 0.31 ^c	10.09 \pm 0.47 ^d
	Control	3 \pm 0.0001 ^a	0.21 \pm 0.09 ^a	0.006 \pm 0.002 ^a	0.05 \pm 0.02 ^a
	Ethanol	8.66 \pm 0.88 ^c	2.73 \pm 0.43 ^b	0.227 \pm 0.009 ^a	0.56 \pm 0.07 ^b
	24 h	17.74 \pm 0.31 ^d	11.45 \pm 0.41 ^d	2.58 \pm 0.22 ^c	2.82 \pm 0.02 ^d
48 h	14.49 \pm 0.33 ^d	5.68 \pm 0.44 ^c	1.07 \pm 0.07 ^b	1.27 \pm 0.05 ^c	
72 h	4.20 \pm 1.68 ^{ab}	0.18 \pm 0.55 ^a	0.20 \pm 0.05 ^a	0.05 \pm 0.15 ^a	
96 h	7.08 \pm 0.11 ^{bc}	1.42 \pm 0.08 ^{ab}	0.13 \pm 0.11 ^a	0.39 \pm 0.02 ^{ab}	

The values given as mean \pm standard error. Different letters (a, b, c, d) with in columns are significantly different (Tukey's test) and signify the effect of different concentrations at different hour of exposure.

and OTM. Different authors used different parameters to study DNA damage and supported the use of different parameters for genotoxicity testing. Some suggested TL as a good indicator of genetic damage (Nwani *et al.* 2010), while the use of TL and TM is supported by Duez *et al.* 2003, use of OTM is emphasized by Sunjoy *et al.* 2013, furthermore use of TL, TI and TM is supported by Adeyemi *et al.* 2015. Blood cells were used in the present study as no cellular dissociation is required. Different degree of DNA damage has been observed in blood cells of *C. punctatus* after treatment with different concentrations of 4-nonylphenol after acute exposure (Fig. 1). Table 1 showed the effect of different concentrations of 4-NP (0.51 mg/litre, 0.31 mg/litre and 0.63 mg/litre) at different hours of exposure on the blood cells. Treatment with 4-NP induced significant change ($P \leq 0.05$) in all the parameters tested when compared to both control groups. High DNA damage was observed at 24 h post treatment (p.t.) followed by a decrease in the values of all the parameters at 48 and 72 h. But at 96 h again the value increased. This might be due to repair of damaged DNA or replacement of highly damaged cells or both (Banu *et al.* 2001). Another reason may be

gene activation like cytochrome p450 which activate the metabolizing enzymes which provide a defensive mechanism against genotoxicants. Similarly, Gulsoy *et al.* (2015) reported that when Zebra fish (*D. rerio*) were exposed to borax, the highest DNA damage was observed at 24 h, followed by a decrease at 48 and 72 h and again increase in the value was observed at 96 h, while treatment with boric acid induced highest effect at 96 h of exposure. The highest DNA damage was seen at 96 h after treatment with 0.31 mg/litre concentration. Decline in the value of higher concentration might be due to the threshold repair theory according to which repair enzyme gets activated only when tissue accumulates the toxicant above a threshold level.

NP is reported to induce DNA adduct formation and mutation or genomic rearrangements (Vazquez-Duhalt *et al.* 2005). Increased DNA damage may also lead to apoptosis and apoptosis was observed in fish sertoli cells when exposed to NP (Makkewy *et al.* 2011). The finding of present study suggests that 4-NP is having the genotoxic effect to fish *C. punctatus* and 24 h show maximum DNA damage at all the three concentrations.

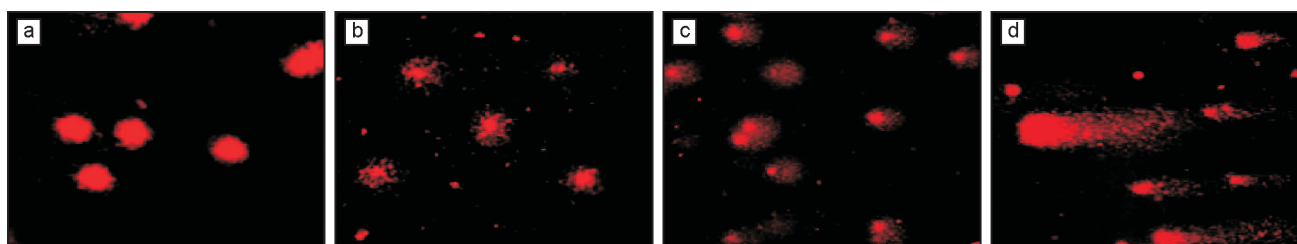


Fig. 1. Showing a different degree of DNA damage in blood cells after acute exposure with 4-NP (a) Control, (b) blood cells with mild damage, (c) blood cells with moderate damage, (d) blood cells with severe damage.

SUMMARY

The DNA damaging effect of 4-nonylphenol was evaluated in blood cells of fish *Channa punctatus* by using single gel electrophoresis assay (SGEA). Fish were exposed to three sublethal concentrations (0.15 mg/l, 0.31 mg/l and 0.63 mg/l) of 4-NP which were calculated after LC₅₀ determination. Exposure was given for 96 hours and blood sampling was done after 24, 48, 72 and 96 hours. Tail moment (TM), tail intensity (TI), tail length (TL) and olive tail moment (OTM) were used as parameters for assessing DNA damage. Comet assay results indicated significant DNA fragmentation in blood cells of *C. punctatus* as a significant increase in the values of all parameters was observed when exposed to different concentrations of 4-NP. Highest damage was observed at 24 h of exposure followed by a decrease in value at 48 and 72 h while at 96 h of exposure increase in the value of all the parameters were observed. On the other hand, after exposure to different concentrations highest damage was seen after treatment with 0.31 mg/l of NP. Owing to the results, the blood cells of *C. punctatus* show great sensitivity for 4-NP and can be used as bio-indicator for genotoxicity testing.

ACKNOWLEDGEMENTS

The authors are sincerely thankful to DST-PURSE for funds and Head, Department of Zoology for providing laboratory facility.

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