4- Nonylphenol induced Genotoxicity assessment in blood cells of fish *Channa punctatus* using Comet Assay

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**Summary**

The present study has been undertaken to study genotoxic effects of endocrine disrupting compound nonylphenol (NP) on *Channa punctatus* after acute exposure using comet assay. Blood cells were used for the study and percent tail DNA was used as biological indicator. Fish were exposed to three sublethal concentrations (0.15 mg/l, 0.31 mg/l and 0.63 mg/l) of 4-NP for 24, 48, 72 and 96 hrs. Blood cell was found to show genotoxic effect and highest genotoxicity was found at 24 hrs of exposure followed by decrease in the value but at later hrs value again increases. So the present study is intended to shed light on the genotoxic potential of 4-NP in fish, *Channa punctatus* and to find the time for maximum induction of genotoxicity.

**Introduction**

Nonylphenol ethoxylates (NPE) are cost effective surfactants used in commercial and household applications such as detergents, dispersing agents and solubilisers (Soares *et al.* 2008). Due to extensive use of NPE, they reach sewage treatment works in substantial amounts where they are incompletely degraded to NP due to microbial action. NP has attracted the attention due to its pervasiveness in the environment. Concerns have increased recently as it can mimic natural hormones and its copious level in environment can disrupt endocrine and reproductive systems. The investigation of the genotoxic potential of various aquatic pollutants has become a major task in the monitoring of environmental pollution. A number of techniques have been developed to test DNA alterations in aquatic organisms. Comet assay has advantage over other tests as these are easy technique, less time and resource consuming.

Blood is highly susceptible to changes in the environment and is a good indicator of environmental toxicity and is used for various genotoxicity studies in the case of fish as it is easy to collect and no cellular dissociation is required. So keeping all these things in mind, this paper aimed at increasing the knowledge and understanding the toxic effects of 4-NP towards an aquatic vertebrates model *Channa punctatus*. *C. punctatus* is distributed throughout India. Such a species is of commercial importance due to its easy maintenance, high food value and availability throughout the year. Moreover *C. punctatus* has been used in fundamental research and considered as an excellent model for toxicological studies.

**Materials and Methods**

Freshwater fish (*C. punctatus*) of an average weight and length of 16.50± 2.14 g and 11.40 ± 2.01 cm respectively, were procured from the local fish market and acclimatized for two weeks under laboratory conditions in glass aquaria of 200 liters capacity. They were fed with boiled eggs and other waste materials were siphoned off daily to reduce the ammonia content in water. The 96 hrs LC$_{50}$ value of 4-NP was determined as 1.27 mg/l for *C. punctatus* following the probit analysis method as described by Finney (1971). Based on the 96 hrs LC$_{50}$ value, the three test concentrations of NP viz; SL-I; 1/8$^{th}$ of LC$_{50}$ = 0.158mg/l, SL-II; 1/4th of LC$_{50}$ =0.317mg/l and SL-III; 1/2nd of LC$_{50}$ =0.635mg/l were estimated and used for the in vivo experiment.

The blood sample was taken at the intervals of 24, 48, 72 and 96 hour at the rate of five fish per interval. The specimens maintained in tap water are considered as negative control while in ethanol as positive control. Comet assay using blood cells were analyzed as per the protocol given by Ahuja and Saran, 1999. DNA damage was assessed using parameter percent tail DNA (T) using Casplab software.

The data was analyzed using Assistat version 7.7 beta (en) using one-way analysis of variance (ANOVA) to assess the effect of concentration and time using. The Tukey-HSD test was considered for multiple comparisons and signify the effect of concentration and time duration.

**Results and Discussion**

Table-1. showed the effect of different concentrations of 4-NP (0.51 mg/l, 0.31 mg/l and 0.63 mg/l) at different hrs of exposure on the blood cells. Treatment with 4-NP induced significant change (p ≤ 0.05) in TI when compared to both control groups. High DNA damage was observed at 24 hrs post treatment (p.t.) followed by a decrease in the TI value at 48 and 72 hrs. But at 96 hrs again the value increased. This might be due to repair of damaged DNA or replacement of highly damaged cells or both. Other 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 hrs, followed by a decrease at 48 and 72 hrs and again increase in the value was observed at 96 hrs, while treatment with boric acid induced highest effect at 96 hrs of exposure. Highest DNA damage was seen at 96 hrs after treatment with 0.31 mg/l concentration. Decline in the value at highest concentration might be due to 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.*, 2008).

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Increased DNA damage may also lead to apoptosis and apoptosis was observed in fish sertoli cells when exposed to NP. The finding of present study suggests that 4-NP is having genotoxic effect to fish *C. punctatus* and 24 hrs show maximum DNA damage at all the three concentrations.

**Table 1.** Percent tail DNA in blood cells of fish *C. punctatus* after exposure to different concentrations of 4-NP for different time intervals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time duration</th>
<th>24 hrs</th>
<th>48 hrs</th>
<th>72 hrs</th>
<th>96 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.11±0.060^a</td>
<td>1.5±0.41^a</td>
<td>0.21±0.09^a</td>
<td>0.60±0.54^a</td>
</tr>
<tr>
<td>Ethanol</td>
<td></td>
<td>0.37±0.16^ab</td>
<td>6.39±2.54^ab</td>
<td>2.73±0.43^ab</td>
<td>1.87±0.36^ab</td>
</tr>
<tr>
<td>0.15 mg/l</td>
<td></td>
<td>12.67±5.87^ab</td>
<td>6.21±1.13^ab</td>
<td>7.3±1.03^ab</td>
<td>9.22±0.28^ab</td>
</tr>
<tr>
<td>0.31 mg/l</td>
<td></td>
<td>11.3±0.80^ab</td>
<td>7.5±0.21^ab</td>
<td>4.49±0.18^ab</td>
<td>25.5±0.70</td>
</tr>
<tr>
<td>0.63 mg/l</td>
<td></td>
<td>5.68±0.44^ab</td>
<td>11.45±0.41^ab</td>
<td>0.18±0.55^ab</td>
<td>1.42±0.08^ab</td>
</tr>
</tbody>
</table>

The values given as mean ±standard error. Different letters a, b, c, d and p, q, r, s are significantly different (Tukey’s test) and signify the effects concentrations and time duration.

**Acknowledgement**

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**References**


