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From Genotoxicity Induction to Recovery in Different Organs in Fish *Channa punctatus* after Sub Chronic Exposure to 4-Nonylphenol

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Abstract

The present study has been undertaken to study the damage and recovery capabilities of different organs (liver, kidney and gill) of fish, *Channa punctatus* after sub chronic exposure to three sublethal concentrations of 4-nonylphenol and 30 days recovery period. To see genotoxic effect and appraise the recovery capabilities Micronucelated Cell (MNC), Binucleated Cells (BNC) and Aberrant Cell (AC) frequency by micronucleus assay and tail moment was evaluated by using the comet assay. The exposure was given for 90 days and the effect was seen after 30, 60 and 90 days of exposure. Three sublethal concentrations were decided after calculating the safe application rate. Exposure results in increased frequency of MNC, BNC and AC in all the tissues. Gill tissue was found to be more sensitive to 4-nonylphenol exposure. The effects show that 4-NP can cause water quality deterioration, resulting in deleterious effect on the health of fish. After 90 days exposure, 30 days recovery was also observed and significant reduction in the values of both the parameters was observed showing a great capacity of *C. punctatus* to restore its DNA integrity.

Keywords: Comet Assay, Genotoxicity, Micronucleus Assay, 4-Nonylphenol

1. Introduction

Release of Industrial and urban waste into rivers has resulted in deposition of a variety of new toxic chemicals and organic compounds in Asian countries. Change in genome caused by pollutants led to mutation and pose a burden to the population of fish. Pollution of the aquatic ecosystems is worldly recognized as a potential threat to all animals, including humans as well as aquatic organisms which interact with aquatic environment[1].

Nonylphenol ethoxylate are non ionic surfactants widely used for domestic and industrial purpose. 4 nonylphenol an estrogen mimicking compound is produced by biodegradation of alkylethoxylates. 4-NP is more lipophilic, persistent and estrogenic as compared to its parent compound [2]. In the developing countries, 90 to 95% of all sewage and 70% of industrial wastes are dumped untreated into surface water[3]. Moreover Jadhav and Singare, (2015) revealed that in India almost all the water bodies contain one or other pollutant due to mismanagement of industrial effluent.

Numerous studies reported the toxicity and oestrogenic activity of nonylphenol little is known about mutagenicity of these compounds. In this paper genotoxicity of 4-NP was evaluated by using the micronucleus assay and comet assay on fish *Channa puntatus* as an experimental

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model. *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[5]. Moreover *C. punctatus* has been used in fundamental research and considered as an excellent model for toxicological studies.

Limbu *et al.*[6] revealed that to get integrated health responses of an organism a battery of biomarkers have proven to be useful. Genotoxicity testing has enjoyed a wide acceptability as can be used in a broad range of species. Comet assay and micronucleus assay are the most promising and accepted methods used for testing cytogenetic damage. These are extensively allied methods among currently available due to proven suitability to fish[7].

Tissue specific response depends upon the properties of cell, tissue and contaminants. Gill is the organ for respiration, plays a role in osmoregulation and nitrogen excretion and is particularly sensitive to adverse environmental conditions. A number of studies have documented the toxic effect of nonylphenol ethoxylate and concluded that gill of fish is particularly sensitive for the surfactants[8]. Kidney play important role in maintaining osmotic homeostasis. It is the main organ of homeostasis and filtration. The liver is the primary target organ for toxicants and is involved in bioaccumulation[9].

We aimed to see tissue specific genotoxic effects of 4-NP in gill, kidney and liver after subchronic exposure of 60 days. We further assessed the repair mechanism in fish *C. punctatus* after 30 days of cessation of exposure. To the best of author knowledge, no such study has been conducted in literature pertaining to 4-NP in fish *C. punctatus*.

2. Materials and Methods

2.1 Chemical

4-nonylphenol used in the present study was obtained from Himedia (India). A stock solution was prepared in ethanol. 1 gm NP/L was used for preparing the stock solution using ethanol as carrier solvent.

2.2 Experimental Fish Specimens and Chemicals

To determine the 96 hours LC_{50} value of 4-Nonylphenol, acute toxicity bioassay was conducted in the static system in the laboratory with the change of test solution for every day to maintain the similar concentration of the chemical. The 96 hours LC_{50} value of 4-nonylphenol was determined as 1.27 mg/l for *C. punctatus*[10] following the probit analysis method as described by Finney[11]. Safe Application Rate (SAR) was calculated using the formula given by Basak and Konar[12]. After calculating the SAR three sublethal concentrations were decided, which were $1/10^{th}$ (0.15 mg/l), $1/15^{th}$ (0.10 mg/l) and $1/20^{th}$ (0.07 mg/l) of SAR.

$$SAR = \frac{LC_0 \times LC_{100}}{LC_{50}}$$

2.3 In Vivo Exposure Experiment

The fish specimens were exposed to the three aforementioned test concentrations of 4-nonylphenol, in tap water and ethanol in a static renewal system with the change of test water on every day to maintain the concentration. In order to eliminate the leaching potential of 4-nonylphenol, plastic material was avoided and 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 CO_2 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[13]. The exposure was continued up to 90 days and blood sample were taken at the intervals of 30, 60 and 90 days at the rate of five different fish per interval. Recovery was observed after 30 days of cessation of exposure. The specimens maintained in tap water are considered as negative control while in ethanol as positive control.

2.4 Micronucleus Assay

For each experimental group as well as control tissue from gill and kidney and liver were taken after dissection. Tissue was kept in 45% acetic acid for tissue maceration for 30 minutes. Then tissue smear was smeared onto the clean slides. Slides were then fixed in absolute ethanol for 10 min and stained with Giemsa 10%[14]. In each group 1000 cells were counted under a binocular microscope (Olympus) using a 100x oil immersion lens.

2.5 Comet Assay

Methodology of Singh *et al.*[15] was carried out with slight modifications to suit the present study. Liver, gill and kidney were obtained from the fish after dissection organs

were kept in PBS immediately and then homogenized. The organ pellet was mixed with the phosphate saline buffer, a procedure used in previous studies (Sunjog *et al.* 2014)[16]. Microscope slides were coated with 1% (w/v) Low-melting-Point Agarose (LMPA) at 37°C. A second layer of organ suspension, LMPA (0.5%), was applied, which contained 15 µl of the tissue suspension and the third layer containing 120 µl of LMPA was also applied. Then, the slides were kept in lysis buffer for 1 h in refrigerator. After lysis, the slides were placed for 20 min in 300 mM NaOH + 1 mM EDTA buffer (pH > 13) and followed by electrophoresis for 20 min at 300 mA and 25 V. The slides were then neutralized with 0.4 M Tris for 15 min. The staining was done with ethidium bromide (0.02 mg ml^{-1}) and the slides were analyzed under a Nikon fluorescence microscope. For each fish, 100 nuclei were analyzed. DNA damage was assessed as tail moment (TM—product of tail length and percentage of tail DNA), using the CASP Lab software.

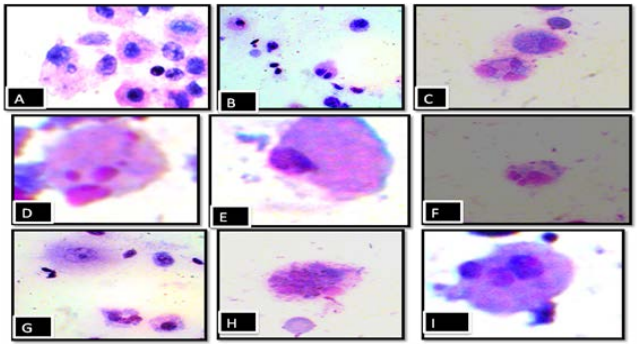


Figure 1. Different nuclear and cytoplasmic changes observed in liver cells (a) Micronucleated cell (b) binucleated cell (c, f, g,h, i) tri, tetra and fragmented nucleus (e) nucleus pushed toward the periphery.

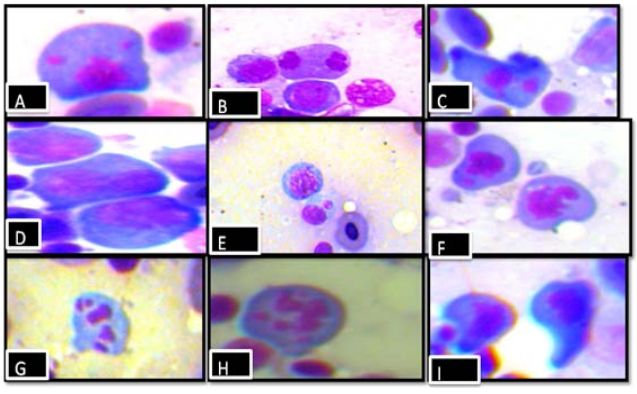


Figure 2. Different nuclear and cytoplasmic changes observed in Kidney cells (a). Micronucleated cell, (b). binucleated cell, (c). tetra nucleus, e. nuclear bud, (f). irregular nucleus. (g,h). fragmented nucleus. (i). Cytoplsmic changes.

2.6 Statistical Analysis

The results are expressed as mean ±S.E. and to study the significance of the difference in frequency of micronucelated and altered erythrocytes among treated and control group One-way Analysis of Variance (ANOVA) followed by Tukey-HSD test was applied with the help of SPSS.

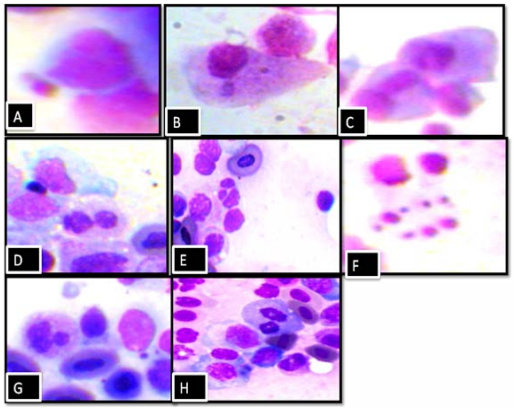


Figure 3. Different nuclear and cytoplasmic changes observed in gill cells (a) Micronucleated cell, (b) Cell with two micronuclei, (c) Binucleated cell, (d,g,h) nuclear bridge, (e) tetranucleated, (f) fragmented nucleus

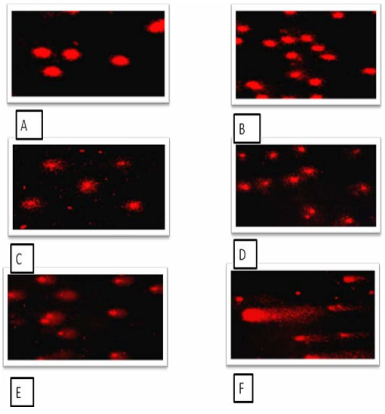


Figure 4. Different level of DNA damage in cells in comet assay (a) control. (b) mild damage. (c,d) moderate damage (e,f) High damage.

Table 1. Frequency of percent MNC, BNC and AC in liver, gill and kidney cells of fish *C. punctatus* after exposure to different concentrations of 4-NP for 30, 60 and 90 days

Tissue	Parameters	Concentration	30 days	60 days	90 days
Kidney	**MNC**	**Control**	0.21±0.01^{a,p}	0.22±0.008^{a,p}	0.21±0.01^{a,p}
		Ethanol	0.32±0.008^{a,p}	0.28±0.01^{a,p}	0.27±0.005^{a,p}
		0.07 mg/l	1.53±0.16^{a,q}	2.22±0.08^{ab,q}	2.87±0.24^{b,q}
		0.10 mg/l	2.19±0.16^{a,r}	3.03±0.03^{b,r}	4.01±0.11^{c,r}
		0.15 mg/l	1.38±0.19^{a,q}	3.03±0.12^{b,r}	5.00±0.11^{c,s}
	BNC	**Control**	0.3±0.01^{a,p}	0.3±0.01^{a,p}	0.27±0.01^{a,p}
		Ethanol	0.39±0.008^{a,p}	0.39±0.005^{a,p}	0.39±0.008^{a,p}
		0.07 mg/l	1.46±0.09^{a,q}	1.87±0.10^{b,q}	2.12±0.05^{b,q}
		0.10 mg/l	2.36±0.17^{a,r}	3.35±0.31^{a,r}	3.19±0.18^{a,r}
		0.15mg/l	2.46±0.28^{a,r}	4.16±0.12^{b,s}	5.46±0.39^{c,s}
	AC	**Control**	3.76±0.14^{a,p}	3.93±0.14^{a,p}	4.06±0.08^{a,p}
		Ethanol	4.85±0.28^{a,p}	4.7±0.20^{a,p}	4.86±0.08^{a,p}
		0.07 mg/l	11.66±0.58^{a,q}	26.84±1.04^{b,q}	29.92±0.31^{b,q}
		0.10 mg/l	18.24±0.64^{a,r}	30.77±0.79^{b,r}	34.88±0.71^{c,r}
		0.15 mg/l	29.51±0.58^{a,s}	33.63±0.57^{b,r}	38.78±0.74^{c,s}
Liver	**MNC**	**Control**	0.16±0.008^{a,p}	0.14±0.01^{a,p}	0.183±0.006^{a,p}
		Ethanol	0.22±0.005^{a,p}	0.22±0.008^{a,p}	0.24±0.006^{a,p}
		0.07 mg/l	1.32±0.06^{a,q}	1.41±0.18^{a,q}	2.42±0.07^{b,q}
		0.10 mg/l	2.09±0.13^{a,r}	3.11±0.10^{ab,r}	4.32±0.53^{b,r}
		0.15 mg/l	2.45±0.26^{a,r}	3.57±0.14^{b,r}	6.28±0.22^{c,s}
	BNC	**Control**	0.2±0.005^{a,p}	0.21±0.01^{a,p}	0.2±0.005^{a,p}
		Ethanol	0.24±0.01^{a,p}	0.23±0.001^{a,p}	0.2±0.005^{a,p}
		0.07 mg/l	1.69±0.42^{a,q}	2.28±0.32^{a,q}	3.89±0.34^{b,qr}
		0.10 mg/l	1.87±0.30^{a,q}	2.89±0.15^{a,q}	5.52±0.24^{b,r}
		0.15 mg/l	1.73±0.26^{a,q}	4.11±0.16^{b,r}	5.31±0.60^{a,qr}
	AC	**Control**	1.13±0.06^{a,p}	1.16±0.08^{a,p}	1.22±0.01^{a,p}
		Ethanol	2.23±0.05^{a,p}	2.40±0.05^{a,p}	2.43±0.04^{a,p}
		0.07 mg/l	22.9±1.18^{a,q}	31.78±1.15^{b,q}	34.78±2.42^{b,q}
		0.10 mg/l	26.05±0.28^{a,q}	32.93±0.71^{b,qr}	35.94±0.31^{c,q}
		0.15 mg/l	31.35±1.53^{a,r}	35.77±0.5^{ab,r}	38.85±1.05^{b,q}
Gill	**MNC**	**Control**	0.07±0.001^{a,p}	0.08±0.002^{a,p}	0.09±0.004^{a,p}
		Ethanol	0.15±0.01^{a,p}	0.14±0.01^{a,p}	0.25±0.025^{b,p}
		0.07 mg/l	1.04±0.081^{a,q}	2.31±0.13^{b,q}	1.96±0.242^{b,q}
		0.10 mg/l	1.24±0.10^{a,qr}	2.06±0.18^{b,q}	3.78±0.13^{c,r}
		0.15 mg/l	1.47±0.06^{a,r}	3.54±0.44^{b,r}	5.77±0.24^{c,s}
	BNC	**Control**	0.21±0.008^{a,p}	0.17±0.02^{a,p}	0.24±0.005^{a,p}
		Ethanol	0.32±0.005^{a,p}	0.3±0.008^{a,q}	0.26±0.04^{a,p}
		0.07 mg/l	1.13±0.07^{a,q}	1.21±0.23^{a,q}	1.11±0.33^{a,p}
		0.10 mg/l	3.03±0.04^{a,r}	3.30±0.32^{a,r}	3.49±0.66^{a,q}
		0.15 mg/l	3.95±0.12^{a,s}	4.91±0.28^{ab,s}	6.49±0.62^{b,r}

	AC	**Control**	1.22±0.13 ^{a,p}	2.07±0.77 ^{b,p}	2.17±0.11 ^{b,p}
		Ethanol	2.29±0.14 ^{a,p}	2.59±0.21 ^{ab,p}	3.03±0.03 ^{b,p}
		0.07 mg/l	16.32±2.29 ^{a,q}	25.25±1.12 ^{b,q}	27.58±1.27 ^{b,q}
		0.10 mg/l	19.74±1.17 ^{a,q}	25.05±1.31 ^{b,q}	29.03±0.99 ^{b,q}
		0.15 mg/l	33.58±1.56 ^{a,r}	37.16±0.89 ^{b,r}	39.93±0.49 ^{b,r}

MNC-Micronucleated cells
BNC- Binucleated cells
AC- Aberrant cells

3. Results

The results of the present study are given in Table 1-4. MNC, BNC and AC frequency in gill, liver and kidney are given in Table 1 and Figures 1, 2 and 3 are showing different changes observed in different tissues. All the concentrations and their respective duration of exposure evoked significant DNA damage when compared with control groups. Highest genotoxicity was observed in gill tissue after 90 days of exposure and at highest concentration followed by liver and kidney. Although the highest MNC frequency was observed in liver tissue, which is 0.51% higher than gill and 1.28% in kidney, but other parameters (BNC and AV) show the highest value in gill tissue. BNC is found to be more 1.03% higher in gill as compared to kidney while 1.18% higher than liver. Table 3 shows the value of the tail moment in all the three tissues after 90 days of exposure with different concentrations of 4-nonylphenol. A significant increase in the value of tail moment was observed in all the tissue with duration and exposure (Figure 4). Similar to micronucleus assay gill show highest DNA damage followed by liver and kidney. Value of tail moment was found to be low at concentration 0.10 mg/l as compared to 0.07 mg/l concentration. But the highest effect is seen at 90 days of exposure and at the highest concentration. Tables 2 and 4 are showing the recovery capabilities of these organs with respect to micronucleus assay and tail movement respectively. The values of all parameters decreased significantly and values at certain concentration almost recover to normal values.

The values given as mean ±standard error. Different letters (a, b, c) between the columns are significantly different (Tukey's test, $p \leq 0.01$) and signify the effect of duration of exposure at each concentration. Similarly, different letters (p, q, r, s) within the columns are significantly different (Tukey's test, $p \leq 0.01$) and signify the effect of different concentrations of 4-nonylphenol at the same time interval.

Table 2. Frequency of percent MNC, BNC and AC in kidney, gill and liver tissue of fish *C. punctatus* after exposure to different concentrations of 4-NP for 90 days and after 30 days recovery

Organs	**Parameters**	**Experimental groups**	**0.07 mg/l**	**0.10 mg/l**	**0.15 mg/l**
Gill	**% MNC**	**Exposed gp**	1. 96±0.24 ^{a}	3. 78±0.13 ^{a}	5.77±0.24 ^{a}
		Recovery gp	0.58±0.01 ^{b}	0.91±0.03 ^{b}	1.02±0.08 ^{b}
	%BNC	**Exposed gp**	1.11±0.33 ^{a}	3.49±0.66 ^{a}	6.49±0.62 ^{a}
		Recovery gp	0.16±0.33 ^{b}	1.5±0.11 ^{b}	1.97±0.03 ^{b}
	%AC	**Exposed gp**	27.58±1.27 ^{a}	29.03±0.99 ^{a}	39.93±0.49 ^{a}
		Recovery gp	10.03±0.31 ^{b}	13.56±0.74 ^{b}	17.28±0.30 ^{b}
Liver	**%MNC**	**Exposed gp**	2.4±0.07 ^{a}	4.32±0.53 ^{a}	6.28±0.22 ^{a}
		Recovery gp	0.82±0.03 ^{b}	1.02±0.02 ^{b}	1.59±0.22 ^{b}
	%BNC	**Exposed gp**	3.89±0.34 ^{a}	5.52±0.24 ^{a}	5.31±0.60 ^{a}
		Recovery gp	0.45±0.05 ^{b}	0.93±0.03 ^{b}	1.3±0.05 ^{b}
	%AC	**Exposed gp**	34.78±2.42 ^{a}	35.94±0.31 ^{a}	38.85±1.05 ^{a}
		Recovery gp	11.91±0.33 ^{b}	16.16±0.57 ^{b}	19.39±0.58 ^{b}
Kidney	**%MNC**	**Exposed gp**	2.87±0.24 ^{a}	4.017±0.119 ^{a}	5.00±0.113 ^{a}

		Recovery gp	0.68±0.01[b]	0.95±0.03[b]	1.18±0.03[b]
	%BNC	**Exposed gp**	2.12±0.05[a]	3.19±0.182[a]	5.46±0.39[a]
		Recovery gp	0.43±0.02[b]	0.96±0.03[b]	1.73±0.14[b]
	%AC	**Exposed gp**	29.92±0.31[a]	34.88±0.71[a]	38.78±0.74[a]
		Recovery gp	11.96±0.33[b]	14.56±0.29[b]	19.45±0.33[b]

MNC-Micronucleated cells
BNC- Binucleated cells
AC- Aberrant cells
Values are given as mean ±standard error. Different letters (a, b) are significantly different (Tukey's test, p≤0.01) and signify the difference between exposed and recovery group.

Table 3. Showing values of different comet parameters Tail Moment (TM) in gill, kidney and liver tissue after exposure to different concentrations of 4-NP for 30, 60 and 90 days

Tail Moment				
Tissue	**Time Interval**	**0.07 mg/l**	**0.10 mg/l**	**0.15 mg/l**
Gill	**Control**	0.92 ± 0.06 [a]	0.85 ± 0.03 [a]	0.981 ± 0.07 [a]
	Ethanol	0.805 ± 0.13 [a]	0.67 ± 0.20 [a]	0.45 ± 0.06 [a]
	30 days	1.1 ± 0.05 [a]	1.97 ± 0.73 [ab]	3.93 ± 0.604 [a]
	60 days	1.59 ± 0.40 [a]	1.07 ± 0.05 [a]	14.59 ± 3.64 [b]
	90 days	5.65 ± 0.34 [b]	3.09 ± 0.45 [b]	15.53 ± 0.06 [b]
Kidney	**Control**	0.16 ± 0.03 [a]	0.09 ± 0.002 [a]	0.26 ± 0.03 [a]
	Ethanol	0.25 ± 0.001 [a]	0.30 ± 0.008 [a]	0.25 ± 0.002 [a]
	30 days	0.3 ± 0.05 [a]	0.99 ± 0.13 [b]	0.7 ± 0.4 [a]
	60 days	0.82 ± 0.25 [a]	4.66 ± 0.15 [c]	2.16 ± 0.29 [b]
	90 days	5.30 ± 0.25 [b]	4.24 ± 0.02 [c]	6.28 ± 0.54 [c]
Liver	**Control**	0.009 ± 0.005 [a]	0.18 ± 0.021 [a]	0.32 ± 0.05 [a]
	Ethanol	0.47 ± 0.05 [b]	0.35 ± 0.03 [a]	0.20 ± 0.01 [a]
	30 days	0.571 ± 0.05 [b]	1.67 ± 0.16 [a]	4.38 ± 0.02 [b]
	60 days	1.84 ± 0.004 [c]	2.70 ± 0.02 [b]	7.79 ± 0.09 [c]
	90 days	1.68 ± 0.05 [c]	5.63 ± 0.51 [c]	8.85 ± 0.12 [d]

**- (p≤0.01)
The values given as mean ±standard error. Different letters (a, b, c, d) with in columns are significantly different (Tukey's test) and signify the effect of 0.15 mg/l concentration at different hours of exposure.

Table 4. Showing values of the tail moment in gill, kidney and liver tissue after exposure to different concentrations of 4-NP for 90 days and after 30 days recovery

		Gill	**Kidney**	**Liver**
0.07 mg/l	**Exposed gp**	5.65 ± 0.34 [a]	5.30 ± 0.25 [a]	1.687 ± 0.05 [a]
	Recovery gp	0.64 ± 0.19[b]	0.31 ± 0.038 [b]	0.91 ± 0.003[a]
0.10 mg/l	**Exposed gp**	3.099 ± 0.45 [a]	4.24 ± 0.028 [a]	5.63 ± 0.51 [a]
	Recovery gp	0.17 ± 0.03 [b]	0.54 ± 0.15 [b]	0.49 ± 0.13[b]
0.15 mg/l	**Exposed gp**	15.53 ± 0.06 [a]	6.28 ± 0.54 [a]	8.85 ± 0.12 [a]
	Recovery gp	0.79 ± 0.16 [b]	0.08 ± 0.01 [b]	0.91 ± 0.41 [b]

Values are given as mean ±standard error. Different letters (a, b) are significantly different (Tukey's test, p≤0.01) and signify the difference between exposed and recovery group.

4. Discussion

Micronucleus test has been used by a number of researchers for assessing the genetic damage[17-19]. Nuclear abnormalities (nuclear abnormalities, including nuclear bud, fragmented nucleus, vacuolated nucleus and binucleated) along with micronucleus are considered as valuable high quality indicators of cytotoxicity[7] though the mechanism of their formation has not yet been fully explained, but according to[20] nuclear abnormalities can be the precursor of MN and may be a process to eliminate amplified genes from the nucleus. These abnormalities are considered as an indicator of genotoxic damage and therefore, may compliment the scoring of MN in routine genotoxicity surveys[21,22]. Under comet assay percent tail DNA was used to assess DNA damage. This is extremely important and sensitive parameter that has been widely used[23,24].

Among aberrant cells, nuclear buds, fragmented nucleus, lobed nucleus, vacuolated cytoplasm, swelled cells are considered. These nuclear abnormalities may be the result of detrimental effects produced by clastogenic pollutants. They may lead to gene amplification and may cause problem in chromosomal attachment. The result of all these changes leads to formation of blebbed, lobed, notched, binucleated, budded and other deformed nuclei[25,26]. Vacuoles in blood cells may be formed due to unequal distribution of hemoglobin and one of the common alterations observed in our study was swelled cells which may be due to necrosis of cellular outline. Similar results were obtained by[27] after treating the fish *C. galipinus* with 4-NP. Osman *et al.*,[28] investigated the genotoxic effect of UV-A radiation by determining MN and BN frequency in erythrocytes of African catfish (*C. gariepinus*). Talapatra *et al.*,[29] also used these parameters for their study using *C. punctatus* exposed to metronidazole. Number of workers have scored nuclear abnormalities along with micronuclei[30-32]. Six types of nuclear lesions were detected along with MN by Osman *et al.*[33] in Nile tilapia (*O. niloticus*) and African catfish (*C. gariepinus*) collected from the course of River Nile.

The present study highlighted intertissue differences regarding the induction of MN. Similar inter-tissue differences in frequency of MN in cod and turbot were observed by[34,35]. During the present investigation gill cells are found to show the highest frequency of micronucleated cells and other aberrant erythrocytes after sub chronic exposures, which increase with dose as well as with time. Gill is the organ which comes in contact with contaminant prior to other organs and exposed constantly. Liver also shows high damage. Zhou *et al.*[36] found that 4-NP show higher hepatic accumulation as compared to gill, muscle and gonads. Various investigations have been conducted where gill is found to be more sensitive than erythrocytes and kidney blood cells[19,37]. Further Cavas and Egene-Gozukara[38] suggested gill tissue as the most sensitive site for MN testing. Similarly MN induction was found 56 times in gill tissue of exposed group as compared the control when Zebrafish (*Danio verio*) was treated with arsenic[39]. A group of researchers has also supported the higher sensitivity of gills than other tissue[19,40,41]. Highest damage in gill and liver due to exposure of detergent in Zebra fish was observed by Figueroa[42]. A decline in ALP enzyme was observed by Sayed *et al.*[43] after treatment with 4-NP which lead to apoptosis and membrane devastation. Reduced activity of antioxidant enzymes has been suggested in liver, gill and spleen of fish in response to 4-nonylphenol and has been suggested to cause oxidative stress, lipid peroxidation and inflammation[44]. A Gang and Han[45] found that exposure of 4-nonylphenol cause accumulation of intracellular reactive oxygen species in rat sertoli cells. 4-nonylphenol treatment may lead to loss of mitochondrial membrane potential. Overwhelming production of ROS may lead to damage to lipid, protein and DNA[46]. Increased cellular damage can result to apoptosis and apoptosis in response to 4-nonylphenol in fish sertoli cells have been observed by Yi *et al.*[47]. Furthermore 4-NP is reported to induce microtubular disrupting activity, DNA adduct formation, point mutation, strand break and genomic rearrangements[8,48].

A significant decrease in DNA damage was found in all the tissues after 30 days recovery. Damage dropped to control value suggested the possibility of complete turnover of fish cells. Similar results were found by Bony *et al.*[49] in Trout in response to vineyard pesticide, in Carp and Bullheads[50] and in Chub[51]. Guilherme *et al.*[52] reported that blood cells of *A. anguilla* showed evident recovery at 14 days after exposure to herbicides based pesticide Roundup®. Further Marques *et al.*[53] found that DNA integrity returned to the control on the first day on cessation of exposure.

5. Conclusion

All the tissues tested show DNA damage after sub chronic exposure. Cells from gill were found to show higher damage as compared to liver and kidney. The various regions for DNA damage, induced by NP may be due to its microtubular disruptive activity, DNA adduct formation, mutation and genomic rearrangement or may be due to the formation of toxic intermediates which may lead to DNA damage. Oxidative stress has been presented not only as an important source of DNA damage, but also as a cause of impairment in the DNA repair system. A significant recovery in DNA damage after 30 days suggest a great capacity of *C. punctatus* to retain its DNA integrity. The study improves the knowledge of the progression of DNA damage upon exposure and post exposure. It is further recommended that to achieve the actual magnitude of risk imposed by a toxicant to aquatic biota recovery experiment should be performed.

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7. References

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