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Original Research
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Effect of 2 Naphthalene Sulfonate on Biochemical Stress Markers as well as Structural Integrity of DNA in Liver and Kidney Tissue of *Channa punctatus* after Acute Exposure

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Abstract

2Naphthalene Sulfonate (2NS) is an intermediate compound used in textile industries but being non-biodegradable, the solicitude regarding its ecotoxicity has risen. Thus, an inquisition was undertaken with the objective of evaluating the oxidative stress and genotoxicity of 2NS in fresh water fish, *Channa punctatus*. Based upon calculated LC50 value, two sublethal doses were selected i.e. 2.38g/L and 4.77g/L for further investigation. In order to study acute effect of 2NS, liver and kidney samples were collected after 24h, 48h, 72h and 96h of exposure. Symbolic elevation in oxidative stress biomarkers and DNA damage was observed revealing the toxic impact of 2NS. The study would be helpful in assessing the risk impose by 2NS and calls for urgency in application of stringent policies against the indiscriminate use of such toxic compounds.

Keywords: 2 Napthalene Sulfonate, Acute Toxicity, Genotoxicity, *Channa punctatus*, Oxidative Stress

Highlights

- 2 Naphthalene sulfonate, a dye inermediate induce genotoxic damage and oxidative stress in fish tissues
- There is a considerable urgent need to develop treatment methods that are effective in eliminating such toxic intermediates from industrial discharge.
- MDA, CAT, SOD and GST and comet assay are suitable biomarkers for analyzing oxidative stress and genotoxicity

1. Introduction

Accelerated growth of industrialization has boosted human interference which leads to high imbalance in virtue of ecosystem leading to environmental pollution[1]. During series of processes, the water comes into contact with harmful chemicals, heavy metals, inorganic wastes and even organic sludge[2]. These are either dumped into rivers or other water bodies which results in accumulation of high amount of industrial waste in them. This affects the status of our eco-system as well as the health of man, plants and animals. Studies showed water pollution to be cause of several dreadful diseases including skin irritation, kidney failure, intestinal tract infection and cancer[3]. Thus aquatic contamination has been considered as one of the critical issues[4,5].

Among several other chemicals being used in various industries, aromatic sulfonates are generated in huge quantities by chemical industries and are widely used in concrete finishing, industrial textile processing and tanning of hides and in manufacture of agrochemicals

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and pharmaceuticals[6,7]. During last few years several sulphonated aromatic compounds are being applied and subsequently discharged into the aquatic environment[4,8] where these chemicals tend to persist for quite a long time making the water unfit for irrigation and drinking purpose. The aquatic organisms which remain in contact with them for a very long time are also prone to toxic damage. 2Naphthalene sulfonate (2NS) belongs to aromatic compounds carrying sulfonate as their functional group and is widely used in industrial and chemical processes. It is used as an intermediate for the production of sulfonated azo dyes, ion exchange resins, pharmaceuticals and pesticides[9]. Studies confirmed its presence in industrial effluents and landfill leachate as a contaminant of soil and water[10–12]. The presence of C-SO_3- imparts polar nature to aromatic sulfonates which obstruct their transport through cell membrane, rendering these compounds resistant to biodegradation by microbial species leading to their accumulation. Despite of various studies showing it as major contaminant present in industrial effluent, scarce studies has been performed on the evaluation of its toxicity on aquatic organisms.

Fish being the first recipient of the contaminants released in aquatic bodies represents the valuable model for toxicity studies. Moreover, *Channa punctatus*, a fresh water fish commonly called as spotted snakehead fish, being available throughout the year serves the most efficient model for the study of aquatic toxicology. Organism uptake xenobiotics via dermal or dietary routes of exposure which tends to accumulate in tissues of aquatic organisms inducing negative effects necessitating the need to study the stress induced in the internal organs. Liver and kidney are considered to be vital organs involved in the process of detoxification, hence target organs of various xenobiotic substances. Thus the present study was performed with the objective of comparing the impact of 2NS on biochemical constituents in liver and kidney of *Channa punctatus* by studying lipid peroxidation and activities of different enzymes like Catalase (CAT), Glutathione S Transferase (GST), Superoxide Dismutase (SOD) and DNA damage.

2. Material and Methods

2.1 Chemical

2NS was purchased from Himedia Research laboratory, Mumbai, India (CAS No. 120-18-3).

2.2 Experimental Design

The freshwater fish *C. punctatus* commonly called as snakehead fish weighing 15 ± 2 g were procured from local outlets and acclimatized in 200-liter capacity glass aquarium for 15 to 20 days under laboratory conditions. The water of aquarium was changed daily in order to avoid the accumulation of waste material and to reduce its ammonia content and fishes were fed with synthetic diet. After determination of LC50 value (9.54g/l)[13], two test concentrations of 2NS viz., 1/4 of LC_{50} and ½ of LC_{50} were selected for the experiment. Kidney and liver samples were collected at intervals of 24, 48, 72, 96h to evaluate detrimental effects induced by acute exposure of 2NS. Experiment was performed in triplicates taking ten fishes in each group for sampling. Fishes maintained in tap water were considered as control group. The physiochemical parameters were checked using standard protocol[14]. The water temperature shifted from control to exposed 26.7 to 28.4 C, pH values ranged from 7.2 to 8.10, dissolved oxygen from 5.63 to 5.34, electrical conductivity 630µs/cm to 1234 µs/cm and total dissolved solids (TDS) 307 mg/l to 612mg/l. Ethical approval for this study was not required as *C. punctatus* is a food fish.

2.3 Oxidative Stress Biomarkers

2.3.1 Sample Preparation

The tissues were blotted dry, weighed and minced very finely with small scissors. 10% homogenate was prepared in ice cold 0.1M Phosphate Buffer Saline (PBS), pH 7.4.

2.3.2 Malondialdehyde (MDA)

The lipid peroxidation was determined according the method of Draper and Hadley[15]. Supernatant was mixed with 1.25 ml TCA, 2 ml TBA (0.67%) and heated for 1 hour at 80^0C.After cooling, the precipitate was removed by centrifugation. The absorbance of the sample was measured at 532 nm using a blank containing all the reagents except the sample. As 99% TBARS are malondialdehyde (MDA), so TBARS concentrations of the samples were calculated using the extinction co-efficient of MDA, which is 1.56×10^5 M^{-1} cm^{-1}.

2.3.3 Catalase

The enzyme catalase converts H_2O_2 into water. The CAT activity in tissue supernatant was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H_2O_2, the substrate of the enzyme[16]. Activity was monitored at 240 nm for 60s. Data is expressed as U/mg protein.

2.3.4 Glutathione-S-Transferase (GST)

Glutathione S-transferase catalyzes the conjugation reaction with glutathione in the first step of mercapturic

acid synthesis. The activity of GST was measured according to the method of Habig *et al.*[17]. One unit of GST activity is defined as 1 mol product formation per minute.

2.3.5 Superoxide Dismutase (SOD)

Superoxide dismutase was assayed according to Kono[18]. One unit of enzyme activity was defined as the amount of the enzyme exhibiting 50% inhibition of auto-oxidation rate of pyrogallol. Data is expressed as U/mg protein.

2.3.6 Estimation of Protein Content

Protein content was estimated according to Bradford assay using BSA as standard[19].

2.4 Genotoxicity

The alkaline SCGE was carried out using the method of Ahuja and Saran[20] with some modifications. Tissues were homogenized in PBS followed by centrifugation at 3000 rpm for 10 minutes. Standard procedures of 1% normal melting agarose coating followed by sample loading mixed with low melting agarose, then incubation, solidification and further lysis in electrophoresis buffer was followed. After lysis, the slides were incubated for 20 minutes in electrophoretic buffer followed by electrophoresis in the same buffer for 20 minutes at 300mA and 24 volts. The slides were then neutralized with the neutralization buffer for 15 minutes and after overnight drying, were stained with ethidium bromide and analyzed under a fluorescence microscope by using excitation filter 515–560 nm with barrier filter of 590 nm at 40X magnification. In total 100 randomly selected comets were scored from each animal. Comet Tail Length (TL) as the distance travelled by DNA fragments and Olive Tail Moment (OTM) were used as standard scoring parameters.

2.5 Statistical Analysis

Statistical analysis was performed using SPSS 16.0. All data were presented as mean ± SE. One-way ANOVA followed by post-hoc Tukey's test was used to study the significant difference between control and treated groups.

3. Results

The toxic effects of 2NS were observed by measuring oxidative stress in liver and kidney samples of fish after acute exposure with both concentrations. Different oxidative stress markers were assessed for acute study till 96h of exposure. The results (Tables 1–4) obtained show dose as well as time dependent significant increase ($p<0.05$) in the biochemical parameters after acute exposure in fish tissues.

In both tissues exposed to 4.77g/l ($^{1/2}LC_{50}$) of 2NS, significant increase ($p<0.05$) in MDA (malondialdehyde) was observed as compared to control after 24h followed by 48h, 72h and 96h in which maximum effect was observed at 96h. Effect of duration was also found to be significant for both tissues. At 96h after exposure of 4.77g/l ($^{1/2}LC_{50}$) of 2NS, the value rises from $0.62\pm0.04^{a,p}$ to $0.90\pm0.054^{b,p}$ in liver and $1.19\pm0.08^{a,p}$ to $1.61\pm0.12^{b,p}$ in kidney tissues respectively when compared to control groups indicating the occurrence of lipid peroxidation induced by 2NS (Table 1).

Table 1. Effect on MDA level in liver and kidney tissues of *C. punctatus* after acute exposure of different concentrations of 2NS for different hours of exposure

		24h	48h	72h	96h	F value
Liver	**Control**	$0.62\pm0.04^{a,p}$	$0.54\pm0.04^{a,p}$	$0.54\pm0.08^{a,p}$	$0.56\pm0.05^{a,p}$	NS
	2.38g/L 2NS	$0.70\pm0.04^{a,p}$	$0.72\pm0.03^{a,p}$	$0.81\pm0.05^{ab,p}$	$1.20\pm0.25^{ab,p}$	NS
	4.77g/L 2NS	$0.90\pm0.054^{b,p}$	$1.00\pm0.058^{b,p}$	$1.13\pm0.11^{b,p}$	$1.51\pm0.11^{b,p}$	8.470^{**}
	F value	10.031^{**}	22.007^{**}	10.41^{**}	8.64^{**}	
Kidney		**24h**	**48h**	**72h**	**96h**	**F value**
	Control	$1.19\pm0.08^{a,p}$	$1.32\pm0.10^{a,p}$	$1.31\pm0.09^{a,p}$	$1.24\pm0.03^{a,p}$	NS
	2.38g/L 2NS	$1.21\pm0.05^{ab,p}$	$1.41\pm0.05^{a,p}$	$1.49\pm0.09^{a,pq}$	$1.72\pm0.02^{b,q}$	10.63^{**}
	4.77g/L 2NS	$1.61\pm0.12^{b,p}$	$1.74\pm0.03^{b,pq}$	$2.04\pm0.09^{b,qr}$	$2.20\pm0.05^{c,r}$	9.56^{**}
	F value	6.43^{*}	9.55^{**}	15.40^{**}	128.70^{**}	

NS- non significant, *($p\leq0.05$); ** ($p\leq0.01$) Values given as mean ± standard error. Different letters (a, b, c) between the columns are significantly different (Tukey's test, $p\leq0.05$) and signify the effect of treatment at the same time interval, and p, q, r signifies the effect of duration of exposure.

Table 2. Effect on GST activity in liver and kidney tissues of *C. punctatus* after acute exposure of different concentrations of 2NS for different hours of exposure

		24h	48h	72h	96h	F value
Liver	**Control**	213.11±1.22^{a,p}	215.51±1.75^{a,p}	212.85±1.16^{a,p}	212.35±1.67^{a,p}	NS
	2.38g/L 2NS	211.54±1.20^{a,p}	210.17±3.07^{a,p}	210.11±1.73^{a,p}	206.04±1.11^{a,p}	NS
	4.77g/L 2NS	230.88±20.5^{a,pq}	258.54±13.21^{b,pq}	203.73±1.23^{b,p}	190.27±9.20^{a,q}	5.37*
	F value	NS	10.20**	11.16**	NS	
Kidney		**24h**	**48h**	**72h**	**96h**	**F value**
	Control	247.63±6.44^{a,p}	248.45±5.36^{a,p}	246.78±18.0 ^{a,p}	246.34±18.19^{a,p}	NS
	2.38g/L 2NS	228.43±14.55^{a,p}	219.20±5.58^{ab,p}	214.74±8.47^{ab,p}	197.87±3.12^{ab,p}	NS
	4.77g/L 2NS	214.88±8.62^{a,p}	189.91±9.64^{b,p}	189.31±9.68^{b,p}	182.78±7.70^{b,p}	NS
	F value	NS	16.804**	5.073*	8.250**	

NS- non significant, *(p≤0.05); ** (p≤0.01) Values given as mean ± standard error. Different letters (a, b, c) between the columns are significantly different (Tukey's test, p≤0. 05) and signify the effect of treatment at the same time interval, and p, q, r signifies the effect of duration of exposure

Table 3. Effect on SOD activity in liver and kidney tissue of *C. punctatus* after acute exposure of different concentrations of 2NS for different hours of exposure

		24h	48h	72h	96h	F value
Liver	**Control**	18.42±0.69^{a,p}	17.90±0.48^{a,p}	18.72±0.53^{a,p}	17.23±1.08^{a,p}	NS
	2.38g/L 2NS	17.61±1.16^{a,p}	16.73±0.46^{a,p}	17.25±0.32^{ab,p}	15.24±0.24^{ab,p}	NS
	4.77g/L 2NS	16.28±0.63^{a,p}	16.03±0.64^{a,p}	15.09±0.69^{b,p}	13.89±0.46^{b,p}	NS
	F value	NS	NS	11.51**	5.795**	
Kidney		**24h**	**48h**	**72h**	**96h**	**F value**
	Control	18.55±0.55^{a,p}	17.98±0.46^{a,p}	18.37±0.69^{a,p}	18.06±0.96^{a,p}	NS
	2.38g/L 2NS	17.47±0.31^{ab,p}	15.64±0.53^{b,p}	14.97±1.04^{a,p}	15.13±0.53^{ab,p}	NS
	4.77g/L 2NS	15.54±0.58^{b,pq}	14.44±0.31^{b,p}	14.49±1.24^{a,q}	13.41±0.65^{b,q}	7.919**
	F value	9.187**	16.161**	NS	10.053**	

NS- non significant, *(p≤0.05); ** (p≤0.01) Values given as mean ± standard error. Different letters (a, b, c) between the columns are significantly different (Tukey's test, p≤0. 05) and signify the effect of treatment at the same time interval, and p, q signifies the effect of duration of exposure.

Tables 2–4 indicate the alterations observed in antioxidant status of kidney and liver. Antioxidant status of tissues was assessed by analyzing Glutathione-s-transferase (GST), Superoxide Dismutase (SOD) and Catalase (CAT) activities. Results presented in Table 2 depicted the altered activities of GST in liver of *C. punctatus* after exposure with different concentrations (2.38g/L and 4.77g/L) of 2NS for different hours of duration. When exposed to 2.38g/L of 2NS the value decreases as compared to control however after exposure to 4.77g/L the activity of GST was found to be increased in 24 hours, however significant increase was observed after 48 hours but after 72 hours the value of dropped significantly as compared to control (ANOVA). Maximum decline in GST activity for both concentrations was found after 96 hours. At 96 hours of exposure of 4.77g/L values of GST drops from 212.35 to 190.27 n moles/mg protein indicating 11.60 % significant reduction ($p<0.05$) in GST activity. Effect of duration of exposure was also found to be significant for 4.77g/L.

The activity of glutathione-s-transferase in kidney of fish after exposure to different concentrations (2.38g/L and 4.77g/L) of 2NS for different time intervals is shown in Table 2. It is clearly indicated from the results that as compared to control group, both exposed groups revealed a symbolic decline in activity of GST at all durations of exposure except for 24h of 2NS exposure. Maximum effect was observed at 96 hours of exposure with 19.67 % and 34.77 % reduction after exposure to 2.38g/L and 4.77g/L 2NS respectively.

Table 4. Effect on CAT activity in liver and kidney tissues of *C. punctatus* after acute exposure of different concentrations of 2NS for different hours of exposure

		24h	48h	72h	96h	F value
Liver	**Control**	954.80±23.01^{a,p}	972.08±10.18^{a,p}	980.74±4.90^{a,p}	958.41±23.35^{a,p}	NS
	2.38g/L 2NS	743.63±20.20^{b,p}	754.88±21.74^{b,p}	754.61±22.02^{b,p}	723.44±16.88^{b,p}	NS
	4.77g/L 2NS	664.64±11.82^{b,p}	641.74±17.34^{c,p}	621.78±15.24^{c,p}	608.11±3.16^{c,p}	NS
	F value	62.644**	96.312**	133.300**	113.789**	
Kidney		**24h**	**48h**	**72h**	**96h**	**F value**
	Control	971.39±9.62^{a,p}	975.33±9.24^{a,p}	955.02±22.81^{a,p}	955.14±23.02^{a,p}	NS
	2.38g/L 2NS	765.44±16.81^{b,p}	766.20±6.01^{b,p}	688.01±19.79^{b,q}	641.73±8.99^{b,q}	18.96**
	4.77g/L 2NS	557.64±26.86^{c,p}	542.90±36.36^{c,p}	469.38±23.46^{c,pq}	414.27±6.30^{c,q}	6.755**
	F value	117.045**	97.139**	121.309**	339.96**	

NS- non significant, *(p≤0.05); ** (p≤0.01) Values given as mean ± standard error. Different letters (a, b, c) between the columns are significantly different (Tukey's test, p≤0. 05) and signify the effect of treatment at the same time interval, and p, q signifies the effect of duration of exposure.

Table 5. Mean value of tail Length (µm) in liver and kidney tissues of *C. punctatus* after acute exposure of different concentrations of 2NS for different hours of exposure

		24h	48h	72h	96h	F value
Liver	**Control**	22.76±0.54^{a,p}	22.76±0.54^{a,p}	23.4±1.17^{a,p}	23.63±0.66^{a,p}	NS
	2.38g/L 2NS	26.00±0.85^{a,p}	25.10±1.75^{a,p}	27.20±0.26^{ab,p}	25.63±1.57^{a,p}	NS
	4.77g/L 2NS	25.76±2.05^{a,p}	27.40±2.86^{a,p}	30.66±1.18^{b,p}	33.76±1.53^{b,p}	NS
	F value	NS	NS	13.81	16.346	
Kidney		**24h**	**48h**	**72h**	**96h**	**F value**
	Control	20.83±0.46^{a,p}	20.7±0.57^{a,p}	21.53±0.42^{a,p}	21.06±0.36^{a,p}	NS
	2.38g/L 2NS	20.96±0.46^{a,p}	21.80.57^{a,p}	24.73±0.60^{ab,q}	25.21±0.77^{b,q}	10.33**
	4.77g/L 2NS	21.8±0.55^{a,p}	24.9±0.10^{b,pq}	27.77±2.09^{b,qr}	30.5±0.84^{c,r}	11.73**
	F value	NS	20.99*	5.91*	45.994**	

NS- non significant, *(p≤0.05); ** (p≤0.01) Values given as mean ± standard error. Different letters (a, b, c) between the columns are significantly different (Tukey's test, p≤0. 05) and signify the effect of treatment at the same time interval, and p, q signifies the effect of duration of exposure.

Table 3 indicates the activity of SOD in both tissues. In liver tissue reduction in values of SOD was noticed at 24 hours of exposure followed by significant reduction was observed after 72 hours for both concentrations as compared to control group (ANOVA). Maximum decrease was observed at 96 h of exposure where 11.54 % and 19.67% decrease was observed after the exposure of 2.38 g/L and 4.77 g/L 2NS value of 2NS respectively. In case of kidney at 24h both the treated groups showed significant decline in activity compared to control groups (ANOVA). 16.22% and 25.74% reduction observed after the exposure of $^{1/4}LC_{50}$ and $^{1/2}LC_{50}$ value of 2NS respectively. Maximum decline was observed at 96h of exposure with the highest concentration (4.77g/L). Further Tukey's test revealed significant changes among different durations after exposure to 4.77g/L 2NS (p≤0.01). Table 4 presents dose and time dependent significant reduction in activity of catalase. Maximum effect was observed at 96h of exposure. In case of liver, the value decreased from 955.14 to 414.27 U/mg proteins whereas in case of kidney value decreased from 958.41 to 608.11U/mg proteins after exposure to $^{1/2}LC_{50}$ of 2NS.

For assessment of genotoxicity, two parameters such as tail length and olive tail moment were studied. Time and dose dependent significant increase in DNA damage was observed after 24h of exposure which was followed by 48h,

72h and 96h of exposure in both tissues (Table 5-6). In case of liver, 42.86% and 8.46% increase in tail length and 33.59% and 10.10% increase in olive tail moment were observed after exposure of $^{1/2}LC_{50}$ and $^{1/4}LC_{50}$ value respectively of 2NS. In case of kidney, after exposure of $^{1/2}LC_{50}$ and $^{1/4}LC_{50}$ value respectively 44.82% and 19.70% increase in tail length and 52.06% and 33.01% increase in olive tail moment was observed indicating genotoxic effect of 2NS. Figure 1 presents the microphotographs of DNA extracted from tissues exposed to 2NS where the DNA found was highly scattered which indicates damage. The image analysis of comets of blood and different organs was carried out using CASPLAB software.

Table 6. Mean value of OTM in liver and kidney tissue of *C. punctatus* after acute exposure of different concentrations of 2NS for different hours of exposure

		24h	48h	72h	96h	F value
Liver	**Control**	$6.41\pm0.41^{a,p}$	$6.61\pm0.47^{a,p}$	$6.46\pm0.56^{a,p}$	$6.63\pm0.73^{a,p}$	NS
	2.38g/L 2NS	$6.81\pm0.55^{a,p}$	$7.18\pm0.23^{ab,p}$	$7.82\pm0.61^{ab,p}$	$7.30\pm0.44^{ab,p}$	NS
	4.77g/L 2NS	$7.06\pm0.92^{a,p}$	$8.41\pm0.35^{b,p}$	$8.92\pm0.48^{b,p}$	$8.99\pm0.14^{b,p}$	NS
	F value	NS	6.263^{*}	4.855^{*}	5.857^{*}	
Kidney		**24h**	**48h**	**72h**	**96h**	**F value**
	Control	$4.61\pm0.2^{a,p}$	$4.74\pm0.14^{a,p}$	$4.63\pm0.06^{a,p}$	$4.61\pm0.20^{a,p}$	NS
	2.38g/L 2NS	$4.87\pm0.12^{a,p}$	$4.91\pm0.11^{a,p}$	$5.16\pm0.30^{a,p}$	$6.04\pm1.03^{a,p}$	NS
	4.77g/L 2NS	$4.94\pm0.12^{a,p}$	$5.24\pm0.24^{a,pq}$	$6.94\pm0.55^{b,qr}$	$7.01\pm0.45^{a,r}$	8.133^{**}
	F value	NS	NS	10.941^{*}	NS	

NS- non significant, *($p\leq0.05$); ** ($p\leq0.01$) Values given as mean ± standard error. Different letters (a, b, c) between the columns are significantly different (Tukey's test, $p\leq0.05$) and signify the effect of treatment at the same time interval, and p, q signifies the effect of duration of exposure.

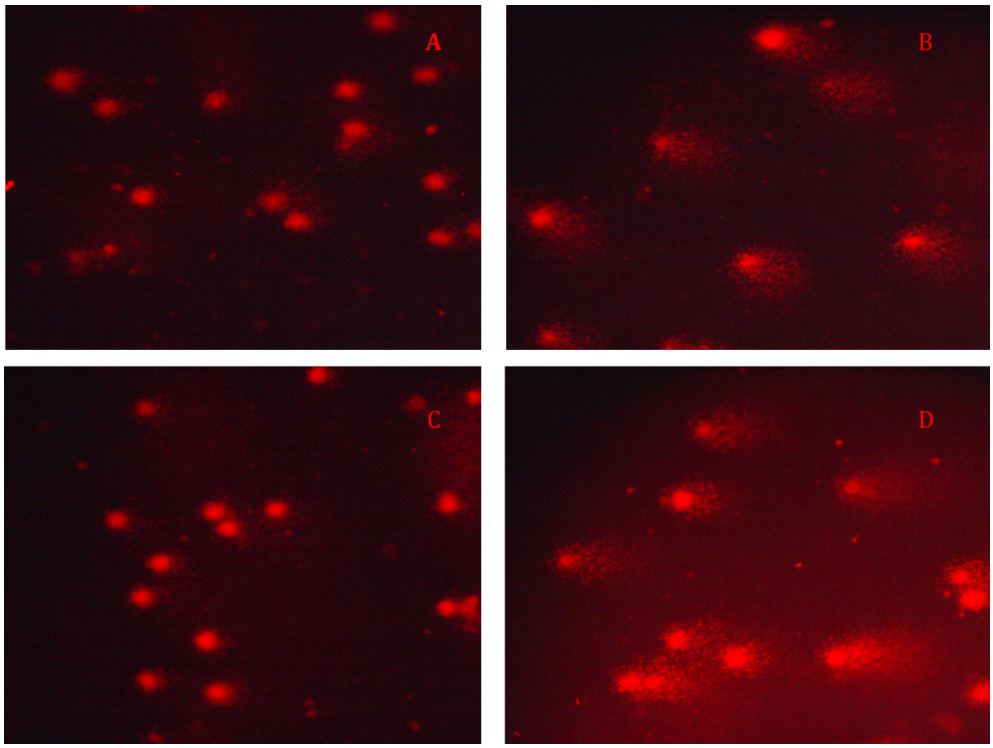

Figure 1. Microphotographs of DNA extracted from **(A)** control liver **(B)** liver of group treated with 2NS (96h) **(C)** control Kidney **(D)** kidney of group treated with 2NS (96h).

4. Discussion

Being non-biodegradable, the occurrence of 2NS in aquatic environment is a matter of serious concern. Long ago, Alonso and Barcelo[10] detected the presence of 50 mg/L of 2NS in Llobregat River in Spain which further found to be varied in different untreated effluents in Sweden, Spain and Portugal. Ignoring the toxic impact of the intermediate compounds, industries are indiscriminately using these chemicals and discharging their effluent in the water bodies. Very few reports are available in the literature regarding toxic impact of intermediate compounds. Keeping in mind the present study was carried out to reveal the acute toxicity of 2NS, a dye intermediate, on fresh water fish *Channa punctatus*. For the purpose, two sub lethal doses were selected after the determination of LC_{50} and the effect was studied on the internal organs such as liver and kidney. Liver is the foremost organ to confront chemicals for their biotransformation. It is critical organ having role in degradation of harmful materials but its regulatory mechanism can be deactivated by the elevation of xenobiotic concentration which may lead to damage in the tissue and affecting its function[21]. Like liver, kidney is also main organ for homeostasis which possess important role in maintaining osmotic balance and filtration. It obtains blood from different organs and serves as primary route for excretion of many types of toxic compounds and their metabolites. Thus being constantly exposed to chemicals, its vulnerability to damage increases.

Oxidative stress is a detrimental state which occurs due to an upsurge in reactive oxygen species (ROS). ROS cause serious damages by having direct negative effect on lipids, DNA and proteins[22]. In our previous study[23] performed revealed that different biomarkers such as MDA, GST, CAT and SOD are best suited for analyzing oxidative stress induced in blood cells. Thus for assessing oxidative stress present study involves the use of MDA, GST, CAT and SOD. Malondialdehyde, MDA is a major product of peroxidised polyunsaturated fatty acids and is considered an important biomarker of lipid per oxidation[24,25]. The elevated content of MDA in the present study revealed the oxidative stress inducing potential of 2NS. In a recent study done by Sharma *et al.*[26], the increased level of MDA in *C. punctatus* after the exposure of Tetrabromobisphenol A (TBBPA) was found. Similar increase in MDA level after the exposure of transition metal-doped titanium dioxide nanoparticles in goldfish and common carp was observed[27]. The study performed by Verma *et al.*[28] also revealed lipid peroxidation in gills of *Channa punctatus* induced by cadmium sulphide nanoparticles. Furthermore, the test chemicals used in the various studies[29–31] have also been reported to cause oxidative stress leading to LPO in different tissues of different fish species revealing the potential of xenobiotic to induce ROS.

The antioxidant enzymes provide a first line of defense to organism being exposed to xenobiotic. These enzymes have proven to carry crucial role against cellular damage[32]. For instance, SOD protects cells against oxidative damage and catalyzes the conversion of superoxide radical to hydrogen peroxide (H_2O_2) while CAT is an effective protective enzyme against lipid peroxidation and converts hydrogen peroxide to water[33]. Dose and time dependent significant decrease in CAT and SOD was observed in the present study after treating fishes with 2NS whereas irregular alteration in the activity of GST was observed. Elevation in GST activity after 24h might indicate the apparent protective behavior of the enzyme against the free radicals being produced against xenobiotic, however, the followed decline in GST activity indicates the suppression of detoxifying system of body against 2NS induced toxicity in both organs. Study performed by Almashhedy *et al.*[34] revealed the adverse effect of textile dyes on antioxidant enzymes. Javed *et al.*[35] also reported alterations in activities of antioxidant enzymes after the exposure of thermal power plant effluent. Similar results were also reported by Mansour *et al.*[36] and Stara *et al.*[37] in freshwater fish treated with profenofos and prometryne, respectively.

The free radicals interact with other biomolecules including DNA leading the generation of DNA adduct thus hindering the replication of DNA. The present study shows the genotoxic potential of 2NS using comet assay. Comet assay is an authentic as well as reliable biomarker for measuring DNA damages, thus, it is considered as the most propitious tool for assessing genotoxicity. Various studies revealed that the Tail Length (TL) and Olive Tail Moment (OTM) are mainly taken as parameters for assessing genotoxicity[38–41]. Thus, in present study, these two parameters were considered for evaluating DNA damage. Dose as well as time dependent remarkable elevation in TL and OTM in both tissues was observed after acute exposure. Mohanty *et al.*[42] also revealed similar trend in fish L. rohita after the exposure to organophosphate pesticide in blood and gill tissue. Likewise, study performed by Sharma *et al.*[26] also revealed genotoxic potential of tetrabromobisphenol A using *C. punctatus* as model. Concentration-dependent significant rise was also observed in DNA damage in profenofos induced *C. punctatus*[43]. Similar study performed by Sharma *et al.*[44] also showed DNA damage in fresh water fish *C. punctatus* after the exposure of non-ionic surfactant, nonylphenol. From the results of present inquisition, it was noted from the comparative analysis that out of both tissues studied; kidney was highly affected followed by liver. Study

performed by Velma and Tchounwou[45] also revealed kidney of goldfish to be more susceptible to damage induced by chromium.

5. Conclusion

Our study concludes that 2NS exposure causes oxidative stress as well as genotoxicity in kidney and liver of *Channa punctatus*. The advancement and application of relevant management policies should be considered as accumulation of these xenobiotic in aquatic environment imposes serious threat to aquatic life. Continuous monitoring of discharge of treated chemicals and their residues in waste water plants are highly recommended. The study also emphasizes the use of MDA, CAT, SOD and GST and comet assay as suitable biomarkers for analyzing oxidative stress and genotoxicity in liver and kidney.

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7. Conflicts of Interest

The authors declare that they have no conflicts of interest.

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