Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(3): 22-25

Received: 13-03-2019
Accepted: 16-04-2019

Madhu Sharma
Department of Fisheries, COVAS, CSKHPKV, Palampur, Himachal Pradesh, India

Subhash Verma
Department of Veterinary Microbiology COVAS, CSKHPKV, Palampur, Himachal Pradesh, India

Pardeep Sharma
Department of Veterinary Medicine, COVAS, CSKHPKV, Palampur, Himachal Pradesh, India

Correspondence
Madhu Sharma
Department of Fisheries, COVAS, CSKHPKV, Palampur, Himachal Pradesh, India

Behavioural and genotoxic effects of paracetamol after subchronic exposure to *Cyprinus carpio*

Madhu Sharma, Subhash Verma and Pardeep Sharma

Abstract
The aim of the present study was to survey the impact of different doses of paracetamol on micronucleus frequency and other morphological deformities of erythrocytes of fish *Cyprinus carpio.* A total of 18 fish were divided in to three groups. Group A served as control while group B and C were given treatment with 5mg/l and 10 mg/l of paracetamol. Behaviour of the exposed fish was observed. Increase in pigmentation, high breathing rate, surface gulfing was observed in treatment groups. Blood samples were taken the from caudal vein after 7 and 14 days of exposure**.** Micronucleated cells, Binucleated cells, fragmented DNA, lobed nucleus, notched nucleus were scored under nuclear deformities and cytoplasmic vacuole and karyolysis were considered as cytoplasmic deformities. A significant increase in values of all the nuclear and cytoplasmic deformities have been observed in dose as well as time dependent manner except for karyolysis cells. Highest effect was observed at the highest concentration and at 14 days of exposure. Study showed that paracetamol have genotoxic effect on fish even at low concentration.

Keywords: Paracetamol, genotoxicity, nuclear abnormalities, cytoplasmic abnormalities

Introduction
Paracetamol also known as acetaminophen is most frequently used analgesic and antipyretic drug [1]. It was first introduced in the year 1955 for its clinical application and since then, it is widely used almost throughout the world. In many countries the drug is readily available over-the-counter without the need of prescription [2]. The consumption of medical drugs and their use in veterinary practice is expected to systematically increase over the coming years, resulting in their increased discharge [3]. It has currently become one of the most emerging pollutants worldwide. Other sources include industrial and hospital discharges, animal waste and improper disposal of expired or unused drugs, landfill leachate and the accidental leakage during manufacturing and distribution [4]. According to the ranking system proposed by environmental agency of England and Wales paracetamol is among the top ten chemical compounds according to their perceived relative risk.

Paracetamol was detected in highest concentrations with an average of 32.4 µg/L, in municipal wastewater in Mardan, 3649 ± 851 ng/L in River Kabul and 175 ± 100 ng/L in River Indus in Pakistan [5]. Based on an exhaustive review of literature, it is observed that the relevance of PCs as an emerging contaminant has not been investigated in the India [6]. Studies by Singh *et al.* 2014 [7] and Shanmugam *et al*., 2014 [8] in India do indicate the presence of paracetamol in rivers contaminated by urban wastewater.

A number of techniques have been developed to test DNA alterations in aquatic organisms. These include, mainly, chromosomal aberrations, sister chromatid exchange, comet assay and micronucleus assay. Micronucleus assay has advantages over other tests as it is technically easier to use, cheaper, and less time-consuming [9, 10]. In fish change in blood cell is correlated with change in the environment. Therefore, in the presence of toxicant or other stressors change in blood cells can be seen.

Fish are both ecologically and environmentally important, which is why most environmental research has focused on them [11]. *C. carpio* is a freshwater species of commercial importance and have great potential for aquaculture. *C. Carpio* was selected considering its high commercial value and a higher rate of consumption. It has been widely used in various studies to check the toxicity of different pollutants [12, 13]. This study aimed at increasing the knowledge and understanding about the toxic effects of paracetamol on aquatic vertebrate model *C. carpio* after sub-chronic exposure of 2 weeks.

Materials and Methods

The experimental tanks were of 50 liters capacity each. The fish chosen were of length 19-25 cm, and weight 110-210 grams. Exposure was semi-static and every 24 hours the drug was replenished to complete the initial concentration. Control animal were submitted to the same water change schedule without the addition of paracetamol. Exposure was given for 14 days. Two sub-lethal concentrations of paracetamol were decided as 5 mg/l and 10 mg/ l of water for the exposure according to the LC_{50} value for *C. carpio* of paracetamol [14]. Blood sampling was done after 7th and 14th day of exposure from all the three groups. The experiment was conducted in triplicate. The fish from both the groups (control and treated) were keenly observed for behavioural inconsistencies and alterations. Different behavioural parameters including moment, equilibrium, operculum beat, air gulfing, jumping, hyper activeness, abrupt swimming, aggression, motionless, vertical position, fast swimming and increased surface activity were profoundly studied.

Frequency of micronuclei and morphological abnormalities in erythrocytes was evaluated by the method used by **11**. Blood was taken from a caudal vein of fish and then stored in EDTA vials. Then smears of blood were made and then they were dried and fixed in alcohol for 10 minutes. Then the slides were stained with 10% Geimsa stain for 15 min and then seen under oil immersion lens (100x) of microscope. The changes in cytoplasm and nucleus in RBC's and number of micronucleus were recorded. The results are expressed as mean ± S.E. and to study the significance of the difference in the values of different parameters between treated and control groups a one-way analysis of variance (ANOVA) and Tukey-HSD test using the statistical software SPSS were conducted.

Results and Discussion

Different behavioural changes have been observed in *C. carpio* after treatment with different concentrations of paracetamol. Initially the fish were active and feeding was normal. After two week there was a moderate decrease in metabolic activity. Change in locomotors activity is due to impairment at neural and neuromuscular levels. Slight change in pigmentation was also observed this is related to a stress condition. Under stress condition increase in pigmentation was observed by **15**. Fishes were showing signs of restlessness, increased respiration rate and were gasping air on the surface with open mouth operculum activity was higher. Paracetamol inhibit enzyme AChE [16] and can lead to behavioural alterations of fish.

Blood cells from peripheral circulation have been used for micronucleus assay as it is easy to isolate and no cellular dissociation is required [17]. The value of all the parameters tested showed a significant increase in treated groups as compared to control group. Dose and time dependent increase in the value of micronuclei, deformed nucleus, vacuolated cytoplasm and caryolised cytoplasm was observed (Table 1).

Table 1: Frequency of percent Micronucleated cell (MNC), deformed nucleus (DN), vacuolated cytoplasm (VC) and karyolysed cell (KC) in blood cells of fish *C. carpio* after exposure to different concentrations of paracetamol for 7 and 14 days.

Parameters	Concentration	Control	7th day	14th day
MNC	5mg/l	0.34 ± 0.47^{aA}	17.33 ± 0.47^{bB}	23.66 ± 0.47^{cC}
	10mg/l	0.66 ± 0.6^{dA}	31.66 ± 0.63^{bC}	42.33 ± 0.63^{eD}
DN	5mg/l	150.00 ± 3.65^{aA}	336.66 ± 5.5^{bA}	510.0 ± 3.61^{cA}
	10mg/l	145.33 ± 1.8^{aA}	436.66 ± 5.5^{bB}	535.00 ± 1.82^{dB}
VC	5mg/l	4.33 ± 2.11^{aA}	34.00 ± 0.73^{cA}	56.6 ± 0.5^{eA}
	10mg/l	4.6 ± 0.5^{bA}	65.6 ± 0.42^{dB}	80.00 ± 0.73^{fB}
KC	5mg/l	6.33 ± 0.89^{aA}	23.33 ± 0.88^{bA}	65.00 ± 0.57^{cA}
	10mg/l	7.00 ± 0.59^{bA}	97.00 ± 1.15^{cC}	60.33 ± 0.89^{dB}

The values given as mean ±standard error. Different letters (a, b, c, d, e, f) between the columns are significantly different (Tukey's test, $p\leq0.01$) and signify the effect of duration of exposure at each concentration. Similarly, different letters (A, B, C, D) within the columns are significantly different (Tukey's test, $p\leq0.01$) and signify the effect of different concentrations of paracetamol at the same time interval.

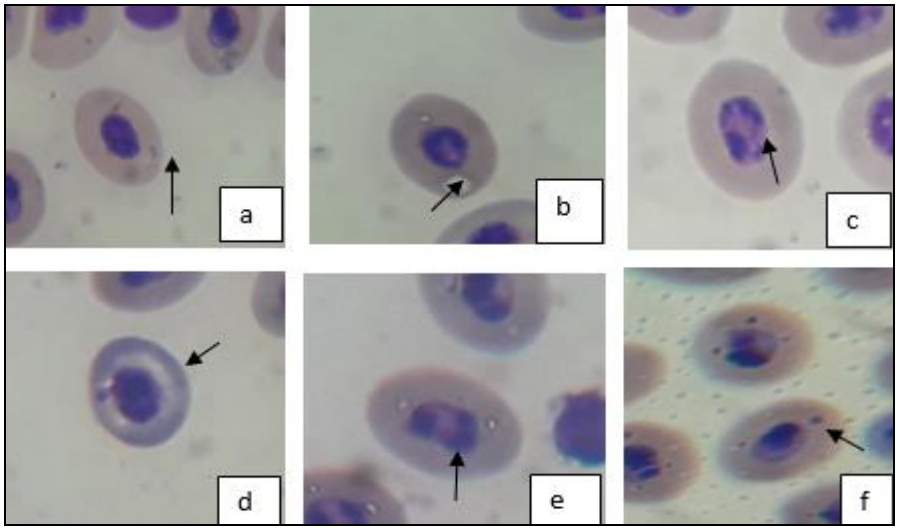

Fig 1: Different nuclear and cytoplasmic abnormalities observed in RBCs of *C. carpio* in response to paracetamol (a) Normal blood cell (b) vacuolated cytoplasm (c) Uneven distribution of nuclear material in nucleus (d) Karyolysis (e) Blebbed nucleus) (f) Micronucleated cell

Under deformed nucleus blebbed, lobed, notched, binucleated, budded and other deformed nuclei were included. Dose dependent increase in different parameters may be due to the accumulation of this hydrophilic compound in body with time and dose. Different reasons for nuclear and cytoplasmic abnormalities in blood cells caused by paracetamol may be its tendency to generate reactive oxygen intermediates (ROI) and reactive nitrogen intermediate (RNI) **[18]** during its metabolism. These reactive metabolites including NADQI (*N*-acetyl-*p*-benzoquinone imine) may react with proteins and nucleic acids [19]. Moreover it has been suggested that unmetabolised paracetamol may inhibit DNA replication and DNA repair by inhibiting the action of specific enzyme ribonuclease reductase [20]. Blood cells in treatment group show uneven distribution of genetic material in nucleus (Figure1c) which may be due to inhibition of DNA replication. Such effect provides a reason for paracetamol to cause sister chromatid exchange, micronuclei, chromosomal aberration and apoptosis in cells [21, 22]. Conducted a similar study on mice and showed that normal and high dose of paracetamol may cause sperm DNA fragmentation [23]. Observed that paracetamol has a high tendency to induce genotoxicity and cytotoxicity [24]. Found that micronuclei only produced if DNA damage is evident.

Vliegenthart *et al.,* 2015 [25] found dramatic decrease in mRNA for histone in response to acetaminophen in mice. So it may be possible that this may be due to altered chromatin structure. One of the cytoplasmic changes observed during this study was vacuolated cytoplasm, vacuoles in blood cells may be formed due to the unequal distribution of haemoglobin. Highest karyolysed cells were observed at 7th days of exposure and after treatment with 10 mg/l concentration while a decrease in the value was observed at 14th day of exposure. As paracetamol induce apoptosis in cell and karyolysed cells may be removed from the circulation can be the reason for less karyolysed cells at the highest duration of exposure.

Conclusion

The cytoplasmic and nuclear abnormalities observed in blood cells of *C. carpio* indicated that paracetamol has behavioural and genotoxic effects. Prudent use and scientific disposal of pharmaceutical compounds is needed so as to curtail toxic effects on human, animal and aquatic fauna health.

Acknowledgement

The authors are sincerely thankful to Dean, College of Veterinary and Animal Sciences for providing laboratory facility.

References

1. Abdel-Daim M, Abushouk AI, Reggi R, Yarla NS, Palmery M, Peluso I. Association of antioxidant nutraceuticals and acetaminophen (Paracetamol): Friend or foe?. 2018; 26(2):S78-S87. DOI: https://doi.org/10.1016/j.jfda.2017.11.004
2. Ibrahim M, Farooq T, Hussain N, Hussain A, Gulzar T, Hussain I *et al*. Acetyl and butyryl cholinesterase inhibitory sesquiterpene lactones from Amberboa ramose. Chemistry Central Journal. 2013; 7:116.
3. Klimaszyk P, Rzymski. Water and aquatic fauna on drugs: What are the impacts of pharmaceutical pollution? Water management and environment: Case Studies. 2018; 255-278.
4. Al-Odaini NA, Zakaria MP, Yaziz MI, Surif S, Abdulghani M. The occurrence of human pharmaceuticals in wastewater effluents and surface water of Langat River and its tributaries, Malaysia. International Journal of Environmental Analytical Chemistry. 2013; 93(3):245-265.
5. Khan GA, Berglund B, Khan KM, Lindgren PE, Fick J. Occurrence and abundance of antibiotics and resistance genes in rivers, canal and near drug formulation facilities-a study in Pakistan. PLoS One. 2018; 8:e62712
6. Chinnaiyan P, Thampi SG, Kumar M, Min KM. Pharmaceutical products as emerging contaminant in water: relevance for developing nation and identification of critical compounds for Indian environment. Environment Monitoring and Assessment. 2018; 190 (288):288. https://doi.org/10.1007/s10661-018-6672-9
7. Singh KP, Rai P, Singh AK *et al*. Occurrance of pharmaceuticals in urban waste water of North Indian cities and risk assessment. Environment Monitoring Assessment. 2014; 186:6663. https://doi.org/10.1007/s10661-014-3881-8.
8. Shanmugam G, Sampath S, Selvaraj KK, Larsson DG, Ramaswamy BR. Non-steroidal anti-inflammatory drugs in Indian rivers. Environment Science Pollution Research. 2014; 21(2):921-31. Doi: 10.1007/s11356-013-1957-6.
9. Bhatnagar A, Yadav AS. Cheema N. Genotoxic Effects of Chlorpyrifos in Freshwater Fish Cirrhinus mrigala Using Micronucleus Assay. Advances in Biology, 2016. http://dx.doi.org/10.1155/2016/9276963
10. Sharma M, Chadha P. 4-Nonylphenol induced DNA damage and repair in fish, Channa punctatus after subchronic exposure. Drug and Chemical Toxicology. 2016; 40(3):1-6
11. Sharma M, Chadha P. Widely used non-inonic surfactant 4-nonylphenol: Showing genotoxic effects in various tissues of Channa punctatus. Environmental Science and Pollution Research. 2017; 24:11331-113339.
12. Chupani L, Niksirat H, Velisek J, Stara A, Hradilova S, Kolarik J *et al*. Chronic dietary toxicity of zinc oxide nanoparticles in common carp (*Cyprinus carpio*): Tissue accumulation and physiological responses. Ecotoxicology and environmental safety. 2018; 147:110-116.
13. Fiorino E, Sehonova P, Plhalova L, Blahova L, Svobodova Z, Faggio C. Effects of glyphosate on early life stages: comparison between Cyprinus carpio and Danio rerio. Environmental Science and Pollution Research. 2018; 25(90):8542-8549.
14. Rao R, Manu B, Thalla AK. Behavioural, physical and Biochemical Responses of Cyprinus carpio for Paracetamol Exposure. International Journal of Emerging Research in Management. 2017; 6(2):215-219.
15. Backstrom T, Brannas E, Nilsson J, Magnhagen C. Behaviour, physiology and carotenoid pigmentation in arctic Charr Salvelinus alpines. Journal of Fish Biology. 20-14; 84:1-9.
16. Nunes B, Verde MF, Soares AM. Biochemical effects of the pharmaceutical drug paracetamol on Anguilla Anguilla. Environmental Science and Pollution research. 2015; 22 (15):11574-11584.
17. Kilemade MF, Hartl MGJ, Sheehan D, Mothersill C, Van Pelt FNAM, O' Halloran J *et al*. Genotoxicity of field collected inter-tidal sediments from Cork Harbor, Ireland, to juvenile trubot (*Scophthalmus maximus* L.) as

measured by the comet assay. Environment Molecular Mutagenesis. 2004; 44:56-64.
18. Hazai E, Vereczkey L, Monostory K. Reduction of toxic metabolite formation of acetaminophen. Biochemistry Biophysics Research Community. 2002; 291:1089-1094.
19. Wang TX, Zhang C, Fu L, Zhang DG, Wang BW, Zhang ZH *et al*. Protective effect of rosiglitazone against acetaminophen-induced acute liver injury is associated with down-regulation of hepatic NADPH oxidases. Toxicological letters. 2016; 265:38-46.
20. Wiger R, Finstad HS, Hongslo JK, Haug K, Holme JA. Paracetamol inhibits cell cycling and induces apoptosis in HL-60 cells. Pharmacology and Toxicology. 1997; 81:285-293.
21. Rodrigues S, Correia AT, Antunes SC, Nunes B. Alerations in gill of *Lepomis gibbosus*, after acute exposure, to several xenobiotics (pesticide, detergent and pharmaceuticals): morphometric and biochemical evaluation. Drug and Chemical toxicology. 2014; 38:1-7
22. Abedi N, Nabi A, Mangoli E, Talebi AR. Short and long term effects of different doses of paracetamol on sperm parameters and DNA integrity in mice. Middle East Fertility Society Journal. 2017; 22(4):323-328
23. Parolini M, Binelli A, Cogni D, Riva C, Provini A. An in vitro biomarker approach for the evaluation of the ecotoxicity of non-steroidal antiinflammatory drugs (NSAIDs). Toxicology *in vitro*. 2009; 23:935-942.
24. Binelli A, Parolini M, Cogni D, Pedriali A, Provini A. A multi-biomarker assessment of the impact of the antibacterial trimethoprim on the non-target organism zebra mussel (*Dreissena polymorpha*). Comparitive Biochemistry and Physiology. 2009; C 150:329-336.
25. Vliegenthart ADB, Antoine DJ, Dear JW. Target biomarker profile for the clinical management of paracetamol overdose. British Journal of Clinical Pharmacology. 2015; 80(3):351-362.