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Investigations on remedial role of *Rauwolfia serpentina* root extract against carbofuran formulation induced genotoxicity in *Channa punctatus*

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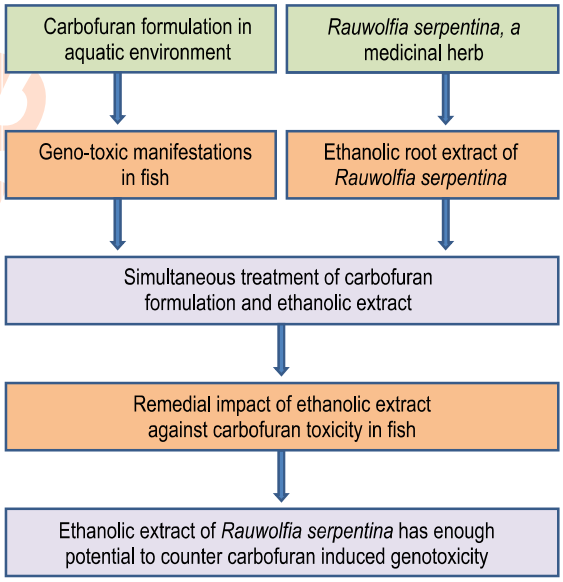
Abstract

Aim : To investigate the remedial potential of *Rauwolfia serpentina* root extract against genotoxic alterations induced by exposure of carbofuran formulation in freshwater teleost, *Channa punctatus*.

Methodology : Ten days acclimatized fish were categorized in three groups, Group 1 (control), Group 2 (0.09 mg l^{-1} carbofuran formulation) and group 3 (0.09 mg l^{-1} carbofuran formulation +10 ppm ethanolic extract of *Rauwolfia serpentina*). Genotoxic alterations were recorded in terms of single cell gel electrophoresis (SCGE) and micronucleus (MN) assay in blood cells. The variation in comet tail length and micronuclei frequencies were compared among Group 1, 2 and 3 after 24, 48, 72 and 96 hr.



Results : A significant ($p<0.05$) increase was observed in comet tail length and micronuclei induction in carbofuran formulation exposed group. The longest comet length and peak of micronuclei frequencies were observed after 96 hr of carbofuran formulation exposure. However, an appreciable and gradual decline in both frequencies of micronuclei and comet tail length were observed in group 3 (combined Carbofuran formulation and *Rauwolfia serpentina* root extract) in comparison to group 2 (Carbofuran formulation).

Interpretation : The study, thus, demonstrates ameliorative potential of *Rauwolfia serpentina* root extract against carbofuran formulation induced genotoxicity in fish.

Key words: *Channa punctatus*, Carbofuran toxicity, Micronucleus assay, *Rauwolfia serpentina*

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Introduction

Water is an unequivocally essential and fundamental constituent for life. It provides a life support system for aquatic organisms, however, aquatic bodies are continuously getting polluted by agrochemicals, used to increase the crop yield. A major portion of agrochemicals comprise of chemical pesticides, in which carbamates have strong impact. As these pesticides are broad spectrum in nature and easily degraded due to their short half-life, they are frequently used against insects and nematodes (Rendón-Von Osten *et al.,* 2005). Carbofuran, a carbamate pesticide, is widely used in paddy fields (Ramesh *et al.,* 2015) and has been detected in ground, surface and rain waters (Pessoa *et al.,* 2011). After reaching the aquatic environment through run off from agricultural fields, carbofuran contaminates water bodies and adversely affects both flora and fauna (Saglio *et al.,* 1996). Similarly, when used in paddy cum fish culture, this pesticide poses risk to the fish, generating various disorders and restricting their growth and population, therefore making them unfit for human consumption. Several studies have been carried out to show its toxic effects on aquatic organisms by evaluating the changes in the morphological, hematological and biochemical parameters in various fish species (Ramesh *et al.,* 2015). Further more, carbofuran inhibits acetylcholine esterase resulting in severe physiological disorders and altering biochemistry in animals (Pessoa *et al.,* 2011). Besides genotoxic impairment, induction of reactive oxygen species due to carbofuran toxicity has also been reported (Hamed and Osman, 2017; Soloneski *et al.,* 2008).

Now a days, secondary plant metabolic compounds are gaining importance for intensive research, as they reduce the undesirable effect of xenobiotic compounds. Alcoholic extract of various plants have been found to be effective and showed amelioration against low dose radiation (Hasan *et al.,* 2016). Bioactive compounds of plants due to their antioxidant properties can delay or inhibit oxidative damage of cell membrane as well as cellular macromolecules by inhibiting the initiation or propagation of oxidizing chain reactions (Velioglu *et al.,* 1998). Several phytochemicals *viz.,* alkaloids, tannins, gums and flavonoids are frequently used for therapeutic purposes. *Rauwolfia serpentina* or "Sarpagandha" is one of the plants rich in bioactive compounds like alkaloids, flavonoids, phenols, saponins and tannins (Kumari *et al.,* 2013; Bhardwaj and Yadav 2016). This has ample scope in therapeutic purposes from ages. For centuries, it has also been used as an antidote against snake venom (Parinitha *et al.,* 2004; Sankaranarayanan *et al.,* 2010).

Further, *Rauwolfia* is rich in many flavonoids (Gupta *et al.,* 2015) which are potent water-soluble antioxidants known for their free radical scavenging capabilities that prevent oxidative cell damage and have also shown anticancer activities (Treml and Šmejkal, 2016). These compounds may be attributed to impart ameliorative role in counteracting toxicity generated by carbofuran in *Channa punctatus.* Due to the presence of potential phytochemicals, *Rauwolfia serpentina* root extract can be used against carbofuran formulation induced toxicity and, therefore, in the present study an attempt was made to explore mitigating potential of ethanolic extract of *Rauwolfia serpentina* root powder against carbofuran formulation induced toxicological alterations in freshwater teleost, *Channa punctatus* in terms of two cytogenotoxic endpoints, micronucleus assay for cytogenetic damage and single cell gel electrophoresis comet assay for DNA damage.

Materials and Methods

Test animal : Apparently, healthy and fresh water teleost, *Channa punctatus* (14.8 ± 1.0 cm and 30 ± 2.0 g) were obtained from local lotic habitats of Lucknow. After prophylactic dip in formalin (0.4%) for 15 min, they were treated with $KMnO_4$ (1 mg l^{-1}) for 1 hr to keep away dermal infections, if any. For acclimatization, they were kept in large glass aquaria (100×40×40 cm^3) containing 7 days-old tap water for 10 days and maintained in laboratory conditions following standard procedure (APHA *et al.,* 2012). During acclimatization in the Environmental Toxicology and Bioremediation Laboratory, Department of Zoology, University of Lucknow, fishes were fed twice a day with fresh minced goat liver and artificial fish food pellets.

Preparations of ethanolic root extract of medicinal plant *Rauwolfia serpentina* : Dried whole plants of *Rauwolfia serpentina* (Family Apocynaceae) were procured from local market of Lucknow, India. The plant samples were sent for taxonomic identification to the Department of Botany, University of Lucknow. The roots were seprated from the plants and washed thoroughly in tap water and shade dried for a minimum period of 10 days. Dried roots were cut into small pieces and then powdered with the help of an electric grinder. For preparation of ethanolic root extract of *Rauwolfia serpentina*, 40 g of powdered root was taken in Soxhlet apparatus and extracted with ethanol (95%) for about 12 hr at 25°C. The obtained filtrate was further concentrated by rotary vacuum evaporator till a semi solid state was achieved. The alcoholic root extract of *Rauwolfia serpentina* was stored in a refrigerator at 4°C for further use.

Experimental design : A total number of 36 fishes, after 10 days of laboratory acclimatization, were randomly divided into three groups, each containing 12 specimens. Group 1 served as untreated control. Group 2 was exposed to a sub-lethal concentration of 0.09 mg l^{-1} (1/10 of 96 hr-LC_{50} of carbofuran formulation (Furadan 3 G) alone while in group 3, fish were treated with 0.09 mg l^{-1} of carbofuran formulation along with 10 ppm of *Rauwolfia serpentina* root extract.

The concentration of technical grade carbofuran with product name Furadan 3G manufactured by FMC India Private Ltd. was taken in terms of fraction of LC_{50}, which was already reported by Tiwari *et al.,* (2016). The test media of all the groups were replenished without disturbing the test animals on alternate day to maintain desired concentration of carbofuran formulation and *Rauwolfia serpentina* root extract.

At the end of itemized period (24, 48, 72 and 96 hr) of exposure, 3 fish were taken from each group and immersed in a solution of tricaine methanesulphonate (MS 222; 0.1 g l^{-1} of water) for anesthetization, blood samples were taken by puncturing caudal vessel by using disposable syringes. The blood was kept in vials without using any anticoagulant and samples were immediately used for assessment of micronuclei induction (MN) and single cell gel electrophoresis (SCGE). Experiments were run in triplicate to verify reproducibility of results.

Micronuclei assay : Freshly collected peripheral blood samples were smeared on dried, cleaned microscopic slides and fixed in methanol for 10 min. Slides were left overnight for air drying at room temperature. After fixation, slides were stained with May-Grunwald's solution 1 for 3 min, followed by solution 2 for 5 min. Finally, slides were stained with second stain, *i.e.,* 5% Giemsa in 0.04 M phosphate buffer (pH 6.8) for 30 min. After dehydration through graded alcohol series and clearing in xylene, slides were finally mounted in DPX and observed under oil immersion microscope (Nikon Corporation K 12432) using 40/100 X objective lenses. To calculate the frequency of micronuclei induction, a minimum of 1000 erythrocytes were examined for each specimen. Micronuclei were scored following the method of Fenech *et al.* (2003).

Comet assay / Single cell gel electrophoresis (SCGE) : Comet assay/ SCGE was performed by the method of Singh *et al.* (1988) with slight modifications. Before SCGE assay, cell viability of erythrocytes was evaluated by trypan blue exclusion test method (Strober, 2015) and samples showing more than 84% cell viability were used. One side frosted slides were coated with 1% molten NMA followed by cell suspension in 0.75% LMA. They were kept at 37°C till solidification. The slides were immersed in lysis solution (2.5M NaCl, 100mM EDTA, 10mM Tris-HCl, pH 10 and 1% Triton X-100 and 10% DMSO, added fresh) and kept in dark at 4°C for 24 hr. After cell-lysis, slides were placed in alkaline electrophoresis buffer (300 mM NaOH, 1mM Na_2-EDTA and 0.2% DMSO, pH>13) for 20 min to allow DNA unwinding. DNA electrophoresis was done (24V, 300mA in the same buffer with pH> 13) for 20 min and placed in neutralizing solution (0.4MTris-HCl buffer with pH 7.5) followed by staining with ethidium bromide (20 µg m l^{-1}). Analyses of comet images were done using a fluorescent microscope (Nikon Corporation K 12432) under 40 X objective. A total number of 25 cells per slide were scored for each group and DNA damage was determined by software, Komet-5.5 Kinetic Imaging, Merseyside, UK.

Statistical analyses : All data are presented as mean ±S.E. Values were analyzed by one-way ANOVA with Tukey's post hock analysis and the significance for all experimental groups measured at $p < 0.05$ using SPSS (version 20.0).

Results and Discussion

During experimentation, significant physio-chemical parameters of test medium *viz.,* alkalinity, hardness, chemical oxygen demand (COD), dissolved oxygen (DO), pH and temperature were recorded up to 96 hr, at an interval of 24hr, for both control and treated groups. These parameters were found well with in the prescribed reference limits (APHA *et al.,* 2012).

In the present study, the frequency of micronuclei and mean comet tail length were recorded every 24 hr up to 96 hr of exposure period. The evaluation of comet was done with intense head and sharp tail only (Fig. 2). Less instance comets were over looked. Micronuclei having staining similar to main nucleus and not connected with it were scored (Fig. 1). After different exposure periods, frequency of micronuclei induction and mean comet tail length were recorded for all three groups. A statistically significant ($p<0.05$) increase in micronuclei induction and average comet length were recorded in fishes of group 2 incomparison to group 1. However, in Group 3, micronuclei frequencies decreased as compared to Group 2 at each subsequent exposure period. Similarly, the average comet tail length in Group 3 also decreased as compared to Group 2 for speculated period. So, the obtained results clearly reflect the cytotoxic as well as genotoxic nature of carbofuran formulation at exposed concentration, and the simultaneous mitigating impact of *Rauwolfia serpentina* root extract in terms of micronuclei and comet assay.

Fishes, placed at higher tropic level in the aquatic ecosystem, are highly sensitive to xenobiotics as they cannot escape the stressed environment they inhabit. Thus, they are excellent models for monitoring toxicants in aquatic ecosystem (Bhatnagar *et al.,* 2016; Luzhna *et al.,* 2013). Ample studies have documented noxious nature of common pesticide contaminants in fishes (Giri *et al.,* 2000; Rai and Sharma, 2007; Ibrahim and Harabawy, 2014; Pandey *et. al.,* 2014, 2018). Among these most of the toxicants, including pesticides and their residues induce genotoxicity by generating oxidative stress (Ratn *et al.,* 2017). Disruptive changes in DNA by over production of ROS as well as electrophilic free radical metabolites have also been documented by Farah *et al.* (2006). Being lipophilic, carbofuran easily accumulate in tissues and hinders biotransformation reactions (phase I and phase II) which generate 3-hydroxycarbofuran and 3-ketocarbofuran resulting in biochemical and enzymological alterations (Ramesh *et al.,* 2015). Moreover, its accumulation accelerate oxidative stress and perturbs cellular biochemistry and genomic stability (Ibrahim and Harabawy, 2014).

Micronuclei and comet assay are commonly employed tools for assessment of genotoxicity in fishes (Yadav and Trivedi 2009; Pandey *et al.,* 2011). Formation of micronuclei results due to improper segregation of chromosomes during meiosis or fragmentation of DNA (Fenech *et al.,* 2011) and other abnormalities induced by spindle failure (Luzhna *et al.,* 2013). Micronuclei may also be induced due to improper functioning of Topoisomerase II enzyme, responsible for double strand breaks (DSBs) (Xu *et al.,* 2011). Further, induction of micronuclei may also be due to deficiencies in chromosome segregation during anaphase, usually caused by defects in the cell cycle control system, mitotic spindle failure, centromeric DNA hypomethylation

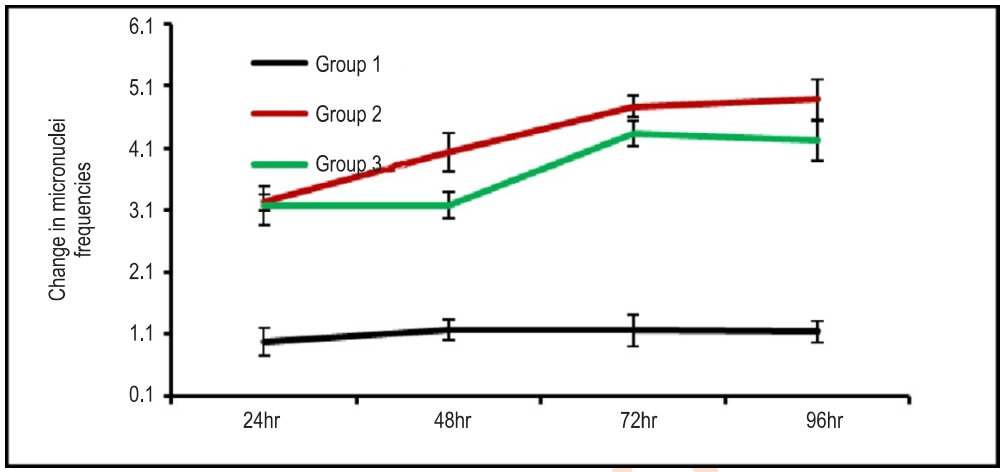

Fig.1 : Graphical representation of alterations in micronuclei frequencies in erythrocytes of *Channa punctatus* in different experimental groups after 24, 48, 72 and 96 hrs of exposure. Group 1 (control), Group 2 (0.09 mg l^{-1} carbofuran formulation) and Group 3 (0.09 mg l^{-1} carbofuran formulation +10 ppm ethanolic extract of *Rauwolfia serpentina*).

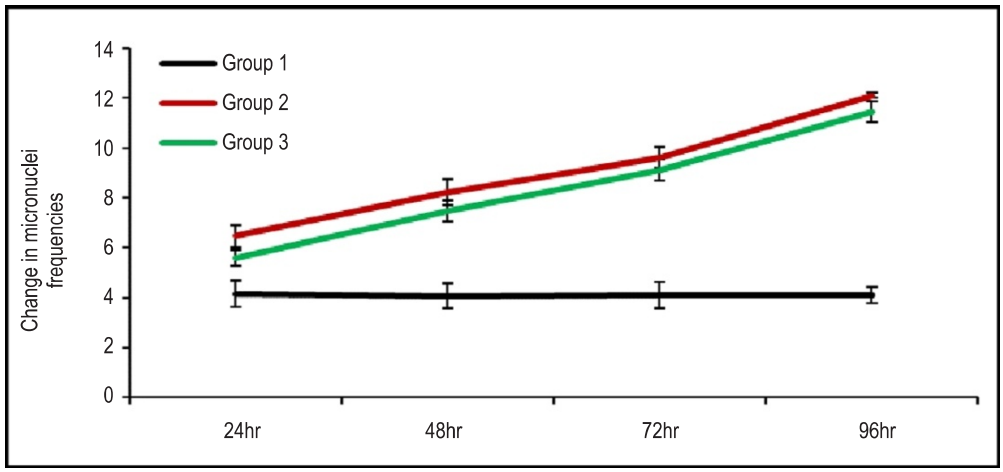

Fig. 2 : Graphical representation of alterations in comet tail length in erythrocytes of *Channa punctatus in* different experimental groups after 24, 48, 72 and 96 hrs of exposure. Group 1 (control), Group 2 (0.09 mg l^{-1} carbofuran formulation) and Group 3 (0.09 mg l^{-1} carbofuran formulation +10 ppm ethanolic extract of *Rauwolfia serpentina*).

and kinetochore damage (Mateuca *et al.,* 2006). The present results are in agreement with the findings of Chauhan *et al.* (2000) and Zeljezic *et al.* (2006) who reported that carbofuran formulation causes induction of micronuclei in mouse and rats, respectively. Moreover, *in vitro* studies on carbofuran formulation toxicity showed that it induces micronuclei formation in Chinese hamster ovary cells (Soloneski *et al.,* 2008) and human lymphocytes (Akyıl *et al.,* 2015). Oxidative stress induced DNA damage is easily detectable by comet assay (Sharma *et al.,* 2012) and can be appreciably applied in toxicogenomics, ecotoxicology and DNA repair research (Pandey *et al.,* 2011). The findings of present study exhibit significant ($p < 0.05$) increment in comet tail length establishing DNA damage in fish of Group 2. However, Group 3 illustrated appreciable reduction in comet tail length recorded in fish co-exposed to both carbofuran formulation and root extract of *Rauwolfia serpentina.* Several phytochemicals have already been proved for their protective role against several genotoxicants. Prasad and Trivedi (2018) reported chromosomal repairing potential of curcumin in *Channa punctatus* against sub lethal exposure of chromium trioxide.

Amelioration against genotoxicity induced by methyl methane sulfonate in mice by aqueous and hydro-methanol extracts of *Spondias mombin, Nymphaea lotus* and *Luffa cylindrical* (Oyeyemi *et al.,* 2015) and *Melissa officinale* against arsenite (As^{3+}) induced oxidative stress and cytogenotoxicity (Dwivedi *et al.,* 2017) has already been established. Similarly, efficacy of *Lawsonia inermis* leaf extract was observed against copper induced toxicity in fish (Kumar and Trivedi, 2015). Flavonoids present in *Rauwolfia serpentina* have high scavenging activities against free radicals (Keshavkant *et al.,* 2008), and thus they exhibit antigenotoxic capabilities (Tiwari *et al.,* 2016). The findings of the present study recorded a decline in both the frequencies of micronuclei induction and mean comet tail length in fish of Group 3 as compared to Group 2.

This suggests the ameliorative potential of *Rauwolfia serpentina* root extract against sub-lethal exposure of carbofuran formulation in fish. This happens due to antioxidant properties of root extract of *Rauwolfia serpentina* which helps in mitigation of cellular and nuclear damages. Flavonoid rich methanolic extract of *Alchemilla mollis* was also estimated for its antioxidant potential against DPPH radicals (Trendafilova *et al.,* 2011). In fish, mitigating properties of *Mellisa officinalis* and *Acacia catechu* have been reported by Tiwari *et al.,* (2017) and Dwivedi *et al.* (2015). Prasad and Trivedi, (2018) also reported the protective role of curcumin against chromium induced chromosomal aberrations in, *Channa punctatus*. *Rauwolfia serpentina* root extract has enough potential to mitigate Carbofuran formulation induced genotoxicity as evident by appreciable reduction in micronuclei frequencies and comet tail length in fish specimens simultaneously treated with *Rauwolfia serpentina* root extract and sub-lethal concentration of carbofuran formulation. Therefore, after proper standardization, *Rauwolfia serpentina* root extract can be used effectively as a safety measure for fish against carbofuran toxicity. The present study clearly demonstrates that carbofuran can impart significant genobiotic impact on *Channa punctatus,* while *Rauwolfia serpentina* root extract has an ameliorative effect and can improve the ability of *C. punctatus* to with stand the severity of exposure. In future, more elaborate analysis with varying levels of root extract in commercially important fishes and also using different toxicants will help to devise a new cultural strategy to counter the negative impact of pesticide toxicity in fishes.

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References

Akyıl, D., A. Özkara, S.F. Erdoğmuş, Y. Eren, M. Konuk and E. Sağlam: Micronucleus assay in human lymphocytes after exposure to alloxydim sodium herbicide *in-vitro. Cytotech.,* **67**, 1059-1066 (2015).

APHA: Standard Methods for Examination of Water and Waste water. 22nd Edn., APHA, AWWA, WPCF, Washington DC, USA (2012).

Bhatnagar, A., A.S. Yadav and N. Cheema: Genotoxic effects of chlorpyrifos in freshwater fish *Cirrhinus mrigala* using micronucleus assay. *Adv. Biol.,* 1–6 (2016). Article ID 9276963

Bhardwaj, N. and M. Yadav: Evaluation of chemical composition of *Rauwolfia serpentina* and *Leucas aspera* – A comparative study. *World J. Pharm. Sci.,* **5**, 10,914-920 (2016).

Chauhan, L.K., N. Pant, S. Gupta and S. Srivastava: Induction of chromosome aberrations, micronucleus formation and sperm abnormalities in mouse following carbofuran exposure. *Mutat. Res. Toxicol. Environ. Mutagen.,* **465**, 123–129 (2000).

Dwivedi, S., V.Tiwari and S.P. Trivedi: Arsenite induced genotoxic effect and its phytoremediation by *Acacia catechu* leaf extract in freshwater fish, *Channa punctatus* (Bloch). *Int. J. Fish. Aquat. Stud.,* **2**, 163–165 (2015).

Dwivedi, S., M. Kumar and S.P. Trivedi: Mitigating potential of *Melissa officinale* against As^{3+} induced cytotoxicity and transcriptional alterations of Hsp 70 and Hsp 27 in fish, *Channa punctatus* (Bloch). *Envir. Monit. Assess.,* **189**, 1-15 (2017).

Farah, M.A., B. Ateeq and W. Ahmad: Antimutagenic effect of neem leaves extract in freshwater fish, *Channa punctatus* evaluated by cytogenetic tests. *Sci. Total Environ.,* **364**, 200–214(2006).

Fenech, M., W.P. Chang, M. Kirsch-Volders, N. Holland , S. Bonassi and E. Zeiger: HUMN project: Detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. *Mutat. Res.,* **534**, 65-75 (2003).

Fenech, M., M. Kirsch-Volders, A.T. Natarajan, J. Surralles, J.W. Crott, J. Parry, H. Norppa, D.A. Eastmond, J.D. Tucker and P. Thomas: Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. *Mutagenesis,* **26**, 125-132 (2011).

Giri, A.N., D.K. Srivastva and S.P. Trivedi: Insecticide besathrin induced histoanatomical insult of ovarian tissue of Indian catfish, *Heteropneustes fossilis. Biological Memoirs,* **26**, 20-24 (2000).

Gupta, J., A. Gupta and A.K. Gupta: Extraction and identification of flavonoid natural antioxidant in the leaves of *Rauwolfia serpentina*. *Int. J. Chem. Stud.,* **35**, 35–37(2015).

Hamed, H.S. and A.G.M. Osman: Modulatory effect of lycopene against carbofuran toxicity in African catfish, *Clarias gariepinus. Fish Physiol. Biochem.,* **43**, 1721–1731(2017).

Hasan, H.F., N.M. Thabet and M.K. Abdel-Rafei: Methanolic extract of *Moringa oleifera* leaf and low doses of gamma radiation alleviated amiodarone-induced lung toxicity in albino rats. *Arch. Biol. Sci.,* **68**, 31–39 (2016).

Ibrahim, A.T. and A.S. Harabawy: Sublethal toxicity of carbofuran pesticide on the African catfish *Clarias gariepinus* (Burchell, 1822): Hematological, biochemical and cytogenetic response. *Ecotoxicol. Environ. Saf.,* **103**, 61–67(2014).

Keshavkant, S., T. Sukhdev, C. Srinivasarao and S.C. Naithani: Antioxident activities, phenols and flavonoid contents of *Withania somnifera* and *Rawolfia serpentina. Indian J. Plant Physiol.,* **13**, 394-399 (2008).

Kumar, V. and S.P. Trivedi: Efficacy of *Lawsonia inermis* leaves extract against copper (Cu^{++}) toxicity induced in fish, *Channa punctatus* (Bloch.): A study based on chromosomal aberrations. *J. Env. Bio-Sci.,* **29**, 87-92 (2015).

Kumari, R., B. Rathi, A. Rani and S. Bhatnagar : *Rauvolfia serpentina* L. Benth. ex Kurz.: Phytochemical, pharmacological and therapeutic aspects. *Int.J. Pharm. Sci. Rev. Res.,* **23**, 348–355 (2013).

Luzhna, L., P. Kathiria and O. Kovalchuk : Micronuclei in genotoxicity assessment: From genetics to epigenetics and beyond. *Front.*

Genet., **4**, 131(2013) doi: 10.3389/fgene

Mateuca, R., N. Lombaert, P. V. Aka, I. Decordier and M. Kirsch-Volders: Chromosomal changes: Induction, detection methods and applicability in human biomonitoring. *Biochimie,* **88**, 1515–1531 (2006).

Oyeyemi, I. T., O.M. Yekeen, P.O. Odusina, T.M. Ologun, O.M. Ogbaide, O. I. Olaleye and A.A. Bakare: Genotoxicity and antigenotoxicity study of aqueous and hydro-methanol extracts of *Spondias mombin* L., *Nymphaea lotus* L. and *Luffa cylindrical* L. using animal bioassays. *Interdiscip. Toxicol.,* **8**, 184–192 (2015).

Pandey, A.K., N.S. Nagpure, S.P. Trivedi, R. Kumar and B. Kushwaha: Profenofos induced DNA damage in freshwater fish, *Channa punctatus* (Bloch) using alkaline single cell gel electrophoresis. *Mutat. Res.Genet. Toxicol. Environ. Mutagen.,* **726**, 209–214 (2011).

Pandey, A.K., N.S. Nagpure and S.P. Trivedi: Genotoxicity assessment of pesticide profenofos in fresh water fish *Channa punctatus* (Bloch) using comet assay and random amplified polymorphic DNA (RAPD). *Chemosphere,* **211**, 316-323 (2018).

Pandey, A.K., N.S. Nagpure and S.P. Trivedi: Evaluation of genotoxicity of profenofos to freshwater fish *Channa punctatus* (Bloch) using micronucleus assay. *African J. Biotech.,* **13**, 3985-3988 (2014).

Parinitha, M., G.U. Harish, N.C. Vivek, T. Mahesh and M.B. Shivanna: Ethno-botanical wealth of Bhadra Wild Life Sanctuary in Karnataka. *Indian J. Tradit. Knowl.,* **3**, 37–50 (2004).

Pessoa, P.C., K.H. Luchmann, A.B. Ribeiro, M.M. Veras, J.R. Correa and A.J. Nogueira: Cholinesterase inhibition and behavioral toxicity of carbofuran on *Oreochromis niloticus* early life stages. *Aquat. Toxicol.,* **105**, 312-320 (2011).

Prasad, R. and S.P. Trivedi: Protective effects of curcumin against Cr (VI) induced chromosomal aberrations in a fresh water fish, *Channa punctatus* (Bloch, 1793). *J. Appl. Biosci.,* **44**, 41-49 (2018).

Rai, D.K. and B. Sharma: Carbofuran-induced oxidative stress in mammalian brain. *Mol. Biotechnol.,* **37**, 66–71(2007).

Ramesh, M., S. Narmadha and R.K. Poopal: Toxicity of furadan (carbofuran 3% g) in *Cyprinus carpio*: Haematological, biochemical and enzymological alterations and recovery response. *Beni-Suef Univ. J. Basic Appl. Sci.,* **4**, 314–326 (2015).

Ratn, A., Y. Awasthi, M. Kumar, S.K. Singh, R. Tripathi and S.P. Trivedi : Phorate induced oxidative stress, DNA damage and differential expression of p53, apaf-1 and cat genes in fish, *Channa punctatus* (Bloch, 1793). *Chemosphere,* **182**, 382-391(2017).

Rendón-Von Osten, J., A.M.V.M. Soares and L. Guilhermino: Black-bellied whistling duck (*Dendrocygna autumnalis*) brain cholinesterase characterization and diagnosis of anticholinesterase pesticide exposure in wild populations from *Mexico. Environ. Toxicol. Chem.,* **24**, 313–317(2005).

Saglio, P., S.Trijasse and D. Azam: Behavioral effects of waterborne carbofuran in goldfish. *Arch. Environ. Contam. Toxicol.,* **31**, 232–238 (1996).

Sankaranarayanan, S., P. Bama, J. Ramachandran, P.T. Kalaichelvan, M. Deccaraman, M. Vijayalakshimi, R. Dhamotharan, B. Dananjeyan and S. Sathya Bama: Ethnobotanical study of medicinal plants used by traditional users in Villupuram district of Tamil Nadu, India. *J. Med. Plants Res.,* **4**, 1089–1101(2010).

Sharma, P., A.B. Jha, R.S. Dubey and M Pessarakli: Reactive oxygen species, oxidative damage and antioxidative defense mechanism in plants under stressful conditions. *J. Bot.,* **2012**, Article ID 217037 1–26 (2012).

Singh, N.P., M.T. McCoy, R.R. Tice and E.L. Schneider: A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell Res.,* **175**, 184–191(1988).

Soloneski, S., M.A. Reigosa, G. Molinari, N.V. González and M.L. Larramendy: Genotoxic and cytotoxic effects of carbofuran and furadan on Chinese hamster ovary (CHOK1) *cells. Mutat. Res.,* **656**, 68–73 (2008).

Strober, W.:Trypan blue exclusion test of cell viability. *Curr. Protoc. Immunol.,* **111**, A3.B.1-A3.B.3 (2015).

Tiwari, V., M. Kumar and S.P. Trivedi: Protective effects of perennial herb, *Melissa officinalis* against furadan 3G induced cytotoxicity in *Channa punctatus. J. Environ. Biol.,* **38**, 1375-1381(2017).

Tiwari, V., M. Kumar and S.P. Trivedi: Amelioration by sarpagandha root extract to counter DNA damage induced by carbofuran in fish using RAPD tool. *J. Env. Bio-Sci.,* **30**, 87–93 (2016).

Trendafilova, A., M. Todorova, M. Nikolova, A. Gavrilova and A. Vitkova: Flavonoid constituents and free radical scavenging activity of *Alchemilla mollis*. *Nat. Prod. Commun.,* **6**, 1851-1854 (2011).

Treml, J. and K. Šmejkal: Flavonoids as potent scavengers of hydroxyl radicals. *Compr. Rev. Food Sci. Food Saf.,* **15**, 720–738(2016).

Velioglu, Y.S., G. Mazza, L. Gao and B.D. Oomah: Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *J. Agric. Food Chem.,* **46**, 4113–4117 (1998).

Xu, B., Z. Sun. Z. Liu, H. Guo, Q. Liu, H. Jinag, Y. Zou, Y. Gong, J.A. Tischfield and C. Shao: Replication stress induces micronuclei comprising of aggregated DNA double-strand breaks. *PloS One,* **6**, 1-11 (2011).

Yadav, K.K. and S.P. Trivedi: Sub lethal exposure of heavy metals induces micronuclei in fish, *Channa punctata. Chemosphere,* **77**, 1495-1500 (2009).

Zeljezic, D., V. Garaj-Vrhovac and P. Perkovic: Evaluation of DNA damage induced by atrazine and atrazine-based herbicide in human lymphocytes *in-vitro* using a comet and DNA diffusion assay. *Toxicol. Vitro.,* **20**, 923–935 (2006).