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Ethanolic root extract of *Rauwolfia serpentina* alleviates copper induced genotoxicity and hepatic impairments in spotted snakehead fish, *Channa punctatus* (Bloch, 1793)

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Abstract

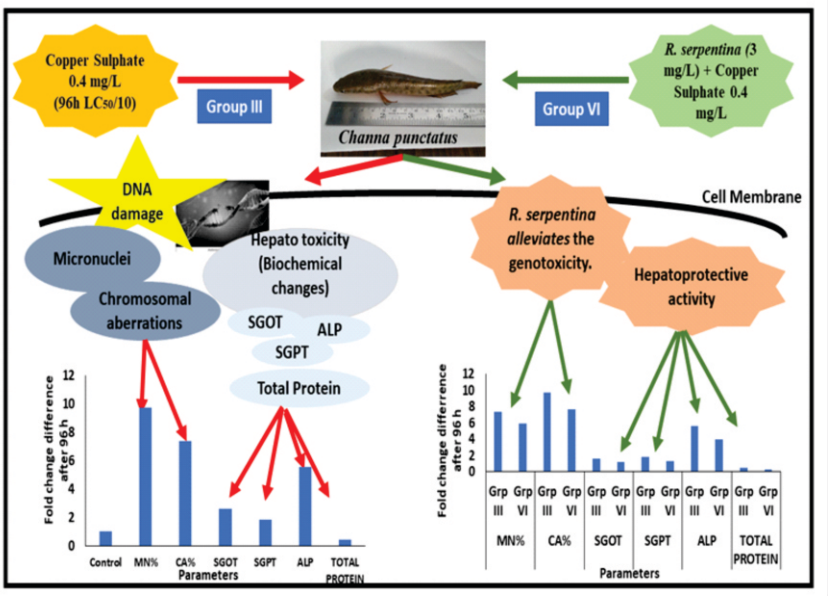
Aim: The present study was designed to evaluate the plausible efficacy of ethanolic extract of *Rauwolfia serpentina* to mitigate copper induced toxicity by investigating cytogenetic biomarkers, *i.e.*, chromosome aberrations (CA) and micronucleus (MN) formation; assessment of biochemical changes in liver enzymes (SGOT, SGPT and ALP) and estimation of protein levels in a freshwater spotted snakehead fish(*Channa punctatus).*

Methodology: The experiment was carried out in six groups, each having 15 specimens for 24, 48, 72, and 96 hr. Group I served as control. Groups II and III were maintained with 3 mg l^{-1} of *Rauwolfia* root extract and 0.4 mg l^{-1} of Cu^{2+}, respectively. Groups IV, V, and VI were simultaneously co-exposed with 0.4 mg l^{-1} of Cu^{2+} and three different concentrations of *Rauwolfia* root extract 1, 2 and 3 mg l^{-1}, respectively. For the genotoxicity assessment, blood and kidney tissues were used. Hepatic impairments were assessed after each exposure period.

Results: A significant increase ($p<0.05$) in chromosomal aberrations, micronuclei frequency, activity of liver enzymes (SGOT, SGPT and ALP) and a decrease in protein level were recorded in Group III in comparison to the control. Groups co-exposed with $Cu^{2+}$and *Rauwolfia serpentine* showed a significant ($p<0.05$) decrease in cytogenetic biomarkers, activity of liver enzymes and an increase in protein levels, as compared to Group III, with respect to control in a dose dependent manner.

Interpretation: Thus, result of the present investigation establish the efficacy of *R. serpentina* root extract against Cu^{2+} induced toxicity in spotted snakehead (*Channa punctatus)*.

Key words: Copper sulphate, *Channa punctatus,* Chromosomal aberrations, Hepatotoxicity, Micronuclei, *Rauwolfia serpentina*



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Introduction

Rauwolfia serpentina, an endangered medicinal plant of Family Apocynaceae is a woody perennial shrub, commonly known with different names- Sarpagandha, snake root plant, 'chotach', and 'Chandrika'etc. (Mallick *et al.*, 2012). The roots of this shrub for long had been known to posses great medicinal value (Mrunalini and Khobragade, 2016). It is used as an anti-fungal, anti-inflammatory, anti-proliferative, anti-cancerous, anti-diuretic, anti-dysenteric, anti-hypertensive, antioxidant, anti-arrhythmic and tranquillizing agent (Azmi and Qureshi, 2016; Gupta and Gupta, 2016). Administration of *R. serpentina* has showed immense therapeutic responses against various diseases in mice (Azmi and Qureshi, 2016) as well as in humans (Lobay, 2015). Further, Qureshi *et al.* (2009) reported that the methanolic root extract of *R. serpentina* was hypoglycemic, hypolipidemic and hepato-protective in alloxan-induced diabetic rats.

Copper (Cu^{2+}) is a naturally occurring metallic element that occurs in soil at an average concentration of about 50 parts per million (ppm). It is present in all animals and plants and is an essential nutrient for organisms in small amount. For humans copper constitutes on an average 15-120 mg of total body content. It is found in surface water, groundwater, seawater and drinking water. Copper is used as an anti-algae treatment in ponds (Watson and Yanong, 2011). Fish and shellfish use it for metabolism of carbohydrate and more than thirty enzymes. It also helps in the formation of hemoglobin and hemocyanin. The problem with the use of copper is that there is a thin line that separates effective treatment levels from overdoses, that can kill fish (Watson and Yanong, 2011). However, if copper level exceeds 20 µg g^{-1} it can be toxic (Solomon, 2009). A high concentration of copper in aquatic regimes adversely affects marine and freshwater organisms such as fish and mollusks (Van Genderen *et al.*, 2005).

Fishes are the sentinel organisms that occupy top most level in the aquatic food chain and are able to accumulate heavy metals in their tissues (Padrilah *et al.*, 2018).*Channa punctatus* has been used as a test model in many pollution studies, because of its easy availability and suitability in adopting laboratory conditions. The large size and small number (2n=32) of chromosomes of *C. punctatus* are suitable for chromosomal aberration study.

Liver, a vital organ of vertebrates, has a wide range of functions, including detoxification, protein synthesis, and production of biochemicals necessary for digestion (Dorcas and Solomon, 2014). Serum liver chemistry tests can be used to provide a practical knowledge for the analysis of hepatotoxicity (Green and Flamm, 2002). Aminotransferases are the most commonly used and most precise indicators of hepatic damage. Serum glutamic oxaloacetic acid transaminase (SGOT) and serum glutamine pyruvate transaminase (SGPT) help in the catalytic transfer of amino acids (Thapa and Walia, 2007). Alkaline phosphatase (ALP) enzyme also occurs mainly in liver cells next to the bile ducts and helps in breakdown of proteins. If the liver is affected, damaged liver cells release increased amount of ALP into the blood.

Chromosomal aberration test (CAT) is one of the most important mutagenicity tests for assessing cytogenetic damage (Ueda *et al.,* 1992). Many water pollutants have cytotoxic properties that can increase the frequency of chromosomal aberrations (CAs) in fishes (Iji and Adeogun, 2014). Fish cells have been used to assess the genotoxicity of contaminated water in the field and in laboratories for various *in-vivo* and *in-vitro* monitoring. CAs were observed after exposure of various chemicals to fish species (Ahmad Dar, 2016). For instance, exposure of fish to dichlorvos (0.01 ppm) induces chromosomal aberrations, namely chromatid gaps, centromere gaps, attenuation, chromatid breaks and additional fragments in kidney cells (Rishi and Grewal, 1995). There are several studies that show the suitability of CAT to evaluate the genotoxic effects of various poisons using fish as a bioindicator (Iji and Adeogun, 2014; Yadav and Trivedi, 2009a). Micronuclei (MN) induction is also a sensitive indicator of DNA damage and, thus, can be effectively utilized to have sight of genotoxicity. MN assay is commonly used for the assessment of genotoxicity induced by aquatic pollutants in fishes (Bolognesi, Cirillo, 2014; Osman, 2014; Ratn *et al.*, 2018; Yadav and Trivedi, 2009b).

The present study evaluated the antimutagenic potential of ethanolic root extract of *Rauwolfia serpentina* by assessing various cytogenetic endpoints *viz.,* chromosome aberration (CA) and micronucleus (MN) formation; activities of liver enzymes (SGOT, SGPT and ALP) and total protein levels against copper toxicity of Cu^{2+} in fish, *Channa punctatus*, used as a vertebrate model. The above parameters are considered very sensitive assays for detecting toxic chemicals and identifying antimutagenic agents and thus, are used extensively in cytogenetic and biochemical investigations.

Materials and Methods

Determination of sub-lethal concentration of Cu^{2+} : For 96 hr LC_{50} acute static toxicity bioassays were performed following the standard methods of APHA *et al.*, 2017. Initially, ten well aerated aquaria were used and in each aquarium ten well acclimatized and randomly picked healthy fish specimens were exposed to different concentrations (3.0, 3.2, 3.4, 3.6, 3.8, 4.0, 4.2, 4.6, 4.8 and 4.10 mg l^{-1}) of Cu^{2+}.The experiment was repeated thrice to obtain the 96 hr-LC_{50} values of the test chemical for the fish. Percentage mortality was noted at different intervals, *i.e.,* 24, 48, 72 and 96 hrs. For determination of 96 hr LC_{50} of Cu^{2+}, mean values of percentage mortalities up to 96 hr against each concentration were analyzed following Trimmed Spearman-Karber method (Hamilton *et al.*, 1977).

Preparation of *Rauwolfia serpentina* root extract : The ethanolicr oot extract of *Rauwolfia* was prepared by following the

modified protocols described by Chattopadhyay (1998) and Azmi and Qureshi (2009). The roots were washed in tap water; air-dried and powdered. Four hundred gram of powdered root was dissolved in 2 l of ethyl alcohol and kept at room temperature for 48 hr. The mixture was filtered and concentrated under reduced pressure in a water bath (temperature 50 ºC) and finally dried in a rotavapour. A thick brown paste was suitably diluted with 0.6% normal saline and used for the experiments.

On the basis of no observed effect concentration (NOEC) method, *R. serpentina* root extract dose was designed (Hutchinson *et al.*, 2009) and 3.0 mg l^{-1} of *R. serpentina* root extract was found to be the safest concentration, above which fish may show harmful effects.

Experimental design and acclimation of fish : From lentic habitats of Lucknow, *Channa punctatus* (35 ± 3.0 g; 14.5 ± 1.0 cm) were procured with the help of fishermen. A treatment of 0.05% $KMnO_4$ solution was given to fish for 2-5 min to remove dermal infections (APHA *et al.*, 2017; Awasthi *et al.*, 2018; Ratn *et al.*, 2017, 2018). Glass aquaria (100 x 40x 40cm^3) of 100 l capacity containing 40 l of 10 days old de-chlorinated water were maintained and fish were acclimatized for 15 days under laboratory conditions (hardness 71 mg l^{-1} as $CaCO_3$, total dissolved solids 310 mg l^{-1}, dissolved oxygen 6.7 mg l^{-1}, alkalinity 74.6 mg l^{-1}, pH 7.2 and temperature 26.5ºC), following APHA *et al.* (2017). Fish were given commercial fish food (Perfect Companion Group Company Limited, Thailand). An exposure was given to the randomly picked non-infectious fish for 96 hr and samples were collected at an interval of 24, 48,72 and 96 hr. Fish were divided into six groups and kept in six separate aquaria each having 15 specimens. Group I served as a control (de-chlorinated tap water). Fish of Group II were exposed to *Rauwolfia* root extract (3 mg l^{-1}) and fish of Group III were challenged with sub-lethal concentration of Cu^{2+} (1/10 of LC_{50} for 96 hr; 0.4 mg l^{-1}). Three different concentrations of *Rauwolfia* root extract (1, 2 and 3 mg l^{-1}, respectively)were given to Groups IV, V and VI simultaneously with Cu^{2+} (1/10 of LC_{50} for 96 hr; 0.4 mg l^{-1}). Each experimental group was maintained in triplicate in separate aquaria. After the completion of desired exposure periods, prior to blood and tissue sampling for genotoxicity and biochemical analysis, three fish from each group were anaesthetized with 0.1 % diethyl ether and sacrificed for the experiment (n = 3 fish from each group).

Assessment of genetic damage

Chromosomal aberration test (CAT): Fish kidney tissue was used to perform the chromosome aberrations test. The techniques of Al-Sabti *et al.* (1983) and Cucchi and Baruffaldi (1990) were followed for preparation of chromosomes. One hour prior to the completion of desired duration, fish were given injection intra-muscularly of freshly prepared colchicine solution at the dosage of 1 mg 100 g^{-1} of body weight to arrest cell division at metaphase stage. After that, kidneys were removed, homogenized in 0.56% KCl solution and incubated for 30–40 min at 32 ºC. Chilled Carnoy's fixative (methanol : glacial acetic acid, 3:1) was added to the homogenate to stop the hypotonic treatment, mixed gently with Pasteur pipette, centrifuged at 1500 rpm for 10 min, and the supernatant was discarded. The pellet was re-suspended in chilled Carnoy's fixative and the above process was repeated till white cell suspension was obtained. Chromosome slides were prepared by dropping a few drops of cell suspension over pre-cooled slides in 70% alcohol. Immediately thereafter, the fixative was burned off using flame drying. The slides were stained in 5 % Giemsa stain prepared in Sorensen's buffer (pH-6.8) for 20–25 min. Brightly stained slides were independently coded and observed using an oil immersion microscope (Nikon Corporation K 12432) with 40/100X objective lenses. A minimum of 100 metaphases (2n=32 chromosomes) in each group, including control, were analyzed.

Micronucleus test (MNT) : For the micronuclear test, blood was taken from cardiac puncture and a thin and uniform smear was prepared on glass slides. Slides were air dried overnight at 37 ºC. Fixation of slides was done in absolute methanol for 5 min and they were stained with May-Grunnwald's dye (solution 1 and 2 for 3 and 5 min, respectively). Slides were again stained with 5% Giemsa (Sigma Aldrich, USA) in a phosphate buffer (pH 6.8) for 30 min.Stained slides were mounted with DPX (a mixture of distyrene, plasticiser and xylene) for microscopic examination (Schmid, 1975). A minimum of 6,000 erythrocytes were scored for recording the induction of MN in each treatment group, including control, using an oil immersion microscope (Nikon Corporation K 12432) with 40/100X objective lenses. The established criteria for scoring MN was followed (Schmid, 1975). The MN frequency was calculated as follows:

MN% = Number of cells containing micronucleus × 100/Total number of cells counted

Examination of biochemical parameters in liver

Estimation of total protein : The total protein content was estimated following the method of Lowry *et al.* (1951). In 5 ml of chilled distilled water, 100 mg of liver tissue was homogenized. Five milliliter of 30% trichloroacetic acid was added immediately for the precipitation of protein. The precipitated product was collected by centrifugation at 3000 rpm for 15 min. The supernatant was removed and the pellet was cleaned repeatedly with distilled water to remove extra deposited TCA. The pellet was dissolved again in 0.1 N NaOH. 0.5 ml of the solution was transferred to a test tube, and 4 ml of alkaline copper sulphate was added to it, followed by addition of 0.4 ml of diluted Folin's Reagent. After 30 min, the solution developed a blue color. Its optical density was determined using a spectrophotometer (Shimadzu, UV-1800 Pharma spec) at 750 nm wavelength. Bovine serum albumin was used as a standard. The content of tissue protein was expressed in µg 100 mg^{-1} of wet tissue.

Activity of liver biomarkers : From freshly drawn blood, serum was obtained by centrifugation at 3,000 rpm for 10 min. Activities

of the enzymes serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT) and *alkaline phosphatase* (ALP) were analyzed by commercially available kits (Robonik India Pvt. Ltd, Navi Mumbai) using Shimadzu UV-1700 pharma spec UV-VIS spectrophotometer.

SGOT and SGPT in liver tissues were estimated by following the method of International Federation of Clinical Chemistry (IFCC))(Schumann and Klauke, 2003) with some modifications. The rate of oxidation of NADH to NAD was measured at 340 nm as a decrease in absorbance consumption photometrically. It was directly proportional to SGOT and SGPT activities in the sample. The enzyme activities were expressed as Units l^{-1}.

ALP in the liver tissue was estimated by following the modified method of Kanakis *et al.*, (2004). The increased absorption was measured at 405 nm due to the formation of 4-nitrophenolate photometrically and was found directly proportional to ALP activity in the sample. Working reagent was prepared by combining enzymatic reagent - R1 (diethanolamine and magnesium chloride) and substrate reagent - R2 (p-nitrophenylphosphate) in 4:1 ratio, respectively, mixed gently and incubated at room temperature for 5 min, then 100 µl sample/control was added, and after 1 min of incubation the change in absorption ($\Delta A\ min^{-1}$) was recorded at an interval of 3 min and its activity is expressed in Units l^{-1}.

Data evaluation and statistical analysis: Data recorded in triplicate, were presented as mean ± standard error mean (S.E.M). One-way analysis of variance (ANOVA) with Tukey's post-hoc test was used to test the significance of each result in the erythrocytes, liver and kidney of fish exposed to the toxicant. Copper induced changes in SGOT, SGPT, ALP, total protein, micronuclei frequency and chromosomal aberrations were analyzed using SPSS software (version 20.0, SPSS Company, Chicago, USA).

Results and Discussion

Genotoxicity was evaluated in terms of micronuclei induction in erythrocytes and chromosomal aberrations (CAs) in kidney cells of the test fish, *Channa punctatus,* after 24, 48, 72 and 96 hrs of exposure to sub-lethal test concentration of copper sulphate (96 h-LC_{50}/10; 0.4 mg l^{-1}). In fact, exposure of Cu^{2+} generates oxidative stress in fish. This culminates into cytogenetic instability including DNA damage. DNA strand breaks are mainly responsible for the formation of MN (Trivedi *et al.*, 2021). The frequency of MN induction was significantly ($p < 0.05$) increased in Group-III with an increasing fold-change of $3.25 < 3.40 < 4.30 < 7.38$ after 24, 48, 72 and 96 hr exposure periods, respectively, as compared to control (Fig. 1). The frequency of MN decreased in groups, IV, V and VI exposed with 96 h-LC_{50}/10 of copper sulphate along with increasing concentrations (1, 2 and 3 mg l^{-1}, respectively) of *R. serpentina* root extract, after 24, 48, 72 and 96 hr exposure periods. In groups IV, V, and VI, the fold-changes of MN induction decreased in comparison to group III after 24, 48, 72 and 96 hr of exposure. An insignificant MN induction was observed in group II (3 mg l^{-1} of *R. serpentina* root extract alone) as compared to group I (control).

The chromosomal aberrations recorded in the kidney tissue of test fish for both control and treated groups are illustrated in Fig. 2. A significant ($p < 0.05$) increase in chromosomal aberrations was recorded in Group III, with an increasing fold-change of $5.3 < 6.36 < 8.00 < 9.70$ after 24, 48, 72 and 96 hr of exposure periods, respectively, as compared to control. The fish co-treated with *R. serpentina* root extract (1, 2 and 3 mg l^{-1}) and copper sulphate (96 hr-LC_{50}/10; 0.4 mg l^{-1}) showed progressively reduced chromosomal aberrations in groups IV, V and VI, in comparison to group III with respect to control after 24, 48, 72 and 96 hr exposure. When fish were co-exposed to a sub-lethal fraction of copper sulphate (0.4 mg l^{-1}) and root extract of *R. serpentina* (3 mg l^{-1}) there was a fold-change difference of just 2.1 between group-III and group-VI, illustrating a decrease in the frequency of chromosomal aberrations after 96 hr. Fish treated with 3 mg l^{-1} of *R. serpentina* root extract showed insignificant chromosomal aberrations as compared to unexposed fish.

A significant ($p<0.05$) increase in chromosomal aberrations and micronuclei frequency in Cu^{2+} exposed fish of group III is indicative of cytogenetic damage. Presrent findings are in accordance with those of Yadav and Trivedi, (2009 a,b), who demonstrated the extent of chromosomal aberrations and micronuclei induction in *C. punctatus* exposed to three heavy metal compounds and found a significant ($p<0.05$) increase in frequency of CAs and MN. Further, this was also noted that *R. serpentina* alleviates the DNA damage as was noticeable by a significant ($p<0.05$) reduction in CAs in the kidney tissue and MN in erythrocytes of fish in a dose-dependent manner (groups IV, V, VI). For all the above-mentioned groups, both CAs and MN induction registered a significant ($p<0.05$) declining trend, as evident by a gradual decrease in the frequency of CAs and MN induction with corresponding fold-changes in a decreasing order. Similarly, Kour *et al.* (2017) also found efficacy of an ethanolic extract of *Equisetum arvense* against cyclophosphamide, by analyzing the frequency of CAs compared to control group. Likewise, the antimutagenic property of neem extract was explained by Arivazhagan *et al.* (2003) in male Wistar rats. Correspondingly, the protective effect of *Melissa officinale* against sub-lethal exposure of arsenic was explained by Dwivedi *et al.* (2017). They elaborated that *M. officinale*, being rich in different phenolic components; quickly scavenge free radicals and consequently the oxidative stress, generated in Channa punctatus after exposure to arsenic. Subsequently, cytogenetic stability challenged by oxidative stress is restored as is evident by significantly ($p<0.05$) alleviated MN induction coupled with expression of stress genes- *Hsp70* and *Hsp27*. Additionally, the present findings are similar to those of Tiwari *et al.* (2017), who observed a significant induction in MN frequency in furadon 3G exposed group, and gradual reduction in MN frequency after exposure of the extract of *Melissa officinale* in *Channa punctatus*.

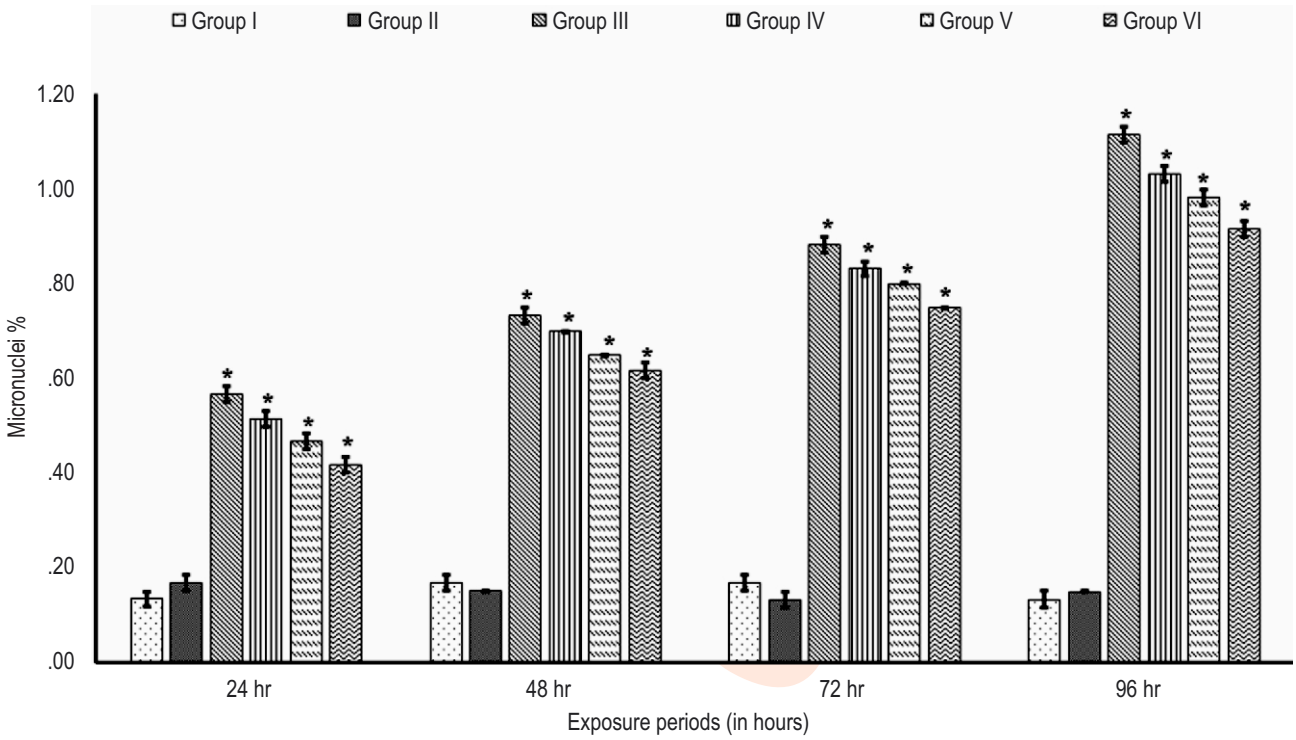

Fig. 1 : Quantitated and integrated micronuclei in erythrocytes of test fish of all experimental groups I, II, III, IV, V and VI after 24, 48, 72 and 96 hrs of exposure. Values are expressed as mean ± S.E.M.; n = 3 fishes in each replicate. * represents significant ($p<0.05$) values in comparison to control fishes; Group I – control, Groups II – 3.0 mg l^{-1} of *R. serpentina* root extract, Group III – 0.4 mg l^{-1} of copper sulphate, Group IV, V, and VI – 0.4 mg l^{-1} of copper sulphate plus 1, 2, 3 mg l^{-1} of *R. serpentina* root extract, respectively.

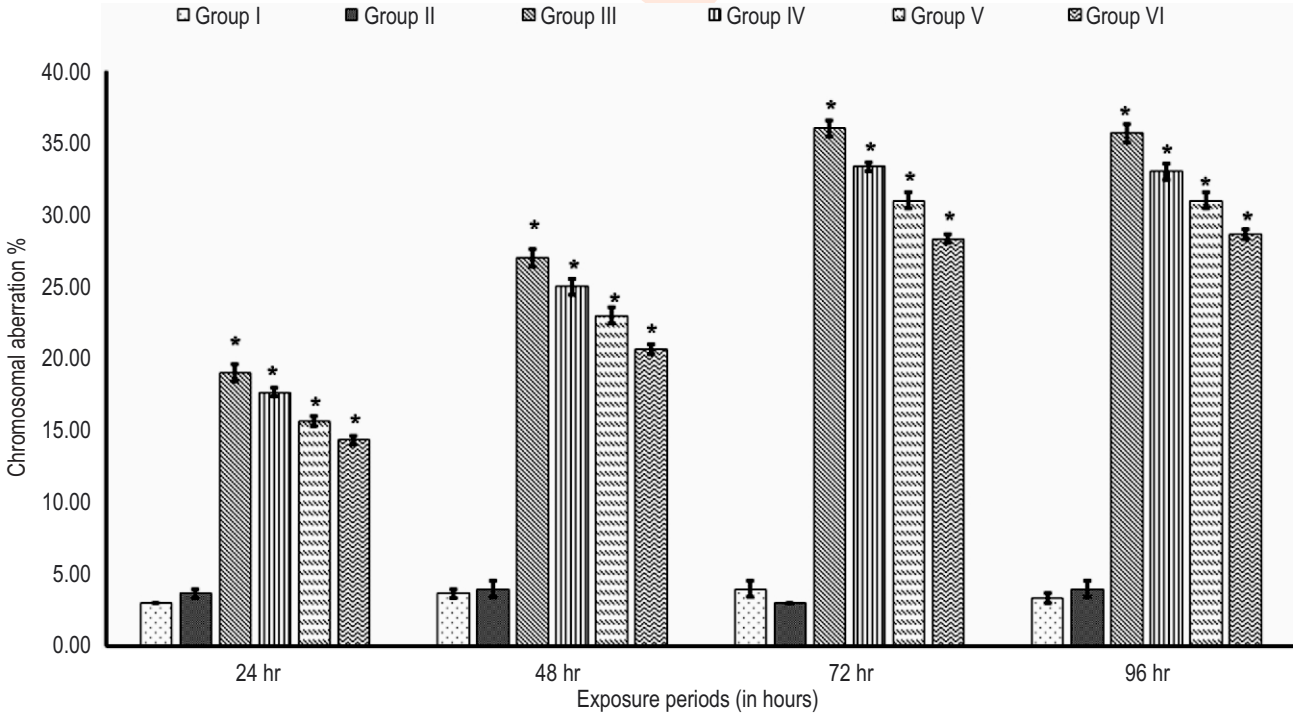

Fig. 2 : Quantitated and integrated chromosomal aberration in test fish of all experimental groups after 24, 48, 72 and 96 hrs of exposure. Values are expressed as mean ± S.E.M.; n = 3 fishes in each replicate. * represents significant ($p<0.05$) values in comparison to control fishes; Group I – control, Group II – 3.0 mg l^{-1} of *R. serpentina* root extract, Group III – 0.4 mg l^{-1} of copper sulphate, Groups IV, V and VI – 0.4 mg l^{-1} of copper sulphate plus 1, 2, 3 mg l^{-1} of *R. serpentina* root extract, respectively.

The variations recorded in protein levels in liver tissue of fish for both control and treated groups are shown in Fig. 3. The fold-change difference in the group treated with copper sulphate alone (Group III) was observed 0.21 < 0.29 < 0.37 < 0.42 after 24, 48, 72 and 96 hrs of exposure, as compared to control. The highest fold-change of 0.42 was noticed after 96 hr of exposure. The fish co-exposed with copper sulphate (96 hr-LC_{50} 10^{-1}; 0.4 mg l^{-1}) and *R. serpentina* root extract (1, 2 and 3 mg l^{-1}) showed variation in fold-change as 0.18 > 0.15 > 0.13 after 24 hr as compared to group III (0.21) with respect to control, and 0.26 > 0.21 > 0.20 after 48 hr as compared to group III (0.29) with respect to control. A similar decreasing trend in fold changes was also observed after 72 and 96 hr of exposure period as 0.29 > 0.26 > 0.20 and 0.32 > 0.30 > 0.28, respectively, as compared to group III (0.37, and 0.42) with respect to control. A fold-change difference of 1.14 was calculated between group-III and group-VI, illustrating a decrease in the protein level after 96 hr of exposure period. Group II showed an insignificant change in protein level as compared to control.

The mean values of SGOT in the liver tissue of *C. punctatus* for all experimental groups are shown in Fig 4 (A). The fold changes in the activities of SGOT were observed as 0.49 < 0.83 < 1.31 < 1.58 for copper sulphate in Group III as compared to control. However, a combined exposure of copper sulphate (0.4 mg l^{-1}) and different concentrations of *R. serpentina* root extract (1, 2 and 3 mg l^{-1}) produced a significant decrement in fold- changes of SGOT activities as 0.44 > 0.33 > 0.24 after 24 hr as compared to group III (0.49) with respect to control. Further, fold-changes of 0.79 > 0.70 > 0.54 were observed after 48 hr as compared to group III (0.83) with respect to control. After 72 hr, in comparison to group III (1.31) with respect to control, fold-changes were observed as 1.06 > 0.91 > 0.90, and after 96 hr 1.42 > 1.32 > 1.20 as compared to group III (1.58) with respect to control.

The activity of SGPT was estimated in the liver of fish for all experimental groups (Fig. 4B). Fish of group III showed distinct fold-changes as 0.80 < 1.30 < 1.56 < 1.83 in the activity of SGPT. A significant decrement ($p < 0.05$) in the activity of SGPT in the groups IV, V and VI exposed to copper sulphate (96 hr-LC_{50}/10; 0.4 mg l^{-1}) and *R. serpentina* root extract (1, 2 and 3 mg l^{-1}) were recorded as 0.67 > 0.57 > 0.40 after 24 hr of exposure period as compared to group III (0.80) with respect to control. After 48 hr of exposure, fold-changes were calculated as 1.07 > 0.93 > 0.74 as compared to group III (1.30) with respect to control. Further, after 72 hr, fold-changes were recorded as 1.30 > 1.19 > 0.85 in comparison to group III (1.56) with respect to control. Similarly, after 96 hr of exposure, decreased values in fold-changes, 1.60 > 1.50 > 1.30 were obtained in comparison to group III (1.83) with respect to control.

The increasing trend of fold-change in ALP activity was observed as 2.82 < 3.84 < 5.31 < 5.54 after 24, 48, 72 and 96 hr of exposure period in the liver of fish treated with copper sulphate

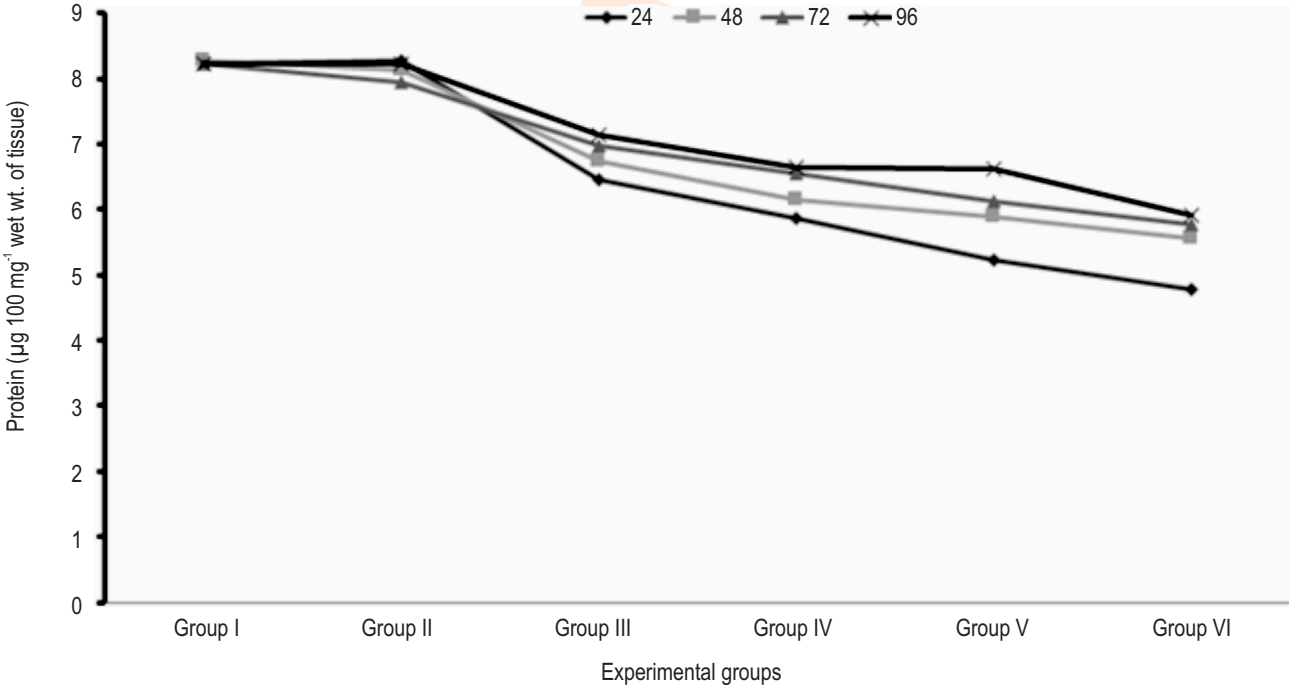

Fig. 3 : Protein level of *Channa punctatus* for experimental groups I, II, III, IV, V and VI after 24, 48, 72, 96 hrs of exposure. Values are expressed as mean ± S.E.M.; n = 3 fishes in each replicate, Group I – Control, Group II – 3.0 mg l^{-1} of *R. serpentina* root extract, Group III – 0.4 mg l^{-1} of copper sulphate, Groups IV, V, and VI – 0.4 mg l^{-1} of copper sulphate plus 1,2,3 mg l^{-1} of *R. serpentina* root extract, respectively.

(96 hr-LC_{50}/10; 0.4 mg l^{-1}) alone as compared to unexposed group. However, after a combined exposure of different doses *i.e.*, 1, 2 and 3 mg l^{-1} of *R. serpentina* along with copper sulphate (96 hr-LC_{50}/10; 0.4 mg l^{-1}) a significant ($p < 0.05$) and gradual reduction in fold- changes of ALP activity was recorded as 2.23 > 2.09 > 2.07 after 24 hr in comparison to group III (2.82), after 48 hr exposure period, similar decreasing trend was noticed as 2.90 > 2.72> 2.76 as compared to group III (3.84). Additionally, the decreasing trend continued after 72 and 96 hr exposure. Fold-changes of 4.88 > 3.99 > 3.77 after 72 hr as compared to group III (5.31) and 4.92 > 4.26 > 3.92 were recorded after 96 hr as compared to group III (5.54) with respect to control. This suggests that a co-treatment with *R. serpentina* was instrumental in bringing down the activities of ALP in corresponding groups.

Total protein levels, and SGOT, SGPT and ALP activities in liver tissues of test fish were evaluated to explain that biochemical alterations are produced due to Cu^{2+} induced cytotoxic stress. In the present study, exposure of Cu^{2+} reduced protein levels significantly ($p<0.05$) in liver tissues of test fish in a concentration and time-dependent manner. The present findings are comparable with those of Vutukuru (2003), who reported the depletion of total protein in the liver, gill and muscle of fish, *Labeo rohita* due to chromium exposure. Nagaraju (2016) also reported that in sub-lethal exposure of profenofos, the maximum percentage (19.34 %) of protein depletion was observed in the liver of *Labeo rohita*. Harit and Srivastava (2017) also found that endosulfan interfered with the enzymatic activities, leading to protein depletion in the muscles of *Channa punctatus.*

Although, the liver plays a crucial role in metabolism and detoxification of many xenobiotics, Cu^{2+}accumulates in the liver and causes biochemical alterations. Present findings show that

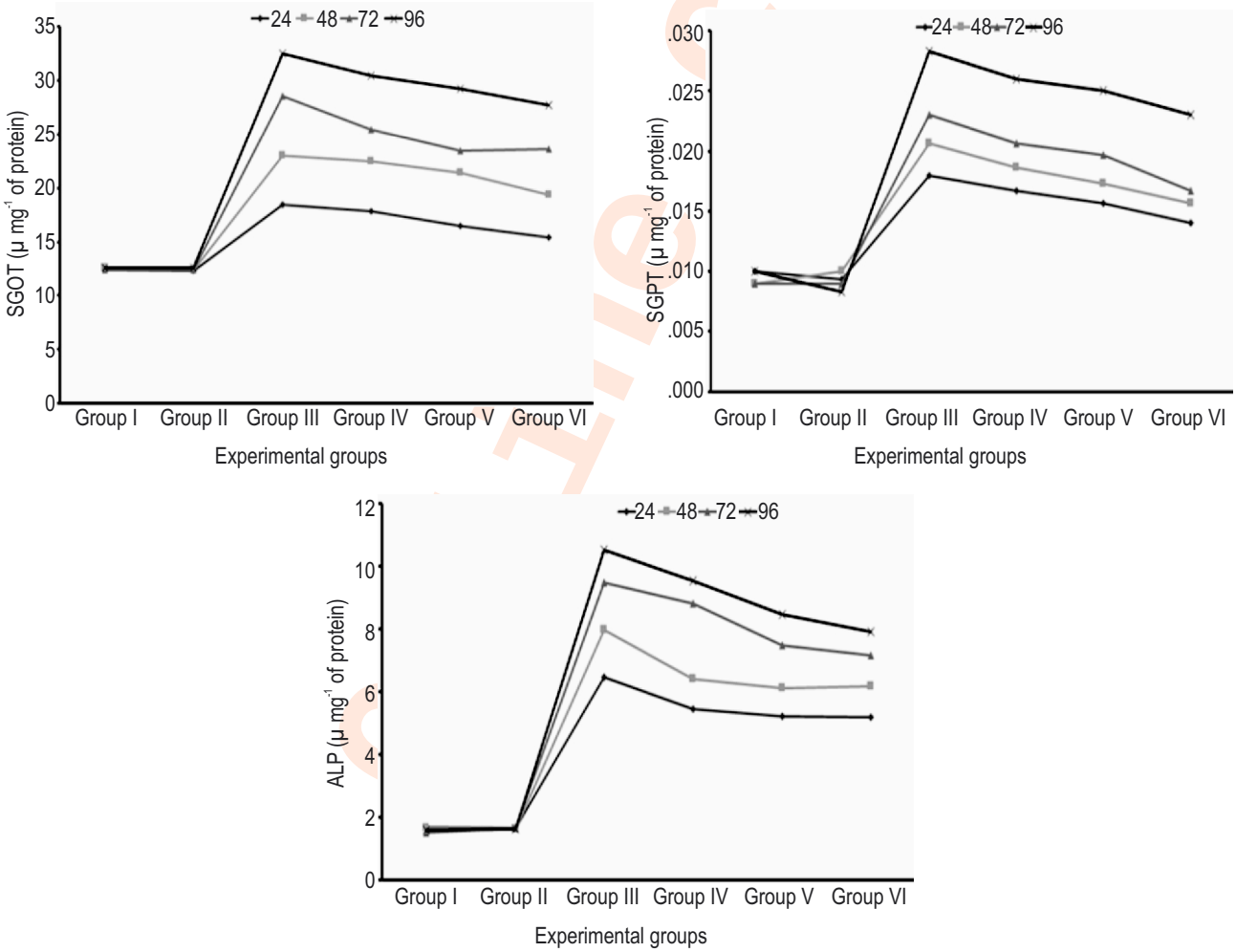

Fig. 4 : Variations in Serum glutamic-oxaloacetic transaminase (SGOT), Serum glutamic-pyruvic transaminase (SGPT) and Alkaline phosphatase (ALP) enzyme activities in liver tissue of fish *Channa punctatus* in experimental Groups I, II, III, IV, V and VI after 24, 48, 72 and 96 hrs of exposure period. Values are expressed as mean ±S.E.M.; n = 3 fishes in each replicate, Group I – control, Group II – 3.0 mg l^{-1} of *R. serpentina* root extract, Group III – 0.4 mg l^{-1} of copper sulphate, Groups IV,V, and VI – 0.4 mg l^{-1} of copper sulphate plus 1, 2, 3 mg l^{-1} of *R. serpentina* root extract, respectively.

exposure to Cu^{2+} resulted in a progressive and significant ($p<0.05$) increment in the activities of SGOT, SGPT and ALP in liver tissue of test fish. The present findings are, thus, in line with observations made by Tiwari *et al.* (2017), who found similar increasing trends in activities of SGOT, SGPT and ALP in liver of *Channa punctatus* exposed to furadan 3G for 24 and 96 hrs. Srivastava and Prakash, (2018) documented similar findings, i.e. a significant elevation in activities of SGPT, SGOT and ALP after exposure to zinc sulphate in *Clariasbat rachus*. Further, activities of SGOT, SGPT and ALP showed a significant ($p<0.05$) declining trend with corresponding decreased fold-changes in comparison to group III with respect to control when fishes were simultaneously exposed to Cu^{2+} and *R. serpentina* root extract. Moreover, the increment in activities of liver biomarkers (SGOT, SGPT and ALP) decreased gradually after an increase in the concentration of *R. serpentina* root extract. This shows the efficacy of *R. serpentina* against Cu^{2+}- induced changes in biochemical activities of liver. These findings are similar to those of Tiwari *et al.* (2017) who documented a significant decrease in the activities of SGOT, SGPT and ALP in liver of *Channa punctatus* after their treatment with *Melissa officinalis*. Laltlanmawia *et al.* (2019), also documented the stimulatory effect of 1.0% *Withania sominifera* and dietary L-ascorbic acid, which reduced the elevated activities of SGOT, SGPT and ALP in *Labeo rohita* as compared to control. Similarly, higher gene expression of immune relevant genes TLR-22 and β2-M was observed down regulated after a single dose treatment of miconazole nitrate in *Labeo rohita* fingerlings (Singh *et al.,* 2018). Results of the present study are in accordance with the findings of Gupta *et al.,* (2006), who documented that aqueous ethanolic extract of *Rauwolfia serpentina* is a promising hepatoprotective agent due to its antioxidant properties in fish.

Findings of the present study amply illustrate that *R. serpentina* root extract substantially alleviates genotoxicological and biochemical impairments induced by the exposure of copper sulphate in fish, *Channa punctatus*. Thus, it can be inferred that referenced polyphenolic medicinal plant can be effectively employed as a remedy to overcome the harmful effects caused by contamination of copper and to preserve fish biodiversity in such aquatic regimes.

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Add-on Information

Author's contribution : S. P. Trivedi: Conceptualization, Experimental designing, final editing of the MS and proof correction; **V.Kumar:** Contribution: Execution of the experiment; **S. Singh:** Statistical analysis of the data; **A. Trivedi:** Plotting of graphs and overall analysis of the data; **M. Kumar:** Supervision of the experiment and preparation of the manuscript.

Research content : The research content of manuscript is original and has not been published elsewhere.

Ethical approval : For entire experimentation and euthanization of fish, necessary guidelines issued by the Institutional Animal Ethics Committee (IAEC, Registration No. 1861/GO/Re/S/16/CPCSEA) were followed.

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References

Dar, S.A., A.R. Yousuf and M.H. Balkhi: An introduction about genotoxicology methods as tools for monitoring aquatic ecosystem: Present status and future perspectives. *Fish Aquac. J.*, **7**, 2 (2016)

APHA: Standard Methods for Examination of Water and Wastewater. 22nd Edn., American Public Health Association (APHA), American Water Works Association (AWWA), and Water Pollution Control Federation (WPCF), Washington DC, USA (2018).

Arivazhagan, S., S. Nagini, S.T. Santhiya and A. Ramesh: N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced *in-vivo* clastogenicity: Protective effects of aqueous neem leaf extract. *Pharmazie.,* **58**, 750-752 (2000)

Awasthi, Y., A. Ratn, R. Prasad, M. Kumar, A. Trivedi, J.P. Shukla and S.P. Trivedi: A protective study of curcumin associated with Cr^{6+} induced oxidative stress, genetic damage, transcription of genes related to apoptosis and histopathology of fish, *Channa punctatus* (Bloch,1793).*Environ.Toxicol.Pharmacol.,* **71**,110 (2019)

Awasthi, Y., A. Ratn, R. Prasad, M. Kumar and S.P. Trivedi: An *in-vivo* analysis of Cr^{6+} induced biochemical, genotoxicological and transcriptional profiling of genes related to oxidative stress, DNA damage and apoptosis in liver of fish, *Channa punctatus* (Bloch, 1793). *Aquat. Toxicol.,* **200**, 158–167 (2018).

Azmi, B.M. and A.S. Qureshi: *Rauwolfia serpentina* improves altered glucose and lipid homeostasis in fructose-induced type 2 diabetic mice. *Pak. J. Pharm. Sci.,* **29**, 1619–1624 (2016).

Bolognesi, C. and S. Cirillo: Genotoxicity biomarkers in aquatic bioindicators. *Curr. Zool.,* **60**, 273–284 (2014).

Flemming, C.A. and J.T. Trevors: Copper toxicity and chemistry in the environment: a review. *Water Air Soil Poll.*, **44**, 143–158 (1989).

Chattopadhyay, R.R.: Possible biochemical mode of anti-inflammatory action of *Azadirachta indica* A. Juss. in rats. *Indian J. Exper. Biol.*, **36**, 418-420 (1998).

Cucchi, C. and A. Baruffaldi: A new method for karyological studies in teleost fishes. *J. Fish Biol.,* **37**, 71–75 (1990).

Dey, A.: Ethnobotanical aspects of *Rauvolfia serpentina* (L). *J. Med. Plants Res.,* **5**, 144–150 (2011).

Dorcas, I.K. and R.J. Solomon: Calculation of liver function test in *Clarias*

gariepinus collected from three commercial fish ponds. *Nat. Sci. Nat Sci.,* **1212**, 107–123 (2013).

Dutta, P.K., I.C. Chopra and L.D. Kapoor: Cultivation of *Rauvolfia serpentina* in India. *Econ. Bot.,* **17**, 243–251 (1963).

Dwivedi, S., M. Kumar and S.P. Trivedi: Mitigating potential of *Melissa officinale* against As^{3+}-induced cytotoxicity and transcriptional alterations of Hsp70 and Hsp27 in fish, *Channa punctatus* (Bloch). *Env. Monit. Assess.,* **189**, 1–15 (2017).

Green, R.M. and S. Flamm: AGA technical review on the evaluation of liver chemistry tests. *Gastroenterology,* **123**, 1367–1384 (2002).

Gupta, J. and A. Gupta: Determination of trace metals in the leaves of *Rauwolfia serpentina* by using atomic absorption spectroscopy. *Int. J. Chem. Stud.,* **4**, 94–96 (2016).

Hamilton, M.A., R.C. Russo and R.V. Thurston: Trimmed Spearman-Karber method forestimating median lethal concentrations in toxicity bioassays. *Environ. Sci. Technol.,* **11**, 714-719 (1977).

Hutchinson, T.H., C. Bögi, M.J. Winter and J.W. Owens: Benefits of the maximumtolerated dose (MTD) and maximum tolerated concentration (MTC) concept in aquatic toxicology. *Aquatic Toxicol.*, **91**, 197-202 (2009).

Iji, O.T. and A.O. Adeogun: Industrial effluent induced chromosomal aberration in catfish from Ogun river, Lagos, Nigeria. *Pakistan J. Biol. Sci.,* **17**, 675–681(2014).

Kanakis, I., M. Nikolaou, D. Pectasides, C. Kiamouris and N. K. Karamanos: Determination and biological relevance of serum cross-linked type I collagen N-telopeptide and bone-specific alkaline phosphatase in breast metastatic cancer. *J. Pharm. Biomed. Anal.*, **34**, 827-832 (2004).

Kour, J., M.N. Ali, H.A. Ganaie and N. Tabassum: Amelioration of the cyclophosphamide induced genotoxic damage in mice by the ethanolic extract of *Equisetum arvense*. *Toxicol,* **4**, 226-233 (2017).

Laltlanmawia, C., R.K. Saha, H. Saha and P. Biswas: Ameliorating effects of dietary mixture of *Withania somnifera* root extract and vitamin C in *Labeo rohita* against low pH and waterborne iron stresses. *Fish Shellfish Immunol.,* **88**, 170–178 (2019).

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall: Protein measurement with the Folin phenol reagent. *J. Biological Chem.*, ***193***, 265-275 (1951).

Mallick, R.S., C.R. Jena, C.K. Samal: Rapid *in-vitro* multiplication of an endangered medicinal plant sarpgandha (*Rauwolfia serpentina)*. *Sci. Res.,* **3**, 437–442 (2012).

Mrunalini, P. and P. Khobragade: Consideration with environmental factors and cultivation techniques. *Int. J. Ayurv. Pharma. Res.,* **4**, 58–62(2016).

Nagaraju, B. and V. Rathnamma: Effect of profenofos an organophosphate on protein levels in some tissues of fresh water fish *Labeo rohita* (Hamilton). *Int. J. Phar. Pharmac.Sci.,* **5**, 276-279 (2013).

Osman, A.G.M.: Genotoxicity tests and their contributions in aquatic environmental research. *J. Environ. Protec. (Irvine,. Calif).,* **5**, 1391–1399 (2014).

Padrilah, S.N., M. Khalizan Sabullah, M. Yunus, A. Shukor, N.A. Yasid, N.A. Shamaan and S.A. Ahmad: Toxicity effects of fish histopathology on copper accumulation. *Pertanika J. Trop. Agric. Sci.,* **41**, 519–540 (2018).

Qureshi, S.A., A. Nawaz, S.K. Udani and B. Azmi: Hypoglycaemic and hypolipidemic activities of *Rauwolfia serpentina* in alloxan-induced diabetic rats. *Int. J. Pharmacol.,* **5**, 323–326 (2009).

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).

Ratn, A., R. Prasad, Y. Awasthi, M. Kumar, A. Misra and S.P Trivedi: Zn^{2+} induced molecular responses associated with oxidative stress, DNA damage and histopathological lesions in liver and kidney of the fish, *Channa punctatus* (Bloch, 1793). *Ecotoxicol. Environ. Saf.,* **151**, 10–20 (2018).

Rishi, K.K. and S. Grewal: Chromosome aberration test for the insecticide, dichlorvos, on fish chromosomes. *Mutat. Res. Toxicol.,* **344**, 1–4 (1995).

Schmid, W.:The micronucleus test. *Mutat. Res. Mutagen. Relat. Subj.,* **31**, 9–15 (1975).

Singh, M., R.K. Saha, H. Saha and J. Parhi: Effect of miconazole nitrate on immunological response and its preventive efficacy in *Labeo rohita* fingerlings against oomycetes *Saprolegnia parasitica. J. Fish Dis.,* **41**, 1539–1548 (2018).

Srivastava, N.K. and S. Prakash: Effect of sublethal concentration of zinc sulphate on the serum biochemical parameters of freshwater cat fish, *Clarias batrachus* (LINN). *Indian J. Biol.,* **5,** 113-119 (2018).

Solomon, F.: Impacts of copper on aquatic ecosystems and human health. *Environ. Comm.,* **25**, 25-28 (2009).

Thapa, B.R. and A. Walia: Liver function tests and their interpretation.*Indian J. Pediatr.,* **74**, 663–671 (2007).

Tiwari, V. and S.P. Trivedi: Investigations on remedial role of *Rauwolfia serpentina* root extract against carbofuran formulation induced genotoxicity in *Channa punctatus. J. Environ. Biol.,* **40**, 1023–1028 (2019).

Trivedi, S.P., A. Ratn, Y. Awasthi, M. Kumar and A. Trivedi: In vivo assessment of dichlorovos induced histological and biochemical impiarements coupled with expression of p53responsive apoptotic genes in the liver and kidney of fish, *Channa punctatus* (Bloch, 1793).Comp. *Biochem. Physiol.*, **Part C 245**, 109032 (2021).

Ueda, T., M. Hayashi, Y. Ohtsuka, T. Nakamura, J. Kobayashi and T. Sofuni: A preliminary study of the micronucleus test by acridine orange fluorescent staining compared with chromosomal aberration test using fish erythropoietic and embryonic cells. *Water Sci. Technol.*, **25**, 235-240 (1992).

Van Genderen, E.J., A.C. Ryan, J.R. Tomasso and S.J. Klaine: Evaluation of acute copper toxicity to larval fathead minnows (*Pimephales promelas)* in soft surface waters. *Environ. Toxicol. Chem.*, **24** , 408–414 (2005)

Vutukuru, S.S.: Chromium induced alterations in some biochemical profiles of the indian major carp,*Labeo rohita* (Hamilton). *Environ. Contam. Toxicol.,* **70**, 118–123 (2003).

Watson, C. and R.P.E. Yanong: Use of copper in freshwater aquaculture and farm ponds. Fact Sheet FA13. Department of Fisheries and Aquatic Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida (2011).

Yadav, K.K. and S.P. Trivedi: Chromosomal aberrations in a fish, *Channa punctata* after in-vivo exposure to three heavy metals. *Mutat. Res. Toxicol. Environ. Mutagen.,* **678**, 7–12 (2009a)

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