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Efficacy evaluation of *Rauwolfia serpentina* against Chromium (VI) toxicity in fish, *Channa punctatus*

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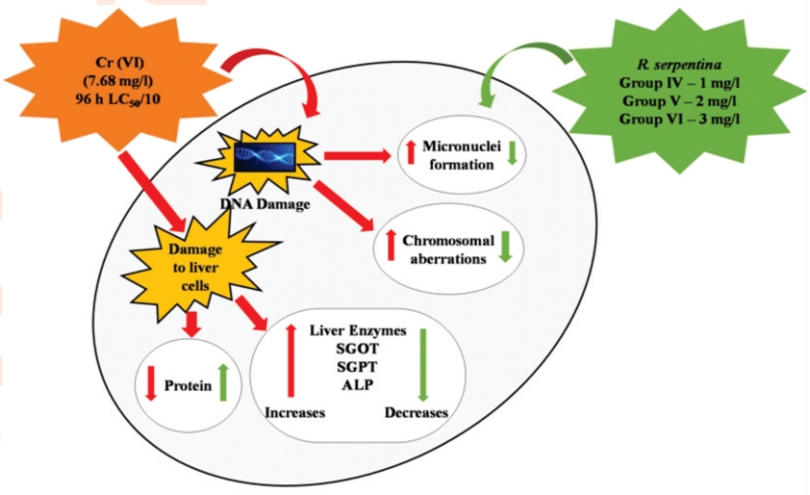
Abstract

Aim: To explore the efficacy potential of ethanolic root extract of *Rauwolfia serpentina* against Chromium (VI) toxicity in fish, *Channa punctatus*.

Methodology: Acclimatized fish were divided into six groups, each having 15 specimens. Group I served as a control, while Group II and III fish were exposed to ethanolic root extract of *Rauwolfia serpentina* (3 mg l^{-1}) and Cr (VI) (96 h LC_{50}/10; 7.68 mg l^{-1}), respectively. Groups IV, V and VI fish were exposed to three different concentrations of ethanolic root extract of *Rauwolfia serpentina* (1, 2 and 3 mg l^{-1}), simultaneously with 7.68 mg l^{-1} of Cr (VI). The induction of micronuclei, chromosomal aberrations, protein levels and liver enzymes-SGOT, SGPT and ALP- were assessed in fish of all six groups after designated exposure periods.



Results: A significant induction ($p<0.05$) in chromosomal aberrations, micronuclei frequency and activities of liver enzymes (SGOT, SGPT and ALP) coupled with reduced protein level was recorded in Group III as compared to the control. Whereas, a significant ($p<0.05$) decrease in the frequency of chromosomal aberrations, micronuclei induction and activities of liver enzymes together with an increase in protein level was observed in Group IV, V and VI with respect to control and Group III.

Interpretation: Present investigation evince the ameliorative potential of ethanolic root extract of *Rauwolfia serpentina* against Cr (VI) induced chromosomal aberrations and micronuclei induction, changes in protein levels and biochemical alterations of liver enzymes in *Channa punctatus*. Thus would help present work helps in saving the aquatic biodiversity and increasing the production of protein rich food fish.

Key words: Chromosomal aberrations, Chromium trioxide, Liver marker enzymes, Micronucleus, *Rauwolfia serpentina*

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Introduction

Rauwolfia serpentina ('Sarpagandha'), inhabiting the foothills of Himalayas at an altitude of 1,300 to 1,400 m. is an important medicinal plant because of its extraordinary therapeutic properties. The plant containing more than 50 different alkaloids finds ample application in modern medical systems, including Ayurveda and Unani. Further, on account of its cultural acceptance, better human body compatibility, and fewer side effects, *Rauwolfia* herb is still used in primary health care by 75-80% of the world's population (Kumari *et al.,* 2013). Rauwolfia is being used as an antidote against snake bites for centuries (Upasani *et al.,* 2017). It is useful in mental diseases, epilepsy, sleeplessness and several other ailments (Ojha and Mishra, 1985). Administration of *R. serpentina* has shown immense therapeutic response against various disorders in mice (Azmi and Qureshi, 2016) and humans (Lobay, 2015). Qureshi *et al.* (2009) emphasized the hypoglycemic, hypolipidemic, and hepatoprotective role of methanolic root extract of *R. serpentine* in alloxan-induced diabetic rats. Its active constituent, reserpine is effectively used as a commercial antihypertensive and antihelminthic drug in modern medicines (Azmi and Qureshi, 2016).

Chromium a metallic element, listed by the US Environmental Protection Agency (USEPA) as one of 129 priority pollutants, occurs naturally in the earth's crust, although it enters the environment due to anthropogenic activities or atmospheric deposition from industrialized areas (Paustenbach *et al.,* 2003; Guertin *et al.,* 2004; Reinds *et al.,* 2006; Krystek and Ritsema, 2007). Chromium (VI), a known carcinogenic and mutagenic agent (Nakajima and Baha, 2004) is a topic of discussion due to its presence in drinking water throughout the world. Keeping this in mind, the State of California has recommended a permissible limit of 0.01 mg l^{-1} for Cr (VI) in drinking water. In humans, Cr (VI) damages DNA leading to lung cancer and reproductive ailments besides liver damage, pulmonary congestion, and skin irritations resulting in formation of ulcers (Kobya, 2004). Hexavalent chromium, due to its redox potential, induces oxidative stress in fishes (Velma and Tchounwou, 2011). Awasthi *et al.,* 2018 and Kumar *et al.* (2019) have adequately reported the genotoxic potential of Cr (VI) in fishes.

Exposure to Cr (VI) results in its accumulation in liver, the prime site for biotransformation of xenobiotics and their detoxification in fish. Further, liver is the main source of serum protein and is the only place for albumin synthesis (Morrison *et al.,* 2019). The changes in mobility and low level of proteins reflect a change in the rate of synthesis and degradation of proteins (Nagaraju, 2016). Elevated activities of liver biochemical markers serum glutamic-oxaloacetic transaminase or aspartate aminotransferase, serum glutamic pyruvic transaminase or alanine aminotransferase (ALT) and alkaline phosphatase in tissue/blood is suggestive of their leakage from the liver due to hepato-cellular impairments (Dorcas and Solomon, 2014). Assessing metal genotoxicity in terrestrial and aquatic ecosystems has become a major area of research. Several methods are employed for this purpose. Nevertheless, simple, reliable, sensitive, and cost-effective methods such as micronucleus test, chromosomal aberration test and DNA damage tests are still popular and being routinely applied to assess the genotoxicity of various chemicals in fishes (Pandey *et al.,* 2011; Ratn *et al.,* 2018; Yadav and Trivedi, 2009a).

Micronuclei induction is a sensitive indicator of DNA damage and can be effectively utilized to have sight of double strand breaks, single strand breaks, base mismatch and DNA adducts. This makes micronucleus test a reliable tool for evaluating genetic damage induced by aquatic pollutants in fishes and other aquatic organisms (Bolognesi, and Cirillo, 2014; Ratn *et al.,* 2018; Yadav and Trivedi, 2009b). Chromosomal aberrations are important cyto-toxic end markers against a number of xenobiotics, including metals. *Channa punctatus* is a suitable fish model owing to its larger and fewer numbers of chromosomes (2N=32). In light of the above, the present study was designed to evaluate the efficacy of ethanolic root extract of Sarpgandha (*Rauwolfia serpentina*) in fish, *Channa punctatus* against exposure of Cr (VI) using chromosomal aberrations in kidney, micronucleus in blood and biochemical parameters in liver.

Materials and Methods

Acclimatization of fish and experimental design: *Channa punctatus* (35 ± 3.0 g; 14.5 ± 1.0 cm), hand netted from nearby freshwater habitats of Lucknow, were brought to the laboratory and treated with 0.05% $KMnO_4$ solution for 2 min to remove external infections. Fish were kept in 100 L glass aquaria (100 x 40x 40 cm^3) containing 10 days old dechlorinated water and acclimatized for 15 days under laboratory conditions. They were fed with commercial aquarium fish food (Perfect Companion Group Company Limited, Thailand). Wastes were removed regularly to reduce the ammonia content in aquaria water during acclimatization. Only healthy and active fish were randomly selected and used in the experiments. Fish were divided in six groups each having 15 specimens. Group I served as control, fish of Group II and III were exposed to ethanolic root extract of *Rauwolfia serpentina* (3 mg l^{-1}) and sublethal concentration of Cr(VI) (1/10 of LC_{50} for 96 h; 7.68 mg l^{-1}), respectively. While fish in Groups- IV, V and VI were exposed to three different concentrations of ethanolic root extract of *Rauwolfia serpentina* (1, 2 and 3 mg l^{-1}) simultaneously with Cr (VI) (7.68 mg l^{-1}). Exposure was continued for 96 hr and the samples were collected at an intervals of 24, 48, 72 and 96 hr. After the termination of desired exposure periods, three fish from each group were sampled and anaesthetized with 0.1 % (v/w) diethyl ether prior to blood and tissue sampling for genotoxicity and biochemical analysis. For genotoxicity evaluation, Micronuclei were analyzed from blood, kidney tissue was employed for chromosomal aberration test, while biochemical parameters were assessed in liver tissues.

Determination of sub lethal concentrations of chromium

trioxide: Acute static toxicity bioassays were performed to determine 96 hr LC_{50} by following the standard methods (APHA, 2017). For determination of 96 hr LC_{50} of Cr(VI), the mean values of percentage mortalities of fish up to 96 hr against each concentration were subjected to Trimmed Spearman-Karber method software (Hamilton *et al.,* 1977).

Preparation of ethanolic extract of *Rauwolfia serpentina* roots: *Rauwolfia* roots were purchased from the market (Manufactured by Tansukh, India). The ethanolic root extract was prepared by following the procedure of Azmi and Qureshi (2012). Roots were cleaned with tap water; air dried and powdered by crushing. About 400 gm of root powder was dissolved in 2l of ethyl alcohol and kept at room temperature for 48 hr. The mixture was filtered and the filtrate was concentrated under reduced pressure in a water bath at 50°C and finally dried in a rotavapour to obtain a thick brown paste, which was suitably diluted with normal saline and used for the experiments. Doses of ethanolic root extract of *Rauwolfia serpentina* were selected on the basis of No observed effect concentration (NOEC) method of Hutchinson *et al.* (2009). Exposure to a dose >3.0 mg l^{-1} of ethanolic root extract of *Rauwolfia serpentina* caused undesirable behavioral symptoms in fish.

Genotoxicity assessment

Chromosomal aberration test: An hour prior to completion of stipulated duration, fish were injected intramuscularly with freshly prepared colchicine solution at a dosage of 1 mg per 100 g body weight to arrest cell division at metaphase. Fish were anaesthetized and their kidneys were taken out to study chromosome aberrations (Al-Sabti *et al.,*1983; Cucchi and Baruffaldi, 1990). Macerated kidneys were suspended in 0.56% KCl solution and incubated for 30–40 min at 32°C. The cell suspension was fixed in chilled Cornoy's fixative, mixed gently with Pasteur pipette, centrifuged at 1500 rpm for 10 min and the supernatant was discarded. The pellet was resuspended in chilled Cornoy's fixative and the above process was repeated twice. Chromosome slides were prepared by dropping a few drops of cell suspension onto pre-cooled slides in 70% alcohol. Immediately, thereafter, the fixative was burned off using flame drying. The slides stained in 5 % Giemsa's stain prepared in Sorensen's buffer (pH-6.8) for 20–25 min was cleared in xylene and permanently mounted in DPX. Slides having well spread metaphase chromosomes were observed using 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 examined.

Micronucleus test: After the termination of stipulated exposure periods, blood was collected by cardiac puncture and immediately a uniform smear was prepared on grease free slides. They were air dried overnight at room temperature, fixed in absolute methanol for 5 min and stained with May-Grunnwald's dye. Slides were again stained with 5% Giemsa (Sigma Aldrich, USA) in phosphate buffer (pH 6.8) for 30 min and mounted in DPX for microscopic examination. A minimum of 6,000 erythrocytes were examined to detect MN in each treatment group including control using oil immersion microscope (Nikon Corporation K 12432) with 40/100X objective lenses. The MN frequency was calculated as follows (Schmid, 1975).

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

Analysis of biochemical parameters

Total protein estimation: For estimation of total protein content (Lowry *et al.,* 1951), 100 mg of liver tissue was homogenized in 5 ml of chilled distilled water. Immediately, 5 ml of 30% trichloro acetic acid was added to it. The protein precipitate was then centrifuged at 3000 rpm for 15 min. After discarding the supernatant, the pellet was washed repeatedly with distilled water to remove traces of deposited TCA. The pellet was dissolved again in 0.1 N NaOH. A 0.5 ml of solution was transferred to a vial and 4 ml of alkaline copper sulphate was added followed by 0.4 ml of diluted commercial Folin's Reagent. After 30 min, the solution developed a blue color, its optical density was determined using spectrophotometer (Schimadzu, UV-1800 Pharma spec) at 750 nm wavelength. Bovine serum albumin was used as a standard. The content of tissue protein was expressed in micrograms 100 mg^{-1} of wet tissue weight.

Estimation of liver biomarkers: 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. A working solution was prepared for sample by combining enzyme reagent - R1 for SGOT (Tris buffer, L-aspartate, malate dehydrogenase) and for SGPT (Tris buffer, L-alanine, Lactate dehydrogenase) with substrate reagent - R2 (α – Ketoglutarate and NADH) in 4:1 ratio, mixed gently and incubated at 37°C for 5 min then 100 µl sample/control was added, mixed and after 1 min of incubation the change in absorption (ΔA/minute) was recorded every 3 min. Their activities are expressed as Units/L. ALP in liver tissues was estimated by following the modified method of Kanakis *et al.*, (2004). The increase in absorption was read at 405 nm. For a sample, a working solution was prepared by combining enzymatic reagent - R1 (Diethanolamine and Magnesium chloride) and substrate reagent - R2 (p-Nitrophenyl phosphate) in 4:1 ratio, respectively, mixed gently and incubated at 37°C for 5 min then 100 µl sample/control was added, mixed and after 1 min of incubation the change in absorption (ΔA min^{-1}) was recorded every 3 min. Its activity is expressed as Units/L.

Data evaluation and statistical analysis: Each treatment was performed in triplicate and data were presented as Mean ± S.E.M. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied to test the significance of each result. Cr (VI) induced biochemical and genotoxicological changes were analyzed by with SPSS software (version 20.0, SPSS Company, Chicago, USA).

Results and Discussion

Sub-lethal exposure of Cr(VI) (7.68 mg l^{-1}) showed an increasing trend of chromosomal aberrations (CA) (Table1). The estimated frequencies of CA) in Group II, treated with ethanolic root extract of *Rauwolfia serpentina* alone were insignificant in comparison to Group I (control). However, the frequency was significantly ($p<0.05$) increased in group III with an increased mean value in comparison to Group I. Interestingly, there was a reduction in the frequency of CA in groups IV, V and VI exposed to 7.68 mg l^{-1} of Cr(VI) along with increasing concentrations (1, 2 and 3 mg l^{-1}) of ethanolic root extract of *Rauwolfia serpentina*, after 24, 48, 72 and 96 hr of exposure in comparison to Group III, with respect to control.

The reduction percentage of chromosomal aberrations in fish treated with aforementioned three different concentrations of ethanolic root extract of *Rauwolfia serpentina* along with chromium trioxide were estimated (Table 4). The highest reduction of chromosomal aberrations (49.0%) was recorded in Group VI after 72 hr of exposure. Exposure to Cr (VI) (7.68 mg l^{-1}) caused a time and dose dependent increase in micronuclei (Table 2) with a slight variation in their size, position and number. The frequency of micronuclei in Group I were recorded as 0.13, 0.16, 0.13 and 0.13 after 24, 48, 72 and 96 hr of exposures, respectively. The micronuclei frequencies in Group II were found insignificant in comparison to Group I (Table 2). However, in comparison to control, the frequency of micronuclei in Group III significantly ($p<0.05$) increased as 0.28, 0.61, 0.76 and 0.94 after 24, 48, 72 and 96 hr, respectively. The maximum frequency (0.94) was recorded after 96 hr of exposure. Significant ($p<0.05$) differences in the frequency of micronuclei were also observed among all successive exposure periods of 24, 48 72 and 96 hr in different groups (Table 2). However, a simultaneous exposure of 7.68 mg l^{-1} Cr(VI) along with ethanolic root extract of *Rauwolfia serpentina* (1, 2 and 3 mg l^{-1}) in Groups IV, V and VI, respectively, showed a significant ($p < 0.05$) reduction in micronuclei percent at all the exposure periods in comparison to Group III, with respect to control. The highest reduction in micronuclei induction (55.5%) was observed in Group VI after 24 hr of exposure (Table 4).

The mean values of total protein content in liver tissue of *C. punctatus* of Group I was recorded as 8.16,7.99,8.23, and 7.99 µg100 mg^{-1} wet tissue weight. after 24, 48, 72 and 96 hr, respectively. However, changes in its levels were insignificant when fish were exposed to ethanolic root extract of *Rauwolfia serpentina* (3 mg l^{-1}) alone (Group II) with respect to control after exposure periods of 24, 48, 72 and 96 hr, respectively. In

Table 1: Quantified values of chromosomal aberrations in kidney tissues of *Channa punctatus* under sub-lethal exposure of chromium trioxide for experimental Groups: I, II, III, IV, V and VI after 24, 48, 72 and 96 hr of exposure

Groups	Treatments (mg l^{-1})	Exposure period (hr)	Total no. of metaphases observed	Total no. of aberrations	Frequency of chromosomal aberrations
Group I	Control	24	300	9	3.00±0.33
		48	300	12	4.00±0.57
		72	300	11	3.66±0.33
		96	300	11	3.66±0.33
Group II	*R. serpentina* (3 mg l^{-1})	24	300	10	3.33±0.56
		48	300	12	4.00±0.57
		72	300	9	3.00±0.50
		96	300	11	3.66±0.33
Group III	Chromium (7.68 mg l^{-1})	24	300	45	15.00±0.57*
		48	300	75	25.00±0.57*
		72	300	108	36.33±0.33*
		96	300	107	35.66±0.66*
Group IV	Chromium (7.68 mg l^{-1}) +	24	300	38	12.66±0.33*
	R. serpentina extract (1 mg l^{-1})	48	300	69	23.00±0.57*
		72	300	91	30.33±0.33*
		96	300	86	28.66±0.66*
Group V	Chromium (7.68 mg l^{-1}) +	24	300	34	11.33±0.33*
	R. serpentina extract (2 mg l^{-1})	48	300	63	21.00±0.57*
		72	300	79	26.33±0.33*
		96	300	77	25.66±0.33*
Group VI	Chromium (7.68 mg l^{-1}) +	24	300	30	10.00±0.00*
	R .serpentina extract (3 mg l^{-1})	48	300	57	19.00±0.57*
		72	300	61	20.33±0.33*
		96	300	62	20.66±0.33*

Values are mean of three replicates±S.E. *represents significant ($p<0.05$) values in comparison to control)

Table 2: Quantified values of micronuclei in erythrocytes of *Channa punctatus* under sub-lethal exposure of chromium trioxide for experimental Groups: I, II, III, IV, V and VI after 24, 48, 72 and 96 hr of exposure

Groups	Treatments (mg l^{-1})	Exposure period (hr)	Total no. of cells studied	Total no. of cells containing micronuclei	Frequency of micronuclei
Group I	Control	24	6019	8	0.13±0.01
		48	6021	10	0.16±0.01
		72	2021	8	0.13±0.01
		96	6016	8	0.13±0.01
Group II	*R. serpentina* (3 mg l^{-1})	24	6027	9	0.14±0.01
		48	6021	10	0.16±0.01
		72	6023	8	0.13±0.01
		96	6022	8	0.13±0.01
Group III	Chromium (7.68 mg l^{-1})	24	6028	17	0.28±0.01*
		48	6021	37	0.61±0.01*
		72	6012	53	0.76±0.01*
		96	6022	57	0.94±0.02*
Group IV	Chromium (7.68 mg l^{-1}) +	24	6020	15	0.24±0.01*
	R. serpentina extract (1 mg l^{-1})	48	6020	35	0.58±0.02*
		72	6026	43	0.71±0.02*
		96	6018	53	0.88±0.01*
Group V	Chromium (7.68 mg l^{-1}) +	24	6024	14	0.24±0.03*
	R. serpentina extract (2 mg l^{-1})	48	6024	33	0.54±0.00*
		72	6019	40	0.66±0.01*
		96	6024	49	0.81±0.00*
Group VI	Chromium (7.68 mg l^{-1}) +	24	6021	12	0.19±0.33*
	R. serpentina extract (3 mg l^{-1})	48	6016	21	0.51±0.01*
		72	6026	37	0.61±0.12*
		96	6012	45	0.74±0.02*

Values are mean of three replicates ±S.E.* represents the significant ($p<0.05$) values in comparison to control)

comparison to unexposed group, a significant ($p<0.05$) decrease in protein level was observed in Group III, after aforementioned periods of exposure to Cr(VI). The maximum decrease in protein level, was recorded in Group III after 96 h of exposure. Moreover, significant ($p<0.05$) differences in protein content were also observed among different exposure periods. (Table 3). However, a combined exposure to Cr(VI) and different concentrations of ethanolic root extract of *Rauwolfia serpentina* (1, 2, 3 mg l^{-1}) produced a significant ($p<0.05$) increase in protein content in comparison to Group III, with respect to control. Activities of liver biomarkers - SGOT, SGPT and ALP were estimated in *Channa punctatus* for all experimental groups (Table 3). A significant ($p<0.05$) increase in the activities of aforementioned biomarkers was observed in Group III exposed to Cr (VI). However, their elevated activities were effectively reduced when fishes were simultaneously treated with combined treatment of ethanolic root extract of *Rauwolfia serpentina* and Cr(VI).

The activities of SGOT, SGPT and ALP in liver tissues of fish in Group II, treated with ethanolic root extract of *Rauwolfia serpentina* alone were insignificant in comparison to Group I. However, a significant increase in ($p < 0.05$) SGOT, SGPT and ALP activities was observed in Group III, at all exposure periods in comparison to Group I. The maximum increase in SGOT, SGPT and ALP activities was, in Group III after 96 h of exposure. Table 3 shows a significant ($p<0.05$) difference in SGOT, SGPT and ALP activities at all exposure periods. However, a combined exposure to Cr (VI) and different concentrations of ethanolic root extract of *Rauwolfia serpentina* produced a significant ($p<0.05$) and gradual reduction in SGOT, SGPT and ALP activities at all exposure periods in comparison to Group III, with respect to control (Table 3). The highest percent reduction in activities of SGOT, (61.3) SGPT (60) and ALP (33.6) was recorded, in Group VI after 24 hr of exposure. The present study demonstrates the protective role of *Rauwolfia serpentina* against Cr (VI) induced toxicity in fish, *Channa punctatus viz*. hexavalent chromium induced chromosomal aberrations, micronuclei, elevated activities of liver marker enzymes and reduced levels of total protein.

These were sufficiently and significantly alleviated in fish co-exposed to 96h-LC_{50}/10 concentration of Cr (VI) and ethanolic root extract of *Rauwolfia serpentina*. This suggests the therapeutic role of aforementioned herb against chromium toxicity. In fact, fish being a resident of top trophic level serve as a sensitive sentinel of aquatic regimes and is frequently exposed to noxious xenobiotics, including heavy metals from different industrial, agricultural and domestic sources. After absorption, these toxicants are accumulated in fish liver, the prime site for detoxification of xenobiotics, and cause biochemical alterations.

Table 3: Alterations in activities of SGOT, SGPT, ALP and protein levels induced by chromium trioxide (CrO_3) and alleviated byethanolic root extract of *Rauwolfia serpentina* in liver tissues of *C. punctatus*. (* represents the significant (p<0.05) values in comparison to control fishes; Group I – control, Group II – 3.0 mg l^{-1} treated with ethanolic root extract of *Rauwolfia serpentina*, Group III – exposed with 7.6 mg l^{-1} of Cr^{6+}, Group IV, V VI – co-exposed with 7.6 mg l^{-1} of Cr^{6+} plus 1, 2, 3 mg l^{-1} of ethanolic root extract of *Rauwolfia serpentina*, respectively)

Groups	Treatments (mg l^{-1})	Exposure period (hr)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Protein level (µg 100 mg^{-1})
Group I	Control	24	12.12 ± 0.01	0.010 ± 0.00	1.626 ± 0.01	8.16 ± 0.01
		48	12.13 ± 0.01	0.009 ± 0.01	1.636 ± 0.02	7.99 ± 0.02
		72	12.14 ± 0.02	0.009 ± 0.01	1.484 ± 0.01	8.23 ± 0.01
		96	12.12 ± 0.01	0.010 ± 0.02	1.625 ± 0.03	7.99 ± 0.01
Group II	*R. Serpentina*	24	12.28 ± 0.03	0.009 ± 0.01	1.636 ± 0.01	7.87 ± 0.01
	(3 mg l^{-1})	48	12.48 ± 0.00	0.010 ± 0.02	1.682 ± 0.02	8.23 ± 0.01
		72	12.93 ± 0.01	0.009 ± 0.01	1.691 ± 0.03	7.93 ± 0.01
		96	12.29 ± 0.02	0.008 ± 0.02	1.601 ± 0.05	8.22 ± 0.01
Group III	Chromium	24	15.29 ± 0.05*	0.015 ± 0.01*	5.372 ± 0.00*	6.67 ± 0.01*
	(7.68 mg l^{-1})	48	24.25 ± 0.02*	0.016 ± 0.03*	5.376 ± 0.01*	5.36 ± 0.01*
		72	27.76 ± 0.01*	0.021 ± 0.02*	8.386 ± 0.02*	5.11 ± 0.00*
		96	31.36 ± 0.01*	0.027 ± 0.00*	9.298 ± 0.01*	4.57 ± 0.01*
Group IV	Chromium (7.68 mg l^{-1}) +	24	13.93 ± 0.00*	0.013 ± 0.01*	5.271 ± 0.00*	6.85 ± 0.01*
	R. serpentina (1 mg l^{-1})	48	20.12 ± 0.03*	0.014 ± 0.00*	5.101 ± 0.02*	5.95 ± 0.01*
		72	24.81 ± 0.02*	0.019 ± 0.02*	7.277 ± 0.03*	5.87 ± 0.01*
		96	26.62 ± 0.00*	0.024 ± 0.01*	8.176 ± 0.05*	4.57 ± 0.02*
Group V	Chromium (7.68 mg l^{-1}) +	24	13.86 ± 0.00*	0.013 ± 0.03*	4.382 ± 0.01*	6.98 ± 0.00*
	R. serpentina (2 mg l^{-1})	48	19.25 ± 0.04*	0.013 ± 0.02*	4.198 ± 0.03*	5.74 ± 0.01*
		72	23.27 ± 0.01*	0.017 ± 0.01*	7.145 ± 0.02*	5.98 ± 0.00*
		96	25.26 ± 0.00*	0.022 ± 0.05*	7.287 ± 0.05*	5.24 ± 0.01*
Group VI	Chromium (7.68 mg l^{-1}) +	24	13.35 ± 0.01*	0.012 ± 0.00*	4.110 ± 0.02*	7.15 ± 0.01*
	R. serpentina (3 mg l^{-1})	48	18.61 ± 0.00*	0.013 ± 0.01*	4.121 ± 0.01*	6.16 ± 0.00*
		72	23.38 ± 0.05*	0.016 ± 0.02*	6.322 ± 0.02*	6.52 ± 0.00*
		96	24.11 ± 0.02*	0.020 ± 0.01*	7.397 ± 0.04*	5.78 ± 0.01*

Values are mean of three replicates ±S.E.

The present findings explored that acute exposure to Cr (VI) significantly increases the activities of liver biomarkers in fish. These observations corroborate with the findings of Vutukuru (2005) who observed similar increasing trend in the activities of SGOT and SGPT in liver of *Labeo rohita* exposed to chromium for 24 and 96 hr. Singh *et al.* (2016) also reported an increase in the activities of SGOT and SGPT and subsequent liver damage in *Heterpneustes fossilis* after famfos intoxication. Balasubramanian and Kumar (2013) also documented almost similar findings, *i.e.*, significant elevation in the activities of SGPT and ALP after exposure of fish *Heteropneustes fossilis* to two different concentrations of sodium arsenite.

Besides biochemical impairments, exposure to Cr (VI) has also been found to impart cyto-genetic damage as evident by a significant (p<0.05) induction in chromosomal aberrations and micronuclei frequency in fish of Group III. Elevated frequencies of micronuclei reflect DNA double strand breaks (Xu *et al.,* 2011). Fish being a significant resource in providing nutritional security to growing population has gained ample attention by fishery biologists for their conservation and productivity. Chemical protection measures against water borne toxicants are not always environmentally sound. Hence, researches are now being carried out to explore the remedial potential of promising plant metabolites against aquatic xenobiotics (Kumar and Trivedi, 2015; Dwivedi *et al.,* 2017; Tiwari *et al.,* 2017; Hamed *et al.,* 2019; Trivedi *et al.,* 2020). Sarpgandha is considered as a promising herb that provides protection against environmental stresses, not only in human beings but also in other animal groups, including fishes. Azmi and Qureshi (2016) and Lobay (2015) emphasized ameliorative potential of *R. serpentina* against environmental toxicants in non-piscean species as well.

The present study also illustrates alleviated SGOT, SGPT and ALP activities when fishes were subjected to simultaneous treatment of Cr(VI) and *R. serpentina* root extract. Further, activities of aforesaid enzymes registered a significant (p<0.05) and gradual declining trend in comparison to Group III, with respect to control. This envisages the efficacy of *R. serpentina* against chromium induced alterations in biochemical activities of liver. The results are in confirmation with the previous studies of Singh and Kaneez (2019) who documented that garlic extract act as a strong antioxidant and when given as supplement significantly reduces elevated activities of SGOT, SGPT and ALP in *Heteropneustes fossilis* exposed to cypermethrin. Similarly, *R. serpentina* restored liver function by normalizing the total protein level and reducing the increased levels of SGOT, SGPT and ALP in alloxan induced diabetic mice (Azmi and Qureshi, 2012). Heavy metals are genotoxic and induce cyto-genetic damage in fishes (Yadav and Trivedi, 2006, 2009b). A significant (p< 0.05)

Table 4: The attenuation potential of *Rauwolfia serpentina* in terms of reduction percentage of micronuclei, chromosomal aberrations and liver marker enzymes in fishes of different groups co-exposed with Cr (VI) (7.68 mg l^{-1}).

Group	Parameters																			
	Reduction % in MN				Reduction % in CA				Reduction% in SGOT				Reduction % in SGPT				Reduction % in ALP			
	24hr	48hr	72hr	96hr	24hr	48hr	72hr	96hr	24hr	48hr	72hr	96hr	24hr	48hr	72hr	96hr	24hr	48hr	72h	96hr
Group IV Chromium (7.68 mg l^{-1}) + *R. serpentina* extract (1 mg l^{-1})	22.0	7.4	8.2	8.1	19.4	9.5	18.4	21.9	43.0	34.0	18.9	24.6	40.0	16.7	16.7	17.6	2.7	7.4	16.1	14.6
Group V Chromium (7.68 mg l^{-1}) + *R. serpentina* extract (2 mg l^{-1})	44.4	14.9	15.9	16.4	30.6	19.0	30.6	31.3	45.2	41.3	28.8	31.7	40.0	33.3	33.3	29.4	26.4	31.5	18.0	26.2
Group VI Chromium (7.68 mg l^{-1}) + *R. serpentina* extract (3 mg l^{-1})	55.5	22.1	23.9	24.3	41.7	28.6	49.0	46.9	61.3	46.5	28.0	37.7	60.0	33.3	41.7	41.2	33.7	33.6	29.9	24.8

increase in the frequency of chromosomal aberrations in Group III (7.68 mg l^{-1} Cr (VI)) with an increased mean value in comparison to Group I was also recorded in the present study. However, a significant ($p<0.05$) declining trend in the frequency of chromosomal aberrations was observed when fishes were co-exposed to 96 h-LC_{50}/10 of Cr (VI) along with increasing concentrations of ethanolic root extract of *Rauwolfia serpentina* in comparison to Group III, with respect to control. Similarly, Prasad and Trivedi (2018) observed alleviation in frequency of chromosomal aberrations in fish after simultaneous exposure to curcumin and chromium trioxide. Kumar and Trivedi (2015) reported the efficacy potential of *Lawsonia inermis* leaf extract in reducing the increased frequency of chromosomal aberrations in *C. punctatus* after exposure to copper sulphate.

Decrease in protein content on exposure to Cr (VI) might be due to tissue destruction, necrosis and disturbance of cellular fraction and consequently impairment in protein synthesis machinery, most probable cause of it is oxidative stress due to accumulation of heavy metals (Ratn *et. al.*, 2018). Oxidative stress in liver tissue due to over production of free radicals disturb the physiology of liver resulting in elevation of SGOT, SGPT and ALP activities. Reduced liver enzyme activities in Group IV, V and VI treated with *R. serpentina* extract may be due to antioxidant property of Sarpgandha, which reduces ROS generation resulting in reduced oxidative stress. This brings the elevated level of liver marker enzymes gradually downward. Interestingly, a significant ($p < 0.05$) reduction in MN frequency in erythrocytes of fish of Groups IV, V and VI, in a concentration-dependent manner also indicates ameliorative potential of 'Sarpgandha' against DNA damage. Similarly, Awasthi *et al.* (2019) also observed a significant decrease in micronuclei frequency in *Channa punctatus* on simultaneous treatment with curcumin and Cr(VI), in a dose dependent manner. Additionally, present findings are in harmony with the study of Tiwari and Trivedi, (2019) who have documented a gradual decrease in frequency of MN after a combined exposure of carbofuran and *R. serpentina* root extract in fish *Channa punctatus*. Thus, it can be inferred that both curcumin and metabolites from root extract of *Rauwolfia serpentina* have the ability for partial restoration of mitochondrial impairments and therefore, havereduce intracellular ROS production in tissues of fish. This has resulted in a progressive reduction in genetic damage in fish after their co-exposurewith a toxicant.

The present findings are in accordance with Trivedi *et al.* (2020) who documented that micronuclei frequency significantly ($p<0.05$) decreased when fish were co-exposed to *Withania somnifera* and chromium. Laboratory microcosm-based studies involving biological screening of raw extracts of promising medicinal plants in alleviating ill effects of noxious xenobiotics in fishes have multidimensional scope. After standardization, they can be incorporated in dietary formulations of fish to achieve better protection, conservation and productivity. Cost effectiveness and easy availability of raw material coupled with least involvement of technological in-puts in preparation of desired plant extracts are other added advantages.

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Add-on Information

Authors' contribution: S. P. Trivedi: Experimental designing and editing of the manuscript; **V. Kumar:** Execution of the experiment and initial drafting of the manuscript; **S. Singh:** Data storage, retrieval and analysis; **M. Kumar:** Observations and their recording.

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