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Toxicity studies on black molly, *Poecilia sphenops* against chromium trioxide

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Abstract
Alteration of biochemical profiles of muscle tissues in chromium trioxide in the ornamental fish Black molly was investigated after exposure to five acute concentrations of chromium trioxide (80, 100, 110,120, 140 mg/l with control and 110.79 ppm of the 96hrs LC_{50}) for 4 days and sub lethal level exposures were maintained as 5, 10, 20, 40, and 60 mg/l (5^{th}, 10th, 15th, 20th, 25th and 30^{th} respectively) with control for 30 days. The results showed that the total biochemical content decreased with increased duration of exposure as well as increase in concentrations of chromium trioxide for four days in acute study period. All the sub acute concentration exposures gradually increase the biochemical contents throughout study period. The consequences of decrease in the fluctuating biochemical levels and compared with control. The gradual decreases were recorded in the biochemical content for the chromium trioxide effects and also highly significant in the entire sub acute levels. It concludes, the metal induced alterations may probably affect the biochemical profile of the fish.

Keywords: Chromium trioxide, sub lethal, biochemical changes, *Poecilia sphenops*

1. Introduction
All living organisms require several essential trace amounts of metals. During biological evolution of prokaryotes and eukaryotes, several metals become incorporated as essential factors in many biochemical functions more or less in accordance with the abundance of these metals on the planet [1].

The toxic pollutant changes water quality and feeding, swimming behaviour of fish and also delays the hatching, the maturation period. Fish may absorb metal directly from contaminated water or indirectly from feeding on living organisms in the contaminated water [2]. Generally, there are four possible routes for metals to enter a fish: food, gills, drinking water, and skin adsorption and the most important one are uptake through gills that have a prominent role in ion uptake and homeostasis [3]. Fishes are moderately sensitive to changes in their surrounding environment. Therefore, fish health may reflect a good indication of the status of the specific aquatic ecosystem [4].

The levels of pollutants such as heavy metals in aquatic environment increased due to an increase in population, widespread use of chemical fertilizers and pesticides and discharge of domestic waste water without refining which in turn becomes a global problem threatening all tropical levels. Metals are unique among pollutants, which cause adverse health effects, in that they occur naturally and in many instances are ambiguities in the environment. Metals are usually dispersed at sub-lethal concentrations around the world. Pollution of rivers, lakes, and coastal and marine waters by metals is frequently reported, and it has resulted in their accumulation in the tissues of aquatic organisms. One of the most serious results of their persistence is biological amplification through the food chain [5-7].

This ultimately affects the biomolecules, growth and reproductive ability of the organisms. They are reducing and their ability to compete for food and habitat. But higher concentrations of heavy metals can cause harmful effects on metabolic, physiological and biochemical systems of fishes [8].

The chromium accumulates mainly in metabolically active organs such as liver, gill and kidney at high concentrations and fish go under some behavioral alterations such as suspending feeding, irregular swimming and accelerated operculum movement when first encountered with chromium. It also caused structural changes such as hypertrophy and by paraplegia at gill epithelium, degeneration in fin rays and weakening of the immune system [9-12].

In the same way at molecular level it can damage DNA in several ways, including DNA Single and Double Strand Breaks (SDSBs) generating chromosomal aberrations, micronucleus formation, sister chromatid exchanges, and alteration in DNA replication and transcription [13-15].

The freshwater fish, *Colisa fasciatus* caught from lake and biochemical parameters was studied in reproductive cycle in comparison to control fish against Zinc sulphate toxicity. Fish liver was analyzed for various biochemical parameters like total protein, total glycogen, nucleic acids (DNA and RNA). Zinc sulphate was lethal to 0, 50 and 100% of test fish which produce absolute mortality for different time intervals [16].

In the present study, investigates the biochemical changes of total free sugar, total protein, DNA, RNA and total lipid in the case of affected by chromium trioxide on *Poecilia sphenops*.

2. Materials and methods

2.1 Collection of animals

The experimental animal, *Poecilia sphenops* (400 ± 0.35mg) were collected from an aquarium at Kadachanenthal, Madurai.

2.2 Water Sample Analysis

Water sample was analyzed from Enviro Care (India P Ltd) at Madurai.

2.3 Maintenance of the experimental animals

Collected fishes were brought to the laboratory and maintained in well-aerated freshwater in a plastic trough of 10 liter capacity. The water parameter such as dissolved oxygen (7.6 mg/l), alkalinity (448 mg/l), pH (7.7) and temperature (27±1ºC) were recorded and monitored at optimal levels. They were fed with readymade fish food. The animals were acclimatized in laboratory condition before starting the toxicological experiments.

2.4 LC_{50} determination

The total number of live organisms after every 24hrs was counted up to 96 hrs. The data obtained were subjected to profit analysis to calculate $LC_{50.}$

2.5 Morphological observations after metal exposure

Morphological applications such as survival, mortality, LC_{50} determination etc., were carried out after heavy metal application.

2.6 Experimental setup

The lab-acclimatized fishes were divided into three main groups and each divided into subgroups. The first subgroups in each group were used as control. The rest of the animals in each group were exposed to heavy metal chromium trioxide (Cr). Based on LC_{50} results the acute toxicity concentrations of chromium were maintained as 80, 100, 110,120, 140 mg/l and sub-acute toxicity levels were 5, 10, 20, 40, 60 mg/l.

2.7 Biochemical estimations of tissue samples

Biochemical components such as total protein, total free sugar, total lipid, Ribonucleic acid, Deoxyribonucleic acid were estimated using standard procedures. This following method was used by Total protein content was estimated according to Lowry *et al*. [17], Roe in 1955, devised the procedure for the estimation of total free sugar, RNA was estimated by Searcy and Maclnnis method [18], Deoxy points were converted into a highly reactive hydroxyl aldehyde, which reacts with diphenylamine to give a blue colored complex and estimation of total lipid was done by Folch method [19].

2.8 Statistical analysis

The data obtained by exposing the Fish *P. Sphenops* the different doses of metals were subjected to statistical analyses such as standard deviation, probit analysis by SPSS (statistical package for social sciences) and one way analysis of variance and their significance expressed at $P<0.01$ and 0.05 levels.

3. Results

3.1 Morphological changes in *P. sphenops* after exposure to Chromium trioxide

A change in survival after exposure to heavy metal was studied during acute and sub acute toxicity levels. The results obtained were compared with control and recorded.

Physico-chemical parameters of fresh water quality including pH values, temperature and the amount of dissolved oxygen of the test aquariums were determined as 7.06 ± 1.24, 27.0 ± 1.02°C and 7.60 ± 1.46 mg l^{-1} respectively during the experiment. Higher metal concentrations caused a significant decrease in pH values, however, in all cases; pH values did not reach the acidic range, which could affect the organism's survival.

The concentrations of Cr measured in the aquariums throughout the experiment were ± 10% of the nominal concentrations. Therefore, nominal concentrations from now on will take this value as the basis. The general agreement of Cr6+ with nominal concentrations confirmed that virtually all of the Cr remained in the Cr6+ form throughout the experiment [20].

3.2 Influence on mortality and LC_{50} determination

P. Sphenops after exposure to different concentration of metals were observed for mortality up to 96 hours and percentage mortality was calculated and recorded (Table 1).

3.3 Chromium trioxide

About 100% survival was observed at the concentration of 0.060gm/l of chromium trioxide in *P. sphenops* while they got reduced to 50% in 0.110gm/l (110.79ppm) were recorded (Table- 2 and figure-1).

Table 1: LC_{50} determination for Chromium trioxide influenced toxicity in *P. sphenops* (day Concentration / probit regression analysis)

Exposure Period hours	LC_{50} ppm	Regression equation (y=a+bx)	Correlation (r)	95% fiducial limits		Confidance Limit (fLC_{50})	Chi-square Test	
				Lower (LFL)	Upper (UFL)		X^2	Critical value
24	154.571	y= -111+21.3x	0.972	127.004	143.20	1.217	27.425	16.266
48	147.544	y= - 109.2+20.7x	0.985	139.958	15.200	1.054	14.590	13.277
72	121.609	y= - 81.66+19x	0.968	116.647	126.873	1.042	15.540	13.131
96	110.79	y = - 47.61+15.57x	0.959	105.576	115.901	1.049	12.396	10.823

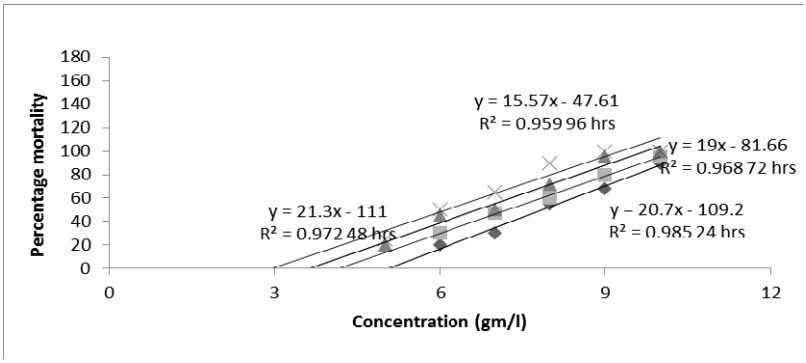

Fig 1: Response curve for obtaining LC_{50} of Chromium trioxide to *P. sphenops*

3.4 Fluctuations in the biochemical components of *P. sphenops* due to metal

Changes in the organic constituents such as total protein, total free sugar, RNA, DNA and total lipid were quantified in Black Molly after exposure to the chromium trioxide. Quantifications were carried out in Black Molly tissue after every 24 hrs up to 96 hrs for acute toxicity. After 10th day sub-acute toxicity was studied.

3.5 Total biochemical changes in the tissue of *P. Sphenops* after exposure to metal

There was a gradual decrease in the total biochemical content of fish tissue. Total protein content decreased with increased duration as well as increase in concentration of chromium trioxide.

The highest concentration of chromium trioxide exposure led to highest decreased in the total protein content, which was 0.242µg/ml (0.517µg/ml for control). After 96 hours and gradually decreased in total free sugar content, this was 0.154µg/ml (0.298µg/ml for control). After 96 hours, similarly DNA and RNA content were quantified to be 0.176 µg/ml (DNA) and 0.250µg/ml for RNA, less against 0.276 µg/ml (DNA) and 0.350 µg/ml (RNA) in control. The chromium exposed fish tissue in lipid content showed the decrease up to 0.326 µg/ml on 96 hours as against 0.600µg/ml in control (Table-2 and; Fig-2). The one-way ANOVA showed the decreased to be statistically significant at $p<0.05$ level.

Table 2: Total Biochemical fluctuations of chromium trioxide exposed *P. sphenops* at acute toxicity (24 ~ 96 hrs)

Chromium trioxide (ppm)	Biochemical composition (µg/ml)				
	Protein	Free sugar	Lipid	DNA	RNA
Control	0.517 ± 0.64	0.298 ± 0.99	0.600 ± 0.22	0.276 ± 0.14	0.350 ± 0.42
80	0.385 ± 0.43	0.259 ± 0.36	0.466 ± 0.13	0.210 ± 0.65	0.326 ±0.53
100	0.379 ± 0.67	0.236 ± 0.08	0.418 ± 0.27	0.203 ± 0.84	0.320 ±0.03
110	0.328 ± 0.32	0.212 ± 0.63	0.400 ± 0.16	0.197 ±0.46	0.290 ±0.35
120	0.297 ± 0.95	0.194 ± 0.87	0.372 ± 0.96	0.183 ± 0.29	0.267 ±0.98
140	0.242 ± 0.76	0.154 ± 0.24	0.326 ± 0.24	0.176 ± 0.56	0.250 ±0.50

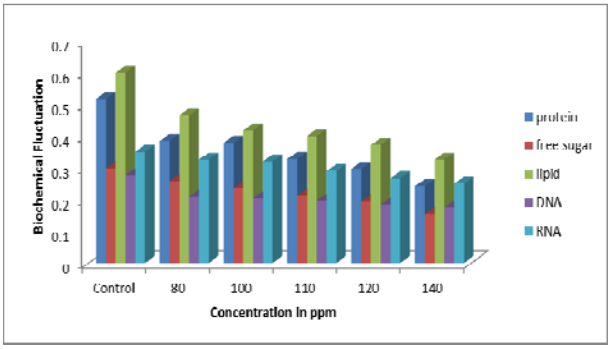

Fig 2: Total biochemical fluctuations of chromium trioxide exposed *P. sphenops* at acute toxicity (24 ~ 96 hrs)

Sub-acute concentrations of chromium trioxide used between 0.005 gm/l – 0.060 gm/l in *P. sphenops*. Total biochemical content quantified in the tissue extract of the black molly of the exposing, therefore the 5th day in sub acute toxicity concentrations displayed interesting results.

Biochemical content starts to increase slightly for all the sub acute concentration exposures gradually from 1 ~ 5 days and 5 ~10 days. For sub acute toxicity effects upto 5th days the total protein content got reduced from 0.663 µg/ml in the control to 0.648 µg/ml. For lowest concentrations of chromium trioxide used (0.005gm/l to 0.400 gm/l of highest concentrations 0.060gm/l) and the results showed total free sugar content decreased up to 0.392 mg/l (0.005gm/l) on the 5th day as against 0.401 µg/ml in control (Table-3 and Fig-3).

Table 3: Total biochemical fluctuations of chromium trioxide exposed *P. sphenops* at sub acute toxicity (1~5 days)

Chromium trioxide (ppm)	Biochemical composition (µg/ml)				
	Protein	Free sugar	Lipid	DNA	RNA
Control	0.663 ± 0.37	0.401 ± 0.61	0.806 ± 0.82	0.370 ± 0.09	0.450 ± 0.79
5	0.648 ± 0.89	0.392 ± 0.70	0.768 ± 0.28	0.313 ± 0.18	0.423 ± 0.68
10	0.591 ± 0.40	0.365 ± 0.66	0.670 ± 0.45	0.279 ± 0.33	0.410 ± 0.34
20	0.551 ± 0.51	0.352 ± 0.83	0.632 ± 0.17	0.250 ± 0.92	0.365 ± 0.23
40	0.466 ± 0.20	0.294 ± 0.25	0.578 ± 0.02	0.246 ± 0.55	0.350 ± 0.72
60	0.400 ± 0.94	0.229 ± 0.75	0.532 ± 0.91	0.188 ± 0.97	0.280 ± 0.77

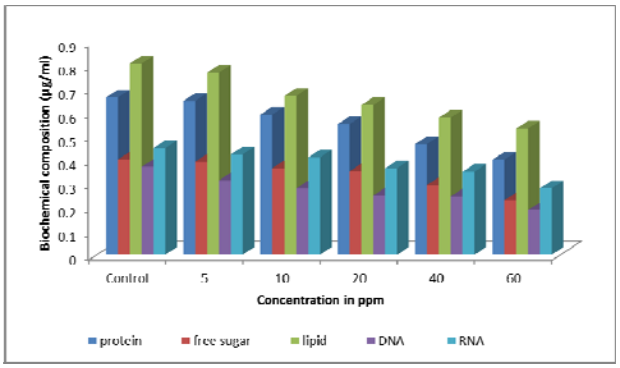

Fig 3: Total biochemical fluctuations of chromium trioxide exposed *P. sphenops* at sub acute toxicity (1~5 days)

Here the minimum concentration exposure showed 0.313 µg/ml (0.005gm/l) of DNA and maximum concentrations showed 0.188 µg/ml (0.060g/l) at the end of 5th day exposure and as against 0.370 µg/ml in control.

The results showed the decrease up to 0.423 µg/ml for RNA (0.005gm/l of chromium trioxide) and as against 0.450 µg/ml in control. Total lipid content 0.768 µg/ml for 0.005 gm/l of chromium trioxide exposure to black molly was recorded. The differences were statistically signified (p<0.05).

The extent of sub acute toxicity studies for the total protein content was reduced from 0.829µg/ml to 0.531 µg/ml on the 10th days (control- 0.932µg/ml). Similarly the total free sugar contents were decreased up to 0.238mg/l (0.060gm/l) at the end of days (Table -4 and Fig-4).

After exposure of chromium trioxide to the black molly on the 10th day and gradually decreased DNA and RNA content that was 0.373µg/ml - 0.210µg/ml and 0.485µg/ml – 0.303µg/ml and compared to the control (for 0.407µg/ml of DNA and 0.510µg/ml of RNA). The total lipid content of fish tissue was decreased up to 0.606µg/ml (0.060gm/l). The differences were statistically signified (Table-4 and Fig-4).

Table 4: Total biochemical fluctuations of chromium trioxide exposed *P. sphenops* at sub acute toxicity (5~10 days)

Chromium trioxide (ppm)	Biochemical composition (µg/ml)				
	Protein	Free sugar	Lipid	DNA	RNA
Control	0.932 ± 0.57	0.378 ± 0.85	0.912 ± 0.86	0.407 ± 0.21	0.510 ± 0.59
5	0.829 ± 0.90	0.372 ± 0.60	0.860 ± 0.38	0.373 ± 0.52	0.485 ± 0.39
10	0.815 ± 0.15	0.305 ± 0.41	0.852 ± 0.19	0.300 ± 0.03	0.476 ± 0.48
20	0.777 ± 0.88	0.282 ± 0.73	0.754 ± 0.93	0.280 ± 0.71	0.380 ± 0.31
40	0.701 ± 0.58	0.263 ± 0.44	0.672 ± 0.78	0.226 ± 0.30	0.321 ± 0.49
60	0.531 ± 0.11	0.238 ± 0.07	0.606 ± 0.47	0.210 ± 0.81	0.303 ± 0.80

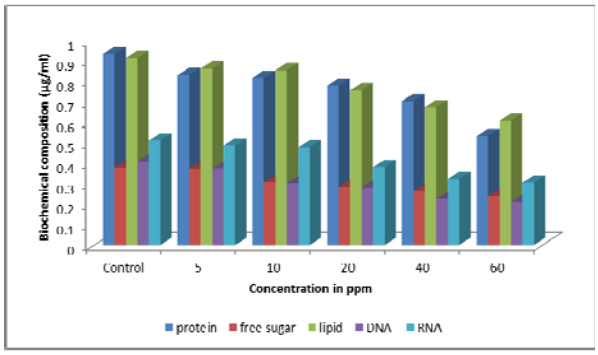

Fig 4: Total biochemical fluctuations of chromium trioxide exposed *P. sphenops* at sub acute toxicity (5~10 days)

4. Discussion

Biochemical methods of diagnosis constitute a promising approach to the problems of detecting the effect of toxic chemicals at possible stages. According to the use of biochemical methods constitute the area viz., detection of stages of stress, suggestion of modes of action and tentatively as tools to explain the metabolic basis for conventional fishery statistics like growth. Since pollution may induce certain biochemical changes in fishes before the drastic cellular and systematic dysfunction manifest, appropriate biochemical parameters could be used effectively as sensitive indicators [2].

The effects of heavy metals depend on the chemical form of the metal, its concentration and exposure period, interaction with other metals, the species of concern and to its developmental stage and also to the chemical and physical properties of water.

There was no mortality was recorded in *Channa punctatus* exposed to chromium over 30 days of exposure which caused 100% mortality after exposure to the same concentration over 120 days [21]. The above findings indicated that heavy metal contamination usually causes depletion in food utilization in fish and such disturbances may result in reduced fish metabolic rate and hence causing reduction in their growth [4, 5, 7]. Similar types of results were found in the present investigation of *P. Sphenops* exposed to 0.080 and 0.100gm/l chromium trioxide over 96 hrs.

Toxic materials, probably due to their effects on gill tissue, causes some behavioral alterations such as moving toward the surface, increase in operculum movement, disorders in swimming coordination and rejection to take of feed. These discrepancies in behavior were observed in *Cyprinus Carpio* exposed to cadmium [22], *Anguilla Anguilla* exposed to lead [23], and *P. Sphenops* exposed to chromium trioxide in the present study which returned to normal with the 1-10 days (sub acute levels) of exposure period.

The serum glucose and cholesterol concentrations reduced with higher dietary chromium supplementation in rainbow trout [24]. However, biochemical changes were observed up to 96hrs for chromium trioxide exposure, the present study of total protein, total free sugar, DNA, RNA, and total lipid concentration levels got reduced among groups and compared to control.

Chromium induced hypoxia probably might have resulted in a shift to anaerobic glycolytic pathway by increased glycogenolysis. Depleted glycogen levels following chromium stress reported in *Cyprinus Carpio* communis [8] under hypoxic conditions also support this view. A consistent decrease in tissue biochemical levels reserve observed in this study suggests an impaired system. Further, the decline in molecular might be partly due to its utilization in the formation of glycoprotein's, glycolipids and amino acids,

which are essential constituents of various cells and other membranes.

Total protein level in serum of common carp exposed to sublethal concentrations of chromium toxicity was found to be decreased moderately in experiment fish than the control. Decreased in tissue lipid and proteins was also observed in *Labeo rohita* exposed to Aluminium. Hence, the toxic effects may result from the bioconcentration of metals and their consequent binding with biologically active constituents of the body such as lipids, amino acids, enzymes and proteins [25, 27].

The decrease in tissue lipid and proteins might be partly due to their utilization in cell repair and tissue organization with the formation of lipoproteins which are important cellular constituents of cell membranes and cell organelles present in cytoplasm [26].

The decrease in the sugar concentration of the tissues of *P. Sphenops* may be due to its enhanced utilization since glucose forms the immediate source of energy to meet energy demands under metallic stress. The previous finding revealed that decreased in glycogen concentration might be due to the prevalence of hypoxic or anoxic conditions, which normally enhances glycogen utilization [26]. Enhanced utilization of glycogen and its consequent depletion in tissues may be attributed to hypoxia since it increases carbohydrate consumption. Under hypoxic conditions, the animal derives its energy from the anaerobic breakdown of glucose, which is available to the cells by the increased glycogenolysis [16, 22, 23].

However, if trivalent chromium has access to the intracellular medium through processes such as pinocytosis and endocytosis or by the reduction of hexavalent chromium inside the cell it acts directly on DNA and causes more damage than when it continues in the chromate form [15]. In addition, that the chromium binds macromolecules, form adducts with thiol groups on proteins, creates DNA adducts and causes DNA strand breakage [13].

In the present investigation, the results shows that there was gradually increased in different biochemical constituents such as total protein, total free sugar, DNA, RNA and total lipid in exposure to sub acute concentration of chromium for 1~10 days. This can be explained by the prevention of metal to cause its toxic effects by excretion, storage and detoxification mechanisms of this species. The results of the one way ANOVA revealed that the decrease in the fluctuation biochemical level of control is significant (p<0.05) in tissues at the end of 5th and 10th day.

5. Conclusion

In the present investigation, the bioassay study shows that Chromium is highly toxic to fish and the toxicity is inversely proportional to exposure time and it also depicts that the mortality percentage is dose dependent. This decrease in the biochemical based on the concentration of chromium trioxide and highly significant in the entire sub acute levels were also recorded. It concludes that the metal induced alterations may probably affect the enzyme mediated bio-defense mechanisms of the fish. Further studies are required to elucidate the impact of chromium on detoxifying enzymes for assessing the fish health and reproduction.

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