

Impact of Chromium Oxide Nanoparticles on Mrigal *Cirrhinus Mrigala*

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Abstract: Chromium is a toxic element in aquatic environments. This study evaluates the impact of chromium oxide nanoparticles on the biochemical and haematological parameters of *Cirrhinus mrigala* (Mrigal). Chromium oxide nanoparticles were synthesized using the co-precipitation method and characterized via UV-visible spectroscopy, SEM, EDAX, and FTIR. Toxicity tests were conducted over 96 hours at varying concentrations of chromium oxide nanoparticles. In survival studies, sub-acute toxicity was assessed over a 14-day period by monitoring mortality rates. Biochemical parameters, including protein, carbohydrate, and lipid levels in the gill, muscle, and liver, were analyzed. Haematological parameters such as RBC, WBC, haemoglobin, hematocrit, platelet count, MCV, MCH, and MCHC were also measured. UV-visible absorption spectra confirmed the presence of chromium oxide nanoparticles within the 200–800 nm range, exhibiting a strong absorption band at 366 nm. SEM imaging at 2 μm revealed a feather-like morphology. The EDAX spectrum displayed two peaks between 0KeV and 10KeV, with a chromium peak at 5.4 KeV and an oxygen peak at 0.5 KeV. FTIR analysis in the 4000–500 cm^{-1} range identified functional groups such as alcohols, alkynes, anhydrides, and sulfonates. Results indicate a significant decrease in total protein content in the muscle, gill, and liver of *C. mrigala* exposed to chromium oxide nanoparticles. Additionally, all haematological parameters declined progressively with increasing nanoparticle concentration..

Keywords: Impact; Chromium Oxide Nanoparticles; Biochemical; Haematology; Mrigal.

1.Introduction

The metal oxide nanoparticles represent a wide class of materials that have been studied largely due to their fascinating catalytic, optical, magnetic, electronic, and medical properties. Among these metal oxide nanoparticles, chromium oxide has great attention, because the exposure of a significant amount of chromium to a wide array of fishes will involve a crucial and harmful impact are histopathology, DNA damage, behavioural changes such as swimming, cell disruption, metabolism, physiological changes etc [1]. Metallic nanoparticles such as iron, iron oxide, selenium, zinc, copper, silver, chromium and magnesium oxide are used in aquaculture [2]. *Cirrhinus mrigala* is a very fast-growing big-sized carp commonly known as Mrigal. Freshwater rivers and reservoirs are the natural habitats of Mrigal. Riverine fisheries are important as it provide nutritional food and employment for millions of people around the world. It is an excellent species for pond culture in India, Burma, Bangladesh, Nepal and Pakistan. Mrigal is the backbone of cultural fishery practices in India [3]. Mrigal *Cirrhinus mrigala* is one of the most abundant cultivated Indian major carp. The achievement of fish culture builds upon the expense of feeds, the toxicity of water etc. *Cirrhinus mrigala* was utilized as the model organism in the study because its wide distribution represents the major contribution to the total mass of the Indian subcontinent and its fishery is strongly dependent on the commercial and recreational demand [4]. In the field of toxicology studies, how chemicals, substances and situations may adversely affect people, animals and the environment. Typically, aquaculture systems are toxic due to the presence of high levels of metabolites such as carbon dioxide, ammonia nitrite, hydrogen sulphide, algal toxins, heavy metals, and agricultural and industrial chemicals [5]. A toxicity test involves the estimation of acute toxicity or subacute toxicity which proved to be lethal causing death to 50% of the tested organisms. The types of toxicity tests which are routinely performed by pharmaceutical manufacturers in the investigation of a new drug involve acute, sub-acute and chronic toxicity. The Probit analysis is commonly used in toxicology to determine the relative toxicity of chemicals to living organisms. The response is always binomial and the relationship between the response and the various concentrations of Nanoparticles toxicity of fish may affect the gill surface because the gill is the primary organ that is directly in contact with aquatic organisms. The discharge of wastewater containing heavy metal nanomaterials affects the aquatic environment [6]. The biochemical characterization of organisms involves the determination of chemical properties such as the

quantity of carbohydrates, lipids, and proteins in tissues, gills, muscles, liver etc. Biochemical parameters could be used as important and sensitive biomarkers in ecotoxicological studies concerning the effects of metal contamination and fish health [7]. The routine haematological analyses include the evaluation of blood cell counts and other cell-related parameters as well as measurements of biochemical indices concentrations or activities of plasma compounds. Red blood parameters like erythrocyte count, and haemoglobin concentration, White blood parameters such as leukocyte count, lymphocytes, neutrophils or heterophils, monocytes, eosinophils, and basophils. Thrombocyte count and blood cell morphology are rarely evaluated. Hematological and biochemical indices provide extensive information about fish oxygen transport capacity, immune potential, level of stress, disease, intoxication, nutritional status etc [8]. The work related to the impact of chromium oxide nanoparticles on biochemical and haematological parameters in Mrigal *Cirrhinus mrigala* is waiting. Hence the present study was carried out.

2. Materials and Methods

2.1. Materials

Synthesis of Chromium Oxide Nanoparticles

0.2 M of chromium chloride was mixed in 100 ml distilled water and kept in a magnetic stirrer for 30 minutes to get a homogenous solution. Liquor ammonia was added dropwise with a constant stirring until the pH of the solution reached 12 and the temperature 50°C. The prepared sample was washed several times (3-5) using distilled water with the help of a centrifuge machine. The obtained precipitate was then dried in a hot air oven at 90 °C for 12 hours and was calcinated at 550°C in a Muffle furnace for about 5 hours. The prepared sample was grained by mortar and pestle. Mrigal *Cirrhinus mrigala* fingerlings (2±0.05 g) were collected from K. V. K Fish farm Vannanduarai, Palani, Tamil Nadu, India, and transported to the laboratory in polythene bags filled with water and oxygen was introduced from an oxygen cylinder. Fish were acclimatized for 15 days, and fed with fish feed in the form of pellets.

2.2. Methods

Characterization of Chromium Oxide Nanoparticles

(a) UV-visible spectroscopy

UV-Vis spectroscopy (UV-Vis) measures the intensity of light reflected from a sample and compares it to the intensity of light reflected from a reference material. NPs have optical properties that are sensitive to size, shape, concentration, agglomeration state and refractive index near the NP surface, which makes UV-Vis spectroscopy an important tool to identify, characterize and investigate these materials, and evaluate the stability of NP colloidal solutions. The absorbance at 200–800 nm wavelength can also be used to measure the chromium oxide nanoparticles (Thermoscientific Genesys 180 UV-Visible spectrophotometer).

(b) Scanning Electron Microscopy

Scanning electron microscopy (SEM) is one of the most versatile and well-known analytical techniques. Compared to conventional optical microscopes, an electron microscope offers advantages including high magnification, large depth of focus, and great resolution. SEM provides different information about the NPs such as size, shape, aggregation, and dispersion of nanoparticles. (VEGA3, TESCAN(Czech Republic)Model SEM).

(c) Energy – Dispersive X-Ray Spectroscopy

The EDAX technique is usually used for the confirmation of the presence of the element in nanoparticles. Each element produces a characteristic X-ray emission pattern due to its unique atomic structure and therefore can be used to perform compositional analysis.(BRUKER Nano,GmbH,D-12489)

(d) Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) is a technique based on the measurement of the absorption of electromagnetic radiation with wavelengths within the mid-infrared region (4000–400 cm^{-1}). If a molecule absorbs IR radiation, the dipole moment is somehow modified and the molecule becomes IR active. A recorded spectrum gives the position of bands related to the strength and nature of bonds, and specific functional groups. (FT/IR -4700 Model).

Acute Toxicity Test

This test was conducted following OECD (Organization for Economic, cooperation and Development guidelines)(1992) [9]. The median lethal concentration (LC_{50}) of Cr_2O_3 nanoparticles was established in five different concentrations such T0 (control), T2(10 mg/l),T3(20mg/l), T4 (30 mg/l)and T5 (40mg/l). Each treatment has been carried out in triplicate. Seven healthy fishes were introduced into each treatment and exposed to various concentrations of Cr_2O_3 nanoparticles. Fishes were not fed during the experimental period Behavioral and mortality were measured in 24, 48,72, and 96 h. The dead fish were taken out of the water right away to prevent any water contamination. Based on fish mortality at 96 hours median lethal concentration (LC_{50}) value was determined by probit analysis using the software SPSS 23.

Sub-Acute Toxicity Test

Sub-acute toxicity test was conducted by taking 1/100 (0.24 mg),1/50 (0.49 mg), and 1/10 (2.4 mg) of LC_{50} values along with control for 14 days. The behavioural changes and mortality were observed twice per day. Blood samples were collected from the fish on the 14th day. The gill, muscle, and liver were dissected for biochemical parameters.

Biochemical Characteristics

Biochemical parameters such as total protein [10] (Lowry *et al.*, 1951), Carbohydrate (Carrol *et al.*, 1959) [11] lipid (Barnes and Blackstock, 1973) [12] content in the gill, muscle and liver and haematological parameters of Mrigal were estimated after 14 days.

Results and Discussion

The UV–Visible absorption spectroscopy is a widely used technique to examine the optical properties of nanosized particles. A range of wavelengths 200-800 nm was used to measure the absorbance spectra of chromium oxide nanoparticles. It exhibits the strong absorption band at 366nm as shown in Figure 1. Chromium oxide nanoparticles are prepared via Allium *sativum* extract. Those nanosized particles are observed in the UV region 420nm [13]. Vivek Sheel Jaswal *et al.*, (2014) [14] reported that the UV-visible spectra of the prepared chromium oxide nano powder in the absorbance mode, and in the wavelength range between 200-90 nm. The morphology and structure of the samples were investigated by scanning electron microscopy. SEM indicates that chromium oxide nanoparticles are feather-like structures (Figure 2). Shujaat Ahmad *et al.*, (2022) [15] reported that the scanning electron microscope (SEM) of the chromium oxide nanoparticles formed irregular round-shaped particles. Zakia Kanwal *et al.*, (2019) [16] reported that the scanning electron microscope appeared spherical and nearly spherical, and cluster formation. The energy dispersive x-ray spectrum recorded on the chromium oxide nanoparticles is shown as two peaks located between 0KeV to 10KeV (Figure 3). The maximum peak located on the spectrum at 5.4 KeV comes from chromium. The second peak located on the spectrum at 0.5 KeV indicates oxygen. Chromium is more abundant than oxygen. Sappani Muthu *et al.*, (2022) [17] reported the Energy Dispersive X-ray spectrum of the chromium oxide nanoparticles along the 14.4% of chromium and 55% of oxygen indicated. Cheah *et al.*, (2022) [18] reported that the EDAX spectrum shows the peak corresponding to chromium located in 56.7% and the oxygen indicated in 43.53%. Fourier transform infrared spectroscopy measurement was carried out to identify the possible chemical responsible for the reduction and capping of chromium oxide synthesized. The FTIR spectrum of chromium oxide nanoparticles were analysed at the wavenumber range of 4000 to 500 cm^{-1} (Figure 4). The spectra show bands at 3424,2914,1634,1251,1014 and 436 cm^{-1} functional groups such as alcohol, phenol, alkaline, and ketone. The peaks observed at 436 cm^{-1} indicate the presence of chromium oxide. Poonam Sangwan and Harish Kumar (2017) [19] reported the chromium oxide nanoparticles using the

FTIR spectroscopy and found the vibration mode in the range 2929, 1072, 952, 902, 550 and 617 cm^{-1} wavenumber indicates the chromium oxide bonds. Rayani Nivethitha and Carolin Jeniba Rachel (2022) [20] reported that FTIR shows absorption peaks at 756.10 cm^{-1} and 640.37 cm^{-1} due to the bending of the metal oxide single bond indicating the formation of chromium oxide.

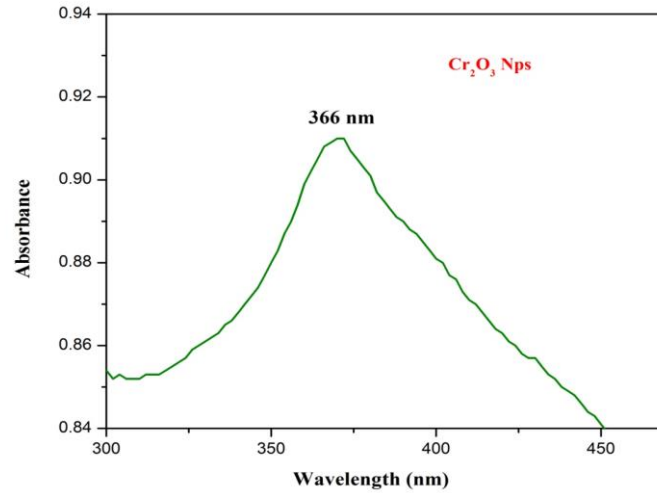


Figure 1: UV-Vis Analysis of Chromium Oxide Nanoparticles

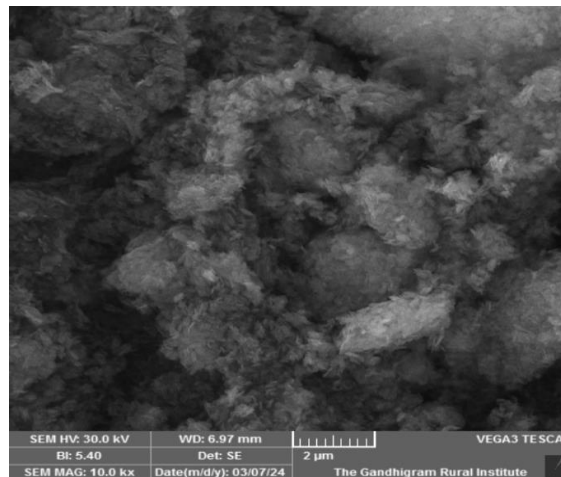


Figure 2: Scanning Electron Microscopic image of Chromium oxide nanoparticles

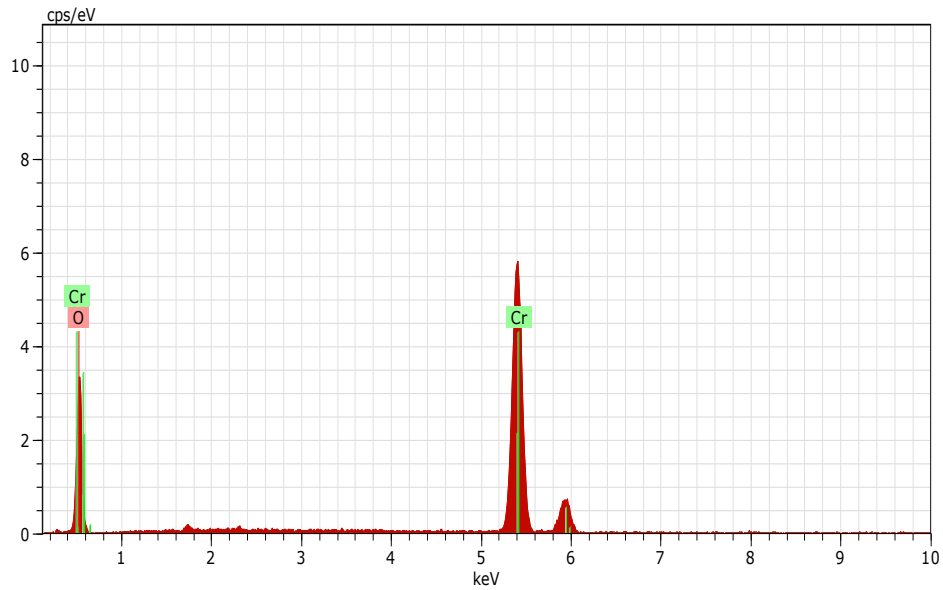


Figure 3: EDAX Analysis of Chromium oxide nanoparticles

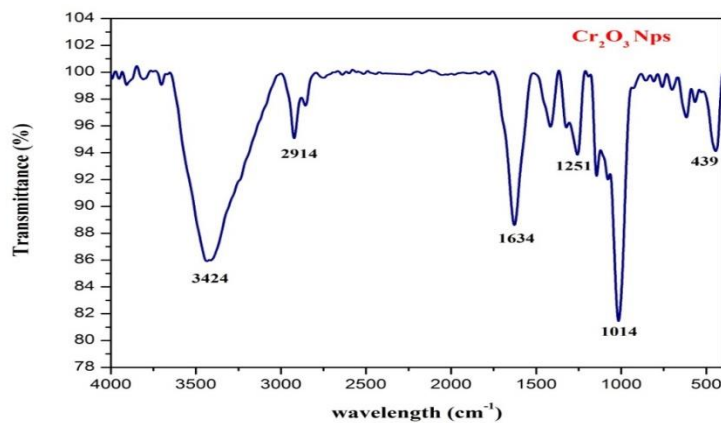


Figure 4: FT-IR Image of chromium oxide nanoparticles

The physicochemical parameters of tap water are presented in Table 1. The results of the acute toxicity test for synthesized Cr₂O₃ nanoparticles are presented in Tables 2 and 3. No mortality was observed in the control group during the experiment. Fish mortality increased significantly when the concentration and the time of exposure were increased. [Holdway \(1988\) \[21\]](#) reported the acute toxicity to fish than hexavalent chromium its mean 96 hours LC₅₀ for trivalent chromium for fishes. The sub-acute test was carried out for 14 days under static conditions. The sub-acute test was carried out for 14 days under static conditions (Water was not changed during the entire exposure period). Based upon the LC₅₀ value (24.839) the different concentrations for synthesized chromium oxide nanoparticles for the sub-acute test were set at 0.24, 0.49 and 2.4 mg respectively. [Deepak et al., \(2021\) \[22\]](#) reported that fish were exposed to Chromium (Cr) at the sub-acute toxicity level at a concentration of 40mg/L in 96 hours. During the observation period, the fish showed some behavioural changes like erratic swimming, slow motility, suffocation, and the scales becoming thin and decolourized. [Majharul Islam et al., \(2020\) \[23\]](#) reported that no mortality was observed up to 10 mg/L, but 90% and 100% mortality was observed at 50 mg/L and 60 mg/L, respectively after a 96 hours exposure period.

Table 1: Physicochemical Parameters of Tap water

S. No	PARAMETERS	VALUES
1	pH	6.9
2	Temperature	27°C
3	Dissolved Oxygen	6.1)
4	Chloride	70
5	Total Hardness	300
6	Dissolved Carbon Dioxide	Nil

* All the values are expressed in mg/l except pH and temperature.

Table 2: Estimation of Median Acute toxicity test of Chromium oxide nanoparticles on Mrigal

Probit	95% Confidence Limits for Concentration			95% Confidence Limits for log(Conc) ^a		
	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
LC1	6.977	1.908	11.259	0.844	0.281	1.052
LC2	8.096	2.531	12.505	0.908	0.403	1.097
LC3	8.898	3.026	13.373	0.949	0.481	1.126
LC4	9.552	3.460	14.071	0.980	0.539	1.148
LC5	10.121	3.858	14.669	1.005	0.586	1.166
LC6	10.631	4.231	15.202	1.027	0.626	1.182
LC7	11.099	4.586	15.688	1.045	0.661	1.196
LC 8	11.536	4.929	16.140	1.062	0.693	1.208
LC9	11.948	5.263	16.564	1.077	0.721	1.219
LC10	12.340	5.589	16.967	1.091	0.747	1.230
LC15	14.107	7.154	18.779	1.149	0.855	1.274
LC.20	15.690	8.679	20.415	1.196	0.938	1.310
LC.25	17.188	10.214	21.998	1.235	1.009	1.342
LC30	18.656	11.784	23.600	1.271	1.071	1.373
LC35	20.127	13.404	25.283	1.304	1.127	1.403
LC40	21.631	15.080	27.108	1.335	1.178	1.433
LC45	23.192	16.816	29.147	1.365	1.226	1.465
LC.50	24.839	18.608	31.489	1.395	1.270	1.498

Table 3: Probit Analysis based upon Acute toxicity

LD ₅₀	Control	1/100	1/50	1/10
Concentration	0 mg/l	0.24	0.49	2.4

The total protein content in the muscle, gill and liver of Mrigal (Table 4) is significantly decreased when reared



in different chromium oxide concentrations. Chromium oxide is the cofactor of protein enzymes and it plays a vital role in the transporting of the same to gills, muscles and liver. Total carbohydrate content in the muscle, gill and liver of Mrigal are significantly decreased when reared to different chromium oxide concentrations in the water. Total lipid content in the muscle, gill, and liver of Mrigal also decreased with increased concentration. Vyshnav *et al.*, (2023)[24] reported the biochemical characteristics such as protein, carbohydrates and lipids in Mrigal are higher in T₃, and T₂ compared to the control. Carbohydrate at T₃ and T₂ is lower than when compared to T₁ and control. Palanisamy *et al.*, (2011)[25] reported that concentration-based increases and decreases in protein, lipids and carbohydrates. The Cu, Zn, Fe, Ca, Mg, Na and K, have improved the synthesis of protein in aquatic animals and the optimum concentration of Mg can improve the synthesis of protein in shrimps and fishes.

Table 4: Total protein, carbohydrate and lipid in muscle, gill and liver of Mrigal

Concentration	Tissues	Protein (mg/g)	Carbohydrate(mg/g)	Lipid(mg/g)
T ₀ (Control)	Muscle	0.300	2.245	0.78
	Gill	0.365	2.248	0.85
	Liver	0.288	1.269	0.61
T ₁	Muscle	0.286	2.109	0.75
	Gill	0.283	2.128	0.60
	Liver	0.202	1.100	0.52
T ₂	Muscle	0.212	1.100	0.60
	Gill	0.256	1.765	0.51
	Liver	0.162	0.685	0.46
T ₃	Muscle	0.161	1.302	0.27
	Gill	0.198	1.406	0.30
	Liver	0.123	0.107	0.31

All blood parameters of Mrigal are gradually decreased from treatment 1 to 3 (Table 5). [Auriana M Walker *et al.*, \(2020\) \[26\]](#) reported the biochemical treatment of the presence of Cr₂O₃ reduced plasma, total cholesterol and triglycerides and haemoglobin values. In both treatments, blood glucose, plasma total cholesterol and triglycerides levels, showed a reduction at 240 min after initial glucose exposure, but haemoglobin values decreased most at 30 and 240 min than at 60 and 120.

Table 5: Haematological parameters of Mrigal

Parameters	T ₀	T ₁	T ₂	T ₃
RBC count (millions/cumm)	0.4	0.28	0.2	0.13
Hemoglobin (gm/dl)	1.0	0.7	0.5	0.2
Haematocrit (PCV) (%)	2.7	2.0	1.4	1.1
WBC count (Cells/ cumm)	7,100	5,000	4,600	2,500
Platelets (Lakhs/cumm)	90	83	75	65
Mean Corpuscular Volume(fi)	78	61	53	40
Mean Corpuscular Haemoglobin (pg)	25	19	17	15
Mean Corpuscular Haemoglobin Concentration (%)	33	26	22	19

Conclusion

The present study concluded that the biochemical characteristics and hematological parameters are decreased with increased concentration of chromium oxide nanoparticles.

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