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# Acute toxicity test of synthesized calcium zincate nanoparticles in common carp *Cyprinus carpio*

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**Abstract**
Aquatic pollution, due to the anthropogenic activity has become one of the serious challenges in biotic communities. Since fish are highly sensitive to pollutants, study of fish leads to the better understanding of the aquatic ecosystems. This paper emphasizes on the determination of 96hr $LC_{50}$ value of calcium zincate nanoparticles for the fish, *Cyprinus carpio*. The acute toxicity of $CaZnO_2$ NP was evaluated by static bioassays and calculation of the $LC_{50}$ (lethality concentration for 50%). The test was performed according to the standard methods in APHA and the value was calculated by probit analysis. The fish specimens were acclimatized in the laboratory conditions for 12 days. The stock solution of $CaZnO_2$ NP was prepared and the fish were treated with various concentrations for 96 hours. The results showed that the median lethal concentration ($LC_{50}$) of $CaZnO_2$ NP for the fish, *Cyprinus carpio* is 35.251 mg/l. Results showed that calcium nanoparticles are toxic for fish species.

**Keywords:** Toxicity, 96-Hr $LC_{50}$, calcium zincate, *Cyprinus carpio*

## 1. Introduction
Nanoparticles are a particle in which at least one dimensions is smaller than100nm in size [1]. Nanomaterials have unique properties as compared to the same material in a conventional formulation [2]. The application of one type of nanoparticle, the metal oxide nanoparticle, has accelerated in the last decade [3].
The application of nanotechnology in support of life, but at the same time there are growing about human exposure to these which may have adverse health effect [4] and its impact on the environment and living organism are becoming an important issue [5]. Human activities are major responsible for water pollution. Water polluted due to pollution is looked upon with disdain. Water pollution affects the fish severely and proves lethal to them. Water pollution imposes this adverse effect on all kinds of aquatic flora and fauna. Fishes are mainly affected from the human nuisance. So, it is the need of time to pay adequate attention to this issue and execute necessary corrective measures [6].
Studies carried out in USA and Europe showed that Ag-NPs, $TiO_2$-NPs, and ZnO-NPs from sewage treatment may be toxic for aquatic organisms [7]. ZnO NPs were strongly cytotoxic at lower concentrations and exhibited strong protein adsorption abilities [8] which may be contribute toward their cytotoxicity.
Lethal Concentration of 50% ($LC_{50}$) tests can measure the susceptibility and survival potential of animals to particular toxic substances such as heavy metals. Higher $LC_{50}$ values are less toxic because greater concentrations are required to produce 50% mortality in animals [9]. Heavy metals such as mercury, cadmium and lead are toxic to aquatic animals at very low concentrations and are never beneficial to living beings [10]. Thus, the aim of the present study was to investigate the acute effect of calcium zincate nanoparticle by assessing the mortality effect on freshwater fish, common carp (*Cyprinus carpio*).

## 2. Materials and Methods
### 2.1 Chemicals and reagents
Calcium nitrate, Zinc nitrate and Acetamide were purchased from Hi-Media chemicals, Mumbai, India. All the reagents used for the synthesis $CaZnO_2$ were analytical grade and used without further purification.

### 2.2 Synthesis and Characterization of calcium zincate nanoparticles

Solution combustion method was adopted for the synthesis of calcium zincate nanoparticles. Stoichiometric compositions of calcium nitrate (8.65g), zinc nitrate (10.90g), and acetamide (3.93g) was taken in a silica crucible (with volume of 100 $cm^3$) for the synthesis of $CaZnO_2$ NPs. Crucible containing the solution was then introduced into the muffle furnace for calcinations which was preheated to 500°C. Finally, calcium zincate nanoparticles ($CaZnO_2$) formed. According to propellant chemistry the reaction is as follows [11].

$$11Ca(NO_3)_2+11Zn(NO_3)_2+20CH_3CONH_2 \longrightarrow 11CaZnO_2+ 40CO_2 + 50H_2O + 32N_2$$

The morphology and composition of $CaZnO_2$ nanoparticles were examined by Scanning Electron Microscopy (SEM), X-Ray Diffraction and Uv-Vis absorption spectra.

### 2.3 The systematic classification of the selected fish is as follows

| | | |
|---|---|---|
| Kingdom | : | Animalia |
| Phylum | : | Chordata |
| Class | : | Actinopterygii |
| Order | : | Cypriniformes |
| Family | : | Cyprinidae |
| Subfamily | : | Cyprininae |
| Genus | : | *Cyprinus* |
| Species | : | *carpio* |

The fresh water fish *Cyprinus carpio* (18.5 ± 2.0g) were procured from the State Fisheries Department, Bhadra Reservoir Project, Bhadravati and acclimatized to laboratory conditions for about twelve days before the commencement of the experiment. During acclimatization, fish were fed with CP 9932 herbivorous fish feed once a day. During acclimatization, the fish stock was maintained at natural photoperiod and ambient temperature. This ensures sufficient oxygen for the fish and the environment is devoid of any accumulated metabolic wastes. The initial body weight and length of the fish were measured in gram and cm respectively.

### 2.4 Calcium zincate ($CaZnO_2$) NP exposure

For assessment of $CaZnO_2$ NP toxicity, plastic trough with 20 L of water was taken. In each plastic troughs, different concentrations of the $CaZnO_2$ nanoparticles (i.e. 20mg, 25mg, 30mg, 35mg, 40mg, 45mg, 50mg, 55mg and 60mg) were added (control was maintained without CaZnO2 nanoparticles. Ten healthy fish, with an average length of 10 ± 2 cm and average weight of 18.5 ± 2.0g were selected and introduced into each trough. The manifestation and survival time of fish was observed in each concentration for 96 hrs.

### 2.5 Acute toxicity and $LC_{50}$ determination

The test organisms (i.e. fish) were randomly distributed in aquaria. The amount of $CaZnO_2$-NP to be added in each aquarium was calculated accurately with reference to the volume of each aquarium. The fish, in batches of 10, were exposed to varying concentrations of $CaZnO_2$-NP with 20 liters of water using three replicates for each concentration. There was simultaneous control group together with the actual experiments. The control group was kept in experimental water without adding the nanoparticle keeping all other conditions constant. The experiments were performed in triplicates. The mortality rate in the control group did not exceed 5% and 95% of the fish looked healthy throughout the experiment.

Acute toxicity tests were carried out for a period of 96 h, and dead fish were removed as and when observed. Acute toxic effects of metal oxide to the fish were determined by the use of Finney Probit Analysis [12].

### 2.6 Statistical analyses

Percent mortality was calculated and the values were transformed into probit scale and analyzed as per Finney, 1971. Regression lines of probit against logarithmic transformation of concentrations were obtained. Slope function (S) and confidential limits (upper and lower) of the regression line with Chi-square test (EPA, 1999) were calculated and $LC_{50}$ value of Calcium oxide nanoparticles were calculated with the help of probit analysis (SPSS software).

## 3. Results

### 3.1 Characterization of calcium zincate nanoparticles ($CaZnO_2$)

Scanning electron microscope (SEM) was used to decide size, location and shape of the calcium zincate nanoparticles. Analysis of the SEM image of synthesized calcium zincate nanoparticles, reveal the rod and cluster like structures (Fig. 1).

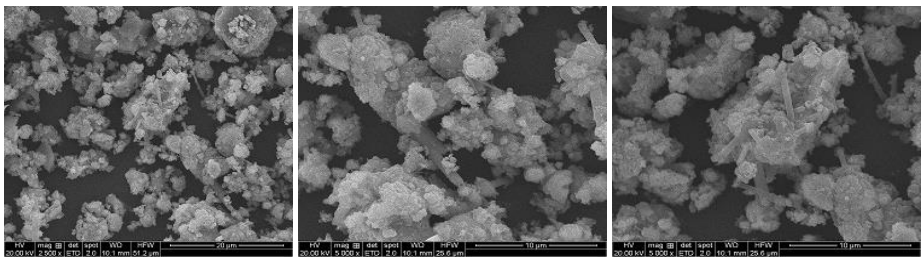


**Fig 1:** SEM images of $CaZnO_2$ NP

The pattern obtained from the XRD analysis of the prepared $CaZnO_2$ NP is presented in Figure 2. According to the Debye-Scherrer's formula $D = K\lambda/(\beta\cos\theta)$, where, K is the Scherrer's constant, λ the X-ray wavelength, β is the full width at half-maximum, and θ is the Bragg diffraction angle. The average percentage of nanoparticles present in the synthesized sample is 43 nm.

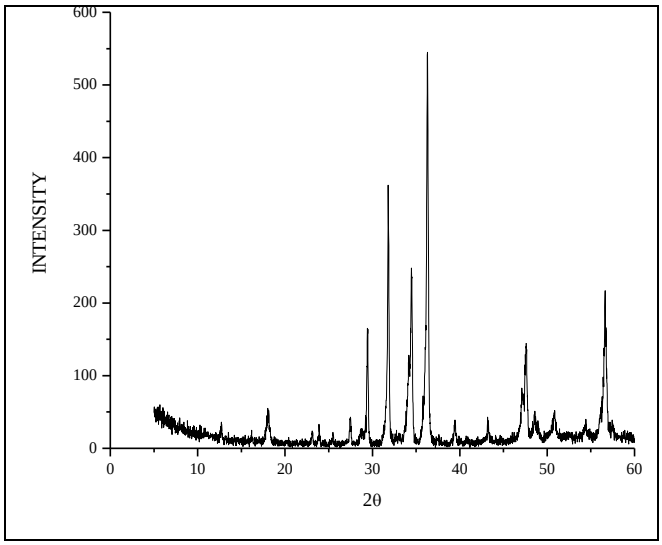



**Fig 2:** XRD of $CaZnO_2$ NP

The UV-absorbance spectra of $CaZnO_2$ NP presented in Figure 3. The UV-Vis absorbance spectrum of synthesized $CaZnO_2$ NPs was taken in the range of 200nm to 1200nm and the band gap energy was found to be 2.6eV. The band gap energy of the $CaZnO_2$ nanoparticle was calculated using the Planck's equation as follows.

$E = hC/\lambda$

h = Planck's constant,

C = Velocity of light,

$\lambda$ = wavelength,

$h = 4.135\times10^{-15}$ eV,

$C = 3\times10^{8}$ m/s,

$\lambda = \text{---}\times10^{-9}$nm

Band gap energy (eV) = $4.135\times10^{-15}\times3\times10^{8}\times10^{9}$

Band gap energy (eV) = 1240/wavelength (nm)

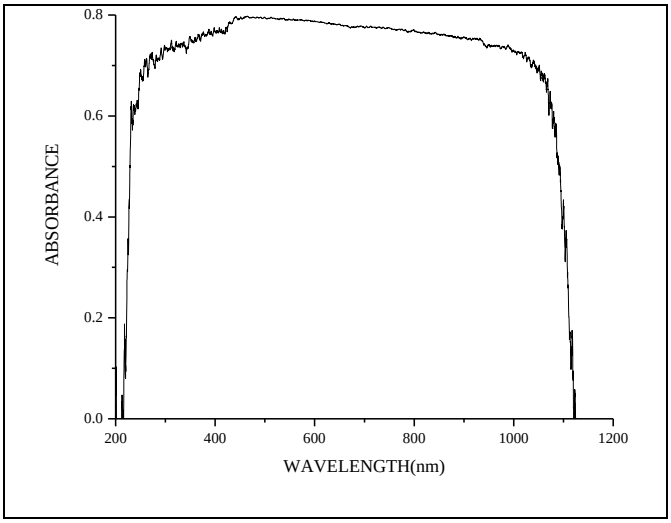



**Fig 3:** UV-absorption spectra of $CaZnO_2$ NP

**Table 1:** Mortality of *Cyprinus carpio* at 96h after treatment of different concentration of $CaZnO_2$ NP

| No. | Conc. ppm | Log Conc | No. of Subjects | Observed Responses | Expected Responses | Residual | Probability |
|---|---|---|---|---|---|---|---|
| 1 | 20 | 1.301 | 10 | 1 | .973 | .327 | .097 |
| 2 | 25 | 1.398 | 10 | 2 | 2.158 | .142 | .216 |
| 3 | 30 | 1.477 | 10 | 4 | 3.560 | .140 | .356 |
| 4 | 35 | 1.544 | 10 | 4 | 4.935 | -.635 | .493 |
| 5 | 40 | 1.602 | 10 | 5 | 6.138 | -.838 | .614 |
| 6 | 45 | 1.653 | 10 | 7 | 7.118 | -.118 | .712 |
| 7 | 50 | 1.699 | 10 | 8 | 7.881 | .119 | .788 |
| 8 | 55 | 1.74 | 10 | 9 | 8.456 | .244 | .846 |
| 9 | 60 | 1.778 | 10 | 9 | 8.882 | .418 | .888 |

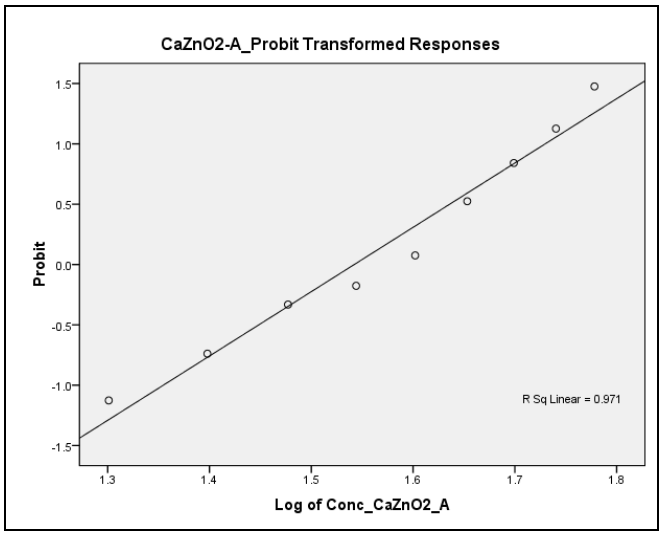
**Table 2:** The relation between the $CaZnO_2$ NP concentration and the mortality rate of *Cyprinus carpio*

| Parameter Estimates | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Parameter | Estimate | Std. Error | Z | Sig. | 95% Confidence Interval | |
| | | | | | | Lower Bound | Upper Bound |
| PROBIT[a] | Conc_$CaZnO_2$_A | 5.269 | 1.076 | 4.898 | .000 | 3.161 | 7.378 |
| | Intercept | -8.152 | 1.700 | -4.796 | .000 | -9.852 | -6.452 |

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

| Chi-Square Tests | | | | |
|---|---|---|---|---|
| | | Chi-Square | df[a] | Sig. |
| PROBIT | Pearson Goodness-of-Fit Test | .836 | 7 | .997[b] |
| a. Statistics based on individual cases differ from statistics based on aggregated cases. | | | | |
| b. Since the significance level is greater than. 150, no heterogeneity factor is used in the calculation of confidence limits. | | | | |

The graph shows linear relationship between probit response and log concentration of $CaZnO_2$ NP on *C. carpio.*




**Graph 1**: Probit vs. Log concentration

**Table 3:** Estimated $CaZnO_2$ NP concentration values and confidence limits

| Probability | 95% Confidence Limits for Conc_CaZnO2_A | | |
|---|---|---|---|
| | Estimate | Lower Bound | Upper Bound |
| 0.01 | 12.754 | 6.098 | 17.68 |
| 0.05 | 17.179 | 9.953 | 22.014 |
| 0.1($LC_{10}$) | 20.135 | 12.894 | 24.801 |
| 0.2 | 24.403 | 17.561 | 28.784 |
| 0.3 | 28.031 | 21.801 | 32.254 |
| 0.4 | 31.556 | 25.997 | 35.863 |
| 0.5($LC_{50}$) | 35.251 | 30.245 | 40.125 |
| 0.6 | 39.378 | 34.548 | 45.725 |
| 0.7 | 44.33 | 39.017 | 53.681 |
| 0.8 | 50.921 | 44.157 | 65.985 |
| 0.9 | 61.715 | 51.566 | 89.311 |
| 0.99($LC_{99}$) | 97.427 | 72.656 | 188.02 |

## 4. Discussion

In the present study the fresh water fish, *Cyprinus carpio* commonly known as common carp was selected as an experimental model as they are highly sensitive to the environmental pollutants found in freshwater. ZnO NPs partially but relatively quickly dissolved in water, and released free zinc ions were the primary source of toxicity [13-15] or induced additional effects [16]. The solubility of nano-ZnO may play a more important role in its toxicity.

Acute toxicity (96 h $LC_{50}$) of different zinc compounds to common carp (*C. carpio*) is reported by the following researchers. Subashkumar and Selvanayagam, 2014 [17] showed acute toxicity of ZnO nanoparticles for common carp (*C. carpio*) at 4.897 mg/L, Aligul *et al*, 2009 [18] showed acute toxicity of $ZnSO_4$ to guppies (*Poecilia reticulata*) at 30.826 mg/L. The mean lethality of *O. mossambicus* was 30% in 100ppm concentration, 10% in 90ppm concentration in oral administration of ZnO NPs [19]. Acute toxicity (96h $LC_{50}$) of ZnO NPs to adult zebra fish reported as 3.97mg/l with primary particle size of 30nm [20]. In carp (*Cyprinus carpio*), up to 50mg/l of ZnO NP was not lethal to the fish, but caused significant oxidative stress [21]. AL-Taee and AL-Hamdani (2013) identified the acute toxicity ($LC_{50}$) of N-ZnO in *Cyprinus carpio* was 30ppm for 24 hrs [22].

However, the median lethal concentration ($LC_{50}$) of the $CaZnO_2$ NP was not yet studied or reported. Therefore, an attempt was made to evaluate the 96 h $LC_{50}$ value of $CaZnO_2$ NP in fresh water fish *C. carpio*. Acute toxicity refers to the damage that happens to the test animal when exposed to toxicant from a single exposure, generally of short duration. In fishes, in order to evaluate the acute toxicity or the medial lethal effect usually fishes were exposed for 96 h duration [23]. In the present study the probit analysis clearly states that 35.251 mg/ L as the mean lethal concentration of $CaZnO_2$ NP to the Carp fish *Cyprinus carpio*.

## 5. Conclusion

The median lethal concentration of $CaZnO_2$ NP ($LC_{50}$) for *Cyprinus carpio* was determined as 35.251 mg/ L for 96 h by probit analysis and when the concentration increased above 55 mg/ L showed 100% mortality. The data reported in the study therefore recommend 35.251 mg/ L as 96 h $LC_{50}$ of $CaZnO_2$ NP for *Cyprinus carpio.*

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