Journal of Population Therapeutics & Clinical Pharmacology

ORIGINAL ARTICLE
DOI: 10.53555/jptcp.v31i5.6255

CHRONIC EXPOSURE OF CARBOFURAN TO *LABEO ROHITA* AND ITS MITIGATION THROUGH BIOLOGICALLY SYNTHESIZED ZINC NANOPARTICLES

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Abstract
The present study was conducted in order to explore the mitigation efficiency of biologically synthesized Zn NPs against carbofuran toxicity in *Labeo rohita*. For this purpose black pepper leaves extract was used Zn NPs were synthesized. Initially 96 hrs LC_{50} for fish fingerlings against carbofuran was determined which was 0.3mg/l. Then 90 days trial was conducted to study the chronic effects of carbofuran in *Labeo rohita* fingerlings and its mitigation through biologically synthesized Zn NPs as supplemented feed. . A total 180 healthy individuals of similar weight and length 30±05 of *Labeo rohita* were maintained and acclimatized prior to the experiment. Fish were randomly distributed into three groups designated as T_1, T_2 and T_3. Treatment 1 was given no carbofuran and was considered as control group while T_2 and T_3 were received $1/5^{th}$ and $1/10^{th}$ carbofuran sublethal concentrations of 96-hr LC_{50} (0.3 mg/l) respectively. Each treatment was be divided into four into four groups, each having fish (n=15). Four levels of supplemented feed i.e, 0 mg/kg, 5 mg/kg, 10 mg/kg and 15 mg/kg Zn BNPs (Biologically synthesized) were given to each group of the three treatments. Among the four feed levels 10mg/kg Zn NPs containing feed was found best in order to mitigated the chronic effects carbofuran by analyzing liver biomarkers and antioxidant enzyme assays.

Key words: Carbofuran, *Labeo rohita*, mitigation, Zn NPs

INTRODUCTION

Pesticide exposure has long been known to cause problems for ecosystem and human health. In extreme cases, pesticide runoff from agriculture can result in large fish kills. These effects are manifested in reduced inland fisheries potential and therefore have potential to affect the billions of people who rely on inland fisheries for food and the millions who rely on them for their livelihood (Li *et al.* 2017). In spite of their usefulness, pesticides pose severe threat to natural resources and poisoning to animals and plants. Humans are indirectly affected via food chain because of the chemical complexity and persistent nature of pesticides (Sun *et al.* 2018). Carbofuran a Carbamate pesticide is extensively used in rice and sugarcane crops to control pests reached to aquatic environment cause acute and chronic toxicities in aquatic organisms (Vani *et al.* 2020). The toxicity of pesticides on aquatic organisms can be studied by evaluating the changes in the acetylecholinesterase activity, haematological, histological, biochemical and enzymes related parameters (Ramesh *et al.* 2015).

Several conventional technologies are used for the removal of carbofuran including physicochemical processing like photo-catalysis, Ozonation/UV- irradiation (Ibrahim and Solpan, 2019), membrane filtration, adsorption (Khuntong *et al.* 2010) and fenton degradation (Ma *et al.* 2010). However, none of these technologies is feasible and cost effective for complete mineralization of carbofuran pollution from the environment. A diverse application of nanomaterial-based technology has opened a new horizon in material science over the past decades because nanomaterials offer a high surface area and other very distinctive physical, chemical, and biological properties compared to their bulk counterparts (Jayachandran *et al.* 2021).

Zinc oxide nanoparticles (ZnO-NPs) are the most commonly used metal oxide nanoparticles because their distinctive optical and chemical properties can be easily modified by altering the morphology and the wide bandgap (3.37 eV) and high excitation binding energy (60 meV) to simulate the ZnO-NPs to be a potent photocatalytic and photo-oxidizing moiety against chemical and biological species (Shaba *et al.* 2021). ZnO-NPs have been shown to reduce the parameters responsible for hepatic fibrosis and nephrotoxicity (Kielbik *et al.* 2017).

The current study is planned to evaluate the chronic effects in *Labeo rohita* induced by carbofuran and ameliorative potential of green synthesized Zinc nanoparticles against carbofuran.

MATERIALS AND METHODS

Green synthesis of Zn NPs by biosynthesis method

Zn BNPs (Zinc biologically synthesized) were prepared by biosynthesis method. For this purpose, the black pepper leaves were collected from the Pattoki plant nursery farms in polythene bags and carried to the laboratory of Department of Fisheries and Aquaculture for further analysis. The leaves were washed with distilled water and shade dried. Then 20 gm crushed dried leaves powder were mixed with 50 ml of distil water. After that, mixture was stirred by magnetic stirrer at 60 °C for 1h, then filtration was done with Whatsman^{TM} filter paper. Zinc stock solution was prepared by dissolving 5g of zinc acetate in 100ml of deionized water while stirring continuously and mixed with 20ml of plant extract while heating at 60 °C for 4 hours (Singh *et al.* 2019). The resulting solution was centrifuged at 1000rpm. After centrifugation gel like product was collected and calcined in muffle furnace for removal of impurities. After that white color nanoparticles were collected for further characterization.

The Zn BNPs was synthesized using the black pepper leaf extract, which comprises of various biological reducing agents. Which were helpful in the synthesis and stabilization of Zn BNPs (Patel *et al.* 2016).

Characterization of Zn NPs

The quartz cuvette was used to observe UV-visible spectra of samples at the 200-800 nm wavelength range on Shimadzu-UV 2600 spectrophotometer (Rocha *et al.* 2018). XRD for Zn nanoparticles was carried out via a PAN Analytical X-ray Diffractometer operating at 45 KV, while using Cu K radiation as the X-ray source by following Bunaciu *et al.* (2015). Field emission SEM (FE-SEM; JXA-8200, JEOL) was used for particles size and surface morphology of NPs (Modena *et al.* 2019).

Study area

The present studies were carried out at the hatchery of Fisheries Research Farms, Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Ravi Campus Pattoki.

LC_{50} for Labeo rohita against carbofuran

Labeo rohita individuals were obtained from grow out ponds, kept in hatchery holding tanks, supplied with aerated flow through water and acclimatized for a period of 15 days (Hunn *et al.*1968). The fish

were fed during the period of acclimatization with commercially available feed of 30% crude protein. Laboratory test was conducted to determine the 96-hr LC_{50} of carbofuran, 100 individuals of *Labeo rohita* were selected randomly from rectangular tanks and equally distributed in ten glass aquaria having 80 litter water capacity. Stock solution of carbofuran was prepared. A total of 10 concentrations in geometric series were set simultaneously to determine 96 hr LC_{50} of carbofuran against *Labeo rohita*. Optimum value of pH, temperature and total hardness of water were maintained at 7.5, 30°C and 200mg/L respectively. Mortality of fish was noted on daily basis. The experiment was conducted by following OECD (2019*)* standard guidelines. Against each test dose probit analyses was performed and mortality were measured by adopting standard protocol (Finney *et al.* 1971).

Experimental design for chronic exposure

The experiments was conducted in glass aquaria of 340 liters volume. Each aquarium was filled with two third of tap water. A total 180 healthy individuals of similar weight and length 30±05 of *Labeo rohita* were maintained and acclimatized prior to the experiment. Fish were randomly distributed into three groups designated as T_1, T_2 and T_3. Treatment 1 was given no carbofuran and was considered as control group while T_2 and T_3 were received $1/5^{th}$ and $1/10^{th}$ carbofuran sub lethal concentrations of LC_{50} value for 96-hr (0.04 mg/l) respectively. Each treatment was be divided into four into four groups, each having fish (n=15). Four levels of supplemented feed i.e, 0 mg/kg, 5 mg/kg, 10 mg/kg and 15 mg/kg Zn BNPs (Biologically synthesized) were given to each group of the three treatments.

pH (7.5 ± 0.5) and water temperature (30^{o}C) of each aquarium were kept constant. Dissolved oxygen (mg/L) was maintained at optimum level by installing aerators. The experimental trial was carried out for a period of 90 days. The parameters were analyzed using factorial analysis of variance (ANOVA) while significant differences among treatment means were compared using Duncan's multiple range test (DMRT) using SAS version 9.1. Significance was tested at 5% level ($p > 0.05$).

Liver biomarker

Fish was randomly selected from each tank and anesthetized with clove oil. Serum AST and ALT were analyzed through serum chemistry analyzer by using bioactive diagnostic kits following the method used by Reitman and Frankel (1957). Serum ASP was analyzed by following Tietz *et al.* (1983).

Antioxidant Enzyme Assays

Samples from liver tissues were homogenized in cold phosphate buffer saline (0.1 M, pH 7.4) using a Potter Elvehjem glass/Teflon homogenizer. After filtration, the homogenate was centrifuged for 10 min at 1600 rpm at 4°C until further analysis the supernatants was kept at -80 °C. SOD was analyzed by following the method given by Nishikimi *et al*. (1972) and absorbance variations were noted at 560nm. Aebi (1984) was followed in order to estimate CAT enzyme.

RESULTS

UV visible analysis

The UV absorption spectra representing a strong absorption band at 373 which the characteristic feature are biologically synthesized Zn NPs (1 µM concentration) presented in figure.1. The d-d transitions are usually forbidden so the absorption band is mostly comprised upon a LMCT (ligand to metal charge transfer) band having absorbance maxima at approximately 1.2.

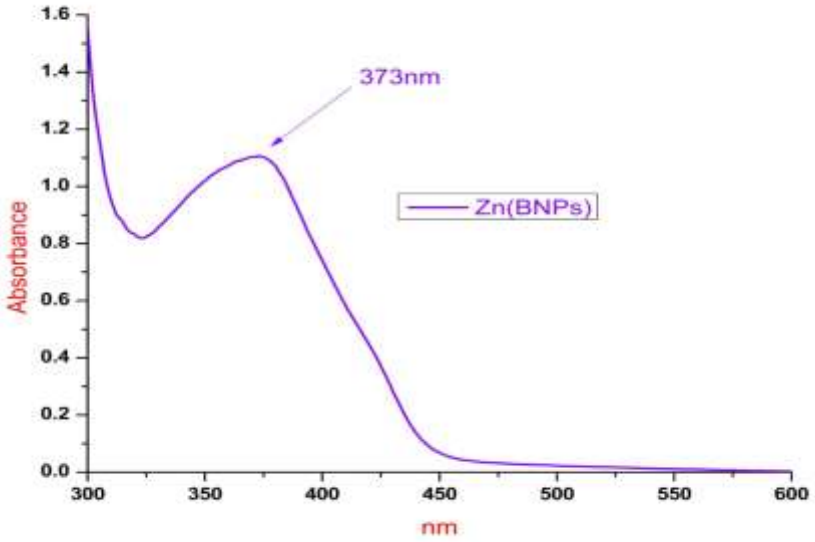

Figure 1. UV visible spectra for biologically synthesized Zn NPs

XRD analysis

Figure. 2 shows the XRD results of biologically synthesized Zn NPs using biological method. The peaks are completely according to the standard. The known peaks and miler indices values for nanoparticles are used in order to measure the average size of the particles through scherrer equation. The average size calculated for biologically synthesized nanoparticles is 15.37 nm.

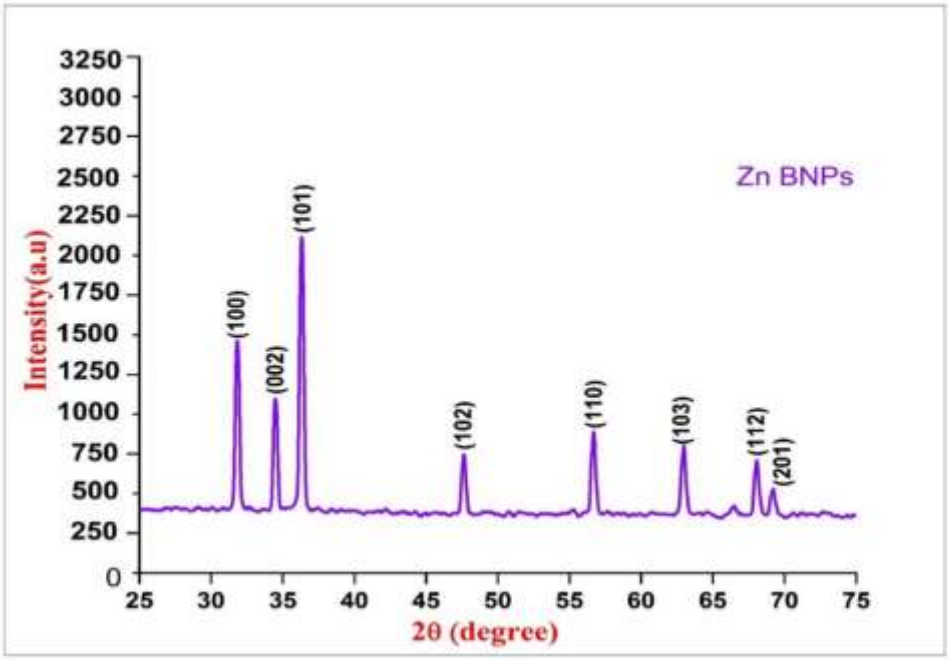

Figure 2. XRD spectra of biologically synthesized Zn NPs.

FE-SEM: Scanning electron microscopy results describe in figure.3 for biologically synthesized nanoparticles. The particles size decoded by a software image j and results shows that the diameter of biologically synthesized nanoparticles are 12 to 22 nm which are clearly backed the XRD results.

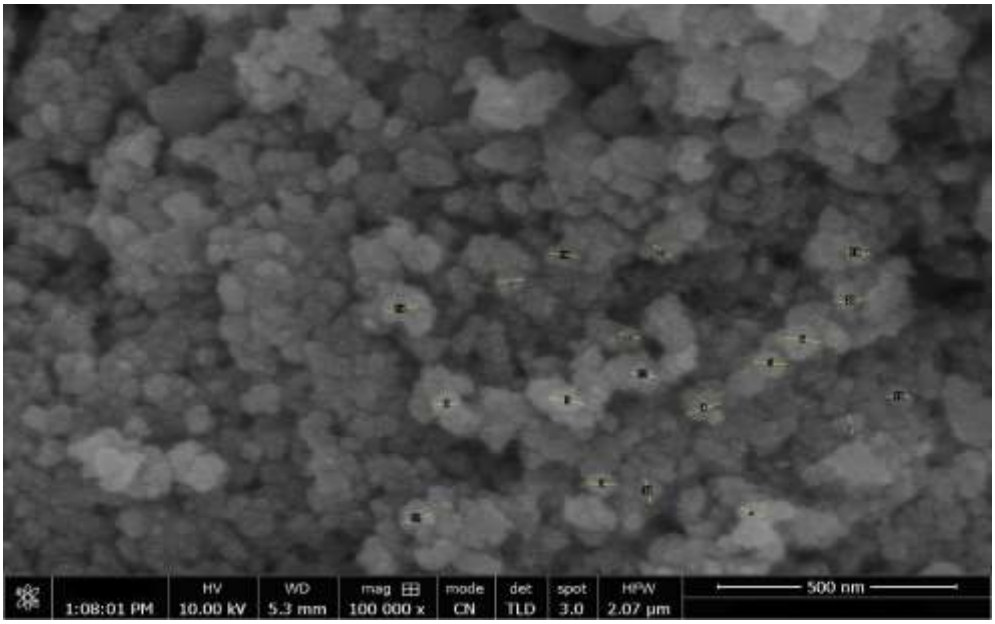

Figure 3. SEM analysis of biologically synthesized nanoparticles

Determination of tolerance limit of *Labeo rohita* against carbofuran

In the present experiment the 96 hours LC_{50} value of carbofuran was calculated through probit analysis as 0.3 mg/l at which 50% of the fish fingerlings were died. The lower and upper bounds through this analysis were calculated as 0.24 and 0.36 respectively. The results were highly significant at $p \leq 0.05$ and the gradual increase in concentrations the rate of mortality also increased.

Liver biomarker

After chronic exposure, AST, ALT and ALP and serum cortisol activities were determined in three treatments T1 (control) with no carbofuran, T2 with $1/5^{th}$ and T3 with $1/10^{th}$ of 96- hours LC_{50} concentrations of carbofuran fed each treatment with four different levels of biologically synthesized zinc nanoparticles supplemented feeds and results are described as:

Aspartate amino transferase (AST)

The results for metabolic enzyme AST varies among treatments fed with different levels of biological synthesized zinc nanoparticles supplemented feed displayed in table no.1. AST activities significantly increased in T2 administered 0mg/kg ZnBNPs ($p<0.05$) while significantly reduced in the same treatment fed with 10mg/kg ZnBNPs supplemented diet. The activities of AST in T3 were found significantly increased that fed with 0mg/kg ZnBNPs supplemented feed and the most reduced activities were recorded in the same treatment fed with 10mg/kg ZnBNPs supplemented feed ($p<0.05$). Activities of AST in control group among different feed levels were recorded non-significant.

Table. 1. Aspartate amino transferase (AST) activity (IU/ml) in serum of *Labeo rohita* across different treatments

Treatments			
Feed levels	**T1 (No carbofuran)**	**T2 ($1/5^{th}$ carbofuran)**	**T3 ($1/10^{th}$ carbofuran)**
1	130 ± 2.30^{A}	385 ± 3.15^{A}	305 ± 2.52^{A}
2	126 ± 2.45^{A}	205 ± 3.12^{B}	198 ± 2.82^{B}
3	121 ± 2.25^{A}	160 ± 2.32^{C}	142 ± 2.05^{C}
4	124 ± 3.12^{A}	168 ± 2.72^{C}	158 ± 2.15^{D}

Means with the same letters in a single column are statistically similar at $p \leq 0.05$.

1= 0mg/kg ZnBNPs, 2= 05 mg/kg ZnBNPs, 3= 10 mg/kg ZnBNPs, 4= 15 mg/kg ZnBNPs

Alanine aminotransferase (ALT)

Table no. 2 and displayed the results for metabolic enzyme ALT varies among three treatments T1 (control), T2 and T3 each fed with four levels of ZnNPs supplemented feed. T1 (control) group shows ALT activities among all four feed levels were remains non-significant at ($p<0.05$). ALT activities were significantly higher in 0mg/kg Zn BNPs supplemented feed level among all four levels in treatment 2 while significant decreased for ALT activities in T2 were found that fed with 10mg/kg Zn BNPs supplemented feed. The same activities results for T3 shows highest and lowest values among all four feed levels were recorded in 0mg/kg Zn BNPs and 10mg/kg Zn BNPs supplemented diets respectively.

Table. 2. Alanine transaminase (ALT) activity (IU/ml) in serum of *Labeo rohita* across different treatments

Treatments			
Feed levels	**T1 (No carbofuran)**	**T2 (1/5th carbofuran)**	**T3 (1/10th carbofuran)**
1	24 ± 2.25^{A}	83 ± 2.23^{A}	72 ± 2.54^{A}
2	21 ± 2.61^{A}	53 ± 2.36^{B}	44 ± 2.18^{B}
3	19 ± 2.22^{A}	35 ± 2.59^{C}	29 ± 2.62^{C}
4	26 ± 2.49^{A}	41 ± 2.05^{C}	34 ± 2.08^{C}

Means with the same letters in a single column are statistically similar at $p \leq 0.05$.

1= 0mg/kg Zn BNPs, 2= 05 mg/kg Zn BNPs, 3= 10 mg/kg Zn BNPs, 4= 15 mg/kg Zn BNPs

Alkaline phosphatase (ALP)

Results for metabolic enzyme ALP were described in table no.3. The results for ALP activities among all treatments were found significantly different and described as T2>T3>T1. T1 (control) consists of four groups fed with four different supplemented feed, ALP activities were found non-significant among all four feed levels while T2 and T3 each having four ZnNPs supplemented feed groups shows ALP activities significant increase in fish fed with 0mg/kg Zn BNPs and significant decrease were recorded for fish fed with 10mg/kg Zn BNPs supplemented feed ($p<0.05$).

Table. 3. Alkaline phosphatase (ALP) activity (IU/ml) in serum of *Labeo rohita* across different treatments

Treatments			
Feed levels	**T1 (No carbofuran)**	**T2 (1/5th carbofuran)**	**T3 (1/10th carbofuran)**
1	75 ± 2.92^{A}	160 ± 2.42^{A}	152 ± 2.66^{A}
2	73 ± 2.15^{A}	120 ± 2.81^{B}	101 ± 2.52^{B}
3	70 ± 3.05^{A}	90 ± 2.73^{C}	79 ± 2.18^{C}
4	$77\pm3,82^{A}$	102 ± 2.06^{D}	97 ± 3.01^{D}

Means with the same letters in a single column statistically similar at $p \leq 0.05$.

1= 0m/gkg Zn BNPs, 2= 05 mg/kg Zn BNPs, 3= 10 mg/kg Zn BNPs, 4= 15 mg/kg Zn BNPs

Cortisol activity

Serum cortisol level in response to carbofuran chronic exposure and its mitigation response with biological synthesized zinc nanoparticles were determined and displayed in table no. 4. Cortisol highest significant increase activities were found in T2 group 1 fed with 10mg/kg Zn BNPs supplemented feed. Cortisol activities between T2 and T3 were found signicantly varied ($p<0.05$). Among all four groups of T3 group 3 fed with 10mg/kg Zn BNPs shows significant decrease and highest increase for cortisol activities were found in group 1 fed with 0mg/kg Zn BNPs supplemented feed and lowest significant decrease were recorded in group 3 fed with 0mg/kg Zn BNPs supplemented feed.

Table. 4. Cortisol activity (ng/ml) in serum of *Labeo rohita* across different treatments

Treatments			
Feed levels	**T1 (No carbofuran)**	**T2 (1/5th carbofuran)**	**T3 (1/10th carbofuran)**
1	188±1.23^{A}	248±2.62^{A}	243±2.19^{A}
2	183±2.05^{A}	226±2.13^{B}	219±2.70^{B}
3	180±2.37^{A}	202±2.18^{C}	192±2.55^{C}
4	179±2.20^{A}	207±2.42^{C}	201±2.38^{C}

Means with the same letters in a single column are statistically similar at p ≤ 0.05.

1= 0mg/kg Zn BNPs, 2= 05 mg/kg Zn BNPs, 3= 10 mg/kg Zn BNPs, 4= 15 mg/kg Zn BNPs

Antioxidant Enzyme Assays

Catalase and SOD activities were analyzed and results are describes as:

Catalase (CAT) activities

Table. 5 presented Catalase activities for three treatments designated as T1 (control) with no carbofuran, T2 with 1/5th and T3 with 1/10th of 96-hours LC_{50} concentrations of carbofuran each divided into four groups fed with four different feeds supplemented with biologically synthesized ZnNPs as 0mg/kg, 5mg/kg, 10mg/kg and 15mg/kg. T1 shows non-significant variations among all four groups for catalase activities. Significant increase were found in group 1 of T2 and T3 fed with 0mg/kg Zn BNPs supplanted feed ($p<0.05$) while catalase activities were significantly reduced in group 3 of T2 and T3 fed with 10mg/kg Zn BNPs supplemented feed.

Table. 5. Catalase activity (IU/mg) in liver of *Labeo rohita* across different treatments

Treatments			
Feed levels	**T1 (No carbofuran)**	**T2 (1/5th carbofuran)**	**T3 (1/10th carbofuran)**
1	22.15±1.25^{A}	75.41±2.55^{A}	71.05±2.59^{A}
2	24.14±1.48^{A}	41.21±2.63^{B}	39.11±2.46^{B}
3	19.23±1.71^{A}	30.23±1.85^{C}	28.15±2.06^{C}
4	26.73±2.03^{A}	33.15±1.72^{C}	31.02±2.13^{C}

Means with the same letters in a single column are statistically similar at p ≤ 0.05.

1= 0mgkg Zn BNPs, 2= 05 mgkg Zn BNPs, 3= 10 mgkg Zn BNPs, 4= 15 mgkg Zn BNPs

Superoxide dismutase (SOD) activity

Table no. 6 described the SOD activities for three treatments T1 (control), T2 and T3. SOD activities determined for T1 (control) having fours groups fed four levels of supplemented feed were normal as highest and lowest values were 15.00±2.43 and 10.25±1.62 respectively fed with 10mg/kg and 15mg/kg. Significant increase in SOD activities were found in group 1 fed with 0mgkg Zn BNPs supplemented feed of T2 and T3 while significant decrease were recorded in group 4 of T2 and T3 fed with 15 mgkg Zn BNPs supplemented feed ($p<0.05$).

Table. 6. SOD activities (IU/mg) in liver of *Labeo rohita* across different treatments

Treatments			
Feed levels	**T1 (No carbofuran)**	**T2 (1/5th carbofuran)**	**T3 (1/10th carbofuran)**
1	12.00±1.05^{A}	27.00±2.11^{A}	25.15±0.08^{A}
2	14.32±1.65^{A}	23.00±2.06^{B}	21.00±2.72^{A}
3	15.00±2.43^{A}	21.00±2.62^{B}	18.09±1.49^{B}
4	10.25±1.62^{A}	20.00±1.52^{B}	14.50±2.12^{C}

Means with the same letters in a single column are statistically similar at p ≤ 0.05.

1= 0mg/kg Zn BNPs, 2= 05 mg/kg Zn BNPs, 3= 10 mg/kg Zn BNPs, 4= 15 mg/kg Zn BNPs

DISCUSSION

In the present experiment the 96 hours LC_{50} value of carbofuran was found as 0.3 mg/l at which 50% of the fish fingerlings were died which was similar to the results of Saglio *et al.* (2003) assessed toxicity of carbofuran against gold fish, the 96-hr LC_{50} was 1 mg/L and 0.92 µmol/L LC_{50} value for common carps against carbofuran was calculated by Assis *et al.* (2010) whereas Mustafa *et al.* (2014) summarized their experiments, where they test different pesticides against *Labeo rohita* with average weight 500±20 g and calculated 96-hr LC_{50} through probit analysis. Among different pesticides the value for carbofuran was 1.4 mg/L. similarly, Mahboob *et al.* (2015) did various experiments in order to evaluate the 96 hr LC_{50} for different pesticides, among various pesticides the tolerance limit for carbofuran was calculated as 0.49 mg/l in *Cirrhinus mrigala.* The absorption spectra of the green synthesized Zn NPs displayed maximum optical absorption bands at 373 nm. The diffraction patterns show that all the peaks are clearly assigned to the hexagonal phase (wurtzite structure) of Zn NPs and are completely matched with standard data (JCPDS 36-1451). For the structural and elemental analysis, the prepared Zn NPs were examined by FE-SEM. The EDX showed strong signal in the zinc and oxygen regions, confirming the formation of Zn NPs. Further, the chemical Zn NPs were spherical with aggregations and these results were much similar to that of Haque and Grayson (2020) prepared two types of Zn NPs by different methods like biosynthesis and sol-gel method. Neem leaf extract was used in order to synthesize biological NPs. Characterization of biologically synthesized Zn NPs reveals wurtzite hexagonal makeup. Particles size of the biosynthesized NPs were smaller (25.97 nm) as compared to that of sol-gel method's NPs (33.20 nm). Range of morphological study of most of the particles have the range between 10 to 70 nm similarly, neem plant extract was used to synthesize Zn NPs by Sohail *et al.* (2020) with an average size of 19.57 ± 1.56 nm and characterization were carried out by using X-ray diffraction, FTIR analysis and surface morphology using SEM. Jhamta and Kaur (2020) used *Sambucus ebulus* plant extract for the green synthesis of Zn NPs and results of X-ray diffraction shows that NPs were highly crystalline structure with an average size around 17 nm.

Carbofuran being the most toxic carbamate cause toxicity by damaging the immune cells and enzymatic activities in aquatic organisms mainly in fish (Rahdar *et al.* 2021). The present study reveals that chronic exposure of carbofuran to *Labeo rohita* cause alteration in liver enzymatic activities. There is an elevation in the AST, ALP, ALT and cortisol activities in group 1 of treatment 2 and 3 that were given $1/5^{th}$ and $1/10^{th}$ of 0.3mg/l (96-hr LC_{50}) of carbofuran while T1(control) shows normal enzymes activities . This increase may be due to the tissue damage cause by carbofuran particularly in the liver cells. All the three treatments and their sub groups that were fed with different ZnNPs supplemented feed in order to mitigate the effect of carbofuran, the best mitigation results were found in group 3 of treatment 2 and 3 that were fed with 10mg/kg Zn NPs supplemented feed shows significant decrease in liver enzymatic activities as compared to group 1 of treatment 2 and 3. Which clearly reveals that Zn NPs mitigate the damaging effect of carbofuran in liver cells of the experimental fish. These results are in agreement with the research reported by Essa *et al.* 2019. Antioxidant enzymes have been reported as biological indicators of pollutant exposure to fish. Like Catalase and SOD in the present study reveals significant increase by exposure of carbofuran to fish. In control group the activities of these enzymes were found normal but treatment 2 and 3 shows significant elevation in activities due to carbofuran exposure. The experimental groups, fed with Zn NPs supplemented feed shows significant reduced the elevated enzymatic activities, may revealed the effective mitigated efficiency of Zn NPs against carbofuran.

Conclusion

The present study explored the beneficial role of biologically synthesized Zn NPs as supplemented feed in the amelioration of toxic effect of carbofuran in fish. We found 10mg/kg Zn NPs supplemented feed was suitable to restored antioxidant enzymes and liver Enzymatic activities against carbofuran.

REFERENCES

1. Aebi H. 1984. Catalase in vitro. In Methods in enzymology. Academic press. 105: 121-126.
2. Amaeze NH, Komolafe BO, Salako AF, Akagha KK, Briggs TM, Olatinwo OO, Femi MA.2020 Comparative assessment of the acute toxicity, haematological and genotoxic effects of ten commonly used pesticides on the African Catfish, *Clarias gariepinus* Burchell 1822. Heli. 6(8): 047-68.
3. Assis CR, Castro PF, Amaral IP, Carvalho EV, CarvalhoJr LB, Bezerra RS. 2010. Characterization of acetylcholinesterase from the brain of the Amazonian tambaqui (*Colossoma macropomum*) and in vitro effect of organophosphorus and carbamate pesticides. Environ Toxico Chem. 29(10): 2243-2248.
4. Bharti S, Rasool F. 2021. Analysis of the biochemical and histopathological impact of a mild dose of commercial malathion on *Channa punctatus* (Bloch) fish. Toxico Rep.8:443-455.
5. Bunaciu AA, UdriŞTioiu EG & Aboul-Enein HY. 2015. X-ray diffraction: instrumentation and applications. Crit rev in ana chem. 45(4): 289-299.
6. El-Asely AM, Abbass AA, Austin B. 2014. Honey bee pollen improves growth, immunity and protection of Nile tilapia (*Oreochromis niloticus*) against infection with *Aeromonas hydrophila*. Fish Shellfish Immunol. 40(2): 500-506.
7. Essa SS, El-Saied EM, El-Tawil OS, Gamal IM, & Abd El-Rahman SS. 2019. Nanoparticles of zinc oxide defeat chlorpyrifos-induced immunotoxic effects and histopathological alterations. Vet W. 12(3): 440.
8. Finney DJ. 1971. Statistical logic in the monitoring of reactions to therapeutic drugs. Methods Inf Med. 10(04): 237-245.
9. Haque FM, Grayson SM. 2020. The synthesis, properties and potential applications of cyclic polymers. Nat Chem. 12 (5): 433-444.
10. Hunn JB, Schoettger RA, Whealdon EW. 1968. Observations on the handling and maintenance of bioassay fish. Progr Fish cult. 30 (3): 164-167
11. Ibrahim KEA, Solpan D, (2019). Removal of carbofuran in aqueous solution by using UV irradiation/ hydrogen peroxide. J. Environ. Chem. Engineer 7: 1-7.
12. Jayachandran A, Aswathy TR, Nair AS (2021). Green synthesis and characterization of zinc oxide nanoparticles using Cayratia pedata leaf extract. Biochem Biophys Rep. 26: 100995.
13. Jhamta R, Kaur, H. 2020. Antidiabetic and antioxidant potential of *Zanthoxylum armatum* DC. leaves (Rutaceae): An endangered medicinal plant. P Sci To. 7(1): 93-100.
14. Khuntong S, Sirivithayapakorn S, Pakkong P, Soralump C. (2010). Adsorption kinetics of carbamate pesticide in rice field soil. Int. J. Environ. A 3: 20-28.
15. Kielbik P, Kaszewski J, Rosowska J, Wolska E, Witkowski B, Gralak M, Gajewski Z, Godlewski M, Godlewski MM. (2017). Biodegradation of the ZnO:Eu nanoparticles in the tissues of adult mouse after alimentary application. Nanomed. Nanotechnol. Biol. Med. 13: 843–852.
16. Li Z. Jennings A. (2017). Worldwide regulations of standard values of pesticides for human health risk control: A review. Int. J. Environ. Res. PH 14: 826.
17. Ma YS, Sung CF, Lin JG (2010). Degradation of carbofuran in aqueous solution by ultrasound and Fenton processes: Effect of system parameters and kinetic study. J. H Mat. 178: 320-325.
18. Mahboob, S, Al-Ghanim KA, Sultana S, Al-Balawi HA, SultanaT, Al-Misned F, Ahmed Z. 2015.A study on acute toxicity of triazophos, profenofos, carbofuran and carbaryl pesticides on *Cirrhinus mrigala*. Pak J of Zool. 47(2): 461-466.
19. Mahmoud AH, Darwish NM, Kim YO, Viayaraghavan P, Kwon JT, Na SW, Lee JC, Kim HJ. 2020. Fenvalerate induced toxicity in Zebra fish, Daniorerio and analysis of biochemical changes and insights of digestive enzymes as important markers in risk assessment. J King Saud Univ Sci. 32 (2):1569-1580.
20. Modena MM, Rühle B, Burg TP & Wuttke S. 2019. Nanoparticle characterization: what to measure? Adv Mat. 31(32): 1901556

21. Mustafa G, Mahboob S, Al-Ghanim KA, Sultana S, Al-Balawi HA, Sultana T, Ahmed Z. 2014. Acute toxicity I: effect of profenofos and triazophos (organophosphates) and carbofuran and carbaryl (carbamates) to *Labeo rohita.* Tox & Envir Chem, 96(3): 466-473.
22. Nishikimi M, Rao NA, Yagi K.1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun. 46(2): 849-854.
23. OECD (Organization for Economic Co-operation and Development). 1992. "OECD Guideline for Testing of Chemicals." Fish AT T 203: 1-9.
24. Patel BH, Channiwala MZ, Chaudhari SB, & Mandot AA. 2016. Biosynthesis of copper nanoparticles; its characterization and efficacy against human pathogenic bacterium. J. of env chem eng. 4(2): 2163-2169.
25. Rahdar A, Hajinezhad MR, Sargazi S, Bilal M, Barani M, Karimi P, Kyzas GZ. 2021. Biochemical effects of deferasirox and deferasirox-loaded nanomicellesin iron-intoxicated rats. Life Sci. 270: 119146.
26. Ramesh M, Narmadha S, & Poopal RK. (2015). Toxicity of furadan (carbofuran 3% g) in Cyprinus carpio: Haematological, biochemical and enzymological alterations and recovery response. Beni-suef uni j of b and app sci. 4(4): 314-326.
27. Reitman S, Frankel S. 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. J Biol Chem. 28(1): 56-63.
28. Rocha FS, Gomes AJ, Lunardi CN, Kaliaguine S & Patience GS. 2018. Experimental methods in chemical engineering: Ultraviolet visible spectroscopy—UV-Vis. The Can J. of Chem Eng. 96(12): 2512-2517.
29. Saglio P, Bretaud S, Rivot E, Olsén KH. 2003. Chemobehavioral changes induced by short-term exposures to prochloraz, nicosulfuron, and carbofuran in goldfish. Arch of environ cont and toxico. 45(4): 515-524.
30. Shaba EY, Jacob JO, Tijani JO, Suleiman MAT. (2021). A critical review of synthesis parameters affecting the properties of zinc oxide nanoparticle and its application in wastewater treatment. Appl. Wat Sci. 11: 48.
31. Silva FF, Silva JM, Silva TD, Tenorio BM, Tenorio FD, Santos EL, Machado SS, Soares EC.2020. Evaluation of Nile tilapia (*Oreochromis niloticus*) fingerlings exposed to the pesticide pyriproxyfen. Lat Am J AquatRes. 48(5):826-835.
32. Singh D, Agusti A, Anzueto A, Barnes PJ, Bourbeau J, Celli BR, Criner GJ, Frith P, Halpin DM, Han M, Varela MV. 2019. Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease: the GOLD science committee report. Eur Respir J. 53(5): 164-190.
33. Sohail M, Naz R, Abdelsalam SI. 2020. On the onset of entropy generation for a nanofluid with thermal radiation and gyrotactic microorganisms through 3D flows. Phy Scripta. 95(4):045206.
34. Sun S, Sidhu V, Rong Y. (2018). Pesticide pollution in agricultural soils and sustainable remediation methods: A review. Curr. Pollut. Rep. 4: 240-250.
35. Tietz NW, Burtis CA, Duncan P, Ervin K, Petitclerc CJ, Rinker AD, Shuey D, Zygowicz ER. 1983. A reference method for measurement of alkaline phosphatase activity in human serum. Clin Chem. 29(5): 751-761.
36. Vani G, Veeraiah K, Kumar MV, Parveen S, & GRao DP. (2020). Biochemical changes induced by cartap hydrochloride (50% SP), carbamate insecticide in freshwater fish Cirrhinus mrigala (Hamilton, 1822). N Env and PoL Tech. 19(5): 1821-1828.