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Preliminary Assessment of the Effects of Probiotic Bacteria on Cadmium-Induced Toxicity in Zebrafish Kidneys and Non-cancer Renal Cell Lines

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Abstract: The objective of the current study was to assess how well the probiotic *Bacillus licheniformis* works in reducing cadmium-induced toxicity in *Danio rerio*, zebrafish. The fish were given dietary *B. licheniformis* $1x10^8$ $CFUL^{-1}$ and/or cadmium (Cd) 100 $ppmL^{-1}$ for 5 weeks. The fish were assessed for their growth and survival rates, protein and amino acid content, lipid peroxidation, enzymatic antioxidant activities, glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), along with hematoxylin and eosin staining (H and E) in the kidney tissues after the fifth week. The study was further extended to investigate the synergistic effects of partially purified probiotic extracellular secondary metabolite (PPPM) and cd-induced cytotoxicity on non-cancer renal cell lines, HEK293 and HEK293T. The results proved a substantial hindrance of Cd-induced toxicity in zebrafish because of enhanced antioxidant activity by supplementing B. *licheniformis* in the diet. The ability of *B. licheniformis* to resist the effects of stress caused by Cd was further supported by histopathology findings. The application of PPPM in Cd-induced cytotoxicity in HEK293 and HEK293T cell lines showed the effectiveness of dietary probiotic use. Our findings suggest dietary treatment with the probiotic *B. licheniformis* may help *D. rerio* combat cadmium toxicity.

Keywords: zebrafish; cadmium; *Bacillus licheniformis*; antioxidants; histopathology; cytotoxicity.

1. Introduction

Heavy metals, which exist in both forms as metals and metalloids, are highly toxic and have a molecular weight 5 times more than water [1,2]. These heavy metals are highly toxic to the human environment and to the ecosystem, considered as a reason for causing lots of damage to the living forms. Their toxicity is influenced by the dosage, route of exposure, chemical compounds, and, to a smaller extent, by age, gender, genes, and nutritional state of those exposed, all of which influence their toxicity. Environmental pollution caused by these metals has been a growing environmental and global public health problem in recent years [3]. Mining, forestry, rapid growth of industries, and chemical effluents are some of the major concerns that

are adding to heavy metal toxicity. Plant growth and development will be incomplete without the presence of some heavy metals such as copper (Cu), zinc (Zn), molybdenum (Mo), manganese (Mn), and iron (Fe), whereas they canbe toxic at higher concentrations [4,5]. Heavy metals usually accumulate in various parts of plants or within the dead decaying matter. This is the beginning of their accumulation in the food chain. Eventually, it is consumed by the human, initiating its deleterious effects on the body [6].

Cadmium (Cd) is a poisonous heavy metal that aids in toxic health concerns to humans. This heavy metal is prevalent in agricultural and industrial areas, particularly in the atmosphere, and is one of the most common environmental risks. Over the last century, several distinct types of cadmium exposure have been documented because of increased man-made activities affecting the environment, with Cd being the highest usage [7]. Cadmium contamination is frequently caused by its utilization in industrial corrosive reagents and stabilizers in PVC material's, colored pigments, and Ni-Cd batteries [8]. Additionally, underground soils were contaminated by exposure to cadmium, which may also occur through household dust [9]. Because of volcanic activity, the gradual abrasion and erosion of rocks and soil, forest fires, the release of Cd metal into the atmosphere, and subsequent soil pollution, Cd concentrations in the living environment are also increasing [10]. The lengthy biological half-life makes it a progressive toxin, meaning prolonged exposures could still cause detrimental consequences from the metal remains [11].

Inhalation of tobacco smoke, contaminated air, and consumption of water and food from polluted areas are considered cadmium exposure routes in humans [12]. Transportation of cadmium within the human body occurs through erythrocytes as well as albumin, and deposition occurs in the kidneys, liver, and gut [13,14]. Excretion of cadmium from the human body occurs through urine, fecal matter, and through breastfeeding in the case of lactating women.

Probiotics are non-pathogenic microbes that promote gut microbial balance by inhibiting pathogen growth when consumed and benefit the host's health. *Bifidobacterium* and *Lactobacilli* species have gained popularity among others [15]. Zebrafish aquaculture has shown encouraging outcomes since using *Bacillus licheniformis* as a probiotic [16]. It is a non-pathogenic, mesophilic, gram-positive bacteria used to control diseases and enhance growth in zebrafish [17,18]. Major sources of probiotics include curd, sour milk, yogurt, and other fermented milk products [19]. Nutraceuticals are considered commercially available probiotics and are frequently prescribed for anti-microbial activity [20].

Zebrafish (*Danio rerio*) have become popular as tropical fish pets and are important model organisms for studying human biology, disease, vertebrate development, and genetics [21,22]. It is recognized as an experimental animal model due to its tiny size, good reproducibility, rapid growth, and embryo transparency[23]. It has been prominent in the embryonic and adolescent stages of toxicological investigations. The purpose of the study was to examine the protective role of the probiotic *Bacillus licheniformis* on Cd-induced toxicity in zebrafish kidneys using antioxidant assays and Hematoxylin and Eosin staining (H and E), as well as *in-vitro* Cd-induced cytotoxicity evaluation in HEK293 and HEK293T cell lines using partially purified probiotic extracellular secondary metabolite (PPPM) through MTT assay.

2. Materials and Methods

2.1. Zebrafish collection and maintenance.

Healthy 16-week-old zebrafish (0.5 ± 0.2 g) with weight were procured [Fig. 1A] from locally commercially certified vendors for this study. The study was planned as a fully randomized experiment with three treatments and a control group. The fish were raised in filtered freshwater in a lab setting by being stocked at a maximum of seven fish per experimental tank for up to a week to acclimate. Fish were fed *Artemia nauplii* after acclimating to the 28°C temperature and 12-hour light/dark cycle of the lab. Water was maintained at a temperature between 22 to 24°C, with a pH range of 6.8 to 7.2 and a dissolved oxygen content of 5-7mg/L. Fish were randomly separated into 4 groups, each with 7 fish after acclimation for a 30-day exposure period [Fig. 1B]. Every day, the water for each group was entirely replenished, and the aquariums were cleaned carefully. Water that contains probiotics has been changed every day, thus reducing the excess feed of probiotics, which may result in contamination. In general, if contamination is detected, antibiotics such as ampicillin or streptomycin can be used based on the type of contamination [24].

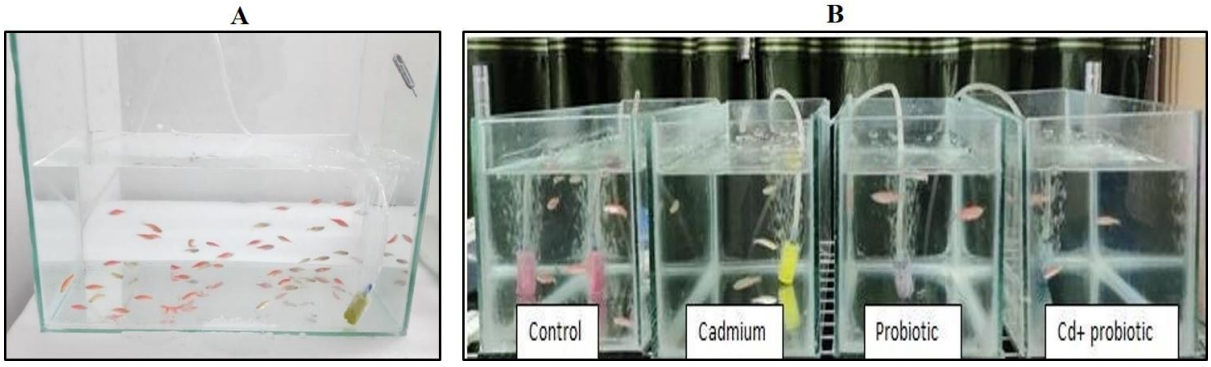

Figure 1. Collection and maintenance of zebrafish in tanks. **A)** Zebra fish collected in a fish tank; **B)** Treatment groups of Zebra fishes.

2.2. Probiotic bacterial collection.

The probiotic bacteria, *Bacillus licheniformis* was kindly donated by Dr. Vijay Paul, Bio Nest, Hyderabad Central University. A saprophytic, gram-positive, endospore-forming microorganism called *Bacillus licheniformis* is found in soil and plants. The probiotic strain is subsequently sub-cultured in MRS broth (55.15g/L) [Fig. 2].

Figure 2. *Subculture of Bacillus licheniformis.2.3. Experimental groups and sample preparation.*

Seven fish from each of the four groups that are acclimatized were utilized to make up one sample (n=7 pools), and seven zebrafish kidneys were used to make up each group, as shown below. Treatment group 1: (Control - Untreated): A group of fishes that were not exposed to cadmium and probioticswas fed only with *Artemia nauplii*. Treatment Group 2: Fish exposed to cadmium (Cd) at a rate of 100 ppm/per liter daily for 30 days). Treatment Group 3: Group of fishes that were treated only with probiotics - *Bacillus licheniformis* (containing $1x10^8$ CFU/L daily for 30 days). Treatment Group 4: Group of fishes treated with Cd (100 ppm/per liter) as well as probiotic (containing $1x10^8$ CFU) daily for 30 days.

2.4. Growth and survival rate estimation.

After 30 days of treatment, quantitative analysis was done on the 'Zebrafish's body length (from the mouth to the caudal peduncle), weight, and survival rate.

2.5. Estimation of protein from the tissue homogenate.

The total protein content of the kidney homogenates from the treated fish was calculated according to the method followed by [25] with (Bovine Serum Albumin) BSA as a reference. The kidney tissues were precisely weighed, dissolved in a predetermined amount of double-distilled water, and centrifuged with the homogenate for 15 minutes at 2500 rpm. The residue was used to estimate structural (water-insoluble) proteins, and to precipitate the protein, an equal amount of 10% trichloroacetic Acid (TCA) was added to the supernatant. The solution was then centrifuged for 30 minutes at 2500 rpm and left to rest for an additional 30 minutes.

The protein fraction that is water soluble is represented by the residue, which was subsequently dissolved in 1ml of 1N sodium hydroxide. This solution was diluted to 0.2ml, and then 4ml of the alkaline copper reagent and 0.4ml of the folin-phenol reagent were added (1:1 folin-phenol water). A spectrophotometer was used to detect the color observed at 600nm compared to the blank. BSA was used to assess the total protein content of the sample, and the results were represented as mg/g wet weight of tissue.

2.6. Estimation of free amino acids (FAA).

Each piece of tissue was homogenized (3%) in 10% TCA and centrifuged for 15 minutes at 600 rpm. 2ml of ninhydrin reagent was added to 0.2ml of the supernatant, boiled for 6 minutes, and then immediately cooled to room temperature. The samples were made up to a volume of 10ml with distilled, and the color was measured at a wavelength of 575nm using a spectrophotometer [26]. The results were represented as M of tyrosine/g wet. wt. in tissues.

2.7. Antioxidative enzyme levels.

2.7.1. Lipid peroxidation.

In tissue homogenates, depending on the reaction with Thio barbituric acid (TBA), the final product of lipid peroxidation, Malondialdehyde (MDA), was measured, producing a pink complex. MDA content was calculated using the absorbance coefficient of the MDA-TBA complex at 550nm using 1, 1, 3, and 3-tetra ethoxy propane (TMP) as the standard.

The following setup was used for the lipid peroxide assay on animal tissues: samples were mixed with less than 200µL of 10% (w/v) tissue homogenate, 200µL of 8.1% SDS, 1.5 ml of 20% acetic acid solution (pH 3.5), and 1.5ml of 0.8% TBA solution. A glass ball served as the condenser while the liquid was heated in an oil bath at 95°C for 60 minutes after being diluted with distilled water to 4.0 ml. The mixture of n-butanol, pyridine (15:1, v/v), and 1.0ml of distilled water was added, cooled with tap water, and shaken rapidly. The organic layer was collected, and its absorbance at 532nm was measured following centrifugation at 4000 rpm for 10 min. The amount of lipid peroxides is determined from the absorbance at 532nm using TMP as an external standard and is represented in terms of nmol MDA/g wet wt. [27].

2.7.2. Glutathione peroxidase and glutathione reductase.

The enzymatic activity of glutathione peroxidase and glutathione reductase was evaluated at 340nm using a spectrophotometer. Oxidized glutathione (GSSG) is converted to reduced glutathione (GSH) by utilizing NADPH. The activity was measured in terms of nmol of NADPH consumed per minute per mg of protein [28].

2.7.3. Test for glutathione reductase.

With a few modifications, referenced in [29], the method was used to measure glutathione reductase. The assay mixture contains 0.1M potassium phosphate buffer (pH 7.4), 0.01M EDTA, 0.01M GSSG, and 0.1mM NADPH, along with the enzyme source at the proper concentration. The NADPH oxidation was measured at 340nm, and the enzyme activity was expressed as nmoL/min per mg protein.

2.7.4. Test for glutathione peroxidase.

The reduction of H_2O_2 and organic hydroperoxides is catalyzed by glutathione peroxidase (GPx). In a total volume of 1ml, the reaction mixture contained the necessary amount of tissue homogenate together with 50mM potassium phosphate buffer (pH 7.0), 1mM EDTA, 1mM NaN_3, 0.2mM NADPH, 1 IU/ml glutathione reductase, 1mM GSH, 1.5mM cumene hydroperoxide or 0.25mM H_2O_2. For five minutes, the change in absorbance of the system at 340nm was noted. The activity's measurement unit was µmol NADPH oxidized per minute per mg of protein [30].

2.7.5. Superoxide dismutase.

Superoxide dismutase activity was assessed in a tissue sample using a modified version of the spectrophotometric NADH-phenazine methosulphate-nitroblue tetrazolium formazan inhibition reaction, measured at 550nm [31].

2.7.6. Catalase.

'Aebi's approach was used to evaluate the spectrophotometric breakdown of H_2O_2 at 240nm to determine catalase's catalytic activity. It was done by extracting 10 µl of testes, 240µl of phosphate buffer containing EDTA, and 250µl H_2O. The enzyme-specific activity was calculated as µmol of decomposed H_2O_2 per min per mg of protein. It was measured in a thermoregulated cuvette holder at 240nm, using a spectrophotometer every 30 sec for 3 min [32].

2.8. Histological examination.

For standard histological investigations, kidney tissues were obtained from all four groups and stored in 10% neutral formal saline. Fixed tissues were processed using the common paraffin embedding method. Hematoxylin and Eosin were used to cut and stain sections of 5-6 µ thickness (H & E).

2.9. Partial purification of extracellular probiotic metabolite (PPPM).

The probiotic bacteria, *Bacillus licheniformis* was kindly donated by Dr. Vijay Paul, BioNest, Hyderabad Central University. *B. Licheniformis* was added to 1L of MRS, and incubated for 16 hours at 37°C. Cells were removed by centrifuging for 3 minutes at 8,000 rpm. A two-step purification procedure was then used for the extracellular metabolites found in the cell-free supernatant. The bacteriocin-containing cell-free supernatant was precipitated by adding ammonium sulfate in amounts of 30, 40, 50, 60, and 70 % [Table 1].

Table 1. Represents partial purification of extracellular probiotic metabolite.

Sample	Volume (ml)	Total Protein (mg)	Total Bacteriocin Activity (AU)	Specific Activity (AU/mg)	Purification (Fold)	Yield (%)
Cell-free supernatant	1000	5985	86,782	14.50	1	100
70% ammonium sulfate precipitation	132	240.28	47,625	198.21	13.66	47.51

2.10. In-vitro assessment of protective effects of probiotic metabolitein reducing cadmium toxicity.

2.10.1. MTT assay.

For the present study, HEK-293 and HEK-293T cell lines were procured from the National Centre for Cell Science (NCCS), Pune. By using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) method, cadmium-induced cytotoxicity and the synergistic effects of a partially purified probiotic extracellular secondary metabolite (PPPM), were evaluated. In a 96-well plate, HEK-293 and HEK-293T cells were planted at a density of 5×10^4 cells/well and placed in the incubator for 24 hrs. For 12 hrs at 37°C in an environment with 5% CO_2, cells were treated to increasing concentrations of Cd and PPPM, either separately or in combination. Cells in media (without any treatment) make up the control groups, which are handled the same way and incubated at the same time as the treated groups. The medium was withdrawn after 12 hrs and changed with 50µL of MTT solution (2mg mL^{-1}), which was then used for another 4 hours before the medium was again removed after the addition of 100µL of dimethyl sulfoxide (DMSO), stirred gently for 5 min at 35-37°C. Using a microplate reader, the absorbance was measured at 552 nm.

2.11. Statistical data analyses.

The means ± SEM of triplicates were used to calculate all experimental data in the results. For multiple comparisons, a one-way analysis of variance (ANOVA) was used along

with Duncan's significant difference post-hoc test. A statistically significant value was considered with a p-value less than 0.05.

3. Results and Discussion

3.1. Growth and survival rate of Zebrafish.

Table 2 compares the zebrafish body weight and length before and after the treatment. The survival percentage of the group of treated fish was also represented. The difference in body weight and length of the treated zebrafishes were notably visible in the graph. The control fishes, which were untreated, showed a normal increase in body weight and length; at the same time, group 2 fishes, which were treated with cadmium, had less growth and development. A large difference of development is observed in group 3 fishes, which were treated with probiotics- *Bacillus licheniformis.* The group 4 fishes show an increase in body weight and length compared to the group 2-cadmium-treated fishes, but it is less than the group 1 and 4 fishes. This indicates the protective effect of probiotic bacteria against the toxicity induced by cadmium. From the survival rate of the fish after treatment, we analyzed the effect of probiotics on increasing the survival of fish treated with cadmium. Later, the kidney tissue sample collected from the fish of all the groups was used for protein, FAA, enzymatic, and H & E staining analysis [Fig. 3].

Figure 3. Dissected zebrafish.

Table 2. Represents growth and survival of zebrafish after treatment.

Parameter		Group 1	Group 2	Group 3	Group 4
Bodyweight	A	0.21^{a}	0.23^{a}	0.21^{a}	0.21^{a}
	B	0.98^{b}	0.78^{b}	1.23^{c}	0.95^{b}
Body Length	A	1.18^{a}	1.23^{a}	1.23^{a}	1.21^{a}
	B	1.72^{b}	1.34^{a}	2.23^{c}	1.72^{b}
Survival % after treatment		100	80	100	95

A and B represent before and after treatments. Significant differences between treatments are marked with lowercase letters ($^{a\,b\,c\,d}$). When $p < 0.05$, the same letter indicates insignificance, whereas a different letter indicates significance, and a comparison between the groups was done in terms of reference ranges.

3.2. Protein and FAA levels in kidney homogenate.

Table 3 shows the amount of protein in the group 2 fishes, i.e., cadmium cadmium-treated ones are comparatively less. Low molecular weight (LMW) proteins are eliminated more often through urine because of 'cadmium's harmful consequences on the kidney's proximal tubular cells, which results in impaired reabsorption of these proteins. The protein level increased in the fishes treated with both cadmium and probiotics when compared with cadmium-treated fishes. This shows the protective effect of probiotics in reducing the toxicity

induced by cadmium. The levels of total proteins, soluble and structural proteins, and free amino acids in the exposed group were much reduced compared to the control groups. However, the percentage of free amino acids has been reduced to half in group II compared to the control groups.

Table 3. Represents proteins and free amino acid levels in kidney homogenate.

Kidney Homogenate	Group-I	Group-II	Group-III	Group-IV
Total Protein (mg/ml)	63.45±2.74^{a}	37.21±1.63^{c}	65.21±3.23^{a}	52.81±2.46^{a}
Structural Proteins (mg/ml)	41.651±2.31^{a}	24.23±0.92^{c}	39.42±1.21^{a}	30.12±2.54^{b}
Soluble Proteins (mg/ml)	18.71±1.91^{a}	10.63±0.23^{b}	21.10±2.14^{a}	18.41±2.21^{a}
Free Amino acids (mg/ml)	2.78±0.04^{a}	1.07±0.02^{b}	2.81±0.28^{a}	1.24±0.04^{b}

Significant treatment differences are marked with lowercase letters ($^{a\ b\ c\ d}$). When $p < 0.05$, the same letter indicates the insignificance, whereas a different letter indicates the significance, and a comparison between the groups was made in terms of reference ranges.

3.3. Antioxidant levels in kidney homogenate.

Table 4 represents the antioxidant activity of all 4 groups of treated zebrafish. The lipid peroxidase activity is maximum in cadmium-treated fishes and minimum in probiotic-treated fishes. The graph shows the level of glutathione peroxidase in the 4 groups of the sample. It was observed that the GPx level is higher in cadmium-treated fishes than in other groups, indicating the high activity of GPx in deactivating the free radicals. The GR levels are high in cadmium-treated fishes, but it was observed that the level of GR comparatively decreased when the fishes were treated with cadmium and probiotic bacteria. A high amount of SOD can be analyzed in the cadmium-treated fishes (group 2) from the graph. The SOD level in probiotic-treated fishes is less when compared to the cadmium-treated ones. Still, it is somewhat high in the group 4 fishes, which contain both cadmium andprobiotics. A high level of catalase is present in the cadmium-treated fishes, and wecan observe the decrease in catalase in group 4 fishes that were treated with cadmium and probiotics.

Table 4. Represents antioxidant levels in kidney tissue homogenate.

Kidney Homogenate	Lipid Peroxidation	Glutathione Peroxidase	Glutathione Reductase	Catalase	Superoxide Dismutase
Group 1	258.72±3.21^{a}	12.78±1.63^{a}	9.71±0.54^{a}	135.24±3.21^{a}	0.14±0.01^{a}
Group 2	327.31±4.25^{b}	17.81±0.67^{b}	16.25±0.28^{b}	210.21±6.91^{b}	0.95±0.13^{c}
Group 3	241.63±5.21^{a}	10.48±0.34^{a}	8.27±0.54^{a}	148.21±3.32^{a}	0.27±0.04^{a}
Group 4	290.63±5.21^{b}	13.10±0.45^{a}	13.14±0.31^{b}	170.23±5.26^{b}	0.45±0.12^{b}

Significant treatment differences are marked with lowercase letters ($^{a\ b\ c\ d}$). When $p < 0.05$, the same letter indicates insignificance, whereas a different letter indicates significance, and a comparison between the groups were done in terms of reference ranges.

3.4. H and E staining.

The changes in internal kidney tissue of all four groups of fishes that have undergone treatment can be visualized through the histological examination [Fig. 4]. A healthy set of tissues containing untreated fish was observed in group 1. A high level of internal tissue damage was visible in the cadmium-treated fishes. We can analyze from the internal tissue structure that group 4 shows less damage when compared to group 2, and it is because probiotics can reduce the harmful effects of cadmium.

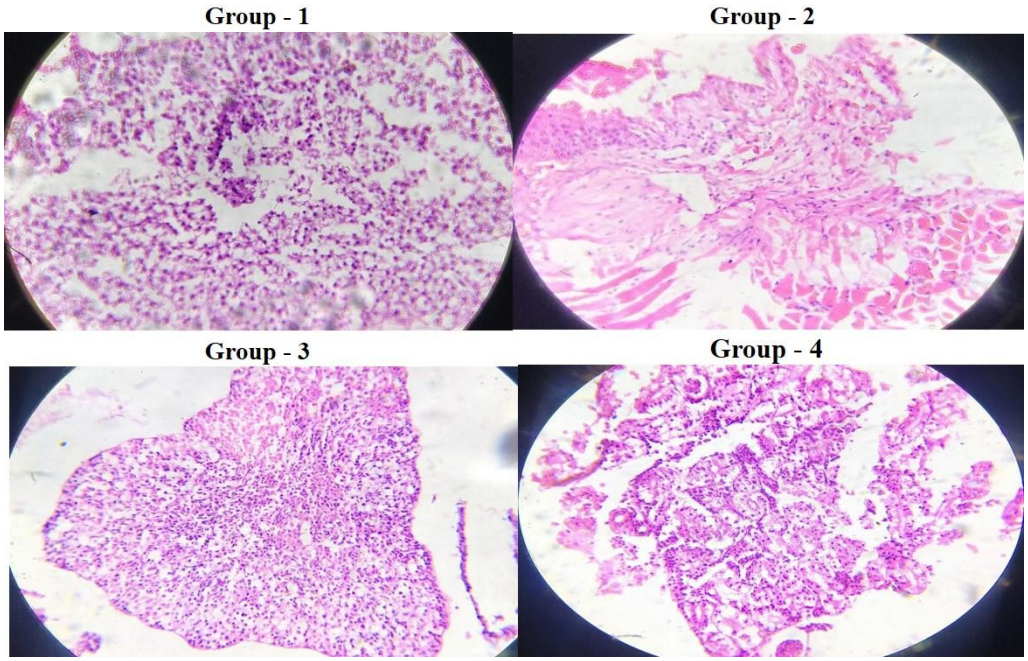

Figure 4. Kidney tissue changes after treatment in control (group - 1) and treated groups (Group 2 to 4).

3.5. Cell viability.

From the graph [Fig. 5], it was observed that the cell viability of cadmium-treated cell lines (HEK-293 and HEK-293T) was almost near zero as the concentration increased, and this cell damage was decreased when we added partially purified extracellular probiotic metabolite along with the cadmium.

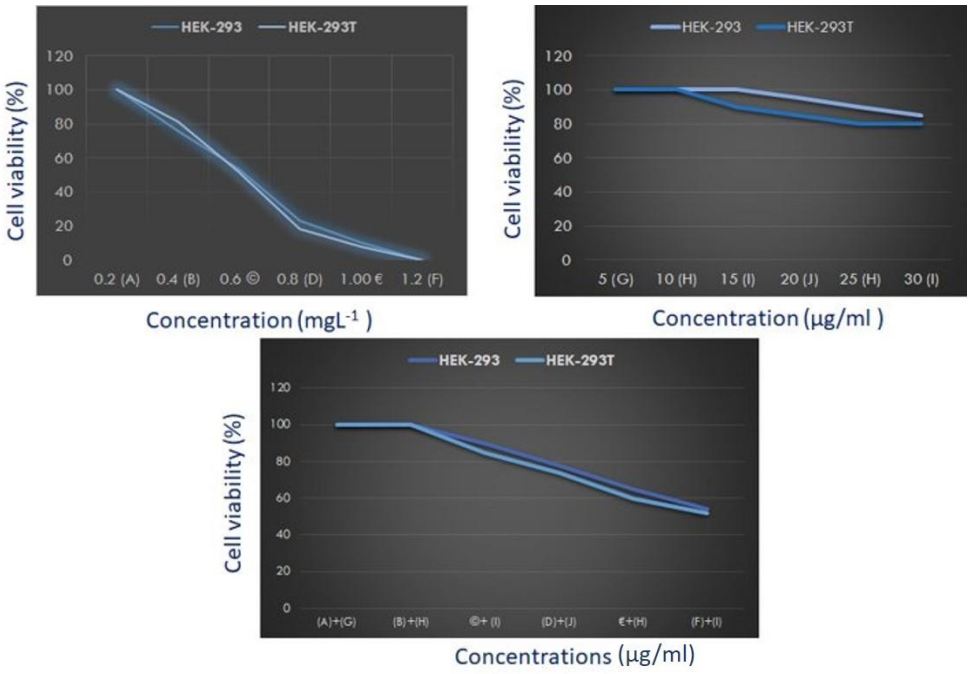

Figure 5. Cell viability (A) after the exposure of Cadmium (Cd) (B) after the treatment with partially purified extracellular probiotic metabolite (PPPM) (C) after the treatment with Cd + PPPM for 12 hours.

4. Discussion

Several research investigations were performed to study and evaluate the toxic effects of heavy metals using zebrafish larvae [33,34]. Heavy metals cause severe toxicity and induce

heavy malfunction at larval stages. Considered as heavy metal, cadmium toxicity has a severe effect on maintaining the zebrafish and has a disturbing effect on the internal organs. Studies from [35] have shown that heavy metals, including lead, cadmium, and iron, have a severe toxicity effect in developing zebrafish. At the lowest concentration of 0.183µg/L concentration, cadmium has displayed an adverse effect of hypoactive larval photo motor response (LPR). Studies by [36] confirmed that cadmium has shown a significant toxic effect on zebrafish larvae and lead, concluding that behavioral rhythm changes occur by heavy metal combinations.

Alongside, studies performed by [37] have shown that cadmium at concentrations of 0.2 µM/L has increased the malformation of the spinal cord, mortality, and induced the hatching rate of zebrafish larvae. Yang group [38] has shown that cadmium combined with cypermethrin potentiates the toxicity of adult zebrafish and their larvae toxicity. Their study confirmed the induction of oxidate stress enzymes in the cadmium-treated groups, and the combination enhances the toxicity levels.

Considering the toxic effects of heavy metals in contaminated water proven to cause severe damage to the kidneys. In our study, our data revealed that after 5 weeks of treatment, either by cadmium exposure or probiotic treatment or both, kidney samples from treated groups and performed protein studies, including soluble protein, structural proteins, total proteins, and free amino acids percentage was reduced in enormous amounts in cadmium exposed group given an explanation of reduced functionality of nephrons in the kidneys due to toxic effect. Our results confirm that cadmium showed a drastic effect on kidney function, which was proven by [39]; in group II, there is a reduction in soluble, total, and structural proteins as well as free amino acids. This confirms that heavy metals cause damage to the kidney and result in the efflux of substrates.

The MTT test, which, despite being frequently employed to determine cytotoxicity, evaluates mitochondrial metabolic activity and suggests proliferative capacity, was used to evaluate cell viability following exposure to cadmium at various concentrations. The generation of harmful ROS may cause decreased viability in both cell lines at the higher dosages of group 2 treated with cadmium alone. Through mitochondrial damage and apoptosis induction, ROS-induced damage causes cytotoxicity. The findings are consistent with Sachdeva and Maret''s team's earlier work [40]. The reduction in cell damage shown in group 4 after treatment with Cd and PPPM suggests that the given 'PPPM's increased antioxidant activity has helped to repair the damage to the cells. This shows that PPPM made from the probiotic *B. licheniformis* has favorable effects. Our findings are supported by Rohith and 'Halami's group [41] research on the protective impact of *B. licheniformis* on cancer cell lines.

5. Conclusions

The current study aimed to examine the cadmium toxicity in zebrafishes exposed to lower concentrations for 30 days and the ability of probiotics to reduce the adverse effects of cadmium. Physical parameters were adversely influenced by cadmium toxicity, and the probiotics helped manage physical parameters. Studies on protein metabolism and antioxidative enzyme levels support the idea that probiotics reduce cadmium toxicity through a protective mechanism. Histopathological findings further support the efficacy of probiotics to reduce cadmium toxicity. The extracellular metabolites were successfully extracted and partially purified, and these managed the cadmium toxicity in HEK-293 and HEK-293T cell lines in a positive matter. To validate probiotics' effectiveness, more research is necessary to reduce cadmium toxicity using the exact mechanisms in animal models.

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Conflicts of Interest

The authors declare no competing interests.

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