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Short Communication

In-vitro Biocompatibility Studies of Mint Oil-Vitamin D Nanoparticles

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Abstract

Mint, a medicinal plant has gained immense attention from food and pharmaceutical industries because of its numerous health benefits while treating Vitamin D (Vit D) deficiency via recommended fortified food always remains the primary objective of nutritionists. We aimed to evaluate the biocompatible nature of our mint oil-Vit D encapsulated β-Cyclodextrin carbon-based nanoparticles before establishing their potential application in the medicine and food industry. The repercussion of different concentrations of nanoparticles was evaluated on various model systems (microbes, cell lines, erythrocytes, plant seeds and zebrafish embryos) and result obtained was subjected to statistical analysis. In our study, synthesized nanoparticles revealed no antimicrobial activity. The cytotoxicity and anticancer potential of the nanoparticles were studied using L929 and HeLa cell lines respectively at various concentrations and divulged the fact that these nanoparticles induce significant cell death at higher concentrations but remain non-detrimental at lower concentrations. Further, exposure of nanoparticles to RBCs presented a dose-dependent induction of hemolysis and lipid peroxide production. A similar trend of toxicity was evident in the zebrafish embryo as well at higher concentrations. Phytotoxicity analysis revealed no effect of nanoparticles on germination of seeds albeit the root and shoot length of seedlings were affected significantly. Overall, our results indicate high biocompatibility of these nanoparticles only at lower concentrations and their further applications in various industries should strictly consider minimal doses.

Keywords: *Antimicrobial, Antioxidant, Hemolysis, Mint oil, Phytotoxicity, Zebrafish.*

1. INTRODUCTION

Aromatic herbs derived essential oil have gained a lot of recognition in food industries which is attributed to their positive health benefits [1, 2, 3]. Mint is one of the most well-documented herbs for its plethora of medicinal uses as well as its antimicrobial, antiviral, and anti-inflammatory activity. There has been accumulating evidence which reveals natural analgesic, anesthetic and anti-inflammatory properties of mint oil [4, 5, 6]. Mint is a calming and soothing herb that has been used for indigestion. The refreshing smell of menthol has been shown to reduce nausea symptoms, upper respiratory complication, coughs and sore throats. Research has also approved use of mint oil to relieve itching related to diabetes, liver disease, and kidney disease [1].

Vitamin D (Vit D), are a group of fat soluble secosteroids and its classic role is to augment the intestinal absorption of calcium,

magnesium and phosphate which is known to manifest multiple other biological effects. Vit D, also known as sunshine vitamin is a precursor to the potent steroid hormone calcitriol, which mediates neuronal activities in the body. There are very few dietary sources of Vit D, therefore humans are dependent on sunlight to maintain Vit D stores [7]. The prevalence of Vit D deficiency raises an unmet need for fortifying various foods with Vit D, through different procedures [8].

In the last few decades, nanomedicine has emerged as a new technology in fortification of edible food products with dietary supplements. Vit D deficiency has been recognized globally as a health risk which intrigued us to develop new functional foods using nanotechnological approaches. Therefore, in order to retain the beneficial medicinal property of mint oil and Vit-D in the same molecule, we have encapsulated both using β-cyclodextrins nanoparticles. β-cyclodextrins is a cyclic polysaccharide made of seven units of glucose (α-D-glucopyranose) linked by α-(1,4) type bonds, and presents a hydrophilic external surface with a hydrophobic internal cavity. This *vitro* models as their fortification with food products can have a direct implication on human health and environment [12,13].

2. MATERIALS AND METHODS

2.1. Materials

Encapsulated nanoparticles containing mint oil and Vit D synthesized as per the protocol of Dima *et al.* 2014 (12) were procured from the Food Science and Technology laboratory of School of Biotechnology and Bioinformatics, D. Y. Patil Deemed to be University, Navi Mumbai. Cell lines (L929 and HeLa) were procured from NCCS Pune. Dulbecco's Modified Eagle's Medium (DMEM), Penicillin-Streptomycin (10,000 Units/ml-10,000 µg/ml) were acquired from Cell Clone, Mumbai. Fetal Bovine Serum (FBS), 0.25% Trypsin-EDTA were obtained from GIBCO, Sodium pyruvate was obtained from Hi-Media. The other reagents required for the procedure were of analytical grade.

2.2. Fabrication of the Microencapsulated Mint Oil Powder

For the preparation of the mint oil/β-cyclodextrin complex, co-precipitation structure enables cyclodextrins to form full inclusion complexes with small molecules or partial inclusion complexes with macromolecules, hence improving the physicochemical and biological properties of the complexed compound [9].

Cyclodextrins are widely utilized for improvement of solubility, stability, dissolution, bioavailability and drug loading. It provides faster onset of action when mixed with drugs and masks bitter taste of drug thereby making the preparation in compliance to patients. Moreover, cyclodextrins are biodegradable and can effectively protect the complexed substances against heat, decomposition and oxidation [10].

Though nanoparticles have been extensively used for different applications, there is an inevitable need to address the safety concerns pertaining to the use of nanoparticles in the health and food industry. Also release of nanoparticles in the environment is viewed as a potential threat to the environment [11]. Thus, the main objective of our study was to evaluate any cytotoxic effect of these synthesized nanoparticles on different *in-*

method was adopted as per the protocol mentioned by *Cristian dima et al*., 2014 [14]. A hydroalcoholic solution of β-cyclodextrin was prepared by dissolving 1g β-CD in 10 ml of water:ethanol solution (2:1v/v), at 55°C. Then 1g of mint essential oil and 0.2mmol of vitamin D (250 mcg, 10,000IU) were added to a mixture of beta-cyclodextrin and water: ethanol solution. The mixtures were then electromagnetically stirred for 30 minutes at 55°C followed by continuous stirring for 4 hours without heating. The samples were then incubated for 12 hours at 4°C. The precipitate formed was separated through vacuum filtration and was dried in an oven at 50°C for 24 hours. Further the resulting powder was dried in open air, at room temperature, for 3 days. The encapsulated mint oil/β- cyclodextrin complex powder was preserved in closed plastic bags at room temperature.

2.3. Antibacterial Activity Determination

The effect of encapsulated nanoparticles comprising mint oil and Vit D on microorganisms was assessed by the method of *Rajinder Singh et al*. [15]. Two Gram-positive (*Streptococcus Pyogenes* and *Corynebacterium Diphtheriae*), and two Gram-negative (*Shigella* and *Bacillus Marcescens*) bacteria were selected for antibacterial activity assay. The microorganisms were inoculated into sterile nutrient agar and incubated at 37ºC for 24 hours (hrs). Loop full of cultures of all the strains was taken and the turbidity of the resulting suspension was diluted with sterile saline. This level of turbidity was adjusted to approximately 3.0 x 10^8 Cfu/ml, equivalent to 0.5 McFarland turbidity standards. The effect of different concentrations of encapsulated nanoparticles (0.001 to 10 mg/ml) was investigated. Ampicillin, tetracycline and distilled water were used as positive and negative controls respectively. Further, the plates were incubated at 37ºC for 24 hrs and the activity was evaluated by measuring the width of the zone of inhibition of growth of organisms in comparison to control.

2.4. Cytotoxicity Analysis Using Cell Lines

The effect of nanoparticles encapsulating mint oil and Vit D was studied on cell lines by the method of *T. Mosmann* [16]. 3500 cells per 200µl of complete medium were seeded in a 96-well plate and the cells were incubated at 37°C in a CO_2 incubator. After 24 hrs, mint oil-Vit D nanoparticles were added to make up the final concentration ranging from 0.001 to 10 mg/ml. All experimental controls were also maintained. The final volume of the test sample added from the stock solution did not exceed 1%. After 24 hrs of incubation with the sample, 20µl of 5 mg/ml MTT stock solution was added to each well and formazan crystals were allowed to form for a period of 4 hrs at 37°C. The supernatant was then carefully discarded without losing the formazan crystals. Further the crystals were dissolved by adding 100µl of DMSO and absorbance was read using a Biorad microplate reader (version 680) at 570nm with a reference wavelength of 655 nm. The optical density (OD) obtained was used to calculate percentage viability when compared to the OD of control.

2.5. Cytotoxicity on Erythrocytes:

2.5.1. Preparation of RBC

Blood sourced from Blood bank was used for this study. Ten ml blood was centrifuged and the plasma along with a buffy coat was removed. The RBCs were washed thrice with phosphate buffered saline (PBS), and the tubes were centrifuged at 3000 rpm for 10

minutes to give an RBC pellet which was used to set up the experiments.

2.5.2. Experimental Setup

10% RBC solution was prepared in phosphate buffered saline (pH 7.4) with 5mM glucose (normoglycemia) and was further used to set up the experiment. 5ml of untreated 10% RBC solution was considered as control and different concentrations of encapsulated nanoparticles i.e. 0.5, 1, 5 and 10 mg/ml were incubated with the RBC solution as test samples. A set of tubes representing 100% hemolysis by adding distilled water to the RBC solution were also maintained. All the tubes were mixed well and incubated at room temperature for 24 hrs on a shaker at 30 rpm.

2.5.3. Estimation of the Effect of Nanoparticles on RBCs

The hemolysis induced by the nanoparticle was determined after 24 hrs of incubation. All the samples were centrifuged at 3000 rpm for 15 min and the absorbance of the supernatant was read at 540 nm. The hemolysate was used for all the other assays. Lipid peroxide level [17] and activities of superoxide dismutase (SOD) [18], catalase (CAT) [19], glutathione (GSH) [20], GSH peroxidase (GPX) [21], GSH reductase (GRD) [22] was determined using standard protocol. Total protein content of the hemolysate was estimated as per *lowry et. al*. [23].

2.6. Zebrafish Husbandry and Fish Embryo Toxicity Test

Wild-type Zebrafishes (*Danio rerio*) were maintained at 28 ± 1˚C with constant light conditions of 14:10 hrs (light: dark). The fishes were fed *Daphnia magna* and commercial dry flake food three times a day. The fish tanks were maintained regularly. Experimental animals were handled according to the method described by Westerfield (24). Breeding tanks were set up prior to the need for embryos. A ratio of 3:2 (females: males) was used for breeding. The healthy embryos were separated and transferred to sterile distilled water. The test was performed according to Organization for Economic Co-operation and Development (OECD) test guideline No.236 [25].

Ten healthy embryos were placed in 6-well culture plates and were exposed to each concentration of mint oil-Vit D nanoparticle i.e. 0.001, 0.005, 0.01, 0.05, 0.5, 1, 5, 10 mg/ml from 0-96 hours post fertilization (hpf) to reveal any possible toxicities of nanoparticles. Embryos in sterile distilled water was maintained as control. The plates were kept in the dark at 26 ± 2ºC. The embryos were observed at an interval of 24 hours for a period of 96 hours using an inverted microscope (Nikon eclipse, TS100) and stereomicroscope (Labovision, Ksf200). The embryos were documented for mortality, hatching, malformations and heart rate. Videos were recorded using high-definition video cameras to determine the heart rate.

2.7. Phytotoxicity Assay

The effect of encapsulated nanoparticles comprising mint oil and Vit D was evaluated for any possible phytotoxicity by the method of *Sarvendra-Kumar et al.* [26]. The seeds were treated with 1.2% sodium hypochlorite for 1 minute and then washed with distilled water three times to ensure surface sterility. Seeds in experimental plates were soaked with mint oil and Vit D coated with cyclodextrin solution at concentration of 10 mg/ml and those in the control plate were soaked in distilled water only. The plants were maintained for 7 days. Seeds were monitored for percent germination, root length and shoot length.

2.8. Statistical Analysis

Data are presented as Mean ± SEM. The significance of difference among the groups was assessed using one-way analysis of variance (ANOVA) test followed by Tukey's honest significant difference (HSD) post-hoc test by Graphpad Statistical Software version 6.0. In the figures and tables, symbols represent statistical significance as indicated: *p<0.05, **p<0.01, ***p< 0.001 and NS Non-significant.

3. RESULTS AND DISCUSSION

3.1. Antimicrobial Activity Assessment of Nanoparticle

Assessment of antimicrobial activity of nanoparticles is of considerable importance because of its probable interaction with biological systems. This has raised a concern because of increasing resistance of bacteria for existing antibiotics. Accumulating evidence suggests the application of nanoparticles as a promising approach to tackle the notorious cases of drug resistance [27, 28].

Thus, in the present study we have evaluated the consequences of varying concentration of our mint oil-vit D nanoparticles on growth of different microorganisms i.e. Corynebacterium diphtheriae, Streptococcus pyogenes, Bacillus marcescens and Shigella. All concentrations of nanoparticles did not exhibit any inhibitory activity against any bacterial species at concentration 0.5, 1, 5, 10 mg/ml after 24 hrs of exposure (Figure 1). The results obtained contradicts the previous studies which reported antimicrobial activity of mint oil against different bacterial strains [15, 29].

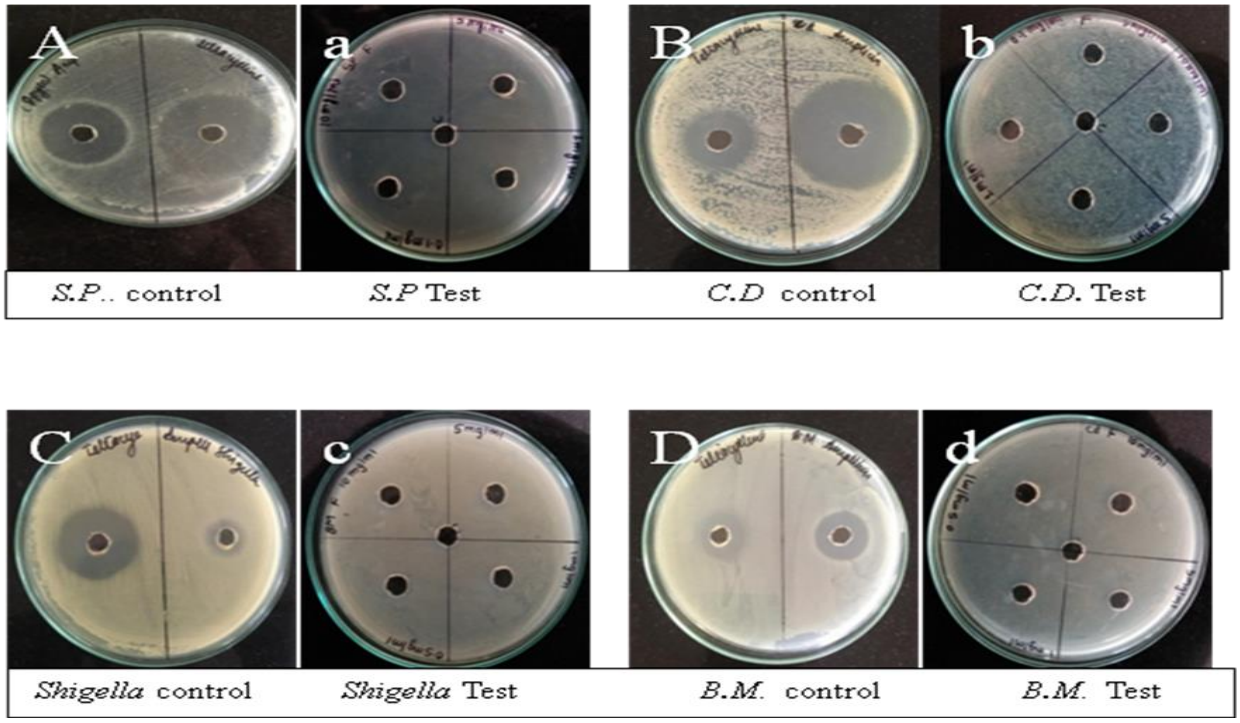

Figure 1. *Representative image of antimicrobial activity assay of mint oil-vit D nanoparticles on different microorganisms. Plate label signify A-a: Control and test plate for Streptococcus pyogenes (SP), B-b: Control and test plate for Corynebacterium diphtheriae (CD), C-c: Control and test plate for Shigella, D-d: Control and test plate for Bacillus marcescens (BM).*

3.2. Effect of Nanoparticles on Cell Lines:

3.2.1. Cytotoxicity Assay Using L929 Cell Line

L929 cells did not exhibit growth inhibition in a dose dependent manner when treated with mint oil-Vit D nanoparticles (Figure 2A). However, cytotoxicity assays illustrated significant ($p<0.001$) decrease in cell viability at higher concentration of nanoparticles i.e. 5 mg/ml and 10 mg/ml which inhibited the cell growth by 64 % and 72 % respectively when compared to control. The results obtained are concurrent with the results of previous studies indicating cytotoxicity of nanoparticles only at very high concentrations [30,31].

3.2.2. Estimating the Anticancer Potential of Nanoparticles:

Antiproliferative potential of mint oil-Vit D nanoparticles on cervical cancer cell line (HeLa) was analyzed *in vitro*. MTT assay determined the cytotoxic effects of nanoparticles by significantly ($p<0.001$) decreasing the cell viability of HeLa cells at higher concentrations i.e 5 and 10 mg/ml which inhibited the growth by 31 % and 37 % respectively when compared to control (Figure 2B). Lower concentration of nanoparticles did not exhibit any significant growth inhibition. The results acquired were analogous with the previous studies indicating the cytotoxic nature of mint extracts on cancerous cell lines at higher concentrations as reported by *Hajighasemi et.al.* [32].

3.3. Cytotoxicity Analysis on Erythrocytes

3.3.1. Effect of Mint Oil-Vit D Nanoparticle on Hemolysis

Hemolysis is a sign of hemoglobin leakage from erythrocytes indicating membrane injury leading to jaundice, anemia and other pathological conditions. Hence, it is important to analyze the hemolytic potential of synthesized nanoparticles. In our study, the percent hemolysis at nanoparticle concentrations of 0.5 and 1 mg/ml was comparable to control but there was a significant increase ($p<0.001$) in hemolysis at higher concentrations indicating the membrane disrupting activity of the nanoparticle (Figure 3A). Previous studies have reported the antihemolytic activity of mint oil which corroborates with our observations [33]. Hemolysis at higher concentrations may be attributed to the nanoparticle (β-cyclodextrin) being hydrophobic in nature can cause damage to the RBC membrane [34].

3.3.2. Effect of Mint Oil-Vit D Nanoparticle on Lipid Peroxidation and Antioxidant Enzymes

Lipid peroxidation is oxidative damage that affects cellular membranes causing RBCs to change their shape and when they squeeze through the smallest capillaries hemolysis takes place. There is a reduction in lipid peroxide levels expressed in terms of MDA at lower concentrations of mint oil-Vit D nanoparticle when compared to control. In contrast augmented levels of lipid peroxide was observed with higher concentrations of mint-Vit D nanoparticle (Figure 3B) which could be attributed to the hydrophobic nature of the nanoparticle. The presence of mint and Vit D may have prevented peroxidation in the lower concentrations since they are well known antioxidants [35].

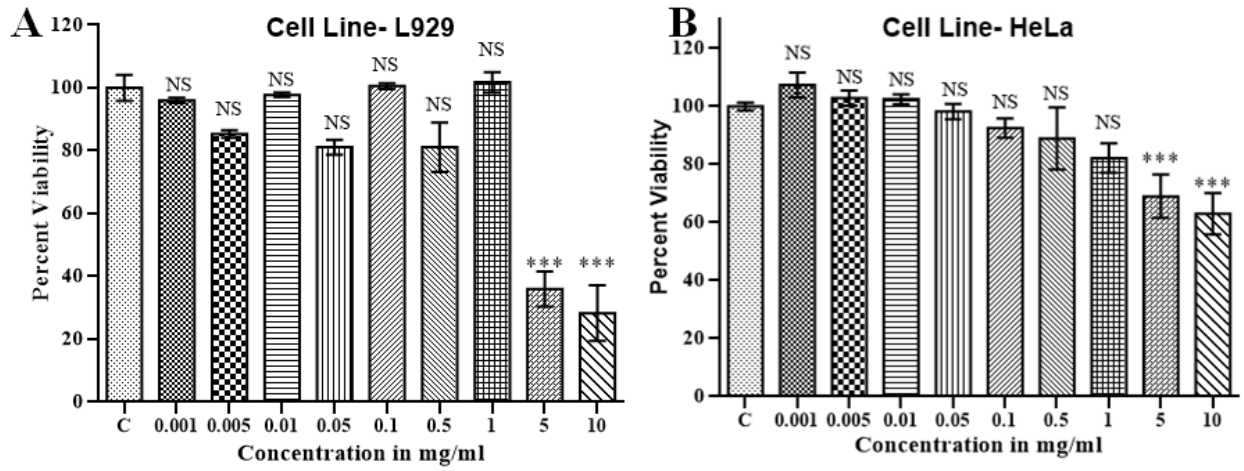

Figure 2. *Bar diagram representing effect of mint oil-vit D nanoparticle on L929 and HeLa cells lines. Data in the figure is expressed as Mean ± SEM and symbols represent statistical significance ***p< 0.001 and NS- Non-significant.*

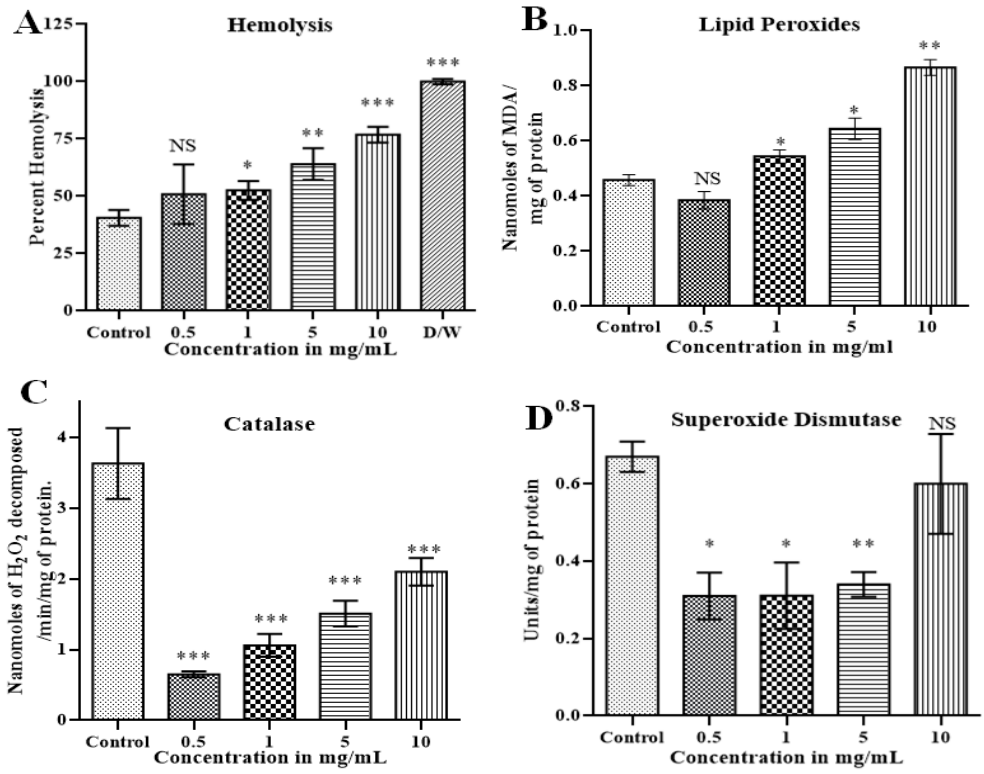

Figure 3. *Effect of mint oil-vit D nanoparticle on Percent hemolysis, levels of lipid peroxides, and antioxidant enzymes. Data is expressed as Mean ± SEM. Symbols in the figure represent statistical significance such *p<0.05, **p<0.01, ***p< 0.001 and NS Non-significant.*

Figure 3C and 3D represent the activities of different antioxidant enzymes SOD and CAT in the RBC hemolysate. SOD, being a primary scavenger of radical oxygen species, catalyzes the conversion of superoxide anions to H_2O_2 and CAT converts H_2O_2 to water. Our results depict significantly reduced activity of these enzymes at lower concentration as compared to control though the activity of enzyme increased with the higher concentration exposure. Bellassoued *et al*. 2018 also have reported a similar trend in rats treated with *Mentha piperita* L. leaf essential oil against CCl4 induced hepatic oxidative damage [36]. Our results suggest a dual role of mint oil- Vit D nanoparticles which at lower concentration inhibits oxidative stress leading to diminished activity of these antioxidative enzymes and vice versa at higher concentrations it led to an increase in reactive oxygen species (ROS) activity as also reported by *Ogaly et al* [37].
One of the classical non-enzymatic system which plays a significant role in protecting cells from excessive ROS generation includes GSH and GPX enzyme system. Figure 4A-4C represents the levels of GSH and activities of various enzymes associated with GSH. There is a significant ($p<0.01$) increase in the levels of GSH as compared to control displaying the mint oil's antioxidant potential has a sparing effect on GSH utilization. Also our results displayed an enhanced activity of GPX only at higher concentrations of the drug. Further there is a significant reduction in the GRD (glutathione reductase maintains adequate levels of reduced GSH) activity in the presence of mint oil-Vit D nanoparticle which suggests antioxidant potential of mint oil-Vit D nanoparticle providing alleviation from oxidative stress. Our studies in the RBC model system indicates that lower concentrations of nanoparticles do not cause toxicity to the erythrocytes but rather protect the cells by supplementing antioxidant activity. However, further studies are warranted at higher concentrations of nanoparticles.

3.4. Studies on Zebrafish Model System

3.4.1. Percentage Survival Assay

The zebrafish is extensively used as a model system for developmental and environmental toxicology studies due to its small size, high fertilization rate, less time consuming, cheap and rapid external development of transparent embryos [38,39]. In our study, a percentage survival assay was performed to investigate the toxicity of mint oil-Vit D nanoparticle on zebrafish embryos. Mortality was investigated considering possible signs of loss such as coagulation of eggs, lack of tail detachment, hatching of the embryos, tail malformation and absence of heartbeat. Mortality was observed for every day of exposure i.e. 24, 48, 72 and 96 hrs time point at all the concentrations of mint oil-Vit D samples. Kaplan- Meier survival curve showed that mortality was dose dependent. In our study, the major lethal effect was coagulation of the egg. Lowest mortality of 8% was recorded in 0.001, 0,005, 0.01, 0.05 mg/ml of mint oil-Vit D nanoparticle sample whereas highest mortality of 100% was recorded at 10mg/ml of the mint oil-Vit D nanoparticle after 24 hrs (Figure 5). Our study was in accordance with *Borges et al.* 2018 that dose dependent mortality of zebrafish embryos are evident when treated with higher concentrations of *Rosmarinus officinalis's* essential oil [40].

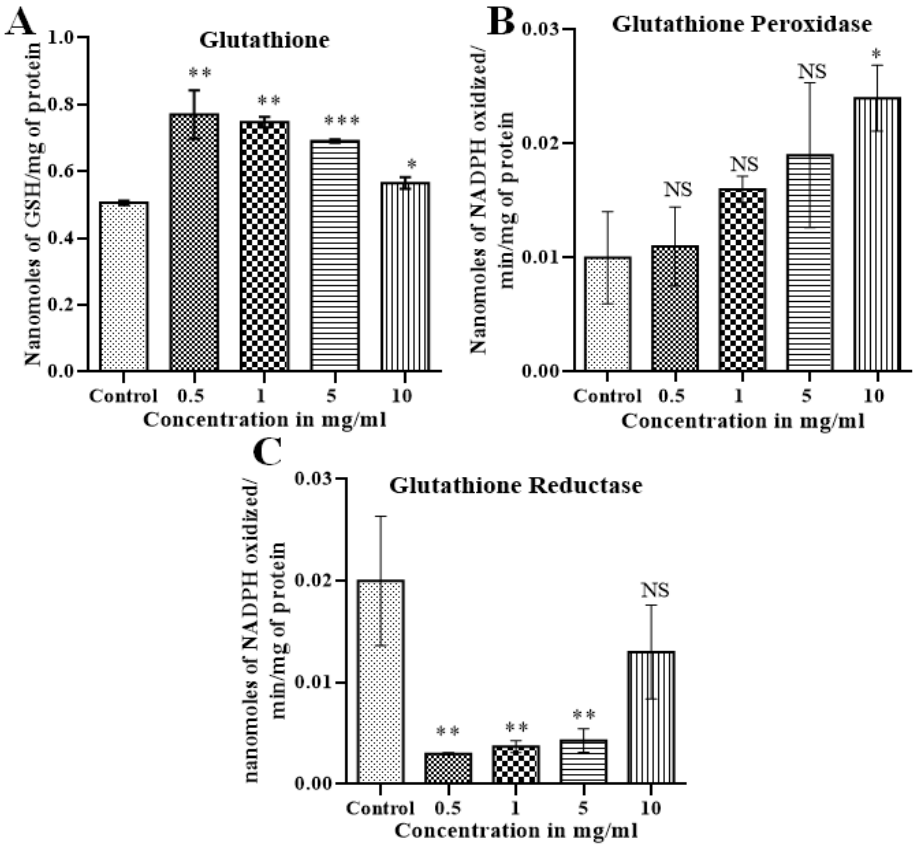

Figure 4. *Effect of mint oil-vit D nanoparticles on levels of GSH and GSH dependent antioxidant enzymes activities in RBCs. Data is expressed as Mean ± SEM. Symbols in the figure indicates statistical significance *p<0.05, **p<0.01, ***p<0.001 and NS Non-significant.*

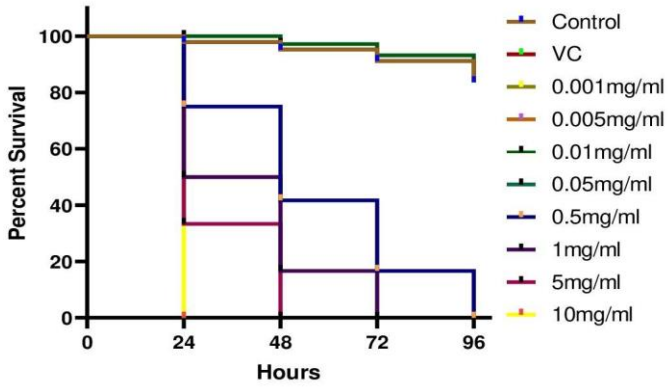

Figure 5. *Kaplan Meier survival graph representing percent survival in zebrafish embryos upon exposure to mint oil-Vit D nanoparticle.*

3.4.2. Hatching Assay

Numerous internal and external factors including presence of various toxic chemicals in the aquatic environment can affect the phenomenon of hatching which is a crucial stage of reproduction in fishes [41]. In our study, the fertilized embryos were treated with different concentrations of nanoparticles and were monitored for hatching at 48, 72 and 96 hrs time points (Figure 6A). The normal embryos had a hatching period from 48 to 72 hpf. At a lower dose of nanoparticle, normal hatching of embryos were observed whereas upon higher dose exposure no hatching of embryos were recorded due to higher percentage mortality.

The percent hatching at 48 and 72 hpf for the lowest dose i.e. 0.001 and 0.005 mg/ml of mint oil-Vit D nanoparticle showed 53% and 94% hatching respectively although at dose 0.01 and 0.05 mg/ml of mint oil-Vit D nanoparticle, it showed 47% and 92% hatching of the embryos respectively. Higher doses (0.5, 1, 5, 10 mg/ml) of mint oil-Vit D nanoparticle showed restricted hatching of embryos.

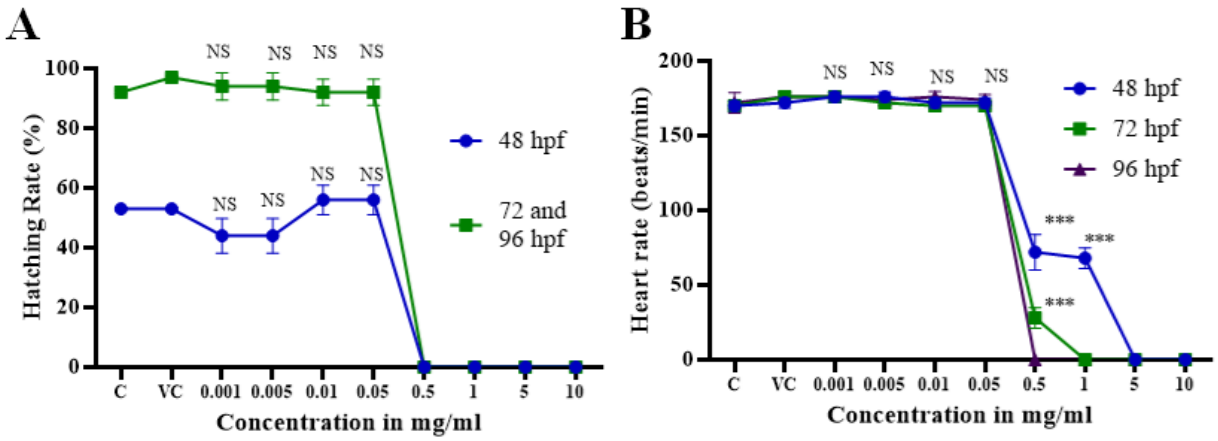

Figure 6. *Effect of mint oil-Vit D nanoparticle on hatching and heartbeat rate of zebrafish embryos. Data is expressed as Mean ± SEM. Symbols represent statistical significance, ***p< 0.001 and NS Non-significant.*

3.4.3. Heartbeat Rate Assay of Zebrafish

The development of the heart is the first step of organogenesis in *Danio rerio*. The assessment of heart beat rate is crucial in evaluating cardiac function since any change in rhythm of the heart is suggestive of the underlying state of the heart. Any variation in heart rate indicates cardio-toxicity, and hence zebrafish embryos are instrumental to assess both cardio and neuro-toxic effects of environmental pollutants or pharmacological compounds during drug discovery. The normal embryonic heart beat rate in zebrafish is 120–180 beats per minute [42]. After 48, 72 and 96 hpf, heart rate seems to be unchanged at the lower concentrations of mint oil-Vit D nanoparticle (0.001, 0,005, 0.01, 0.05 mg/ml) whereas at the highest

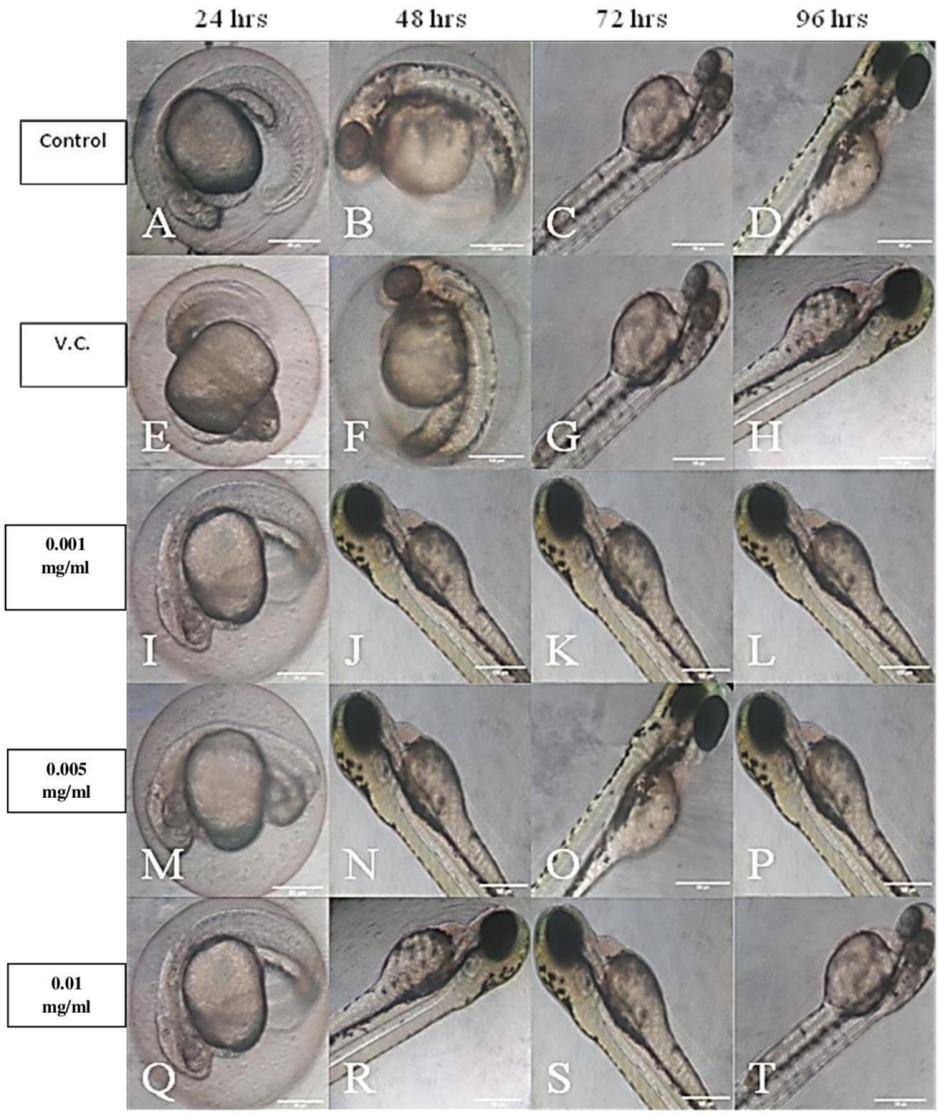

Figure 7A. *Photomicrograph of early embryonic development of Zebrafish embryo exposed to various concentrations of mint oil-Vit D nanoparticles at various time points. The picture in the plate represents: A-D: Control; E-H: Vehicle Control; I-L: 0.001 mg/ml; M-P: 0.005 mg/ml; Q-T: 0.01 mg/ml at 24, 48, 72 and 96 hrs respectively.*

concentrations i.e. 5 and 10 mg/ml of nanoparticle, there was 100% larval mortality and hence no heartbeat was recorded for this time point, at 48 hpf. Though at the concentrations 1 and 0.5 mg/ml there was a decrease in heart rate which seems to be significant at 72 and 96 hpf respectively (Figure 6B). Similarly, a report by *Kim et al.* 2012 demonstrates the teratogenicity of ajowan oil in zebrafish [42].

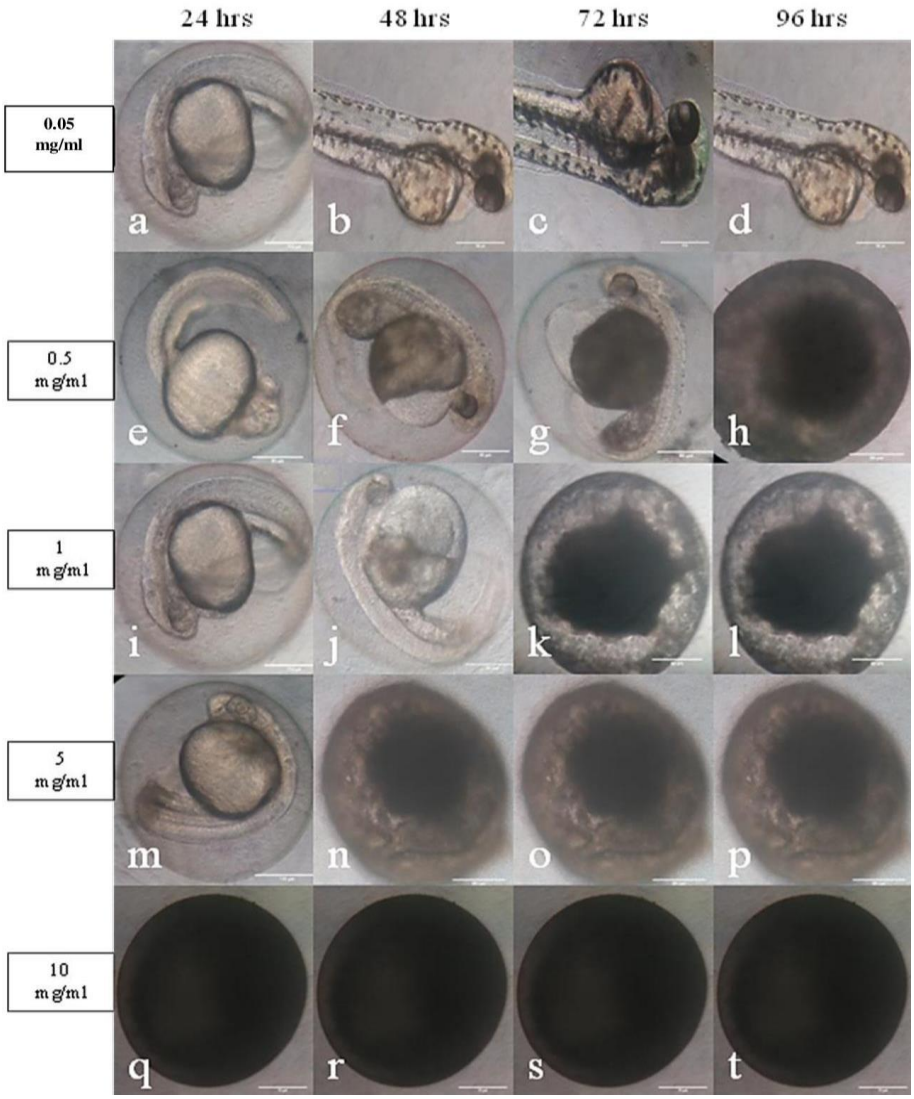

Figure 7B. *Photomicrograph of early embryonic development of Zebrafish embryo exposed to various concentrations of mint oil-Vit D nanoparticles at various time points. The picture in the plate represents: a-d: 0.05 mg/ml; e-h: 0.5 mg/ml; i-l: 1 mg/ml; m-p: 5 mg/ml; q-t: 10 mg/ml at 24, 48, 72 and 96 hrs respectively.*

3.4.4. Pericardial Edema

A pericardial edema is an accumulation of fluid connecting the heart and the sac which is encompassing the heart, known as the pericardium. Due to space limitation in the pericardial cavity, fluid accumulation results in enhanced intrapericardial pressure which can affect heart function negatively [43].

Edema was monitored in the live embryos using microscopic photographs. From our results, pericardial edema seemed to be classic cardiac toxicity induced by mint oil-Vit D nanoparticle. The pericardial edema of the embryos was significant at the concentrations 1 and 0.5 mg/ml as compared to the control. The pericardial edema was observed in 1 mg/ml at 48 hpf although in 0.5 mg/ml at 48 and 72 hpf. (Figure 7A and 7B).

3.5. Phytotoxicity Assay

The potential of environmental impacts of synthesized nanoparticles has to be addressed by a suitable phytotoxicity assay. The toxicity of mint oil-Vit D nanoparticle at highest concentration (10 mg/ml) on percent seed germination of *Triticum aestivum, Zea mays* and *Pisum sativum* seeds was not evident. (Figure 8).

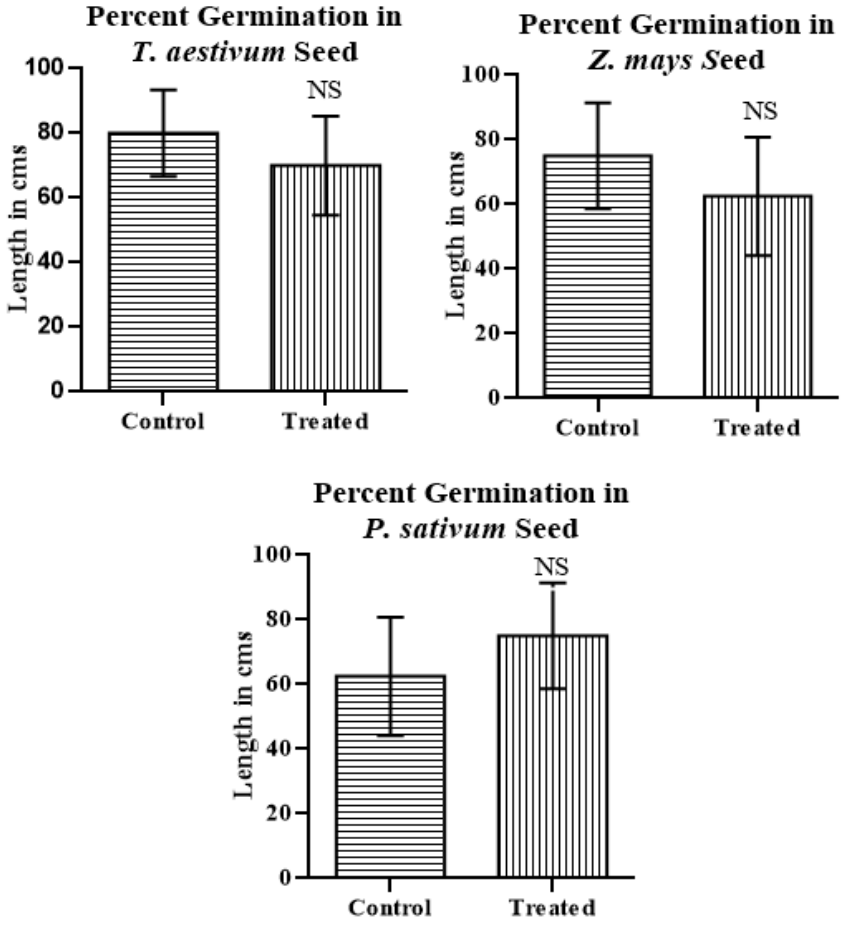

Figure 8. *Bar diagram representing effect of mint oil-Vit D nanoparticle at 10 mg/ml concentration on percent germination of Triticum aestivum, Zea mays and Pisum sativum seeds. Data is expressed as Mean ± SEM. Symbols represent statistical significance NS - Non-significant.*

Table 1*. Repercussion of mint oil-Vit D nanoparticle at 10 mg/ml concentration on shoot and root length of Triticum aestivum, Zea mays and Pisum sativum seeds. Values were expressed as mean ± SEM. Symbols in the table represents statistical significance ***p< 0.001 and NS indicates non-significant.*

Seeds	Control shoot length (cm)	Test shoot length (cm)	Control root length (cm)	Test root length (cm)
Triticum aestivum	2.63 ± 0.74	0.11 ± 0.001***	3.44 ± 1.10	1.018 ± 0.32***
Zea mays	7.49 ± 1.28	2.66 ± 1.125***	4.007 ± 2.53	1.740 ± 1.0***
Pisum sativum	2.23 ± 1.24	2.37 ± 0.94 NS	2.43 ± 1.67	2.340 ± 1.04 NS

Reduction in the root and shoot length is considered to be a more sensitive parameter as compared to seed germination. Further the toxicity on root and shoot length of all the treated and control seeds were monitored for the period of seven days. The results suggested that the exposure of 10 mg/ml concentration significantly reduced the growth of *T. aestivum* and *Z. mays* seedlings whereas the seedlings of *P. sativum* were found to be resistant in the presence of nanoparticles (Table 1). The results obtained were synchronous with the previous studies indicating the phytotoxicity of essential oils on selected food crops by *Ibanez et al.* [44].

4. CONCLUSIONS

Our present research findings suggest that the synthesized mint oil-Vit D nanoparticles possess no antimicrobial activity. Albeit exposure to higher concentrations of these nanoparticles proved to be cytotoxic, phytotoxic, hemolytic and lethal in nature and lower concentrations were found to have a zilch effect. Thus, it will be very prudent to exploit the applications of these mint oil-Vit D encapsulated nanoparticles at lower concentrations. These results confer additional evidence that essential oils- Vit D loaded nanoparticles can cause functional damage if applied at non-recommended doses. Further studies to establish safe fortification of these nanoparticles in food industries in order to retain medicinal property from mint oil along with recommended dietary levels of Vit D are imperative and warranted.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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