Research Article

Synthesis Characterization of Vitamin C Doped MgS Nanoparticles and Toxicity Assessment against Freshwater Fish *Oreochromis mossambicus*

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ABSTRACT

The nanoparticle used for drug delivery and other medical related are increasing widely. But their toxic nature should be evaluated for prescribing them to medical usage. In the present study, an attempt was made to analyze the toxic nature of vitamin C doped MgS nanoparticle against freshwater fish *Oreochromis mossambicus.* Vitamin C doped MgS nanoparticle was synthesized by chemical method and its characteristic feature was analyzed by Scanning Electron Microscope. The size of particles was in the range of 191 nm to 405nm. Biochemical parameters such as carbohydrate, protein and DNA levels were estimated in muscle, gill and liver tissues of vitamin C doped MgS nanoparticle treated fishes *Oreochromis mossambicus*. An increased level of biochemical parameters such as carbohydrate, protein, DNA and liver marker enzymes such as ALP, ACP and bilirubin was observed and it confirmed that vitamin C doped NgS nanoparticle was toxic to freshwater fish *Oreochromis mossambicus.* The overall study concludes that the vitamin C doped MgS nanoparticle is toxic to the fresh water fish *Oreochromis mossambicus* at lethal dose.

Keywords: Nanotechnology, Nanoparticles, Toxicity, MgS and *Oreochromis mossambicus.*

INTRODUCTION

Nanotechnology is an emerging field with application in the field of Science and Technology for the purpose of manufacturing new materials at the nanoscale level[1]. Nanotechnology is an interdisciplinary field and by the convergence of Physics, Chemistry and Biology. When the size of the particles is reduced the physicochemical parameters of the particles is changed favorably. In fact, surface area to volume ratio and quantum behavior is the most important characteristics in the field of nanotechnology that are seen when the particles to shrink at nano scale level[2]. Nanoparticles have beneficial effects for many purposes but due to the toxic nature their utilization in medical field needs to be thoroughly evaluated. Many nanoparticles are proved for their anticancer effect, antibiotic effect and efficient drug delivery mechanism.

Accidentally or intentionally nanoparticles are released into the environment has potential ecological effects[3, 4]. The unique physical and chemical properties of NPs are often related with ecological toxic effects in environment and unexpected health hazards[5, 6]. Moreover, increased releasing of NPs into the environment, it is essential to assess the toxicity of NPs using various tests organisms[7, 8]. Evaluation of their environmental impact accurately is necessary to adopt organisms, as well as understanding the effects of NP mixtures. Some of the research has explained the toxic nature of NPs using bioassays[9, 10]. For example, the widely used NPs TiO2 and ZnO have toxic effect to microalgae[11, 12]. Many investigations were conducted on the toxicity of silver and platinum NPs and carbon nanotubes on terrestrial animals and bacteria[13, 14]. Thus, the present study was made an attempt to evaluate the toxic nature of vitamin C doped MgS nanoparticle on fresh water fish *Oreochromis mossambicus*.

MATERIALS AND METHODS

Synthesis of vitamin C doped MgS Nanoparticles

The Vitamin C doped MgS nanoparticles were synthesized by using chemical method[15].

Vitamin C doped MgS nanoparticle characterization

The characterization study of Vitamin C doped MgS nanoparticle done through the SEM study method.

Collection of stock Fishes

Tilapia fishes (*Oreochromis mossambicus*) were collected from the local pond in Thiruvellarai, Trichy District. The fishes were fed regularly with artificial feed and excess of feeds were removed by transferring water for every day morning.

Toxicity studies

The fishes were divided into six groups and each group contains six fishes. The experimental set up was

Group I was served as control.

Group II: Treated with 0.1 mg of Vitamin C doped MgS nanoparticles.

Group III: Treated with 0.3 mg of Vitamin C doped MgS nanoparticles.

Group IV: Treated with 0.5 mg of Vitamin C doped MgS nanoparticles.

Group V: Treated with 0.7 mg of Vitamin C doped MgS nanoparticles.

Group VI: Treated with 0.9 mg of Vitamin C doped MgS nanoparticles respectively.

The behavior changes of MgS nanoparticles toxicity injected fishes were observed at regular interval and recorded.

Toxic effect on Biochemical profiles

Toxic effect of MgS nanoparticlse in *O. mossambicus* was evaluated by measuring ALP, ACP level[16] and bilirubin, carbohydrate[17], and protein[18] in liver with the different concentrations 0.1mg, 0.3mg, 0.5mg 0.7mg and 0.9mg (lethal dose). Further DNA levels in liver, gills and muscle were estimated by the method of Burton[19] and bilirubin level in kidney tissue was also estimated by the method of Malloy and Evelyn[20].

RESULTS AND DISCUSSION

The figures of vitamin C doped MgS nanoparticles were easy to notice that the examined particles consist of a number of smaller objects however, the size varied from 191nm to 405nm (figure 1).

Table 1: The LD_{50} value of Vitamin C doped MgS nanoparticle treated freshwater fish *Oreochromis mossambicus*

Concentration (mg)	No. of Fish	Observed mortality (%)	Expected mortality (%)	LD_{50}	95% confidence limit		Chi square
					Lower	Upper	
0.1	6	33.5	27.0	3.301	-0.3902	5.736	23.188
0.3	6	50	46.7				
0.5	6	50	67.2				
0.7	6	83	83.6				
0.9	6	100	93.4				

Table 2: Estimation of Carbohydrate levels of fresh water fish *Oreochromis mossambicus* treated with Vitamin C doped MgS nanoparticle

Groups (mg/ml)	Muscle (mg/gm)	Gill (mg/gm)	Liver (mg/gm)
Control	3.8 ± 0.21	2.6 ± 0.18	5.5 ± 0.20
0.1	3.4 ± 0.15	2.5 ±0.13	5.5 ± 0.18
0.3	3.3 ±0.12	2.2 ±0.08	4.3 ± 0.15
0.5	2.9 ± 0.06	2.2 ± 0.11	3.3 ± 0.13
0.7	2.7 ± 0.05	2.1 ±0.07	3.0 ± 0.06
0.9	2.3 ± 0.09	1.9 ± 0.06	2.0 ± 0.05

Table 3: Estimation of Protein levels of fresh water fish *Oreochromis mossambicus* treated with Vitamin C doped MgS nanoparticle

Groups (mg/ml)	Muscle (mg/gm)	Gill (mg/gm)	Liver (mg/gm)
Control	5.7 ± 0.19	4.4 ± 0.15	6.2 ± 0.06
0.1	5.2 ± 0.07	4.1 ± 0.08	6.0 ± 0.04
0.3	5.1 ± 0.08	3.9 ± 0.07	5.9 ± 0.05
0.5	4.8 ± 0.05	3.8 ± 0.08	5.5 ± 0.09
0.7	4.7 ± 0.03	3.7 ± 0.04	4.9 ± 0.09
0.9	4.2 ± 0.07	3.3 ± 0.08	4.3 ± 0.03

There are many types of nanomaterials are synthesized by scientific community and are making observation on nanoparticle ecotoxicity, risks and benefits. High concentrations of nanoparticles have been showed inhibition of growth of algae[21] and aggregation of nanoparticles can found in the cell walls[22]. The health effects induced by metal oxide nanoparticles are ability to cause oxidative stress[23, 24]. This mechanism is believed to important in the toxic nature of manufactured nanoparticles. Furthermore, oxidative stress is directly associated with DNA damage, mutations and also cancer.

In the present study, LD_{50} value and biochemical changes caused by vitamin C doped MgS nanoparticle treated on fresh water fish *Oreochromis mossambicus* were evaluated. The median lethal dose of (LD_{50}) vitamin C doped MgS nanoparticle in tilapia fish was about 0.5mg/10mg of fish (table 1 and figure 2). The toxicity of silver, gold and other nanoparticles were proved to animals by Ganapathi and Alikunhi[25]. High level chemicals led to mortality in fishes by coagulating the gill mucus. Nanoparticles are considered as a problematic in the toxicity assessment for several reasons[24, 25, 26].

Table 4: Estimation of DNA content of fresh water fish *Oreochromis mossambicus* treated with Vitamin C doped MgS nanoparticle

Groups (mg/ml)	Muscle (µg/gm)	Gill (µg/gm)	Liver (µg/gm)
Control	2.8 ± 0.03	1.9 ± 0.04	3.7 ± 0.06
0.1	2.4 ± 0.05	1.6 ± 0.06	3.4 ± 0.10
0.3	2.2 ± 0.05	1.3 ±0.04	2.9 ± 0.05
0.5	2.2 ± 0.09	1.2 ± 0.07	2.4 ± 0.05
0.7	1.9 ±0.08	0.9 ± 0.05	2.1 ± 0.04
0.9	1.9 ± 0.03	0.8 ± 0.04	1.9 ± 0.11

Table 5: Results of One way Analysis of Variance (ANOVA) show the effect on biochemical parameters of vitamin C doped MgS nanoaprticles treated fishes.

Parameters	Sources	Sum of Squares	Df	Mean Square	F	Sig.
Carbohydrate	Between Groups	22.651	5	4.53	5.650	0.000*
	Within Groups	37.683	47	0.80		
	Total	60.334	52			
Protein	Between Groups	13.411	5	2.68	4.906	0.001*
	Within Groups	25.696	47	0.55		
	Total	39.107	52			
DNA	Between Groups	10.483	5	2.09	4.676	0.002*
	Within Groups	21.074	47	0.45		
	Total	31.557	52			

Significant level @0.05

Table 6: Estimation of Liver enzymes of fresh water fish *Oreochromis mossambicus* treated with Vitamin C doped MgS nanoparticle

Groups (mg/ml)	ACP (U/L)	ALP (U/L)	Bilirubin (U/L)
Control	7.3 ± 0.08	31.6 ± 0.72	8.4 ± 0.04
0.1	7.7 ± 0.07	32.5 ± 0.59	7.4 ± 0.09
0.3	8.3 ± 0.12	32.5 ± 0.27	11.2 ± 0.15
0.5	9.1 ± 0.09	33.8 ± 0.14	11.9 ± 0.06
0.7	11.4 ± 0.27	34.27 ±0.10	12.8 ±0.06
0.9	14.3 ± 0.14	35.9 ± 0.30	13.2 ± 0.13

Table 7: Results of One way Analysis of Variance (ANOVA) show the effect on liver marker enzymes of vitamin C doped MgS nanoaprticles treated fishes.

Parameters	Sources	Sum of Squares	Df	Mean Square	F	Sig.
ACP	Between Groups	107.843	5	21.57	343.29	0.000*
	Within Groups	0.754	12	0.06		
	Total	108.597	17			
ALP	Between Groups	37.709	5	7.54	14.12	0.000*
	Within Groups	6.411	12	0.53		
	Total	44.120	17			
Bilirubin	Between Groups	83.845	5	16.77	595.23	0.000*
	Within Groups	0.338	12	0.03		
	Total	84.183	17			

Significant level @0.05

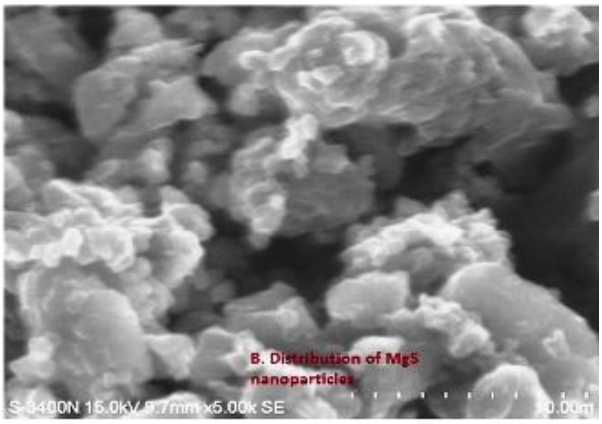

Figure 1: SEM image of Vitamin C doped MgS nanoparticles

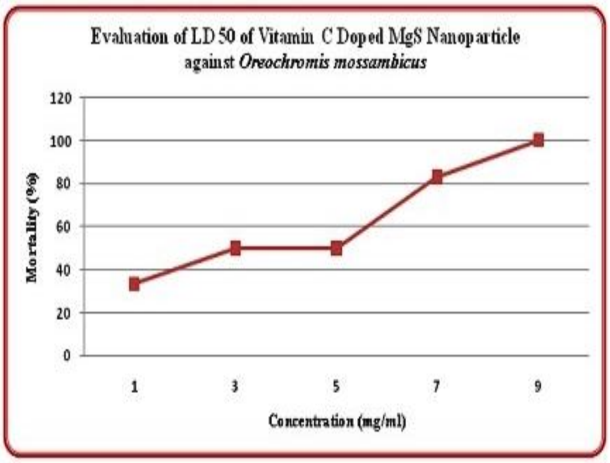

Figure 2: LC_{50} value of Vitamin C doped MgS nanoparticles against fresh water fish *Oreochromis mossambicus*

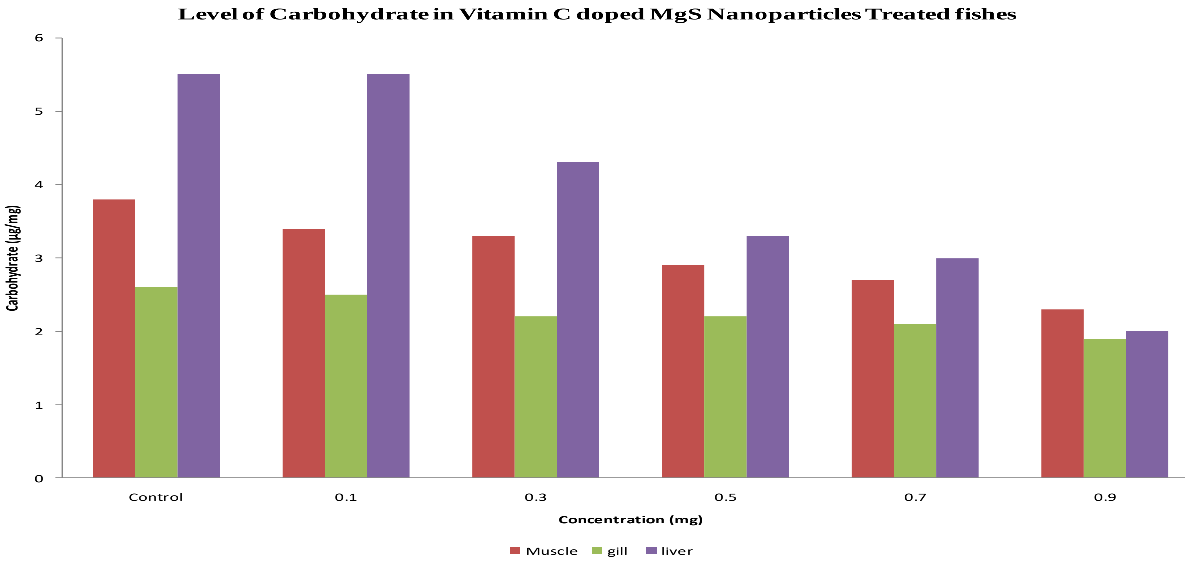

Figure 3: Carbohydrate level in different tissues of vitamin C doped MgS nanoaprtices treated fish *Oreochromis mossambicuss*

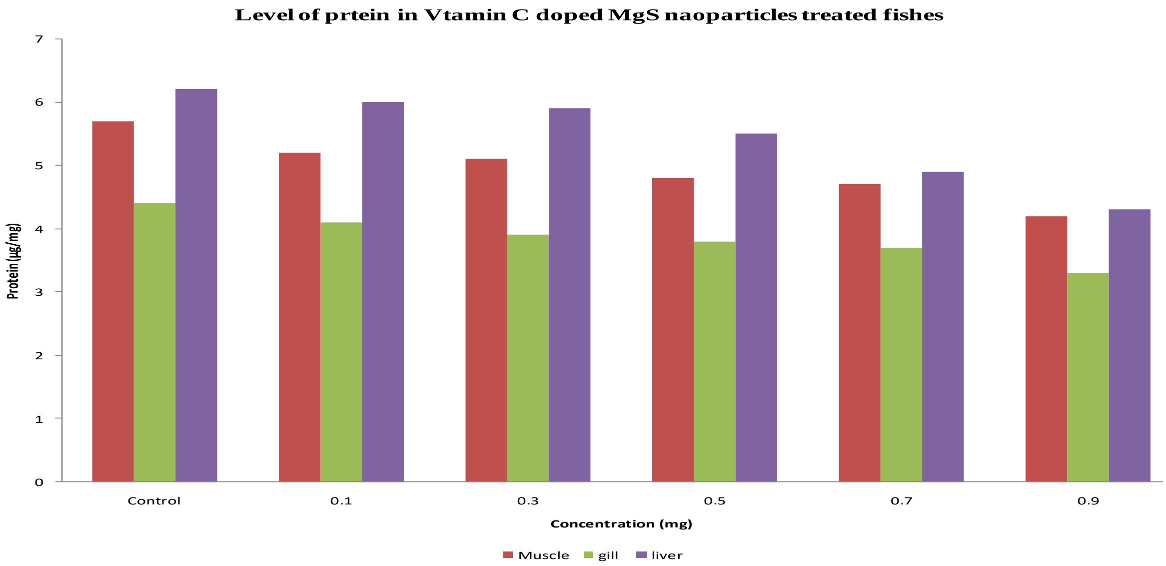

Figure 4: Protein level in different tissues of vitamin C doped MgS nanoaprtices treated fish *Oreochromis mossambicuss*

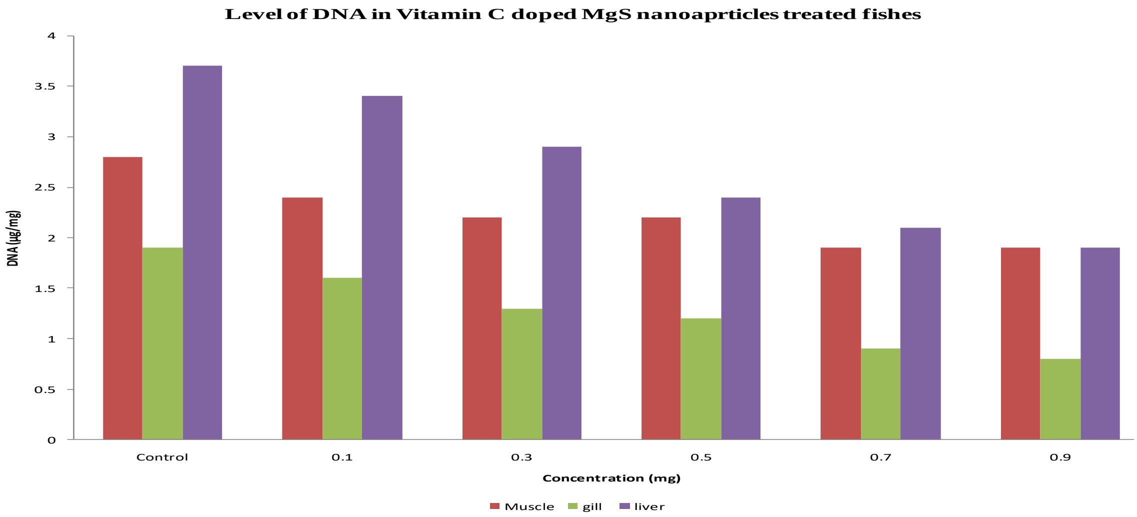

Figure 5: DNA level in different tissues of vitamin C doped MgS nanoaprtices treated fish *Oreochromis mossambicuss*

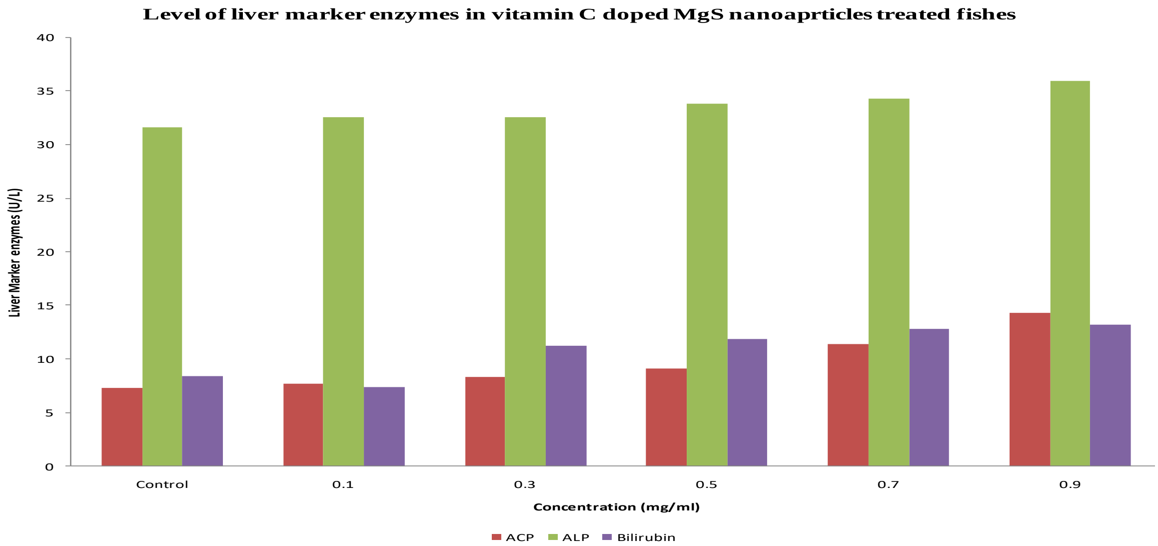

Figure 6: Level of liver marker enzymes in different tissues of vitamin C doped MgS nanoaprtices treated fish *Oreochromis mossambicuss*

The nano size and relatively large surface area have been suggested increase in toxicity, as compared to micrometer sized particles; however, it is not clear the toxicity of nanoparticles is a common feature of all kinds of nanoparticles with different chemical composition. Many nanoparticles known as an antibacterial agent have been shown to toxic to aquatic organisms. Silver nanoparticles are possessed medicinal values however they having toxic nature to fishes. Metal nanoparticles have unique properties due to their size, aggregation characteristics, and chemical composition[27, 28, 29] that differ from their respective soluble metal.

In the present study, a decreased amount of carbohydrate, protein and DNA content in the tissues like muscle, gill and liver of *Oreochromis mossambicus* observed when treated with vitamin C doped MgS nanoparticle. The one way ANOVA test showed that there was no significant different among the all concentrations of vitamin C doped MgS nanoparticles (Table 2-5). When concentration of nanoparticle increases the biomolecules such as carbohydrate, protein, DNA contents decreases (figurer 3- 5).

On the other hand, an increase in ALP and ACP were observed in vitamin C doped MgS nanoparticle dosed fishes which denotes the toxic that impair bile formation and to a lesser extent in hepatocellular disease (table 6,7 and figure 6). One way ANOVA test also proved the similarities among the all concentration of vitamin C doped MgS nanoparticles. In the gills of *Notopterus notopterus,* maximum significant inhibition (80.76%) in acid phosphatase occurred *in vitro* effects of four sub lethal concentrations of mercury[30]. The phenol, dinitrophenol and mixtures of both, inhibited the activity of acid phosphatase in the kidney, heart, brain, gills, muscles, stomach and pyloric caeca[30]. Sastry *et al*[31] have reported that acid phosphatase activity was inhibited in liver, ovary and gills following exposure to 0.26 mg/l of cadmium. In the present study, the liver enzymes ACP, ALP and bilirubin levels were increased when the fishes are treated with vitamin C doped MgS nanoparticle.

CONCLUSION

From the present study, it is concluded that the vitamin C doped MgS particles are about 191 to 405 nm further they are toxic to *Oreochromis mossambicus* at some extend. Hence, it is very important to resynthesize the MgS nanoparticle with some other inert materials to avoid the toxicity of nanoparticle.

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