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IN VIVO TOXICITY ASSESSMENT OF SILICA NANOPARTICLES IN CHICK EMBRYO

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Abstract

Silica is one of the most effective stored seedand food grain protecting pesticide agent, usedfor ages Silica nanoparticles are hard and strong andresistant to brittle fracture under an imposed stress. The aim of our study is to synthesize and evaluate the toxicity of silica nanoparticles at various concentrations using chick embryo, as it serves as a bridging model between in vivo and ex vivo studies. Silica nanoparticles of 70 nm sizewith concentrations of 10, 20, 40 and 80 PPM were injected in vivo and incubated for 19 days as per IACUC guidelines. The study was carried out on dissected chick embryo after the 19th day of incubation. Vital tissues such as liver and heart tissues were subjected to toxicity assays. Biochemical assay of antioxidant enzymes like superoxide dismutase and glutathione peroxide were assessed. The results showed a decreased level of MDA (Malondialdehyde), an end product of lipid Peroxidation, whereas increased activity of superoxide dismutase and glutathione peroxide were observed. Therefore silica nanoparticles may be used as a pesticide and also as a biomaterial for therapeutic application in the field of medicine.

Keywords *Silica nanoparticles, Chick embryos, lipid Peroxidation, Superoxide dismutase, Glutathione peroxidase.*

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Introduction

Nanoparticles are now being employed in various consumer goods, increasing exposurenot only at work place, but also in homes. Increasing attention has been given to food safety worldwide due to the important link between food and health. The unique property found in nanoparticles comparatively to its counterpart ultrafine particles favors the exponential growth and the demand for nanosized particles especially in industrial applications. There has been increasing concern regarding the safety of nanoparticles due to their size and exposure to humans, with the growing commercialization of nanotechnology products. The pure silica is colorless and transparent and has a vitreous luster. They are nonconductors of electricity and are diamagnetic. Synthesis of silica nanoparticles (SNPs) is one of the recent developments for various applications like drug delivery for various biomolecules, due to their unique properties andthe mesoporous and amorphous nature. The large surface area of the pore in the particles allows the particles to be filled with a drug and targeted particularly in mesoporous form. The nature of the SNPs allow them to be used as biosensors,in this case the fluorescent dye filled in the particlesbeing able to pass through the cell walls because of the optically transparent nature of the particles. These particles would display unique chemical and physical properties in nanoscale size, that could interfere with the biological activity and may cause potential adverse health effects in living organisms. There is no reported study that assesses the effect of particle size on a comprehensive set of biological responses. No conclusive data have been established to prove that the nanomaterials are either safe or harmful to human health, even though engineered nanomaterials have been found to induce toxicity. These aspects areurging the necessity of

studies for establishing a safety archetype. Chick embryo serves as a bridging model between *in vivo* and *ex vivo* studies due to its high gene homology to humans. Chicken embryo also is a unique biological model because it has no external nutrient supply as it is independent from its mother. Moreover, the embryo's development is very fast, intensive and quite familiarly described [1]. Even very small amounts of toxic substance are found to be harmful to the embryo making them really sensitive. This avian model has been used in medical, toxicological and also nutritional experiment as a primary investigation, prior to experiments with animals or humans [2].Thus, in order to detect possible adverse health effects due to nanomaterials application there is an urgent need to find their toxicological impact and develop early indicators. Toxicity tests are carried out routinely for chemical compounds prior to their release to the public and for nanomaterials no such procedure currently exists [3]. Simple pre-screening tests should be included in the proactive development of nano based materials [4]. We therefore evaluated an experimental model based on chick embryo for the study of SNPs toxicity.

Experiment Details

Silica Nanoparticles (SNPs). The nanoparticles used in the present study were synthesized in our laboratory. Synthesis and characterization of SiO_2 nanoparticles were performed by stober method and characterization was done as per the protocol of [5]. SNPs size ranged from 70-80 nm, with a purity >98.0% were used in the present study. The SNPs suspensions were sonicated for 30mns in a sonicator for 30 mns (100 W, 40KHZ) to disperse the particles. The concentrations of 10,20,40,80 PPM of Silica nanoparticles were used in the present study.

Chick Embryo Collection. The chick embryos of breed BV 380 were collected from Regal Poultry Farms, Coimbatore and acclimatized to lab conditions for a day or two.

Treatment. Thirty fertilized 3-days old chick embryos were taken and randomly divided into 5 groups, each with 6 eggs; group I (control) – not treated, group II – 10ppm concentration of SNPs with 0.9% saline, group III- 20ppm concentration ofSNPs with 0.9% saline, group IV - 40ppm concentration of silica nanoparticles with 0.9% saline, group V- 80ppm concentration of SNPs with 0.9% saline. Experimental solutions were given *in vivo* by injecting into albumen (Plate 2). Eggs were hold by hand in a vertical position, the area of injection (at 2/3 of egg's high from blunt ends) was disinfected with 70% ethanol, a little hole was made and 0.3 ml of SNPs solutions with different concentration were injected using sterile 1ml tuberculin syringe and 26 gauge, 1/2 inch needle. After being injected , the injection holes were sealed with wax, and eggs were placed in the incubator and incubated at standard conditions (temperature 37.7°C, humidity 60%, turn once per hour in first 18 days, and later at temperature 37°C and humidity 70%).

Collection of samples. The incubation was done till the embryo reached the19th day The Heart and Liver tissues of the embryos were dissected out and frozen with liquid nitrogen and stored at -20°C until usage. The frozen tissues were washed with phosphate buffer solution. The homogenates were centrifuged at 10,000RPM at 4^{o}C for 20 mns and the supernatant was stored in Eppendorf tubes at 4°C. The samples were used for further analysis.

Biochemical studies. Protein content was determined using bovine serum albumin using a standard method described earlier [6].The enzyme activities were expressed as units of enzyme activity per milligram of protein. Lipid Peroxidation was assayed by the method of [7], superoxide dismutase (SOD) activity was determined by pyrogallol method [8], Glutathione peroxidase activity was measured by the procedure of [9].

Statistical analysis. The biochemical parameters were carried out in triplicate. The data are presented as mean ± SD value n=6 comparisons of the means of control and treated groups by one-way ANOVA test followed by Student-Newman-Keuls test. The level of significance was established at $P \leq 0.001$.

Results and Discussions

The results recorded from our present study showed that the concentration of protein in liver was high in group V (80ppm concentration of SNPs) when compared with others, as well as with the untreated control (Fig 1). The protein concentration was increasing as the concentration of the nanoparticles treated increases. But in the case of Heart tissue the level of protein was decreasing in 80ppm (7.5 mg/ml) treated embryo when compared to the control. The concentration of protein in heart was high in group III (20ppm concentration of silica nanoparticles) (18.5 mg/ml) when compared with others as well as with untreated control (Table 1). The increased rate of protein metabolism was recorded in liver tissue when compared to the heart tissue. This increase was also in parallel with the concentration of the nanoparticles. Alteration in liver protein concentration also plays a critical role during detoxification progression. The result was in accordance to our earlier studies [1].

Table 1: *Protein Concentration in Liver and Heart Tissue Treated with Sio_2 Nanoparticles*

Groups	Treatment	Protein concentration in liver (mg/ml)	Protein concentration in heart (mg/ml)
Group I	No Nanoparticles	17.5±0.414	16.2±0.414
Group II	SiO_2 (10ppm)	40.0±0.286	12.5±0.396
Group III	SiO_2 (20ppm)	55.0±0.397	18.5±0.349***
Group IV	SiO_2 (40ppm)	57.5±0.397	13.0±0.326
Group V	SiO_2 (80ppm)	95.0±0.380***	7.5±0.246

Results are expressed as mean ± S.D (n=6) *** indicates significant difference ($p<0.001$) compared with the control group

The level of lipid peroxidation of the silica nanoparticles treated tissues were significantly ($p>0.001$) decreasing compared with the control. The level of lipid peroxidation in liver was observed to be very low in group IV (40ppm concentration of silica nanoparticles) when compared with others as well as with untreated control (Fig 2). The level of lipid peroxidation in heart is very low in group II (10ppm concentration of silica nanoparticles) when compared with others as well as with untreated control (Table 2).

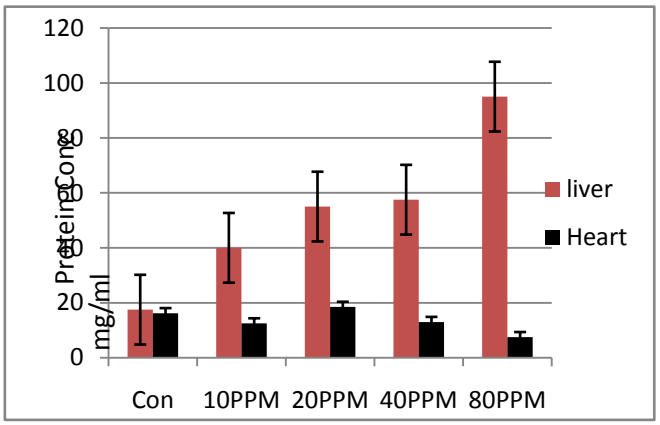

Figure 1: *Protein Concentration in Liver and Heart Tissue treated with Sio_2 Nanoparticles*

Table 2: *Livel of lipid Peroxidation in Liver and Heart Tissue Treated with Sio_2 Nanoparticles*

Groups	Treatment	Liver (n mol MDA/mg protein)	Heart (n mol MDA/mg protein)
Group I (Control)	-	6.53±0.063	6.42±0.063
Group II	SiO_2 (10ppm)	6.29±0.180	6.17±0.065***
Group III	SiO_2 (20ppm)	6.14±0.179	6.19±0.094
Group IV	SiO_2 (40ppm)	6.12±0.079***	6.40±0.054
Group V	SiO_2 (80ppm)	6.15±0.109	6.12±0.090

Results are expressed as mean ± S.D (n=6) *** indicates significant difference ($p<0.001$) compared with the control group.

Lipid Peroxidation rate in the heart tissue is very low in group II (10ppm concentration of silica nanoparticles) when compared with others as well as with control. This suggests that there was no oxidative degeneration within the cell membranes. In the groups treated with 20ppm and 80 ppm the increase in level of lipid peroxidation was recorded both in the heart and liver tissue and this may be due to the degradation of cell membrane initiated by the enhanced reactive

oxygen species production which is directly proportional to the presence of thiobarbituric acid reactive substances (TBARS)[10].

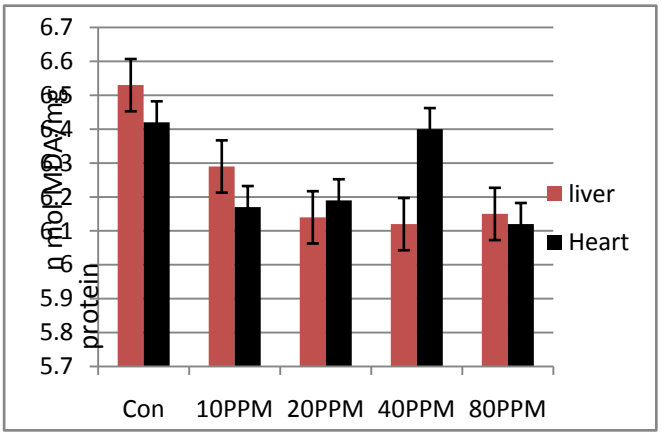

Figure 2: *Effect of silica nanoparticles on the level of lipid Peroxidation*

TiO_2 Nanoparticles when treated in Zebra fish recorded increased level of MDA which indicated that the tissues were undergoing oxidative stress [11]. In our present study, it was interesting to note that the level of lipid peroxidation was not higher in the treated groupseven in highest concentration of nanoparticles, which may be due to the less toxicity of SNPS.

Table 3: *The Activity of Superoxide dismutase in Liver and Heart Tissue Treated with Sio_2 Nanoparticles*

Groups	Treatment	Liver (units/mg protein)	Heart (units/mg protein)
GroupI (Control)	-	6.28±0.065	3.62±0.084
Group II	SiO_2 (10ppm)	7.47±0.075	3.64±0.109
Group III	SiO_2 (20ppm)	8.47±0.057	6.13±0.121***
Group IV	SiO_2 (40ppm)	6.83±0.094	5.48±0.069
Group V	SiO_2 (80ppm)	9.18±0.088***	4.11±0.079

Results are expressed as mean ± S.D (n=6) *** indicates significant difference (p<0.001) compared with the control group

Superoxide dismutase activity of the control and treated tissues of liver and heart are represented in the Table 3.The results were recorded that in the control the activity of SOD was higher in the liver tissue than in the heart tissue. in 10 ppm treated sample 6.29 n mol mda/mg protein was observed. it was recorded lesser in heart tissue (6.17 n mol/ mg protein) (Fig 3).

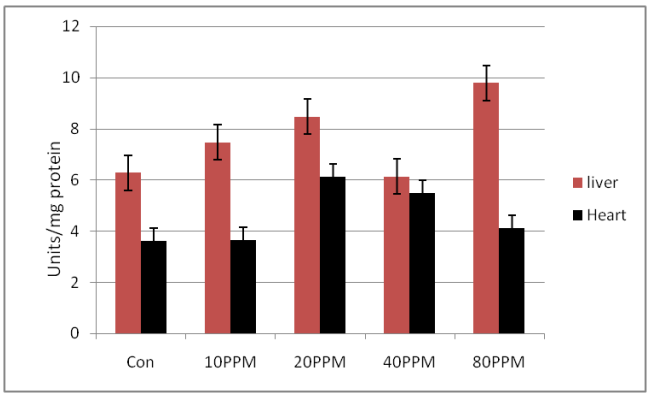

Figure 3: *Effect of silica nanoparticles on the activity of superoxide dismutase*

This increase in the level of SOD in liver may be dew to the levels of two enzymes in the liver which is cytosolic Cu Zn and mitochondrial Mn enzymes play an important role in scavenging the superoxide radicals in the body system [12]. Activity of SOD of the silica nanoparticles treated tissues is significantly (p>0.001) increasing compared with the control. The activity of superoxide dismutase in liver is high in group V (80ppm concentration of silica nanoparticles) when compared with others as well as with untreated control. The activity of superoxide dismutase in heart is high in group III (20ppm concentration of silica nanoparticles) when compared with others as well as with untreated control. The level of antioxidant enzyme activity was higher with the nanoparticles treated samples of liver and heart tissue. This will not allow the cellular damage of the tissues and also plays an important role in preventing hydrogen peroxide damage by scavenging superoxide and inhibiting the generation of toxic species [12] and [13].

Glutathione peroxidase activity of the control and treated tissues of liver and heart tissues ware represented in the Table.4. Glutathione peroxidase activity of the silica nanoparticles treated tissues was significantly (p>0.001) increasing compared with the control. The activity of glutathione peroxidase in liver is high in group V (80ppm concentration of silica

nanoparticles) (9.17 Units/mg Protein) when compared with others as well as with untreated control (Fig 4).

Table 4. *The Activity of Glutothione peroxidase in Liver and Heart Tissue Treated with Sio_2 Nanoparticles*

Groups	Treatment	Liver (Units/mg protein)	Heart (Units/mg protein)
GroupI (Control)	-	5.78±0.082	2.18±0.018
Group II	SiO_2 (10ppm)	7.48±0.079	3.68±0.070
Group III	SiO_2 (20ppm)	6.88±0.062	5.47±0.068
Group IV	SiO_2 (40ppm)	8.41±0.055	6.11±0.089***
Group V	SiO_2 (80ppm)	9.17±0.066***	4.13±0.121

Results are expressed as mean ± S.D (n=6) *** indicates significant difference ($p<0.001$) compared with the control group

The activity of glutathione peroxidase in heart is high in group IV (40ppm concentration of silica nanoparticles) 6.11 (Units/mg Protein), when compared with others as well as with untreated control.

The increased level activity of glutathione peroxidase was recorded in the liver and heart tissue in the silica nanoparticles treated samples when compared to the control. This result proves the increased activity of glutathione peroxidase, which is a well known selenoenzyme functions as an important antioxidant enzyme first line of defensive system. The selenoproteins catalyzes the reduction of harmful peroxides by glutathione and helps in the protection of lipid membranes in the cell against oxidative damage [14] as well.

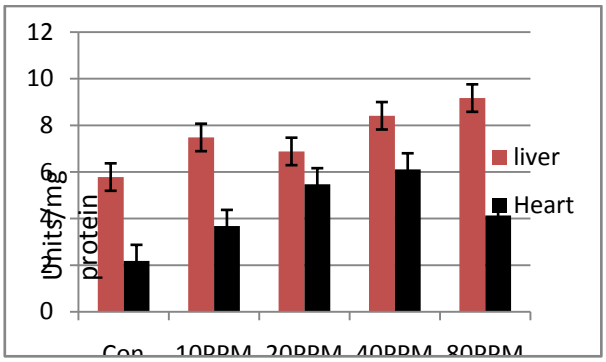

Figure 4: *Activity of Glutothione peroxidase in Liver and Heart Tissue Treated with Sio_2 Nanoparticles*

The increased activity of the enzymes superoxide dismutase and glutathione peroxidase and lower level of lipid peroxidation confirms that the silica nanoparticles are non-toxic because they are not accounting for oxidative stress induction, which is the main indicator of toxicity [15]. Therefore, our studies suggest that silica nanoparticles are non-toxic, and can be used as seed protecting agent. This study could lead to open up newer pathways of using nanomaterials based technology in pesticide industry.

Conclusions

The linear accumulation of SNPs suggests that the nanoparticles presence in the tissues of chick embryo did not bring any lethality or toxicity during the course of our study. The defensive mechanism of the used biosystem despite themild variations among the antioxidant processesdid not induce any change in MDA level, that represents an evidence for the intact membrane. Hence, this study on avian model is a good forerunner in confirming the nontoxic nature of SNPs, and can highly be recommended for testing storage pesticide protecting agents and also for those addressed to therapeutic applications.

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