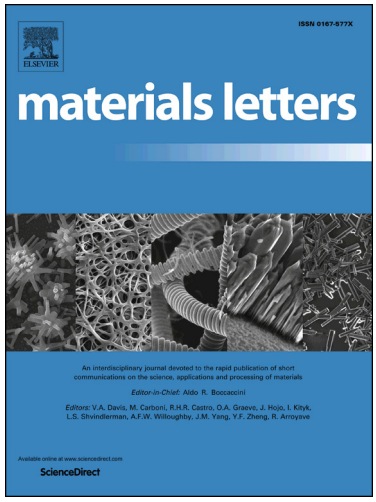


Synthesis of zinc oxide nanoparticles (ZnONPs) by aqueous extract of *Amaranthus caudatus* and evaluation of their toxicity and antimicrobial activity

Subhaschandrabose Jeyabharathi, Kalimuthu Kalishwaralal, Krishnan Sundar, Azhaguchamy Muthukumaran

PII: S0167-577X(17)31221-1
DOI: http://dx.doi.org/10.1016/j.matlet.2017.08.030
Reference: MLBLUE 23007

To appear in: *Materials Letters*

Received Date: 25 January 2017
Revised Date: 23 June 2017
Accepted Date: 8 August 2017

Please cite this article as: S. Jeyabharathi, K. Kalishwaralal, K. Sundar, A. Muthukumaran, Synthesis of zinc oxide nanoparticles (ZnONPs) by aqueous extract of *Amaranthus caudatus* and evaluation of their toxicity and antimicrobial activity, *Materials Letters* (2017), doi: http://dx.doi.org/10.1016/j.matlet.2017.08.030

Synthesis of zinc oxide nanoparticles (ZnONPs) by aqueous extract of *Amaranthus caudatus* and evaluation of their toxicity and antimicrobial activity

Subhaschandrabose Jeyabharathi[1], Kalimuthu Kalishwaralal[1], Krishnan Sundar[1,2] and Azhaguchamy Muthukumaran[1,2]*

[1]Department of Biotechnology, Kalasalingam University, Krishnankoil - 626126, Tamilnadu, India

[2]International Research Centre, Kalasalingam University, Krishnankoil - 626126, Tamilnadu, India

Abstract

In the present study zinc oxide nanoparticles (ZnONPs) were synthesized by the extract of the plant, *Amaranthus caudatus.* ZnONPs were characterized by FTIR and SEM coupled with EDX. Among the various concentrations of ZnONPs administered, 10mg/ml or more caused deformities and less survival rate. ZnONPs were found to influence the normal development of zebrafish embryos in a dose dependent manner. The ZnONPs were found to exhibit anti-bacterial activity; the activity was more towards Gram positive bacteria (*S. epidermidis)* than the Gram negative bacteria *(E. aerogenes*). The low concentrations of ZnONPs could be used as a therapeutic agent for diabetes mellitus.

Key words: *Amaranthus caudatus*, SEM-EDX mapping, Zinc Oxide Nanoparticles (ZnONPs), zebrafish, toxicity, Diabetes Mellitus

* Corresponding Author

A. Muthukumaran

Assistant Professor, Department of Biotechnology

Kalasalingam University, Anand Nagar, Krishnankoil- 626126, India

a.muthukumaran@klu.ac.in

1. Introduction

Zinc plays an indispensable role in over 300 different types of enzymes and was proved for their potential effects in the biological system [1]. Unfortunately over 80% of pregnant women have inadequate zinc intake, which is considered as a risk factor for their maternal condition [2]. The highest level of zinc is located in the insulin secretary granules (ISGs) which may contain upto 70% of total β-cell zinc [3]. However, transportation of zinc into insulin granules is carried out by ZnT8 and it is transported through ZIP4 into pancreatic β-cells [4]. Zinc is also involved in various metabolic pathways, beta cell growth and also in insulin production [3]. Low concentration of zinc in serum leads to reduce level of placental zinc transport, thus it distresses the supply of zinc to the fetus. Many research studies indicated that the deficiency of zinc may leads to poor birth outcomes [4-6]. *A. caudatus* is a well known medicinal plant; it can be used as an antidiabetic, astringent, diuretic and anti-helmintic agent [7]. *A. caudatus* protect pancreatic beta cells in streptozotocin (STZ) treated rats. This was already proved in Indian traditional system of medicine [8]. The antibacterial potential ZnONPs is already studied [9-11]. In the present study, ZnONPs were synthesized using *A. caudatus* leaf extracts which act as a reducing and capping agent for the precipitation of zinc. The antibacterial effects of ZnONPs were studied in gram positive *Staphylococcus epidermidis* and gram negative *Enterobacter aerogenes*. Further, the toxicity of the ZnONPs was evaluated using zebrafish embryos.

Experimental

2.1 Extraction & Purification of the sample

The *A. caudatus* leaves were shade dried and it was finely powdered using a mortar & pestle. Deionizer water (100ml) was added to the powder (25g) and kept in a water bath for 20 mins at 80°C. One ml of the extract was added to 1mM of zinc acetate (100µl). To this added 9 ml of deionized water and the mixture was sterilized at 121°C for 20mins. The color changes in the reaction mixture indicated the synthesis of ZnONPs. The ZnONPs were purified by

centrifugation at 10,000 rpm for 10 mins in a refrigerated centrifuge. The aggregated particles were dispersed by sonication process.

2.2 Characterization of ZnONPs

2.2.1 FTIR analysis

The synthesized ZnONPs were analyzed for the presence of functional groups using Fourier Transformer Infrared Spectrophotometer (IR Tracer, Shimadzu, Japan) by ATR method [12].

2.2.2 Size determination with SEM

The size and shape of the ZnONPs were measured using a Scanning Electron Microscope (EVO18 Bruker, Germany). Selected areas within the SEM sections were subjected to elemental composition analysis using an Energy Dispersive X-ray spectroscopy (Bruker 6130, Germany) microanalysis system.

2.3 Antimicrobial assay

The antimicrobial activity of the ZnONPs was carried out by well-diffusion method described by Perez et al. [13] with minor modifications**.** 0.1 ml of diluted inoculum of *S. epidermidis* and *E. aerogenes* were swabbed on the LB agar plates. Wells of 5 mm diameter were punched into the agar plates with the help of sterilized punch cutter. Using a micropipette, 100µl of the plant extract and ZnONPs were added to the separate well made in the plate. The plates were incubated aerobically in an upright position at 37 °C for 24-48 h. Antimicrobial activity was evaluated by measuring the zone of inhibition (mm) against the bacterial strains.

2.4. Treatment of ZnONPs on zebrafish embryos

The zebrafish embryos with early blastula stage were treated to ZnONPs at various concentrations (1, 5, 10, 25, 50 and 100 mg/ml) for 48hrs. After 72 hour post fertilization (hpf) the survival and malformations of the embryos were studied [14].

3. Results and Discussion

3.1 Characterization of ZnONPs

ZnONPs synthesized by *A. caudatus* extract exhibited peaks at 1631, 1409, 956, 584, 468 and 449 cm^{-1} in the FTIR spectrum. The peak at 449 cm^{-1} confirmed the presence of ZnONPs (Fig 1b). The very strong peak observed at 1631 cm^{-1} representing to C=O carbonyl group, and the C-H hydroxyl group peak at 1409 cm^{-1}, which were sharper and broader for ZnONPs. In IR spectra the peak at 499 cm^{-1} (Fig 1b) showed a high intensity very broad band due to the stretching mode of the zinc and oxygen bond [15-18]. The SEM images showed the presence of numerous ZnONPs formed due to the reduction of zinc acetate by *A. caudatus* extract (Fig. 1a). The reaction temperature of 121°C was found to be favourable for the reduction of zinc acetate to ZnONPs. The shape of ZnONPs obtained was spherical, and these results corroborate with the results obtained in earlier studies [18-19]. The EDX spectra clearly show the equal distribution of Zn and O indicating the presence of ZnO in the particles analyzed (Fig 1c-f). The EDX mapping revealed a homogeneous distribution of Zn (Red color) (Fig 1d) into the O (Green color) (Fig 1e).

3.2 Antimicrobial Effect

The antibacterial activity of ZnONPs was investigated against both gram positive *Staphylococcus epidermidis* (MTCC9040) and gram negative *Enterobacter aerogenes* (MTCC8100) by well diffusion method. The ZnONPs were found to exhibit a higher antimicrobial activity against *S. epidermidis* (14mm) compared to *E. aerogene*s (9mm) as measured by the zone of inhibition. The results are presented in Fig.3a, b.

3.3 Evaluation of ZnONPs on zebrafish embryos

Zebrafish embryos at an early blastula stage were exposed to ZnONPs for 24 hrs. After 96 hpf of hatching, survival and malformations of the embryos were studied [Fig2a-f]. Concentration of ZnONPs up to 10mg/ml did not have any significant effect on the survival and malformation in zebrafish embryos, whereas a concentration dependent effect on the survival of embryos was observed when the embryos were exposed to 25 mg/ml and higher [20]. At 96 hpf, high level of mortalities were noted at 50 and 100 mg/ml concentration (Fig 2e). The results of the present

study corroborate with the earlier reports on the toxicity of ZnONPs and Zn ions to zebrafish embryos during the 96 hpf period [21, 22].

4 Conclusions

The biological synthesis of zinc oxide nanoparticles from *A. caudatus*, is advantageous over other physical and chemical methods. The results of the present study conclude that the ZnONPs are toxic at high concentrations when tested in zebrafish embryos. The results also suggest that lower concentrations of ZnONPs can be evaluated as a possible therapeutic agent for diabetes mellitus.

5 Acknowledgments

The authors thank Kalasalingam University for the facilities provided. Mr. P. Kathirval of International Research Centre (IRC), Kalasalingam University is acknowledged for his help with SEM and EDX analysis.

6 References

1 Christianson, D. W.: 'The structural biology of zinc', *Adv. Prot. Chem.*, 1991, **42**, pp. 281-335.

2 Parr, R.M.: 'Assessment of dietary intakes. In: Trace elements in human nutrition and health. Geneva', World Health Organization, 1996, pp. 265-88.

3 Chausmer, A.B.: 'Zinc, insulin and diabetes', *J. Am. Coll. Nutr*., 1998, **17**,pp. 109-115

4 Black, R.E.: 'Micronutrients in pregnancy', Br. J. Nutr., 2001, **85**,193S197S

5 Chaffee, B.W., King, J.C.: 'Effect of zinc supplementation on pregnancy and infant outcomes: a systematic review. Paediatric and Perinatal Epidemiology', 2012, **26**, pp. 118-137.

6 Jansen, J., Karges, W., Rink, L.: 'Zinc and diabetes-Clinical links and molecular mechanisms', *J. Nutr. Biochem*., 2009, **20**, pp. 399 – 417.

7 Gian, F.C., Monica, R.L.: 'In vitro antioxidant effect and inhibition of amylase of two varieties of Amaranthus caudatus seeds', *Biol Pharm Bull*., 2005, **28**, pp. 1098-1102.

8 Girija, K., Lakshman, K., Udaya Chandrika, Sabhya Sachi Ghosh, Divya, T.: 'Anti-diabetic and anti-cholesterolemic activity of methanol extracts of three species of Amaranthus', *Asian Pac. J. Trop Biomed*., 2011, **1**, pp. 133-138.

9. Mason.C, Vivekanandhan.S, M. Misra, A.K. Mohanty, *World J. Nano Sci. Eng*. 2012, **2**, pp. 47–52.

10. Sangeetha.G, Rajeshwari.S, Venckatesh.R, *Prog. Nat. Sci. Mater. Int*. 2012, **22**, pp. 693-700.

11. M.J. Divya, K. Sowmia, K. Joona, K.P. Dhanya, *Res. J. Pharm. Biol. Chem. Sci.* 2013, **4**, 1137–1142.

12 Narmato, K.: 'Infrared and Raman spectra of Inorganic and Coordination compounds', New York: John Wiley & Sons., 1997, pp.296-346.

13 GRM Perez, JG Avila, MA Zavala, GS Perez, GC Perez. *Phytomed*. 1990,**3**,186.

14 Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B., Schilling, T.F.: 'Stages of embryonic development of the zebrafish', Dev. Dyn., 1995, **203**, pp. 253-310.

15 Zheng, J., Nagashima, K., Parmiter, D., De Cruz J., Patri, A.K.: 'SEM X-ray microanalysis of nanoparticles present in tissue or cultured cell thin sections', Methods Mol Biol, (Clifton, N.J.)., 2011, **697**, pp. 93-99

16 Kwon, Y.J., Kim, K.H., Lim, C.S., Shim, K.B.: 'Characterization of ZnO nanopowders synthesized by the polymerized complex method via an organo chemical route', *J. Ceram Proc Res.,* 2002, **3**, pp. 146-149

17 Li, H., Wang, J., Liu, H., Yang, C., Xu, H., Li, X., Cui, H.: 'Sol-gel preparation of transparent zinc oxide films with highly preferential crystal orientation', Vacuum., 2004, **77**, pp.57-62

18 Nagarajan, Sangeetha, and Kumaraguru Arumugam Kuppusamy.: 'Extracellular synthesis of zinc oxide nanoparticle using seaweeds of gulf of Mannar, India'. *Journal of Nanobiotechnology*, 2013, **1,** pp. 39.

19 Silva, R.F., Zaniquelli, MED.: 'Morphology of nanometric size particulate aluminium doped zinc oxide films', Colliod Surf Physicochem Eng Aspect., 2002, **198-200**, pp. 551-558

20 Vicario-Parés, Unai, Luis Castañaga, Jose Maria Lacave, Miriam Oron, Paul Reip, Deborah Berhanu, Eugenia Valsami-Jones, Miren P. Cajaraville, and Amaia Orbea.: 'Comparative toxicity of metal oxide nanoparticles (CuO, ZnO and TiO2) to developing zebrafish embryos', *Journal of Nanoparticle Research*., 2014, **8**,pp. 1-16

21 Wei Bai, Zhiyong Zhang , Wenjing Tian , Xiao He ,Yuhui Ma , Yuliang Zhao , Zhifang Chai.: 'Toxicity of zinc oxide nanoparticles to zebrafish embryo: a physicochemical study of toxicity mechanism'. *J Nanopart Res*., 2010, **12**, pp. 1645-1654

22 Jeyabharathi, S., Kalishwaralal, K., Sundar, K., and Muthukumaran, A.: 'Green synthesis of zinc oxide nano particles (ZnONPs) from the leaves of *Eugenia jambolana* and its toxicty effects in embryos of zebrafish (*Danio rerio*)', In Bio-Nanomaterials for Biomedical Technology., V. Rajendran, P. Prabu and K.E. Geckeler (eds.), 2015, pp. 135–140.

Figure 1.

A. SEM micrograph of *A. caudatus* mediated synthesis of ZnONPs at higher resolution; B. FTIR analyses of ZnONPs; C. ZnONPs shown by scale bar of 30 µm (With our EDX); D. EDX image - red color spot indicated the presence of zinc element; E. Green color spot indicated the presence of oxygen; F. Optical absorption band peak of ZnONPs

Figure 2. Zebrafish embryos exposed to different concentrations of ZnONPs

A. Control embryo; B. 50mg/ml; C. Control hatched out larva; D. Deformed larva with cured body, pericardial edema and blood clot (50 mg/ml); E. Survival of zebrafish embryos exposed to ZnONPs after 96 hpf; F. Malformation of zebrafish exposed due to ZnONPs.

Figure 3.Antimicrobial activity of ZnONPs synthesis from *Amaranthus caudatus* leaf extract.

A. *Enterobacter aerogenes* G (-) ve. ; B. *Staphylococcus epidermidis* G (+) ve.

Figure1.

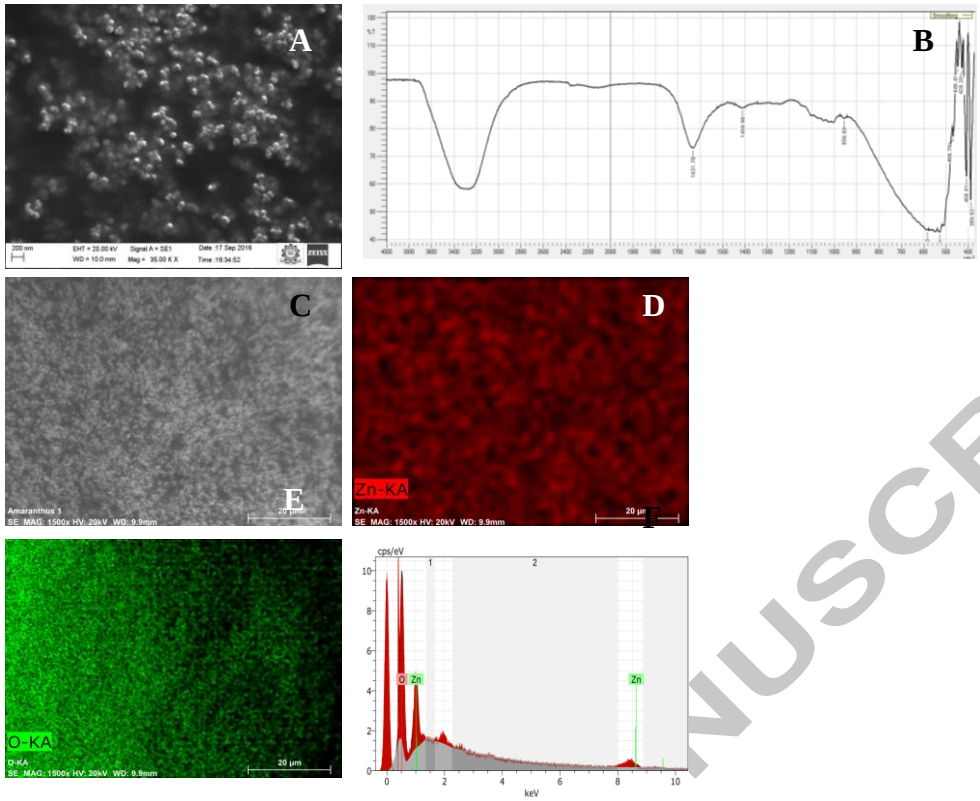


Figure 2.

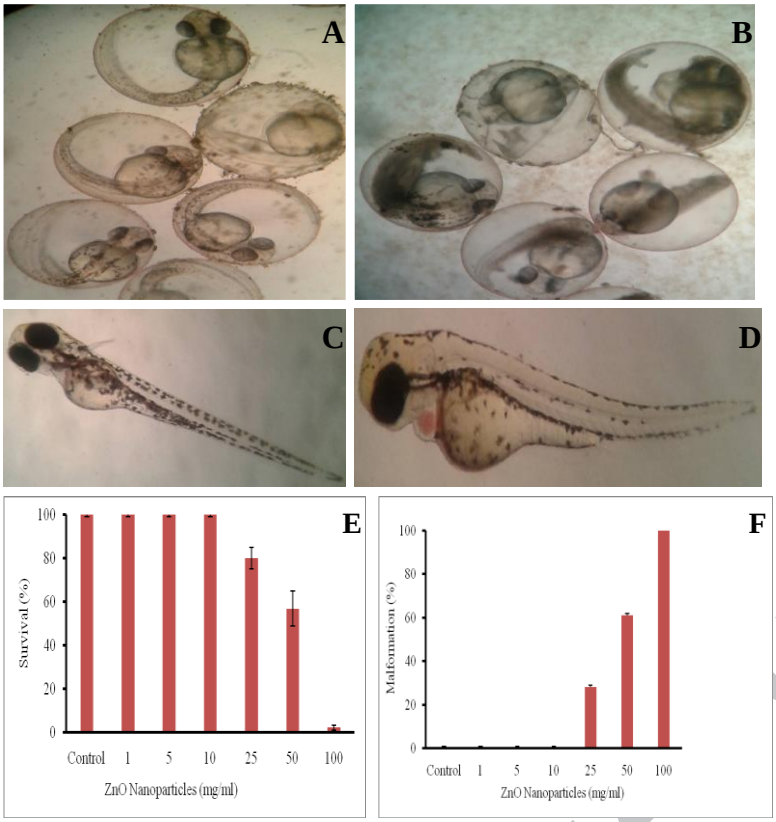
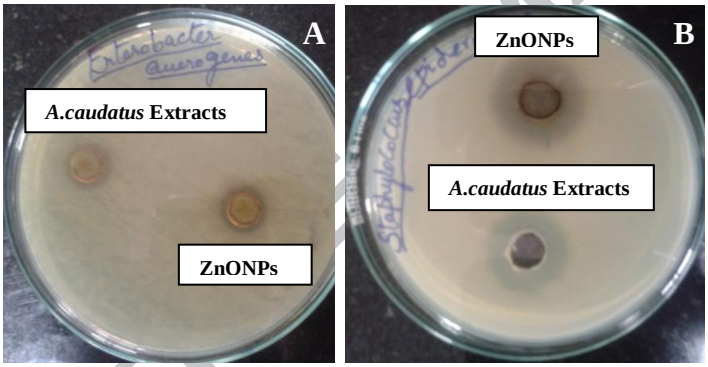


Figure 3.



- ZnONPs were synthesized by the extract of the medicinal plant, *A. caudatus*
- The toxicity of ZnONPs was evaluated in zebrafish embryos
- ZnONPs have anti-bacterial activity against *S. epidermidis* and *E. aerogene*s
- The low concentrations of ZnONPs could be used as a therapeutic agent