Green Synthesis of Zinc Sulfide Nanoparticles Using Abrus precatorius *and Its Effect on Coelomic Fluid Protein Profile and Enzymatic Activity of the Earthworm,* Eudrilus eugeniae

Muniyandi Biruntha, Jayaprakasam Archana, Kurumban Kavitha, Balan Karunai Selvi, et al.

BioNanoScience

ISSN 2191-1630
Volume 10
Number 1

BioNanoSci. (2020) 10:149-156
DOI 10.1007/s12668-019-00694-0

Springer

BioNanoScience (2020) 10:149–156
https://doi.org/10.1007/s12668-019-00694-0

Green Synthesis of Zinc Sulfide Nanoparticles Using *Abrus precatorius* and Its Effect on Coelomic Fluid Protein Profile and Enzymatic Activity of the Earthworm, *Eudrilus eugeniae*

Muniyandi Biruntha[1] · Jayaprakasam Archana[1] · Kurumban Kavitha[1] · Balan Karunai Selvi[2] · James Arockia John Paul[3] · Ramalingam Balachandar[4] · Muthupandian Saravanan[5] · Natchimuthu Karmegam[6]

Published online: 28 November 2019

Abstract

In the present study, green synthesized zinc sulfide nanoparticles (ZnS NPs) from the leaves of the medicinal plant, *Abrus precatorius*, were characterized and tested for toxicity using *Eudrilus eugeniae*. The formation of ZnS NPs through green synthesis was confirmed by using UV-Vis spectroscopy, FE-SEM, FT-IR, and XRD analyses. The clitellate earthworms were used to assess the effect of ZnS NPs by exposing to ZnS NPs 300 mg ZnS NPs per kg of OECD soil. On 0, 7, and 14 days of exposures, the coelomic fluid of the earthworms were separated and analyzed for total protein and protein profile, activities of enzyme markers such as superoxide dismutase (SOD), catalase (CAT), and protease. The results on the analysis of coelomic fluid of *E. eugeniae* after exposure to ZnS NPs showed a significant increase in protein content from the initial levels of 1.51 to 2.32 mg/mL with a protein profile of 25–40 kDa size. The activity of SOD was significantly ($P < 0.05$) declined from initial levels whereas CAT and protease showed a significant ($P < 0.05$) increase on 7th day of exposure then declined. The results indicate that the ZnS NPs in OECD soil significantly interfere with the protein and enzyme markers, SOD, CAT, and protease in earthworm coelomic fluid.

Keywords ZnS nanoparticles · *Abrus precatorius* · *Eudrilus eugeniae* · Coelomic fluid · Protein profile

1 Introduction

Metal nanoparticles (NPs) are well-established for their biological and pharmacological activities [1–4]. Among the metal nanoparticles, the synthesis and application of zinc sulfide NPs (ZnS NPs) are rapidly increasing because of their multifaceted applications such as field emitters, field-effect transistors (FETs), p-type conductors, catalyzators, UV-light sensors, chemical sensors (including gas sensors), biosensors, nanogenerators, and pharmaceutical and cosmetic industries [5, 6]. The Zn NPs are found to possess a modulator effect on plant growth [7]. Importantly, the application of ZnS NPs in the field of medicine is widening fast, due to its promising results. For instance, ZnS NPs synthesized from *Phoenix dactylifera* showed antioxidant, cytotoxic, and antileishmanial activities [8]. The green synthesis of emerging NPs from plant resources is an advanced phytonanotechnology [9, 10]. The green synthesis of ZnS NPs is a cost-effective eco-friendly method for the production of ZnS NPs with a range of biological properties including anticancer, antibacterial, and antifungal activities [11–13]. The biosynthesis of ZnS NPs using plant extracts is emerging nanoscience field, and the recent studies expose green synthesis, characterization, and application of ZnS NPs. The ZnS NPs synthesized from flower bud extract of *Syzygium aromaticum* and from the leaf extracts of *Tridax procumbens* showed good antimicrobial activity [14,

✉ Natchimuthu Karmegam
kanishkarmegam@gmail.com

[1] Department of Animal Health and Management, Alagappa University, Karaikudi, Tamil Nadu 630003, India

[2] Department of Botany, V. V. Vanniaperumal College for Women (Autonomous), Virudhunagar, Tamil Nadu 626001, India

[3] Department of Zoology, Arumugam Pillai Seethai Ammal College, Tiruppattur, Tamil Nadu 630211, India

[4] Department of Biotechnology, Aarupadai Veedu Institute of Technology, Vinayaka Missions' Research Foundation (Deemed to be University), Paiyanoor, Chennai, Tamil Nadu 603104, India

[5] Department of Microbiology and Immunology, Division of Biomedical Science, School of Medicine, College of Health Sciences, Mekelle University, 1871 Mekelle, Ethiopia

[6] Department of Botany, Government Arts College (Autonomous), Salem, Tamil Nadu 636007, India

15]. *Abrus precatorius* L. (rosary pea) is an important medicinal plant possessing various ethnobotanical and pharmacological activities in roots, leaves, and seeds [16]. The efficacy of petroleum ether extract of the leaves of *A. precatorius* against *Culex quinquefasciatus* larvae showed a LC_{50} value of 359.02 ppm [17]. The biosynthesized silver (Ag) NPs from the leaves of *A. precatorius* has been shown to possess antimicrobial activity against Gram-positive and Gram-negative bacteria [18]. A recent study revealed that the leaf extract of *A. precatorius* is owned cytotoxic, antimicrobial, and antidiabetic activities [19]. However, the synthesis of ZnS NPs from *Abrus precatorius* leaves is narrowly studied.

Earthworms are natural engineers of the soil and largely employed for vermicompost production and commonly used test organisms for toxicity studies [20, 21]. The environmental impact of NPs using animal models is gaining momentum as the toxicity levels of synthesized NPs for their environment-friendly exploitation. Invertebrate organisms are used to study the environmental effects of nanomaterials; modeling and route of bioaccumulation of such materials are well represented [22]. The zinc-derived NPs, ZnO NPs had been assessed for toxicity in earthworms [23, 24]. The green synthesis of ZnS NPs from the leaf extracts of *A. precatorius* and their toxicity to soil organisms like earthworms is least studied. The coelomocytes in earthworms are found to be more sensitive than gut cells to NP toxicity [25]. Antioxidant enzymes, including SOD, CAT, and protease activity, are used as potential biomarkers to assess the ecotoxicological effects of NPs on earthworms and the revelation of NPs induced oxidative stress, and also to get insight that the earthworms could be used as important bioindicator organisms for monitoring chemical toxicity in the soil ecosystem. Hence, the present study was aimed to find out the effect of green synthesized ZnS NPs from *A. precatorius* leaves on protein profile, activities of super oxide dismutase (SOD), CAT and protease in the coelomic fluid of the earthworm, *Eudrilus eugeniae* by soil contamination.

2 Materials and Methods

2.1 Green Synthesis of ZnS NPs and Characterization

The leaves of *A. precatorius* were collected from Tirupattur, Sivagangai District, Tamil Nadu, India (GPS co-ordinates: latitude 10° 11′ 25.50″ N; longitude 78° 59′ 94.00″ E). For the preparation of plant extract, 5 g of fresh leaves was washed with running tap water followed by Milli-Q water and then cut and soaked in a 250-mL Erlenmeyer flask containing 100 mL Milli-Q. The solution was boiled at 70 °C for 8 min. The leaf extract was allowed to cool to room temperature, filtered through Whatman no. 1 filter paper, and the filtrate was stored for further experimental use. For the green synthesis of ZnS NPs, 0.1 mole of 5.750 g zinc sulfate was dissolved into 100 mL of distilled water and stirred vigorously using magnetic stirrer for 20 min. Precipitation was achieved by adding 20 mL plant extract, drop by drop, under vigorous stirring. The precipitation process was continued until obtaining light brown color precipitate, and then centrifuged at 2000 rpm for 15 min. The precipitate was dried and calcinated at 500 °C for 3 h. A light brown precipitate resulted was then dried at 60 °C overnight. Precipitation was observed by increasing the pH to 14. The ZnS NPs formed were preserved for further studies. The physicochemical properties of resulting ZnS NPs were subjected to UV-Vis spectroscopic analysis, XRD, FT-IR, and FE-SEM examinations. The optical property of ZnS NPs was determined by the result of UV-Vis Spectrophotometer (Systronics Double Beam Spectrophotometer-2205, Haryana, India). After the addition of zinc sulfate to extract, the spectra were taken in time intervals up to 3 h between 200 and 600 nm. The supernatant ratios of 30:1 and 120:1 were collected and dried at 75 °C and the dried powder was taken for FTIR analysis. FTIR was obtained in the range 4000–400 cm^{-1} using KBR pellet method in Thermo Nicolet 380 FTIR spectrophotometer. The SEM slide was prepared by making a smear on a slide. Then, the slide was set for FE-SEM analysis after coating the slide with platinum and the FE-SEM image was taken in FE-SEM with EDAX, Quanta FEG 250. ZnS NPs were examined by X-ray diffractometer. For this, the powdered ZnS NPs were cohered to the cubes of XRD and the final output was taken in the XRD equipment (Powder X-Ray Diffractometer, X' Pert Pro–PAnalytical, USA), at university instrumentation center (USIC) Alagappa university with the scan rate of 0.1 s.

2.2 Experimental Animals and Vermibed Preparation

The earthworm, *E. eugeniae*, for the present study was procured from Vermiculture Unit, Alagappa University, Karaikudi. The earthworms were cultured in plastic containers (diameter 30 cm, height 20 cm) with 5:1 ratio (*w*/*w*) of cowdung and garden soil. The earthworms were exposed to ZnS NPs used in this study in artificial soil as described by OECD guidelines [26]. An artificial soil was prepared using 70% soil, 20% kaolin clay, and 10% sphagnum peat moss and the pH was adjusted to 6.0 ± 0.5 by the addition of $CaCO_3$. The dry artificial soil was moistened with distilled water to hold 60 ± 5%. All the experiments were conducted under laboratory conditions in triplicate; the temperature maintained was 28 ± 2 °C. The dry OECD test soil mix was contaminated at the rate of 300 mg ZnS NPs/kg of soil by thorough mixing to ensure a homogenous mixture. After 24 h of stabilization, the earthworms were introduced in to the OECD soil with ZnS NPs. For the experiments, clitellate adult earthworms were sorted out from the culture beds and fed with tissue paper for overnight to clean the gut. The earthworms were then

washed with saline solution and blotted on Whatman No. 1 filter paper and used for the experiments.

2.3 Collection and Analysis of Coelomic Fluid from Earthworms

On 0, 7, and 14 days of exposure to ZnS NPs, the earthworms were collected from experimental soil and subjected to coelomic fluid extraction. The coelomic fluid was collected using whole body dissection method [27]. The worms were placed on a dissection tray and their gut contents were evacuated. The earthworm muscles were ground with 0.1 M phosphate buffer. The samples were centrifuged for 10 min at 13×*g* and the supernatant was collected and re-centrifuged for 10 min at 13×*g* to exclude any remaining particulates in the coelomic fluid, followed by 5-min centrifugation at 16×*g* to ensure solid free samples. Total crude coelomic fluid protein concentration for samples collected from control and experimental organisms was determined according to the Lowry's method [28]. The protein separation was accomplished by SDS-PAGE by using 10% SDS run at 100 V for 2 h. The gels were removed from the plate carrier following separation and placement in a plastic tray containing staining solution (80 mL methanol, 20 mL acetic acid, 100 mL distilled water, and 0.24 g of Coomassie brilliant blue) for 2 h. Then, the staining solution was discarded, and destaining solution (80 mL methanol, 20 mL acetic acid, and 100 mL distilled water) was added. The destaining solution was changed 2–3 times until the bands get clearly visible. The destaining solution was removed and gels were washed with distilled water to remove any remaining destaining solution. Then, the gel was stored in 10% acetic acid and photographed.

2.4 Enzymatic Assays

The activity of SOD (EC 1.15.1.1) was determined by the method described by Magwere et al. [29]. This involved measuring the SOD inhibition of the auto-oxidation activity of epinephrine at pH 10.2 and 30 °C. One unit of superoxide activity is defined as the amount of SOD necessary to cause 50% inhibition of epinephrine auto-oxidation. The analysis was performed in 0.02 mL of the sample and 3.0 mL of 50 M Na_2CO_3 buffer. This was followed by the addition of 0.03 mL of epinephrine stock solution before taking the absorbance reading at 480 nm for 3–5 min. A blank devoid of the sample (but containing all reagents) was used for background correction. CAT (EC 1.11.1.6) activity was determined as a degradation of H_2O_2 by the enzyme. The enzymatic activity was expressed in micromoles of H_2O_2 produced per minute per milligram of protein [30], and the protein content was measured using standard bovine albumin [31]. The protease activity was measured by using skim milk agar plates following the procedure of Cappuccino and Sherman [32]. To the solidified skim milk agar medium in Petri plates, wells of 0.63 mm were cut and filled with 35 μL of 0-, 7-, and 14-day samples in separate wells. The Petri dishes were then incubated for 6 h at 37 °C and the diameter of the zone of clearance around each well was measured.

2.5 Statistical Analysis

The results on total protein and enzymatic analyses were expressed as mean ± standard deviation (SD). The differences in coelomic fluid enzyme activity between 0-, 7-, and 14-day treated earthworms were statistically interpreted using ANOVA. The statistical significance of various treatments was assessed by one-way analysis of variance (ANOVA) with SPSS version 18.0 (SPSS Inc., Chicago, USA). When there was a significant difference, Tukey's honestly significant different (HSD) multiple comparison tests were performed at $P < 0.05$ significance level. The correlation coefficient of determination (R^2) was derived to know the effect of number of days of ZnS NPs exposure on protease activity in the coelomic fluid of earthworm, *E. eugeniae*.

3 Results and Discussion

3.1 Characterization of ZnS NPs

The complete conversion to ZnS NPs took place during drying and the precipitation was observed by increasing the pH to 14. The ZnS NPs were light brown in color (Fig. 1). The UV-Visible spectra showed an absorption peak at 280 nm (after 2 h) which remained stable after 3 h of reaction (Fig. 2). It has been reported that the UV-Visible spectra of ZnS NPs exhibited a sharp absorbance of UV-Vis region at 270 nm and then decreased due to the optical absorption of the colloidal solution of ZnS [15]. The FE-SEM analysis showed the high density of green synthesized spherical ZnS NPs, and further

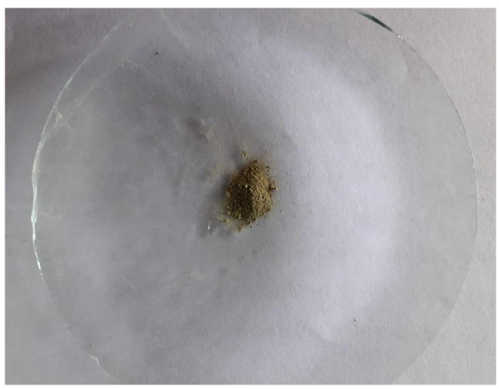

Fig. 1 The powder of ZnS NPs synthesized from leaf extract of *Abrus precatorius*

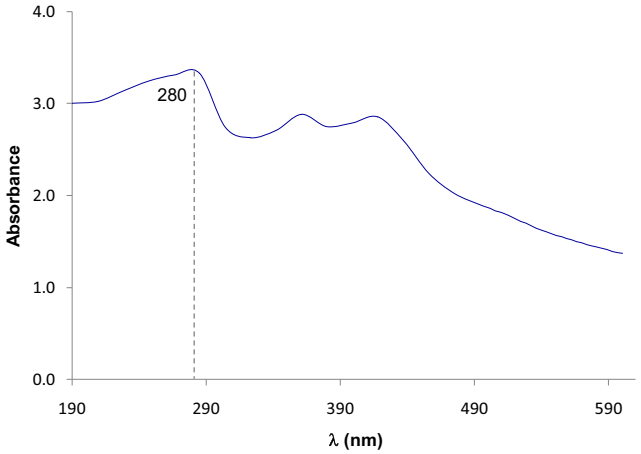

Fig. 2 UV-Visible spectroscopic analysis of ZnS NPs synthesized from leaf extract of *Abrus precatorius*

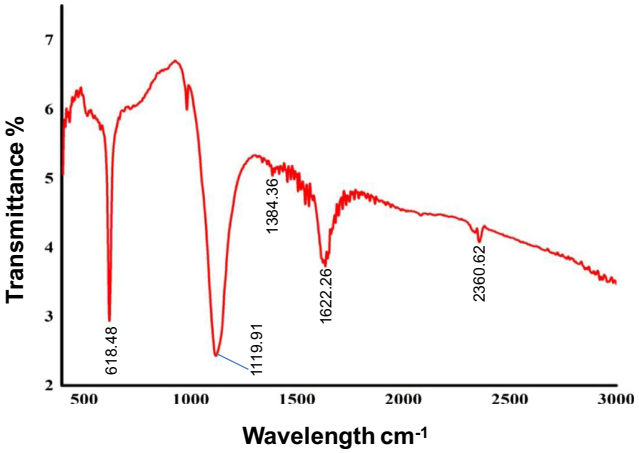

Fig. 4 FTIR spectrum of ZnS NPs synthesized from leaf extract of *Abrus precatorius*

confirmed the development of ZnS nanostructures. The FE-SEM image shows (Fig. 3) that the ZnS NPs appear as discrete particles but form much larger dendrite flocks whose size reached micron-scale size range about 9.55 nm. FTIR measurements carried out to identify the possible functional groups responsible for the reduction of the Zn ions in green synthesized ZnS NPs confined the formation (Fig. 4). The FTIR spectrum of ZnS NPs showed that the peaks were found in the range between 3000 and 500 cm^{-1} (Fig. 4) and bands observed at 3565.14, 2360.62, 1622.26, 1384.36, 1119.91, and 618.48 which were associated with amide B: N–H stretching of proteins, PO_2^- symmetric stretch: mainly nucleic acids, COO^- symmetric stretch: fatty acids and amino acids, C–O asymmetric stretching of glycogen, carbonate, ion, and aliphatic iodo compounds. Figure 5 shows an XRD pattern of biosynthesized ZnS NPs. The 2theta peak position at 28.50, 35.10, 47.51, and 56.81 showed the corresponding plane of index of (111), (200), (220), and (311), respectively. These peaks matching with JCPDS card number (05-0566) which shows the confirmation of cubic structure. Similar results have been reported while synthesizing ZnS NPs from the plant, *Stevia rebaudiana* [6]. All diffraction peaks in the present study were indexed according to the cubic structure of ZnS. No characteristic peaks of impurity phases except ZnS were found which revealed the good crystalline nature of the samples. The broadening of the peaks in the XRD pattern can be attributed to the small particle size of the synthesized ZnS NPs. The present study results fall in line with the results of Sathishkumar et al. [15] during the green synthesis of ZnS NPs using the extracts of the plant, *Syzygium aromaticum*.

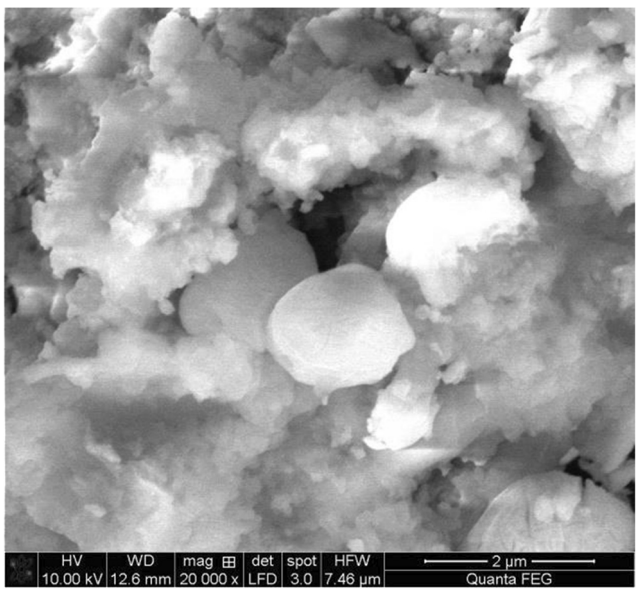

Fig. 3 FE-SEM image of ZnS NPs synthesized from leaf extract of *Abrus precatorius*

3.2 Effect of ZnS NPs Exposure on Earthworm Coelomic Fluid Protein and Enzymes

The toxicity tests conducted for a period 14 days with ZnS NPs on the earthworm *E. eugeniae* showed no mortality. The

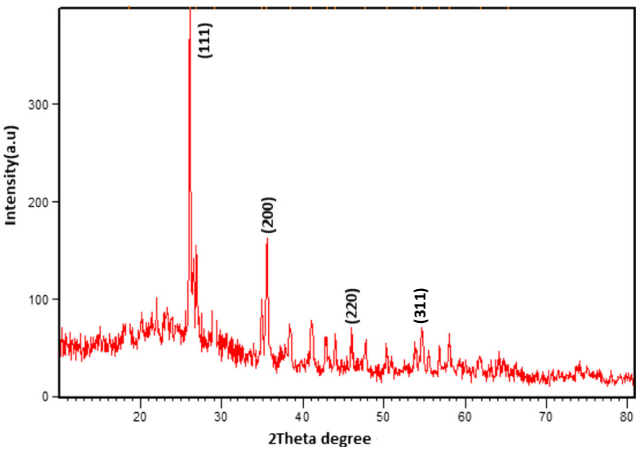

Fig. 5 XRD pattern of ZnS NPs synthesized from leaf extract of *Abrus precatorius*

protein expression profile of coelomic fluid collected from ZnS NPs-exposed *E. eugeniae* showed that the number of proteins expressed was varied between normal and stress-induced earthworms. The protein bands observed were in the range of 25–40 kDa (Fig. 6a). The exposure of Zn NPs to the earthworms showed significant increase in protein content from the initial levels of 1.51 to 2.32 mg/mL (Fig. 6b). Further, the regression analysis showed a significant positive correlation between coelomic fluid protein content of *E. eugeniae* and number of days of exposure ($y = 0.41x + 1.113$, $R^2 = 0.990$) indicating that the period of exposure of ZnS NPs increases the total protein content in the coelomic fluid of *E. eugeniae*. However, contrasting results have been obtained by the exposure of copper sulfate and copper NPs to the earthworm, *Metaphire posthuma* where the protein content showed significant decline upon exposure of experimental concentrations of copper NPs and copper sulfate per kg of soil for 7 and 14 days [33]. This could have been attributed to the immunological response of the earthworms to different chemical substances including NPs in soil.

The SOD activity of 3.95, 2.48, and 2.33 U/mg was recorded, respectively, on 0, 7, and 14th day of treatment (Fig. 7a). A

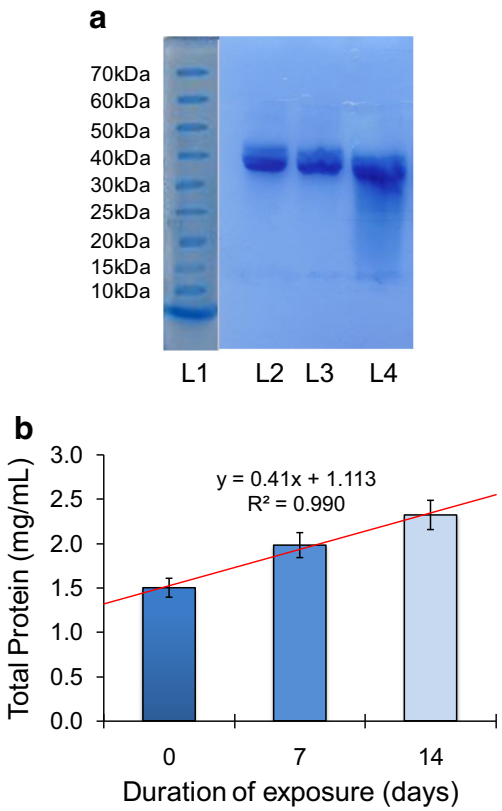

Fig. 6 **a** SDS-PAGE showing protein profile and **b** total protein content of the coelomic fluid of *Eudrilus eugeniae* exposed to ZnS NPs [Fig. 5a: L1-standard protein markers; L2, L3, and L4 are the coelomic fluid samples collected from control (0 day), 7th day and 14th day, respectively]

significant reduction ($P < 0.05$) in SOD activity was observed in the coelomic fluid of the earthworms treated with ZnS NPs on the 7th and 14th day after exposure in comparison with the initial levels, i.e., before exposure (0th day). However, the difference in SOD activity between 7th and 14th day did not differ significantly ($P > 0.05$). This shows that the SOD activity was triggered after exposure to ZnS NPs and the activity was maintained at lower levels thereafter (14th day). The antioxidant enzymes protect the cells from the reactive oxygen species produced by the interference of environmental contaminants in the metabolic processes and these enzymes can be used as biomarkers for assessing the level of environmental contaminants [34]. A significant decline in SOD activity has been reported in the earthworms, *E. eugeniae*, *P. ceylanensis*, and *P. excavatus* exposed to lead (Pb) at a concentration of 150 mg/kg [35] which is supportive to the reduction of SOD activity in the coelomic fluid of *E. eugeniae* exposed to ZnS NPs in the present study.

The activity of CAT in coelomic fluid of *E. eugeniae* showed fluctuation during the experimental period. From the initial level of 17.3 U/mg, CAT activity was significantly increased on 7th day (25.6 U/mg) and then decreased on 14th day (15.4 U/mg) after exposure to ZnS NPs (Fig. 7b). The protease activity determined as the formation of clear zone on the skim milk agar plates followed the same trend to that of CAT (Fig. 6c, d). Garcia-Velasco et al. [36] reported that the activity of CAT in the earthworm, *Eisenia fetida* exposed to Ag NPs, showed increase on 3rd day. Further, the study concluded that the activity of CAT and DNA damage could be the result of oxidative stress-mediated toxicity of Ag NPs. The zone formation by protease in coelomic fluid of *E. eugeniae* on 0th day was 6.2 mm which increased significantly to 9.6 mm on 7th day of exposure to ZnS NPs and declined thereafter to 6.5 mm. The relative changes in the activities of SOD, CAT, and protease are depicted in Fig. 8 where it is apparently clear that the CAT and protease activity in the coelomic fluid followed same trend of changes during the period of exposure to ZnS NPs. The soils contaminated with metals and pesticides deplete the antioxidant enzymes like SOD and CAT in turn results in the development of stress in earthworms and retards the normal activity, growth, and survival of earthworms [35, 37]. Correspondingly in the present study, the contamination of soil with ZnS NPs at 300 mg/kg caused abnormal fluctuations in coelomic fluid enzyme activities (SOD, CAT, and protease) accompanied by increased protein content.

4 Conclusion

The ZnS NPs green synthesized in eco-friendly technique using the leaf extracts of the medicinal plant, *A. precatorius* were confirmed with UV-Visible spectroscopy, FE-SEM, FT-

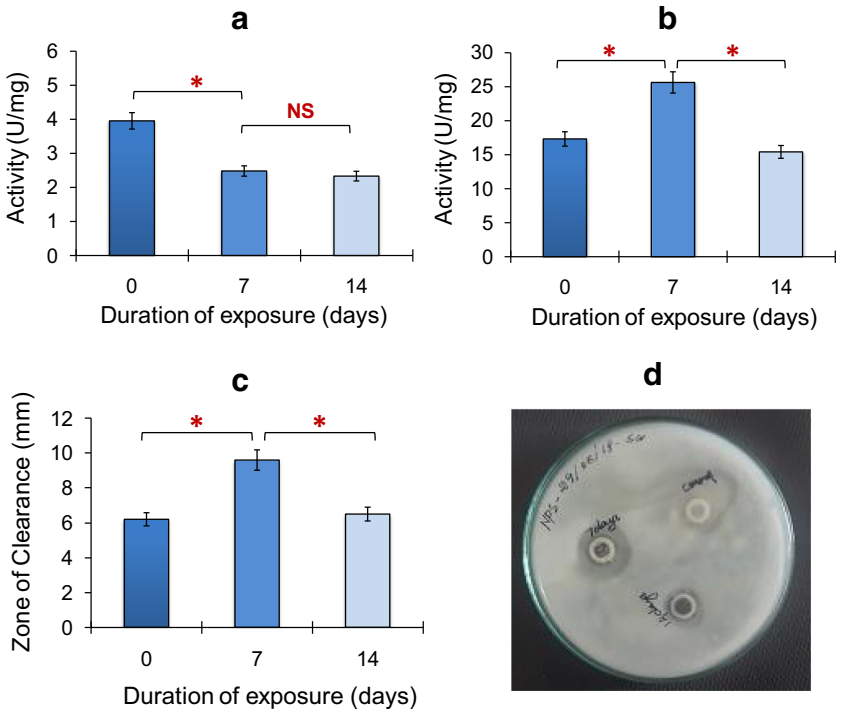

Fig. 7 **a** SOD, **b** CAT, and **c**, **d** protease activity in the coelomic fluid of *Eudrilus eugeniae* exposed to ZnS NPs

IR, and XRD. The total protein content increased from 0th day, 7th day, and 14th day of exposure to ZnS NPs in OECD soil and the protein profile by SDS-PAGE showed variation in comparison to unexposed worms, and this might probably due to the interference of ZnS NPs to protein metabolism of *E. eugeniae* which requires further insight. The activity of SOD showed relentless decline until 14th day whereas CAT and protease showed significant enhancement on 7th day of exposure and declined sharply on 14th day, indicating that the earthworm is able to retain normalcy. Further studies on different levels of ZnS NPs for longer duration and analyses of coelomocyte activity related to immune mechanism of the *E. eugeniae* may provide pathways of effects and adaptive mechanism to the exposure of NPs.

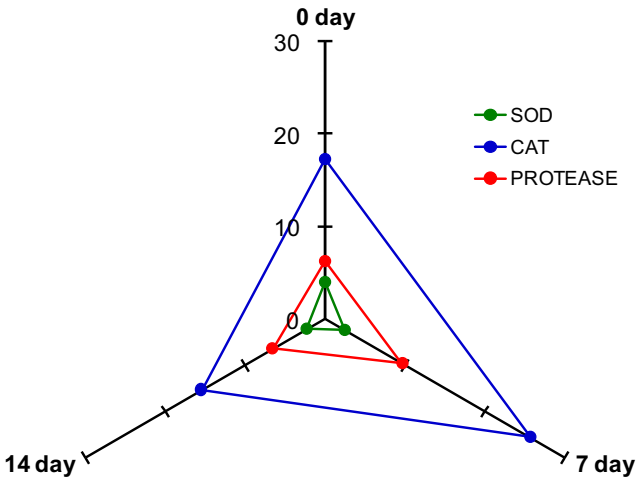

Fig. 8 The relation of enzyme activities in the coelomic fluid of *E. eugeniae* exposed to ZnS NPs (refer to Fig. 7 for units)

Acknowledgments The authors are thankful to RUSA Scheme Phase 2.0 [F-24-51/2014–U, Policy (TNMulti-Gen), Dept of Edn., Govt. of India, dated 09.10.2018] for the facilities and support. The authors duly acknowledge Dr. (Mrs.) Thilagavathy Daniel, Professor (Retd.), Department of Biology, The Gandhigram Rural Institute (Deemed University), Gandhigram, Tamil Nadu, India, for going through the manuscript and critical comments.

Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Barabadi, H., Damavandi Kamali, K., Jazayeri Shoushtari, F., Tajani, B., Mahjoub, M. A., Alizadeh, A., & Saravanan, M. (2019). Emerging Theranostic Silver and Gold Nanomaterials to Combat Prostate Cancer: A Systematic Review. *Journal of Cluster Science*, 1–8. https://doi.org/10.1007/s10876-019-01588-7.
2. Anandan, M., Poorani, G., Boomi, P., Varunkumar, K., Anand, K., Chuturgoon, A. A., et al. (2019). Green synthesis of anisotropic silver nanoparticles from the aqueous leaf extract of Dodonaea viscosa with their antibacterial and anticancer activities. *Process Biochemistry, 80*, 80–88. https://doi.org/10.1016/J.PROCBIO.2019.02.014.
3. Miri, A., Khatami, M., & Sarani, M. (2019). Biosynthesis, magnetic and cytotoxic studies of hematite nanoparticles. *Journal of Inorganic and Organometallic Polymers and Materials*, 1–8. https://doi.org/10.1007/s10904-019-01245-6.

4. Miri, A., Sarani, M., Hashemzadeh, A., Mardani, Z., & Darroudi, M. (2018). Biosynthesis and cytotoxic activity of lead oxide nanoparticles. *Green Chemistry Letters and Reviews, 11*(4), 567–572. https://doi.org/10.1080/17518253.2018.1547926.
5. Fang, X., Zhai, T., Gautam, U. K., Li, L., Wu, L., Bando, Y., & Golberg, D. (2011). ZnS nanostructures: From synthesis to applications. *Progress in Materials Science*. https://doi.org/10.1016/j.pmatsci.2010.10.001.
6. Grasset, F., Saito, N., Li, D., Park, D., Sakaguchi, I., Ohashi, N., et al. (2003). Surface modification of zinc oxide nanoparticles by aminopropyltriethoxysilane. *Journal of Alloys and Compounds, 360*(1–2), 298–311. https://doi.org/10.1016/S0925-8388(03)00371-2.
7. Thapa, M., Singh, M., Ghosh, C. K., Biswas, P. K., & Mukherjee, A. (2019). Zinc sulphide nanoparticle (nZnS): A novel nanomodulator for plant growth. *Plant Physiology and Biochemistry, 142*, 73–83. https://doi.org/10.1016/J.PLAPHY.2019.06.031.
8. Sharififar, F., Sharifi, F., Alijani, I. Q., & Hajar Khatami, M. (2003). *IEE proceedings. Nanobiotechnology. IET Nanobiotechnology* (Vol. 12). [Institution of Electrical Engineers]. Retrieved from. https://doi.org/10.1049/iet-nbt.2017.0204.
9. Balachandar, R., Gurumoorthy, P., Karmegam, N., Barabadi, H., Subbaiya, R., Anand, K., et al. (2019). Plant-Mediated Synthesis, Characterization and Bactericidal Potential of Emerging Silver Nanoparticles Using Stem Extract of Phyllanthus pinnatus: A Recent Advance in Phytonanotechnology. *Journal of Cluster Science*, 1–8. https://doi.org/10.1007/s10876-019-01591-y.
10. Khatami, M., Alijani, H., Nejad, M., & Varma, R. (2018). Core@shell nanoparticles: Greener synthesis using natural plant products. *Applied Sciences, 8*(3), 411. https://doi.org/10.3390/app8030411.
11. Alijani, H. Q., Pourseyedi, S., Torkzadeh Mahani, M., & Khatami, M. (2019). Green synthesis of zinc sulfide (ZnS) nanoparticles using Stevia rebaudiana Bertoni and evaluation of its cytotoxic properties. *Journal of Molecular Structure, 1175*, 214–218. https://doi.org/10.1016/j.molstruc.2018.07.103.
12. Chaliha, C., Nath, B. K., Verma, P. K., & Kalita, E. (2019). Synthesis of functionalized Cu:ZnS nanosystems and its antibacterial potential. *Arabian Journal of Chemistry*. https://doi.org/10.1016/j.arabjc.2016.05.002.
13. Labiadh, H., Lahbib, K., Hidouri, S., Touil, S., & Chaabane, T. B. E. N. (2016). Insight of ZnS nanoparticles contribution in different biological uses. *Asian Pacific Journal of Tropical Medicine*. https://doi.org/10.1016/j.apjtm.2016.06.008.
14. Sathishkumar, M., Saroja, M., Venkatachalam, M., Parthasarathy, G., & Rajamanickam, A. T. (2017). Biosynthesis and characterization of zinc sulphide nanoparticles using leaf extracts of Tridax procumbens. *Oriental Journal of Chemistry*, *33*(2), 903–909. doi: 10.13005/ojc/330240
15. Sathishkumar, M., Saroja, M., & Venkatachalam, M. (2017). Green synthesis, characterization and antimicrobial activity of ZnS using Syzygium aromaticum extracts. *International Journal of ChemTech Research, 10*(9), 443–449 Retrieved from https://pdfs.semanticscholar.org/431f/593306457fecd6cffcd4a92c7642d9bf9c40.pdf.
16. Garaniya, N., & Bapodra, A. (2014). Ethno botanical and Phytophrmacological potential of Abrus precatorius L.: A review. *Asian Pacific Journal of Tropical Biomedicine, 4*(Suppl 1), S27–S34. https://doi.org/10.12980/APJTB.4.2014C1069.
17. Karmegam, N., Sakthivadivel, M., Anuradha, V., & Daniel, T. (1997). Indigenous-plant extracts as larvicidal agents against Culex quinquefasciatus Say. *Bioresource Technology, 59*(2–3), 137–140. https://doi.org/10.1016/S0960-8524(96)00157-5.
18. Gaddala, B., & Nataru, S. (2015). Synthesis, characterization and evaluation of silver nanoparticles through leaves of Abrus precatorius L.: an important medicinal plant. *Applied Nanoscience, 5*(1), 99–104. https://doi.org/10.1007/s13204-014-0295-4.
19. Adekunle Alayande, K., Sabiu, S., & Ashafa, O. T. A. (2017). Medicinal properties of *Abrus precatorius* L. leaf extract: antimicrobial, cytotoxicity and carbohydrate metabolising enzymes' inhibitory potential. *Transactions of the Royal Society of South Africa, 72*(3), 242–250. https://doi.org/10.1080/0035919X.2017.1303797.
20. Li, M., Yang, Y., Xie, J., Xu, G., & Yu, Y. (2019). In-vivo and in-vitro tests to assess toxic mechanisms of nano ZnO to earthworms. *Science of the Total Environment, 687*, 71–76. https://doi.org/10.1016/J.SCITOTENV.2019.05.476.
21. Karmegam, N., Vijayan, P., Prakash, M., & John Paul, J. A. (2019). Vermicomposting of paper industry sludge with cowdung and green manure plants using Eisenia fetida: A viable option for cleaner and enriched vermicompost production. *Journal of Cleaner Production, 228*, 718–728. https://doi.org/10.1016/j.jclepro.2019.04.313.
22. van den Brink, N. W., Jemec Kokalj, A., Silva, P. V., Lahive, E., Norrfors, K., Baccaro, M., et al. (2019). Tools and rules for modelling uptake and bioaccumulation of nanomaterials in invertebrate organisms. *Environmental Science: Nano*. https://doi.org/10.1039/c8en01122b.
23. Hu, C. W., Li, M., Cui, Y. B., Li, D. S., Chen, J., & Yang, L. Y. (2010). Toxicological effects of TiO2 and ZnO nanoparticles in soil on earthworm Eisenia fetida. *Soil Biology and Biochemistry, 42*(4), 586–591. https://doi.org/10.1016/J.SOILBIO.2009.12.007.
24. Li, L.-Z., Zhou, D.-M., Peijnenburg, W. J. G. M., van Gestel, C. A. M., Jin, S.-Y., Wang, Y.-J., & Wang, P. (2011). Toxicity of zinc oxide nanoparticles in the earthworm, Eisenia fetida and subcellular fractionation of Zn. *Environment International, 37*(6), 1098–1104. https://doi.org/10.1016/J.ENVINT.2011.01.008.
25. Nahrul Hayawin, Z., Abdul Khalil, H. P. S., Jawaid, M., Hakimi Ibrahim, M., & Astimar, A. A. (2010). Exploring chemical analysis of vermicompost of various oil palm fibre wastes. *Environmentalist, 30*(3), 273–278. https://doi.org/10.1007/s10669-010-9274-9.
26. OECD. (1984). *Earthworm, acute toxicity tests, OECD guideline for testing of chemicals-207*. https://doi.org/10.1787/9789264070042-en.
27. Herring, R. (2010). *Modulation of the Coelomic Fluid Protein Profile in the Earthworm, Lumbricus Terrestris, After Exposure to Copper as Copper Sulfate*. Thesis prepared for the degree of Master of Science, submitted to University of North Texas, America. Retrieved from https://digital.library.unt.edu/ark:/67531/metadc28428/%60
28. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry, 193*(1), 265–275 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14907713.
29. Magwere, T., Naik, Y. S., & Hasler, J. A. (1997). Effects of chloroquine treatment on antioxidant enzymes in rat liver and kidney. *Free Radical Biology & Medicine, 22*(1–2), 321–327 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8958157.
30. Wilczek, G., Babczyńska, A., & Wilczek, P. (2013). Antioxidative responses in females and males of the spider Xerolycosa nemoralis (Lycosidae) exposed to natural and anthropogenic stressors. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 157*(2), 119–131. https://doi.org/10.1016/j.cbpc.2012.10.005.
31. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*(1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.
32. Cappuccino, J. G., & Sherman, N. (1999). *Microbiology: A Laboratory Manual* (5th ed.). Menlo Park/California: Benjamin/Cummings.

33. Gautam, A., Ray, A., Mukherjee, S., Das, S., Pal, K., Das, S., et al. (2018). Immunotoxicity of copper nanoparticle and copper sulfate in a common Indian earthworm. *Ecotoxicology and Environmental Safety, 148*, 620–631. https://doi.org/10.1016/j.ecoenv.2017.11.008.
34. Feng, L., Zhang, L., Zhang, Y., Zhang, P., & Jiang, H. (2015). Inhibition and recovery of biomarkers of earthworm Eisenia fetida after exposure to thiacloprid. *Environmental Science and Pollution Research, 22*(12), 9475–9482. https://doi.org/10.1007/s11356-015-4122-6.
35. Jeyanthi, V., John Paul, J. A., Karunai Selvi, B., & Karmegam, N. (2016). Comparative Study of Biochemical Responses in Three Species of Earthworms Exposed to Pesticide and Metal Contaminated Soil. *Environmental Processes, 3*(1). https://doi.org/10.1007/s40710-016-0131-9.
36. Garcia-Velasco, N., Peña-Cearra, A., Bilbao, E., Zaldibar, B., & Soto, M. (2017). Integrative assessment of the effects produced by Ag nanoparticles at different levels of biological complexity in Eisenia fetida maintained in two standard soils (OECD and LUFA 2.3). *Chemosphere, 181*, 747–758. https://doi.org/10.1016/J.CHEMOSPHERE.2017.04.143.
37. Du, L., Li, G., Liu, M., Li, Y., Yin, S., & Zhao, J. (2015). Biomarker responses in earthworms (Eisenia fetida) to soils contaminated with di-n-butyl phthalates. *Environmental Science and Pollution Research, 22*(6), 4660–4669. https://doi.org/10.1007/s11356-014-3716-8.