Full Length Research Article

© 2023, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences **14**(6): 1937–1941 (Nov-Dec 2023)
DOI: http://rjas.org/Article/Article/5713

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Gut Biochemical and Antioxidant Enzyme Analysis of *Eudrilus eugeniae* Treated with CuO Nanoparticles

MOHAMAD RAFEEK J.[1], MEERAMAIDEEN M.*[2], RAJASEKAR P.[3] and SANESH P. K.[4]

[1-4] PG and Research Department of Zoology, Jamal Mohamed College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli - 620 020, Tamil Nadu, India

Received: 04 Oct 2023; Revised accepted: 26 Nov 2023; Published online: 15 Dec 2023

Abstract

Nanoparticles (NPs) are widely used material due to their typical size (<100nm) and potential interaction mechanism. Even though various precautionary steps were maintained during synthesis and handling of nanoparticles, aquatic and terrestrial pollution were developed due to either intentionally or accidentally releasing of NPs. Three different concentration (120, 150 and 180ppm) of copper oxide (CuO) NPs were exposed to the adult *Eudrilus eugeniae* earthworm for 14 days (n=20). Gut tissues were subjected to biochemical (AST, ALT, ACP, ALP, Total protein) and enzymatic (SOD, GSH, GPx, GST, and CAT). Considerably increased exposure of CuO NPs resulted in the increased ($P<0.05$ and $P<0.01$) tissue damaging enzymes such as transaminases and phosphatases in the Gut tissues of the treated earthworms. This condition was evidenced in the reduction of decreased total protein levels in the treated worms than control. Decreased antioxidant enzyme levels were observed in the treated earthworms than control. Our study concluded that exposure of CuO NPs to the *E. eugeniae* resulted in the significantly varied biochemical and antioxidant enzymes in the gut tissues.

***Key words*:** Copper oxide nanoparticle, Gut tissues, Biochemical, Antioxidant enzymes, Earthworm

Nanotechnology is considered as one of the quickest growing fields. Nanoparticles sizes are always less than 100 nm. Almost all recent technologies, including computers and medicine delivery systems, have used nanoparticles [1]. In addition to being used in wastewater treatment [2], nanoparticles have also been used in agriculture for sustained growth of plants where they act as carrier ions, magnetic resonance imaging, magnetic sensing and ultrasonography [3-6]. Nanoparticles (NPs) can be used for a number of additional beneficial purposes, but when handled improperly, they can also have negative effects on the environment and living things, including pollution and poisonous effects. From 2005 to 2010 there was a sharp increase in the improper disposal of nanoparticles [7].

Copper oxide (CuO) nanoparticles has unique characteristics such as conductivity, stability, catalytic activity, antibacterial and anticancerous activities, has attracted greater interest than other nanomaterials. Due to its accessibility, affordability than compared to the costliest noble metals like gold and silver, and also has effective potential as microbial agents, researchers are gaining increased attention towards CuO NPs [8-9].

Even though there are numerous safety precautions that must be taken when creating and managing nanoparticles, disposing of trash, and cleaning up, inappropriate removal of nanomaterials is still a common practice. According to MacCormack and Goss [10] and Boraschi *et al*. [11], enhanced utilization and inopportune removal of nanoproducts will certainly cause the spread of nano-waste into the ecosystems, having a negative impact on soil-dwelling organisms and even possibly causing the extinction of some species, particularly earthworms.

Several vertebrates such as mice and fish are often used, utilizing smaller species like earthworms [12] can save time and aid in the investigation of the effects of NPs. Earthworms are the most common soil invertebrate animals and constantly exposed to viruses, bacterium and fungi. The hazardous effects of nanoparticles have been widely studied using earthworms, and they provide an overview of the consequences of chemically synthesized CuO NPs.

Due to discerning rummaging, the gut regions are frequently more concentrated in few soil particles, nutrients, organic matter and water, which can support the enhanced microbial activities. Various biochemical measures are employed to evaluate the enzyme action in earthworms, and AST and ALT levels operate as indicators of chloragogen cell activity [13]. A change in the metabolic pathway for carbohydrates that favours the anaerobic breakdown of glycogen, which is typically observed in organisms under stress. Though aspartate transaminase (AST or L-aspartate 2-oxoglutarate aminotransferase) and alanine transaminase (ALT or L-alanine 2-oxoglutarate aminotransferase) enzymes are known to be important in transporting L-amino acids for gluconeogenesis process and serve as bridges between the metabolism of carbohydrates and proteins [14].

Alterations in physiology, disease, and artificial environmental stress. Numerous enzymes, including transaminases and alkaline and acid phosphates (ALP, ACP),

***Correspondence to:** Meeramaideen M., E-mail: mmeera@jmc.edu; Tel: +91 9500980766

Citation: Mohamad Rafeek J, Meeramaideen M, Rajasekar P, Sanesh PK. 2023. Gut biochemical and antioxidant enzyme analysis of *Eudrilus eugeniae* treated with CuO nanoparticles. *Res. Jr. Agril. Sci*. 14(6): 1937-1941.

are tested as helpful indicators to identify cellular deterioration and cell rupture. Two transaminases that are crucial for protein and carbohydrate metabolism, serve as markers for tissue injury [15]. The main objective was to study the effect of CuO NPs on *E. euginea* gut tissues biochemical parameters (AST, ALT, ACP, ALP and total protein) and antioxidant enzymes (GSH, GST, GPx, SOD and CAT).

MATERIALS AND METHODS

Experimental animal

The earthworm, *Eudrilus eugeniae* is an epigeic alien species acquired from Periyar University (Vallam, Tanjore, Tamil Nadu) and moved to Jamal Mohamed College (Tiruchirappalli) for acclimatization. After 5 weeks, the adult earthworms are gathered and divided into groups [16]. Groups of 20 are kept in separate pots with 10 kg of soil each. Chemically synthesized CuO NPs acute toxicity (14 days) on *E. eugeniae* resulted in earthworm mortality, with LC50 of 1100-1200ppm. For this study, three different concentrations of CuO NPs (120, 150, and 180ppm) were chosen.

Biochemical parameters

The tissue samples from the dissected earthworms are homogenized in cold phosphate buffer (0.1M, pH 7.2), followed by centrifugation at 4°C for 20min at 10,000 rpm. The ALT, AST, ALP, and ACP enzymes are measured in the clear supernatants [17]. The Diagnostic laboratory examined each biochemical parameter. The approach of Lowry *et al.* [18] is used to assess the protein content in different earthworm tissues.

Antioxidant enzyme studies

Samples of earthworm gut tissue were centrifuged at 10,000 g for 20 minutes at 4°C after being homogenized in cold phosphate buffer (0.1M, pH 7.2). The GSH, SOD, GST, CAT and GPx activities were determined using the clear supernatants, or they were stored at 80°C for later use [17].

Superoxide dismutase (SOD) activity

According to Giannopolitis and Ries [19] method, efficiency of the SOD which prevent the photochemical denaturation of nitroblue tetrazolium chloride (NBT) was estimated. The sample extract was mixed with EDTA (100mM), phosphate buffer (pH 7.8, 50 mM), riboflavin (20 mM), NBT (750 mM), and methionine (130 mM) were allowed to react for 15 minutes. At 560 nm, absorbance of the mixture was measured. One unit of enzyme activity (U) was defined as the amount of enzyme that reduced the rate of NBT autooxidation by 50% and the values were expressed as U/mg protein.

Reduced glutathione (GSH) and GPx activities

Anderson [20] method was used to test reduced glutathione. The sample extract was mixed with sodium phosphate (125mM), disodium EDTA (6.3mM), NADPH (0.3mM) (pH 7.5), and 5,5'dithiobis-2-nitrobenzoic acid (DTNB) solution. To convert all GSSG (oxidized glutathione) into GSH, to the mixture GSH reductase (GR) one unit was added. GSH concentration was measured at 412 nm absorbance after 5 minutes of incubation and values expressed as $mg100g^{-1}$. Clair and Chow [21] method used to measure GPx activity. In Tris-HCl buffer (50 mM, pH 7.5), the sample extract was mixed with disodium EDTA (2mM), sodium azide (1mM), GSH (1mM), NADPH (0.2mM) and GR one unit. For the initiation of reaction, cumene hydroperoxide (1.2 mM), and GPx were added by measuring the optical density (340 nm). The enzyme activity data were represented as $mg100g^{-1}$ of tissue.

Glutathione-S-transferase (GST) and catalase (CAT) activities

GST activity was determined by using Habig *et al.* [22] method. Potassium phosphate buffer (100 mM, pH 6.9), GSH (1 mM), 1-chloro-2,4-dinitrobenzene (1 mM), and the sample added to the reaction mixture. At 340 nm, optical density of the solution was measured and represented as $mg100g^{-1}$ of tissue. Claiborne [23] method was used to measure catalase (CAT) activity. The sample extract was mixed with phosphate buffer (pH 7.0, 66 mM) and H_2O_2 (3%). At 240 nm, the optical density of the catalase enzyme was measured and are reported as Umg^{-1} of protein.

Statistical analysis

For the examination of the statistical difference in biochemical and antioxidant enzyme levels in earthworms subjected to nanoparticles compared to the control sets. $P<0.05$ was used to signify a significant difference. Values were evaluated for the one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Ingestion of soil particles along with CuO NPs were augmented into the earthworm's body through gut tissues. Treated worms gut tissues showed significantly varied tissue damaging enzymes and antioxidant enzymes than control group worms. The physiological condition, age, and microorganisms all have an impact on the region-specific specialization of earthworm enzyme activity [24].

Earthworms treated with ZnO NPs had altered enzyme levels as a result of the altered physiological state brought on by the organs' lower biochemical makeup. *Eisenia fetida* treated with ZnO NPs and found that increasing accumulation occurs as NPs concentration rises [25], supporting the findings of this study. Many various forms of environmental contaminants disrupt the cell's normal metabolic process, causing in the generation of reactive oxygen species (ROS), which leads to cell and tissue denaturation; thus, a high level of antioxidant enzymes may involve in defending biosystem [26].

Biochemical parameters

Total protein and tissues damaging enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), acid phosphatase (ACP) and alkaline phosphatase (ALP) levels were analyzed. Significantly decreased total protein levels ($F=0.90$, $P<0.05$) were observed as 0.90±0.02, 0.75±0.03 and 0.58±0.03gm/dL for 120, 150 and 180ppm respectively than control group (1.01±0.02gm/dL) earthworm gut tissues (Table 1). In Control group, the ALT and AST levels were observed as 56.06±4.88 and 98.11±6.08IU/L. Treated groups ALT levels were found as 61.12±2.34, 67.80±2.74 and 75.27±3.56IU/L for 120, 150 and 180ppm groups respectively. Similarly, increased AST levels were observed as 102.34±5.35, 114.47±4.35 and 123.58±4.73IU/L for 120, 150 and 180ppm respectively. Significantly increased ALT ($F=1.11$, $P<0.05$) and AST ($F=2.02$, $P<0.05$) were observed in treated groups than control due to the deamination of the proteins and other macromolecular degradation.

Membrane damages leads to the release of phosphatase enzymes such as ACP and ALP. Control ALP levels was observed as 101.01±3.58IU/L whereas 119.94±2.16, 131.35±1.68 and 145.68±1.38IU/L for 120, 150 and 180ppm respectively (Table 1, Fig 1). Control ACP levels was observed as 123.45±3.63IU/L whereas 138.01±1.70, 149.74±2.14 and

160.53±2.11IU/L for 120, 150 and 180ppm respectively. Significantly (P<0.01) increased ALP (F=2.11) and ACP (F=1.98) were observed in treated groups than control due to the membrane integration damages and releases.

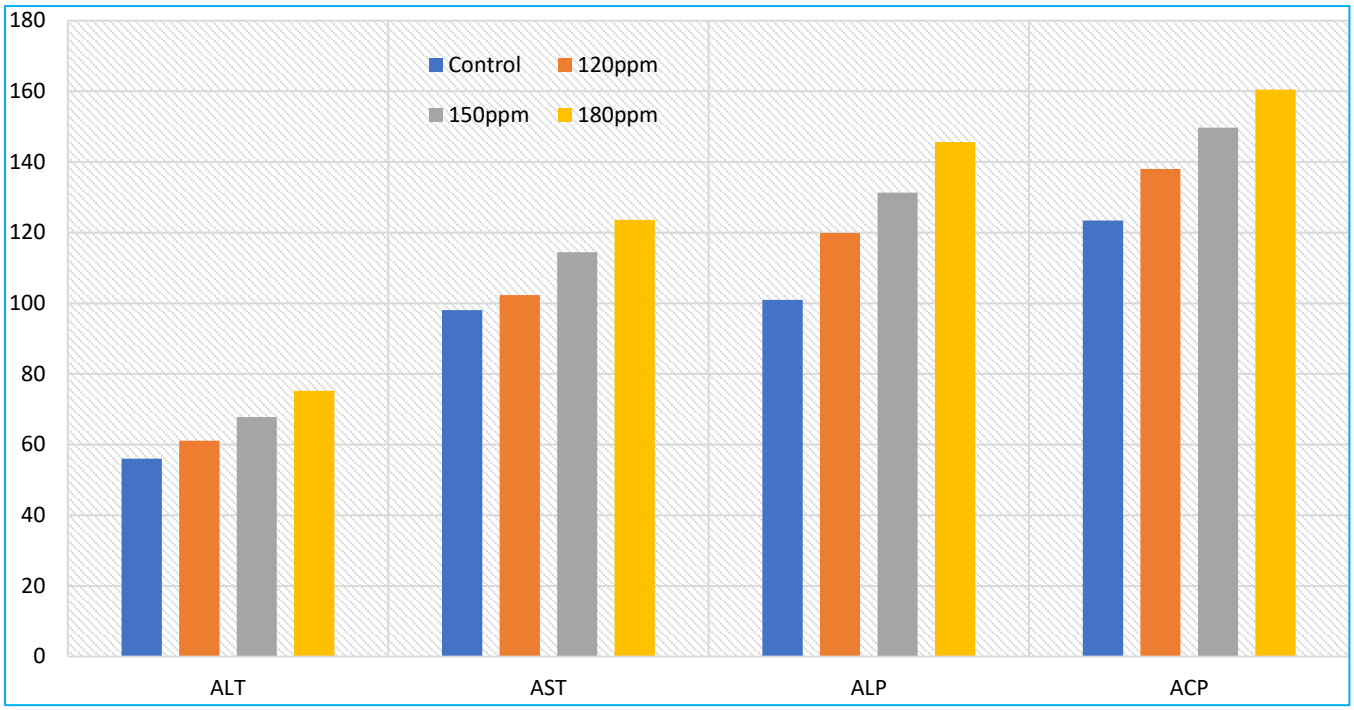

Fig 1 Transaminases and phosphatase enzyme analysis in CuO NPs treated earthworm *E. eugeniae*

Two essential enzymes known for their roles in the utilization of proteins and carbohydrates are GOT and GPT. Transamination chemical reactions are common in stressful environments, and the enzymes are generated following cellular damage or lysis, they can be used to gauge how much stress an organism is experiencing as a result of exposure to a toxicant [27]. ALP is a chemical that is used as an intermediary step in the production of glycogen and the breakdown of glycogen.

Table 1 Control and CuO NPs treated earthworm gut tissues biochemical and antioxidant enzyme studies

Groups	Biochemical parameters				
	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	ACP (IU/L)	Total protein (gm/dL)
Control	56.06±4.88	98.11±6.08	101.01±3.58	123.45±3.63	1.01±0.02
120ppm	61.12±2.34	102.34±5.35	119.94±2.16	138.01±1.70	0.90±0.02
150ppm	67.80±2.74	114.47±4.35	131.35±1.68	149.74±2.14	0.75±0.03
180ppm	75.27±3.56	123.58±4.73	145.68±1.38	160.53±2.11	0.58±0.03
F value	1.11*	2.02*	2.11**	1.98**	0.90*
Groups	Antioxidant enzymes				
	GSH (mg.100g^{-1})	GPx (mg.100g^{-1})	GST (mg.100g^{-1})	SOD (U.mg^{-1})	CAT (U.mg^{-1})
Control	7.06±0.11	3.09±0.08	198.05±1.23	3.29±0.21	20.3±0.11
120ppm	6.56±0.23	2.98±0.06	154.30±0.99	3.11±0.14	17.6±0.07
150ppm	6.00±0.31	2.45±0.05	112.81±1.03	2.58±0.26	11.7±0.04
180ppm	5.69±0.29	2.30±0.06	97.69±0.91	2.32±0.11	9.80±0.08
F value	2.16*	1.64*	2.89**	1.24*	1.99*

*P<0.05; **P<0.01 significantly varied from the control earthworm (n=20), GPx: Glutathione peroxidase, GSH: Reduced glutathione, GST: Glutathione-S-transferase, SOD: - Superoxide dismutase, CAT: Catalase

ACP is a hydrolytic lysosomal enzyme that fights invading, poisonous foreign compounds by releasing it during hydrolysis process. The foreign substance is cleansed by ACP [28]. Similar to our findings, Ahmed *et al*. [29], Habiba and Ismail [30] observed increased transaminases, acid and alkaline phosphatases in relation to lower protein levels in earthworm tissues exposed to various pollutants. There are no significant modifications in antioxidant enzyme levels in the three different earthworm species following carbaryl (12 and 25 mg/kg) and Pb (75 and 150 mg/kg) treatment.

Antioxidant parameters

Antioxidant enzymes are basically involved in the elimination of oxidative species or oxygen radical. Reduced glutathione levels were significantly (F=2.16, P<0.05) reduced CuO NPs treated 6.56±0.23, 6.00±0.31 and 5.69±0.29mg.100g^{-1} for 120, 150 and 180ppm groups respectively than control worm levels as 7.06±0.11mg.100g^{-1}. Similarly, Glutathione peroxidase (F=1.64, P<0.05) and GST (F=2.89, P<0.01) levels were also significantly decreased as 2.30±0.06 and 97.69±0.91mg.100g^{-1} in 180ppm (Table 1) groups respectively.

Control worms GST levels were observed as 198.05±1.23mg.100g^{-1} whereas CuO NPs treated groups 154.30±0.99, 112.81±1.03 and 97.69±0.91 mg.100g^{-1} for 120, 150 and 180ppm groups respectively. Control group SOD and CAT levels were observed as 3.29±0.21 and 20.3±0.11 U.mg^{-1} respectively. SOD levels of the CuO NPs treated groups (120, 150 and 180ppm) were observed as 3.11±0.14, 2.58±0.26 and

2.32±0.11U.mg^{-1} respectively. CAT levels in the CuO NPs treated groups (120, 150 and 180ppm) were found as 17.6±0.07, 11.7±0.04 and 9.80±0.08 U.mg^{-1} respectively. Significantly decreased GST (F=2.89), SOD (F=1.24) and CAT (F=1.99) levels were observed as increasing concentrations of the CuO NPs.

Acute exposure (14 days) of lead resulted in the significantly varied antioxidant enzyme levels in *E. eugeniae*. 150ppm Pb treated earthworm showed a substantial decrease in SOD activity. Significantly decreased levels of catalase was also reported in earthworms *P. excavatus* and *E. eugeniae* treated to the similar quantity of Pb. Our studies are corroborated by Liu *et al*. [31], who observed suppression of SOD and CAT enzyme activity in *E. fetida* following 7 days of exposure to HHCB (1,3,4,6,7,8,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-c-2-benzopyran) at 50-100ppm. One probable explanation is that H_2O_2 inhibits SOD [32]. Du *et al*. [33] also found that *E. fetida* was significantly lower in SOD and CAT levels after being exposed to 50-100 ppm of DBP (Din-butyl phthalates) for 28 days. Several research on the association between the cellular GST levels and metal pollutants revealed that the chemical glutathione has a cell protective mechanism against metal pollutant induced toxicity [34].

P. ceylanensis treated to carbaryl and Pb of 50 mg/kg and 150 mg/kg for 10 and 14 days respectively, showed reduced GSH. This is consistent with recent research on *Lampito mauritii*, which showed that depletion of GSH level occurred after exposure to enhancing concentrations of lead (75, 150, and 300 mgkg^{-1}) for one week [35]. *P. excavatus* and *E. eugeniae* exposed to Pb showed a considerable rise in GST levels, and under carbaryl stress conditions, *E. eugeniae* and *P. ceylanensis* showed a decrease in the activity of the same enzyme. Both Pb and carbaryl treatment decreased the GPx activity in all three earthworm species [36]. Maity *et al*. [35] also evidenced that *L. mauritii* subjected to soil contaminated with 75, 150, and 300 mg kg^{-1} of Pb had higher GST and GPx levels. The enzymatic profiles of GPx, GST, GSH, CAT and SOD provided a strong indication of the earthworms' ability to tolerate long-term exposure to agro pesticides and metal-contaminated soil. Similar enzymatic and biochemical results were reported by Biruntha *et al*. [37] in *Eudrilus eugeniae* treated with zinc nanoparticles.

CONCLUSION

Copper oxide nanoparticles treated earthworm gut tissues showed significantly varied tissue damaging enzymes and antioxidant enzymes than control group earthworms. NPs augmented through soil entered into the gut tissues which resulted in the altered biochemical and antioxidant enzymes in the treated earthworms.

Acknowledgement

Authors are thankful to Dr. A. K. Khaja Nazeemudeen, Secretary and Correspondent, Dr. S. Ismail Mohideen, Principal and Dr. I. Antony Joseph Jerald, Head, P.G. and Research Department of Zoology (DBT STAR Funded), Jamal Mohamed College (Autonomous), Tiruchirappalli for Institutional support.

Conflict of interest

Authors have no any conflict of interest.

LITERATURE CITED

1. Yu J, Zhou Y, Chen W, Ren J, Zhang L, Lu L, Luo G, Huang H. 2015. Preparation, characterization and evaluation of α-tocopherol succinate-modified dextran micelles as potential drug carriers. *Materials* 8: 6685-6696.
2. Tiwari DK, Behari J, Sen P. 2008. Application of nanoparticles in waste water treatment. *World Applied Science Journal* 3(3): 417-433.
3. Pankhurst QA, Connolly J, Jones SK, Dobson J. 2003. Applications of magnetic nanoparticles in biomedicine. *Jr. Phys. D Applied Physics* 36: R167-R181.
4. Mukhopadhya SS. 2014. Nanotechnology in agriculture: prospects and constraints. *Nanotechnol. Sci. Appl*. 7: 63-71.
5. Gautam A, Ray A, Mukherjee S, Santanu Das S, Pal K., Subhadeep Das P, Karmakar M, Ray S. 2018. Immunotoxicity of copper nanoparticle and copper sulfate in a common Indian earthworm. *Ecotoxicology Environ. Saf.* 148: 620-631.
6. Thurn-Albrecht T, Schotter J, Kastle GA, Ley N. 2000. Ultrahigh-density nanowire arrays grown in self-assembled diblock copolymer templates. *Science* 290: 2126-2129.
7. Lee WM, Kim SW, Kwak JI, Nam SH, Shin YJ, An YJ. 2010. Research trends of ecotoxicity of nanoparticles in soil environment. *Toxicol. Research* 26(4): 253-259.
8. Sankar R, Manikandan P, Malarvizhi V, Fathima T, Shivashangari Ravikumar KSV. 2014. Green synthesis of colloidal copper oxide nanoparticles using Carica papaya and its application in photocatalytic dye degradation. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc*. 121: 746-750.
9. Singh D, Jain D, Rajpurohit D, Jat G, Kushwaha HS, Singh A, Mohanty SR, Al-Sadoon MK, Zaman W, Upadhyay SK. 2023. Bacteria assisted green synthesis of copper oxide nanoparticles and their potential applications as antimicrobial agents and plant growth stimulants. *Front. Chem*. 11: 1154128.
10. MacCormack TJ, Goss GG. 2008. Identifying and predicting biological risks associated with manufactured nanoparticles in aquatic ecosystems. *Jr. Ind. Ecology* 12(3): 286-296.
11. Boraschi D, Alijagic A, Auguste M, Barbero F, Ferrari E, Hernadi S, Mayall C, Michelini S, Navarro Pacheco NI, Prinelli A, Swart E, Swartzwelter BJ, Bastús NG, Canesi L, Drobne D, Duschl A, Ewart MA, Horejs-Hoeck J, Italiani P, Kemmerling B, Kille P, Prochazkova P, Puntes VF, Spurgeon DJ, Svendsen CJ, Wilde AP. 2020. Addressing nanomaterial immune safety by evaluating innate immunity across living species. *Small* 16: Article e2000598.
12. Exbrayat JE, Moudilou EN, Lapied E 2015. Harmful effects of nanoparticles on animals. *Jr. Nanotechnology* 2015: 10.
13. Tiwari R, Singh S, Pandey R, Sharma B. 2016. Enzymes of earthworm as indicators of pesticide pollution in soil. *Adv. Enz. Research* 4: 113-124.
14. Qi B. 2009. Acute and reproductive toxicity of nano-sized metal oxides (ZnO and TiO_2) to earthworms (*Eisenia fetida*). M.D. Dissertation, Texas Tech University, Texas.

15. Song Z, Cawthon D, Beers K, Bottje WG. 2000. Hepatic and extra-hepatic stimulation of glutathione release into plasma by norepinephrine in vivo. *Poul. Science* 79: 1632-1639.
16. Mohamad Rafeek J, Meeramaideen M, Suganthi P, Rajasekar P, Jafar Ahamed A. 2022. Synthesis and characterization of CuO nanoparticles and acute lethal studies in *Eudrilus eugeniae* earthworm. *Asian Jr. Biol. Life Science* 11(3): 731-736.
17. Lala V, Zubair M, Minter DA. 2023. *Liver Function Tests*. *In*: Stat Pearls [Internet]. Treasure Island (FL): Stat Pearls Publishing; 2023 Jan- Available from: https://www.ncbi.nlm.nih.gov/books/NBK482489/
18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. *Jr. Biol. Chem*. 193(1): 265-275.
19. Giannopolitis CN, Ries SK. 1977. Superoxide Dismutases I. Occurrence in higher plants. *Plant Physiology* 59: 309-314.
20. Anderson M. 1996. *Glutathione in Free radicals*: *A Practical Approach*. Oxford University Press, New York.
21. Clair DK, Chow CK. 1996. Chapter 16: Glutathione peroxidase: activity and steady-state level of mRNA. *In*: (Eds) Punchard NA and Kelly FJ. Free Radicals a Practical Approach, Oxford University Press, New York. pp 227-240.
22. Habig W, Pabst M, Jakoby W. 1974. Glutathion-Stransferases- The first step in mercapturic acid formation. *Jr. Biol. Chem*. 249(22): 7130-7139.
23. Claiborne A. 1985. *Catalase Activity. Handbook Methods for Oxygen Radical Research*. 1st Edition. CRC Press. pp 2. ISBN:9781351072922
24. Prabha P, Li YL, Usha RK. 2008. An experimental study of vermi-biowaste composting for agricultural soil improvement. *Bioresource Technology* 99(6): 1672-1681.
25. Hu CW, Li M, Cui YB, Li DS, Chen J, Yang LY. 2010. Toxicological effects of TiO_2 and ZnO nanoparticles in soil on earthworm *Eisenia fetida*. *Soil, Biol. Biochem*. 42: 586–591.
26. Hayashi Y, Engelmann P, Foldbjerg R, Szabó M, Somogyi I, Pollák E, Molnár L, Autrup H, Sutherland DS, Scott-Fordsmand J, Heckmann LH. 2012. Earthworms and humans in vitro: characterizing evolutionarily conserved stress and immune responses to silver nanoparticles. *Environ. Sci. Technology* 46: 4166-4173.
27. Nchumbeni H, Kulkarni DR, Bhavita Chavan BG. 2007 Effect of arsenic on the enzymes of the rohu carp, *Labeo rohita* (Hamilton, 1822). *The Raffles Bull. of Zoo. Suppl*. 14: 17-19.
28. Jaroli DP, Sharma BL. 2005. Effect of organophosphate insecticide on the organic constituents in liver of *Channa punctatus*. *Asian Jr. Exp. Sci*. 19: 121-129.
29. Ahmed YM, Ismail SM, Shoukry A. 1991. An assessment of diflubenzuron to earthworms. 4th National Congress on Pests and Diseases of Vegetables and Fruits in Egypt; Ismailia, Egypt. pp 341-351.
30. Habiba RA, Ismail SM. 1992. Biochemical effects of profenfos in the New Zealand white rabbit. *Jr. Pest. Control and Environ. Science* 4: 15-29.
31. Liu J, Levine AL, Matton JS, Yamaguchi M, Lee RJ, Pan X, Rosol TJ. 2006. Nanoparticles as image enhancing agents for ultrasonography. *Phys. Med. Biol*. 51: 2179-189.
32. Sandalio LM, Dalurzo HC, Gómez M, Romero-Puertas MC, del Río LA. 2001. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *Jr. Exp. Botany* 52(364): 2115-2126.
33. Du L, Li G, Liu M, Li Y, Yin S, Zhao J. 2014. Biomarker responses in earthworms (*Eisenia fetida*) to soils contaminated with di-n-butyl phthalates. *Env. Sci. Pol. Res. Int*. 22(6): 133-145.
34. Saint-Denisa M, Narbonne JF, Arnaud C, Ribera D. 2001. Biochemical responses of the earthworm *Eisenia fetida* andrei exposed to contaminated artificial soil: effects of lead acetate. *Soil Bio and Biochemistry* 33: 395-404.
35. Maity S, Roy S, Chaudhury S, Bhattacharya S. 2008. Antioxidant responses of the earthworm *Lampito mauritii* exposed to Pb and Zn contaminated soil. *Environ. Pollution* 151: 1-7.
36. Jeyanthi V, James Arockia JP, Karunai S, Karmegam N. 2016. Comparative study of biochemical responses in three species of earthworms exposed to pesticide and metal contaminated soil. *Environ. Process* 3: 167-178.
37. Biruntha M, Archana J, Kavitha K. 2020. 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*. *BioNano Science* 10: 149-156.