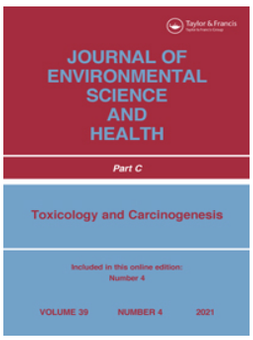
Taylor & Francis
Taylor & Francis Group



Journal of Environmental Science and Health, Part C
Toxicology and Carcinogenesis

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/lesc21

Ecotoxicological effects of dietary titanium dioxide nanoparticles on metabolic and biochemical parameters of model organism *Galleria mellonella* (Lepidoptera: Pyralidae)

Serkan Sugeçti, Benay Tunçsoy, Ender Büyükgüzel, Pınar Özalp & Kemal Büyükgüzel

To cite this article: Serkan Sugeçti, Benay Tunçsoy, Ender Büyükgüzel, Pınar Özalp & Kemal Büyükgüzel (2021) Ecotoxicological effects of dietary titanium dioxide nanoparticles on metabolic and biochemical parameters of model organism *Galleria mellonella* (Lepidoptera: Pyralidae), Journal of Environmental Science and Health, Part C, 39:4, 423-434, DOI: 10.1080/26896583.2021.1969846

To link to this article: https://doi.org/10.1080/26896583.2021.1969846

Published online: 09 Sep 2021.

Submit your article to this journal

Article views: 287

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=lesc21

JOURNAL OF ENVIRONMENTAL SCIENCE AND HEALTH, PART C, TOXICOLOGY AND CARCINOGENESIS
2021, VOL. 39, NO. 4, 423–434
https://doi.org/10.1080/26896583.2021.1969846

Taylor & Francis
Taylor & Francis Group

Check for updates

Ecotoxicological effects of dietary titanium dioxide nanoparticles on metabolic and biochemical parameters of model organism *Galleria mellonella* (Lepidoptera: Pyralidae)

Serkan Sugeçti[a], Benay Tunçsoy[b], Ender Büyükgüzel[c], Pınar Özalp[d], and Kemal Büyükgüzel[e]

[a]Department of Veterinary Medicine, Çaycuma Food and Agriculture Vocational School, Zonguldak Bülent Ecevit University, Zonguldak, Turkey; [b]Faculty of Engineering, Department of Bioengineering, Adana Alparslan Turkeş Science and Technology University, Adana, Turkey; [c]Science and Art Faculty, Department of Molecular Biology and Genetics, Zonguldak Bülent Ecevit University, Zonguldak, Turkey; [d]Science and Art Faculty Department of Biology, Çukurova University, Adana, Turkey; [e]Science and Art Faculty, Department of Biology, Zonguldak Bülent Ecevit University, Zonguldak, Turkey

ABSTRACT

Nanoparticles (NPs) are now being used in many industrial activities, such as mining, paint and glass industries. The frequent industrial use of NPs contributes to environmental pollution and may cause cellular and oxidative damage in native organisms. In this study, the toxic effects of titanium dioxide nanoparticles (TiO_2 NPs) were investigated using *Galleria mellonella* larvae as a model insect species. Alterations in cell damage indicators, such as alanine transferase, aspartate transferase, lactate dehydrogenase, non-enzymatic antioxidants and biochemical parameters, were determined in the hemolymph of *G. mellonella* larvae exposed to TiO_2 NPs at different concentrations (5, 50, 250 and 1250 μg/mL) in their diets. TiO_2 NPs caused concentration-dependent cellular damage in the hemolymph of *G. mellonella* larvae and increased the levels of the non-enzymatic antioxidants uric acid and bilirubin. In addition, total protein in hemolymph significantly decreased at the highest concentration (1250 μg/mL) of TiO_2 NPs. Level of the urea increased at the highest concentration (1250 μg/mL) of TiO_2 NPs, whereas the amount of glucose was not affected. These findings demonstrated that TiO_2 NPs caused concentration-dependent toxic effects on *G. mellonella* larvae.

KEYWORDS

Nanoparticles; environmental pollution; non-enzymatic antioxidants; transferase enzymes; model organism

Introduction

Nanoparticles (NPs) are being used in many applications by mining, paint, glass, and other industries and their widespread use causes environmental pollution. Therefore, the ecotoxicological effects of NPs are an important

CONTACT Serkan Sugeçti, serkan.sugecti@hotmail.com Department of Veterinary Medicine, Çaycuma Food and Agriculture Vocational School, Zonguldak Bülent Ecevit University, Zonguldak, Turkey

© 2021 Taylor & Francis Group, LLC

problem.[1] Nanoparticles that pass through biological membranes can cause adverse physiological and biochemical effects.[1] Metal oxide nanoparticles, such as titanium dioxide nanoparticles (TiO_2 NPs) and copper oxide nanoparticles (CuO NPs), are the most widely used nanoparticles in industry and could cause adverse effects on the environment and human health.[2,3]

TiO_2 NPs are also present in cosmetics and antibacterial products that are widely used by consumers; NPs in these products then contaminate wastewater where additional TiO_2 NPs may be added to act as photocatalysts for the degradation of organic pollutants, further contributing to the environmental release of these materials.[4–6] TiO_2 NPs represents the highest produced nanomaterilas worldwide, with annual production predicted to reach 2.5 million tonnes by 2025.[7–9] TiO_2 may also be used as to adsorb organic pollutants and metals in water ecosystems by nano remediation.[7] It has also been shown that TiO_2 NPs react with other NPs or metal pollutants and therefore accumulate in organisms to cause oxidative stress.[10,11] Intense use of TiO_2 NPs in many industrial areas poses a danger to the ecosystem.

Organisms are affected by environmental pollution due to toxic substances such as pesticides and heavy metals. Biochemical and physiological changes occur in the tissues of organisms exposed to these toxic substances. In studies, it has been stated that cell damage occurs in many organisms such as fish and insects due to increasing environmental pollution.[9,10] In another study conducted with terrestrial invertebrates, it was reported that TiO_2 NPs ingested through diet cause toxic effects.[11] Metabolic enzymes such as alanine transferase (ALT), aspartate transferase (AST), lactate dehydrogenase (LDH) are used as indicators of cell damage.[12] Enzymatic and non-enzymatic antioxidant defense systems protect organisms against oxidative damage. In many studies, it has been reported that there is an increase in the amount of enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase and non-enzymatic antioxidants such as bilirubin (BIL), glutathione (GSH), uric acid (UA), Vitamin E due to environmental pollution.[13–15] In addition, various biochemical parameters are negatively affected in different tissues of organisms due to oxidative stress.[16]

Galleria mellonella is an important agricultural pest that damages beehives, bee combs and beeswax.[17] In recent years, *G. mellonella* has been used as a model organism in ecotoxicology studies, determination of virulence effects of pathogenic bacteria and insecticide research.[16,18–20] Large numbers of *G. mellonella* larvae can be produced easily under laboratory conditions, their use avoids many ethical problems associated with other laboratory species, and their short life cycle represent significant advantages for their use as model organisms for studies in environmental toxicology.[18]

In addition, the immune system of *G. mellonella* resembles the innate immune response of mammals and is therefore also used for immunology studies.[21,22]

The widespread use of TiO_2 NPs in industrial areas could introduce potentially toxic effects for humans, fish and insects.[22,23] In the present study we hypothesized that TiO_2 NPs induce adverse effects leading to deteriorated metabolic and biochemical parameters of *G. mellonella* as a model insect. For this reason, we aimed to determine the effects of dietary TiO_2 NPs on amounts of non-enzymatic, metabolic enzymes and biochemical parameters in hemolymph of *G. mellonella* larvae.

Material and methods

Insect culture

G. mellonella larvae were obtained from the Insect Culture Laboratory in Biology department of Çukurova University. *G. mellonella* larvae were reared in artificial diet in an incubator set at 28 ± 2 °C and 65 ± 5% humidity. Artificial diets were prepared using 420 g wheat bran, 150 mL filtered honey, 150 mL glycerol (Merck, Darmstadt, Germany), 20 g ground dark old honeycomb and 30 mL pure water.[24]

Characterization of TiO_2 NPs

TiO_2 nanopowder (TiO_2 Degussa P25, powder form) was purchased from Sigma–Aldrich (St. Louis, Missouri, USA). TiO_2 nanopowder was used in the preparation of experimental solutions. The method developed by Tunçsoy and Meşe[22] was used for the preparation and characterization of the suspensions of the nanoparticles. Mean Ti levels in exposure media were determined as 4.946 ± 0.05 mg/L, 49.93 ± 0.065, 248.1 ± 1.95 and 1247.86 ± 2.85 Ti, respectively.

Experimental design and biochemical assay

Newly hatched larvae were reared to the last instar larvae (7^{th} instar larvae) with the artificial diet in an incubator set at 28 ± 2 °C and 65 ± 5% humidity. TiO_2 NPs was directly incorporated into the artificial diet at the concentration of 5, 50, 250 and 1250 µg/mL. No TiO_2 NPs was added to the control diet. Experiments were repeated four times and 40 last instar larvae of *G. mellonella* were used in each replicate.

Last-instar larvae of *G. mellonella* were kept on ice for 5 minutes for anesthesia. The surface of the larvae was disinfected with 95% ethanol. Hemolymph from the abdomen of the larvae of *G. mellonella* with

Hamilton needle was collected into Eppendorf tubes (1.5 mL) kept in ice. A few phenylthiourea (PTU) (Sigma Aldrich, Missouri, USA) crystals were placed in the Eppendorf tubes to prevent melanization. Samples were stored at −80 °C until analysis.

Extracts of hemolymph were obtained for biochemical analysis prepared at 4 °C by an ultrasonic homogenizer (Bandelin Sonoplus, HD2070, Germany) at 50 W, 50 s in homogenization buffer (w/v 1.15% KCl, 25 mM K_2HPO_4, 5 mM ethylenediaminetetraacetic acid, 2 mM phenylmethylsulphonyl fluoride, 2 mM dithiotreitol, pH 7.0) followed by centrifugation at 10.000 g for 10 min at 4 °C.[13] The resulting cell-free supernatant extracts of hemolymph were collected for biochemical analyses. The analyses were performed with the Roche Hitachi Cobas c501 instrument (Roche, Germany) using appropriate kits. ALT, AST, LDH, creatine kinase (CK), BIL, glucose (GLU), total protein (TP), UA and urea. Biochemical assays were performed according to manufacturer's instructions.

Statistical analysis

A one-way analysis of variance (ANOVA) test was used to evaluate differences between metabolic enzyme activities and biochemical parameters. Tukey's HSD test was used to determine the importance of the difference between the averages. All analyses were performed in SPSS v.15.0 (SPSS, Chicago, IL, USA). A probability level of 0.05 was used to check the significance of the difference between the averages.

Results

Characterization of TiO_2 NPs

The particle size was characterized using transmission electron microscopy (TEM). TiO_2 NPs were also characterized by X-ray diffraction (XRD). More details on the characterization methods are described in the previous study.[25] Characterizations of TiO_2 NPs were as follows: particle size distribution 28.82 nm ± 11.07 nm, specific surface area 46.45 ± 2.32 m^2/g, density 4.3384 ± 0.0057 g/cm^3.

Effect of dietary TiO_2 NPs on metabolic enzymes in hemolymph of G. mellonella

In the present study, ALT level statistically decreased at 5, 50, 1250 µg/mL concentrations of TiO_2 NPs in hemolymph of *G. mellonella* larvae ($F_{4,15}$: 63.439, $p < 0.001$). ALT activity increased at a concentration of 250 µg/mL, but there was no statistically significant difference ($p > 0.05$) (Fig. 1a). AST level significantly decreased at 5, 50, 1250 µg/mL concentrations of TiO_2

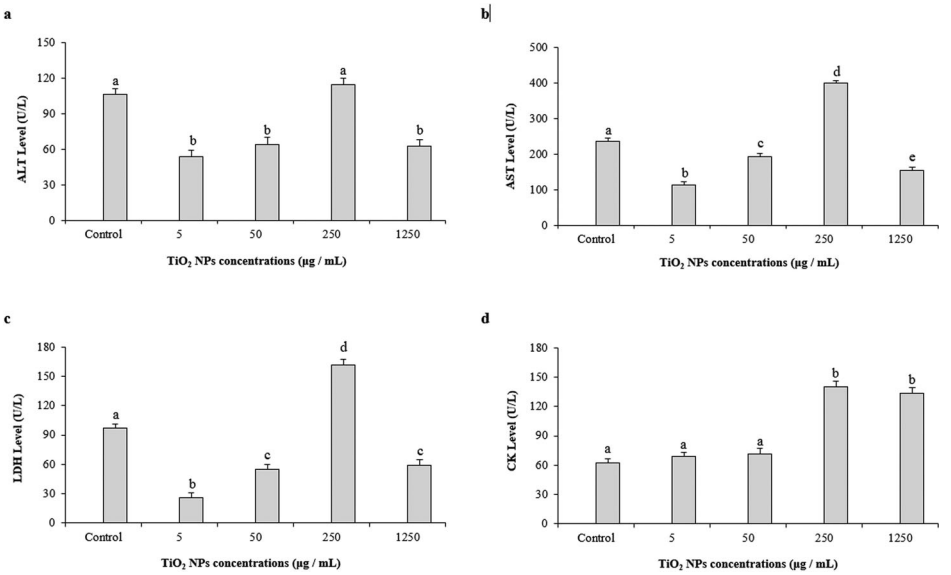


Figure 1. Effects of dietary TiO_2 NPs on metabolic enzymes in hemolymph of *G. mellonella*. Bars represent the means (± S.E.) of four replicates. Means followed by the same letter are not significantly different (Tukey's HSD test: $p < 0.05$) (a: alanine transferase, b: aspartate transferase, c: lactate dehydrogenase, d: creatine kinase).

NPs in hemolymph of *G. mellonella* larvae, while it was statistically increased at 250 µg/mL concentrations ($F_{4,15}$: 832.594, $p < 0.001$) (Figure 1b). The other cell damage indicator, LDH activity, significantly decreased at the highest concentration (1250 µg/mL), although it was statistically increased at a concentration of 250 µg/mL ($F_{4,15}$: 520.453, $p < 0.001$) (Figure 1c). CK activity increased approximately 2-fold at 250 and 1250 µg/mL concentrations of TiO_2 NPs when compared to the control group ($F_{4,15}$: 301.517, $p < 0.001$) (Figure 1d).

Effect of dietary TiO_2 NPs on biochemical paremters in hemolymph of G. mellonella

The findings from this study demonstrated that the amount of the non-enzymatic antioxidant UA increased significantly at 5, 50, 250 and 1250 µg/mL concentrations of TiO_2 NPs when compared to the control group ($F_{4,15}$: 23.756, $p < 0.001$) (Figure 2a). The amount of non-enzymatic antioxidant BIL significantly increased from 0.018 ± 0.002 to 0.034 ± 0.004 mg/dL at the highest concentration of TiO_2 NPs (1250 µg/mL) when compared to the control group ($F_{4,15}$: 106.073, $p < 0.001$) (Figure 2b). Urea levels significantly decreased at 5 and 50 µg/mL concentrations of TiO_2 NPs in hemolymph of *G. mellonella* larvae, while it was statistically increased at 250 and 1250 µg/mL concentrations of TiO_2 NPs ($F_{4,15}$: 141.1755, $p < 0.001$) (Figure 2c). The amount of GLU significantly increased at

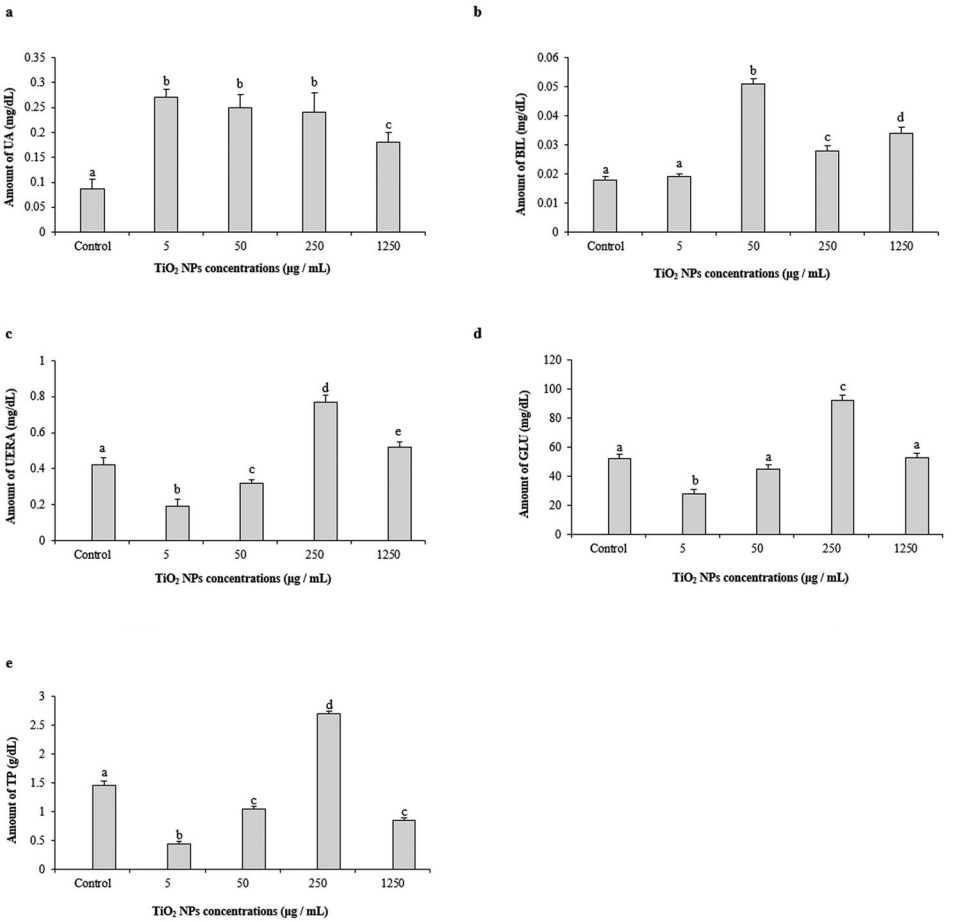


Figure 2. Effects of dietary TiO_2 NPs on biochemical parameters in hemolymph of *G. mellonella*. Bars represent the means (± S.E.) of four replicates. Means followed by the same letter are not significantly different (Tukey's HSD test: $p < 0.05$) (a: uric acid, b: bilirubin, c: urea, d: glucose, e: total protein).

250 µg/mL concentration of TiO_2 NPs, whereas significant decrease was observed at 5 µg/mL concentration when compared to the control group ($F_{4,15}$: 172.555, $p < 0.001$). The amount of GLU increased at the highest concentration of TiO_2 NPs, but there was no statistically significant difference ($p > 0.05$) (Figure 2d). Amount of TP significantly decreased at 5, 50 and 1250 µg/mL concentrations of TiO_2 NPs in hemolymph of *G. mellonella* larvae, while it was statistically increased at 250 and 1250 µg/mL concentrations ($F_{4,15}$: 1132.533, $p < 0.001$) (Figure 2e).

Discussion

This study provides information about the toxicity of dietary TiO_2 NPs on the model organism *G. mellonella*. The findings showed that metabolic

enzymes and biochemical parameters in hemolymph of *G. mellonella* were altered by exposure to TiO_2 NPs.

The metabolic and biochemical parameters of exposed organisms are significantly affected by toxic metals.[26–29] Environmental pollution caused by toxic metals can cause lipid and protein oxidation, cell damage and disruption of energy metabolism in insects.[12,30,31] AST, ALT and LDH enzyme activities are used as bioindicators in determining the oxidative effects of toxic chemicals on insect tissues.[12] AST and ALT transfer the amino group from amino acids to α-keto acids to switch between energy source molecules in the tissues of insects, as in mammals.[32] Another cell damage indicator LDH, converts pyruvate formed in the last step of glycolysis to lactate.[33] CK participates in adenosine triphosphate homeostasis, which is the main energy source in vertebrate and invertebrate animals.[34] In this study, it was determined that TiO_2 NPs added to artificial diet (250 µg/mL) increased AST, ALT, LDH and CK levels in the hemolymph of *G. mellonella*. The reason for the increase in metabolic enzyme activities in the hemolymph of *G. mellonella* may be cell damage and disruption of energy metabolism due to TiO_2 NPs. It is known that chemical agents increase AST and ALT levels by causing tissue damage.[12,16,35] On the contrary, the activity of cell damage indicators AST, ALT and LDH was significantly decreased at 1250 µg/mL concentrations of TiO_2 NPs in hemolymph of *G. mellonella* larvae. In addition, it was determined that the amount of TP significantly decreased at 1250 µg/mL concentrations. The reason for this may be disruption of protein and enzyme metabolism due to the toxic effect of high concentrations of TiO_2 NPs. On the other hand, the increase in the amount of non-enzymatic antioxidants at 1250 µg/mL concentrations may be an adaptation developed by the *G. mellonella* due to the toxic effect of TiO_2 NPs. Adamski et al.[36] reported that leaf potato extract and its component, α-solanine caused protein damage in fat body of *G. mellonella* larvae and enzyme metabolism was impaired due to protein oxidation. In another study, it was determined that AST, ALT, LDH and CK activities increased in hemolymph of *G. mellonella* larvae exposed to Diaquabis-Bis-Cobalt metal complex after 8 hours.[16] Sugeçti and Büyükgüzel[12] reported that the activity of cell damage indicators as AST, ALT and LDH increased due to the oxidative effect of oxfendazole in hemolymph of *G. mellonella*. In another study, cell damage indicators such as AST, ALT, LDH levels were significantly increased due to *Escherichia coli* infection in the hemolymph of *G. mellonella* larvae.[37] Sugeçti[38] reported that AST, ALT, LDH and CK levels significantly increased due to *Klebsiella pneumoniae* infection in the hemolymph of *G. mellonella* larvae. Similarly, LDH level also increased due to cell damage after infection with *K. pneumoniae* (different strains) increased in greater wax moth larvae.[39]

Antioxidant levels are increased to eliminate oxidative stress in organisms exposed to environmental pollutants such as pesticides, heavy metals, metal oxide nanoparticles, etc.[23,40] In this study, the amount of non-enzymatic antioxidants UA and BIL altered due to the toxic effect of TiO_2 NPs. UA and BIL levels statistically increased at 50, 250 and 1250 µg/mL concentration of TiO2 NPs. The reason for this increase may be the adaptation of the insect to the toxic effect of TiO2 NPs. In another study, the effect of Diaquabis (N, N-diethylnicotinamide-$_{kappa}$ N1) bis (4-formylbenzoato-$_{kappa}$ O) cobalt (II) metal complex on the non- enzymatic antioxidants such as UA and BIL in the hemolymph of *G. mellonella* larvae was investigated. UA and BIL levels increased in the hemolymph of *G. mellonella* larvae 8 hours after exposure to the metal complex.[16] Zorlu et al.[23] reported that TiO_2 NPs increased enzymatic antioxidants such as SOD, CAT and GST to prevent oxidative damage in model insect *G. mellonella* larvae. In another study CAT and GST activity increased in fat body of *G. mellonella* larvae exposed to CuO NPs.[3] In other studies, it was determined that the antioxidant levels of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) exposed to silver nanoparticles (Ag NPs) and zinc oxide nanoparticles (ZnO NPs) alters due to oxidative stress.[41,42] Moreover, in a study conducted with *Hydropsyche exocellata* Dufour, it was reported that as a result of the application of TiO_2 NPs at a concentration of 50 mg/L, there was an increase in the survival rate and lipid peroxidation level in dose depend manner, while a decrease in CAT activity occurred.[43] In another study was reported that the antioxidant response was altered in *Musca domestica* larvae fed a diet containing different concentrations of silver and chitosan nanoparticles.[44] Studies have shown that metal compounds with the potential to cause environmental pollution alter the antioxidant levels of the model insect. These results support our hypothesis.

Environmental pollutants such as pesticides, heavy metals, nanomaterials cause adverse effects on the physiological and biochemical of insects.[45,46] In studies, the increase in the amount of protein in insects has been reported as an adaptation developed against oxidative damage caused by environmental pollutants.[47] In this study, the amount of TP significantly increased at 250 µg/mL concentrations of TiO_2 NPs. Increased activity of AST, ALT and LDH at 250 µg/mL concentrations of TiO_2 NPs indicated cellular damage. The amount of TP may have been increased against this damage. Sertçelik et al.[16] reported that the amount of TP in hemolymph of *G. mellonella* larvae exposed to the Diaquabis bis cobalt (II) metal complex altered due to the cellular level. In another study, it was stated that the dietary oxyclozanide (0.003, 0.03 and 0.3%) has adverse effects on *G. mellonella* larvae, increasing the amount of TP to eliminate these effects.[47] The amount of GLU significantly increased at 250 µg/mL concentrations of TiO_2 NPs. Increase of GLU exposed at 250 µg/mL concentrations of TiO_2

NPs could be due to physiological stress or a response to suppress the stress. Also, the insect's feeding activity may have increased as an adaptation to the toxic effect of TiO_2 NPs. Increasing the amount of GLU, TP and UREA at 250 µg/mL concentrations of TiO_2 NPs supports this hypothesis. In another study, it was reported that the feeding activity and the amount of GLU, TP and UREA of *Bombyx mori* larvae changed due to the toxic effect of pyriproxyfen.[48]

In conclusion, the results of the present study revealed toxic effects of titanium dioxide nanoparticles for *G. mellonella* larvae. TiO_2 NPs caused alterations to metabolic enzymes and biochemical parameters in the hemolymph of *G. mellonella* larvae, showing that *G. mellonella* is a useful model organism for the investigation of toxic effects of environmental NP pollutants in insects. In recent years the intensive use of NPs has created a need to investigate their toxicological effects on native organisms present in the environment, such as insects. In addition, the adverse effects of NPs that may occur in lower organisms may affect other organisms in the ecosystem by disrupting the food chain.

References

[1] Schrand AM, Rahman MF, Hussain SM, Schlager JJ, Smith DA, Syed AF. Metal-based nanoparticles and their toxicity assessment. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 2010; 2 (5):544–568. doi:10.1002/wnan.103.

[2] Aschberger K, Micheletti C, Sokull-Klüttgen B, Christensen FM. Analysis of currently available data for characterising the risk of engineered nanomaterials to the environment and human health-lessons learned from four case studies. *Environ Int*. 2011;37(6):1143–1156. doi:10.1016/j.envint.2011.02.005.

[3] Sezer Tuncsoy B, Tuncsoy M, Gomes T, et al. Effects of copper oxide nanoparticles on tissue accumulation and antioxidant enzymes of *Galleria mellonella* L. *Bull Environ Contam Toxicol*. 2019; 102 (3):341–346. doi:10.1007/s00128-018-2529-8.

[4] Lachheb H, Puzenat E, Houas A, et al. Photocatalytic degradation of various types of dyes (alizarin s, crocein orange g, methyl red, congo red, methylene blue) in water by UV-irradiated titania. *Appl Catal B*. 2002;39(1):75–90. doi:10.1016/S0926-3373(02)00078-4.

[5] Hu R, Gong X, Duan Y, et al. Neurotoxicological effects and the impairment of spatial recognition memory in mice caused by exposure to TiO_2 nanoparticles. *Biomaterials*. 2010;31(31):8043–8050. doi:10.1016/j.biomaterials.2010.07.011.

[6] Ghasemi Z, Younesi H, Zinatizadeh AA. Preparation, characterization and photocatalytic application of TiO_2/Fe-ZSM-5 nanocomposite for the treatment of petroleum refinery wastewater: Optimization of process parameters by response surface methodology. *Chemosphere*. 2016; 159:552–564. doi:10.1016/j.chemosphere.2016.06.058.

[7] Vance ME, Kuiken T, Vejerano EP, et al. Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. *Beilstein J Nanotechnol*. 2015; 6:1769–1780. doi:10.3762/bjnano.6.181.

[8] Gottschalk F, Sonderer T, Scholz RW, Nowack B. Modeled environmental concentrations of engineered nanomaterials (TiO(2), ZnO, Ag, CNT, Fullerenes) for different regions. *Environ Sci Technol*. 2009; 43(24):9216–9222. doi:10.1021/es9015553.

[9] Dedman CJ, King AM, Christie-Oleza JA, Davies GL. Environmentally relevant concentrations of titanium dioxide nanoparticles pose negligible risk to marine microbes. *Environ Sci Nano*. 2021;8(5):1236–1255. doi:10.1039/d0en00883d.

[10] Nigro M, Bernardeschi M, Costagliola D, et al. n-TiO_2 and $CdCl_2$ co-exposure to titanium dioxide nanoparticles and cadmium: Genomic, DNA and chromosomal damage evaluation in the marine fish *European sea bass* (*Dicentrarchus labrax*). *Aquat Toxicol*. 2015; 168:72–77. doi:10.1016/j.aquatox.2015.09.013.

[11] Hu S, Han J, Yang L, et al. Impact of co-exposure to titanium dioxide nanoparticles and Pb on zebrafish embryos. *Chemosphere*. 2019; 233:579–589. doi:10.1016/j.chemosphere.2019.06.009.

[12] Sugeçti S, Büyükgüzel K. Effects of Oxfendazole on Metabolic Enzymes in Hemolymph of *Galleria mellonella* L. (Lepidoptera: Pyralidae) Larvae Reared on Artificial Diet. *Karaelmas Sci. Eng. J*. 2018; 8 (2):590–594. doi:10.7212/2Fzkufbd.v8i2.1380.

[13] Hyršl P, Büyükgüzel E, Büyükgüzel K. The effects of boric acid-induced oxidative stress on antioxidant enzymes and survivorship in Galleria mellonella . *Arch Insect Biochem Physiol*. 2007; 66 (1):23–31. doi:10.1002/arch.20194.

[14] Farhat Z, Brown RW, Bonner MR, et al. How do glutathione antioxidant enzymes and total antioxidant status respond to air pollution exposure? *Environ Int*. 2018; 112:287–293. doi:10.1016/j.envint.2017.12.033.

[15] Yucel MS, Kayis T. Imidacloprid induced alterations in oxidative stress, biochemical, genotoxic, and immunotoxic biomarkers in non-mammalian model organism *Galleria mellonella* L. (Lepidoptera: Pyralidae). *J Environ Sci Health B*. 2019; 54 (1): 27–34. doi:10.1080/03601234.2018.1530545.

[16] Sertçelik M, Sugeçti S, Büyükgüzel E, Necefoğlu H, Büyükgüzel K. Diaquabis N, N-dietilnikotinamid-lN1 bis 4-formilbenzoato-lO kobalt II Kompleksinin Model Organizma *Galleria mellonella* L. Lepidoptera: Pyralidae Üzerindeki Toksikolojik ve Fizyolojik Etkileri. *Karaelmas Sci. Eng. J*. 2018; 8 (1):359–364. doi:10.7212/2Fzkufbd.v8i1.1207.

[17] Kwadha CA, Ong'amo GO, Ndegwa PN, Raina SK, Fombong AT. The biology and control of the greater wax moth, *Galleria mellonella*. *Insects*. 2017; 8 (2):61. doi:10.3390/insects8020061.

[18] Moya-Andérico L, Vukomanovic M, del Mar Cendra M, et al. Utility of *Galleria mellonella* larvae for evaluating nanoparticle toxicology. *Chemosphere*. 2021; 266: 129235 doi:10.1016/j.chemosphere.2020.129235.

[19] Sugeçti S, Büyükgüzel K, Büyükgüzel E. Laboratory assays of the effects of oxfendazole on biological parameters of *Galleria mellonella* (Lepidoptera: Pyralidae). *J.Entomol. Sci*. 2016; 51 (2):129–137. doi:10.18474/JES15-36.1.

[20] Perini HF, Moralez AT, Almeida RS, Panagio LA, Junior AO, et al. Phenotypic switching in *Candida tropicalis* alters host-pathogen interactions in a *Galleria mellonella* infection model. *Sci Rep*. 2019; 9 (1):12555–12510. doi:10.1038/s41598-019-49080-6.

[21] Büyükgüzel E, Hyršl P, Büyükgüzel K. Eicosanoids mediate hemolymph oxidative and antioxidative response in larvae of *Galleria mellonella* L. *Comp Biochem Physiol A Mol Integr Physiol*. 2010; 156 (2):176–183. doi:10.1016/j.cbpa.2010.01.020.

[22] Tuncsoy B, Mese Y. Influence of titanium dioxide nanoparticles on bioaccumulation, antioxidant defense and immune system of *Galleria mellonella* L. *Environ Sci Pollut Res Int*. 2021;28(28):38007–38015. doi:10.1007/s11356-021-13409-4.

[23] Zorlu T, Nurullahoğlu ZU, Altuntaş H. Influence of dietary titanium dioxide nanoparticles on the biology and antioxidant system of model insect, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). *J. Èntomol. Res. Soc.* 2018; 20 (3):89–103.

[24] Bronskill J. A cage to simplify the rearing of the greater wax moth, *Galleria mellonella* (Pralidae). *J. Lep. Soc.* 1961; 15 (2):102–104.

[25] Kastamonuluoğlu S, Büyükgüzel K, Büyükgüzel E. The use of dietary antifungal agent terbinafine in artificial diet and Its Effects on Some Biological and Biochemical Parameters of the Model Organism *Galleria mellonella* (Lepidoptera: Pyralidae)). *J Econ Entomol.* 2020; 113 (3):1110–1117. doi:10.1093/jee/toaa039.

[26] Srikanth K, Pereira E, Duarte AC, Ahmad I. Glutathione and its dependent enzymes' modulatory responses to toxic metals and metalloids in fish-a review. *Environ Sci Pollut Res Int.* 2013; 20(4):2133–2149. doi: 0.1007/s11356-012-1459-y doi:10.1007/s11356-012-1459-y.

[27] Ahn S, Ardoña HAM, Lind JU, et al. Mussel-inspired 3D fiber scaffolds for heart-on-a-chip toxicity studies of engineered nanomaterials. *Anal Bioanal Chem.* 2018; 410(24):6141–6154. doi:10.1007/s00216-018-1106-7.

[28] Kissoum N, Bensafi-Gheraibia H, Hamida ZC, Soltani N. Evaluation of the pesticide Oberon on a model organism *Drosophila melanogaster* via topical toxicity test on biochemical and reproductive parameters. *Comp Biochem Physiol C Toxicol Pharmacol.* 2020; 228:108666. doi:10.1016/j.cbpc.2019.108666.

[29] Ismael NE, Abd El-Hameed SA, Salama AM, Naiel MA, Abdel-Latif HM. The effects of dietary clinoptilolite and chitosan nanoparticles on growth, body composition, haemato-biochemical parameters, immune responses, and antioxidative status of *Nile tilapia* exposed to imidacloprid. *Environ Sci Pollut Res Int.* 2021;28(23): 29535–29550. doi:10.1007/s11356-021-12693-4.

[30] Aslan N, Büyükgüzel E, Büyükgüzel K. Oxidative effects of gemifloxacin on some biological traits of *Drosophila melanogaster* (Diptera: Drosophilidae). *Environ Entomol.* 2019; 48 (3):667–673. doi:10.1093/ee/nvz039.

[31] Sheeja CC, Anusri A, Levna C, Aneesh PM, Lekha D. MoS_2 nanoparticles induce behavioral alteration and oxidative stress mediated cellular toxicity in the social insect *Oecophylla smaragdina* (Asian weaver ant). *J Hazard Mater.* 2020; 385:121624 doi:10.1016/j.jhazmat.2019.121624.

[32] Zibaee A, Sendi JJ, Etebari K, Alinia F, Ghadamyari M. The effect of diazinon on some biochemical characteristics of *Chilo suppressalis* Walker (Lepidoptera: Pyralidae), rice striped stem borer. *Mun Entomol Zool.* 2008; 3(1):255–265.

[33] Young A, Oldford C, Mailloux RJ. Lactate dehydrogenase supports lactate oxidation in mitochondria isolated from different mouse tissues. *Redox Biol.* 2020; 28: 101339–101337. doi:10.1016/j.redox.2019.101339.

[34] Rojas V, Morales-Lange B, Avendaño-Herrera R, et al. Detection of muscle-specific creatine kinase expression as physiological indicator for Atlantic Salmon (*Salmo salar* L) skeletal muscle damage. *Aquaculture.* 2018; 496 (1):66–72. doi:10.1016/j.aquaculture.2018.07.006.

[35] İçen E, Armutçu F, Büyükgüzel K, Gürel A. Biochemical stress indicators of greater wax moth exposure to organophosphorus insecticides. *J Econ Entomol.* 2005; 98 (2): 358–366. doi:10.1093/jee/98.2.358.

[36] Adamski Z, Adamski Z, Marciniak P, et al. Potato leaf extract and its component, α-solanine, exert similar impacts on development and oxidative stress in Galleria mellonella L. *Arch Insect Biochem Physiol.* 2014; 87 (1):26–39. doi:10.1002/arch.21177.

[37] Sugeçti S. Biochemical and immune responses of model organism *Galleria mellonella* after infection with *Escherichia coli*. *Entomol Exp Appl*. 2021; doi:10.1111/eea.13092.

[38] Sugeçti S. Pathophysiological effects of *Klebsiella pneumoniae* infection on *Galleria mellonella* as an invertebrate model organism. *Arch Microbiol*. 2021;203(6): 3509–3517. doi:10.1007/s00203-021-02346-y.

[39] Wand ME, McCowen JW, Nugent PG, Sutton JM. Complex interactions of *Klebsiella pneumoniae* with the host immune system in a *Galleria mellonella* infection model. *J Med Microbiol*. 2013;62(Pt 12):1790–1798. doi:10.1099/jmm.0.063032-0.

[40] Gripp HS, Freitas JS, Almeida EA, Bisinoti MC, Moreira AB. Biochemical effects of fipronil and its metabolites on lipid peroxidation and enzymatic antioxidant defense in tadpoles (*Eupemphix nattereri*: Leiuperidae). *Ecotoxicol Environ Saf*. 2017; 136: 173–179. doi:10.1016/j.ecoenv.2016.10.027.

[41] Yasur J, Pathipati UR. Lepidopteran insect susceptibility to silver nanoparticles and measurement of changes in their growth, development and physiology. *Chemosphere*. 2015; 124:92–102. doi:10.1016/j.chemosphere.2014.11.029.

[42] Ibrahim MA, Ali M. Silver and zinc oxide nanoparticles induce developmental and physiological changes in the larval and pupal stages of *Spodoptera littoralis* (Lepidoptera: Noctuidae). *J Asia Pac Entomol*. 2018;21(4):1373–1378. doi:10.1016/j.aspen.2018.10.018.

[43] Torres-Garcia D, Faria M, Soares AMVM, et al. Lethal and sub-lethal effects of nanosized titanium dioxide particles on *Hydropsyche exocellata* Dufour, 1841. *Aquat. Insects*. 2020; 41(1):85–103. doi:10.1080/01650424.2020.1712422.

[44] Abdel-Meguid AD, Ramadan RH, Emara MM. Effects of synthesized silver and chitosan nanoparticles using Nerium oleander and aloe vera on antioxidant enzymes in Musca domestica. *Egypt Soc Environ Sci*. 2020; 21(1):9–14.

[45] Kayis T, Coskun M, Dursun O, Emre I. Alterations in antioxidant enzyme activity, lipid peroxidation, and ion balance induced by dichlorvos in *Galleria mellonella* (Lepidoptera: Pyralidae). *Ann Entomol Soc Am*. 2015; 108 (4):570–574. doi:10.1093/aesa/sav038.

[46] Wu G, Yi Y. Effects of dietary heavy metals on the immune and antioxidant systems of *Galleria mellonella* larvae. *Comp Biochem Physiol C Toxicol Pharmacol*. 2015; 167: 131–139. doi:10.1016/j.cbpc.2014.10.004.

[47] Çelik C, Büyükgüzel K, Büyükgüzel E. The effects of oxyclozanide on survival, development and total protein of *Galleria mellonella* L. (Lepidoptera: Pyralidae). *J. Èntomol. Res. Soc*. 2019; 21 (1):95–108.

[48] Etebari K, Bizhannia AR, Sorati R, Matindoost L. Biochemical changes in haemolymph of silkworm larvae due to pyriproxyfen residue. *Pestic Biochem Physiol*. 2007; 88(1):14–19. doi:10.1016/j.pestbp.2006.08.005.