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The role of oleuropein against nanocomposite toxicity in fruit fly: evidence for lifespan extension

Tuğba Atıcı[1], Deniz Altun Çolak[2,*]

[1] *Institute of Natural and Applied Science, Erzincan Binali Yıldırım University, Erzincan, 24100 Turkey*
[2] *Department of Biology, Faculty of Art and Science, Erzincan Binali Yıldırım University, Erzincan, 24100 Turkey*
* Corresponding author. E-mail: dnz_altun@yahoo.com

Abstract. The effect of zinc oxide/titanium dioxide nanocomposite the lifespan of the fruit fly *Drosophila melanogaster* and the protective role of oleuropein, a strong antioxidant, against the zinc oxide/titanium dioxide were investigated. Chemicals prepared in different concentrations (0.005, 0.1, 0.5, and 1 g/L for zinc oxide/titanium dioxide; 0.1 mmol/L for oleuropein) have been separately applied to female and male populations of *D. melanogaster* for the control and application groups. In both female and male populations, it has been observed that zinc oxide/titanium dioxide has decreased the lifespan and oleuropein has increased the lifespan according to the control group, depending on the concentration. These findings demonstrate the beneficial effect of oleuropein, suggested as a protective role in the prevention of zinc oxide/titanium dioxide induced developmental toxicity.

Keywords: *Drosophila melanogaster*, lifespan, oleuropein, toxicity, zinc oxide/titanium dioxide nanoparticles.

INTRODUCTION

Nanotechnology has a great potential for applications in many fields advancements in nanotechnology are increasing rapidly (Karahalil 2013). So, this technology is appearing as a new research field that is investigating the potential risks of nanoparticles (NPs) on human and environmental health (Landsiedel *et al.* 2009). In this respect, many reports have demonstrated various effects of NPs exposure on different animals, plants, and microorganisms; depending on their species, growth conditions, NPs type, and exposure concentrations. Also, the *in vitro* and *in vivo* studies using different experimental models indicate that nanoparticles may cause genotoxic effects that involve oxidative stress and inflammation (Kang *et al.* 2008; Xu *et al.* 2009; Petković *et al.* 2011). However, the mechanisms involved in NPs-induced toxicity have not been clearly explained and are poorly studied *in vivo*.

Metal NPs are the most widely used among nanoparticles. Titanium dioxide (TiO_2) and zinc oxide (ZnO) NPs are of special concern since they used in food products, plastics, paper, drugs, cosmetics, sunscreens, paints, and medical materials and are two of the fastest-growing product categories in the nanotechnology industry (Yeber *et al.* 2000). As a result of this widespread use, increased environmental release and a higher potential for human exposure will appear. The data from several *in vitro* studies demonstrate that TiO_2NPs cause various adverse effects at the cellular level, such as oxidative stress and DNA damage (Wang *et al.* 2007).

The aging process is generally described as the progressive decline of homeostatic maintenance functions and physiological fitness (Sohal *et al.* 2002). *Drosophila* or fruit fly is commonly used for studying in the aging model system because the genetic background, developmental processes, and some more aspects are well known. They are also highly similar to mammalians in terms of genetic structure (Çakır and Bozcuk 2000). The production of free radicals and other oxidants produced during aerobic respiration is the most reason for mechanistic explanations for aging links (Barja 2002). Oxidative stress is supposed to be an important mechanism underlying nanoparticle toxicity. Thus, it is believed that nanoparticle toxicity can be prevented by antioxidants (Nel *et al.* 2006; Mocan *et al.* 2010). From this point of view, the effects of zinc oxide/titanium dioxide (ZnO-TiO_2) nanocomposite on the lifespan of *D. melanogaster* and the protective role of oleuropein (OLE, Olive Leaf Extract), a strong antioxidant, on these effects.

MATERIALS AND METHODS

Experimental organism

Drosophila melanogaster (Diptera; Drosophilidae) *Oregon R* strain was reared in Standard *Drosophila* Medium (SDM) containing 15 g sucrose, 17 g cornmeal, 3 g agar-agar, and 9 g yeast in 360 mL distilled water within environmental chamber maintained at 25±1 °C and 40-60% relative humidity in darkness. Further addition of 1 mL propionic acid as a preservative/fungicide was done. The flies used in the experiments were at the same age (1-3 days) and the females were virgins.

Chemicals and solutions

Oleuropein (98% pure; St. Louis, MO, USA) and titanium dioxide (99% pure, anatase, Steinheim, Germany) were purchased from Sigma Aldrich. ZnO-TiO_2 (zinc oxide-titanium dioxide) nanoparticles were synthesized in the chemistry laboratory of Black Sea Technical University. ZnO was loaded into the TiO_2 photocatalyst with a 1% ratio. TiO_2 catalyst (10 g) and water (10 mL) were mixed to have a slurry. ZnO (0.3355 g) was added to the slurry and calcined at 400 °C for 6 h. After this process, it was cooled in a desiccator and stored in a closed dark bottle. For the stock solutions, $ZnOTiO_2$ NPs powder was dispersed in deionized water. Then, this solution was vortexed for 20 seconds, and sonicated for 30 min in an ultrasonic bath (Sonorex, Bandelin Electronic, Berlin, Germany) at a frequency of 60 kHz, to ensure uniform suspension of NPs. Finally, the nanoparticles concentrations were prepared by diluting the stock solution.

Characterization of $ZnOTiO_2$NPs

$ZnOTiO_2$NPs size distribution and morphology were represented by scanning electron microscope (SEM, (JEOL JSM 5600 LV, Tokyo, Japan) at the magnification of 100×. The hydrodynamic diameter was characterized by a master sizer (Malvern, Zetasizer ver. 7.02, Malvern Instruments Ltd, Worcestershire, UK) using the dynamic light scatter (DLS) technique.

Lifespan assay

The lifespan experiments were studied separately in female and male populations. For this purpose, about 100 individuals were collected from among the same aged (1-3 days) female and male flies which were not mated and obtained from the pupa. The gathered individuals were then put into the empty culture bottles and left hungry for 2 hours before $ZnOTiO_2$ and OLE applications. For the application, two layers of blotting papers were placed into each culture vial; $ZnOTiO_2$ and OLE in different concentrations (0.005, 0.1, 0.5, and 1 g/L for $ZnOTiO_2$, 0.1 mmol/L for OLE) were absorbed into these papers. Afterward, the flies were put into these application vials and were left for 2 hours. After 2 hours, the individuals (separately female and male flies) were placed into the culture vials containing only SDM as 25 × 25. The experiments for both the control and application groups were started at the same time. All the vials were kept in appropriate thermal cabins. During the experiments, food was replaced with fresh food twice a week. The number of individuals was controlled both at the beginning and the end of each application day and the dead individuals were counted. The application was carried out until the last individual died. The experiments were repeated three times.

Statistical analyses

The obtained data were analyzed with SPSS version 16.0 (Statistical Package for the Social Sciences Software, SPSS, Chicago, IL). The mean lifespan values of the control and application groups were subjected to Duncan's one-way range test ($p<0.05$).

RESULTS

Characterization of ZnOTiO$_2$NPs

The size of the nanoparticles is an important parameter that determines activity in biomedical applications. DLS is an analytical method that estimates the hydrodynamic diameter while SEM is used for the estimation of the actual diameter of nanoparticles. DLS studies revealed that the hydrodynamic diameter of ZnO-TiO$_2$NPs was 42.5±1.2 nm. The morphological characterization of ZnOTiO$_2$NPs was performed by SEM to visualize the actual particle size and the overall size distribution (Figure 1). SEM image indicates that the nanoparticles formed aggregates of different sizes and these aggregations have a porous structure.

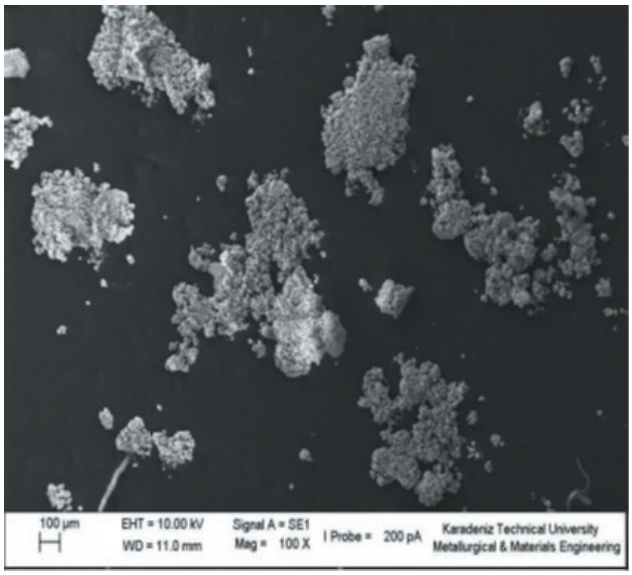

Figure 1. SEM images of ZnOTiO$_2$NPs in dry form.

Lifespan assay

In this study, it was observed that ZnOTiO$_2$, depending on the concentration, has decreased the lifespan of the male and female population according to the control group. It was also determined that OLE has increased the lifespan according to the control group. The maximum female lifespan of the control and application groups was observed for 78 days while the maximum male lifespan belonging to the control and application groups was 76 days, respectively. The difference between the control and application groups is not statistically significant ($p>0.05$) (Table 1).

In the female population applied with ZnOTiO$_2$, the maximum lifespan for the lowest concentration (0.005 g/L) was 70 days however for the highest concentration (1 g/L) the maximum lifespan was 54 days. Also, in the 0.1 and 0.5 g/L ZnOTiO$_2$ application groups, the female maximum lifespan was 68 and 62 days, respectively (Figure 2).

According to results, in the male population applied with ZnOTiO$_2$, the maximum lifespan for the lowest concentration (0.005 g/L) was 71 days however for the highest concentration (1 g/L) the maximum lifespan was 51 days. Also, in the 0.1 and 0.5 g/L ZnOTiO$_2$ applica-

Table 1. The longevity of male and female populations of *D. melanogaster*

Experimental group (No)	Female number	Maximum lifespan	Mean lifespan	Probability level	Male number	Maximum lifespan	Mean lifespan	Probability level
CONTROL - (1)	100	72	56.58±1.10		100	74	55.79±1.12	
OLE (0.1 mmol/L)- (2)	100	78	57.64±1.23		100	76	55.51±1.17	
ZnOTiO$_2$ 5 mg/L- (3)	100	70	49.48±1.45	1-2*	100	71	44.12±1.84	1-2* 3-4*
0.1 g/L- (4)	100	68	45.17±1.59	4-7*	100	66	41.03±1.85	3-7* 4-5*
0.5 g/L- (5)	100	62	35.41±1.29	5-6*	100	60	39.11±1.70	4-7*
1 g/L- (6)	100	54	34.19±1.65		100	51	35.50±1.45	5-6*
ZnOTiO$_2$ +OLE (1 g/L+0.1 mmol/L)- (7)	100	64	43.77±1.79		100	62	43.80±1.69	

*The mean difference is not significant at the 0.05 level.

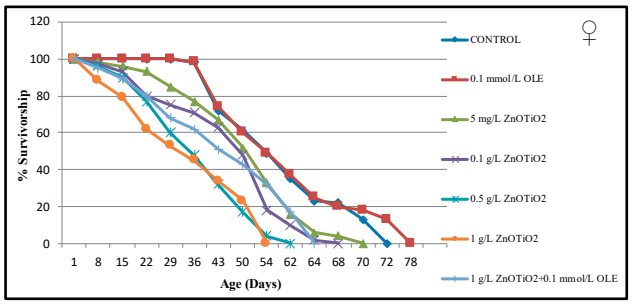

Figure 2. Exposure to $ZnOTiO_2$ and OLE in female adult *D. melanogaster* leads to lifespan alteration.

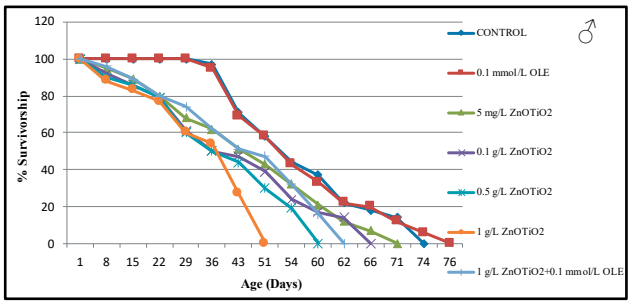

Figure 3. Exposure to $ZnOTiO_2$ and OLE in male adult *D. melanogaster* leads to lifespan alteration.

tion groups, the male maximum lifespan was 66 and 60 days, respectively (**Figure 3**). Also in every group, it is determined that female individuals live longer in comparison to male individuals (Table 1).

DISCUSSION

In recent years, studies about the toxic risks of NPs are increasing (Landsiedel *et al.* 2009; Yilmaz Öztürk 2019). First reports about the toxicity of some nanoparticles show that they can affect biological systems at the organ, tissue, cellular, subcellular, and protein levels (Braydich-Stolle *et al.* 2009; Khajavi *et al.* 2019). *In vivo* toxicity studies have demonstrated that inhalation of TiO_2 NPs causes pulmonary inflammation in rats and mice (Bermudez *et al.* 2004) and TiO_2 NPs induce DNA damage and genetic instability in mice (Trouiller *et al.* 2009). Reeves *et al.* (2008) showed oxidative stress-related effects, including inflammation, cytotoxicity, and genomic instability, either alone or in the presence of UVA irradiation, in mammalian studies. Also, zinc oxide nanoparticles have been reported to be cytotoxic and exhibited strong protein adsorption abilities (Horie *et al.* 2009). Toxicity of ZnO NPs on human bronchial epithelial cells was investigated and suggested that oxidative stress is a mechanism of toxicity (Heng *et al.* 2010). The production of free radicals has been supposed to be one of the primary mechanisms of NPs toxicity (Nel *et al.* 2006; Yang *et al.* 2009). It may result in oxidative stress, inflammation, and consequent damage to proteins, membranes, and DNA (Bhabra *et al.* 2009; Hu *et al.* 2009). Thus, in our study, we investigated the toxic effects of $ZnOTiO_2$ nanocomposite on the lifespan of *D. melanogaster* and the protective role of oleuropein (OLE), a strong antioxidant. Oleuropein is the major phenolic constituent of olive leaves (*Olea europaea*) and is also present in the fruit and oil (van Acker *et al.* 1998). Many studies demonstrated that OLE has an antiinflammatory activities (Carluccio *et al.* 2003), free-radical scavenging properties (Le Tutour and Guedon 1992; Manna *et al.* 1997) and inhibit the growth of different tumor cell types (Hamdi and Castellon 2005). In our preliminary study, it was determined that $ZnOTiO_2$NPs were relatively increased levels of total oxidant status (TOS) and was decreased level of total antioxidant capacity (TAC) compared to the control group. But, in a $ZnOTiO_2$+OLE group, it was observed that vice versa (Çolak *et al.* 2016).

CONCLUSION

These experiments provide evidence that $ZnOTiO_2$ nanocomposite can shorten the lifespan of *Drosophila* which is partially or completely prevented by oleuropein. This means oxidative stress is the major contributor to $ZnOTiO_2$ toxicity. As a result, although the emerging technologies and their opportunities are desirable to be implemented into daily life, before their applications, regulations should be defined to protect human health and the environment.

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