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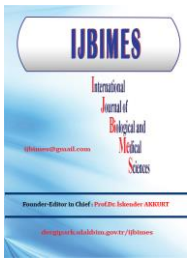

Research Article

Role of Oleuropein on *Drosophila* Induced $ZnOTiO_2$ Nanocomposite[#]

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Oxidative stress
Drosophila melanogaster

Abstract: Today, in parallel with the development of nanotechnology, the increase in the concentration of the nanoparticles in the nature has gained importance and daily the risks and effects of nanoparticles on the environment and human health are increasing. In this study, the possible toxic effects of increasing usage and applications of nanocomposite $ZnOTiO_2$ on *Drosophila melanogaster* larvae and the protective role of Oleuropein (OLE) in order to eliminate this effect have been investigated. For this purpose, two separate sets of experiment were prepared, one a control group including $ZnOTiO_2$ with distilled water and OLE and another $ZnOTiO_2$+OLE application group. In order to determine the oxidative parameters in mature flies, total oxidant status (TOS), total antioxidant capacity (TAC) and oxidative stress index (OSI) were measured in mature flies subjected $ZnOTiO_2$ and $ZnOTiO_2$+OLE in larvae state. In $ZnOTiO_2$ applications groups the level of TOS was relatively high and TAC was low compared to control group; in $ZnOTiO_2$+OLE group while TOS was decreasing and TAC was increasing in compared with the only $ZnOTiO_2$ application group. The observed TAC and TOS changes compared to control group values were statistically significant (p<0.05). These toxic effects of $ZnOTiO_2$ nanocomposite observed in *Drosophila* is being provided through the increasing the formation of reactive oxygen species, leading to oxidative stress and lipid peroxidation. OLE is believed to play a protective role against the harmful effects of $ZnOTiO_2$ nanocomposites thanks to OLE's antioxidant role by preventing the formation of free radicals, inhibiting lipid peroxidation and stimulating the detoxification enzymes.

1. Introduction

Although a detailed mechanism of the toxicity caused by the nanoparticles (NPs) has not been clarified yet, basic and possible mechanism of the NPs are cause the toxicity by introducing reactive oxygen species (ROS) [1]. The resulting free radical species damage the cells by causing oxidation of lipids, structural changes in proteins, damage to DNA, signaling systems and degradation of gene transcription [2]. Oleuropein (OLE) is the major phenolic constituent of olive leaves (*Olea europaea*) and is also present in the fruit and oil. Many studies demonstrated that OLE has an anti-inflammatory activities, free-radical scavenging properties and inhibits the growth of different tumour cell types [3, 4]. The aim of this study was to investigate the effects of $ZnOTiO_2$ application on oxidant-antioxidant systems on *Drosophila melanogaster*, and protective roles of Oleuropein on these effects.

2. Material and Metods

2.1. Chemical and Strains

$ZnOTiO_2$ obtained from the Black Sea Technical University, titanium dioxide (TiO_2) and zinc oxide (ZnO) was synthesized. Oleuropein was obtained from MP Biomedicals. *Oregon R wild type (w.t)*

strain *of Drosophila melanogaster* (Diptera; Drosophilidae) have been used in the experiments. composed of maize flour, agar, sugar, dried yeast and propionic acid (Standart Drosophila Medium, SDM). The humidity of the experimental chamber was %40-60. The flies used in the experiments were at the same age and the females were virgins. The flies were kept at a constant temperature of 25±1°C and in darkness, on a standard medium in the TOS and TAS values for control group, are significant statistically in $p<0.05$ level.

2.2. Experimental Procedures

Biochemical analyzes which were carried out to determine whether associated with downregulation of antioxidant systems possible toxic effect observed in adult subjects by depending on the growing concentration of $ZnOTiO_2$ larvae obtained from the application groups. Two separate set of experiments were prepared as $ZnOTiO_2$ and $ZnOTiO_2$+OLE application groups by using 0.005; 0.1; 0.5 and 1mg/mL doses for $ZnOTiO_2$, 100 µM dose for OLE. Total oxidant status (TOS) and total antioxidant capacity (TAC) of tissue were measured using a novel automated colorimetric measurement method for TOS developed by Erel [5, 6]. The ratio of TOS to TAC was accepted as the oxidative stress index (OSI) [7]. The results were presented as mean±standard deviation (SD) or percentages, where appropriate. To be able to determine the statistical significance of the results, Duncan's one-way range test was applied. Data were analyzed using the SPSS (ver. 15 for Windows) software.

3. Results

Total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI) values of individuals that can be grown up with larvae which is applied $ZnOTiO_2$ was examined in order to determine the oxidative parameters. It is determined that in $ZnOTiO_2$ group TOS value is higher and TAS value is lower than control group (Table 1, Figure 1a-b). These differences which are observed

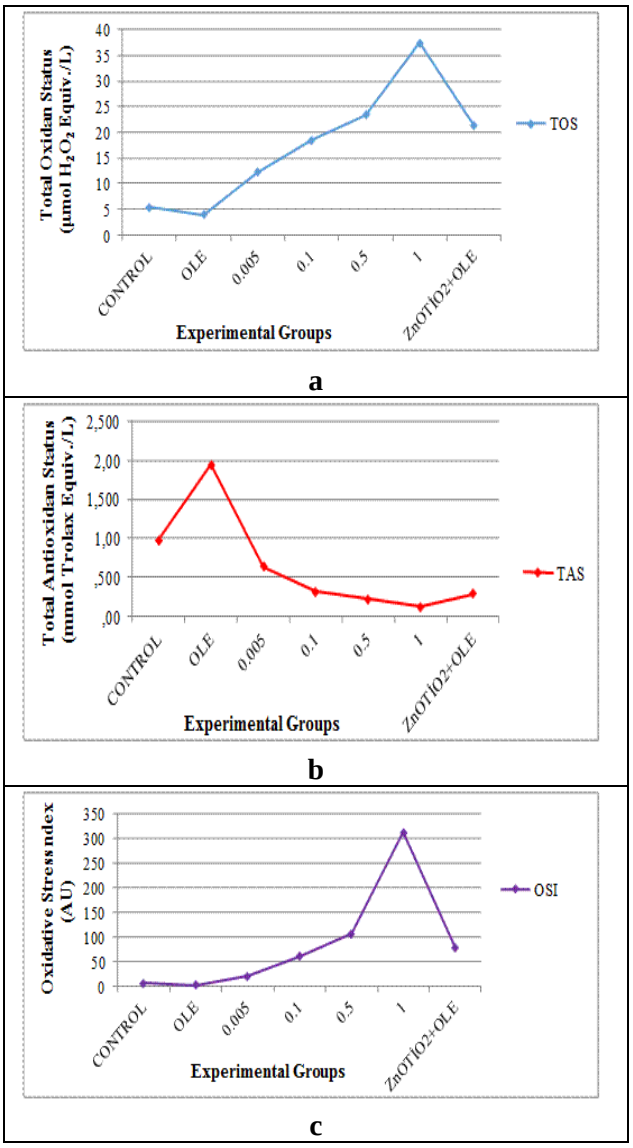

Figure 1. *Oxidative parameters data of $ZnOTiO_2$ and $ZnOTiO_2$+OLE groups*

Table 1. *Oxidative parameters data obtained from D. melanogaster for experimental groups with $ZnOTiO_2$ and $ZnOTiO_2$+OLE*

Experimental groups			TOS (µmol H_2O_2 Eqv./L)	TAS (mmol Trolox Eqv./L)	OSI (AU)
Control groups		Distilled water	5.37±0.09[a]	0.97±0.03[b]	5.54±0.09[a]
		OLE (100µM)	3.83±0.46[a]	1.94±0.54[a]	2.20±0.42[a]
Application groups	$ZnOTiO_2$	0.005mg/mL	12.18±0.77[b]	0.63±0.06[bc]	19.53±0.92[a]
		0.1mg/mL	18.51±0.41[c]	0.31±0.04[bc]	61.48±6.84[ab]
		0.5mg/mL	23.40±0.16[d]	0.22±0.08[c]	153.71±68.65[b]
		1mg/mL	37.41±1.05[e]	0.12±0.28[c]	350.30±82.36[c]
	$ZnOTiO_2$+OLE	1mg/mL+100µM	21.27±0.44[f]	0.28±0.04[c]	78.85±10.10[ab]

[a-e]: The values in the same column showed significant different at %5 level.

Furthermore, higher OSI level obtained by dividing the total oxidant activity, total antioxidant capacity are indicate that the presence of toxic effects (Table 1, Figure 1c).

TOS, TAS and OSI values were measured in $ZnOTiO_2$+OLE groups. In application groups, includes OLE antioxidant used as a exogenous are found singnificant statically (p<0.05) that TOS values are falling while TAS values are increasing according to application groups includes only $ZnOTiO_2$ (Table 1, Figure 1a-b). Again for this group obtaining of oxidative stress index is lower than the control group is shown that toxic effect we have observed depending on $ZnOTiO_2$ in studies can be inhibited by OLE (Table 1, Figure 1c). Total oxidant status, total antioxidant status and oxidative stress index data of individuals obtained from *Drosophila melanogaster*'s larvae, exposed to $ZnOTiO_2$ and $ZnOTiO_2$+OLE applications is given in Table 1.

4. Discussion and Çonclusions

Researches in the recent years have shown that NPs may interact with the biological system and exhibit some toxicity. Some reports suggested that ZnO and TiO_2 NPs in mice, rat and mammalian tissues caused damages leading to the generation of reactive oxygen species (ROS), oxidant injury, excitation of inflammation, and cell death [8- 11]. Furthermore, Kumar *et al.* [12] reported that cytotoxic and genotoxic effects of ZnO and TiO_2 occurred in *E. coli* at higher doses. On the other hand, Oleuropein shows anti-clastogenic and anti-inflammatory activities, free-radical scavenging properties, and inhibits the growth of various tumour cell types [13- 15].

It is observed clearly that Oleuropein antioxidants substantially eliminate to peroxidation and oxidative damage occured in *Drosophila*. Also, antioxidants taken from the outside increases the activity of endogenous antioxidants by showing supports the feature antioxidant defense system in the body and considerably increasing the values of total antioxidant status.

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