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RESEARCH REPORT

Effects of Magnetic Iron Oxide Nanoparticles (Fe_3O_4 NPs) on *Galleria mellonella* Hemocytes

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Abstract

In this study, we examined the harmful effects of magnetic iron oxide nanoparticles (Fe_3O_4 NPs) on the Greater Wax Moth *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). For this aim, *G. mellonella* larvae were administered Fe_3O_4 nanoparticles (NPs) at concentrations of 0.4, 2, 10, 50, and 250 µg/10 µl. Subsequently, the impact of these concentrations on total hemocyte counts (THCs) and various hemocyte indices, namely viable, mitotic, apoptotic, necrotic, and micronucleated cells, was assessed using hematoxylin and eosin (H&E) staining. Compared to the control group, *G. mellonella* larvae exposed to Fe_3O_4 NPs at concentrations of 10, 50, and 250 µg/10 µl exhibited a statistically significant reduction in THCs. Additionally, in these experimental groups exposed to different concentrations of Fe_3O_4 NPs, the percentage of viable cells significantly decreased, while the percentage of apoptotic cells increased in comparison to the control group. Likewise, the percentage of necrotic hemocytes was significantly higher in the larvae exposed to 50 and 250 µg/10 µl Fe_3O_4 NPs than that in the control group. Furthermore, the frequency of micronucleated hemocytes was significantly elevated at Fe_3O_4 NP doses of 10 and 50 µg/10 µl when compared to the control group.

Key Words: *Galleria mellonella*; hemocyte; iron oxide nanoparticle; micronucleus

Introduction

Magnetic iron oxide is the most frequently chosen nanoparticle among magnetic particles. Nano-sized iron oxide is utilized in areas like catalysis and biomedicine due to its magnetic properties, resulting in a wider range of applications in recent times (Fu and Ravindra, 2012). Nano-sized iron oxide typically falls within the 1-100 nm range in size and has been utilized for the purposes of in-vitro diagnostic research for approximately four decades (Rahman *et al*., 2011; Sakallıoğlu, 2013). During their widespread use, NPs come into contact with water. Subsequently, they are separated from the materials that are included in and pass into the water environment. As a result, they can turn into toxic substances (Ünşar and Perendeci, 2016).

There is a growing body of research on the acute toxicity of iron oxide nanoparticles (Fe_3O_4 NPs) in various fields, including their impact on reactive oxygen species, cellular morphology, and cell proliferation as well as understanding how they break down internal cells (Soenen *et al*., 2012). The interaction of NPs with cell fate pathways (necrosis/necroptosis, apoptosis, and autophagy) continues to be a topic of discussion today (Mohammadinejad *et al*., 2019). The removal of damaged and waste cells from the tissue environment by genetically planned cell death, or apoptosis, occurs without upsetting the balance of the tissue (Qiang *et al*., 2005). Previous studies conducted by Qiang *et al*., (2005) and Eskin (2017) put forth that apoptotic cells undergo conversion into apoptotic bodies, which are then phagocytosed by the surrounding cells. Unlike apoptosis, necrosis is an unpredictable phenomenon that happens spontaneously without theinterference of genetics. Hypoxia is the most frequent underlying reason for this process. Toxic elements like arsenic, cyanide, and insecticides, along with heavy metals, can result in necrosis during which an increase in mitochondrial reactive reactive oxygen species (ROS) production, a decrease in ATP production, activation of non-apoptotic proteases, and opening of Ca^{++} channels occur (Nicotera *et al*., 2004; Golstein and Kroemer, 2007; Coşkun and Özgür, 2011). Micronuclei are formations that arise during the mitotic division of a cell, originating from complete chromosomes or

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acentric chromosome fragments that are not included in the main nucleus. The micronucleus test is utilized for assessing the genotoxic impacts of physical and chemical substances on cells (Atlı and Şekeroğlu, 2011). Force-feeding involves delivering essential nutrients including calories, protein, macro and micro elements, and vitamins into a living organism's body without its consent. This technique is commonly applied in research on toxicity (Ramarao *et al*., 2012).

The Greater Wax Moth, scientifically known as *Galleria mellonella* (L.), is a species of moth (Lepidoptera: Pyralidae). The presence of this species results in significant harm to the honeycombs in hives that they share with *Apis mellifera* L. and *A. cerana* Fabricius honeybees. Damage happens when the larvae of the pest create nests on the edges of brood and uncapped comb cells and consume the middle layer of the comb. These damages lead to a reduction in the population of the honeybees, *A. mellifera* and *A. Ceran* (Kwadha *et al*., 2017). Though being costly, *G. mellonella* is used as a model organism in the experiments thanks to its fast growth, high reproductive rate, small size, and brief lifespan. Furthermore, the larvae exhibit robust immune defenses, making them ideal for comparative research on mammalian immune models (Harding *et al*., 2013; Mikulak *et al*., 2018).

Experiments conducted on *G. mellonella* larvae will never fully replace research conducted on mammals, but they will enable future research with regulated animals to be more skillfully designed and refined (Senior and Titbal, 2020). Accordingly, the main goal of this study is to ascertain the harmful impacts of Fe_3O_4 NPs on the total hemocyte counts (THCs) of the final instar *G. mellonella* larvae via the force-feeding method. In addition, evaluating the effect of Fe_3O_4 NPs on the percentage of apoptotic, necrotic, and micronucleus-containing hemocytes in the hemolymph circulation of *G. mellonella* larvae is the second major goal of this study. The data in this study were obtained from the first author's master's thesis (Eskin, 2022)

Materials and Methods

Insects

To ensure the continuation of *G. mellonella*, an in-vitro (ready-made) insect diet was prepared under laboratory conditions. The artificial insect diet was prepared using the method from Avan and Uğur (2019) containing ingredients such as corn flour, bran, milk powder, honey, glycerin, yeast, and wax. This diet was first placed into 20 glass jars, each filled up to one-third of its capacity. Then, *G. mellonella* eggs were placed into the jars one-third full of the prepared food. Next, the jars were covered with two layers of American fabric and secured with rubber bands. After that, equal-sized holes were made in the jar lids before sealing them. After having been covered and drilled in their lids properly, these jars including the prepared insect diet and the larva were finally placed in a dark laboratory setting at 27±4 °C with an average humidity of 55±5%. These procedures ensured both the continuity of insect culture and the provision of larvae to be used in experiments. For the experiments, the larvae that developed from the eggs were waited to reach the final stage (180 ± 20 mg).

Characterization of Fe_3O_4 NPs

In a prior investigation, we examined the Fe_3O_4 NPs using field emission scanning electron microscopy (FESEM) and X-ray diffraction (XRD) analysis (Eskin *et al*., 2021b). Based on the results of this study, we defined nanoparticles as spherical (FESEM) and having magnetic properties (XRD) (Eskin *et al*., 2021b).

Preparation of a Fe_3O_4 NP mixture for experiments

A prepared product for commercial use, Fe_3O_4 NP from Nanokar (Turkey) with CAS number 1317-61-9, was employed, possessing a surface area of 6-8 m²/g and 97% metal-based characteristics. The Fe_3O_4 NP concentration unit was stated as 'µg / 10 µl'. In our prior investigation, Fe_3O_4 NPs were tested on *G. mellonella* larvae to determine LC_{50} and LC_{90} values, which were found to be 482.72 µg/10 µl and 1843.89 µg/10 µl respectively, over a 30-day period (Eskin *et al*., 2021b). As a result, concentrations of Fe_3O_4 NPs at 0.4, 2, 10, 50, and 250 µg/10 µl were identified as the experimental levels used for all studies related to hemocytes. While setting up the experimental group, various Fe_3O_4 NP solutions (0.4, 2, 10, 50, 250 µg /10 µl) were thoroughly mixed using a bath-type sonicator at 40 °C for 10 minutes. Larvae in the control group (0) were given just 10 µl of distilled water.

Determining the effect of magnetic Fe_3O_4 NPs on the hemocyte count of G. mellonella

The experimental methods were adapted from the research conducted by Eskin and Bozdoğan (2021) in order to investigate how magnetic Fe_3O_4 NPs affect the total hemocyte counts (THCs) of *G. mellonella*. Hemolymph was extracted from 180 ± 20 mg of *G. mellonella* larvae 96 hours after the NP treatments. In each of the experimental and control groups, five larvae were used. Three duplicates of the experiment were conducted at various times. 4 µl of hemolymph was extracted from the first hind leg segment of *G. mellonella* larvae using a micropipette in order to calculate the THCs in the experimental and control groups. Following the hemolymph collection, 36 µl of PBS (Phosphate Buffer Saline), an anticoagulant solution, was added to 1.5 ml tubes (Teramoto *et al*., 2004; Er, 2011; Yılmaz, 2013; Eskin, 2017). The diluted cell mixture (1:10) was combined using a micropipette. The mixed cell solution (10 µl) was taken with a 10 µl micropipette and then loaded onto a Neubauer hemocytometer (Figure 1). "Cell count / ml = number of cells counted in the large square x dilution factor (10) x 10^4)" was the formula used to determine the THC per milliliter. Photographs were taken with a SWIFT microscope camera, and the cell mixture was counted using a SWIFT SW380T microscope before being placed onto the Neubauer hemocytometer (Figure 1).

Micronucleus status and necrotic-apoptotic-mitotic indices

The staining sequence, methods, and procedures developed by Richardson, (2018) and Eskin and Bozdoğan (2021) were applied to calculate the apoptotic, necrotic, and mitotic indices and the percentages of micronucleus-containing hemocytes in *G. mellonella* hemocytes. Larvae and slides were

cleaned with sterile cloths soaked in 70% ethanol and allowed to dry. The first segment of the hind legs of the larvae was punctured with a No. 1 insect pin. Five µl of hemolymph was collected using a micropipette and spread onto a cleaned and dried slide, which was left to dry for 30 minutes. To facilitate the adhesion of hemocytes to the slide, 70% ethanol was dropped onto the slide with hemolymph, and allowed to dry for 5 minutes. Hematoxylin stain was then applied to the dried slide to stain the cell nuclei and kept there for 1 minute. The slide stained with hematoxylin was rinsed under running water for 1 minute to remove excess hematoxylin. After that, the eosin stain was immediately applied to the slide to stain the cytoplasm of the hemocytes. The eosin-stained slide was left so for 45 seconds and then rinsed under running water for 1 minute. The stained slides were dried at room temperature for 30 minutes. Finally, a mounting medium (Entellan) was applied to the stained hemocytes on the slide, and covered with a coverslip. From each preparation of the experimental and control groups, 1000 hemocytes were randomly selected. Each preparation was examined using a SWIFT SW380T microscope at 1000X magnification, and images were captured using a SWIFT microscope camera. Each experimental and control group was analyzed with 5 larvae and 3 replicates. Viable, apoptotic, necrotic, mitotic, and micronucleus-containing hemocytes in the preparations were counted. The criteria for identifying viable, apoptotic, necrotic, mitotic, and micronucleus-containing hemocytes in the prepared slides are presented in detail below.

Viable cells: Cells without any anomalies in the cytoplasm or nucleus, such as dense cytoplasmic vacuoles, nuclear fragments, or micronucleus formation, were considered viable cells.

Micronucleus: Micronuclei are nuclear fragments that stain with the same color and intensity as the cell nucleus and are up to 1/3 the size of the cell nucleus (Venier *et al*.**,** 1997).

Necrotic hemocytes: Cells with large vacuoles in the nucleus and cytoplasm and a dull appearance of the cytoplasm, were classified as necrotic hemocytes (Bolognesi, 2019).

Apoptotic hemocytes: Cells with darker cytoplasm compared to viable cells, showing chromatin condensation in the early stages and nuclear fragments in the later stages, were classified as apoptotic hemocytes (Bolognesi, 2019).

Mitotic hemocytes: Cells undergoing mitosis were classified as mitotic hemocytes. The counting of cells in different stages of mitosis was conducted through the methods introduced by Amaral *et al*. (2010) and Ghasemi *et al*. (2013).

Statistical analysis

In order to conduct statistical analyses, we initially checked whether the data on THCs, viable cells, mitotic, apoptotic, and necrotic cell percentages, and micronucleus-containing hemocytes of *G. mellonella* exhibited a normal distribution. If the collected data displayed a normal distribution, the group means were compared through the One-Way Analysis of Variance (ANOVA). The Tukey test was used to compare differences between means in cases of homogenous variances. If a normal distribution was not found in the data, the Kruskal-Wallis test was utilized to examine group differences, and it was followed by Mann-Whitney U tests to identify specific differences among groups. A significance level of $P<0.05$ was considered statistically significant. In the statistical result tables of all the experiments conducted, the control group was labeled as "0." Before conducting statistical analysis with SPSS software, all percentage values were normalized by taking the arcsine square roots (SPSS, version 11.0, SPSS Science, Chicago, IL).

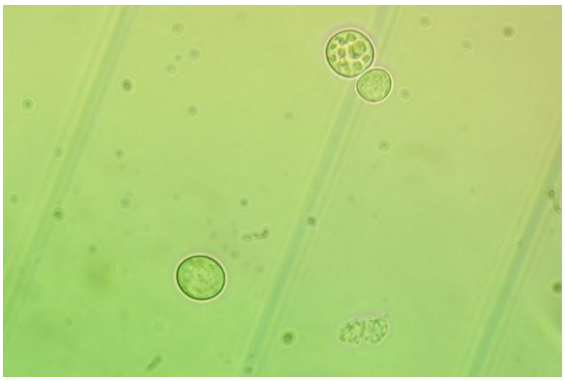

Fig. 1 Hemocytes loaded onto a Neubauer hemocytometer (1000x)

Results

Table 1 shows the findings on THCs of *G. mellonella* larvae exposed to different magnetic Fe_3O_4 NP concentrations using the forced-feeding technique below.

Table 1 demonstrates that there is a statistically significant difference ($\chi^2 = 27.848$, df = 5, $P = 0.00$) between the mean hemocyte counts of the larvae exposed to 10, 50, and 250 µg/10 µl Fe_3O_4 NP concentrations and that of the larvae in the control group. The comparison of the experimental group to the control group revealed that the mean hemocyte counts of the larvae exposed to the NP concentrations exhibited a substantial decrease (Table 1). Figure 2 displays the images of live, mitotic, apoptotic, necrotic, and micronucleus-containing hemocytes. Following this figure, the percentages of *G. mellonella* cells that are viable, mitotic, apoptotic, necrotic, and micronucleated are shown in Table 2 below (96 hours after force-feeding).

As shown in Table 2, the application of Fe_3O_4 NPs caused a significant difference in the percentage of viable cells (91.66%) in the larvae exposed to 50 µg/10 µl Fe_3O_4 NP concentration compared to the control group (94.18%) ($\chi^2 = 8.324$, df = 5, $P = 0.00$). Despite the fact that all experimental groups' mean mitotic indices were lower than those of the control

group, this difference was not statistically significant (χ^2 = 1.502, df = 5, *P* = 0.198) (Table 2). When larvae were exposed to 10, 50, and 250 µg/10 µl Fe_3O_4 NP doses, the percentage of apoptotic cells increased significantly in comparison to the control group (χ^2 = 6.572, df = 5, *P* = 0.00) (Table 2). A statistically significant difference in necrotic cells was observed between the control group and the larvae subjected to 250 µg/10 µl Fe_3O_4 NP concentration (χ^2 = 6.941, df = 5, *P* = 0.00) (Table 2). In conclusion, 1.14 percent of the control group's hemocytes contained micronuclei. At the 50 µg/10 µl Fe_3O_4 concentration (2,77%), the percentage of hemocytes with micronuclei was the highest. Table 2 indicates that there was a statistically significant 2,42-fold increase in the percentage of hemocytes with micronuclei at this concentration compared to the control group (χ^2 = 11.709, df = 5, *P* = 0.00).

Table 1 The mean number of larval hemocytes of *G. mellonella* larva by the applied concentrations (96 h after the-force-feeding treatment)

Fe_3O_4 NP concentrations (µg/10 µl)	**Hemocyte count / ml (THCs) (Mean ± SE)[a]**
0	34.64 ± 1.88[a]
0.4	33.66 ± 2.88[ab]
2	34.85 ± 2.08[a]
10	24.66 ± 1.79[bc]
50	26.46 ± 1.56[bc]
250	22.66 ± 2.49[c]

[a]Data are means ± standard errors of three replicates using five larvae per replicate. Different letters in the same vertical column indicated significant differences within different concentrations (Kruskal Wallis, Man Whitney U Test *P*<0.05

Discussion

In our study, a statistically significant difference was observed between the THCs of larvae exposed to 10, 50, and 250 µg/10 µl concentrations of iron oxide nanoparticle (NP) and the THCs of the control group larvae (Table 1). In a study conducted by Park *et al*. (2014), the cellular uptake process and toxic mechanism of magnetic iron oxide nanoparticles (M-FeNPs) were investigated using a murine peritoneal macrophage cell line, RAW264.7. The study reported that M-FeNPs significantly increased the cell mobility. Twenty-four hours after the exposure to NP, M-FeNPs were found to localize freely in the cytosol or in the organelles containing autolysosomes such as the endoplasmic reticulum (ER). The study showed a dose-dependent decrease in the cell viability associated with the S phase arrest. ATP production rapidly decreased along with mitochondrial damage, while the number of ROS-producing cells increased. The levels of genes related to oxidative stress and ER stress were upregulated, while the levels of transcription-related genes were downregulated. (Park *et al*, 2014).

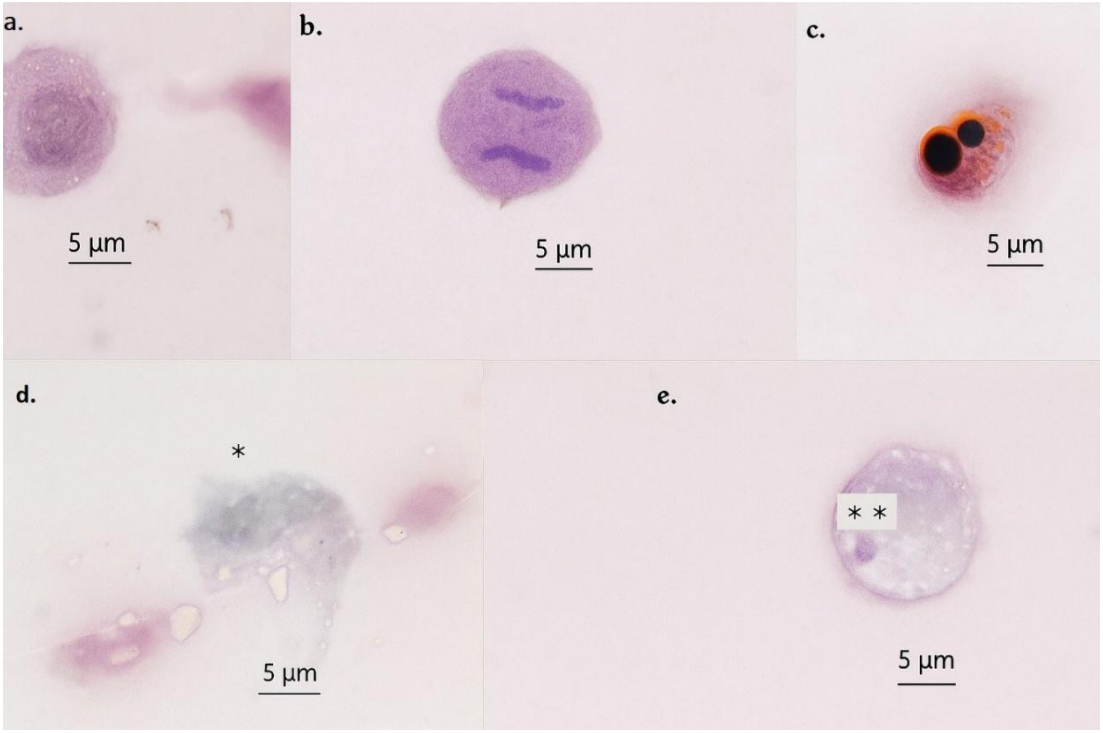

Fig. 2 The light microscope images of viable hemocyte-control (a), mitotic hemocyte (b), apoptotic hemocyte (c), necrotic hemocyte (d), and micronucleated hemocyte (e). *shows the necrotic hemocyte (d), **shows micronucleus (e) (1000×). The scale bar shows 5 µm

Table 2 Percentages of viable, mitotic, apoptotic, necrotic, and micronucleated cells of *Galleria mellonella* larvae (96 h after the force-feeding treatment)

Fe_3O_4 NP concentrations (µg/10 µl)	Viable cells (%)*	Mitotic cells (%)*	Apoptotic cells (%)*	Necrotic cells (%)*	Micronucleated cells (%)*
0	94.18^{ab}	3.25^{a}	0.66^{a}	0.72^{ab}	1.14^{ab}
0,4	95.13^{a}	2.36^{a}	0.70^{ab}	0.75^{ab}	1.04^{a}
2	94.81^{a}	2.22^{a}	1.00^{ab}	1.10^{ab}	0.86^{a}
10	93.48^{bc}	2.41^{a}	1.72^{b}	0.50^{a}	1,90^{bc}
50	91.66^{c}	2.31^{a}	1.76^{b}	1.49^{bc}	2.77^{c}
250	92.44^{bc}	2.14^{a}	1.82^{b}	2.12^{c}	1.47^{ab}

*Data are means ± standard errors of three replicates using five larvae per replicate. Different letters in the same vertical column indicated significant differences within different concentrations (Tukey Test, P<0.05)

Similar toxic effects observed in the THCs (Table 1) are associated with statistically significant increases in apoptotic cell percentages as shown in Table 2. It is evident in this table that the percentage of apoptotic cells in larvae exposed to 10, 50, and 250 µg/10 µl Fe_3O_4 NP concentrations increased significantly compared to the percentage in the control group. Consequently, the significant reduction in mean hemocyte counts in the larvae exposed to 10, 50, and 250 µg/10 µl iron oxide NP concentrations, as observed in Table 1, may be attributed to the increase in apoptotic cell numbers at the same concentrations as shown in Table 2. The increase in apoptotic cell numbers related to oxidative stress, ER stress, and subsequent apoptosis described in Park *et al*. (2014) may have also occurred in the hemocytes in this study, as supported by the increase in apoptotic cell numbers in Table 2. Furthermore, Table 2 shows that when the larvae are exposed to the same iron oxide NP concentrations, the increase in apoptotic and necrotic cell percentages leads to a significant decrease in the percentage of viable cells due to the increased concentration.

In a study investigating the effects of copper sulfate pentahydrate on the THC of the freshwater prawn *Macrobrachium dayanum* (Crustacea: Decapoda), it was found that exposure to copper sulfate pentahydrate resulted in significant fluctuations in THC during acute exposure periods (24, 48, 72, and 96 hours) (Lodhi *et al*., 2008). There are also recommendations for cytotoxic studies to be performed within 4-48 hours (Fröhlich and Meindl, 2015; Eskin and Bozdoğan 2021). When the studies carried out in this context are evaluated, in a study investigating the effects of copper phosphate nanoflowers (p-CPnfs) on the hemocytes of *G. mellonella*, it was found that exposure to p-CPnfs for 24 hours led to a significant increase in the THC of *G. mellonella* larvae at concentrations of 1000, 2000, and 6000 ppm (Eskin *et al*., 2021a). The researchers attributed this increase in the hemocyte counts to several potential factors: the insect's defensive response to detoxify the toxic substance and possibly the increase in circulating granulocytes, mitotic cell counts, or autonomous cell division (Eskin and Bozdoğan, 2021); King and Hillyer, 2013). In our study, although the percentage of mitotic cells showed a decrease relative to the control group with increasing concentrations, this decrease was not statistically significant (Table 2).

In another study investigating the effects of copper oxide nanoparticles (NPs) on the THC of *G. mellonella* larvae, it was reported that copper oxide NPs caused a decrease in the THC by inducing toxic effects on the insect's immune system (Tunçsoy, 2020). The researcher explained this reduction in the THC with the suppression of hemocyte release from hematopoietic organs as a result of exposure to toxic substances such as heavy metals or insecticides, which leads to the inhibition of these organs' functions and subsequently results in cytotoxic effects. Additionally, the same researcher suggestedthat metal oxide nanoparticles entering the insect's body might cause encapsulation and nodule formation, leading to the clustering of hemocytes and a reduction in the THC (Tunçsoy, 2020). In the current study, the significant decrease in the mean hemocyte counts observed in the larvae exposed to 10, 50, and 250 µg/10 µl iron oxide NP concentrations, as shown in Table 1, may be attributed to the physiological reasons described by Tunçsoy (2020).

The findings of this study showed a notable decrease in the percentage of live cells in the larvae exposed to 10, 50, and 250 µg/10 µl Fe_3O_4 NP concentrations compared to the control group after 96 hours. The number of cells undergoing apoptosis significantly rose in the larvae exposed to Fe_3O_4 NP concentrations of 10, 50, and 250 µg/10 µl compared to the number of apoptotic cells in the control group larvae (Table 2). Significant variation in necrotic cells

was noticed between the larvae in the control group and the larvae exposed to 50 and 250 µg/10 µl Fe_3O_4 NP concentrations (Table 2). At 50 µg/10 µl Fe_3O_4 concentration, the largest percentage of micronuclei was finally found, and the percentage of hemocytes having micronuclei at this concentration increased 2,42-fold in comparison to the control group, which was a statistically significant finding in the current study (Table 2).

Eskin and Bozdoğan (2021) conducted a study to reveal the impact of copper oxide nanoparticles (NPs) on *G. mellonella* larvae and obtained results similar to those found in this study. During their study, *G. mellonella* larvae in their fourth stage were given copper oxide nanoparticles (NPs). They were fed using force-feeding technique with concentrations of 10, 50, 100, and 150 µg/10 µl per larva. The percentage of viable hemocytes decreased in the larvae exposed to 50, 100, and 150 µg/10 µl doses within 24 and 72 hours in comparison to the control group. There was a statistically significant increase in the percentage of micronucleated hemocytes in 24 hours following the administering of the copper oxide nanoparticle at the 150 µg/10 µl dose. The findings indicated that the elevated levels of copper oxide NPs (>10 µg/10 µl) raised the percentage of hemocytes undergoing apoptosis within a 24-hour period. The percentage of necrotic hemocytes in the *G. mellonella* larvae also significantly increased within 24 hours following the exposure to copper oxide NPs at concentrations of 100 and 150 µg/10 µl. The percentage of apoptotic hemocytes in larvae exposed to copper oxide NPs at 100 and 150 µg/10 µl was higher after 72 hours than that in the control group larvae (Eskin and Bozdoğan, 2021). Consequently, the outcomes of that study and findings from our current investigation are consistent (Eskin and Bozdoğan, 2021) (Table 2).

The researchers proposed that the rise in apoptotic and necrotic indices could be caused by molecular mechanisms like DNA damage, ROS molecules, death receptors, and intrinsic mitochondrial pathways. They also mentioned that the notable increase in micronucleus-containing hemocytes in *G. mellonella* at higher concentrations might be linked to chromosomal damage (Eskin and Bozdoğan, 2021). We also think that the higher rates of cell death and abnormal cells found in hemocytes based on the higher iron oxide NP levels could be attrifbuted to the factors discussed by Eskin and Bozdoğan (2021) (Tables 1 and 2).

In the current study, the effects of spherical magnetic iron oxide nanoparticles (Fe_3O_4 NPs) on various parameters of *G. mellonella* were investigated, including hemocyte count, apoptotic, necrotic, and mitotic indices in hemocytes, hemocyte viability, and micronucleus formation. We think that the early information presented in this study will be useful for further research.

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References

Amaral IMR, Neto JFM, Pereira GB, Franco MB, Beletti ME, Kerr WE*, et al*. Circulating hemocytes from larvae of *Melipona scutellaris* (Hymenoptera, Apidae, Meliponini): Cell types and their role in phagocytosis. Micron 41: 123-129, 2010.

Atlı-Şekeroğlu Z, Şekeroğlu V. Genetik toksisite testleri. TÜBAV Bilim Dergisi 4(3): 221-229, 2011.

Avan M, Uğur A. Farklı yapay besin ortamlarının *Galleria mellonella* (L.) (Lepidoptera: Galleriidae)'nın gelişimine etkisi. ÇOMÜ Ziraat Fakültesi Dergisi 7(1): 39-46, 2019.

Bolognesi C. Micronucleus experiments with bivalve mollusks. In: S. Knasmüller and M. Fenech, eds. Royal Society of Chemistry, London, UK, pp 273-289, 2019.

Coşkun G, Özgür H. Apoptoz ve nekrozun moleküler mekanizması. Arşiv Kaynak Tarama Dergisi 20(3): 145-158, 2011.

Er A. Endoparazitoit *Pimpla turionellae* (L.) (Hymenoptera: Ichneumonidae) zehiri ve parazitlemesinin konak hemositlerine etkileri. Balıkesir Üniversitesi, Fen Bilimleri Enstitüsü, Biyoloji Anabilim Dalı, Doktora Tezi, Balıkesir, 2011.

Eskin A. Çinko Oksit (ZnO) ve gümüş (Ag) nanopartiküllerinin *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) üzerindeki toksik etkilerinin belirlenmesi. Marmara Üniversitesi, Fen Bilimleri Enstitüsü, Biyoloji Anabilim Dalı, Doktora Tezi, İstanbul, 2017.

Eskin A, Bozdoğan H. Effects of the copper oxide nanoparticles (CuO NPs) on *Galleria mellonella* hemocytes. Drug and Chemical Toxicology 45(4): 1870-1880, 2021.

Eskin A, Ekremoglu M, Altinkaynak C, Özdemir N. International Journal of Tropical Insect Science 42: 333-344, 2021a.

Eskin AN, Öztürk Ş, Eskin A. The effects of magnetic iron oxide nanoparticles (Fe_3O_4) on some biological aspects of *Galleria mellonella* L. (Lepidoptera: Pyralidae). Celal Bayar University Journal of Science 17(3): 319-324, 2021b.

Eskin AN. Demir oksit nanopartiküllerinin *Galleria mellonella* (L.) (Lepidoptera: Pyralidae)'nın biyolojisine ve hemositlerine etkisi. Nevşehir Hacı Bektaş Veli Üniversitesi, Fen Bilimleri Enstitüsü, Çevre Mühendisliği Anabilim Dalı, Yüksek Lisans Tezi, Nevşehir, 2022.

Fröhlich, E, Meindl, C. *In vitro* assessment of chronic nanoparticle effects on respiratory cells. In: M.L. Larramendy and S. Soloneski, eds. Nanomaterials, toxicity and risk assessment. London: Intech Open, Chapter 4, 69-92, 2015.

Fu C, Ravindra NM. Magnetic iron oxide nanoparticles: synthesis and applications. Bioinspired, Biomimetic and Nanobiomaterials 1(4): 229-244, 2012.

Ghasemi V, Moharramipour S, Sendi, JJ. Circulating hemocytes of mediterranean flour moth, *Ephestia kuehniella* Zell. (Lep: Pyralidae) and their response to thermal stress. Inv Surviv J 10(1): 128-140, 2013.

Golstein P, Kroemer G. Cell death by necrosis: towards a molecular definition. Trends in Biochemical Sciences 32(1): 37-43, 2007.

Harding CR, Schroeder GN, Collins JW, Frankel G. Use of *Galleria mellonella* as a model organism to study *Legionella pneumophila* infection. Journal of Visualized Experiments (81): 1-10, 2013.

IBM-SPSS Statistics for Windows. Elsevier, London, UK.IBM Corp. Released. Armonk, NY: IBM Corp, 2011.

King JG, Hillyer JF. Spatial and temporal in vivo analysis of circulating and sessile immune cells in mosquitoes: hemocyte mitosis following infection. BMC Biology 11(55): 1-15, 2013.

Kwadha CA, Ong'amo GO, Ndegwa PN, Raina SK, Fombong AT. The biology and control of the Greater Wax Moth, *Galleria mellonella*. Insects 8(2): 61, 1-17, 2017.

Lodhi HS, Tiwari KJ, Shukla, S and Sharma UD. Alternations in total haemocyte counts (THCs) of freshwater prawn, *Macrobranchium dayanum* (Crustacea-Decapoda) after copper sulphate exposure. Journal of Advanced Zoology 29(2): 92-98, 2008.

Mikulak E, Gliniewicz A, Przygodzka M, Solecka J. *Galleria mellonella* L. as model organism used in biomedical and other studies. Przegl Epidemiol 72(1): 57-73, 2018.

Mohammadinejad R, Moosavi MA, Tavakol S, Vardar DÖ, Hosseini A, Rahmati M,*et al*. Necrotic, apoptotic and autophagic cell fates triggered by nanoparticles. Autophagy 15(1): 4-33, 2019.

Nicotera P, Bernassola F, Melino G. Regulation of the apoptosis-necrosis switch. Oncogene 23(16): 2757-2765, 2004.

Park EJ, Choi DH, Kim Y, Lee EW, Song J, Cho MH, *et al*. Magnetic iron oxide nanoparticles induce autophagy preceding apoptosis through mitochondrial damage and ER stress in RAW264. 7 cells. Toxicology in Vitro 28(8): 1402-1412, 2014.

Qiang X, Hongxia S, Yinghua S, Dexiang UG, Zhang G. Apoptosis of *Spodoptera litura* larval hemocytes induced by heavy metal zinc. Chinese Science Bulletin 50(24): 2856-2860, 2005.

Rahman MM, Khan SB, Jamal A, Faisal M, Aisiri AM. Iron oxide nanoparticles. Nanomaterials 3: 43-67, 2011.

Ramarao N, Nielsen-Leroux C, Lereclus D. The insect *Galleria mellonella* as a powerful infection model to investigate bacterial pathogenesis. Journal of Visualized Experiments 11(70), 1-7, 2012.

Richardson T. Morphological and functional characterization of honey bee, *Apis mellifera*, hemocyte cell communities. Apidologie 49(3): 397-410, 2018.

Sakallıoğlu H. Manyetik nanopartiküller üzerine desteklenmiş Schiff bazi türevi metal komplekslerinin sentezleri ve katalitik etkinliklerinin incelenmesi. Çukurova Üniversitesi Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, Adana, 2013.

Senior NJ, Titball RW. Isolation and primary culture of *Galleria mellonella* hemocytes for infection studies. F1000Research 9: 1-17, 2020.

Soenen SJ, De Cuyper M, De Smedt SC, Braeckmans K. Investigating the toxic effects of iron oxide nanoparticles. Methods Enzymology 509: 195-224, 2012.

Teramoto T, Tanaka T. Mechanism of reduction in the number of the circulating hemocytes in the *Pseudaletia separata* host parasitized by *Cotesia kariyai*. Journal of Insect Physiology, 50: 1103-1111, 2004.

Tunçsoy B. Bakır oksit nanopartiküllerinin *Galleria mellonella* larvaları üzerine immün ve metabolik etkileri. Karaelmas Fen ve Mühendislik Dergisi 10(1): 53-60, 2020.

Ünşar EK, Perendeci NA. Nanopartiküllerin çevresel akıbetleri ve anaerobik parçalanma prosesine etkileri. Pamukkale Üniversitesi Mühendislik Bilimleri Dergisi 22(6): 503-512, 2016.

Venier P, Maron S, Canova S. Detection of micronuclei in gill cells and haemocytes of mussels exposed to benzo[a]pyrene. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 390(1-2), 33-44, 1997.

Yılmaz E. Farklı dozlardaki alüminyum klorür'ün *Galleria mellonella* (l.) (Lepidoptera: Pyralidae)'nın biyolojisine ve hemosit sayılarına etkileri. Marmara Üniversitesi, Fen Bilimleri Enstitüsü, Biyoloji Anabilim Dalı, Yüksek Lisans Tezi, İstanbul, 2013.