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ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

INFLUENCE OF ALUMINUM OXIDE NANOPARTICLES ON BIOLOGICAL FEATURES AND HOST HEMOCYTES OF *Galleria mellonella* L. (Lepidoptera: Pyralidae) WITH ITS ENDOPARASITOID *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae)

Alüminyum Oksit Nanopartiküllerinin *Galleria mellonella* L. (Lepidoptera: Pyralidae) ile Endoparazitoiti *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae)'nın Biyolojik Özellikleri ve Konak Hemositleri Üzerine Etkisi

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ABSTRACT

Nanoparticles (NPs) are released directly or indirectly into nature with increased production and consumption, and their effects on insects, which occupy a large place in the ecosystem, are of interest. There is also interest in the potentially toxic effects of NPs applied to hive pests on parasitoids, honey bees, and host-parasitoid relationships. The influence of aluminum oxide (Al_2O_3) NPs on the biological features of the hive pest *Galleria mellonella*, total counts of hemocyte, and hemocyte types; as well as on the biological features of the endoparasitoid *Pimpla turionellae* were investigated. The data obtained revealed that Al_2O_3 NPs caused a decrease in the larval, pupal, and adult development time of *G. mellonella*. The immature developmental time of *P. turionellae* was reduced. It was also demonstrated that Al_2O_3 NPs decreased the total counts of hemocytes in *G. mellonella* larvae; granulocyte, spherulocyte, oenocytoid, and prohemocyte counts decreased at all NP concentrations, while plasmatocyte counts increased. The data showed that Al_2O_3 NPs affected the biological properties of the hive pest model organism *G. mellonella* and indirectly affected its endoparasitoid *P. turionellae*. In addition, Al_2O_3 NPs showed a suppressive effect on cellular immune system responses, decreasing hemocyte counts. Our study results suggest that honey bees, honeycomb pests, and parasitoids may be negatively affected by NPs, which have increased in recent years as environmental pollutants, and that NPs may have insecticidal effects.

Keywords: Aluminium oxide nanoparticle, Biological features, *Galleria mellonella*, Hemocyte, *Pimpla turionellae*

ÖZ

Dünya çapında üretim ve tüketimin artmasıyla birlikte nanopartiküller (NP'ler) doğrudan ya da dolaylı olarak doğaya salınmaktadır ve ekosistemde büyük bir yer kaplayan böceklerde etkileri merak uyandırmaktadır. Ayrıca kovan zararlısına uygulanan NP'lerin parazitoitler üzerinde muhtemel toksik etkileri, diğer bir deyişle bal arıları ve konak-parazitoit ilişkileri ilgi çekmektedir. Bu nedenle alüminyum oksit (Al_2O_3) NP'lerin kovan zararlısı *Galleria mellonella*'nın biyolojik özellikleri, toplam hemosit sayısı

ve hemosit tipleri ile endoparazitoid *Pimpla turionellae*'nın biyolojik özellikleri üzerindeki etkisi araştırıldı. Elde edilen veriler, Al_2O_3 NP'lerin *G. mellonella*'nın larva, pupa ve ergin gelişim sürelerinde azalmaya neden olduğunu ortaya koydu. *P. turionellae*'nın ise olgunlaşma öncesi gelişim süresi kısaldı. Aynı zamanda Al_2O_3 NP'lerin *G. mellonella* larvalarındaki toplam hemosit sayısını azalttığı; granülosit, sferülosit, önositoid ve prohemosit sayılarının tüm NP konsantrasyonlarında azaldığı, plazmatosit sayılarının ise arttığı tespit edildi. Bulgular, Al_2O_3 NP'lerin kovan zararlısı model organizma *G. mellonella*'nın biyolojik özelliklerini etkilediğini ve endoparazitoiti *P. turionellae*'nın dolaylı olarak etkilendiğini gösterdi. Ayrıca Al_2O_3 NP'lerin hücresel bağışıklık sistemi tepkileri arasında yer alan hemosit sayılarının azalması ile sonuçlanarak baskılayıcı etki gösterdiği görüldü. Çalışma sonuçlarımız bal arılarının, petek zararlılarının ve parazitoitlerinin çevresel kirleticiler olarak son yıllarda artan NP'lerden olumsuz etkileneceği ve NP'lerin insektisidal etki gösterebileceği düşüncesini ortaya koymaktadır.

Anahtar Kelimeler: Alüminyum oksit nanopartikülü, Biyolojik özellikler, *Galleria mellonella*, Hemosit, *Pimpla turionellae*

GENİŞLETİLMİŞ ÖZET

Amaç: Ağır metaller ve bu metallerin oksitlenmiş nano yapıları günlük hayatta sıklıkla karşılaşılan toksik maddelerden biridir. Ağır metaller arasında yer alan alüminyum zorlu çevresel ve iklim koşullarına olan dayanıklılığı, hafif ve sünek yapılarından dolayı kolay şekil alabilmesi nedeniyle sıklıkla üretimde tercih edilmektedir. Alüminyumun oksijen ile tepkimesi sonucu oluşan alüminyum oksit (Al_2O_3) nanopartikülleri (NP) fiziksel ve kimyasal özellikleri nedeniyle birçok uygulama alanında diğer NP'lere kıyasla daha fazla ilgi görmektedir. Metallerin özellikle Al_2O_3 NP'ler gibi nanoparçacık formları vücuda beslenme, solunum ve deri yoluyla kolaylıkla alınmaktadırlar. Biyolojik olarak vücuttan atılımları kolay olmayan bu nano yapılı metal oksit türevleri canlı sağlığını tehdit etmektedir. Bununla birlikte son yıllarda Al_2O_3 NP'lerin böceklerde insektisit etkileri merak konusu olmuştur. Bu nedenle böcekler ve insanlar dahil tüm ekolojik sistemler üzerinde oluşturabileceği etkilerin belirlenmesine ihtiyaç duyulmaktadır. Bal arısı, *Apis mellifera* ve *Apis cerana*'nın bir zararlısı olan büyük balmumu güvesi *Galleria mellonella* bal arısı popülasyonlarında azalmaya neden olur ve bu zararlı türlerle mücadele etmek arıcılık endüstrisi için önemli bir sorun haline gelmiştir. Diğer yandan *Pimpla turionellae*, bu zararlıların endoparazitoidi olarak tanımlanır ve biyolojik mücadelede etkilidir. Çevrede artan NP konsantrasyonları direkt veya konak ile etkileşimleri sonucu dolaylı olarak endoparazitoitleri etkileyebilir. Bu nedenle çalışmada farklı konsantrasyonlarda Al_2O_3 NP'lerin konak *Galleria mellonella*'nın ve endoparazitoiti *Pimpla turionellae*'nın biyolojik özelliklerine etkisini incelemek amaçlandı. Aynı zamanda bu NP'lerin konak türün hemosit aracılı immün sistemine etkileri de belirlendi.

Gereç-Yöntem: *Galleria mellonella* larvaları 50, 100, 500 ve 1000 ppm konsantrasyonlarında Al_2O_3 NP içeren solüsyonlar hazırlanarak sentetik besinin su içeriğine eklendi ve ilk evre larvalardan son evre larvalara gelişinceye kadar beslendi. Al_2O_3 NP'ler sadece larval gelişim süresince uygulandı. Al_2O_3 NP'lerin *G. mellonella*'nın larva, pupa ve ergin gelişim süreleri ile ağırlık ve uzunlukları gibi yaşam döngüsü parametrelerine etkisi belirlendi. Pupaların bir kısmı parazitleme için kullanıldı. Parazitlemenin ardından bu NP'lerin endoparazitoit *P. turionellae*'nın olgunlaşma öncesi gelişim süresi ve ergin ömrü gibi yaşam döngüsü parametrelerine etkileri gözlendi. Bununla birlikte *P. turionellae*'nın ağırlık ve uzunluk gibi morfolojik özellikleri de kaydedildi. Konak türün tüm deney gruplarını oluşturan son evre larvalardan alınan hemolenf süspansiyonları Neubauer hemositometresine yüklendi ve total hemosit sayısındaki değişiklikler faz kontrast mikroskobunda gözlemlendi. Son olarak Al_2O_3 NP kaynaklı hemosit tiplerindeki değişiklikler Giemsa boyama yöntemi kullanılarak faz kontrast mikroskobunda belirlendi.

Bulgular ve Sonuç: *G. mellonella*'da Al_2O_3 NP'ler larval, pupal ve ergin gelişim sürelerini (gün) doza bağlı bir şekilde azalttı. *P. turionellae*'nın olgunlaşma öncesi gelişim süresinde doza bağlı bir şekilde azalma düşük dozlardan itibaren görüldü. Diğer taraftan *P. turionellae*'nın ergin ömrü, ağırlığı ve uzunluğunda önemli bir farklılık görülmedi. Çalışmamız metal Al_2O_3 NP'lere kronik olarak maruz kalan konak *G. mellonella*'nın biyolojik kontrol ajanı parazitoit *P. turionellae* ile etkileşimleri sonucu

gelişim sürelerini etkilediğini ortaya koymaktadır. Ancak bu NP'lerin konak ve parazitoitlerin morfolojik özelliklerine önemli etkileri gözlenmedi.

Al_2O_3 NP'lerin tüm deney gruplarında toplam hemosit sayısında azalmaya neden olduğu tespit edildi. Hemosit tiplerinden granülosit, sferülosit, önositoid ve prohemosit sayılarının tüm dozlarda azaldığı, plazmatosit sayılarının ise arttığı belirlendi. Elde edilen sonuçlar ile Al_2O_3 NP'lerin model organizma *G. mellonella*'nın hücre-aracılı bağışıklık sistemi üzerinde baskılayıcı etkileri olduğunu ortaya koymaktadır. Verilerimiz metal türevli Al_2O_3 NP'lerin *G. mellonella*'da insektisidal etki gösterebileceğini ve potansiyel insektisit olabileceğini vurgulamaktadır. Ancak ekosistemde önemli bir biyolojik kontrol ajanı olan *P. turionellae*'nın da NP kaynaklı toksisiteden etkilenebileceği belirlendi. Bu sonuçlar insan beslenmesinin önemli kaynağı olan bal arılarının da nanokirleticilerden etklenebileceğini ortaya koymaktadır. Bu nedenle Al_2O_3 NP'lerin daha iyi anlaşılması ve yönetimine dikkat edilmesi son derece önemli olacaktır.

INTRODUCTION

Since nanomaterials are increasingly used in a wide variety of fields, their toxic effects are a matter of curiosity. Among them, nanoparticles (NPs) are utilized in many industries from biomedicine to engineering (Bankier et al. 2019). They have an exceptional place in the industrial field due to their properties such as their nano size, high reactivity, and physical and chemical features. Metal and metal oxide NPs constitute more than 30% of the entire NP-containing products (Kumar et al. 2018, López-Muñoz et al. 2019). At the same time, metallic nano and microparticles, which can be comprised of both natural periods and anthropogenic factors are among the major causes of environmental pollution (Yanar et al. 2022). Nano-sized NPs may be more toxic to living organisms than their ionic forms (Tunçsoy 2018, Eskin and Bozdoğan 2022) and micro / macro-sized materials (Das et al. 2019). NPs may enter organisms through the respiratory or digestive system and may be carried by circulation to several organs and tissues. They can enter the cell through biological membranes through endocytotic transport processes such as phagocytosis, pinocytosis, or receptor-mediated endocytosis (Ahmad et al. 2019, Assar et al. 2022, Tuncsoy and Mese 2021). After that, they can cause cellular toxicity and become lethal factors (Assar et al. 2022, Tuncsoy and Mese 2021). Both *in vitro* and *in vivo* investigations have shown that metal oxide NPs have genotoxic (Sharma et al. 2009), carcinogenic, and mutagenic (Kumar et al. 2011, Pan et al. 2010) potentials.

Aluminum constitutes nearly 8% of the elements in the Earth's crust (Barabasz et al. 2002, Kara et al. 2020). Aluminum oxidizes spontaneously in the air to form aluminum oxide (Al_2O_3) NP with a prooxidant feature (Fricault 2018). Since metals are prone to hydrolysis in an aqueous environment, they can affect oxidoreduction processes in biological systems. A metal with a redox potential may produce reactive oxygen species by interfering with reactions such as Fenton that occur in living cells (Egorova and Ananikov 2017). Their toxicity on organisms is not adequately understood; research on their acute, chronic, and environmental toxicity is also inadequate (Ismail et al. 2021). Al_2O_3 NPs' impact on biological systems, including insects, has raised concerns. Studies indicate that these nanoparticles can induce oxidative stress, alter enzymatic activities, and affect cellular structures in insects (Kara et al. 2020; Demirtürk et al. 2023). For example, exposure to Al_2O_3 NPs may lead to changes in hemocyte counts and function, which are critical components of the insect immune system. However, some of them have been recommended as possible biopesticides for seed conservation apart from their normal usage (López-Muñoz et al. 2019, Sahayaraj 2017). Therefore, Al_2O_3 NPs may have an essential role in agricultural applications (López-Muñoz et al. 2019, Poborilova et al. 2013, Willhite et al. 2014) and may be used instead of traditional insecticides (Ismail et al. 2021). Such research results may contribute to its use in Integrated Pest Management.

Honey bees have a crucial role in ecosystem function as well as in agricultural production and are considered as essential pollinators. However, they face an increasing number of stressors, especially xenobiotics, directly and indirectly (Hung et al. 2018, O'Connell et al. 2024). The greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), is a natural pest of honey bee (*Apis mellifera* L.) colonies. By inactivating honey bee colonies, they may periodically cause significant losses to the beekeeping industry (O'Connell et al. 2024). On the other hand, NPs of xenobiotic origin can affect the hive pest *G. mellonella* and indirectly honey bees. Additionally, insects are considered bioindicators in studying the toxicity, bioaccumulation, and

biotransfer of metals in the ecosystem and determining their effect on environmental pollution (Banville et al. 2012, Kara et al. 2020, Wu and Yi 2015). The immune system of *G. mellonella*, which is among the larvae of Lepidopteran species, resembles the mammalian immune system both in structure and function (Gwokyalya and Altuntaş 2019, Tunçsoy et al. 2021). *G. mellonella* is hence of interest for physiological, immunological, and toxicological investigations (Altuntaş 2015, Uçkan et al. 2021). Hemocytes of *G. mellonella* recognize foreign substances and phagocytose them, similar to neutrophils in the mammalian immune system (Browne et al. 2013, Tunçsoy et al. 2021). Species of the Lepidoptera generally have particular hemocytes: granulocytes, plasmatocytes, spherulocytes, oenocytoids, prohemocytes, and adipohemocytes (Lavine and Strand 2002, Altuntaş et al. 2012). Nanotoxicological studies with this type of insect may help to detect the potential impacts on human health and the ecosystem (Eskin and Bozdoğan 2022, Zorlu et al. 2018). *Pimpla turionellae* (Hymenoptera: Ichneumonidae) is one of the endoparasitoids of the host *G. mellonella* (Kansu and Uğur 1984, Uçkan et al. 2011). Nanomaterials that affect the host are likely to indirectly affect parasitoids, and these particles could potentially disrupt their host- parasitoids interactions. As hemocytes in *G. mellonella* play an crucial role in defence against parasitoid invasion, NPs may compromise this defence by affecting hemocyte viability and function, making the host more susceptible to parasitism. Conversely, nanoparticles may also affect the parasitoid's ability to successfully parasitise the host by interfering with its biological processes. The introduction of nanoparticles into the environment, including agricultural settings, may pose a risk to non- target organisms like beneficial insects. Therefore, Al_2O_3 NPs were given to *G. mellonella* larvae via diet, and their influence on the life cycle of host and endoparasitoid *P. turionellae*, and total hemocyte counts and hemocyte types of host larvae were investigated. Our results provide information on the influence of Al_2O_3 NPs on biological and hemocyte-mediated immunity in host-parasitoid insects. The study aimed to reveal the effects of Al_2O_3 NPs on the life cycle and immune system of *G. mellonella* due to their environmental toxicity (with nutrition) their impact on host-parasitoid interactions.

MATERIALS AND METHODS

Host and Parasitoid Rearing

The host greater wax moth *G. mellonella* was reared at 25 ± 3 ºC, a humidity of 60 ± 3%, and 24 hours of darkness. A synthetic diet was prepared from honeycomb, bran, honey, glycerin, and distilled water for feeding during larval stages (Bronskill 1961, Sak et al. 2006). The endoparasite *P. turionellae* was reared at a temperature of 25 ± 3 ºC, a humidity of 60 ± 3%, and 12: 12 h (Light: Dark) lighting conditions. They were fed with sterile cotton wool soaked with honey solution (30%, V: V) diluted with distilled water and pupal hemolymph of *G. mellonella* (two pupae for five females) three times a week.

Nanoparticles

Aluminum oxide nanoparticles (nanopowder Al_2O_3 NPs, TEM particle size < 50 nm) were purchased from Sigma Aldrich (Al_2O_3 NPs reference: 544833). For the preparation of NP solutions, a bath-type sonicator was used at 40 °C for 10 min. Data (scanning electron microscope images and Zeta potentials of Al_2O_3 NPs) on the characterization of Al_2O_3 NPs were given in our previous study (Demirtürk et al. 2023).

Bioassays

Determination of Al_2O_3 NP lethal concentrations (LC_{50}, probit analysis), preparation of larvae diets, and application of NPs to the diet were performed as in the previous study (Demirtürk et al. 2023). Al_2O_3 NP solutions prepared at different concentrations were used instead of water content and fed until the larvae grew to the last instar. As a result of Probit analysis, three concentrations below and one concentration above the LC_{50} value were selected and 50, 100, 500 and 1000 ppm Al_2O_3 doses were used (Demirtürk et al. 2023). The time required to complete the larval, pupal, and adult stages was recorded. Adult weights and sizes were measured. In addition, the pupae were parasitized by reproductively mature *P. turionellae* females. The time from the parasitism of the host *G. mellonella* pupae by *P. turionellae* to the formation of adult parasitoids (immature developmental time) and the longevity time of adult parasitoids were regularly observed and recorded. The weight and size data of *P. turionellae* were also measured.

Hemolymph Collection

To determine the total and differential hemocyte counts of host larvae, firstly, the last instars of *G. mellonella* (0.21 ± 0.01 g) from the experimental groups were chosen randomly. Larvae were anesthetized on ice for approximately 4-6 minutes and sterilized with ethanol (70%). The hind leg of the larvae was punctured with a needle and hemolymph was removed with a micropipette (Eppendorf, St. Louis, MO).

Total and Differential Hemocyte Counts

To determine the influence of NPs on circulating total hemocyte counts (THCs), the protocol recommended by Altuntaş et al. (2012) was applied. Hemocytes were counted under 60 × magnification in a phase contrast microscope (Nikon Eclipse Ti-U Phase contrast microscopy). Results expressed as THCs 10^6 cells / mL hemolymph (Altuntaş et al. 2012).

Giemsa staining protocol was used for differential hemocyte counts (DHCs) (Uçkan and Sak 2010). DHCs in hemolymph preparations obtained from larvae were observed under phase contrast microscopy. For DHCs, 500 cells from a single larva were counted on each slide. Hemocyte types were identified using the morphological characters described by Altuntaş et al. (2012).

Statistical Analysis

The means of data were analyzed by using the Independent Samples T-test and One-Way ANOVA in SPSS version 27. Levene's test analyzed concentration-dependent changes in the means for the normality of the data distribution. In One-way variance analysis, Tukey's HSD (Tukey's Honestly Significant Difference) was applied if the means were homogenous and Tamhane's t2 post hoc test was used if the means were not homogenous. For all the statistical tests, the p-value was taken as 0.05.

RESULTS

Biological Features of *Galleria mellonella* and *Pimpla turionellae*

All Al_2O_3 NP concentrations caused shortening of the larval and pupal developmental time of *G. mellonella* (df1, df2 = 4,70, F = 37.15, p = 0.00 < 0.001; df1, df2 = 4,70, F = 17.09, p = 0.00 < 0.001). For both groups, the greatest decrease occurred at the concentration of 1000 ppm. Adult longevity was shortened at concentrations of 50, 100, and 500 ppm Al_2O_3 NPs. Besides, adult longevity did not become different in the 1000 ppm group (df1, df2 = 4,70, F = 26.76, p = 0.00 < 0.001). Adult weights of larvae in the group treated with 500 ppm Al_2O_3 NP concentration increased, while there was no significant change in the other groups (df1, df2 = 4,70, F = 7.70, p = 0.00 < 0.001). In addition, no changes in adult size were observed (df1, df2 = 4,70, F = 1.12, p = 0.35, Table 1).

Table 1. Aluminum oxide nanoparticles (Al_2O_3 NPs)-associated changes in larval, pupal, and adult development time (day), adult weight (mg) - size (mm) of *Galleria mellonella*

Concentrations of Al_2O_3 NPs (ppm)	Larval Developmental Time (day) *	Pupal Developmental Time (day) *	Adult Longevity Time (day) *	Adult Weight (mg) *	Adult Size (mm) *
Control	27.1 ± 0.33^{a}	14.2 ± 0.42^{a}	14.8 ± 0.43^{a}	79.7 ± 0.40^{a}	13.7 ± 0.38^{a}
50	23.6 ± 0.37^{bc}	12.4 ± 0.41^{b}	10.7 ± 0.43^{bc}	81.1 ± 0.42^{a}	14.4 ± 0.32^{a}
100	24.7 ± 0.33^{b}	12.9 ± 0.50^{ab}	9.33 ± 0.34^{c}	80.3 ± 0.34^{a}	13.9 ± 0.35^{a}
500	22.4 ± 0.43^{c}	10.8 ± 0.41^{c}	11.8 ± 0.32^{b}	82.6 ± 0.42^{b}	14.2 ± 0.34^{a}
1000	20.9 ± 0.44^{d}	9.66 ± 0.39^{c}	12.0 ± 0.31^{a}	80.8 ± 0.30^{a}	13.2 ± 0.33^{a}

* All data for each group are represented as Means ± Standard Errors. In each group, the mean of 15 individuals was given and three replicates were analyzed. Means following the same letter in each column is not substantially different, but particular letters (a-d) are significant (p < 0.001).

The immature developmental time of *P. turionellae* decreased at all concentrations (df1, df2 = 4,70, F = 5.12, p = 0.001 < 0.05). However, on average, the maximum decrease of 12.9% occurred at concentrations of 500 and 1000 ppm. No significant differences were observed in longevity, weight, and size of adult parasitoids (df1, df2 = 4,70, F = 17.09, p = 0.06; df1, df2 = 4,70, F = 26.76, p = 0.92; df1, df2 = 4,70, F = 7.70, p = 0.88, Table 2). Even if there were changes in "Mean ± Standard Errors" values between the groups, they were not statistically significant.

Table 2. Aluminum oxide nanoparticles (Al_2O_3 NPs)-associated changes in immature and adult development time (day), adult weight (mg) - size (mm) of *Pimpla turionellae*

Concentrations of Al_2O_3 NPs (ppm)	Immature Developmental Time (day) *	Adult Longevity Time (day) *	Adult Weight (mg) *	Adult Size (mm) *
Control	20.9 ± 0.33[a]	24.5 ± 0.30[a]	18.3 ± 0.26[a]	11.3 ± 0.23[a]
50	19.6 ± 0.41[ab]	23.0 ± 0.38[a]	17.9 ± 0.23[a]	10.9 ± 0.18[a]
100	19.3 ± 0.36[b]	23.3 ± 0.34[a]	17.6 ± 0.26[a]	10.6 ± 0.37[a]
500	18.4 ± 0.34[b]	23.8 ± 0.22[a]	17.7 ± 0.31[a]	11.0 ± 0.22[a]
1000	18.6 ± 0.31[b]	24.2 ± 0.26[a]	18.0 ± 0.30[a]	10.8 ± 0.26[a]

* All data for each group are represented as Means ± Standard Errors. In each group, the mean of 15 individuals was given and three replicates were analyzed. Means following the same letter in each column is not substantially different, but particular letters (a-d) are significant ($p < 0.05$).

Total and Differential Hemocyte Counts

Hemocyte counts in larvae were decreased at all Al_2O_3 NP concentrations (df1, df2 = 4,70, F = 3.59, p = 0.01 < 0.05). At the lowest concentrations of 50 and 100 ppm NP, THCs decreased by 30.6% and 44.9%, respectively. In the higher concentrations of 500 and 1000 ppm NP groups, THCs decreased by 58.9% and 62.7%, respectively (Figure 1).

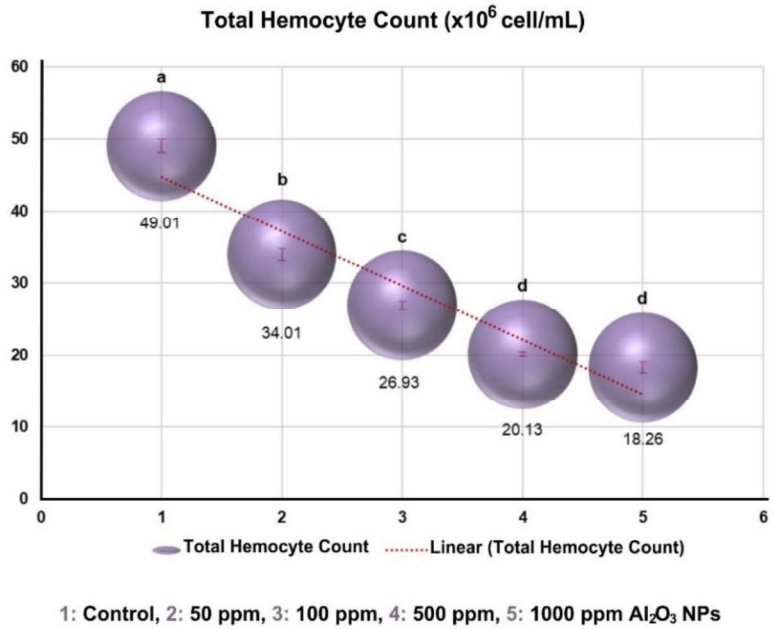

Figure 1. Chronic toxic effects of aluminum oxide nanoparticles (Al_2O_3 NPs) on total hemocyte count (× 10^6 cells / mL) of *Galleria mellonella* Data represent "Mean ± Standart Error" of 15 larvae in total ($p < 0.05$; One-way ANOVA, Tukey's HSD).

The differences in differential hemocyte counts (cells/500) of *G. mellonella* larvae associated with Al_2O_3 NPs are given in Figure 2. Plasmatocyte counts were significantly higher at all concentrations compared to the control group (df1, df2 = 4,70, F = 402.56, p = 0.00 < 0.05). In each treatment group,

important changes were noticed equated to the control (98.75 ± 2.16) and the maximum plasmatocyte increase in the 1000 ppm group was 165.9%. The major hemocyte type in *G. mellonella* larvae was plasmatocyte cells, which constituted 147.03 ± 2.22, 172.23 ± 3.51, 233.11 ± 3.25, and 262.62 ± 4.63 of the total hemocyte population at all concentrations (50, 100, 500 and 1000 ppm), respectively. On the contrary, significant decreases in granulocyte counts were observed in all groups with Al_2O_3 NP concentrations (df1, df2 = 4,70, F = 121.48, p = 0.00 < 0.05). Granulocyte count was 195.03 ± 1.70 in the control group and a 56.5% decrease was observed at the highest (1000 ppm) Al_2O_3 NP concentration. Means ± Standard Errors (Means ± SE) values of granulocyte counts from low to high concentrations were 181.60 ± 2.19, 169.92 ± 2.64, 134.51 ± 2.37, and 124.65 ± 4.21, respectively. Similarly, significant decreases in spherulocyte, oenocytoid, and prohemocyte counts have been observed at all low and high concentrations (df1, df2 = 4,70, F = 14.81, p = 0.00 < 0.05; df1, df2 = 4,70, F = 34.94, p = 0.00 < 0.05; df1, df2 = 4,70, F = 18.58, p = 0.00 < 0.05). Maximum reduction rates (at 1000 ppm concentration) were 73.2% (spherulocyte), 88.8% (oenocytoid), and 85.6% (prohemocyte).

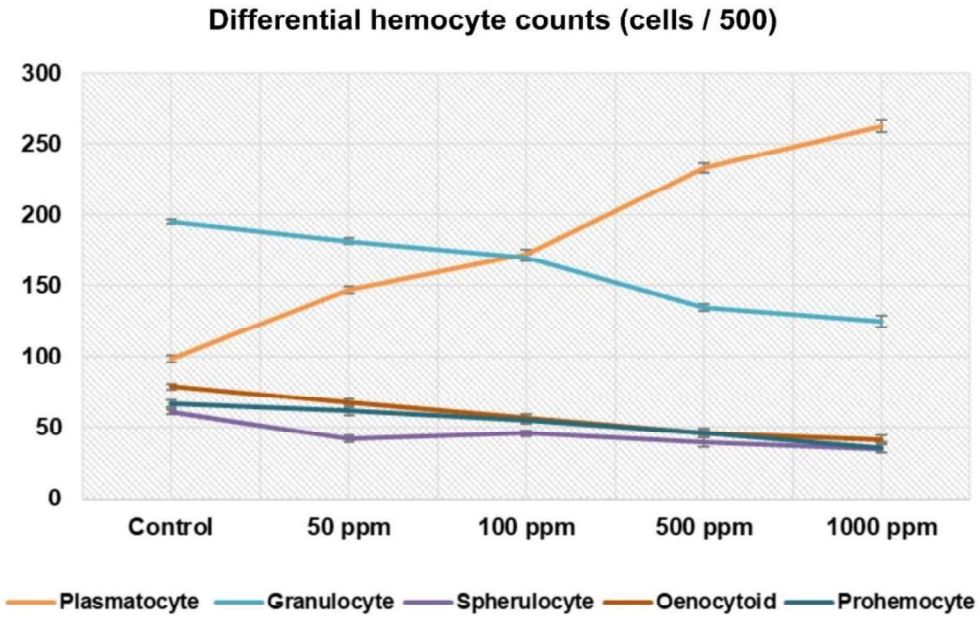

Figure 2. Influence of Aluminum oxide nanoparticles (Al_2O_3 NPs) on plasmatocyte, granulocyte, spherulocyte, oenocytoid, and prohemocyte counts *Galleria mellonella* larvae. Data represent "Mean ± Standart Error" of 15 larvae in total (p < 0.05; One-way ANOVA, Tukey's HSD).

DISCUSSION

The expanding use of NPs in numerous industries and their accumulation in the environment is critical for host-parasitoid relationships, which are the life vests of the ecosystem (Uçkan and Gülel 2002). The effects of NPs on the hive pest *G. mellonella*, which adversely may affect honey bees (pollinators and honey producers), and its endoparasitoid *P. turionellae* species are particularly vital presently. Research on the relation between the biological features of insects and metal oxide NPs is restricted and gives varying results. Especially studies on Al_2O_3 NPs are few (Assar et al. 2022, Kara et al. 2020). In a study conducted on the house fly model "*Musca domestica* L.", silver (Ag), Al_2O_3, and ZnO NPs were administered to larvae with diet for 72 hours, and it was reported that all NPs caused elongation in larval and pupal developmental time (Assar et al. 2022). In *G. mellonella*, it was found that pupal development time and pupal weights were not different from iron oxide NPs (Fe_3O_4) NP application (Eskin et al. 2021). In another study investigating the biological properties of *G. mellonella*, it was reported

that TiO_2 NPs extended the development time of larvae and pupae (Zorlu et al. 2018). The research shows that Al_2O_3 NPs can alter developmental timelines, with larvae and pupae of *G. mellonella* experiencing shortened developmental stages at various concentrations of Al_2O_3 NPs. This contrasts with other studies showing that different metal oxide NPs extend development times. Such findings indicate that Al_2O_3 NPs have a unique impact on insect physiology, potentially by accelerating metabolic processes or through toxicological stress that prompts faster development as a survival response.

Adult longevity is an important parameter in insect biology and studies on nanotoxicity show highly variable results. Eskin et al. (2021) reported that Fe_3O_4 NPs did not differ in adult weights and lifespan of *G. mellonella*. It has been documented that 5000 ppm ZnO NPs extend the adult lifespan of *G. mellonella* (Eskin and Nurullahoğlu 2022). In contrast, it has been observed that different ZnO NPs applied on *Spodoptera frugiperda* shortened female and male adult lifespans in a dose-dependent manner (Pittarate et al. 2021). It has been reported that TiO_2 NPs shorten adult lifespan even at low concentrations in *G. mellonella* (Zorlu et al. 2018). We observed that Al_2O_3 NPs (50, 100, and 500 ppm), like ZnO and TiO_2 NPs mentioned in previous research, also shorten adult longevity. In addition, only the 500 ppm Al_2O_3 NP group showed an increase in adult weight, while no change was observed in adult size. Heavy metals such as copper and zinc have been expressed to decrease the longevity of insects (Coskun et al. 2021, Sang et al. 2018). The reduction in adult longevity for *G. mellonella* exposed to Al_2O_3 NPs, along with an increase in adult weight at higher NP concentrations, suggests that these NPs might be influencing energy allocation and stress responses. The observed shortening of lifespans parallels findings with other NPs, indicating a potential universal stress response across different NP types. According to the study of Uçkan et al. (2015) with the parasitoid *P. turionellae*, treatment with indole-3-acetic acid (IAA) did not affect adult weights, only 5000 ppm IAA decreased female weights. In addition, while the size of adult females did not change, declines in immature developmental time and, adult longevity have been observed at IAA doses ≥ 1000 ppm (Uçkan et al. 2015). Unlike these data, the immature developmental time of *P. turionellae* parasitoids decreased at all Al_2O_3 NP concentrations, while no change was observed in the longevity of adult parasitoids. The unchanged adult longevity and weight of *P. turionellae* despite its host's altered development time and immune responses indicate a complex interaction where the parasitoid might be indirectly affected by the host's exposure to NPs. This relationship is critical for understanding ecosystem dynamics and potential cascading effects within food webs.

Hemocytes have a substantial role in the cellular and humoral immune systems of insects. The functioning of many systems in the organism is related to the immunity and hemocytes of insects (Kaya et al. 2021). As a response to immune defense, the number and morphology of hemocytes may change depending on toxic substances (Coskun et al. 2021, Yucel and Kayis 2019). Kara et al. (2020) have stated that Al_2O_3 NPs applied at different concentrations and for different periods decreased THCs in *G. mellonella* larvae. It was found that ZnO NPs significantly reduced the counts of hemocytes in *G. mellonella* (Nurullahoğlu et al. 2015). Likewise, it has been stated that TiO_2 NPs reduced the counts of hemocytes in the hemolymph of *G. mellonella* (Zorlu et al. 2018). Tuncsoy and Mese (2021) have documented that there were significant decreases in THCs in the groups where the lowest and highest concentrations of TiO_2 NPs were applied. The same researchers have found significant decreases in THCs at high CuO concentrations, another metal oxide NP (Tunçsoy et al. 2021). Our data showed that Al_2O_3 NPs can cause changes in total and differential hemocyte counts in *G. mellonella* hemolymph. Earlier reports showing the interactions between THCs and metal oxide NPs in insects support our study with Al_2O_3 NPs. It was observed that all concentrations of Al_2O_3 NPs were effective and THCs decreased in *G. mellonella* larval hemolymph. These decreases in THCs may be associated with an increase in apoptosis in hemocytes or inhibition of hemocyte production and release by affecting the deterioration of hematopoietic function due to the toxic influence of Al_2O_3.

Although their influence is various in the insect immune system, the roles of hemocyte types are crucial. Metal oxide NPs are effective in THCs, but can also be effective in the number of hemocyte types. Tunçsoy et al. (2021) have observed substantial increases in the counts of granulocytes in LC_{10} group larvae as a result of CuO NP application. Plasmatocyte counts were high in all

CuO NP groups, and the biggest increments were detected in prohemocyte and spherulocyte counts as a result of LC_{10} application and in oenocytoid counts in 1000 mg / L application (Tunçsoy et al. 2021). Administration of ZnO NPs treated with mulberry leaves to *Bombyx mori* for 12 and 24 h increased the number of granulocytes and plasmatocytes, while the population of prohemocytes and spherulocytes decreased (Mir et al. 2020). Similarly, it was shown that the population of DHCs was significantly decreased, while the count of oenocytoid was increased significantly in *B. mori* larvae fed with mulberry leaves treated with ZnO NPs (Belal and Gad 2023). Plasmatocye counts increased at all Al_2O_3 NP concentrations, while granulocyte, spherulocyte, oenocytoid, and prohemocyte counts decreased. These changes in hemocyte counts may be due to increased cell division rate, the release of bound hemocytes, or the attendance of hemocytes in the cellular responses including phagocytosis, encapsulation, and melanization. Since plasmatocyte has an essential function in the formation of these cellular responses. Furthermore, the population of hemocytes is affected as a result of mitotic division of prohemocytes (Er et al. 2011). It is considered that the presence of Al_2O_3 NPs as a threat to the organism may have resulted in the differentiation of prohemocytes into plasmatocytes and the decrease in the prohemocyte population may be related to these conditions. In our previous study, we also investigated encapsulation and melanization data related to cellular immunity. We observed that Al_2O_3 NPs decreased larvae's strong encapsulation and melanization responses at certain times (at 4 and 24 h) in a concentration-dependent (Demirtürk et al. 2023).

Compared with the results, the increase in plasmatocyte counts observed in encapsulation and melanization responses supports this hypothesis. However, the decrease in other hemocyte counts does not confirm this. At that point, decreases in other hemocyte counts may be associated with apoptosis or necrosis. This is also a curiosity about other influences of Al_2O_3 NPs. Al_2O_3 NPs affect the immune system of *G. mellonella* by altering hemocyte counts. Decreases in THCs and changes in specific hemocyte types suggest that these NPs could weaken the insect's immune defense, making them more susceptible to pathogens and parasites. This has broader implications for insect health and survival in environments contaminated with NPs.

Conclusion: Collected data demonstrate that Al_2O_3 NPs cause significant changes in the life cycle of *G. mellonella* and *P. turionellae* and the total, and differential hemocyte means of the host species. Therefore, this study may contribute to accumulating of knowledge about the lifetime and cellular immunity of nano Al_2O_3, which is among the metal oxide NPs. A material that may cause nanotoxicity in a living species in ecosystems may affect the food chain or other living species due to its interaction with other species. It is concluded that it may represent the influence of Al_2O_3 NPs and may be helpful for future research or insight into potential influences on humans. Similar to *G. mellonella*, bees exposed to NPs can experience adverse effects. NPs can accumulate in bee tissues, causing oxidative stress, disrupting metabolism, and impairing immunity. This has significant implications for bee health and hive stability, crucial for pollination and ecosystem balance. Studies on the environmental impact of NPs highlight their potential to contaminate nectar and pollen, which bees collect. This contamination can lead to bioaccumulation and magnification of NP effects within the hive, affecting brood development and overall colony health. Understanding these impacts is essential for developing safer agricultural practices that minimize harm to beneficial insects while leveraging the advantages of nanotechnology.

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