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RESEARCH

Evaluation of *Galleria mellonella* immune response as a key step toward plastic degradation

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Abstract

Background Plastic's remarkable durability presents a significant challenge for our planet, leading to widespread environmental damage. However, some organisms, such as *Galleria mellonella* larvae, have shown a unique capability to consume and degrade plastic, offering potential solutions to plastic pollution. In this study, we investigated the response of *G. mellonella* larvae to different diets, including artificial diet (AD), polyethylene low density mixed with AD (PELD+AD), and PELD alone. Using various microscopy techniques, we examined the larvae's hemocyte hemogram and mid-gut characteristics to understand their immune response and digestive system when exposed to plastic.

Results The results revealed that PELD-only feeding negatively impacted hemocyte immunity, resulting in a significant decrease in total hemocyte counts compared to AD and AD+PELD feeding. Moreover, plastic consumption induced differential hemocyte alterations, affecting specific cell types. The presence of phagosomes in larval hemocytes and mid-gut cells during PELD-only feeding suggested active involvement in plastic breakdown.

Conclusions These findings highlight the potential of *G. mellonella* larvae as a model organism to study responses to pollutants, emphasizing the urgent need to address plastic pollution's global threat. Further investigation is warranted to explore larval deformities, weight loss, and appetite changes, potentially influencing mortality rates and enzyme biochemistry. Understanding the impacts of plastic ingestion on *G. mellonella* larvae is crucial to develop effective strategies for mitigating plastic pollution's ecological implications.

Keywords *Galleria mellonella*, Plastic feeding, Polyethylene low density, Cellular immunity, Ultrastructure, Hemocytes, Mid-gut

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Background

Plastic degradation is a very serious problem globally. Petroleum-derived polymers such as polyethylene (PE), polyethylene terephthalate (PET), polyurethane (PU), polystyrene (PS), polypropylene (PP), and polyvinyl chloride (PVC) are extremely disobedient to natural biodegradation pathways (Mohanan et al., 2020). Polyethylene PE is mostly used in packaging. Living organisms, mostly marine animals, can be injured by ingestion of plastic wastes or via exposure to chemicals in plastics that interfere with their body physiology. Also, humans are affected by plastic pollution, via

disruption of various hormonal mechanisms (Godswill & Gospel, 2019). Biodegradation is the friendliest way for the environment to eliminate its negativity (Sheth et al., 2019). Fortunately, there are micro- and macrocellular organisms that can degrade plastics to simple forms (Lee & Liew, 2020). Microbial and enzymatic degradation of waste petro-plastics is a promising strategy for depolymerization to convert waste plastics into higher-value bioproducts. The natural gut bacteria of some insects can degrade plastics (Jang & Kikuchi, 2020). Many strains of bacteria that were suggested to be incorporated in PE biodegradation were isolated from the gut of the larvae of *Tenebrio molitor, Tenebrio obscurus, and Plodia interpunctella* (Liaqat, 2020). *G. mellonella* can digest wax and plastic molecules with or without the intestinal microbiome (Kong et al., 2019). Bombelli et al., 2017 recorded the rapid biodegradation of PE by *G. mellonella* (Bombelli et al., 2017). The saliva of *G. mellonella* larvae can overcome the biodegradation of PE via the oxidation process as the first step in degradation. Two enzymes were identified and characterized by Sanluis-Verdes et al., (2022). Hemocytes play a pivotal role in innate immunity (Wu et al., 2016). Innate immunity is the first line of defense in our bodies against infection. Innate immunity is the instant and only response of invertebrates to invaders. In insects, it depends on cellular and humoral responses (Tsakas & Marmaras, 2010). The cellular immune responses include phagocytosis, nodulation, and encapsulation (Al Mutawa et al., 2020). Kurt and Kayiş (2015) reported that the hemocytes of insects are similar to the white blood cells of mammals in some features including morphology, embryonic origin, ameboid movement, and phagocytic activity. The immune response in insects differs from that of vertebrates in the lack of cell specificity, immunoglobulins, and response memory. However, similar responses to immune memory in insects have been described as a phenomenon called "immune priming," where the insect exposed to a low dose of a pathogen becomes more resistant when later exposed to a high dose of the same pathogen (Gálvez & Chapuisat, 2014). The insect gut is a single layer of epithelial cells supported by a basal lamina surrounded by muscles. The gut is divided into three regions with different features, functions, and embryonic origin, i.e., the foregut, midgut, and hindgut (Caccia et al., 2019). The mid-gut is the functional core of the alimentary canal since it accomplishes a significant role in food digestion and nutrient absorption. To our knowledge, it is the first time to investigate the impact of plastic feeding on the cellular immune response as well as the mid-gut characteristics of *G. mellonella* larvae.

Methods

Insect

G. mellonella larvae were sourced from the Parasitology and Animal Diseases Department, at the Veterinary Institute, of the National Research Center (Dokki, Egypt). The larvae were reared in cylindrical glass jars with metal lids, which were perforated with tiny pores for proper ventilation. The glass jars, each with a capacity of 500 ml, were placed in an incubator with transparent glass doors, maintaining a constant temperature of 28 ± 2 °C and a relative humidity of 60 ± 3%. The larvae experienced natural day–night cycles within the incubator.

For their diet, the larvae were fed with an artificial diet (AD) based on the formulation by Jorjão et al. (2018) with minor adjustments. The AD consisted of 600 g of corn flour, 300 g of whole wheat flour, 60 g of full cream milk powder, 60 g of active yeast, 100 ml of glycerol, and 200 ml of honey. The larvae were continuously reared on this AD for six consecutive generations throughout the year 2020 before being used in the subsequent experimental procedures. The polyethylene low density (PELD) used in all experiments was commercially obtained in a package meant for electrical instruments, as shown in Fig. 1b.

The experimental design

The newly hatched larvae were continuously reared on the artificial diet (AD) from the year 2020 until April 2023. Once the larvae reached the fifth instar, they were weighed, with an average weight of approximately 60 to 85 mg. For the experimental setup, a total of 90 larvae were utilized and divided into three groups; each group was repeated three times:

1. (Control) AD Group: This group served as the control and was fed with different series of feeding doses of the AD, consisting of 50, 25, 15, 10, 5, and 2.5 g.
2. AD + PE-LD Group: The second group received a combination of AD and polyethylene low density (PE-LD) in equal feeding weights.
3. PE-LD Only Group: The third group was exclusively fed with the same weight of PE-LD without the addition of AD.

Each group of larvae was maintained for a period of ten days, allowing them to grow and develop until they reached the last instar stage with weight ranging from 150 to 300 mg.

Hemolymph collection

For hemolymph collection, the last instar *G. mellonella* larvae were subjected to anesthesia by exposing them to a temperature of − 20 °C for 5–10 min until they became
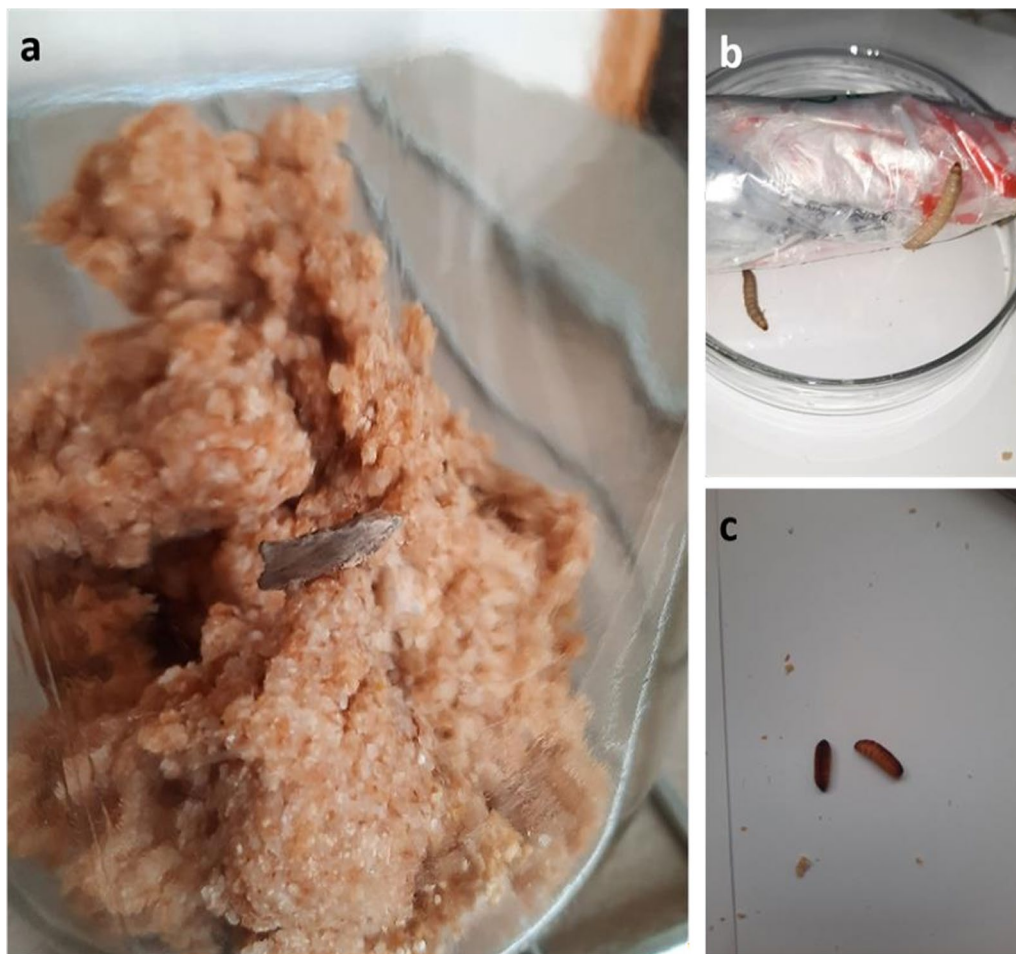
Fig. 1 **a** The female moth of *G. mellonella* on the artificial diet in the cylindrical glass jar, **b** the last instar of *G. mellonella* larvae eating PELD (plastic package covering), **c** the pupae of *G. mellonella*

motionless. Afterward, the larvae were surface-sterilized using 70% ethyl alcohol. A fine surgical scissor was used to carefully pierce the ventral side of the larvae at the second proleg, following the method described by El Deeb and Hassan ([1990](#)). Exuding drops of hemolymph (HL) were collected from each larva on a parafilm sheet. Subsequently, the collected hemolymph was diluted with phosphate buffer saline (PBS) at a 1:10 (v/v) ratio. The diluted hemolymph was then transferred to an Eppendorf tube using a micropipette. To precipitate the delicate hemocytes, the tube was centrifuged at 1400*g* for 10 min under cooling conditions. The supernatant was further centrifuged at 3000*g* for 10 min to separate the plasma from the hemocytes, following the method proposed by Stoepler et al., ([2012](#)).

Monolayer preparation and Giemsa staining of hemocytes
To prepare a monolayer of hemocytes for analysis, a drop of hemolymph (2 μl) was carefully placed on a microscope

slide that had been cleaned with alcohol. Another clean dry slide was then used to smear the hemolymph drop evenly. The slide with the smears was left to air dry before being subjected to methanol fixation for 2 min. Next, the slide was stained with Giemsa stain for approximately 20 min, followed by a thorough wash with distilled water. After the staining process, the slide was once again air-dried. To preserve the preparation, the slide was mounted in Distyrene Plastic Xylene (DPX) mounting medium. Subsequently, the slides were examined using a light microscope at a magnification of 1000. This allowed for the counting and evaluation of the total hemocytes as well as the differential hemocyte percentage, as described by Al Mutawa et al. ([2020](#)).

Total hemocytes count (THC)
Hemolymph was collected from the last instar larvae of *G. mellonella* fed on AD, AD+PELD and PELD. Then it was diluted 10 times with PBS (pH:7) to avoid hemocyte

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aggregation. And the total number of hemocytes was counted using a Neubauer hemocytometer (Marienfeld Germany). After discarding the first three drops near the edge of a coverslip of a Neubauer counting chamber, it was filled by capillary action. Four white-cell squares from both the upper and lower chambers were counted. THC was calculated using the formula suggested by Jones (1962). Each treatment was replicated three times.

$$Total\ hemocytes\ count/ml\ of\ hemolymph = \frac{Hemocyte - 1\ mm\ squares \times dilution \times depth\ of\ chamber}{Number\ of\ 1\ mm\ counted\ squares} \times 10^3$$

Percentage of differential hemocytes count % (DHC)

The DHC was determined based on the percentage of each hemocyte type in the total cells counted, following the method proposed by Jones (1962). Fresh monolayer preparations were carefully examined, and the percentage of the different hemocyte types was recorded for a fixed number of hemocytes, typically ranging from 100 to 200.

To aid in hemocyte identification, Giemsa-stained smears were prepared using the same method described earlier. The shapes and diameters of the cells were assessed to distinguish and characterize the various hemocyte types present. For observation and analysis, the prepared smears were examined using an optical microscope from Nikon, Japan, equipped with a digital camera (lCMOS05100KPA USB 2.0 Microscope Camera). Hemocytes derived from larvae fed on the artificial diet (AD) were used as the control group for comparison.

By calculating the percentage of different hemocyte types, this analysis facilitated a deeper understanding of the differential immune response of *G. mellonella* larvae to the various experimental diets, as observed and reported by Al Mutawa et al., (2020).

Transmission electron microscopy (TEM)

Mid-gut and hemocytes preparation

For TEM microscopy, the last instar larvae of *G. mellonella*, weighing between 150 and 300 mg, were dissected after being fed on different diets, including AD (control), AD + PE-LD, and PE-LD for a duration of ten days. Many larvae were dissected in phosphate buffer saline (PBS) at a pH of 7 to isolate the mid-gut tissues (Al Mutawa et al., 2020).

The hemolymph of the larvae was collected and centrifuged as described previously to remove the plasma. The resulting hemocyte pellets and mid-gut tissues were separately fixed in a 0.1 M cacodylate buffer at a pH of 7.2, containing 5% glutaraldehyde for a period of

2 h. Subsequently, both the hemocyte pellets and mid-gut tissues underwent multiple washes in the same buffer (pH 7.2) for 1 h.

Following the washing steps, each preparation was fixed in cacodylate sucrose buffer for at least 12 h. Later, they were suspended in a 2% osmium tetraoxide fixative (pH 7.2) for 2 h at 4 °C. The specimens were then dehydrated using a series of ethyl alcohol, and propylene oxide was used to replace the alcohol with two changes of 2 min each.

Each preparation was embedded in epon and treated for semi-thin sections at a thickness of 1 μm. The sections were stained with toluidine blue for examination under a light microscope.

Ultra-thin sections were obtained from selected areas of the trimmed blocks and collected on copper grids. These ultra-thin sections were contrasted using uranyl acetate for 10 min and lead citrate for 5 min, after which they were ready for examination under the transmission electron microscope (TEM), specifically the 100 CX11 TEM, at the Electron Microscope Unit at Assiut University.

Scanning electron microscopy (SEM)

For SEM microscopy, the hemocyte pellets collected from larvae fed on the normal diet (AD) and those fed on AD + PE-LD were fixed in 3% glutaraldehyde after the centrifugation process. The fixed hemocytes were then sputtered with gold and examined using a JEOL GM 5200 microscope at the experimental research station Faculty of Agriculture, Cairo University, Giza, Egypt.

Statistical analysis

Data were represented by a series of analyses of variance and independent-samples *T*-test using the SPSS program. Differences were significant when ($p \leq 0.05$).

Results

Hemocytes with Giemsa staining

In the Giemsa-stained light microscopic preparation, Fig. 2 illustrates the distinct morphologies of the hemocyte types in *G. mellonella* under normal feeding conditions. The prohemocytes were observed as round cells with an approximate diameter of 6.7–8 μm, featuring a central nucleus measuring around 6–4.7 μm in diameter (Fig. 2b). As for the plasmatocytes, they exhibited poorly stained cytoplasm, assumed a spindle-shaped appearance when spreading on glass slides, and had tapered
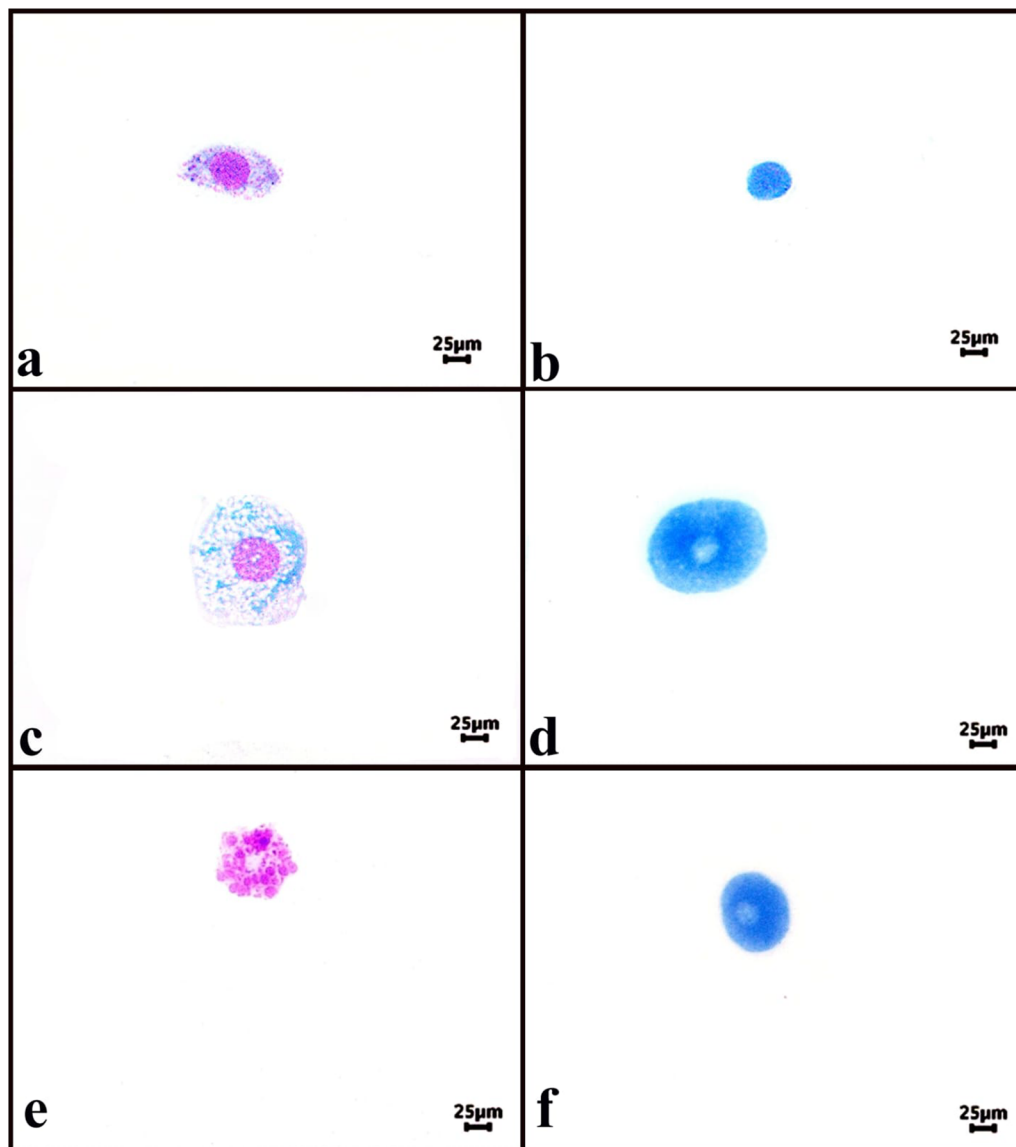
Fig. 2 Light microscopic Giemsa stained photomicrograph (×1000) of *G. mellonella* hemocytes of larvae fed on the AD, **a** plasmatocyte, **b** prohemocyte, **c** granular cell, **d** large oenocytoid, **e** spherule cell, **f** small oenocytoid

ends ranging from 7 to 15 μm in width and 15–20 μm in length. The nucleus of plasmatocytes measured approximately 5–10 μm in diameter (Fig. 2a). The granular cells showed granules with different sizes in dark cytoplasm with an 8–10 μm diameter, with round nuclei of 5 μm diameter, approximately (Fig. 2c). The oenocytoids were large cells with homogenous cytoplasm ranging from 10 to 19 μm in length and 7–10 μm in width, with an eccentric nucleus; its diameter was 6–7 μm (Fig. 2d, f). The spherule cells were oval with vesicular cytoplasm of round spherules masking the nucleus (Fig. 2e). Ten days after feeding on AD + PE-LD (Treatment 1),

some changes were observed in hemocyte morphology compared to those of the control (AD)-fed larvae. Cell deformities and increased mitosis in prohemocytes, the granular cells were degranulated and vacuolated. The cytoplasm of the oenocytoids becomes dense blue. Enlargement in the plasmatocytes nucleus, the spherule cells appeared with denser spherules (Fig. 3). After ten days of *G. mellonella* larvae feeding on PE-LD only, the hemocyte types were completely undifferentiated, vacuolated, with shrinking cell membranes, bubbling, the cytoplasm of oenocytoids appeared deeply stained with Giemsa with structureless nuclei, as shown in Fig. 4c.
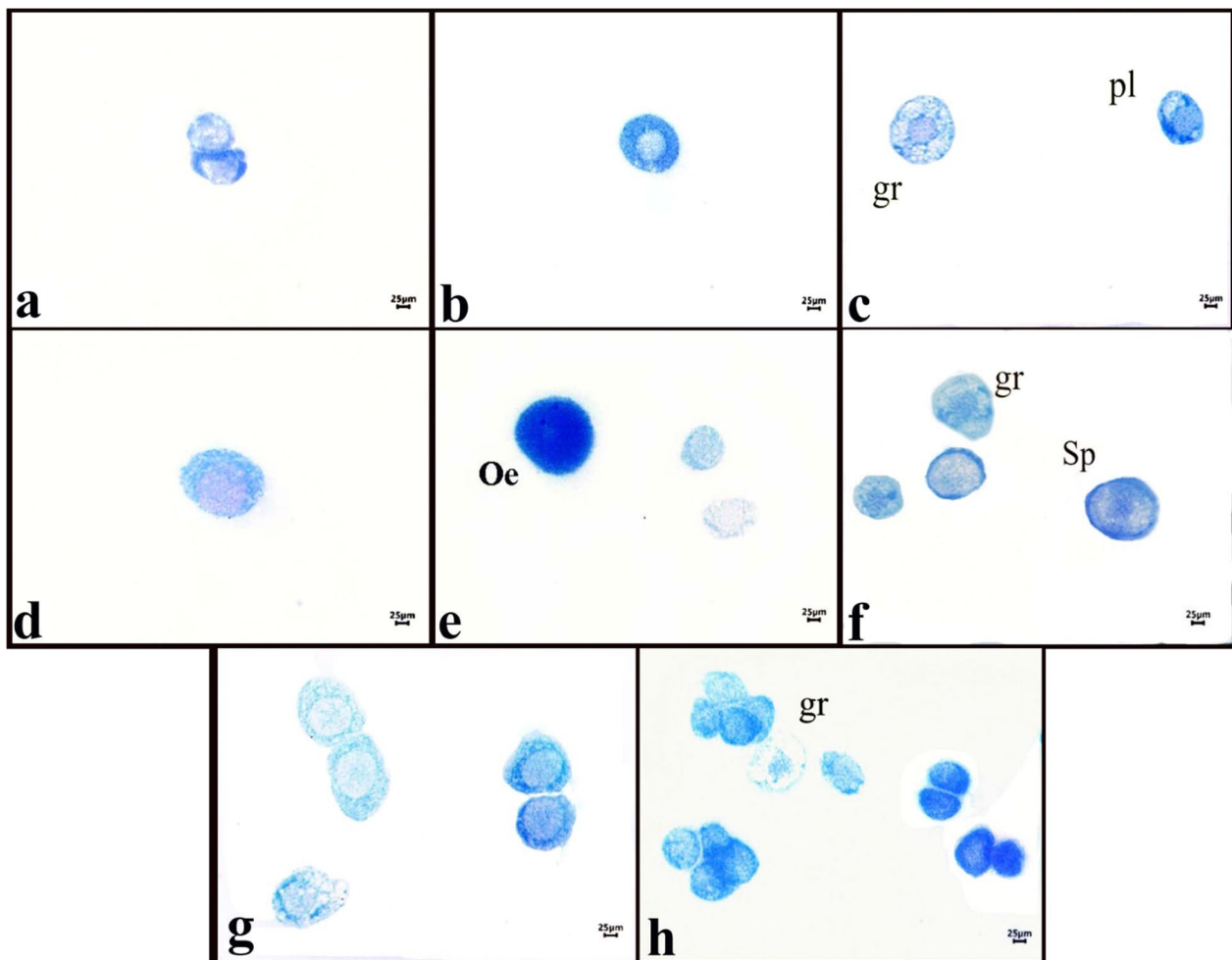
Fig. 3 Light microscopic Giemsa stained photomicrograph (×1000) of *G. mellonella* hemocytes of larvae fed on AD + PELD, **a** mitotic prohemocyte, **b** plasmatocyte spherical shape, **c** granular cell (gr), plasmatocytes (pl), **d** plasmatocyte oval shape, **e** oenocytoid (Oe), **f** spherule cell (Sp), **g** mitosis of plasmatocytes and granular cells, **h** structureless, degranulated, aggregation of cells

SEM of *G. mellonella* larval hemocytes

Five types of circulating hemocytes could be differentiated in the hemolymph of *G. mellonella* larvae, under SEM: granular cell, plasmatocyte, prohemocyte, and oenocytoid; granular cells are always spherical or ovoid as shown in Figure 5a. Plasmatocytes are usually round- or spindle-shaped and the plasma membrane exhibits irregular processes, filopodia, and pseudopodia, as shown in Fig. 5b, c. The larvae fed on AD + PE-LD (Treatment 1), granular cells and plasmatocytes were undifferentiated as in Fig. 5h. Prohemocytes are small and round in larvae fed on AD, but when larvae fed on AD + PE- LD the prohemocyte was ruptured and undifferentiated. Oenocytoids are represented as big and elongated, as shown in Fig. 5e. In the case of larvae fed on AD + PELD, all the cells appeared with irregular deformed and degranulated shapes, increased pseudopodia with ruptured cell membranes. Cells become aggregated with either increased swollen or shrunk appearance, as shown in Fig. 5f–i.

TEM of *G. mellonella* larvae
The effects of PELD feeding on the hemocytes

The hemocytes of *G. mellonella* larvae fed on normal diet (AD) are presented in Fig. 6. The prohemocyte appeared as small round cell with very big nucleus and a small rim cytoplasm (Fig. 6a). Figure 6b represents the plasmatocytes with pseudopodia and very big nucleus. However the hemocytes of *G. mellonella* fed on AD + PELD for ten days as presented in Fig. 6c, d appeared enlarged in size and vacuolated and aggregated. In the larvae of *G. mellonella* fed on PELD for ten days, the hemocytes were paled, undifferentiated, and appeared with dark particles
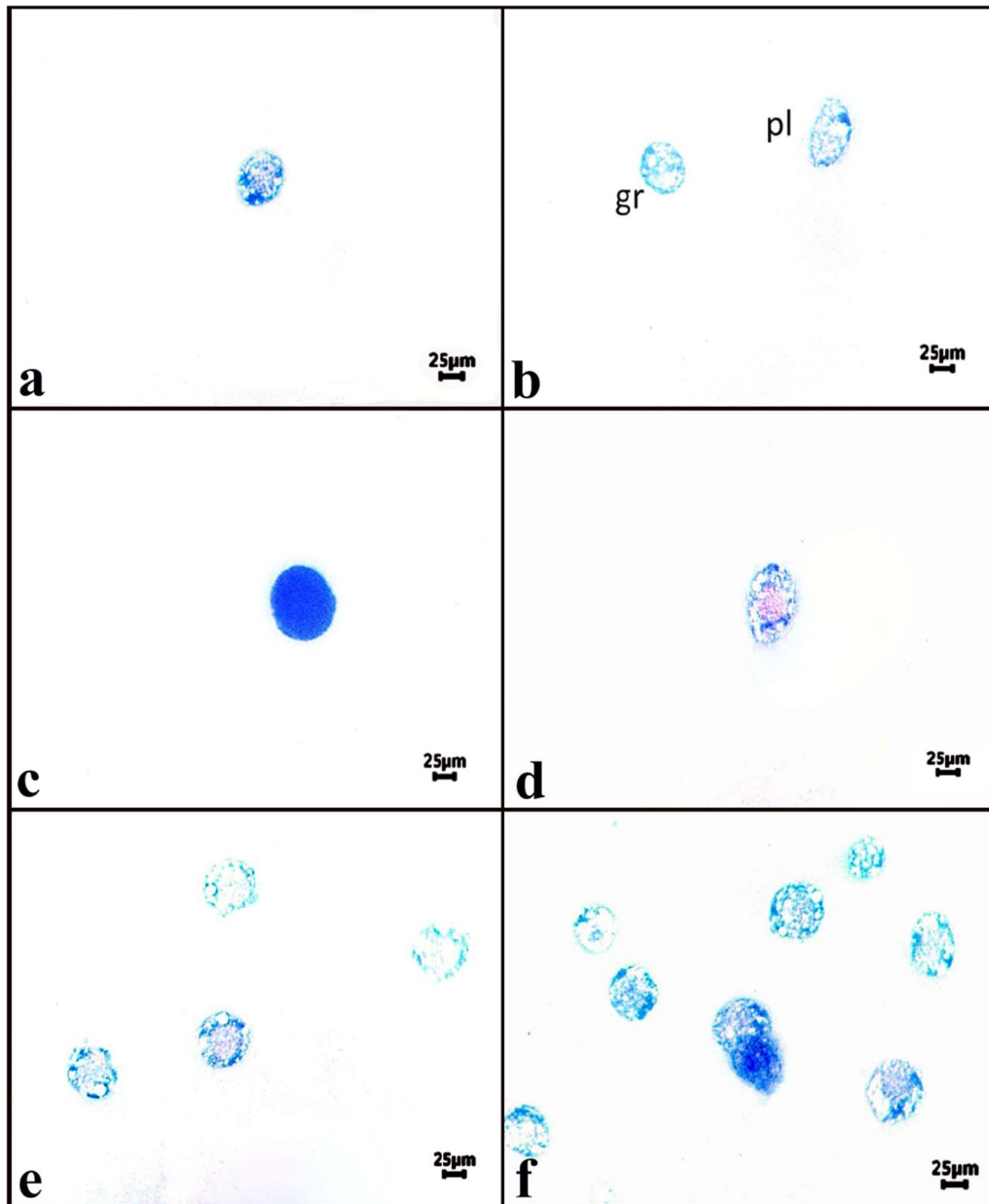
Fig. 4 Light microscopic Giemsa stained photomicrograph (×1000) of *G. mellonella* hemocytes of larvae fed on PE-LD only **a** prohemocyte, **b** granular cell (gr), plasmatocyte (pl), **c** oenocytoid, **d** plasmatocyte, **e** spherule cells, **f** types of undifferentiated hemocytes

on them, as in Fig. 6e, f, and that was most likely due to the phagocytosis of PELD pieces.

The effects of PELD feeding on the mid-gut characteristics

The ultrastructure of the mid-gut tissue of *G. mellonella* last instar larvae fed on AD (control) revealed four types of cells: columnar cells (cl) are the most prominent cell type, which is characterized by a central nucleus and a deeply folded apical membrane, representing the microvilli (Fig. 7A, C). Several phagosomes

of undigested PELD pieces appeared in the columnar cells of the mid-gut of *G. mellonella* last instar larvae fed on PELD for ten days as shown in Fig. 7G, H. This cell type is responsible for digestive enzyme production as well as the absorption of nutrients (Caccia et al., 2019). As for the fine structure of the organelles, there were not any changes that appeared in this cell configuration type (cl) compared to those of normal AD feeding (Fig. 7E). Many electron-dense granules in their cytoplasm, as in Fig. 7C, evidence the secretory nature
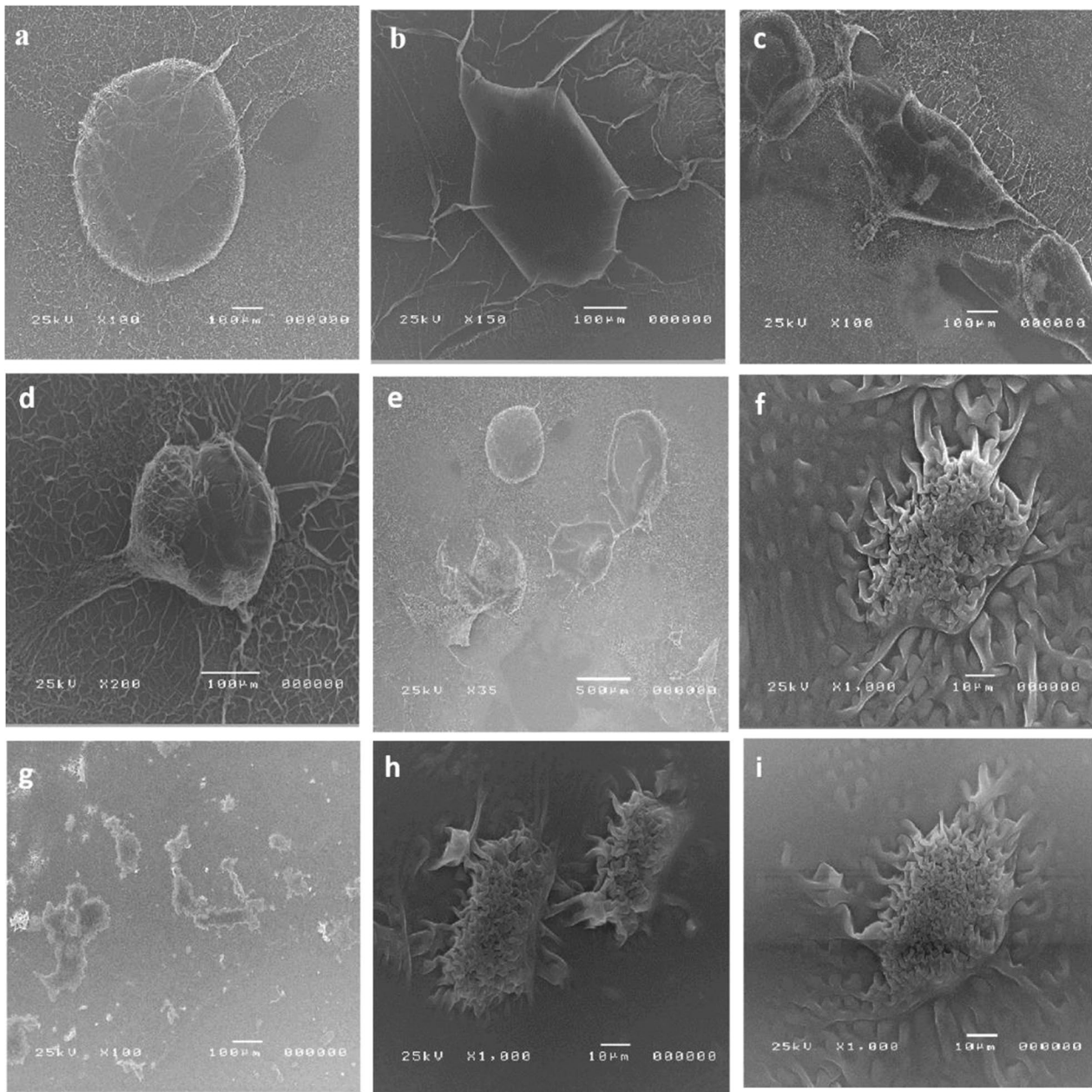
Fig. 5 SEM of *G.mellonella* hemocytes (**a–e**) larvae fed on AD (control), **a** granular cell, **b** plasmatocyte, **c** plasmatocytes, **d** prohemocyte, **e** different types of *G. mellonella* hemocyte, **f–i** hemocytes of larvae fed on AD + PELD appeared bubbling with structureless cell membranes and undifferentiated pseudopodia

of ECs. There was not any significant change in EC in the mid-gut of the last instar *G. mellonella* larvae fed on AD + PELD, as in Fig. 7E. Goblet cell (G) is with special morphology and functions, and usually, the nucleus is basally located as shown in the mid-gut of *G. mellonella* larvae fed on AD (Fig. 7B). There was no goblet cell noticed in the mid-gut of *G. mellonella* last instar

larvae fed on AD + PELD or PELD, separately. A stem cell (SC) known as a regenerative cell, SC, is characterized by a blast-like morphology, with limited, dense cytoplasm, and few organelles. They can be scattered as single cells along the gut. The presence of glycogen granules and lipid droplets scattered in the cytoplasm of SCs was observed in the mid-gut epithelium
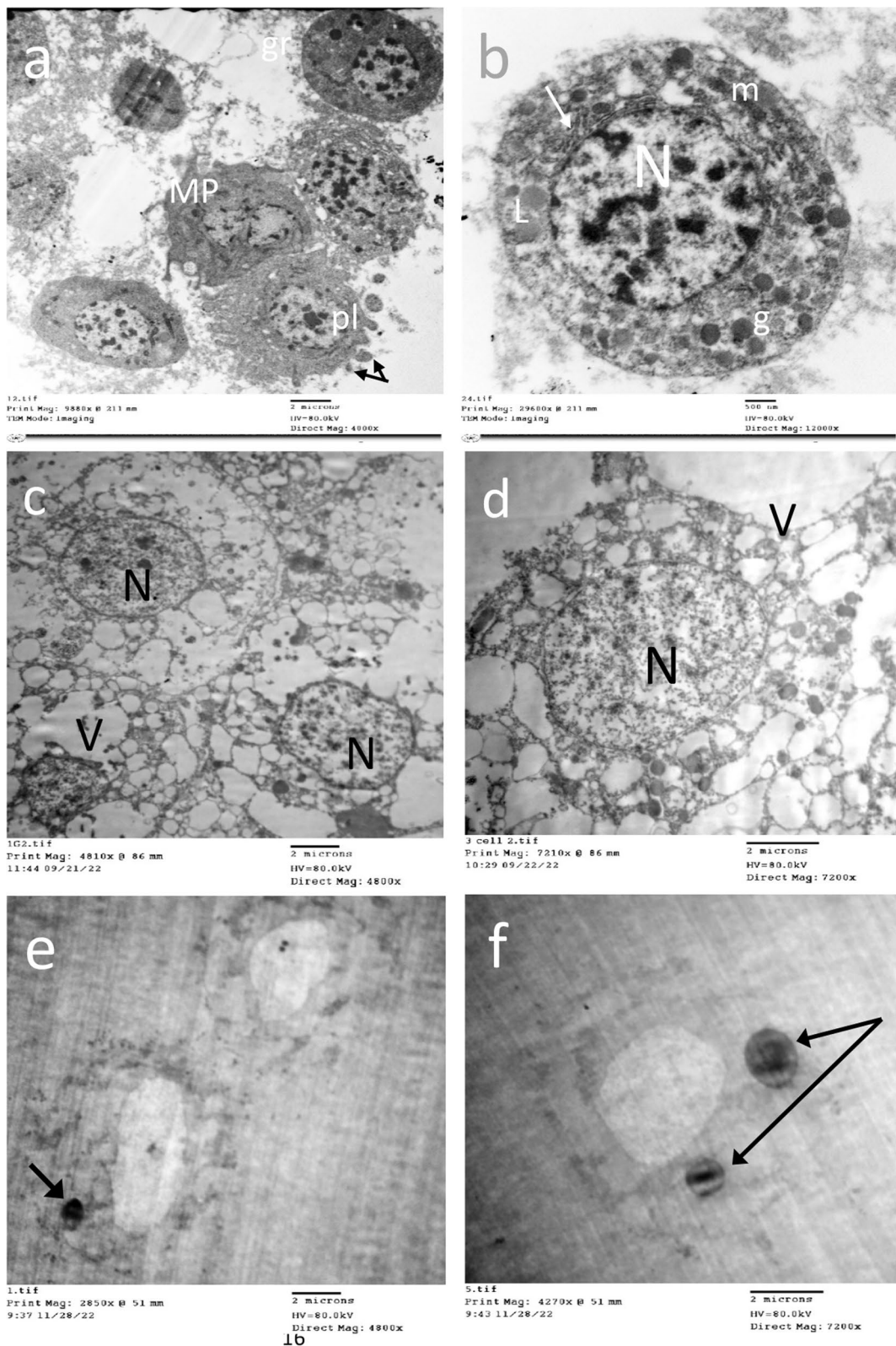
Fig. 6 TEM of hemocytes of *G. mellonella*, **a**, **b** fed on AD (control), {a}: granular cell (gr), mitotic prohemocyte (MP), plasmatocyte (pl), pseudopodia (arrow), {b}: nucleus (N), lysosome (L), endoplasmic reticulum (arrow), mitochondria (m), granules (g). **c**, **d** hemocytes of *G. mellonella* fed on AD + PELD, vacuolated hemocytes (v), aggregated, with structureless cytoplasm (c) and uncharacterized nucleus (N). **e**, **f** *G. mellonella* hemocytes of larvae fed on PELD, melanized phagosome surround engulfed pieces of plastic (arrow)
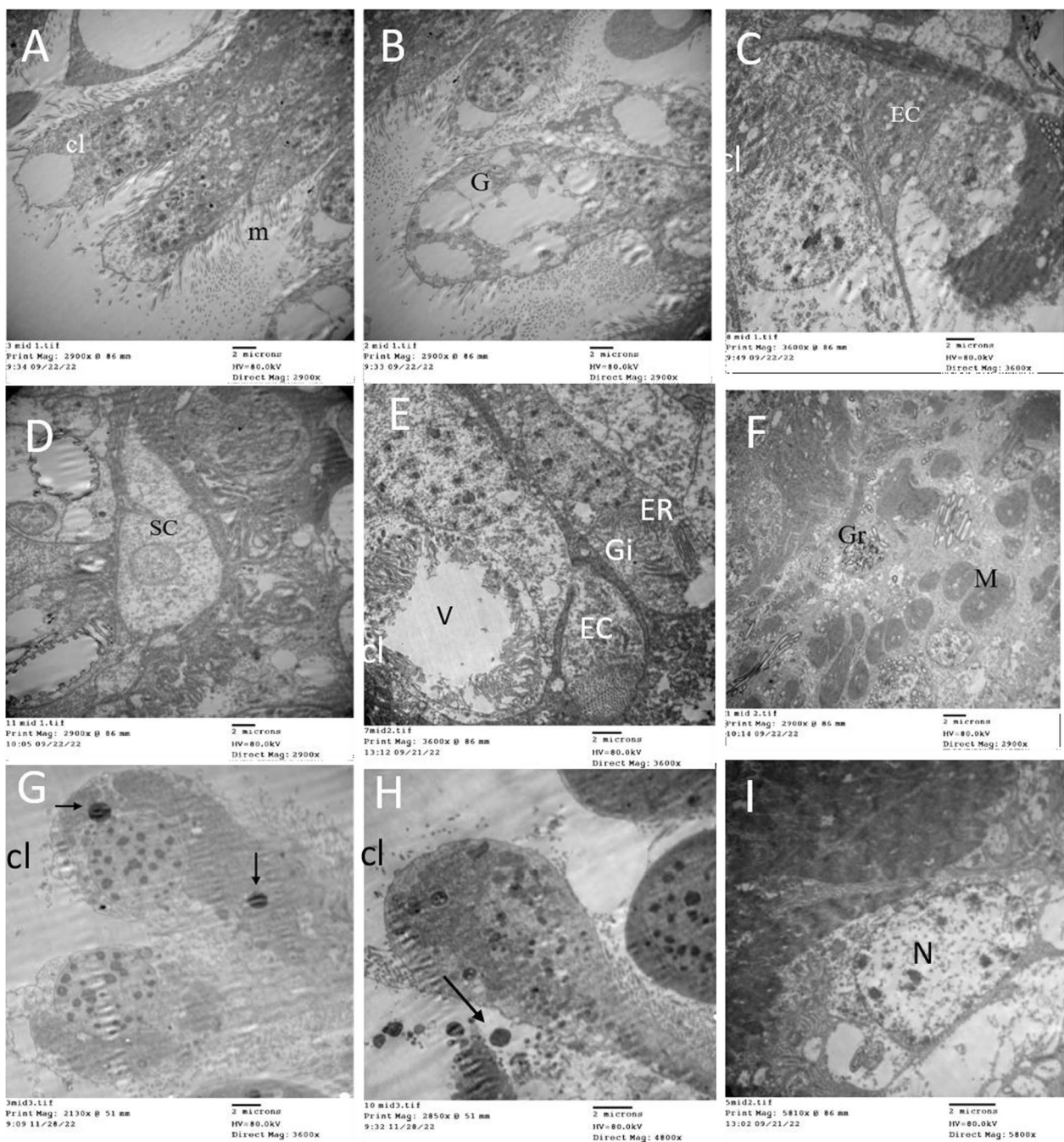
Fig. 7 **A–D** TEM photomicrograph of mid-gut cells of *G. mellonella* larvae fed on AD (control) shows columnar epithelium (cl), goblet cell (G), endocrine cell (EC), stem cell (SC). **E**, **F** Mid-gut cells of larvae fed on AD + PELD show the endoplasmic reticulum (ER), Golgi apparatus (Gi), and vacuoles (V). **F** the electron-dense granules (Gr), and the mitochondria (M). **G**, **H** the mid-gut cells of *G. mellonella* larvae fed on PELD show the phagocytosis of PELD pieces in the columnar cell (cl). **I** represents the stem cell (SC) in the mid-gut of *G. mellonella* larvae fed on AD + PELD, and its nucleus (N)

of *G. mellonella* larvae fed on AD (Fig. 7D), as well as those larvae fed on AD + PELD (Fig. 7I). However, in the mid-gut cells of larvae fed on PELD, there was no SC, observed (Fig. 7G, H). Moreover, mid-gut cells of

larvae fed on AD + PELD revealed dense scattered granules and mitochondria with a great increase in size and number (Fig. 7F). And the other mid-gut organelles as the endoplasmic reticulum, and the Golgi apparatus
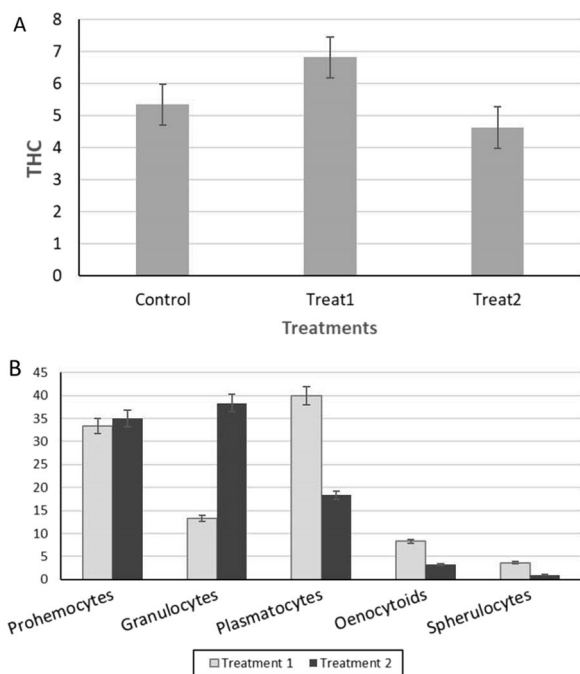
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Fig. 8 A Total hemocyte counts of *G. mellonella* last instar larvae fed on AD (control), fed on AD + PELD (Treat 1), and fed on PELD (Treat 2). **B** Differential hemocyte counts of *G. mellonella* last instar larvae fed on AD (Treatment 1) and fed on AD + PELD (Treatment 2)

were seen in the columnar epithelium (cl) cells with swollen shape (Fig. 7E) compared to normal feeding mid-gut cells of Fig. 7A–D.

Total hemocytes count
The total hemocyte number of the last instar of *G. mellonella* fed on the AD (control) was determined (mean $= 5.333 \pm 1.61 \times 10^3$) Fig. 8A, which was increased when larvae fed on AD + PELD for ten days (Treat 1) $6.81 \pm 1.32 \times 10^3$; conversely, when the larvae fed on PELD, the total hemocyte counts were decreased (Treat2) $4.62 \pm 1.95 \times 10^3$.

Percentages of differential hemocyte counts (%DHC)
The light microscopy differentiation revealed that five hemocyte types were identified in *G. mellonella* larvae fed on the AD (control): the prohemocyte occupied approximately 33% of the hemocytes population, the granular cell formed 13% approximately of the hemocyte counts, the plasmatocyte were 40%, the most prevalent hemocyte type, oenocytoid 9.33% and 4.67% spherule cell. In the larvae fed on AD + PELD (Treatment 2) in Fig. 8B, the prohemocytes increased insignificantly (P>0.05) compared to control (36%), but granulocytes (granular cells) remarkably increased

compared to control (38.333), however plasmatocytes, oenocytoids, and spherule cells decreased significantly representing 19.33, 5.33, and 2.00, respectively. However, in those larvae that were fed on PELD only, the hemocyte shapes and consistencies were greatly undifferentiated.

Discussion
Plastics have become an integral part of our lives, but their accumulation in the environment poses serious challenges, causing damage to ecosystems and wildlife due to their low biodegradability. Among insects, the greater wax moth (*G. mellonella*) is a notorious pest that inflicts significant harm to beehive farms, contributing to honeybee colony collapse disorder. Despite this negative impact, *G. mellonella* possesses a unique ability to digest both bee wax and polyethylene (PE), making it a valuable model for studying innate immunity (Junqueira et al., 2021). In addition to being easy to rear, low maintenance cost, and having many offspring in a short lifecycle, with no ethical restrictions, the *G. mellonella* genome was published recently (Coates et al., 2019; Wojda et al., 2020). In this study, we explored the sensitivity of *G. mellonella* larvae to different diets, including artificial diet (AD), AD mixed with PE-LD (polyethylene low density), and PE-LD alone. To assess the immune response toward plastic feeding, we evaluated the circulating hemocyte hemogram using light, scanning, and transmission electron microscopy (TEM). Additionally, we examined the mid-gut cells using TEM.

G. mellonella larvae were successfully reared on an artificial diet consisting of cereal products, milk powder, yeast, honey, and glycerol, making them suitable for laboratory studies. The investigation revealed distinct hemocyte types in the last instar larvae of *G. mellonella* fed on AD compared to those on diets containing plastic pieces. Hemocytes play a critical role in various physiological processes throughout an insect's life cycle. They originate from the lymph gland, a specialized hematopoietic organ, during the larval stages of Lepidoptera and Diptera orders (Hartenstein, 2006; Wang et al., 2014). This gland was prominent in the last instar larvae of *G. mellonella* in the present investigation as detected with the TEM results during normal feeding AD diets. The presence of prominent hemocyte types, such as prohemocytes, plasmatocytes, granular cells, spherule cells, and oenocytoid, was consistent with previous findings (Altunta et al., 2012). Hemocytes are involved in cell migration, chemotaxis, inflammation regulation, cell apoptosis, and embryogenesis (Wood & Jacinto, 2007). They also mediate the cellular innate immune response, which includes processes like cell spreading, aggregation, nodulation, phagocytosis,

and encapsulation of foreign invaders (Strand, 2008). They also orchestrate specific insect humoral defenses during infection, such as the production of antimicrobial peptides and other effector mechanisms (Fauvarque & Williams, 2011; Nehme et al., 2011). The results in the current study indicated that the total number of circulating hemocytes increased markedly when the PELD was added to the artificial diet (AD) in a ratio of 1:1 over ten days (AD + PELD) by weight. However, the total hemocyte number decreased when the larvae were fed on PELD alone. The percentage of different types of hemocytes changed significantly when the larvae were fed on PELD + AD. Conversely, in the case of the larvae fed on PELD only, the circulating hemocytes were greatly damaged and become undifferentiated, vacuolated, and aggregated. Phagosomes of adherent undigested plastic pieces were observed in melanized aggregates of the oenocytoid hemocytes that are apparent as the biggest-sized cell was confirmed by Altincicek et al., 2008. The same insect species have discovered this hemocyte type to be responsible for the production of the nucleic acids that are indicated as immune-responsive molecules relevant to antimicrobial peptides toward bacterial pathogens, and they are also related to the crystal cells in the fruit fly *Drosophila melanogaster* where both types of cells are large, regular in shape, contain phenoloxidases, and rupture upon immune activation (Strand, 2008; Wood & Jacinto, 2007). And also this specific type of hemocytes is responsible for producing nucleic acids as immune-responsive molecules, particularly antimicrobial peptides that combat bacterial pathogens. Comparisons with Drosophila melanogaster, which lacks granulocytes and the apolipoprotein III gene present in many other insects, suggest significant differences between insect species (Smith et al., 1994). Moreover, the examination of mid-gut epithelial cells using TEM revealed various cell types, including columnar cells responsible for digestive enzyme production and nutrient absorption, endocrine cells (EC) involved in maintaining insect homeostasis through the release of bioactive peptides, and goblet cells (G) with distinct morphology and functions. Stem cells (SC) were also present in the mid-gut, known for their regenerative capacity. Unfortunately, feeding on PE-LD caused severe impairment in the mid-gut epithelium, leading to structureless differentiation, electron-dense granulation, and enlargement of deformed mitochondria (Caccia et al., 2019). These findings align with studies exploring the effect of other substances on *Spodoptera littoralis* larvae mid-gut (Shaurub et al., 2020). Spochacz et al., (2021) have also highlighted the toxicity of glycoalkaloids, the secondary metabolites of plants belonging to the Solanaceae family that clearly showed the disturbance of the structure of the mid-gut of *G. mellonella*.

Conclusions

PELD has a serious adverse effect on the immune system of living organisms, manifested through the phagocytosis of plastic particles by both hemocytes and mid-gut cells. The alterations in total and differential hemocyte counts were evident, indicating a significant immune response to PELD exposure. Our findings align with the investigation by Sanluis-Verdes et al. (2022), affirming that *G. mellonella* larvae are attracted to PELD and digest it with their saliva, indicating a feeding behavior rather than a mere preference. Moreover, it is worth noting that PELD lacks essential nutritional components and carries harmful chemical constituents, which may exacerbate its detrimental effects. Remarkably, our study highlights the potential of *Galleria mellonella* as an excellent model to explore the responses of living organisms to pollutants, which pose severe global threats to human life. This model offers valuable insights into blood cell-dependent innate immune responses, shedding light on both conserved and derived molecular mechanisms. Future research in this direction holds great promise in enhancing our understanding of the impact of pollutants on biological systems and the broader implications for human health.

Abbreviations

G. mellonella	*Galleria mellonella*
AD	Artificial diet
PELD	Polyethylene low density
TEM	Transmission electron microscopy
PET	Polyethylene terephthalate
PU	Polyurethane
PS	Polystyrene
PP	Polypropylene
PVC	Polyvinyl chloride
THC	Total hemocytes count
DHC	Differential hemocytes count
gr	Granular cell
pl	Plasmatocytes
Oe	Oenocytoid
Sp	Spherule cell
MP	Mitotic prohemocyte
N	Nucleus
L	Lysosome
cl	Columnar epithelium
G	Goblet cell
EC	Endocrine cell
SC	Stem cell
ER	Endoplasmic reticulum
Gi	Golgi apparatus
Gr	Electron-dense granules
m	Mitochondria
g	Granules
v	Vacuoles
c	Cytoplasm

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Declarations

References

Al Mutawa, M. Y., Ayaad, T. H., & Shaurub, E. H. (2020). Hemocyte profile, phagocytosis, and antibacterial activity in response to immune challenge of the date fruit stalk borer, *Oryctes elegans*. *ISJ, 17*, 147–162.

Altincicek, B., Stötzel, S., Wygrecka, M., Preissner, K. T., & Vilcinskas, A. (2008). Host-Derived extracellular nucleic acids enhance innate immune responses, induce coagulation, and prolong survival upon infection in insects extracellular nucleic acids play important roles in human immunity and hemostasis by inducing IFN production, entrapping pathogens in neutrophil extracellular traps, and providing procoagulant cofactor templates for induced contact activation during mammalian blood clotting. *The Journal of Immunology, 181*. http://journals.aai.org/jimmunol/article-pdf/181/4/2705/1261539/zim01608002705.pdf

Altunta, H., Kili, A. Y., Ukan, F., & Ergin, E. (2012). Effects of gibberellic acid on hemocytes of *Galleria mellonella* L. (lepidoptera: Pyralidae). *Environmental Entomology, 41*(3), 688–696. https://doi.org/10.1603/EN11307

Bombelli, P., Howe, C. J., & Bertocchini, F. (2017). Polyethylene bio-degradation by caterpillars of the wax moth *Galleria mellonella*. *Current Biology, 27*(8), R292–R293. https://doi.org/10.1016/j.cub.2017.02.060

Caccia, S., Casartelli, M., & Tettamanti, G. (2019). The amazing complexity of insect midgut cells: Types, peculiarities, and functions. In *Cell and tissue research* (Vol. 377, Issue 3, pp. 505–525). Springer. https://doi.org/10.1007/s00441-019-03076-w

Coates, C. J., Lim, J., Harman, K., Rowley, A. F., Griffiths, D. J., Emery, H., & Layton, W. (2019). The insect, *Galleria mellonella*, is a compatible model for evaluating the toxicology of okadaic acid. *Cell Biology and Toxicology, 35*(3), 219–232. https://doi.org/10.1007/s10565-018-09448-2

El Deeb, S. O., Hassan, T. H., Cooper, E. I., & Saad, A. H. M. (1990). Characterization of diverse hemolymph lectins in the cotton caterpilar *Spodoptera littoralis*. *Comparative Biochemistry and Physiology, 97*, 321–325.

Fauvarque, M. O., & Williams, M. J. (2011). Drosophila cellular immunity: A story of migration and adhesion. *Journal of Cell Science, 124*(9), 1373–1382. https://doi.org/10.1242/jcs.064592

Gálvez, D., & Chapuisat, M. (2014). Immune priming and pathogen resistance in ant queens. *Ecology and Evolution, 4*(10), 1761–1767. https://doi.org/10.1002/ece3.1070

Godswill, C., & Gospel, C. (2019). Impacts of plastic pollution on the sustainability of seafood value chain and human health. *International Journal of Advanced Academic Research, 5*(11), 46–138.

Hartenstein, V. (2006). Blood cells and blood cell development in the animal kingdom. *Annual Review of Cell and Developmental Biology, 22*, 677–712. https://doi.org/10.1146/annurev.cellbio.22.010605.093317

Jang, S., & Kikuchi, Y. (2020). Impact of the insect gut microbiota on ecology, evolution, and industry. *Current Opinion in Insect Science, 41*, 33–39. https://doi.org/10.1016/j.cois.2020.06.004

Jones, J. C. (1962). Current concepts concerning insect hemocytes. In *Source: American zoologist* (Vol. 2, Issue 2). https://www.jstor.org/stable/3881211

Jorjão, A. L., Oliveira, L. D., Scorzoni, L., Figueiredo-Godoi, L. M. A., Prata, M. C. A., Jorge, A. O. C., & Junqueira, J. C. (2018). From moths to caterpillars: Ideal conditions for *Galleria mellonella* rearing for in vivo microbiological studies. *Virulence, 9*(1), 383–389. https://doi.org/10.1080/21505594.2017.1397871

Junqueira, J. C., Mylonakis, E., & Borghi, E. (2021). *Galleria mellonella* experimental model: Advances and future directions. In *Pathogens and disease* (Vol. 79, Issue 5). Oxford University Press. https://doi.org/10.1093/femspd/ftab021

Kong, H. G., Kim, H. H., Chung, J., & JunLeeKimJeonParkBhakRyu, J. H. S. H. M. S. S. G. J. C. M. (2019). The *Galleria mellonella* Hologenome supports microbiota-independent metabolism of long-chain hydrocarbon beeswax. *Cell Reports, 26*(9), 2451-2464.e5. https://doi.org/10.1016/j.celrep.2019.02.018

Kurt, D., & Kayiş, T. (2015). Effects of the pyrethroid insecticide deltamethrin on the hemocytes of *Galleria mellonella*. *Turkish Journal of Zoology, 39*(3), 452–457. https://doi.org/10.3906/zoo-1405-66

Lee, A., & Liew, M. S. (2020). Ecologically derived waste management of conventional plastics. *Journal of Material Cycles and Waste Management, 22*(1), 1–10. https://doi.org/10.1007/s10163-019-00931-4

Liaqat, S. (2020). Microbial ecology: A new perspective of plastic degradation. *Pure and Applied Biology*. https://doi.org/10.19045/bspab.2020.90228

Mohanan, N., Montazer, Z., Sharma, P. K., & Levin, D. B. (2020). Microbial and enzymatic degradation of synthetic plastics. *Frontiers in Microbiology*. https://doi.org/10.3389/fmicb.2020.580709

Nehme, N. T., Quintin, J., Cho, J. H., Lee, J., Lafarge, M. C., Kocks, C., & Ferrandon, D. (2011). Relative roles of the cellular and humoral responses in the *Drosophila* host defense against three gram-positive bacterial infections. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0014743

Sanluis-Verdes, A., Colomer-Vidal, P., Rodríguez-Ventura, F., Bello-Villarino, M., Spinola-Amilibia, M., Ruiz-L, E., Illanes-Vicioso, R., Castroviejo, P., Aiese Cigliano, R., Montoya, M., Falabella, P., Pesquera, C., GonzálezGonz, L., Arias-Palomo, E., Soi, M., Torroba, T., Arias, C., & Bertocchini, F. (2022). Wax worm saliva and the enzymes therein are the key to polyethylene degradation by *Galleria mellonella*. Preprint. 1–27. https://doi.org/10.1101/2022.04.08.487620

Shaurub, E. H., El-Sayed, A. M., Ali, A. M., & Mohamed, D. S. (2020). Some plant essential oils induce variations in the physiological aspects and midgut ultrastructure of larvae of *Spodoptera littoralis* (Lepidoptera: Noctuidae). *African Entomology, 28*(2), 349–358. https://doi.org/10.4001/003.028.0349

Sheth, M. U., Kwartler, S. K., Schmaltz, E. R., Hoskinson, S. M., Martz, E. J., Dunphy-Daly, M. M., Schultz, T. F., Read, A. J., Eward, W. C., & Somarelli, J. A. (2019). Bioengineering a future free of marine plastic waste. *Frontiers in Marine Science, 6*(October), 1–10. https://doi.org/10.3389/fmars.2019.00624

Smith, A. F., Owen, L. M., Strobel, L. M., Chen, H., Kanost, M. R., Hanneman, E., & Wells, M. A. (1994). *Exchangeable apolipoproteins of insects share a common structural motif*. Journal of Lipid Research.

Spochacz, M., Chowá Nski, S., Szymczak-Cendlak, M., Marciniak, P., Lelario, F., Salvia, R., Nardiello, M., Scieuzo, C., Scrano, L., Bufo, S. A., Adamski, Z., & Falabella, P. (2021). Solanum nigrum extract and solasonine affected hemolymph metabolites and ultrastructure of the fat body and the midgut in *Galleria mellonella*. *Toxins*. https://doi.org/10.3390/toxins

Stoepler, T. M., Castillo, J. C., Lill, J. T., & Eleftherianos, I. (2012). A simple protocol for extracting hemocytes from wild caterpillars. *Journal of Visualized Experiments*. https://doi.org/10.3791/4173

Strand, M. R. (2008). The insect cellular immune response. *Insect Science, 15*(1), 1–14. https://doi.org/10.1111/j.1744-7917.2008.00183.x

Tsakas, S., & Marmaras, V. J. (2010). Insect immunity and its signalling: An overview. *ISJ, 7*, 228–238.

Wang, L., Kounatidis, I., & Ligoxygakis, P. (2014). Drosophila as a model to study the role of blood cells in inflammation, innate immunity and cancer. *Frontiers in Cellular and Infection Microbiology, 3*, 1–17. https://doi.org/10.3389/fcimb.2013.00113

Wojda, I., Staniec, B., Sułek, M., & Kordaczuk, J. (2020). The greater wax moth *Galleria mellonella*: Biology and use in immune studies. *Pathogens and Disease, 78*(9), 1–15. https://doi.org/10.1093/femspd/ftaa057

Wood, W., & Jacinto, A. (2007). Drosophila melanogaster embryonic haemocytes: Masters of multitasking. *Nature Reviews Molecular Cell Biology, 8*(7), 542–551. https://doi.org/10.1038/nrm2202

Wu, G., Liu, Y., Ding, Y., & Yi, Y. (2016). Ultrastructural and functional characterization of circulating hemocytes from *Galleria mellonella* larva: Cell types and their role in the innate immunity. *Tissue and Cell, 48*(4), 297–304. https://doi.org/10.1016/j.tice.2016.06.007

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