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Research Article

Comparing the Histological Structure of the Fat Body and Malpighian Tubules in Different Phases of Honeybees, *Apis mellifera jemenatica* (Hymenoptera: Apidae)

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Abstract

Background and Objective: The honey bee has high economic importance as a successful pollinator in several agricultural crops worldwide and as a source of valued products. The objective of the current study was to and determine the effect of the difference in nutrition in the embryonic stages on the fat body and malpighian tubules of *Apis mellifera jemenatica* range in different ages. **Materials and Methods:** In the larval stage of which two ages, the third and fifth have been studied in both the queen and the worker; as queen larvae were fed an intensive diet of royal jelly throughout the larval stage evolution, while the worker larvae were fed royal gel in the first three days only, but in fifth age, it was fed a mixture of honey and bee bread. **Results:** Several differences were found from one developmental stage to another. Fatty bodies spread throughout the body and fill the internal spaces between the organs. Where fatty bodies increase the size of trophocyte cells with the accumulation of fatty substances and waste inside them with gaps. Either in the malpighian tubules in worker were characterized by the presence of cubic cells with clear nuclei and the composition of these cells based on the basement membrane with the presence of gaps within the cells and the inner cavity is narrow, but in the queen is characterized by the size of more than the worker. **Conclusion:** It seem very useful to link the study of histological structure recorded at the cellular or tissue levels with the development of age and with changes at the community levels.

Key words: Honeybee, histology, fat body, malpighian tubules, queen, worker

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The honey bee *Apis mellifera* (Linnaeus 1758) (Hymenoptera: Apidae) has high economic importance. This species has been used as a successful pollinator in several agricultural crops worldwide and as a source of valued products such as wax, honey, propolis and royal jelly[1]. *Apis mellifera jemenatica* is the main honey bee strain of the Arabian Peninsula, also spread in some parts of Africa[2]. Many organisms undergo distinct shifts in the history of life that are associated with changes in physiology[3]. Honeybee life is characterized by a profound age-related change in behavior phenotype. Usually, the hive bees remain inside the nest where they eat and feed and tend to the queen and keep the colon clean. After that, bees become slave laborers and leave the colony on daily trips to collect food[4]. This behavioral change is related to structural and biochemical changes in the body[3].

The insect fat body is a multifunctional device that has a role in a variety of metabolic processes, including storage of proteins, fats and carbohydrates, which are precursors to metabolism in other organs[5-7]. This tissue is a major organ in the medium metabolism of insects, the majority of hemolymph proteins are synthesized in this tissue, which also displays the functions of fat, carbohydrate and protein storage[8]. In addition, the obese body of insects displays other cell specialties, such as urinary cells, hemoglobin cells, melanocytes and chromatic cells[8]. The fatty body is divided into a vital part, surrounds the viscera and occupies the abdominal cavity of the insects and a wall part[9,10].

All in multicellular organisms there is a device that collects and disposes of waste outside the body. Production systems vary in various animals, including insects and mammals. Malpighian tubules are the main devices for insect removal. It is similar to that of the kidney invertebrate animals[10,11]. In addition to osmoregulation, malpighian tubules are also involved in organic material transfer, body defense system and chemical detoxification processes[12]. The malpighian tubules (located at the anterior end of the intestine) opens in the gate cavity directly behind the pyloric valve area[13-15]. Morphologically, are thin, finger-like extensions connected to the intestinal tract between the midgut and hindgut[11]. Urine is produced in the insects in its initial stages of malpighian tubules, by the isosmotic filtrate from the hemolymph, which carries the metabolic and toxic compounds of the gut. The final stage of the rectal output process usually occurs, where some ions and water are reabsorbed[16]. The excretion occurs by the secretion of ions and organic molecules from the dermal into the lumen of the tubule. Of the lumen, the contents are modified as they pass through the Malpighian and hindgut tubules until they are secreted[11]. Also, it is responsible for producing isosmotic filtrate of dermal, main urine, which carries excretory products of metabolism and toxic compounds in the backside[11]. Which are responsible for the secretion of substances in the body. In most types of insects, the output system involves a variable number of sediments. These play an important role in the detoxification process by actively functioning to dispose of substances that are not metabolized and are in excess of the organism[17]. In addition, to excretion and their impairment could help contribute to increased colony losses in the face of environmental stressors[14]. However, this needs further exploration. The Malpighian tubules play a variety of protective roles[18]. The malpighian tubules can also mount an immune response independent of the fat body[19]. Many studies have examined the structure and function of these organisms, but there is no scientific study that seriously addressed the study of the effect of nutrition in the stages of embryonic development, especially in the larval stage where the nutrition is different in the queen in the workers. This difference is followed by several changes in the external appearance or internal structure. Current study to trace differences in the histological structure of the fat body and malpighian tubules of *Apis mellifera jemenatica* range in different ages.

MATERIALS AND METHODS

The individuals used in the current study was transferred from the apiary directly to the laboratory where the study was conducted under laboratory conditions. Local bee race (*Apis mellifera jemenatica*)[20], which is the smallest race of yellow bees, were selected for the present study. The most obvious distinguishing morphological characteristics in this race are that worker bees have relatively short wings and legs and have yellow rings on their abdomens, whereas queens have relatively large yellow abdomens. Two medium-sized hives of local pure bees were used to collect individuals from different castes at different ages for histological studies of fat body and malpighian tubules.

Individuals used in the study

- **Larval stage:** In the larval stage; of which two ages, the third and fifth have been studied in both the queen and the worker; as queen larvae were fed an intensive diet of royal jelly throughout the larval stage evolution, while the worker larvae were fed royal gel in the first three days only, but in the fifth age, it was fed a mixture of honey and bee bread[21]
- **Pupa stage:** The age (in days) of the pupa stages was determined by changes in body and eye color

For queens, one and three-day-old pupae were selected and for workers, one and seven- day-old pupae were selected.

- **Adult stage:**
  - Queen's adult queens (virgin queens) were selected for this study
  - **Workers:** Adult workers were examined at three age levels that varied by the type of food consumed. For newly emerged workers, the age levels were as follows: 0-12 h prior to the onset of feeding, nurse workers which consume large amounts of the pollen stored inside the hexagonal cells, particularly over days 3-6 (protein feeding) and foragers which collect and feed on nectar and pollen and they consume a larger amount of nectar than the nurse bees (carbohydrate feeding)

Determining ages of worker bees: One comb was selected and dated, containing one- day-old eggs. After three days, the eggs hatched and larvae continued to develop over five instars (13 days after oviposition or 6 days after the hexagonal cells are covered), then became pupae (one-day-old pupae). Worker bee age was determined by identifying newly emerged worker bees (either during exit from a cell or immediately after exit) on a brood frame. A temperature of 33°C and a relative humidity of 65% was used to obtain 24 h old workers. The newly emerging worker bees were marked with colored markers to track the different ages.

Honeybee queen breeding: Waxy cups grafting method (also known as the Doolittle), the method was used to breed and obtain queens of different ages.

Histology: For the histological studies, the samples were prepared according to the historesin technique as follows: individuals of each age were collected and placed in a preservative solution. Were fixed in 10% paraformaldehyde in 0.1 M phosphate buffer solution (pH 7.2). The material was dehydrated in an ascending series of 70, 80, 90 and 95% ethanol baths lasting 20 min each and transferred to the resin solution for 72 h at 4°C. Finally, the material was transferred to molds which were then filled with resin containing a catalyst and sealed with a metal support for microtome. After polymerization, the blocks were sliced using a microtome. The sections were mounted on slides and stained with hematoxylin and eosin. Samples were then examined and photographed using an Olympus microscope (Olympus-Bx41)[17,11,22].

RESULTS AND DISCUSSION

Fat body

Larval stage: In the results of the present study in the larval instar of honey bees, the fatty bodies are characterized by two types of cells: Trophocyte of fat body (Tr) and Oenocyte of fat body (On) (Fig. 1). In the 3rd larval instar of honey bees, queen fatty bodies spread throughout the body and fill the internal spaces between the organs. Where fatty bodies increase the size of fat cells (Tr) with the accumulation of fatty substances and waste inside them with gaps in Fig. 1a. But, in the 3rd larval instar of honey bee's worker the fat cells of (Tr) large size and the presence of spaces or gaps in the internal small with the clarity of the nuclei. On cells are characterized by their small numbers, which are distributed among the (Tr) cells (Fig. 1b). In the 5fh larval instar of honey bees queen, the boundaries between the fat cells (Tr) are clearly marked, the gaps inside the are few, the accumulation of the external substances inside and the lack of clarity of the nuclei (Fig. 1c). For the worker, it is similar to honey bees queen (Fig. 1d). This similar to what was found[6] the insect fat body is a diffuse organ that fills the body cavity and consists of mesodermal cells known as trophocytes or fat body cells. As well as to trophocytes, bees contain oenocytes, another cell type of ectodermal origin found scattered throughout the fat body, this agrees with Martins *et al.*[23]. Also, agrees with Ahmed *et al.*[24], when he found that the trophocyte is the main cell type of the insect fat body. The shape, appearance and volume of the trophocytes vary and are widely depending on the development stage and nutritional state of the insect, however, a marked characteristic of this type of the cell is a cytoplasm filled with lipidic deposits except for a thin portion at the cell's periphery, a ring surrounding the nucleus and dispersed islands between the lipid droplets. Few inclusions are present at these regions of the cytoplasm. The trophocytes increase in size and probably also in number during the postembryonic feeding period. When they become the largest cells of the insect's body. In addition to during the larval phase, it is involved in the metabolism of lipids and carbohydrates and in the synthesis and secretion of proteins, which are released in large quantities into the hemolymph in the last larval stage to drive and carry out the metamorphosis[25]. Both in the pre-embryonic and adult phases, the fat body is involved in the accumulation of toxic materials, such as urate[26]. The fat body is more prominent in immature insects, particularly holometabolous insects in *Apis mellifera* larvae[27]. Maybe that's because the abdomen contains the bulk of the honey bee's fat body, which is a site

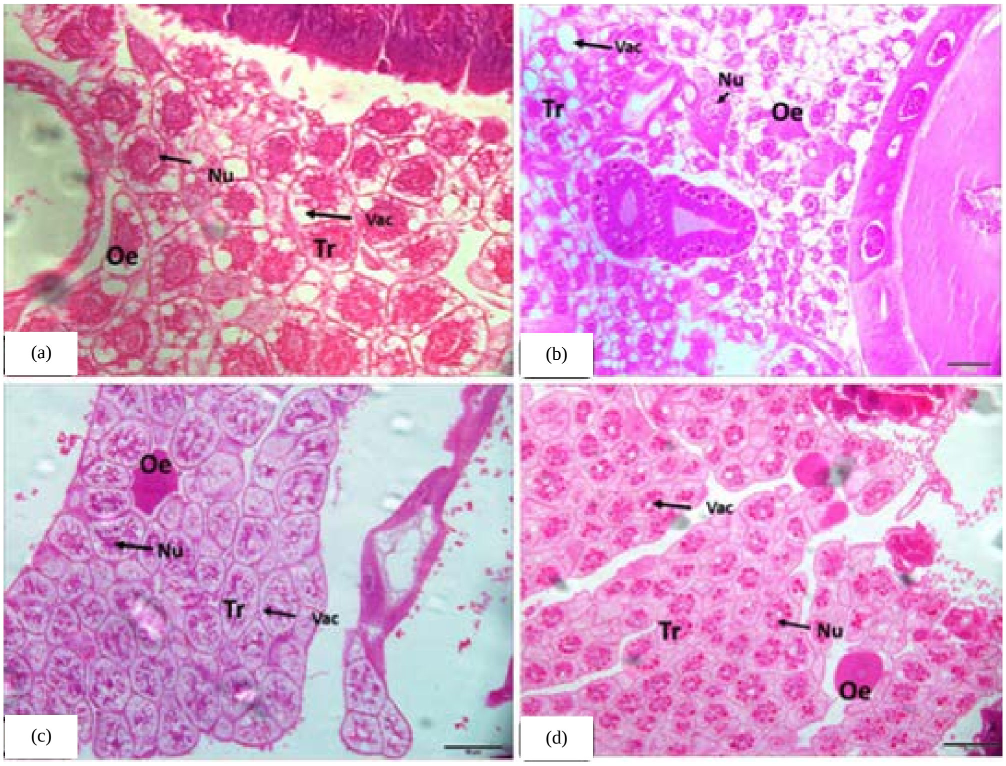

Fig. 1(a-d): Cross section of the fat body in the honey bee larva, (a) 3rd day old honey bee queen larva (20 μm), (b) 3rd day old honey bee worker larva (20 μm), (c) 5th day old honey bee queen larva (50 μm) and (d) 5th day old honey bee worker larva (50 μm)

of nutrient synthesis and storage, including carbohydrate, protein, lipid macronutrients and important micronutrients. The insect peripheral fat body is located just beneath the cuticle and extends over most of the body[28]. The results of previous studies have proved a high level of protein accumulation in the form of vitellogenin as a major constituent of the honeybee fat body[29]. Additionally, the fatty body consists of thin layers or filaments. Generally, one or two thick cells or the small nodules are attached to the hemocele through connective tissue and trachea. Within a species, the structure of this tissue is fairly stable, but it can have significant differences between insects of different orders[8]. The fatty body is spread in most of the body cavity. This tissue extends into all regions of the body, including the external appendages of the insects. The fat body is more abundant in the abdominal region and is formed by thin strings or nodules that are suspended in the insect hemocoel and involve connective tissue and the trachea[30].

Pupal stage: In the results of the present study, it was found that the honey bee pupa in 1st day old honey bee queen pupa the cellular boundary between the fat cells tr is dissipated with the accumulation of waste inside (Fig. 2a) and in 1st day old honey bee worker pupa the distances between the (Tr) cells are spaced, filled with internal debris and the gaps are disintegrated (Fig. 2b). Also, in the 3rd day old honey bee queen pupa, similar to what existed in the first age of the pupa. Also, it is clear that the waste is decreased and the spacing of cells is wide Fig. 2c. But, in the 7th day old honey bee worker pupa the accumulation and disintegration of fat cells in the (Tr) and the gaps inside them are obvious (Fig. 2d). The fat body contains impurities consisting of several compounds, mainly fats, proteins and glycogen. The amount and quality of the material accumulated in the cytoplasm vary according to the functional stage of the organism[31]. In a study by Zara and Caetano[32], they show three different cell types: trophocyte, urate and the oenocyte. The trophocyte is the most abundant cell type in the fat body; the cells are rounded and show nuclei with varying sizes and shapes. Several electron-lucid spheres, lipid droplets and some eosinophil granules can be observed in their cytoplasm. The urate cells, seemingly more abundant next to the ventriculus, are distributed among the trophocytes throughout the body cavity. These cells, recognizable based on their slightly

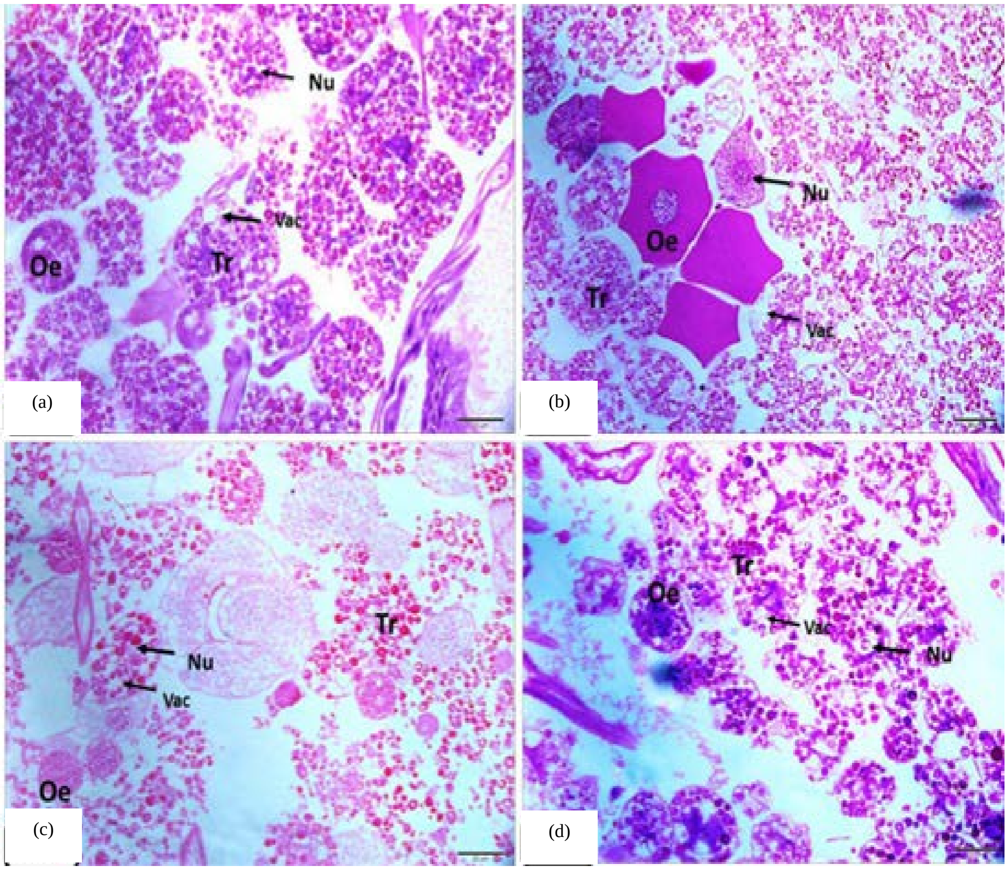

Fig. 2(a-d): Cross section of the fat body in the honey bee pupa, (a) 1st day old honey bee queen pupa (20 μm), (b) 1st day old honey bee worker pupa (20 μm), (c) 3rd day old honey bee queen pupa (20 μm) and (d) 7th day old honey bee worker pupa (20 μm)

polygonal morphology, show a central nucleus with a large amount of heterochromatin. Several small and lucid vesicles can be observed in their cytoplasm. The last cellular type found in the fat body the oenocytes, present a rounded shape although they can be slightly deformed due to pressure exerted by neighboring cells. They show a rounded and large, centrally located nucleus, with a great amount of heterochromatin. The cytoplasm is strongly stained by both hematoxylin and eosin (HE); it appears flocculated, similar to small vacuoles.

Adult stage: The results of present study showed that the adult honey bee queen, the size of the cells in the triglyceride cells increased and elongated and the gaps inside them were clear (Fig. 3a, b). In the honey bee worker, the adult stage the increase of (Tr) fat cells and internal gaps as well as accumulation of waste, which works on the cells (On) to remove toxins from the body (Fig. 3c, d). This is consistent with what is mentioned[33] when they found the oenocytes can act in detoxification processes. However, there is a specific region where the fat body is associated with two or more different cell types, playing the role of only one and in bees, it may play functions homologous with the hepatocytes and nephrocytes of vertebrates[34,35]. The fat body of insects consists of two main types of cells: trophocyte and oenocyte. Although these cells were apparently associated in a mass of tissue, the former has a mesodermal origin and the latter, ectodermal, presenting different morphology and function[9,31].

In the results of the current study found that the histological structure of fatty body varies between the worker and queen honeybees in various stages and may be due to the nature of the tasks performed by individuals in the colony. This is consistent with what many researchers have thought honeybee workers are essentially sterile female helpers that make up the majority of individuals in a colony. Workers display a marked change in physiology when they transition from in-nest tasks to foraging. Recent technological advances have made it possible to unravel the metabolic modifications

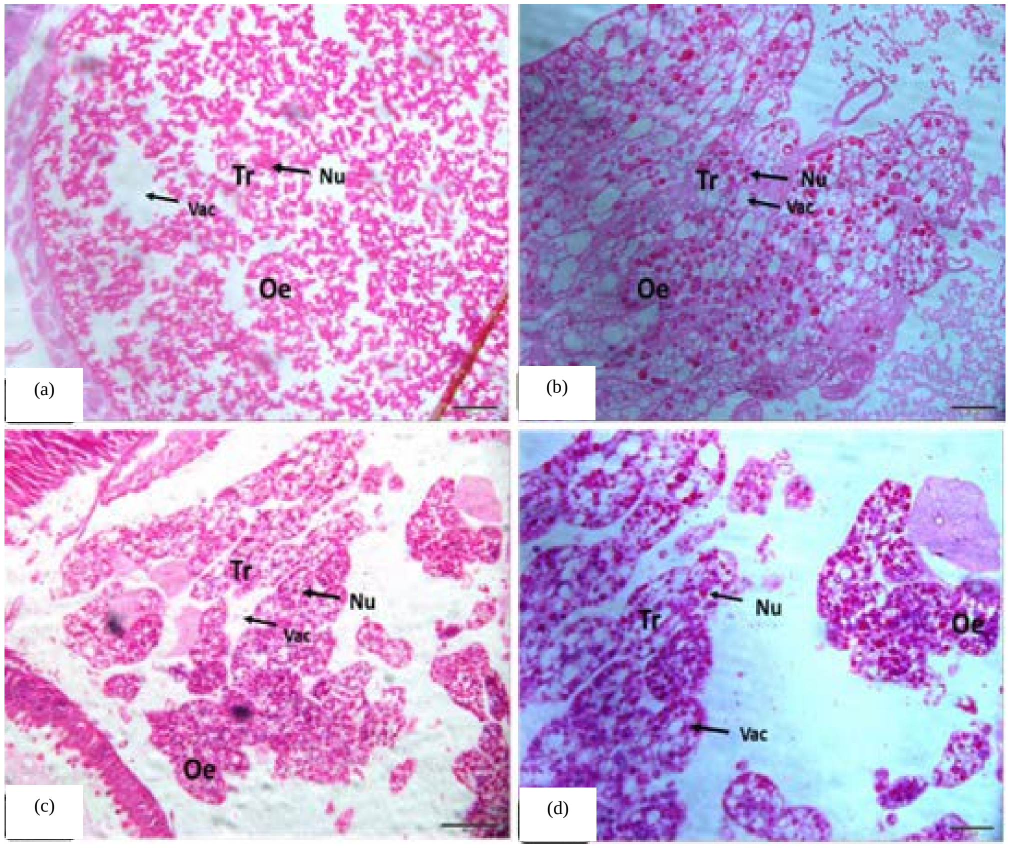

Fig. 3(a-d): Cross section of the fat body in the adult honey bee, (a) 20 µm, (b) 20 µm adult queen, (c) 50 µm and (d) 20 µm adult worker

associated with this transition. The honeybee fat body is more developed in nest bees compared to forager bees[4] and a decrease in abdominal lipids has been shown to precede the onset of foraging[4]. These observations indicate that the biochemistry of the abdomen is profoundly remodeled during the transition from in-nest tasks to foraging[4]. For nurse workers, the trophocytes of fat body incubated only in culture media (control) were IO-positive around the mitochondria and in heterogeneous inclusions of the cytoplasm. For nurse workers, the trophocytes of fatty body cultured only in plain media (control) were AS-positive with a powdery granulation scattered in the cytoplasm and around inclusions of low electron-density, which are probably lipids. For the virgin queen, the AP activity on control fatty body trophocytes appeared as powdery granules scattered in the cytoplasm and condensed around lipid droplets[31]. Lipids are the main compounds stored in the fatty body trophocytes of nurse workers and virgin queens. In the controls, lipids appeared as small dots around mitochondria in both cases, suggesting a role for these organelles in lipid metabolism as seen in several kinds of tissues, primarily the ones that store lipids and glands that secrete lipid-like substances. In a study by Seehuus *et al.*[36], they observation than both foragers, treated bees and the reversion bees all showed a high proteolysis activity. This result probably reflects a degradation of protein storage in the forager fat body. As a preparation for foraging, workers use their fat bodies as a source of energy, fat and proteins and thereby reduce their demand for nutrients from the hive. Protein synthesis is one of the most energy consuming cellular processes, devouring an estimated 50% of total cellular energy. Limiting the protein synthesis in foragers, therefore, seems like a good energy saving solution on both the individual and colony level. A reduced translation rate would reduce not only normal protein production but also the flux of damaged proteins through various pathways.

Malpighian tubules

Larval stage: In the results of the present study, it was found in the 3rd day old honey bee queen larva was characterized by the existence of cubic cells in large size and a clear nucleus and the presence of gaps inside the cells and the inner cavity is narrow (Fig. 4a). The 3rd day old larva in honey bee's worker,

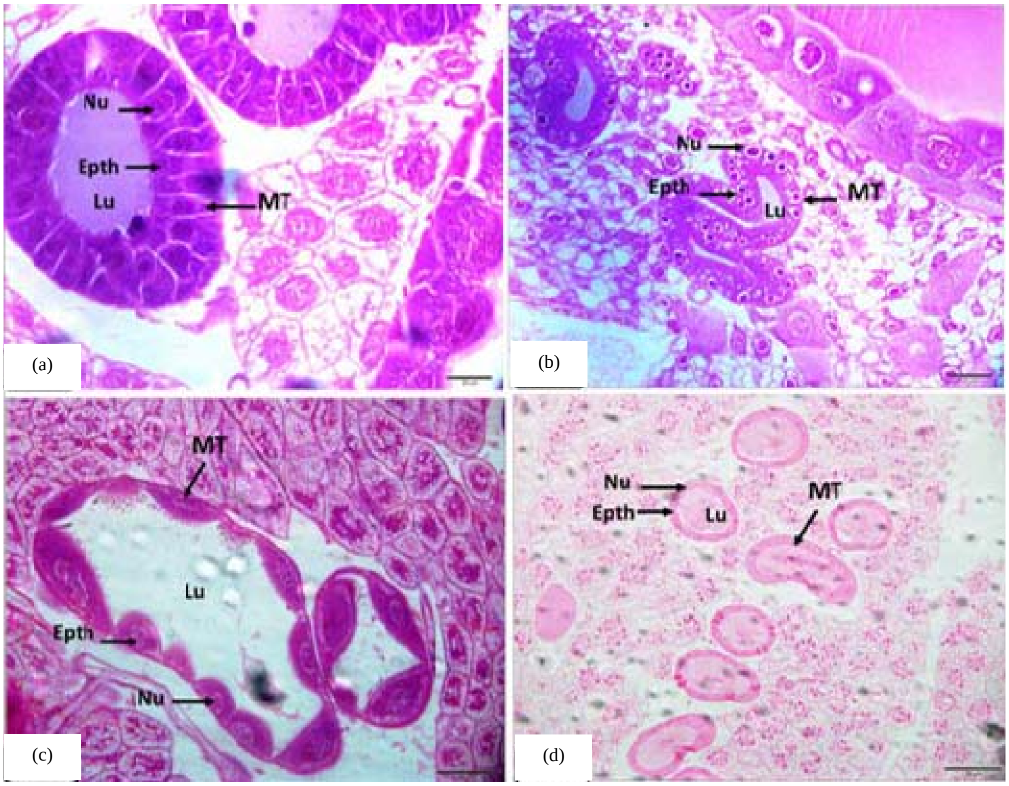

Fig. 4(a-d): Cross section of the malpighian tubules in the honey bee larva, (a) 3rd day old honey bee queen larva (20 μm), (b) 3rd day old honey bee worker larva (20 μm), (c) 5th day old honey bee queen larva (50 μm) and (d) 5th day old honey bee worker larva (50 μm)

it was characterized by the presence of cubic cells with clear nuclei and the composition of these cells based on the basement membrane with the presence of gaps within the cells and the inner cavity was narrow. But in the queen was characterized by the size of more than worker (Fig. 4b). Also, the 5th day old larva in honey bees queen epithelial cells elongation with the accumulation of waste and its accumulation in the inner cavity and secondary is larger in size but its boundaries are unclear (Fig. 4c). In the 5th day old honey bee worker larva was similar to the larva of the third age, but the cells have become the border between them is not clear and the inner cavity was wide (Fig. 4d). Therefore, current observations strongly suggested that the during the larval stage of bees, the products absorbed from the hemolymph by Malpighian tubules are accumulated in their lumen until the end of the larval feeding phase. The Malpighian tubules finally excrete their accumulated products when there is a connection between the midgut and the hindgut preceding metamorphosis[11]. At the end of the larval stage of bees, Malpighian tubules become inoperative and begin their degenerative process. During metamorphosis of bees, new Malpighian tubules differentiate from stem cells located at the insertion of the tubules into the digestive tract[11]. In a study by Scholes *et al.*[37] and Rangel *et al.*[38], tissue-specific differences in ploidy levels are also documented in the Hymenoptera. Using the honey bee as a model to study endoreduplication in eusocial insects. In accordance with previous findings, malpighian tubules exhibited relatively high ploidy levels, which increased with worker age, this relatively high ploidy in the Malpighian tubules highlights the essential role of endopolyploidy in insect secretory cells[39].

Pupal stage: In the 1st day old honey bee queen pupa the epithelial cells are adjoining and overlapping as if they are cellular and the nanoparticles are unclear and the inner cavity is virtually devoid of any components (Fig. 5a). But, in the 1st -day-old honey bee worker pupa the epithelial cells of the malpighian tubules became adjacent and the secondary was unclear and the inner cavity was very round (Fig. 5b). In the 3rd day old honey bee queen pupa similar to the first age of the pupa as it increases as the age approaches its end (Fig. 5c). Also, in the 7th day old honey bee worker pupa the epithelial cells of the malpighian tubules have become dilapidated, the nuclei are unclear, the internal cavity is almost solid (Fig. 5d).

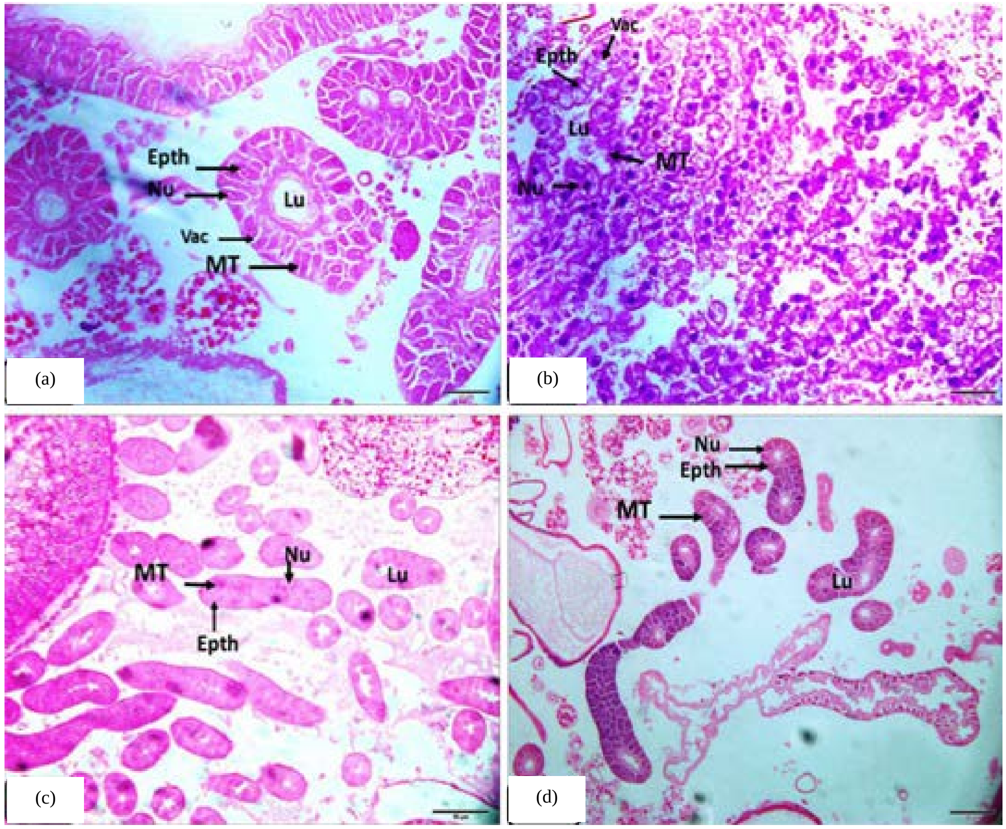

Fig. 5(a-d): Cross section of the malpighian tubules in the honey bee pupa, (a) 1st day old honey bee queen pupa (20 μm), (b) 1st day old honey bee worker pupa (20 μm), (c) 3rd day old honey bee queen pupa (50 μm) and (d) 7th day old honey bee worker pupa (20 μm)

From the results of the present study, it can conclude that the stage of the pupa phase clearly shows the re-formation of tissues where the bees are holometabolous insects and therefore undergo a complete reorganization of organs during metamorphosis, during pupae phase. In Hymenoptera changes in the midgut begin in the prepupa with the larval epithelium degenerating, leaving only the basement membrane and the regenerative cells. At metamorphosis, the larval midgut epithelium is reabsorbed and substituted for cells from regenerative cells[27]. From the results of the present study, It was concluded that the stage of the pupa phase clearly shows the re-formation of tissue in the bees.

Adult stage: In the adult queen, the epithelial cells in malpighian tubules between them are unclear and the nuclei scattered. Also, the internal cavity is almost solid (Fig. 6a, b). In general, the results obtained in the present study showed in the adult worker similar to the seventh age in the pupa (Fig. 6c, d). The malpighian tubules of bees showed structural integrity and spherical-shaped nuclei; the interior of the nucleus was filled with euchromatin with some regions presenting heterochromatin around the nuclear envelope and the presence of a nucleolus was observed. Mitochondria exhibited cristae and were found throughout the cell, with a higher concentration in the apical region and within the microvilli; additionally, intact microvilli were observed[40]. Typical ultrastructural characteristics were observed in the Malpighian tubules for the epithelium appeared to be well preserved and showed no alterations in the basal labyrinth and the normal presence of microvilli in the apical portion. Many mitochondria with cristae could be visualized in the cells[41]. The results of previous studies have proved, following the metabolism of thiamethoxam, in the body of the bee, the digested products or the undigested molecule will reach the hemolymph and subsequently, the brain and the Malpighian tubules. This organ was responsible for the maintenance of homeostasis and for the detoxification process that eliminates excess substances, even those that have not been metabolized by the body[42]. Maybe that's because the endocycle is also active in adult tissues such as those present in the malpighian tubules all of which exhibit high levels of endoreduplication[43].

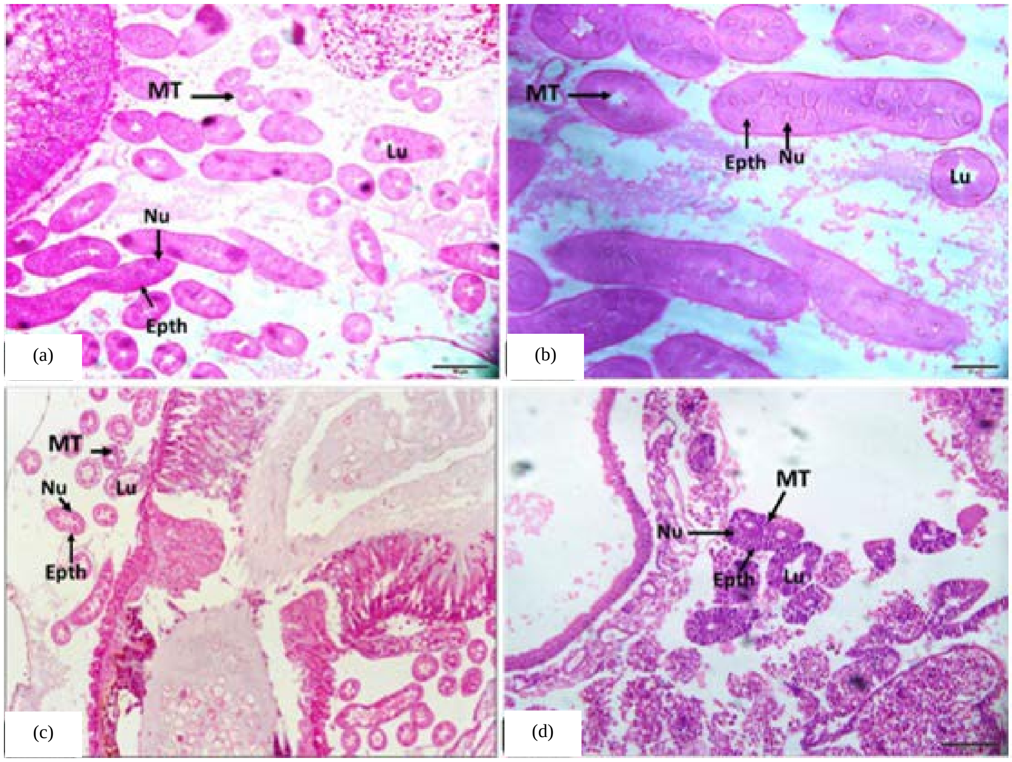

Fig. 6(a-d): Cross section of the malpighian tubules in the honey bee, (a) 50 μm, (b) 20 μm adult queen, (c) 50 μm and (d) 50 μm adult worker

CONCLUSION

The results showed the accuracy and miracles in the composition of such an insect and that changes in nutrition in embryonic stages have a clear effect on cellular structure, as it has been in the histological structure of the fat body and malpighian tubules of queen and worker of the local honey bees races, *Apis mellifera jemenitica* the various ages of the workers found differences between the honey bee queen and the worker as well as the difference in the different ages.

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