Histological Changes Of Bee Worker (*Apis mellifera* L.) By Bee Venom And Amino Acids Feeding

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Abstract

The effect of feeding honey bee colonies on a sugar solution to which bee venom has been added at a concentration of 50 mg/liter sugar solution and on amino acids extracted from clover 4 cm amino acids/liter sugar solution on the amount of food consumed by honey bee workers as well as the effect on gland tissues Hypopharyngeal and maxillary glands and histological changes that occur in them. Nine honeybee colonies were tested in an apiary of the Bee Research Department, Plant Protection Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt, during 2020-2021. Statistically, there are no significant differences in the amount of food consumed by honeybee workers, whether the bee venom solution or the amino acid solution, compared to the control colonies that fed on the sugar solution only. No histological changes were observed in the hypopharyngeal (HPGs) and mandibular glands (MGs) in workers fed sugary solution (control colonies). Feeding with amino acids resulted in histological changes in the HPGs and MGs, as most of the acini were atrophic and smaller in size than usual, with the loss of vesicles in the cytoplasm. As for the bees that fed on a solution of bee venom, histological changes in the HPGs and MGs were observed, where severe atrophy and contraction of the acini with an irregular outline were noted. Nuclear thickening of the epithelial cell lining was observed. The histological structure of the lower glands showed atrophy in some individual cells, which led to damage to the development of both the HPGs and MGs.

Keywords: amino acids, bee venom, feeding, honeybee, histological changes

Introduction

Apiculture has a significant economic impact; therefore, many countries consider the health status of honeybees to be of utmost importance. The honeybee *Apis mellifera* plays an important role in pollination, and therefore, in agricultural processes. Besides, they are the main source of many products like honey, wax, propolis, royal jelly, pollens, and venom that have been widely used in food and medicine (27).

Excessive use of chemicals and pesticides in the treatment of diseases affecting honey bee colonies leads to great danger to the health of consumers as well as the health of honey bees and reduces the effectiveness of honey bee products. The use of natural materials, such as bee venom secreted by honey bee workers, and amino acids extracted from plants to combat diseases and pests of honey bee colonies to obtain honey bee products that are free from harmful substances, such as pesticide residues and antibiotics (5, 10).

A bee venom solution was sprayed onto wire cages containing honey bees inside the laboratory, which increased the longevity of workers and improved some characteristics and behaviors, such as hoarding behavior, bee population, brood rearing, pollen storage, and storage of honey, healthy behavior, and foraging activity (6, 17, 37).

Bee venom is a colorless, transparent, viscous liquid that maintains a strong aromatic bitter taste and is an off-white or off-white powder in the dry state. Bee venom consists of about 40 constituents, elements, minerals, volatile organic acids, and some antibiotics, and it is a complex mixture of proteins such as amino acids, solutes, and phospholipase A2. These substances cause anti-bacterial, anti-fungal, and anti-viral diseases that affect honey bee colonies and maintain the health of bees, which increases bee activity. (34, 5, 7).

Bee venom improves and strengthens the immune system in a manner similar to that of vertebrates, which is necessary for the growth, development, and activity of honeybees (17, 1). Feeding with bee venom solution resulted in the longest longevity of honeybee workers (37).

In the honeybee colony, *A. mellifera* requires a protein from pollen, primarily as a source of essential amino acids that are necessary for building different tissues of bee workers and drones, and from the beginning of the fall season (dearth period), will support these colonies to stimulate brood rearing early in the spring. This, in turn, will positively reflect the adult bee population during the following spring and summer (28). Therefore, the products that could be produced from these colonies could increase, and the potential productivity, as income net gain, rose. When compared with colonies fed with sugar syrup alone, this may encourage beekeepers to reconsider the importance of protein in nutrition to stimulate egg production and brood rearing (32, 23, 28, 20).

Amino acids extracted from some plants are the basic unit for building proteins, that is, when proteins are digested inside the body they are converted to amino acids, which are absorbed in the bee body so that it is of great importance to the body, they enter the building of cells and tissue repair, In the formation of the basic material from which the antibodies are built, which attack

foreign objects entering the body, in addition to the great impact on vitamins and minerals, so that they perform their functions to the fullest when digested (16, 21).

Feeding with amino acids extracted from soya bean and clover improves the productivity of honeybee colonies, particularly colonies fed on amino acids extracted from clover, which increases the brood rearing, and adult worker population within honeybee colonies fed on amino acids compared with control colonies. The amount of honey obtained from colonies-fed amino acids extracted from the two plants was higher than that obtained from the control colonies. Feeding with amino acids reduced the swarming of colonies and resulted in the production of new wax, which was white to light yellow in color in broods and honey covers. (28).

(HGPs) and (MGs) present in the header of queens and workers. Young nurses of *A. mellifera* produce royal jelly that is fed to larvae. (18, 24) they are on both sides of the head, directly above the mandibles; their secretion is discharged on the inner side of the mandible bases; the greater part of this secretion is fluid, It has a remarkable effect on the behavior and physiology of honey bees.

Therefore, this study aimed to at the effect of honeybee colonies with bee venom and amino acids feeding on the determination of hoarding behavior and studies histological of honeybee workers such as the changes that occur in the tissues of the hypopharyngeal glands and mandibular glands.

Materials and Methods

At the apiary, nine colonies of almost equal strength were ready for feeding trials. A total of 1.125 kg. of adult bees, or roughly 12300 bees, were placed within each Langstroth brood chamber during the autumn. Each chamber held two sealed and unsealed brood combs, two combs containing honey and pollen, and one empty fully drawn comb. Each colony was split up into the following three groups:

First group: 3 replicates of honeybee colonies were fed with a mixture of bee venom with a sugar syrup solution of 50 mg bee venom/ 1000 ml sugar syrup.

Second group: colonies were fed on amino acids extracted from clover at the level of 4 cm/1000 ml sugar syrup was weekly introduced in each colony, The most important amino acids extracted from clover are as follows (phenylalanine, Valine, Threonine, Tryptophan, Methionine, Leucine, Isoleucine, Lysine, and Histidine) (28, 29).

Third group: each colony was fed with one liter of sugar syrup (1:1) as a control treatment.

Determination of the consumption behavior of honeybee workers fed on sugar syrup with bee venom and amino acids:

The consumption behavior of honeybee workers was evaluated under field conditions. This behavior was estimated by measuring the quantity of sugar syrup+ amino acid added to the colony in graduated feeders at a rate of 1000 ml/colony and sugar syrup+ bee venom 5 mg added to the colony at 1000 ml/colony. The quantities of hoarded (sucked) sugar syrup withdrawn were measured after six hours for each experimental colony of offering the syrup to the test colonies. The metabolic sugar consumption of the group

was estimated by subtracting the amount consumed from the total sugar syrup with bee venom and amino acids taken from the feeder (37).

Histopathological studies

Histological studies were performed at the Central Laboratory of the Agriculture Faculty of Cairo University. Ten nurse honeybee workers (9 days old) were used for each treatment, anesthetized by cold exposure (4°C), for 3-5 minutes, and carefully dissected (12). Samples from the hypopharyngeal glands (HPGs), of treated bees, were taken, were fixed in 10% formol saline for twenty-four hours. Tap water was used for washing and serial dilutions of alcohol (methyl, ethyl, and absolute ethyl) were used for dehydration. The samples were cleared in xylene and embedded in paraffin at 56°C in a hot air oven for 24 h. Paraffin beeswax tissue blocks were prepared for sectioning at a 4-micron thickness in a sliding microtome. Then, the tissue sections were collected on slides, deparaffinized, and stained by hematoxylin-eosin for examination through the electric light microscope (6).

Statistical analysis

Data from all treatments were analyzed in a randomized complete block design (ANOVA) by MSTAT-C version 1.41 (36) and using graph pad Prisma version 3.03 for Windows, software. All means were compared by Duncan's multiple range test at level 0.05 (14)

Results and Discussion

Determination of the hoarding behavior of honeybee workers fed on sugar syrup with bee venom and amino acids

The results in Fig. (1) the means of consumption amount sugar syrup by treated colonies with bee venom 50 mg venom/liter of sugar syrup, amino acids (4 ml/L) extracted from clover, sugar syrup (control) which were 100, 100, and 100 % respectively, (25, 33, 5, 29) they mentioned that feeding with bee venom and amino acids increases the worker's hoarding behavior, worker longevity, brood rearing, and bee health, Particularly colonies fed on 50 mg bee venom/liter, 4 cm amino/L. and sugar syrup was hoarded at 1000 ml (100%).

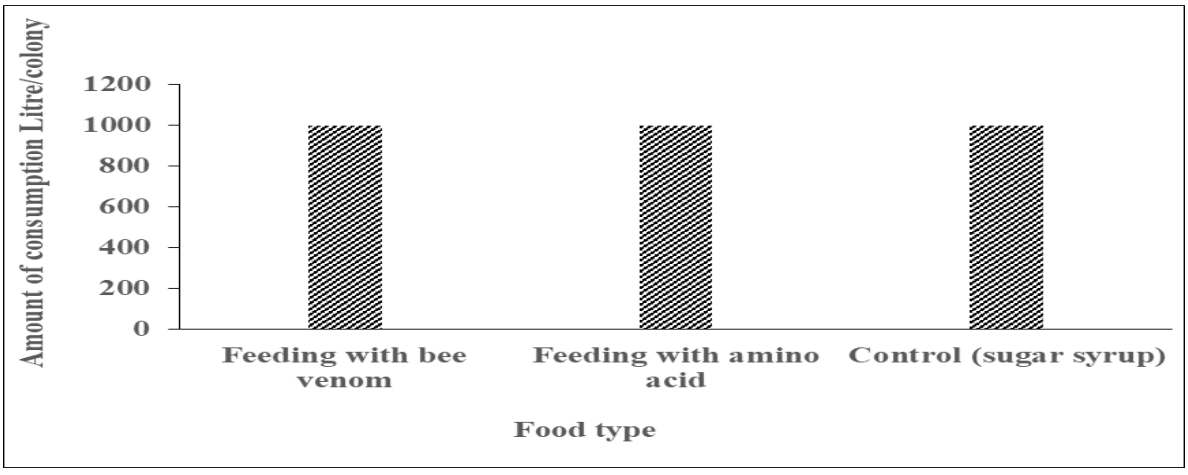

Fig 1. Impact of sugar syrup feeding, amino acids (ml/col), and bee venom (mg/col) on the consumption behavior of honeybee workers

Honey bee colonies fed on bee venom and consumed amino acids, Dussutour (15) and Marzouk *et al*., (29) reported that honeybee colonies fed on bee venom and amino acids encourage workers to produce new wax, which is white to light yellow and appear in brood and honey covers. This improves the productivity of honeybee colonies, which increases brood rearing, adult worker population, and extraction of honey, and reduces swarming within honeybee colonies fed on bee venom and amino acids compared to control colonies. Feeding honeybee colonies with a solution of bee venom as a treatment for some diseases that affect bee colonies and adding bee venom does not affect the amount of sugar solution consumed by honeybees, Badr *et al.*, (5) and Wahba *et al.*, (37) reported that feeding with bee venom reduced the incidence of American foulbrood compared with other treatments and reduced the percentage of honey bee workers infected with *Nosema apis* and Varroa mite.

Histopathological studies

Histopathological findings of control bees:

Fig 1. hypopharyngeal gland (HPGs) in control bees (x 40)

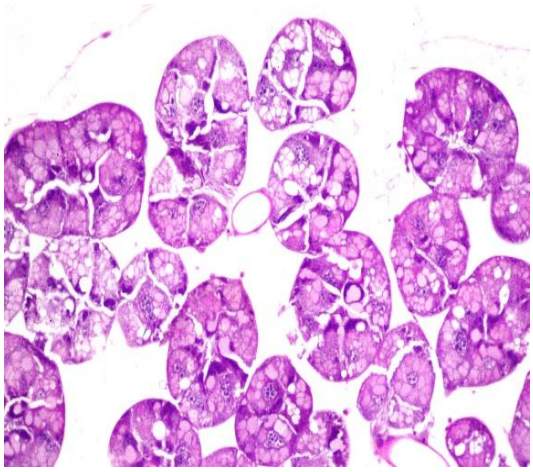

Fig 2. hypopharyngeal gland (HPGs) in control bees (x 60)

Fig 3. mandibular glands (MGs) in control bees (x 40)

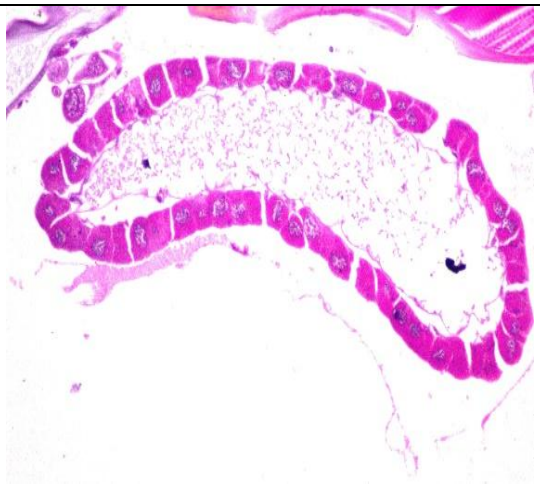

Fig 4. mandibular glands (MGs) in control bees (x 60)

Histological changes were assessed in worker bees (HPGs and MGs) (Tab.1). There was no histopathological alteration, and the normal histological structure of the acini with lining epithelial cells and nuclei and vesicular cytoplasm with compact cytoplasm and round central nuclei were recorded (Fig. 1, 2, 3, 4). Consumption of food mixed with amino acids or bee venom by honeybees is positively associated with the development of the hypopharyngeal and maxillary glands (11, 22, 9). Two glands were assessed for sugar feeding, no histological changes occurred in the glands (HPGs and MGs) and the regularity of the shape and size of the glands as feeding on the sugar solution honeybee workers reared under normal conditions (control) (2).

Histopathological findings of bees feeding with amino acids:

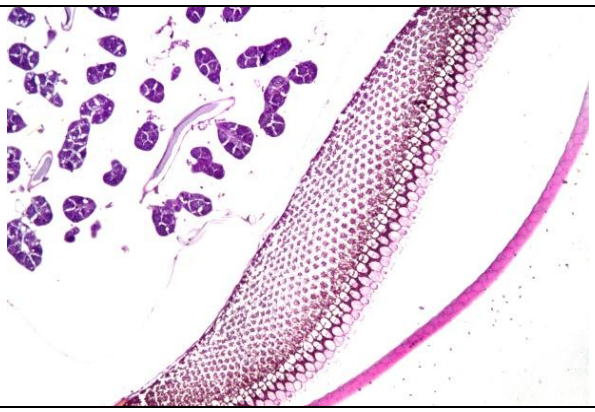

Fig 5. HGPs in bees feeding with amino acids (x 40)

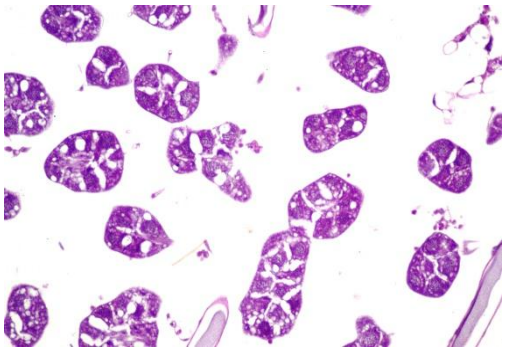

Fig 6. HGPs in bees feeding with amino acids (x 60)

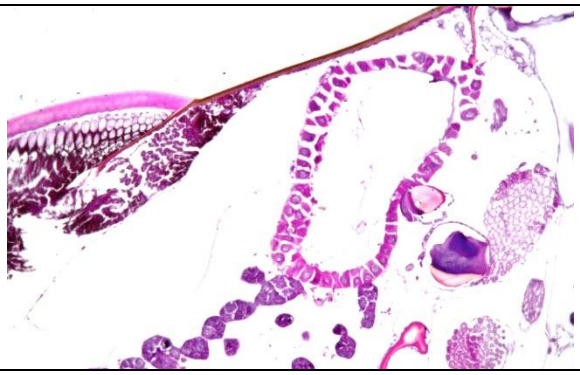

Fig 7. MGs in bees feeding with amino acids (x 40)

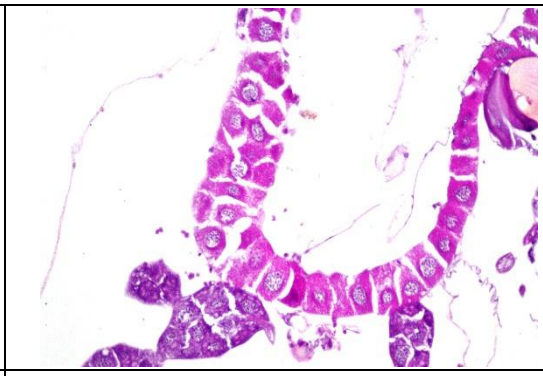

Fig 8. MGs in bees feeding with amino acids (x 60)

The assessment of histological changes in worker bees (HPGs and MGs) fed amino acids is presented in (Tab.2). The hypopharyngeal gland in bees fed with amino acids showed that most of the acini were atrophied and smaller in size than normal, with clear loss of vesicles in the cytoplasm (Fig. 5,6), while there was no histopathological alteration in the mandibular glands, as shown in (Fig. 7, 8). The gland activity related to its size and royal jelly secreted amounts (4, 13, 35). The finding is that the volume of HPG decreased with the consumption of amino acids because the amino acids increase the activity of the glands and thus increase the secretion of royal jelly, which affects the physiological effort of the gland and leads to a decrease in its size, that feeding bees with protein diets decrease the size of their HPGs as measured on day nine, twelve, fifteen, and eighteen this matches the age at which worker bees' exhibited smaller acini than the bees sampled (31, 3, 26).

Histopathological findings of bees feeding with bee venom:

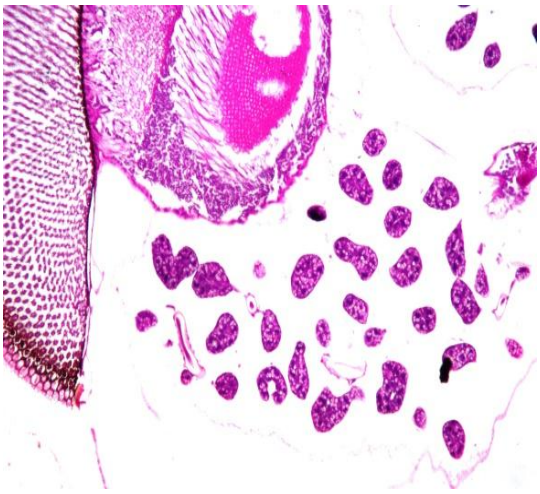

Fig 9. HGPs in bees feeding with bee venom

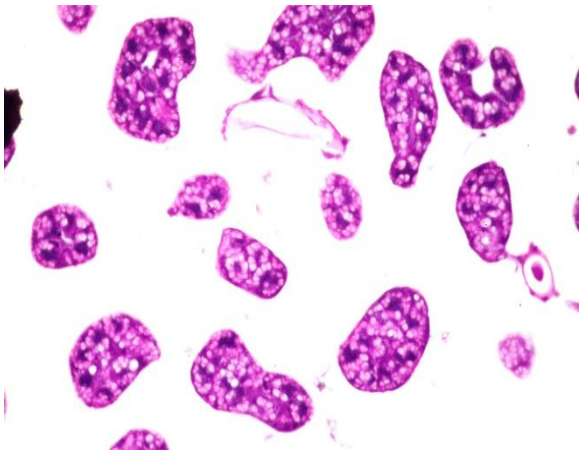

Fig 10. HGPs in bees feeding with bee

(x 40)	venom (x 60)
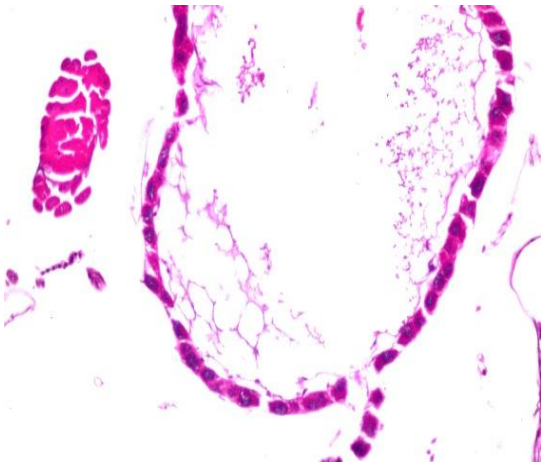	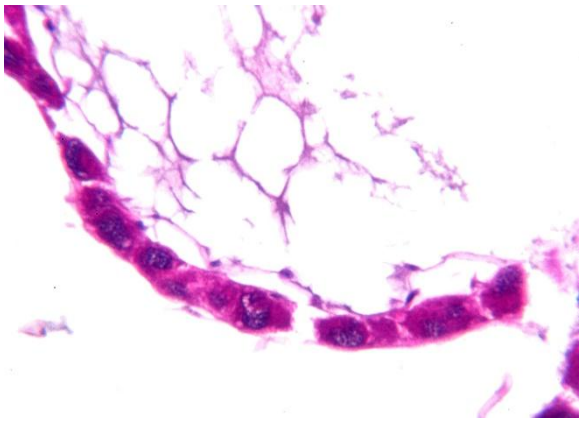
Fig 11. MGs in bees feeding with bee venom (x 40)	**Fig 12. MGs in bees feeding with bee venom (x 60)**

The assessment of histological changes in worker bees (HPGs and MGs) fed bee venom is shown in (Tab. 3). In the hypopharyngeal gland, severe atrophy and shrinkage were observed in the acini, with irregular outlines (Fig. 9). Nuclear pyknosis was observed in the epithelial lining cells (Fig.10). The histological structure of the mandibular glands showed atrophy of some individual cells, as shown in (Fig. 11 and 12). Feeding with bee venom can cause physiological changes in bees, such as damage to the development of both the (HPGs and MGs) and reduction in the number and area of acini of honey bee glands subjected to stress induced by feeding with bee venom solution. Thus, This experiment showed a negative effect on the growth and development of the glands, although feeding on the bee venom solution was short this is in accordance with (30, 8) they explained that collecting bee venom from colonies leads to their weakness.

Conclusion

Generally, the feeding with amino acids and bee venom showed there are no differences in the amounts of food consumed such as bee venom solution and amino acids feeding, The occurrence of some histological changes in the shape and size of the (HPGs and MGs) in worker honeybees as a result of colonies feeding on a solution of bee venom or amino acids compared to control cells that fed on a sugar solution. Therefore, we advise beekeepers when using bee venom in the treatment of colon diseases or using amino acids in the food of bee colonies as an alternative to pollen that the use is not excessive, at specific times and when necessary.

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Conflict of interest

The authors declared that they have no competing interests.

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