Sabuncuoglu Serefeddin Health Science (SSHS)

ISSN: 2667-6338, 2020/Vol.2:2

EFFECTS OF PERGA (BEE BREAD) ON METAMORPHOSIS, MORTALITY, AND FECUNDITY IN *DROSOPHILA MELANOGASTER*

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Research Article

Received: 06/08/2020, Accepted: 30/08/2020

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Abstract

This study aimed to investigate the effects of Perga, whose high biological activity inhibits mold and fungal growth and increases the level of breeding hormones, on the rate of larva-pupa-adult metamorphosis, survival and fecundity in *Drosophila melanogaster*.

The study determined the metamorphosis and mortality rates of 3rd stage *Drosophila melanogaster* larvae placed in media containing Perga at different concentrations (5, 2.5, 1.25, 0.625, and 0.3125 mg/ml) and in Perga-free media as well as the fecundity rates of female individuals developing from larvae. The obtained data revealed that there was a significant increase in the fecundity rates of female individuals grown in media containing Perga at 5 different concentrations ($p < 0.05$). While the mean fecundity rate was 57±0.88 in female individuals grown in media containing 2.5mg/ml Perga, this rate was found to be 47±1.15 in the standard *Drosophila* medium containing no Perga, which was the control group. Considering Perga's effects on larval mortality in 3rd stage *Drosophila melanogaster* larvae, while the percentage of survival was 94% in the control group, it was determined as 98% in the larvae fed

with Perga ($p < 0.05$). Considering the metamorphosis rates, no significant increase was observed in the number of individuals who could develop from larva to adult ($p > 0.05$). This study revealed the positive effects of this precious apitherapic product, whose effects and importance are not well known, on vital parameters in *Drosophila melanogaster*, a eukaryotic model organism.

Keywords: Bee bread, Perga, *Drosophila melanogaster*, Fecundity, Mortality, Metamorphosis

Özet

Çalışmada Perga'nın yüksek biyolojik aktivitesinin küf ve mantar gelişimini inhibe etmesi ve üreme hormanlarının seviyesini artırıcı etkisi nedeniyle *Drosophila melanogaster*'in hayatta kalış yüzdesi, dişi bireylerde yumurta verimi, larva-pupa ve ergin dönüşüm yüzdesi üzerine etkileri araştırılmıştır. Farklı konsantrasyonlarda (5, 2.5, 1.25, 0.625 ve 0.3125 mg/mL) Perga içeren ve Perga içermeyen besiyerlerine yerleştirilen *Drosophila melanogaster* 3.evre larvalarının metamorfoz ve mortalite oranları ile larvalardan gelişen dişi bireylerin fekundite oranları tespit edilmiştir. Elde edilen veriler incelendiğinde özellikle 5 farklı konsantrasyonda Perga içeren besiyerinde yetiştirilen dişi bireylerin yumurta veriminde anlamlı derecede artış olduğu gözlenmiştir ($p<0.05$). 2.5mg/ml Perga içeren besin ortamında yetiştirilen dişi bireylerde ortalama yumurta verimi 57±0.88 iken kontrol grubu olan Perga içermeyen standart *Drosophila* besiyerinde bu oran 47±1.15 olarak tespit edilmiştir. Perganın *Drosophila melanogaster*'in 3.evre larvalarında larval mortaliteye etkisi incelendiğinde kontrol grubunda yaşam yüzdesi %94 iken Perga ile beslenen larvalarda %98 olarak tespit edilmiştir ($p<0.05$). Metamorfoz oranlarına bakıldığında ise yine anlamlı derecede larvadan ergine gelişebilen birey sayısında artışlar gözlemlenmemiştir ($p>0.05$). Etkileri ve önemi çok fazla bilinmeyen bu çok değerli apiterapik ürünün ökaryotik bir model organizma olan *Drosophila melanogaster*'de yaşamsal parametreler üzerine olumlu etkileri bu çalışmayla belirlenmiştir.

Anahtar Kelimeler: Arı ekmeği, Perga, *Drosophila melanogaster*, fekundite, mortalite, metamorfoz

1. Introduction

Bee products, especially honey, have been used by people for centuries in the treatment of many diseases. Based on the results of recent scientific research, treatment methods with bee products have started to be accepted in the medical world under the name of "Apitherapy". The number of clinics and Apitherapy centers that treat diseases using only bee products is increasing day by day. Our country, which is very rich in biological resources, also has a great potential in terms of honey and other bee products. However, although it is known that bee products are beneficial for health, Apitherapy centers have not been established and scientific studies on this issue have not started yet in our country (Albayrak and Albayrak, 2008). Honey, pollen, propolis, and royal jelly are generally used in apitherapy practices (Özkan and Sancar 2015). Bee bread (Perga), which is not included in apitherapy practices, but whose important features have been revealed by studies, is one of the other bee products.

Perga, besides being the main foodstuff of the queen bee, is consumed by adult bees and used for feeding the larvae(Krell, 1996; Karaman et al., 2016). Honey bees store the pollen they collect in honeycombs in the form of bee bread. While bee bread is produced in the hive, it is mixed with pollen, honey, and other bee secretions and subjected to lactic acid fermentation. The mixture becomes bee bread in about two weeks, and bee bread, which is a fermented product, can thus be preserved for a long time in the hive (Bogdanov, 2011).

Besides containing all the minerals and valuable nutrients contained in the pollen, Perga has at least three times higher bioactive properties than pollen. The main reason for this is that, unlike pollen, Perga is fermented with special enzymes of the bee, making the minerals in it useful (Gilliam et al., 1989).

Perga contains essential amino acids, monosaccharides, vitamins C, B1, B2, E, H, various minerals (cobalt, phosphorus, iron, calcium, etc.), carotenoids and anthocyanins, sucrose, amylase and phosphatase enzymes and phytohormones(Vasquez and Olofsson, 2009). Perga is much richer than pollen in terms of its content and variety of amino acids (Hoffman et al., 2013). Compared to bee pollen, Perga contains 6 times more lactic acid, which allows it to protect itself and not to be as much open to yeast development as pollen. (Mutsaers et al., 2005).

The transformation of pollen into Perga and biochemical changes are the results of lactic acid fermentation and microbial activity caused by bacteria and yeast (Haydak, 1958). The high biological activity of Perga inhibits mold and fungal growth, providing better protection for itself (Nagai et al., 2005). Due to its outer shell, only 60% of the pollen taken from outside can be

digested in the stomach, whereas Perga, which is the melted form of this indigestible shell with special enzymes of the bee, is digested 100% in the stomach (Pascoal et al., 2014).

Drosophila melanogaster, which was used as a model organism in our study, is an organism that is frequently used in biological studies. It was first used in experimental studies by Thomas Morgan in 1911. It is a eukaryotic organism with a short generation time, it is easy and economical to produce large numbers in laboratory culture, has a wide variety of morphological characters and mutant lineages that can be genetically controlled, the larvae have giant chromosomes in salivary gland cells that allow chromosome maps and chromosome function analysis, and Since many substances that are carcinogenic to humans also give positive results in Drosophila tests, it has many advantages in terms of use in such studies (Graf et al.,1992; Uysal et al., 2006; Gui and Grant, 2008; Falakalı, 2010; Llyold and Taylor, 2010).

This study aimed to investigate the effects of Perga, whose high biological activity inhibits mold and fungal growth and increases the level of breeding hormones, on the larva-pupa-adult metamorphosis, larval mortality, and fecundity in *Drosophila melanogaster*.

2. Material and Methods

2.1. *Material*

Perga (bee bread) used in the study was obtained from a bee business operating in our region. The supplied Perga was kept in closed conditions until it was used in the study (Figure 1).

Figure 1. Bee bread (Perga) obtained from a commercial firm for use in the study

D. melanogaster, on which we investigated the effects of Perga, has been cross-bred and reproduced for years in Amasya University, Faculty of Arts and Sciences, Biological Research Laboratory (Figure 2).

Figure 2. Oregon (Wild type) lineage of *Drosophila melanogaster* used in the study

Perga, whose effects we investigated, was ground by a blender in order to be added to the *Drosophila* medium. Powdered Perga was dissolved in water to prepare a 100 ml stock solution as 5mg/ml (Figure 3).

Figure 3. Stock solution of Perga prepared by grinding

Oregon (R) (wild type) lineage of *Drosophila melanogaster* was used to determine fecundity in this study. *It*'s Oregon R lineage is a wild-type lineage with normal round, red eyes, and no mutant character. *Drosophila* stock cultures are kept in vials containing standard *Drosophila* medium (SDM) in heated and cooled containers at 40% -60% relative humidity, 25±1 °C temperature, and under continuously dark conditions.

2.2. Methods

2.2.1. *Preparation of experimental setups and application of Perga to the medium*

During the studies, instant media were used for the storage, reproduction, and crossbreeding of *Drosophila* cultures. Instant *Drosophila* Medium was purchased from Carolina Biological Supply Company. 1.5 g *Drosophila* instant medium and 5 ml distilled water were added to 50 ml falcon tubes. In the study, the standard *Drosophila* medium was used to determine the fecundity rate. In order to count the eggs, the medium was prepared as a thin layer in Petri dishes (Figure 4).

Figure 4. Preparation of media in Petri dishes to determine fecundity

In our study, 3rd stage *Drosophila melanogaster* larvae were used to determine larval mortality, and the female *Drosophila melanogaster* individuals were used to determine fecundity. Female and male individuals of it's Oregon R lineage (Figure 2) were crossbred in 250 ml culture vials containing SDM to create preliminary stocks.

The same-aged (1-3 days/72±4 hours) unbred female individuals emerging from the pupae were collected once every 5 hours for 3 days and kept in culture vials for use in experiments. The female flies thus obtained were placed for egg counting in the media in Petri dishes as 3♀♀ X 3♂♂. Egg counting was performed for 3 days. The data obtained during these three days were averaged. Egg counting was performed under a stereo microscope and at the same time every day. Following the counting process, the flies were transferred to fresh media for 3 days. The flies were subjected to CO_2 etherization during transfer to the new media.

In order to obtain 3rd stage larvae, 40 female individuals and 40 male individuals were placed in food-containing vials. These individuals were kept in the same environment for at least 1 day to allow them to mate. Individuals were then transferred to new vials containing food and

allowed for 8 hours to lay eggs in the medium. Later, individuals were transferred to other vials. The purpose of the 8-hour egg collection was to obtain individuals in the same larval stage. Individuals that reached the 3rd larval stage after 72±4 hours were separated under tap water with the help of fine-pore sieves. 3rd stage larvae collected with the help of a sieve were transferred to plastic vials containing *Drosophila* instant medium, to which 5 ml of Perga concentration was added. 1-2 spatula-full larvae (approximately 100 larvae) were placed in each application medium. Food for *Drosophila melanogaster* larvae was prepared by adding 5 ml solution containing Perga at different proportions to 1.5 g instant medium. The larvae, fed with this mixture, were expected to develop into adult flies. All experiments were carried out in 3 replicates.

Before Perga was added to the medium, it was ground by a blender. Then 5 mg/ml stock solution was prepared with distilled water. Dilution was performed to obtain other ratios used in the study. The study was carried out in 3 replicates. The obtained results were averaged.

2.2.2. *Statistical analyses*

Statistical analyses of all obtained data were carried out with the SPSS (Statistical Package for the Social Sciences) 15.0 program.

3. Results

In this study, possible toxic or beneficial effect of Perga at different concentrations (5; 2.5; 1.25; 0.625 and 0.3125 mg/ml) on *Drosophila melanogaster* larvae were investigated. Also, the study examined the effect of Perga on the metamorphosis rate and duration of *Drosophila melanogaster*. Furthermore, the effect of Perga on fecundity in female *Drosophila melanogaster* individuals was examined.

First of all, in order to detect possible toxic or beneficial effect of Perga on *Drosophila melanogaster*, 3rd stage larvae were chronically fed with Perga at different concentrations (5; 2.5; 1.25; 0.625 and 0.3125 mg/ml). This preliminary study demonstrated that Perga had no toxic effects at any concentration and did not increase larval mortality (Table 1). Also, it was found that even at the highest Perga concentration (5 mg/ml), the number of individuals who could not mature from the larva was lower than that in the negative control group.

Table 1. Survival and mortality rates of larvae chronically fed with Perga at different concentrations

Study Groups	Concentration (mg/ml)	Number of Larvae	Mortality Rate (%)	Survival Rate (%)
Control (Distilled Water)		100	6	94[a]
	0.3125	100	4	96[a]
	0.625	100	4	96[a]
Perga (Bee Bread)	1.25	100	5	95[a]
	2.5	100	2	98[a]
	5	100	2	98[a]

S.E.: Standard Error; Values indicated by different letters in the same column are significant at the level of $p < 0.05$.

Five different Perga concentrations (5; 2.5; 1.25; 0.625; and 0.3125 mg/ml) were used to determine the effects of Perga on *Drosophila melanogaster*'s fecundity. Unpaired, same-aged female individuals obtained as a result of preliminary crossbreeding were grown chronically in media containing Perga at different concentrations. Fecundity of female individuals whose eggs were counted at the same time for 3 days was determined (Table 2). The over-time change in daily average fecundity per female for all study groups is shown in Figure 1.

Table 2. Average of the data obtained from the 3-day egg counting in female *Drosophila melanogaster* individuals after Perga application

Study Groups	Day(1) Fecundity ± S.E	Day(2) Fecundity ± S.E	Day(3) Fecundity ± S.E	Mean Fecundity ± S.E
Control (Distilled Water)	45.0±1.15	48.6±0.33	47.0±1.15	47.0±1.15[a]
0.3125 mg/ml	46.6±0.33	49.3±0.33	49.0±1.15	48.3±1.15[a]
0.625 mg/ml	51.0±1.15	49.0±1.15	53.6±0.33	51.0±1.15[a]
1.25 mg/ml	49.0±1.15	54.0±0.33	59.0±1.15	54.0±2.88[a]
2.5 mg/ml	56.3±0.33	56.6±0.33	58.6±0.33	57.0±0.88[b]
5 mg/ml	53.6±0.33	56.0±1.15	56.0±1.15	55.0±1.15[a]

S.E.: Standard Error; Values indicated by different letters in the same column are significant at the level of $p < 0.05$.

Pupation and maturation duration and rates in *Drosophila melanogaster* wild type (Oregon) lineage were also investigated. The effects of Perga on the rate and duration of transformation from larva to pupa were compared for each Perga concentration. By counting the individuals transforming from larva to pupa in both Perga-fed and control groups of *Drosophila melanogaster* wild-type (Oregon) lineage, percentages of transformation from larva to pupa were determined (Table 3). As can be inferred from Table 3, there is an increase in P1, P2, P3, P4, and P5 groups in the number of pupated larvae compared to the control group. However, the results of one-way analysis of variance revealed that the effect of Perga was statistically insignificant in terms of the number of pupated larvae ($p > 0.05$).

Table 3. Changes in the percentages of transformation from larva to pupa according to different Perga concentrations

Study Groups	Group No	Number of larvae	Number of pupae	Percentage of pupation ± S.E
Control Group	C	100	94	94.0±0.57
0.3125 mg/ml	P1	100	94	94.0±1.15
0.625 mg/ml	P2	100	95	95.6±0.33
1.25 mg/ml	P3	100	97	97.3±0.66
2.5 mg/ml	P4	100	97	97.6±0.33
5 mg/ml	P5	100	97	97.0±1.15

S.E.: Standard Error, $p<0.05$, P: Perga

In order to investigate the effect of Perga on the duration of transformation from larva to pupa in the *Drosophila melanogaster* wild type (Oregon) lineage, the results of the counts performed at 4-hour intervals were compared statistically (Table 4). As can be inferred from Table 4, the mean pupation durations are less in all Perga concentrations than in the control group. However, the results of the one-way analysis of variance revealed that this difference was statistically significant only in P4 and P5 concentrations ($p<0.05$).

Table 4. Changes in the duration of transformation from larva to pupa for all Perga concentrations

Study Groups	Group No	Number of larvae	Mean Pupation Duration (hours) ±S.E

Control Group	C	100	66.6±1.68
0.3125 mg/ml	P1	100	65.1±1.78
0.625 mg/ml	P2	100	63.2±1.69
1.25 mg/ml	P3	100	62.3±1.15
2.5 mg/ml	P4	100	61.4±1.77
5 mg/ml	P5	100	60.7±1.64

S.E.: Standard Error, p<0.05, P: Perga

In Perga-fed and control groups, the percentages of pupae that could develop into adults were determined and statistically analyzed. The analysis results revealed that in the control group, 97.8% of pupae could develop into adults, whereas in P1 (03125 mg/ml), P2 (0.625 mg/ml), P3 (1.25mg/ml), and P5 (5mg/ml) concentrations, 96.8%, 98.9%, 96.9%, and 97.9% of pupae could develop into adults, respectively (Table 5). As can be inferred from Table 5, the number of pupae that could grow into adults decreased in P1 and P3 concentrations compared to the control group, while an increase was observed in all other Perga concentrations. However, these decreases and increases were not statistically significant (p> 0.05). As can be seen in Table 4, according to the results of variance analysis (at 95% confidence interval), the number of pupae that could grow into adults was not affected by the Perga application (p> 0.05).

Table 5. Changes in the percentages of transformation from pupa to adult according to different Perga concentrations

Study Groups	**Group No**	**Number of pupae**	**Number of adults**	**Percentage of maturation ±S.E**
Control Group	C	94	92	97.8±1.45
0.3125 mg/ml	P1	94	91	96.8±1.45
0.625 mg/ml	P2	95	94	98.9±1.20
1.25 mg/ml	P3	97	94	96.9±0.88
2.5 mg/ml	P4	97	96	98.9±0.57
5 mg/ml	P5	97	95	97.9±0.66

S.E.: Standard Error, p<0.05, P: Perga

In order to investigate the effect of Perga on the duration of transformation from pupa to adult in the *Drosophila melanogaster* wild type (Oregon) lineage, the results of the counts

performed at 4-hour intervals were compared statistically. As can be inferred from Table 6, the mean maturation duration of individuals grown in medium containing Perga was shorter in all Perga concentrations than in the control group. However, the results of one-way analysis of variance revealed that these differences were not statistically significant ($p > 0.05$).

Table 6. Changes in the duration of transformation from pupa to adult for all Perga concentrations

Study Groups	Number of pupae	Mean maturation duration (hours) ±S.H
Control Group	94	73±1.88
0.3125 mg/ml	94	73±1.68
0.625 mg/ml	95	71±1.49
1.25 mg/ml	97	70±1.63
2.5 mg/ml	97	69±0.66
5 mg/ml	97	69±0.49

S.E.: Standard Error, $p<0.05$

4. Discussion and Conclusion

Perga, which has a wide range of use in the health industry, is a very important bee product that can be used especially for metabolism problems, diet regulation, and allergies. The medical world is in favor of avoiding drug therapy as much as possible so that disease-causing microorganisms can develop less resistance to drugs. In this context, one of the best options is natural foods that contain substances that destroy these microorganisms. One of them is bee bread (Perga), which is still underappreciated (Karaman et al., 2016).

The results obtained in the study demonstrated that Perga had no toxic effect on the 3rd stage *Drosophila melanogaster* larvae (Table 1). In addition, it was found that Perga increased fecundity rates in female *Drosophila melanogaster* individuals in all concentration groups compared to the control group (Table 2). Considering the effects of Perga on mean daily fecundity, it was found that Perga caused a significant increase in all concentration groups (0.3125 mg/ml, 0.625 mg/ml, 1.25 mg/ml, 2.5 mg/ml, and 5 mg/ml).

While no Perga concentration caused a statistically significant difference in terms of transformation from larva to pupa and from pupa to adult as well as in terms of duration of

transformation from pupa to adult, administration of only 2.5 mg/ml and 5mg/ml Perga concentrations caused a developmental acceleration, that is, a significant decrease in the duration of transformation from larva to pupa.

Considering the studies on Perga, no study in the literature has investigated the effect of Perga on *D. melanogaster*. The studies on Perga have mostly focused on the content of Perga, which is known as a new bee-product, and on the comparison of Perga with pollen, which is another bee product. Bobiş et al. (2017) found that like pollen, Perga also contains proteins, amino acids, fatty acids, lipids, sterols, enzymes, minerals, vitamins, and phenolic compounds. In addition, it was found that due to the honey in its content, bee bread contains more carbohydrates than pollen as well as containing honey bee hormones.

Pascoal et al. (2014) found that Perga has rich biochemical and antioxidant content and has many therapeutic properties such as antimicrobial, antitumoral, antibacterial, immunomodulatory, anti-inflammatory properties. Kowalski et al. (2017) stated that due to its contribution to reproductive hormones, it is used for improving sexual performance and increasing muscle strength and volume.

Although there are no studies in the literature examining the effects of Perga on *Drosophila melanogaster*, some studies on other apitherapy products have been conducted. Based on the fact that water extract of propolis (WEP) contains high levels of antioxidants and captures free radicals, Valaderes et al. (2007) measured the protective effects of a mixture containing doxorubicin (DXR), an anti-tumor agent, and propolis on the wing cells of *Drosophila melanogaster* by the somatic mutation and recombination test. They found that the recombination frequency when WEP and DXR were co-administered was lower than when DXR was administered alone. Özcan (2011) investigated the antigenotoxic effect of ethanol extracts of propolis (EEP) on the genotoxic effect of NaNO2 on *Drosophila melanogaster*. In the group to which NaNO2 and EEP were co-administered, a decrease in clone induction frequency was observed for all EEP doses. In the pretreatment groups where NaNO3 and EEP were added to the medium, it was determined that EEP had an antigenotoxic effect for all doses, and a significant decrease in clone induction frequency was determined. It was observed that the antigenotoxic effect of PEE varied depending on the dose in the group to which PEE and NaNO3 were co-administered. In addition, the antigenotoxic effect of EEP was determined to be higher in the pretreatment group than in the co-administration group.

Studies on royal jelly, another product used in apitherapy, have also reported antitumoral and antioxidant activities. Jamnik et al. (2007) investigated the effects of royal jelly on tumor growth and formation. In their study, rats exposed to oxidative DNA degradation were given royal jelly for 16 weeks, as a result of which DNA degradation was reduced and their life span was prolonged.

We think that the results obtained in this study are related to the antiseptic and germicidal properties of Perga and its ability to increase the level of reproductive hormones.

In conclusion, due to its extremely rich composition and high bioactive usefulness, Perga is not only a superior food product but also a unique source of healing. The proper utilization of this valuable bee product will not only be an important alternative for apitherapy and healthy nutrition but also create a very important added value for the beekeeping industry. Perga is an important bee product, although it is not as widely known as other bee products. Although it is a challenging process to separate Perga from the honeycomb, it can be easily separated by some apparatus developed recently. This, in turn, expands its availability to more and more people. Due to its extremely rich composition and high bioactive usefulness, Perga is not only a superior food product but also a unique source of healing. The proper utilization of this valuable bee product will not only be an important alternative for apitherapy and healthy nutrition but also create a very important added value for the beekeeping industry.

Conflicts of interest

The author declare that there are no potential conflicts of interest relevant to this article.

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