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Drone Larvae Homogenate (Apilarnil) as Natural Remedy: Scientific Review

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ABSTRACT

For centuries, honey bee products such as honey, bee pollen, royal jelly, bee bread (Perga), and bee venom have been utilized in natural medicine due to their beneficial properties. A great deal of scientific research has been dedicated to exploring their physico-chemical properties and therapeutic effects. Despite this, drone larvae have not received as much attention from the scientific community. Within a honey bee colony, drones are responsible solely for fertilizing queen bee eggs and consuming food reserves collected by worker honey bees. As a result, beekeepers commonly remove excess drone brood from the hive, which is crucial for preventing and treating varroasis. Lyophilization is the most effective method for preserving drone larvae, and the physicochemical properties of fresh and lyophilized drone larvae were compared. The therapeutic effects of drone larvae, such as androgenic, hepatoprotective, immunostimulatory, and hypolipidemic effects in humans and experimental animals, were summarized. This study aims to summarize current scientific knowledge on drone larvae (apilarnil). The author utilized well-known publication databases like SCOPUS, Google Scholar, and Pub Med to gather research on drone larvae. Furthermore, this review collected information on the chemical composition preservation and bioactive action of drone larvae. Thanks to their high levels of amino acids, fatty acids, vitamins, minerals, and hormones, drone larvae can be considered a potential potency-raising agent.

Keywords: Drone larvae, Apilarnil, Drone brood, Physicochemical properties, Lyophilize, Natural remedy, Varroa control, Hormone

1. Introduction

People prefer to take preventive measures instead of struggling with health problems in order to increase the quality and duration of life. Functional foods attract consumers because they would rather prevent a disease than cure it. Side effects of drugs and increased medical costs are among other important reasons. In addition, in industrialized countries, functional foods are preferred in order to be protected from the pollution in water, air and food, the use of chemicals and hormones or the health risks caused by environmental factors. Similarly, increasing health awareness is important in today's world where access to information becomes easier with developing technologies. As a matter of fact, the increase in demand for these reasons has revitalized the sector and paved the way for the introduction and marketing of novel and different products every day. As a result, the desire of consumers to seek a healthier and higher quality life cannot be ignored.

Apitherapy, which has existed since the beginning of humanity and whose importance has increased with scientific studies in recent years, draws attention in terms of preventing many diseases and supporting treatment. Natural bee products such as honey, pollen, Perga, propolis, royal jelly and apilarnil have high antioxidant capacity as well as rich nutritional content (Kumazawa et al. 2004; Leja et al. 2007; Eraslan et al. 2017; Özkök & Silici 2017). These valuable products, which honey bees process by collecting nectar and pollen from plants in nature, consist of many bioactive compounds such as protein, carbohydrates, vitamins, enzymes, phenolic compounds, aroma compounds, phytosterols, terpene and terpenoids, fatty acids and aliphatic compounds. Many different biologically active components of honey bee products have been held responsible for different effects such as antioxidant, antimicrobial, antifungal, immunostimulant and anticarcinogenic activities (Eraslan et al. 2008; Koc et al. 2009; Nassar et al. 2012; Miyata & Sakai 2018). The number of scientific studies on Apilarnil a recently discovered bee among the bee products, is very limited. Thus, this review summarizes the current knowledge on the structure, physicochemical content and biological functions of apilarnil, and points out and promotes further research directions.

1.1. Biology of the drone

The order Hymenoptera is known for its haplodiploid system. In the case of honey bees (*Apis mellifera* L.), a colony typically includes a queen bee, numerous sterile female workers, and several hundred drones during the breeding season (Palmer & Oldroyd 2000; Collison 2004). In all castes, developmental stages consist of egg, larva, pupa and adult. A queen bee that has completed mating lays two types of eggs; fertilized and unfertilized. Queen and worker bees hatch from fertilized eggs, while drones hatch from unfertilized eggs. All hatchlings are fed with royal jelly for the first three days. While queen bee larvae were fed with royal jelly throughout the entire developmental period, the nutrition of worker and drone larvae consisted of a mixture of honey, bee bread and royal jelly (Isidorov 2021). Drones are reared in honeycomb cells that are larger than worker bee cells. In a typical colony's annual cycle, drone production begins 3-4 weeks before the production of new queens at the start of the breeding season. Unlike drones, which mate only once, queen bees mate with an average of 12-14 drones (Rhodes 2002; Tarpy & Page 2000). The development of drones from egg to adult is greater than that of queen (16 days) and workers (21 days), lasting approximately 24 days (De Grandi-Hoffman et al. 1998). Like the males in other Hymenoptera species, spermatogenesis in honey bee drones begins at the larval stage and ends at the pupal stage. The volume of sperm ejaculate in the drone ranges from approximately 0.91 to 1.7 μL per drone, and 3.6-12 million sperm cells are produced. Sperm counts are strongly affected by drone size, larval diet and season (Collins & Pettis 2001; Rhodes et al. 2011). In the first week after hatching, sexual maturation in drones is completed with the development of a pair of mucous glands that protect and nourish sperm along with the migration of sperm to the seminal vesicles (Johnson et al. 2013). Sperm cells provide protection against pathogens due to the proteins found in the seminal fluid. Environmental factors such as climate and diet affect the timing of sexual maturation (Peng et al. 2016). Successful mating ends with the death of the drones, because the drones die soon after mating due to the ejaculation of sperm with great force, and the endophallus remains in the genital tract of the queen. Drones do not have a long proboscis, mouth structure, corbicula, and stingers, which are found in worker bees to collect and transport nutrients. Much of the research examining drone biology and health has been on reproductive quality and ability. Since the only role of drones in the hive is related to reproduction and although they are in the hive temporarily, they have an important function in mating with their queen. The only function of drones in a honey bee colony is to gather hundreds of drones from many different colonies in the air (in drone gathering areas) and mate with the queen bee in flight. It is clear that drones with large, high flight capacity and good maneuverability have a competitive advantage. Thus, a honeybee colony can actually increase reproductive success by producing higher quality and competitive males for reproduction (Winston 1987).

When the colony is without a queen, the worker bees lay unfertilized eggs in the cells of the comb, as the pheromone pressure that prevents the development of the ovaries of the worker bees is removed. These eggs are not drone cells but worker bee cells. The drones grown in these honeycomb cells are smaller. Goins & Schneider (2013) investigated the effects of drones reared in drone cells (DC) and drones reared in worker cells (WC) on potential reproductive quality and caste interactions. Although worker bees initially observed different size and quality potential within the two drone types, they stated that this distinction was not related to acceptance decisions. Utaipanon et al. (2019) showed that drones reared in worker honeybee cells also contribute to mating.

Drone brood production depends on pollen supply to meet protein needs. Colonies regulate drone production according to season and food availability (Hrassnigg & Crailsheim 2005). Optimal drone production depends on climate, colony conditions (size and queen age) and food availability (Boes 2010). Regular removal of drones from a colony helps to regulate drone production. In the Northern Hemisphere, drone production is typically concentrated between May and August. However, the production of drones is ultimately determined by the number of drone honeycomb cells in the colony (Boes 2010). During the drone breeding season, beekeepers can place empty frames in the colony to stimulate the production of drone broods. Removing drone larvae from these combs where Varroa spp. mites laying their eggs can help control their population in the hive. This method has been found to be effective in managing *Varroa* spp. infestations, which can be detrimental to the colony's health (Calderone 2005).

1.2. Functional components of drone larvae

A drone larva (Apilarnil) is obtained by collecting drone larvae 3 to 11 days after hatching. This valuable bee product was discovered by the Romanian scientist Nicolae V. Iliesiu. This name consists of “api” for bee, “lar” for larva and “nil” which is a shortened form of explorer name. However, while drone larvae are collected from the honeycomb cell, they can be collected together with the larval food (drone milk) or it can be obtained by eliminating the larval food. Drone jelly (DM) is a highly nutritious food produced by the hypopharyngeal and mandibular glands of worker bees. It serves as the primary food source for developing drone larvae, and its composition is believed to be linked to the reproductive abilities of drones. While not as well researched as other honeybee products such as honey and royal jelly, drone royal jelly has gained attention in recent years for its potential health benefits. In addition to its nutritional value, drone milk has been shown to have antimicrobial and immunomodulatory properties that may be beneficial in the treatment of various human diseases. Understanding the chemical composition and biological properties of male royal jelly is an important area of research and could lead to the development of new therapeutic agents (Mutsaers et al. 2005).

Larva homogenate has the consistency of creamy milk. Its colour can vary from white to yellowish and it has a distinctive slightly acidic taste (Isidorov 2021). Storage and shelf life information is required for food products. Since apilarnil contains important nutrients and is in a natural state, it is a suitable environment for the development of microorganisms and fungi. Therefore, it can deteriorate under inappropriate storage conditions. The most convenient way to stabilize unstable moist media is lyophilization. Larvae can be stored for 6 days at -2 ºC and up to 10 months at -18 ºC without losing their biological activity (Barnutiu et al. 2013). It can be preserved for 6 months by adding 1-2% volume of honey, which is another method. Krylow et al. (2007) reported that the addition of homogenate to honey at a concentration of 3-5% preserves its biological properties for up to 6 months at 6-12 °C. Other than that, the larvae can be mixed with 40% ethyl alcohol at a ratio of 1:1 (Bak & Wilde 2002) or dried in air-circulating dryers and stored for 7 months. Using adsorbent is another method. In this method, it has been reported that the final product obtained with the combination of 1:1 glucose and lactose, larvae and adsorbent (1:6) can be stored for 3 years at room temperature and 3 months in the refrigerator (Lebiediew & Legowicz 2003). The content of main components and physicochemical properties of drone larvae are presented in Table 1 (Finke 2005; Barnutiu et al. 2013; Balkanska et al. 2014; Bogdanov 2016; Isidorov et al. 2016; Margaoan et al. 2017; Sawczuk et al. 2019; Silici 2019; Prikhodko et al. 2020; Koşum et al. 2022). The greatest differences in the physicochemical composition were reported between fresh and lyophilized homogenate in terms of water content. While the water content of fresh apilarnil is between 70.30-76.8%, it decreases to 3.0-5.0% when apilarnil is lyophilized.

Table 1- Chemical composition of drone larvae (fresh and lyophilized) (Balkanska et al. 2014; Silici 2019; Koşum et al. 2022; Margaoan et al. 2017; Barnutiu et al. 2013; Prikhodko et al. 2020; Isidorov et al. 2016; Finke 2005; Bogdanov 2016; Sawczuk et al. 2019)

Characteristics	*Drone larvae*	
	Fresh *Range (Min-Max)*	*Lyophilized* *Range (Min-Max)*
Water, %	65.0-78.5	3.0-5.0
Protein, %	4.6-13.2	32.0-52.4
Lipid, %	1.2-8.38	4.8-24.2
Carbohydrates, %	6.22-12.2	9.30-38.9
Fructose	0-0.38	-
Glucose	3.55-7.88	-
Sucrose	0-0.18	-
Ash, %	0.7-4.1	2.7-4.1
pH	5.8-6.63	7.0
Acidity, mL 0.1 NaOH g^{-1}	0.74-2.61	-
Energy value, kJ100g^{-1}	111.9-503.3	501.4-2097.9

Apilarnil is a rich source of protein and amino acids as they are the most abundant nutrients in its composition (Table 2). The protein content of drone larvae is very important for the evaluation of their nutritive properties. According to the analyzes made to date, the protein content is approximately 3.5 times higher in lyophilized apilarnil than the fresh one (Table 1). Studies of chemical analysis of apilarnil showed that relatively low molecular mass proteins consisting of globulin and albumin predominate (Lazaryan et al. 2003; Xu & Gao 2013). It was demonstrated that protein profiles depend on the bee's stages and during larval development its content increases (Ghosh et al. 2016). Comprehensive analysis proved that the total content of amino acids in brood larvae is 37.57-40.57% (Lazaryan et al. 2002). The amino acid composition was characterized by high levels of glutamic acid, valine, aspartic acid, lysine and leucine (Lazaryan et al. 2002; Isidorov 2016). Lazaryan et al. (2002) demonstrated that the content of essential amino acid to be 15.45-16.28. Among all of the amino acids reported in lyophilized apilarnil, the greatest amounts are glutamic acids, leucine, proline, arginine and aspartic acids. Also, valine and lysine are the most abundant (Ghosh et al. 2016). The amino acid lysine contributes to the absorption of calcium in the body and plays an important role in the formation of collagen in bones and connective tissues (Civitelli et al. 1992). Valine, leucine, and isoleucine are essential amino acids that play a crucial role in muscle protein synthesis and repair (Bifari & Nisoli 2017). Animal proteins are reported to have higher nutritional quality than plant-based proteins. This superiority is due to their amino acid composition, digestibility, and ability to carry nutrients such as calcium and iron (Kim et al. 2020). Food proteins are broken down into smaller peptides and individual amino acids by digestive enzymes during the process of digestion. These amino acids are absorbed and utilized for various metabolic processes, including the synthesis of tissue proteins for growth and repair. Amino acids can also be used to synthesize other nitrogen-containing compounds such as neurotransmitters and nucleotides or catabolized for energy production when needed (Atherton et al. 2010). The human body cannot synthesize essential amino acids (EAA), which include isoleucine, leucine, histidine, lysine, methionine, threonine, tryptophan, phenylalanine, and valine. Other amino acids like glycine, proline, arginine, glutamine, and serine are considered "conditionally essential" since they are more important in specific stages of life or conditions such as illness, early development, or stress. The remaining amino acids are classified as "non-essential" because the human body can synthesize them. Therefore, the only way to obtain essential amino acids is through the diet. Animal-based protein sources like meat, eggs, and milk are known as "complete sources of protein" since they contain all nine essential amino acids in sufficient amounts for the body's needs (Wu 2009). Apilarnil is an acceptable product in this group because it contains both high protein content and all nine essential amino acids.

Table 2- Amino acid composition of drone larvae (Lazaryan, 2002; Silici 2019; Ghosh et al. 2016; Fınke 2005; Isıdorov et al. 2016)

Amino acids (g/100g)	*Fresh Range (Min-Max)*	*Lyophilised Range (Min-Max)*	*Amino acids (g/100g)*	*Fresh Range (Min-Max)*	*Lyophilised Range (Min-Max)*
Valine* (Val)	0.49-1.7	0.02-2.27	Asparagine	2.6	3.5
Alanine (Ala)	0.45-1.6	0.08-1.83	Lysine* (Lys)	0.1-1.9	7.2
Glycine (Gly)	0.1-1.4	0.1-1.66	Methionine* (Met)	0.15-2.0	0.5
Leucine* (Leu)	0.43-2.5	0.06-4.0	Tyrosine (Tyr)	0.32-1.5	0.2-2.02
Isoleucine* (Ileu)	0.25-1.6	0.2-2.02	Histidine* (His)	0.18-0.7	0.2-0.99
Proline (Pro)	0.3-2.15	2.0-8.8	Tryptophane*	0.09	0.2
Threonine*(Thr)	0.31-1.6	1.30	Arginine (Arg)	0.4-1.6	3.0
Serine (Ser)	0.21-1.4	0.04-1.61	Cysteine	0.1-0.3	-
Aspartic acid (Asp)	0.76	3.57	Asparaginic acid	0.04	-
Phenylanalanine* (Phe)	0.17-0.33	0.1-1.84	Taurine	0.08	-
Glutamic acid	0.25-1.29	0.1-5.6	Phosphoserine	0.12	-

*: Indicates essential amino acid for human

Proteins are followed by carbohydrates. Among analyzed carbohydrates in apilarnil, fructose and sucrose are at very low levels compared to glucose. Fructose, glucose, sucrose, turanose, maltose, trehalose and isolmaltose contents of fresh apilarnil were determined as 0.6, 3.61,0.14, 0.05, 0.33, 0.44, and 0.11, respectively (Barnitiu et al. 2013). Apilarnil is a rich source of carbohydrates (Balkanska et al. 2014) and is composed of glucose trehalose and glycogen (Lipinski et al. 2008). The carbohydrate level is related to the developmental stage of the larvae. For example, the glycogen level is highest in the youngest larvae (92.2 mg/kg) and decrease by 50% by the fourth day (Lipinksi et al. 2008). The glucose level is low throughout the entire developmental period of the drone. According to Barnutiu et al. (2013) the concentration of glucose in fresh homogenate is 3.61 g/100g. Furthermore, other mono and disaccharides such as α and β-glucopyranose, β-fructofuranose, turanose, glucitol, and maltose have been identified in drone larvae (Barnutiu et al. 2013; Isidorov et al. 2016).

According to the analysis results obtained from the research; the lipid level of apilarnil is approximately 3 times higher in lyophilized form than in fresh one (Table 1). It is an important group of nutrients consisting of lipids, free fatty acids, tri-di- and monoacylglycerols, sterols, phospholipids, and vitamins. Especially the composition and content of fatty acids have an important effect on the functional properties of foods. Alpha linoleic acid (omega 3) and linoleic acid (omega 6) are known as essential fatty acids. The analysis results provided show that drone larvae are a rich source of lipids and fatty acids. (Calder 2015) and lipid contents include free fatty acids, sterols, triacylglycerols, and phospholipids (Isidorov et al. 2016). Fatty acids are energy sources and cell membrane components. It can affect numerous cell characteristics such as metabolism, gene expression, hormone sensitivity and production of biologically active substances. Therefore, they can affect people's health, physiological function, well-being, and disease risk. They are also a source of energy (Calder 2015). The main lipids found in apilarnil include free fatty acids, triglycerides, fatty acid esters and decanoic acids. Apilarnil is rich in fatty acids. Palmitic and stearic acids, which make up almost 50% of the fatty acid content, are unsaturated fatty acids. Apilarnil is also a source of polyunsaturated fatty acids of which linoleic acid (Table 3). In an analysis with GCMS-RI, the oleic acid content of fresh apilarnil was 64.75% and the palmitic acid content was 26.08%, while the saturated fatty acid content was 34.35% and the unsaturated fatty acid content was 65.26% (Koşum et al. 2022). In studies on the fatty acid content of drone larvae, oleic acid and palimitic acid content were determined as 47.5% and 37.3% (Ghosh et al. 2016), and Prikhodko et al. (2020) determined 28.2% and 27.5%. They determined that the basic fatty acids were oleic, palmitic and stearic acids, while the saturated fatty acids were 51.75% and the unsaturated fatty acid level is 46.25%. Otherwise, Ghosh et al. (2016) in oven-dried larvae, lauric acid, myristic acid, palmitic acid, and stearic acid contents were found as 15.5, 116.6, 1844 (37.3%), 584.9 mg/100g, respectively, while hexadecanoic acid and oleic acid contents were 35.1 and 2346.1 (47.5%) detected. Palmitic acid, stearic acid and myristic acid are the most common fatty acids in the diet. Saturated fatty acids are synthesized de novo in humans. The precursor to these fatty acids is acetyl CoA, which is produced in carbohydrate or amino acid metabolism. Saturated fatty acids (lauric, myristic and palmitic acid) in the diet increase LDL concentration, coagulation, inflammation and insulin resistance. Oleic acid is the most common monounsaturated fatty acid (MUFA). Its consumption causes a decrease in low-density lipoprotein (LDL) cholesterol and an increase in high-density lipoprotein (HDL) cholesterol (Morlok 2010). Linoleic acid is an essential fatty acid that lowers blood cholesterol, has an important place in brain development function and skin barrier (Calder 2015). In addition, chemical analyses showed the presence of plant sterols in apilarnil. These sterols are campesterol (5.5 mg/100 g), beta-sitosterol (1.3 mg/100 g), 5-hydroxysitosterol (1.3 mg/100 g), and stigmasterol (0.2 mg/100 g) (Kedzia & Kedzia 2017).

Table 3- Fatty acid content of Apilarnil (Finke 2005; Ghosh et al. 2016)

Fatty acids (mg/100g)	*Range (Min.-Max.)*	*Fatty acids (mg/100g)*	*Range (Min.-Max.)*
Lauric acid (C12:0)	0.2-28.2	Hexadecanoic acid (C16:1)	0.2-72.3
Myrsitic acid (C14:0)	1.2-379.3	Oleic acid (C18:1)	18.2-4701.8
Palmitic acid (C16:0)	14.7-4847.7	Eicosenoic acid (C20:1)	0.1-7.3
Stearic acid (C18:0)	4.3-1277.9	Myristoleic acid (C14:1	2.4-3.1
Arachidic acid (C20:0)	0.2-46.8	Linoleic acid (C18:2)	0.3-46.6
Behenic acid (C22:0)	0.1-16.9	Lİnolenic acid (C18:3)	0.4-153.0
Capric acid (C10:0)	2.0	Eicosadienoic acid (20:2)	0.1
Heptadecanoic acid (C17:0)	4.2-4.3		

Ash analysis provides information on the total mineral content in a food sample. While the ash content was 0.7-4.1 in fresh apilarnil, it was 2.1-4.1 mg/100g in lyophilized samples. Apilarnil is also rich in mineral and vitamin content. When the analyses made to date are evaluated, it is seen that apilarnil contains the highest amount of potassium (140-656 mg/100g) according to the maximum detection limits. The second element's sulfur content is (392.37 mg/100 g). It is followed by magnesium, phosphorus, zinc and calcium. These elements were detected in the range of 20-424, 179-330, 1.5-225.2, and 13.8-139.5 mg/100g, respectively. Na, Fe, Cu and Se contents of other elements analyzed in fresh apilarnil were found in the range of 6.45-106, 1.17-3.2, 0.29-2.4, and 0.01-0.06 mg/100 g, respectively. Minerals are needed for maintaining health and normal functioning of the body. Macrominerals such as Na, Cl, K, Ca, P, Mg, S and trace minerals needed in much smaller amounts are required for normal functions such as cell division, cell metabolism and growth, intracellular K concentration, DNA synthesis, optimal enzyme function and acid-base balance. necessary for cell functions. Magnesium is a cofactor of many enzymes. It is also necessary for synthesis (protein, RNA and DNA), energy metabolism, and maintenance of the electrical potential of cell membranes and nerve tissues in the human body. Selenium is a mineral that protects against the harmful effects of free radicals during stress, infections and tissue injuries. Zinc, which is the basic component of many enzymes, has a function in the synthesis and breakdown of lipids, carbohydrates, proteins and nucleic acids. It is involved in the molecular structure of cellular components and membranes and has an important role in the immune system. Phosphate is an essential component of cell membranes, bones and nucleic acids. It is required in intracellular signalling, cellular energy metabolism, and oxygen release from hemoglobin (Food & Nutrition Board 1997; 2002) Another important mineral is calcium, an element that plays a vital role in blood coagulation, neuromuscular function, and provides hardness to the skeleton together with phosphate salts (Food & Nutrition Board 1997).

Vitamins are needed for a normal and healthy metabolism, their deficiency can lead to serious diseases and even death. Apilarnil is rich in fat and water-soluble vitamins. Data on the vitamin and mineral content of apilarnil are summarized in Table 4. The analyzes have reported that the vitamin A content of drone larvae is 0.01-0.05 mg/100 g. Vitamin A (retinol) is a vitamin that is needed in small amounts. Beta carotene is the main source of provitamin A in the diet. It is necessary for the maintenance of vision and immune function, growth and development, epithelial cell integrity and reproduction. The vitamin D content of apilarnil has been reported to be between 0.03-0.9 mg/kg. Vitamin D is needed in all cells of the body for muscle contraction, mineralization of bone, nerve conduction, and maintenance of blood calcium and phosphate levels (Nordin 1976). Fat-soluble vitamin E, which we can only get through diet, is the main antioxidant in the cell (Food & Nutrition Board 2000). Vitamin C is a water-soluble vitamin that plays an important role in the antioxidant system (Sies 1993). Vitamin E content of apilarnil has been reported as 0.4-1.6 mg/kg. Among the B complex vitamins, the highest amount of B vitamin detected is choline (44.3-68.1 mg/100 g). It was followed by nicotinic acid (Vit B3), riboflavin (Vit B2), pantothenic acid (Vit B5), thiamin (Vit B1) and pyridoxine (Vit B6) and biotin. The need for vitamins and minerals in human nutrition has been determined for water-soluble (vitamin C; 45 mg/day, thiamine; 1.1-1.2 mg/day, riboflavin; 1.1-1.3 mg/day, niacin; 14-16 /NE/day, vitamin B6; 1.3 mg/day, pantothenate; 5mg/day, Vit B12; 2.4 µg/day, folate; 400 µgDFE/day) and fat-soluble vitamins (Vitamin A 500-600 µgRE/day, Vitamin D 5-10 µg/day, Vitamin E is 7.5 mg alpha-TE/day, Vitamin K is 55 µg/day) (WHO 2004). Considering these values, it can be said that apilarnil is an important source of vitamins and minerals.

Table 4- Vitamin and mineral contents in drone larvae (Hu & Li 2001; Bogdanov 2016; Hyrniewicka et al. 2016; Sidor et al. 2021; Prikhodko et al. 2020)

	fresh	*lyophilised*		*fresh*	*lyophilised*
Minerals mg/100g	***Range (Min-Max)***	***Range (Min-Max)***	***Vitamins mg/100g***	***Range (Min-Max)***	***Range (Min-Max)***
Calcium (Ca)	13.8-139.5	556	Retinol (Vitamin A)	0.01-0.05	0.31-14.70
Phosphorus (P)	179-330	-	Beta-carotene (provit A)	0.02-0.9	0.940
Magnesium (Mg)	20-424	126.4	Calciferol (Vitamin D)	0.39-0.6	-
Sodium (Na)	6.45-106	424	α-tocopherol (Vitamin E)	0.4-8	0.53-24.10
Potassium (K)	140-656	-	Thiamin (Vit B1)	0.58-4.1	2.320
Iron (Fe)	1.17-3.2	-	Riboflavin (Vit B2)	0.95-9.1	3.824
Zinc (Zn)	1.5-225.2	900.4	Pantothenic acid (Vit B5)	2.6-13.4	13.396
Manganese (Mn)	0.06-4.4	2.4	Pyridoxine (vit B6)	0.05-1.2	0.220
Copper (Cu)	0.29-2.4	15.2	Choline (Vit B4)	44.3-68.1	-
Selenium (Se)	0.01-0.06	-	Nicotinic acid (Vit B3)	0.06-15.8	0.256
S	392.37	-	Vitamin C	4.02	-
Cr	0.01	-	Coenzyme Q10	2.0	-
Al	0.39	-	Niacin	36.7	-
Cd	0.003	-	Biotine	0.23	-
Pb	0.003	-	Choline	1.68	-
			Ascorbic acid (mg/g)		0.65-3.36
			Coenzyme Q10	20	0.03-114

Scientific research shows that reactive free radicals play a role in many diseases such as heart disease, diabetes and cancer. The cell contains potentially oxidizable substrates such as proteins, fatty acids and DNA (Sies 1993). Therefore, the antioxidant defense system protects it from the harmful effects of free radicals, which are normally produced endogenously in the cell, as well as pollutants and exogenous species such as cigarette smoke. If exposure to free radicals, called oxidative stress, exceeds the protective capacity of the antioxidant defense system, damage to biological molecules may occur. Consuming foods with antioxidant activity that can potentially eliminate or neutralize free radicals may play an important role in disease prevention. Lipophilic molecules such as alpha-tocopherol, retinol and coenzyme Q10 are antioxidants with basic regulatory and metabolic functions in living organism cells. Vitamin E, an endogenous antioxidant, protects lipids in the cell membrane against peroxidation. Along with vitamin E, vitamin A and beta-carotene also protect against oxidation. Another cellular antioxidant is Coenzyme Q10 which acts as an electron carrier in the mitochondrial respiratory chain (Sies 1993). Hryniewicka et al. (2016) determined the content of alpha-tocopherol and coenzyme Q10 in honey bee-derived animal products such as royal jelly, bee bread and drone larvae homogenates by liquid chromatography-tandem mass spectrometry (LC/MS/MS). They found that apilarnil is a rich source of coenzyme Q10. The drone homogenate contained only 8±1 μg/g α-tocopherol and 20±2 μg/g coenzyme Q10.

In addition, there are studies on the bioactivity of Apilarnil. In one of these studies, total phenolic content was 14.35 mgGAE/100 g, DPPH value was 4.93 SC50 mg/mL, ABTS 35.89% and FRAP 0.59 mmol/100 g (Kurtdede & Sevim 2022). In this study, in which the antioxidant capacity of honey, bee pollen, bee bread and apilarnil was evaluated, honey showed the highest antioxidant capacity among the products tested. Bioactivity of a total of 139 apilarnils, 3 times a season, was analyzed from 7 different apilaries in Poland for 3 years. Among the antioxidant tests, DPPH analysis was determined in the range of 2.5-80.40%. In another study, the DPPH value was found to be between 9.2-16.36% in two different antioxidant activity tests, and the FRAP test was 0.80-1.16 (μmolTE/100g), while the total phenolic content was found to be 23462-268.84 mgGAE/100g (Sidor et al. 2021). Haber et al. (2019) reported that the powder of honey bee larvae has high antioxidant activity and polyphenol content.

Apilarnil is a natural substance produced by honeybee larvae, which contains a range of hormones. These hormones include testosterone, which is a male sex hormone, as well as female sex hormones such as estradiol, progesterone, and prolactin. These hormones are important for the development and maintenance of reproductive functions in both males and females, and they have been used in traditional medicine for their potential health benefits (Burmistrova 1999; Budnikowa 2009; Bolatovna et al. 2015). It is known that sex hormones such as testosterone, progesterone and estrogen play important roles in various physiological processes besides reproductive functions and the formation of secondary sex characteristics (Rider & Abdou 2001; Roof & Hall 2000). Testosterone is produced in the ovaries and testicles. Androgens have important roles in muscle development, bone density, production of red blood cells, maturation during puberty, libido and sexual function in both men and women. It also has roles such as regulating menstruation and preventing osteoporosis in women. The other sex hormone, progesterone, receptors have been identified in the brain, cortical and subcortical regions (Woolley & McEwen 1993). This hormone has neuroprotective effects and is effective in promoting nerve regeneration and myelination (Schumacher et al. 2014). Estradiol is an effective hormone in the modulation of neurotransmitter synthesis, release and metabolism, while prolactin is a hormone responsible for breast tissue development and milk production (Glasier et al. 1984; Barth et al. 2015). The content of the hormone of drone larvae is presented in Table 5. Fresh drone homogenate was found to contain 0.31 nmol/100g testosterone, 51.3 nmol/100g progesterone, 410 nmol/100g prolactin and 677.6 nmol/100g estradiol Budnikowa (2009) revealed the dynamics of sex hormones from larva to pupa, while five-day-old larvae contained 8.2 nmol/l testosterone and 2745 nmol/l, 15-17-day-old pupae contained 15.6 nmol/l testosterone and 343.5 nmol/l estradiol. Drone larvae were found to have more pronounced gonadotropic activity than royal jelly. It has the highest amounts of estradiol and prolactin while the lowest levels of testosterone. The testosterone content of apilarnil is about 0.03 nmol/mL has been reported.

Table 5- Hormone content of apilarnil (Sidor et al. 2021; Bogdanov 2016)

	Fresh	Lyophilised
Hormones (nmol/100g)	**Range (Min-Max)**	**Range (Min-Max)**
Testosterone (nmol/100 g)	0.31-1.10	0.04
Estradiol (nmol/100g)	653.70-680.26	100.3
Progesterone	51.32	8.1
Prolactine	410	79.8

Table 6 of a study shows the enzyme composition of drone larvae. The amylase enzyme, which breaks down starch into glucose, is responsible for amylolytic activity. High amylase activity was found in drone brood based on the study's results (Sidor et al. 2021).

Table 6- Enzyme content of drone larvae* (Sidor et al. 2021)

Enzyme(U/100g w/w)	*Range (Min-Max)*	*Enzyme*	*Range (Min-Max)*
α-D-Glucosidase	47.0-47.8	β- D-Manosidase	0.6-1.0
β- D-Glucosidase	2.2-3.9	N-acetyl-D-hexomaminidase	59.0-68.3
α-D-Galactosidase	0.4-1.4	Alkaline phosphatase	0.1-1.2
β- D- Galactosidase	1.6-2.4	Acid phosphatase	1.6-3.4
α-D-Mannosidase	21.0-22.8	alpha-amylase (U7g)	14.0-17.60

*; 7 days old larvae (n=3)

Semiochemicals (chemical signaling molecules) are naturally produced substances that enable interaction between organisms. Zhang et al. (2019) reported that the only volatile chemical (E)-β-Okimene released by *A. mellifera* larvae. Furthermore, 2 and 3 methyl diacetyl, nonanal, dimethyl sulfide and okimen were determined as active odor compounds. In honey bees (*Apis mellifera*), methylpalmitate (MP), methyloleate (MO), methylololol (ML) and methyl linoleate (MLN) are important pheromone components that trigger the behavior of bees (Haber et al. 2019) Qin et al. (2019) compared these four pheromone components in larvae of worker and drone bees before and after glazing of the comb cells. It was determined that MP, MO and MLN levels were preserved in the closing phase of the eyes and ML was at the highest level in worker bee larvae in glazed cells. They reported that the sum of the four pheromone components increased with aging in worker bee larvae as well as in drone larvae.

1.3. Pharmaceutical activity of drone larvae

Studies conducted to date on the therapeutic activities of apilarnil; estrogenic and androgenic effects, antioxidant capacity, protecting testicular damage, reducing sexual dysfunction, protecting testicular toxicity and liver injury, neurprotective effect, stimulating immune system, antiatherosclreotic activity, etc. (Figure 1).

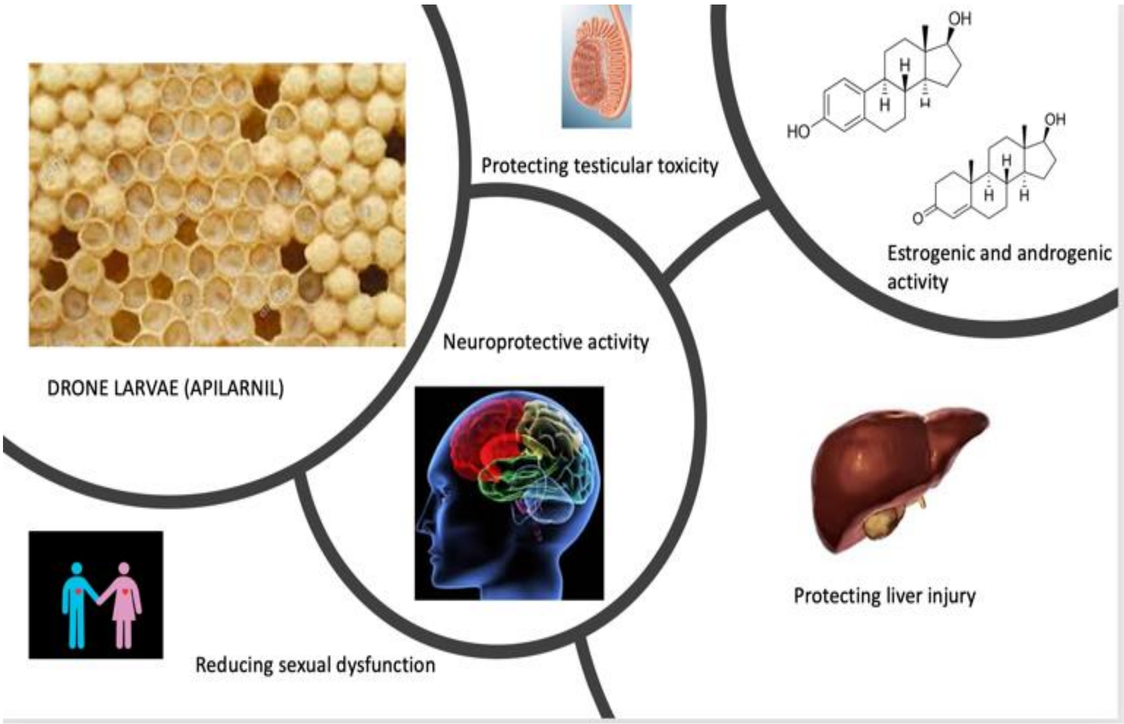

Figure 1- Biological activities of drone larvae (apilarnil)

Androgens have an anabolic effect on skeletal muscle and bone. They increase calcium binding to bones and accelerate protein production. Androgens, together with growth hormone (GH), are the most important endocrine factors affecting bone anabolism. Estrogens are steroid hormones. They are synthesized largely from cholesterol, but also from acetyl CoA. Progesterone and testosterone are synthesized first. All of the testosterone and most of the progesterone are then converted to estrogens in the granulosa cells. Estrogen in most mammalian tissues; has important roles in the growth of cells, embryological development and continuity of life (Fadini et al. 2009). Recent studies have shown that drone larvae have both estrogenic and androgenic effects. Seres et al. (2014) found that DM exhibits significant sexual hormonal effects in rats. Drone milk displayed marked androgenic activity in castrated male rats. They identified the compounds methyl oleate and methyl palmitate which are responsible for its androgenic effect. In a study of the estrogenic effect of apilarnil, this effect was attributed to E-dec-2-enedioic acid (Seres et al. 2013). Another study on drone milk by Seres et al. (2014b) showed that the combination of drone milk and spironolactone has a potent gestagenic effect.

Anabolic substances change the metabolism in the direction of increasing the formation of muscle mass and bone tissue, and in the direction of consumption of fat stores. It has been determined that the administration of drone brood homogenate to pigs affects the hormonal status and increases the growth rate (Zdorovyeva et al. 2018). The addition of drone brood homogenate (25 mg/kg feed) to the pig diet showed an anabolic effect and significantly stimulated the growth rate of the animals (Boryayev et al. 2017). In another study, drone homogenate improved the characteristics of the ejaculate, and had a stimulating effect on the reproductive function of rams (Shoinbayeva 2017). Kosum et al. (2022) showed that drone larvae provide sexual function restoration for Saanen male goat kids. In addition, it was reported that testicular growth and an increase in the production of androgen hormone were obtained in the study. In a study on pigs, it was shown that the supplementation with drone brood homogenate stimulates the early stages of folliculogenesis in gilts, but provokes atresia of follicular development (Kistanova et al. 2020). In male and female broilers, apilarnil did not have a positive effect on growth performance, but it reduced blood glucose and cholesterol levels (Altan et al. 2013). An increase in testicular weight, and testosterone level has been shown to stimulate sexual maturation in the early stages. It was found that dietary apilarnil did not have a positive effect on growth performance in male and female chickens, and apilarnil did not show an anabolic effect.

It is reported that eighty million people worldwide are affected by the inability to have children. It has been reported that the high amount of free oxygen products (ROS), such as hydrogen peroxide (H_2O_2), nitric oxide (NO), and peroxynitrite, in spermatozoa, is associated with male infertility. In scientific studies, it has been determined that many antioxidants improve sperm quality and prevent sperm damage; such as vitamins E and C, coenzyme Q10, glutathione, folic acid, zinc and selenium, (Agarwal & Sekhon 2010). Coenzyme Q10 has been shown to protect the cell membrane against oxidative stress (Bentinger et al. 2010), folic acid plays a role in DNA synthesis and scavenges free radicals (Joshi et al. 2001). It has been reported that lycopene, vitamins A, C and E are antioxidants used to improve sperm quality, and vitamin E, for example, significantly reduces DNA fragmentation rates and improves sperm quality (Ebisch et al. 2007; Rolf et al. 2009). In recent studies, it has been determined that n-3 PUFA deficiency in the diet affects spermatids (Roqueta-Rivera et al. 2011). Nutrition affects sperm quantity, semen quality, and fertility status (Stevermer et al. 1961). Protein quality is highly dependent on amino acid content and amino acid bioavailability (Kim et al. 2009). Different amino acid ratios have significant effects on reproductive performance (Ren et al. 2015). In studies on different experimental and farm animals; dietary lysine (Lys) has been shown to improve semen quality from 0.86% to 1.03% (Rupanova 2007). The amino acid composition of seminal plasma significantly affects sperm motility. Li et al. (2003) determined that the addition of amino acids proline, glutamine and glycine) improved sperm membrane and acrosome integrity as well as sperm motility in monkey semen. Research findings suggest that apilarnil has the potential to function as a natural stimulant in the animal endocrine system, which could be beneficial for restoring and improving male sexual desire (Vakina et al. 2020). A preparation containing drone larvae homogenate has been utilized to regulate androgenic activity in females (Elistratov et al. 2017). Androgen deficiency syndrome causes a decrease in the development of the penis and testicles at an early age and prevents puberty. In young people, gynecomastia causes weakness in facial, body or pubic hair and voice development, while in adults, it causes problems such as mood changes, decreased muscle strength, increase in body fat, decreased libido, difficulty in erection, low sperm volume and gynecomastia. Doganyigit et al. (2019) in their study, which tested the protective effect of apilarnil on endotoxic shock, reported that apilarnil reduced testicular damage caused by LPS and this effect was due to the antioxidant capacity of apilarnil.

The biological activity of apilarnil is not limited to its effects on the reproductive system. Doganyigit et al. (2019) reported that apilarnil administered in rats prevented lipopolysaccharide (LPS)-induced liver damage by inhibiting the TLR4/ HMGB-1/ NF-kB signaling pathway. In addition, apilarnil showed protective effect against DNA damage and oxidative stress caused by LPS (Doganyigit et al. 2020). In another study, apilarnil promoted potential renoprotective effects, by the modulation of important markers of the local immune response in the model of LPS-induced sepsis (Inandiklioglu et al. 2021). Similar experimental model, tumor necrosis factor-alpha (TNF-alpha) and brain natriuretic peptide (BNP) expressions were significantly increased in the LPS group, and co-administration of LPS and apilarnil suppressed increased expression levels (Okan et al. 2022). Furthermore, apilarnil increased the activity of the autophagy pathway and showed potential positive effects by providing a significant decrease in protein expression increased by LPS (Doganyigit et al. 2020b).

Vasilenko et al. (2002) demonstrated that the administration of lyophilized apilarnil decreased cholesterol and triglyceride levels. They also demonstrated its hepatoprotective activity and stimulated the immune system. The administration of drone homogenate (Apilarnil) to mutant mice with hereditary hemolytic anemia resulted in a significant increase in the survival rates of the experimental animals. (Andritou et al. 2012). Hamamci et al. (2020) demonstrated the neuroprotective potential of apilarnil.

In another animal experiment, apilarnil proved to have a significant effect on energy production. Animals fed apilarnil have been shown to have greater resistance to fatigue and improve vital parameters. It caused an increase in glycogen depletion and synthesis of glucocorticoid hormones. In another study, it was shown that apilarnil is a powerful energizer, has a strong catabolic effect and stimulates the oxidative process (Kogalniceanu et al. 2010). Apilarnil is used in beverages and in the prophylaxis and treatment of fatigue (Trifonov et al. 2014).

2. Conclusions

Drone larvae (Apilarnil), is a little-known honey bee product rich in nutrients and exhibits many healing and therapeutic properties. It has been used as a cheap, safe and effective natural food against different diseases. Its high protein and essential amino acid content, fatty acid composition, vitamin and mineral richness, and hormonal content differentiate it from among other bee products with apilarnil. The biological and therapeutic activities of drone larvae have been confirmed by performing laboratory and animal/human in vivo experiments. In addition to the many biological activities of this product, it is thought that it may lead to important developments in the future, especially in the field of infertility.

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