TOXIC RISKS ASSOCIATED WITH APITHERAPY PRODUCTS

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1. Introduction

Alternative medical therapies include a broad spectrum of practices and beliefs. Biologic-based practices (dietary therapy, herbal medicine, and dietary supplements – nutraceuticals) are one of the broadest categories, relying on the use of chemical substances or dietary alterations to promote healing. Apitherapy is a form of Complementary and Alternative Medicine that uses honeybee products, such as bees’ pollen, honey, royal jelly, propolis, beeswax, and bee venom, for therapeutic purposes (Diehl and Eisenberg, 2000). The history of honeybee products use for medicinal applications dates back thousands of years. Today, honey is classified as a functional food. Scientific research demonstrated the antimicrobial, anti-inflammatory, and antioxidant potential of the product, investigating its efficiency in wound healing (Oryan et al., 2016), hepatic and renal protection against different aggressive agents (eq. CCl4) (El-Haskoury et al., 2018), gastrointestinal protection (Mundo et al., 2004; Nasuti et al., 2006) and immunostimulatory effect (Ota et al., 2019). Propolis is a complex resinous substance collected by bees, also containing salivary secretions and enzymes, and it is used for protection against invading insects and microorganisms and in beehives repair. It is a rich source of essential elements (Ca, Mg, Fe, Cu, Zn, Mn, Ni), vitamins, and phenolic compounds (caffeic acid phenethyl ester being one of the most studied compounds found in propolis), but the composition varies according to specific flora at the site of collection (Rufatto et al., 2017). Scientific data revealed that propolis exhibits antibacterial, antifungal, anti-inflammatory, and cytotoxic activities (Rufatto et al., 2017; Dobrowolski et al., 1991). Bee pollen is a product that results from the agglutination of flower pollens with nectar, combined with salivary bees’ secretions. It represents a good source of bioactive substances and energy and also possesses many therapeutic and protective effects: antimicrobial, antifungal, antioxidant, anti-inflammatory, anti-carcinogenic, anti-allergic, hepatoprotective,
improving the cardiovascular and digestive systems, immunity booster, and aging delaying (Pascoal et al., 2014; Huang et al., 2017; Li et al., 2018). Royal jelly is a honeybee secretion product used to feed the larvae and adult queens. Studies have indicated antioxidant, anti-inflammatory, anti-hepatotoxic, and anticancer activities for royal jelly (Pasupuleti et al., 2017). There is substantial scientific data to sustain the use of apitherapy as a prophylactic tool, as well as treatment for several medical conditions, but one very important aspect that needs to be assessed is the safety of these products. Quality parameters must be imposed for all nutraceuticals and dietary supplements.

2. Contaminants and toxic compounds in honeybee products

2.1. Phytotoxins

The composition of honeybee products varies greatly depending on the flora at the site of collection. Some secondary plant metabolites (pyrrolizidine alkaloids, grayanotoxins, hyoscyamine, hyoscine, saponin, strychnine, gelsemine, tutin, hyenanchin, oleandrin, and oleandrigenin) can be transferred to honey and related products, leading to toxic effects in humans (Figure 1) (Grigoryan, 2016).

Pyrrolizidine alkaloids are one of the most common natural toxins, being identified in over 6000 plants. They possess genotoxic and hepatotoxic effects. Contaminated honey is a possible source of intoxication. The ingested dose is usually not high enough to cause acute poisoning, but a long-time consumption of low doses of pyrrolizidine alkaloids can cause liver fibrosis, pulmonary arterial hypertension, somatic mutation, and liver cancer. Fetuses and neonates are more susceptible to pyrrolizidine alkaloids poisoning, even at extremely low levels (Zhu et al., 2018).

Tutin is a plant-derived neurotoxin, responsible for many intoxication cases associated with honey consumption; clinical signs include nausea, headache, vomiting, dizziness, and in severe cases seizures and coma. These cases are common in New Zealand, where Coriaria arborea (tutu), which is known to contain tutin, grows (Fields et al., 2014).

Grayanotoxins are often found in “mad honey”, produced by bees from the nectar of Rhododendron ponticum, a member of the Ericaceae family, which is used in indigenous medicine, especially in the treatment of hypertension and sexual dysfunctions. This phenomenon was known from ancient times (Xenophon recorded the intoxication of Greek soldiers stationed on the Black Sea coast), but its prevalence is still high, especially in Turkey, where “mad honey” can be purchased in local markets. The common symptoms of intoxication are dizziness, bradycardia, nausea, vomiting, presyncope and syncope, blurred vision, hypotension, and fainting (Silici et al., 2015; Demircan et al., 2009). Besides “mad honey poisoning” described in Turkey, Nepal, and Korea, and “Tutin honey poisoning” described in New Zealand, there is another type of honey poisoning caused by Tripterygium wilfordii Hook F. It occurs mainly in southwestern China, and it is characterized by multiple-organ damage and high mortality. The toxic potential of the plant is mainly related to the presence of triptolide – a highly toxic substance – whose major target is the kidney, acute renal failure being the main cause of death (Zhang et al., 2016; Zhang et al., 2017).

![Figure 1. Phytotoxins commonly found in honeybee products](image_url)
2.2. Toxic elements – Heavy metals and metalloids

Heavy metals pollution is a serious problem nowadays around the world, affecting the quality of the atmosphere and waters and also posing a threat to the health and life of human beings and animals via the food chain (Aghamirlou et al., 2015).

The content of toxic elements (heavy metals and metalloids – Table 1) in honeybee products depends on many factors: floral source (some plant species can selectively accumulate toxic elements), environmental contamination and conditions (geo-climatic conditions, geochemical characteristics of the soil, anthropogenic activities – eq. chemical industries in the vicinity) and production methods. Therefore, bees and their products can often serve as bio-indicators for heavy metal contamination. Many studies on this matter were conducted and the results showed significant differences in element concentrations between honey products of different botanical origins and from different geographic areas (Czipa et al., 2015; Bilandžić et al., 2017). Two of the most frequently studied metals are lead and cadmium. Lead originates mainly from motor traffic, contaminates the air, and then nectar, and honeydew. It is not generally transported by plants, unlike cadmium, which is transported from the soil to plants, also contaminating nectar and honeydew. Lead pollution is expected to diminish, due to the reduction of toxic car emissions worldwide, associated with the introduction of car-engine catalysts (Bogdanov, 2006).

The quantities of heavy metals accumulated in different apiculture products can vary. Honey usually has a lower degree of contamination than other products, probably due to bees’ “filtering” capacity, but lead residues found in propolis are often high, indicating that the harvest area should not be placed near heavy traffic roads (Bogdanov, 2006).

Table 1. Toxic elements identified in honeybee products

<table>
<thead>
<tr>
<th>Toxic element</th>
<th>Toxic effects</th>
<th>References</th>
</tr>
</thead>
</table>
| Pb            | • neurotoxicity  
               • gastrointestinal and renal dysfunctions | Aldgini et al., 2019; Ru et al., 2013; Aljedani, 2017; Talk Gajger et al., 2016; Kieliszek et al., 2018 |
| Cd            | • gastrointestinal and renal dysfunctions  
               • osteomalacia and osteoporosis  
               • carcinogenic effects | Aldgini et al., 2019; Ru et al., 2013; Aljedani, 2017; Kieliszek et al., 2018 |
| Hg            | • gastrointestinal, hepatic and renal damage  
               • neurotoxicity | Aghamirlou et al., 2015; Ru et al., 2013; Talk Gajger et al., 2016; Kieliszek et al., 2018 |
| Al            | • toxic effects on the central nervous, skeletal and hematopoietic systems | Bilandžić et al., 2017 |
| Cr            | • carcinogenic and teratogenic effects  
               • nephrotoxic and hepatotoxic effects | Harmanescu et al., 2007 |
| Ni            | • sensitization and allergic contact dermatitis  
               • hepatotoxic and nephrotoxic | Bilandžić et al., 2017; Aldgini et al., 2019 |
| As            | • carcinogen (skin, kidney, lung, bladder, and liver cancers) | Aldgini et al., 2019; Talk Gajger et al., 2016; Kieliszek et al., 2018; Fiorentini et al., 2019 |
| Ba            | • acute toxicity: low potassium levels, cardiac arrhythmia, gastrointestinal dysfunction or paralysis, muscle twitching, and high blood pressure  
               • chronic exposure: kidney damage, respiratory failure, the development of neurodegenerative diseases, including multiple sclerosis | Bilandžić et al., 2017 |
| Be            | • carcinogen to humans | Bilandžić et al., 2017 |
| Sb            | • acute cardiac toxicity and myocarditis  
               • prolonged skin contact – dermatitis kidney and liver damage, vomiting | Bilandžić et al., 2017 |
2.3. Pesticides

Pesticides are indispensable for today’s agriculture, increasing crop productivity and minimizing losses due to uncontrollable pests, but exposure to pesticides has several negative consequences for all living beings, including humans. Pesticide residues present in the environment, but mostly in food products, can cause skin rashes, respiratory disorders (asthma attacks); chronic exposure is often associated with cancer, neurological and reproductive dysfunctions, and birth defects (Gil et al., 2016).

Bee products can also be a source of pesticide exposure, due to both environmental pollution (honeybees are exposed by consumption of contaminated pollen and water or by contact with plants and soil) and wrong beekeeping practices (administration of pesticides and antibiotics, in order to control hive infestations).

Organohalogen, organophosphates, organonitrogens, pyrethroids, carbamates are examples of pesticide residues most frequently reported in honey and related products (Figure 2), in countries like Brazil, Turkey, Spain, Colombia, China, France, Portugal, and India (López et al., 2014). Organophosphates and organohalogens occupy the first positions in terms of detection frequency. Although organochlorine pesticides (eq. lindane) have been prohibited by law for decades in most countries, their residues are still present as pollutants in water, soil, air, and food products, due to their high persistence. Organochlorine pesticides are lipophilic substances, soluble and stable in beeswax, and an amount of these substances gradually migrates from wax into the stored honey (Gawel et al., 2019). Bee pollen samples are also a good indicator for environmental monitoring, many studies being focused on quantifying pesticide residues in pollen samples (de Oliveira et al., 2016). However, the distribution of pesticides in different apiculture products is heterogeneous. There are studies indicating that, in some cases, pesticides, especially herbicides, can contaminate bees and pollen and rarely appear in honey. This effect probably appears due to the filtering capacity of bees (Fléché et al., 1997). Similar results were obtained when pesticide levels in wax, pollen, and honey were compared. Beeswax was the most contaminated hive compartment regarding quantities of pesticides detected (Jan et al., 1993; Calatayud-Vernich et al., 2018). Some authors also argued whether honey consumption represents an important source of pesticide exposure and a threat to human health, contributing substantially to the daily intake of pesticides, based on the consumed quantities and the degree of contamination, which is generally very low. However, different national regulations have established maximum concentrations of pesticide residues permitted in honey and related products, but the lack of homogeneity causes problems in international trade. The maximum limits of pesticide residues in honey are not included in the Codex Alimentarius (Grigoryan, 2016; Al-Waili et al., 2012). Maximum residue limits (MRL) were also established in the European Union for several pesticides used in agricultural and beekeeping practices (Table 2).

In the EU, an action level of 0.01 mg/kg is often considered for pesticides with no fixed MRL (***, 2005).

It is important to control bees’ exposure to pesticides because bees end up inhabiting a toxic hive, exposed to different pesticides cocktails, and their health is seriously affected. Another concern is related to the safety of bee products consumers. In this context, Yuan et al. investigated the photodegradation phenomenon of organophosphorus pesticides in honey medium, concluding that photodegradation could become an accepted method for organophosphorus pesticides removal from honey (Yuan et al., 2014).
Table 2. Maximum levels of pesticide residues for honey and other apiculture products – Regulation (EC) No 396/2005

<table>
<thead>
<tr>
<th>No</th>
<th>Pesticide</th>
<th>MRL (mg/kg)</th>
<th>No</th>
<th>Pesticide</th>
<th>MRL (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Asulam</td>
<td>0.05*</td>
<td>20</td>
<td>Fenpyroximate</td>
<td>0.01*</td>
</tr>
<tr>
<td>2</td>
<td>Azoxystrobin</td>
<td>0.05*</td>
<td>21</td>
<td>Fipronil</td>
<td>0.01</td>
</tr>
<tr>
<td>3</td>
<td>Bicyclopyrone</td>
<td>0.05*</td>
<td>22</td>
<td>Fluopyram</td>
<td>0.05*</td>
</tr>
<tr>
<td>4</td>
<td>Boscalid</td>
<td>0.5</td>
<td>23</td>
<td>Fluoxastrobine</td>
<td>0.01*</td>
</tr>
<tr>
<td>5</td>
<td>Bromopropylate</td>
<td>0.1</td>
<td>24</td>
<td>Flutolamid</td>
<td>0.02*</td>
</tr>
<tr>
<td>6</td>
<td>Cabendazin + Benonyl</td>
<td>1</td>
<td>25</td>
<td>Fosetyl</td>
<td>0.5*</td>
</tr>
<tr>
<td>7</td>
<td>Chlordane</td>
<td>0.01</td>
<td>26</td>
<td>Fosetyl-Al</td>
<td>0.5*</td>
</tr>
<tr>
<td>8</td>
<td>Chloromequat</td>
<td>0.05*</td>
<td>27</td>
<td>Gibberellic acid</td>
<td>0.1</td>
</tr>
<tr>
<td>9</td>
<td>Cyprodinil</td>
<td>0.05*</td>
<td>28</td>
<td>Haloxyfop</td>
<td>0.05</td>
</tr>
<tr>
<td>10</td>
<td>DDT</td>
<td>0.05</td>
<td>29</td>
<td>Heptachlor</td>
<td>0.01</td>
</tr>
<tr>
<td>11</td>
<td>Diclofop</td>
<td>0.01*</td>
<td>30</td>
<td>Isoprothiolide</td>
<td>0.05*</td>
</tr>
<tr>
<td>12</td>
<td>Difenconazole</td>
<td>0.05*</td>
<td>31</td>
<td>Isopyrazam</td>
<td>0.05*</td>
</tr>
<tr>
<td>13</td>
<td>Endosulfan</td>
<td>0.01*</td>
<td>32</td>
<td>Lindane</td>
<td>0.01*</td>
</tr>
<tr>
<td>14</td>
<td>Endrin</td>
<td>0.01</td>
<td>33</td>
<td>Mepiquat</td>
<td>0.05*</td>
</tr>
<tr>
<td>15</td>
<td>Epoxiconazole</td>
<td>0.05</td>
<td>34</td>
<td>Oxamyl</td>
<td>0.05*</td>
</tr>
<tr>
<td>16</td>
<td>Etofenprox</td>
<td>0.05</td>
<td>35</td>
<td>Prothioconazole</td>
<td>0.05*</td>
</tr>
<tr>
<td>17</td>
<td>Fenoxaprop-P</td>
<td>0.05</td>
<td>36</td>
<td>Spinetoram</td>
<td>0.05*</td>
</tr>
<tr>
<td>18</td>
<td>Fenpropidin</td>
<td>0.02*</td>
<td>37</td>
<td>Trifloxystrobin</td>
<td>0.05*</td>
</tr>
<tr>
<td>19</td>
<td>Fenpropimorph</td>
<td>0.05*</td>
<td>38</td>
<td>Triflumezopyrim</td>
<td>0.05*</td>
</tr>
</tbody>
</table>

Figure 2. Common pesticides found in bee products

2.4. Residues of veterinary medicinal products – Antibiotic residues

Exposure of the general population to antibiotics can have negative effects, like allergenic reactions, or even contributing to the expansion of the increased bacterial resistance phenomenon. Therefore, the use of antibiotics is forbidden in beekeeping practice in the EU, due to the risk of antibiotic residues in honey. There are many studies regarding the monitoring of antibiotic residues in honey. Although EU directive 2377/90 states that honey should be free of antibiotic contamination, research in this domain revealed that many analyzed samples of honey contained traces of antibiotics, generally used against bacterial plant pests. Typically found antibiotics residues include streptomycin, sulphonamides, chloramphenicol, macrolides,
tetracycline, streptomycin, and nitrofurans (Grigoryan, 2016; Al-Waili et al., 2012).

2.5. 5-Hydroxymethylfurfural

5-Hydroxymethylfurfural is an indicator of the poor quality of food products, and it is usually formed during the heating or preservation of honey. There are several factors that favor the formation of 5-hydroxymethylfurfural: use of metallic containers for storage, physicochemical properties of honey (e.g. acidity), humidity, and thermal and photochemical stress. Due to the mutagenic, carcinogenic, and cytotoxic potential, the amount of 5-hydroxymethylfurfural is limited by EU regulations to 40 mg/kg (exceptions: 80 mg/kg in honey from regions with tropical temperatures, and 15 mg/kg in honey with low enzyme levels) (***, 2001). Ample research was performed in this direction, investigating the level of 5-hydroxymethylfurfural in honey samples from different countries (like Spain or The Czech Republic), the results indicating that honey is unlikely to exceed the 40 mg/kg limit unless it is mishandled, commercial honey-processing methods (e.g. heating above 15°C) being usually incriminated for the appearance of undesirable compounds, including 5-hydroxymethylfurfural (Grigoryan, 2016).

2.6. Genetically modified organisms (GMOs)

The appearance of genetically modified plants, grown in some countries, has raised new concerns about the impact of this phenomenon on apiculture, in terms of the marketability of bee products. In the European Union, it is compulsory to notify the consumers if the GMO content in food is above 1%. Pollen is the most affected bee product, because it contains genetic material, while honey contains only a small percentage of pollen (less than 0.1% pollen, if the honey is sieved) and probably will not require any specific appellations (Grigoryan, 2016; Bogdanov, 2006).

2.7. Microbiological contaminants and mycotoxins

In terms of microbiological contaminants, bacterial risks associated with honey consumption are usually low, but honey has been identified as a dietary risk factor for infant botulism. Clostridium botulinum is a Gram-positive, anaerobic, spore-forming bacterium that produces a neurotoxin. Honey samples can contain Clostridium botulinum spores, but botulinum toxin is not found in honey, because the natural antimicrobial potential and the high osmotic pressure prevent spores’ germination. However, the spores can colonize the undeveloped gut of infants, leading to infant botulism, a rare neuroparalytic disease that can occur among babies under 1 year of age (Grigoryan, 2016).

Aside from C. botulinum, mold, yeasts, and other spore-forming bacteria can be found in honey. The main sources of contamination are the environment and not respecting good manufacturing practices, contamination with fungi and bacteria usually indicates inadequate hygiene conditions during collection, processing, and storage (Grigoryan, 2016; Bogdanov, 2006). Mycotoxins are toxic compounds produced by fungi that colonize different nutritional substrates. Bee products represent an adequate medium for the development of fungi, thus being subjected to the risk of accumulating mycotoxins. Among mycotoxins, aflatoxins and ochratoxin A are the most common, being involved in both acute and chronic intoxications (González et al., 2005).

Aflatoxins, produced by species of Aspergillus, are hepatotoxic, teratogenic, mutagenic, and carcinogenic mycotoxins. Ochratoxin A, also produced by species of Aspergillus (eq. Aspergillus ochraceus), is nephrotoxic, hepatotoxic, teratogenic, and immunotoxic, being associated with fatal endemic human nephropathies (González et al., 2005). Unprocessed honey is a poor medium for synthesis of mycotoxins, and it is relatively safe, from this point of view (Martins et al., 2003;
Eissa et al., 2014), but bee pollen was found to be a substrate that stimulates ochratoxin A production by Aspergillus ochraceus. The levels of mycotoxin found in the incubation medium containing bee pollen was significantly higher than in corn, wheat, and rice grains (Medina et al., 2004). González G et al. analyzed the presence of mycotoxins and mycotoxin-producing fungi in bee pollen and stressed the importance of application of good manufacturing practices, sanitation procedures, and analysis of risks and critical control points to honeybee products (González et al., 2005).

3. Conclusions
The benefits associated with the consumption of honey and other related bee products are unquestionable. As highlighted above, scientific research revealed antimicrobial, anti-inflammatory, antioxidant, immunostimulatory activities for these products, promoting general health and well-being. Despite these considerations, it is also important to point out the possibility of contamination with several toxic compounds, which can lead to grave outcomes in some cases. If for honey, the most popular bee product, intensely consumed around the world, quality criteria are usually clearly specified, when it comes to other related products, many quality regulation gaps can be found. Another problematic issue is represented by the existence of different national standards for honey produced and commercialized in different parts of the world (European countries, the United States, Canada, Australia, and India have separate standards), making difficult the elaboration of general guidelines for quality assessment.

4. References


elements in Hungarian honeys. *Food Chemistry*, 175, 536-42.


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