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Importance of Pyrrolizidine Alkaloids in Bee Products

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ABSTRACT

Pyrrolizidine alkaloids are one of the groups of harmful chemicals of plants, which are natural toxins. Pyrrolizidine alkaloids found in about 3% of all flowering plants of widespread geographical distribution are known as one of the components of the hepatotoxic group of plant origin and referred as hepatotoxic pyrrolizidine alkaloids. According to researches, bee products is regarded as one of the main food sources in the exposure of people to pyrrolizidine alkaloids. Consumption of pyrrolizidine alkaloids containing bee products, such as honey, pollen, propolis and royal jelly, is a potential threat to human health, especially for infant and fetuses. Besides the acute toxic effects, the genotoxic effects and tumorigenicity potential of pyrrolizidine alkaloids was demonstrated. This manuscript gives an overview about bee products containing pyrrolizidine alkaloids and toxification processes in humans.

Key words: Pyrrolizidine alkaloids, honey, pollen, bee products, human health.

Introduction

Plants which are primary producers are defenseless to consumers and they have evolved toxic substances as a defense system for protection. Honey is a complex mixture of these numerous substances. One such group of toxic substance that threaten public health like grayanotoxin is pyrrolizidine alkaloids (PAs) [1, 2]. Indicating widespread distribution, PAs are secondary metabolites of plants and presumedly the most important natural toxins affecting livestock and humans. Mainly located in the *Fabaceae* (genus *Crotalaria*), *Boraginaceae* (all genera) and *Asteraceae* (tribes *Senecioneae* and *Eupatorieae*) families, PAs are estimated to be present in approximately 6000 plant species, over 350 PAs have been identified. The flowering and the seeds portion of the plants contains toxins at the highest rate [3]. Despite their rich variety they all share a common general molecule formula which composed of a necine base esterified to one or more necic acids. Taking into account chemosystematics and biogenetical analysis, almost all pyrrolizidine alkaloids can be classified into five different large structural types (Fig. 1) [4].

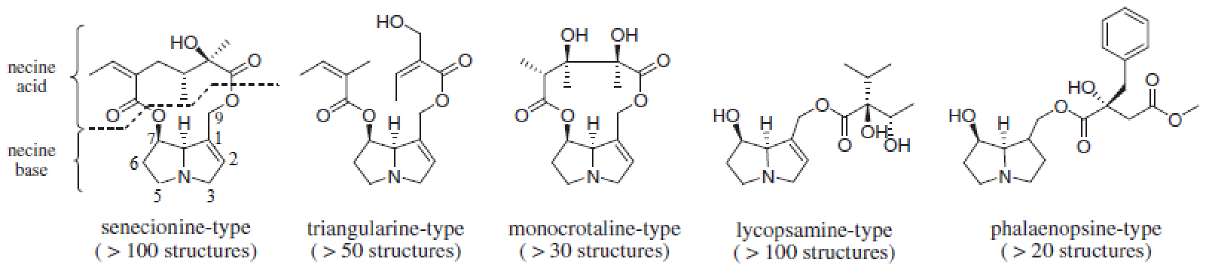

Figure 1. Major structural types of naturally occurring plant PAs [4].

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As with the majority of toxic chemicals, PAs also require metabolic activation to show their toxicity. However, the presence of a carbon-carbon double bond in 1,2-unsaturated position of the necine base is a pre-requisite for toxicity. An ester functionality in location C-7 or C-9 or both positions is also required. If these requirements are met, PA structures can converted into 6,7-dihydropyrrolizine ester metabolites (pyrroles), mainly by liver cytochrome P-450 monooxygenases located primarily in hepatocytes. These pyrroles are initial toxic metabolites and highly electrophilic compounds that irreversibly and readily react with negatively charged nucleophilic functional groups on proteins, DNA or amino acids to form adducts or are hydrolysed to produce more stable 6,7-dihydropyrrolizine alcohols. Alcohols are common and ultimate toxic metabolites, and are thought to play an important role in PAs toxicity [5]. PAs are known to cause damage primarily in the liver, lungs and blood vessels, secondary in the kidney, gastrointestinal tract, pancreas and bone marrow. According to cases of poisoning it is seen that especially children and neonates are affected to PAs intoxication due to their high sensitivity [6].

Among the bee products, one of the most important source of dietary exposure to PAs is honey. According to published risk assessments when PAs containing-honeys are regularly consumed about 15–25 g which are recommended serving sizes or at higher consumption levels, acute and chronic toxicity may occur in risk group of humans. Genotoxicity, carcinogenicity and teratogenic effects were also seen due to the consumption of contaminated foods with PAs [5, 7].

The detection of pyrrolizidine alkaloids in a sensitive and reliable manner is of great importance in terms of public health. Chromatographic methods used in determination of PAs are Thin-layer chromatography, Gas chromatography, Gas chromatography–mass spectrometry (GC–MS), High-performance liquid chromatography (HPLC) and High-performance liquid chromatography-mass spectrometry (LC–MS). However HPLC is more preferred since the determination of both the free base form and the *N*-oxides can be done more practically [8].

Pyrrolizidine Alkaloids in Honey

Beehives are placed around the most efficient flowering plants. While it is expected that the nominated floral sources represent these plants, other flowering plants in the area also contribute to the honey produced. This situation causes a variation in the level of pollen from the nominated source in the honey and results in the transfer of pollen of undesirable plants, such as PAs containing plants, into the honey. On the other hand apiarists utilize voluntarily a series of PAs-containing plants for honey production in many countries. These plants consist of plant species of *Echium, Senecio, Eupatorium, Heliotropium, Borago, Myosotis, Chromolaena, Petasites, Ageratum, Cynoglosum, Crotalaria, Tussilago* and *Symphytum* [5, 9].

In a study conducted by Betteridge et al. [10], nine floral honeys were analyzed and five of which were attributed to *Echium vulgare.* As published by Beales et al. [11], 63 honey samples which attributed to floral sources known to produce pyrrolizidine alkaloids were analyzed. As a result of the analysis 13 samples were *Echium plantagineum*, 9 samples were *Echium plantagineum* mix, 4 samples were *Heliotropium amplexicaule*, 2 samples were *Heliotropium europaeum* and 35 samples were attributed to floral sources with no known association with PAs. Levels of pyrrolizidine alkaloids differ from 800 to 2000 ppb in the samples. There were no pyrrolizidine alkaloids in 30% of the samples. Moreover five retail samples of honey were analyzed. The two are derived from bees foraging on *Eucryphia lucida* and *Echium vulgare*, respectively. According to Dubecke et al. [12] a total of 3917 honey samples were analyzed for PAs which were found in 66% of the raw honeys and in 94% of retail honeys available in supermarkets. In a study by Griffin et al. [13], the 50 retail samples surveyed were categorized by their origin. Eight samples tested positive for one or two PAs, predominantly *lycopsamine* and *echimidine*. Detected PAs concentrations ranged from 182 to 4078 µg/kg. In a study researched by Martinello et al. [3], the amounts of PAs that consumers are exposed to were investigated whether it is dangerous to humans or not. The nine PAs were determined simultaneously in 70 retail honey samples purchased in local supermarkets. 64% of honey samples were found positive for at least one of the PAs. The concentrations detected ranged from 1 to 169 mg PAs/kg. This study reveals that many samples tested would exceed the tolerable daily intake suggested for these substances and they could be a hazard to human health. According to Orantes-Bermejo et al. [14] the incidence and concentration of PAs from *Echium spp*. have been defined in 103 Spanish honey samples. PAs were found in 94.2% of the raw honey samples analyzed, in the range of 1 to 237 µg/kg. The study conducted by Bodi et al. [15] comprised the analysis of 87 honeys which originate from German/Austrian beekeepers, brand products, discount products and other forms of sale in the Berlin retail market. The total PAs concentration ranged from 6.1 to 14 µg/kg honey in samples from beekeepers and discount products, while 15 µg/kg in branded honeys. According to EFSA (European Food Safety Authority) report [16], 14604 honey samples were analyzed for PAs. 13280 of the samples are concerned bulk honey which has been found to be positive in terms of PAs, predominantly *echiumine* (71%), followed by *echimidine* (56%) and *lycopsamine* (49%). For the samples of which 1324 covered retail honey, the situation was slightly different in terms of positive PAs, predominantly *echiumine* (45%), followed by *lycopsamine* (36%) and *echimidine* (16 %).

Pyrrolizidine Alkaloids in Other Bee Products

Pyrrolizidine alkaloids in pollen

Most of plants produced pyrrolizidine alkaloids does not affect the pollinators. This situation raises the idea that nectar of the plants does not contain PAs or contain only small concentrations of PAs. This rationale allowed the hypothesis that PAs in honey originate not from nectar but from pollen [1]. In a study conducted by Boppre et al. [9] pure pollen collected from the anthers of *Echium vulgare* were investigated in terms of PAs and detected 8000 to 14000 ppm. Kempf et al. [17] investigated the question whether honey filtration method can be reduced the level of PAs or not. But there is showed no decrease in PAs levels. It is appeared that when pollen grains come into contact with nectar, PAs rapidly transfer from pollen to honey due to the fact that PAs are very soluble in water. The study by Boppre et al. [18] freshly collected pollen from *Senecio ovatus*, *Senecio jacobaea*, *Echium plantagineum* and *Eupatorium cannabinum*, and pollen loads from bees that foraged on those plants was examined in terms of PAs. In PAs levels of collected samples from pollen loads lower than the PAs level (8000-14000 µg) of pure pollen collected directly from plants. The difference is based on the dilution effect of regurgitate fluid used excreting pollen from their honey stomachs by nectar collecting bees.

Pollen collected by honeybees is consumed as food supplements for its nutritional and health benefits. According to Kempf et al. [19] 55 commercially available pollen products were investigated in terms of PAs. 31% of samples were found PAs positive which are range from 1.08 to 16.35 mg/g. In another study 119 pollen samples collected by honeybees were analyzed for PAs. 60% of the samples contained PAs [12].

Pyrrolizidine alkaloids in products containing honey and bee products

In recent years, there has been a steadily growing number of published data on PAs in honey and pollen due to the use of honey and pollen as ingredients in food processing. In the survey, several food types containing honey between 5-37%, which are mead, candy, fennel honey, soft drinks, power bars and cereals, jelly babies, baby food, supplements and fruit sauce were investigated. Positive samples are mead, candy and fennel honey [17]. A study conducted by Cao et al. [20] has investigated the PAs level in mead, or honey wine. In this study 1 kg of honey and two bottle of mead made from the honey, have been analyzed. While PAs level of honey is found 780 ng/g, level of PAs in meads are measured 236 ng/ml and 540 ng/ml.

Honey and bee pollen are not only consumed for breakfast but also used in alternative medical treatments. In recent years, there has been an increasing interest in antibacterial use of honey for wound treatment [1]. Several recent studies demonstrated that honey for human consumption was contaminated with natural occurring, plant derived pyrrolizidine alkaloids. In a study by Cramer and Beuerle [21] 19 different medical honey samples were analyzed for PAs. All medical honeys, except one, were found PAs positive (from 10.6 to 494.5 µg/kg).

According to EFSA report [22], a total of 29 samples of food supplements containing bee products are analyzed for the presence of PAs. The samples consist of pollen, propolis and royal jelly which are 12 samples, 9 samples and 8 samples, respectively. The samples were collected in supermarkets, retail shops and internet in France, Germany, Greece, Italy, the Netherlands and Spain. While concentrations of PAs were found 576.0 µg/kg in 11 of the 12 pollen products, 0.6 µg/kg and 15.5 µg/kg are quantified in propolis and royal jelly products. Bee products mainly contained PAs of the *lycopsamine*-type.

Toxicity of Pyrrolizidine Alkaloids

Toxicity caused by pyrrolizidine alkaloids has become more common in developed countries because of the increase in interest in alternative medicine methods in the last 25 years, while it pose a problem in developing countries in previous years. Using of herbal products containing PAs increased the number of fatal intoxication in the European Union (EU), The United Kingdom (UK) and The United States Of America (USA) [23]. After the consumption of PAs containing products, toxic effects begin to appear and toxicity of PAs occur via cytochrome P450 [5]. It is know that PAs causes damage primarily in the liver, lungs and blood vessels; secondary in the kidney, gastrointestinal tract, pancreas and bone marrow. Depending on the amount of intake PAs poisoning in humans occurs in three forms: acute, sub-acute and chronic. Acute toxicity has been observed in very rare cases. It is often occurred in newborns and infants due to their high sensitivity. This is characterized by ascites, hepatomegaly and hemorrhagic necrosis. Death occurs as a result of liver failure. Sub-acute poisoning is characterized by endothelial proliferation and medial hypertrophy which causing obstruction of the hepatic veins and resulting in hepatic sinusoidal obstruction syndrome (VOD). Furthermore hepatomegaly and recurrent ascites is also seen in this forms. VOD is proceed with epigastric pain associated with abdominal swelling originated from ascites. Other organs may be also affected by the PAs as well as liver. The pyrrolic metabolites can

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pass from the liver into pulmonary arterioles where they can produce damage similar to the VOD-changes in the liver. The first changes seen in pulmonary vessels are thrombus in vessels, acute inflammation and thickening of the vessel wall causing obstruction. These effects lead to hypertension inter alveolar and septal fibrosis. As a result of weakening of pulmonary blood flow, the operation of the right ventricle increases. This situation leads to the hypertrophy and congestive heart failure eventually. It is estimated that the pulmonary damage are observed in the low dose exposure for prolonged periods [4, 6, 24]. Although there are a few cases which recorded exposure level varied between acute and chronic levels in people exposed to PAs, there are no reports of cancer associated with such exposures by far. But the carcinogenic potential of some pyrrolizidine alkaloids has been proven in rodents, and the National Toxicology Program recently has accepted *riddelliine* as a human carcinogen. Some plant species are known to cause cancer in rodents: *S. longilobus, Petasites japanicus Maxim, Tussilago farfara L., Symphytum officinale, Farfugium japonicum, Ligularia dentata* and *S. cannabifolis* [7, 24].

PAs intoxication in humans is not only related to exposure time and amount of taken, but also age and sex are important. Men are more susceptible than women. Fetuses and children (especially newborns and infants) show the highest sensitivity. There are some cases about PAs intoxications. For example a pregnant woman consumed herbal spices contaminated with PAs about 7 mg daily, during her pregnancy in 2003. While it is not seen any toxic effects in the mother's liver, newborns died 2 days later after he born due to damage in the fetal liver. The toxic compounds leads to the formation of hepatic failure in the fetus through the placenta due to the highly lipophilic properties of this compounds. Another VOD case has been reported in newborn baby of pregnant woman who consume brewed herbal tea with leaves of *T. farfara* containing 0.6 mg/kg *senecionine* [6, 24, 25]. However none of the cases of toxicity in humans are associated with PAs toxicity based on honey consumption despite the statistics [5].

Tolerable Levels of Pyrrolizidine Alkaloids

Although some cases of acute intoxication in humans have been reported after consumption of high doses of PAs containing foods, the amount of cases is still not enough to suggest whether how much concentrations of PAs ingestion would pose a health risk to consumers. Rarity of information on pollen markets and lack of chemical screening of commercial bee product samples currently makes risk assessments uncertain. Because of that there is no legal limit about the concentration of PAs in bee products. However there are several countries which have regulations about the amount of PAs in some other food products or food supplements [26, 27].

According to Germany the Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung – BfR) both acute and chronic toxic effects of PAs have been taken into account. Therefore ingestion of PAs containing foods is to be kept as low as possible [27]. In 1992 The German Federal Ministry of Health established regulations about the sale of herbal products containing PAs. While use of herbal products containing PAs which have no demonstrated health benefits are banned by the German regulations, the dose of herbal products which provide some health benefits is restricted to 1μg of PAs per day for oral administration or 100 μg of PAs per day for external use. However such use is applied just for a maximum of six weeks per year. If the herbal medicine is to be taken for longer than 6 weeks per year, the allowed dose for pyrrolizidine alkaloids is reduced to 0.1 μg per daily for oral use or 10 μg for external use. On the other hand The German regulations have mandated a zero tolerance for PAs containing herbal products by women in the lactation period and pregnant women. They also require prescribed level and legal tag which is “Not to be used in pregnancy and during the lactation period” for all PAs containing herbal products [28].

The Netherlands Food and Consumer Product Safety Authority (NVWA) advise limit value of 1 µg/kg for consumption of PAs. The same limit value also carries out to beverages containing herbal extracts contaminated with PAs, such as soft drinks or sweets containing, as well as herbal teas and food supplements [29]. According the assessment published by the Australian New Zealand Food Authority (ANZFA) it is stated that hepatocellular injury, liver cirrhosis and VOD are the major effects in humans, but information related to the formation of cancer causing PAs ingestion remains insufficient. Therefore, according to available human data, the PAs level which does not cause VOD is stated about 10 µg/kg. Thus the ANZFA proposed a provisional tolerable daily intake of 1 µg/kg for PAs [30]. On the other hand in Belgium, use of *Borago officinalis* plant in foods and food supplements was accepted if the final product does not contain PAs [3].

The investigations are stated that consumption rates of *echimidine* and related alkaloids (equivalent to 9 µg *heliotrine*/kg per day) above 15 µg/kg per day over a period of four to six months may cause acute or sub-acute liver disease. Therefore International Programme on Chemical Safety of The World Health Organization (WHO-IPCS) conclude that a dose equivalent to 10 µg *heliotrine*/kg per day may lead to disease in humans. In terms of equivalent doses of *heliotrine*, the total doses in the known outbreaks or cases of VOD, nonfatal cases and fatal cases are estimated to range from 1000 to 167,000 µg/kg, 1000 to 120,000 µg/kg and 6000 to 167,000 µg/kg, respectively [16].

Conclusion

Honey is known as natural food with high nutritional value and safe for human consumption. In addition to providing nutritional benefits of honey, it is also vital importance in terms of medical terms. Bee pollen which is another bee product is consumed as a positive food support recently. Although it has been known that hazardous levels of PAs may be presented in honey, importance of using PAs containing honey and pollen as food additives is not yet fully comprehended. Recent chemoecological research has shown that pollen from PAs producing plants contains very high levels of PAs and it carries significant health risks for consumers if this pollen is consumed as a health food supplement; the possibility that PAs in honey originate from contaminating pollen is also suggested by this finding. Consumers who take pollen based food supplements should be aware that such products can contain PAs in higher concentrations and regular consumption of them would pose a health risk to consumers. When such products are mainly consumed by children and pregnant women, a dangerous situation emerges. Because studies have revealed that fetuses and children show the highest sensitivity. Multidisciplinary action is required at international level; however, it should avoid creating public concern about honey and bee products.

Arı Ürünlerinde Bulunan Pirolizidin Alkaloidlerinin Önemi

Öz: Pirolizidin alkaloidleri bitkilerde bulunan doğal toksinlerdir ve zararlı bitki kimyasalları gruplarından birini oluşturmaktadır. Dünyadaki çiçekli bitkilerin %3’ünde bulunan bu alkaloidler bitki orijinli komponentlerin en hepatotoksik gruplarından biri olarak bilinirler ve hepatotoksik pirolizidin alkaloidleri olarak adlandırılırlar. Yapılan

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araştırmalara göre, arı ürünleri insanların pirolizidin alkaloidine maruz kalmasında temel gıda kaynaklarından biri olarak kabul edilmektedir. Bal, polen, propolis ve arı sütü gibi pirolizidin alkaloidi içeren arı ürünlerinin tüketilmesi başta fetuslar ve yenidoğan bebekler olmak üzere insanlar için sağlık tehlikesi oluşturmaktadır. Pirolizidin alkaloidlerinin akut toksik etkilerinin yanı sıra genotoksik ve tümörejenik etkilerinin olma potansiyelleri olduğu da bildirilmiştir. Bu makale, arı ürünlerinde bulunan pirolizidin alkaloidleri ve bunların insanlarda meydana getiridiği toksisite ile ilgili genel bilgi vermektedir.

Anahtar kelimeler: Pirolizidin alkaloidleri, bal, polen, arı ürünleri, insan sağlığı

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