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Natural plant toxins in honey: An ignored threat to human health

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Abbreviations: ABT, aminobenzotriazole; APCI, atmospheric pressure chemical ionization; ATR-FTIR, attenuated total internal reflectance Fourier transform infrared; CH, *Coriaria arborea* honey; CYP, cytochrome P450; DHP, dehydropyrrolizidine; dSPE, dispersive solid-phase extraction; ESI, electrospray ionization; GC, gas chromatography; GlyR, glycine receptor; GTX, grayanotoxin; HGE, 14-(R)-hydroxy-gelsenicine; HPLC, high-performance liquid chromatography; HR, high resolution; HSOS, hepatic sinusoid occlusion syndrome; IR, infrared radiation; LC, liquid chromatography; LOD, limit of detection; LOQ, limit of quantitation; MRLs, maximum residue limits; MS, mass spectrometer; NMR, nuclear magnetic resonance; PAs, pyrrolizidine alkaloids; PM, *Polygonum multiflorum*; Q-TOF, quadrupole time-of-flight; QuEChERS, quick-easy-cheap-effective-rugged-safe; RH, Rhododendron honey; RSD, relative standard deviation; SPE, solid-phase extraction; SRM, selected reaction monitoring; TwHf, *Tripterygium wilfordii* Hook f.; UHPLC, ultrahigh-performance liquid chromatography; UV, ultraviolet detector.

ABSTRACT

Consumers often believe that "natural food" is harmless, however naturally occurring toxins in food represent a health risk to humans. Honey as a natural, nutritious sweetener, is one of the most commonly consumed foods throughout the world. However, food safety concerns for honey arise when honeybees collect nectar from poisonous plants such as *Rhododendron* sp., *Coriaria arborea,* and *Tripterygium wilfordii* Hook F. Such honey contains natural plant toxins. Humans may develop intoxication symptoms after consuming toxic honey; in some cases, it can be fatal. As a result, toxic honey poses an often-ignored threat to public health. Typical plant toxins such as grayanotoxins, triptolides, tutin and pyrrolizidine alkaloids, have been identified in toxic honey. Although different toxic honeys elicit similar symptoms, such as vomiting, nausea, and dizziness, the mechanism of toxicity may be different. Thus, it is necessary to determine the exact toxicity mechanism of different toxins to further develop effective antidotes and cures. Another important challenge is preventing toxic honey from entering the food chain. Liquid chromatography-mass spectrometry has a wide range of applications in the detection of different toxins due to its accuracy and simplicity. More methods, however, are urgently needed to detect multiple plant-derived toxins in honey and its derivatives. Developing uniform international standards for toxin detection during quarantine using advanced techniques is critical for preventing human consumption of toxic honey.

KEYWORDS: Honey, Plant toxins, Human health, Toxicological mechanisms, Detection technologies, Metabolomics

1. Introduction

Honey is consumed on a daily basis throughout the world as a nutritious, natural sweetener, and in some cases as a potent nutraceutical (Misirlioglu et al., 2003; Molan, 2001). It contains a variety of vitamins, minerals, and polyphenolic compounds, that provide myriad health benefits via antimicrobial, antioxidant, and anti-inflammatory activities. Honey's characteristics are closely related to the specific local environment and botanical origins (Soares et al., 2017; EI-Sofany et al., 2020). Some high-quality honeys, such as Manuka honey from New Zealand, can sell a premium price and contribute to the economies of local communities worldwide. Global production and consumption of honey are increasing year after year; significant efforts to ensure the quality and safety of honey are therefore critical to the development of the industries.

Honey has been used not only as a natural nutritive product but also as traditional medicine in ancient and modern times (Misirlioglu et al., 2003; Molan, 2001). Honey containing bioactive ingredients, such as polyphenol and methylglyoxal, is used in both clinical trials and niche health circles, which makes people usually think that 'natural food is safe'. However, it has been frequently reported that after consuming toxic honey, people experience toxic symptoms, such as nausea, vomiting, dizziness or seizures, and even death in some cases. The toxic *Rhododendron* sp. is widely distributed in Turkey and Nepal (Fig. 1a and 1b). Honeybees collect toxic *Rhododendron* sp. nectar to make what is known as "mad honey" (Yang et al., 2020). Rhododendron honey (RH) is one of a typical toxic honey types. Mad honey, on the other hand, contains grayanotoxins (GTXs), which, in small doses, alleviates gastric pain, bowel disorders, hypertension and aphrodisiac properties (Silici et al., 2008). Thus, RH is used as a folk remedy in some regions. Consumption of unknown quantities of GTXs usually results in intoxication (both deseried and unwanted) and sometimes hospitalization but rarely, if ever, death (Yang et al., 2020).

Toxic honeys from *Tripterygium wilfordii* Hook. f. (TwHf), which contains triptolide are another lesser-known global market that is popular in China (Zhang et al., 2016). Triptolide is a kind of plant toxin that causes poisoning symptoms and, in some cases, death after ingestion. In extreme cases, however, this compound can be used for immunosuppressive, anti-inflammatory and anticancer therapies (Ziaei & Halaby, 2016). Minnelide, a water-soluble prodrug of triptolide, entered phase II clinical trials for pancreatic cancer treatment after completing a phase I clinical trial (Noel et al., 2017). Because it has no distinct taste, it is sometimes consumed unintentionally. These honeys may contain unknown levels of toxins due to a major lack of international regulations. Medical professionals may be unaware of the toxicity symptoms caused by these compounds, especially if the honey is not locally sourced.

Honey contains other hazards that have no health benefits but are heavily regulated. Other potential hazardous components (reviewed elsewhere) include antibiotics (Al-Waili et al., 2012), pesticides (Tette et al., 2016), heavy metals (e.g., lead (Pb), arsenic (As), and mercury (Hg) (Solayman et al., 2016)), microbial contaminants, and byproducts during honey storage/processing (e.g., 5-hydroxymethylfurfural (Shapla et al., 2018)). Toxins in honey derived from plants, pose a threat to food safety and human health, but are largely ignored. Tutin is an example of regulated plant-derived/nectar-derived honey. New Zealand has set up regulations to preserve its honey industry with a maximum permissible level of tutin in both honey and honeycomb of 0.7 mg/kg (Beasley et al., 2018). This could help with the prevention and monitoring of other plant-based toxins in honey. Pyrrolizidine alkaloids (PAs) are distributed globally and are believed to be toxicologically relevant secondary metabolites of plants. Honey was previously regarded as the main dietary source for humans, but recent evidence suggests that tea samples and herbal preparations are most likely to be contaminated by PAs (Brugnerotto et al., 2020). To avoid potential health risks, regulations on the most insidious chemicals found in toxic honey are required.

To fully understand natural toxins from in some honeys and to reduce the risk to human health, this review provides insight into the current situation of toxic honey, including *Rhododendron* sp.-derived toxic honey, *Tripterygium*-derived toxic honey, *Coriaria arborea*-derived toxic honey, and PA-contaminated honey. These four types of toxic honey account for the majority of poisoning cases and provide the most comprehensive sets of data from which to learn. The risks of these four types of honey to human health are discussed in detail, as well as the mode of action, safe levels, and antidotes. The most up-to-date detection methods for monitoring raw or retail honey samples as well as for clinical use are discussed. Future insights and recommended directions are also provided. While this review does not cover all toxic honeys, it does provide a useful framework for examining other honeys that contain plant-derived toxins. This review supports the viewpoint that honey products containing plant toxins are an underappreciated health risk that requires further attention.

2. Literature screening and data extraction

2.1. Scope of this review and reference selection

Honey is a natural sweetener that is widely used on a regular basis. When it is contaminated with plant toxins that threaten food safety, it is defined as “toxic honey”. The intake of toxic honey is a potential threat to human health. Some research papers have reported poisoning cases, biological properties and detection methods for specific types of toxic honey. However, to the best of our knowledge, a comprehensive review of toxic honey is lacking. Therefore, the focus of this review is to systematically and comprehensively summarize information on toxic honey, the risks to human health, toxicological evaluation, mechanisms of toxicity, and analytical techniques for detection and characterization of toxic honey. The study was based on firsthand information from 148 sources, with the selection procedure for

these studies represented in Fig. 2. The following selection criteria are satisfied by the majority of the publications' citations:

1. Most of the references should report on one of the four kinds of toxic honey or relevant toxins (i,e., *Rhododendron* sp.-derived toxic honey, *Tripterygium*-derived toxic honey, *Coriaria arborea*-derived toxic honey, and PA-contaminated honey). In addition, toxicological mechanisms, metabolomics and detection technologies are included in the search keywords to provide a comprehensive assessment and enrich research prospective.
2. These references need to be published in Web of Science, PubMed and Scopus indexed journals. Web pages, graduate theses and nonrefereed conference papers were excluded.
3. It should present original research findings (experimental or observational) and, or it should be a collection of literatures on the topic's background and general information.
4. We didn't set definite delineate periods for information searching to introduce the history and background knowledge, as well as to state the research activities for toxic honey for a long time. These articles from the previous decade, on the other hand, have gotten greater attention.

2.2. Data extraction

For this review, data from these articles that met all of the prior criteria were compiled. The level ranges of four different types of toxins were retrieved in this work. The risk of toxic honey including toxins to human health was also assessed using these data. All of the data for the analysis was taken from peer-reviewed journals.

3. Toxic honey and its risks to human health

3.1. Rhododendron sp.-derived toxic honey

RH, called “Deli Bal” in Turkey, is widely known as “bitter honey” because of its slightly sharp taste when consumed. It is often called “mad honey” due to its neuroactive and intoxicating effects (Gunduz et al., 2008a; Sibel et al., 2014). It has been used as an indigenous medicine in various countries for ages to alleviate ailments, such as stomachache, digestive disorders, hypertension, and sexual stimulation/dysfunction (Gunduz et al., 2007b; Harissis & Mavrofridis, 2013; Koca & Koca, 2007). An ancient report on honey intoxication by RH was reported by Xenophon (B.C. 434–354), and the report showed that nearly 10,000 soldiers experienced honey intoxication during their deployment on the Black Sea coast with the Greek Army (Gunduz et al., 2011; Kelhoffer, 2005).

The genus *Rhododendron* L. (Ericaeceae) comprises 8 subgenera with more than 800 species. Rose trees are grown naturally, especially in Turkey, Japan, Nepal, Brazil, Europe and some regions in the United States. The majority of those species, however, have been shown to be nontoxic (Bhattacharyya, 2011; Dossey, 2015). In Turkey, five *Rhododendron* sp. can make toxic honey, and the most common are *R. ponticum*, *R. flavum*, and *R. leutem* (Fig. 1a and 1b) (Gunduz et al., 2008b). Nepal tends to be an origin for RH due to the widespread use of *Rhododendron* sp. in the wild. The intoxicating, purported healing, and toxic effects are attribuated to *Rhododendron* nectar and honey which contain GTX (i.e., acetylandromedol, andromedotoxin).

To date, more than 25 GTX isoforms have been reported from *Rhododendron* sp. (Qiang et al., 2011). Among them, GTX-I, GTX-II, and GTX-III are the most common toxic diterpenoids in honey (mainly from nectar, Table 1); GTX-III generally has the highest concentration (Qiang et al., 2011; Sahin et al., 2016; Yavuz et al., 1991). Along the Black Sea coast, widespread purple-(*R. ponticum*) and yellow-flowered (*R. luteum*) rhododendrons result in honey colloquially known as ‘black poison’ and ‘yellow poison’, respectively, and are known to contain relatively high levels of GTX-I and GTX-II (Gunduz et al., 2007a). GTXs

cause neurotoxicity by interacting with sodium ion channels (as described in detail in the toxicology section). However, death is rarely reported from toxic honey, and the most severe symptoms usually disappear within 24 h.

Typical clinical symptoms relating to RH are dizziness, hypotension (low blood pressure), and bradycardia (slow heart rate), and the severity of poisoning is directly proportional to the dosage of certain GTXs eaten. Some studies have reported rare symptoms associated with higher dosage consumption, such as excessive salivation and perspiration, impaired consciousness and seizures, and atrioventricular block (impaired electrical signaling in the heart) (Jansen et al., 2012; Sogut et al., 2009; Sohn et al., 2014). The bradycardia and hypotensive symptoms observed in patients with toxic honey poisoning were studied in rats after an 800 µg/kg b.w. dose was administered (Türkmen et al., 2013). Clinical cases of RH poisoning have been reported worlwide, with the majority of cases coming from Nepal or Turkey over the last three decades (Salici & Atayoglu, 2015).

People tend to consume RH because it is considered a potential medical use for some diseases, particularly in folk remedies, and its toxicity and consequences have been widely reported (Akca & Kahveci, 2012). A study on the impact of hospitalization and the behaviors of patients toward the consumption of toxic honey after hospitalization found that 12 (75%) out of 16 patients discharged from emergency unit observation or their family members began consuming toxic honey again, regardless of their previous clinical experience (Eroğlu et al., 2013). However, 16 (88.9%) out of 18 patients who were discharged from intensive care observation or their family members stopped consuming toxic honey. The greater the clinical impact on RH consumption, the less likely patients were to consume RH for any other reason, indicating that clinical manifestation does play a psychological role in determining RH consumption after hospitalization. Due to the limited/no oversight of GTX in RH, consuming

GTX-containing RH carries the danger of causing unwanted intoxication and adverse health issues. Without increasing oversight, the risk does not outweigh the benefits.

3.2. Tripterygium-derived toxic honey

While poisoning from honey originating from *Tripterygium* species (Fig. 1c) is less commonly reported in the literature than RH, poisoning by *Tripterygium* honey has been occurring for a long time in China, and it is often fatal after heavy consumption (Zhang et al., 2016). *Tripterygium* plants are used in traditional Chinese medicine for the treatment of rheumatoid arthritis, autoimmune disorders, and cancer, but the correct dosage is critical to avoid accidental poisoning (Zhang et al., 2016; Chen et al., 2000; Matsui et al., 2008). Triptolide, a diterpenoid triepoxide, is the primary active compound found in *Tripterygium*. It is found in the pollen, roots and leaves of *Tripterygium wilfordii* and *Tripterygium hypoglaucum*, also known as thunder god vine.

The use of *Tripterygium* honey for medicine is problematic, and triptolide is thought to be the key compound responsible for liver and kidney toxicity from *T. wilfordii*; however, studies on mice revealed that other key active compounds, triptoline and alkaloids (wilforgine, wilforine, euonine and peritassine A), were not toxic at 20-fold their therapeutic dose, even when administered in combination with the cytochrome P450 (CYP) inhibitor aminobenzotriazole (ABT) (Li et al., 2015). Other compounds identified from TwHf include wilforsinine C, D, E, F, G, and H (detailed information on these active compounds see Table 2), but their role in toxicity requires further investigation (Xu et al., 2011; Lv et al., 2019).

The incidences of poisoning by *T. hypoglaucum* honey were relatively lower than those caused by RH. Zhang et al. identified 31 honey poisoning cases reported between 2007 and 2012 from the southwest region of China (Zhang et al., 2017). Honey poisoning was reported in all incidences after consuming at least 100 g of honey. All 1199 patients experienced the

most common symptoms, such as nausea and vomiting (100%), abdominal pain (90%), diarrhea (74%), palpitations (61%), dizziness (55%), chest congestion (48%) and dyspnea (48%) (Silici et al., 2015). In those cases, oliguria/anuria, twitch, hematuria or hematochezia were also observed. Despite the fact that 20 patients (65%) consumed farmed honey, and 11 (36%) consumed wild honey, the mortality rates of patients in farmed and wild honey were 30% and 18%, respectively. One possible explanation for the mortality observed in farmed honey is that honey farming in areas where *T. hypoglaucum* is predominant results in the presence of its pollen in honey. *T. hypoglaucum* pollen was found in 22 of 29 honey samples collected from a local farming area in Lanping County in Southwest China between July and August 2013 (Zhang et al., 2017).

A previous study found that TwHf poisoning in honey caused acute renal failure, which in some cases results in death (Jing, 2011). In regions with predominant growth of *T. wilfordii* or *T. hypoglaucum*, honey production using beehive farming should be strictly monitored, and beekeepers should be educated with the technology of identifying *Tripterygium* pollen in honey before it is marketed to the local people to avoid health risks to customers.

Triptolide was also toxic to Eastern honeybees, *Apis cerana*, which foraged solely on *T. hypoglaucum*, during seasons when nectar from other sources was scarce (Tan et al., 2007). Bee aversion to *T. hypoglaucum* nectar when an alternative food source is available is critical for protecting the hive from long-term exposure effects (Zhang et al., 2018). Asian hive bees modulate their dance behavior depending on alternative nectar availability; *i.e.*, when nectar was scarce, the foragers danced for *T. hypoglaucum* in a manner similar to that of common vetch, their preferred food source (Tan et al., 2012). Triptolide poisoning in honey occurs only when bees feed on the nectars of TwHf or *T. hypoglaucum*, which is commenly found in honey from wild honeybee hives. Thus, inadvertent ingestion of triptolide-containing honey could be avoided during the late summer months (from July to September), particularly when adverse

conditions cause *Tripterygium* to be the only nectar source for bees in Southwest China (Zhang et al., 2017).

The good news is that honey contaminated with triptolide tends to lose its triptolide content over time. It has been demonstrated that the triptolide content decreases by 50% after 3 months of storage. This could be due to the role of oxidoreductase (Cao et al., 2014). Triptolide's primary degradation pathway involves decomposition of the C12 and C13 epoxy groups (Mao et al., 2010). Large-scale studies are required to determine whether the triptolide content completely disappears, without affecting the nutritive value of honey when it is stored for an extended period of time. This information has aided in the resolution of issues involving triptolide-contaminated honey, while legislation and monitoring continue.

3.3. Coriaria arborea-derived toxic honey

Honey toxicity and deaths from honey consumption have been reported in New Zealand on an irregular basis since the introduction of honeybees in the mid-nineteenth century. Due to the obvious similarities with tutu bush poisoning, it was assumed that there was a link between this bush and toxic honey. Tutu is a Mori-derived common name for plants in the genus *Coriaria* in New Zealand. However, it wasn't until the mid-20th century that Paterson noticed bees collecting honeydew excreted on the leaves of the tutu tree (Fig. 1d) by a passion vine hopper that the link between the bush and the toxic honey became clear (Paterson 1947).

Tutin and hyenanchin are the two most toxic components of *Coriaria arborea* honey (CH). The molecular structure of tutin was discovered through X-ray structure analysis of α-bromoisotutin (Craven, 1963). Because it could not be found in tutu leaves, young shoots, or stems, hyenanchin, a hydroxy derivative of tutin and the most abundant of the two in the honeydew excreted by *S. australis*, was thought to be a metabolic product produced by the insect (Hodges & White, 1966). Later, dihydrotutin and dihydrohyenanchin were discovered

to be trace components (8-fold less abundant than tutin and hyenanchin in toxic honeydew honey by ^{13}C NMR), but their toxicity was unkown (Blunt et al., 1979).

Although hyenanchin is much more abundant than tutin, it is also much less toxic. Thus, to prevent outbreaks of toxic honey, the government strictly regulates the allowable level of tutin in New Zealand honey. However, it was noted that even honey with little or no tutin could be toxic. A summary of tutin and its derivatives in toxic honey is shown in Table 3. It was hypothesized that acetone extraction of tutin would miss contaminants that evade acetone extraction, such as conjugated forms of tutin with glycine, glucose, glucuronic acid, etc. (Sutherland, 1992). Indeed, a pharmacokinetic analysis of toxic honey exposure revealed two spikes in tutin in blood, an early peak at approximately 7 h and a much later peak (~18 h), indicating metabolism of a tutin conjugate (Fields et al., 2014). A subsequent investigation confirmed the presence of two additional components in honey: tutin monoglucoside (2-(β-D-glucopyranosyl) tutin) and tutin diglucoside (2-[6′-(α-D-glucopyranosyl)-β-D-glucopyranosyl] tutin) (Larsen et al., 2015).

The discovery of two tutin conjugates prompted a reduction in the maximum amount of tutin permissible in honey, from 2 mg/kg to 0.7 mg/kg (Beasley et al., 2016). More research is being conducted to develop guidelines for ‘total tutin' regulations and to synthesize standards in order to improve monitoring efforts (Larsen et al., 2015). Thus, New Zealand's approach is a good example of regulating toxic components in honey to prevent illness and protect their multibillion-dollar agricultural industry.

A recent study found that all four picrotoxanes (tutin, hyenanchin, tutin monoglucoside, and tutin diglucoside) are plant-derived rather than insect-derived, as previously thought (Watkins et al., 2018). Although hyenanchin was barely detectable and diglucosides were not detectable in *C. arborea* leaves, the tutu phloem sap contained all four at a concentration similar to the highest found in honeydew (Watkins et al., 2018). According to the findings,

diglucosides may act as a tutin transport form, similar to sucrose transport in the phloem. Other *Coriaria* species containing tutin have been reported from various parts of the world, including *C. nepalensis*, *C. japonica*, and *C. ruscifolia* (Fuentealba et al., 2007).

Common clinical symptoms of CH poisoning are similar to those of RH poisoning, and include nausea, headache, vomiting and dizziness, as well as seizures and coma in severe cases. The onset of the first clinical symptoms ranged from 0.5 to 17 h, with a median of 7.5 h after consumption (Fields et al., 2014). Tutu berry (*Coriaria arborea*) ingestion caused tonic-clonic seizures in two patients and mild symptoms in the third (Belcher & Morton, 2013). Such symptoms, however, were not observed in CH intake cases. Based on a clinical case definition, a retrospective case identified 22 possible or probable cases.Ten of the 13 honey samples linked to symptomatic individuals, tested positive for tutin and its hydroxy metabolite hyenanchin (hydroxytutin), while one tested positive for hyenanchin alone (Beasley et al., 2018). A pharmacokinetic study was conducted in 6 healthy males who received a single oral dose of tutin-containing honey with a tutin dose of 1.8l g/kg body weight. Two subjects reported mild, transient headache at a time postdose corresponding to maximum tutin concentrations, but no typical symptoms such as nausea, vomiting, dizziness or seizures were observed. As a result, a low tutin concentration does not result in any significant clinical signs of CH poisoning (Fields et al., 2014).

3.4. Toxic honey contaminated with pyrrolizidine alkaloids (PAs)

PAs are very common natural toxins (Table 4); they are prevalent in pollen and nectar and thus can be found in honey. In addition to honeys, other bee products, including bee pollen, propolis and bee wax, have chances to be contaminated by PAs (reviewed in Brugnerotto et al., 2020). PAs are thought to be present in 3% of flowering plants, which are found all over the plant kingdom in up to 13 distantly related angiosperm families (Fu et al., 2004). The majority

of PAs, however, are derivedfrom four plant families, Asteraceae (*Senecio* spp. and *Eupatorium* spp.), Boraginaceae, Apocynaceae, and Fabaceae (*Crotalaria* spp.) (Fig. 1e, 1f, 1 g, 1h and 1i) (Valese et al., 2016).

PAs are esters of amino alcohols with a pyrrolizidinic core (necine) and aliphatic acids (necic acids). PAs are generally di- and monoesters of three types: retronecine type, otonecine type, and platynecine type (Zhu et al., 2018). A double bond at the C1 and C2 positions of the necine base is highly hepatotoxic, as seen in retronecine- and otonecine-type PAs, whereas a saturated necine base, as seen in platynecine-type PAs, makes it less toxic (Zhu et al., 2018).

An increasing number of publications have focused on the health risks of PAs in honeys, but it is unsurprising that PAs are prevalent in honeys with significant differences in their chemical types, geography, nectar sources and storage method/period. Griffin et al. used liquid chromatography-ion trap mass spectrometry to detect PAs in commercial honey, and positive samples contained an average PA concentration of 1260 μg/kg of honey, with eight tested positive in 50 samples for one or two PAs, primarily lycopsamine and echimidine (Griffin et al., 2013).

A study on 3917 honey samples from European countries found that PAs were present in 66% of raw (bulk honey yet to be packed and sold in retail shops) and 94% of retail honey (1–267 μg/kg) samples (Dubecke et al., 2011). PAs were found in 68 percent of 2839 raw honey samples from Argentina, Brazil, Chile, Cuba, El Salvador, Guatemala, Mexico, and Uruguay, with concentrations ranging from 1–1087 μg/kg. It was found in all of the honey samples from Uruguay, with an average concentration of 105 μg/kg. While 76 percent of Guatemala honeys were PA positive, the amounts were relatively low (8 μg/kg PAs) (Dübecke et al., 2011). In a Latin American study, 91% of the tested honeys contained one or two senecionine-type PAs, 67% had lycopsamine-type PAs, and 76% had monocrotaline (Moreirac et al., 2018). In China, 58% of the 120 tested samples contained PAs (0.04–288 μg/kg) (Zhu et al., 2018). In their

study, PAs in Hong Kong and Macau honey were significantly higher than those from Beijing and Shanghai. Lycopsamine and echimidine were found in high concentrations. Surprisingly, the authors discovered high levels of chlorine in PA-contaminated honey, which had never been reported before (Zhu et al., 2018).

Interestingly, a study indicated that honey samples from German and Austrian beekeepers had a lower rate of positive samples than those purchased from supermarkets and other sources (Bodi et al., 2014). The average PA concentrations ranged from 6.1 μg/kg honey from beekeeper samples, 14 μg/kg in discount products and 15 μg/kg in branded honey. Furthermore, storing honey for a longer period of time affects the PA concentration. PA and PA with N-oxides in stored honey samples decreased by 20% after storage for 1 d and by 34% after 7 d. However, tertiary amine PA remained stable during 6 months of storage, whereas PA with N-oxides steadily decreased over that time until only trace amounts remained (Kaltner et al., 2018).

The German Federal Health Bureau has set the PA intake threshold at 1.0 μg for daily intake limits of up to 6 weeks and 0.1 μg for a longer period of consumption (Zhu et al., 2018). According to a recent review, honey contaminated with PA poses toxicity risks, with a maximum daily intake of 13 g/day by a 60 kg adult (Brugnerotto et al., 2020). Nevertheless, no regulations on minimum tolerable limits for PAs in honey are currently available.

RH contains GTX, which has over 25 different isoforms. There have been more reports of RH, and poisoning symptoms are mild when compared to honey from *Tripterygium* species. *Tripterygium* honey containing triptolide is fatal if consumed in large quantities. Tutin is the main toxin found in *Coriaria* arborea-derived toxic honey, and its clinical poisoning symptoms are similar to those of RH. PAs are more prevalent in honey samples from around the world than the other three toxins. PAs are highly hepatotoxic, and toxicity decreases with increasing

saturation. Although the symptoms of various toxins are similar, the toxicity mechanism is not. As a result, determining the precise toxicity mechanism is required.

4. Toxicological evaluation and mechanisms of toxicity

As previously stated, toxic honey poisoning has emerged as a new food safety and human health concern with increasing accidences worldwide. The greatest risk of human exposure to toxic honey comes from isolated toxins derived from plants, such as GTXs, triptolides, tutin and PAs. To understand the acute and/or subacute toxicity of these toxins, as well as the mechanism of toxicity, it is helpful to distinguish between toxicity and medicinal purposes.

4.1. Grayanotoxins

The major toxins of toxic honey collected from rhododendrons and other Ericaceae family plants are GTXs (Salici & Atayoglu, 2015). In animals, GTX poisoning causes neurotoxicity. The mean LD_{50} (lethal dose, 50%) of GTXs (intraperitoneal injection) in male albino mice was 1.28 mg/kg for GTX-I and 0.908 mg/kg for GTX-III, according to toxicological tests. In the same experiment, GTX-II was found to be non-lethal at 4 mg/kg (Medveditskova, 1969). In a subsequent study, however, mice died quickly after receiving intraperitoneal injections of 10 and 100 mg/kg GTX-II (Terai et al., 2003). The lethal dosage of GTX-III in rats was 50 μg/kg/bw and higher (Doganyigit et al., 2019), and dose-dependent acute and chronic effects were observed after three weeks with a single i. p. dose of GTX-III. A recent study determined the dose-dependent toxic effects of toxic honey (25, 50 and 75 mg/kg) and GTX-III (0.01 mg/kg) on mouse liver, using attenuated total internal reflectance Fourier transform infrared (ATR-FTIR) spectroscopy, and lipid peroxidation with increased membrane fluidity was noticed (Cakmak-Arslan et al., 2020).

GTX's toxic effects are primarily mediated by the cells' voltage-dependent sodium channels ($Na_v1{\cdot}x$) (Fig. 3a). These effects can prevent inactivation, and excitable cells are kept

in a depolarized state, allowing calcium to enter the cells. All of the observed symptoms are related to skeletal and heart muscles as well as the central nervous system (Koca et al., 2007). GTX, like batrachotoxin, veratridine, and aconitine, was classified as a site 2 toxin with selective binding affinities on Nav1x. GTX binds to the sodium channel in the open state, causing the channel's inactivation function to be lost, resulting in a hyperpolarizing alteration of the modified sodium channel. The affinity of GTX to $Na_v1{\cdot}4$ sodium channels was mediated by Phe and Tyr residues at positions Phe-1579 and Tyr-1586, which regulated access and binding of GTX to its receptor, respectively, according to site-directed mutagenesis of $Na_v1{\cdot}4$ (Maejima et al., 2003). Nevertheless, whether other sodium channel family members interact with GTX still needs to be explored.

4.2. Triptolide

Triptolide is found in toxic honey originating from TwHf (Zhao et al., 2018). This diterpenoid toxin has immunosuppresive and cytotoxic properties. Although triptolide has been used for the treatment of rheumatic and immunological diseases, it has been linked to multiorgan toxicity and extremely toxic reactions. The LD_{50} for triptolide in mice is 0.83 to 1.93 mg/kg (*i.p.*) and 1.278 mg/kg (*p.o.*) (Ziaei & Halaby, 2016; Chen et al., 2000; Xu et al., 2013). Toxicity by triptolide is dose- and time-dependent, primarily affecting the liver, reproductive organs, kidney, heart, spleen, gastrointestinal organs and circulatory system (Li et al., 2014). Triptolide detoxification metabolic pathways have also been reported (Mei et al., 2005). CYP, the key superfamily members that contribute to the biotransformation of drug metabolism in the liver, are thought to be responsible for the hydroxylation of triptolide *in vitro* (Xue et al., 2011).

The main target of triptolide has been investigated for over 40 years, and studies have revealed that it mainly participates in several cellular signaling pathways (Fig. 3b). Triptolide

was found to be capable of inducing autophagy by stimulating endoplasmic reticulum stress and activating the CaMKK β -AMPK pathway (Tara et al., 2013). In Triptolide toxicity, there was a interaction between apoptosis and autophagy. Triptolide-induced liver injury apoptosis was characterized by apoptosis rather than necroptosis, with the PI3K/AKT, MAPK, TNFα and p53 signaling pathways all involved (Zhao et al., 2020). Triptolide cytotoxicity was also investigated in HepaRG cells, with the results indicating that triptolide inhibits cell proliferation and induces apoptosis via the Fas death pathway. Triptolide can cause cellular apoptosis by altering the Caspase-3 pathway (Xi et al., 2017).

Triptolide was found to cause nephrotoxicity and oxidative stress by suppressing NF-E2-related factor 2 (Nrf2) activity and decreasing superoxide dismutase and glutathione expression (Li et al., 2012). Other oxidative stress pathways included FXR signaling, SIRT3 signaling and mitochondrial damage (Xi et al., 2017). Proximal tubular damage is also associated with loss of epithelial cell barrier function after triptolide treatment as evidenced by disrupted paracellular permeability and decreased tight junction protein expression (Sun et al., 2013). Additionally, studies have also shown that metabolic pathways involving hydroxylation and glutathione conjugation could lead to triptolide detoxification (Li et al., 2015). Triptolide has also been shown to interact with protein translation signaling, which can reduce the transcription of the larger subunit of RNA polymerase II. Triptolide increased the expression of Th17 cell-transcription factors, and aggravated liver injury (Wang et al., 2016). Because triptolide disrupted the cAMP/PKA pathway, the level of cellular cAMP decreased, resulting in reproductive dysfunction (Zhang et al., 2012). All of these cases proved that triptolide can cause cell death through a variety of signaling pathways.

4.3. Tutin

Tutin, as mentioned earlier, is a plant-derived neurotoxin that is found in honeys produced by an insect that feeds on sap of the New Zealand native shrub plant, *Coriaria arborea* ("tutu"). Tutin toxicity was reported in female mice with an LD_{50} of 3.0 mg/kg (i.p.) and Palmer-Jones observed oral toxicity in rats with an LD_{50} of 20 mg/kg (p.o) (Palmer-jones, 1947). Another study found that at a dose of 55 mg/kg, tutin was toxic to Norway rats (*Rattus norvegicus*), and both male and female rats died within 60 min. Tutin-treated rats, become unconscious within 5–10 min after neurological poisoning symptoms began. Therefore, the authors proposed that tutin can be developed as a novel, culturally acceptable rodenticide in New Zealand (Ogilvie et al., 2019). In a human pharmacokinetic study, 6 healthy males were given a single oral dose of tutin-containing honey (equiv to 1.8 μg tutin/kg b.w.); the serum concentration–time curve for participants showed two discrete peaks, with the second peak being higher and occurring approximately 15 h after dosing (Fields et al., 2014).

Toxicological mechanisms by tutin were quite similar to those of strychnine, a well-known plant-derived terpenoid indole alkaloid first isolated in 1818. Both of these typical neurotoxins were found to have strong convulsant effects, which have been demonstrated to antagonize the glycine receptor (GlyR). GlyR is made up of five units (α1–4, β) with a putative 2α:3β stoichiometry. Glycine receptors are the main inhibitory receptors in nervous organs, such as the brain stem and spinal cord. Tutin inhibited GlyR causing pain and debilitating muscle spasms and pathologically exaggerated startle responses with preserved consciousness (Fig. 3c). In spinal neurons, Fuentealba and colleagues found that tutin inhibited the glycinergic evoked current at doses of mg/kg. Enhanced neuronal excitability has also been noticed with an increased frequency of spontaneous Ca^{2+} spikes and spontaneous synaptic activity, which is associated with the phosphorylation of cAMP-response element binding protein (Fuentealba et al., 2007). Furthermore, tutin inhibited α1 and α2 homomeric GlyRs in a dose-dependent manner. Coexpression of αβ subunits reduced the potency of tutin in HEK 293 cells. Tutin's

agonist affinity on the α2 subunit is greater than on the α1 subunit, which explains why tutin is more toxic in children than in adults (Fuentealba et al., 2011).

4.4. Pyrrolizidine Alkaloids

PAs are widely distributed in over 6,000 flowering plants worldwide. Poisoning cases and their hepatotoxic effects have received a lot of attention. Among over 300 identified PAs, not all are toxic, but toxic PAs have typical structures with an esterified hydroxyl group and a double bond in the ring nucleus with a branched carbon in at least one of the ester side chains (Neuman et al., 2015). For example, senecionine, retrorsine, and riddelliine are three of the major toxic PAs that fully meet the aforementioned criteria.

Human intake of PA-contaminated foods is recognized as the main cause of hepatic sinusoid occlusion syndrome (HSOS). Animal studies have indicated that chronic exposure to certain PAs leads to hepatic carcinoma and hemangioendothelial sarcoma. Through the metabolism of PA *in vivo* into its oxidative form, dehydropyrrolizidine (DHP) esters form adducts with proteins and DNA, which is attributed to the carcinogenic effects of PAs (Fig. 3d). Other toxicities, including cytotoxic effects in cell lines, genotoxic effects in *Drosophila* and pneumo toxicity in rodents, have been reported (Song et al., 2020). A recent study revealed that 14-(R)-hydroxy-gelsenicine (HGE), a novel poisonous PA of toxic honey from *Gelsmium elegans* nectar, caused neurological toxicities with LD_{50} values of 0.125 mg/kg (for female mice) and 0.295 mg/kg (for male mice). Distinct neurotoxicity was shown by activating GABA receptors (Yang et al., 2020).

4.5. Detoxification and antidotes of toxins from toxic honey

Honey poisoning occurs frequently, and it may be life-threatening if not treated properly. Understanding the toxicity mechanism is thus crucial. Presently, most of research has focused on the cellular level, and the toxicity mechanisms for the representive four kinds of toxins in

honey samples have been summaried. For GTX, the toxic effects mainly act on the voltage-dependent sodium channels (Nav1·x) of the cells. Triptolide is involved in a variety of cellular signaling pathways, including oxidative stress, apoptosis, autophagy, gene and protein expression and others. Due to the effect on glycine receptor, tutin can cause neurotoxicity. PA metabolits can form adducts with proteins and DNA, causing toxity. Although some signaling pathways involved in the toxicity of cells have been identified, many mechanisms participating in the processes remain unknown. These toxins induced signaling pathways may interact with one another. As a result, more research into the toxicity mechanism of toxins is required. Toxins with different toxicity mechanisms produce similar poisoning symptoms, increasing the difficulty of determining toxins and detoxification methods. Furthermore, the target organs and poisoning mechanisms of toxins in the body are unknown. To develop an effective therapy method, we must first better understand the mechanism of action, metabolism, and biotransformation of toxins found in honey on body organs.

According to animal experiments, the LD_{50} by injection is lower than that by oral administration. Toxin concentrations in plasma are high after injection administration and can directly act on target organs. The severity of poisoning is determined by the amount of toxins consumed rather than the consumption of toxic honey. Thus, it is necessary to develop an accurate determination method to analyze the content of toxins from toxic honey and set the criteria for the permissible and prohibited ranges of toxins from toxic honey. It is advantageous to establish criteria and strengthen detection of these toxins in honey in order to prevent poisoning cases caused by the consumption of toxic honey.

General and cardiac symptoms usually last for less than 24 h after consuming toxic honey (Lennerz et al., 2012; Osken et al., 2012). Nevertheless, after a few days, poisoning signs of mental status and visualizations gradually recovered (Fukumoto, 1993; Işık et al., 2016). Previous clinical practices have been successful for the treatment of toxic honey poisoning,

and antidotes have also been applied for toxins from toxic honey (Erenler, 2016). Atropine sulfate can be used for patients with bradycardia and hypotension symptoms, requiring a dosage of 0.5–2 mg/adult. Intravenous fluid infusion with normal saline was shown to help relieve hypotension and dizziness. It should be noted that antiplatelet therapy is not recommended since toxic honey leads to a decreased oxygen supply in the heart secondary to bradycardia (Karabag et al., 2015). In addition, if patients consume excessive amount of toxic honeys, gastric decontamination and supportive care are required (Oguzturk et al., 2012). A case study was reported in which the patient did not respond to atropine or saline but developed severe bradyarrhythmia after ingesting *Rhododendron brachycarpum* (Choi et al., 2002). Overall, patients need careful planning of therapies after toxic honey poisoning, and specific attention should be given to lethal dysrhythmia symptoms.

5. Analytical techniques for toxins in toxic honey

5.1. Grayanotoxins

GTXs and other potential markers for detecting RH or minor contamination in natural honey product using advanced methods have not been widely reported. One of the early methods to detect RH was by analyzing the pollen content in honey using common standard procedures (Sumerkan et al., 2011). Another study (Onat et al., 1991) reported microscopic examination of *R. ponticum* pollen tetrades in honey consumed by patients to confirm RH. Nearly 72 volatile compounds have been identified in RH in recent years, and it was proposed that 1,2-benzene dicarboxylic acid, tributyl phosphate, stearic acid, propanoic acid, benzene, ethyl phenyl acetate and benzophenone could potentially act as specific floral origin markers for RH (Silici, 2010). Thin-layer chromatography was one of the earliest common methods of detecting RH, and the study identified GTX-II and GTX-III in honey samples from Grouse

548 Mountain, British Columbia. In addition, the study identified other compounds closely related
549 to GTX-I (andromedotoxin), as well as desacetyl pieristoxin B (Scott et al., 1971).
550 Using high-performance liquid chromatography (HPLC) with an ultraviolet (UV) detector
551 poses a major challenge in detecting GXTs, as it does not have any UV-active sites (Sahin et
552 al., 2015). There have been few reports on the quantification of GXTs using LC-MS/MS-based
553 analysis. A simple and robust LC-MS/MS-based method for the quantitation and confirmation
554 of GXTs in honey using a "dilute-and-shoot" sample preparation approach has been reported
555 (Kaplan et al., 2014). Honey samples were diluted 10-fold in methanol-water (1:4 v/v) before
556 analysis by LC-MS/MS method. The authors used a water-methanol gradient with 0.1% acetic
557 acid to achieve chromatographic separation on a reversed-phase HPLC column. GTX residues
558 ranging from 0.1 to 39 mg/kg were found in ten real honey samples. In another study, after
559 extraction with C_{18} solid-phase extraction (SPE), a Thermo-Scientific LC coupled with a TSQ
560 Quantum Access Max triple-stage quadruple-mass spectrometer (LC-MS/MS) could detect
561 GTX-III in various toxic honey samples (Sahin et al., 2015). GTX-III was eluted under isocratic
562 conditions using a mobile phase consisting of 50:50 water/methanol solution containing 1%
563 acetic acid at a flow rate of 0.3 mL/min for 8 min. The authors acquired MS data by running
564 electrospray ionization (ESI) in negative ion mode using selected reaction monitoring (SRM)
565 after indicating the real molecular weight of GTX-III by full scan in the range of m/z 200–500.
566 Overall, from different honey samples, the concentration of GTX-III ranged from 0.701 to
567 68.754 µg/g, and honey samples from the Artvin/Hopa region in Turkey recorded the highest
568 GTX-III level. Previously, Oasis HLB SPE in conjugation with LC-MS/MS was used to
569 quantify GTX-I and GTX-III in toxic honey samples from *R. ponticum* nectar collected for
570 three consecutive years (Kurtoglu et al., 2014). Although the GTX-I and GTX-III total average
571 contents were 20.4 ± 1.69 and 8.20 ± 1.93 mg/kg, respectively, their contents varied every year.
572 Interestingly, GTX concentrations did not vary significantly during the 6-month storage of

honey, indicating that long-term storage does not reduce the GTX level. Another similar study detected GTX-I and GTX-III levels by LC-MS/MS combined with SPE cartridges preconditioned from four honey samples brought by respective patients to the hospital after health issues, and the average amounts detected were 4.683 mg/g and 8.423 mg/g, respectively, but they were higher in only one sample (Aygun et al., 2015).

Several techniques have been reported for the detection of GTX in biological samples, allowing for the identification of human blood samples when toxic RH is consumed. Following SPE cleanup, a rapid LC-MS/MS method was used to quantify GTX-I, GTX-II, and GTX-III in biological samples, such as rumen contents, feces, and urine (Holstege et al., 2001). Another study reported a sensitive, reliable, and validated quantitative SPE combined with LC-MS/MS method in rat whole blood (Cho et al., 2014). This method was applied to human blood samples, and the study could detect GTX-I and GTX-III in patients' blood and urine, which were 30.62 and 4.917 ng/mL and 0.447 and 1.998 mg/mL, respectively (Aygun et al., 2015). This was the first study to report GTX levels in human body fluids, even though fewer human samples were detected by LC-MS (Aygun et al., 2015). In another similar study on 25 cases, by LC-MS/MS with SPE, the mean GTX-I and GTX-III concentrations in blood were 4.82 ng/mL and 6.56 ng/mL, respectively, in urine were 0.036 μg/mL and 0.391 μg/mL and in honeys consumed were 8.73 μg/g and 27.60 μg/g (Aygun et al., 2018).

5.2. Triptolide

To date, the detection of *Tripterygium* pollen has been used as the standard procedure to identify toxic honey. The pollen content of honey was examined under microscopy at 100× magnification using standard procedures, and the absolute pollen counts were calculated using a simple formula (Song et al., 2012). Rapid detection of triptonide or its metabolites in honey samples using advanced techniques such as LC-MS/MS or quadrupole-time of flight (Q-TOF)

is highly essential. Whole flower extracts and honey from TwHf using quick-easy-cheap-effective-rugged-safe (QuEChERS) sample preparation and UHPLC/Q-TOF-MS revealed that triptolide and protopine could act as potential markers for identifying toxic honey. The developed method detected different honey varieties that were spiked with 5% TwHf honey samples, and the study tested 90 commercial honey samples but found no contamination (Sun et al., 2019).

5.3. Tutin

There are very few technical reports on topics relevant to the detection of tutin and its derivatives. LC-MS was one of the most commonly methods. One of the earlier studies used ^{13}C NMR to identify the derivates of tutin in toxic honeydew honeys and identified dihydrotutin and dihydrohyenanchin as trace components (Blunt et al., 1979). A study from China reported the use of SPE combined with ultra high-performance liquid chromatography (UHPLC) with high resolution (HR)-MS for three coriaria lactones in honey, and the RSDs were 3.0%–8.4% (Yin et al., 2015). Recently, a quantitative triple-quadrupole LC-MS method was used to identify tutin and its derivatives in honey, and it was capable of simultaneously identifying tutin and its derivatives hyenanchin, 2-(β-D-glucopyranosyl) tutin, and 2-[6′-(α-D-glucopyranosyl)-β-D-glucopyranosyl] tutin simultaneously (Larsen et al., 2015). In real samples, the study identified tutin (3.6 ± 0.1 μg/g honey), hyenanchin (19.3 ± 0.5), tutin glycoside (4.9 ± 0.4), and tutin diglycoside (4.9 ± 0.1) in one honey sample. The authors indicated that the ratios of tutin glycoside and tutin diglycoside to tutin varied greatly in other tutin-containing honeys.

5.4. Pyrrolizidine Alkaloids

One of the most common detection methods for PAs is HPLC-based techniques coupled with MS/MS, but the elution of PAs from honey through different columns varies. A study

identified PAs and their N-oxides using HPLC coupled to ESI MS, but the authors used "Strata" SCX SPE columns for efficient pyrrolizidine alkaloid/N-oxide capture from honey-based matrices (Betteridge et al., 2005). Another method proved to be fast and simple in determining PA in honey samples, as the authors used liquid chromatography tandem mass spectrometry (LC-MS/MS) with ESI (Valese et al., 2016). This method had a higher limit of detection (0.1–1.0 µg/kg) and limit of quantification (0.2–1.5 µg/kg) with effective detection of senecionine, senecionine-N-oxide, echimidine, intermedine, lycopsamine, retrorsine, monocrotaline and retrorsine-N-oxide in honey, and these eight PAs could be determined within 11 min (Valese et al., 2016). Among the 92 samples tested, 84 were positive for one or two senecionine-type PAs, lycopsamine-type PAs were positive in 62 samples, and monocrotaline was positive in 70 samples. Their extraction procedure was simple requiring only diluting with water and no additional clean-up steps.

However, other reported methods for the determination of PAs in honey adopt solid-phase extraction (SPE) or QuEChERS (Beales et al., 2004; Edgar et al., 2011; Griffin et al., 2013; Martinello et al., 2014). A study detected retail honeys by LC-MS/MS, but Strata-X-C SPE cartridges for the clean-up process were used, and crotaline, lycopsamine, retrorsine, heliotrine, trichodesmine, otosenine, seneciphylline, senecionine, echimidine, and senkirkine (LOD: 0.0134–0.0293 µg/ml) were detected (Griffin et al., 2013). Recently, a study indicated that freezing the raw extract and performing the water/acetonitrile washing steps in a SPE column could efficiently remove complex matrices; the LOQ of different PAs ranged from 0.010–0.087 µg/kg for the LC-MS/MS method, with 15 PAs and 13 PA N-oxides identified in foodstuffs including honey (Chung et al., 2018).

The application of QuEChERS sample treatment and liquid chromatography coupled to hybrid HR-MS resulted in good linearity ($R^2 > 0.99$) and relatively high recoveries ranging from 92.3 to 114.8%, with LODs and LOQs ranging from 0.04 to 0.2 µg/kg and from 0.1 to

646 0.7 μg/kg, respectively (Martinello et al., 2017). Nine different PAs (echimidine, heliotrine,
647 intermedine, lycopsamine, lasiocarpine, retrorsine, seneciphylline, senecionine, and senkirkine)
648 in honey based on QuEChERS sample extraction and ultrafast liquid chromatography coupled
649 with MS detection showed LODs ranging from 0.021 to 1.39 μg/kg and LOQs ranging from
650 0.081 to 4.35 μg/kg (Martinello et al., 2017).

651 To determine 1,2-unsaturated PAs in honey, gas chromatography (GC) coupled with MS
652 was developed, and the samples were purified on MCX cartridges. PAs were eluted with a
653 solvent mixture consisting of ethyl acetate, methanol, ammonia, and triethylamine, and the
654 limit of quantification was 1 μg/kg (Kowalczyk et al., 2018).

655 *5.5. Comparison of detection methods for different toxins*

656 Based on present reports, the comparison of detection methods for four kinds of toxins is
657 shown in Fig. 4. Microscopic examination is a common and simple method to detect
658 contaminated toxic honey samples, including GTXs and triptolide, and there are standard
659 procedures for the two kinds of toxins. However, this method does not accurately quantify and
660 evaluate the extent of contamination by toxins. Microscopic examination is not an appropriate
661 detection method for toxic honey containing tutin or PAs. NMR is not only useful for structural
662 identification of compounds, but also suitable for rapid and non-destructive testing. It is
663 currently used in tutin and triptolide, and it is being considered for use in other plant toxins.
664 Exception of GTXs, most of toxins cannot be directly determined by GC approach. LC-UV is
665 a simple detection method to identify the honey with plant toxins, howover, it requires the
666 target compounds have UV-active. Among these toxins, only triptolide is applicable for UV
667 detection. LC-MS technology has wide applicability to the four kinds of toxins with the
668 characteristics of accuracy and simplicity. Generally, before chromatographic analysis, SPE is

669 the necessary procedure to extract and perform clean-up for the determination of most toxins.
670 However, there is a lack of a method to detect multiple toxins from toxic honey simultaneously.
671 LC-MS/MS has been widely applied in drug monitoring and toxicology analysis (Leung
672 et al, 2014). To begin, different toxins can be chromatographically separated due to polarity
673 differences. Second, using the characterized ions of toxin molecules, a database for plant toxins
674 can be established and developed multiple reaction monitoring (MRM) detection method to
675 monitor these toxins simultaneously. LC-MS/MS is a simple, time-saving, accurate and easy
676 to generalize and standardize approach to detect plant toxins in honey samples. HR-MS, with
677 TOF MS and TOF MS/MS modes, can provide accurate mass for toxin compounds, especially
678 unknown substances (Wu et al., 2017). Full-scan accurate mass for product ions in MS/MS
679 mode is a feasible tools to perform sensitive and accurate identification. HR-MS has been
680 applied to analyze pesticides, drugs and mycotoxins using a generic procedure (Wong et al.,
681 2018). By HR-MS, a database of plant toxins with accurate mass and ion fragments is generated,
682 which can provide specific metabolites or markers to identify the presence of toxins. Based on
683 these identified metabolites or derivets of toxins, a potential role and molecular mechanism can
684 be explained. Additionally, the database based on HR-MS results, is important reference to
685 select characterized ions for LC-MS/MS method. For a given toxin, the results would vary with
686 different detection conditions. Therefore, it is necessary to develop standard methods to detect
687 these toxins so that it is helpful to establish maximum residue limits (MRLs) and effective
688 legislation and rules to prevent toxic honey from entering the food chain. LC-MS/MS and LC-
689 HR-MS are available and promising approaches to establish standard methods to detect plant
690 toxins in honey samples.

691 **6. Implications for future research and perspectives**

692 Several studies have been published on honey toxicity in different parts of the world,
693 including from Turkey, other parts of Europe, New Zealand, China, Nepal, and Latin America,
694 however, no reports on toxic honeys from the African continent have been published.
695 *Rhododendron* sp. honey poisoning, concerning GTXs, has been reported widely in the last
696 two decades, with several cases in Nepal or Turkey, among other places, while honey from
697 these places was consumed without knowing its origin. Despite this, no legislation or a
698 minimum detection limit concerning the presence of GTXs has been developed. International
699 organizations have not taken any concrete steps concerning the aspects of GTX poisoning in
700 honey. A possible step would be that individual countries, such as Turkey or Nepal, come up
701 with an effective standard to check honey toxicity. This would be a great breakthrough in
702 preventing GTXs from entering the food chain through honey. Proper rapid GTX analysis
703 techniques that could be used for controlling the security of honey are essential. The clinical
704 treatment for GTX honey poisoning is well established and widely reported.

705 Studies on *Tripterygium* sp. honey poisoning have rarely been reported. Although several
706 papers have reported its detection by pollen analysis in honey, a proper standardizing technique
707 for identifying its toxin has yet to be published. Although few clinical cases have been reported
708 in Asia for *Tripterygium* sp. honey poisoning, the toxicity is not well reported and should be
709 given attention.

710 *Coriaria arborea* poisoning of honey by tutin and its derivatives has been reported only
711 in New Zealand. Few detection techniques have been established to identify tutin and its
712 derivatives in honey. There are few reports on the presence of tutin in exported New Zealand
713 honey, and New Zealand has already established minimum tolerable limits for tutin presence
714 in honey. New Zealand honey is distributed globally for its taste and quality.

715 The presence of PAs in honey is widely published, yet there is no widely accepted standard
716 for the minimal tolerable limit, except in the EU. No reports on PA-contaminated honey

causing health issues were recorded or published. PA contamination in honey can be easily identified, as several LC-MS/MS methods have been published. The consumption of honey is associated with a risk of poisoning, and toxic compounds vary widely. Despite many reports on toxins of toxic honey and all published detection methods, the specific poisoning mechanisms and symptomatic treatment have not been fully understood. More research into toxin metabolism and the organism's reaction processes to a toxin is urgently needed.

Toxin contamination, such as alkaloids, mycotoxins and heavy metals, poses a global challenge due to their wide presence in various foods. Due to the high mortality and morbidity resulting from toxin exposure, it is crucial to determine the exact poisoning mechanism and find effective treatment methods. To better solve this problem, it is important to know the related metabolic mechanism of toxic chemicals and the influence of endogenous host metabolism (Wen et al., 2021). Metabolomics can dynamically and efficiently determine the changes in toxins (from the intermediates to the end products) and describe the interaction among multiple molecules in pathological processes. Based on high-throughput, rapid and specific characteristics, recent improvements in metabolomics technologies can analyze thousands of metabolites and help to identify unique metabolic traits that are potential markers of some toxins. The determination of toxin biomarkers is helpful for rapid diagnosis and immediate treatment.

There are several examples of how metabolomics can be used to characterize toxins. Long-term exposure to Cr (VI) can result in organ cancer. The potential marker substances identified in a metabonomic analysis of hepatic injury caused by chromium poisoning were oleic acid amide, galactinol, choline, betaine, farnesyl lactone, and taurine, as well as several important metabolic pathways. This finding may aid in the development of a method to prevent and treat chromium poisoning (Zhao et al., 2019). The herbicide paraquat is highly toxic to humans. Gao et al. used a serum metabolomic method combined with multivariate statistical

analysis to explore mechanisms of paraquat poisoning. They found biomarkers in paraquat poisoning mice, including creatinine, palmitoleic acid, α-tocopherol, citric acid, succinic acid, glycine, succinic acid, 6-phosphogluconic acid and fumaric acid (Gao et al., 2019). These findings provide a detector for the clinical diagnosis and treatment of paraquat poisoning. The root of *Polygonum multiflorum* (PM), a traditional Chinese medicine, is frequently used to treat various diseases. However, the toxicity of PM cannot be ignored, especially hepatotoxicity (Han et al., 2019). Han et al. identified emodin-8-O-glucoside and torachrysone-O-hexose as potential toxic markers to evaluate different processing methods through metabolite profile analysis at the cellular level. Another example of toxic Chinese medicine is *Aconitum Kusnezoffii*. Six toxic diester diterpenoid alkaloids were identified from the sera of patients by HR-MS. Additionally, among 32 altered metabolites after poisoning, hydroxyeicosatetraenoic acids and some dicarboxylic acids, which are related to Aconitum alkaloid toxicity, may be potential markers for clinical diagnosis (Zhang et al., 2019).

Taken together, metabolomics technology, as an important tool to screen effective biomarkers, has been applied to the analysis of a variety of toxins. To the best of our knowledge, there have been no relevant references to explore the poisoning mechanism caused by toxic honey by applying metabolomics. Metabolomics provides a better understanding of organism reactions to toxins from toxic honey and characterizes the poisoning process and toxic components. Thus, the application of metabolomics to identify the characteristic biomarkers reflecting physiological or pathophysiological responses to toxins from toxic honey contributes to establishing accurate clinical diagnostic methods and developing effective antidote drugs.

7. Conclusions

Honey intoxication caused by various toxins is an emerging and complex global issue. More studies on their presence are needed to gain a better understanding of their role in human

life. Toxic honey from *Rhododendron* sp., *Tripterygium*, *Coriaria* arborea, and PA contaminated honey are highly reported in poisoning cases, and the relevant toxins have also been studied more frequently than other honey toxins. Intake of these toxic honeys commonly causes typical clinical poisoning symptoms, including dizziness, hypotension and bradycardia. Different toxins from toxic honey show different toxicity mechanisms. GTX mainly acts on the voltage-dependent sodium channels of cells. Triptolide interacts with several different pathways, including those associated with inflammation and protein translation. The toxicological mechanism of tutin is similar to that of strychnine and acts by antagonizing the glycine receptor. Intake of PA can cause hepatic sinusoid occlusion syndrome. Accurately understanding the toxicity mechanism of these toxic honey samples is beneficial for distinguishing different kinds of toxic honeys and developing antidotes.

Honey contamination with toxins has become an issue in the food chain and should be addressed and handled by regulatory agencies to prevent them from entering the food chain through effective legislation and rules. Clinical cases globally reported due to honey poisoning provide insight that honey samples need to be carefully examined before allowing them to the market. Most detection methods for toxins from toxic honey have just been performed in the laboratory. Some standard, simple, accurate and time-saving analysis methods should be developed worldwide, and international MRLs for natural toxins in bee products should be established in the future. In addition, improved clinical diagnosis and novel treatment strategies for different types of toxic honey ingestion should be evaluated and developed in the near future. Developed analytical chemistry methods, including metabonomic approaches, are promising and effective to characterize the potential biomarkers for toxins from toxic honey because of their robust application.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table legends

Table 1 Summary of grayanotoxins and their main derivatives

Table 2 Summary of triptolide and its derivatives

Table 3 Summary of tutin and its derivatives

Table 4 Summary of different PAs found in contaminated honey

1262

1263 **Table 1 Summary of grayanotoxins and their main derivatives**

Compound Name	CAS number	Identification or Analysis method	Concentration range in honey (mg/kg)	Reference
Grayanotoxin-I	4720-09-6	SPE, LC-MS/MS	0.61–9.895	Aygun et al., 2015
		SPE, LC-MS/MS	15.1–26.0	Kurtoglu et al., 2014
Grayanotoxin-III	4678-45-9	SPE, LC-MS/MS	2.977–16.890	Aygun et al., 2015
		pollen analysis, SPE, LC-MS/MS	2.114–11.371	Sibel et al., 2014
		SPE, LC-MS/MS	2.53–12.1	Kurtoglu et al., 2014
Grayanotoxin-XIX	75829-08-2	SPE, LC-HR-MS	5.8	These et al., 2015
Grayanotoxin-IV	30272-17-4		10.5	
Grayanotoxin-VI	30460-36-7		2.0	
Grayanotoxin-VII	30460-59-4		116.3	
Grayanotoxin-XIII	35928-07-5		12.2	
Grayanotoxin-XVII	/		18.3	
Grayanotoxin-XVIII	70474-76-9		39.8	

Kalmitoxin-I	56663-60-6	9.5
Rhodojaponin-VI	37720-87-9	1.4
Rhodomollein-XIII	54781-72-5	58.3
Rhodomollein-XIX	/	8.3

"/" means no available information.

1290

Table 2 Summary of triptolide and its derivatives

Compound Name	CAS number	Identification or Analysis method	Concentration range in honey (mg/kg)	Reference
Triptolide	38748-32-2	HPLC-UV	/	Li et al., 2015
		UHPLC/Q-TOF-MS	0.396–0.633	Sun et al., 2019
Triptonide	38647-11-9	HPLC-UV	/	Li et al., 2015
		UHPLC/Q-TOF-MS	0.062–0.322	Sun et al., 2019
Triptolide	38748-32-2	UHLC-MS/MS	/	Lei et al., 2015
Wilforine	11088-09-8		/	
Wilforgine	37239-47-7	HPLC-UV, HR-MS, NMR	/	Li et al., 2015
Wilforine	11088-09-8		/	
Euonine	41758-69-4		/	
Peritassine A	262601-67-2		/	
Triptobenzene H	146900-55-2	IR, NMR, HR-ESI-MS	/	Xu et al., 2011
Triptinin B	189389-05-7		/	
Triptobenzene A	170557-25-2		/	
Blumenol C	36151-02-7		/	
Wilfosinine A, B, C, D, E, F, G, H	/		/	
Ejap-3, 4	/		/	

Celastrine A	/	/
Celangulatin F	/	/

"/" means no available information.

1306

Table 3 Summary of tutin and its derivatives

Compound Name	CAS number	Identification or Analysis method	Concentration range in honey (mg/kg)	Reference
Tutin	2571-22-4	SPE, LC–MS/MS	5.1	Fields et al., 2014
		NMR, LC–MS/MS	0.13–52.18	Larsen et al., 2015
		LC-MS	84−3868	Watkins et al., 2018
		SPE, UPLC-HR-MS	0.3	Yin et al., 2015
Corianin	35481-77-7	SPE, UPLC-HR-MS	ND	
Coriatin	91653-75-7		ND	
Picrotin	21416-53-5	LC-MS/MS	ND	Fields et al., 2014
Picrotoxinin	17617-45-7		ND	
Tutin monoglucoside		LC-MS	0−553	Watkins et al., 2018
Tutin diglucoside			0−923	
Hyenanchin	3484-46-6	LC–MS	0−340	
		SPE, LC-MS/MS	23	Fields et al., 2014
		NMR, LC–MS/MS	0.97–405.53	Larsen et al., 2015
Tutin diglycoside 5	/	NMR, LC-MS/MS	0.65–4.27	

Tutin diglycoside peracetate 7	/	ND
Tutin glycoside 4	/	0.12–2.86
Tutin monoglucoside 4	/	ND
Tutin monoglycoside peracetate 6	/	ND
Dihydrohyenanchin	/	ND
Dihydrotutin	/	ND
Tutin diglucoside 5	/	ND

1307 ND, not detected. "/" means no available information.

1308

Table 4 Summary of different PAs found in contaminated honey

Compound Name	CAS number	Nectar origin	Identification or Analysis method	Concentration range in honey (μg/kg)	Reference
7-O-acetylvulgarine	/	borage crystalline, borage clarified, clover crystalline, blue borage herbal, New Zealand alpine borage, clover blend, blue borage, rata, multifloral with manuka	SPE, HPLC-ESI-MS	ND	Betteridge et al., 2005
7-O-acetylvulgarine-N-oxide	/	borage crystalline, borage clarified, clover crystalline, blue borage herbal, New Zealand alpine borage, clover blend, blue borage, rata, multifloral with manuka	SPE, HPLC-ESI-MS	ND	
acetylechimidine	/	borage crystalline, borage clarified, clover crystalline, blue borage herbal, New Zealand alpine borage, clover blend, blue borage, rata, multifloral with manuka	SPE, HPLC-ESI-MS	0–96	

acetylechimidine-N-oxide	/	borage crystalline, borage clarified, clover crystalline, blue borage herbal, New Zealand alpine borage, clover blend, blue borage, rata, multifloral with manuka	SPE, HPLC-ESI-MS	0–104	
Crotaline	315-22-0	commercial honey from Ireland, EU and non-EU countries	SPE, LC-MS/MS	ND	Griffin et al., 2013
Echimidine	520-68-3	commercial honey from Ireland, EU and non-EU countries	SPE, LC-MS/MS	190–1746	
		Echium vulgare from Basel, Verzasca and other regions of Switzerland	UHPLC-HR-MS	1 – 153	Lucchetti et al., 2016
		acacia (Robinia pseudoacacia) or multifloral honey from Italia or Europea	QuEChERS, dSPE, LC-HR-MS	0.4–3.3	Martinello et al., 2014
		borage crystalline, borage clarified, clover crystalline, blue borage herbal, New Zealand alpine borage, clover blend, blue borage, rata, multifloral with manuka	SPE, HPLC-ESI-MS	0–299	Betteridge et al., 2005

echimidine N-oxide	/	borage crystalline, borage clarified, clover crystalline, blue borage herbal, New Zealand alpine borage, clover blend, blue borage, rata, multifloral with manuka	SPE, HPLC-ESI-MS	0–291
echimiplatine-N-oxide	/	borage crystalline, borage clarified, clover crystalline, blue borage herbal, New Zealand alpine borage, clover blend, blue borage, rata, multifloral with manuka	SPE, HPLC-ESI-MS	0–10
echiuplatine	/	borage crystalline, borage clarified, clover crystalline, blue borage herbal, New Zealand alpine borage, clover blend, blue borage, rata, multifloral with manuka	SPE, HPLC-ESI-MS	0–194
echiuplatine-N-oxide	/	borage crystalline, borage clarified, clover crystalline, blue borage herbal, New Zealand alpine borage, clover blend, blue borage, rata, multifloral with manuka	SPE, HPLC-ESI-MS	0–140

echivulgarine	/	borage crystalline, borage clarified, clover crystalline, blue borage herbal, New Zealand alpine borage, clover blend, blue borage, rata, multifloral with manuka	SPE, HPLC-ESI-MS	0–879	
echivulgarine-N-oxide	/	borage crystalline, borage clarified, clover crystalline, blue borage herbal, New Zealand alpine borage, clover blend, blue borage, rata, multifloral with manuka	SPE, HPLC-ESI-MS	0–558	
erucifoline	40158-95-0	forest honey, floral honey and rape honey from Germany	SPE, LC-MS/MS	ND	Kaltner et al., 2018
erucifoline N-oxide	/	forest honey, floral honey and rape honey from Germany	SPE, LC-MS/MS	ND	
europine	570-19-4	Non-pyrrolizidine alkaloid-producing floral sources, pyrrolizidine alkaloid-producing floral sources and retail honey from Australia	HPLC-APCI-MS	ND	Beales et al., 2004
Heliotrine	303-33-3	Non-pyrrolizidine alkaloid-producing floral sources, pyrrolizidine alkaloid-producing floral sources and retail honey from Australia	HPLC-APCI-MS	ND	

		retail honey from Italia, European Union, European and non-European Union	QuEChERS, SPE, UPLC-MS	ND	Martinello et al., 2014
		Echium (New Zealand), raw honeys (Germany), *Jacobaea vulgaris* (Netherlands)	QuEChERS, online-SPE, LC-MS, GC-MS	ND	Kempf et al., 2011
Heliotrine-N-oxide	6209-65-0	forest honey, floral honey and rape honey from Germany	SPE, LC-MS/MS	ND	Kaltner et al., 2018
		Echium (New Zealand), raw honeys (Germmany), *Jacobaea vulgaris* (Netherlands)	QuEChERS, online-SPE, LC-MS, GC-MS	ND	Kempf et al., 2011
Intermedine	10285-06-0	retail honey from Italia, European Union, European and non-European Union	QuEChERS, SPE, UPLC-MS	11 (mean)	Martinello et al., 2014
		forest honey, floral honey and rape honey from Germany	SPE, LC-MS/MS	ND	Kaltner et al., 2018
intermedine N-oxide	95462-14-9	forest honey, floral honey and rape honey from Germany	SPE, LC-MS/MS	ND	
Jacobine	10285-07-1	forest honey, floral honey and rape honey from Germany	SPE, LC-MS/MS	ND	
		specific hive sites and retail honey from UK	HPLC-MS	0 – 25	Crews et al.,1997

Jacolline	/	specific hive sites and retail honey from UK	HPLC-MS	0 – 2	
Jacozine	/	specific hive sites and retail honey from UK	HPLC-MS	0 – 9	
jacobine N-oxide	/	forest honey, floral honey and rape honey from Germany	SPE, LC-MS/MS	ND	
Lasiocarpine	303-34-4	retail honey from Italia, European Union, European and non-European Union	QuEChERS, SPE, UPLC-MS	ND	Martinello et al., 2014
		Non-pyrrolizidine alkaloid-producing floral sources, pyrrolizidine alkaloid-producing floral sources and retail honey from Australia	HPLC-APCI-MS	ND	Beales et al., 2004
leptanthine-N-oxide	/	borage crystalline, borage clarified, clover crystalline, blue borage herbal, New Zealand alpine borage, clover blend, blue borage, rata, multifloral with manuka	SPE, HPLC-ESI-MS	0–14	Betteridge et al., 2005
Lycopsamine	10285-07-1	commercial honey from Ireland, EU and non-EU countries	SPE, HPLC-ESI-MS	182–4078	Griffin et al., 2013
		acacia (*Robinia pseudoacacia*) or multifloral honey from Italia or Europea	QuEChERS, dSPE, LC-HR-MS	0.2–74.7	Martinello et al., 2014

		retail honey from Italia, European Union, European and non-European Union	QuEChERS, SPE, UPLC-MS	12 (mean)	
lycopsamine N-oxide	95462-15-0	Echium (New Zealand), raw honeys (Germany), *Jacobaea vulgaris* (Netherlands)	QuEChERS, online-SPE, LC-MS, GC-MS	ND	Kempf et al., 2011
Monocrotaline-N-oxide	35337-98-5	EC countries, Non-EC countries and blends from EC and non-EC countries	SPE, LC-ESI-MS/MS	ND	Bodi et al., 2014
Monocrotaline	315-22-0	92 commercial honey samples from Brazil	LC-ESI-MS/MS	0–16	Valese et al., 2016
Retrorsine	480-54-6	92 commercial honey samples from Brazil	LC-ESI-MS/MS	0–45	
Retrorsine-N-oxide	15503-86-3	92 commercial honey samples from Brazil	LC-ESI-MS/MS	0–55.7	
Senecionine	130-01-8	92 commercial honey samples from Brazil	LC-ESI-MS/MS	1.2–180	
		specific hive sites and retail honey from UK	UPLC-MS	0 – 13	Crews et al., 1997
Senecionine-N-oxide	13268-67-2	92 commercial honey samples from Brazil	LC-ESI-MS/MS	0–248	Valese et al., 2016
Seneciphylline	480-81-9	Echium (New Zealand), raw honeys (Germany), *Jacobaea vulgaris* (Netherlands)	QuEChERS, online-SPE, LC-MS, GC-MS	ND	Kempf et al., 2011

		retail honey from Italia, European Union, European and non-European Union	QuEChERS, SPE, UPLC-MS	3 (mean)	Martinello et al., 2014
		specific hive sites and retail honey from UK	HPLC-MS	0 – 9	Crews et al., 1997
Seneciphylline-N-oxide	38710-26-8	EC countries, Non-EC countries and blends from EC and non-EC countries	SPE, LC-ESI-MS/MS	ND	Bodi et al., 2014
senecivernine	72755-25-0	forest honey, floral honey and rape honey from Germany	SPE, LC-MS/MS	ND	Kaltner et al., 2018
senecivernine N-oxide	101687-28-9	forest honey, floral honey and rape honey from Germany	SPE, LC-MS/MS	ND	
Senkirkine	2318-18-5	Echium (New Zealand), raw honeys (Germany), *Jacobaea vulgaris* (Netherlands)	QuEChERS, online-SPE, LC-MS, GC-MS	ND	Kempf et al., 2011
		retail honey from Italia, European Union, European and non-European Union	QuEChERS, SPE, UPLC-MS	4 (mean)	Martinello et al., 2014
Trichodesmine	548-90-3	EC countries, Non-EC countries and blends from EC and non-EC countries	SPE, LC-ESI-MS/MS	ND	Bodi et al., 2014
uplandicine-N-oxide	/	borage crystalline, borage clarified, clover crystalline, blue borage herbal, New Zealand alpine borage, clover blend, blue borage, rata, multifloral with manuka	SPE, HPLC-ESI-MS	0–15	Betteridge et al., 2005

vulgarine	/	borage crystalline, borage clarified, clover crystalline, blue borage herbal, New Zealand alpine borage, clover blend, blue borage, rata, multifloral with manuka	SPE, HPLC-ESI-MS	0–84
vulgarine-N-oxide	/	borage crystalline, borage clarified, clover crystalline, blue borage herbal, New Zealand alpine borage, clover blend, blue borage, rata, multifloral with manuka	SPE, HPLC-ESI-MS	0–166

ND, not detected. "/" means no available information.

Figure captions

Fig. 1. Varieties of plants producing toxic honey

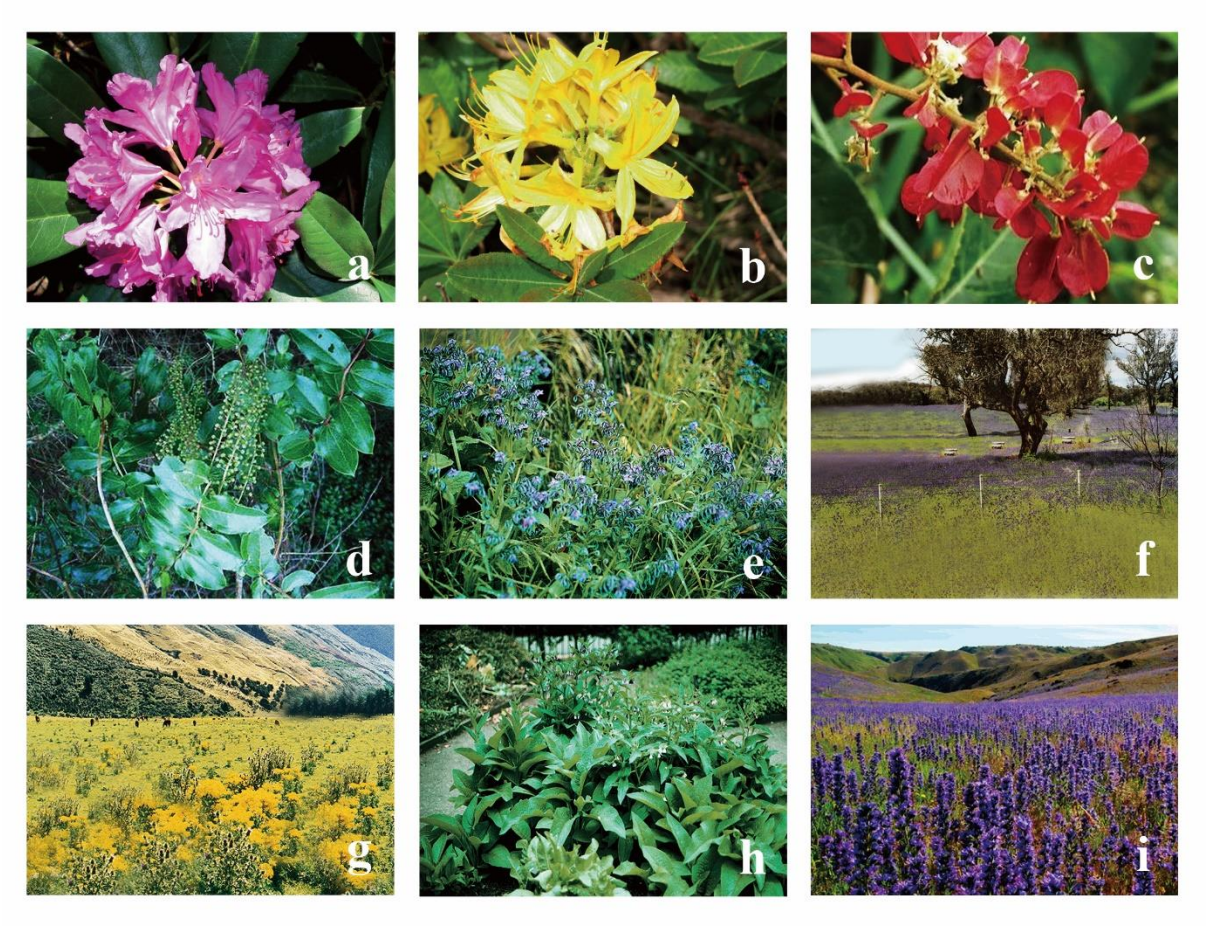
a: The purple-flowered *Rhododendron ponticum* is also known as the "mountain rose" (Gunduz et al., 2008b); b: The yellow-flowered Rhododendron flavum, also known as *Rhododendron flavum* (Gunduz et al., 2008b); c: TwHf (Zhang et al., 2016); d: Tutu Bush Picture - Jim Edwards: From the references Guidance Document: Compliance Guide to the Food Standard: Tutin in Honey (28 November 2016); e: Common borage (*Borago officinalis*; Boraginaceae) cultivated in garden, Berkeley, California (Edgar et al., 2002); f: Paterson's curse (*Echium plantigineum*; Boraginaceae) infesting paddock in Victoria, Australia; the common name indicates the antipathy with which this plant is regarded by livestock producers (Edgar et al., 2002); g: Tansy ragwort (*Senecio jacobaea*; Compositae, tribe Senecioneae) growing in pastureland in New Zealand (Edgar et al., 2002); h: Comfrey (Symphytum officinale; Boraginaceae) cultivated in garden (Captain Cook's Cottage), Melbourne, Victoria, Australia (Edgar et al., 2002); i: Vista of *E. vulgare* in New Zealand showing the monofloral opportunity for foraging bees. (Photo courtesy of Barrie Wills.) (Betteridge et al., 2005).

Fig. 2. Process of selecting and screening literatures

Fig. 3. Mechanisms of toxicity of four kinds of plant toxins found in honey

a: Schematic representation of the functional α-subunit of voltage-gated ion channels and binding sites of grayanotoxin I (GTX-I) (Maejima et al., 2003); b: The schematic diagram of toxicity by triptolide (Xi et al., 2017); c: Tutin acts as an antagonist of the glycine receptor; d: Metabolism of toxic pyrrolizidine alkaloids (Song et al., 2020).

Fig. 4. Comparison of detection methods for different plant toxins in honey samples



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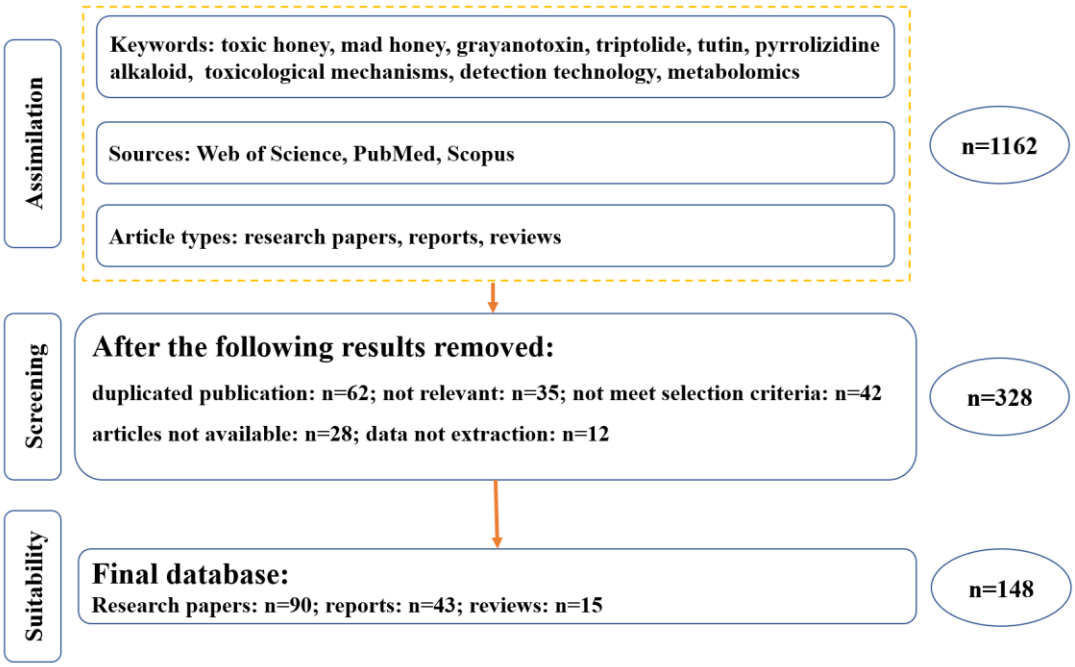

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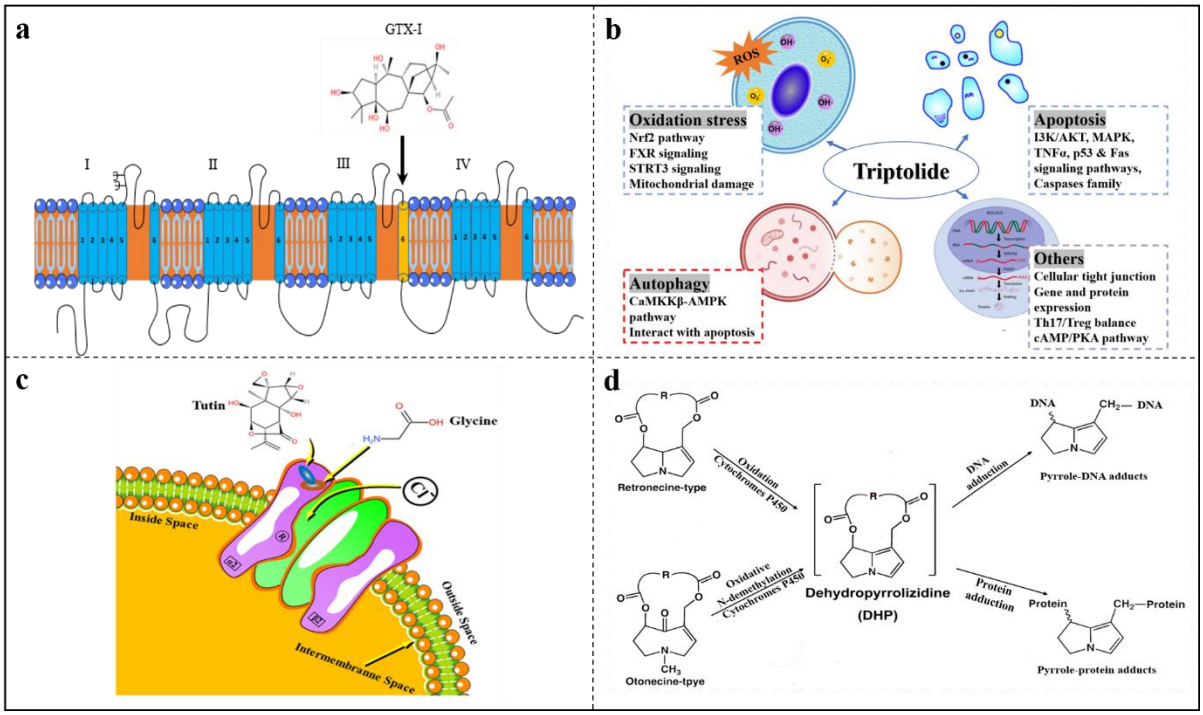

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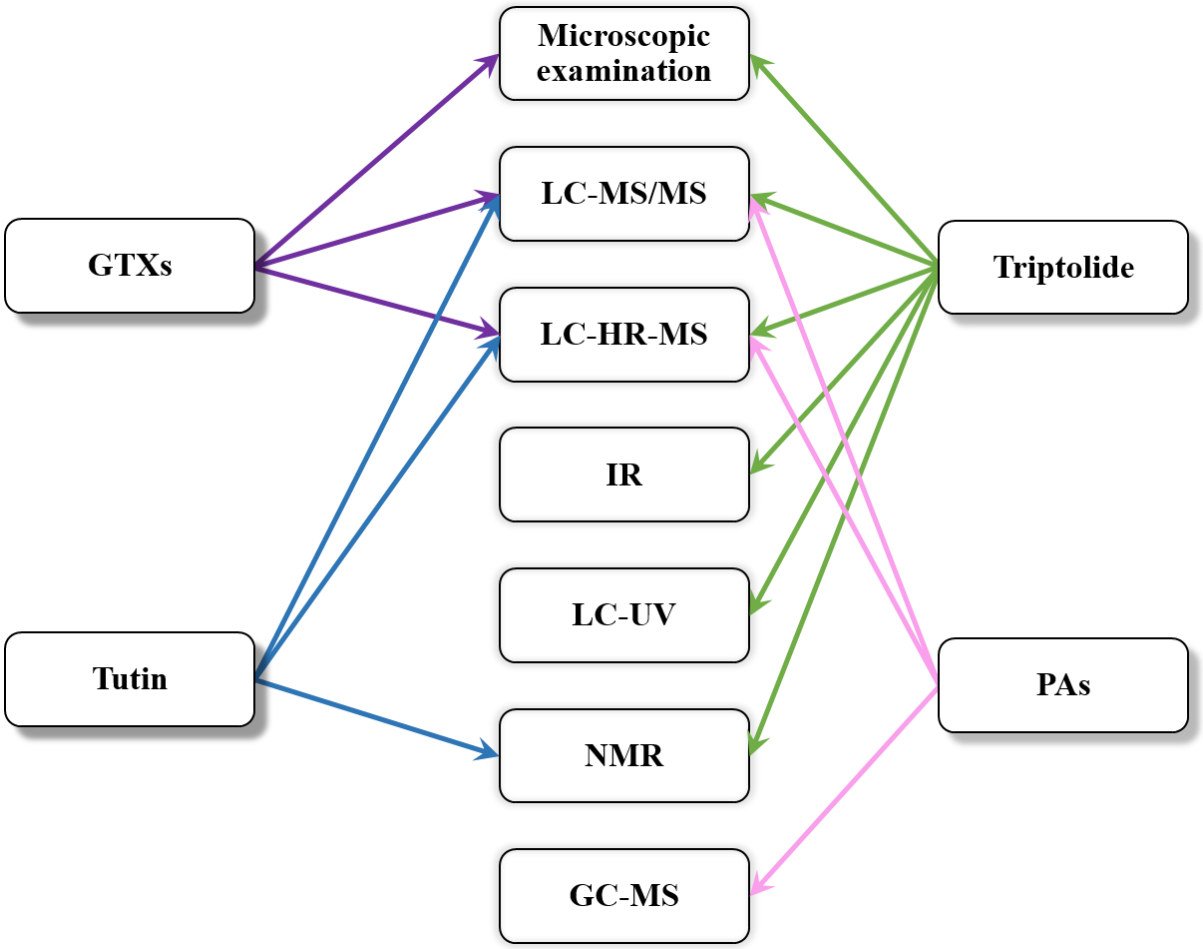

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