FATAL HONEY POISONING IN SOUTHWEST CHINA: A CASE SERIES OF 31 CASES

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Abstract. Honey poisoning cases occur in southwestern China. In this case series, we attempted to determine the symptoms and causes of honey poisoning from 2007 to 2012 in southwestern China. We also conducted a quantitative melissopalynological analysis of honey samples. During the study period, 31 honey poisoning cases occurred in the study location, all during July to August. All the cases occurred after consuming at least 100 grams of honey. The most frequent symptoms were nausea and vomiting (100%), abdominal pain (90.3%), diarrhea (74.2%), palpitations (61.3%), dizziness (54.8%), chest congestion (48.4%) and dyspnea (48.4%). Severe cases developed oliguria/anuria, twitch, hematuria, ecchymosis or hematochezia. The median time from ingestion to onset of symptoms was 29 hours. Eight patients died (mortality rate: 25.8%). The pollen of *Tripterygium hypoglaucum* (a plant with poisonous nectar and pollen) was detected in 22 of 29 honey samples examined (75.9%). The results of pollen analysis were consistent with the clinical findings of previous cases. *T. hypoglaucum* appears to be the cause of honey poisoning in southwestern China. Honey poisoning should be included in the differential diagnosis of patients who consume honey in this region and develop symptoms of food poisoning.

Keywords: honey, intoxication, triptolide, pollen analysis

INTRODUCTION

Ingestion of honey made from the nectar and pollen of poisonous plants may cause acute honey poisoning (Islam *et al*, 2014). Honey poisoning was first described in 401 BC (Leach, 1972). Two types of honey poisoning are the most well-known: "mad honey poisoning" described from Turkey, Nepal and Korea and "Tutin honey poisoning" described from New Zealand (Yavuz *et al*, 1991; Choo *et al*, 2008; Dubey *et al*, 2009; Chancellor, 2013). "Mad honey poisoning" is caused by Grayanotoxins from toxic *Rhododendron* species and "Tutin honey poisoning" is caused by the neurotoxin Tutin from shrubs in the genus *Coriaria* (Gunduz *et al*, 2008; Mcnaughton and Goodwin, 2008). Honey poisoning depends on the distribu-

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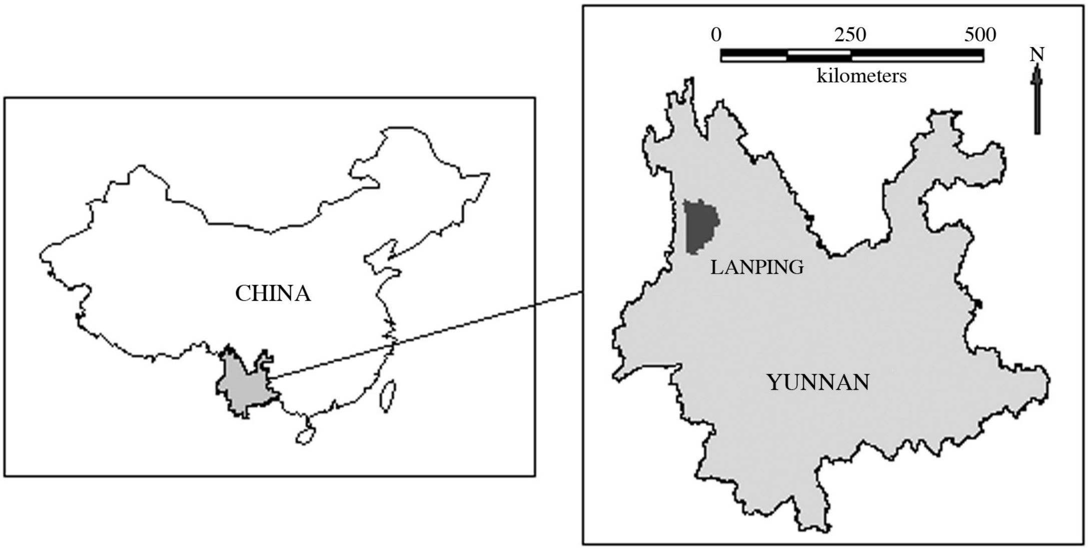

Fig 1–Location map showing study area.

tion of toxic plants. Honey poisoning may occur in areas without the toxic plants due to export of honey (Ocak *et al*, 2013; Sohn *et al*, 2014). With the popularity of natural food in recent years, more attention has been paid to the safety of honey.

Effective prevention and treatment require better understanding of honey poisoning. Pollen analysis is used to determine the cause of honey poisoning (Cagli *et al*, 2009; Sumerkan *et al*, 2011). By studying the pollen contained in the honey, the botanical and geographic origin of that honey can be identified (Oliveira *et al*, 2010). The clinical presentations and outcomes of honey poisoning depend on the toxic compounds in the honey. For example, "mad honey poisoning" caused by grayanotoxins is characterized by dizziness, hypotension and atrial-ventricular block (Jansen *et al*, 2012). Patients usually recover within 24 hours and no deaths due to this type of honey poisoning have been reported in the modern literature (Silici and Atayoglu, 2015). However, fatal honey poisoning has been occurring for a long time in southwestern China. This poisoning is characterized by multiple-organ damage and high mortality (Liu *et al*, 2013; Zhang *et al*, 2016). However, studies of the clinical features and cause of this honey poisoning have not been reported. Recognition of this fatal honey poisoning by physicians could be lifesaving. The aims of this study were to determine the clinical characteristics and etiology of honey poisoning in southwestern China.

MATERIALS AND METHODS

We selected Lanping County, Yunnan Province, China as our study area, because this area is the most affected by honey poisoning in southwestern China (Fig 1). The honey poisoning cases reported from Lanping account for half of those in the province. Thirty-one patients were diagnosed with honey poisoning from 2007 to 2012; all were included in this study. A questionnaire was filled by all patients or their relatives or guardians. The questionnaire asked for demographic information, dietary history, main symptoms and time to onset of symptoms after consumption of the honey.

Between July and August 2013, 29 honey samples were collected in Lanping County, which has an altitude 1,500 to 2,700 meters in a local farming area. These samples were collected directly from the comb in beekeeping households. The method recommended by the International Commission for Bee Botany was used to analyze honey samples to identify the types of pollen (Louveaux *et al*, 1978; Yang *et al*, 2014). Twenty-five grams of honey from each sample was dissolved in 50 ml of warm water (40°C). The solution was centrifuged for 10 minutes at 3,000 revolutions/minute (rpm). The supernatant solution was decanted and the sediment collected in a conical tube and treated with an acetolysis mixture (acetic anhydride: conc. sulphuric acid = 9:1 V/V) for approximately 30 minutes at room temperature (Erdtman, 1960). After acetolysis, the sediment was rinsed three times with distilled water, centrifuged for 10 minutes at 3,000 rpm and placed on a slide for analysis. The slides were photographed under a Leica DM2500 light microscope (Leica, Wetzlar, Germany). Pollen types were identified using reference slides and palynological literature (Liu, 1995, 1996, 2000). The absolute pollen counts (APC) of the honey samples were calculated using a high microscope at 100×magnification (Song *et al*, 2012). The percentages of the types of pollen were calculated as follows:

$$\text{Pollen percentages} = \frac{\text{APC of certain pollen grains in 10 microscopic fields}}{\text{APC of all pollen grains in 10 microscopic fields}} \times 100\%$$

Statistical analysis

The study area was displayed with MapInfo 7.0 (MapInfo, Harrison, NJ). The demographic characteristics and clinical symptoms of the 31 cases were analyzed statistically. Numeric variables (*eg*, age) were calculated as means ± standard deviation (SD) and means with ranges. Categorical variables (*eg*, gender) were calculated as percentages. All data were analyzed using SAS software (Version 9.4; SAS Institute, Cary, NC).

RESULTS

All 31 cases occurred in July or August. There were 14 males (45.2%). The cases denied any previous medical problems, trauma or food or drug allergies. The mean age of the cases was 30±16 (ranging: 3-73) years. The most frequently observed symptoms among cases were: nausea and vomiting (100%), abdominal pain (90.3%), diarrhea (74.2%), palpitations (61.3%), dizziness (54.8%), chest congestion (48.4%) and dyspnea (48.4%). Severe cases had oliguria/anuria, twitch, hematuria, ecchymosis and hematochezia. The median time to onset of symptoms after ingestion of the honey was 29 hours. Eight patients died (mortality of 25.8%).

Twenty patients (64.5%) consumed farmed honey and 11 (35.5%) consumed wild honey. The mortality rates for the farmed and wild honey were 30.0% and 18.2%, respectively. The average honey consumed was 100.0 ± 70.0 g (Table 1).

The pollen of *T. hypoglaucum*, a plant with poisonous nectar and pollen (Fig 2) was detected in 22 samples (75.9%). In 18 samples, *T. hypoglaucum* pollen was the predominant pollen (pollen percentage >45%). No significant associations were observed between the presence of *T. hypoglaucum* pollen and altitudes or colors of the honey (Table 2).

Table 1
Demographic and clinical findings of the patients diagnosed with honey poisoning.

Characteristics	Findings
Mean age in years (range)	28.6 (3-73)
Sex, *n* (%)	
Male	14 (45.2)
Female	17 (54.8)
Symptoms, *n* (%)	
Nausea and vomiting	31 (100)
Abdominal pain	28 (90)
Diarrhea	23 (74)
Palpitations	19 (61)
Dizziness	17 (55)
Chest congestion	15 (48)
Dyspnea	15 (48)
Oliguria/anuria	8 (26)
Twitch	4 (13)
Hematuria	4 (13)
Ecchymosis	2 (7)
Hematochezia	2 (7)
Median time after ingestion to onset of symptoms in hours (range)	29 (6-48)
Deaths, *n* (%)	8 (26)
Mean amount of honey ingested in grams ± SD	100 ± 70
Source of honey, *n* (%)	
Farmed	20 (64.5)
Wild	11 (35.5)

SD, standard deviation.

DISCUSSION

The honey poisoning cases in the series had symptoms similar to common food poisoning cases (*eg*, nausea and vomiting), but also presented with organ compromise (*eg*, oliguria) in severe cases. The signs and symptoms of the cases are consistent with the *T. hypoglaucum* identified from the honey samples tested. This information is important for honey poisoning prevention and treatment in southwestern China, and where ever the toxic honey is exported.

Honey poisoning in southwestern China was fatal, unlike the "mad honey poisoning" reported from Turkey (Zhang *et al*, 2016). Mad honey poisoning symptoms generally begin within several minutes to hours of ingestion but do not last more than 24 hours (Yavuz *et al*, 1991; Silici and Atayoglu, 2015). Most patients with mad honey poisoning can be adequately treated with intravenous atropine and normal saline infusion and will recover (Gunduz *et al*, 2009). However, the incubation period with honey poisoning in southwestern China could vary from several hours to several days, and the early symptoms are not specific. Because of this, early diagnosis and treatment are difficult. Severe patients develop multi-organ

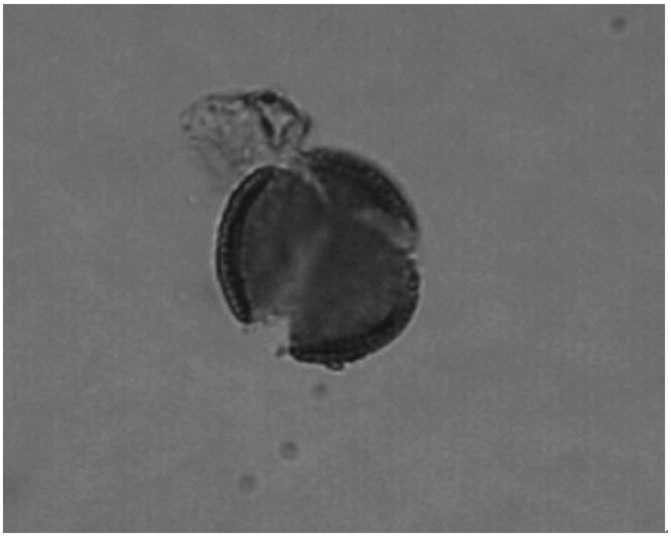

Fig 2–Photomicrograph of *T. hypoglaucum* pollen grains.

involvement and die within 3-4 days (Zhang *et al*, 2016). Toxic myocarditis and acute renal failure are the most common causes of death (Yang *et al*, 2012). As with other types of food poisoning, reducing toxin absorption and promoting excretion are crucial (WHO, 2008). Hemodialysis is effective in treating critical patients with honey poisoning (Liu *et al*, 2013). However, treatment may be more effective if the cause of poisoning is known (Sumerkan *et al*, 2011).

T. hypoglaucum is a plant from the genus *Tripterygium wilfordii* and is found distributed in southern China (Wu *et al*, 2003). *T. hypoglaucum* and other plants in the genus *Tripterygium wilfordii* contain a variety of alkaloids (Deng, 2001). The most active alkaloid is triptolide, a diterpenoid triepoxide which could be found in the leaf, root and pollen (Tan *et al*, 2007). Triptolide is highly toxic and the LD50 administrated intravenously in mice was 0.83 mg/kg (Xu *et al*, 2013). Triptolide can induce oxidative stress by suppressing superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and result in apoptosis of cells (Yang *et al*, 2012). The kidneys, liver and heart are all injured by triptolide (Xu *et al,* 2013). Acute poisoning caused by *T. hypoglaucum* is characterized by gastrointestinal symptoms at the beginning and then symptoms of cardiac, kidney, liver and nervous system damage, such as palpitations, twitch, oliguria and hematuria (Deng, 2001). Toxic myocarditis and acute renal failure (ARF) are the main causes of death (Huang *et al*, 2009). *T. hypoglaucum* has long been used in Chinese traditional medicine for treating autoimmune disease (Xu *et al*, 2013). Due to its narrow therapeutic window, *T. hypoglaucum* often results in serious poisoning (Deng, 2001). Clinical cases of acute *T. hypoglaucum* poisoning are usually caused by over or mistaken ingestion (Deng, 2001). Intoxication caused by honey ingestion is seldomly reported. In this study, all the cases occurred during July or August, which is the blooming period for *T. hypoglaucum.* Approximately 30% of the patients (10/31) developed oliguria, anuria or hematuria. A large proportion of the pollen detected in the studied honey was *T. hypoglaucum*. This suggests *T. hypoglaucum* may have been the cause of the poisoning cases described here and indicates ongoing risk for future poisoning cases.

Previous studies have found the honeybee (*Apis cerana*) will forage on *T. hypoglaucum* when regular nectar plants are less available due to prolonged dry weather or other reasons (Tan *et al*, 2007). Honey may be contaminated with *T. hypoglaucum* pollen in southern China. The risk of honey poisoning is related to climatic

Table 2
Percent of pollen that is *T. hypoglaucum* among studied samples.

Sample No.	Altitude (meters)	Honey color	Percentage of *T. hypoglaucum*
1	1,429	Light amber	-
2	1,441	Light amber	100
3	1,493	Light amber	5
4	2,329	Light amber	9
5	2,359	Dark amber	-
6	2,359	Dark amber	81
7	2,359	Dark amber	97
8	2,359	Dark amber	100
9	2,359	Dark amber	-
10	2,401	Light amber	46
11	2,486	Light amber	100
12	2,486	Light amber	93
13	2,486	Light amber	12
14	2,523	Dark amber	76
15	2,523	Dark amber	98
16	2,523	Dark amber	85
17	2,523	Dark amber	98
18	2,523	Dark amber	91
19	2,523	Dark amber	89
20	2,523	Dark amber	-
21	2,523	Dark amber	94
22	2,523	Dark amber	98
23	2,529	Dark amber	86
24	2,529	Dark amber	50
25	2,529	Dark amber	-
26	2,589	Light amber	-
27	2,630	Light amber	50
28	2,630	Amber	33
29	2,630	Amber	-

-, not detected.

conditions and plant resources. A recent study found the content of triptolide in honey decreased by 50% after storing for 3 months (Cao *et al*, 2014). Honey poisoning caused by triptolide generally occurs when the honey is consumed soon after it is made, during the bloom period for *T. hypoglaucum,* between June and August. Local villagers mistakenly believe light colored honey is not made from *T. hypoglaucum*, but we found this to be untrue. We also found *T. hypoglaucum* pollen to be in higher concentrations in some parts of the hive. This may explain why a dose-response relation was not found among the reported cases. The high detection rate of *T. hypoglaucum* pollen in the studied honey samples indicates widespread risk of honey poisoning in this region. *T. hypoglaucum* is a plant with poisonous nectar and pollen prohibited for use by the latest national food safety standards of China (GB 14963-2011) (SAC/MOHC, 2011). More testing and standard limits are needed for prevention this potentially fatal honey poisoning (Lambert *et al*, 2013).

In summary, *T. hypoglaucum* is the probable cause of honey poisoning in this case series from southwestern China. Patients who consume honey from this region and patient with symptoms of food poisoning should be examined to determine for the possibility of honey poisoning, especially during July and August.

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REFERENCES

Cagli K E, Tufekcioglu O, Sen N, *et al*. Atrioventricular block induced by mad-honey intoxication: confirmation of diagnosis by pollen analysis. *Tex Heart Inst J* 2009; 36: 342-4.

Cao C, Dong S, Liu X, Gong Z, Tan K. Deactivation of triptolide in *Trypterygium hypoglaucum* honey. *Apicult China* 2014; 65: 51-2.

Chancellor AM. A bitter-sweet tale from the land of milk and honey. *Pract Neurol* 2013; 13: 185-7.

Choo YK, Kang HY, Lim SH. Cardiac problems in mad-honey intoxication. *Circ J* 2008; 72: 1210-1.

Deng W. Review: toxic side effects of *Tripterygium wilfordii*. *Pharmacol Clin Chine Mater Medica* 2001; 17: 42-7.

Dubey L, Maskey A, Regmi S. Bradycardia and severe hypotension caused by wild honey poisoning. *Hellenic J Cardiol* 2009; 50: 426-8.

Erdtman G. The acetolysis method, a revised description. *Svensk Bot Tidsk* 1960; 54: 561-4.

Gunduz A, Merice ES, Baydin A, *et al*. Does mad honey poisoning require hospital admission? *Am J Emerg Med* 2009; 27: 424-7.

Gunduz A, Turedi S, Russell RM, Ayaz FA. Clinical review of grayanotoxin/mad honey poisoning past and present. *Clin Toxicol (Phila)* 2008; 46: 437-42.

Huang G, Li L, Liu L, Wei D. [Pathological study on autopsy died of tripterygium intoxication— report of 4 cases]. *Chin J Intergr Tradit West Medic* 2009; 29: 165-8.

Islam MN, Khalil MI, Islam MA, Gan SH. Toxic compounds in honey. *J Appl Toxicol* 2014; 34: 733-42.

Jansen SA, Kleerekooper I, Hofman ZL, Kappen IF, Stary-Weinzinger A, Van Der Heyden MA. Grayanotoxin poisoning: 'Mad honey disease' and beyond. *Cardiovasc Toxicol* 2012; 12: 208-15.

Lambert O, Piroux M, Puyo S, *et al*. Widespread occurrence of chemical residues in beehive matrices from apiaries located in different landscapes of western France. *PLOS One* 2013; 8: e67007.

Leach DG. Ancient curse of the rhododendron. *Am Hort Mag* 1972; 51: 20-9.

Liu B. [Morphology of poisonous nectar and pollen plants in China]. *Apicult China* 1995; 3: 21-3.

Liu B. [Poisonous nectar and pollen plants of ranunculaceae family and their pollen morphology]. *Apicult Sci Technol* 1996; 3: 5-7.

Liu B. [Poisonous nectar and pollen plants and their pollen morphology in China]. *Apicult Sci Technol* 2000; 6: 4-5.

Liu L, Qin L, He M, *et al*. [Successful rescue of seven acute honey poisoning by blood purification]. *Chin J Blood Purif* 2013; 3: 154-5.

Louveaux J, Maurizio A, Vorwohl G. Methods of melissopalynology. *Bee World* 1970; 51: 125-38.

Mcnaughton D, Goodwin R. Reducing the threat tutu toxic tutu honey poses to the New Zealand beekeeping industry and consumers. *Horticult Food Res Inst New Zealand* Ltd, 2008.

Ocak T, Duran A, Basturk M, Sahin I. Mad honey poisoning presenting with ventricular tachycardia and acute myocardial infarction: a case report. *Hong Kong J Emerg Med* 2013; 20: 299-300.

Oliveira P, van den Berg C, Santos F. Pollen analysis of honeys from Caatinga vegetation of the State of Bahia, Brazil. *Grana* 2010; 49: 66-75.

Silici S, Atayoglu AT. Mad honey intoxication: a systematic review on the 1199 cases. *Food Chem Toxicol* 2015; 86: 282-90.

Sohn CH, Seo DW, Ryoo SM, *et al*. Clinical characteristics and outcomes of patients with grayanotoxin poisoning after the ingestion of mad honey from nepal. *Intern Emerg Med* 2014; 9: 207-11.

Song X, Yao Y, Yang W. Pollen analysis of natural honeys from the central region of shanxi, North China. *PLOS One* 2012; 7: 11.

Sumerkan M C, Agirbasli M, Altundag E, Bulur S. Mad-honey intoxication confirmed by pollen analysis. *Clin Toxicol (Phila)* 2011; 49: 872-3.

Tan K, Guo YH, Nicolson SW, Radloff SE, Song QS, Hepburn H R. Honeybee (*Apis cerana*) foraging responses to the toxic honey of *Tripterygium hypoglaucum* (Celastraceae): Changing threshold of nectar acceptability. *J Chem Ecol* 2007; 33: 2209-17.

World Health Organization (WHO). Foodborne disease outbreaks: guidelines for investigation and control [online]. Geneva: WHO, 2008. [Cited 2016 Jul 17]. Available from: http://apps.who.int/publications/list/9787117113977/zh/index.html

Wu Z, Raven PH, Hong D. Flora of China. Vol 45(3). Celastraceae. Beijing: Science Press, 2003.

Xu L, Qiu Y, Xu H, Ao W, Lam W, Yang X. Acute and subacute toxicity studies on triptolide and triptolide-loaded polymeric micelles following intravenous administration in rodents. *Food Chem Toxicol* 2013; 57: 371-9.

Yang F, Ren L, Zhuo L, Ananda S, Liu L. Involvement of oxidative stress in the mechanism of triptolide-induced acute nephrotoxicity in rats. *Exp Toxicol Pathol* 2012; 64: 905-11.

Yang Y, Battesti M-J, Costa J, Paolini J. Characterization of botanical and geographical origin of corsican "spring" honeys by melissopalynological and volatile analysis. *Foods* 2014; 3: 128-48.

Yavuz H, Ozel A, Akkus I , Erkul I. Honey poisoning in Turkey. *Lancet* 1991; 337 (8744): 789-90.

Zhang Q, Chen X, Chen S, Liu Z, Wan R, Li J. Fatal honey poisoning caused by *Tripterygium wilfordii* Hook F in Southwest China: a case series. *Wilderness Environ Med* 2016; 27: 271-3.