International Journal of Nutrition and Food Sciences
2023; 12(6): 166-172
http://www.sciencepublishinggroup.com/j/ijnfs
doi: 10.11648/j.ijnfs.20231206.11
ISSN: 2327-2694 (Print); ISSN: 2327-2716 (Online)

Investigation of *Galerina sulciceps*-Induced Food Poisoning: A Case Study

He Zhifan[1, †], Li Xiaohui[1, *, †], Ma Haiying[2], Zhang Qiang[2], Luo Chunying[1], Feng Min[1], Wang Yao[1], Wang Xixi[1]

[1]Chengdu Center for Disease Control and Prevention, Chengdu, China
[2]Wenjiang Center for Disease Control and Prevention, Chengdu, China

Email address:
lxhqj1@163.com (Li Xiaohui), 504574004@qq.com (He Zhifan)
[*]Corresponding author
† He Zhifan and Li Xiaohui are co-first authors.

To cite this article:
He Zhifan, Li Xiaohui, Ma Haiying, Zhang Qiang, Luo Chunying et al. (2023). Investigation of Galerina sulciceps-Induced Food Poisoning: A Case Study. *International Journal of Nutrition and Food Sciences*, *12*(6), 166-172. https://doi.org/10.11648/j.ijnfs.20231206.11

Received: September 6, 2023; **Accepted**: October 27, 2023; **Published**: November 24, 2023

Abstract: *Objective*: This study aimed to investigate a poisoning incident resulting from the consumption of wild mushrooms, understand the poisoning process, identify the cause of poisoning, and determine potential influencing factors. *Methods*: The investigation employed epidemiological methods, morphological and molecular identification of mushroom samples, and toxin detection. *Results*: A 65-year-old male and a 62-year-old female consumed approximately 300g of wild mushrooms, while a 3-year-old boy only consumed mushroom soup. Symptoms including abdominal pain, vomiting, and diarrhea appeared in the three individuals 14-17 hours after consumption. The patients were correctly diagnosed and treated at approximately 19 hours, 43 hours, and 72 hours after consuming the wild mushrooms. Upon admission, the liver function indicators of the three patients were: 102U/L (ALT)/141U/L (AST), 1186U/L (ALT)/795U/L (AST), and 15446U/L (ALT)/18033U/L (AST), respectively. The 65-year-old male and 62-year-old female were discharged on the 7th and 9th day after treatment, respectively, while the 3-year-old boy died on the 6th day. Morphological and molecular identification of fresh toxic mushrooms revealed *Galerina sulciceps* as the causative agent. Laboratory testing detected three types of amatoxins, including α-amanitin, β-amanitin, and γ-amanitin. *Conclusion*: The outbreak of this incident was caused by the ingestion of toxic mushrooms *Galerina sulciceps* containing amatoxins. The prognosis of amatoxin-induced poisoning is associated with timely and accurate diagnosis and treatment. It is recommended to strengthen public education and market supervision to prevent the picking and consumption of wild mushrooms. The public should be reminded to seek medical attention promptly if symptoms appear 6-7 hours after consuming wild mushrooms and inform the healthcare providers about their history of wild mushroom consumption.

Keywords: Investigation, Mushroom, Food Poisoning, *Galerina Sulciceps*

1. Introduction

Mushroom poisoning poses a significant threat to public health, being the leading cause of death resulting from foodborne illnesses [1-2]. Data from the "Outbreak Public Health Event Reporting and Management Information System" by the Chinese Center for Disease Control and Prevention reveals that mushroom poisoning accounted for 35.6% of all foodborne illness-related deaths in China between 2004 and 2014 [3]. Particularly within Sichuan Province, toxic mushrooms not only pose a major risk for fatal food poisoning but also contribute significantly to foodborne illness incidents. From 2010 to 2018, mushroom-related food poisoning events and fatalities in Sichuan Province accounted for 61.5% and 50.0% respectively, of the total incidents and deaths within households [4]. Notably, in 2017, mushroom-induced food poisoning events in Chengdu City constituted 75.9% of the overall incidents [5].

In October 2019, a specific incident occurred in Chengdu City where three individuals experienced poisoning due to the inadvertent consumption of *Galerina sulciceps*, a highly toxic species of mushrooms. This study aimed to investigate the circumstances surrounding this incident through comprehensive methods including epidemiological investigation, laboratory testing, morphological identification, and molecular biological identification.

2. Materials and Methods

Epidemiological Investigation

The Chengdu Center for Disease Control and Prevention collaborated with the local center for disease control and prevention at the incident site to investigate and confirm the occurrence of mushroom poisoning. Interviews were conducted with attending physicians to gather information on clinical manifestations and treatment provided to the affected patients. Medical records were collected for further analysis. Patients and their family members were interviewed regarding the collection, processing, and consumption of the suspected mushrooms. The collection site was identified, and photographs and samples of the mushrooms were taken for examination and identification.

Morphological Identification

The suspected mushrooms were photographed and the images were forwarded to the Sichuan Academy of Forestry for morphological identification.

Molecular Biology Identification

For DNA extraction, a plant DNA extraction kit (TSP101-200, Beijing TsingKe Biological Technology Co., Ltd.) was utilized to extract DNA from the collected mushroom samples. The internal transcribed spacer (ITS) region was amplified and sequenced using polymerase chain reaction (PCR) technology. Following electrophoresis at 150V and 100mA for 20 minutes, the PCR products were observed, photographed, and the target DNA bands were excised, purified, and directly sequenced with primers. The resulting ITS sequences were compared with the GenBank nucleotide sequence database at the National Center for Biotechnology Information (NCBI) using Basic Local Alignment Search Tool (BLAST) [6]. To construct phylogenetic trees, Mega software was employed.

Laboratory Detection of Amanita Phalloides Toxins:

To detect toxins, high-performance liquid chromatography (HPLC) coupled with Q-Orbitrap mass spectrometry was employed. Ultra-pure water was used throughout the experimental process. Standard solutions of α-amanitin, β-amanitin, γ-amanitin, phalloidin, phallacidin, and phallolysin were procured from Fuzhou Qinpeng Biotechnology Co., Ltd. Sample pretreatment and analysis were performed according to the recommended procedures outlined in the "Manual for Food Safety Risk Monitoring".

3. Results

Case reports

On October 5, 2019, a 62-year-old female in Chengdu City, China collected approximately 300 g of wild mushrooms under a Cryptomeria tree located in her residence's courtyard. Subsequently, the harvested mushrooms were cooked and consumed by three family members. The woman ate approximately 100 g, her 65-year-old husband had around 200 g, and their 3-year-old grandson didn't directly consume any mushrooms but had about 50 g of mushroom soup. Within 14 to 17 hours, all three individuals experienced similar gastroenteritis symptoms.

Case 1: The 62-year-old female

Approximately 7 hours after experiencing nausea, vomiting, abdominal pain, and diarrhea, the woman purchased antibiotics from a pharmacy for self-medication. As her symptoms did not improve, she sought medical attention at the local township health center and was admitted to the hospital on October 7th. The initial examination revealed abnormal liver function, with ALT levels measured at 1186 U/L and AST levels at 795 U/L. Despite treatment, her AST levels continued to rise, reaching 2103 U/L on October 9th. Consequently, she was transferred to a higher-level hospital along with her husband. With appropriate treatment, she recovered and was discharged on October 14th.

Case 2: the 65-year-old *husband*

Around 4 hours after experiencing nausea, abdominal pain and diarrhea, the husband was admitted to the local township health center due to a history of consuming wild mushrooms. He was diagnosed with "acute gastritis" and "possible mushroom poisoning." The initial examination upon admission revealed abnormal liver function, with elevated levels of alanine aminotransferase (ALT) at 102 U/T (normal range: 5-45) and aspartate aminotransferase (AST) at 141 U/T (normal range: 5-45). Despite treatment, including liver protection measures, the patient experienced persistent abdominal pain, bloating, and fatigue. On October 9th, he was transferred to a higher-level hospital for further management. With appropriate treatment, he recovered and was discharged on October 12th.

Case 3: the 3 years and 10 months old grandson

On the early morning of October 6th, their grandson experienced temporary abdominal pain and vomiting. Initially, the family did not consider it a serious issue. However, when the child experienced another episode of abdominal pain and vomiting in the afternoon, they decided to seek medical attention at a pediatric healthcare clinic. Unfortunately, the family failed to inform the doctor about the child's consumption of mushroom soup. Consequently, the doctor initially diagnosed the child with "acute gastritis" due to "unexplained vomiting" and provided symptomatic treatment using Lactobacillus bifidus granules.

As the child's symptoms persisted on October 8th, a follow-up visit was scheduled, and intravenous infusion therapy was administered. Regrettably, during the treatment, the child suddenly developed a disorder of consciousness, prompting an immediate transfer to a higher-level hospital. It was at this point that the medical team became aware of the child's dietary history involving wild mushroom soup ingestion. The diagnosis was subsequently revised to "mushroom poisoning," accompanied by "hepatic failure," "hepatic encephalopathy," and "increased intracranial pressure syndrome.

Laboratory tests conducted upon admission revealed severe liver damage, reflected by significantly elevated ALT levels of 15,446 U/T and AST levels of 18,033 U/T. Despite receiving supportive treatment, including liver protection measures, the grandson's ALT and AST levels displayed some decrease, reaching 2856 U/T and 2995.5 U/T, respectively, on October 10th. However, these values remained dozens of times higher than the normal range. Additionally, signs of renal dysfunction emerged, indicated by elevated creatinine (CREA) levels of 242.1 umol/L and uric acid (UA) levels of 517.7 umol/L.

The medical consultation and progression of the three poisoned patients are shown in Figure 1, while the laboratory test results can be found in Table 1.

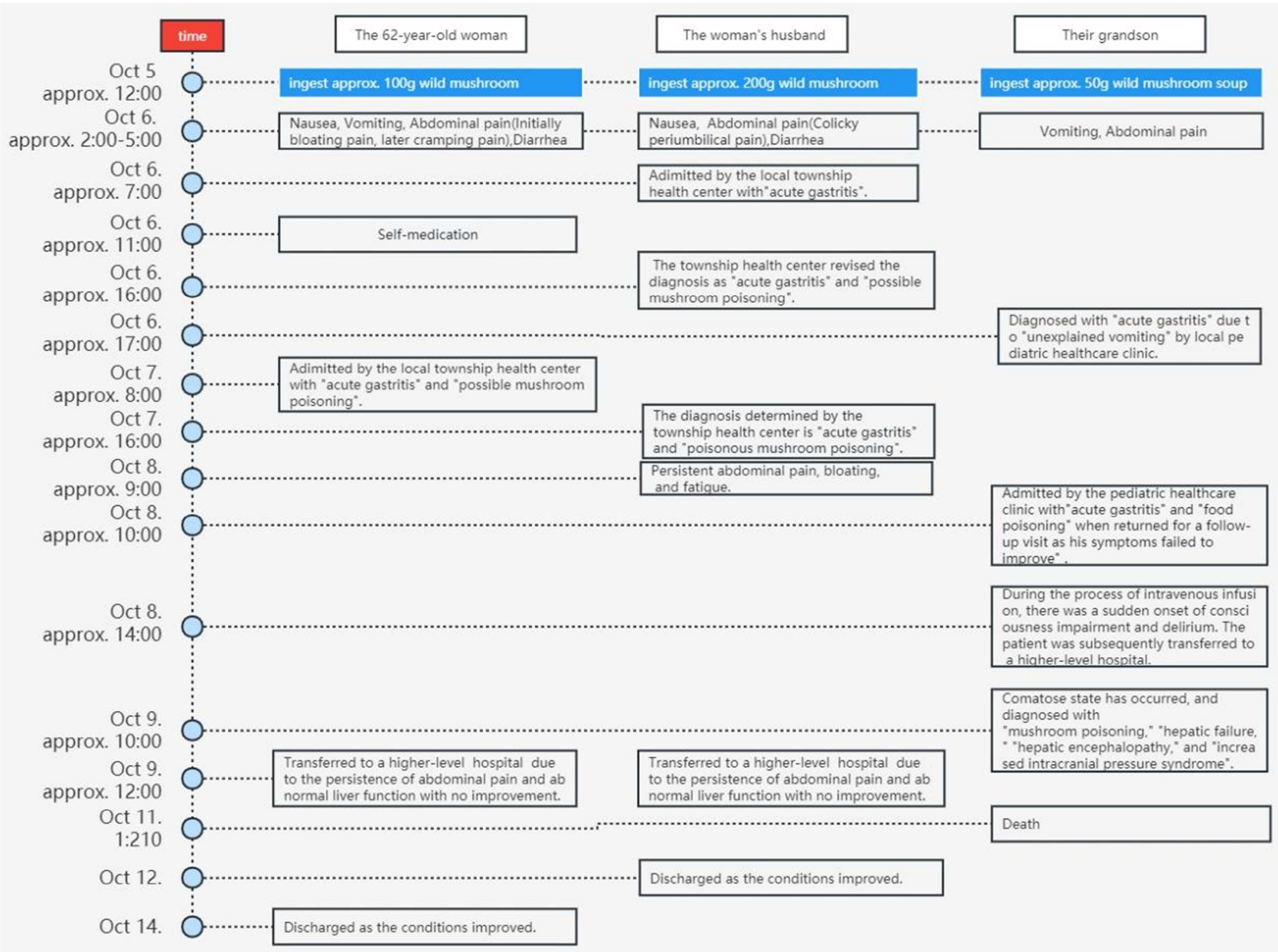

Figure 1. *Graph depicting the onset, medical consultation, and progression of the three poisoned patients.*

Table 1. *Laboratory test results of key indicators for the three poisoned patients.*

Laboratory test parameters	**The 62-year-old woman**				**The woman's husband**		**Reference range for adults**	**The 3-year-old grandson**				
	Oct 7.	**Oct 8.**	**Oct 9.**	**Oct 11.**	**Oct 6.**	**Oct 12.**		**Oct 6.**	**Oct 8.**	**Oct 9.**	**Oct 10.**	**Reference range**
ALT (U/L)	1186	1221	2103	831	102	-	5~45	-	15446	7150.2	2856	0~40
AST (U/L)	795	813	499	108	141	-	8~40	-	18033	8906.8	2995.5	0~40
CREA (umol/L)	132.7	142.5	52.5	57.2	125	86.69	12~133	-	35.3	56.6	242.1	17.3~54.6
UA (umol/L)	404	409	243	255	397	276	37~363	-	340.2	510	517.7	210~430
WBC/ (*10^9/L)	10.98	-	-	8.73	8.9	4.04	3.50~9.50	8.9	12.76	5.7	13.76	3.6~13
NE%/ (%)	85.2	-	-	82.2	83.8	61.9	40.0~75.0	82.81	82.6	84.6	75	12.9~56.7
Ly%/ (%)	11.4	-	-	13.1	2	26.2	20.0~50.0	10.32	4.5	6.3	16.7	35.4~78.6

Note: ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; CREA: Creatinine; UA: Uric Acid; "-" indicates not detected; WBC: White Blood Cell; NE%: Neutrophil Percentage; LY%: Lymphocyte Percentage; Reference ranges may vary across different medical institutions, and the widest range is used here; The reported values represent the highest levels recorded on the day.

Morphological identification

A sample of poisonous mushroom was collected at the scene (Figure 2). The fruiting body was small, with a cap diameter ranging from 1.3 to 4.5 cm. It exhibited a hemispherical to bell-shaped flattened convex shape, with a darker center and indistinct fine radial ridges along the margin. The cap surface was sticky when wet. The flesh of the mushroom was pale brown. The gills were initially yellow and later turned yellow-brown, with a close arrangement and varying lengths. The stem measured 5.4 to 8.3 cm in length and 0.3 to 0.7 cm in thickness. The upper part of the stem was yellow, while the lower part was black-brown, and it had a hollow interior. The ring membrane was present on the upper part of the stem. These morphological characteristics were consistent with those of *Galerina sulciceps*, as identified by an expert in forestry from the Sichuan Academy of Forestry.

Figure 2. *Galerina sulciceps under the Cryptomeria tree.*

Molecular biology identification

Comparison of the ITS sequence of the mushroom sample with the GenBank nucleotide sequence database revealed a 99% similarity to the sequence with the accession number KX214585.1. The species identified by this accession number is *Galerina sulciceps*.

A phylogenetic tree was constructed using the MEGA software for phylogenetic analysis. The newly generated sequence in this study (GenBank No. MT150619) clustered with *Galerina sulciceps* in the same branch and showed extremely high bootstrap support (Figure 3). Therefore, the sample was identified as *Galerina sulciceps*.

Based on the above results, it can be concluded that the wild mushroom responsible for the food poisoning incident is *Galerina sulciceps*.

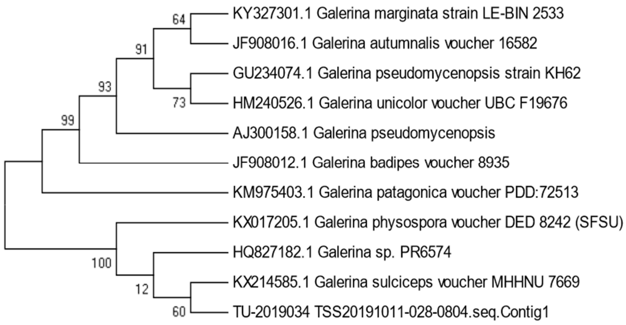

Figure 3. *phylogenetic relation tree of Galerina sulciceps.*

Detection of Amatoxins

The laboratory test results revealed the presence of α-amanitin, β-amanitin, and γ-amanitin in the mushroom sample, with detected levels of 131μg/kg, 64.8μg/kg, and 7.46μg/kg (fresh weight), respectively. PhaLLoidin, PhaLIacidin, and PhaLLisacin were not detected. The mass spectra of α-amanitin, β-amanitin, and γ-amanitin are shown in Figures 4 to 6.

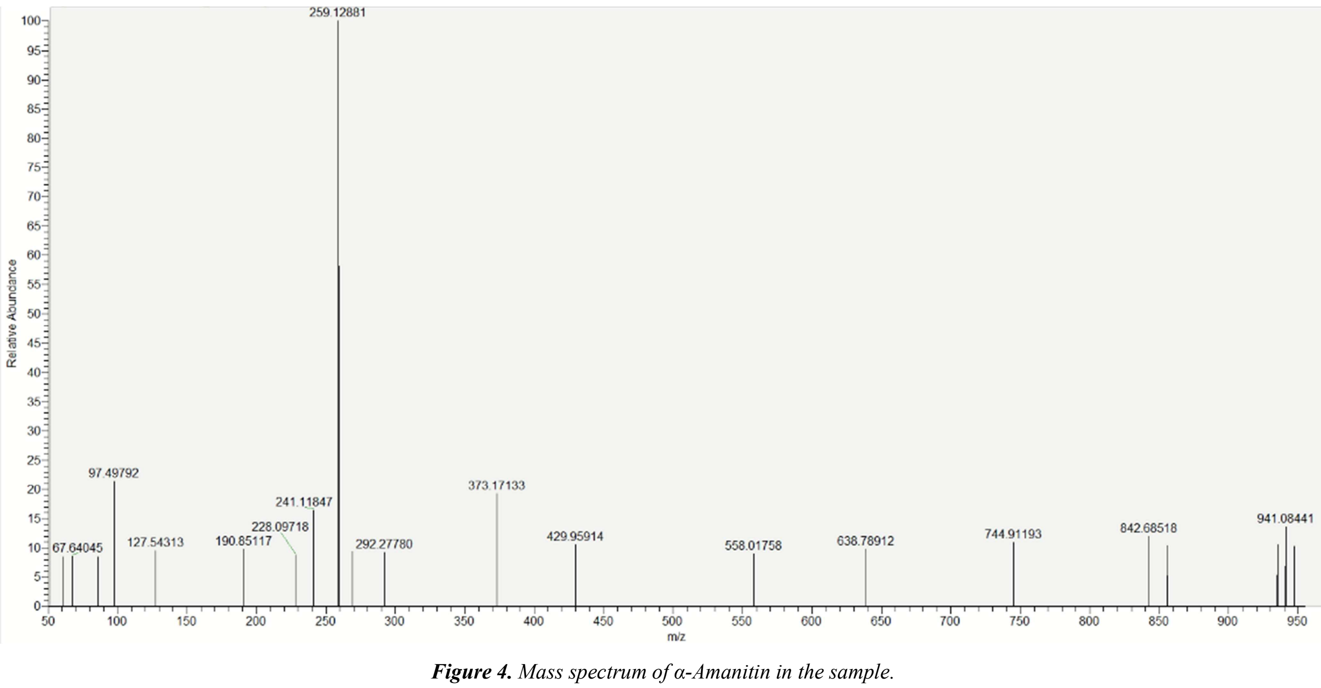

Figure 4. *Mass spectrum of α-Amanitin in the sample.*

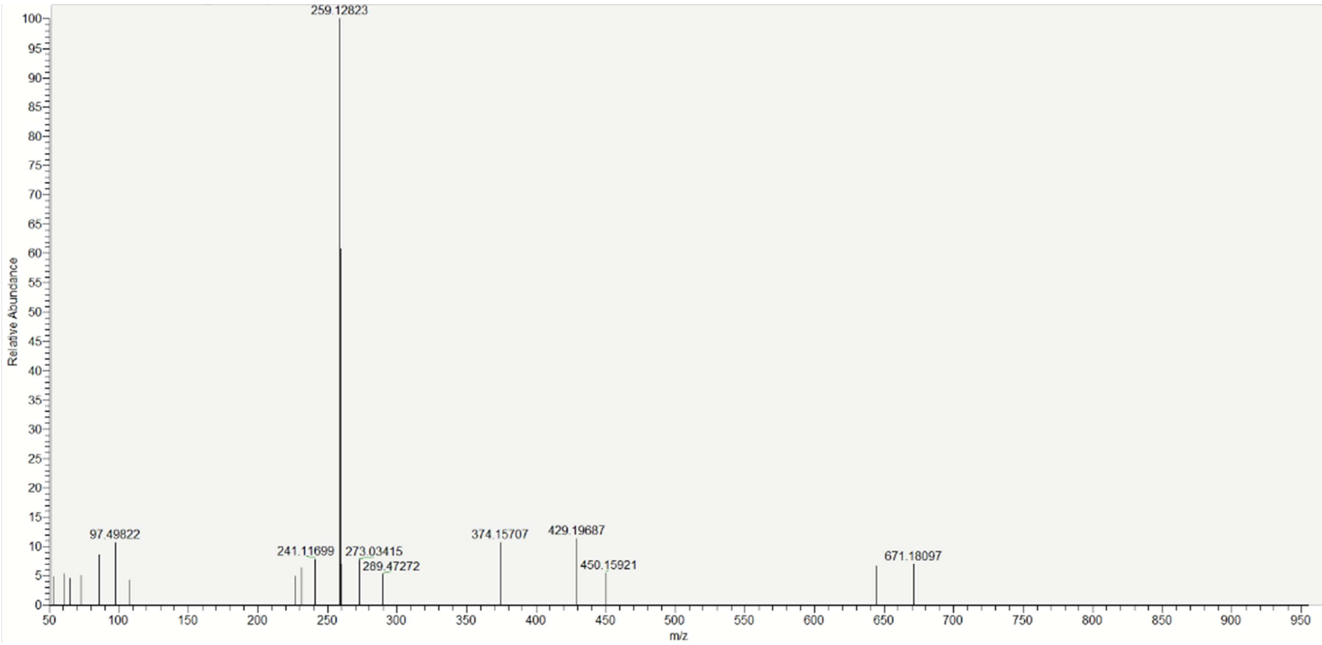

Figure 5. *Mass spectrum of β-Amanitin in the sample.*

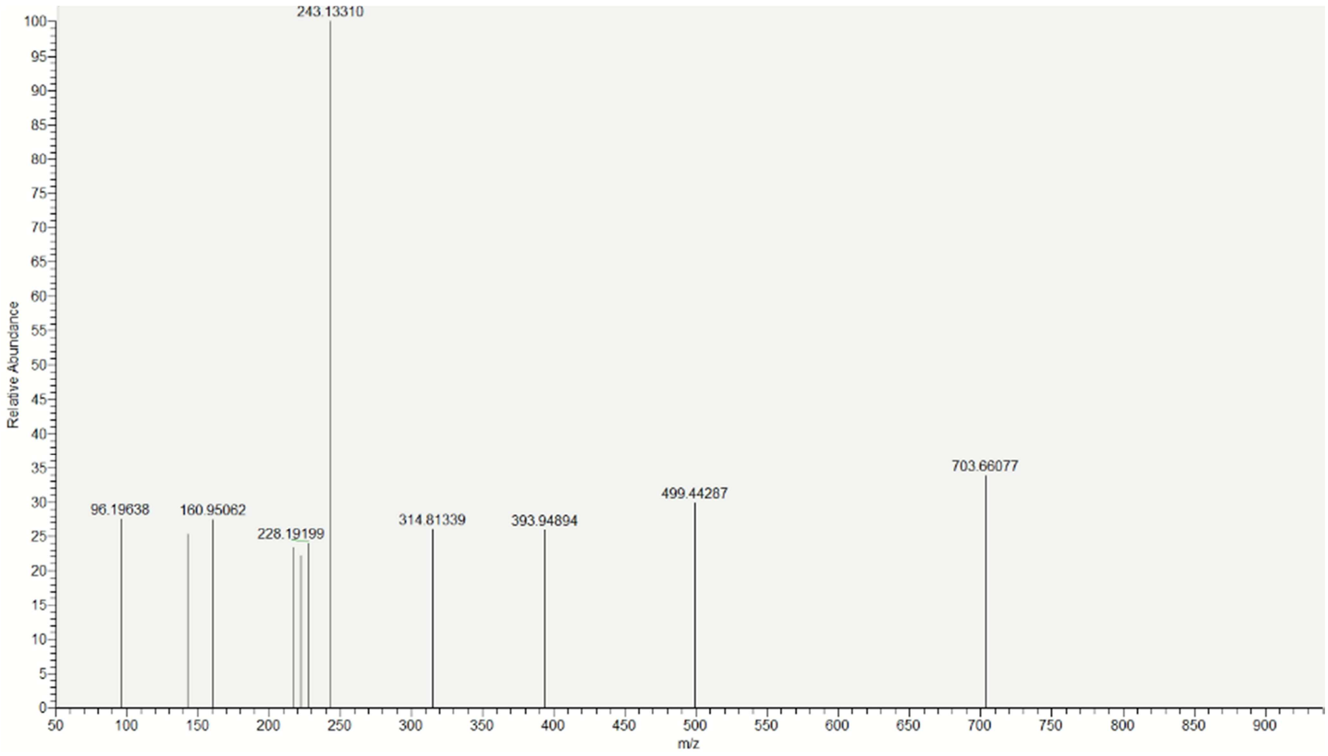

Figure 6. *Mass spectrum of γ-Amanitin in the sample.*

4. Discussion

In recent years, there have been a series of incidents across the country where people have been poisoned or even died after consuming the deadly mushroom *Galerina sulciceps* [7-8]. *Galerina sulciceps* contains potent toxins such as amanitin and phalloides [9-11]. The poisoning caused by these toxins can manifest in different types, including hemolytic, hepatotoxic, respiratory-circulatory failure, and gastrointestinal [10].

In this particular incident, the presence of α, β, and γ-amanitins was detected in the samples. Research has shown that amatoxins primarily damage liver tissue and cells by inhibiting the activity of eukaryotic RNA polymerase II, inducing cell apoptosis, and causing oxidative stress [12-13]. The clinical manifestations of amatoxins poisoning can be categorized into five distinct stages. The first stage is the latent period lasting for 6-24 hours, during which no symptoms are present. The second stage is characterized by gastroenteritis symptoms, such as nausea, vomiting, abdominal pain, and diarrhea, lasting for approximately 24-48 hours. The third stage is known as the ‘pseudo-remission period’, where gastroenteritis symptoms appear to alleviate, but in reality, this marks the initiation of liver and kidney impairment. The fourth stage is the organ damage period, spanning 72-96 hours, which carries a high risk of mortality. Surviving this stage leads to the recovery period [14]. In this reported incident, two elderly patients progressed through the latent period, gastroenteritis period, organ damage period, and recovery period, while an infant succumbed during the organ damage period.

While some literature suggests that the prognosis of patients may be linked to the amount of amatoxins ingested [15], our findings in this case series did not confirm this hypothesis. Instead, we found a significant association between the prompt recognition along with treatment and prognosis, this is similar to many other studies [16, 17]. Delayed diagnosis and treatment initiation were consistently associated with more severe liver and kidney damage, leading to a worse prognosis. For example, Case 2 sought medical attention approximately 19 hours after ingesting the wild mushroom. Laboratory tests revealed mild liver dysfunction, with ALT and AST levels at 2.3-fold and 3.5-fold the upper limit of normal, respectively. In contrast, Case 1 sought medical attention approximately 43 hours post-ingestion and exhibited severe liver impairment, with ALT and AST levels at 26.4-fold and 19.9-fold the upper limit of normal, respectively. Case 3 experienced a significant delay in the correct diagnosis for over three days, leading to hepatic failure, with ALT and AST levels at 386.2-fold and 450.8-fold the upper limit of normal, respectively. These findings underscore the importance of timely initiation of appropriate treatment, as it significantly impacts patient prognosis. Case 2 and Case 1 demonstrated recovery and were discharged after 7 and 9 days, respectively. However, Case 3 tragically succumbed to the illness on the sixth day.

In this incident, the delayed treatment of Case 3 was primarily attributed to the family's failure in promptly providing accurate information regarding the consumption of wild mushrooms. It is worth noting that poisoning can occur even through the ingestion of mushroom soup, as amatoxins are soluble in water [16, 18]. Unfortunately, the family seemed to be unaware of this fact.

Another contributing factor can be attributed to the initial attending doctor at the township health clinic. Although Case 2 sought medical attention in a timely manner, the doctor suspected wild mushroom poisoning but neglected to inquire about shared meals and inform other individuals who might be at risk, potentially preventing further cases. Furthermore, the first doctor at the pediatric healthcare clinic missed an opportunity for a correct diagnosis by neglecting to thoroughly inquire about the child's dietary history carefully during the initial examination. Additionally, children are particularly vulnerable to such incidents. As a result of these overlapping factors, the child's death occurred.

The frequent occurrence of wild mushroom poisonings can be attributed to several reasons. Firstly, in the mountainous regions of Chengdu, it is a common practice for villagers to gather and consume wild mushrooms. They heavily rely on their own experience to determine the edibility of the mushrooms. Secondly, the internet is filled with numerous articles on "how to identify poisonous wild mushrooms," which mislead the general public into believing that following some simple steps can prevent the consumption of toxic mushrooms. However, such methods have proven to be unreliable. Thirdly, there is a significant demand for wild delicacies that are free from additives and pesticides, which drives people to pick and purchase wild mushrooms. Some even sun-dry the mushrooms for easy storage. Once sliced and dried, it becomes even more difficult to identify if toxic mushrooms are mixed in. In the past two years, several incidents of poisoning have occurred in Chengdu due to the consumption of dried wild mushrooms. Lastly, rural areas often lack access to information, and there are many left-behind elderly and children who are particularly vulnerable, just like this case. The dissemination of relevant educational information through conventional channels is often challenging.

Therefore, in order to prevent such tragedies from happening again, a comprehensive approach should be taken. Firstly, health education should be provided to warn the public against buying, gathering, and consuming wild mushrooms. Since mushrooms are relatively easy to grow and reproduce, not only in rural mountainous areas but also in places like roadsides, parks, and flower beds at home, wild mushrooms can be found almost anywhere. For example, in this case, the wild mushrooms were gathered from the yard of the victim's house. In addition, attention should be paid to the accessibility of remote mountainous areas. Promotional methods can use traditional means such as posters, leaflets, and lectures, as well as new media such as TikTok and WeChat. Moreover, the public should be made aware of the potential hazards of mushroom poisoning. If symptoms of gastrointestinal inflammation occur within 4 hours of consuming wild mushrooms, it indicates that the consequences would not be severe. However, if more than 6-7 hours have passed, serious toxicity or even death may occur, and timely medical attention is necessary [19]. Secondly, relevant authorities should strengthen regulatory efforts and increase inspections to strictly prohibit the sale and production of wild mushrooms. Thirdly, it is necessary to enhance the diagnosis and treatment capabilities for mushroom poisoning and ensure proper inquiry and documentation of suspicious food cases. This requires strengthening the training of doctors at grassroots medical institutions to master the key points of local surveillance of foodborne diseases and understand effective questioning methods.

5. Conclusion

The outbreak of this incident was caused by the ingestion of toxic mushrooms, *Galerina sulciceps*, containing amatoxins. The death of the 3-year-old child was due to the healthcare provider’s failure to accurately obtain the history of wild mushroom consumption, leading to a delay in diagnosis and treatment.

It is important to highlight that the prognosis of amatoxin-induced poisoning is closely associated with timely and accurate diagnosis and treatment. To prevent the recurrence of such incidents, it is necessary to enhance health education and market supervision, discouraging the picking and selling of wild mushrooms. Additionally, the public should be reminded to promptly seek medical attention if symptoms occur 6-7 hours after consuming wild mushrooms and inform healthcare providers about their history of wild mushroom consumption.

References

[1] General Office of the State Health Commission. Notice on the situation of food poisoning incidents nationwide in 2015. Chinese Journal of Food Hygiene, 2016, 28 (3): 290, 391, 408.

[2] Jiang K, Ao YP, Luo HB. Analysis of the Food Poisoning in China from 1999 to 2015. Journal of Anhui Agricultural Sciences, 2018, 46 (28): 147-150, 154.

[3] Zhou J, Yuan Y, Lang N, et al. Analysis of mushroom poisoning incidents and hazards in mainland China. Chinese Journal of Emergency Medicine, 2016, 25 (6): 724-728. DOI: 10.3760/cma.j.issn.1671-0282.2016.06.008.

[4] Chen W, Lan Z, Cheng G, et al. Foodborne disease outbreaks in family in Sichuan, 2010-2018. Modern Preventive Medicine, 2019, 46 (23): 4391-4395.

[5] Liu Z, Li XH, Wang Y, et al. Analysis of poisoning caused by poisonous plant in Chengdu, 2017. Modern Preventive Medicine, 2019, 46 (7): 1308-1320.

[6] National Center for Biotechnology Information. https://www.ncbi.nlm.nih.gov/

[7] Guo C, Yang CL, Li XH, et al. Investigation and analysis of a poisoning event caused by Galerina sulciceps. Adverse Drug Reactions Journal, 15 (1): 22-26. DOI: 10.3760/cma.j.issn.1008-5734.2013.01.007.

[8] Zhang HM, Ye JS, Wang W, et al. Investigation and analysis of the first poisoning incident caused by Galerina sulciceps in Guizhou Province. Journal of Qiannan Medical College for Nationalities, 2015, 28 (3): 195-198.

[9] Chen ZH. New advances in researches on poisonous mushrooms since 2000. Mycosystema, 2014, 33 (3): 493-516. DOI: 10.13346/j.mycosystema.140041.

[10] Tu LGE, Bao HY, Li Y. A revised checklist of poisonous mushrooms in China. Mycosystema, 2014, 33 (3): 517-548. DOI: 10.13346/j.mycosystema.130256.

[11] Huang S, Chen ZH, Zhang P. Detection of Amanitin Toxins from Fruiting Body and Pure Culture of Galerina sulciceps. Fungal Science, 2015, 13 (3): 164-167. DOI: 10.13341/j.jfr.2014.1058.

[12] Mas A. Mushrooms, amatoxins and the liver. J Hepatol. 2005; 42: 166–169.

[13] Zheleva A, Tolekova A, Zhelev M, et al. Free radical reactions might contribute to severe alpha amanitin hepatotoxicity--a hypothesis. Med Hypotheses. 2007; 69: 361–7.

[14] Sun J, Sun CY, Li HJ, et al. Research progress on amanita peptide toxins. Chinese Journal of Emergency Medicine, 2006, 25 (8): 1076-1081. DOI: 10.3760/cma.j.issn.1671-0282.2016.08.025.

[15] Yilmaz I, Ermis F, Akata I, et al. A Case Study: What Doses of Amanita phalloides and Amatoxins Are Lethal to Humans?. Wilderness & Environmental Medicine, 2015, 26 (4): 491-496. DOI: 10.1016/j.wem.2015.08.002.

[16] Afrah Thiab Hlail. Mushroom (Amanita phalloides) Poisoning: Mechanisms, Pathogenesis, Prognosis and Strategies of Treatment. University of Thi-QarJournalof Science (UTsci). 2021; 8 (2): 16-22. DOI: 10.32792/utq/utjsci.v8i2.807.

[17] Wang LL, Lin D, Gao SH, et al. Investigation and analysis of a food poisoning incident caused by consumption of poisonous mushrooms resulting in 6 deaths. Chinese Journal of Food Hygiene, 2017, 29 (4): 505-507.

[18] Sun YB, Cao RM, Liu S, et al. Investigation of a poisoning incident caused by ingestion of Amanita subpallidorosea. Preventive medicine tribune, 2012, 18 (10): 778-782. DOI: 10.16406/j.pmt.issn.1672-9153.2012.10.004.

[19] Satora L, Pach D, Ciszowski K, et al. Panther cap Amanita pantherina poisoning case report and review. Toxicon, 2006, 47 (5): 605-607. DOI: 10.1016/j.toxicon.2006.01.008.