

POISONING WITH *AMANITA PHALLOIDES* (VAILL. EX FR.) LINK - A 25-YEAR RETROSPECTIVE ANALYSIS IN VARNA REGION, BULGARIA

Dobri Ivanov¹, Petko Marinov², Snezha Zlateva², Tsonka Dimitrova¹

¹*Department of Biology, Faculty of Pharmacy, Medical University of Varna*

²*Department of Pharmacology, Toxicology and Pharmacotherapy, Faculty of Pharmacy, Medical University of Varna*

ABSTRACT

Fungal intoxications are important in the ethology of acute exogenous intoxications and *A. phalloides* mushrooms intoxications are the most important for clinical toxicology. Poisonings with *A. phalloides* mushrooms are infrequent, but they have high lethality due to their high toxicity. A retrospective analysis of poisonings with *A. phalloides* mushrooms in Varna region for a period of 25 years (1991-2015) was conducted and 147 patients have been registered. The frequency relative to all hospitalised patients with acute poisoning is 0.8%. Such intoxications are more common in males and the ratio of men to women is 1.62:1. The largest number of intoxications was registered in the age group of 45 to 60 years - 62 (42.2%). All intoxications occurred following oral ingestion of *A. phalloides* mushrooms by mistake instead of edible mushrooms such as field mushroom - *Agaricus campestris* L. A lethal outcome was registered in 25 patients (17%). *A. phalloides* mushrooms account for 11.4% of the lethality in acute poisonings.

Keywords: *Amanita phalloides*, poisoning, amatoxin, liver failure

INTRODUCTION

Mushrooms are the main reason for hospitalization due to plant and fungi poisonings in Bulgaria. In Turkey the main portion of such toxicities comprises mushroom poisoning (1). The most important mushroom poisonings for toxicological practice in Southeast Europe are those with *A. phalloides*

mushrooms. *A. phalloides* poisonings are a worldwide problem. Most reported *A. phalloides* poisonings occur in Europe (2). Approximately 50-100 fatal cases are reported every year in Western Europe with them being less common in the United States. Cases of amatoxin poisoning from Africa, Asia, Australia and Central and South America have also been reported (3,4). Mushroom poisoning by *A. phalloides* is a rare but potentially fatal condition (5). Poisoning with Death cap (*Amanita phalloides* (Vaill. ex Fr.) Link) is a serious medical condition, causing organ failure with potential fatal outcome (6). Death cap is a toxic mushroom responsible for the majority of deaths occurring after mushrooms ingestion (2,7). *A. phalloides* is the most dangerous, poisonous mushroom species. It has been involved in the ma-

Address for correspondence:

Dobri Ivanov
Faculty of Pharmacy
Medical University of Varna
84 Tzar Osvoboditel Blvd
9000 Varna
e-mail: d.ivanov@mu-varna.bg

Received: May 18, 2018

Accepted: May 31, 2018

majority of human deaths from mushroom poisoning (90-95%) (3,8).

In this regard, we aimed to examine the *A. phalloides* poisonings in Varna region for a period of 25 years - from 1991 to 2015, analysing their frequency, distribution by sex and age, clinical course and therapeutic behaviour and lethality.

MATERIALS AND METHODS

The objects of the study were 147 patients treated at the Clinic of Toxicology of Military hospital Varna, the only Clinic of Toxicology in the Eastern Bulgaria, for a period of 25 years. The study was retrospective. The disease history, personal medical records of the admitted for treatment patients and medico-legal protocols from autopsies of the deceased patients were analysed. The diagnosis was based on:

- ◆ a history of consumption of wild mushrooms
- ◆ prolonged latent period of more than six hours
- ◆ severe gastrointestinal syndrome with repeated vomiting and watery diarrhoea stools
- ◆ expressed astheno-dynamic syndrome
- ◆ abnormal liver tests

We did not use radio-immunological or chromatographic determination of fungal toxins in serum and urine. The diagnosis was based on the combination of those clinical criteria.

RESULTS AND DISCUSSION

During the analysed period, 147 patients with *A. phalloides* poisoning have received treatment. Their relative share to all patients with acute intoxication is low - 0.8% confirming the low frequency of *A. phalloides* poisoning. In the neighbouring Turkey a significantly higher frequency with relative share of about 3% was reported (9). All *A. phalloides* poisonings result from consumption of wild Death cap mushrooms instead of edible mushrooms. The toxicity of *A. phalloides* is due to two groups of toxins - phallotoxins and amatoxins. Phallotoxins damage the cell membrane of enterocytes and cause gastrointestinal symptoms, such as nausea, vomiting, and diarrhoea. They are not absorbed by the intestine and do not reach the liver (10). Amatoxins are bicyclic octapeptides and are potentially lethal substances (11, 12). Amatoxins - alpha-, beta- and gamma-Amanitin are resistant to high temperatures with alpha-Amanitin having the highest toxicity (1,10,13,14). They

are not destroyed by cooking and can be present after long periods of storage (13,15). Amatoxins are easily absorbed through the intestinal epithelial cells and quickly leave the plasma because of a weak binding to plasma proteins up to the 48th hour after ingesting *A. phalloides* mushrooms, but are included in the enterohepatic circulation up to the 4th day (16,17). About 60% of alpha-Amanitin is excreted in bile and included in the enterohepatic circulation, which extends its effect to the liver cells (18). In addition to hepatotoxicity amatoxin possesses nephrotoxicity, damaging the proximal and distal tubules (19). Intestinal mucosa, hepatocytes and proximal tubules of the kidney are the main target structures, which are damaged by the toxins of *A. phalloides* mushrooms (4,11,16,20,21). Amatoxins directly interact with the enzyme RNA polymerase II in eukaryotic cells, inhibit transcription, prevent protein synthesis and cause cell death (16,22). The lethal amatoxin dose for humans is 0.1 mg/kg body weight (8) and may be contained in one single mushroom (16,22). The consumption of about 50 g fresh mushroom may be lethal (12).

Most of the patients who received treatment were men - 91 (61.9%). Women were 56 (38.1%) and the ratio of men to women was 1.62:1. The average age of patients with *A. phalloides* poisoning was 52.5 years (16-86). The analysis of the distribution of *A. phalloides* intoxications by age indicated that the most affected age group was from 45 to 60 years - 62 cases (42.2%) and their frequency in patients up to 24 years was very low (4.1%) - Table. 1.

A. phalloides poisonings in Bulgaria are most common in late summer and autumn, as in the period from August to November 136 cases (92.5%) were registered. The peak of intoxication was in October, when more than half of the poisonings were registered (Table. 2).

Table 1. Distribution of *A. phalloides* intoxication by age

Age	Number of patients	Percent
up to 24	6	4.1
25 - 44	35	23.8
45 - 60	62	42.2
above 60	44	29.9

Table 2. Distribution of *A. phalloides* intoxication by months

Month	Number of cases	Percent
May	2	1.4
June	3	2
July	5	3.4
August	29	19.7
September	11	7.5
October	76	51.7
November	20	13.6
December	1	0.7

The clinical picture of *A. phalloides* intoxication can be viewed in four phases.

1. Asymptomatic lag phase - from the time of ingestion of mushrooms to the appearance of the first symptoms. It continues from 6 to 24 hours (18,22), while according to other authors, duration can reach 40 hours (16) and most often lasts 10-12 hours (13,16,22,23). The average duration of the latency period in our study was 11 hours (7-20).
2. Gastrointestinal phase - characterised by the appearance of nausea, abdominal pain, repeated vomiting and watery diarrhoea stools, causing dehydration, arterial hypotension and electrolyte abnormalities. It lasts for about 12-24 hours. During this phase serum transaminases are already abnormal.
3. Apparent convalescence - develops 36-48 hours after ingestion of mushrooms. Gastrointestinal symptoms diminish or disappear, but serum transaminases continued to increase and jaundice may occur.
4. Acute liver failure - characterised by a prolonged increase in transaminase concentrations, whose values may reach several thousand units, hyperbilirubinemia, coagulopathy, hepatic encephalopathy, hepatorenal syndrome and acute renal failure. The hepatorenal phase developed after 2-3 days and acute renal failure was registered on 3rd-5th day (13). The renal biopsy indicated acute massive necrosis of the proximal tubule (24). Multi-organ failure, disseminated intravascular coagulation, seizures

and death may occur one to three weeks after ingestion (16).

The diagnosis is made based on the combination of a history of consumption of wild mushrooms, prolonged latent period (over 6 hours), clinical data and increased liver enzymes. Patients were admitted for treatment most often at the end of the first day after the consumption of mushrooms (15-38), according to our data - after 21 hours. The majority of these patients did not ask for medical assistance immediately after the appearance of the gastrointestinal syndrome because they did not associate the symptoms with mushroom consumption. This is the main reason for the delay of the hospitalisation and comparatively late initiation of treatment. At the end of the first day after the mushrooms consumption liver enzymes are already increased several times the upper limit of the reference values and this facilitated the establishment of the correct diagnosis. There are difficulties in rare cases of coincidence between the consumption of wild non-poisonous (edible) mushrooms by patients with chronic liver diseases and complaints of vomiting and diarrhoea due to another reason - e.g. enterovirus infection that appeared after the sixth hour from the mushroom consumption. In these cases, the initial transaminase levels were abnormal, but not very high and we recommended retesting of hepatic transaminases after 3 hours, as their values should have been identical or very similar to the initially measured. In the case of *A. phalloides* intoxication transaminase levels are significantly increasing during this 3-hour interval. We adhere to this scheme, because radio-immunological and chromatographic determination of the amatoxins in serum or urine is not available for clinical practice in Bulgaria.

Active treatment starts immediately after the patient is hospitalized and the diagnosis is made. Severe mushroom poisoning caused by amanitin remains an unresolved problem in clinical toxicology because no specific and fully efficient antidote is ready available (1,16,25). The recommended therapeutic regimen in *A. phalloides* poisoning includes gastrointestinal decontamination procedures, rehydration, correction of metabolic acidosis and electrolyte abnormalities, specific therapies (silibinin, N-acetylcystein, Penicillin G, MARS) and liver transplantation (3,4,13,16,26,27,28). In Bulgaria, Silibinin,

MARS and urgent liver transplantation were not available during this period. All patients hospitalised within 36 hours after consumption received extracorporeal treatment methods - hemodialysis hemodesorption or plasma filtration, although in the literature there is no convincing evidence of their effectiveness (29,30). We applied hyperbaric oxygenation for faster regeneration of hepatocytes for all patients in the recovery stage. Our experience indicates that the protocol used in our Toxicology Unit is effective for amatoxin poisoning, because although not all recommended methods were available in Bulgaria the percentage of cured patients was as much as the average for Europe. A lethal outcome was registered in 25 (17%) patients - 13 women and 12 men at the average age 56.8 years (26-85). The relative share of *A. phalloides* poisoning is 0.6% of all acute intoxications, but they cause 11.4% of lethality of acute poisonings. Despite the optimal treatment performed, lethality of *A. phalloides* intoxication is high and reaches 20% (3,22,31,32), or 30% in adults and 50% in children (4, 8). *A. phalloides* mushrooms frequently cause acute liver failure, and sometimes acute renal failure and are associated with high lethality (2,33,34).

CONCLUSION

A. phalloides poisonings have a low frequency - 0.8% of all intoxication, but are characterised by the development of life-threatening organ failures and high mortality - 17%. They cause 11.4% of lethality in acute intoxications. They are more common in men and the ratio of men to women is 1.62: 1. The share of patients over 45 years of age is 72.1%. *A. phalloides* intoxications in Bulgaria are mostly in late summer and autumn. A therapeutic option to reduce the high lethality is the implementation into clinical practice in Bulgaria of liver transplantation as a routine method in patients who develop acute liver failure when other treatment methods have proven ineffective.

REFERENCES

1. Erden A, Esmeray K, Gumuscu H, Basak M, Cetinkaya A, Avci D, et al. Acute liver failure caused by mushroom poisoning: a case report and review of the literature. *Int Med Case Rep J*. 2013; 6:85-90. doi: 10.2147/IMCRJ.S53773.
2. Roberts D, Hall M, Falkland M, Strasser S, Buckley N. *Amanita phalloides* poisoning and treatment with silibinin in the Australian Capital Territory and New South Wales. *Med J Aust*. 2013; 198(1):43-7.
3. Enjalbert F, Rapior S, Nouguiet J, Guillon S, Amouroux N, Cabot C. Treatment of amatoxin poisoning: 20-year retrospective analysis. *J Toxicol Clin Toxicol* 2002; 40(6):715-57.
4. Karlson-Stiber C, Persson H. Cytotoxic fungi – an overview. *Toxicon*. 2003; 42(4):339-49.
5. Vanooteghem S, Arts J, Decock S, Pieraerts P, Meersseman W, Verslype C, et al. Four patients with *Amanita phalloides* poisoning. *Acta Gastroenterol Belg*. 2014; 77(3):353-6.
6. Rudbaek TR, Kofoed P, Bove J, Hastrup P, Ebbehøj N. Management of poisoning with *Amanita phalloides*. *Ugeskr Laeger*. 2014; Mar 31;176(7): Pii:V01130022.
7. Garcia J, Oliveira A, de Pinho PG, Freitas V, Carvalho A, Baptista P, et al. Determination of amatoxins and phallotoxins in *Amanita phalloides* mushrooms from northeastern Portugal by HPLC-DAD-MS. *Mycologia*. 2015; 107(4):679-87. doi: 10.3852/14-253.
8. Ferenz T, Lukasiewicz B, Cieciewicz J, Kowalczyk E. Poisoning with *Amanita phalloides*. *Med Pr*. 2009; 60(5):415-26.
9. Yilmaz I, Kaya E, Sinirlioglu ZA, Bayram R, Surmen MG, Colakoglu S. Clinical importance of toxin concentration in *Amanita verna* mushroom. *Toxicon*. 2014; 87:68-75. doi: 10.1016/j.toxicon.2014.05.019.
10. Broussard CN, Aggarwal A, Lacey SR, Post AB, Gramlich T, Henderson JM, et al. Mushroom poisoning-from diarrhea to liver transplantation. *Am J Gastroenterol*. 2001; 96(11):3195-8. doi: 10.1111/j.1572-0241.2001.05283.x.
11. Vetter J. Toxins of *Amanita phalloides*. *Toxicon*. 1998; 36(1):13-24.
12. Mengs U, Pohl RT, Mitchell T. Legalon® SIL: The antidote of choice in patients with *Amanita phalloides* poisoning. *Curr Pharm Biotechnol*. 2012; 13(10):1964-70.
13. Mas A. Mushrooms, amatoxins and the liver. *J Hepatol*. 2005; 42(2):166-9. doi: 10.1016/j.jhep.2004.12.003.
14. Diaz JH. Syndromic diagnosis and management of confirmed mushroom poisonings. *Crit Care Med*. 2005; 33(2):427-36.

15. Himmelmann A, Mang G, Schnorf-Huber S. Lethal ingestion of stores *Amanita phalloides* mushrooms. *Swiss Med Wkly.* 2001; 131(41-42):616-7. doi: 2001/41/smw-09831.
16. Santi L, Maggioli C, Mastroroberto M, Tufoni M, Napoli L, Caraceni P. Acute liver failure caused by *Amanita phalloides* poisoning. *Int J Hepatol.* 2012; doi:10.1155/2012/487480.
17. Magdalan J, Ostrowska A, Piotrowska A, Gomulkiewicz A, Podhorska-Okolow M, Patrzalek D, et al. Benzylpenicillin, acetylcysteine and silibinin as antidotes in human hepatocytes intoxicated with alpha-amanitin. *Exp Toxicol Pathol.* 2010 Jul; 62(4):367-73. doi: 10.1016/j.etp.2009.05.003.
18. Smith MR, Davis RL. Mycetismus: a review. *Gastroenterol Rep (Oxf).* 2016; 4(2):107-12. doi: 10.1093/gastro/gov062.
19. Baniasad N, Oghabian Z, Mehrpour O. Hepatotoxicity due to mushroom poisoning: A case report. *Int J Med Tox Foren Med.* 2014; 4(2):68-73. doi: 10.22037/ijmtfm.v4i2(Spring).4915
20. Escudie L, Francoz C, Vinel JP, Moucari R, Cournot M, Paradis V, et al. *Amanita phalloides* poisoning: reassessment of prognostic factors and indications for emergency liver transplantation. *J Hepatol.* 2007; 46(3):466-73. doi: 10.1016/j.jhep.2006.10.013.
21. Garcia J, Costa VM, Carvalho A, Baptista P, de Pinho PG, de Lourdes Bastos M, et al. *Amanita phalloides* poisoning: Mechanisms of toxicity and treatment. *Food Chem Toxicol.* 2015; 86:41-55. doi: 10.1016/j.fct.2015.09.008.
22. Bergis D, Friedrich-Rust M, Zeuzem S, Betz C, Sarrazin C, Bojunga J. Treatment of *Amanita phalloides* intoxication by fractionated plasma separation and adsorption (Prometheus®). *J Gastrointestin Liver Dis.* 2012; 21(2):171-6.
23. French LK, Hendrickson RG, Horowitz BZ. *Amanita phalloides* poisoning. *Clin Toxicol (Phila).* 2011; 49(2):128-9. doi: 10.3109/15563650.2011.557663.
24. Garrouste C, Hemery M, Boudat AM, Kamar N. *Amanita phalloides* poisoning-induced end-stage renal failure. *Clin Nephrol.* 2009; 71(5):571-4.
25. Lukasik-Glebocka M, Popiolek A, Nawrocka K. Some issues of death cap (*Amanita phalloides*) poisoning based on cases treated in Poznan Department of Toxicology in 2010. *Przegl Lek.* 2011; 68(8):444-8.
26. Hydzik P, Gawlikowski T, Ciszowski K, Kwella N, Sein Anand J, Wojcicki M, et al. Liver albumin dialysis (MARS)-treatment of choice in *Amanita phalloides* poisoning? *Przegl Lek.* 2005; 62(6):475-9.
27. Ganzert M, Felgenhauer N, Schuster T, Eyer F, Gourdin C, Zilker T. *Amanita* poisoning-comparison of silibinin with a combination of silibinin and penicillin. *Dtsch Med Wochenschr.* 2008; 133(44):2261-7. doi: 10.1055/s-0028-1091268.
28. Wittebole X, Hanston P. Use of the molecular adsorbent recirculating system (MARS) for the treatment of acute poisoning with or without liver failure. *Clin Toxicol.* 2011; 49(9):782-93. doi: 10.3109/15563650.2011.624102.
29. Mullins ME, Horowitz BZ. The futility of hemodialysis in *Amanita phalloides* poisoning. *Vet Hum Toxicol.* 2000; 42(2):90-1.
30. Ward J, Kapadia K, Brush E, Salhanick SD. Amatoxin poisoning: Case report and review of current therapies. *J Emerg Med.* 2013; 44(1):116-21. doi: 10.1016/j.jemermed.2012.02.020.
31. Ganzert M, Felgenhauer N, Zilker T. Indication of liver transplantation following amatoxin intoxication. *J Hepatol.* 2005; 42(2):202-9. DOI: 10.1016/j.jhep.2004.10.023.
32. Sorodoc L, Lionte C, Sorodoc V, Petris O, Jaba I. Is MARS system enough for *A. phalloides*-induced liver failure treatment? *Hum Exp Toxicol.* 2010; 29(10): 823-32. doi: 10.1177/0960327110363327.
33. Oeckinghaus R, Cuneo A, Brockmeier J, Oeckinghaus GS, Drewek-Platena S, Hochreuther S, et al. Acute hepatic failure after ingestion of mushrooms. *Internist (Berl).* 2012 May; 53(5):619-24. doi: 10.1007/s00108-011-3008-z.
34. Li P, Deng W, Li T. The molecular diversity of toxin gene families in lethal *Amanita* mushrooms. *Toxicon.* 2014; 83:59-68. doi: 10.1016/j.toxicon.2014.02.020.