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# Toxins of the Death Cap: *Amanita phalloides*

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**Abstract**

A quick insight into one of the most poisonous mushrooms i.e. *Amanita phalloides* and its toxicology. The mechanisms of toxicity and treatment as well as the methods of identifying Amanitins. include chromatographic and non-chromatographic techniques that require additional study to produce a more accurate and sophisticated means of detecting the presence of toxins in cases of mortality. Along with the toxokinetics and mechanism of action, this article also covers symptoms of poisoning and how to identify them to stop the poison from spreading throughout the body.

**Keywords*:*** Amanitin poisoning, HPTLC, TLC, Meixner's Test.

**Introduction:**

The majority of the deaths due to mushroom poisoning worldwide are caused by *Amanita phalloides*, the death cap. It belongs to the family- Amanitaceae and order – Agaricales. Sebastien Vaillant, a French botanist, made the initial discovery and named it in 1727. In 1833, Johann Heinrich Friedrich Link ultimately decided to call the species Amanita phalloides. Death caps are native to Europe but now they have become ubiquitous except Antarctica. The active substance "amanitin" from *A. phalloides* was successfully crystallized, and this was first reported by R. Hallermayer (Hallermayer, 1940). There are three primary categories of toxins found in this species: virotoxins, phallotoxins, and amatoxins (Vetter, 1998). Of these three, amatoxins containing α-amanitin are the primary cause of the elevated death rate in both humans and animals.

**Morphology:**

Death cap often resembles the other species of mushrooms which are non-poisonous. This leads to unintentional poisoning in humans as they consume them. *Amanita phalloides* caps vary in colors ranging from greenish yellow to brown, tan, or rarely white with a metallic shine with age or drying. The caps are round or oval in shape and turn convex or flat with

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aging. The flesh inside is white and does not show any color-changing properties when cut, thus it may lack psilocybin and psilocin, the primary chemicals to affect the central nervous system and cause psychoactive effects in humans. The volva does not appear above the ground and sometimes digging up the mushroom is an identifying characteristic.

The annulus is preset on the stipe and the stipe may be white or yellowish in color, 4-18 cm long. It is either even or tapers upward. The gills are white, adjacent to each other, and free. The spores are smooth and ellipsoid, and the spore print is white. When young, death caps smell sweet and nice, like fresh fruits or potatoes, but as they get older, they have an unpleasant stench. They taste great when eaten as described, but they are extremely toxic.

Death caps are ectomycorrhizal fungi that live in symbiotic relationships with tree roots, taking sugars from the roots in return for helping the tree absorb nutrients from the soil, such as phosphorus and nitrogen, and sometimes even improving the plant's resistance to disease and drought. Death caps form the symbiosis with deciduous trees, oaks, birches, etc. Sometimes they are also found to be associated with pine trees.

**Toxicology:**

α and β amanitin were the most important chemicals on which toxicological studies were carried out. Amatoxins are often insoluble in ether, chloroform, and other organic solvents but readily soluble in water, methanol, and other organic solvents (Kaya et al., 2012). Because of its unique chemical structure—bicyclic octapeptides—the amanitin group is stable and resistant to heat treatments, even at elevated temperatures. That is why even after cooking roasting or even frying the mushrooms, the toxins remain in them and lead to poisoning. It has been observed that preserving them in cold temperatures had a higher amount of toxins present in them (91% in water and 97% in methanol at 4 $^{0}$C after 6 months) rather than heating them or leaving them at room temperature for the same period (Sharma et al., 2021). However, the amatoxin levels were observed to be the lowest when the mushrooms were boiled. Boiling amanitins release poisons into the cooking water, which is the most likely explanation as amanitins are water-soluble. Even in that case, eating them continues to be hazardous as their lethality does not decrease. Since the molecules do not break down in the digestive tract and are persistent even at acidic pH levels (Vetter, 1998; Vetter & Vetter, 2014), amanitin inhibits RNA polymerase II when toxins enter the body, leading to a protein shortage and eventually cell death. The primary organ of toxicity is the liver, which is followed by the kidneys.

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According to the $LD_{50}$ values of amatoxins, the human value of 0.1 mg/kg body weight is the same as the sensitivity of rabbits and guinea pigs (0.1-02 and 0.05-0.1 mg/kg, respectively). Poisoning in humans is alike, i.e., the spread of toxins is faster and post-mortem examinations have shown symptoms of cell death in liver and kidney tubules, gastritis, and hemorrhages (Vetter, & Vetter, 2014).

**Toxicokinetic**

The gastrointestinal tract absorbs amatoxins quickly since the liver is the first organ they target, and toxins can be detected in urine 90–120 minutes after consuming mushrooms (Garcia J et al., 2015 & Le Dare B. et al., 2021). A few toxins that remain in the bile are reabsorbed by the digestive tract, but the majority of amatoxins are eliminated during the first 72 hours of poisoning. It was made quite evident that the poisoned individuals had liver damage, centrilobular necrosis, and fatty degeneration. Kidney cells have 6-90 times higher detectable toxin concentrations than liver cells. (Le Dare B. et al., 2021).

**Mechanism of action and effects:**

RNA polymerase II is known to be inhibited by □-amanitin, which causes a steady and dramatic reduction in RNA levels within the first 24 hours of poisoning. Thus, there is a decrease in both mRNA and protein synthesis. It has been found that amanitin at concentrations of just 10 ng/mL can decrease RNAP II enzyme activity by 60–70%. It is significant to note that non-mammalian polymerase enzymes have slower susceptibility to α-amanitin toxicity, while only RNAP II, which is derived from mammalian cells, is particularly susceptible to this toxicity.

The effect of amanitins can be described in 2 stages: A series of cellular damage occurs after the initial functional harm, which is the suppression of protein production. Amatoxins cause necrosis in the livers of all animals during the second stage, which is characterized by necrotic or apoptotic cell death. (Vetter, 1998; Vetter & Vetter, 2014).

**Determining the amatoxins:**

The first and foremost method to determine amanitins was the Meixner Test (Fiedziukiewicz, 2013 & Meixner 1979). The color response of the lignin and cyclopeptides in the paper (wood) in the presence of strong HCl acid is the basis for this test. When the juice extract of the mushroom was added to the high lignin content paper and then HCl, it resulted in the formation of a blue spot on the paper.

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This was done by Wieland while Meixner also experienced a similar reaction on the paper although the color was greenish-blue. Even though the test was simple, fast, and does not require equipment, it is still unpopular among the researchers due to its many drawbacks and chances of giving a false-positive test result. (Garcia et al., 2015; Fiedziukiewicz, 2013; Beuhler et al., 2004).

**Chromatographic Methods:**

Paper chromatography can identify all amanita poisons, but in the 1960s, a more accurate technique known as Thin Layer Chromatography (TLC) was developed as a solution to the method's poor resolution and quantitative property accuracy. Because TLC had a higher resolving capacity, it started to be frequently used in the examination of toxins in mushrooms.

The first to create a quick and accurate method for measuring alpha and beta amanitin in *A. phalloides* methanolic extracts was Stijve et al. (Barbosa et al., 2023). This technique is known as High-Performance Thin Layer Chromatography (HPTLC). The stationary phase of the column in the HPLC method, as described by Bentler and Marderosian (Beutler et al., 1981), keeps lipophilic amatoxins and separates distinct compounds, which is a property shared by the HPTLC method. Nevertheless, this approach was unsuitable because the results reported contamination and trace levels of poisons.

Wieland utilized cellulose powder as a medium in a liquid chromatography-related procedure. (Wieland, 1986). Barbosa and coworkers (2023) also used the method which had a common feature as Wieland's method. The feature was; that the toxin separation was carried out by reversed-phase liquid chromatography under gradient conditions. However, these methods do not always give assured results.

A report published by Bambauer et al (2020), mentioned the usage of Liquid Chromatography (LC) high-resolution tandem mass spectrometry (HRMS/MS) method. This technique is particularly important since it can identify amanitin at concentrations ranging from 37 to 2890 pg/mL in blood plasma samples. It is highly important since urine has a larger concentration of toxins than other bodily fluids in the case of amatoxin poisoning. However, if there is acute kidney failure and urine production is stopped, blood samples can still be tested using the previously described procedure.

**Non-chromatographic Methods:**

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Non-chromatographic methods include RIA (Radio Immuno Assay), ELISA (Enzyme Linked Immuno Sorbent Assay), and the most recent- LFIA (Lateral Flow Immuno Assay). RIA was first used to identify amanitin, however, it is a time-consuming and demanding process. The ELISA technique is straightforward and sensitive, but laborious (Staack et al., 2000). It is a laborious process that requires specialized chemicals and numerous cycles of incubation and cleaning. Thus, the most recent approach, LFIA, was presented. It is quickly gaining popularity because of its simplicity, speed, and low cost. With a detection limit of 10 ng/mL for alpha and gamma amanitin and 100 ng/mL for beta amanitin, it swiftly detects amatoxins in urine samples (Bever et al., 2020). Another noteworthy benefit is that, unlike ELISA, it doesn't require pretreatments, and no specialized equipment is needed to evaluate the results.

**Symptoms:**

It can be divided into 4 stages:

- **Stage 1:** It is also called the Lag stage. It is long and latent for an average of about 8-10hr and in a few cases 24-36hr. There are no signs of poisoning and the consumer often forgets about the consumption of the mushroom (till 1-2 days after consumption).
- **Stage 2:** This stage occurs after 1-2 days of consumption. The gastrointestinal phase (GI stage) is characterized by symptoms such as recurrent vomiting, diarrhea, and abdominal discomfort, which can result in hypovolemic shock, dehydration, and hypoglycemia. When a patient is released after receiving a false diagnosis, it becomes dangerous (Sezer et al., 2021).
- **Stage 3:** The symptoms of the GI phase may slowly go away but the toxins present in the body attack the liver and kidneys more strongly. The coagulation of blood is disturbed which may induce internal bleeding and result in septicemia.
- **Stage 4:** This is when the toxins level in the blood skyrockets resulting in blood infection and multiorgan failure syndrome. Thus, this result is also called as Multiorgan failure stage indicating the increased quantities of toxins in bilirubin, liver transaminase, LDH, coagulation problems, hemorrhage, metabolic acidosis, hypoglycemia, and encephalopathy. Numerous and maybe partially irreversible liver and kidney damage is indicated by physiological markers. Death may happen six to ten days after ingestion.

**Treatment:**

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Staying hydrated, replacing fluid and electrolyte losses, alleviates symptoms, sometimes leading the victim to delay seeking medical treatment as the GI phase leads to dehydration. The problem begins in the delayed recognition of the GI phase (the majority of the cause of misdiagnosis).

Removal of the toxins should begin as soon as possible by using methods like hemodialysis, hemoperfusion, and plasmapheresis. Recently, a purification technology known as the Molecular Adsorbent Recirculating technology, or MARS, has been employed. By doing this, the hazardous metabolites are transported from the bloodstream into a dialysate compartment. But this should also be done before the toxin affects the heart as it results in low blood pressure (a patient having low BP will fail to tolerate dialysis and ultimately go into septic shock and die). In short, Detoxification outside of the body works best when it starts very early.

Chemotherapy may be used but should be given cautiously as it may alter the results and increase the mortality rate of the patient. High doses of antibiotics like penicillin G, antioxidants (Vitamin C, thioctic acid), hormones, and steroids (Santi et al., 2012) have been prescribed.

One common treatment for amatoxin poisoning is milk thistle, or *Silybum marianum*, a medicinal herb of the Asteraceae family. The chemical compound is silibinin-containing flavonoids found in mature seeds. Silibinin consists of silibin A and silibin B, two diastereomers together. The effect of silibinin is as follows: -

- It stabilizes the liver cells' membranes, which significantly reduces the ability of poisons to enter the cells.
- Has a strong antioxidant activity, inhibiting lipid peroxidation and reducing the formation of substances that cause inflammation.
- Promotes protein synthesis, aids in the regeneration of damaged membranes, and aids in the regeneration of liver cells (Radko et al., 2007).

It is recommended to provide the patient with Silibinin treatment within 48 hours of mushroom consumption (20-50 mg/kg/day, intravenously) for 2-3 days at least.

Treatment with silibinin is advised not only for mushroom poisoning but also for normal liver function protection and accelerated liver regeneration. It is used in therapeutic procedures and is not considered an antidote as it can only delay the poisoning but not cure it fully. Liver

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transplantation is considered if the situation becomes worse, although transplantation is executed only if the patient can handle the procedure (considering the state of the patient, hypoglycemia, conditions of metabolic acidosis, etc.)

On the other hand, in the middle of May 2023, Indocyanine green (ICG), a substance that was once employed as an antidote for α-amanitin, has been shown by a few Chinese researchers to have no negative effects when taken at a dose of 0.5 mg/kg. The same Chinese studies also noted that ICG could limit the amanitin-induced cell in-vitro and in-vivo and that STT3B enzyme (catalytic enzyme for N-glucan biosynthesis) is required for α-amanitin toxicity, indicating that ICG may have some use as a treatment for amanitin poisoning. (Wang et al. 2023). The team had only stated the possibility of ICG as an antidote but further research is needed to bring about new treatments and procedures for this poisoning.

**Conclusion:**

Amanita poisoning mostly occurs due to the accidental consumption, as they resemble the non-poisonous mushrooms. Amatoxins containing □-amanitin is the most lethal toxin for humans and animals. These bicyclic octapeptides are stable in heat and cold temperatures and do not lose their lethality easily which makes them extremely dangerous. □-amanitin inhibits the RNAP II which reduces the mRNA and protein synthesis. Necrosis is observed in the liver and kidneys are also affected. In case of poisoning, Milk thistle mushrooms may be provided for 2-3 days within 48hr of consumption. Milk thistle mushrooms are not an antidote but are given as a therapy that can delay the poisoning effect. Symptoms are gastrointestinal problems like watery diarrhea, frequent vomiting, and dehydration. Liver transplantations since the 1990s and MARS in 2023 are the few measures adapted to slow down the mortality rate due to amatoxin but they only prolong the life span of the patient and do not fully cure him/her In recent times, ICG has some potential to be used as an antidote for this amatoxin poisoning as it inhibits the □-amanitin chain reaction but meticulous and in-depth research is required to ensure that ICG applies to humans and animals in amanitin poisoning.

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