ORIGINAL RESEARCH PAPER

Pharmacology

MUSHROOM POISONING: IDENTIFICATION AND QUANTIFICATION OF TOXINS WILL TOXINS IDENTIFICATION HELP IN PREVENTION OF ACCIDENTAL POISONING?

KEY WORDS: Amatoxins, Amanitin phalloides, Amanitin, HPLC Analysis

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ABSTRACT

Amatoxins commonly found in Amanita phalloides is the main constituents of toxins present in most toxic mushroom specimens containing α-amanitin, β-amanitin, γ-amanitin, ε-amanitin, amanin, amaninamide, amanullin, amanullinic acid, and proamanullin. RP-HPLC analysis of toxins Chromatography: The method of Ismail Yilmaz et. al, is followed using C18 (Agilent Technologies) at UV detection 303nm for amatoxins and 291nm for phallotoxins. The mobile phase in isocratic pump with a flow rate of 1ml/min consisting of 0.05M ammonium acetate (pH 5.5 with acetic acid) and acetonitrile (90:10v/v). The geographical variations determine the content of the toxins and it brings a landmark to create awareness to the community for such mushroom specimens. We hope that analyzing the toxin content in the coming years will be of great service to the Physician and the community as well.

INTRODUCTION

Amatoxins commonly found in Amanita phalloides is the main constituents of toxins present in most toxic mushroom specimens. It is further classified into three classes of cyclic peptide known as amatoxins, phallotoxins and virotoxins.

Among the three toxins identified the most lethal toxin is amatoxin with a lethal dose 50 (LD50) 0.4–0.8 mg/kg causing death in few days time. The phallotoxins is less toxic with an LD50 of less than 20mg/kg; however death may occur at a much faster rate than Amatoxins (Vetter, 1998).

Overview of Amatoxins

Vetter (1998) first identified and classified the amatoxins as bicyclic octapeptides containing nine subgroups: α-amanitin, β-amanitin, γ-amanitin, ε-amanitin, amanin, amaninamide, amanullin, amanullinic acid, and proamanullin. The compounds of amatoxins have high water solubility and heat stable with resistance to cold temperature; which make them highly toxic (Himmelmann et al., 2001). A. phalloides contains seven subgroups: phalloidin, phalloin, prophallin, phallisin, phallacin, phallacidin, and phallisacin (Vetter, 1998).

The kinetics of these toxins after human ingestion has been investigated in recent years. The reports have shown that they are readily absorbed orally and are excreted in the urine as early as two hours within forty eight hours after ingestion. (Faulstich et al.). Similarly, Karlson (2003) and Letschert (2006) have reported that the liver is the first organ of contact after absorption with increased accumulation in the sinusoidal membranes of (OATP1B3- uptake) hepatocytes. Enteroheptic circulation has been reported as some amount (6.3 mg) of alpha amnitin has been found to be eliminated in feces. Alarming amount of the toxin is excreted through the kidney (90 times) higher than in the liver, showing the potential nephrotoxicity of amanitin(Garcia et al., 2016) .

Toxicity of amatoxins has been explained to be the binding and inhibition of RNA polymerase (RNAP II). Bushnell et al., (2002) identified the binding site of the α-amanita binding site at the interface of subunits Rpb1 and Rpb2 following RNAP II/α-amanitin interactions. In another study Garcia (2014) showed that α-amanitin interferes with the bridge helix and trigger loop, causing derangement of the elongation process with possible inhibition of mRNA and ultimately decrease protein synthesis resulting in cell death. Future studies regarding the activation of cytokines especially TNF-α leading to hepatotoxicity of amanitin needs to be explored.

Clinically all patients with mushroom poisoning present to the emergency setting within 12 – 36 hours as symptoms of nausea, vomiting, diarrhea, abdominal pain, and hematuria . Latent phase reaction is presented with signs and symptoms of liver and kidney dysfunction ranging from jaundice, hypoglycemia, oliguria, delirium, and confusion. Clinical laboratory findings of raised aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH). Deranged blood coagulopathy may lead to internal bleeding in some patients and hence gives a poor prognosis of such cases.

IDENTIFICATION AND QUANTIFICATION OF AMATOXINS

If A. phalloides-type mushrooms has been ingested, gastric content as well as mushroom samples should be analyzed as soon as possible for identifying and quantification of the presence of amatoxins and phallotoxins (Becker et al., 1976).

Methods for identification and quantification of amatoxins

1. Evaluation of these toxins in mushrooms has been performed using reversed-phase high-performance liquid chromatography (RP-HPLC) (Enjalbert et al., 2004 ; Garcia et al., 2015). RP-HPLC is the most commonly used method, although the LC-MS method which provide the most reliable and sensitive results.
2. Capillary electrophoresis coupled to mass spectrometry (MS) (Rittgen et al., 2008)
3. Liquid chromatography (LC) coupled to MS or to tandem Mass Spectometry (Garcia et al., 2015)
4. UPLC-MS/MS Combined with PRiME HLB Elution (Shuo

Zhang, 2016)

5. The Meixner test is run from the juice of the grounded fresh mushroom tissue onto a piece of newsprint, allowing the spot to dry, and one drop of concentrated hydrochloric acid is added where a blue color indicates a positive test.
6. Methods for urine analysis: Radioimmunoassay, Enzyme Linked Immunosorbent Assay (ELISA) and HPLC (Barceloux, 2008).

RP-HPLC analysis of toxins Chromatography: The method of Ismail Yilmaz et. al, is followed using C18 (Agilent Technologies) at UV detection 303nm for amatoxins and 291nm for phallotoxins. The mobile phase in isocratic pump with a flow rate of 1ml/min consisting of 0.05M ammonium acetate (pH 5.5 with acetic acid) and acetonitrile (90:10v/v)[fig 1 &2].

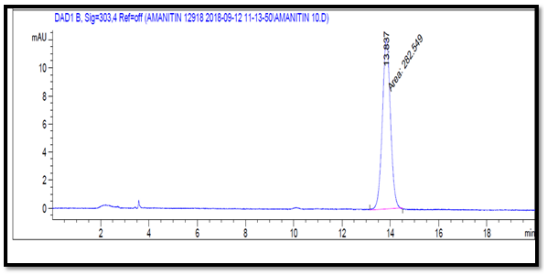

Fig 1

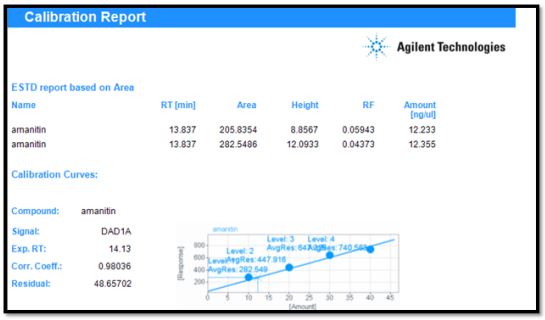

Fig 2

CONCLUSION

Amatoxins are one of the most toxic mushrooms leading to human fatal cases of mushroom poisoning. Treatment often aims decontamination with drugs and supportive measures. Physicians today faced hurdles in the prognosis of such poisoning cases mainly due to lack of quantification of the toxins present in the mushroom. The geographical variations determine the content of the toxins and it brings a landmark to create awareness to the community for such mushroom specimens. We hope that analyzing the toxin content in the coming years will be of great service to the Physician and the community as well.

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