Toxicon 90 (2014) 273–285

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Lethal protein in mass consumption edible mushroom *Agrocybe aegerita* linked to strong hepatic toxicity

Yanxia Jin [a], Tao Che [a], Yalin Yin [a], Guojun Yu [a], Qing Yang [a], Wei Liu [a], Xiangdong Ye [a], Wenhui Yu [a], Shrestha Alok [a], Yijie Chen [a], Barry Hon Cheung Wong [a], Pengfei Cheng [c], Hui Sun [a, b, d, *]

[a] *State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, 430072, Hubei Province, PR China*
[b] *Key Laboratory of Combinatorial Biosynthesis and Drug Discovery, Ministry of Education, Wuhan University, Wuhan, 430072, Hubei Province, PR China*
[c] *Wuhan University Hospital, Wuhan University, Wuhan, 430072, Hubei Province, PR China*
[d] *Hubei Provincial Cooperative Innovation Center of Industrial Fermentation, Key Laboratory of Fermentation Engineering (Ministry of Education), Hubei University of Technology, Wuhan, 430068, Hubei Province, PR China*

ARTICLE INFO

Article history:
Received 26 June 2014
Received in revised form 10 August 2014
Accepted 19 August 2014
Available online 28 August 2014

Keywords:
Agrocybe aegerita
Hepatotoxicity
Lectin AAL
Stability
Total protein

ABSTRACT

Edible mushrooms are well-known for their health and nutritional benefits, however, undesirable effects have been reported in animals fed with these types of edible mushrooms (Nieminen et al., 2009). For health and safety reason, it is necessary to evaluate the toxicity of edible mushrooms, especially those that have been artificially cultured in recent decades. The aim of this study was to assess the safety of the edible mushroom *Agrocybe aegerita*, which is also known as *Agrocybe cylindracea* in Europe and America. Components from *A. aegerita* (Yt) were extracted in water and unexpectedly displayed lethal effect and median lethal dose (LD_{50}) at 8.77 g/kg. Strong hepatic toxicity in BALB/c mice was observed when mice were administered with 25 and 250 mg/kg body weight/day of Yt for 6 days. To identify the hepatotoxic components, Yt was further separated into two components by Diaion HP-20 column chromatography to produce the proteins (Yp) and small molecules (Ys) fractions. Biochemical and histopathological analysis showed that Yp could induce liver injury. LC–MS/MS analysis of Yp identified the main causative agent as AAL (*A. aegerita* lectin), which was shown to have similar hepatotoxicity in the Yt and Yp fractions. In addition, proteinase treatment assays indicated that AAL is resistant to the degradation by digestive enzymes. We have shown that the strong hepatic toxicity is due to a lectin in *A. aegerita*. This study suggests that correct consumption of *A. aegerita* can avoid human health risk and help us better understand its nutritional and medicinal value.

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1. Introduction

The consumption of edible mushrooms as a delicacy is popular worldwide with a reported consumption exceeding 10 kg per capita per year (Isildak et al., 2004). It is generally accepted that edible mushrooms are nutritious and beneficial to people's health as a rich source for protein, dietary fiber and medicinal purposes (Cheung, 2008; Reguła, 2007.).

Abbreviations: Yt, total components; Yp, total protein compounds; Ys, small molecule compounds; AAL, *Agrocybe aegerita* lectin; ALT, serum alanine aminotransferase; AST, serum aspartate aminotransferase; HE, hematoxylin and eosin; pK, proteinase K; HRP, horse radish peroxidase; IPTG, isopropyl-β-D-thiogalactoside.

* Corresponding author. State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, 430072, Hubei Province, PR China. Tel./fax: +86 27 68754401.

E-mail addresses: sunhui@whu.edu.cn, 2011202040157@whu.edu.cn (H. Sun).

http://dx.doi.org/10.1016/j.toxicon.2014.08.066
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However, in recent years there have been reports demonstrating the toxicity of edible mushrooms such as *Pleurotus ostreatus*, which is toxic to mice when fed for long time periods (Al-Deen et al., 1987). Mass consumption of edible mushrooms has been shown to cause various signs and symptoms, such as gastrointestinal, hepatic and cardiac toxicity (Al-Deen et al., 1987; Nieminen et al., 2008, 2009). Components purified from edible mushrooms have also been shown to possess toxicity as the below reports. In 1973, a cardiotoxic protein was isolated from the edible mushroom *Volvariella volvacea* (Lin et al., 1973). Two years later, the cardiotoxic protein "flammutoxin" was isolated from the edible mushroom *Flammulina velutipes* (Lin et al., 1975) as a cytolytic toxin (Bernheimer and Oppenheim, 1987). A purified fraction from *Lactarius necator* was shown to be mutagenic (Suortti and von Wright, 1983), and showed similar effects to those found in the edible mushroom *Agaricus bisporus* (Papaparaskeva-Petrides et al., 1993). Ostreolysin, a protein from oyster mushrooms (*P. ostreatus*) can cause hyperkalaemia and myocardial ischemia in rodents (Zuzek et al., 2006). It has also been reported that ostreolysin can produce aorta ring tension in rats, resulting in induced cardiotoxicity (Rebolj et al., 2007). The ribosome-inactivating proteins (RIPs) such as lyophyllin were isolated from *Lyophyllum shimeji*, and was the first report showing teratogenicity by altering embryonic development (Chan et al., 2010).

The large consumption of edible mushrooms can cause disease to humans. For example, an outbreak of encephalopathy occurred in Japan in the autumn of 2004 as the patients had a history of consuming *Sugihiratake* (*Pleurocybella porrigens*) as a Japanese delicacy (Kato et al., 2004). Further studies indicated that the accumulation of vitamin D-like compounds from *Sugihiratake* were the cause of the increased encephalopathy outbreaks (Sasaki et al., 2006).

With the rapid increasing consumption of edible mushrooms, little is known about the mechanistic toxicity of various edible mushrooms especially from varieties that have been artificially cultivated in recent decades.

Agrocybe aegerita, an edible aromatic and flavorsome mushroom, is popular in Asia as a nutritional delicacy (Diyabalanage et al., 2008; Li et al., 2014). *A. aegerita* is also known as *chaxingu* in China and is a synonym of *Agrocybe cylindracea* in America and Europe with popular consumption (Uhart and Albertó, 2007; Uhart et al., 2008). *A. aegerita* is rich in nutrient value with high protein and reduced fat content, and containing 8 kinds of essential amino acids and abundant vitamins and minerals like selenium, potassium. The fresh and dried fruiting bodies are eaten traditionally in meals or administered to patients with hypertension, cardiovascular disease and obesity in Chinese traditional medicine. In addition, co-products from this mushroom are used in the food industry (Brennan et al., 2012).

Before artificial cultivation in the 1970's, *A. aegerita* was a valuable food in diets (Philippoussis et al., 2001; Uhart et al., 2008), however the food safety of *A. aegerita* has never been previously reported. With the fast rising consumption of *A. aegerita* for nutrition, the food safety of *A. aegerita* needs to be urgently evaluated for human health. In this study, aqueous extracts from *A. aegerita* unexpectedly resulted in hepatotoxicity in mice and even caused death in mice. Further investigation showed that AAL (*A. aegerita* lectin) as the main protein components to play an important role in the inflammation and induction of liver injury in mice. In the current work, we provide a better understanding on the toxicity of the edible mushroom *A. aegerita* and provide suggestions how this should be consumed in human diets.

2. Materials and methods

2.1. Extraction of components from the edible mushroom A. aegerita

Components (Yt) were isolated from *A. aegerita* by water extraction as previously described (Liang et al., 2011). In brief, fresh fruiting bodies were dried at 60 °C and crushed into powder. Approximately 200 g powder was extracted four times with 1 L distilled water at 4 °C overnight. The aqueous extract of *A. aegerita* was obtained by filtration and the collected filtrate was added to $(NH_4)_2SO_4$ to achieve 80% saturation and sedimentation at 4 °C overnight. The precipitate was collected by centrifugation at 8000 × g for 20 min, dissolved in a small volume of distilled water, and dialyzed four times at 4 °C overnight and lyophilized.

The total extraction component was named as Yt and separated by using a Diaion HP-20 chromatographic column (10 × 1.5 cm), and elution was performed with 75% aqueous ethanol at a flow rate of 1 mL/min. The fractions were collected and lyophilized and stored at −20 °C until further analysis.

2.2. Animal experiments

Female BALB/c mice were obtained from the animal center of the epidemic prevention sector in Hubei province (permission number: SCXK 2008-0005) and kept in a pathogen-free animal facility in laminar airflow cabinets, with a 12-h light/12 h dark schedule. The animals were fed with an autoclaved rodent diet *ad libitum*. Mice between 7 and 8 weeks old were used in experiments. All procedures followed the institutional and national guidelines for the care and use of laboratory animals.

In acute toxicity assays, mice were singly orally administered with four different dosages of Yt (2.5, 5.0, 10.0, 15.0 g/kg body weight) ($n = 6$–10 mice per group), respectively. The mice were continuously observed for two weeks and analyzed for mortality. The median lethal dose (LD_{50}) was analyzed as described previously (Estork et al., 2014). LD_{50} was calculated from a plotted graph of X represented by $\log_{10}^{(dosage)}$ and the probability unit of death (Y) represented by the function of NORMSINV to mortality rate in Excel.

For hepatic toxic assessment, the mice were randomly distributed into control and treatment groups ($n = 5$ mice per group) and dosed (25 mg/kg body weight, 50 mg/kg body weight, 250 mg/kg body weight) with different components (Yt, Yp, Ys, nAAL, rAAL) by intragastric (i.g.) administration, once a day for six days. After the dosing period, the mice were withdrawn from the study and euthanized. Blood was collected from each mouse for clinical chemistry testing. All the hepatic toxic experiments were repeated more than three times.

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