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# **Grifola frondosa Extract Induced Acute Hepatic Injury**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors RS and PT managed the literature searches and acquired data. Authors RS, PT and CA were involved in analyzing and interpreting data. Author SR wrote the final manuscript, aided by author PT. Authors RS, PT, CA were involved in critical revision of the manuscript and contributed to the intellectual content. This was supervised by author CA. All authors read and approved the final manuscript.*

**Original Research Article**

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## **ABSTRACT**

**Aim:** To describe a case of acute hepatic injury related to the use of *Grifola frondosa* in a patient with colon cancer.

**Case Presentation:** Patient is a 67 year old female with stage IV poorly differentiated adenocarcinoma of the colon, who presented with epigastric pain one month after resection of her primary tumor. A staging PET scan revealed metastasis to regional lymph nodes without solid organ involvement. Her home medications include longstanding amlodipine and losartan, and a recently started *Grifola frondosa* derivative. Her laboratory data was significant only for acute transaminitis (AST:967 U/L, ALT:768 U/L) without hyperbilirubinemia. Alcohol, acetaminophen, and a viral panel (EBV, CMV, hepatitis A/B/C) were all negative. A CT scan revealed heterogenous liver parenchyma without focal lesions. A subsequent liver biopsy demonstrated active portal inflammation with eosinophilic infiltration.

**Discussion:** The etiologies of significant acute transaminitis include viral hepatitis, ischemic liver injury, acetaminophen toxicity and drug-induced liver injury (DILI). Viral and ischemic hepatitis and acetaminophen toxicity were excluded based on laboratory

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analysis and imaging studies. Liver biopsy findings demonstrating the characteristic eosinophilic infiltration of a drug reaction favored DILI as the etiology of transaminitis in this case. With a RUCAM score of 7 calculated based on history, clinical course, and objective data, DILI was concluded to be probably attributed to the patient's recent use of the *Grifola frondosa* extract.

**Conclusion:** A diagnosis of drug induced liver injury probably secondary to the use of *Grifola frondosa* extract was made after excluding all other causes of significant acute transaminitis.

**Keywords:** *Grifola frondosa*; Maitake mushroom; acute hepatic injury; drug induced liver injury.

## 1. INTRODUCTION

*Grifola frondosa* is a mushroom found in the mountains of northeastern Japan [1]. It has been consumed for centuries as food but in recent years has made its way into the pharmaceutical industry with promises of improving various disease processes from hypertension to a compromised immune system [2]. Most supplements derived from *Grifola Frondosa* are comprised of two active isolates; a glycoprotein moiety known as the SX fraction and a protein bound  $\beta$ -glucan called the Maitake D (MD)-fraction [2]. While the SX fraction has been reported to have health benefits such as improving insulin sensitivity [3], it is the MD-fraction that has garnered popularity, particularly in patients diagnosed with cancer.

Recent literature reports that administration of MD-fraction into murine tumor models has immune modulating effects. Oral MD-fraction is described to boost the host immune response against tumor cells by increasing the percentage of activated antigen presenting cells (APCs), natural killer cells, CD4+ and CD8+ T cells and mature B cells in circulation, via immune stimulating cytokines like tumor necrosis factor-alpha and interleukin-12 [4,5]. It has also been shown in vivo to decrease the immunosuppressive effects of the tumor microenvironment by down regulating myeloid-derived suppressor cells and regulatory T (T-reg) cells that hinder activation of APCs responsible for alerting T lymphocytes [4].

Despite the progress in elucidating the anti-oncogenic properties of the MD-fraction, much still remains to be discovered. Most of the current understanding of the therapeutic benefits of this fraction is from pre-clinical studies, without adequate human clinical trials evaluating its safety and efficacy. Nevertheless, several variations of the *Grifola frondosa* supplement composed of the concentrated MD-fraction have emerged, with advertised benefits of tumor reduction [4,5], enhancement of cellular immunity [4,5], and alleviation of chemotherapeutic side effects [6,7]. With little to no adverse effects reported for these supplements, their use continues to increase in this niche population. Therefore, the prompt recognition and publication of potential side effects is vital for patient safety.

## 2. CASE PRESENTATION

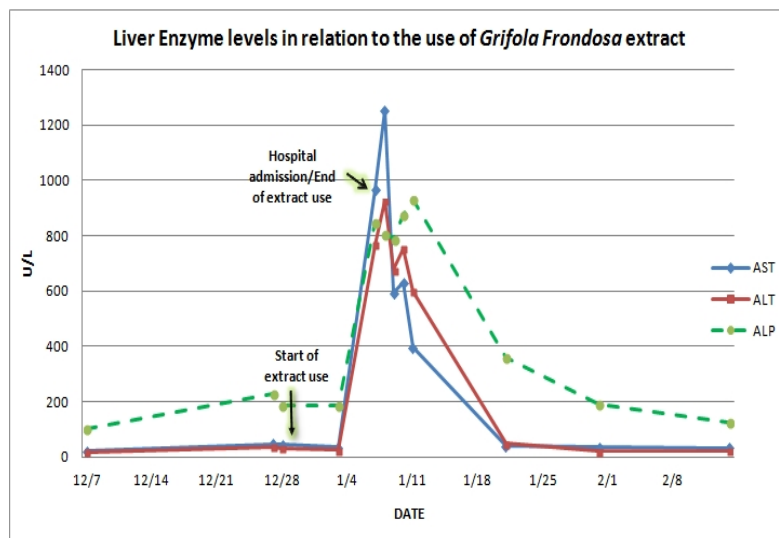
Patient is a 67-year-old female with history of hypertension who was recently diagnosed with poorly differentiated adenocarcinoma of the colon through surgical pathology from an uncomplicated resection of her primary sigmoid tumor. A positron emission tomography (PET) scan post-surgery revealed retroperitoneal lymph node involvement without solid

organ metastasis. Patient had not been started on any chemotherapy or radiation therapy at the time this liver complication was detected.

Approximately one week after her staging PET scan, patient presented with dull, non-radiating epigastric discomfort of two days duration. She denied nausea, vomiting, fevers, chills, and recent weight changes. She reported rare alcohol use and denied smoking, intravenous drug use or history of sexually transmitted infections.

Her medications at the time included amlodipine, losartan, and a recently started *Grifola frondosa* extract. This particular product contained Maitake D-Fraction in a standard 4 oz. bottle containing 3000 mg of PD-Fraction, which is standardized to contain more than 900 mg of pure active proteoglycan. Every 20 drops contain approximately 5.5 mg of pure active proteoglycan extracted from the fruiting body of high quality Maitake mushrooms. The extract was started nine days prior to admission, upon recommendation from a friend, with a dosing regimen of 2 mL (11mg) three times daily. Physical exam was pertinent only for mild, localized, epigastric tenderness to palpation without evidence of jaundice, ascites, hepatomegaly or splenomegaly.

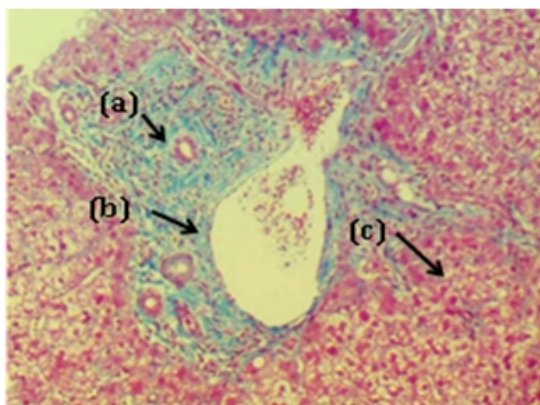
Her admission lab work up was significant for acute transaminitis with aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of 967 U/L (Reference range: 0-45 U/L) and 768 U/L (Reference range: 0-60 U/L) respectively. Alkaline phosphatase (ALP) was 849 U/L (Reference range: 0-133 U/L), though it was mildly elevated to 186 U/L a week prior to admission (Fig. 1).



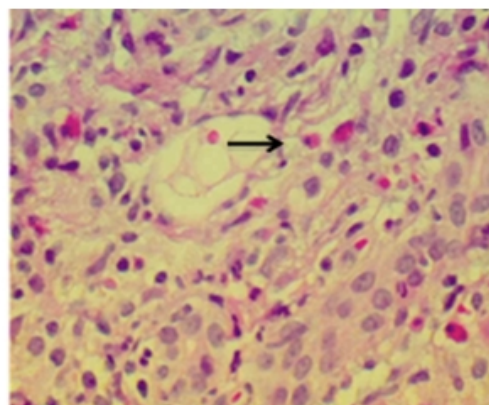
**Fig. 1. AST/ALT/ALP enzyme levels prior to, during, and after cessation of *Grifola frondosa* extract**

There was no hyperbilirubinemia. Pro thrombin time (PT) and activated partial thromboplastin time (aPTT) were both within normal limits. Acetaminophen levels were undetectable. Further workup with a viral panel revealed a non-reactive hepatitis A antibody, hepatitis B core antibody, and hepatitis B surface antigen with an undetectable hepatitis C RNA. Cytomegalovirus DNA was undetectable. Epstein Barr viral IgG was positive but IgM was negative consistent with past exposure.

Imaging with a right upper quadrant ultrasound was negative for focal hepatic lesions and ductal dilatation. An abdominal computed tomography (CT) scan revealed a heterogenous pattern throughout the liver parenchyma without any evidence of hepatomegaly or focal lesions. A closer look at the liver with a biopsy showed minimally active portal inflammation, grade 1 out of 4, with a mixture of lymphocytes, plasma cells, and eosinophils (Fig. 3). There was no portal or bridging fibrosis, cirrhosis or steatosis (Fig. 2,3). The transaminitis gradually resolved after discontinuation of the *Grifola frondosa* extract upon hospital admission.



**Fig. 2. Portal Triad area (a): Bile Duct (b) Portal Vein (c): eosinophilic infiltration. There is no evidence of portal or bridging fibrosis, steatosis or necrosis**



**Fig. 3. Portal Inflammation with arrow pointing to eosinophilic and lymphocytic infiltration of portal area.**

### 3. RESULTS AND DISCUSSION

Drug induced liver injury, also known as DILI, is the most common cause of acute liver failure in the United States [8,9,10]. It accounts for approximately 10 percent of adverse drug reactions and is seen in up to 50 percent of patients presenting with acute liver failure[10].

The pathogenesis of DILI is complex but there exist three commonly postulated mechanisms; direct mitochondrial injury, increased intracellular stress and immune (allergic) mediated reactions [9,10,11]. Direct mitochondrial injury occurs when the accumulation of toxic drug metabolites is significant enough to alter cellular biochemistry, thus resulting in necrosis or apoptosis [11,12]. Increased intracellular stress may catalyze hepatic and biliary cell death by affecting gene expression, transcription and translation [11]. Finally, immune mediated drug reactions may occur when a drug metabolite acts as a hapten, binding to and altering the structure of macromolecules in such a way that they are then perceived by the host immune system as neoantigens and destroyed via an autoimmune attack [9,10,11]. The immune mediated pathway is less understood but it is characterized by the classic symptoms of an allergic reaction such as eosinophilia [11].

Similar to the pathogenesis, DILI also has a wide and complex clinical spectrum depending on the site of hepatic injury. A pure hepatocellular injury, with histological signs of liver cell necrosis and portal inflammation, may present as asymptomatic biochemical abnormalities or severe transaminitis with poor prognostic factors like jaundice [10]. This pattern of isolated hepatitis is most associated with impaired liver function and acute liver failure, thus carrying

a mortality rate of approximately 7% [10]. Injury concentrated to the biliary canaliculi or ducts represent a cholestasis pattern of DILI with marked bile stasis and symptoms resembling that of extrahepatic obstructive jaundice [9,10]. Lastly, the clinical and histological presentation may be mixed with findings of both hepatocellular and cholestatic injury. The mixed pattern of DILI carries the lowest mortality at around 2% [10].

As with any cause of acute hepatic injury, prompt recognition and management is needed given its overall excessive mortality rate [8]. However, DILI is difficult to diagnose and requires a high index of suspicion. Unlike most diseases, DILI is a diagnosis of exclusion as there is no widely clinically applicable gold standard for diagnosis. The only confirmatory test is a positive “re-challenge” fulfilling Koch’s postulate, which is rarely done due to ethical and legal concerns [9].

In order to aid in the diagnosis of DILI, a number of tools have been developed, with the most widely used being the Roussel Uclaf Causality Assessment Method (RUCAM) scale (Fig. 4) created by the Council for International Organizations of Medical Sciences [8,9,10,11]. It has demonstrated 86% sensitivity and 89% specificity with a positive and negative predictive value of 93% and 78% respectively [9]. The accuracy of the RUCAM scale is only exceeded by the Digestive Disease Week-Japan (DDW-J) scale, which requires the use of an in vitro drug lymphocyte stimulation test and the expert opinion panel established by the Drug-Induced Liver Injury Network (DILIN) [9], both of which are impractical in daily clinical setting.

With the use of the above-mentioned diagnostic scale, and a high degree of suspicion, DILI may be quickly identified and managed. This is essential since untreated DILI may rapidly progress to acute hepatic failure, which is associated with a mortality of 80 percent in the absence of liver transplantation [13]. But this is easily prevented with early recognition because DILI is often reversible with cessation of the offending drug. While there is vigilance in thoroughly reviewing medications in a case of suspected DILI, it has become challenging in recent years due to increasing incorporation of alternative therapies with mainstream medicine.

Such is the case with the patient presented here who began taking a *Grifola Frondosa* extract to augment her cancer therapy. We arrived at the conclusion of DILI attributable to this supplement after excluding common causes of acute hepatitis. The culprits of significantly elevated transaminitis (AST, ALT  $\geq$  1000) include viral hepatitis, ischemic hepatitis or “shock liver”, acetaminophen toxicity and other drug-induced liver injury.

A pan viral hepatitis panel that included testing for hepatitis A, B, and C, CMV, and EBV was negative. This, along with virtually undetectable serum levels of acetaminophen excluded these two processes as etiologies of the patient’s acute transaminitis. Ischemic liver injury was ruled out after a right upper quadrant ultrasound and abdominal CT scan were negative for pathognomonic signs of a hypoxic insult such as hepatic congestion, or dilatation of supra hepatic veins [14]. Moreover, she lacked clinical risk factors for developing ischemic end organ damage such as a history of cardiac or renal impairment or signs of hypotension upon admission [14]. In accordance with the imaging findings and clinical presentation, the liver biopsy also did not reveal the characteristic finding of centrilobular liver cell necrosis seen in ischemic hepatitis [14].

In addition to the above etiologies, factors such as organ metastasis and hypercoagulability were also explored to explain the transaminitis given the patient’s stage IV adenocarcinoma

of the colon. However, the abdominal CT with contrast and recent PET scan excluded concerns for hepatic metastasis, obstructive lesions and vascular processes such as portal vein thrombosis. Imaging and biopsy also excluded any granulomas or other concerns of rare autoimmune mediated causes of acute transaminitis.

With most etiologies of significant transaminitis excluded, the attention was then focused on possible drug-related liver injury. The patient was on three medications at the time of admission, low dose amlodipine, losartan and the *Grifola frondosa* extract. Amlodipine and losartan were previously well-tolerated chronic medications without any recent dose changes. Thus the suspicion for DILI was with the use of the herbal supplement. This was supported by the fact that her AST and ALT increased within 9 days of starting the supplement and trended downward quickly upon cessation of the drug during her hospital stay. These two characteristics represent the hallmarks of a typical drug-reaction [15]. There are rare reports relating amlodipine with acute hepatitis [16] but it is unlikely that amlodipine or losartan played a role in this patient's acute transaminitis given that both medications were continued throughout the hospitalization as well as after discharge without any effects on the patient's liver enzymes. The resolution of the patient's transaminitis occurred only after cessation of the *Grifola frondosa* extract.

In order to corroborate our suspicion of DILI, the RUCAM score was calculated based on ALT (926 U/L) and ALP (807 U/L) values within 24 hours of admission, history of exposure between 5 to 90 days, more than 50% improvement in 30 days of drug withdrawal, age over 55 years as the only one identified risk factor, no concomitant hepatotoxic drug use, no identifiable non-drug causes of hepatitis and lack of re-challenge with the same drug, which resulted in a score of 7 (Appendix A). This, along with a calculated R ratio (Appendix B) of 2.54 suggesting a mixed pattern of hepatocellular and cholestatic injury revealed that a DILI as the etiology for this hepatic injury was probable. The subsequent liver biopsy displaying the classic findings of portal inflammation with increased eosinophils (Fig.3) strongly advocated for the suspicion of DILI. The lack of cellular necrosis, fibrosis and steatosis (Fig. 2) highlights the mixed pattern of injury that is associated with the lowest mortality. This correlated well with the patient's clinical picture of isolated transaminitis with retained hepatic function (normal PT and PTT) in the absence of jaundice and signs of cholestasis. Based on our high index of suspicion, the RUCAM score, and liver biopsy findings, a confident diagnosis of DILI was established, most likely attributable to the *Grifola frondosa* extract. Not surprisingly, a post-hospital outpatient follow up approximately 2 weeks after discontinuation of the *Grifola frondosa* extract revealed complete resolution of patient symptoms and normalization of liver function tests.

The commercial product consumed in this case contained a combination of the Maitake-D fraction and a compound called N,N-Dimethylglycine (DMG). This is worth mentioning, as the possibility exists that the hepatic injury in this case may also be due in part to the DMG component. However, this is less likely as DMG is a natural intermediate in the synthesis of the amino acid glycine, and any exogenous consumption of DMG is rapidly metabolized to glycine and utilized for various bodily reactions [17]. Also, numerous DMG products have been in existence since the 1960s without any reported incidents of drug interactions, adverse effects, or overdose from its consumption.

#### 4. CONCLUSION

Currently, there is growing interest in the Maitake-D (MD) fraction derived from *Grifola frondosa* based on initial studies highlighting its anti-oncogenic, and immune stimulating

effects [4,5,18]. Given the magnitude of these findings, some phase I/II human clinical trials have been completed [19] with several more underway in large cancer centers across the world. One major ongoing clinical trial in the United States involves evaluating the added therapeutic benefit of the MD-fraction when combined with traditional chemotherapeutic drugs in advanced cancer [20]. As research into the therapeutic effects of this extract progresses, it becomes increasingly important to report any adverse effects from its use. To date, there are no dose-limiting toxicities or adverse effects related to the *Grifola frondosa* extract. However, we present here the first published case of a rare, yet significant adverse effect of drug induced acute hepatic injury associated with *Grifola frondosa* MD-fraction use.

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## CONSENT

Not applicable.

## ETHICAL APPROVAL

Not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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**APPENDIX A. Roussel Uclaf Causality Assessment Method (RUCAM) criteria [15]**

<b>Criteria</b>	<b>Explanation</b>	<b>Possible Score</b>
Initial exposure to time of onset of liver injury	- 5 to 90 days: +2 - <5 or >90 days: +1	+1 to +2
Course of the reaction	- >50% improvement in 8 days: +3 - >50% improvement in 30 days (hepatocellular) or in 180 days (cholestatic/mixed): +2 - Lack of information/ no improvement: 0 - Worsening / <50%improvement in 30 days: -1	-1 to +3
Risk factors for DILI: alcohol use or age≥55	- None: 0 - One risk factor: 1 - Two or more: 2	0 to 2
Concomitant drug exposure	- None/no information/incompatible time of onset: 0 - Time compatible but no known hepatotoxicity: -1 - Time compatible and known hepatotoxicity: -2 - Definitely caused by other drug: -3	0 to -3
Exclusion of nondrug-related causes like hepatitis A, B, C, alcohol induced, gallstone induced, shock liver.	- Rule out: +2 - Possible/not investigated: -2 to +1 - Probable: -3	-3 to +2
Previous information on drug hepatotoxicity	- Reaction unknown: 0 - Reaction published: +1 - Reaction labeled: +2	0 to +2
Response to rechallenge	- Positive: +3 - Compatible: +1 - Not available: 0 - Negative: -2	-2 to +3

**Score Analysis**

0 or less – relation with the drug excluded  
 1-2 – unlikely  
 3-5 – possible  
 6-8 – probable  
 >8 – highly probable

**APPENDIX B. Different types of acute hepatic injury based on serum liver function tests (LFTs) [15].**

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**Ratio (R) of serum ALT/ALP, in x ULN, measured together at the time of liver injury first recognized.**

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Hepatocellular	$R \geq 5$ , or ALT>2xULN with normal ALP
Cholestatic	$R \leq 2$ , or ALP>2xULN with normal ALT
Mixed	$2 < R < 5$ and ALT>2xULN and ALP>ULN

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*Note: ALT: alanine aminotransferase; ALP: alkaline phosphatase; xULN: multiples of the upper limit of normal range. International Consensus (1990), J Hepatol11, 272-6*

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