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Article

Examining the correlation between Massa Medicata Fermentata and Crohn's disease

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SUMMARY

Crohn's disease, a chronic inflammatory bowel disease (IBD), is characterized by inflammation of the gastrointestinal tract. This condition commonly presents symptoms such as abdominal pain, overwhelming tiredness, weight loss, severe diarrhea, and malnutrition due to impaired nutrient absorption. While no curative treatment currently exists for Crohn's disease, several therapeutic strategies have been shown to reduce its signs and symptoms. Previous studies have established a connection between the presence of Saccharomyces cerevisiae (S. cerevisiae) and Crohn's disease, although the exact nature of this relationship requires further investigation. Additionally, S. cerevisiae has frequently been utilized in the fermentation process of Chinese herbal medicine (CHMs). We hypothesized that consuming a specific CHM, Massa Medicata Fermentata (MMF), would trigger certain CHM mechanisms to exacerbate Crohn's disease patients' condition. We observed the fermentation process of Massa Medicata Fermentata under the microscope to identify S. cerevisiae, examined the effect of adding MMF to S. cerevisiae experimentally, followed by online interviews to validate our findings. Our research suggests a possibility where Massa Medicata Fermentata potentially plays two competing roles. On the one hand, it suppresses yeast development, thereby improving patients' illness states. On the other hand, it has the potential to provide an environment conducive to the survival of microbes initially present in MMF due to the fermentation process. Our findings provide insights into the safety and efficacy of CHM for treating this chronic disease. While the study advances understanding of MMF's complex biological interactions, further studies and clinical investigations are essential to determine the broader implications of our findings for the use of CHMs in treating chronic inflammatory conditions.

INTRODUCTION

Crohn's disease (CD), a chronic, relapsing inflammatory disorder of the gastrointestinal system, imposes a significant clinical burden due to its well-established association with high morbidity and significant economic burden on the healthcare system (1, 2). As a principal subtype of inflammatory bowel disease (IBD), CD may involve any segment of the gastrointestinal tract and is frequently associated with extra-intestinal infections (3, 4). Clinical characteristics vary widely but typically include abdominal pain, hematochezia or mucoid diarrhea, nausea, vomiting, unintended weight loss, and symptoms such as fever (1, 5). Disease progression often leads to complications such as bowel obstructions (both colonic and small intestinal), fistulas, intraperitoneal abscesses, and perianal pathologies (3, 6).

Chinese herbal medicine (CHM) is a branch of traditional Chinese medicine that dates back at least 23 centuries. It seeks to prevent or treat illness by preserving or reestablishing the balance between yin and yang (7). CHM encompasses a wide array of medicinal herbs, each with distinct qualities and tastes (8). CHMs address issues like moisture, humid heat, and disturbances in air (qi) and blood circulation and offer a holistic approach to disease management, with various therapies known to modulate a patient's response to illness (9). Prior research has found that Massa Medicata Fermentata (MMF) and other CHMs (including Bai-zhu and Licorice) are capable of relieving visceral hypersensitivity symptoms such as abdominal tension, abdominal pain, and diarrhea in Irritable Bowel Syndrome with Diarrhea (IBS-D) patients (10-12). Mechanisms of CHMs include immune modulation, gastrointestinal irritation, and microbiota disruption (13). This can be beneficial in certain health contexts, since an overactive or dysregulated immune response is a key feature of CD (14). Prior studies have shed light on the complications of CHMs. Specifically, the efficiency of CHM in CD treatment might be complicated by two factors: the compositional complexity typical of CHMs and the fermentation process undergone by some CHMs. The complex composition of CHM formulations, which frequently incorporate various herbal compounds with diverse physiological effects, may contribute to the disruption of gut microbiota, as some of the CHM formulations are reported to have antimicrobial properties (15).

Fermentation also contributes to the complication of the efficiency of CHM. Fermentation, a naturally occurring anaerobic process, involves the enzymatic breakdown of energy-rich molecules mediated by microorganisms such as bacteria or yeast. This process is widely utilized in industrial production processes, primarily through the regulation of microbial enzymatic activity to achieve desired outcomes (16). In CHM, fermentation plays a crucial role in the enhancement of the original characteristics of medicinal herbs and/or produces new effects by manipulating the factors of temperature, humidity, and moisture conditions (17). The mechanism underlying fermentation is that raw materials for fermentation, such as rice, sorghum, corn, barley, and

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gluten, act as a substrate in the reaction and provide essential nutrients to promote microbial growth and reproduction (17). The primary medicinal effect of fermentation is the increase in potency and bioavailability of the CHM (9).

Recent studies have explored the various aspects of MMF fermentation. Earlier research reported shifts in physical and chemical characteristics of MMF, emphasizing dynamic variations in components such as substrate, volatile organic components, and lactic acid throughout the fermentation process. A notable study classified three distinct stages of MMF fermentation and recommended an optimal duration of four days for the procedure (18). Another study found that the initial components of MMF differ from its pharmacodynamic components and highlighted the role of amylase activity in MMF's pharmacological effects, while noting that enzyme efficacy is lost during processing (19). A third study showed that microbial fermentation increases enzyme activity and alters volatile compounds in MMF, with significant differences depending on the microbes used (20). However, as seen above, most research has primarily focused on the mechanistic aspects of fermentation and the properties of medicinal ingredients and was conducted on mice with limited attention to patient responses and associated side effects. In this study, we aimed to investigate patient responses to MMF, providing experimental evidence to support the notion that its effects may vary. Our findings could inform careful considerations of CHM's (including MMF) benefits and potential risks before use.

In this study, we focused on Massa Medicata Fermentata (MMF), a CHM for treating indigestion and related disorders (21). We chose to investigate MMF because it is frequently used as a traditional Chinese herbal treatment for those suffering from digestive problems and gastrointestinal pain, which are symptoms of CD (10). More importantly, MMF was identified by the patients interviewed in the present study as the most common and standardized treatment received. Fermentation of MMF is also a common practice. This process typically involves the introduction of microbial agents like *Saccharomyces cerevisiae* (*S. cerevisiae*) (22).

S. cerevisiae has been shown to significantly elevate the IgG (Immunoglobulin G) and IgA (Immunoglobulin A) values of patients with established CD (23). IgG and IgA values are the typical characteristics of exacerbations of inflammatory bowel diseases (23). In contrast, the antibody values of patients suffering ulcerative colitis (a type of IBD) were found to be comparable to those of healthy controls (23). Ulcerative colitis shares many inflammatory and symptomatic similarities with CD, although it affects different parts of the digestive tract (12). Similar observations were made in a more recent study that tested several substances (yeast, milk, peanut, cereal, citrus, and cabbage) to determine their impact on CD: nutritional yeast had the most severe effect on inflammation in the digestive tract (24).

Even when the *S. cerevisiae* fungus is dead, e.g., that contained in nutritional yeast, its protein structure remains recognizable to the human body (25). Nearly 1 in 20 people in the U.S. have anti-yeast antibodies in their bloodstream (26). Approximately 60-70% of people with CD have anti-yeast *S. cerevisiae* antibodies (26). The antibodies attack the yeast protein even when it is deactivated because its structure resembles *Candida albicans*, which causes thrush and vaginal yeast infections (26). Therefore, we investigated whether MMF, which is fermented by *S. cerevisiae,* would worsen the symptom presentation of the patient who carries CD and other disadvantages that may support the inefficiency of CHMs in treating CD.

Overall, our study aims to examine the relationship between MMF and CD, addressing the need for more scientific scrutiny in this aspect. We investigated the presence of yeast in MMF following fermentation and its potential impact on CD patients. There have been no specific papers discussing the side effects of MMF, and most relevant experiments have been conducted on mice, which may not fully reveal the potential side effects in humans. However, our informant doctors who work with patients extensively have reported that patients experienced side effects after ingesting MMF, including nausea, vomiting, diarrhea, skin rash, and, in some cases, depression. Inspired by the doctors' observations, in our study, we hypothesized that ingesting Massa Medicata Fermentata would correlate with the worsening state of illness of the patient carrying CD. We will also further delve into the dual role of MMF on CD patients in the Discussion section.

RESULTS

Our first step in the examination of the potential effects of MMF on CD was monitoring the fermentation of MMF in a Petri dish. Specifically, we conducted *in vitro* culturing experiments to assess the microbial composition of Massa Medicata Fermentata (MMF) samples used by CD patients, who were later interviewed. To achieve this, we mixed MMF samples (the same MMF consumed by the majority of the patients) with PDA, spread them onto Petri dishes, and incubated them under controlled conditions to facilitate microbial growth. We observed the growth of both certain microbes and mold growth on the Petri dishes (**Figure 1B**). The colonies displayed a milky yellow and pink substance resembling a paste adhering to the substrate. Intriguingly, despite the robust growth, no visible presence of spores or hyphae was observed in the microscopic images (**Figure 1C**). To ensure the specificity of our observations, we included a control Petri dish in which no growth was observed (**Figure 1A**). From these *in vitro* data, we concluded that certain microbes were present in the MMF used by participants in this study, which is consistent with a prior study (17).

Furthermore, we explored the impact of MMF on *S. cerevisiae* growth to understand its potential role in modulating

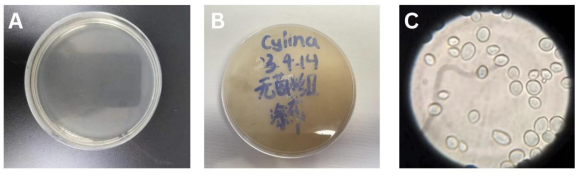

Figure 1: Yeast growth comparison on Petri dishes. (A) A clear Petri dish as the control for assessing microbial growth. (B) Growth of yeast on Petri dish. The figure shows a milky yellow and brown substance resembling a paste that adheres to the surface of the substrate. Image was captured using an iPhone camera. The culture medium used was 5 g of Massa Medicata Fermentata (MMF) mixed with a culture solution. (C) Optical microscope images of yeast growth on Petri dish. Yeast cells were observed under a light microscope. The cells ranged from 3 to 6 micrometers in diameter, with a smooth, spherical shape and transparent appearance. (Magnification: Eyepiece 10x; Objective Lens 100x).

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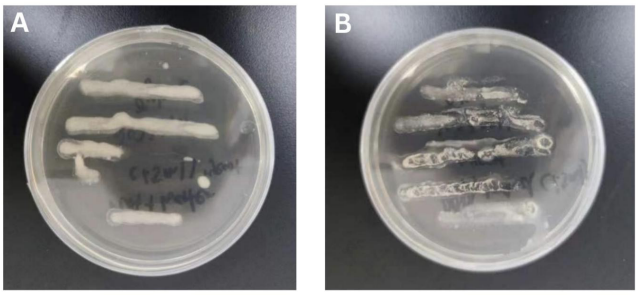

Figure 2: Comparison of S. cerevisiae growth in the presence and absence of MMF. (A) Yeast growth in the absence of MMF. A dense yeast colony formed on the Petri dish. The culture medium comprised 1.5 mL of water thoroughly mixed with a culture solution. (B) Yeast growth with MMF. The yeast growth on the Petri dish was notably sparse. The culture medium consisted of 1.5 mL of 20% (0.2 g/mL) MMF solution thoroughly mixed with a culture solution.

yeast development. To be more specific, we conducted *in vitro* culturing experiments with *S. cerevisiae* to assess MMF's effect on the growth of yeast. In conducting this experiment, we inoculated plates with MMF with *S. cerevisiae* and incubated them under identical conditions. We initially hypothesized that MMF would have a promotive effect on yeast growth, which would align with the observation of the growth of microbes under the microscope (**Figure 1**). Contrary to our initial hypothesis, MMF exhibited a suppressive effect on yeast growth. This is evidenced by notably sparse colonies in the presence of MMF compared to the densely packed colonies without MMF (**Figure 2**). From this finding, we concluded that MMF plays a dual role in suppressing the development of yeasts like *S. cerevisiae* and has the potential to provide an environment conducive to the survival and further growth of microbes initially present in MMF due to the fermentation process.

Simultaneously, we interviewed 65 CD patients for a more comprehensive view of the outcomes experienced by patients who had ingested MMF as part of their CHM regimen (**Figure 3**, **Table 1**). There were self-reported improvements in 21% of patients and no change in 21%, with 58% reporting worsening symptoms after MMF ingestion (**Figure 3B**). A summary table of the survey results of CD patients with specifics in gender, age, type of CHM ingested, duration of illness, CD symptoms, disease state, duration of the CHM treatment, length of time that CHM was taken, other concurrent treatments, and the improved or worsened symptoms after CHM is also presented (**Table 1**). Additionally, to delve deeper into the relationship between disease states and the effects of MMF ingestion, we allowed the patients to self-report their understanding of their disease states as mild, moderate, or severe.

Mild CD is characterized by occasional symptoms, such as abdominal pain and diarrhea (27). Despite experiencing these symptoms, individuals with mild CD generally have minimal disruption to their daily lives. Furthermore, individuals with mild CD can usually maintain their nutritional status without experiencing weight loss. Moderate CD is characterized by more frequent and pronounced symptoms, including abdominal pain, diarrhea, and fever (27). The impact on daily life becomes more noticeable, and there may be some interference with regular activities. Inflammation is moderate, with more extensive mucosal involvement, and

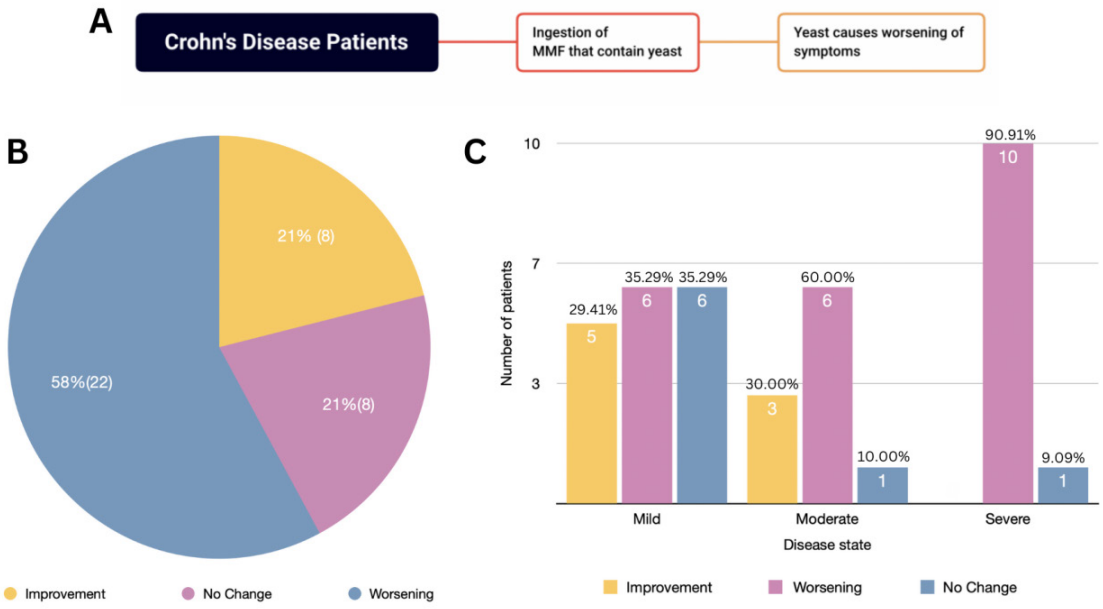

Figure 3: Comprehensive analysis of patient responses to MMF ingestion. (A) Hypothesized model: If Crohn's disease patients ingest MMF that contains yeast, their symptoms will worsen. (B) Patient self-reported outcomes after ingesting MMF. The chart displays the percentages of different outcomes (improvement, worsening, or no change) reported by the patients. Numbers in parentheses indicate the number of patients in each category. (C) Responses of patients in different disease states to MMF ingestion. Patient responses are categorized by disease state, with three distinct states defined as mild (n=17), moderate (n=10), and severe (n=11). The percentages were calculated by dividing the number of patients with the specific reported effect of MMF by the total number of patients in that disease state.

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General Information		Mean /Number	Percentage
Age (years)		34	/
Disease condition after CHM	Improvement	24	36.92%
	Worsening	27	41.54%
	No change	14	21.54%
Disease condition after MMF	Improvement	4	13.33%
	Worsening	22	73.33%
	No change	4	13.33%
Potential Factors		Number	Percentage /frequency
Gender	Female	31	47.69%
	Male	34	52.31%
Crohn's Disease Symptoms	Abdominal pain	36	55.38%
	Diarrhea	16	24.62%
	Weight loss	17	26.15%
	Nausea	13	20.00%
	Fever	12	18.46%
	Anemia	10	15.38%
	Tiredness	6	9.23%
	Feeling of a filled abdomen	5	7.69%
	Constipation	3	4.62%
Time since diagnosed with Crohn's Disease	1-3 years	47	72.31%
	3-6 years	13	20.00%
	6-8 years	5	7.69%
Type of CHM ingested (main components)	MMF	38	58.46%
	Angelica	21	32.31%
	Pulsatilla	18	27.69%
	Phellodendron	14	21.54%
	Sophora flavescens	13	20.00%
	Amomum villosum	12	18.46%
	Agastache	12	18.46%
	Psoralen	11	16.92%
Length of time that CHM was taken	Less than 1 year	53	81.54%
	1-2 years	8	12.31%
	Over 3 years	4	6.15%
Patients using other treatment methods	Western medicine	53	81.54%
Disease condition	Mild	35	53.85%
	Moderate	23	35.38%
	Severe	8	12.31%
Disease State	Crohn's Disease	43	66.15%
	Ulcerative colitis	22	33.85%

Table 1: Survey results of the Crohn's disease (CD) patients with specifics in gender, age, type of CHM ingested, duration of illness, CD Symptoms, disease state, duration of treatment, length of time that CHM was taken, other treatments, and whether symptoms improved or worsened after CHM ingestion.

there may be a risk of weight loss and nutritional deficiencies (27). Lastly, in severe cases of CD, symptoms are severe and persistent, encompassing abdominal pain, diarrhea, significant weight loss (>10%), and fever (27). The impact on daily life is significant, with a notable impairment of daily activities and overall quality of life. Severe inflammation is present, often leading to complications such as strictures, fistulas, or abscesses. Individuals with severe CD are at risk of substantial weight loss and malnutrition (27). It is worth noting that patients were provided with these specific definitions; however, they were told to describe their specific symptoms first. After receiving the definitions, they classified their condition, and we subsequently reclassified it based on the descriptions received.

Of patients with mild disease who consumed MMF (n = 17), 29.41% reported improvements, 35.49% noted worsening symptoms, and the remaining 35.49% observed no change (**Figure 3C**). For those with moderate disease (n = 10), 30% of the patients experienced improvements, 60% reported worsening symptoms, and 10% observed no change (**Figure 3C**). In contrast, for patients with severe disease (n = 11), 90.91% reported worsening symptoms after MMF ingestion, and 9.1% observed no change (**Figure 3C**). We concluded that there is a negative correlation between CD severity and the effectiveness of MMF, which raises intriguing questions about the intricate dynamics between MMF and CD. It is important to note that the focus on MMF in **Figure 3** is intentional, as these 38 individuals exclusively consumed or prioritized MMF in their treatments. The remaining 27 participants did not emphasize MMF as the primary component in their treatment, providing a nuanced perspective on the diverse treatment approaches within the study cohort.

During our interviews, we also uncovered a range of side effects experienced by patients with CD who had been consuming CHMs as part of their treatment regimen. These side effects included sensations of stomach discomfort characterized by a "stomach drop" feeling (n=24), noticeable trembling (n=5), persistent lethargy (n=13), uncontrolled bouts of vomiting (n=14), hallucinations (n=2), and hair loss (n=8). These side effects provide a sobering perspective on the real-world impact of CHM treatment on patients' lives and underscore the critical need to evaluate the safety and efficacy of these remedies, particularly in conditions like CD.

DISCUSSION

The results indicate that MMF plays a dual role in suppressing yeast development and potentially creating an environment conducive to the survival of microbes originally present in MMF due to the fermentation process. As observed, there appears to be a continued growth of microbes originally present in MMF, a deterrence of *S. cerevisiae* development by MMF, as well as a reduced medicinal effect of MMF and increased side effects for CD patients suffering more severe conditions.

It is important to note that these implications of MMF's effect on CD patients are based on the lab culturing results and surveys. Due to the distinct environments of the culturing experiments and the human body, there is no direct evidence connecting our experimental observations with the information elicited through surveys. However, we hypothesize that the observation in lab culturing may contribute to the patients' experiences due to the fact that microbial agents in MMF have been observed in the literature to reduce inflammation.

According to previous studies, the predominant microbes present in MMF include *Pediococcus acidilactici*, *Aspergillus oryzae*, and *Rhizopus oryzae* (28). Using polymerase chain reaction/denaturing gradient gel electrophoresis (PCR/DGGE) analysis, researchers identified dominant microbial populations within the genera *Enterobacter*, *Pediococcus*, *Pseudomonas*, *Mucor*, and *Saccharomyce* (28). These microbes, like *Pediococcus acidilactici*, *Aspergillus oryzae*, and *Rhizopus oryzae,* could all grow in the human body system (29, 30, 31). Regarding specific effects of these microbes' development on CD patients: *Pediococcus Acidilactici* administration can reduce inflammation and improve the symptoms of patients (29); *Aspergillus oryzae* also has an anti-inflammatory effect (28); *Rhizopus oryzae* exhibits probiotic and antioxidant properties and have an anti-stress effect on gut microbiota (31, 32). Contrarily, microbes from the *Saccharomyce* genera may exacerbate CD symptoms and affect gut barrier permeability (33).

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The presence of beneficial microbes such as *Pediococcus acidilactici* and *Aspergillus oryzae* in MMF may have contributed to the reported medicinal effects observed in surveys, with these microbes likely reducing inflammation by decreasing the presence of yeast (see the culturing experiment) and improving symptoms in CD patients. In contrast, the potential exacerbation of symptoms by microbes from the MMF, like the *Rhizopus oryzae*, suggests that the efficacy of MMF is complicated by multiple factors: the worsened symptoms experienced by some patients may be influenced and explained by the presence of other microbes in the MMF.

Our study has shed light on the intriguing connection between MMF and yeast growth. While we did not delve deeply into the specific mechanisms underlying how MMF can both harbor yeast and suppress yeast growth in this study, we acknowledge the significance of this question. We intend to explore the potential mechanisms at play, acknowledge the limitations of our study, and point to potential future experiments. Our primary research focus was to investigate the correlation between MMF and the status of CD patients, which we believe is essential for providing insights into the clinical aspects of MMF consumption.

Our initial hypothesis was that if CD patients ingest MMF that contains yeast, their symptoms will worsen (**Figure 3**). While some data initially supported this hypothesis, our investigation revealed a more complicated reality. Though our data analysis did not unveil any clear patterns based on gender or age, a trend emerged concerning the relationship between disease severity and the effects of MMF: patients with mild to moderate disease states appeared to benefit from MMF ingestion, and patients with severe disease states appeared to have worsening symptoms as they ingest MMF (**Figure 4**).

To elaborate on our discoveries, we conducted *in vitro* studies to examine MMF. We observed both the growth of microbes that are initially present in MMF on the Petri dishes and the microscopic observation of the plates (**Figure 1**). On another experimental plate (of which the figure is not included), we observed mold. This observation is not uncommon, as traditional Chinese fermentation of medicinal herbs often involves growing molds (34).

Based on the informant doctors' observations, we hypothesized that the use of CHMs may lead to digestive tract inflammation and could potentially exacerbate the symptoms of CD. To explain the rationale behind this hypothesis, the growth of microbes that are initially present in MMF may disturb the normobiosis, or microbiota balance, within the digestive tracts. We analyzed the effect of adding MMF to the media on *S. cerevisiae* growth. The result was contrary to our initial hypothesis and revealed a striking and unexpected outcome: MMF exhibited a suppressive effect on yeast growth (**Figure 4**). This striking phenomenon can be observed in **Figure 2B**, where yeast colonies are notably sparse in the presence of MMF, in stark contrast to the densely packed yeast colonies seen in **Figure 2A** when MMF was absent. This revelation may potentially challenge the hypothesis that MMF may exacerbate CD by promoting yeast proliferation, since the suppression of MMF on the growth of *S. cerevisiae* is observed. Yet, further development of the microbes in MMF and the medicinal effects of MMF are not considered in this study, and these limitations may hinder us from delving into the complications of the mechanism of CHMs.

Our qualitative data emphasizes the link between disease severity and the effectiveness of MMF, suggesting that MMF may suppress the growth of yeast under specific conditions, but this effect weakens as the severity of the patient's condition increases (**Figure 3C**). Our findings here highlight the complex nature of CD and its interactions with treatments like MMF. It is important to acknowledge the varied experiences among patients and the noticeable shift in responses based on the severity of their disease. While many patients with mild to moderate disease states appeared to derive benefits from MMF ingestion, the overall negative response of patients in severe disease states raised questions about the suitability of this treatment for individuals with more severe conditions. This also suggests that the influence of MMF on CD is specifically tied to the individual patient's conditions.

In order to explore the broader applicability of our study, additional experiments could be conducted with a wider range of CHMs. While our initial findings illustrated promising effects of a specific CHM — MMF — used in our research, delving into the various effects of different CHMs could provide valuable insights into their potential applications in various contexts.

The findings of this study, however, should be considered with several limitations and potential confounding factors. Firstly, our sample size of 65 CD patients may limit the comprehensiveness of our data and its generalizability to a broader population. This also affects our ability to survey with an equal number of participants from each disease state, leading to a smaller group of patients with severe conditions compared to the other two. It is also worth noting that the differences in the concurrent treatments (other than MMF) received by the participants are not considered when interpreting the findings, leaving the complexity of the interplays between CHMs and CHMs with Western medicine

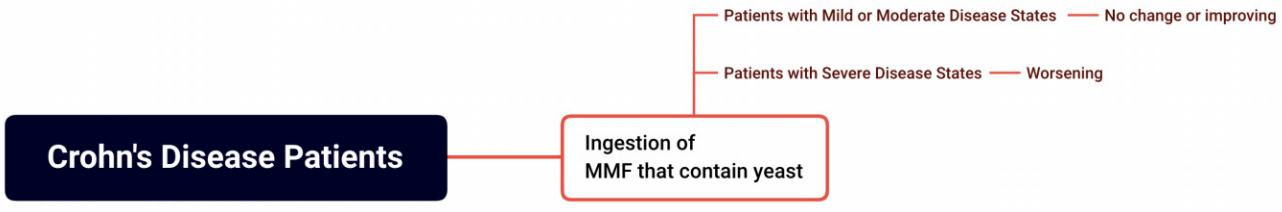

Figure 4: Concluded model regarding the Impact of MMF ingestion on Crohn's disease. Patients with mild disease states were more likely to benefit from MMF ingestion, and patients with severe disease states worsened as they ingested MMF.

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unaddressed. Thirdly, our reliance on patient self-reports introduces potential biases such as recall bias and the subjective interpretation of symptoms. Additionally, we did not fully explore the specific mechanisms underlying MMF's dual roles of harboring and suppressing yeast growth. Furthermore, the diversity in the course and characteristics of CD within severity groups complicates attributing MMF effects solely to disease severity. These factors may include age, sex, medical history such as diabetes, anemia, hypertension, and chronic kidney disease, smoking, alcohol consumption, physical activity levels, body mass index (35). Finally, differences exist between the microbe culturing condition and the actual environment in the gastrointestinal system. The two culturing experiments are attempts to understand the mechanism of MMF in human bodies, and they were conducted as an approximation because of the infeasibility of conducting animal experiments with resources available to us. However, the difference limits the confidence one should have when considering similar phenomena (further growth of microbes or the suppression effect) that would appear in human bodies.

Future research should focus on unraveling the biological pathways through which CHM exerts its effects, including gene expression and signaling pathways. Other potential focuses may include studying the dose-response relationship of CHM and assessing its long-term effects through longitudinal studies to evaluate safety and sustainability and translating preclinical study results into human trials with rigid methodology to assess CHM's effectiveness in real-world settings.

In summary, our study provides evidence both supporting and challenging the effectiveness of the CHM used in our research-MMF. However, the possibility of other CHMs exerting similar or even stronger effects on the growth of microbes and CD symptoms warrants further exploration. By investigating a broader range of CHMs, we can enhance our understanding of the underlying biological pathways and expand the scope of potential applications of CHMs in health interventions. Such findings would have profound implications for the development of novel therapeutic approaches and contribute to improving human well-being on a larger scale.

MATERIALS AND METHODS

Interviewing doctors

As part of the pre-study preparation, we conducted interviews with medical professionals to gather clinical insights on the side effects of MMF. A total of 3 doctors, specializing in gastroenterology, were interviewed using a structured questionnaire. These interviews aimed to capture their observations regarding adverse reactions in patients treated with MMF, as these effects were not widely reported in existing literature. The doctors reported several side effects, including nausea, vomiting, diarrhea, skin rash, and in some cases, central nervous system depression. These qualitative findings informed the design of our study, particularly in assessing patient responses to MMF.

Culturing and fermentation studies

To initiate these studies, a sample of MMF solution at a concentration of 20% (0.2 g/mL) was obtained from an offline store based in China, as suggested by patients who were surveyed in this study (details about the survey will be explained later). Three Petri dishes were prepared to create a controlled environment for observing microbial behavior. Sterilization measures, including the use of an alcohol lamp and a sterilized spreader, were employed to prevent contamination. MMF solution was evenly spread and mixed across the surfaces of two Petri dishes containing potato dextrose agar (PDA) growth media, while the third served as a control plate also containing PDA growth media.

Cultures were allowed to incubate at 27°C in the incubator for 24 hours to facilitate the observation of microbial growth, as the optimal growth temperature range for most laboratory yeasts typically falls between 20-30°C (36). Microbial growth was examined using an optical microscope, and microscope slides were employed. Samples were prepared by mounting them on sterile microscope slides. Thin slices of the samples were carefully cut and prepared to enhance visibility under the microscope. This enabled the identification of the growth of microbes based on morphology and colony size on the Petri dish, allowing us to characterize the microbial growth.

In addition to our primary experiments, we conducted a parallel study to explore the impact of adding MMF to the PDA growth medium for *S. cerevisiae*. In this specific experiment, we designed two distinct growth media to assess the influence of MMF. For the experimental plate, we introduced 1.5 mL of MMF at a concentration of 20% (0.2 g/mL) into the growth medium, creating a test environment where yeast growth would occur in the presence of MMF. Conversely, for the control plate, we substituted the 1.5 mL of MMF with an equal volume of water, providing an environment for yeast growth in the absence of MMF. Both plates were then inoculated with *S. cerevisiae* and incubated under identical conditions in an incubator for 24 hours at a controlled temperature of 27°C, as in the first culturing experiment. Similarly, colony size and morphology were assessed to characterize the microbial growth.

Survey data collection

For information gathering, we reached out to potential participants through social media platforms. We posted and sent direct messages to individuals who have CD and may have used CHMs, including MMF, to manage their condition. In total, we engaged with 65 willing participants who provided consent to participate in our study, with 92 participants contacted. Systematic questioning and recording of responses were employed via oral interviews on video calls, and the data collected were organized and analyzed using tables to facilitate the identification of trends from participant responses.

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APPENDIX

1. What are the main components of the traditional Chinese medicine you take?
2. How long has it been since you took the traditional Chinese medicine? Do you feel that your condition has been controlled or improved?
3. Have you experienced any discomfort while taking traditional Chinese medicine?
4. Have you ever used Western medicine before receiving treatment with Chinese medicine? If so, please briefly describe the effect of Western medicine on you.
5. What are the treatment options?
6. Do you think the overall effect is better than the previous Western medicine treatment, or is there no better effect or no effect?
7. Have you tried some non-herbal treatments (such as moxibustion, suppositories, etc.)? If yes, does it play a role in relieving the condition?
8. Could you briefly explain the side effects of Chinese and Western medical treatments and compare the severity of the side effects? (If there is only Chinese medicine, just say Chinese medicine)
9. Are there any side effects of Western medicine?
10. What are your controls on diet and other factors:
11. Are the traditional Chinese medicines fermented or boiled?
12. Have you ever taken traditional Chinese medicine similar to Liushenqu (MMF)? If yes, have you had any reaction?
13. During the period of taking traditional Chinese medicine, did the doctor reduce the dosage of any medicine when the condition worsened? What specific medicine's dosage was reduced?
14. What prompted you to switch from Chinese medicine to Western medicine?
15. After the negative impacts of Chinese/Western medicine, did Chinese/Western medicine improve this situation?