Mechanism of Chinese Medicinal-Medicated Leaven for Preventing and Treating Gastrointestinal Tract Diseases

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Keywords

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Abstract

In recent times, there has been an increased attention on gastrointestinal tract (GIT) diseases, with GIT diseases becoming one of the greatest crises to human health. In China, traditional Chinese medicine, Medicated leaven (ML), has been applied to treat gastrointestinal diseases for thousands of years. However, its mechanism remains unclear till date. Here we review both the antibacterial and antiviral effects of ML, the mechanisms for improving human immunity, antioxidant and anti-inflammatory activities, the role of digestive function, and the role of its secondary metabolite. ML may be a new hope for GIT diseases.

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Records of medicated leaven (ML) in medicinal theory (Yao xinglun) dates back to 1612 A.D. Indeed, a large number of papers have been published in Chinese jour-

nals regarding ML. Also, a few of these articles about ML have been written in English. In fact, it is one of the most important drugs for the treatment of gastrointestinal diseases in China.

Due to the discovery of artemisinin, Tu Youyou, a phytochemist more than 80 years old at the China Academy of Traditional Chinese Medicine, was awarded the 2011 Lasker-DeBakey Clinical Medical Research Award and the 2015 Nobel Prize in Medicine. Artemisinin as a drug for treating malaria has saved millions of lives in the world, particularly in developing countries [1, 2].

ML is composed of six Chinese medicines, including *Artemisia annua*, *Xanthium sibiricum*, *Polygonum hydropiper*, adzuki bean, bitter apricot kernels, and flour. ML is a processed product, obtained from the fermentation of a mixture of fresh Chinese herbs in appropriate temperature and humidity. It contains yeast, vitamin B complex, ergosterol, essential oil (volatile oil), enzymes, glycosides, etc., and inhibits and kills bacteria and viruses and improves the function of the gastrointestinal tract (GIT). How does ML exert this miraculous effect for GIT?

Antibacterial and Antiviral Effect in GIT

In ML, *A. annua*, *X. sibiricum*, *P. hydropiper*, which contain the antibacterial active component for GIT, are combined with flour, bitter apricot kernels, and adzuki bean; and this mixture helps in the regulation of gastrointestinal microflora balance. ML is able to inhibit and kill different kinds of bacteria in GIT, such as dysentery bacillus, methicillin-resistant *Staphylococcus aureus* (MRSA), lemon *S. aureus*, *Escherichia coli*, beta hemolytic streptococcus etc [3].

In *A. annua*, the antimalarial property of Artemisinin was discovered by Chinese scientists, which is considered as one of the great discoveries in Medicine in the 20th century. Through inhibiting of toll-like receptors 2 and Nod2 mRNA expressions and nuclear transcription factor kappa B (NF- κ B) activation, Artesunate was associated with the reduction on tumor necrosis factor-alpha (TNF- α) release to mediate protection on septic mice with MRSA [4]. What's more, this increased the antibiotic accumulation within methicillin-resistant MRSA. Artesunate enhances the antibacterial activity of β -lactams [5]. Dihydroartemisinin and artemether also have antibacterial and antiviral active components in *A. annua*. The essential oil of *A. annua* has an antipathogenic activity such as gram-positive, gram-negative bacterial strains and yeast strains and can interfere with the expression of cell-associated and soluble virulence factors [6]. There are several active fractions and components exhibiting activity against *Clostridium perfringens* with different minimum inhibitory concentrations [7]. *A. annua* L. extracts exhibit antimicrobial activity against the periodontopathic microorganisms *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum* subsp. *animalis*, *F. nucleatum* subsp. *polymorphum*, and *Prevotella intermedia* [8]. *A. annua* leaves (AAL) showed significant leishmanicidal activity against *Leishmania donovani* in vitro and in vivo [9].

In many experiments, *X. sibiricum* showed antibacterial activity against MRSA [10]. In addition, the constituents of *X. sibiricum* Patr er Widd were found to have effective antibacterial activity against methicillin-resistant MRSA. In *X. sibiricum*, the main component xanthatin has an exceptional activity against strains of MRSA [11], xanthinin and xanthatin were found to have antibacterial and cytotoxic activities [12]. Compounds 8, 11, 12, 23, and 24 were isolated from the fruits of *X. sibiricum* which exhibited potent activity against influenza A virus (A/FM/1/47, H1N1) [13]. Compound 1 of the chemical constituents of the roots

of *X. sibiricum* showed moderate antibacterial activity against *Bacillus subtilis*, *E. coli*, MRSA, and *Micrococcus tetragenus* [14].

Early in the 20th century, people were aware that *Helicobacter pylori* infections can cause gastric cancer and peptic ulcer, and the antimalarial artemisinin from *A. annua* acts remarkably against the pathogen responsible for peptic ulcer diseases, *H. pylori* [15, 16].

Methanol and water extracts of bitter kernels had the highest activity, which showed a broad spectrum of antimicrobial activity against gram-negative, gram-positive, and *Candida* strains. The higher activity of bitter kernels was found for both the methanol and water extracts against *S. aureus*. The methanol extracts of bitter kernels were very potent against the gram-negative bacteria *E. coli*. Additionally, the methanol extract of bitter apricot kernels had significant anti-*Candida albicans* activity [17].

Using column chromatography, Confertifolin was isolated from the essential oil of *Polygonum hydropiper* L. Confertifolin showed activity both in bacteria and fungi including *Enterococcus faecalis* [18]. Polygodial and (2E)-hexenal possessed antibacterial activity against *Salmonella choleraesuis*. In this food-borne bacterium at any stage of growth, these 2 α , β -unsaturated aldehydes showed antibacterial activity from the time kill curve study. However, they showed different effects at the stage of growth of *S. choleraesuis* [19].

Artemisinins can inhibit hepatitis B and C viruses, a range of human herpes viruses [20], HIV-1 [21], influenza virus A [20, 22], and a bovine viral diarrhea virus [23] in the low micromolar range. In addition, Artemisinins possessed antibacterial activity against other herpes viruses from all subfamilies (α , β , and γ), namely, Epstein-Barr virus, herpes simplex virus 1, and human herpes virus 6A [24, 25]. Importantly, during preemptive antiviral therapy after stem cell transplantation has been described, this is the first time artesunate was used for the treatment of human cytomegalovirus (HCMV), a drug-resistant infection [27]. Artesunate can reduce the level of NF- κ B and Sp1 in HCMV through inducing protein synthesis and reducing the DNA binding activity of NF- κ B and Sp1 [24]. Its activity is not restricted to human cytomegalovirus (HCMV), but it also includes some animal CMVs, such as rat CMV [25].

Isoquercitrin in *P. hydropiper* suppresses the adipogenesis of 3T3-L1 cells via the inhibition of Wnt/ β -catenin signaling. *P. hydropiper* and isoquercitrin may therefore be potential therapeutic agents for obesity and its associated disorders [26]. These components produce antibacterial and antiviral effects together (Table 1).

Table 1. Antibacterial and antiviral effect for GIT

Author	Year	No.	Mechanism
Wang et al. [3]	2009	ML	Inhibiting and killing dysentery bacillus, MRSA, lemon <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , beta hemolytic streptococcus etc
<i>Artemisia annua</i>			
Efferth et al. [20, 21], Qian et al. [22], Romero et al. [23]	2002/1982/2006	Artemisinin	Inhibiting hepatitis B and C viruses, human herpes viruses, HIV-1, influenza virus A, a bovine viral diarrhea virus
Efferth et al. [24], Kaptein et al. [25]	2002/2006	Artemisinin	Possessing antibacterial activity against other herpes viruses from all subfamilies (α , β , and γ), namely, Epstein-Barr virus, herpes simplex virus 1, and human herpes virus 6A
Li et al. [4]	2010	Artesunate	Inhibiting of TLR2 and Nod2 mRNA expressions and transcription factor NF-kappaB activation; reducing TNF-alpha release to mediate protection of septic mice with MRSA
Jiang et al. [5]	2013	Artesunate	Enhancing antibacterial activity of β -lactams
Shapira et al. [27]	2008	Artesunate	Treating for HCMV about drug-resistant infection
Efferth et al. [24]	2002	Artesunate	Reducing the level of NF-kB and Sp1 in HCMV through inducing protein synthesis and reducing the DNA binding activity of NF-kB and Sp1
Kaptein et al. [25]	2006	Artesunate	Restricting to HCMVs, but also includes some animal CMVs
Kim et al. [8]	2015	<i>Artemisia annua</i> extracts	Restricting to the periodontopathic microorganisms <i>Aggregatibacter actinomycetemcomitans</i> , <i>Fusobacterium nucleatum</i> subsp. animalis, <i>Fusobacterium nucleatum</i> subsp. polymorphum, and <i>Prevotella intermedia</i>
Islamuddin et al. [9]	2014	<i>Artemisia annua</i> leaves	Showing significant leishmanicidal activity against <i>Leishmania donovani</i> in vitro and in vivo
Marinas et al. [6]	2015	The essential oil	Restricting to gram-positive, gram-negative bacterial strains and yeast strains and interfering with the expression of cell-associated and soluble virulence factors
Cumanda et al. [10]	1991	<i>Xanthium sibiricum</i>	Inhibiting the MRSA
Tsankova et al. [12]	1994	Xanthinin and xanthatin	Showing antibacterial and cytotoxic activities
Shi et al. [13]	2015	Compounds 8, 11, 12, 23, and 24	Exhibiting potent activity against influenza A virus (A/FM/1/47, H1N1)
Chen et al. [14]	2015	Compound 1	Inhibiting <i>Bacillus subtilis</i> , <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> and <i>Micrococcus tetragenus</i>
Chen et al. [14]	2015	Compound 6 and compound 12	Exhibiting stronger antibacterial activity against <i>E. coli</i> , with minimum inhibitory concentration values of 0.17 and 0.35 $\mu\text{g/mL}$, respectively
<i>Polygonum hydropiper</i>			
Duraipandiyan et al. [18]	2010	Confertifolin	Showing activity both in bacteria and fungi including <i>Enterococcus faecalis</i>
Kubo et al. [19]	2001	Polygodial and (2E)-hexenal	Possessing antibacterial activity against <i>Salmonella choleraesuis</i>
Kubo et al. [19]	2001	Two α , β -unsaturated aldehydes	Showing different inhibited effects at stage of growth of <i>Staphylococcus aureus</i>
Lee et al. [26]	2011	Isoquercitrin	Suppressing the adipogenesis of 3T3-L1 cells via the inhibition of Wnt/ β -catenin signaling
<i>Bitter apricot kernels</i>			
Shapira et al. [27]	2008	Methanol and water extracts	Exhibiting antibacterial activity against gram-negative, gram-positive and <i>Candida</i> strains
Shapira et al. [27]	2008	Methanol extracts	Inhibiting the gram-negative bacteria <i>E. coli</i> and significant anti- <i>Candida albicans</i> activity

Antioxidant and Anti-Inflammatory Activity

Free radicals may cause a number of diseases, such as cancer and atherosclerosis [28–30]. And antioxidants can neutralize free radicals, which may be of central importance in the prevention of these diseases [31].

Some flavonoids that are frequent components of the human diet have shown strong antioxidant activities [32]. In fact, higher plants exhibit significant potency against human bacterial and fungal pathogens. A role for oxidative stress suggest that antioxidant defenses act in combination to affect the cellular response to ART in tumor cells [33]. Some isolated flavonoids from the dried leaves of *P. hydropiper* L possess strong antioxidative capabilities. 2"-O-(3,4,5-trihydroxybenzoyl) quercitrin (Galloyl quercitrin) has the most effective antioxidant activities [34]. Antioxidative flavonoids and flavonoid glucoside were isolated from the leaves of *P. hydropiper* on the basis of chemical and spectroscopic evidence [35]. By various chromatographic methods, some new phenylpropanoid esters which were isolated from the methanolic extract of the whole plant of *P. hydropiper* L were found to possess effective antioxidant activity [36].

Furthermore, the results show adzuki bean extract is a potential antioxidant [37]. Amygdalin has anti-inflammatory and analgesic effects by suppressing the mRNA expressions of COX-2 and iNOS [38]. Amygdalin may have analgesic property for relieving inflammatory pain, by effectively alleviating responses to lipopolysaccharide (LPS) treatment in RAW 264.7 cells and carrageenan-induced arthritis in rats [39].

A. annua leaf extracts have potent antioxidant activities that significantly inhibit lipid peroxidation and erythrocyte hemolysis [40].

The flavonoids casticin and chrysosplenol D inhibited croton oil-induced ear edema. In addition, casticin and chrysosplenol D inhibited LPS-induced systemic inflammatory response syndrome in mice. LPS-induced release of interleukin-1 beta (IL-1 β), IL-6, and MCP-1 could be suppressed by casticin and chrysosplenol D; they inhibited cell migration and in Raw 264.7 cells, reduced LPS-induced I κ B and c-JUN phosphorylation [41]. Through altering of the expression level of flavonoids, biosynthetic pathway genes, the flavonoid content of *A. annua* can be increased, which enhances the antioxidant and cytotoxic potential of the plant [42].

A. annua L. extract reduces insulin resistance and hepatic steatosis in HFD-fed mice and attenuates connective tissue growth factor and fibrosis-related transform-

ing growth factor- β 1 and HFD-induced collagen deposition [43].

Endotoxin, TNF- α , and IL-6 levels were remarkably decreased and protein and mRNA expression of α -smooth muscle actin, toll-like receptors 4, myeloid differentiation factor 88 and transforming growth factor- β 1 were significantly downregulated by Artesunate (Art). In addition, Artesunate (Art) also significantly inhibited the NF- κ B p65 translocation into the nucleus [44]. The various parts of *A. annua* L., including flowers, leaves, stems, and roots have the polyphenolic profile and antioxidant effects [45]. The acetone extract of *A. annua* L. inhibited prostaglandin E2, LPS-induced nitric oxide, and proinflammatory cytokine (IL-1 β , IL-6, and IL-10) production. In addition, ethanol extract had the highest free radical scavenging activity [8]. By suppressing Src/Syk/NF- κ B and IRAK/AP-1/CREB pathways, *P. hydropiper* L. showed strong anti-inflammatory activity, which contributed to an anti-gastritis remedy [46]. *Daphniphyllum calycinum* Benth and *P. hydropiper* L. extracts can significantly improve the dextran sulfate sodium-induced colitis in mice, reduce myeloperoxidase activity in colonic mucosa and content of IL-1 β , TNF- α , malondialdehyde and nitric monoxide in colonic homogenate [47].

The Role of Immune Function

Artemisinin and its derivatives can enhance the DNA synthesis of mouse spleen cells, which had been activated with alloantigens or T cell mitogen Con A in vitro and in vivo. The results suggested that artemisinin and its derivatives promote T cell function selectively, and had a potential application for the recovery of immune function [48]. In addition, artesunate with low toxicity had a relatively high immunosuppressive activity and could inhibit T lymphocyte proliferation induced by mitogen and alloantigen. Meanwhile, artesunate could restrain DTH response significantly. Moreover, artesunate could also enhance the secretion of TGF- β , coupling with the striking increase of NF- κ B/p65 and Smad2/3 signaling [49]. A monoclonal antibody (mAb) 3H2 had sensitive and specific indirect competitive enzyme-linked immunosorbent assay for artemisinin. The mAb 3H2 recognized dihydroartemisinin, artesunate, and artemether with cross-reactivity of 650, 57, and 3%, respectively [50]. Meanwhile, the artemisinin-thyroglobulin conjugate generated polyclonal antibodies [51]. The flavonoids of *ALLs* can suppress CYP450 enzymes by altering the absorption and metabolism of artemisinin in the body, they have also

been linked to a beneficial immunomodulatory activity on chronic diseases [52]. Artemether could enhance DTH reaction, and increase the production of hemagglutinating antibody in normal mice. As well, artemether was significantly effective in the depletion of splenic CD4+ CD25+ Foxp3+ Treg cells. Artemether also enhanced the production of IL-4 and IFN- γ . So artemether has both cytotoxic and immunomodulatory properties in vivo [53].

After thorough stir-frying as an ingredient, the dried seeds of *Xanthium strumarium* are used in traditional Chinese medicines for relieving allergy. A compound of them exhibited significant selective inhibition of superoxide anion generation [54]. Xanthatin and xanthosin, which can inhibit nitric oxide synthesis, restrained the expression of iNOS and COX-2 and the activity of NF- κ B through the inhibition of LPS-induced I- κ B- α degradation [55]. A new antifungal peptide from rice beans suppressed the activity of human immunodeficiency virus type 1 reverse transcriptase and inhibited the translation by rabbit reticulocyte lysate [56].

The proliferation of CD4(+) T cells and the production of IL-2, T cell growth factor were inhibited by artesunate. Moreover, the expression of cell surface protein CD25 (IL-2 receptor α chain) and CD69 on CD4(+) T cells were reduced. In addition, artesunate increased the production of IFN- γ and IL-4 under Th1 and Th2 skewed condition, respectively, and inhibited the activation of naïve T cell [57].

AAL and seeds treated mice induced the strongest DTH response, which decreases three-fold in IgG1 levels and increases 2-fold in IgG2a levels when compared to infected controls. Upon antigen recall in vitro, AALs and seeds enhanced lymphoproliferative response, IFN- γ producing CD4+ and CD8+ T lymphocytes and nitrite levels [58] (Fig. 1).

The Role of Digestive Function

A trypsin was purified from 4-day old germinated seeds of rice bean; the activity of the enzyme is a serine protease which benefits the digestive function [59]. ML which can promote the secretion of digestive juice in vivo and in vitro in intestinal canal rabbit, especially for the contraction of rabbit intestinal fatigue has an activation function. ML could adjust the amount of intestinal tract micro-flora in patients with irritable bowel syndrome and improve the clinical symptoms [60]. ML which impelled functions and intestines of hungry mice, promot-

ed digestion for invigorating the function of the spleen [61]. The pathologic changes of gastric mucosa were improved greatly, and inflammatory degree in treated groups was significantly decreased compared to untreated group. It was found that amygdalin could reduce the activity of gastric pepsin and has significant effect in treating chronic gastritis and chronic atrophic gastritis [62]. It was found that amygdalin inhibited the acute gastric mucosa injury induced by restraint-cold stress, as well as improved the healing process of chronic gastric ulcer by acetic acid in rats and inhibited the formation of gastric ulcer in rats induced by pylorus ligation. Moreover, amygdalin plays a significant role in treating gastric ulcer [63].

The Role of Secondary Metabolites

Flour, bitter apricot seed, adzuki bean form the solid culture medium; while fresh juice of *A. annua*, *X. sibiricum* Patr, *P. hydropiper* form the liquid nutrient medium. They are suitable for different kinds of probiotic bacteria growth and provide adequate nutrition.

By morphology and molecular biology identification, 3 strains of fungi *Aspergillus flavus*, *Penicillium chrysogenum* and *Cladosporium* fungus were found in the ML, and some species were isolated and identified in ML samples including the bacteria *Pantoea agglomerans*, *Staphylococcus haemolyticus*, *Enterobacter asburiae*, *Bacillus subtilis*, *Bacillus cereus*, *Pediococcus pentosaceus*; the yeasts *Hyphopichia burtonii*, *Debaryomyces hansenii*, *Pichia kudriavzevii* and the filamentous fungi *Mucor circinelloides*, *Mucor racemosus*, *Penicillium camemberti*, *Aspergillus candidus* [11, 64].

Yeast strains were isolated from fresh Massa Medicata Fermentata samples that sold in markets. After fermentation, the Massa Medicata Fermentata samples contained a variety of yeast species. Yeasts were the main microorganism of the fermentation process. The isolated strains Y1, Y3, Y4, Y5 were *Cryptococcus albidus*, *Saccharomyces cerevisiae*, *Pichia kudriavzevii*, *Endomyces fibuligera*, respectively [65].

Probiotic bifidobacteria, in combination with prebiotic carbohydrates, have documented positive effects on human health regarding gastrointestinal disorders and improved immunity, however, the selective routes of uptake remain unknown for most candidate prebiotics [66]. Stachyose can improve the intestinal environment, and regulate the function of the intestinal imbalance flora.

Fig. 1. The role of immune function of ML. ML, Medicated leaven; AAL, *Artemisia annua* leaves; AAS, *Artemisia annua* seeds; LPS, lipopolysaccharide; NF-κB/p65, nuclear transcription factor kappa B p65; IL-4, interleukin-4; GIT, gastrointestinal tract.

Discussion

With rapid development, the incidence of gastrointestinal diseases has also increased significantly. In 1612 A.D., ML was recorded in medicinal theory (Yao xinglun) as having an effect on gastrointestinal diseases. Although ML has been used for the treatment of gastrointestinal diseases for thousands of years, the system studies rarely explain its mechanism. In this review, we attempted to clarify the real reason for the magical efficacy of ML. *A. annua*, *X. sibiricum*, *P. hydropiper* together with flour, adzuki bean, and bitter apricot kernels help in the prevention and treatment of gastrointestinal diseases. In fact, these investigations clearly show that it has recently been a hot target of many research studies related to a strong relationship among the biological, chemical, pharmacological, and

medical properties of these plants. Interestingly, studies indicate that ML has potential disease-modifying properties, including its well proven effects of antibacterial and antiviral effect, the mechanism of improving human immunity, antioxidant and anti-inflammatory activities, the role of digestive function and the role of its secondary metabolite.

In the clinical field, Shenqu oral liquid can accelerate the patient's recovery, relieve clinical symptoms, improve the patient's quality of life, safety, reliability, good taste, and achieve significant therapeutic effects, and has clinical application value [67].

After treatment the counts of Bifidobacterium were increased ($p < 0.05$) and *Lactobacillus* increased remarkably ($p < 0.01$) in patients of the Shenqu group. It could adjust the amount of intestinal tract microflora in pa-

tients with irritable bowel syndrome and improve the clinical symptoms [68]. Therefore, it contributes not only to symptomatic improvement, but also plays an important role in the treatment of gastrointestinal diseases. ML may be the new hope for GIT diseases.

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Conflict of Interest Statement

The authors have no conflicts of interest.

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