Effects of traditional Chinese medicines on intestinal bacteria: A review

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Traditional Chinese medicines (TCM) are increasingly used throughout the world for their effective and few side-effects. Though many studies have successfully proved their therapeutic effects both \textit{in vivo} and \textit{in vitro}, little researches were carried out to evaluate the interactions between TCM and intestinal microflora, which maybe the key point to reveal the beneficial efficacies of TCM. In this review, the adjustment effects of TCM on intestinal microflora published in recent decades were summarized and the mechanism between TCM and intestinal bacteria, as well as the development trend were proposed, too.

\textbf{Keywords:} Traditional Chinese medicines; Probiotics; Intestinal microflora; Microecology

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Traditional Chinese medicine (TCM) is an empirical healthcare system based on human experience dating back several thousand years ago, and stands out as the only one with a long uninterrupted history of extensive documentation among the world’s traditional medical systems\textsuperscript{1}. As a unique historic healthcare system, TCM aims to restore and maintain the dynamic balance of a person, thus achieving a harmony between the person and nature. In addition, TCM does not focus solely on the disease defined by specific pathological changes, but instead concentrates on the overall functional state of the patient. Unlike modern allopathic medicine, the development of TCM started directly with humans by trial and error, and the results, their efficacy and toxicity were eventually documented in writing since 3000 yrs ago. While a modern drug may have undergone an extensive development and approval process in a short time before it is finally being used on humans, whose true potential (better or worse) will not be known until it is actually used by humans over time.

As an ancient healthcare system, the concepts of theory and practice of TCM are difficult for modern scientists to comprehend and accept. These concepts often seem superstitious, nonsensical and unscientific to those who do not understand the Chinese language or the nature of the Chinese psyche. Consequently, the Western-educated researchers tend to opt for the obvious easy-access approach, which is to concentrate on the physical and chemical aspects of the TCM materials themselves, and their traditional aspects were largely ignored or avoided. So, when conducting research on Chinese herbs, most investigators tend to apply the approach and research methodology rooted in chemistry and molecular biology in a reductionism manner, and treat TCM as if they were single-entity chemical drugs with little concern of the documented traditional explanations and ignoring the seemingly inaccessible ancient Chinese theories. In the recent decades, enormous numbers of “single components” have been isolated, and chemical structures and pharmacological activities have been studied and thousands of research papers have been published elsewhere, while the success stories unfortunately have been few and far between.

With the rapid developments of micro-ecology theory, we find many phenomenons in micro-ecology theory can be well explained by traditional Chinese medicine theories (e.g. yin-yang theory, integrity concept, good-evil theory and visceral manifestation theory)\textsuperscript{2}. Moreover, the beneficial effects on diarrhea, urinary tract infections, immune disorders, hypercholesterolemia and food allergy, obesity and

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cancer can also been shared by TCM and probiotics (e.g. *Lactobacilli* and *Bifidobacteria*). Above all, the published literatures suggest that an extensive connection has been observed between the efficacy of TCM and probiotics. So, published articles on interactions between TCM and intestinal bacteria from the years 1979-2012 were analyzed to unveil the mystery of TCM in a new way.

**The relationship of intestinal microflora and disease**

The intestinal microflora is closely related to the human health, and many diseases in the development process are accompanied with the imbalance of intestinal microflora, including their colonization sites, number and specie changes. Usually, the intestinal microflora is mainly composed by *Bifidobacteria* and *Lactobacilli* which benefit human health as followings: (1) probiotics can colonize to the human intestinal surface which inhibits the pathogens’ invasion and colonization. (2) metabolisms such as short chain fatty acids (SCFAs) and peroxide can lower the pH and oxidation reduction electro-dynamic potential of the intestinal contents, which have inhibited the growth of pathogens or directly kill them. (3) probiotics can synthesize the nutrients of vitamins and folic acids, as well as the digestive enzymes which participate the digestion, absorption and metabolism of protein, carbohydrate and fat. (4) probiotics can improve the human immunity, especially the human intestinal immunity. (5) probiotics can reduce the body's absorption of harmful substances.

When gastrointestinal tract disease caused by stress, food mutation, antibiotics and other risk factors happened, the dysbacteriosis phenomenon in human intestine were observed characterized by the overgrowth of the opportunistic pathogens and the serious reduction of probiotics (e.g. *Lactobacilli*, *Bacteroides* and *Bifidobacteria*). In the same time, the microflora imbalance will conversely lower the nutrients absorption, immune capability and disrupt the colonization resistance to pathogens, which further aggravating the illness. Now, the ratio of probiotics and pathogens had been regarded as one of the important standards to evaluate the human health in Chinese hospitals.

**The adjustment effects of TCM on intestinal microflora**

Some experiments showed that many TCM were useless for the inhibition of pathogens in vitro, while possessed sound effects on probiotics promoting and pathogen inhibition in vivo. These results inspired us that probiotics may play a significant role in TCM treatment. So, more and more researches are carried out to study the relationship between TCM and intestinal microflora in China, and this is maybe a key point to explain the mechanism of TCM. The effects between TCM and intestinal microflora are listed in Tables 1 & 2.

Till now, the adjustment effect of TCM on intestinal microflora has been studied in the form of herb combination, single herb and the single component isolated from herb. In fact, papers published in high level journals were most about the isolated components which proved a clear background for safety evaluation and experimental repeatability. In our opinions, the advanced chemical, pharmacological and biological technologies do have facilitated an increasing number of researchers in the search for possible ways to explore the potential healthcare benefits of this “mysterious” millennia-old healing system, while the single component is just a part of a herb, even maybe a negligible part for the herb combination, and its healing effect on illness maybe largely different from the herb, not to mention the herb combination. Just like the relationship of DNA and human being: we can identify human beings by base sequence, but the DNAs can’t totally represent all the human characters, e.g. thinking, laughing and moving. So, a suitable evaluation system for traditional herbal medicine was urgently required and should be established.

**The effects of different kind of single herb on intestinal microflora**

TCM originate from natural plants and have the extremely complex compositions. Besides the active ingredient possessing the direct medical value, TCM also contain proteins, polysaccharides, trace elements and vitamins, which make the TCM, especially the herbal combination possess a variety of pharmacological effects. In short, the tonic TCM containing polysaccharides can significantly promote the growth of probiotics, and these polysaccharides not only can served as the bifidus factors which is beneficial for the growth of *Bifidobacteria*, but also can influence the glucose metabolism and sugar utilization.

To our knowledge, various types of TCM have different effects on intestinal microflora. *Donkey-hide gelatin*, *Schisandra*, *Wolfberry* and *Acanthopanax*
Table 1-The effect of single herb on intestinal microflora

<table>
<thead>
<tr>
<th>Name</th>
<th>Acting form</th>
<th>Models</th>
<th>Probiotics</th>
<th>Pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coptis</td>
<td>Water decoction</td>
<td>Antibiotic interference mice</td>
<td>Bifidobacteria ↑↑</td>
<td>E. coli ↑↑</td>
</tr>
<tr>
<td>Loquat leaf</td>
<td>Water decoction</td>
<td>Antibiotic interference mice</td>
<td>Bifidobacteria ↑↑</td>
<td>E. coli ↑↑</td>
</tr>
<tr>
<td>Alum</td>
<td>/</td>
<td>Normal mice</td>
<td>Bifidobacteria ↓</td>
<td>E. coli ↑↑</td>
</tr>
<tr>
<td>Burdock</td>
<td>Polysaccharides</td>
<td>Normal mice</td>
<td>Bifidobacteria ↑↑</td>
<td>E. coli</td>
</tr>
<tr>
<td>Rehmanniae</td>
<td>Oligosaccharides</td>
<td>Diabetic rats</td>
<td>Bifidobacteria ↓</td>
<td>E. coli ↓</td>
</tr>
<tr>
<td>Loquat pit</td>
<td>Water extract</td>
<td>Diarrhea mice</td>
<td>Bifidobacteria ↑↑</td>
<td>Enterococcus↑↑</td>
</tr>
<tr>
<td>Deer blood</td>
<td>/</td>
<td>Antibiotic interference mice</td>
<td>Bifidobacteria ↑↑</td>
<td>E. coli ↑↑</td>
</tr>
<tr>
<td>Kelp</td>
<td>Polysaccharides</td>
<td>Normal mice</td>
<td>Bifidobacteria ↑↑</td>
<td>E. coli</td>
</tr>
<tr>
<td>Rhubarb</td>
<td>/</td>
<td>Human volunteers</td>
<td>Lactobacilli ↑↑</td>
<td>E. coli ↓</td>
</tr>
<tr>
<td>Schisandra</td>
<td>/</td>
<td>Cirrhotic rats</td>
<td>Bifidobacteria ↑</td>
<td>E. coli ↑</td>
</tr>
<tr>
<td>Tibetan linggu</td>
<td>Milk soaking</td>
<td>Diarrhea mice</td>
<td>Bifidobacteria ↑↑</td>
<td>Enterococcus♀</td>
</tr>
<tr>
<td>Honeysuckle</td>
<td>Water extract</td>
<td>Allergic mice</td>
<td>Bifidobacteria ↑↑</td>
<td>E. coli ↓</td>
</tr>
<tr>
<td>Polyporus pecipes</td>
<td>/</td>
<td>Colon cancer mice</td>
<td>Bifidobacteria ↑</td>
<td>E. coli ↓</td>
</tr>
</tbody>
</table>

↑: significantly increased (P<0.05); ↓: significantly reduced (P<0.05); ↑↑: extremely significantly increased (P<0.01); ↓↓: extremely significantly reduced (P<0.01)

promoted the growth of *Bifidobacterium infantis* by 75.8%, 50.0%, 40.5% and 21.7%, respectively; Garlic, Fennel and Pepper all could promote the growth of *L. plantarum* and *Leuconostoc*, and the *Leuconostoc* grew better than *L. plantarum* when cultured at the same medicine dose; Effects of 23 different kinds of herbs on the *Bifidobacteria* in vitro were studied, and Astragalus, Reed rhizome and Ya gourd were found that they could promote the bacterial growth at the level of 12.5%, and the effects of medicine in different concentrations varied on the growth of *Bifidobacteria*. In conclusions, the herbs belong to tonic class could significantly promote the probiotics growth and their beneficial effect were enhanced as dose increased; but for the bitter cold herbs, the stimulating ingredients of emodin play an inhibit effect on the intestinal probiotics, and their inhibit effect was enhanced when higher dose of drug was used. Further, low dose of *Coptis decoction* had a good adjustment effect on intestinal microflora, but the antibiotics effect was also observed when *Coptis decoction* was heavily used for a long time. In addition, the different doses of the same herb can promote growths of different probiotics, it was showed that 500 ug/g of *Laminaria* could just only promote the growth of *Lactobacilli*, while 1000 ug/g of *Laminaria* could increase the biomass of *Lactobacilli* and *Bifidobacteria*; also, the different active ingredients in same herb may have different effects on intestinal microflora: the water-soluble
polysaccharides (scα) in Cassia can promote the probiotics growth and competitive inhibit the growth of invading bacteria and indigenous pathogens; otherwise, the anthraquinones (sc I) containing in Cassia don’t have such a nature 11.

The effects of different kinds of herb combination on intestinal microflora

In order to get the best effect, most of Chinese medicines were used by herb combinations. In this way, the herbs involved can complement each other and enhance their original role; in addition, for some partial properties or toxic drugs, the combination can adjust their side effects and toxicity, eliminate or mitigate their adverse effects. Some herb combination can well adjust the microflora in human intestine, enhance the growth and activity of probiotics, especially the growth of Bifidobacteria.

The extract mixture of Astragalus, Pueraria and Huang Ling can enhance the growth of probiotics and Bifidobacteria, and can adjust the intestinal microflora notably. It can also improve the health and recovery rate of mice that have undergone irradiation.

Table 2-The effect of herb combination on intestinal microflora

<table>
<thead>
<tr>
<th>Name</th>
<th>Herb combination</th>
<th>Models</th>
<th>Probiotics</th>
<th>Pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sijunzi Tang 13</td>
<td>Ginseng, Atractylodes, Poria, Licorice</td>
<td>Radiation mice</td>
<td>Bifidobacteria ↑↑</td>
<td>E. coli ↓</td>
</tr>
<tr>
<td>Wuwei Xiaodu Drink 27</td>
<td>Honeysuckle, Wild chrysanthemum, Dandelion, Violet etc</td>
<td>Antibiotic</td>
<td>Lactobacilli ↑↑</td>
<td>E. coli ↑</td>
</tr>
<tr>
<td>Gui Zhu Gan Tang 20</td>
<td>Poria, Cinnamon Twig, Atractylodes, Zhigancao, France Pinellia, Jujube, etc</td>
<td>Human volunteers</td>
<td>Bifidobacteria ↑↑</td>
<td>E. coli ↓</td>
</tr>
<tr>
<td>Jianpishengshi Tang 15</td>
<td>Codonopsis, Atractylodes, Poria, Licorice root, Yams, Lentils etc</td>
<td>Human volunteers</td>
<td>Bifidobacteria ↑↑</td>
<td>E. coli ↓</td>
</tr>
<tr>
<td>Dachengqi Tang 14</td>
<td>Rhubarb, Glauber's salt, Magnolia, Citrus aurantium</td>
<td>Interior-excess mice</td>
<td>Bifidobacteria ↑↑</td>
<td>Enterococcus ↑</td>
</tr>
<tr>
<td>Xiaochengqi Tang 14</td>
<td>Rhubarb, Magnolia bark, Citrus aurantium</td>
<td>Interior-excess mice</td>
<td>Lactobacilli ↑</td>
<td>Enterococcus ↓</td>
</tr>
<tr>
<td>Tiaowei Decoction 14</td>
<td>Rhubarb, Licorice, Glauber's salt</td>
<td>Interior-excess mice</td>
<td>Lactobacilli ↑↑</td>
<td>Enterococcus ↓</td>
</tr>
<tr>
<td>Yinchen Mixture 26</td>
<td>Capillaris, Astragalus and Codonopsis</td>
<td>Human volunteers</td>
<td>Bifidobacteria ↑↑</td>
<td>E. coli ↓</td>
</tr>
<tr>
<td>Decoction of Rehabilitation 36</td>
<td>IntestinalCodonopsis, Atractylodes coke, Magnolia, Coix Seed, Chinese yam, Poria, etc</td>
<td>Human volunteers</td>
<td>Bifidobacteria ↑↑</td>
<td>Enterococcus ↓</td>
</tr>
<tr>
<td>Bufe Spleen Particles 21</td>
<td>Astragalus, Ganoderma, Poria, Jujube, Licorice, etc.</td>
<td>Spleen-deficiency mice</td>
<td>Lactobacilli ↑↑</td>
<td>Enterococcus ↓</td>
</tr>
<tr>
<td>Coptis Decoction 9</td>
<td>Coptis, Scutellaria, Phellodendron, Gardenia</td>
<td>Normal mice</td>
<td>Bifidobacteria ↓</td>
<td>E. coli ↑</td>
</tr>
<tr>
<td>Shenling Baizhu San 37</td>
<td>Ginseng, Poria, Atractylodes (fried), Yam, Lotus seeds etc.</td>
<td>Spleen-deficiency mice</td>
<td>Lactobacilli ↑↑</td>
<td>Enterococcus ↓</td>
</tr>
<tr>
<td>Sishen Wan 22</td>
<td>Psoralen, Nutmeg, Schisandra, Evodia</td>
<td>Spleen-deficiency mice</td>
<td>Bifidobacteria ↑↑</td>
<td>E. coli ↓</td>
</tr>
<tr>
<td>Nano-medicine Preparations 30</td>
<td>Ligustrum lucidum, Ginseng, Atractylodes, Poria, etc.</td>
<td>Antibiotic</td>
<td>Bifidobacteria ↑↑</td>
<td>Enterococcus ↓</td>
</tr>
</tbody>
</table>

↑: significantly increased (P<0.05); ↓: significantly reduced (P<0.05); ↑↑: extremely significantly increased (P<0.01); ↓↓: extremely significantly reduced (P<0.01)
**Bifidobacteria, Lactobacilli and Bacteroides** in diabetic rats’ intestine\(^{12}\), *Shi Junzhitian* can promote the growth of *Bifidobacteria* and *Lactobacilli*, and inhibit the growth of *Enterococcus* and *Enterobacteriaceae* \(^{13}\). While some herb combinations, such as *Three Chenqi Decoctions*, not only inhibit the growth of *Enterococcus* and *Enterobacteriaceae*, but also the probiotics \(^{14}\).

**The adjustment mechanism of TCM on intestinal microflora**

In recent years, there is an increasing interest to study the adjustment effect of TCM on intestinal microflora in China, and these researches are mostly focus on the bacterial growth impact. Based on the current data, the adjustment mechanism was concluded as followings: (1) some ingredients of TCM can directly inhibit the growth of intestinal microflora. For example, some bitter cold medicines possessed the capability of inhibiting and killing effects in vitro \(^{9,15}\), and their related experiments also proved that the high dose of *Coptis Decoction* could lead to the reduction of *Lactobacilli* and *Bifidobacteria*, as well as *Enterococcus* and *Enterobacteriaceae* \(^{9}\). (2) polysaccharides in TCM can stimulate the growth of probiotics and pathogens, but they have a better effects on probiotics growth than pathogens. And, the metabolites (e.g. SCFA, peroxide, bacteriocin and lipoteichoic acid) produced by probiotics indirectly inhibit the growth of pathogens \(^{9}\). Now, a few references showed that some TCM were useless to inhibit the growth of pathogens in vitro, but they can significantly lower the biomass of pathogens in vitro, which indirectly verify this hypothesis \(^{16}\). (3) Some TCM possess the probiotics promoting and pathogens inhibition characters. The bifidus factors isolated from *Honesuckle* can promote the growths of *Lactobacilli* and *Bifidobacteria*, while the chlorogenic acid, different chlorogenic acid and luteolin in *Honesuckle* could successfully inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* \(^{17}\).

**The research methods and models**

Now, researches in China studied the interaction of TCM and intestinal microflora were mainly depend on the traditional viable cell count, and only few studies have referred to pathology testing, cell response and molecular biology techniques (e.g. ERIC-PCR) \(^{18,19}\), besides the inhibit experiment in vitro, some in vivo models such as antibiotic interference model, spleen model, diarrhea model and diabetic model have been established \(^{20,21}\). But till now, the research technologies used to study the interaction of TCM and intestinal microflora is relatively backward and the research models are relatively single.

Besides the adjustment effects introduced above, *Shi Junzhitian*, *Sishen Pill*, *Linggu Milk* and *Medicated Leaven* \(^{9}\) can promote the recovery of intestinal mucosal injury and increase the thickness of intestinal wall and the amounts of goblet cell. In addition, *Schisandra* and *Yinchen Mixture* \(^{25,26}\) can regulate cytokine and promote the liver cells’ regeneration by adjusting intestinal microflora; *Wuwei Xiaodu Drink* and *Laminaria* \(^{18,27}\) can significantly increase the mean of hemolytic plaque, lymphocyte transformation rate, phagocytic rate and phagocytosis index of macrophages, and enhance the biomass of intestinal microflora and human immunity; *Lonicera* can ease the food allergic inflammation and *Brown polypore* can inhibit the growth of colorectal cancer \(^{17}\). Also, *Ginkgo Biloba Extract* \(^{29}\) may take part in the lipid metabolism and play a role in lipid-lowering. In short, the beneficial aspects of TCM to human are extremely similar to probiotics, which inspire us much more advanced technologies and models should be applied to reveal the relationship between TCM and intestinal microflora.

**Conclusion**

Researches indicated that many TCMs are not the effective drug to disease treatment, but play their roles used as prodrugs. These TCM were orally administrated and interacted with various types of digestive enzymes in human intestine, and then were processed and transformed by probiotics, and eventually been selectively aborted by human body. During this progress, the TCM had also promoted the growth of probiotics. Till now, many active ingredients in TCM were found to possess stronger pharmacological activity when metabolized by probiotics, especially for the water-soluble sugar component parts of the glucosides, which had a low bioavailability and hard to absorb, and its long intestinal residence time also made it possible for probiotics to make use of. For example, the senna glycosides existing in *Rhubarb* and *Senna* had a good effect on diarrhea, but no effect was observed when subcutaneous injected to normal mice and orally administered to sterile mice; the data also showed that the glycoside compounds in *Peony* and *Angelica*...
cannot be decomposed by intestinal digestive enzymes, but only can be hydrolyzed by β-glucosidase produced by probiotics, which then turned into aglycone and play their roles in ill treatment and probiotics promoting.

The interaction explication of TCM and intestinal microflora was the basis to develop new medicine. Though some published papers in China have revealed the adjustment effects of TCM on intestinal microflora, but the molecular mechanisms of TCM and intestinal microflora are rarely studied yet. So, the current data can’t clearly explain the practical effect of TCM on intestinal microflora and human health. In order to fully understand the interaction of TCM and intestinal microflora, much more work should be done, which should include these aspects:

1. Besides the detection of microflora biomass, much more advanced technologies, such as denaturing gradient gel electrophoresis/temperature denaturing gradient gel electrophoresis (DGGE/TGGE), terminal restriction fragment length polymorphism (T-RFLP) and single strand conformation polymorphism (SSCP), should be used to monitor the diversity changes of human intestinal microflora.

2. Some advanced molecular biology techniques, such as in vivo expression technology (IVET), labels mutation technique (STM) and gene chip technology, should be applied to observe the effect of TCM on the gene expressions of probiotics or pathogens from the molecular level.

3. Much more animal models should been established based on the characters of TCM considering the drug dose, duration and types, which maybe better reveal the mechanisms of TCM on curing the diarrhea, allergies, cancer, hypertension, diabetes and obesity.

Acknowledgement

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