Effect of Mongolian Herbal Tea on Growth of Candida albicans

Murun Davaadorj\(^1\), Tungalag Ser-Od\(^2\), Akram Al-Wahabi\(^1\), Eitoyo Kokubu\(^3\) and Takashi Inoue\(^1,2\)

\(^1\) Department of Clinical Pathophysiology, Tokyo Dental College, 2-9-18 Kanda-Misakicho, Chiyoda-ku, Tokyo 101-0061, Japan  
\(^2\) Oral Health Science Center, Tokyo Dental College, 2-9-18 Kanda-Misakicho, Chiyoda-ku, Tokyo 101-0061, Japan  
\(^3\) Department of Microbiology, Tokyo Dental College, 2-9-14 Kanda-Misakicho, Chiyoda-ku, Tokyo 101-0061, Japan

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Abstract

Candida albicans, one of the main pathogens in the oral cavity, is involved in the development of oral candidiasis. Various components of tea, and especially polyphenols, are believed to be effective against the growth of yeast or bacteria. The purpose of this study was to investigate the effect of polyphenols in Mongolian herbal tea on growth of C. albicans. Tea extract was prepared from Mongolian herbal tea and diluted with distilled water (DW) at concentrations of 10, 20, 30, 40, or 50%. Distilled water was used as the control. Acidification of the medium was determined by measuring its pH; the presence of polyphenols by the Folin-Ciocalteau colorimetric method; and growth of C. albicans by absorbance at a wavelength of 630 nm at 0-, 6-, 12-, and 24-hr intervals. The pH of the medium was 5.2 to 5.27 in all experimental groups compared with 7.1 in the control group. Polyphenols were present in all experimental groups, and at significantly higher levels than in the control group. Growth of C. albicans showed a significant and time-dependent increase in the control and all experimental groups. Growth of C. albicans in all the experimental groups was higher than that in the control group. These results suggest that Mongolian herbal tea promotes the growth of C. albicans, despite the presence of polyphenols.

Key words: Mongolian tea — Herbal tea — Polyphenol — Candida albicans

Introduction

Candida albicans is a fungal pathogen found in the oral cavity. It is believed to be one of the most important constituents of the normal microbial flora of the host in terms of its virulence and rate of occurrence\(^10,24\). The most prevalent oral infections involving C. albicans
are oral candidiasis, in which it generally accounts for around 50% of such cases\(^{21,26}\), and Candida-associated denture stomatitis, which occurs in up to 65% of denture wearers\(^{23}\). One way to prevent these infections is by consuming tea with anti-fungal properties.

There are three types of Mongolian tea—black, herbal, and green—and all are widely consumed among the Mongolian population\(^{2}\). Herbal tea has various health-promoting components, and is, therefore, often consumed for the various medical advantages it offers\(^{6,13}\). The type of herbal tea chosen for the present study is made from 6 medicinal plants, including rosa acicularis, vaccinium vitis-idaea, betula platyphylla, taraxacum officinale, and plantago major. These plants contain a wide variety of polyphenols, including catechins, as well as glucose and ascorbic acid\(^{25}\).

Polyphenols are believed to be an important constituent of tea leaves due to their high relative abundance and bioactive properties\(^{15}\). Those present in green tea inhibit oxidative stress, and have been used against bacterial or candidal growth\(^{7,8}\). The polyphenol content of such tea has been demonstrated to be influenced by a range of factors involved in its production, including the type of tea used, the temperature and concentration at which it is prepared, and time allowed for brewing\(^{1,5}\). To our knowledge, however, no studies to date have investigated the effects of Mongolian herbal tea on the growth of \textit{C. albicans}. The purpose of this study, therefore, was to investigate how growth of \textit{C. albicans} is influenced by exposure to Mongolian herbal tea.

Materials and Methods

1. Preparation of herbal tea extract

The herbal tea Ikh Taiga (Khuvsgul Taiga Co., Ltd., Ulaanbaatar, Mongolia) was used in this study, as it is a well-known and representative brand of Mongolian herbal tea. The leaves were ground and filtered and tea extract prepared by adding 100 ml boiling water to 5 g ground leaves. After 30 min of infusion, the extract was centrifuged at 300 rpm for 5 min and filtered. The resulting tea extract (hereafter, stock) was stored at room temperature and used for the experiment.

2. Measurement of medium acidification

The stock was diluted with distilled water (DW) in a safe-lock tube at a concentration of 10, 20, 30, 40, or 50%. Six groups were created, with DW alone as the control group, and the remaining 5 experimental groups comprising the different concentrations described above. The pH in each group was measured immediately on preparation.

3. Polyphenol content

The Folin-Ciocalteau colorimetric method was used to measure total polyphenol content\(^{12,20}\). All 5 concentrations of stock were used and compared with DW. Ten microliters Folin-Ciocalteu’s phenol reagent (Merck Milipore, Darmstadt, Germany) was added to 100 µl each stock concentration or DW. Absorbance was measured at 760 nm after 30 min of incubation at room temperature.

4. Preparation of \textit{Candida albicans}

\textit{Candida albicans} strain (SC5314) was kindly provided by the Department of Microbiology at Tokyo Dental College. It was first transferred to Brain Heart Infusion (BHI) broth (VWR, Radnor, PA, USA) containing 2% glucose and then incubated for 24 hr. To adjust the optical density of the plate reader so that it was closer to 0.1, approximately \(1 \times 10^6\) cells/ml cultured in fresh BHI broth was used for the experiment.

5. \textit{Candida} growth assay

A total of 0.1 ml of DW, or 10, 20, 30, 40, or 50% concentration of stock was added to 0.1 ml diluted solution of \textit{C. albicans} in a safe-lock tube. After gently shaking each tube, assays were performed in 96-well plates to evaluate rate of absorbance. Immediately after checking absorbance at a wavelength of 630 nm using a Spectra Max M5e (Molecular Devices LLC, Sunnyvale, CA, USA), the 96-well plates were incubated at 28°C for later evaluations at
6, 12, and 24 hr.

6. Statistical analysis

The data reported were obtained from 4 independent experiments. An ANOVA and the Post-Hoc Tukey’s test were used for the statistical analysis. A p value of <0.05 was defined as significant.

Results

1. Medium acidification

Immediately after infusion, the pH in the control group was 7.1 (Fig. 1), while that in the 30 and 50% concentrations of stock was 5.21. The pH in the 10, 20, and 40% concentrations was 5.23, 5.27, and 5.2, respectively (Fig. 1). A significant difference was found between the control and experimental groups.

2. Polyphenol check

As shown in Fig. 2, polyphenols were present in all groups. The data show that the amount of polyphenols depended on the concentration of the stock, with the highest concentration yielding the highest amount of polyphenols. A significant difference was found among all groups, except between the 30 and 40% groups and 40 and 50% groups.

3. Candida growth

Immediately after incubation, Candida growth was significantly higher in the 40 and 50% concentrations as compared to in the other groups. Significant differences in Candida growth were found between the control versus all of the experimental groups at 12 hr and 24 hr (Fig. 3). These results showed that growth of *C. albicans* increased in a time-dependent manner (Fig. 3).

Discussion

As a domestic product of Mongolia, herbal tea is widely consumed among the Mongolian population. Various components of such tea, including polyphenols, are believed to exert an anti-fungal effect. In the present study, the anti-fungal efficacy of Mongolian herbal tea was evaluated by culturing *C. albicans* with various concentrations of stock prepared from its leaves. *Candida albicans* is one of the main pathogens involved in the development of oral candidiasis. Therefore, the goal of this study was to investigate the potential utility of Mongolian herbal tea in the prevention of oral candidiasis.

One reason for the success of *C. albicans* as an opportunistic pathogen is its ability to adapt to the environment, and especially with regard to different pHs. It is able to grow in media with pHs ranging from 2 to 10. The pH of the Mongolian herbal tea stock used in

![Fig. 1 pH of herbal tea extract at various concentrations immediately after infusion](image1)

Control (diluted water, DW) vs experimental groups consisting of 10, 20, 30, 40, or 50% tea stock. Error bars indicate mean (SD). Means with same letters indicate no significant difference (n = 3).

![Fig. 2 Polyphenol content at different concentrations of Mongolian herbal tea extract by Folin-Ciocalteau method](image2)

Error bars indicate mean (SD). Means with same letters indicate no significant difference (n = 3).
the present study, which was approximately 5.2, showed no significant difference among the concentrations used. This indicates that the environmental pH had no effect on the results observed here.

Tea polyphenols, as a natural extract of tea, have been shown to exert an inhibitory effect on the growth of fungi. It has been reported that tea catechins demonstrate antibacterial activity against various pathogenic bacteria, as well. The results of the present study showed that polyphenols were present in the tea extract, and that the higher the concentration, the higher the amount of polyphenols, that is to say, amount was concentration-dependent. According to Toyo-shima et al. and Farhad et al., tea polyphenols inhibit the growth of C. albicans. Nevertheless, the results of the present study indicate otherwise. Here, Mongolian herbal tea promoted the growth of C. albicans.

Herbal tea has many components, each with its own unique bioactivities, including antioxidant, anticarcinogenic, antimicrobial, antifungal, hemostatic, and diuretic effects. According to one earlier study, maximum inhibition of growth of Candida species by green tea stock occurred at a concentration of 50% with 48 hr incubation.

This suggests that higher concentrations elicit statistically significant inhibition of growth of C. albicans. The results of the present study, however, suggest otherwise. This may be explained by the presence of glucose and ascorbic acid as components of the tea used. Glucose has been shown to be one of the main factors aiding growth of Candida species in the oral cavity, including C. albicans. Among other components, sugar also plays a supportive role in the growth of C. albicans. One study reported a delay in the growth of C. albicans with increasing concentration of ascorbic acid. In the present study, Mongolian herbal tea stock did not inhibit growth of C. albicans, which may be attributable to the presence of glucose and ascorbic acid amongst its components.

**Conclusion**

The present findings showed that Mongolian herbal tea stock promoted growth of C. albicans over time, despite the presence of polyphenol in the tea.
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Correspondence:
Dr. Tungalag Ser-Od
Oral Health Science Center,
Tokyo Dental College,
2-9-18 Kanda-Misakicho, Chiyoda-ku,
Tokyo 101-0061, Japan
E-mail: tunga@tdc.ac.jp