INTRODUCTION

Tannic Acid or Hydrolysable Tannins are naturally occurring complex phenol-rich polymers; prevalent in a wide variety of herbs, plants and fruits. Tannic Acid’s antimicrobial activity against food-borne pathogens has been recognized and it is generally considered a safe food additive. The present study was carried out to investigate the effect of Tannic acid (TA) on the growth and acid production of C. albicans. Various concentrations of TA prepared with Sabourand’s dextrose broth were inoculated with C. albicans and incubated at 37°C. Growth was monitored by optical density (OD) at 560nm wavelength and measured at specific intervals over a period of 24 hours. C. albicans cells were used as a non-growing cell suspension in a buffer solution of 40mM Sodium bicarbonate (NaHCO₃) and 6mM Dipotassium phosphate (K₂HPO₄) to which various concentrations of TA were added. Acid production was stimulated by the addition of 1% glucose. The data obtained showed that TA was fungistatic at concentrations of 10, 50, 300 and 500 µg/ml and fungicidal at 700 µg/ml. Acid production of C. albicans was inhibited at concentrations of 100, 300 and 500 µg/ml. The inhibitory activity of TA on growth and acid production of C. albicans suggests that TA could play an important role in prevention and treatment of Oral Candidosis and in reducing caries activity.

Key Words: Tannic Acid, growth inhibition, Candida albicans, acid production and inhibition.

METHODOLOGY

Microorganism

An oral isolate of Candida albicans was obtained from the laboratories of King Khalid University Hospital, Riyadh. The strain was identified and tested by sugar assimilation and fermentation techniques and by the germ tube test. Stock culture was maintained on Sabouraud’s dextrose agar slope at 4°C and sub cultured monthly.
Tested Antimicrobial Agent

Tannic Acid (M.Wt. 1701.23) was obtained from Mallinckrodt Chemical Works, St. Louis, MO. The required concentrations were prepared just before use and filter-sterilized with 0.22 µm GSFT membrane filter system (Millipore Corp., Bedford, Mass).

Effect of Tannic Acid (TA) on Growth

Sabouraud’s dextrose medium (5ml) was prepared and dispensed in tubes, and were set up in triplicate. Concentrations of TA were established at 10, 50, 100, 300 and 700 µg/ml. The inoculums were prepared by growing the yeast in Sabouraud’s dextrose medium at 37°C for 15 hours. The culture was diluted to an optical density (OD) of 2.0 at 560 nm wavelength with a spectrophotometer; this dilution contained 4.5 x 107 cells/ml. In order to inoculate tubes of Tannic Acid, 0.1 ml of diluted culture was used and the OD reading at 560nm wavelength was immediately taken. The tubes then were incubated at 37°C in a water bath for 24 hours and OD readings were recorded at 0, 5, 6, 7, 8 and 24 hours.

Effect of Tannic Acid (TA) on Acid Production

Cells of C. albicans to be used as a non-growing cell suspension were grown in Sabouraud’s dextrose medium for 15 hours at 37°C. The cells were harvested by centrifugation at 8,000 rpm for 5 minutes (Heraeus Labofuge GL); and washed in a buffer system consisting of 40 mM Sodium bicarbonate (NaHCO3) and 6mM of Dipotassium phosphate (K2HPO4) at pH 6.8-7.0 Harvesting and washing processes were repeated three times. The cells were then resuspended in the same buffer to a final concentration of 4.5 x 107 cells/ml.

The effect of 100, 300 and 500 µg/ml concentrations of Tannic Acid on acid production by C. albicans strain was determined by incubating 2.0 ml of the yeast suspension with various concentrations of Tannic Acid in sterile 16x15 mm metal capped tubes. The initial pH was determined for each mixture. To stimulate acid production, a 1% final concentration of glucose was added. Control tubes were included and these consisted of the cells suspension without any addition, yeast suspension with 1% glucose. The tubes were incubated at 37°C and the pH was measured at 30 minutes intervals using Cole Parmer pH meter for 2 hours.

RESULTS

The Effect of Tannic Acid on Growth

Tannic acid was found to be fungistatic at concentrations of 10, 50, 300 and 500 µg/ml, and produced fungicidal effect at 700µg/ml concentration. The inhibitory effect of 10 and 50 µg/ml was slight and equal. However, the inhibitory effect of 500 µg/ml was profound to the extent that the growth was only observed after 24 hours (Table 1 and Figure 1).

The Effect of Tannic Acid on Acid Production

Tannic Acid inhibited acid production of C. albicans at concentrations of 100, 300 and 500 µg/ml. This inhibitory effect was observed at a pH of 6.8-7.0 (Table 2).

<table>
<thead>
<tr>
<th>C. albicans in Sabouraud’s Dextrose Medium Plus</th>
<th>Optical Density (OD) At 0, 5h, 6h, 7h, 8h, 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans only</td>
<td>0.01 0.03 0.04 0.05 0.37</td>
</tr>
<tr>
<td>+ 10 µg/ml</td>
<td>0.0 0.02 0.02 0.03 0.32</td>
</tr>
<tr>
<td>+ 50 µg/ml</td>
<td>0.01 0.02 0.02 0.03 0.31</td>
</tr>
<tr>
<td>+ 300 µg/ml</td>
<td>0 0.01 0.01 0.02 0.22</td>
</tr>
<tr>
<td>+ 500 µg/ml</td>
<td>0 0 0 0 0.03</td>
</tr>
<tr>
<td>+ 700 µg/ml</td>
<td>0 0 0 0 0</td>
</tr>
</tbody>
</table>

TABLE 2: EFFECT OF TANNIC ACID ON ACID PRODUCTION OF C. ALBICANS

<table>
<thead>
<tr>
<th>C. albicans suspension plus glucose</th>
<th>pH Changes At 0 min, 30 min, 60 min, 90 min, 120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (alone)</td>
<td>7 5.5 5.3 4.8 4.5</td>
</tr>
<tr>
<td>+ 100 µg/ml TA</td>
<td>7 5.8 5.5 5 4.7</td>
</tr>
<tr>
<td>+ 300 µg/ml TA</td>
<td>7 6 5.7 5.4 5.1</td>
</tr>
<tr>
<td>+ 500 µg/ml TA</td>
<td>7 6.3 6.1 5.8 5.6</td>
</tr>
<tr>
<td>300 µg/ml TA (alone)</td>
<td>7 6.9 6.8 6.8 6.8</td>
</tr>
</tbody>
</table>

Table 2: Effect of Tannic Acid on Acid Production of C. albicans

Fig 1: Effect of Tannic Acid on the Growth of C. albicans
The inhibitory effect of Tannic Acid on microbial acid production was also supported by a study which showed that Tannic Acid encapsulated in Calcium- alginate microspheres effectively inhibited carbohydrate digestion in Gastro-intestinal tract. The mechanisms through which Tannic Acid exhibits its antibacterial and antifungal effect have not been fully elucidated. However, Tannins are known to bind proteins especially those with open-box structure with a high proportion of hydrophobic amino acids and proline content. Another possible mechanism of action of Tannins and Tannic Acid is attributed to their strong ability to form chelating complexes with metal ions and hence inhibiting candidal growth and metabolism by depriving it from such essential metal ions. In conclusion, the inhibitory activity of Tannic Acid on growth and acid production of C. albicans suggest that Tannic Acid could play an important role in prevention and treatment of Oral Candidosis and in reducing caries activity.

The findings of the present work and other related studies suggest that introducing Tannic Acid into toothpastes or mouthwashes might be advantageous to oral health. Moreover, plants and fruits that contain Tannic Acid such as tea, cocoa beans, grapes, strawberry, and persimmon could also be recommended in diet.

REFERENCES:


