Comparative Preventive Efficacy of Aqueous Extracts from *Lycium Barbarum* Bark and Fruit on Estrogen Receptor Positive Human Mammary Carcinoma MCF-7 Cells

Telang, NT¹, Li, G², Sepkovic, DW³, Bradlow, HL³ and Wong, GYC², ⁴
¹Cancer Prevention Research Program, Palindrome Liaisons, New Jersey, ²American Foundation for Chinese Medicine, New York, ³Hackensack University Medical Center, New Jersey, ⁴Department of Integrative Medicine, Beth Israel Medical Center, New York.
Study Background - I

- Selective estrogen receptor modulators Tamoxifen (TAM) and Raloxifene (RAL) represent conventional strategies for treatment of endocrine therapy responsive estrogen receptor positive (ER\(^+\)) clinical breast cancer (1, 2).

- Treatment for invasive ER\(^+\) breast cancer traditionally involves cytotoxic chemotherapy in combination with endocrine therapy (3).

- Long-term chemo-endocrine therapy is associated with acquired tumor resistance and/or adverse systemic toxicity, compromising patient compliance (4, 5).
Study Background – II

- Herbal medicinal products are in extensive use either as independent agents or as adjuvants to chemo-endocrine therapy (6, 7).

- Human mammary carcinoma derived ER\(^+\) MCF-7 cells represent an extensively utilized preclinical model for clinical breast cancer (8).

- We have reported the preventive efficacy of non-fractionated aqueous extracts from medicinal plants Lycium barbarum, Tabebuia avellende and Fructus corni, also known as Cornus officinalis, in the MCF-7 cell culture model (9-12).
Experimental Model, Test Compounds and Biomarkers

• **Model:**
  MCF-7 cells adapted for growth in chemically defined serum-depleted culture medium.

• **Test Agents:**
  Non-fractionated aqueous extracts from Lycium barbarum Bark (LBB) and Lycium barbarum Fruit (LBF).

• **Biomarkers and End points:**
  Anchorage dependent growth (viable cell number).
  Anchorage independent growth (anchorage independent colony number).
  Cellular metabolism of 17β-estradiol (generation of E₂ metabolites, 2-OHE₁:16α-OHE₁ and E₃: 16α-OHE₁+E₃ ratios).
## Study Results

### Table-1:

Endocrine Responsiveness of MCF-7 Cells in Serum Depleted Medium

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>Biomarker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Population Doubling (hr)</td>
</tr>
<tr>
<td>Serum</td>
<td>0.7%</td>
<td>34.4</td>
</tr>
<tr>
<td>E₂</td>
<td>20 nM</td>
<td>27.2</td>
</tr>
</tbody>
</table>

^a Means ± SD, N=6 per treatment group.  ^d-p=0.04.

^b Means ± SD, N=12 per treatment group.  ^e-f p= 0.03.
Figure 1A
Dose Response of Lycium barbarum Bark (LBB) Extract on MCF-7 Cells

Viable Cell Number ($\times 10^5$) at Day 7 post-seeding

<table>
<thead>
<tr>
<th>LBB (%)</th>
<th>ISD</th>
<th>E$_2$ 20 nM</th>
<th>0.005</th>
<th>0.025</th>
<th>0.05</th>
<th>0.1</th>
</tr>
</thead>
</table>

[IC$_{50}$: 0.02%]
Figure 1B
Dose Response of Lycium barbarum Fruit (LBF) Extract on MCF-7 Cells

Viable Cell number \( (\times 10^5) \) at Day Post-seeding

<table>
<thead>
<tr>
<th>LBF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISD</td>
</tr>
<tr>
<td>E₂ 20 nM</td>
</tr>
<tr>
<td>0.1</td>
</tr>
<tr>
<td>0.5</td>
</tr>
<tr>
<td>1.0</td>
</tr>
<tr>
<td>2.0</td>
</tr>
</tbody>
</table>

\[ [IC_{50} : 0.38\%] \]
Figure 2
Inhibition of Anchorage independent Growth by Lycium barbarum Bark (LBB) and Lycium barbarum Fruit (LBF) Extracts

<table>
<thead>
<tr>
<th>Anchorage independent (AI) Colony Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
</tr>
<tr>
<td>0.7%</td>
</tr>
</tbody>
</table>

![Bar chart showing Anchorage independent colony numbers for different conditions.](chart.png)
Biosynthesis and Metabolism of Estrogens in Man

- Androstenedione (AD) is metabolized by aromatase to Estrone (E1)
- Estrone (E1) is metabolized by 2-hydroxylase to 2-hydroxyestrone (20HE1)
- 2-hydroxyestrone (20HE1) is metabolized by COMT to 2-methoxyestrone (2MeOE1)
- Testosterone (T) is metabolized by aromatase to Estradiol (E2)
- Estradiol (E2) is metabolized by 16alpha-hydroxylase to 16alpha-hydroxyestrone (16OHE1)
- 16alpha-hydroxyestrone (16OHE1) is metabolized by 17beta-reductase to Estriol (E3)
Table 2
Effect of Lycium Barbarum Bark (LBB) Extract on Metabolism of 17β-estradiol (E₂) in MCF-7 Cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>E₂ Metabolite a, b (ng / 10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E₁</td>
</tr>
<tr>
<td>E₂</td>
<td>20 nM</td>
<td>2.8±0.1⁺</td>
</tr>
<tr>
<td>E₂+LBB</td>
<td>0.05%</td>
<td>15.1±0.6⁺</td>
</tr>
</tbody>
</table>

One day old cultures treated with E₂ alone or E₂+LBB. E₂ metabolites determined after a 48hr. treatment.

b Means ± SD, N=3 per treatment group. c-d p=0.004, e-f, i-j p=0.02, g-h p=0.04.
Table 3
Effect of Lycium barbarum Fruit (LBF) Extract on Metabolism of 17β-estradiol (E_2) in MCF-7 Cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>E_2 Metabolite a, b (ng / 10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E_1</td>
</tr>
<tr>
<td>E_2</td>
<td>20 nM</td>
<td>2.6±0.1 c</td>
</tr>
<tr>
<td>E_2 + LBF</td>
<td>1.0%</td>
<td>8.3±0.4 d</td>
</tr>
</tbody>
</table>

a One day old cultures treated with E_2 alone or E_2+LBF. E_2 metabolites determined after a 48 hr. treatment.
b Means ± SD, N=3 per treatment group. c-d, e-f p=0.04, g-h p=0.01.
Figure 3A
Modulation of 2-OHE$_1$:16α-OHE$_1$ Ratio by LBB and LBF Extracts in MCF-7 Cells

2-OHE$_1$:16α-OHE$_1$ Ratio

<table>
<thead>
<tr>
<th></th>
<th>E$_2$</th>
<th>LBB</th>
<th>E$_2$</th>
<th>LBF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20 nM</td>
<td>0.05%</td>
<td>20 nM</td>
<td>1.0%</td>
</tr>
</tbody>
</table>
Figure 3B
Modulation of E$_3$:16α-OHE$_1$ Ratio by LBB and LBF Extracts in MCF-7 Cells

E$_3$:16α-OHE$_1$ Ratio

<table>
<thead>
<tr>
<th></th>
<th>E$_2$ 20 nM</th>
<th>LBB 0.05%</th>
<th>E$_2$ 20 nM</th>
<th>LBF 1.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>0.8</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td>0.7</td>
<td></td>
</tr>
</tbody>
</table>
Study Outcome - I

- MCF-7 cells in a serum-depleted medium maintained their response to E₂ as evidenced by a 20.9 % decrease in population doubling time, a 67.9 % increase in saturation density and a 113.4 % increase in the number of anchorage independent colonies.

- Non-fractionated aqueous extracts of LBB and LBF inhibited E₂ induced growth. LBB relative to LBF exhibited a 19 fold greater potency for cytostatic growth arrest.

- At their respective maximum cytostatic concentrations LBB (0.05%) and LBF (1.0%) produced a 93.2% and an 89.2% reduction in the number of anchorage independent colonies.
Study Outcome - II

- LBB produced a **6.8 fold increase** in 2-OHE$_1$ formation, a **40% decrease** in 16α-OHE$_1$ formation, and a **3.7 fold increase** in E$_3$ formation.

- LBF produced a **3.9 fold increase** in 2-OHE$_1$ formation, a **33% decrease** in 16α-OHE$_1$ formation, and a **10.5 fold increase** in E$_3$ formation.

- LBB produced a **16.3 fold increase** in 2-OHE$_1$:16α-OHE$_1$ ratio, while LBF produced a **6 fold increase** in this endocrine biomarker.

- LBB produced a **2 fold increase** in E$_3$:16α-OHE$_1$ ratio, while LBF produced a **2.9 fold increase** in this endocrine biomarker.
Study Conclusions

• MCF-7 cells adapted for growth in chemically defined serum depleted culture medium retain their responsiveness to E₂.

• Non-fractionated aqueous extracts from LBB and LBF at their respective maximum cytostatic concentrations exhibit distinct differences in anchorage independent colony formation, cellular metabolism of E₂, and E₂ metabolite ratios.

• Higher growth inhibitory potency of LBB compared to that of LBF, functioning via distinct modes of action, offers proof of concept for a strategy to combine the two extracts for more effective prevention regimen against hormone responsive breast cancer.
Acknowledgements

Major funding for this study was provided by philanthropic contributions to the American Foundation for Chinese Medicine by

• Peter Cheney
• Suzanne Hoyt
• The family of Daniel and Kathleen Mezzalingua
• The family of Hakan and Marie Ledin
• The Issac and Laura Perlmutter Fund.
This research is dedicated to the memory of Laurie Mezzalingua (1968-2009). Laurie fought gallantly against her breast cancer from 1993 to July 4, 2009. During that period, she also selflessly and generously devoted herself to helping many others suffering from breast cancer.
References