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

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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 E$_2$ metabolites,

  $2\text{-OHE}_1:16\alpha\text{-OHE}_1$ and $E_3: 16\alpha\text{-OHE}_1+E_3$ 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</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Doubling (hr)</td>
</tr>
<tr>
<td>Serum</td>
<td>0.7%</td>
<td>34.4</td>
</tr>
<tr>
<td>E_2</td>
<td>20 nM</td>
<td>27.2</td>
</tr>
</tbody>
</table>

^a Means ± SD, N=6 per treatment group. ^c-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 (x10^5) at Day 7 post-seeding

<table>
<thead>
<tr>
<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

<table>
<thead>
<tr>
<th></th>
<th>Viable Cell number (x10^5) at Day Post-seeding</th>
<th>LBF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISD</td>
<td>E2 20 nM</td>
<td>0.1</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>Serum</th>
<th>$E_2$</th>
<th>LBB</th>
<th>LBF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7%</td>
<td>20 nM</td>
<td>0.05%</td>
<td>1.0%</td>
</tr>
</tbody>
</table>
Biosynthesis and Metabolism of Estrogens in Man

Androstenedione (AD) → Estrone (E₁) → 2-hydroxyestrone (2OH-E₁) → 2-methoxyestrone (2MeOE₁)

Testosterone (T) → Estradiol (E₂) → 16α-hydroxyestrone (16OH-E₁) → Estriol (E₃)

Aromatase -1%
2-hydroxylation -35%
COMT CH₃O
17β-oxidase -88%
17β-reductase -88%
16α-hydroxylase -100%
### Table 2
Effect of Lycium Barbarum Bark (LBB) Extract on Metabolism of 17ß-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>
<th>E_1</th>
<th>2-OHE_1 16α-</th>
</tr>
</thead>
<tbody>
<tr>
<td>OHE_1 E_3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E_2</td>
<td>20 nM</td>
<td>2.8±0.1 c</td>
<td>0.4±0.1 e</td>
<td>1.0±0.1 g</td>
</tr>
<tr>
<td>E_2+LBB</td>
<td>0.05%</td>
<td>15.1±0.6 d</td>
<td>3.1±0.2 f</td>
<td>0.6±0.1 h</td>
</tr>
</tbody>
</table>

One day old cultures treated with E_2 alone or E_2+LBB. E_2 metabolites determined after a 48hr. treatment.
Table 3
Effect of Lycium barbarum Fruit (LBF) 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  2-OHE₁  16α-OHE₁  E₃</td>
</tr>
<tr>
<td>E₂</td>
<td>20 nM</td>
<td>2.6±0.1 c  1.1±0.6 e  2.7±0.8</td>
</tr>
<tr>
<td>E₂ + LBF</td>
<td>1.0%</td>
<td>8.3±0.4 d  5.4±0.5 f  1.8±0.2</td>
</tr>
</tbody>
</table>

a One day old cultures treated with E₂ alone or E₂+LBF. E₂ 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

<table>
<thead>
<tr>
<th>E$_2$</th>
<th>LBB</th>
<th>E$_2$</th>
<th>LBF</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 nM</td>
<td>0.05%</td>
<td>20 nM</td>
<td>1.0%</td>
</tr>
</tbody>
</table>
Figure 3B
Modulation of $E_3:16\alpha$-OHE$_1$ Ratio by LBB and LBF Extracts in MCF-7 Cells

$E_3:16\alpha$-OHE$_1$ Ratio

<table>
<thead>
<tr>
<th></th>
<th>LBB</th>
<th></th>
<th></th>
<th>LBF</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_2$</td>
<td></td>
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<tr>
<td>20 nM</td>
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<td>1.0%</td>
<td></td>
</tr>
</tbody>
</table>
Study Outcome - I

- MCF-7 cells in a serum-depleted medium maintained their response to $E_2$ 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 $E_2$ 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-OHE₁ formation, a **40% decrease** in 16α-OHE₁ formation, and a **3.7 fold increase** in E₃ formation.

- LBF produced a **3.9 fold increase** in 2-OHE₁ formation, a **33% decrease** in 16α-OHE₁ formation, and a **10.5 fold increase** in E₃ formation.

- LBB produced a **16.3 fold increase** in 2-OHE₁:16α-OHE₁ ratio, while LBF produced a **6 fold increase** in this endocrine biomarker.

- LBB produced a **2 fold increase** in E₃:16α-OHE₁ 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 E$_2$.

• 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 E$_2$, and E$_2$ 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

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• Peter Cheney
• Suzanne Hoyt
• The family of Daniel and Kathleen Mezzalingua
• The family of Hakan and Marie Ledin
• The Issac and Laura Perlmutter Fund.
Dedication

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