Efficacy of Dipsacus asperoides (DA) in a Model for Triple Negative Breast Cancer

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Abstract

**Background:** The triple negative breast cancer (TNBC) is characterized by the absence of estrogen receptor-α (ER-α), Progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2). This molecular subtype responds only to conventional cytotoxic chemotherapy and to small molecule based targeted therapy. These therapeutic options are associated with long-term systemic toxicity and acquired tumor resistance leading to compromised efficacy. These limitations emphasize identification of efficacious non-toxic agents for secondary prevention/therapy of TNBC. *Dipsacus asperoides* (DA) is a Chinese herb of a nutritional nature. The root of this herb represents an ingredient that is commonly included in the traditional Chinese herbal formulations for health management purposes. The present study examines the growth inhibitory effects of DA and identifies possible mechanistic leads for their efficacy in a preclinical cell culture model for TNBC.

**Experimental Model, Herbal Extract and Biomarkers:** The human mammary carcinoma derived ER-α - , PR - and HER-2 - MDA-MB-231 cell line represents the model for TNBC. Non-fractionated aqueous extract from DA represents the test agent. Anchorage dependent growth, anchorage independent (AI) colony formation, cell cycle progression and relevant pathway specific mechanistic assays represent the quantitative biomarkers for efficacy.

**Results:** Treatment of MDA-MB-231 cells with DA extract induces a dose dependent cytostatic growth arrest (IC\(_{50}\): 0.0015%, IC\(_{90}\): 0.0030%), and strongly inhibits AI colony formation. Within the cytostatic concentration range, DA treatment inhibits cell cycle progression via progressive G2/M arrest, suggesting abrogation of the G2/M checkpoint affecting DNA damage repair pathway. The induction of cellular apoptosis by DA is indicated by a dose dependent up-regulation of pro-apoptotic Caspase 3/7 activity.

**Conclusion:** These data identify potential mechanistic leads for the efficacy of DA in the present model. The present study validates a mechanism based approach to prioritize efficacious non-toxic herbal extracts for secondary prevention/therapy of the TNBC molecular subtype of clinical breast cancer.
Study Rationale - I

• The triple negative breast cancer (TNBC) lacks the expression of estrogen receptor-α (ER-α), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2) [1].

• The treatment options for TNBC include conventional Anthracyclin/Taxol based chemotherapy and PARP, PI3K and mTOR pathway small molecule inhibitor based targeted therapy [2, 3].

• Current treatment options are associated with long-term systemic toxicity and acquired tumor resistance, compromising therapeutic efficacy [4, 5].

• Non-toxic natural phytochemicals and herbal extracts may represent testable therapeutic alternatives for clinical TNBC [6].
• Nutritional herbs are commonly used in traditional Chinese herbal medicine for health management purposes. Dipsacus asperoides (DA) is an example of such an herb [7, 8].

• Non-fractionated aqueous extracts from several mechanistically distinct nutritional herbs have documented growth inhibitory efficacy in a model for Luminal A molecular subtype of clinical breast cancer [9-12].

• Extracts from select nutritional herbs have exhibited growth inhibitory effects on a model for clinical TNBC [13-15].
Study Objectives

• To examine the growth inhibitory effects of *Dipsacus asperoides* (DA) on triple negative MDA-MB-231 cells.

• To identify mechanistic leads and potential molecular targets for the efficacy of DA.
### Status of Homeostatic Growth Control in MDA-MB-231 Cells

<table>
<thead>
<tr>
<th>End point</th>
<th>Non-tumorigenic</th>
<th>Tumorigenic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>184-B5</td>
<td>MDA-MB-231</td>
</tr>
<tr>
<td>Population doubling (hr.)</td>
<td>34</td>
<td>15</td>
</tr>
<tr>
<td>Saturation Density (x10^5)</td>
<td>22.3±1.2</td>
<td>32.9±2.3</td>
</tr>
<tr>
<td>G(_1): S+G(_2)/M Ratio</td>
<td>2.3±0.4</td>
<td>0.6±0.3</td>
</tr>
<tr>
<td>S+G(_2)/M: Sub G(_0) Ratio</td>
<td>1.6±0.3</td>
<td>16.8±3.2</td>
</tr>
</tbody>
</table>
Dose Response of Dipsacus asperoides (DA)
in MDA-MB-231 Cells

Control
DA (%)
0.0002                 0.001                 0.002                   0.004                  0.005

IC$_{50}$: 0.0015%; IC$_{90}$: 0.003%

% Cell Viability
**Effect of Dipsacus asperoides (DA) on Anchorage Independent Growth in MDA-MB-231 Cells**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (%)</th>
<th>Anchorage independent Colonies b</th>
<th>Inhibition (% Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>------</td>
<td>325±78</td>
<td>-----</td>
</tr>
<tr>
<td>DA</td>
<td>0.00025</td>
<td>240±74</td>
<td>26.1</td>
</tr>
<tr>
<td></td>
<td>0.0015</td>
<td>139±82</td>
<td>57.2</td>
</tr>
<tr>
<td></td>
<td>0.0030</td>
<td>27±12</td>
<td>91.7</td>
</tr>
</tbody>
</table>

a Non-fractionated aqueous extract. b Colony counts at day 21 post-seeding. Mean ± SD, N=3 per treatment group.
Effect of Dipsacus asperoides (DA) on Cell Cycle Progression in MDA-MB-231 Cells

% G1 and G2/M Phase

<table>
<thead>
<tr>
<th></th>
<th>G1</th>
<th>G2/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.00025</td>
<td></td>
</tr>
<tr>
<td>DA (%)</td>
<td>0.0015</td>
<td>0.0030</td>
</tr>
</tbody>
</table>

The diagram shows the percentage of cells in G1 and G2/M phases for the control and different concentrations of DA.
Effect of Dipsacus asperoides (DA) on Caspase 3/7 Activity in MDA-MB-231 Cells

Relative Luminescent Units (RLU)

<table>
<thead>
<tr>
<th>Control</th>
<th>DA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001</td>
<td>0.00025</td>
</tr>
<tr>
<td>0.0015</td>
<td>0.0030</td>
</tr>
</tbody>
</table>
Effect of Dipsacus asperoides (DA) on RAF-ERK-MEK and RB Signaling Pathways in MDA-MB-231 Cells
Study Outcome - I

Dose Response of Dipsacus asperoides (DA)

- Non-fractionated aqueous extract from the root of DA exhibits a dose dependent cytostatic growth arrest in triple negative MDA-MB-231 cells (IC$_{50}$: 0.0015%, IC$_{90}$: 0.0030%).

- In response to treatment with DA within the cytostatic concentration range, MDA-MB-231 cells exhibit reduction in the number of anchorage independent colonies in a dose dependent manner.
Effect of Dipsacus asperoides (DA) on cell cycle progression and apoptosis

- Treatment with DA within the cytostatic concentration range inhibits cell cycle progression via a G$_2$/M phase arrest of the cells.

- DA induces p21 mediated cellular apoptosis

- Induction of cellular apoptosis is also indicated by a progressive dose dependent increase in the pro-apoptotic Caspase 3/7 activity.
Study Outcome-III

• DA down-regulates the BRAF/ERK/MEK/pathway proteins.

• DA Inhibits the RB signaling pathway via modulation of p21 mediated regulation of CDK4/6 complex and phosphorylation of RB.
Study Conclusions

• Dipsacus asperoides (DA) induces cytostatic growth arrest and inhibits anchorage independent colony formation in the triple negative breast cancer (TNBC) model.

• DA inhibits cell cycle progression and induces cellular apoptosis via G2/M arrest and upregulation of p21 mediated pro-apoptotic Caspase 3/7 activity.

• DA down-regulates the RAS/RAF/ERK/MEK and RB pathways.

• This study validates a mechanistic approach to prioritize efficacious herbal extracts for secondary prevention/therapy of clinical TNBC.
Acknowledgements

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