

Investigations into the effect of Black Cohosh extracts on the growth of the MCF-7 breast cancer cell line

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Biology Capstone
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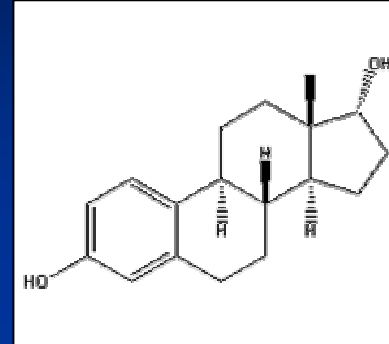
Overview

- Welcome
- Background and Introduction
- Materials and Methods
- Results
- Discussion
- Future Research
- Acknowledgments
- Questions

Background

- Estrogen

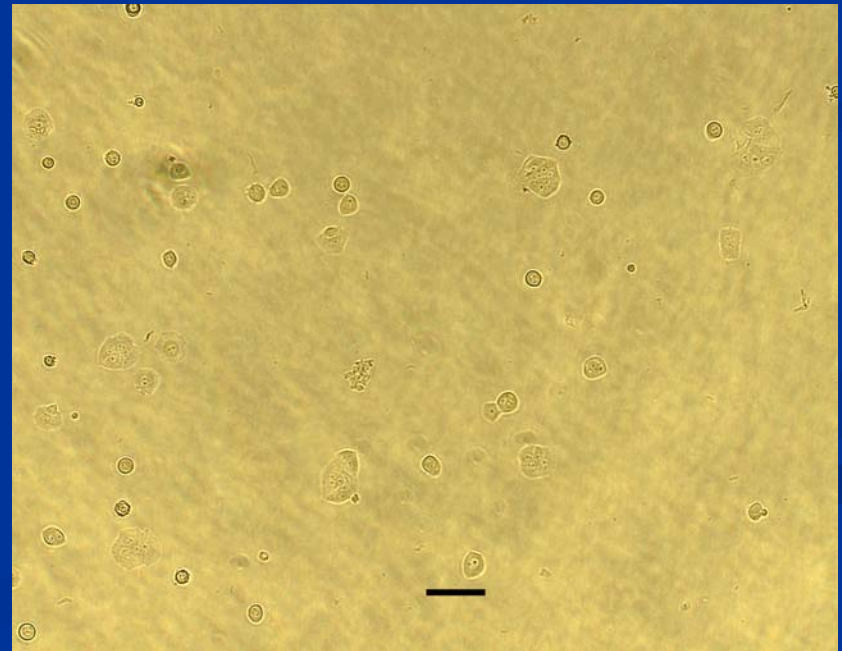
- MCF-7



<http://chemfinder.cambridgesoft.com/result.asp?molid=517-04-4>

- Phytoestrogens

- Definition
- Actions
- Mechanisms



Anna Bock, 2004

Background (cont'd)

- Black Cohosh Root
 - Uses
 - Daily Intake
 - 20mg vs. 525mg
 - Research



<http://www.catskillginseng.com/html/native.htm>

Why did I choose to research this herbal supplement?



<http://www.walgreens.com/store/product.jhtml?CATID=100163&PRODID=352371>

Original Hypothesis

- Black Cohosh Root contains phytoestrogens;
- Phytoestrogens exhibit estrogenic behavior;
- Therefore, Black Cohosh Root should have an effect on the growth of an estrogen-receptor positive (ERP) cancer.

Initial Research

- Dietary intake of phytoestrogens has been associated with:
 - reduced incidence of cancer
 - reduced risk of cardiovascular disease and osteoporosis
- Some show anti-estrogenic properties
- Some show estrogenic activity
- Some have no estrogenic effects

Materials and Methods

■ MCF-7

- RPMI 1640, L-glutamine, 8% FBS, 1% penn/strep
- 37°C and 5% CO₂
- 25cm² cell culture flasks
- Tests performed in phenol-red free DMEM



http://www.aofoundation.org/ari/research/interface/Zetos/zetos_advantages.shtml



Materials and Methods (cont'd)

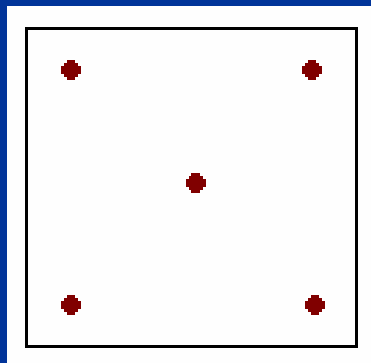
- Growth Curves
 - counted each day for 7 days

- Cell Counts
 - Hemacytometer vs. photography
 - Incomplete harvests in past

Materials and Methods (cont'd)

■ Photography

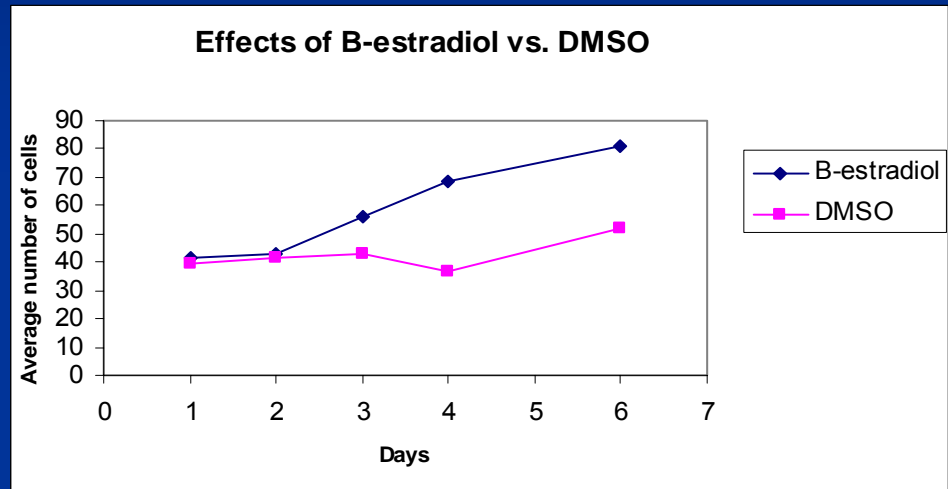
- Olympus camera on inverted microscope
- 100x magnification
- averaged counts for 5 fields of view per flask



Materials and Methods (cont'd)

Initial Tests

- β -estradiol vs. DMSO
 - Testing methods
- Standards:
 - β -estradiol,
 - Tamoxifen,
 - β -estradiol: tamoxifen mixture,
 - DMSO



* Results showed that methods used produce reasonable results

New Pieces to the Puzzle:

■ Past Research

- Solvents: Isopropanol, Ethanol, Methanol, Isopropanolic/aqueous combo.

■ Remifemin

- Past identification of formononetin
 - No longer found in Remifemin!
- Originally made from isopropanolic extract
- Now produced from ethanolic extract

Materials and Methods (cont'd)

- Extraction Method
 - 100 mg combined with 20 mL solvent
 - Redux for 45 minutes
 - Cool
 - Rotovap to remove solvent

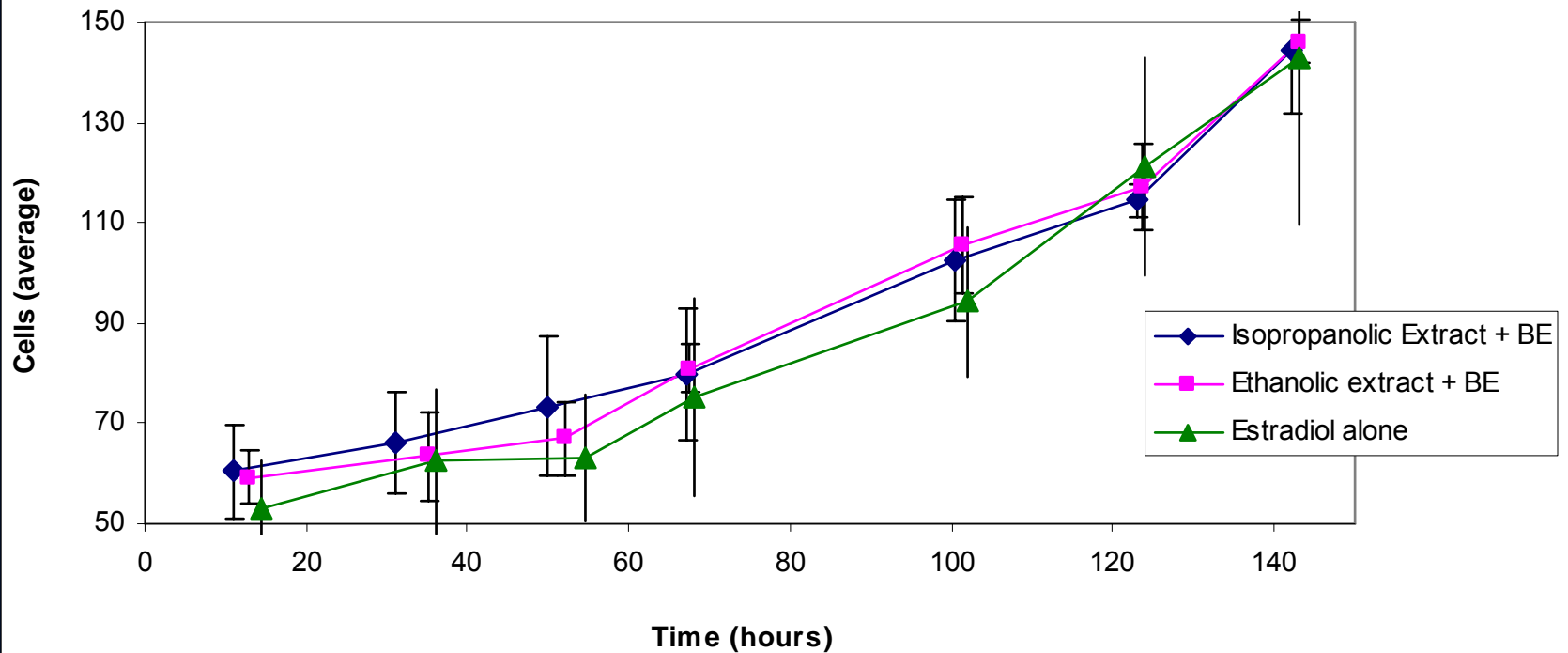
- Retrieved solid was dissolved in DMSO

Materials and Methods (cont'd)

- Growth Curve #3:
 - Isopropanolic extract (15 μ g/mL) plus β -estradiol
 - Ethanolic extract (15 μ g/mL) plus β -estradiol
 - β -estradiol (100pM)
- Antiestrogenic properties?
- If so, presence of extract would decrease growth rate as compared to β -estradiol

Results

Effects of ethanolic and isopropanolic extracts of Black Cohosh with Beta-estradiol on growth of MCF-7



Results (cont'd)

- 3 test conditions show nearly identical growth rates
- B-E curve similar to previous B-E curve
- $R^2 > 0.93$
- Slopes: 0.5 to 0.6
- T-test shows no significant difference in data sets

Discussion of Results

- Neither extract exhibits anti-estrogenic behavior in the presence of 100 pM B-estradiol
- B-estradiol concentrations
- Black Cohosh concentrations

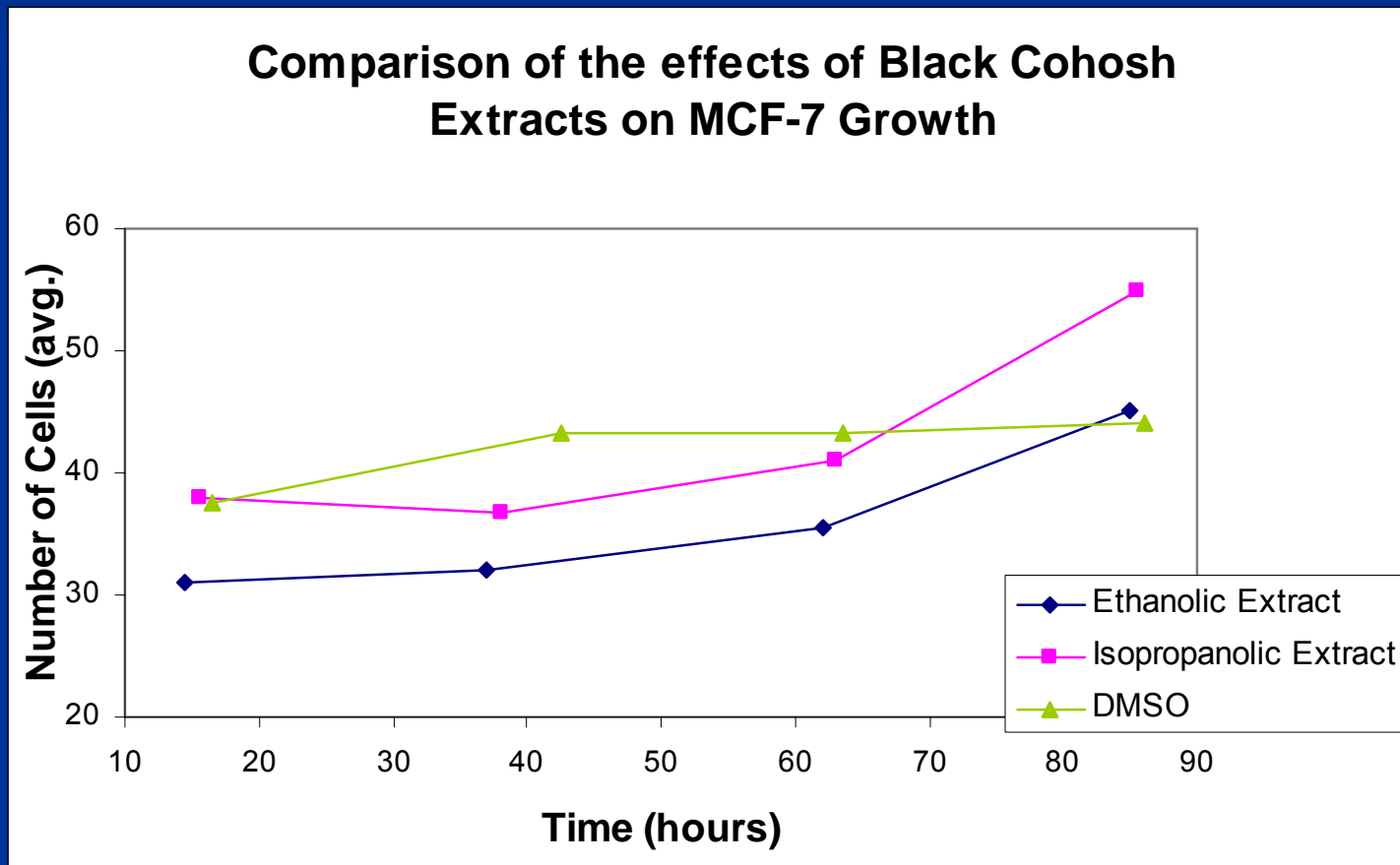
525mg tablets BID = 1050mg/day

Avg. woman = 160 pounds = 72kg

Equivalent to 15ug/g (or mL of cell culture medium)

More Research In Progress:

- Effects of Black Cohosh Extracts alone



Future Research

Black Cohosh:

- Induces growth of MCF-7 in absence of estradiol?
- Estrogenic mechanism or other pathways?
 - Tamoxifen

Phytoestrogens:

- Relevant concentrations
- Different actions by different categories?
 - Coumestins, lignans, and isoflavones*

Future Research (cont'd)

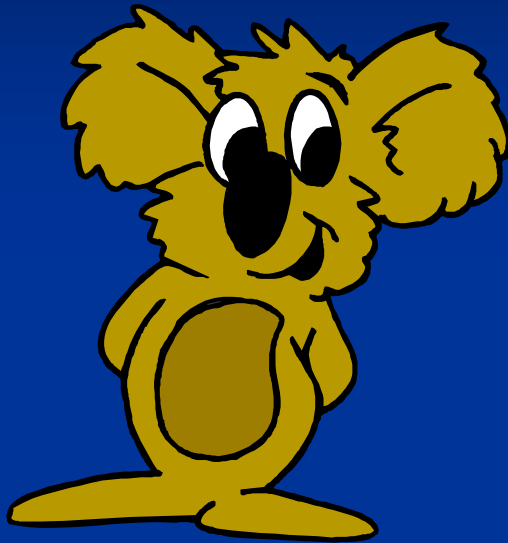
Photography:

- Accuracy?
- Increase time efficiency
- Improving image quality and accuracy

Future Research (cont'd)

- TONS of questions left to answer
- Hopefully to be carried on by future capstone students

Acknowledgements



- Dr. Brown
- Racheal Desmone
- Dr. Spilatro and the capstone class

(esp. Beth, Dominika, Racheal)

- My mom

Questions

