What is the Evidence for Supplement Use for Menopausal Symptoms?

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Global Health Challenge: Ageing

- By 2025 >800 m >65 yrs
- Two-thirds in developing countries
- Population ageing has immense health consequences for all countries, including the USA
- Healthcare goals:
  - Prevent and postpone disease and disability
  - Maintain the health, independence and mobility of our ageing population
Gender and Ageing

- Women make up the majority of the older populations in all countries
- Average age 80 yrs old
- Live an average of eight extra years
  - Generally have more health problems than men
  - Increase in chronic diseases after menopause
  - After the age of 55, 1 in 5 live in disability
Menopause

- Y2000-80 million women entering menopause
  - 1990 467 million postmenopause
  - 2030 1.2 billion postmenopause
  - 55-75% of women experience vasomotor symptoms or a # of other sequelae
  - depression, mood, sleep disorders, vaginal dryness, joint pain
- 25% of women seek treatment from their provider
Increase in Chronic Disease Postmenopause

- Cardiovascular disease-CHD and stroke-60% of all adult female deaths
  - Risk increases after menopause
    - 1 in 50 (45-64 years)
    - 1 in 3 (> 65 years)

- Osteoporosis and associated fractures are major causes of death, illness and disability
  - Bone loss accelerates after menopause- 30% PM
  - 80% of hip fractures in women

- Dementia/Alzheimer’s Disease
  - May be accelerated with the onset on menopause
  - Clinical studies suggest improvement in cognition with ERT as well as ERT and Tacrine combinations

- Lower urogenital dysfunction
  - Polyuria, nocturia, urgency, increased incidence of UTI’s, incontinence
BDS Use in Menopausal Women

79% of women surveyed used one or more BDS, diverse ethnicity

Soy, green tea, ginkgo, ginseng, echinacea

Most women thought BDS were relatively safe and effective

The majority of respondents were not getting their BDS information from healthcare providers

Not informing their physician about their BDS use. (70%) - soy
Black Cohosh (*Cimicifuga racemosa*)

- Native American plant used by native American Indians - *squawroot*
  - Root/Rhizome
  - Distribution - Eastern U.S.
  - *Ranunculaceae* (Buttercup)
- Synonyms: *Bugbane* (to drive away bugs), *Black Snakeroot*, *Rattleroot*, *Squaw-weed*, *Actaea racemosa*, *Macrotyis actaeoides*
- U.S.P-1820-1936, N.F. 1936
- *Pinkham’s Vegetable Compound*
BLACK COHOSH

- Treatment of menopausal symptoms such as anxiety, depression, hot flushes, profuse sweating, insomnia and vaginal atrophy
- 11 clinical trials - 7 controlled, 4 open, some double-blinded, randomized
  - 40-60% alcohol extracts of the rhizome-40-80 mg of the extract daily
- Reduced vasomotor symptoms, Kupperman index, HAMA scale reduced, in some reduces LH levels,
  - as effective as 0.625 mg of conjugated estrogens
Jacobson JS et al., 2001

- Patients: 85(68)-breast cancer diagnosis (59 taking tamoxifen)
- Design: Randomized, Placebo-controlled, double-blind
- Efficacy Criteria: hot flashes, FSH, LH
- Dose: 40% Isopropanol extract, 40 mg daily
- Outcomes: Improvement of symptoms but not significant as compared with placebo
- Problems-
Tamoxifen versus Black cohosh

- Tamoxifen-mechanism of action not completely elucidated
  - Partial agonist/antagonist
  - down regulates ER expression
  - Brain-estrogen is a serotonin agonist up-regulates SERT and 5HTR
  - Serotonergic mechanism-LHRH secretion-LH release
  - Tamoxifen-estrogen-serotonin antagonist-SERT and 5HTR

- Black cohosh-mechanism has not been completely elucidated
  - Estrogenic effects????
  - Serotonergic mechanism of action
Liske E et al., 2001

- RCT (n=150) peri and post menopausal women
- Design: Randomized, double-blind
- Dose: 40% Isopropanol extract, Remifemin
- Compared 40 mg of BC with 127 mg/day for 24 weeks
- Efficacy Criteria: KMI symptoms, vaginal cytology, hormone levels
- Outcomes: 70% and 72%, Improvement of KMI and SDS, no estrogenic effects (NO PLACEBO)
- 40 mg/day dosing
- *J Women’s Health and Gender Based Medicine, 2002, 11:163-173
Is Black Cohosh Estrogenic?

- 1985-1995-in vitro and in vivo-Jarry et al
- Formononetin-CHCL3
- Adulteration of plant material
- In vivo study
  - ovx rats, 3wks, extract not described, uterine wt
- 1999 In vitro-ERalpha upregulation in MCF 7
  - Jarry et al.

- No formonentin
- No ER binding
- No estrogenic effects in mice
- Bodinet 2002-Breast Can Res & Treat-ER+breast cancer cell lines-inhibited proliferation
- Freudenstein 2002 Cancer Res-mammary tumor if rats, no stimulation of tumor proliferation
## Competitive Binding of Extracts to Estrogen Receptors (ER) and Their Estrogenic Activity in Ishikawa Cells

<table>
<thead>
<tr>
<th>Methanol Extracts</th>
<th>200 µg/ml (%)</th>
<th>20 µg/ml (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ER&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ER&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Estrogenic</td>
<td>Antiestrogenic</td>
</tr>
<tr>
<td>Angelica sinensis (Angelica)</td>
<td>27</td>
<td>30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cimicifuga racemosa (Black cohosh)</td>
<td>19</td>
<td>16</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycyrrhiza glabra (Licorice)</td>
<td>33</td>
<td>29</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Humulus lupulus (Hops)</td>
<td>70</td>
<td>79</td>
<td>25</td>
<td>42</td>
</tr>
<tr>
<td>Panax ginseng (Ginseng)</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Panax ginseng (White ginseng)</td>
<td>8</td>
<td>18</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Panax quinquefolius (Am. ginseng)</td>
<td>5</td>
<td>11</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Trifolium pratense (Red clover)</td>
<td>73</td>
<td>74</td>
<td>56</td>
<td>-</td>
</tr>
<tr>
<td>Vitex agnus-castus (Chaste berry)</td>
<td>57</td>
<td>67</td>
<td>40</td>
<td>-</td>
</tr>
</tbody>
</table>

* Shows cytotoxicity at the concentration tested. J. Agric Food Chem, 2001, 49:2472-2477
# INDUCTION OF PS2 EXPRESSION BY PLANT EXTRACTS

<table>
<thead>
<tr>
<th>Agents/Extracts</th>
<th>Expression of PS 2 Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S30</td>
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<tr>
<td>Estrodiol (1 nM)</td>
<td>++++</td>
</tr>
<tr>
<td>DMSO (1 µL)</td>
<td>+</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Genistein (1 µM)</td>
<td>++++</td>
</tr>
<tr>
<td><em>Angelica sinensis</em> (20 µg)</td>
<td></td>
</tr>
<tr>
<td><em>Cimifuga racemosa</em> (20 µg)</td>
<td></td>
</tr>
<tr>
<td><em>Humulus lupulus</em> (20 µg)</td>
<td>+++</td>
</tr>
<tr>
<td><em>Trifolium repens</em> (20 µg)</td>
<td>+++</td>
</tr>
<tr>
<td><em>Vitex agnus castus</em> (20 µg)</td>
<td>+++</td>
</tr>
<tr>
<td><em>Panax ginseng</em> (20 µg)</td>
<td>+++</td>
</tr>
<tr>
<td><em>Panax ginseng</em> (white) (20 µg)</td>
<td>+++</td>
</tr>
<tr>
<td><em>Glycyrrhiza glabra</em> (20 µg)</td>
<td></td>
</tr>
<tr>
<td><em>Trifolium pratense</em> (20 µg)</td>
<td>++</td>
</tr>
</tbody>
</table>
DPPH Free Radical Scavenging Ability and Inhibition of Xanthine/Xanththine Oxidase Activity of Extracts and Fractions

<table>
<thead>
<tr>
<th>EXTRACTS</th>
<th>DPPH (%)</th>
<th>OXIDASE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200 µg/ml (IC$_{50}$)</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>Angelica sinensis (Angelica)</td>
<td>36</td>
<td>-</td>
</tr>
<tr>
<td>Cimicifuga racemosa</td>
<td>79 (99)</td>
<td>-</td>
</tr>
<tr>
<td>Glycyrrhiza glabra (Licorice)</td>
<td>53</td>
<td>-</td>
</tr>
<tr>
<td>Humulus lupulus (Hops)</td>
<td>49</td>
<td>-</td>
</tr>
<tr>
<td>Panax ginseng</td>
<td>26</td>
<td>-</td>
</tr>
<tr>
<td>Panax quinquefolius</td>
<td>26</td>
<td>-</td>
</tr>
<tr>
<td>Trifolium pratense (Red clover)</td>
<td>74 (99)</td>
<td>-</td>
</tr>
<tr>
<td>Vitex agnus-castus (Chaste berry)</td>
<td>51</td>
<td>-</td>
</tr>
</tbody>
</table>
Prevention of Osteoporosis

- Increase in serum calcium and bone mineral density in ovx rats (Li, 1996 -EtOAc fraction from a methanol extract of the rhizome)

- Anti-osteoporosis effects (Li, 1995, MeOH ext.); Li 1996 (EtOAc fraction from MeOH extract)
“Each Tablet Contains:
20 mg extract
Dose: 40-80 mg/day
“One-two tablets twice a day.”
Red Clover (*Trifolium pratense*)

- Symptoms of menopause
- Standardized extract-200-230 mg containing 40 mg of four isoflavones (biochanin A, formononetin, daidzein and genistein 20:12:1:1)

- Four small RCTs
  - Two R, DB, PC trials - 40 -160 mg/day for 3 months
  - no effect on menopausal symptoms (2)
  - R, DB, PC trial (n=30), 80 mg/d for 12 weeks - 44% decrease in hot flushes (1)

Red Clover

One uncontrolled trial (n=46) women, 28.5 mg, 57 mg or 85.5 mg/d for 6 months
- Increased HDL-C; increased bone mineral density with the higher doses
  - Menopause 2001, 8:259-265

R, DB study in 66 women with hypercholesterolemia-12 week
- 50 mg isoflavones did not affect total plasma cholesterol, LDL, HDL or triglycerides

80 mg/day for 10 weeks (n=17) improved arterial compliance by 28%, a risk factor for CVD (1)
## Competitive Binding of Red Clover Extracts and Fractions to Estrogen Receptors (ER) and Their Estrogenic Activity in Ishikawa Cells

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<tr>
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<th>20 µg/ml (%)</th>
<th>Estrogenic (IC$_{50}$)</th>
<th>Antiestrogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ER$^\alpha$</td>
<td>ER$^\beta$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP-MeOH</td>
<td>78</td>
<td>72</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>TP-MeOH/H$_2$O 5:5</td>
<td>84</td>
<td>99</td>
<td>94</td>
<td>-</td>
</tr>
<tr>
<td>TP-EtOH/H$_2$O 5:5</td>
<td>89</td>
<td>98</td>
<td>65</td>
<td>-</td>
</tr>
<tr>
<td>TP-PE</td>
<td>61</td>
<td>77</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>TP - CHCl$_3$</td>
<td>83</td>
<td>93</td>
<td>33 (2.6 ± 0.14)</td>
<td>70 (17 ± 2.0) *</td>
</tr>
<tr>
<td>TP – BuOH</td>
<td>28</td>
<td>34</td>
<td>77 (8.0 ± 1.4)</td>
<td>-</td>
</tr>
<tr>
<td>TP - H$_2$O</td>
<td>7</td>
<td>0</td>
<td>8</td>
<td>-</td>
</tr>
</tbody>
</table>

* Shows cytotoxicity at the concentration tested.
Estrogenic activities of Isoflavones Present in Red Clover and Their Concentration in Extracts and Fractions

<table>
<thead>
<tr>
<th></th>
<th>EC$_{50}$ (µM)</th>
<th>ER$^\alpha$ (µM)</th>
<th>ER$^\beta$ (µM)</th>
<th>E (µM)</th>
<th>Concentration (% w/w)</th>
<th>M</th>
<th>M/H</th>
<th>E/H</th>
<th>PE</th>
<th>CHCl$_3$</th>
<th>BuOH</th>
<th>H$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochanin A</td>
<td>8.1</td>
<td>2.8</td>
<td>5.12</td>
<td>0.04</td>
<td>0.12</td>
<td>0.08</td>
<td>2.77</td>
<td>5.73</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daidzein</td>
<td>5.5</td>
<td>1.0</td>
<td>1.24</td>
<td>0.16</td>
<td>0.05</td>
<td>0.02</td>
<td>0.27</td>
<td>0.62</td>
<td>3.21</td>
<td>0.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formononetin</td>
<td>104.5</td>
<td>59.7</td>
<td>8.32</td>
<td>0.06</td>
<td>0.22</td>
<td>0.14</td>
<td>0.66</td>
<td>10.4</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genistein</td>
<td>0.63</td>
<td>0.012</td>
<td>0.31</td>
<td>0.10</td>
<td>0.03</td>
<td>0.01</td>
<td>0.19</td>
<td>0.57</td>
<td>2.02</td>
<td>0.44</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

E - Estrogenic activity in Ishikawa cell test; M - MeOH; E - EtOH; H - H$_2$O.
Red Clover-Estrogenic?

- Isoflavones-0-15% depends on the extract
- 9-15% extract binds to the ER
- Estrogenic effects in rodents
  - Increased uterine weights
  - Vaginal cornification
- DB, R, PC study (n=30) 50 mg/d, 3 months
  - No effect on the endometrium
  - However, may need at least 80 mg/d for pharmacological effects
This work was supported by a grant from

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National Institutes of Health
U.S. Department of Health and Human Services
www.nccam.nih.gov

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