The Toxicity and Pathology of Dietary Herbals, Botanicals & Supplements

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National Toxicology Program (NTP)

Society of Toxicologic Pathology
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Presentation Outline

I. Herbal medicine use in the U. S.

II. NTP 2-Year cancer studies of herbal medicines
   - Herbal medicine studies with carcinogenic activity
   - Herbal medicine without clear evidence of carcinogenic activity

III. NTP Studies of Cardiotoxicity
    - Ephedrine/Caffeine Studies

IV. NTP Herbal medicine studies – treatment-related lesions (A. Nyska)
# I. Herbal Medicine Use in the U. S.

<table>
<thead>
<tr>
<th>Herb</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goldenseal</td>
<td>Skin disease, ulcers, colds, and other infections</td>
</tr>
<tr>
<td>Ginkgo biloba extract</td>
<td>Asthma, bronchitis, fatigue, memory loss</td>
</tr>
<tr>
<td>Kava kava</td>
<td>Anxiety, insomnia, menopausal symptoms</td>
</tr>
<tr>
<td>Aloe Vera whole leaf nondecolorized extract</td>
<td>In laxatives</td>
</tr>
<tr>
<td>Milk thistle extract</td>
<td>Lower cholesterol levels, Proposed anticancer agent</td>
</tr>
<tr>
<td>Tumeric Oleoresin</td>
<td>Proposed anticancer agent</td>
</tr>
<tr>
<td>Ginseng</td>
<td>Proposed anticancer agent</td>
</tr>
<tr>
<td>Ephedrine</td>
<td>In weight loss products</td>
</tr>
</tbody>
</table>
Herbal Medicines are Complex Mixtures

- Milk thistle – Flavolignan – silymarin, silidyanin, & silychristin
  - Inhibit CYP activity

- Curcumin – Major component in turmeric oleoresin
  - Inhibit CYP activity

- Ginseng – Gingeosides
  - Inhibit CYP activities

- Ginkgo – Terpenoids and flavonoids
  - Inhibit CYP activities

Chen et al., Current Medicinal Chemistry 2011; 18:3190
FDA Guidelines

- 1994 – Dietary Supplement Health and Education Act of 1994 (DSHEA) which defines the term "dietary supplement"
  - A dietary supplement
    - is ingested
    - supplements the diet
    - not represented as a conventional food or as a sole item of a meal or the diet, and contains a "dietary ingredient"
  - "dietary ingredients"
    - may include vitamins, minerals, herbs or other botanicals, amino acids, and dietary substances such as enzymes
    - also can be metabolites, constituents, extracts, concentrates, or combinations of the preceding types of ingredients
- DSHEA placed dietary supplements in a special category under the general umbrella of "foods," except where the product meets the drug definition
- http://www.fda.gov/NewsEvents/Testimony/ucm115163.htm
FDA Guidelines

- Under DSHEA, a dietary supplement is adulterated if, among other things, it or any of its ingredients presents "a significant or unreasonable risk of illness or injury" when used as directed on the label, or under normal conditions of use if there are no directions. FDA bears the burden of proof to show that a product or ingredient presents such a risk. In addition, the Secretary of Health and Human Services (HHS) has the authority to declare that a dietary supplement or dietary ingredient poses an "imminent hazard" to public health or safety.

- [http://www.fda.gov/NewsEvents/Testimony/ucm115163.htm](http://www.fda.gov/NewsEvents/Testimony/ucm115163.htm)
Center for Disease Control and Prevention
National Health and Nutrition Examination Survey

- The dietary supplements section provides personal interview data on the use of supplements and herb in the U. S.

Age-adjusted percent of adults who have used complementary and alternative medicine: United States, 2002

Note: CAM is complementary and alternative medicine.
Data Source: National Health Interview Survey, 2002.
II. NTP 2-Year Cancer Studies of Herbal Medicines

• Liver carcinogens
  – Goldenseal – rats and mice (TR 562)
  – Ginkgo biloba extract – mice (TR 578)
  – Kava kava extract – mice (TR 571)

• Intestinal carcinogen
  – Aloe vera whole leaf nondecolorized extract – rats (TR 577)
    (Noncolorized whole leaf extract Aloe barbadensis Miller)

• No or equivocal evidence for carcinogenic activity
  – Milk thistle extract – rats and mice (TR 565)
  – Tumeric oleoresin – rats and mice (TR 427)
  – Ginseng – rats and mice (TR 567)
II. NTP 2-Year Cancer Studies of Herbal Medicines

- **Liver carcinogens**
  - Goldenseal – J. Dunnick & J. Peckham, NIEHS/NTP
  - Ginkgo biloba extract – C. Rider, P. Chan, A. Nyska, NIEHS/NTP
  - Kava kava extract – M. Behl, P. Chan, A. Nyska, NIEHS/NTP

- **Intestinal carcinogen**
  - Aloe vera whole leaf nondecolorized extract – M. Boudreux & F. Beland, NCTR/FDA/NTP

- **No or equivocal evidence for carcinogenic activity**
  - Milk thistle extract – J. Dunnick & A. Nyska, NIEHS/NTP
  - Tumeric oleoresin – J. Dunnick & R. Sills, NIEHS/NTP
  - Ginseng – P. Chan & J. Peckham, NIEHS/NTP

- **Heart toxicity**
  - Ephedrine/caffeine – J. Dunnick & A. Nyska, NIEHS/NTP
Goldenseal – TR 562 Feed 0, 3,000, 9,000, 25,000 ppm

- **Male F344/N rats**: clear evidence of carcinogenic activity
  - Hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined)

- **Female F344/N rats**: clear evidence of carcinogenic activity
  - Hepatocellular adenoma

- **Male B6C3F1 mice**: some evidence of carcinogenic activity
  - Hepatoblastoma and multiple hepatocellular adenoma

- **Female B6C3F1 mice**: no evidence of carcinogenic activity

- Goldenseal – negative in gentox tests
- Major active component: Berberine – positive in gentox test; topoisomerase inhibition (enzyme essential in DNA repair processes)
Goldenseal Active Ingredients

Berberine

Canadine

Hydrastine
## Goldenseal – 2-year Dietary Feeding Study in F344/N Rats and B6C3F1 Mice

<table>
<thead>
<tr>
<th>Dose (ppm)</th>
<th>0</th>
<th>3000</th>
<th>9000</th>
<th>25,000</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatocellular adenoma, multiple</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Hepatocellular adenoma (includes multiple)</td>
<td>1**a</td>
<td>1</td>
<td>2</td>
<td>10**b</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Hepatocellular adenoma or carcinoma</td>
<td>1**</td>
<td>1</td>
<td>2</td>
<td>11**</td>
</tr>
<tr>
<td><strong>Female rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatocellular adenoma</td>
<td>0**</td>
<td>0</td>
<td>1</td>
<td>8**</td>
</tr>
<tr>
<td><strong>Male mice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatoblastoma (multiple)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Hepatoblastoma (includes multiple)</td>
<td>1*</td>
<td>2</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Hepatocellular adenoma (multiple)</td>
<td>3</td>
<td>5</td>
<td>11*</td>
<td>18**</td>
</tr>
<tr>
<td>Hepatocellular adenoma (includes multiple)</td>
<td>22*</td>
<td>16</td>
<td>23</td>
<td>29</td>
</tr>
</tbody>
</table>

*a*Trend statistic  
*b*Pairwise statistic  
*p ≤ 0.05  
**p ≤ 0.01  
*N=50*
Berberine Metabolites in Rats and Humans
Ginkgo Biloba Extract – 2-year Oral Gavage (Corn Oil) Study in F344/N Rats (0, 100, 300, 1,000 mg/kg) and B6C3F1 Mice (0, 200, 600, 2,000 mg/kg)

- **Male F344/N rats:** some evidence of carcinogenic activity
  - Thyroid gland follicular cell adenoma
  - Mononuclear cell leukemia & hepatocellular adenoma may have been related to treatment

- **Female F344/N rats:** some evidence of carcinogenic activity
  - Thyroid gland follicular cell neoplasms
  - Respiratory epithelium adenoma may have been related to treatment

- **Male B6C3F1 mice:** clear evidence of carcinogenic activity
  - Hepatocellular carcinoma and hepatoblastoma
  - Thyroid follicular cell adenoma were also related to treatment

- **Female B6C3F1 mice:** clear evidence of carcinogenic activity
  - hepatocellular adenoma and carcinoma, hepatoblastoma

- Positive in Salmonella assays with/without activation
Ginkgo Components

- Terpene trilatones and flavonal glycosides
- Ginkgolic acids shown to mutagenic and cytotoxic components
## Ginkgo Biloba Extract – 2-year Oral Gavage (Corn Oil) Study in F344/N Rats and B6C3F1 Mice

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>0</th>
<th>200</th>
<th>600</th>
<th>2000</th>
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</thead>
<tbody>
<tr>
<td><strong>Male mice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatoblastoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3**a</td>
<td>28**</td>
<td>36**</td>
<td>38**b</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22**</td>
<td>31*</td>
<td>41**</td>
<td>47**</td>
</tr>
<tr>
<td>Hepatocellular adenoma or carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>39**</td>
<td>46**</td>
<td>46**</td>
<td>49**</td>
</tr>
<tr>
<td><strong>Female mice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatoblastoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1**</td>
<td>1</td>
<td>8**</td>
<td>11**</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9**</td>
<td>10</td>
<td>15</td>
<td>44**</td>
</tr>
<tr>
<td>Hepatocellular adenoma or carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20**</td>
<td>39**</td>
<td>41**</td>
<td>49**</td>
</tr>
</tbody>
</table>

*aTrend statistic  bPairwise statistic  *p ≤ 0.05  **p ≤ 0.01  N=50
Nonneoplastic and Neoplastic Lesions in Thyroid of Rats in the 2-years Gavage Study of Ginkgo Biloba Extract (N=50)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>100 mg/kg</th>
<th>300 mg/kg</th>
<th>1000 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular cell hypertrophy</td>
<td>13(1.0)</td>
<td>37**(1.2)</td>
<td>41**(1.3)</td>
<td>41**(1.8)</td>
</tr>
<tr>
<td>Follicular cell hyperplasia</td>
<td>0</td>
<td>7**(1.3)</td>
<td>9**(2.0)</td>
<td>5*(2.8)</td>
</tr>
<tr>
<td>Follicular cell adenoma</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td><strong>Female rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular cell hypertrophy</td>
<td>15(1.0)</td>
<td>41**(1.0)</td>
<td>45**(1.1)</td>
<td>48**(2.0)</td>
</tr>
<tr>
<td>Follicular cell adenoma</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Follicular cell carcinoma</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*significantly different (p ≤ 0.05) from vehicle control group by the Poly-3 test
**p ≤ 0.01
Nonneoplastic and Neoplastic Lesions in Thyroid Mice in the 2-years Gavage Study of Ginkgo Biloba Extract (N=50)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>100 mg/kg</th>
<th>300 mg/kg</th>
<th>1000 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male mice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular cell hypertrophy</td>
<td>2(1.0)</td>
<td>0</td>
<td>2(1.5)</td>
<td>38**(1.2)</td>
</tr>
<tr>
<td>Follicular cell hyperplasia</td>
<td>2(1.0)</td>
<td>1(1.0)</td>
<td>7(1.1)</td>
<td>25**(1.4)</td>
</tr>
<tr>
<td>Follicular cell adenoma</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Female mice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular cell hypertrophy</td>
<td>1(3.0)</td>
<td>5(1.4)</td>
<td>9*(1.0)</td>
<td>39**(1.0)</td>
</tr>
</tbody>
</table>

*Significantly different (p \leq 0.05) from vehicle control group by the Poly-3 test

**p \leq 0.01
Nonneoplastic Lesions in the Nose of Rats in the 2-years Gavage Study of Ginkgo Biloba Extract (N=50)

<table>
<thead>
<tr>
<th>Lesion Description</th>
<th>Control</th>
<th>100 mg/kg</th>
<th>300 mg/kg</th>
<th>1000 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olfactory epithelium, atrophy</td>
<td>1(1.0)</td>
<td>26**(1.3)</td>
<td>37**(1.6)</td>
<td>31**(2.2)</td>
</tr>
<tr>
<td>Nerve, olfactory epithelium, atrophy</td>
<td>0</td>
<td>17**(1.4)</td>
<td>14**(2.1)</td>
<td>23**(2.5)</td>
</tr>
<tr>
<td>Olfactory epithelium, respiratory metaplasia</td>
<td>9(1.3)</td>
<td>30**(1.5)</td>
<td>40**(2.0)</td>
<td>32**(1.5)</td>
</tr>
<tr>
<td>Chronic active inflammation</td>
<td>33(1.2)</td>
<td>32(1.3)</td>
<td>38(1.9)</td>
<td>46**(2.2)</td>
</tr>
<tr>
<td>Female rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olfactory epithelium, atrophy</td>
<td>0</td>
<td>18**(1.1)</td>
<td>25**(1.6)</td>
<td>37**(2.1)</td>
</tr>
<tr>
<td>Nerve, olfactory epithelium, atrophy</td>
<td>0</td>
<td>15**(1.1)</td>
<td>22**(1.6)</td>
<td>33**(2.2)</td>
</tr>
<tr>
<td>Olfactory epithelium, respiratory metaplasia</td>
<td>8(1.3)</td>
<td>4(1.3)</td>
<td>32**(2.0)</td>
<td>37**(2.5)</td>
</tr>
<tr>
<td>Chronic active inflammation</td>
<td>22(1.0)</td>
<td>1691.2</td>
<td>26(1.5)</td>
<td>38**(1.9)</td>
</tr>
<tr>
<td>Respiratory epithelium, adenoma</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

*significantly different \( p \leq 0.05 \) from vehicle control group by the Poly-3 test

**\( p \leq 0.01 \)
Nonneoplastic Lesions in the Nose of Mice in the 2-years Gavage Study of Ginkgo Biloba Extract (N=50)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>100 mg/kg</th>
<th>300 mg/kg</th>
<th>1000 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male mice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olfactory epithelium,</td>
<td>18(1.4)</td>
<td>16(1.9)</td>
<td>15(1.8)</td>
<td>28*(1.8)</td>
</tr>
<tr>
<td>hyaline droplet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>accumulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olfactory epithelium,</td>
<td>0</td>
<td>1(1.0)</td>
<td>3(1.0)</td>
<td>13**(1.1)</td>
</tr>
<tr>
<td>pigmentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Female mice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olfactory epithelium,</td>
<td>5(1.0)</td>
<td>3(1.7)</td>
<td>12(1.2)</td>
<td>17**(1.6)</td>
</tr>
<tr>
<td>hyaline droplet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>accumulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olfactory epithelium,</td>
<td>0</td>
<td>1(1.0)</td>
<td>6*(1.5)</td>
<td>13**(1.2)</td>
</tr>
<tr>
<td>pigmentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*significantly different (p \leq 0.05) from vehicle control group by the Poly-3 test
**p \leq 0.01
Kava Kava Extract – TR 571 2-year Oral Gavage (Corn Oil) Study in F344/N Rats (0, 100, 300 1000 mg/kg) and B6C3F1 Mice (0, 250, 500, 1,000 mg/kg)

- **Male F344/N rats**: equivocal evidence of carcinogenic activity
  - Marginal increase in testicular adenomas

- **Female F344/N rats**: no evidence of carcinogenic activity

- **Male B6C3F1 mice**: clear evidence of carcinogenic activity
  - Hepatocellular tumors and hepatoblastomas

- **Female B6C3F1 mice**: some evidence of carcinogenic activity
  - Hepatocellular adenomas and carcinomas (combined)

- Negative in Salmonella assay
Kava Kava – 2-year Oral Gavage (Corn Oil) Study in F344/N Rats and B6C3F1 Mice

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>0</th>
<th>250</th>
<th>500</th>
<th>1000</th>
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</thead>
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<td></td>
<td></td>
</tr>
<tr>
<td>Hepatoblastoma</td>
<td>0**a</td>
<td>4</td>
<td>9**</td>
<td>12**b</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>20</td>
<td>18</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>Hepatocellular carcinoma or</td>
<td>20</td>
<td>21</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>hepatoblastoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Female mice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>3</td>
<td>13**</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Hepatocellular adenoma or</td>
<td>10</td>
<td>21*</td>
<td>20*</td>
<td>13</td>
</tr>
<tr>
<td>carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a*Trend statistic      *b*Pairwise statistic  *p ≤ 0.05  **p ≤ 0.01  N=50
Kava Kava Extract Comprises 30% Total Kavalactones – Consisting of 6 Major Kavalactones

- Kavain
- Yangonin
- Methysticin
- 7,8 Dihydrokavain
- Desmethoxyyangonin/5,6 dehydrokavain
- Dihydromethysticin

R1, R2, R3, R4 = H
Aloe Vera – TR 577 Noncolorized Whole Leaf Extract
Drinking Water 0, 500, 1,000, 1,500 ppm

• Male F344/N rats: **clear** evidence of carcinogenic activity
  – Adenoma and carcinoma of the large intestine

• Female F344/N rats: **clear** evidence of carcinogenic activity
  – Adenoma and carcinoma of the large intestine

• Male B6C3F1 mice: **no** evidence of carcinogenic activity

• Female B6C3F1 mice: **no** evidence of carcinogenic activity

• Aloe emodin positive in Salmonella assays
Aloe Vera – 2-year Drinking Water Study in F344/N Rats and B6C3F1 Mice

<table>
<thead>
<tr>
<th>Dose (ppm)</th>
<th>0%</th>
<th>0.5%</th>
<th>1.0%</th>
<th>1.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large Intestinal adenoma or carcinomas</td>
<td>0*a</td>
<td>0</td>
<td>28**</td>
<td>31**b</td>
</tr>
<tr>
<td><strong>Female rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large Intestinal adenoma/carcinoma</td>
<td>0**</td>
<td>0</td>
<td>8**</td>
<td>15**</td>
</tr>
<tr>
<td><strong>Male and female mice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No evidence of carcinogenic activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aTrend statistic  
*bPairwise statistic  
*p ≤ 0.05  
**p ≤ 0.01  
N=48
Aloe Active Ingredient – Aloin A & B – Metabolized to Aloe Emodin in the Intestinal Tract

Structures of Aloe vera Latex-derived Anthraquinone C-glycosides, Anthrone, and Anthraquinone

Aloin A (Barbaloin)  Aloin B (Isobarbaloin)  Aloesin  Aloeresin A

Hydrolysis of the β-glycosidic bond by intestinal bacteria

Aloe-Emodin-9-anthrone  Aloe-Emodin
Intestinal Lesions/Tumors Occur in Rat (Drinking Water or Feed) Bioassays of Hydroxyanthraquinones or Herbals Containing Anthraquinones

<table>
<thead>
<tr>
<th>Bioassay/Representative Anthraquinone</th>
<th>Cancer Study in Mice</th>
<th>Cancer Study in Rats</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Hydroxyanthraquinone</td>
<td>No study</td>
<td>ACI/N rats Intestinal tumors (also liver and stomach tumors) (feed study)</td>
<td>Mori et al., 1990</td>
</tr>
<tr>
<td>Danthron</td>
<td>C3H/HeN mice Intestinal hyperplasia (no tumors) (feed study)</td>
<td>ACI/N rats Intestinal tumors (feed study)</td>
<td>Mori et al., 1986 (mice) Mori et al., 1985 (rats)</td>
</tr>
<tr>
<td>1,8-dihydroxyanthraquinone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aloe vera leaf extract/ Aloe emodin</td>
<td>B6C3F1 mice Intestinal hyperplasia (no tumors) (drinking water study)</td>
<td>F344/N rats Intestinal tumors (drinking water)</td>
<td>NTP TR 577</td>
</tr>
<tr>
<td>1,8-dihydroxy-3-hydroxymethyl-anthraquinone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emodin</td>
<td>B6C3F1 mice No intestinal lesions or tumors (feed study)</td>
<td>F344/N rats No intestinal lesions (feed study)</td>
<td>NTP TR 493</td>
</tr>
<tr>
<td>1,3,8-trihydroxy-6-methylanthraquinone</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Summary of Point Mutations in Aloe Vera Intestinal Tumors in F344/N Rats

- Point mutations in Kras (codon 13) - 2/12
- Point mutations in Kras (codon 12) - 1/12
- Point mutations in Ctnnb1 (exon 2) - 4/12
- No point mutations in p53 (exon 5 -8) - 0/12
- Molecular pathways involved in carcinogenic process – WNT, MAPK, TGF-β

Pandiri et al. Aloe vera Non-Decolorized Whole Leaf Extract-Induced Large Intestinal Tumors in F344 Rats Share Similar Molecular Pathways with Human Sporadic Colorectal Tumors, ToxPath 39: 1065-1074, 2011
Milk Thistle – TR 565
Feed 0, 12,500, 25,000, 50,000 ppm

• Male F344/N Rats: **No** evidence of carcinogenic activity

• Female F344/N Rats: **No** evidence of carcinogenic activity

• Male B6C3F1 Mice: **No** evidence of carcinogenic activity

• Female B6C3F1 Mice: **No** evidence of carcinogenic activity

• Milk thistle extract: negative in Salmonella
# Milk Thistle Extract – 2-year Dietary Feeding Study in F344/N Rats and B6C3F1 Mice

<table>
<thead>
<tr>
<th>Dose (ppm)</th>
<th>0</th>
<th>12,500</th>
<th>25,000</th>
<th>50,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bile duct hyperplasia</td>
<td>50(2.5)**</td>
<td>32(1.0)</td>
<td>27(1.1)**</td>
<td>15(1.0)**</td>
</tr>
<tr>
<td>Female rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bile duct hyperplasia</td>
<td>37(1.4**)</td>
<td>10(1.7)**</td>
<td>10(1.3)**</td>
<td>8(1.1**)</td>
</tr>
<tr>
<td>Mammary gland fibroadenoma</td>
<td>28**</td>
<td>28</td>
<td>17*</td>
<td>18*</td>
</tr>
<tr>
<td>Male mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatocellular adenoma/carcinoma</td>
<td>26**</td>
<td>22</td>
<td>16*</td>
<td>8**</td>
</tr>
</tbody>
</table>

*aTrend statistic  bPairwise statistic  *p ≤ 0.05  **p ≤ 0.01  N=50
Milk Thistle Extract – Active Ingredients – Metabolites of Active Ingredients Similar in Humans and Animals

Berberine

Canadine

Hydrastine
Tumeric Oleoresin – TR 427
Feed 0, 2,000, 10,000, 50,000 ppm

• **Male rats:** no evidence of carcinogenic activity
  – Increased incidences of preputial gland neoplasms

• **Female rats:** equivocal evidence of carcinogenic activity
  – Clitoral gland adenoma

• **Male mice:** equivocal evidence of carcinogenic activity
  – Hepatocellular adenoma

• **Female mice:** equivocal evidence of carcinogenic activity
  – Hepatocellular adenoma

• Tumeric oleoresin – negative in Salmonella
Ginseng – TR 567

• **Male F344/N rat:** no evidence of carcinogenic activity

• **Female F344/N rat:** no evidence of carcinogenic activity

• **Male B6C3F1 mouse:** no evidence of carcinogenic activity

• **Female B6C3F1 mouse:** no evidence of carcinogenic activity

• Ginseng – negative in Salmonella
III. Cardiotoxicity Studies: Ephedrine/Ephedra (Ma Huang)

- Ephedrine (active ingredient in Ma Huang) binds to adrenergic receptors
- Ephedrine in combination with caffeine is more toxic than exposure to either compound alone
  - Ephedrine and caffeine in combination alter ion flow (calcium)
- Ephedrine/caffeine exposure increases heart rate and temperature within one hour after a single oral gavage study in rats and mice
- Ephedrine/caffeine exposure cause hemorrhage and necrosis in moribund rats and mice
- Both ephedrine/caffeine and the Herb (Ma Huang)/caffeine exposures cause similar cardiac toxicity
Epinephrine (non-selective AR agonist)

Phenylephrine ($\alpha_1$-AR selective agonist)

Methoxamine ($\alpha_{1\alpha}$-AR selective agonist)

Oxymetazoline ($\alpha_{1\alpha}$-AR selective agonist)

Clonidine ($\alpha_{1\alpha}$-AR selective agonist)

(-)-Isoproterenol (non-selective $\beta$-AR agonist)

Dichloroisoproterenol (non-selective $\beta$-AR agonist)

Ephedrine (non-selective $\beta$-AR agonist)

Albuterol (non-selective $\beta$-AR agonist)

Nylidrin (non-selective $\beta$-AR agonist)

Waugh et al JBC 275;11698, 2000
## Ephedrine/Caffeine ECG Parameters – 14 Week F344/N Rats One Oral Gavage Dose

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time Point (hour)</th>
<th>HR Beats/min</th>
<th>QT&lt;sub&gt;c&lt;/sub&gt;, ms</th>
<th>R-amp, mV</th>
<th>Temp °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Baseline</td>
<td>355±5</td>
<td>0.112±0.001</td>
<td>0.401±0.015</td>
<td>36.9±0.01</td>
</tr>
<tr>
<td>25 Eph + 30 Caff</td>
<td>1</td>
<td>478±5*</td>
<td>0.192±0.004*</td>
<td>0.460±0.028*</td>
<td>39.2±0.6*</td>
</tr>
<tr>
<td>25 Eph + 30 Caff</td>
<td>3</td>
<td>485±16*</td>
<td>0.182±0.007*</td>
<td>0.409±0.0371*</td>
<td>38.1±0.2*</td>
</tr>
</tbody>
</table>

*<sup>p<0.05</sup>
Proposed Mechanism of Ephedrine/Caffeine Heart Toxicity

Oral Ephedrine Exposure → Rapid absorption so that toxic levels are obtained within one hour of dosing → α/β adrenergic agonist - release of catecholamines - Calcium influx → Vasoconstriction (V) → Increase HR and QTc interval → Myocardial ischemia → Necrosis and Apoptosis (N) → Hemorrhage (H) → Sudden Death → Resolution by Inflammation Fibrosis
Serum Biomarkers Detect Ephedrine/Caffeine Cardiotoxicity Even in the Absence of Histopathologic Lesions (Studies in B6C3F1 Mice – One Oral Dose)

MyL3 – Day One

With Dr. G. Travlos & Dr. S. Borgdorf
Serum Biomarkers Detect Ephedrine/Caffeine Cardiotoxicity Even in the Absence of Histopathologic Lesions (Studies in B6C3F1 Mice – One Oral Dose)

With Dr. G. Travlos & Dr. S. Borgdorf

NCCP: new onset of chest pain
UAP: unstable angina pectoris
AMI: acute myocardial infarction

With Dr. G. Travlos & Dr. S. Borgdorf
Summary of NTP Herbal Medicine Findings

• Diverse biologic response among herbs and supplements

• Some are carcinogenic, some are not

• Individual components have biologic activities that help explain the carcinogenic findings

• NIH clinical trials underway for anticancer activity of turmeric (curcumin), milk thistle, ginseng
  – http://www.clinicaltrials.gov/
IV. NTP Herbal Medicine Studies – Treatment-related Lesions (A. Nyska)

- Liver nonneoplastic and neoplastic lesions
- Thyroid nonneoplastic and neoplastic lesions
- Intestinal nonneoplastic and neoplastic lesions
- Heart lesions
Ginkgo Biloba Extract
NTP Technical Report TR 578

Histopathology Findings
2-Year Studies – Rats
Focal Fatty Change Associated with Microgranulomas in a Female Rat Treated with 1000 mg/kg of Ginkgo Biloba
Thyroid Follicular Hypertrophy in a Female Rat Treated for 2 Years with 1000 mg/kg of Ginkgo Biloba, Comparing to the Aspect in a Concurrent Control Animal
Thyroid Follicular Adenoma in a Male Rat Treated for 2 Years with 1000 mg/kg of Ginkgo Biloba
Thyroid Follicular Carcinoma in a Female Rat Treated for 2 Years with 300 mg/kg of Ginkgo Biloba
Nose, Level 3: Chronic Active Inflammation and Respiratory Metaplasia of the Olfactory Epithelium in a Female Rat Treated for 2 Years with 1000 mg/kg of Ginkgo Biloba
Nose, Level 3: Atrophy of the Olfactory Epithelium in a Female Rat Treated for 2 Years with 1000 mg/kg of Ginkgo Biloba

Control Rat

Treated Rat
Histopathology Findings
2-Year Studies – Mice
Hepatocellular Adenoma in a Male Mouse Treated with 2000 mg/kg of Ginkgo Biloba for Two Years
Hepatocellular Carcinoma in a Female Mouse Treated with 2000 mg/kg of Ginkgo Biloba for Two Years
Hepatoblastoma in a Male Mouse Treated with 200 mg/kg of Ginkgo Biloba for Two Years
Erythrophagocytosis in a Male Mouse Treated with 200 mg/kg of Ginkgo Biloba for Two Years
Thyroid Follicular cell hyperplasia (left) and follicular cell adenoma (right) in Male Mice Treated with 2000 mg/kg of Ginkgo Biloba for Two Years
Kava Kava Extract
NTP Technical Report TR 571

Histopathology Findings in 3 Month Study in rats
Three-month Study in Rats

- Increase in liver weights of $\geq 0.25$ g/kg males and $\geq 0.5$ g/kg females
- Increase in hepatocellular hypertrophy in 2 g/kg females
- Clinical pathology findings considered unremarkable
Immunohistochemical Analysis of CYPs Expression in the Liver Treated with Kava Kava Extract for 3-month in Rats
Fig. 1: Centrilobular area, control female rat. Note relatively smaller size of hepatocytes with cytoplasmic basophilic stippling.

Fig. 2: Mild hepatocytic hypertrophy in female rat treated with 2.0 g/kg kava kava extract. Centrilobular hepatocytes contain more homogeneous eosinophilic cytoplasm.
Fig’s. 3 & 4: 
Strong CYP2D1 expression (intensity: grade 3) in centrilobular area, control female rat; CYP2D1 detected diffusely in cytoplasm of hepatocytes of controls

Fig’s. 5&6: 
Moderate expression (intensity: grade 2) of CYP2D1 in centrilobular area in female rat treated with 2.0 g/kg kava kava extract by gavage for 3 months
Fig’s. 11&12: Weak expression (relative area: grade 1) of CYP3A1 only in centrilobular area, detected locally in cytoplasm of hepatocytes around central vein, control female rat.

Fig’s. 13&14: Strong expression (relative area: grade 4) of CYP3A1 in almost all of centrilobular area in a female rat treated with 2.0 g/kg of kava kava extract by gavage for 3 month.
Nondecolorized Whole Leaf Extract of *Aloe Barbadensis* Miller (Aloe Vera) – NTP Technical Report TR 577

Histopathology Finding
F344/N Rats Administered Aloe Vera Nondecolorized Whole Leaf Extract for 13-Weeks

**Goblet Cell Hyperplasia** seen in the cecum, colon and rectum
The goblet cell hyperplasia may indicate the presence of epithelial cell dysplasia, a precancerous change

**Goblet Cell Hyperplasia in the Colon**

- **Control 4x**
- **1% Aloe vera whole leaf 4x**
- **2% Aloe vera whole leaf 4x**
- **3% Aloe vera whole leaf 4x**
B6C3F1 Mice Administered Aloe Vera Nondecolorized Whole Leaf Extract for 13-Weeks

Goblet Cell Hyperplasia was seen in the cecum, colon and rectum.

Goblet Cell Hyperplasia in the colon

Control

1% whole leaf extract

2% whole leaf extract

3% whole leaf extract
Lesions of the Gastro-intestinal Tract in F344/N Rats Administered Aloe Vera Nondecolorized Whole Leaf Extract for 2 Years

- **Mucosal hyperplasia**
  - Characterized by thickening of the mucosa due to increased length and complexity of mucosal glands, with no cellular atypia and minimal inflammation
  - Dose-related increased incidences in glandular stomach, small intestine, large intestine, and rectum of male and female rats
  - It is uncertain whether the observed changes represent one step in a multistep process of carcinogenesis
Mucosa Hyperplasia of the Large intestinal Tract in F344/N Rats Administered Aloe Vera Nondecolorized Whole Leaf Extract for 2 Years
Neoplasms in the Large Intestine of F344/N Rats Administered Aloe Vera Nondecolorized Whole Leaf Extract for 2 Years

- Adenomas – identified as either pedunculated nodules that protruded into lumen or sessile lesions that caused focal thickening of the mucosal wall
- Carcinomas – identified by the invasion of epithelial cells into the stroma of the stalk or into the submucosa and/or muscularis of the intestinal wall
Ephedrine + Caffeine

Histopathologic Changes in the Heart of Male F344/N Rats

I-Ephedrine hydrochloride

C_{10}H_{15}NO_4HCl

mw (free base) 201.7 (165.2)

C_{10}H_{15}NO_4HCl

C_{8}H_{10}NO_4O_2

mw 194.19

Cas No. 299-42.3

Cas No. 58-08-2
Rat Treated with Ephedrine (25 mg/kg) and Caffeine (30 mg/kg), Died Few Hours After Dosing

Hemorrhage (H) and Myofiber Necrosis (N) Associated with Macrophages Infiltration in the Left Ventricle
Higher Magnification of the Previous Photo:
Myofiber apoptosis and Macrophages Infiltration

- Apoptotic Bodies
- Macrophages
Rat Treated with Ephedrine (25 mg/kg) and Caffeine (30 mg/kg), Sacrificed Animal Few Hours After Dosing

Deeply Basophilic Fragments of Nuclear Debris, Mixed with Some Macrophages
Rat Treated with Ephedrine (25 mg/kg) and Caffeine (30 mg/kg), Died Few Hours After Dosing.

Apoptotic Bodies (TUNEL Staining)
Rat Treated with Ephedrine (25 mg/kg) and Caffeine (30 mg/kg), Died Few Hours After Dosing

Cleaved Caspase-3 staining

Negative Control (No Antibody for Caspase 3 was Added)
Barbeitto-López Trichrome Stain
Myofiber Degeneration and Necrosis

25 mg/kg ephedrine 30 mg/kg caffeine – degenerating and necrotic myofibers are stained yellow

Control rat
Acknowledgements

- Dr. Mamta Behl
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