CANCER CHEMOPREVENTIVE POTENTIAL OF PHYTOCHEMICALS DERIVED FROM MEDICINAL PLANTS

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Key Causes of Cancer
Reactive oxygen species induced oxidative stress and pathogenesis

CME-2015
Chemoprevention of Cancer

- About 40% of cancers may be avoidable or preventable.

- Risk of cancer may be reduced by:
  - Consumption of nutraceuticals, fruits & vegetables (rich in antioxidants)
  - High-fat content
  - Intake of tobacco & alcohol
  - Regular exercise
Stages of carcinogenesis and their intervention using chemo-preventive agents

**Blocking Activities**

- **Initiation**
  - Normal Cell
- **Promotion**
  - Initiated Cell
- **Progression**
  - Preneoplastic Cells
- **Neoplastic Cells**

**Suppressing Activities**

- **Primary prevention**
  1. Enhance carcinogen detoxification
  2. Detoxification of ROS
  3. Antioxidative effect
  4. Alter carcinogen metabolism
  5. Enhance DNA repair

- **Secondary prevention**
  1. Inhibition of proliferation
  2. Induction of apoptosis
  3. Induction of differentiation
  4. Decreasing inflammation
  5. Enhancing immunity
Anticancer Activity of Antioxidants

$O_2^- + O_2$-Derived Free Radicals

Lipid peroxidation in cell membranes

Cell repair
Exposure of DNA
Cell Death
Lipid Peroxides

Compensatory Cellular Hypoproliferation

No Repair

Loss of differentiation
DNA Oxidative damage

Antioxidants - Free Radical Scavengers & Enzyme Systems

Oxidized bases in DNA

Mutations

DNA Repair

Normal Cells

Cancer

ANTICANCER CME-2015
Why herbal medicine?

- Prevalence of Herbal Medicine in over 75% of the world population, it is preferred over chemicals because
  - Better cultural acceptability
  - better efficacy
  - better compatibility with the human body
  - lesser side effects

- A systemic approach is needed for identifying active constituents from different medicinal plants, using modern techniques.
Phytochemicals in Dietary Vegetables & Fruits

In the newest and most promising area of research, these phytochemicals appear to prevent some cancers and inhibit spread of malignant cells.
General protocol for the induction of skin tumor

1. Clipping of Hair
2. 3 Days
3. Application of DMBA
4. 14 Days
5. Application of Croton Oil
6. 16 Weeks
7. Autopsy
General protocol for the induction of stomach tumor

1. Oral administration of BaP by feeding needle
2. 4 weeks
3. Autopsy after 14 weeks
4. Remove stomach for the experiment
5. Biochemical Study
6. Histopathological Study
General protocol for the induction of hepatic tumor

3 weeks old Animals

↓

Single i.p. injection of Diethyl nitrosamine (DENA) in normal saline

↓

After 2 weeks

↓

Oral administration of CCl₄, 3 times in a week by gavage with 1:1 dilution in corn oil

↓

Necropsy after 24 weeks
Medicinal Plants & Natural Products Screened/under Screening in our Laboratory for the Management of Cancer by using Skin, Hepatic & Gastric Cancer Models
Emblica officinalis (Fruit)
Rosemarinus officinalis (leaves)
Aloe vera (leaves)
Trigonella foenum (leaves)
Alstonia scholaris (Bark)
Syzygium cumuni (Seed)
Tinospora cordifolia (Root)
Panax ginseng (Root)
Aegle marmelos (Fruit)
Linum usitatissimum (Seed oil)

Averrhoa carambola (Fruit)

Carissa carandas (Fruit)

Capparis decidua (Fruit)

Trachyspermum ammi (Seed)
Chemopreventive Potential of Flax/Linseed oil against chemical induced skin carcinogenesis

Materials & Methods

Animals
Swiss Albino Mice
6-8 weeks old

Carcinogen/Promoter
7, 12-Dimethyl Benz (a) anthracene (DMBA)
Croton oil

Plant Material
Cold pressed Flaxseed oil
Chemical constituents present in flaxseed oil

- Alpha-linolenic acid (omega-3 fatty acid)
- Phytoestrogen
- Non-volatile carbonyl compounds
- Tocopherol
- Plastochochromanol-8
- Flavanoids
- Phenolic acid
- Niacine
Flaxseed oil contains:

1. **OMEGA-3 FATTY ACIDS**
   (Improve circulation, strengthen body and reduce cholesterol, diabetes)

2. **PHYTOESTROGENS**
   (Maintain hormonal levels)

3. **ANTI-INFLAMMATORY PROPERTIES**
   (Reduce cysts in breasts and inflammation in bowels, digestive tracts & joints)
## Parameters Studied

### Morphological
- Tumor Incidence
- Cumulative no. of tumors
- Tumor yield
- Tumor burden
- Average latent period
- Inhibition of tumor multiplicity

### Biochemical
- Lipid peroxidation (LPO) (Ohkhawa *et al.* 1979)
- Reduced glutathione (GSH) (Moron *et al.* 1979)
- Superoxide dismutase (Marklund & marklund, 1974)
- Catalase (Aebi, 1984)
- Total Proteins (Lowry *et. al.*, 1951)

### Histopathological
- Skin
- Tumor

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Fig. The gross appearance of skin tumors in mice of different groups during chemical induced skin carcinogenesis with or without flaxseed oil.
- Gr. I: DDW alone; Gr. II: FSO alone
- Gr. III: DMBA+ Croton oil
- Gr. IV: Peri-initiation (FSO-50µl/animal/day)
- Gr. V: Post-initiation (FSO-50µl/animal/day)
- Gr. VI: Peri-Post initiation (FSO-50µl/animal/day)
Gr. I: DDW alone; Gr. II: FSO alone
Gr. III: DMBA + Croton oil
Gr. IV: Peri-initiation (FSO-50μl/animal/day)
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- Gr. V: Post-initiation (FSO-50µl/animal/day)
- Gr. VI: Peri-Post initiation (FSO-50µl/animal/day)
Significance level:
\( a_{p \leq 0.05}, \quad b_{p \leq 0.01}, \quad c_{p \leq 0.001} \)
Significance level:
$ap \leq 0.05$, $bp \leq 0.01$, $cp \leq 0.001$
Significance level:

\[ a_p \leq 0.05, \quad b_p \leq 0.01, \quad c_p \leq 0.001 \]
Plate- 1
Photomicrograph of skin in DMBA/croton oil treated mice illustrating skin lesions in the form of:

X 200

Severe:
(i) ER - Erosion
(ii) HK - Hyperkeratosis
(iii) EH - Epidermal hyperplasia
(iv) DI - Dermal invasion
(v) LDF - Loosening of dermal fibers, and

Severely damaged:
(i) SG - Sebaceous gland
(ii) HF - Hair follicle

Plate- 2
Photomicrograph of T. S. of tumor in DMBA/croton oil treated mice illustrating:
Ac- Acanthosis, Sr- reduced stroma with lymphocytes, KP- Keratinous Pearl,
AN- Atypic nuclei
Plate- 3
Photomicrograph of V.S. of skin in mice, received FSO (50 µl/animal/day) during peri-initiation stage.

X 200

Plate- 4
Photomicrograph of T. S. of tumor in mice, received FSO (50 µl/animal/day) during peri-initiation stage.

X 200
Plate- 5
Photomicrograph of V.S. of skin in mice, received FSO (50 µl/animal/day) during post-initiation stage.
X 100

Plate- 6
Photomicrograph of T. S. of tumor in mice, received FSO (50 µl/animal/day) during post-initiation stage.
X200
Plate- 7
Photomicrograph of V.S. of skin in mice, received FSO (50 µl/animal/day) during peri- & post-initiation stage.

\[ X \ 100 \]

Plate- 8
Photomicrograph of T.S. of Tumor in mice, received FSO (50 µl/animal/day) during peri- & post-initiation stage.

\[ X \ 100 \]
# Tumor Statistics with Different Plant Extracts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumor Incidence (%)</th>
<th>Tumor Burden</th>
<th>Tumor Yield</th>
<th>Average Latent Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinogen treated control (DMBA+ Croton oil)</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>-</td>
</tr>
<tr>
<td><em>Tinospora cordifolia</em> (100mg/ kg.b. wt.)</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
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<tr>
<td>+ DMBA+ Croton oil</td>
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<tr>
<td><em>Syzygium cumini</em> ((125mg/kg. b.wt.)</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
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<tr>
<td>+ DMBA+ Croton oil</td>
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<tr>
<td><em>Phyllanthus niruri</em> (1000mg/ kg.b. wt.)</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
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<td>+ DMBA+ Croton oil</td>
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<tr>
<td><em>Alstonia scholaris</em> (100mg/ kg.b. wt.)</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
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<tr>
<td>+ DMBA+ Croton oil</td>
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<tr>
<td><em>Rosmarinus officinalis</em> (750mg/kg. b.wt)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+ + +</td>
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<tr>
<td>+ DMBA + Croton oil</td>
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<tr>
<td><em>Aegle marmelos</em> (100mg/ kg. b.wt)</td>
<td>+ +</td>
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<td>+ +</td>
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</tr>
<tr>
<td>+ DMBA+ Croton oil</td>
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</tr>
<tr>
<td>Flaxseed Oil (100mg/ kg. b.wt)</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>+ DMBA + Croton oil</td>
<td></td>
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</tr>
<tr>
<td>Treatment</td>
<td>LPO (nmole/mg tissue)</td>
<td>GSH (µmole/gm tissue)</td>
<td>CAT (U/mg tissue)</td>
<td>Protein (mg/ml)</td>
</tr>
<tr>
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<td>- - -</td>
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<tr>
<td><em>Tinospora cordifolia</em> (100mg/ kg.b. wt.)+ DMBA+ Croton oil</td>
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<tr>
<td>Flaxseed Oil (50 µl/ kg. b.wt)+ DMBA+ Croton oil</td>
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<td>-</td>
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ROS induced oxidative stress leading to carcinogenesis
Free radicals and their neutralization by antioxidants
Chemo preventive mechanism by Natural Products
Cancer Economics: Cancer Prevention

- High expenditure in cancer treatments
- Cancer is preventable
- Therefore, cancer prevention is necessary
- Most cancers likely develop from a complex interplay of diet, lifestyle and environmental factors
- Nutrition and diet are the most important way for cancer prevention.
- Dietary Prevention: Edible vegetables/fruits and medicinal plants
What can we do to prevent cancer?

Be good
- healthy diet
- exercise regularly
- maintain healthy weight
- don’t smoke
- alcohol in moderation

Know your risk
- seek appropriate evaluation or therapy for your risk level

Support prevention research
Conclusions

Cancer is an important health problem in the world these days. It is the second largest non-communicable disease that has a sizable contribution in the total number of deaths.

The increasing trend of cancer incidence has forced the humanity to work more on the cancer prevention and treatments.

The quest of identifying new chemopreventive agents from plant sources has become an ideal strategic paradigm to combat cancer.

Natural compounds have practical advantages with regard to availability, suitability for oral application, low toxicity, regulatory approval and mechanisms of action.

Much progress has been made in this field, but more work remains before widespread use and practice of cancer prevention by natural products.

Various medicinal plants extracts demonstrate their ability in reducing the chemical induced tumors and oxidative stress.

These findings suggest the possible preventive and therapeutic use of herbal medicine in the management of cancer.

It may also be recommended that more medicinal plants and natural products should be trialed in the laboratory to access their anticarcinogenic potential.
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