Suppression of colon tumorigenesis in rats by low dose dietary caraway essential oils is mediated by hepatic xenobiotic metabolizing enzymes

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Caraway (Carum carvi L.)

- A widely used spice known as meridian fennel or Persian cumin
- used as crude or essence in various food products for its pleasant flavor and anti-spoilage properties.
- A medicinal herb
- Caraway seeds contain essential oils rich in nutraceutical compounds used as food supplements and plant-based medicine. Caraway is one of the richest plant in carvone.
- Essential oils (essence) contains active ingredients such as carvone Known for antioxidant and anti-microbial properties.
- Essential oils when used as mixture are more effective suggesting the additive effects of the oil components.
Caraway (seeds)
Medicinal application of caraway products

Galactogogue (Lactation)
Carminative (Flatulence)
Anti-inflammatory
Antispoilage
Digestion
Antipasmodic

Medicinal application
Use of caraway products in industry

- Food additive/fish
- Condiment/Spice
- Inhibition of potatoes sprouting
- Natural coating for processed food
- Additive for chewing gum, toothpaste
- Flavoring of Cheese, bread, etc.
Essential oils composition of caraway

- **Compounds:**
  - Monoterpene hydrocarbons (γ-terpinene)
  - Aldehydes (Trans-limonene oxide)
  - Ketones (Carvone)
  - Oxygenated monoterpenes (Carveol)
  - Sesquiterpenes hydrocarbons (β-elemene)
  - Oxygenated Sesquiterpenes (Carvacrol)
  - Esters (Linalyl acetate)
  - Others
Caraway oils (*Carvioleum*) as Anticancer agent

- Colon cancer as the target:
- Experimental Model of Colon Carcinogenesis.
- The mechanism of action
Strategies for studying anticancer activity of a phytochemical

1. Tumor Initiation
   Carcinogen activation/metabolite detoxification

2. Tumor promotion/progression

3. Free radical scavenging/antioxidant

4. Alter gene expression

5. Antiinflammatory

6. Induce apoptosis
Experimental procedure

36 rats (6 animals/group)
- Group 1 (Control); 0.5 ml EDTA (the vehicle), 5 weeks
- Group 2 (Sham-treated) low dose dietary essential oils
- Group 3 (Sham-treated) high dose dietary essential oils
- Group 4 DMH-induced colon carcinogenesis
- Group 5 DMH+ Low dose dietary essential oils
- Group 6 DMH+High dose dietary essential oils
Preparation of caraway essential oils
Clevenger-type apparatus (2 h)
Protocol of DMH-induced carcinogenesis

• Albino rats of Wistar strain
• DMH dissolved in EDTA (20 mg/kg b.w).
  Five s.c injections of 0.5 ml DMH once/week for 5 weeks.
Dietary caraway essential oils

• Rat regimen was supplemented with low or high levels of essential oils (0.01%) or high level essential oils (0.1%) for 5 weeks.

• Rats were sacrificed after 16 weeks.
## Experimental design

<table>
<thead>
<tr>
<th>Rats</th>
<th>Vehicle</th>
<th>DMH (Carcinogen)</th>
<th>LD 0.01% essential oils</th>
<th>HD 0.1% essential oils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sham-group-1</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sham-group-2</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>DMH (Carcinogen)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DMH+ Essential oils</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>DMH+Essential oils</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Assays

- **Plasma** antioxidant status (FRAP assay)
- **Colon and Liver**
  - Lipid peroxidation (TBARS)
  - Reduced Glutathione
  - Superoxide Dismutase (SOD)
  - Catalase
  - Glutathione S-transferase (GST)
  - Cytochrome P450 1A1 (CYP1A1)
Experimental procedure

- An animal model of colon carcinogenesis was developed using 1,2-dimethylhydrazine (DMH) as a carcinogen. Thirty-six young rats were divided into 6 groups (6 rats/group) and treated as follows; animals in group 1 received 0.5 ml of EDTA—the vehicle of DMH, once a week for 5 weeks and considered as controls. Groups 2 and 3 treated with caraway oil (0.01 or 0.1% in feed), respectively, and considered as sham groups. Group 4 rats received DMH (20 mg/kg b.w) injected once a week for 5 wk. Groups 5 and 6, treated with DMH (20 mg/kg b.w.) for 5 weeks and given dietary caraway oils (0.01 or 0.1 %) as treated groups. At the end of the experiment (16 weeks), blood was collected; liver and colon tissues were removed and processed for biochemical and histological examinations.
Results
Effects of dietary essential oils on total antioxidant capacity of plasma
Effects of dietary essential oils on Lipid peroxidation in rat liver
Effects of dietary essential oils on rat Liver glutathione levels
Effects of dietary essential oils on rat Liver superoxide dismutase (SOD)
Effects of dietary essential oils on rat Liver catalase activity
Changes in cytochrom P4501A1 activity in rat liver treated with caraway essential oils
Changes in cytochrom P4501A1 activity in rat colon treated with caraway essential oils
Comparison of hepatic Glutathione S-transferase activity in rats treated with caraway essential oils
Comparison of hepatic Glutathione S-transferase activity in rat colon tissue treated with caraway essential oils.
Histology of colon tissue of experimental groups. A=normal; B&C=Sham; D=DMH; E&F=Essential oils
Prevention of Aberrant Crypt Foci (ACF) in rat colon treated with the essential oils
Methylene blue staining: A=normal crypt, X100. B=ACF, X200
Table: Inhibition of DMH-induced Aberrant Crypt Foci (ACF) in rat colon treated with dietary caraway oils

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total number of ACF</th>
<th>Total number of aberrant crypt (AC)</th>
<th>Crypt multiplicity (AC/ACF)</th>
<th>Inhibition of ACF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>0.01 % essential oil (Sham 1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>0.1 % essential oil (Sham 2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>DMH</td>
<td>8±2.2*</td>
<td>6.75±2.1*</td>
<td>0.84±0.95*</td>
<td>-</td>
</tr>
<tr>
<td>DMH + 0.01 % essential oil</td>
<td>1±0**</td>
<td>5±0</td>
<td>5±0</td>
<td>87.5**</td>
</tr>
<tr>
<td>DMH + 0.1 % essential oil</td>
<td>2.2±0.9**</td>
<td>1.25±0.25**</td>
<td>0.56±0.27**</td>
<td>72.5**</td>
</tr>
</tbody>
</table>
Highlights

1. Low dose (0.01 v/w) dietary essential oils derived from caraway can efficiently suppress DMH-induced premalignant lesions in colon.

2. The dietary essential oils given to rats could not modulate oxidative stress/antioxidant factors induced by DMH.

3. Cancer prevention properties of caraway oils is more likely by modification of hepatic xenobiotic enzymes and inhibition of carcinogenic metabolite.
Precautions

• **Essential oils**: 0.06-0.2 ml /dose. Don't use essential oils for internal use, it is highly concentrated. Not recommended for children and pregnant and lactating woman.

• **Caraway powder**: A drink prepared by dissolving 300-600 mg (10 fruits) in water or milk.

• **Infusion**: Suspension prepared by soaking 2 grams seeds or powder in water or milk.
Book Chapter

• Caraway: usage and applications in traditional medicine and food sciences.
• Book "Essential Oils in Food Production, Preservation, Flavour and Safety". Edited by Victor Preedy.
• Imprint: Academic Press
• Number of Pages: 896
• ISBN: 978-0-12-416641-7
Inhibitory Effects of Dietary Caraway Essential Oils on 1,2-Dimethylhydrazine-Induced Colon Carcinogenesis is Mediated by Liver Xenobiotic Metabolizing Enzymes

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Effect of dietary caraway essential oils on expression of β-catenin during 1,2-dimethylhydrazine-induced colonic carcinogenesis

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Suppressive effects of caraway (Carum carvi) extracts on 2, 3, 7, 8-tetrachloro-dibenzo-p-dioxin-dependent gene expression of cytochrome P450 1A1 in the rat H4IIE cells

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BIOCHEMICAL PROPERTIES OF γ-IRRADIATED CARAWAY ESSENTIAL OILS

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Flowering caraway plant

Thank you for your attention
Results

- DMH-treated rats developed premalignant lesions in colon with the number of 8±2.2 aberrant crypt foci (ACF) in colon tissue. Whereas in rats fed 0.01% caraway essential oils the number of ACF was significantly decreased to 1.0±0 ACF (>87% inhibition in ACF formation). Inhibition of premalignant lesions was associated with changes in hepatic cytochrome P450 1A1 (CYP1A1) and glutathione S-transferase (GST) activity. Feeding rats with low dose oils for 16 weeks reversed the DMH-related changes in these enzymes particularly in liver tissue. However, this regimen failed to alter antioxidant factors namely; catalase, superoxide dismutase, glutathione and total antioxidant status.