Effects of Essential Oils on Glutathione S-Transferase Activity in Mice

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A total of 30 essential oils and 16 of their processing byproducts were assayed for their ability to induce increased activity of the detoxifying enzyme system glutathione S-transferase in A/J mice. Enzyme induction in the cytosols of the liver, forestomach, and small intestinal mucosa was examined. Test samples that elicited enzyme activity greater than 2.5 times the control level in at least one of the three tissues studied were considered active. By use of this criterion, 26 active substances were obtained. They were categorized into three groups according to their enzyme-inducing activity in different tissues. The first group had nine samples that induced enzyme activity significantly higher than that of the control in all three tissues examined. A second group of nine samples induced 3 times the enzyme activity in the mucosa. A third group of eight samples was found to increase enzyme activity greater than 2.5 times the control in either the liver or mucosa. Since anticarcinogenic activity of compounds has been found to correlate with their ability to induce increased activity of detoxifying enzymes, the active samples are good candidates for the isolation of potential chemopreventive agents.

INTRODUCTION

Many secondary metabolites in edible plants that antagonize the noxious effects of chemical carcinogens have been isolated and identified (Loub et al., 1975; Wattenberg et al., 1976; Pantuck et al., 1976). Indoles, isolated from cruciferous vegetables, have been shown to inhibit chemically induced tumorigenesis in animal models (Loub et al., 1975). The diterpenes cafestol and kahweol, isolated from green coffee beans, have been found to inhibit dimethylbenz[a]anthracene-induced mammary tumors in Sprague-Dawley rats (Wattenberg et al., 1984). Limonin and nomilin isolated from citrus have been determined to inhibit tumorigenesis in mice and hamsters (Lam and Hasegawa, 1989b; Miller et al., 1989). Sulfur-containing compounds from onion and garlic oils were found to possess anticarcinogenic properties (Belman, 1983; Wargovich, 1987).

A majority of essential oils, by nature of their manufacturing processes (steam distillation followed by simple distillation), are made up of rather volatile compounds from the monoterpene family. Some sesquiterpenes and low molecular weight aromatic compounds are often present as part of the complex mixture. A few of these compounds appear to be effective inhibitors of carcinogenesis. d-Limonene, which is a major constituent of orange peel oil, has been determined to inhibit mammary tumors in rats (Elegbede et al., 1984). d-Carvone, which is present in high concentration in caraway oil and dillweed oil, was found to inhibit nitrosamine-induced carcinogenesis in mice (Wattenberg et al., 1989). These and other discoveries suggest the possibility that more potent inhibitors may be discovered in essential oils and other diet components that have yet to be studied.

A biochemical method developed as a rapid screening assay for potential inhibitors of chemical carcinogenesis has been used successfully in the isolation of active natural products. This screening technique is based on the induction of the detoxifying enzyme system glutathione S-transferase (GST) (Habig et al., 1974; Jakoby and Habig, 1980).

The GST enzymes catalyze the conjugation of glutathione with electrophilic species to form less toxic, water-soluble substances that are readily excreted (Chasseaud, 1979). An enhancement of the activity of GST suggests an increase in the host's ability to detoxify xenobiotics, including carcinogens. Thus, any substance that can elicit increased activity of GST and other detoxifying enzymes may be a potential anticarcinogen. A positive correlation has been established for several classes of inhibitors of benzo[a]pyrene-induced neoplasia of the mouse forestomach and their GST activity including effects in the same tissue (Lam and Hasegawa, 1989b; Lam and Zheng, 1990; Sparnins and Wattenberg, 1981).

Table I. Alphabetical List of Essential Oils and Byproducts Tested in This Study

ambrette musk residues	lemon oil terpenes
angelica root oil	lime oil tails
anise oil	lime oil terpenes
bergamot oil	mandarin oil
bergamot terpenes	marjoram oil
camomile oil	nutmeg oil
caraway oil	nutmeg terpenes
celery seed oil	orange bitter oil
clove terpenes	orange oil residues
coriander oil	origanum oil
dillweed oil	parsley leaf oil
dillweed terpenes	peppermint oil
eucalyptus oil	peppermint tail fractions
eucalyptus terpenes	pimento leaf oil
fennel oil	rosemary oil
fennel terpenes	sage oil
galanga root oil	spearmint residues
ginger oil	spearmint terpenes
grapefruit oil	sweet basil oil
grapefruit terpenes	taragon oil
hops oil	tea tree oil
lemongrass oil	thyme oil
lemon oil	

The diterpene inhibitors cafestol and kahweol, as the palmitates and other esters in green coffee beans, were detected by their ability to induce the activity of GST. Biochemical assay directed fractionation led to the isolation of the active principles (Lam et al., 1982). The pure compounds thus isolated were found to inhibit chemically induced mammary tumors in rats (Wattenberg and Lam, 1984).

Recently, using this enzyme assay we have determined the GST-inducing activity of citrus limonoids (Lam and Hasegawa, 1989a). Tumor protection experiments confirmed the inhibitory potential of these natural products (Lam and Hasegawa, 1989b). This study reports the induction of GST activity as a preliminary screening assay for the detection of essential oils and byproducts that are potential inhibitors of tumorigenesis.

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Table II. List of Essential Oils and Byproducts That Induced GST Enzyme Activity Significantly Higher than That of the Control Level in Small Intestinal Mucosa, Liver, and Forestomach (Test/Control > 1.30) of A/J Mice

	small intestinal mucosa		liver		forestomach	
chemical	GST act.,[a] μmol min^{-1} (mg of protein)$^{-1}$	ratio test/control	GST act.,[a] μmol min^{-1} (mg of protein)$^{-1}$	ratio test/control	GST act.,[a] μmol min^{-1} (mg of protein)$^{-1}$	ratio test/control
caraway oil	2.01 ± 0.17	3.13	5.99 ± 1.53[c]	3.01	3.08 ± 0.52[b]	1.91
celery seed oil	2.83 ± 0.43	3.29	5.06 ± 1.03	3.28	1.14 ± 0.10	1.49
clove terpenes	3.76 ± 1.21[b]	4.30	5.55 ± 1.21	2.99	2.14 ± 0.23[c]	1.36
dillweed oil	3.97 ± 0.49	4.82	7.61 ± 0.01	3.31	2.46 ± 0.04	2.08
dillweed terpenes	4.98 ± 0.85	5.71	8.38 ± 0.13	4.52	3.23 ± 0.51	2.05
eucalyptus terpenes	3.47 ± 0.57	4.13	4.97 ± 0.57	2.69	2.26 ± 0.55[b]	2.05
fennel terpenes	3.27 ± 0.26	3.89	6.33 ± 0.79	3.43	1.63 ± 0.24[b]	1.48
lemongrass oil	3.18 ± 0.77[b]	4.09	2.65 ± 0.19[c]	1.22	2.23 ± 0.13[c]	1.33
spearmint residues	2.66 ± 0.30	3.37	3.85 ± 0.32	2.06	2.25 ± 0.28[c]	1.31

[a] All P values obtained by the two-tailed Student's t-test ($n = 3$); $P < 0.005$ unless otherwise indicated. [b] $P < 0.01$. [c] $P < 0.05$.

Table III. List of Essential Oils and Byproducts That Induced GST Enzyme Activity Greater than 3 Times the Control Level ($T/C > 3.0$) in the Small Intestinal Mucosa of A/J Mice

	small intestinal mucosa		liver		forestomach	
chemical	GST act.,[a] μmol min^{-1} (mg of protein)$^{-1}$	ratio test/control	GST act.,[a] μmol min^{-1} (mg of protein)$^{-1}$	ratio test/control	GST act.,[b] μmol min^{-1} (mg of protein)$^{-1}$	ratio test/control
ambrette musk residues	5.12 ± 0.85	5.87	7.15 ± 0.00	3.85	1.79 ± 0.18	1.13
angelica root oil	2.41 ± 0.27	3.74	4.66 ± 0.26	2.34	1.93 ± 0.14[c]	1.19
camomile oil	2.14 ± 0.12	3.33	5.28 ± 0.26	2.65	1.87 ± 0.13[c]	1.16
fennel oil	2.53 ± 0.42	3.07	5.47 ± 0.12	2.38	1.67 ± 0.30	1.37
galanga root oil	2.68 ± 0.25	3.25	4.53 ± 0.26	1.97	1.76 ± 0.32	1.45
hops oil	3.24 ± 0.25	3.76	5.52 ± 0.72	2.95	0.92 ± 0.06[c]	1.20
lime oil tails	2.72 ± 0.32	3.45	4.37 ± 0.90	2.34	1.29 ± 0.17	0.75
origanum oil	2.75 ± 0.29	3.54	6.10 ± 0.90	2.81	1.67 ± 0.32	1.11
parsley leaf oil	2.70 ± 0.11	3.75	4.75 ± 0.78	2.29	1.57 ± 0.13	0.89

[a] All P values obtained by the two-tailed Student's t-test ($n = 3$); $P < 0.005$ unless otherwise indicated. [b] All P values >0.05 unless otherwise noted. [c] $P < 0.05$.

MATERIALS AND METHODS

Chemicals. All essential oils and byproducts were generous gifts from Berje, Inc., Bloomfield, NJ. Glutathione (GSH) was purchased from Sigma Chemical Co., St. Louis, MO. 1-Chloro-2,4-dinitrobenzene (CDNB) was obtained from Aldrich Chemical Co., Milwaukee, WI.

Animals. Female A/J mice, 7 weeks of age, were obtained from the Jackson Laboratory (Indianapolis, IN). They were acclimated for 1 week after arrival before they were fed semipurified diet (ICN Nutritional Biochemicals, Cleveland, OH) until the end of the experiment. One week after the start of the semipurified diet they were divided into experimental and control groups with three mice per group. The experimental groups were given 30 mg per dose of the test compounds, suspended in 0.3 mL of cottonseed oil, by gavage once every 2 days for a total of three doses. The control group was given cottonseed oil alone. Twenty-four hours after the last administration, the mice were killed by cervical dislocation and the forestomach, the proximal one-third of the small intestinal mucosa, and the liver were removed for enzyme preparation. The tissues were homogenized in 1.15% KCl solution by means of a Brinkman homogenizer. The cytosol after 100000g centrifugation for 1 h was obtained and frozen at −80 °C until used. Each sample represents one tissue from each individual animal.

Glutathione *S*-Transferase Assay. The activity of cytosolic GST was assayed according to the method of Habig et al. using CDNB as the substrate (Habig et al., 1974). The reaction was monitored at 340 nm in a Beckman DU64 spectrophotometer. Assays were performed at 30 °C in 0.1 M phosphate buffer, pH 6.5, in the presence of 5 mM GSH and 1 mM CDNB. Complete assay mixture without enzyme was used as the control. Data were analyzed by Student's t-test, and P values were obtained in comparison to the control.

RESULTS

A total of 30 essential oils and 16 byproducts (Table I) were tested for their ability to induce increased activity of GST in the forestomach, liver, and small bowel mucosa of female A/J mice. A large number of the samples tested showed activity as inducers of GST. Table II lists nine of the most active ones that induced GST enzyme activity significantly higher than that of the control level in all three tissues examined. With this group the GST activity in the cytosols of small intestinal mucosa was increased to greater than 3 times the control level. In the liver cytosols, the value of increased activity (T/C) ranged from a high of 4.52 to a low of 1.22. The low value was, nevertheless, significantly different from the control ($P < 0.05$). A greater than 30% increase of enzyme activity in the forestomach cytosols was set as one of the criteria of activity for this group.

A second group of nine test samples that induced increased GST activity less than 30% in the forestomach are listed in Table III. The induced GST activity was greater than 3 times the control in the small intestinal mucosa and generally greater than 2 times in the liver.

A third group of eight test samples that were considered active are listed in Table IV. These materials did not induce increased GST activity significantly in the forestomach (except for bergamot terpenes which had $T/C = 1.22$, $P < 0.05$). The increased GST activity in one of the cytosols of the small intestinal mucosa or the liver was greater than 2.5 times that of the respective controls. The separation of group 2 from group 3 (T/C ratio of 3 vs 2.5 in the small intestinal mucosa) was done to prioritize future fractionation decisions rather than to indicate significant difference between the two groups.

The rest of the samples did not increase GST activity greater than 2.5 times the control level in any of the tissues examined. This last group of samples may contain active compounds that are present in low concentrations or compounds that are not very active and yet are present in moderately high concentrations. The values of the GSTspecific activity of all the test samples are given as supplementary material.

Table IV. List of Essential Oils and Byproducts That Induced GST Enzyme Activity Greater than 2.5 Times the Control Level ($T/C > 2.5$) in either Small Intestinal Mucosa or Liver of A/J Mice

	small intestinal mucosa		liver		forestomach	
chemical	GST act.,[a] μmol min^{-1} (mg of protein)$^{-1}$	ratio test/control	GST act.,[a] μmol min^{-1} (mg of protein)$^{-1}$	ratio test/control	GST act.,[b] μmol min^{-1} (mg of protein)$^{-1}$	ratio test/control
bergamot terpenes	2.49 ± 0.69	2.85	4.22 ± 0.56	2.27	1.90 ± 0.14[c]	1.21
ginger oil	2.37 ± 0.23	2.88	4.01 ± 0.30	1.74	1.14 ± 0.07	0.94
lemon oil terpenes	1.98 ± 0.28	2.36	4.25 ± 1.37	2.65	1.35 ± 0.17	1.23
lime oil terpenes	1.71 ± 0.09	2.04	5.14 ± 0.58	2.79	1.37 ± 0.20	1.24
orange oil residues	2.16 ± 0.41	2.74	4.76 ± 0.11	2.55	1.53 ± 0.17	0.89
peppermint tail fractions	2.14 ± 0.22	2.71	4.46 ± 0.59	2.39	1.58 ± 0.09	0.92
sweet basil oil	1.90 ± 0.33	2.95	5.66 ± 1.92***	2.84	1.64 ± 0.17	1.02
thyme oil	1.80 ± 0.48	2.50	4.62 ± 0.73	2.22	1.62 ± 0.09	0.92

[a] All P values obtained by the two-tailed Student's t-test ($n = 3$); $P < 0.005$ unless otherwise indicated. [b] All P values >0.05 unless otherwise noted. [c] $P < 0.05$.

DISCUSSION

The present results indicated a large number of essential oils are capable of inducing increased activity of the detoxifying enzyme GST when given orally. Since an increase of GST activity suggests anticarcinogenic potential, the essential oils that are listed in Tables II–IV are expected to contain ingredients that may be useful as chemopreventive agents. A preliminary check on the most common essential oil constituents such as *trans*-anethole, cineole, and linalool did not show any enzyme-inducing activity. On the other hand, *d*-limonene ($T/C = 1.39$) and *d*-carvone ($T/C = 2.37$), two known inhibitors of carcinogenesis (Elegbede et al., 1984; Wattenberg et al., 1989), were found to induce increased GST activity significantly higher than that of the control (unpublished results). Since *d*-limonene and *d*-carvone constitute more than 95% of caraway oil (Formacek and Kubeczka, 1982), it is not surprising to find high GST-inducing activity with the oil. The activity of some other oils and byproducts cannot be correlated readily with known common constituents. For example, the terpenes (dillweed terpenes, etc.) and the residues (ambrette musk residue, etc.) do not contain appreciable amount of volatiles such as limonene and carvone. To determine the active ingredients that are responsible for the enzyme-inducing activity of these essential oils, detailed fractionation experiments are required.

The essential oils and other natural products that provide most of the raw materials for flavoring needs are being consumed in huge quantities. Commercial use of these materials includes soft drinks, cake mix, cosmetics, detergents, and many other products that are ingested by or come in contact with the general population. The consumption of any one oil on a daily basis is estimated to be quite low. They are, nevertheless, interesting prospects as potential sources of biologically active substances. Since these natural oils are the essence of plants, they are most suitable for the detection of active chemicals without the interference of tissues and other unwanted substances. Previous experience strongly suggests that potentially useful chemopreventive agents may be isolated from these naturally occurring substances.

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Supplementary Material Available: Tables of the GST activity of all test samples (3 pages). Ordering information is given on any current masthead page.

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