JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 1323–1329

Received February 21, 2001
Accepted June 25, 2001
ISSN 0022-3573

Effect of herbal teas on hepatic drug metabolizing enzymes in rats

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Abstract

We have investigated the effect of herbal teas (peppermint, chamomile and dandelion) on the activity of hepatic phase I and phase II metabolizing enzymes using rat liver microsomes. Female Wistar rats were divided into six groups (n = 5 each). Three groups had free access to a tea solution (2 %) while the control group had water. Two groups received either green tea extract (0.1 %) or aqueous caffeine solution (0.0625 %). After four weeks of pretreatment, different cytochrome P450 (CYP) isoforms and phase II enzyme activities were determined by incubation of liver microsomes or cytosol with appropriate substrates. Activity of CYP1A2 in the liver microsomes of rats receiving dandelion, peppermint or chamomile tea was significantly decreased ($P < 0.05$) to 15 %, 24 % and 39 % of the control value, respectively. CYP1A2 activity was significantly increased by pretreatment with caffeine solution. No alterations were observed in the activities of CYP2D and CYP3A in any group of the pretreated rats. Activity of CYP2E in rats receiving dandelion or peppermint tea was significantly lower than in the control group, 48 % and 60 % of the control, respectively. There was a dramatic increase (244 % of control) in the activity of phase II detoxifying enzyme UDP-glucuronosyl transferase in the dandelion tea-pretreated group. There was no change in the activity of glutathione-*S*-transferase. The results suggested that, like green and black teas, certain herbal teas can cause modulation of phase I and phase II drug metabolizing enzymes.

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Funding: The study was supported by a grant from the New Zealand Pharmacy Education and Research Foundation, New Zealand.

Introduction

Herbal teas, beverages made from the leaves, flowers and other parts of various herbs other than the black or green tea plant, *Camellia sinensis*, are consumed by millions of people around the world. Although many people use the term 'tea', herbal teas are not 'real' teas (Camellia tea). Herbal teas are used as general dietary supplements, as a general tonic, as a prophylactic against illness, as well as treatments for ailments. Herbal medicine is the main component of traditional medicine. It has been used for thousands of years and has made a significant contribution to human health. Recently in New Zealand there has been an increasing demand for herbal teas (on a par with its demand worldwide). Some of the major herbal teas available in New Zealand markets are ginseng tea, chamomile tea, peppermint tea, blackcurrant tea, dandelion tea, lemongrass tea, and spearmint tea. One question always asked about traditional medicines and herbal remedies is: "Do they work?". This is not known and will not be known until clinical evaluations of these remedies are carried out. Some of the herbalists recommend dandelion tea as one of the best herbal teas for regular use to purge out toxic entities from the body. So this herbal tea was selected for studying its effect on hepatic

drug metabolizing enzymes. Dandelion tea is prepared from the dried ground roots of *Taraxacum officinale* (Compositae). Dandelion root consists of a vertical rhizome and taproot, but is now obsolete as a drug in western allopathic medicine. Chemical investigation of Taraxacum indicated the presence of triterpenoids (e.g. taraxasterol, taraxacin, taraxarol), inulin, pectin, asparagin and phenolic compounds (Zhu et al 1999). It finds considerable use in herbal medicine as a diuretic, laxative, and antirheumatic (Newall et al 1996). Dandelion is also consumed as a food – even as a beverage in the form of a coffee substitute. In-vitro antitumour activity has been documented for an aqueous extract, given by intraperitoneal injection into the tumour system (Baba et al 1981). In a human study, dandelion was one of nine herbal ingredients in a proprietary preparation used to treat viral hepatitis (Sankaran 1977).

Peppermint tea and chamomile tea were chosen for screening of their effect on hepatic drug metabolizing enzymes due to their popularity in New Zealand and many people use them regularly in place of green or black tea. Chamomile tea is made from the dried flower heads of *Matricaria chamomillla Linn./Matriacaria recutita Linn.* (Compositae) and constitutes the drug known as German Chamomile or Matricaria. Chamomile is extensively cultivated in Europe where it is widely used in folk medicine for its carminative, spasmolytic and anti-inflammatory properties. Peppermint tea is prepared from the leaves of *Mentha piperata L.* (Lamiaceae). Leaves contain 0.5–4% essential oil consisting of menthol and menthol esters, menthone, menthofuran and other monoterpenes (Bisset 1994). Peppermint is used as a spasmolytic, carminative, and cholagogue; mixed with other herbal drugs, it is also a sedative.

The modulation of drug metabolizing enzymes by dietary anutrients as a possible mechanism of their anticarcinogenic potential is of current interest. Although many studies have reported the modulation of these enzyme systems following exposure to green and black teas (Bu-Abbas et al 1994; Sohn et al 1994; Chen et al 1996; Yang et al 1996; Maliakal et al 2001), to our knowledge there is no information on herbal teas. This study has investigated the effects of three different herbal teas (peppermint, chamomile and dandelion) on the hepatic drug metabolizing enzymes i.e. cytochrome P450 (CYP) isoforms and phase II conjugating enzymes, in rats. We have investigated the effect of caffeine as a low concentration of 0.1% green tea extract on CYP enzymes also. Due to the large consumption of caffeine in the population, knowledge of its effect on drug-metabolizing enzymes is important. Green tea and black tea solutions (2%, w/v) contain caffeine in a range from 0.056% to 0.068% (Chen et al 1996). In this study a 0.0625% aqueous caffeine solution was used.

Materials and Methods

Chemicals

All chemicals and reagents used were of HPLC analytical grade. Caffeine was purchased from Sigma Chemical Co. (St Louis, MO). Sodium dodecyl sulfate, hexan-1-ol, sodium hydroxide, potassium dihydrogen orthophosphate, potassium chloride, HPLC-grade acetonitrile, tert-methylbutyl ether, diethyl ether and methanol were purchased from BDH Chemicals (Poole, UK). Tetrabutylammonium bromide, NADPH, sodium dithionite, *p*-nitrophenol, phenacetin, paracetamol, 2-aminophenol, glutathione, and 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from Sigma Chemical Co. (St Louis, MO). Quinine hydrochloride was purchased from Merck-Schuchardt, Schuchardt, Germany. 3-Hydroxyquinine was a gift from Dr P. Winstanley, University of Liverpool, UK. Debrisoquine and 4-hydroxydebrisoquine were kindly donated by Roche (Auckland, New Zealand). Guanoxan hemisulfate was supplied by Pfizer (Auckland, New Zealand).

Chamomile tea (Vita-fit Nutrition, Christchurch, New Zealand), peppermint tea (Megavitamin Laboratories Ltd, Christchurch, New Zealand) and dandelion tea (Vita-fit Nutrition, Christchurch, New Zealand) were purchased from Sunray Health Products, Dunedin, New Zealand.

Treatment of rats with tea

The study was approved by the University of Otago Animal Ethics Committee (protocol number 5/97), Dunedin, New Zealand. Female Wistar rats, aged eight to nine weeks, were divided into six groups of five rats each. They were housed in hanging wire cages in a temperature-controlled room with a 12-h light–dark cycle and had free access to a commercial rodent diet (R-94, Reliance Stock Food, Dunedin). The animals were allowed to acclimatize for five days before the study.

Herbal tea solutions (2%, w/v) were prepared simulating the usual way of brewing tea. Boiled tap water was added to the required weight of tea powder with intermittent stirring for 10 min. The tea brew was then strained through filters to obtain a clear solution, poured into clean drinking bottles, which were wrapped with

light resistant polyethylene sheets, and were given to three groups of rats. Another two groups of rats were given either a 0.1% w/v aqueous solution of green tea extract (courtesy Dr Masami Suganuma, Saitama Cancer, Saitama-Ken 362, Japan) or an aqueous caffeine solution (0.0625%, w/v). The control group received water only. Rats were pretreated with a tea or appropriate solution for four weeks. Animals were weighed at the start of the experiment and then at the end of each week to monitor any influence of tea on growth rate. The green tea extract used was a standard commercial preparation obtained from American Tea Association. The composition of the green tea extract was as follows: epicatechin (3.36%), epigallocatechin (11.7%), epicatechin gallate (3.32%), epigallocatechin gallate (3.32%), catechin (0.75%), gallocatechin (2.01%), gallocatechin gallate (1.06%), gallic acid (0.86%), caffeine (5.44%) and theobromine (0.32%).

After four weeks of treatment with the appropriate solution, rats were killed by CO_2 asphyxiation. Livers were removed and washed with ice-cold saline, the excess saline was blotted off using absorbent gauze and then the livers were kept frozen at −80°C until used. Liver microsomes and cytosols were prepared by a differential centrifugation method (Robson et al 1987) and stored in phosphate buffer (pH 7.4) containing 20% w/v glycerol at −80°C until required. The content of microsomal protein was determined by the method of Lowry et al (1951). The P450 content was determined as described by Omura & Sato (1964).

Determination of CYP1A2 activity

Activity of CYP1A2 was determined by using phenacetin as the specific substrate probe (Boobis et al 1981; Tassaneeyakul et al 1993a). The activity of high affinity component (CYP1A2) of phenacetin-*O*-deethylase was determined by incubating 5 μM phenacetin with liver microsomes (0.5 mg mL^{-1}) for 30 min. The reaction was terminated by the addition of 1 M NaOH. Formation of the metabolite, paracetamol, was measured by a specific HPLC method (Tassaneeyakul et al 1993a).

Determination of CYP3A activity

Quinine was used as a marker for determining CYP3A activity. The reaction mixture (0.5 mL) containing 30 μM quinine, NADPH, and rat liver microsomes (0.5 mg protein mL^{-1}) was incubated at 37°C for 12 min. The reaction was stopped by the addition of cold methanol (1 mL). Formation of the 3-hydroxyquinine metabolite was monitored by an HPLC method as described by Wanwimolruk et al (1996).

Determination of CYP2E activity

This was determined by using an HPLC method previously developed in this laboratory using *p*-nitrophenol as a substrate. In brief, the reaction mixture containing *p*-nitrophenol (140 μM), rat liver microsomes (0.8 mg mL^{-1}), ascorbic acid (1 mM) and NADPH (1 mM) in a total volume of 0.5 mL phosphate buffer (pH 7.4) was incubated at 37°C for 25 min. After terminating the reaction using perchloric acid (0.6 M), an internal standard (phenacetin 1.5 μg mL^{-1}) was added. The metabolite formed, *p*-nitrocatechol, and the internal standard were extracted into *tert*-methylbutyl ether after alkalinizing with ammonium sulfate. The residue obtained after evaporating off the ether was analysed using an HPLC system consisting of a reversed-phase column (C18, 5 μm, 150 mm × 4.6 mm i.d.), a mobile phase of acetonitrile:water (16:84, v/v, pH 3.0) and a UV detector (243 nm).

Determination of CYP2D activity

Debrisoquine 4-hydroxylase (CYP2D) activity was measured using the method described by Wanwimolruk et al (1995). Briefly, each reaction mixture consisted of debrisoquine (substrate, 60 μM), rat liver microsomal protein (1 mg mL^{-1}) and NADPH (1.2 mM) in a final volume of 1 mL, and was incubated at 37°C for 12 min. The addition of 4 M NaOH stopped the reaction. Guanoxan hemisulfate (98 μM) was used as the internal standard. Analysis of the metabolite formed (4-hydroxydebrisoquine) was performed using an HPLC system consisting of a CN column (Spherisorb-CN, 5 μm, 150 mm × 4.6 mm i.d.), a mobile phase of acetonitrile:phosphate buffer (12:88, v/v, pH 5) and a UV detector (214 nm).

Determination of glucuronosyl transferase activity

Microsomal glucuronosyl transferase activity was determined using two different substrates, *p*-nitrophenol and 2-aminophenol. UDP-glucuronosyl transferase activity towards *p*-nitrophenol was determined using a spectrophotometric method (Luquita et al 1994) with slight modifications. *p*-Nitrophenol (800 μM) was used

as a substrate for the assay. A membrane perturbant Triton-X 100 (0.05 mg/protein) and a β-glucuronidase inhibitor, D-saccharic acid lactone (0.5 mM) were systematically incorporated in the reaction medium. The UDP-glucuronosyl transferase activity towards 2-aminophenol was determined using a method described by Burchell (1974).

Determination of glutathione-*S*-transferase activity

Hepatic cytosolic glutathione-*S*-transferase activity was determined using a spectrophotometric method (Habig et al 1974). This procedure was based on the enzyme-catalysed condensation of glutathione with the model substrate 1-chloro-2,4-dinitrobenzene.

Analysis imprecision

While validating assays for all the CYP and phase II enzymes in our laboratory conditions, the linearity of the enzymatic reactions with respect to microsomal protein concentration and the incubation time were determined. Subsequently, the microsomal protein concentration and the incubation time used were shown to be in the linear range. The reproducibility of the assays was well within the acceptable standards with the coefficients of variation ranging between 5–10%.

Statistical analysis

Results are expressed as mean $\pm$ s.d. Statistical analysis was performed using the SPSS (version 8.0) program. The differences between groups were evaluated using one-way analysis of variance, followed by Dunnett's test for pair-wise comparison and Tukey's family error rate. $P < 0.05$ was regarded as statistically significant.

Results

The rats in each group had similar body weight before treatment. Four weeks of pretreatment with different herbal tea solutions (2%, w/v), green tea extract or caffeine solution did not significantly influence body weight. There was no significant difference in the volume of tea solution consumed by the treatment groups and the water consumed by the control group. None of the animals showed any signs of gross pathology of the internal organs. Liver weight/body weight ratios of rats in the treatment groups were not significantly different from the controls. There was no significant difference in hepatic P450 content between the treatment groups as compared with controls (Table 1).

Hepatic CYP activity

There was a significant decrease in hepatic CYP1A2 activity in the groups of rats pretreated with chamomile, peppermint, or dandelion teas (Figure 1A). The dandelion tea group was the most affected, with an observed activity reduced to 15% of the control, and this was followed by the peppermint tea-treated (24%) and chamomile tea-treated (39%) groups. CYP1A2 activity in the group receiving caffeine solution showed statistically significant enhancement (157%) whereas the green tea extract group produced only a non-significant increase in activity (Figure 1A).

Pretreatment with dandelion and peppermint tea solutions for four weeks caused a significant decrease in

Table 1 Effect of treatment for four weeks with herbal tea solutions (2%, w/w), green tea extract (0.1%) and caffeine (0.0625%) on hepatic CYP content, CYP2D and CYP3A activity.

Group	CYP content (nmol (mg protein)$^{-1}$)	CYP2D activity (nmol mg^{-1} min^{-1})	CYP3A activity (nmol mg^{-1} min^{-1})
Control	0.24 $\pm$ 0.04	0.21 $\pm$ 0.04	0.16 $\pm$ 0.04
Green tea extract	0.23 $\pm$ 0.06	0.20 $\pm$ 0.03	0.17 $\pm$ 0.04
Caffeine	0.25 $\pm$ 0.05	0.16 $\pm$ 0.02	0.14 $\pm$ 0.03
Chamomile tea	0.23 $\pm$ 0.03	0.16 $\pm$ 0.02	0.16 $\pm$ 0.05
Peppermint tea	0.25 $\pm$ 0.03	0.17 $\pm$ 0.02	0.12 $\pm$ 0.001
Dandelion tea	0.25 $\pm$ 0.06	0.19 $\pm$ 0.02	0.11 $\pm$ 0.03

Data are presented as mean $\pm$ s.d. of five individual rats. There were no significant differences between the groups studied.

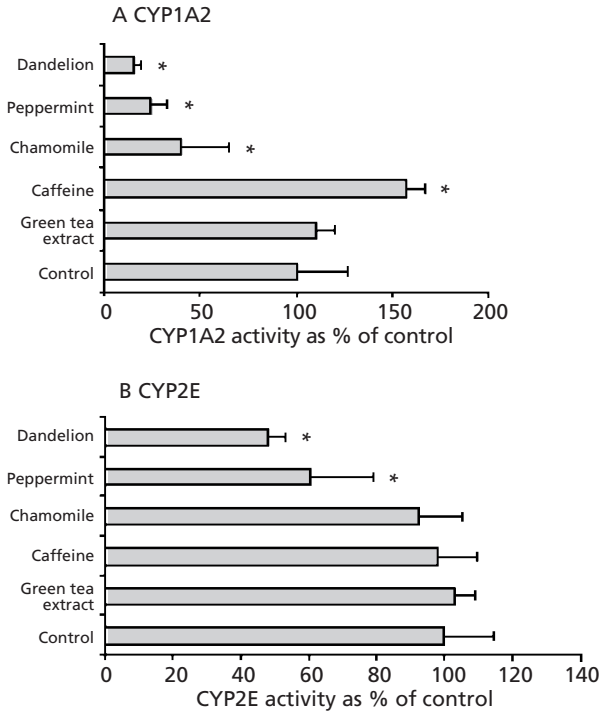

Figure 1 Effect of pre-treatment with herbal tea solutions (2%, w/w), green tea extract (0.1%) and caffeine (0.0625%) on (A) CYP 1A2 and (B) CYP2E activity. Data are presented as mean ± s.d. (n = 5). $*P < 0.05$. The control CYP1A2 and CYP2E activities were 0.174 and 0.501 nmol mg^{-1} min^{-1}, respectively.

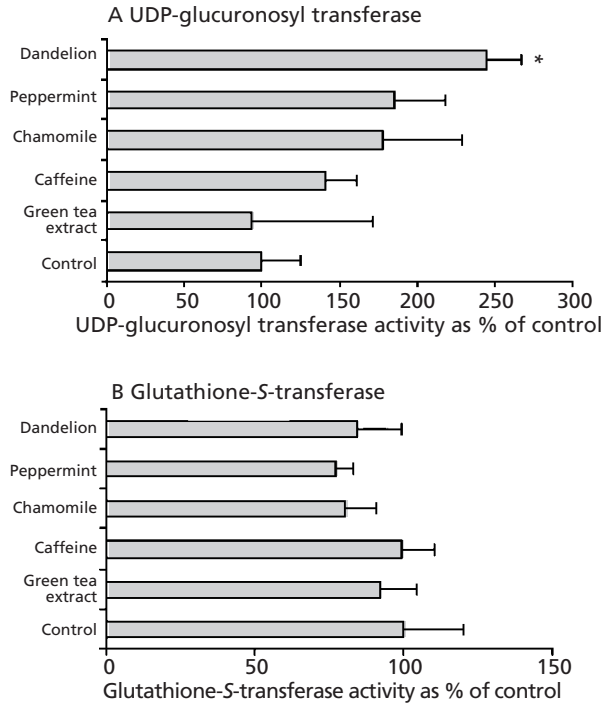

Figure 2 Effect of treatment with herbal tea solutions (2%, w/w), green tea extract (0.1%) and caffeine (0.0625%) on (A) hepatic UDP-glucuronosyl transferase activity toward *p*-nitrophenol and (B) hepatic glutathione-*S*-transferase activity. Data are presented as mean ± s.d. (n = 5). $*P < 0.05$. The control UDP-glucuronosyl transferase and glutathione-*S*-transferase activity was 6.09 nmol mg^{-1} min^{-1} and 0.31 μmol mg^{-1} min^{-1}, respectively.

the activity of hepatic CYP2E to 48% and 60%, respectively, of the controls (Figure 1B). Only negligible changes occurred in the activity of CYP2D and CYP3A in all of the treatment groups (Table 1), which were not statistically different.

Phase II enzyme activity

UDP-glucuronosyl transferase activity

Of all rats in the treatment groups, dandelion tea showed a significant increase in the activity of UDP-glucuronosyl transferase catalysing the conjugation of *p*-nitrophenol (Figure 2A). Dandelion tea produced the maximum increase (to 244% of the control, $P < 0.05$). Although there was a trend for increase in enzyme activity in the groups pretreated either with caffeine, chamomile tea or peppermint tea, these changes were not statistically significant. The activity of UDP-glucuronosyl transferase towards 2-aminophenol was not affected by the treatment with herbal tea, green tea extract or caffeine solution (data not shown).

Glutathione-S-transferase activity

The activity of cytosolic glutathione-*S*-transferase in all treatment groups was not significantly different from that of the control group (Figure 2B).

Discussion

It has long been recognized that modulation of drug metabolizing enzyme activity by dietary components may be important in terms of human health, as this can activate and deactivate a wide range of xenobiotics. There is some evidence relating to metabolic enzyme modulation by green tea and black tea in various rodent models (Wang et al 1994; Yang et al 1996; Weisburger 1999), but there is a dearth of such documented information for various commercially-available herbal teas. In fact, no reports appear in the literature pertaining to the effects of chamomile tea, peppermint tea or dandelion tea on drug metabolizing enzymes either in laboratory animal models or man. Even though

many people use the term 'tea', herbal teas are not 'real' teas. Herbal teas are quite different from black and green tea in that they do not contain antioxidants but they contain other important constituents. In this study, a marked inhibition of hepatic metabolism of phenacetin to paracetamol mediated by CYP1A2 has been observed in rats given three different kinds of herbal teas, chamomile, peppermint and dandelion tea. CYP1A2 plays an important role in the activation of aromatic and heterocyclic amine precarcinogens, including food mutagens such as 2-amino-3-methylimidazo[4,5]quinoline and 2-amino-1-methyl-6-phenylimidazo-[4,5]pyridine (Chen et al 1996). Furthermore, CYP1A isozymes are involved in the metabolism of various other food components such as caffeine, and drugs such as theophylline. Therefore, drinking herbal teas such as peppermint, chamomile or dandelion could have effects on the metabolism of the caffeine-containing preparations as well as other drugs metabolized by CYP1A2. Consequently, there could be an increased level of caffeine in the body or of those drugs metabolized mainly by CYP1A2. However, this in-vitro data alone would be quite insufficient to predict that herbal tea drinking could lead to exaggerated pharmacological effects of drugs metabolized mainly by CYP1A2 enzymes.

The high mineral content of dandelion tea has been reported to greatly increase the chance of drug–drug interactions with conventional medicines that are sensitive to cations (Zhu et al 1999). Those authors have reported a drug interaction between ciprofloxacin and dandelion (whole dried plant), involving changes in the disposition and relative bioavailability of ciprofloxacin in rats. Consequently, those authors cautioned over the possible clinical implications of dosing ciprofloxacin or other quinolone antibiotics when dandelion was being consumed.

The induction of CYP1A2 isozyme after pretreatment with caffeine observed in this study is consistent with previous reports (Sohn et al 1994; Chen et al 1996). Caffeine 3-demethylation, the major pathway for caffeine metabolism, is mainly catalysed by CYP1A (Tassaneeyakul et al 1994). Our observed induction of CYP1A2 by caffeine in rats is in agreement with the concept that many CYP enzymes are induced by their substrates (Yang et al 1996). For example, alcohol and acetone are both substrates and inducers of CYP2E. It is intriguing to note that green tea extract (0.1%) did not cause a significant induction of CYP1A2. In a previous experiment in our laboratory, a significant increase in CYP1A2 activity was observed with green tea extract 0.5% solution (Maliakal et al 2001). With respect to CYP1A2, such a marked modulation was not observed with the low concentration of green tea extract used in this study. Probably the induction of CYP1A caused by green tea extract in rats was dose-dependent.

The significant inhibitory effect on *p*-nitrophenol hydroxylation (CYP2E) exhibited by dandelion tea and peppermint tea is of interest. CYP2E is involved in bioactivation of several nitrosamines and other precarcinogens (Ryan et al 1986). This ethanol-inducible CYP2E1 isoenzyme is implicated in the catalytic bioactivation of many carcinogenic compounds with diverse chemical structure, and other toxins. These include nitrosamines (e.g. nitrosodimethylamine), halogenated alkanes (e.g. chloroform, carbon tetrachloride), anaesthetics such as enflurane, solvents such as benzene, pentane, ethers, ketones, heterocyclics, and vinyl monomers (Tassaneeyakul et al 1993b). Therefore, inhibition of this particular CYP isoform could indicate the usefulness of these teas or their constituents as chemopreventive agents. There is experimental evidence of several dietary components having the potential to inhibit CYP2E enzymes and their possible role in cancer chemoprevention. For example, diallyl sulfide, a thioether and common volatile principle present in garlic and onion, was shown to be an inhibitor of CYP2E1 and a potential inhibitor of nitrosobenzylamine-induced oesophageal cancer in rats as well as experimentally-induced colon cancer in mice (Wargovich 1997).

The increase in the UDP-glucuronosyl transferase activity observed in the rat liver microsomes after pretreatment with the herbal teas could also be an important effect. This stimulation of phase II conjugating enzymes may enhance detoxification of chemical carcinogens. Several dietary phytochemicals active in this regard are found in garlic, onion, cruciferous vegetables and other spices (Wargovich 1997). This observation was also seen with administration of green and black teas in rats (Bu-Abbas et al 1994). Interference in the process of metabolic activation and detoxification of carcinogens is possible through ingestion of certain foods or beverages. However, it is as yet unknown whether short-term effects (such as inhibition of CYP1A and CYP2E) are consistent in the long-term. At least one study has reported that suppression of CYP isozymes was reversed upon long-term dietary exposure to a metabolism-modifying agent, dietary phenylhexyl isothiocyanate (Stoner et al 1995).

In summary, this study has demonstrated that pretreatment with herbal teas in rats caused significant inhibition of some CYP isoforms, CYP1A2 and CYP2E, whereas the activity of phase II glucuronosyl transferase was activated. These results suggested that certain herbal

teas might possess a cancer-prevention potential through reducing activation of carcinogens by CYP and facilitating their detoxification by enhancing phase II conjugating enzymes.

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