VARIABLE INHIBITORY EFFECT OF DIFFERENT BRANDS OF COMMERCIAL HERBAL SUPPLEMENTS ON HUMAN CYTOCHROME P-450 CYP3A4

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SUMMARY

The use of herbal supplements has increased dramatically, making drug interactions with these supplements a major concern. Because herbal supplements are not subject to the same regulations as prescription drugs, we hypothesize that the content of their active ingredients may vary among manufacturers, potentially causing a large variation in therapeutic outcome. The present study aimed to test this hypothesis on commonly used herbal products, i.e. black cohosh (BC), grape seed extract (GSE), green tea extract (GTE) and ginseng. Activity of human CYP3A4 enzyme was used as a parameter to determine the effect of these selected herbal supplements from various manufacturers. The contents of an herbal product, equivalent to one capsule, was extracted with methanol. Aliquots of the extract were tested for their ability to inhibit the metabolism of the human CYP3A4 probe quinine, using an *in vitro* liver microsomal technique. Human liver microsomes and quinine were incubated at 37°C with or without (i.e. control) herbal extract. Formation of quinine's metabolite 3-hydroxyquinine, generated by the CYP3A4-mediated reaction, was measured by HPLC. Seven products of BC were tested, and inhibition

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of CYP3A4 was not observed. Four brands of GSE had no effect, while another five produced mild to moderate but variable inhibition of CYP3A4, ranging from 6.4% by Country Life GSE to 26.8% by Loma Linda Market brand. Among the supplements tested, GTE produced the most pronounced inhibition of CYP3A4, which ranged from 5.6% by Nature's Resource to 89.9% by Natrol GTE product. Nine ginseng products studied had little to no effect on the activity of CYP3A4. This study suggests that GTE use may cause significant interactions with drugs metabolized by CYP3A4. However, the effect on CYP3A4 varied among different brands of GTE, possibly due to variations in their content of the herbal product's active ingredients.

KEY WORDS

dietary supplements, black cohosh, grape seed extract, green tea, ginseng, CYP3A4, drug interactions

INTRODUCTION

For many years it has been recognized that the medicinal use of herbs plays an important role in nearly every culture, including Asia, Africa, Europe and the Americas. Recent surveys suggest that one in three Americans use dietary supplements daily. It is interesting that the rate of herbal usage is much higher in cancer patients /1-4/: in some cases, up to 50% of patients treated in cancer centers. Many of these supplements are herbal in nature /4/. Herbal supplements are generally taken for two main reasons. In the first case they are used to alleviate symptoms of illness - for instance, the widespread use of St. John's wort for relief of depression, the use of echinacea for relief of cold symptoms and the use of *Ginkgo biloba* for improvement in cognition /5/. In the second case, herbal supplements are used specifically with the hope of preventing disease or reducing the risk for certain diseases - for example, the consumption of green tea, grape seed extract and other flavonoid-rich botanicals to take advantage of the natural antioxidants in these products. The use of garlic and its supplement preparation is another example, as it has been demonstrated, at least in animals, to prevent cancer /6,7/.

Cytochrome P450 (CYP) is a family of isoenzymes, mainly found in liver and gut wall. In humans, more than 40 CYP isoforms have

been identified /8-10/. CYP enzymes are responsible for detoxification of a wide range of foreign compounds, including drugs, environmental pollutants and cancer-causing agents (i.e., carcinogens). CYP3A4 is the most important human CYP isozyme as it is involved in the metabolism of more than 40% of all prescription drugs and some environmental toxicants /9-11/. Human CYP3A4 is expressed in the prostate, breast, gut, colon and small intestine. However, its expression is most abundant in the liver and it accounts for 30% of the total CYP protein content /11-16/. Activity of CYP3A4 can be inhibited or induced by drugs, herbs, pesticides, and carcinogens; thus the use of herbal supplements is important in respect to herbal-drug interactions.

Black cohosh (*Cimicifuga racemosa*) is a shrub-like plant native to the eastern forests of North America. It has been used by Native Americans for menopausal symptoms, pre-menstrual discomfort, dysmenorrhea and for a variety of other indications. Several preparations of black cohosh are available from drug stores, herbalists and traditional healers, and are highly recommended as a safe and effective natural remedy for menopausal symptoms. Women who have been advised by their physicians to avoid hormone replacement therapy (HRT), as they are at high risk for breast cancer, or have discontinued HRT after a diagnosis of breast cancer, often use black cohosh as treatment /17/.

Grapes are one of the most consumed fruits in the world and grape seed extract is one of the top-selling herbal supplements in the United States /18/. Commercial preparations of grape seed polyphenols, widely referred to as 'grape seed extract' (GSE), are standardized to contain 95% procyanidins and are marketed in the USA as a dietary supplement, due to its health benefits, particularly the strong antioxidant activity. There are several studies reporting that GSE could be a potential cancer chemopreventive agent /19-22/ and could also prevent heart attack and skin aging /23-25/.

Green tea and tea polyphenols have been investigated extensively because tea polyphenols have strong antioxidant properties and show inhibitory activity against carcinogenesis. Consumption of green tea has been reported to have potential health benefits, such as the prevention of cancer and cardiovascular diseases /26-28/. The active ingredients of green tea are believed to be the polyphenols which are commonly referred to as catechins. Catechins usually account for 30-42% of the dry weight of tea leaves /29/. Since tea is the most popular

beverage in the world and because of the absence of toxicity, tea is an excellent candidate for cancer prevention /28/.

The root of ginseng (*Panax ginseng* C.A. Meyer) has traditionally been used as an herbal medicine in East Asia for at least 2,000 years. It is often used for the treatment of cancer, cardiovascular diseases, hypertension, diabetes mellitus, and liver dysfunction /30/. Ginseng was the second highest selling herbal supplement in the United States in 2000, with gross retail sales of $US 62 million /31/.

Since dietary herbal supplements are not subject to the same FDA regulations as prescription drugs and over-the-counter medications, herbal products generally lack quality control and the regulatory oversight of therapeutic products. Because of this, we hypothesize that the content of active ingredients in herbal supplements may vary among different manufacturers. This variation in the active ingredients may cause variation in therapeutic outcomes and extent of herbal drug interactions. This hypothesis is based on the following observations. First, it has been found with a few herbal supplements, including St. John's wort, ginseng and ephedra, that the content of active ingredients varied widely between brands, and in some cases content variation was also observed between batches of the same herbal product /32-34/. Second, with respect to the antioxidant activity of grape polyphenols, there was a correlation between antioxidant activity and the content of polyphenols in grape seed extracts /22/. Therefore, the current study was designed to investigate this hypothesis with regard to commonly used herbal supplements: black cohosh, grape seed extract, green tea extract and ginseng. Activity of human CYP3A4 was employed as a parameter to determine the effect of these herbal supplements purchased from various manufacturers.

MATERIALS AND METHODS

Chemicals and dietary supplements

All chemicals and reagents used were of analytical grade and distilled water was MilliQ® filtered. Quinine hydrochloride dehydrate, NADPH and sodium dodecyl sulfate were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). 3-Hydroxyquinine was a gift from Dr. Peter Winstanley, Department of Pharmacology and Therapeutics, University of Liverpool, UK.

Four different commercial herbal supplements, namely black cohosh (BC), ginseng, grape seed extract (GSE) and green tea extract (GTE), were randomly purchased from pharmacy stores in California, USA, during the year 2005. There were seven brands of BC, nine brands of ginseng, nine brands of GSE and 12 brands of GTE products. Details of manufacturers, content of each capsule and indications are listed in Table 1.

Preparation of human liver microsomes

A human liver was obtained from a Caucasian male donor aged 50 years who had met traumatic death. He was neither taking medication nor had significant past medical history. The use of human liver was approved by the Southern Regional Health Authority (Otago) Ethics Committee, Dunedin, New Zealand. Human liver microsomes were prepared by standard differential ultracentrifugation as previously described /35/.

Preparation of herbal supplement methanolic extract

The content of herbal supplement studied was determined by weighing the actual content in each capsule. This was performed with six replicates (n = 6 capsules). The contents from these six capsules were combined. The mean (average) content of the capsule was determined. Content of each herbal supplement equivalent to the average content in one capsule was extracted with approximately 40 ml of methanol using sonication for 1 hour. The mixture was adjusted to a final volume of 50 ml with methanol. An aliquot of this mixture was then centrifuged at 2,500 *g* for 15 min. The supernatant was collected and referred to as methanolic extract. This methanolic extract was used to test the dietary supplements' effect on the activity of CYP3A4 enzyme.

CYP3A4 assay procedure

Aliquots (5 µl) of the herbal methanolic extract were tested for their ability to inhibit the metabolism of a CYP3A4 marker substrate using human liver microsomes. All experiments were performed in four replicates. Quinine was used a marker substrate for human CYP3A4 /35/. A substrate concentration of 100 µM quinine was used.

TABLE 1

Product information of the herbal supplements used in this study

Brand name	Manufacturer	mg/ capsule	Indication*
Black Cohosh:			
GNC Herbal Plus	General Nutrition Corp.	50 mg	(no claim)
GNC Nature's Fingerprint	General Nutrition Corp.	540 mg	Support for menopause symptoms
Natural Factors	Natural Factors, Canada	40 mg	Herbal support for hot flashes & night sweats
Nature's Resource	Nature's Resource Products	40 mg	May help alleviate menopausal discomfort
Nature's Way	Nature's Way Products, Inc.	540 mg	Traditional herb for women with mild estrogen-like activity. It is especially helpful during menstruation and menopause.
Spring Valley	IdeaSphere, Inc.	300 mg	Menopause support
Sundown	Sundown, Inc.	540 mg	Menopause support
Ginseng:			
Action Labs	Nutraceutical Corp.	250 mg	Boost your energy to the max
GNC	General Nutrition Corp.	550 mg	Support vitality and overall well-being
GNC Herbal Plus	General Nutrition Corp.	500 mg	(no claim)
Imperial Elixir	GINCO International	600 mg	(no claim)
Life Time	LifeTime Nutritional Specialties, Inc.	650 mg	General tonic and to increase energy and vitality.

Brand name	Manufacturer	mg/ capsule	Indication*
Ginseng:			
Nature's Resource	Nature's Resource Products	250 mg	Promotes physical stamina
Root to Health	Root to Health	500 mg	Provides a 'thermal' effect
Solaray	Nutraceutical Corp.	535 mg	Highly regarded for its adaptogenic properties
Spring Valley	Nature's Bounty, Inc.	100 mg	Ability to revitalize and rejuvenate the entire body, play a role in well-being, ability to support physical performance. Ginseng is also gaining a reputation as an immune support herb.
Grape Seed Extract:			
Country Life	Country Life	50 mg	Helps maintain capillary health
GNC Herbal Plus	General Nutrition Corp.	50 mg	(no claim)
GNC Nature's Fingerprint	General Nutrition Corp.	100 mg	Provides antioxidant support
Jarrow Formulas	Jarrow Formulas	50 mg	A highly potent phyto-antioxidant
Loma Linda Market	Loma Linda Wholesome Foods	100 mg	(no claim)
MRM	Metabolic Response Modifiers	120 mg	Superior antioxidant protection
Sundown	Sundown, Inc.	30 mg	Antioxidants to protect against different types of free radicals
VegLife	Nutraceutical Corp.	105 mg	(no claim)

Table 1 continued

Brand name	Manufacturer	mg/ capsule	Indication*
Grape Seed Extract:			
Walgreens Finest Natural	Walgreen Co.	50 mg	Superior antioxidant protection
Green Tea Extract:			
Country Life	Country Life	300 mg	(no claim)
GNC Herbal Plus	General Nutrition Corp.	150 mg	(no claim)
GNC Natural Brand	General Nutrition Corp.	315 mg	Provides antioxidant support
Henry's Farmers Market	Henry's Marketplace	250 mg	Promotes antioxidant activity
Jarrow Formulas	Jarrow Formulas	500 mg	Antioxidant
Natrol	Natrol, Inc.	500 mg	Helps boost energy, antioxidants
Natural Factors	Natural Factors, Canada	300 mg	Powerful antioxidant support
Natural Factors	Natural Factors, Canada	50 mg	Natural antioxidant protection
Nature's Resource	Nature's Resource Products	150 mg	Premier source of antioxidants
Paradise Herbs	Paradise Herbs, Inc.	250 mg	Powerful antioxidant and revitalizing properties. It also helps support a slim and healthy figure.
Rexall	Rexall, Inc.	315 mg	Provides antioxidant support and health promoting properties
Spring Valley	Nature's Bounty, Inc.	100 mg	Antioxidant support

* These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent disease

This is approximately the apparent K_m value for quinine 3-hydroxylation determined previously in this liver. Human liver microsomes (0.5 mg/ml) were incubated with quinine (100 μM) in the presence or absence (i.e. control) of herbal methanolic extract with 1 mM NADPH in phosphate buffer (0.067 M, pH 7.4) at a final volume of 0.5 ml. Each incubation was performed at 37°C in a shaking water bath for 30 min. The reaction was terminated by the addition of cold methanol solution (1 ml). The samples were vortexed briefly and centrifuged at 2,500 *g* for 10 min. The resultant supernatant (30 μl) was injected onto an HPLC column.

HPLC analysis of 3-hydroxyquinine

The formation of the quinine metabolite, 3-hydroxyquinine, was assayed by a reversed-phase HPLC method as described previously /36/. The detection limit of this assay was 0.1 μM (0.034 μg/ml). The inter- and intra-assay coefficients of variation were <7% over the concentration range of 0.1 to 30 μM.

Statistical analysis

Results were presented as means and standard deviation (SD). Data were analyzed by one-way ANOVA, followed by multiple comparisons utilizing Tukey's test (SPSS version 15.0, SPSS Inc., Chicago, IL, USA).

RESULTS

A preliminary study had shown that the herbal supplements of interest did not include any compounds that interfered with the HPLC assay of 3-hydroxyquinine. This was verified by the results obtained from incubation of each herbal supplement extract with human liver microsomes under the same experimental conditions used, without CYP3A4 substrate (quinine). After incubation, none of the herbal supplement extracts had peaks that interfered with 3-hydroxyquinine in the HPLC analysis.

The seven brands of BC had no effect or caused a slight activation or inhibition of CYP3A4 (Fig. 1). The effect ranged from 7.1% activation by Nature's Resource brand to 5% inhibition by the Spring

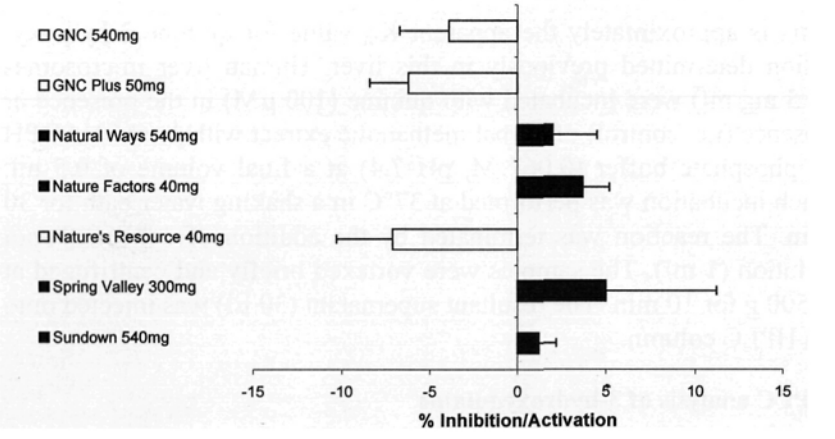

Fig. 1: Effects of different brands of black cohosh dietary supplement on the activity of CYP3A4 measured as the formation of 3-hydroxyquinine (see Methods). Each bar represents mean (and standard deviation) percentage of inhibition or activation of human CYP3A4 activity, obtained from at least four different measurements.

Valley product. The actual activities of CYP3A4 in the presence of all black cohosh extracts were not statistically significantly different from the control value ($p > 0.05$). Of nine brands of grape seed extract tested, four of these caused minor activation of CYP3A4 activity; these were GNC, GNC Plus, Sundown and Walgreen (Fig. 2). The other five brands of GSE, including Country Life, Jarrow, Loma Linda Market, MRM and Veg Life, produced mild to moderate inhibition of CYP3A4. The inhibitory effect produced by GSE ranged from 6.4% by Country Life brand to 26.5% by the Loma Linda Market product. The effect of GSE on CYP3A4 activity did not appear to correlate with the GSE content claimed in these products ($r = 0.5971$, $p > 0.05$). For example, among the products containing 100 mg GSE, the effect varied from no inhibitory effect (or slight activation) by GNC 100 mg to moderate inhibition of 26.5% by Loma Linda Market brand. Nine different brands of ginseng products were studied. Most of them had very little or no effect on the activity of human CYP3A4 (Fig. 3). The inhibitory effect on CYP3A4 produced by ginseng was minimal and ranged from 0.1% by Spring Valley (100 mg) to 9.1% by Action Lab (350 mg) brand. There was no significant correlation between the inhibitory effect of CYP3A4 and the content of ginseng ($r = 0.0374$, $p > 0.5$).

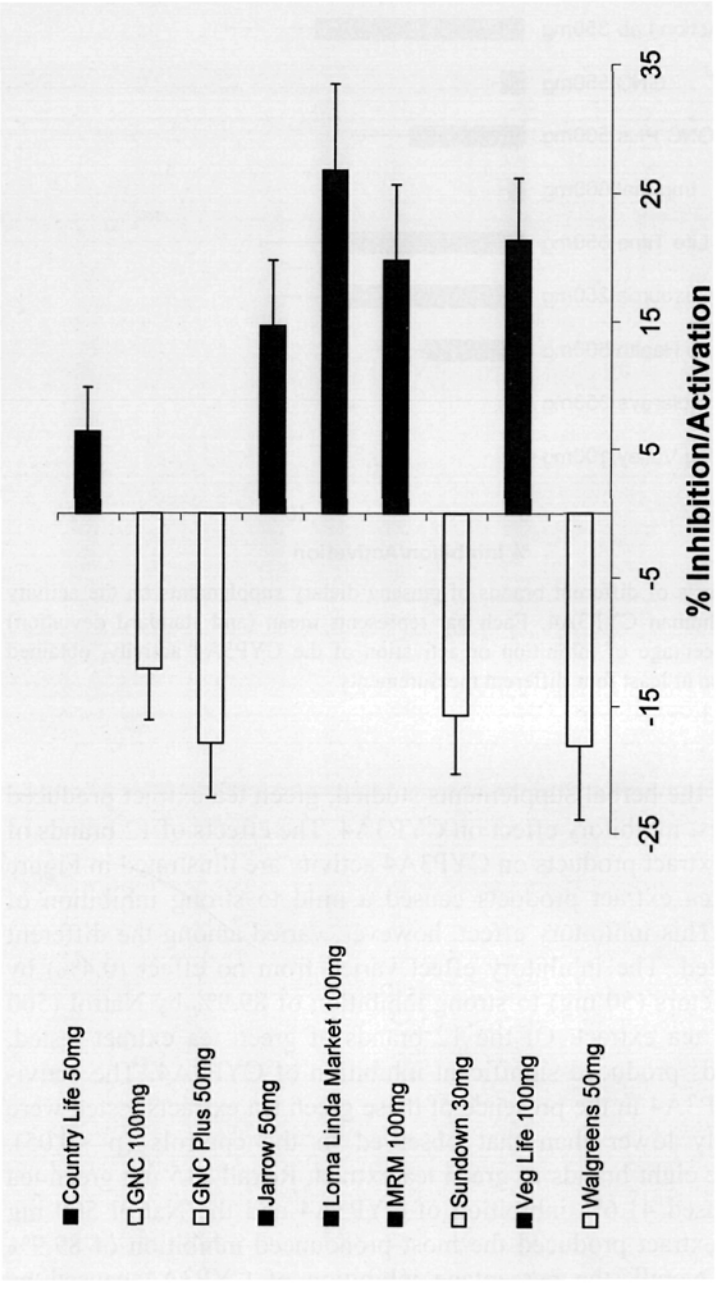

Fig. 2: Effects of different brands of grape seed extract dietary supplements on the activity of human CYP3A4. Each bar represents mean (and standard deviation) percentage of inhibition or activation of CYP3A4, obtained from at least four different measurements.

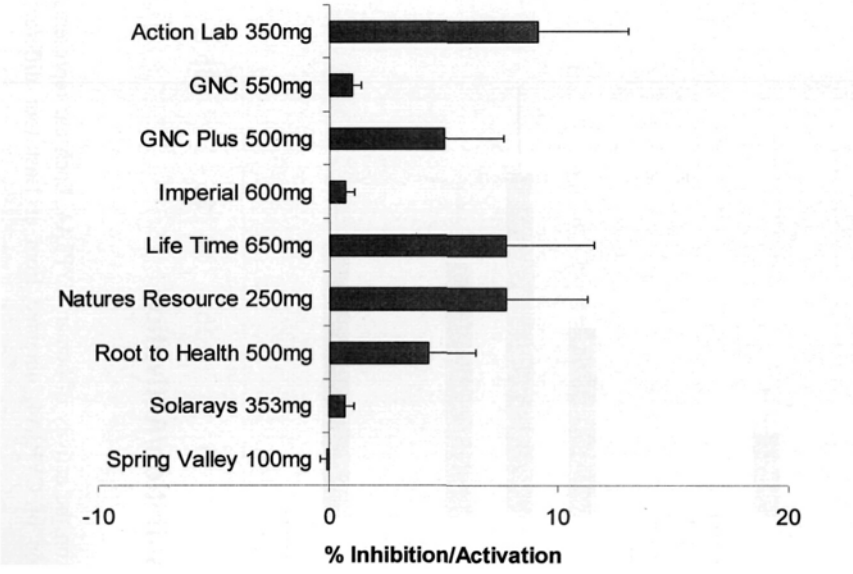

Fig. 3: Effects of different brands of ginseng dietary supplements on the activity of human CYP3A4. Each bar represents mean (and standard deviation) percentage of inhibition or activation of the CYP3A4 activity, obtained from at least four different measurements.

Among the herbal supplements studied, green tea extract produced the strongest inhibitory effect on CYP3A4. The effects of 12 brands of green tea extract products on CYP3A4 activity are illustrated in Figure 4. Green tea extract products caused a mild to strong inhibition of CYP3A4. This inhibitory effect, however, varied among the different brands tested. The inhibitory effect varied from no effect (0.4%) by Natural Factors (50 mg) to strong inhibition of 89.9% by Natrol (500 mg) green tea extract. Of the 12 brands of green tea extract tested, eight brands produced significant inhibition of CYP3A4. The activities of CYP3A4 in the presence of these green tea extracts tested were significantly lower than that observed in the controls ($p < 0.05$). Among the eight brands of green tea extract, Rexall 315 mg green tea extract caused 41.6% inhibition of CYP3A4 and the Natrol 500 mg green tea extract produced the most pronounced inhibition of 89.9% (Fig. 4). Overall, the percentage inhibition of CYP3A4 caused by these products did not correlate ($r = 0.4941$, $p > 0.1$) with the content of green tea extract specified.

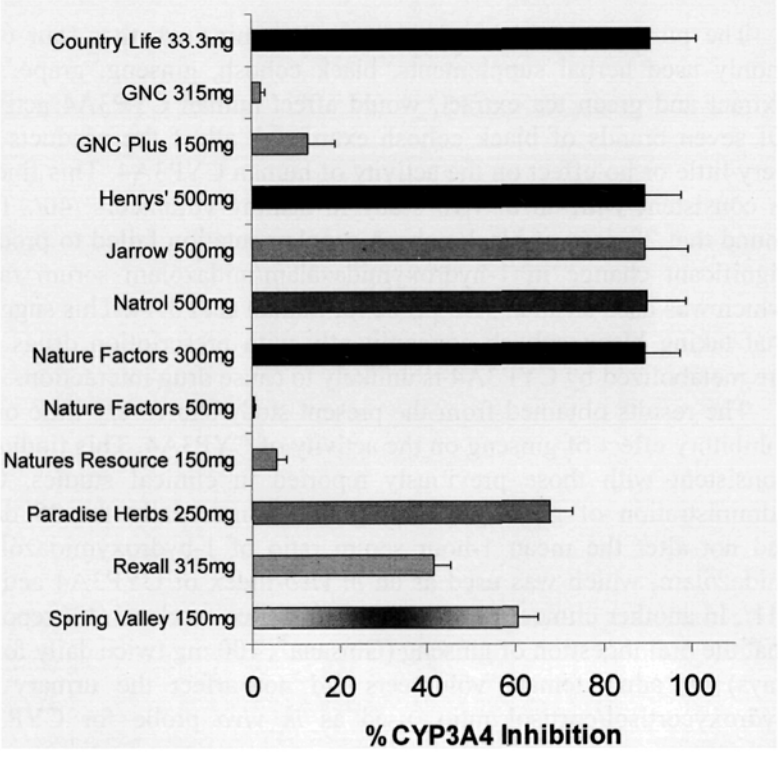

Fig. 4: Effects of green tea dietary supplements on human CYP3A4 activity. Each bar represents mean (and standard deviation) percentage inhibition of CYP3A4, obtained from at least four different measurements.

DISCUSSION

Despite the recent widespread use of herbal supplements, limited information is available on the safety and efficacy of herbal products used alone or in combination with prescription drugs. Further studies are needed to characterize the safety and efficacy of herbal products in humans /37-39/. In the meantime, caution must be taken in prescribing drugs to patients who take herbal supplements. Patients and health care professionals must be encouraged to discuss the use of herbal products and be educated about the potential interactions between herbs and prescription drugs.

The purpose of this study was to determine whether four commonly used herbal supplements, black cohosh, ginseng, grape seed extract and green tea extract, would affect human CYP3A4 activity. Of seven brands of black cohosh examined, all of the products had very little or no effect on the activity of human CYP3A4. This finding is consistent with an *in vivo* study in healthy volunteers /40/. They found that 28 days of black cohosh supplementation failed to produce significant change in 1-hydroxymidazalam/midazolam serum ratios which was used as an *in vivo* probe for human CYP3A4. This suggests that taking black cohosh concomitantly with prescription drugs that are metabolized by CYP3A4 is unlikely to cause drug interaction.

The results obtained from the present study show very little or no inhibitory effect of ginseng on the activity of CYP3A4. This finding is consistent with those previously reported in clinical studies. Oral administration of ginseng (500 mg three times daily for 28 days) did not alter the mean 1-hour serum ratio of 1-hydroxymidazolam/midazolam, which was used as an *in vivo* index of CYP3A4 activity /41/. In another clinical study, Anderson and co-workers /42/ reported that the oral ingestion of ginseng (Ginsana®, 100 mg twice daily for 14 days) by adult female volunteers did not affect the urinary 6β-hydroxycortisol/cortisol ratio, used as *in vivo* probe for CYP3A4 activity.

Our finding with green tea complements a previous study that showed a reduction in CYP3A4 after 4 weeks of green tea catechin pretreatment in healthy volunteers /43/. The most abundant component in the green tea extract used in this study was (-)epigallocatechin-3-gallate (EGCG). EGCG has been shown to be a strong inhibitor of human CYP3A4 /44/. It was expected that EGCG and epicatechin-3-gallate would be the main components responsible for the inhibitory effect of green tea extract /42,43/. The consumption of green tea is especially popular in Asian cultures, and its association with human health benefits has resulted in the inclusion of green tea extracts as common botanical ingredients in dietary supplements, nutraceuticals, and functional foods /45/.

The differences in the effect of herbal supplementary products studied on human CYP3A4 are likely to be due to the variation in active ingredients in these herbal supplements. Even though the active ingredients in each herbal supplement were not measured (due to the complexity and difficulty of the analytical methods), our findings are

consistent with previous studies showing a large variation in the active ingredients in a number of herbal products. These examples include findings reported by many investigators: (i) inconsistencies between actual product composition and labeled content have been reported with St. John's wort, ginseng, echinacea, Ma Huang and androstenedione /32,34,37,38,46/. Analysis of 28 brands of St. John's wort capsules revealed large variations in the total content of active ingredients (sum of hypericin and pseudohypericin). The percentage of label claim varied from 0-109% /32/. Similarly, the Good Housekeeping Institute has also reported a 17-fold difference in the hypericin content and a 13-fold difference in pseudohypericin content present in ten St. John's wort preparations tested /47/. Of 25 commercial ginseng preparations tested, the content of active ingredients (ginsenosides and eleutherosides) differed significantly from labeled amounts /34/. The contents were found to range from 11-328% of the labeled content. There was also significant product-to-product variability.

In conclusion, the findings in the present study indicate that there are small and large variations of the effect of herbal dietary supplements on human CYP3A4. For green tea extract supplements in particular the variations among different brands were remarkable. This questions the quality of the dietary supplements which many patients take presuming these dietary supplement products to be safe. It also supports the recommendation previously made by other investigators /32,38,45-48/ that reliable labeling information and standardized manufacturing practices using biological and phytochemical assays be employed for the quality control of herbal/botanical dietary supplements. In addition, the lack of inhibitory effects of black cohosh, grape seed extract and ginseng on CYP3A4 activity suggests that there is no clinically relevant herbal drug interaction due to enzyme inhibition to be expected upon co-administration of these herbal supplements with prescription drugs metabolized by CYP3A4. However, from our results we cannot exclude that co-administration of green tea extract supplement with CYP3A4-metabolized drugs may cause an increase in the concentrations of such drugs. Clinical studies are warranted to clarify this issue. On the other hand, a possible *in vivo* inhibition of CYP3A4 by green tea extract might contribute to protection against prohepatotoxins, such as aflatoxin B1, that are activated by this isozyme, as postulated for other flavonoids /49/.

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