International Journal of Nutrition and Metabolism Vol. 2(3), pp. 056-062, March 2010
Available online http://www.academicjournals.org/ijnam

Full Length Research Paper

Modulation of xenobiotic metabolism in ginger (*Zingiber officinale* Roscoe) fed rats

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Accepted Date 15 February, 2010.

An association between diet and cancer has been documented only in recent decades. Biotransformation enzymes play an important role in the metabolism of xenobiotics that may be bioactivated or bioinactivated. An *in vivo* experiment was conducted to study the effect of ginger feeding on drug metabolizing enzymes using NIN/male wistar rats. NIN/male wistar rats that were fed with ginger(G) incorporated diet [Control, 0.1, 0.5 and 5%G] for a month and half the animals from all the groups were given 5 mg Benzo(a)pyrene intraperitoneally and after 24 h all the animals were sacrificed and different organs were collected. The drug metabolizing enzymes namely glutathione-s-transferase (GST) and quinone reductase (QR) were estimated in cytosol whereas aryl hydrocarbon hydroxylase (AHH) and uridine diphosphoglucuronyl transferase (UDPGT) were analyzed in microsomes. The enzyme levels were significantly higher in all the carcinogen treated animals compared to their respective controls. Stimulation of GST activity was seen in liver ($p < 0.001$) of all the carcinogen and ginger treated groups compared to control. However, significant activity was observed in lungs ($p < 0.05$), in kidney ($p < 0.01$) and in intestine ($p < 0.001$) at 1 and 5% level of ginger feeding compared to control. Significant stimulation of QR activity was observed in liver ($p < 0.05$) of 1 and 5% ginger fed groups. In lung and kidney increase in the activity was seen in 5% level of ginger feeding. However, there was no significant activity in the levels of UDPGT and AHH. The results of this study demonstrate that ginger intake can stimulate the xenobiotic detoxification. Therefore regular consumption of ginger through diet can confer protective effect against the toxic effect of xenobiotics.

Key words: Ginger, drug metabolizing enzymes, benzo(a)pyrene, detoxification.

INTRODUCTION

One of the major mechanisms of protection against carcinogenesis, mutagenesis and other forms of toxicity mediated by carcinogens is the induction of enzymes involved in their metabolism particularly phase II enzymes such as glutathione-s-transferases, UDP-glucuronoyl transferases and quinone reductases (Manson et al., 1997). Several types of conjugation reactions are present in the body, including glucuronidation, sulfation, glutathione and amino acid conjugation and these reactions require cofactors that can be replenished through dietary sources. Animal studies indicate that stimulation of phase II enzymes is a sufficient condition for obtaining chemoprevention and this can be achieved by administering any of the diverse arrays of naturally occurring and synthetic chemopreventive agents. Numerous observational studies point to the likelihood that diet is a significant determinant of cancer risk (Weisberger, 2000; Van and Pivonka, 2000; Wargovich, 1999). Numerous reviews revealing the merits and possible risk of compounds of diverse categories such as carotenoids, dithiolthiones, flavonoids, glucosinolates, isothiocyanates, allylsulfhydryls, fermentable fibres and other bioactive food components have been found to influence experimentally induced cancers in recent years (Gill and Cross, 2000; Abdullah and Gruber, 2000).

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Plants have the capacity to synthesize a wide array of chemicals to understand the mechanism of function of phytochemicals, which benefit humans. In humans they can have complimentary and overlapping actions including antioxidant effects, modulation of detoxification enzymes, stimulation of immune system, reduction of inflammation, modulation of steroid metabolism and antibacterial and antiviral effects (Lampe, 2003).

The underlying molecular mechanisms by which dietary factors influence the development of cancer are poorly understood. However, a large number of naturally occurring chemicals have been shown to protect against carcinogenesis. Indoles and isothiocyanates (found in cruciferous vegetables) flavonoids (in citrus fruits), coumarins (found in legumes) and organosulphurs (in garlic and onion) are some of the phytochemicals that can prevent chemical carcinogenesis (Kelly et al., 2000, Cristina et al., 2009). These compounds appear to confer resistance against carcinogenesis through their ability to generate a chemical signal that stimulates increased expression of protective antioxidant and detoxification enzymes in specific organs of the host. Chemopreventers in the diet are known to inhibit is carcino-genesis/mutagenesis by inhibiting the activity of enzymes involved in converting xenobiotics to electrophilic moieties (Phase I) or stimulating the activity of detoxifying enzymes involved in the conversion of electrophilic non polar compounds to polar molecules that can be excreted from the body of the host (Phase II). Animal studies indicate that stimulation of phase II enzymes are good markers for chemoprevention. Ginger, the rhizome of *zingiber officinale* is the most commonly used condiment for various foods and beverages. Some pungent constituents present in ginger have potent antioxidant, anti-inflammatory, and cancer preventive properties and are also found to be immunomodulatory, antiapoptotic, and antitumorigenic (Shukla and Singh, 2007, Ali et al., 2008). The major constituents of ginger are the gingerols and shogaols (Chairat and Anchalee, 2008). Regular intake of chemopreventers present in the plant products through diet will play an important role in reducing the risk of developing cancers. Therefore studies were taken up to evaluate the stimulatory effect on the levels of drug metabolizing enzymes, after feeding the rats with ginger through diet.

MATERIALS AND METHODS

Preparation of test diets

Ginger was purchased from local market, peeled, washed, coarsely minced, air dried and pulverized with a blender to a fine powder. This was added (0.5, 1.0 and 5 g) to stock diet and mixed so as to get diets of three compositions containing 0.5, 1 and 5% ginger respectively. The amount of ginger powder present in 15 g diet which the rat consumed daily corresponds to 0.075, 0.15 and 0.75 g which in turn correspond to 0.25, 0.5 and 2.5 g of fresh ginger /15 g of rat diet. The stock diet contained wheat flour 15%, roasted Bengal gram flour 58%, groundnut flour 10%, skimmed milk powder 5%, casein 4%, refined oil 4%, salt mixture 4% and vitamin mixture0.2%. The nutritive value of the diets fed to control and experimental groups were identical.

Study design

The procurement of animals for experiment was undertaken by following the Institutes Animal Ethical Clearance Committee (IAEC) under Committee for the purpose of control and supervision on experiments on animals (CPCSEA), Ministry of Environment and Forests, Government of India. Inbred male NIN/Wistar rats, aged about 8 - 10 weeks, were used for the study. Age matched and weight matched rats were divided into four groups containing 12 animals per group. The control group received stock diet through out the experimental period whereas the experimental groups were fed with the diet containing ginger powder at 0.5%, 1% and 5% levels for a period of one month. The rats were maintained at 22 ± 1.2°C with 50 - 55% relative humidity and 12 h light/dark cycle. Food and water were given adlibitum. The weekly food intake and the body weight of each animal were recorded at the beginning and end of the experiment.

At the end of 1 month feeding, half of the animals were euthanised by placing them in a chamber filled with carbon dioxide. The organs namely liver, kidney, lung and intestine were collected and frozen under liquid nitrogen immediately and stored at -80°C.The remaining rats were given Benzo(a)pyrene (5 mg/rat) intraperitoneally and after 24 h were sacrificed by euthanisation and organs were collected. They were suitably processed as per the standard procedures for enzyme analysis.

The following conventional methods were used to evaluate the detoxification mechanisms of ginger.

Isolation of microsomes and cytosol

The tissues were excised and rinsed with cold 0.154 M KCl. and weights of the tissues were recorded. The tissues were then minced and a 20% (W/V) homogenate was prepared in cold using a polytron homogenizer (Kinematica) for a minute. Homogenate was centrifuged at 15000 x g for 20 min. in a Sorvall OTD-65 B ultracentrifuge using a 50.1 type rotor (fixed angle) to sediment the cell debris, unbroken cells, nuclei, erythrocytes and heavy mitochondria. The supernatant was decanted and centrifuged at 1,00,000 x g for one hour. The supernatant thus obtained (cytosolic fraction) was carefully decanted into small vials and was used to estimate Glutathione-s-transferase (GST) and Quinone reductase (QR).The microsomal pellet was gently suspended in ice cold 0.154 M KCl for the estimation of Aryl hydrocarbon hydroxylase (AHH) and Uridine diphosphoglucuronyl transferase (UDPGT).

Isolation of intestinal mucosal microsomes

Microsomes were prepared from intestinal mucosa by the method of (Stohs et al., 1976). An intestinal segment distal to pylorus was excised, washed and made free of its contents by flushing ice cold saline (0.9% NaCl) through it. The intestinal segment was cut open, the upper layer mucosa was scraped out with the edge of a glass slide and was suspended in 20 ml of ice cold KCl. To this suspension, trypsin inhibitor (5 mg/g weight of small intestine), glycerol (20%,V/V final conc.) and heparin (3 IU/ml) was added to decrease agglutination and degradation of monooxygenase system and was homogenized for 1 min. in a polytron homogeniser and immediately centrifuged at 15,000 x g for 20 min. The supernatant was centrifuged at 105,000 x g for 1 h at 4°C. The supernatant (cytosolic fraction) was used for the assay of GST and QR and the microsomal pellet was suspended in ice cold KCl and was used for

AHH and UDPGT.

Enzyme assays

Aryl hydrocarbon hydroxylase (EC1.14.14.2)

The assay was estimated by the method of Cantrell et al. (1976). Microsomal suspension (50 µl) was added to the incubation mixture containing phosphate buffer (0.2 M, pH 7.5), Ethylene diamene tetra acetic acid (1 mM), Magnesium chloride (30 mM), NADPH (4 mM), Benzo(a)pyrene (5 mM in DMSO) in a final volume of 1 ml. The mixture was incubated for 10min. at 37°C in a shaking water bath and the reaction was arrested by the addition of 37% neutralized formaldehyde and after 5 min. 1 ml of NaOH (1 N) was added to suspend the microsomes. The fluorescent metabolites were then measured in spectroflourimeter using excitation and emission wavelengths of 465 and 522 nm respectively. A standard graph was prepared by using different concentrations of 3-OH B(a)P.

Uridine diphosphoglucuronyl transferase (EC 1.4.1.17)

This assay was done using PNP as substrate according to the method of (Wood Cock and Wood, 1971). The assay was carried out at 37°C in a shaking water bath for 10min The reaction mixture was in a total volume of 0.4 ml containing phosphate buffer [0.4 M, pH 7.4), magnesium chloride (40 mM), PNP (1.6 mM), UDPGA (20 mM), microsomal suspension (50 µl) .The reaction was arrested by the addition of an equal volume of trichloroacetic acid (0.2N TCA). After centrifugation, 0.5 ml of aliquot of the supernatant was added to 1.5 ml of NaOH (0.5 N). The amount of PNP disappeared was monitored at 405 nm in a Gilford Spectrophotometer and were read against PNP standard graph. UDPGT in microsomes of intestinal mucosa was same except for the amounts of microsomal protein, UDPGA (36 mM) and PNP (2.8 mM) and the incubation time were increased from 10 - 30 min.

Glutathione-s-transferase (EC 2.5.1.18)

GST was estimated using 1-chloro-2,4,dinitrobenzene (CDNB) as the substrate according to the method of (Habig et al., 1974). The assay was initiated with the addition of cytosolic enzyme (100 µl) in a reaction mixture of total volume of 3 ml containing potassium phosphate buffer (0.3 M, pH 6.5), reduced glutathione (30 mM), CDNB (30 mM). The reaction was continuously monitored for 5 min. at 37°C in Gilford Spectrophotometer at 340 nm.

NAD(P)H quinone reductase (EC 1.6.99.2)

QR was estimated by the method of (Benson et al., 1990). Cytosolic protein (100µl) was added to the reaction mixture containing Tris HCl (25 mM, pH 7.4), bovine serum albumin (0.7 mg), Tween-20 (1%), Flavin adenine dinucleotide (5 µM) and dicoumarol (10 µM). The activity was measured using NA(D)PH as electron donor and the substrate 2,6 dichlorophenolindophenol (DCPIP) was added to initiate the reaction. Reduction of DCPIP was measured at 600 nm and dicoumarol sensitive portion of the activity was taken as the measure of quinone reductase activity.

Protein concentrations (microsomal and cytosol fractions) were estimated by the method of Lowry et al. (1951)

Statistical analysis

Data analyses were performed using SPSS software version 14.0 for windows. All data were expressed as mean ± SD. Analysis of variance was used to test for differences between the groups. Duncan's multiple range tests was used to determine significant differences among the mean values at $p < 0.05$ (Middle Brooks, 1977).

RESULTS

Table 1 indicates the initial and final body weights of animals fed with ginger through diet at various levels. No changes were observed in the final body weights between any of the groups. At all the levels of ginger feeding (0.5, 1 and 5%), stimulation of GST activity was seen in liver ($p < 0.001$). In other organs namely lungs ($p < 0.05$) kidney ($p < 0.01$) and intestine ($p < 0.001$) significant increase in the enzyme activity was seen at 1 and 5% levels (Table 2). At 0.5% level also increase was observed as compared to control although statistically not significant. In the B(a)P treated groups higher GST activity was observed compared to control. B(a)P treated groups showed a dose response relationship at all the levels of ginger feeding in all the organs (liver $p < 0.001$; lung $p < 0.05$; kidney $p < 0.01$ and intestine $p< 0.001$). There was some increase in the activity of UDPGT in liver, lung, kidney and intestine tissues of rat given ginger through diet, though not statistically significant. The levels of AHH were similar in all the rat tissues. No differences were observed between control and ginger fed groups (Table 3). However the enzyme levels of AHH and UDPGT did not show any significant differences between B(a)P and ginger treated groups though the enzyme levels were more compared to normal control(Table 4). Significantly elevated dose response quinone reductase (QR) enzyme levels ($p < 0.05$) were noted in 1 and 5% ginger fed groups compared to control. Although higher activity was observed in 1%G+B(a)P and 5%G+B(a)P, this increase was significant for 1% B(a)P only as compared to their respective carcinogen untreated ginger fed groups. Results suggest stimulation in liver QR in response to ginger feeding suggesting possible protective role of ginger against xenobiotics. A stimulation in lung QR activity was demonstrated in rats fed with ginger ($p < 0.05$). A dose response trend was observed with various levels of ginger feeding namely 0.5, 1 and 5%. The ginger groups showed no differences in QR levels as compared to their respective groups which were given B(a)P following ginger feeding. Kidney QR levels were also significantly higher in all the carcinogen treated animals compared to their respective controls. The B(A)P treated rats not fed with ginger exhibited enzyme activity similar to B(a)P +ginger fed groups. No dose response to different levels of ginger feeding was observed. The increased levels may be due to tissue mobilization as kidney is one of the site of carcinogen excretion. Intestine QR did not show any significant differences either in the B(a)P treated or untreated groups except at 5% level compared to control, 0.5% and 1%. This difference was significant at $p < 0.05$ in ginger fed B(a)P treated group as compared to all other groups suggesting a synergistic response (Table 5).

Table 1. Initial and final body weights of rats fed diet with ginger (mean ± SD) of 6 rats/group.

Groups	Initial (g)	Final (g)
Control (stock diet)	112.3 ± 5.34	216.4 ± 8.13
Stock diet + 0.5% ginger	115.8 ± 6.26	222.5 ± 11.74
Stock diet + 1.0% ginger	117.1 ± 4.95	229.1 ± 10.86
Stock diet + 5.0% ginger	114.5 ± 5.85	214.4 ± 8.52

Table 2. Effect of ginger (g) on GST activity in tissues of rats.

Treatment	Liver	Lung	Kidney	Intestine
Control	533.2 ± 66.51[a]	105.9 ± 11.28[a]	108.0 ± 14.16[a]	125.4 ± 26.73[a]
0.5% G	682.2 ± 133.8[b]	122.4 ± 21.48[b]	141.6 ± 41.07[a,c]	143.9 ± 41.82[a,c]
% G	773.2 ± 53.58[b]	145.5 ± 36.42[b]	209.4 ± 50.19[b,c]	193.9 ± 14.73[b]
5.0% G	774.0 ± 78.39[b]	167.4 ± 44.82[b]	223.5 ± 82.51[b]	209.4 ± 31.41[b,c]
B(a)P	657.9 ± 48.37[c]	158.9 ± 16.84[c]	171.2 ± 23.2[d]	313.6 ± 70.43 [d]
0.5% G + B(a)P	809.1±61.00[d]	187.9 ± 34.32[d]	223.1 ± 29.80[d,f]	360.8 ±99.22[d,f]
1.0% G + B(a)P	850.2±70.95[d]	214.5 ± 48.63[d]	362.4 ± 45.81[e,f]	485.3 ± 43.65[e]
5.0% G + B(a)P	905.2 ±87.03[d]	251.1 ± 57.16[d]	393.8 ±55.84[e]	523.1±78.07[e,f]

Values are mean ± SD of 6 rats per group. Values are expressed as CDNB units conjugated/min/mg protein. Data were analysed by ANOVA and within each column different letters indicate statistically different values at $p < 0.001$(liver), $p < 0.05$ (Lung), $p < 0.01$(Kidney) and $p < 0.001$(Intestine) compared to control vs ginger fed groups and B(a)P vs B(a)P +ginger fed groups.

Table 3. Effect of ginger (G) on UDPGT activity in rat tissues.

Treatment	Liver	Lung	Kidney	Intestine
Control	5.10 ± 1.343	0.45 ± 0.382	1.55 ± 0.903	0.88 ± 0.920
0.5% G	6.20 ± 1.830	0.62 ± 0.160	2.29 ± 1.102	1.14 ± 0.706
1.0% G	5.65 ± 2.372	0.56 ± 0.167	2.22 ± 1.503	1.82 ± 0.822
5.0% G	6.93 ± 2.077	0.64 ± 0.185	2.52 ± 0.575	1.22 ± 0.394
B(a)P	6.24 ± 1.714	0.696 ± 0.158	1.84 ± 0.967	1.51 ± 1.025
0.5% G+B(a)P	6.64 ± 1.977	0.82 ± 0.395	2.21 ± 0.577	1.92 ± 0.981
% G+B(a)P	7.04 ± 2.433	0.87 ± 0.273	2.62 ± 1.064	2.08 ± 0.822
5.0% G+B(a)P	7.12 ± 1.782	0.78 ± 0.250	2.49 ± 0.989	2.09 ± 0.764

Values are mean ± SD of 6 rats per group. Activity is expressed as nmoles/mg protein.

DISCUSSION

Literature evidences support that naturally occurring nutritive and non-nutritive components of the diets are important, as they possess several biologically beneficial properties. They can delay or counteract onset of chronic diseases including cancer. They are known to play an important role in the inhibition of tumor production (Surh, 2003). The inhibitory action of these compounds are related to several steps involved in the carcinogenic process namely prevention of formation of carcinogens from procarcinogens, induction of coordinated enzyme response and scavenging the active metabolite of the carcinogen.

Dietary factors influence xenobiotic metabolism by inducing specific enzymes responsible for detoxification of xenobiotics. Among them the Glutathione-s-transferases (GST's) are a family of detoxification enzymes involved in cellular protection (Yang, 2006). They catalyze the conjugation of electrophilic compound with reduced glutathione. Substances that specifically increase conjugation systems are considered to be more potent inhibitors of carcinogenesis. The results reported in this study demonstrate stimulation of GST enzymes in liver at all levels of ginger feeding. Since liver is the major site of xenobiotic metabolism and transformation, stimulator

Table 4. Effect of ginger (g) on AHH activity in rats.

Treatment	Liver	Lung	Kidney	Intestine
Control	1.10 ± 0.273	0.40 ± 0.218	0.46 ± 0.127	0.50 ± 0.120
0.5 % G	1.26 ± 0.181	0.40 ± 0.244	0.47 ± 0.212	0.58 ± 0.312
1.0 % G	1.41 ± 0.325	0.42 ± 0.213	0.51 ± 0.208	0.64 ± 0.160
5.0 % G	1.28 ± 0.269	0.51 ± 0.221	0.63 ± 0.253	0.74 ± 0.246
B(a)P	2.59±0.593	0.32±0.138	0.564±0.213	0.64±0.337
0.5 G+B(a)P	2.23±0.523	0.41±0.087	0.45±0.189	0.74±0.409
G+B(a)P	2.19±0.762	0.38±0.151	0.46±0.258	0.77±0.354
5.0 G+B(a)P	2.01±0.800	0.38±0.078	0.46±0.146	0.70±0.374

Values are mean ± SD of 6 rats per group. Activity is expressed as nmoles/mg protein.

Table 5. Effect of ginger on quinone reductase activity in rats.

Treatment	Liver	Lung	Kidney	Intestine
Control	135 ± 29.1^{a}	105 ± 37.8^{a}	142 ± 56.1^{a}	734 ± 116.9^{a}
0.5 % ginger	149 ± 29.6^{a,b}	97 ± 9.2^{a,c}	170 ± 69.7^{a}	671 ± 54.4^{a}
1.0 % ginger	191 ± 52.2^{c,b}	116 ± 30.8^{a,b}	157 ± 27.6^{a}	770 ± 163.1^{a}
5.0% ginger	217 ± 72.4^{c}	132 ± 32.1^{b,d}	190 ± 46.4^{a,b}	846 ± 182.2^{a}
B(a)P	156 ± 31.2^{a}	75±17.6^{c}	241 ± 43.2$^{b, c}$	790±103.5^{a}
0.5 % G+B(a)P	172 ± 42.9^{a}	97±9.67^{a,c}	255 ± 46.5^{c}	802 ± 130.6^{a}
1.0 % G+B(a)P	261 ± 42.1^{c}	106±15.4^{a,b}	241 ± 40.1$^{b, c}$	785 ± 180.4^{a}
5.0 % G+B(a)P	265 ± 52.4^{c}	145±14.7^{d}	248 ±28.4^{c}	1010 ± 211.7^{b}

Values are mean ± SD of 6 rats per group. Activity is expressed as n moles/min/mg protein. Within each column different letters indicate statistically different values at $p < 0.05$ by Duncan's Multiple Range Test between control vs ginger fed groups and B(a)P vs B(a)P+ginger fed groups.

effect of ginger feeding on liver was observed. Other tissues namely kidney, intestine and lungs also participate in the detoxification process and elimination of xenobiotics. Experimental studies in animals have shown that GST's are induced by isothiocyanates, garlic oil (Zhang et al., 1995; Wu et al., 2002). GST levels were increased in persons consuming Brussels sprouts 300 gms/day daily for 3 weeks compared with persons consuming similar diet without glucosinolates (Bogaards et al., 1994). Experiments in Sprague Dawley rats showed that feeding broccoli, cabbage, Brussels sprouts protected against 7, 12-dimethylbenz(a)anthracene (DMBA) induced mammary tumors (Stoewsand, 1988). Similar observations have been made with garlic. Oral administration of diallyldisulphide (DADS) increased liver and colonic GST activity. DADS increased the activity of NADPH and NADH dependent hepatic microsomal mixed function oxidases (Devasagayam et al., 1982). Dietary garlic supplementation has shown to protect against experimentally induced cancers at various sites in animal models (Ip et al., 1992). Some studies have shown that commonly consumed vegetables and spices bring about significant increase in xenobiotic metabolizing enzymes while decreasing B(a)P binding to hepatic DNA (Ramesh and Krishnaswamy, 1994; Conney, 2003).Turmeric and curcumin administration to rats resulted in the stimulation of GST and UDPGT. Turmeric is now being considered as a chemopreventer (Goud et al., 1993; Russo et al., 2005).

Ginger feeding showed some increase in UDPGT activity in target tissues namely liver, lung, kidney and intestine although not statistically significant. There was no effect on AHH activity in any of the tissues. AHH, a component of the microsomal Cyt P450 enzyme complex, catalyses the activation of PAH to ultimate carcinogens. So this effect indicates that ginger feeding is unlikely to effect the phase I pathway involved in drug transformation and metabolism. The bifunctional agents like ethoxyquin (EQ), BHT (butylated hydroxy toluene), PEITC (phenethyl isothiocyanate) are powerful and comprehensive inducers of phase II reactions including an increased capacity of microsomes derived from treated livers to activate AFB_1 to potentially carcinogenic 8,9 epoxide. The capacity for increased formation of this epoxide does not necessarily result in an adverse biological effect, since agents like I-3C (indole 3 carbinol). EQ have been found to be protective against AFB_1 carcinogenicity in long term studies *in vivo* (Manson et

al., 1987). This reflects the balance between activation and detoxification in determining the net effect.

Ginger has shown activity similar to compounds like garlic oil, sinigrin, and caffeine with respect to monofunctional induction of phase II metabolism since it did not show effect on AHH activity. Third group of compounds like α-tocopherol induce some of the phase II parameters while decreasing certain phase I activities. Among both the categories monofunctional inducers of phase II metabolism is considered to be the ideal agent for producing resistance against wide range of chemical insults without adverse effects (Manson et al., 1997).

Type1 NAD(P)H quinone oxidoreductase (NQO) also known as DT diaphorase catalyses the two electron reduction of quinines and prevent formation of free radical oxygen metabolites. It is widely distributed and is primarily cytosolic and catalyses reduction of variety of quinones. Quinone reductase is induced coordinately with phase II metabolizing enzymes by variety of compounds that protect rodents from toxic and mutagenic effects of carcinogens. There is evidence suggesting that monitoring these enzymes is convenient screening method for the anticarcinogenic/antigenotoxic activity (Prochaska and Santamaria, 1988). Increases in activities of NQO1, GST's and other phase II enzymes are known to provide protection against the onset of redox cycling, increase in oxidative stress, neoplastic, mutagenic and other toxic effects of many carcinogens (Rushmore and Pickett, 1993).

BP, a promotogenic and procarcinogenic prototype PAH's requires metabolic activation by CYP 1A1 to exert toxicity. Its metabolite BP 7,8 dihydrodiol-9,10 epoxide (BPDE) and BP quinines binds to CYT P450 A1 = CYP1A1 cellular macromolecules with high affinity to produce carcinogenic transformation (Marshall et al., 1984). Monkey kidney COS 1 cells transiently transfected individually, or in combination with PMT2-NAD[P]H: quinone oxidoreductase (NQO1 or DT diaphorase), PMT2 Cyt P450 1A1, (Cyp1A1), PMT2 Cyt P450 reductase) expressed significant amount of the enzymes were incubated with B(a)P. DNA B(a)P adducts were measured using 32P post labeling and protein adduct detection. Inclusion of cDNA encoding NQO1 along with Cyp1A1 and P450 reductase in transfection reduced the number of DNA adducts from eight to six (Joseph and Jaiswal, 1994). The increase in QR activity in liver with 1 and 5% ginger feeding and stimulation of QR activity in lung and kidney tissues of 5% ginger fed rats could be one of the mechanism through which ginger may be exerting its antimutagenicity towards benzo(a)pyrene. The liver plays a central role in detoxification process. GST and QR enzyme levels were significantly higher in all the carcinogen treated animals as compared to their respective controls. In a study on animals which were pre-treated with dietary curcumin and challenged with B(a)P showed significant enhancement in GST's and QR activities (Rachana et al., 2008). Increases in hepatic QR and GST in ginger fed rats has shown in this study suggest that ginger feeding stimulate detoxification enzymes in target tissue. Potentially genotoxic adducts have to overcome protective events such as DNA repair to exert the ultimate mutagenic and carcinogenic event. Our earlier findings in an *in vitro* study it was shown that DNA damage was inhibited when induced by Benzo(a)pyrene in human peripheral blood lymphocytes of male smokers, male non-smokers and non-smoking females (Nirmala et al., 2007). *In vivo* antimutagenic effect of ginger was also observed in rats that were fed with ginger through diet (Nirmala et al., 2007). These effects could be due to induction of drug metabolizing enzymes. Ginger is also known to possess antioxidant and anti-inflammatory properties due to the presence of active constituents like gingerol and shogaol. Quantification of these constituents were determined by HPLC and it was found that dry ginger powder used in this experiment contained gingerol (183 ± 25.4 mg/g) and shogaols(24.03 ± 2.1 mg/g) respectively. Pharmaco-kinetics of the ginger constituents were studied in a clinical trial and they were absorbed and present as glucuronide and sulfate conjugates (Tao et al., 2009 and Zick et al., 2008).

The results observed demonstrate that ginger intake can stimulate the xenobiotic detoxification enzymes in host tissue. Many non-nutrients in diet are known to induce protective enzymes in animals and humans and show anticancer property. Although literature is replete with reports on in vitro antioxidant activity of dietary substances limited information is available to demonstrate this protective effect when consumed naturally through diet. In a diet survey study conducted by (Thimmayamma et al., 1983), it has been shown that human intake of ginger (2.6 g/consumption unit/day) corresponds to the levels of ginger that has been used in this study. The regular consumption is therefore beneficial to counteract the adverse effects due to exposure to environmentally present genotoxicants.

ACKNOWLEDGEMENT

The authors wish to thank Spices Board, Cochin, India for supporting in part by grants.

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