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Evaluation of *Cissampelos pareira* Against Gastric Cancer and Enzymes Associated with Carcinogen Metabolism

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Abstract

The current study is an effort to identify the effect of a hydroalcohol (50% ethanol) extract of roots of *Cissampelos pareira* (L.) Hirsuta (Menispermaceae) (CPE) in forestomach cancer and on carcinogen metabolizing phase I and phase II enzymes along with antioxidant enzymes. In forestomach, the activities of glutathione *S*-transferase (GST), DT-diaphorase (DTD), and superoxide dismutase (SOD) increased significantly and dose-dependently. The protective effect of CPE was studied against benzo(*a*)pyrene [B(*a*)P]-induced gastric cancer in mice, and the tumor incidence was reduced and the mean number of tumors and the tumor multiplicity were reduced significantly and dose-dependently. The modulatory effect of CPE was also examined on carcinogen metabolizing phase I and phase II enzymes, antioxidant enzymes, glutathione content, lactate dehydrogenase, and lipid peroxidation in liver. Significant increases in the levels of acid-soluble sulfhydryl (–SH) and cytochrome P_{450} contents and in enzyme activities of cytochrome P_{450} reductase, cytochrome b_5 reductase, GST, DTD, SOD, catalase, glutathione (GSH) peroxidase, and GSH reductase but decreased malondialdehyde (MDA) were observed. Butylated hydroxyanisole (BHA) showed an increase in hepatic levels of GSH content, cytochrome b_5, DTD, GST, glutathione reductase (GR), and catalase, whereas MDA formation was inhibited significantly. BHA also showed increased levels of DTD, GST, and SOD significantly in forestomach. The enhanced GSH level and enzyme activities involved in xenobiotic metabolism and maintaining antioxidant status of cells are suggestive of a chemopreventive efficacy of *Cissampelos pareira* against chemotoxicity, including carcinogenicity.

Keywords: Antioxidant enzymes, benzo(*a*)pyrene, carcinogen metabolizing enzymes, chemoprevention, *Cissampelos pareira*, gastric cancer.

Introduction

Gastric cancer may be regarded as a series of malignant diseases characterized by abnormal growth of cells into neoplasm, ability to invade adjacent and even distant tissues, and eventually the death of the patient (Park, 2002). The role of free radicals and active oxygen in the pathogenesis of human diseases including gastric cancer, aging, and atherosclerosis has been recognized (Halliwell et al., 1987). Natural products represent a reservoir of diverse templates, and increasingly, medicinal plants are being tapped to source novel anticancer agents (Harvey, 2000; Morse & Stoner, 1993). *Cissampelos pareira* (L.) Hirsuta (Menispermaceae) is a wound healer and antidote; paste of the roots is externally used for fistula, pruritis, skin disorders, and snake poison. Internally, it is useful in anorexia, indigestion, abdominal pain, inflammation, diarrhea, and dysentery (Anonymous, 1992). It is also used in cough and as it purifies breast milk, it is used in various disorders of breast milk secretion. The material is frequently prescribed for cough, dyspepsia, dropsy, urogenital troubles such as prolapsus uteri, cystitis, hemorrhage and menorrhagia, and calcular nephritis (Kirtikar & Basu, 2001). The roots show significant antibacterial activity against Gram-positive organisms (Adesina, 1982), are used for diarrhea (Amresh et al., 2004), have potent diuretic properties

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(Feng et al., 1962), are used for cardiovascular disorders (Yao & Jiang, 2002), and have antibiotic activities (George & Pandalai, 1949).

The roots and leaves contain several alkaloids and essential oil. The methiodide and methochloride derivatives of hayatine (alkaloid) were reported to be potent neuromuscular blocking agents and produce varying degrees of reduced blood pressure (Patnaik et al., 1973). As per the British Pharmaceutical Codex, the chief constituent of *Cissampelos* is the crystalline alkaloid bebeerine, $C_{18}H_{21}NO_3$, which was formerly called pelosine, and is distinct from buxine, with which it was long supposed to be identical. A small quantity of a crystalline substance, deyamettin, is also present in the plant. The methiodide was found to be one-third as potent as tubocurarine chloride and 1.5-times as potent as gallamine. Hayatine methocholoride has a direct ionotropic effect on the isolated cardiac muscle (Anonymous, 1992). Cissampareine, a *bis*-benzyl-isoquinoline alkaloid, showed a significant and reproducible inhibitory activity against human carcinoma cells of the nasopharynx in cell culture (Morita et al., 1993).

The current experiment was designed to study the efficacy of *Cissampelos pareira* extract (CPE) against benzo(*a*)pyrene-induced forestomach cancer and to evaluate the levels/activities of biochemical markers, namely, phase I and II carcinogen/drug metabolizing enzymes, and antioxidative parameters in mice. The synthetic phenolic antioxidant BHA lowers the incidence of cancer caused by chemical compounds and is used as a standard for the study.

Materials and Methods

Plant material and preparation of extracts

Roots of *Cissampelos pareira* (L.) Hirsuta (Menispermaceae) were collected locally in September 2004 and were identified and authenticated taxonomically at the National Botanical Research Institute (Lucknow, India). They were washed with distilled water to remove dirt and soil and shade-dried. The dried materials were powdered and passed through a 10-mesh sieve. The coarsely powdered material (500 g) was extracted three-times with ethanol (50% v/v). The extracts were filtered, pooled, and concentrated at reduced temperature (-5°C) on a rotary evaporator (Buchi, New Castle, DE, USA) and then freeze-dried (Freezone 4.5; Labconco, Santa Clara, CA, USA) at high vacuum and at a temperature of $-40 \pm 2^{\circ}$C (yield 3.4%, w/w). The dry extracts (CPE) were subjected to various chemical tests to detect the presence of different phytoconstituents (Amresh et al., 2003).

Chemicals

Butylated hydroxyanisole (BHA), 1-chloro-2,4-dinitrobenzene (CDNB), 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), reduced glutathione (GSH), oxidized GSH (GSSG), pyrogallol, 2,6-dichlorophenol-indophenol (DCPIP), potassium ferricyanide, Triton X-100, ethylenediamine tetraacetic acid (EDTA), bovine serum albumin (BSA), sodium pyruvate, thiobarbituric acid (TBA), reduced nicotinamide adenine dinucleotide (NADH), reduced nicotinamide adenine dinucleotide phosphate (NADPH), and benzo(*a*)pyrene [B(*a*)P] were procured from Sigma Chemical Co. (St. Louis, MO, USA). The rest of the chemicals used were obtained from local firms (India) and were of highest grade purity.

Animals

Albino mice (18–24 g) of either sex were purchased from the animal house of the Central Drug Research Institute (Lucknow, India). They were kept in the departmental animal house in a well cross-ventilated room at $27 \pm 2^{\circ}$C, relative humidity 44–56%, light and dark cycles of 10 and 14 h, respectively, for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet (Amrut, India), and the food was with drawn 18–24 h before the experiment; water was allowed *ad libitum*. All the experiments were performed in the morning according to current guidelines for the care of laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals (Zimmerman, 1983).

B(*a*)P-induced gastric cancer

B(*a*)P-induced stomach tumor in mice was performed according to Wattenberg et al. (1980) with minor modifications (Nagabhushan & Bhide, 1987). Group 1 received 1 mg B(*a*)P in 100 μL sesame oil p.o. by gavage twice a week for 4 weeks (a total of eight administrations) to induce the cancer. Two different doses of the CPE (50, 100 mg/kg body weight) were administered p.o. by gavage twice a day to different groups of animals for 6 weeks [2 weeks before the treatment with B(*a*)P and 4 weeks along with the B(*a*)P as in group 1]. The experiment was terminated 14 weeks after the administration of the last dose of B(*a*)P, and all the animals were sacrificed by cervical dislocation after an overnight fast. Ten percent phosphate-buffered formalin was immediately injected into the stomach so that it would be distended and fixed. The forestomach was cut open longitudinally and kept in 10% buffered formalin for 24 h for fixation. Stomach papillomas that were 1 mm or larger in diameter were counted under a stereozoom microscope. Formalin-fixed forestomach was embedded in paraffin wax and processed for histology. The hematoxylin and eosin–stained slides were scored in blind fashion.

The chemopreventive tumor response was assessed on the basis of tumor incidence, mean and multiplicity of tumors as follows:

Tumor incidence = Number of animals having tumors
Mean = (Lowest number of tumors + Highest number of tumors)/2
Tumor multiplicity = Total number of tumors scored/ Total number of animals with tumors

Treatment protocol for biochemical parameters

Group 1 received basal diet and tap water along with 1% Carboxymethylcellulose (CMC) twice a day throughout the experiment and served as the untreated control, and the animals of groups 2 and 3 were administered CPE (50 and 100 mg/kg body weight) p.o. gavage twice a day for 2 weeks along with 1 mg of B(*a*)P in 100 µL sesame oil by p.o. gavage twice a week for 2 weeks. Animals of group 4 were treated with the diet containing 0.75% BHA for 2 weeks with B(*a*)P as administered in groups 2 and 3, which served as positive control for phase II enzymes and antioxidative parameters. Body weights of mice were recorded initially, at weekly intervals, and at the end of the experiment. Animals were fasted overnight prior to the day of termination of the experiment.

Preparation of homogenate, cytosol, and microsome fractions

Animals were sacrificed by cervical dislocation, and the forestomach and liver were blotted dry, weighed quickly, and homogenized in ice-cold 0.15 M Tris-KCl buffer (pH 7.4) to yield a 10% (w/v) homogenate. The forestomach homogenate was then subjected to centrifugation at 15,000 rpm for 30 min at 4°C. The resulting supernatant was used for assaying GST, DTD, superoxide dismutase (SOD), and catalase. An aliquot of this liver homogenate (0.5 mL) was used for assaying the acid-soluble sulfhydryl group (–SH), and the remainder was centrifuged at 10,000 rpm for 20 min. The resultant supernatant was transferred into precooled ultracentrifugation tubes and centrifuged at 105,000 × g for 60 min. The resulting supernatant (cytosolic fraction) was used for assaying total cytosolic glutathione *S*-transferase (GST), DT-diaphorase (DTD), lactate dehydrogenase (LDH), and antioxidant enzymes. The pellet representing microsomes was used for assaying cytochrome P_{450}, cytochrome b_5, cytochrome P_{450} reductase, cytochrome b_5 reductase, and lipid peroxidation.

Estimation of cytochrome P_{450} and cytochrome b_5

Cytochrome P_{450} was determined using carbon monoxide difference spectra. Both cytochrome P_{450} and cytochrome b_5 contents were estimated in microsomal suspension (Omura & Sato 1964) using absorption coefficients of 91 and 185 cm^2/mmol, respectively. They were expressed as nmol/mg protein.

Determination of NADPH–cytochrome P_{450} reductase and NADH–cytochrome b_5 reductase activity

Assay of NADPH–cytochrome P_{450} reductase was determined by measuring the rate of oxidation of NADPH at 340 nm (Omura & Takesue, 1970). The enzyme activity was calculated using the extinction coefficient 6.22 mM/cm. One unit of enzyme activity is defined as that causing the oxidation of 1 mol NADPH per min per mg protein. Assay of NADH–cytochrome b_5 reductase enzyme activity was calculated using the extinction coefficient of 1.02 mM/cm. One unit of enzyme activity is defined as that causing the reduction of 1 mol ferricyanide per min (Mihara & Sato, 1972) and was expressed as µmol NADH oxidized per min per mg.

Determination of glutathione *S*-transferase and DT-diaphorase activity

The specific activity of cytosolic GST was determined and expressed as mmol GSH-CDNB conjugate formed per min per mg protein using an extinction coefficient of 9.6 mM/cm (Habig et al., 1974). DTD activity was calculated using the extinction coefficient 21 mM/cm. One unit of enzyme activity is defined as the amount of enzyme required to reduce 1 µmol DCPIP per min (Ernster et al., 1962). GST was expressed as µmol CDNB-GSH conjugate formed per min per mg protein, and DTD was expressed as µmol DCPIP reduced per min per mg protein

Estimation of reduced glutathione and determination of glutathione reductase and glutathione peroxidase activity

The level of reduced GSH was estimated as an acid-soluble nonprotein sulfhydryl group (Moron et al., 1979). Reduced GSH was used as a standard to calculate µmol –SH per nmol content per g tissue. GSH reductase was determined by the procedure described by Anderson (1985). One unit of enzyme activity has been defined as nmol NADPH consumed per min per mg protein based on an extinction coefficient of 6.22 mM/cm. GSH peroxidase activity (GPx) was measured by the coupled assay method (Rotruck & Pope, 1973). One unit of enzyme activity has been defined as nmol NADPH consumed per min per mg protein based on an extinction coefficient of 6.22 mM/cm.

Determination of catalase and superoxide dismutase activity, and estimation of lipid peroxidation

Catalase was assayed on a UV spectrophotometer at 240 nm by monitoring the decomposition of H_2O_2 (Aebi, 1984). The specific activity of catalase has been expressed as μmol H_2O_2 reduced per min per mg protein. A single unit of enzyme SOD is defined as the quantity of SOD required to produce 50% inhibition of autoxidation (Marklund & Marklund, 1974) and is expressed as units per mg protein. Lipid peroxidation in microsomes, prepared from liver, was estimated using the thiobarbituric acid reactive substances (Das & Banerjee, 1993) and expressed in terms of nmol malondialdehyde (MDA) formed per mg protein.

Determination of lactate dehydrogenase activity and estimation of protein

LDH was assayed by measuring the rate of oxidation of NADH at 340 nm (Bergeyer & Bernt, 1971). The enzyme activity was calculated using the extinction coefficient 6.22 mM/cm. One unit of enzyme activity is defined as that causing the oxidation of μmol NADH per min per mg protein. Protein was determined by using bovine serum albumin (BSA) as standard at 660 nm (Lowry et al.,1951) and expressed as mg/mL.

Statistical analysis

All the data were presented as mean ± SEM and analyzed by Wilcoxon sum rank test (Padmanabha et al., 1982) for the possible significant interrelation between the various groups. A value of $p < 0.05$ was considered statistically significant.

Results

B(*a*)P-induced gastric cancer

Although it has been reported that *Cissampelos pareira* is used in treating various gastric disorders in folk medicine, no experimental evidence has been reported to prevent gastric cancer and antioxidant activity. Thus, in the current study, the protective effects of two doses (50 and 100 mg/kg body weight) of CPE against B(*a*)P-induced gastric cancer in mice were studied. The 50 and 100 mg/kg body weight doses of CPE showed protective effect against B(*a*)P-induced gastric cancer. The tumor incidence was reduced to 90% while the mean number of tumors was reduced significantly ($p < 0.02$) and dose-dependently from 7 to 3.8 (54.29% inhibition). The tumor multiplicity was reduced significantly ($p < 0.02$) only at the higher dose (100 mg/kg). The percentage reduction in the tumor multiplicity was 38.71% (Fig. 1).

Effect on organ and body weight

There were no adverse effects on normal animal health at the given dose levels of *C. pareira*, as there were no decreases in body weight and body weight gain. The relative stomach weights were not varied significantly in mice treated with CPE or BHA. The microsomal protein content was elevated by 1.13-fold in stomach at 50 mg/kg and only 1.09-fold at 100 mg/kg dose of CPE (Table 1). The relative liver weights were increased in mice treated with BHA and higher doses of CPE. The microsomal protein content was elevated significantly after following treatment with BHA as well as CPE ($p < 0.001$). The cytosolic protein level was enhanced ($p < 0.05$) only in the BHA-treated group (Table 2). These results indicated a possible inducing effect of both agents on protein synthesis.

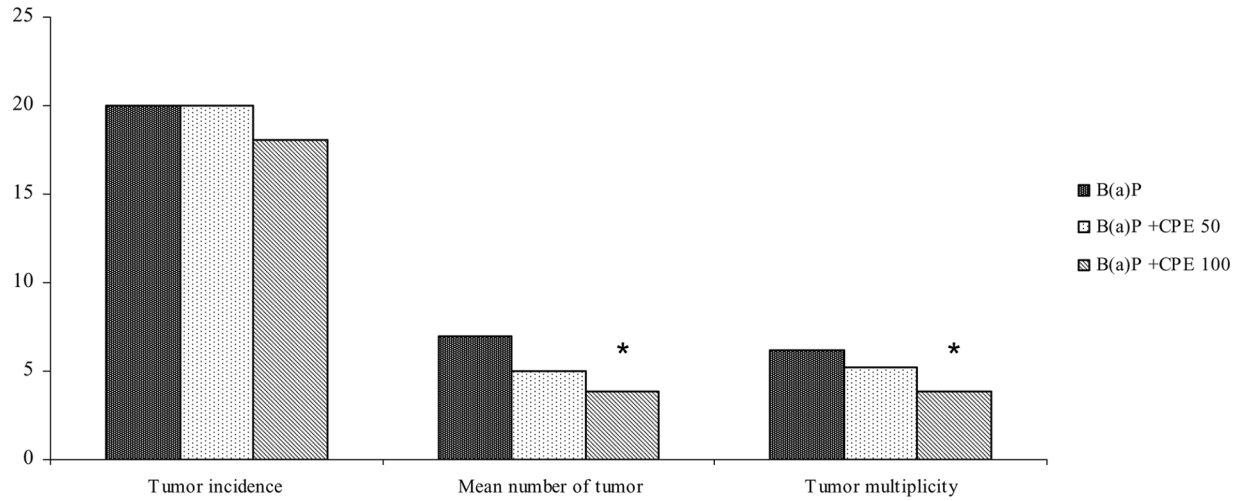

Figure 1. Chemopreventive effect of *Cissampelos pareira* extract (CPE) on benzo(*a*)pyrene [B(*a*)P]-induced gastric cancer in mice. (Values are mean ± SEM, n = 6 mice. *$p < 0.02$ compared with respective B(*a*)P group.)

Table 1. Influence of *Cissampelos pareira* extract (CPE) and BHA on the detoxifying and antioxidant enzymes profile in mouse forestomach.

Treatment	Relative weight of forestomach (%)	GST	DTD	SOD	Protein
Group 1	0.346 ± 0.034	0.198 ± 0.016	0.024 ± 0.0008	5.10 ± 0.434	2.78 ± 0.380
Group 2	0.331 ± 0.025	$0.309 \pm 0.024^{**}$	$0.026 \pm 0.0013^{***}$	$7.20 \pm 0.794^{*}$	3.02 ± 0.256
Group 3	0.392 ± 0.018	$0.267 \pm 0.019^{*}$	$0.028 \pm 0.0017^{*}$	6.30 ± 0.815	3.04 ± 0.300
Group 4	0.346 ± 0.021	$0.294 \pm 0.025^{**}$	$0.027 \pm 0.0013^{*}$	$9.15 \pm 0.990^{**}$	2.74 ± 0.298

Values are expressed as mean ± SEM of six animals.
Group 1, control (only vehicle); group 2, CPE (50 mg/kg) + B(*a*)P; group 3, CPE (100 mg/kg) + B(*a*)P; group 4, BHA (0.75% in diet) + B(*a*)P.
$^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with the respective control group.

Cytochrome P_{450} and cytochrome b_5

These hemeproteins determined in the liver microsomal fraction presented; with the exception of cytochrome b_5, a dose-dependent induction relative to control. In animals treated with CPE extract 50 and 100 mg/kg body weight, the cytochrome P_{450} level was elevated 1.48-($p < 0.001$) and 1.69-($p < 0.001$) fold, respectively. However, in BHA-fed mice, the cytochrome P_{450} level was comparable with that of the control group, whereas the cytochrome b_5 level was significantly elevated 1.29-fold (Table 3).

NADPH-cytochrome P_{450} reductase and NADH cytochrome b_5 reductase

The higher dose of CPE caused a significant increase ($p < 0.05$) in the activities of NADPH-cytochrome P_{450} reductase enzymes, whereas 100 mg/kg body weight CPE caused significant increase (1.1-fold, $p < 0.05$) only in cytochrome b_5 reductase relative to its control. BHA administration did not produce any significant alteration in activities of both these enzymes as compared with control values (Table 3).

DT-diaphorase

CPE increased the specific activity of DTD in the forestomach, in a dose-dependent manner, by 1.08-fold ($p < 0.001$) and 1.17-fold ($p < 0.05$) at 50 and 100 mg/kg kg body weight, respectively, while in the BHA-treated group, DTD-specific activity was elevated significantly by 1.13-fold ($p < 0.05$). Interestingly, the magnitude of induction by BHA was comparatively low in the forestomach (1.13-fold, $p < 0.05$) compared with the level of induction elicited by the higher dose of CPE (1.17-fold, $p < 0.05$) (Table 1). DTD-specific activity was increased in liver by 1.30-fold ($p < 0.05$) and 1.48-fold ($p < 0.01$) at 50 and 100 mg/kg body weight compared to with control, respectively. In the BHA-treated group, the enzyme level was increased by 1.70-fold ($p < 0.001$).

Glutathione *S*-transferase

GST-specific activity was induced significantly in the forestomach of mice after treatment through CPE. It was found induced at the lower dose, the magnitude of elevation being 1.56-fold ($p < 0.01$) in the forestomach. In the BHA-treated group of mice, GST-specific activity was significantly increased ($p < 0.001$) in forestomach (Table 1). In liver, the specific activity of GST was increased by using CPE by 1.55- and 1.72-fold ($p < 0.01$) at a dose of 50 and 100 mg/kg body weight, respectively, while BHA increased the hepatic GST-specific activity by 3.11-fold ($p < 0.001$) (Table 3).

Table 2. Effect of *Cissampelos pareira* extract (CPE) and BHA on body weight gain and toxicity-related parameters in mouse.

Treatment	Body weight		Liver weight × 100/final body weight	LDH	Protein	
	Initial	Final			Microsomes	Cytosol
Group 1	27.5 ± 1.35	27.8 ± 0.85	4.46 ± 0.36	1.83 ± 0.110	6.02 ± 0.541	8.65 ± 0.61
Group 2	27.9 ± 1.03	29.3 ± 1.34	4.49 ± 0.23	1.75 ± 0.149	$8.22 \pm 0.503^{**}$	8.30 ± 0.52
Group 3	28.5 ± 0.63	29.5 ± 1.37	4.83 ± 0.01	$1.41 \pm 0.170^{**}$	$9.75 \pm 0.601^{***}$	9.33 ± 0.75
Group 4	27.3 ± 0.84	28.9 ± 0.95	4.75 ± 0.45	$1.12 \pm 0.090^{***}$	6.32 ± 0.854	$10.43 \pm 0.51^{*}$

Values are expressed as mean ± SEM of six animals.
Group 1, control (only vehicle); group 2, CPE (50 mg/kg) + B(*a*)P; group 3, CPE (100 mg/kg) + B(*a*)P; group 4, BHA (0.75% in diet) + B(*a*)P.
$^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with respective control group.

Table 3. Influence of *Cissampelos pareira* extract (CPE) and BHA on mouse hepatic phases I and II drug metabolizing enzyme levels.

Treatment	Cyt P_{450}	Cyt b_5	Cyt P_{450} R	Cyt b_5 R	GST	DTD
Group 1	0.388 ± 0.013	0.218 ± 0.007	0.146 ± 0.009	3.67 ± 0.169	1.80 ± 0.235	0.040 ± 0.0020
Group 2	$0.576 \pm 0.037^{***}$	0.229 ± 0.013	0.163 ± 0.017	4.16 ± 0.290	$2.79 \pm 0.251^{*}$	$0.052 \pm 0.0039^{*}$
Group 3	$0.654 \pm 0.052^{***}$	$0.250 \pm 0.012^{*}$	$0.170 \pm 0.005^{*}$	4.09 ± 0.332	$3.11 \pm 0.299^{**}$	$0.059 \pm 0.0051^{**}$
Group 4	0.412 ± 0.031	$0.281 \pm 0.017^{**}$	0.161 ± 0.015	3.46 ± 0.275	$5.61 \pm 0.514^{***}$	$0.068 \pm 0.0042^{***}$

Values are expressed as mean ± SEM of six animals.
Group 1, control (only vehicle); group 2, CPE (50 mg/kg) + B(*a*)P; group 3, CPE (100 mg/kg) + B(*a*)P; group 4, BHA (0.75% in diet) + B(*a*)P.
$^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with respective control group.

Reduced glutathione, glutathione peroxidase, and glutathione reductase

At dose levels of 50 and 100 mg/kg body weight CPE treatment, the content of –SH group was elevated significantly ($p < 0.005$) by 1.32-($p < 0.05$) and 1.91-fold ($p < 0.001$), respectively, whereas BHA treatment increased the–SH content by 2.09-fold ($p < 0.001$). Only 50 mg/kg body weight CPE extract was effective in elevating GPx and GSH reductase (GR) activities significantly ($p < 0.001$). Furthermore, BHA elevated only GR significantly ($p < 0.05$) and not GPx (Table 4).

Superoxide dismutase and catalase

The forestomach revealed an increase in the SOD level specific activity by CPE; the lower dose rather than the higher dose appeared to be more potent in inducing enzyme activity. CPE increased the SOD by 1.41-fold ($p < 0.05$) and 1.24-fold at 50 and 100 mg/kg body weight, while BHA increased the SOD by 1.79-fold ($p < 0.01$) (Table 1). The specific activity of SOD was also increased significantly in liver by 1.25-fold at 50 mg/kg body weight. Treatment with CPE at 50 and 100 mg/kg body weight increased the catalase level in liver in a dose-dependent manner by 1.40-($p < 0.05$) and 1.62-fold ($p < 0.05$), respectively. On the other hand, BHA treatment decreased the hepatic catalase level by 5.3% (Table 4).

Lipid peroxidation and lactate dehydrogenase

In the current study, lipid peroxidation was estimated from MDA production. CPE inhibited lipid peroxidation in a dose-dependent manner, that is, by 25% ($p < 0.001$) and 39.28% ($p < 0.001$) at 50 and 100 mg/kg body weight. BHA-treated mice also presented a 32.14% decrease in lipid peroxidation ($p < 0.01$) (Table 4). The specific activity of LDH was inhibited significantly in mice treated with BHA (38.79%; $p < 0.001$) and a higher dose of CPE (22.95%; $p < 0.01$) (Table 2).

Discussion

Chemoprevention is the means of cancer control in which the occurrence of disease, as a consequence of exposure to carcinogens, can be slowed, blocked, or reversed by the administration of one or more naturally occurring or synthetic compounds (Morse & Stoner, 1993). The administration of the carcinogen B(*a*)P caused 100% incidence of forestomach tumors, whereas the oil treatment did not induce any tumors in the recipient animals. The tumor multiplicity in the B(*a*)P alone group was also high (Nagabhushan & Bhide, 1987). The administration of *Cissampelos pareira* extract (CPE) before and during the carcinogen treatment suppressed B(*a*)P-induced forestomach tumorigenesis significantly (Wattenberg 1997). The synthetic phenolic

Table 4. Influence of *Cissampelos pareira* extract (CPE) and BHA on mouse hepatic antioxidant-related parameters and lipid peroxidation.

Treatment	Sulfhydryl content	GPx	GR	SOD	CAT	LPO
Group 1	25.6 ± 2.53	53.9 ± 4.43	36.7 ± 4.18	6.28 ± 0.659	50.5 ± 3.80	0.28 ± 0.010
Group 2	$33.9 \pm 2.15^{*}$	59.8 ± 3.79	$58.9 \pm 2.15^{***}$	7.87 ± 0.537	$70.5 \pm 4.21^{**}$	$0.21 \pm 0.009^{***}$
Group 3	$48.9 \pm 4.04^{***}$	57.6 ± 3.53	$49.2 \pm 2.41^{*}$	6.53 ± 0.674	$81.9 \pm 3.74^{***}$	$0.17 \pm 0.020^{***}$
Group 4	$53.6 \pm 5.31^{***}$	57.1 ± 3.69	$53.4 \pm 4.95^{*}$	5.97 ± 0.579	47.8 ± 4.33	$0.19 \pm 0.018^{**}$

Values are expressed as mean ± SEM of six animals.
Group 1, control (only vehicle); group 2, CPE (50 mg/kg) + B(*a*)P; group 3, CPE (100 mg/kg) + B(*a*)P; group 4, BHA (0.75% in diet) + B(*a*)P.
$^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with respective control group.

antioxidant BHA added to human and animal food is able to lengthen the life of organisms and lower the incidence of cancer caused by chemical compounds and was used as a standard for the study. BHA, a known antioxidant compound, was used to verify our protocols and response in the animal model system. It has already been shown to be effective in cancer chemoprevention (Hocman, 1988). The findings of the current investigation are based on an examination of the inducibility of enzymes involved in carcinogen/drug (xenobiotic) metabolism and maintaining the antioxidant status of the cell. There were no adverse effects on normal animal health at the given dose levels of *C. pareira*. Measurement of LDH as an index of cell damage also supports this observation. Thus, even the higher dose used had a safety margin well clear of the toxic range. An increase in the levels of microsomal protein is indicative of induced protein synthesis and possibly that associated with endoplasmic reticulum.

The microsomal cytochrome P_{450} plays a key role in oxidative activation, inactivation, and the promotion of excretion of most carcinogens as well as in modulating the duration and intensity of their toxicity (Guengerich, 1988). An increase in the levels of different cytochromes with CPE treatment was observed. During oxidative metabolism in the microsomal microenvironment involving the cytochrome P_{450} system, the electron flows from NADPH or NADH through a flavoprotein P_{450} reductase or cytochrome b_5 reductase to different isomorphic forms of cytochrome P_{450} and cytochrome b_5 (Gibson & Skett, 1994). As no inhibition in the activity of these reductases or b_5 content by CPE was noted, it may be that no inhibition in microsomal electron transfer critical for cytochrome P_{450} functional capability is operational.

The protective effects of many naturally occurring chemopreventive agents against carcinogenesis have been ascribed to decreased bioavailability of potential DNA-damaging entities and their destruction into excretable metabolites, facilitated through induction of GST (Coles & Ketterer, 1990). Under the current experimental conditions, CPE treatment elevated gastric and hepatic GST activity significantly, is a critical detoxification enzyme that functions primarily in conjugating "functionalized P_{450} metabolites" with endogenous ligand (GSH), favoring their elimination from the organism (Hartman & Shankel, 1990). There is persuasive evidence to support induction of GST and protection against a wide spectrum of cytotoxic, mutagenic, and carcinogenic chemicals (Lu, 1999). DTD is generally induced in coordination with other phase II detoxifying enzymes (Talalay, 1989). Induction of DTD has been evaluated as a means for determining the potency of many anticarcinogenic substances (DeLong et al., 1986). This enzyme protects against the toxicity of quinones and their metabolic precursors, such as polycyclic aromatic hydrocarbons [benzo(*a*)pyrene] and benzene (Smart & Zannoni, 1984). Our findings indicate a significant induction in the activity of DTD in gastric as well as hepatic region by CPE treatment. Mechanistically, at the cellular level, induced DTD activity circumvents the formation of semiquinone into superoxide anion and results in a stable hydroquinone, thus affording protection from any reactive intermediate (Lind et al., 1982).

It is evident from the current investigation that CPE acts as a "bifunctional" enzyme inducer, as it induces phase I as well as phase II enzyme systems. The current investigation also reveals that CPE can attenuate oxidative stress significantly by modulating cellular enzymatic and nonenzymatic antioxidant defense systems. GSH content in liver was found to be elevated markedly over its basal level. The elevated level of GSH induced by CPE protects cellular proteins against oxidation and also directly detoxifies reactive oxygen species and/or neutralizes reactive intermediate species generated from exposure to xenobiotics, including chemical carcinogens (Ketterer, 1988). The depletion of GSH below its basal level promotes the generation of reactive oxygen species and oxidative stress with a cascade of effects on the functional and structural integrity of cells and organelle membranes (DeLeve et al., 1996).

The induction of GPx and catalase, which are of central importance in the detoxification of peroxides and hydroperoxides, was measured in the hepatic cytosol, where these processes have fundamental importance (Gaetani et al., 1989). The liver plays a major role in the interorgan homoeostasis of glutathione. The liver it is rich in GSH and supplies it to various extrahepatic tissues, such as stomach. GPx uses GSH as a substrate to catalyze the reduction of organic hydroperoxide and hydrogen peroxides (Meister, 1994). The attenuated GR level observed in the current study plays a significant role in the reduction of oxidized GSH to GSH at the expense of NADPH and regulates the GSH-GSSG cycle in the cell (Vanoni et al., 1991). It has been proposed that GPx is responsible for the detoxification of hydrogen peroxide in low concentration, whereas catalase comes into play when the GPx pathway is reaching saturation with the substrate. Under the current experimental conditions, CPE may provide sufficient protection against any pro-oxidant–mediated injury, including tissue damage, owing to intracellular and/or extracellular hydrogen peroxide accumulation. Another antioxidant enzyme, SOD, the activity of which has been found to be augmented by CPE, accelerates dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen. Hydrogen peroxide produced is further removed by catalase (Aebi, 1984). Therefore, CPE-induced SOD activity, in conjunction with catalase, antagonizes any free radical–induced injury. CPE significantly reduced lipid peroxidation, as measured by MDA production, and eliminates the possibility of oxidative stress due to the administration of extract to mice (Rao et al., 2004).

However, CPE showed a protective effect on gastric cancer induced by B(*a*)P, which may be due to its phenolic and/or nonphenolic contents such as flavonoids, and so forth. The current study also demonstrates that CPE increases the activities of phase I and II enzymes and enzymes involved in alleviating oxidative stress. Treatment with CPE resulted in a striking induction in the specific activities of detoxifying enzymes in the stomach as well as in the liver, which strongly suggests the possible cancer chemopreventive potential of *Cissampelos pareira*. Further studies are therefore focused on the fractionation and isolation of the crude extracts of CPE containing the active components responsible for the anticancer activities.

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