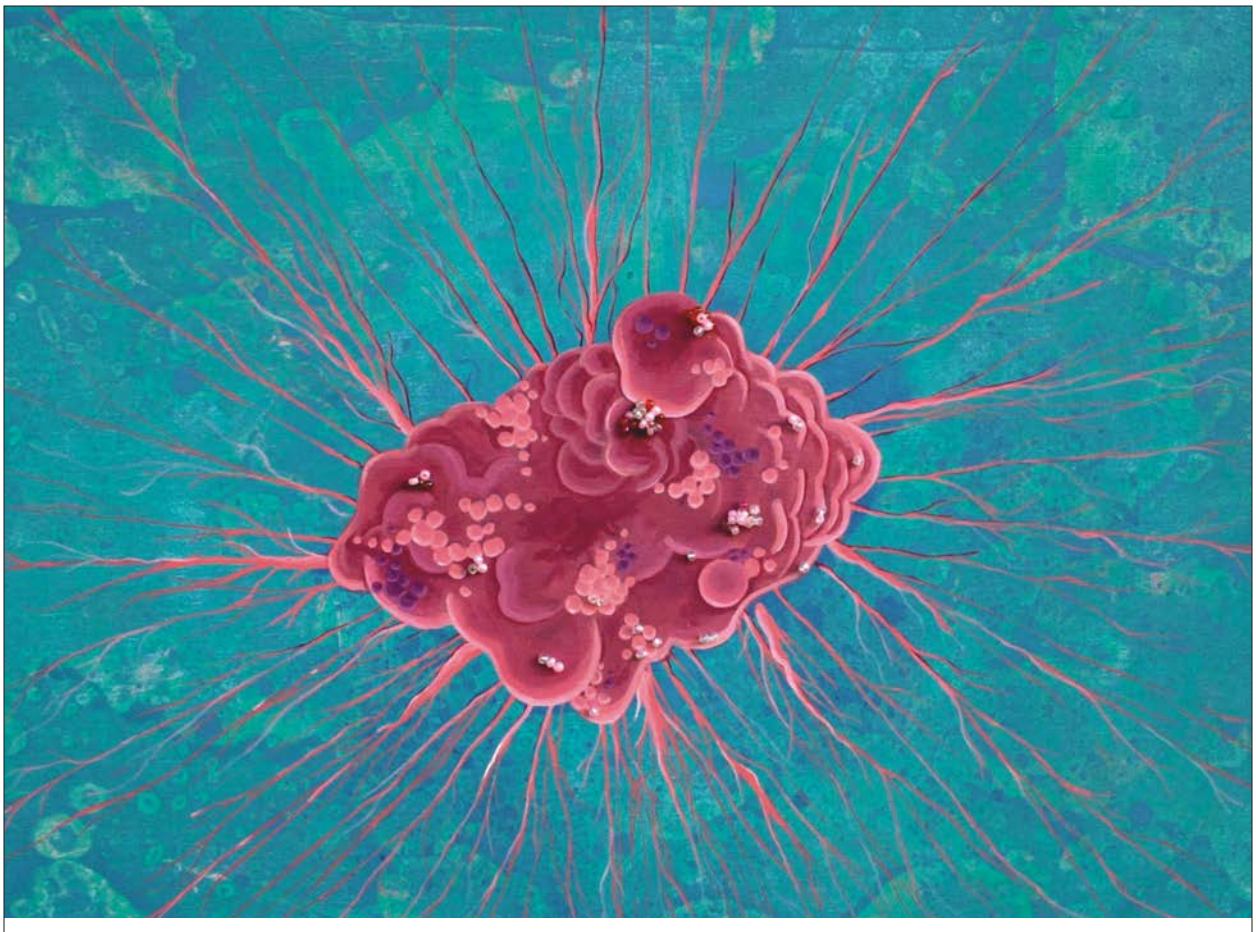

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Determination of Total Phenolics, Flavonoids and Antioxidant and Chemopreventive Potential of Basil (*Ocimum basilicum* L. and *Ocimum tenuiflorum* L.)

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Abstract: Basil (*Ocimum basilicum* L. and *Ocimum tenuiflorum* L.) contains important phytochemicals that have been reported to afford protection against several chronic diseases due to their anti-inflammatory and antioxidant activities. The purpose of this study was to determine the effects of three accessions of *Ocimum tenuiflorum* (Holy Basil) Denmark (HBD), Cuba (HBC), India (HBI)) and one accession of *Ocimum basilicum* (Culinary Basil) (CB) at 1 and 2% levels on azoxymethane (AOM) induced Aberrant Crypt Foci (ACF) in Fisher 344 male rats and to determine the effect of oven drying on total phenolics, flavonoids and anthocyanins of Basil and antioxidative activity. Fifty four rats were divided into 9 groups (n = 6) after a 1 week period of acclimatization. Group 1 was fed a control (C) diet (AIN-93 G) and remaining groups were fed C+1 or 2% CB, HBD, HBC and HBI. All rats received s/c injections of AOM in saline at 16 mg kg^{-1} b.wt. at 7 and 8 week of age. Rats were killed by CO_2 asphyxiation at 17 week of age. The ACF in rats fed C (158.1) was higher than in rats fed C+1% CB, HBD, HBC, HBI (77, 86, 76, 73) and C+2% CB, HBD, HBC, HBI (65, 78, 61, 67). The GST and CAT activities (μmol mg^{-1}) in rats fed C+1 and 2% CB, HBD, HBC and HBI were significantly ($p<0.05$) higher compared to C. Results showed that feeding culinary and Holy Basil leaves reduced the number of AOM-induced ACF and therefore may have implications in the food industry as a potential chemopreventive agent.

Key words: Basil, azoxymethane, aberrant crypt foci, phytochemicals, colon cancer, drying

INTRODUCTION

Cancer is a major health problem in the United States. World colon cancer is one of the leading causes of cancer mortality and the second cause of cancer in the United States (Bianchi and Burke, 2008). The incidence of colon cancer is higher in Australia, Europe, New Zealand and North America, however lower in India, Africa and South America (Johnson and Mukhtar, 2007).

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Epidemiological studies have shown that about 75% of colon cancer cases are directly influenced by diet modification (Coates *et al*., 2007). Consumption of functional foods or beverages may have implications in the prevention of certain diseases (Scalbert and Williamson, 2000) due to the biological activity of dietary fiber, vitamins, minerals and phytochemicals (Coates *et al*., 2007).

Basil (*Ocimum tenuiflorum* L. and *Ocimum basillicum* L.) is one of the oldest herbs/spices within the ocimum genus in the Lamiaceae family and well known for its medicinal value. It is also popular as a kitchen herb. Basil has many uses including culinary, ornamental, aromatic and medicinal. It is grown as a perennial in tropical and subtropical regions of Asia, Africa, Central and South America (Suppakul *et al*., 2003).

Basil is a rich source of essential oils and has been used in confectionaries, condiments, sausages and meats, salad dressings, nonalcoholic beverages and ice cream. The various parts of the Basil plant namely leaves, flowers and stems are being used in the treatment of various disorders such as skin diseases, cold, cough, fever, vomiting, swelling etc. In addition to this, Basil is reported to have antiallergic, anticancer (Hakkim *et al*., 2007), antimicrobial, antiseptic, antispasmodic (Suppakul *et al*., 2003), antifungal, antiviral, anti-inflammatory, analgesic and immuno-stimulatory properties (Umadevi, 2001). Rosmarinic acid is the predominant phytochemical found in *O. basilicum* (Kim *et al*., 2005). Basil also contains potent antioxidants such as caffeic acid (3, 4-di hydroxy cinnamic acid) (Gulcin *et al*., 2007), terpenoids (Loughrin and Kasperbauer, 2001), sinapic acid, ferulic acid (Chamila *et al*., 2003) and radio protective flavonoids such as orientin and vicenin and isoeugenol (Umadevi, 2001). Basil is also a good source of acylated and glycosylated anthocyanins (Mazza and Miniati, 1993). Fourteen anthocyanins have been identified of which eleven of the pigments are cyanidin-based with cyanidin-3-(di-p-coumarylglucoside)-5-glucoside as the major pigment and three minor pigments based on peonidin (Simon *et al*., 1999).

Azoxymethane is a potent carcinogen and has been extensively used in many studies to identify potential chemopreventive agents (Sohn *et al*., 2001). Aberrant crypts are important biomarkers, used for identifying characteristics of colon carcinogenesis (Naoyuki *et al*., 1994). Aberrant crypts exist as early putative lesions in the colon treated with a colon specific carcinogen (Bird, 1987).

Natural antioxidants present in medicinal and aromatic plants may be useful in preventing the deleterious consequences of oxidative damage and are therefore considered as potential chemopreventive agents (Beric *et al*., 2008). Various effects of *Ocimum* sp., including bactericidal, anti-inflammatory, antioxidative, antiulcer, antidiarrheal, chemopreventive, blood-sugar lowering, nervous system stimulation and radiation protection have been reported by Prakash and Gupta (2000) and Umadevi (2001). However, the effect of Basil on detoxification and antioxidant enzymes has not been conducted and there is no available data on its chemopreventive activity against colon cancer. Hence, the main objective of this study was to determine the chemopreventive potential of Basil against azoxymethane induced aberrant crypt foci in Fisher 344 male rats.

MATERIALS AND METHODS

Experimental Design and Animals Housing

Fifty four Fisher 344 male weanling (3 week old) rats were obtained from Harlan, Indiana and were housed in stainless steel wire cages, 2 rats per cage in August 2006. The temperature and relative humidity were maintained at 21°C and 50%, respectively. Light and

dark cycles were maintained at 12 h each. All rats were given free access to potable water. Rats in the control group and rats were fed control [American Institute of Nutrition 93 Growth (AIN 93 G) and rats in the treatment group were fed treatment diets containing Basil (Table 1). All diets were based on the American Institute of Nutrition 93 Growth (AIN 93 G) diet (Reeves *et al*., 1993). After a one-week acclimatization period, rats were divided into 9 groups (6 rats each) (Fig. 1).

Control rats were given free access to AIN 93 G diet (Table 2) throughout the experimental period (13 week).

Preparation of Diet

Four accessions of Basil leaves namely Culinary Basil, Holy Basil Denmark, Holy Basil Cuba and Holy Basil India (Table 1) were obtained from the Winfred Thomas Agricultural Research Station (WTARS), Alabama A and M University, dried using a cabinet drier (Proctor and Schwartz SCM Corporation, Horsham, PA, USA), grounded to a fine powder and mixed in the diet at 1 and 2% levels.

Table 1: Nomenclature and origin of four Basil accessions

Accession No.	Plant introduction No.	Scientific name	Distinguishing features	Origin
31	358463	*Ocimum Basilicum* (Culinary Basil)	Sweet Basil	Macedonia
76	Ames 23154	*Ocimum tenuiflorum*	Hint of Basil	Denmark
77	Ames 23155	*Ocimum tenuiflorum* (Holy Basil)	Clove Basil (Mild)	Cuba
79	288779	*Ocimum tenuiflorum* (Holy Basil)	Clove Basil (Strong)	Gujrat, India

Table 2: Composition of the diets[a]

Ingredients ($g\ kg^{-1}$)	Control (C)	CB	HBD	HBC	HBI	CB	HBD	HBC	HBI
		----------(1%)----------				----------(2%)----------			
Corn starch	397.5	387.5	387.5	387.5	387.5	377.5	377.5	377.5	377.5
Basil	-	10.0	10.0	10.0	10.0	20.0	20.0	20.0	20.0
Common ingredients[b]	602.5	602.5	602.5	602.5	602.5	602.5	602.5	602.5	602.5

CB: Culinary Basil, HBD: Holy Basil Denmark, HBC: Holy Basil Cuba, HBI: Holy Basil India. [a]Formulations of diets based on AIN-93G: American Institute of Nutrition (Reeves and others, 1993). [b]Common ingredients (g): Casein, 200; Dextrose, 132; Sucrose, 100; Soybean oil, 70; Fiber, 50; Mineral mix (AIN-93G), 35; Vitamin mix, 10; L-cystein, 3; Choline bitartrate, 2.5

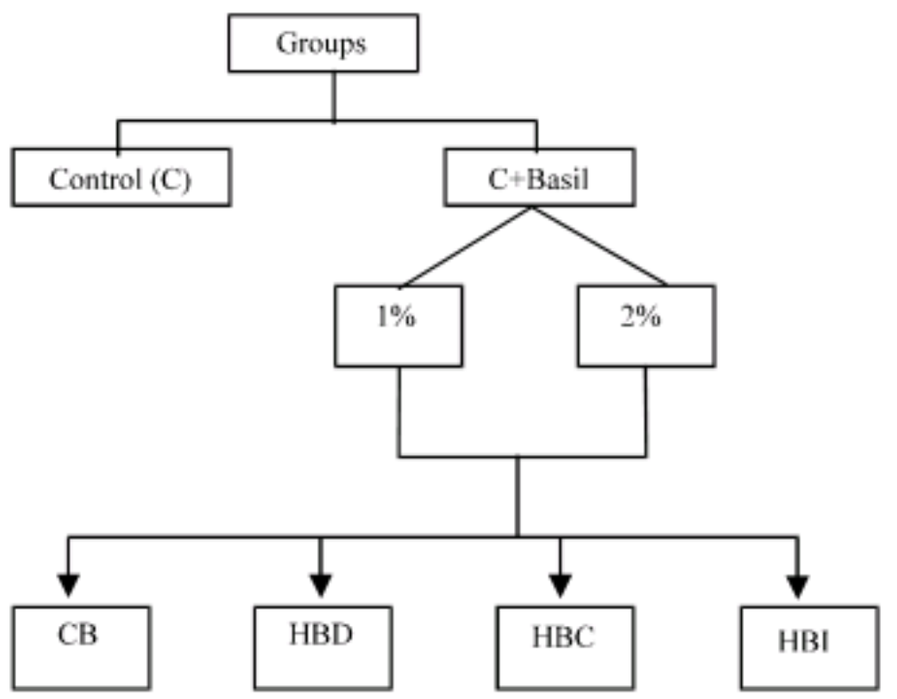

Fig. 1: Experimental design. CB: Culinary Basil; HBD: Holy Basil Denmark; HBC: Holy Basil Cuba and HBI: Holy Basil India. AIN 93 G-American Institution of Nutrition 93-Growth diet

Feed Intake and Body Weights

Daily feed intakes and biweekly body weights were recorded throughout the experiment.

Carcinogen Injection

For induction of ACF, all rats were given s/c injections of Azoxymethane (AOM), (NCI Chemical Repository, Kansas City, MO) in saline @16 mg kg^{-1} b.wt. at 7th week and another at 8th week of age.

Sample Collection

Rats were killed by CO_2 asphyxiation at 17 weeks of age. The colons from rats were removed and flushed with phosphate buffer solution (0.1 M, pH 7.2) and prepared for counting ACF. Liver samples were collected and immediately frozen using liquid nitrogen and stored at -80°C for analysis of enzymes (Glutathione S-transferase and Catalase) (Verghese *et al*., 2002).

Enumeration of Aberrant Crypt Foci (ACF)

Each colon was divided into 2 equal segments (proximal and distal sections). Each respective segment was further divided into 2 cm segments, stained with 0.2% methylene blue for 5-10 min and examined under a light microscope. Enumeration of ACF was performed as described by Bird (1987). The ACF as well as crypts/focus were scored.

Analysis of Cecal Content

Ceca was flushed with potassium phosphate buffer 0.1 M, pH 7.2 and blotted on filter paper to measure cecal weight. Cecal contents were removed and pH was noted.

Glutathione-S-Transferase (GST) Activity

The GST in the liver was assayed by the procedure outlined by Habig *et al*. (1974). Liver samples (1 g) were homogenized in 10 volumes of potassium phosphate buffer (pH 7.0, 0.1 M). The homogenate was centrifuged at 10,000x g for 30 min. The supernatant was centrifuged for a second time at 10,000x g for 10 min. The assay mixture (1 mL) contained potassium phosphate buffer (0.1 M, pH 6.5), 1-chloro 2, 4-dinitrobenzene (1 mM) and glutathione (1 mM). Reactions were started by the addition of 100 µL of sample and change in absorbance at 340 nm as a function of time was monitored in a Cary 1/3 UV/VIS dual beam spectrophotometer. Total enzyme activity was measured at the end of 5 min of reaction.

Determination of Catalase Activity

Liver catalase was estimated in a UV recording spectrophotometer at 240 nm by monitoring the decomposition of H_2O_2 as described by Aebi (1984). The reaction mixture (1 mL) contained 0.02 mL suitably diluted cytosol in phosphate buffer (50 mM, pH = 7.0) and 0.1 mL of 30 mM H_2O_2 in phosphate buffer. The specific activity of catalase was expressed as moles of H_2O_2 reduced.

Preparation of Basil Extracts

Basil leaves of four accessions were collected from the Winfred Thomas Agricultural Research Station (WTARS) and dried using a cabinet drier (Proctor and Schwartz subsidiary of SCM corporation, Horsham, PA, USA), using an alternative upward-downward air circulating pilot plant drier. The drying temperature was set at 50° C, with a 1.5 m sec^{-1} air flow. The drying process was carried out for 3 h soven drying method. They were ground to a fine powder in a grinder and used for extraction. One hundred mililiter of 80% methanol

was added to 10 g of Basil powder. The extracts were filtered using Whatman No.1 paper and the filtrate was collected and dried using a rotary evaporator (Buchi Rotavapor R-205 equipped with self cleaning dry vacuum system™ Model 2025) at 40°C for 15 min and stored at -20°C (Dewanto *et al*., 2002).

Determination of Total Phenolic Content

Total phenolic content of Basil was measured using a modified Folin-Ciocalteu colorimetric method (Singelton *et al*., 1999). Briefly, 12.5 µL of appropriately diluted sample was added to 50 µL of distilled water. Then, 12.5 µL of Folin-Ciocalteu's phenol reagent was added to the mixture. After 5 min, 125 µL of 7% $NaCO_3$ solution was added to the mixture. Prior to spectrometric analysis, the samples were incubated for 90 min at 25°C. The absorbance of the diluted solution was measured at 750 nm versus a blank consisting of all the reaction agents except the extract using a micro plate reader (Synergy HT, BioTek instruments, USA). A standard curve for total phenolics was developed using gallic acid standard solution. The results are expressed as means (mg gallic acid equivalents g^{-1} dry Basil)±SEM for 3 replications.

Determination of Total Flavonoid Content

A colorimetric assay (Kim *et al*., 2003) with some modifications was used to quantify total flavonoid content. Briefly, 25 µL of the diluted Basil sample was added to 125 µL of ddH_2O. Subsequently, 7.5 µL of 5% $NaNO_2$ was added to the mixture. After the mixture was allowed to stand for 5 min, 15 µL of 10% $AlCl_3$ was added. The mixture was incubated at ambient temperature (25°C) for an additional 5 min. Following that and 50 µL of 1 M NaOH was then added to the mixture. The mixture was immediately diluted by the addition of 27.5 µL of ddH_2O and the absorbance of the mixture was measured at 510 nm against a blank prepared with ddH_2O using a microplate reader (Synergy HT, BioTek instruments, USA). (+)-Catechin was used as standard and the results are expressed as means (mg of catechin equivalents 1 g^{-1} dry Basil)±SEM for three replications.

Determination of Total Anthocyanin Content

The anthocyanin content in Basil was determined using a modified pH differential method (Wolfe *et al*., 2003). Basil extracts were diluted with two buffers separately, one with potassium chloride buffer (0.025 M, pH 1.0) and the other with sodium acetate buffer (0.4 M, pH 4.5). Final concentration of the dilutions was around 20 mg mL^{-1}. After equilibration for 15 min in the dark, the absorbance of each dilution was measured at 510 nm and at 700 nm (or 760 nm) against a blank cell filled with acidified distilled water. Total monomeric anthocyanins content was calculated as follows.

$$\text{Total monomeric anthocyanin (mg g}^{-1}\text{ basi)} = A \times MW \times 1000 / (\varepsilon \times C)$$

Where:

A	= Absorbance
C	= Extracts concentration
Molecular weight	= 449.2
ε	= 26.900

The results were recorded as mean (mg of cyaniding-3-glucoside equivalent/100 g Basil)±SEM for three replications.

Free Radical Scavenging Activity

Briefly, 0.1 mM solution of DPPH (1, 1-Diphenyl-2-picryl-hydrazyl) in methanol was prepared and 70 µL of this solution was added to 210 µL of methanolic extracts of Basil in water at different concentrations (1-5 mg mL^{-1}). The absorbance was measured using a micro plate reader at 517 nm after 30 min (Gulcin *et al*., 2007). A lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Free radical scavenging activity was measured as the amount of extract required to decrease the initial absorbance (517 nm) of DPPH radical concentration by 50% (IC_{50}) as compared to the control according to the equation below.

$$\text{DPPH (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Statistical Analysis

The experimental design is shown Fig. 1. Data were analyzed using the SAS system version 9.0 (SAS, 2004) by Analysis of variance. Values are given as Means±SEM and means were separated using Tukey's studentized range test. The significance was tested at the $p<0.05$ level.

RESULTS

Daily Feed Intake, Weight Gain, Cecal Weight and Cecal pH in Rats Fed Basil

Feed intake and weight gain were significantly ($p<0.05$) higher in rats fed Basil (1 and 2%) diets compared to rats fed the control (AIN -93G) diet (Table 3). However, feed efficiency ratio (weight gain per gram of feed intake) in treatment groups was similar to the control rats. There were no significant ($p<0.05$) differences observed in cecal weight and cecal pH among the rats fed control (AIN-93G) and Basil (1 and 2%) diets.

Incidence of Aberrant Crypt Foci (ACF) in Proximal and Distal Colon of Rats

The number of ACF was significantly ($p<0.05$) lower in all groups fed Basil (1 and 2%) diets compared to the control group (Fig. 2). Higher numbers of ACF were observed in the distal colon compared to the proximal colon in all groups. Reductions in number of ACF in the distal colon were 60% (2% HBC), 59% (2% CB), 58% (2% HBI) and 56% (1% HBI), respectively compared to rats fed the control diet. Rats fed 2% Basil diets had lower number of ACF in the distal colon compared to those fed 1% Basil diets.

Crypt Multiplicity

The size of the ACF is expressed as the number of aberrant crypts/ACF or crypt multiplicity. Rats fed Basil diets had significantly ($p<0.05$) lower number of aberrant crypts

Table 3: Basil effect on feed intake, weight gain, cecal weight and cecal pH in Fisher 344 male rats

Groups	Weight gain (g 13 $week^{-1}$)	Feed intake (g day^{-1})	Cecal weight (g)	Cecal pH
Control (C)	170.00±12.79^{c}	12.73±2.26^{b}	1.90±0.20	7.30±0.90
C+1% CB	192.00±10.84^{b}	16.60±1.40^{a}	2.01±0.20	6.86±0.40
C+2% CB	206.12±7.46^{b}	17.04±1.28^{a}	2.06±0.31	6.90±0.70
C+1% HBD	231.33±11.80^{a}	17.22±1.52^{a}	2.10±0.34	7.02±1.10
C+2% HBD	236.42±10.40^{a}	17.96±2.20^{a}	2.16±0.26	7.14±0.80
C+1% HBC	207.67±4.18^{b}	17.53±1.07^{a}	2.22±0.50	7.22±1.00
C+2% HBC	228.14±8.22^{a}	16.98±2.04^{a}	2.30±0.96	7.24±0.60
C+1% HBI	224.30±11.36^{a}	16.67±1.78^{a}	2.16±0.48	7.09±0.50
C+2% HBI	238.00±9.47^{a}	16.84±1.80^{a}	2.20±0.72	7.11±0.90

CB: Culinary Basil; HBD: Holy Basil Denmark; HBC: Holy Basil Cuba; HBI: Holy Basil India. Values are Means±SEM; n = 6. Values not sharing a common superscript are significantly different ($p<0.05$) using Tukey's studentized range test

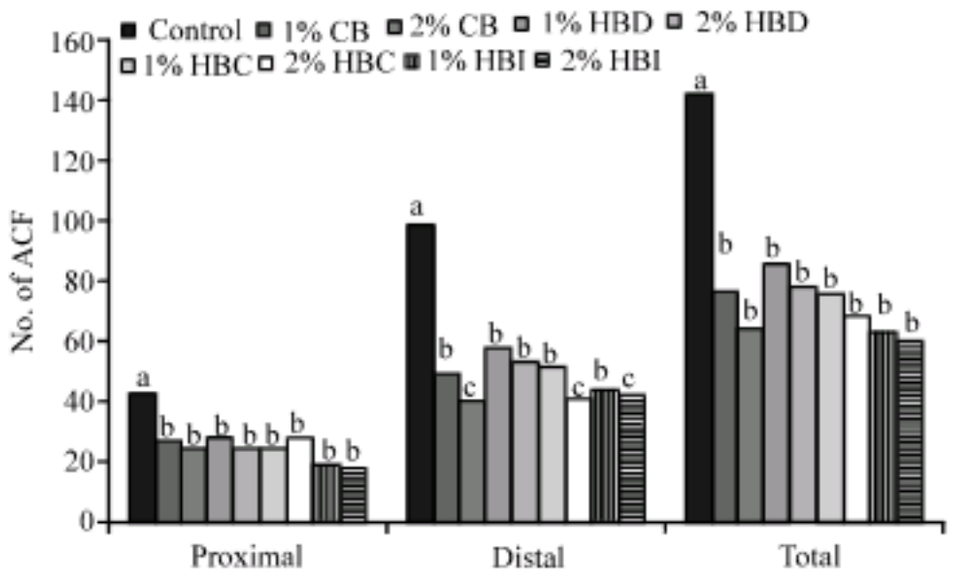

Fig. 2: Basil on AOM induced aberrant crypt foci. CB: Culinary Basil; HBD: Holy Basil Denmark; HBC: Holy Basil Cuba and HBI: Holy Basil India. Values are Mean±SEM; Bars not sharing a common superscript are significantly different ($p<0.05$) using Tukey's studentized range test

Table 4: Basil effect on crypt multiplicity

Groups	1	2	3	4	5
Control (C)	9^{b}	33^{a}	57^{a}	25^{a}	18^{a}
C+1% CB	8^{a}	20^{b}	21^{b}	12^{b}	6^{b}
C+2% CB	17^{a}	18^{b}	17^{b}	9^{b}	4^{c}
C+1% HBD	15^{a}	19^{b}	29^{b}	14^{b}	9^{b}
C+2% HBD	19^{a}	17^{b}	19^{b}	12^{b}	8^{b}
C+1% HBC	18^{a}	22^{b}	17^{b}	11^{b}	8^{b}
C+2% HBC	15^{a}	17^{b}	22^{b}	9^{b}	5^{b}
C+1% HBI	19^{a}	23^{b}	12^{c}	6^{c}	3^{c}
C+2% HBI	21^{a}	25^{b}	8^{c}	4^{c}	2^{c}

CB: Culinary Basil; HBD: Holy Basil Denmark; HBC: Holy Basil Cuba; HBI: Holy Basil India. Values are Means; Bars not sharing a common superscript are significantly different ($p<0.05$) using Tukey's studentized range test

Table 5: Number of total aberrant crypts

Groups	Total crypts
Control (C)	436 ± 26^{a}
C+1% CB	199 ± 18^{c}
C+2% CB	160 ± 12^{e}
C+1% HBD	241 ± 20^{b}
C+2% HBD	204 ± 16^{c}
C+1% HBC	197 ± 12^{c}
C+2% HBC	176 ± 14^{d}
C+1% HBI	140 ± 10^{f}
C+2% HBI	121 ± 15^{g}

CB: Culinary Basil; HBD: Holy Basil Denmark; HBC: Holy Basil Cuba; HBI: Holy Basil India. Values are Means±SEM; n = 6. Values not sharing a common superscript are significantly different ($p<0.05$) using Tukey's studentized range test

with 2, 3, 4 and 5 crypts/focus compared to rats fed the control (AIN 93 G) diet (Table 4). A higher reduction (%) in the incidence of large ACF (crypts = 4) was observed in all rats fed Basil diets compared to the small ACF (crypts = 3). The total aberrant crypts followed the similar trend (Table 5).

Glutathione-S-Transferase Activity

Glutathione-S-transferase is a crucial enzyme involved in the conjugation and detoxification of carcinogens. Glutathione-S-transferase activity (μmol mg^{-1}) was

significantly ($p<0.05$) higher in rats fed Basil diets compared to rats fed the control diet. Glutathione-S-transferase activity in rats fed Basil diets was 2-3 times higher than control rats. Among rats fed Basil diets, rats fed 2% HBI had the highest GST activity. GST activity was 32-66% higher in rats fed Basil diets compared to the control (Table 6).

Catalase Activity

Catalase is an important antioxidative enzyme which detoxifies hydrogen peroxide into water and oxygen. Catalase activity (μmol mg^{-1}) was significantly ($p<0.05$) higher in rats fed Basil diet compared to rats fed the control diet (Table 6). There were no significant differences observed among rats fed Basil diets. However, rats fed 2% HBD had higher catalase activity compared to other treatment groups, although differences were not significant.

Correlation Between the Incidence of ACF and Selected Enzyme Activities

Table 7 shows the correlation between the incidence of ACF and activity of hepatic enzymes such as GST and CAT in rats fed Basil (1 and 2%) diets. There was a positive correlation ($R^2 = 0.86$) between number of ACF and dose of CB (1 and 2%). A similar dose dependant relationship was seen in the rats fed HBD, HBC and HBI at 1 and 2% levels ($R^2 = 0.8421$, $R^2 = 0.83$, $R^2 = 0.77$). The activity of hepatic enzymes (GST and CAT) was compared against ACF incidence in order to determine the role of the detoxification and antioxidative enzymes in colon cancer. There was a strong correlation seen between GST activity and ACF incidence, with an increased GST activity resulting in a lower ACF incidence. There was a strong correlation seen in groups fed CB ($R^2 = 0.9278$), HBD ($R^2 = 0.9812$), HBC ($R^2 = 0.9325$) and HBI ($R^2 = 0.9065$) (Fig. 3-6). Similar but lower correlations were seen in the activity of the antioxidative enzyme (CAT) and the incidence of ACF with R^2 values of 0.8257, 0.8537, 0.6544 and 0.781 in rats fed CB, HBD, HBC and HBI at 1 and 2% levels, respectively.

Total Phenolics, Flavonoids, Anthocyanins and Free Radical Scavenging Activity of Basil

Plant phenolics constitute one of the major groups of compounds that may function as antioxidants. Therefore, it was beneficial to determine the amount of phenolics, flavonoids

Table 6: Feeding Basil on Glutathione-S-transferase and catalase activities

Groups	GST (μmol mg^{-1})	Catalase (μmol mg^{-1})
Control (C)	10.35 ± 1.87^{d}	12.73 ± 2.26^{b}
C+1% CB	23.75 ± 2.48^{c}	16.60 ± 1.40^{a}
C+2% CB	28.42 ± 2.20^{a}	17.04 ± 1.28^{a}
C+1% HBD	20.12 ± 3.10^{c}	17.22 ± 1.52^{a}
C+2% HBD	26.11 ± 3.14^{b}	17.96 ± 2.20^{a}
C+1% HBC	21.44 ± 1.95^{c}	17.53 ± 1.07^{a}
C+2% HBC	25.48 ± 2.19^{b}	16.98 ± 2.04^{a}
C+1% HBI	25.96 ± 2.66^{b}	16.67 ± 1.78^{a}
C+2% HBI	30.41 ± 2.94^{a}	16.84 ± 1.80^{a}

CB: Culinary Basil; HBD: Holy Basil Denmark; HBC: Holy Basil Cuba; HBI: Holy Basil India. Values are Means±SEM; n = 6. Values not sharing a common superscript are significantly different ($p<0.05$) using Tukey's studentized range test

Table 7: Correlation between the activity of selected detoxification and antioxidative enzymes and aberrant crypt foci in rats fed Basil

	CB	HBD	HBC	HBI
ACF	0.8636	0.8421	0.8300	0.7774
GST	0.9278	0.9812	0.9325	0.9065
CAT	0.8257	0.8537	0.6544	0.7810

ACF: Aberrant crypt foci; GST: Glutathione S-Transferase; CAT Catalase; CB: Culinary Basil; HBD: Holy Basil Denmark; HBC: Holy Basil Cuba; HBI: Holy Basil India

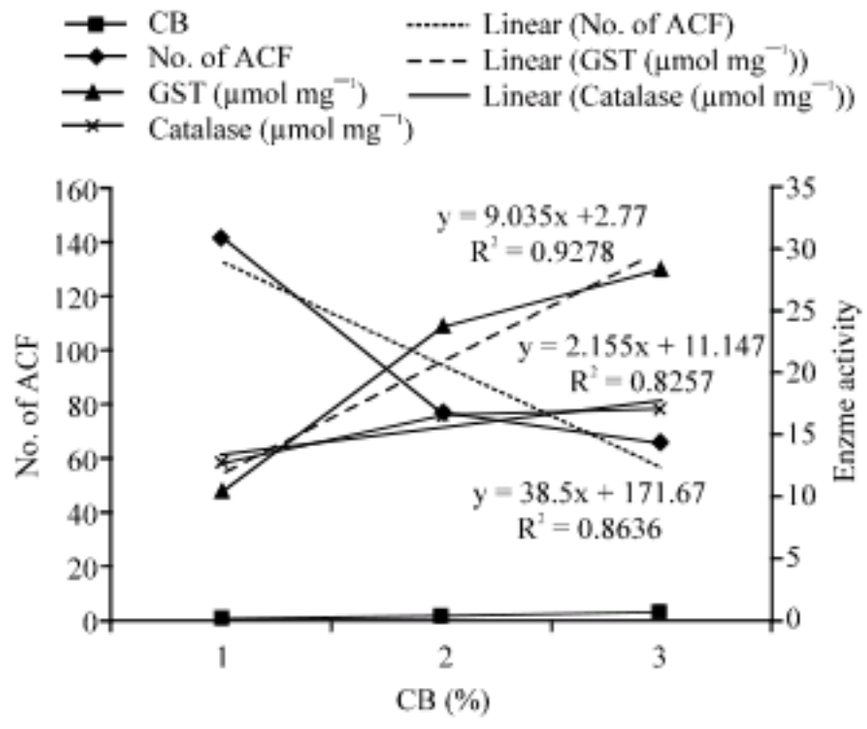

Fig. 3: Correlation between t he activity of selected detoxification and antioxidative enzymes and Aberrant crypt foci in rats fed CB. ACF: Aberrant crypt foci; GST: Glutathione S-Transferase; CAT: Catalase; CB: Culinary Basil. X-axis shows levels of CB (%) 1 = 0% Basil diet (CB), 2 = 1% Basil diet (CB) and 3 = 2% Basil diet (CB)

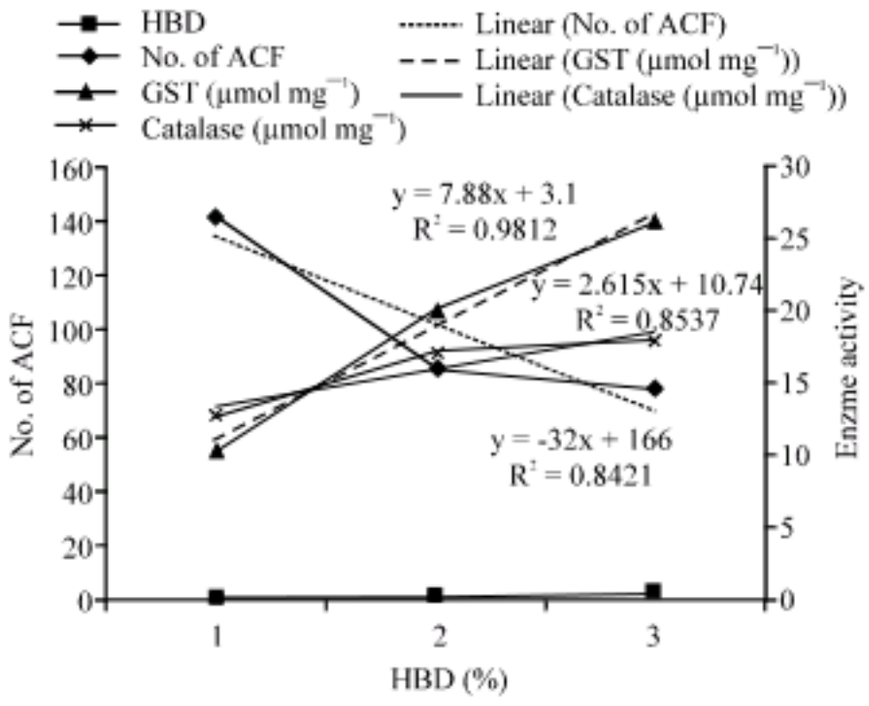

Fig. 4: Correlation between the activity of selected detoxification and antioxidative enzymes and Aberrant crypt foci in rats fed HBD. ACF: Aberrant crypt foci; GST: Glutathione S-Transferase; CAT: Catalase; HBD: Holy Basil Denmark. X-axis shows levels of HBD (%). 1 = 0 % Basil diet (HBD), 2 = 1% Basil diet (HBD) and 3 = 2% Basil diet (HBD)

and anthocyanins in Basil extracts. The amount of total phenolics, flavonoids and anthocyanins varied in different accessions and ranged from 31.4 to 60.5 mg GAE g^{-1}, 4.64 to 6.21 mg CE g^{-1} and 0.28 to 0.64 mg g^{-1}, respectively (Table 8). The DPPH scavenging activity of Basil extracts significantly ($p<0.05$) increased (30-60%) as the concentration of extracts increased (1-5 mg mL^{-1}) (Fig. 7). Also, the ability of scavenging free radicals varied with the Basil variety.

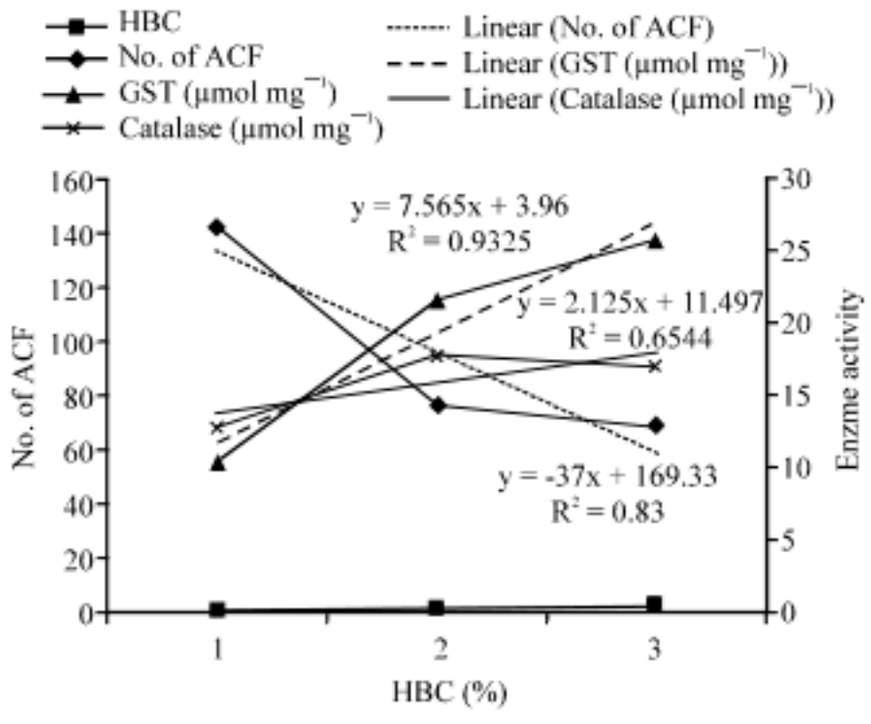

Fig. 5: Correlation between the activity of selected detoxification and antioxidative enzymes and Aberrant crypt foci in rats fed HBC. ACF: Aberrant crypt foci; GST: Glutathione S-Transferase; CAT: Catalase; HBC: Holy Basil Cuba. X-axis shows levels of HBC (%). 1 = 0 % Basil diet (HBC), 2 = 1% Basil diet (HBC) and 3 = 2% Basil diet (HBC)

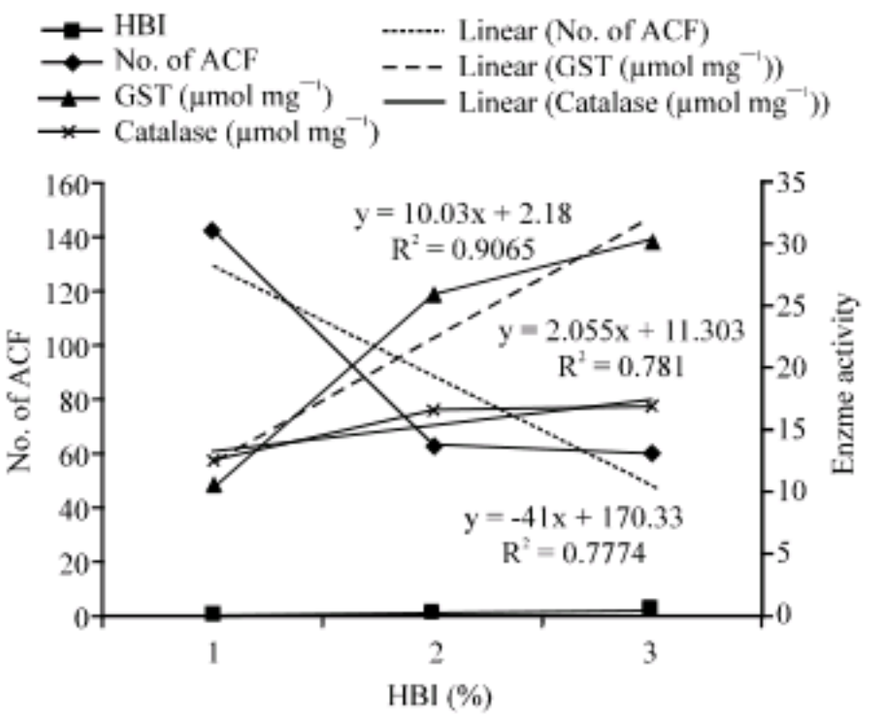

Fig. 6: Correlation between the activity of selected detoxification and antioxidative enzymes and Aberrant crypt foci in rats fed HBI. ACF: Aberrant Crypt Foci; GST: Glutathione S-Transferase; CAT: Catalase; HBI: Holy Basil India. X-axis shows levels of HBI (%). 1 = 0 % Basil diet (HBI), 2 = 1% Basil diet (HBI) and 3 = 2% Basil diet (HBI)

Table 8: Total phenolics, flavonoids and anthocyanin content in Basil accessions

Treatments	Phenolics (mg GAE g of dry Basil^{-1})	Flavonoids (mg CE g of dry Basil^{-1})	Anthocyanins (mg g of dry Basil^{-1})
CB	41.12±6.88^{b}	4.80±0.73	0.55±0.17
HBD	31.37±1.29^{b}	5.84±0.64	0.28±0.92
HBC	60.47±2.19^{a}	4.64±0.05	0.64±0.15
HBI	43.56±2.13^{ab}	6.21±0.70	0.43±0.19

Values are Means±SEM; n = 3. Values not sharing a common superscript in a column are significantly different. ($p<0.05$) with Tukey's studentized range test. CE: Catechin equivalents; GAE: Gallic acid equivalents; CB: Culinary Basil; HBD: Holy Basil Denmark; HBC: Holy Basil Cuba; HBI: Holy Basil India

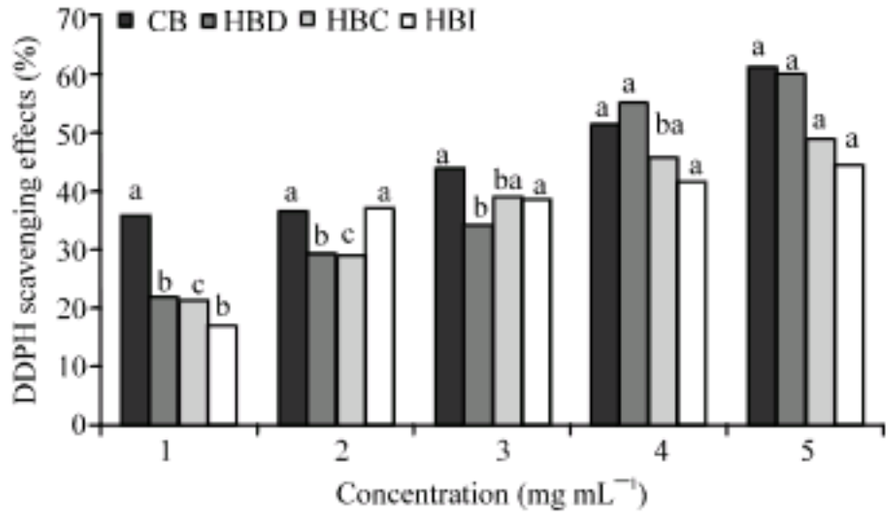

Fig. 7: Free radical scavenging activity of Basil measured at 517 nm using the DPPH assay. CB: Culinary Basil; HBD: Holy Basil Denmark; HBC: Holy Basil Cuba; HBI: Holy Basil India. Bars with different superscripts are significantly different ($p<0.05$). All values are mean of three replicates

Table 9: Correlation between the incidence of Aberrant Crypt Foci and phenolics and flavonoids in rats fed Basil

	CB	HBD	HBC	HBI
ACF	0.8636	0.8421	0.8300	0.7774
Phenolics	0.7500	0.7500	0.7500	0.7500
Flavonoids	0.7500	0.7500	0.7500	0.7500

ACF: Aberrant crypt foci; CB: Culinary Basil; HBD: Holy Basil Denmark; HBC: Holy Basil Cuba; HBI: Holy Basil India

Correlation Between the Incidence of ACF and Phenolics and Flavonoids

Table 9 shows the correlation between incidence of ACF and phenolics and flavonoids in rats fed Basil (1 and 2%) diets. The total phenolics and flavonoids were compared against ACF incidence in order to determine the effect of phytochemicals in colon cancer. A low correlation was seen between ACF incidence and phenolics and flavonoid content. Lower correlations were seen with R^2 values of 0.7500 in rats fed CB, HBD, HBC and HBI at 1 and 2% levels.

DISCUSSION

The present study was conducted to evaluate the chemopreventive potential of Basil at selected concentrations (1 and 2%) on azoxymethane induced aberrant crypt foci formation in Fisher-344 male rats.

Aberrant Crypt Foci (ACF) are identified as precancerous lesions in chemically induced colon cancer. All rats fed Basil diet had significantly ($p<0.05$) lower numbers of proximal and distal, indicating that Basil may have significant chemopreventive properties due to the presence of phytochemicals such as phenolic acids, flavonoids, anthocyanins and carotenoids (Simon *et al*., 1999; Phippen and Simon, 1998). Higher number of ACF was seen in the distal colon compared to the proximal colon in all groups, which is also seen in humans with colon cancer.

The number of crypts/focus was significantly lower in rats fed Basil (1 and 2%) diets compared to the control diet. The ACF with higher number of crypts (≥4 crypts/focus) are more likely to develop into tumors over a period of time, whereas, those with smaller number of crypts (≤3 crypts/focus) may dissolve and disappear as time progresses (Bird, 1987; Shirtliff and Bird, 1996). There was a higher reduction of ACF with ≥4 crypts/focus compared to ACF with ≤3 crypts/focus (Table 4) which may be due to the anti-proliferative activity of Basil or due to the induction of apoptosis in the colonic crypts.

It has been shown that phytochemicals have the ability to induce or inhibit phase I or phase II metabolic enzymes (Block and Gyllenhaal, 2002). Several studies have reported the induction of phase II detoxification enzymes by food phytochemicals offering protection against toxicity and chemical carcinogenesis, especially during the initiation phase. Among the phase II detoxification enzymes, Glutathione-S-transferase is a crucial enzyme involved in conjugation processes to increase the polarity of the compound, hence facilitates its clearance. In this study, we observed a 2-3 fold increase in hepatic GST activity in rats fed Basil (1 and 2%) diets compared to rats fed the control diet indicating that phytochemicals present in Basil may have the ability to induce phase II detoxification enzymes such as GST. Aruna and Sivaramakrishnan (1990) reported that Basil (*Ocimum Basilicum*) suppressed benzo(a)pyrine-induced chromosomal aberrations in bone marrow and increased glutathione (GSH) and Glutathione-S-Transferase (GST) activities in the liver of mice, suggested a possible protective role against cancer. Dasguptha *et al*. (2004) also reported that treatment with Basil leaf extracts augmented the activity of GST in the liver, lung, kidney and the forestomach. Catalase is one of the important antioxidative enzymes involved in removal of hydrogen peroxide produced by the action of superoxide dismutase (SOD). In this experiment, rats fed Basil (1 and 2%) diets had significantly ($p<0.05$) higher catalase activities compared to rats fed control diet indicating that Basil may have the potential to play a role in modulating the oxidation process. Dasguptha *et al*. (2004) also observed similar results with catalase activity. Hepatic activities of SOD, CAT, glutathione peroxidase (GPx) and Glutathione Reductase (GR) were significantly increased with administration of luteolin (1.2 mg kg^{-1}) in mice induced with AOM (Ashokkumar and Sudhandiran, 2008). These results support the data observed in this study.

We observed that the phenolic content was significantly ($p<0.05$) higher in Holy Basil Cuba compared to other Basil accessions (CB, HBD and HBI) used. This may be due to the fact that phytochemical composition varies for each herb within the same family. The phenolic content observed in this study were lower than those reported Suppakal *et al*., 2003) as numerous factors such as extraction procedures, drying methods, growing and harvesting seasons may affect the phytochemical composition (Suppakal *et al*., 2003).

Microscopic studies indicated that the slightly purplish color of Holy Basil results from the accumulation of anthocyanins in the epidermal layer of cells in leaves (Phippen and Simon, 1998). However, there were no significant differences observed in the total anthocyanin content among the four Basil varieties tested.

The antioxidative effect is mainly due to the phenolic acids, flavonoids and anthocyanins present in Basil. Free radical scavenging activity is one of the important antioxidant properties because of the deleterious role of free radicals in food and biological systems. Excessive formation of free radicals accelerates the oxidation of lipids in foods and decreases food quality and consumer acceptance (Issa *et al*., 2006). In this study, the antioxidant activity of Basil extracts was determined using a DPPH method. The antioxidants were able to reduce the radical (DPPH) to the yellow-coloured diphenyl-picrylhydrazine. The method is based on the reduction of methanolic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H (Gulcin *et al*., 2007). Figure 6 shows a significant increase ($p<0.05$) in the scavenging of the DPPH radical due to the scavenging effects of Basil extracts. Different accessions of Basil demonstrated maximum DPPH activity at different concentrations.

CONCLUSION

Results of this study suggest that feeding dietary Basil at 1 and 2% levels significantly ($p<0.05$) reduced azoxymethane-induced aberrant crypt foci formation in Fisher 344 male rats.

However, further end point tumor model and human clinical trials need to be conducted for more conclusive evidence. This study also reveals that the potential of 4 Basil accessions tested as a natural antioxidant may be due to prevalence of phenolic compounds.

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