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Basil (*Ocimum basilicum* and *Ocimum tenuiflorum*) Reduces Azoxymethane Induced Colon Tumors in Fisher 344 Male Rats

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Abstract: The objective of this study was to determine the effects of three varieties of *ocimum tenuiflorum* (Holy Basil) (Denmark (HBD), Cuba (HBC), India (HBI)) and one variety of *ocimum basilicum* (Culinary Basil) (CB) on Azoxymethane (AOM)-induced colon tumors in Fisher 344 male rats. After a 1 week period of acclimatization, rats were divided into groups. Basil leaf powder was mixed at 1% level in an AIN 93G/M based diet. Rats were administered 2 injections of AOM (s/c injections at 16 mg kg^{-1} body weight in saline) at 7 and 8 week of age to induce colon carcinogenesis. Rats were killed by CO_2 asphyxiation and samples of colon, cecum and liver, were collected. Colon tumors were characterized according to number, size, location and tumors per tumor bearing rat ratio. Feeding Basil (1%) resulted in significantly lower tumor incidence compared to rats fed the control diet. Tumors/tumor bearing rat ratio was reduced by 78% in rats fed Basil diets compared to rats fed the control diet. Tumor size (mm) was significantly ($p<0.05$) smaller in treatment diets (CB: 1.20, HBD: 0.8, HBC: 0.8, HBI: 0.6 and control: 3.72) compared to control diet. Selected hepatic enzyme activities (Glutathione-S-Transferase, Superoxide dismutase and Catalase) were significantly ($p<0.05$) higher in the rats fed Basil compared to rats fed the control diet. Results showed that feeding Holy and Culinary Basil significantly ($p<0.05$) reduced the number of AOM-induced colon tumors in Fisher 344 male rats and therefore may have implications in food industry as a potential chemopreventive agent.

Key words: Chemoprevention, Basil, Azoxymethane, colon tumors, phytochemicals

INTRODUCTION

Colon cancer is one of the leading causes of cancer mortality and the third form of cancer in US (Jemal *et al*., 2007) with about 108, 070 new cases of colon cancer and 49, 960 deaths expected in 2008 (Bianchi and Burke, 2008). Epidemiological studies indicate that human diseases such as cardiovascular diseases and cancers can be prevented by consuming fruits and vegetables and 20-40% of cancer deaths in the US are preventable by diet modifications (Coates *et al*., 2007). Among the cancers of the digestive tract, colon cancer is most responsive to diet modification (Block and Gyllenhaal, 2002). In order to reduce the risk of cancer, American Cancer Society (ACS) recommending the inclusion of

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whole grains, fruits and vegetables and limited consumption of red meats in the diet. Nowadays food industry is becoming more and more interested in herbs and spices because of their flavoring, medicinal and/or antioxidant activities.

Basil (*Ocimum basillicum* L. and *Ocimum tenuiflorum*) is well known for its medicinal value and one of the oldest spices within the ocimum genus in Lamiaceae family. It is also popular as a culinary herb. The different parts of the plant are traditionally used for the treatment of various disorders and as an antidote for snake bites and scorpion stings (Umadevi, 2001). Basil has been used in Turkish folk medicine for many years and has several beneficial effects for conditions such as digestive and appetite enhancing effects. It was reported that the leafy parts of basil had tonic, antiseptic (Kosekia *et al*., 2002) and insecticidal properties (Umerie *et al*., 1998). In addition, Basil is used for treatment of inflammation, dyspepsia, aches and pains (Umadevi, 2001).

According to the Asian Indian traditional system of medicine called Ayurveda, Holy Basil (*Ocimum tenuiflorum*) is considered as one of the most effective and has been widely studied in relation to diabetes and its complications medicinal herb among the other medicinal plants (Mentreddy, 2007). *Ocimum tenuiflorum* has shown both hypoglycaemic and antihyperglycaemic properties and lowered blood glucose levels in Type 2 diabetic patients. Aqueous extracts of the leaves and pure eugenol, which is a constituent of the extract, were reported to reduce the biochemical and membrane changes induced by restraint stress in rats (Sen *et al*., 1992). Oral feeding of Basil leaf powder in rats for a period of one month significantly reduced fasting blood sugar and cholesterol levels in blood, liver and heart (Rai *et al*., 1997). Basil contains phytochemicals such as phenolic acids, flavonoids, anthocyanins and carotenoids. Rosmarinic acid is the predominant phytochemical found in *O. basilicum* (Gerhardt and Schroter, 1983). Orientin and vicenin, the radio protective flavonoids from *Ocimum tenuiflorum* have shown strong inhibitory effects against OH radical activity (Umadevi, 2001). Rajakumar and Rao (1993) have reported that isoeugenol, which has a double bond similar to that found in oreintin and vicenin, is a good free radical scavenger.

Numerous studies reported various effects of *Ocimum* sp., including anti-inflammatory, antioxidative, chemopreventive, blood-sugar lowering, nervous system stimulation and radiation protection have been reported (Chattopadhyay, 1999; Prakash and Gupta, 2000; Umadevi, 2001). However, there are no reported studies evaluating the chemopreventive potential of Basil on colon tumorgenesis. In a recent study conducted by our laboratory, we evaluated the chemopreventive potential of Basil on azoxymethane-induced Aberrant Crypt Foci (ACF) using Fisher 344 male rat model. Therefore, we have conducted a long-term study (end point tumor model) on the chemopreventive potential of Basil on modulating colon tumorgenesis using the Fisher 344 rodent model.

MATERIALS AND METHODS

Animals Housing and Diet

Fifty two Fisher 344 male weanling (3 weeks old) rats were obtained from Harlan, Indiana and were housed in stainless steel wire cages @ 2 rats per cage in September 2007. 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 and rats were fed control (American Institute of Nutrition 93 Growth (AIN 93 G)) and treatment diets (Reeves *et al*., 1993). After a one-week acclimatization period, rats were divided into 5 groups (10 rats each in treatment groups and 12 rats in control group). Control rats were given free access to AIN 93 G/M diet throughout the experimental period (41 week).

Table 1: Composition of the diets[a]

Ingredients (g kg^{-1})	Control (C)	CB 1%	HBD 1%	HBC 1%	HBI 1%
Corn starch	465.7	455.7	455.7	455.7	455.7
Basil	0	10	10	10	10
Common ingredients[b]	534.3	534.3	534.3	534.3	534.3

CB: Culinary Basil; HBD: Holy Basil Denmark; HBC: Holy Basil Cuba; HBI: Holy Basil India. [a]Formulations of diets based on AIN-93M: American institute of nutrition (Reeves *et al*., 1993). [b]Common ingredients (g): casein, 140: Dextrose, 155: Sucrose, 100: Soybean oil, 40: Fiber, 50: Mineral mix (AIN-93G), 35L Vitamin mix, 10: L-cystein, 1.8: Choline bitartrate, 2.5

Preparation of Diet

Four accessions of Basil leaves namely Culinary Basil, Holy Basil Denmark, Holy Basil Cuba and Holy Basil India 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), ground to a fine powder and mixed in the diet at 1% level (Table 1).

Feed Intake and Body Weights

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

Chemicals and Dietary Ingredients

All biochemicals, except azoxymethane (Midwestern Research Institute, NCI Repository, Kansas, MO) were obtained from Sigma Chemical, St Louis, MO. Basil was obtained from Winfred Thomas Agricultural Research Station, Alabama A and M University, Normal, Alabama.

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 45 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, Catalase and Superoxide dismutase).

Analysis of Cecal Contents

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.

Tumor Characterization

Tumors were characterized based on location (proximal and distal), tumor number, tumor size and TBR ratio (Tumor per tumor bearing rat ratio). The TBR ratio gives a good picture of chemopreventive benefits of a particular food, as it indicates the number of tumors in rats that developed tumors. Tumor incidence (%) in the proximal and distal regions was also reported. Tumors were characterized as described by Shackelford *et al*. (1983).

Glutathione-S-Transferase (GST) Activity

The GST in the liver and colonic mucosa was assayed with some modifications to the procedure as 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 homogenates were 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 using a micro plate reader. Total enzyme activity was measured at the end of 5 min of reaction.

Determination of Catalase Activity

Liver catalase was estimated in a micro plate reader 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 per min per mg protein.

Determination of Superoxide Dismutase (SOD) Assay

Liver superoxide dismutase was assayed by the technique of Fridovich (1989). One gram of liver sample was diluted in 9 mL of distilled water. An aliquot of 2.0 mL of the diluted aliquot was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrometer and the reaction started by the addition of 0.3 mL freshly prepared 0.3 mM adrenaline to the mixture which was mixed by inversion. The reference cuvette contained 2.5 mL buffer, 0.3 mL of substrate (adrenaline) and 0.2 mL of water. The increase in absorbance at 480 nm was monitored every 30 sec for 150 sec. A single unit of enzyme is defined as the quantity of SOD required to produce 50% inhibition of autoxidation.

Statistical Analysis

Data were analyzed using the SAS system version 9.0 (SAS Institute, Cary, NC) 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

Effect of Basil on Weight Gain, Feed intake, Cecal Weight and Cecal pH

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

Tumor Incidence

Tumor incidence in the colon of rats fed the control diet was higher compared to the rats fed the Basil (1%) diets (Table 3). Most tumors developed in the distal colon in all the rats in the control and treatment groups. Distal tumor incidence (%) was higher in rats fed CB (100%) followed by HBD (83.3%), HBC (85.7%) and HBI (50%), where as proximal tumor incidence (%) was higher in rats fed HBI (50%) compared to the other treatment groups [CB (0%), HBD (16.7%) and HBC (14.3%]. However, total tumor incidence was lower in the rats fed the Basil (1%) diets compared to the rats fed the control diet. Rats fed HBI (30%) had

Table 2: Effect of feeding basil on feed intake, weight gain, cecal weight and cecal pH in fisher 344 male rats

Groups	Weight gain (g)	Feed intake (g)	Cecal weight (g)	Cecal pH
Control (C)	310.20±08.32[b]	16.04±0.44[b]	3.76±0.22	7.82±0.03
C+1% CB	347.10±07.23[a]	17.86±0.43[a]	4.00±0.36	7.68±0.04
C+1% HBD	332.50±07.87[a]	17.99±0.31[a]	3.53±0.33	7.83±0.06
C+1% HBC	350.60±10.86[a]	18.49±0.49[a]	3.58±0.29	7.88±0.06
C+1% HBI	332.40±28.86[a]	17.60±0.46[a]	3.45±0.39	7.89±0.08

Values are Means±SEM; n = 10. Values not sharing a common superscript are significantly different. ($p<0.05$) using Tukey's studentized range test. CB: Culinary Basil, HBD: Holy Basil Denmark, HBC: Holy Basil Cuba, HBI: Holy Basil India

Table 3: Effect of Basil on tnmor incidence (%) in Fisher 344 male rats

Groups	N1/N2	Proximal	Distal	Total
Control (C)	12/12	31.2	100	100
C+1% CB	6/10	0	100	50
C+1% HBD	5/10	16.67	83.33	50
C+1% HBC	4/10	14.28	85.72	40
C+1% HBI	3/10	50	50	30

N1/N2 (rats with tumors/number of rats in the group). CB: Culinary Basil, HBD: Holy Basil Denmark, HBC: Holy Basil Cuba, HBI: Holy Basil India

Table 4: Effect of Basil on tumor nnmbers in fisher 344 male rats

Groups	N1/N2	Proximal	Distal	Total
Control (C)	12/12	18	36	54
C+1% CB	6/10	0	6	6
C+1% HBD	5/10	1	5	6
C+1% HBC	4/10	1	6	7
C+1% HBI	3/10	2	2	4

N1/N2 (rats with tnmors/number of rats in the group). Abbreviations: CB: Culinary Basil, HBD: Holy Basil Denmark, HBC: Holy Basil Cuba, HBI: Holy Basil India

the lowest tumor incidence followed by HBC (40%), CB and HBD (50%). Over all, 50-70% reductions were seen in tumor incidence in the rats fed Basil diets compared to the rats fed the control diet.

Tumor Numbers

The rats in the control group had the highest number of tumors in both the distal (36) and proximal (18) sections of the colon (Table 4). Tumor numbers were higher in the control group compared to the rats fed the Basil (1%) diets. Among the treatment groups, the number of tumors was lower in the proximal colon compared to the distal colon. The number of tumors in the proximal colon was higher in the rats fed HBI (2) compared to the rats fed HBD (1) and HBC (1), however, the rats fed CB did not develop any tumors in the proximal colon. In the distal colon, the number of tumors was lower in the rats fed HBI (2) compared to the rats fed HBD (5), CB (6) and HBC (6). The total number of tumors was lower in the rats fed HBI (4) compared to the other treatment groups CB (6), HBD (6) and HBC (7). The reductions (%) in tumor numbers compared to the control were 93, 89, 89 and 87 in the treatment groups fed CB, HBD, HBC and HBI.

Tumor Size

Rats fed Basil (1%) diets had significantly ($p<0.05$) smaller tumors (mm) compared to the rats fed the control diet in both the proximal and distal colon (Table 5). Among the treatment groups, rats fed HBD (0.20) and HBC (0.20) had smaller tumors compared to the rats fed HBI (0.60) in the proximal colon. In the distal colon, rats fed HBI had significantly ($p<0.05$) smaller tumors (0.60) compared to the rats fed HBD and HBC (1.40) and CB (2.40). Reductions (%)

Table 5: Basil effect on tumor size (mm) in Fisher 344 male rats

Groups	N1/N2	Proximal	Distal	Total
Control (C)	12/12	2.40^{a}	5.04^{a}	3.72
C+1% CB	6/10	0.00^{b}	2.40^{b}	1.20
C+1%HBD	5/10	0.20^{b}	1.40^{bc}	0.80
C+1%HBC	4/10	0.20^{b}	1.40^{bc}	0.80
C+1% HBI	3/10	0.60^{b}	0.60^{c}	0.60

N1/N2 (rats with tumors/number of rats in the group). Values are means. Values not sharing a common superscript are significantly ($p<0.05$) different using Tukey's studentized range test. CB: Culinary Basil, HBD: Holy Basil Deumark, HBC: Holy Basil Cuba, HBI: Holy Basil India

Table 6: Basil effect on tumors per tumor bearing rat ratio (TBR) in Fisher 344 male rats

Groups	N1/N2	T/TBR
Control (C)	12/12	4.5
C+1% CB	6/10	1.0
C+1% HBD	5/10	1.2
C+1% HBC	4/10	1.4
C+1% HBI	3/10	1.3

N1/N2 (rats with tumors/number of rats in the group). CB: Culinary Basil, HBD: Holy Basil Denmark, HBC: Holy Basil Cuba, HBI: Holy Basil India

in tumor size compared to control rats were highest in the rats fed HBI (88.1), followed by HBD (72.2), HBC (72.2) and CB (52.4). The total (proximal and distal) average tumor size (mm) in rats fed the control diet was the highest (3.72). The reductions (%) in tumor size compared to the control in rats fed HBI (83.8) was the highest, followed by HBD and HBC (78.5) and lowest in the group fed CB (67.7).

Tumors/Tumor Bearing Rat Ratio (TBR)

Tumors per Tumor Bearing Rat ratio (TBR) is the ratio of the number of tumors in rats that developed tumors. The TBR ratios ranged from a high of 4.5 in the rats fed the control diet to a low of 1.0 in the rats fed CB and HBI diets. (TBR ratio was higher in rats fed the control (AIN-93G/M) diet compared to the rats fed Basil (1%) diets as shown in Table 6). The greatest reduction (%) in TBR ratio was observed in rats fed CB and HBI (77.8) followed by HBD (73.3) and HBC (68.9) compared to the rats fed the control diet.

Hepatic Glutathione S-Transferase (GST) Activity

The GST activity (Units mg^{-1}) in the liver and colonic mucosa were significantly ($p<0.05$) higher in rats fed the Basil (1%) diets compared to rats fed the control (AIN-93G/M) diet. The GST activity in the liver (Units mg^{-1}) ranged from a low of 6.15 in the rats fed the control diet to a high of 22.05 in rats fed 1% HBI diet. Among the rats fed the Basil (1%) diets, those fed HBI had the highest GST activity in the liver (22.05) followed by HBC (15.24), HBD (14.84) and CB (12.97). A 2-3 fold increase was observed in GST activity in rats fed Basil (1%) diets compared to the rats fed control (AIN-93G/M) diet (Table 7).

Catalase (CAT) and Super Oxide Dismutase (SOD) Activity

The effect of feeding Basil on hepatic antioxidant enzymes (SOD and CAT) in rats is shown in Table 8. The activities of these enzymes were found to be significantly ($p<0.05$) higher in the liver of Basil fed rats compared to the control rats. The CAT activity (μmol mL^{-1}) ranged from a low of 21.13 in rats fed the control diet to a high of 103.26 in rats fed 1% HBD diet. There were no significant ($p<0.05$) differences observed with in the treatment groups. A 3.5-4.9 fold increase in CAT activity was observed in the treatment groups compared to the control group.

Table 7: Hepatic and colonic mucosa Glutathione-S-transferase activity in the Fisher 344 male rats fed Basil

Groups	Hepatic GST activity (units mg^{-1})	CMS GST activity (units mg^{-1})
Control (C)	6.15±0.37^{c}	3.37±0.03^{c}
C+1% CB	12.97±1.15^{b}	8.32±0.25^{ab}
C+1% HBD	14.84±0.56^{b}	9.51±0.59^{a}
C+1% HBC	15.24±2.05^{b}	6.84±0.11^{b}
C+1% HBI	22.05±1.81^{a}	8.10±0.48^{ab}

CB: Culinary Basil, HBD: Holy Basil Denmark, HBC: Holy Basil Cuba, HBI: Holy Basil India, CMS: Colonic Mucosal Scrapings. Values are Means±SEM; n = 10. Values not sharing a common superscript are significantly different ($p<0.05$) using Tukey's studentized range test.

Table 8: Effect of Basil on Catalase (CAT) and Superoxide Dismutase (SOD) activities in Fisher 344 male rats

Groups	CAT (μmol mL^{-1})	SOD (units mL^{-1})
Control (C)	21.13±11.71^{b}	2.9345±0.0914^{c}
C+1% CB	73.48±4.22^{a}	6.3421±0.0354^{ab}
C+1% HBD	103.26±9.22^{a}	6.2391±0.1269^{ab}
C+1% HBC	90.64±5.37^{a}	6.4538±0.1572^{a}
C+1% HBI	85.93±7.50^{a}	5.9317±0.0664^{b}

CB: Culinary Basil, HBD: Holy Basil Denmark, HBC: Holy Basil Cuba, HBI: Holy Basil India. Values are Means±SEM; n = 10. Values not sharing a common superscript are significantly different ($p<0.05$) using Tukey's studentized range test

The activity of SOD (Units mL^{-1}) ranged from a low of 2.93 in the rats fed control diet to a high of 6.45 in rats fed 1% HBC. Among the treatment groups, rats fed 1% HBI had significantly ($p<0.05$) lower SOD activity compared to the rats fed 1% HBC. A 2.0-2.2 fold increase in SOD activity was seen in the rats fed the Basil (1%) diets compared to the control group.

DISCUSSION

The objective of this study was to determine the long term effects of feeding Basil (*Ocimum basilicum* and *Ocimum tenuiflorum*) on colon tumorigenesis in azoxymethane-induced colon carcinogenesis in Fisher 344 male rats. To our knowledge this is the first animal model experiment studying the antitumorigenic effects of Basil against colon cancer. In previous studies, Basil (*Ocimum basilicum)* has been shown to reduce the risk of skin and forestomach papillomagenesis (Dasguptha *et al*., 2004).

In this study, feed intake (g day^{-1}) was significant ($p<0.05$) at the end of the 45 week study. This may due to the strong aroma and flavor of the volatile oils present in Basil. Because the rats fed Basil (1%) consumed more diet, we observed a significantly ($p<0.05$) higher weight gain compared to the control group. However, there were no significant ($p<0.05$) differences observed in cecal weight and cecal pH.

Feeding Basil (1%) resulted in significantly lower tumor incidence compared to the rats fed the control diet. Tumor incidence and number of tumors were higher in the control fed rats. Basil (1%) diets reduced tumor incidence by 50-70% compared to the control diet. The results of this study are comparable to the lower tumor incidence (25%) reported following administration of Basil leaves for 12 weeks on 3'-methyl-4-dimethylaminoazobenzene (3'MeDAB) induced neoplasia in mice stomachs (Aruna and Sivaramakrishnan, 1992). We observed a greater reduction in tumor incidence compared to the report by Dasguptha *et al*. (2004), where feeding Basil (*Ocimum basilicum*) leaves reduced tumor incidence by 20-40% in benzo(a)pyrene-induced forestomach papillomagenesis in mice.

Slower growth of tumors with longer induction time was seen in mice supplemented with *Ocimum tenuiflorum* seed (essential oil) compared to MCA (20-methylcholanthrene) injected mice which may also have accounted for the enhanced survival rate of the mice

(Prakash and Gupta, 2000). In present study, feeding Basil (1%) resulted in lower tumors per tumor bearing rat ratio (TBR) (the number of tumors in rats that developed tumors), which may suggest the chemopreventive potential of Basil. Rats fed Basil (1%) diets had significantly ($p<0.05$) smaller tumors compared to the rats fed the control diet in both the proximal and distal colon indicating that Basil (*Ocimum basilicum* and *Ocimum tenuiflorum*) may have anti-inflammatory and anti angiogenic properties. The essential oils extracted from Sweet Basil (*Ocimum basilicum*) were reported to have higher antiproliferative activity (12 times more potent) than 5-FluroUracil (Manosroi *et al*., 2005). The hepatic antioxidant enzymes, Super Oxide Dismutase (SOD) and Catalase activities were significantly ($p<0.05$) higher in the rats fed Basil (1%) diets compared to the rats fed the control diet. SOD plays an important role in the antioxidant enzyme defense system by converting superoxide radicals into hydrogen peroxide (Li *et al*., 2000). The induction of SOD activity in the rats fed the Basil (1%) diets may have caused the scavenging of Reactive Oxygen Species (ROS) and the dismutation of superoxide radicals. Catalase activity was significantly ($p<0.05$) higher after feeding Basil (1%) diets. Catalase may have assisted in removing the hydrogen peroxide radicals produced by the action of SOD. An increase in SOD activity, along with that of catalase, may have resulted in lower oxidative stress and damage to cells thus resulting in reduction of initiation of cancer. Glutathione-S-Transferase (GST) activity was significantly ($p<0.05$) increased in the rats fed Basil (1%) diets compared to the rats fed the control diet. The elevated levels of GST induced by the Basil (1%) diet may contribute to its anticarcinogenic effects. GST enzymes are involved in the metabolism of a wide variety of electrophilic carcinogens (Alexandrov *et al*., 2002). GSTs are a family of enzymes that assist in the excretion of carcinogens by making them soluble via conjugation with GSH (Sundberg *et al*., 2002). Thus, GSTs are used as a biomarker to investigate the chemopreventive effects of agents (Salinas and Wong, 1999).

CONCLUSIONS

The results of this study show that dietary Basil may have significant implications as a chemopreventive agent. Although, all the mechanisms of action are not fully known, there is evidence suggesting its apoptotic, anti-inflammatory, anti-oxidative and anti-proliferative roles. Induction of critical detoxification and antioxidative enzymes may also have played a significant role in reducing Azoxymethane-induced colon tumors. Human clinical trials will need to be conducted to further explain its chemopreventive action.

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REFERENCES

Aebi, H., 1984. Catalase *in vitro*. Methods Enzymol., 105: 121-126.

Alexandrov, A., M.R. Martzen and E.M. Phizicky, 2002. Two proteins that form a complex are required for 7-methylguanosine modification of yeast tRNA. RNA, 8: 1253-1266.

Aruna, K. and V.M. Sivaramakrishnan, 1990. Plant products as protective agent against cancer. Indian J. Exp. Biol., 28: 1008-1011.

Bianchi, L.K. and C.A. Burke, 2008. Understanding current guidelines for colorectal cancer screening: A case-based approach. Cleve Clin. J. Med., 75: 441-448.

Block, K.I. and C. Gyllenhaal, 2002. Clinical corner: Herb-drug interactions in cancer chemotherapy: Theoretical concerns regarding drug metabolizing enzymes. Integrative Cancer Ther., 1: 83-89.

Chattopadhyay, R.R., 1999. A comparative evaluation of some blood sugar lowering agents of plant origin. J. Ethnopharmacol., 67: 367-372.

Coates, E.M, G. Popa, C.I.R. Gill, M.J. McCann, G.J. McDougall, D. Stewart and I. Rowland, 2007. Colon-available raspberry polyphenols exhibit anti-cancer effects on *in vitro* models of colon cancer. J. Carcinog., 6: 4-4.

Dasguptha, T., R. Rao and P.K. Yadava, 2004. Chemomodulatory efficacy of basil leaf (*Ocimum basilicum*) on drug metabolizing and antioxidant enzymes and on carcinogen-induced skin and forestomach papillomagenesis. Phytomedicine, 11: 139-151.

Fridovich, I., 1989. Superoxide dismutases. An adaptation to a paramagnetic gas. J. Biol. Chem., 264: 7761-7764.

Gerhardt, U. and A. Schroter, 1983. Rosmarinic acid-an antioxidant occurring naturally in herbs. Fleischwirtschaft, 63: 1628-1630.

Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione-S-transferases, the first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.

Jemal, A., R. Siegel, E. Ward, T. Murray, J. Xu and M.J. Thun, 2007. Cancer statistics, 2007. CA Cancer J. Clin., 57: 43-66.

Kosekia, P.M., A.L.C.H. Villavicencioa, M.S. Britoa, L.C. Nahme and K.I. Sebastiao *et al*., 2002. Effects of irradiation in medicinal and eatable herbs. Radiation Phys. Chem., 63: 681-684.

Li, S., T. Yan, J.Q. Yang, T.D. Oberley and L.W. Oberley, 2000. The role of cellular glutathione peroxidase redox regulation in the suppression of tumor cell growth by manganese superoxide dismutase. Cancer Res., 60: 3927-3939.

Manosroi, J., P. Dhumtanom and A. Manosroi, 2005. Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. Cancer Lett., 235: 114-120.

Mentreddy, S.R., 2007. Medicinal plant species with potential antidiabetic properties. J. Sci. Food Agric., 87: 743-750.

Prakash, J. and S.K. Gupta, 2000. Chemopreventive activity of *Ocimum sanctum* seed oil. J. Ethanopharmacol., 72: 29-34.

Rai, V., U. Lyer and U.V. Mani, 1997. Effect of Tulasi (*Ocimum sanctum*) leaf powder supplementation on blood sugar levels serum lipids and tissue lipids in diabetic rats. Plant Foods Hum. Nutr., 50: 9-16.

Rajakumar, D.V. and M.N. Rao, 1993. Dehydrozingerone and isoeugenol as inhibitors of lipid peroxidation and as free radical scavengers. Biochem. Pharmaco., 46: 2067-2072.

Reeves, P.G., F.H. Nielsen and Jr. G.C. Fahey, 1993. AIN-93 purified diets for laboratory rodents: Final report of the American institute of nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr., 123: 1939-1951.

Salinas, A.E. and M.G. Wong, 1999. Glutathione S-transferases-a review. Curr. Med. Chem., 6: 279-309.

Sen, P., P.K. Mediratta and A. Ray, 1992. Effects of Azadirachta indica a juss on some biochemical, immunological and visceral parameters in normal and stressed rats. Indian J. Exp. Biol., 30: 1170-1175.

Shackelford, L.A., D.R. Rao, C.B. Chawan and S.R. Pulusani, 1983. Effect of feeding fermented milk on the incidence of chemically-induced colon tumors in rats. Nutr. Cancer, 5: 159-164.

Sundberg, K., K. Dreij, A. Seidel and B. Jernström, 2002. Glutathione conjugation and DNA adduct formation of dibenzo[a,l]pyrene and benzo[a]pyrene diol epoxides in V79 cells stably expressing different human glutathione transferases. Chem. Res. Toxicol., 15: 170-179.

Umadevi, P., 2001. Radioprotective, anticarcinogenic and antioxidant properties of the Indian holy basil, *Ocimum Sanctum* (Tulasi). Indian J. Exp. biol., 39: 185-190.

Umerie, S.C., H.U. Anaso and L.J.C. Anyasoro, 1998. Insecticidal potentials of *Ocimum basilicum* leaf-extract. Bioresour. Technol., 64: 237-239.