

## RESEARCH COMMUNICATION

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# Inhibition of Lipid Peroxidation and Enhancement of GST Activity by Cardamom and Cinnamon During Chemically Induced Colon Carcinogenesis in Swiss Albino Mice

Shamee Bhattacharjee, Tapasi Rana, Archana Sengupta\*

### Abstract

Globally, colorectal cancer is the third commonest cancer in men since 1975. The present study focuses on the preventive strategies aimed at reducing the incidences and mortality of large bowel cancer. Chemoprevention of colon cancer appears to be a very realistic possibility because various intermediate stages have been identified preceding the development of malignant colonic tumors. Several studies have demonstrated that generous consumption of vegetables reduces the risk of colon cancer. This idea has prompted the present investigation to search for some novel plant products, which may have possible anticarcinogenic activity. It has already been proved from various experiments that chemopreventive agents, by virtue of their anti-oxidant, anti-inflammatory, anti-proliferative, apoptosis-inducing activity, act at various levels including molecular, cellular, tissue and organ levels to interfere with carcinogens. Previous studies from our laboratory have already reported the inhibitory effect of cinnamon and cardamom on azoxymethane induced colon carcinogenesis by virtue of their anti-inflammatory, anti-proliferative and pro-apoptotic activity. This particular experiment was carried out to assess the anti-oxidative potential of these spices. Aqueous suspensions of cinnamon and cardamom have been shown to enhance the level of detoxifying enzyme (GST activity) with simultaneous decrease in lipid peroxidation levels in the treatment groups when compared to that of the carcinogen control group.

**Key Words:** Colon carcinogenesis - GST - lipid peroxidation - anti-oxidant - cinnamon - cardamom

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### Introduction

Colon cancer is one of the leading causes of cancer death in Western countries. It is the third most common malignant neoplasm worldwide (Rose et al., 1986). Genetics, experimental, and epidemiological studies suggest that colorectal cancer results from complex interactions between inherited susceptibility and environmental factors including life style factors such as diet and nutrition (Bjelke, 1980; Hirayama et al., 1981; Phillips and Snowdon, 1985; Potter and McMichael, 1986; Bingham, 1990; Willett et al., 1990; Goldbohm et al., 1994).

Prevention of disease is an old and important concept. It has been realized that the focus and emphasis should be shifted to the control of carcinogenesis, rather than attempting to cure end-stage disease. Chemoprevention, which is a pharmacological approach to intervention in order to arrest or reverse the process of carcinogenesis, attempts to address this issue. Recently, considerable attention has been focused on identifying naturally occurring chemopreventive substances capable of inhibiting, retarding, or reversing the preneoplastic lesion

of carcinogenesis. Chemopreventive substances are identified on the basis of their antioxidant, antimutagenic and anti-inflammatory activities capable of arresting proliferation and enhancing apoptosis and these are the major criteria for their anticarcinogenic activity. Scientific evidence supports that spices and condiments, which are an essential part of the Indian diet, have chemical constituents, which have antioxidant, antimutagenic and anticarcinogenic properties (Krishnaswamy, 1996). So growing interests are now being focused on dietary modulations that can contribute to inhibition of carcinogenesis by blocking or reversing the process of development of preneoplastic lesions. Aberrant crypt foci (ACF), constellation of morphologically abnormal crypts have been recognized to precede the development of colonic adenoma (Hill, 2000; Srivastava et al., 2001). Pretlow et al have shown that these lesions are present in the colonic mucosa of patients with colon cancer and have suggested that aberrant crypts are putative precursor lesions from which adenomas and carcinomas develop in the colon.

The search for an effective chemopreventive regimen

*Dept. of Cancer Chemoprevention, Chittaranjan National Cancer Institute, 37, S. P. Mukherjee Road, Kolkata-700026, India.\*Corresponding author: E-mail: archana\_sen@yahoo.com*

based upon natural food substances has led us to use two commonly consumed spices, viz., cinnamon (*Cinnamomum zeylanicum*), and cardamom (*Elettaria cardamomum*) as treatment agents in our experiments.

The primary mission of our study was to identify the effect of these spices against azoxymethane (AOM) induced colonic aberrant crypts in Swiss Albino mice. Mice were administered aqueous suspensions of the three spices orally from the first day of AOM treatment. It had already been reported earlier that at the end of 8 weeks of spice treatment, after 1st AOM injection, the total number of ACF and number of ACF with four or more aberrant crypts (ACs) were significantly reduced by treatment with 0.5% and 0.25% aqueous suspensions of cardamom and cinnamon respectively (Sengupta et al., 2005; Bhattacharjee et al., 2006). In order to assess the underlying pathway of the inhibitory effect of these spices on colonic ACF, certain biochemical studies were carried out to throw some light on the anti-oxidant potential of these spices. Propagative lipid peroxidation is a degenerative process that affects cell membranes and other lipid-containing structures under conditions of oxidative stress (Kappus, 1985; Girotti, 1985; Halliwell and Gutteridge, 1995). Endogenous DNA adducts derived from oxidative stress, lipid peroxidation, or other endogenous processes have been proposed as contributors to the etiology of human cancer (Marnett, 2000.). So, the level of hepatic and colonic lipid peroxidation was assessed in order to evaluate the antioxidant activity of these spices in the present investigation. Induction of GSTs, phase II enzymes that detoxify certain carcinogens, is regarded as a potential mechanism of blockade of the early stages of carcinogenesis (Sharma et al., 2001.). Moreover, GSTa, an intracellular non-seleno GSH-S-transferase have been implicated in lipid hydroperoxide detoxification (Flohe, 1982; Ursini and Bindoli, 1984). Hence, the modulatory influence of cinnamon and cardamom on this xenobiotic detoxifying enzyme was assessed.

## Materials and Methods

### Experimental Design

The experiment was designed in 2 sets according to the treatment agents used: Set I & Set II for testing the chemopreventive efficacy of 0.5% aqueous suspension of cardamom and 0.25% cinnamon respectively. Each set, having 30 mice, was divided into 3 groups - Normal, Carcinogen Control and 0.5% Cardamom (in case of Set I) / 0.25% cinnamon (in case of Set II). Each group in a set consisted of 10 mice. All the groups except the Normal were initiated with intraperitoneal injections (5mg / kg body weight) of AOM (Sigma Chemicals Co. MO, USA) once a week for two weeks. The Normal group received no treatment. The carcinogen control group (CC) was given AOM alone; whereas the cinnamon and cardamom treated groups received oral administration of 0.25% cinnamon and 0.5% cardamom at a dose of 100ml/mouse/day, continuously starting from 1st day of AOM injection. The treatment was continued for 8 weeks from the 1st day of AOM injection and the two parameters were studied thereafter.

### Estimation of Glutathione-S-transferase (GST) Activity

Spectrophotometric method ((Habig, et.al., 1974)) was adopted to evaluate the activity of GST in rat liver and colon tissues. After 8 weeks of 1st AOM injection, livers and colons were excised immediately after sacrifice. GST activities were measured in tissue cytosol by determining the increase in absorbance at 340 nm with 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate and the specific activity of the enzyme was expressed as formation of CDNB-GSH conjugate per minute per milligram of protein.

### Estimation of the Level of Lipid Peroxidation

Spectrophotometric method (Okawa, et.al., 1979) was also applied to estimate the level of lipid peroxidation by measuring the formation of lipid peroxides using Thiobarbituric acid. After 8 weeks of 1st AOM injection, livers and colons were excised immediately after sacrifice. The level of lipid peroxidation was determined by measuring the formation of lipid peroxides using Thiobarbituric acid, [TBA (Sigma)] and expressed as Thiobarbituric Acid Reactive Substances (TBARS) formed per milligram of protein using an absorbance of 532nm.

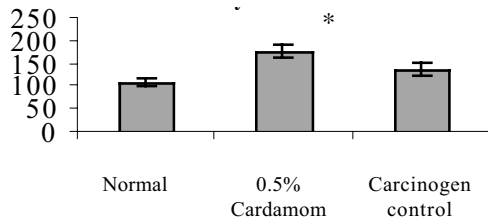
## Results

### Cardamom and Cinnamon increase the level of glutathione-S-transferase (GST) activity in liver and colon

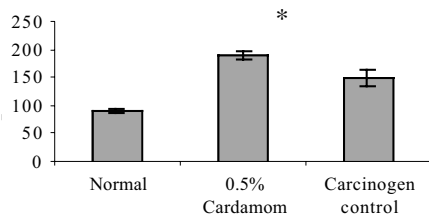
It is quite evident from the results that treatment with cardamom and cinnamon suspension has led to an elevation in the level of GST (expressed as nanomoles of CDNB-GSH conjugate formed per minute per milligram protein) in both liver and colon.

In the Set I after 8 weeks of carcinogen exposure the mean value of GST activity was found to increase in the colon of the carcinogen control group (138.240±13.009 nM CDNB-GSH conjugate /min/mg protein) when compared to that of normal mice (110.289±9.334). The activity of this phase II xenobiotic metabolizing enzyme was found to increase further in the cardamom -treated group (Figure 1.1). In Set II, the mean level of GST in colon was 49.067 nM CDNB GSH Conjugate/min/mg protein in the carcinogen control group, which had increased to 105.326 nM CDNB GSH Conjugate/min/mg protein as a result of treatment with 0.25% cinnamon (Figure 2.1).

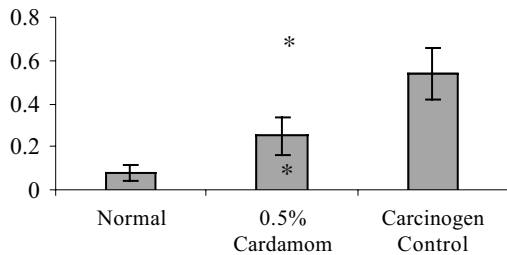
In liver an elevation of GST activity was noted in (Figure 1.2) the carcinogen control group (151.344±15.321 nM CDNB-GSH conjugate/min/mg protein) when compared to the corresponding value of the normal group (92.311±15.321 nM CDNB-GSH conjugate/min/mg protein) in Set I. Treatment with 0.5% cardamom caused the mean GST activity to increase further up to 191.074±7.614 nM CDNB-GSH conjugate/min/mg protein. Similarly, treatment with 0.25% aqueous suspension of cinnamon resulted in a significant increase in the GST activity in comparison the carcinogen control group, the values being 198.35 nM CDNB GSH Conjugate/min/mg protein and 82.063 nM CDNB GSH Conjugate/min/mg protein respectively in the treated and



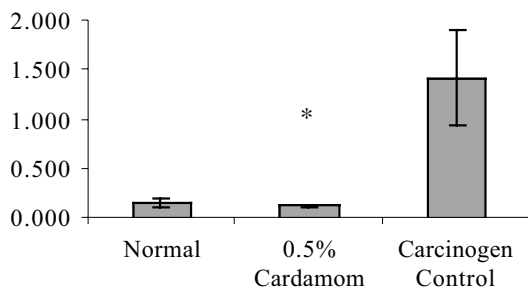
**Figure 1. a) Activity of GST in Mice Colon after 8 Weeks of 1st AOM Injection.** \*Significantly different from carcinogen control group [p=0.0445]



**Figure 1. b) Activity of GST (nM CDNB-GSH/ min/ mg protein) in Liver after 8 Weeks of 1st AOM Injection.** \* Significantly different from carcinogen control group (p=0.0479)



**Figure 1. c) Levels of Lipid Peroxidation (nM TBARS formed/ mg protein) in Mouse Colon after 8 Weeks of 1st AOM Injection** \*significantly different from carcinogen control group (p=0.0282)

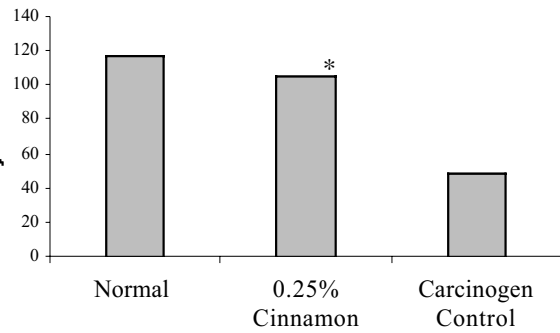


**Figure 1. d) Levels of Lipid Peroxidation (nM TBARS formed/ mg protein) in Mouse Liver after 8 Weeks of 1st AOM Injection.** \*significantly different from carcinogen control group (p=0.03)

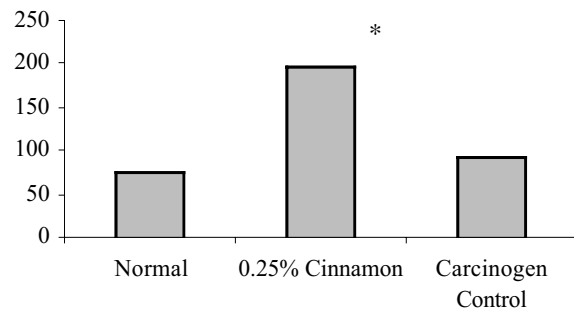
carcinogen control group (Figure 2.2).

*Cardamom and Cinnamon decreases the Levels of lipid peroxidation in Liver and Colon*

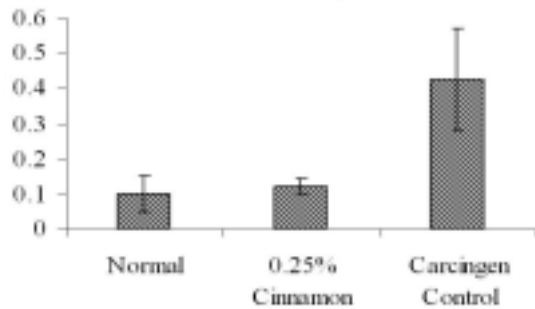
Results suggest that in both the experimental sets, levels of hepatic and colonic lipid peroxidation had increased quite significantly in the carcinogen control groups with respect to that of normal mice. In other words, exposure to azoxymethane has lead to a significant increase in the free radical mediated damage to membrane lipids when compared to that of normal mice. However,



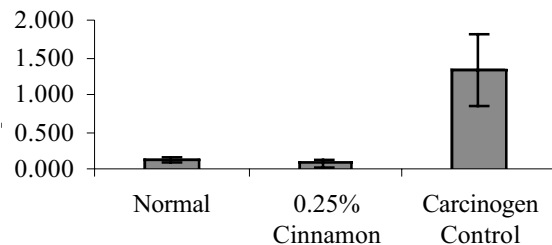
**Figure 2. a) Glutathione-S-Transferase (GST) Activity (nM CDNB-GSH) in Mouse Colon after 8 Weeks of 1st AOM Injection.** \* Significantly different from carcinogen control group (p=0.0365)



**Figure 2. b) Activity of GST (nM CDNB-GSH/min/ mg protein) in Mice liver at 8 Weeks after 1st AOM Injection.** \*Significantly different from carcinogen control group ( p=0.0452)



**Figure 2. c) Lipid Peroxidation Level in Mice Colon after 8 Weeks of 1st AOM Injection.** \* Significantly different from carcinogen control group ( p=0.0262 with respect to the normal group; p=0.0362 with respect to the carcinogen control group)



**Figure 2. d) Lipid Peroxidation Level, Expressed as Nanomoles of Thiobarbituric Acid Reactive Substances (TBARS) Formed Per Milligram Protein, in Mice Liver after 8 Weeks of 1st AOM Injection.** \*Significantly different from carcinogen control group (p=0.0220 with respect to the normal group; p= 0.0196 with respect to the carcinogen control group)

after 8 weeks of treatment, cardamom and cinnamon could counteract this oxidative damage to cellular membranes quite effectively as is evidenced by comparing the levels of thiobarbituric acid reacting substances (TBARS) in the treated groups and the carcinogen control group. The values for Set I being  $0.540 \pm 0.119$  nM TBARS/mg protein and  $0.255 \pm 0.088$  nM TBARS/mg protein respectively in the colon of carcinogen control group and cardamom-treated group (Figure 1.3). For Set II the values are  $0.422$ -nMTBARS/mg protein and  $0.121$  nM TBARS/mg protein for respectively (Figure 2.3).

Assay of lipid peroxidation in the liver reflected a similar observation as in the colon in both the experimental sets (Figures 1.4 and 2.4).

## Discussion

The study conducted showed that the levels of both hepatic and colonic GST levels were considerably increased following treatment with cinnamon and cardamom. GSTs are a family of phase II detoxifying enzymes, synthesized in liver, and catalyze the conjugation of reduced Glutathione (GSH) to a variety of electrophilic compounds (Talalay, 1992). During chemically induced carcinogenesis, AOM is metabolized into an electrophilic methyl diazonium ion, which can methylate cellular nucleophiles including DNA (Fiala et al., 1987). Induced GST may act at this point to inhibit the process. The ability to induce GST levels proves the anti-oxidant property of the spices. This effect is probably a consequence of the modulatory influence of the spice suspensions on the intermediate metabolites resulted from the bioactivation of AOM during the process of carcinogenesis. The anti-oxidant property of cinnamon has been demonstrated in several other studies too (Mancini-Filho et al., 1998; Dhuley, 1999), which may be attributed to its high flavonoid content (Nair et al., 1998).

However, during our present investigation, in the Experimental Set I, the GST activity was found to increase in the carcinogen control group when compared with that of normal group. This may be the effect of natural anti-oxidant defense of the body, which tries to combat the effect of carcinogen exposure (Ketterer, 1988). However, treatment with cardamom in our experiments gives a further boost to this defense system. Chemical analysis of cardamom has shown it to contain anti-oxidant flavonoids (Nair et al., 1998). The observed induction of GST and reduction of lipid peroxidation by cardamom may be due to the anti-oxidant activity of its flavonoid constituents.

AOM was reported to cause oxidative stress in the colonic mucosae (Tanaka et al., 1998). During the process of induced carcinogenesis oxidative stress leads to the formation of reactive oxygen species, viz., superoxide ion, hydrogen peroxide, hydroperoxyl and hydroxyl radicals, which can cause DNA damage by initiation of lipid peroxidation (Hruszkewycz, 1988). Increased level of lipid peroxidation during colon carcinogenesis, as observed in liver and colon of carcinogen control mice during the present experiment, may cause DNA damage including DNA adduct formation (Bartsch et al., 2002). Treatment

with cardamom and cinnamon suspensions, however, resulted in a significant decrease in the levels of both colonic and hepatic lipid peroxidation. In accordance with our findings, experiments by Shobana and Naidu also showed that cinnamon extract was able to dose-dependently inhibit enzymatic lipid peroxidation (Shobana and Naidu, 2000). Moreover, an in vivo study has also shown cardamom to enhance anti-oxidant enzyme activities in rats fed a fat diet. It is noteworthy that, cardamom was found to partially counteract the increase in lipid conjugated dienes and hydroperoxides (Dhuley, 1999).

To summarize, our present study clearly demonstrates the anti-oxidant activity of both cardamom and cinnamon. Apart from that, previous studies in our laboratory have proved the cancer preventive action of these spices against AOM induced colon carcinogenesis in Swiss albino mice by virtue of their anti-inflammatory, antiproliferative and apoptotic actions. Nevertheless, these are only preliminary studies. Moreover, to establish cinnamon and cardamom as potent chemopreventive agents, the properties of their active constituents, including their metabolism and toxicity, need to be tested.

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