Sambar, an Indian Dish Prevents the Development of Dimethyl Hydrazine–Induced Colon Cancer: A Preclinical Study

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ABSTRACT

Background: Colon cancer (CC) is the third commonly diagnosed cancer and the second leading cause of mortality in the US when compared to India where prevalence is less. Possible reason could be the vegetarian diet comprising spices used in curry powders. Researchers believe that 70% of the cases are associated with diet. Spices have inherited a rich tradition for their flavor and medicinal properties. Researchers have been oriented towards spices present in food items for their antitumorigenic properties. Objective: We investigated the effects of sambar as a preventive measure for 1,2-dimethyl hydrazine (DMH)-induced CC in Wistar albino rats. Materials and Methods: The animals were divided into three groups (n = 6) namely control, DMH, and sambar. At the end of the experimental period, the animals were killed using anesthesia and the colons and livers were examined. Results: All the treatment groups exhibited a significant change in the number of aberrant crypt foci (ACF). Sambar group showed a significant change in the colonic GSH when compared to both normal and DMH groups. A significant reduction in the liver GSH was noted in the sambar group. Only sambar group showed a significant change in the liver catalase levels when compared to DMH. There was a significant reduction in the colonic nitrite in the sambar-treated group; 2.94 ± 0.29 when compared to DMH control at 8.09 ± 1.32. On the contrary, a significant rise in the liver nitrite levels was observed in the sambar-treated rats. Conclusion: Sambar may prevent the risk of CC when consumed in dietary proportions.

Key words: Aberrant crypt foci, dimethyl hydrazine, sambar, spices

SUMMARY

• Consumption of sambar significantly reduced aberrant crypt foci in DMH-induced colon cancer model
• Sambar treatment prevented DMH-induced oxidative changes in the colonic tissue, indicating its antioxidant role

INTRODUCTION

Cancer is a threat to the developed and developing nations and is the second most common cause of death. It results from abnormalities in normal mechanisms governing cell behavior. Cells being the smallest and most basic structural and functional unit of the human body undergo proliferation, differentiation, and several other biological processes to maintain the normal cycle of life. This whole phenomenon is controlled by various proteins and cell signaling mechanisms at the cellular and molecular level. Irregularity in any one step results in unregulated proliferation of cells. Thus, we can say that cancer represents a group of diseases that involves unregulated proliferation of cells leading to formation of tumor.

Sambar is a very popular South Indian dish that is eaten with rice and/or rice preparations like idlis, dosas, etc. Many versions of Sambar powder (ingredients differ) exist, varying from region to region. Indian spices that go into the making of this powder are coriander seeds, fenugreek seeds, turmeric rhizomes, black pepper, curry leaves, cumin seeds, and asafoetida. Spices are generally considered to be safe by the USFDA in the proportions used in food. In Asia, the intake of turmeric, a component of curry powder, has been reported to lower the incidence of colon cancer (CC).[1-3]

There are scientific reports stating the beneficial effects of Indian spices and condiments in cancer prevention.[4] CC is the third most commonly diagnosed cancer in the US, whereas in India the incidence is the lowest.[1,2] Almost 70% of the incidence of CC is linked to diet. This may also be attributed to lifestyle factors.[5] A possible explanation is the vegetarian diet comprising spices used in curry powders. Researchers believe that 70% of the cases are associated with diet. Spices have inherited a rich tradition for their flavor and medicinal properties. Researchers have been oriented towards spices present in food items for their antitumorigenic properties.

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diet comprising spices used in curry powders. Hence, the study was proposed to investigate the activity of sambar in 1,2-dimethyl hydrazine (DMH)-induced CC models in albino rats.

**MATERIALS AND METHODS**

**Chemicals**

DMH was procured from Sigma-Aldrich Co. LLC, St. Louis, MO, USA. Glutathione, trichloroacetic acid, and 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) were purchased from HiMedia Laboratories, Mumbai, Maharashtra, India. TNF-alpha kits (Catalog # KRC3011) were procured from Life Technologies, Carlsbad, CA, USA. Carboxy methyl cellulose was purchased from S D Fine-Chem Limited, Mumbai, Maharashtra, India. Potassium chloride and hydrogen peroxide (LR grade) were bought from Merck KGaA, Darmstadt, Germany. BCA reagent was from ThermoFisher Scientific India Pvt. Ltd., Mumbai, Maharashtra, India, and sodium nitrite was procured from LOBA Chemie Pvt. Ltd., Mumbai, Maharashtra, India.

**Animals**

Male Wistar rats inbred at Central Animal Research Facility, Manipal University, Manipal, were used in the study. Animals were acclimatized to the experimental room having temperature 23 ± 3°C, controlled humidity 75%, and 12:12 hour light and dark cycle. Rats were housed in sterile polypropylene cages containing sterile paddy husk as a bedding material. The animals were fed with autoclaved feed and water. Eight- to ten-weeks old male rats weighing 120 ± 10 g were used for the study. The animal care and handling was carried out in accordance to guidelines issued by Institutional Animal Ethics Committee (IAEC), Manipal University, Manipal. The protocol was approved by IAEC with reference no. IAEKC/KMC/79/2014.

**Grouping and dosing regimen**

Animals were divided into three groups (n = 6) namely, normal control, DMH control, and sambar. The normal control received 0.25% CMC, p.o. CC in rats was induced using DMH as per our previously standardized protocol. In brief, rats were pretreated with only sambar (6 mL/kg, p.o.) or vehicle (water; 6 mL/kg, p.o.) for an initial period of 8 weeks. After 8 weeks, DMH was administered weekly once for 20 weeks (dose of 20 mg/kg, i.p. for 10 weeks and thereafter 30 mg/kg, i.p. for next 10 weeks). Sambar/vehicle treatment was continued for an additional 20 weeks.

**Composition of sambar**

The ingredients present in sambar powder used for the study are listed below:

- Dried long fruit of *Capsicum annum* var. annum (dried chilli pepper) = 1000 g
- Dried seeds of *Coriandrum sativum* seeds (coriander seeds) = 1000 g
- Cooked and dried unripe drupes of the *Piper nigrum* (black pepper) = 500 g
- Dried seeds of *Cuminum cyminum* (cumin) = 400 g
- Dried seeds of *Trigonella foenum graecum* (fenugreek) = 30 g
- Dried rhizome of *Curcuma caesia* (black turmeric) = 100 g
- *Oryza sativa* var. ponni (rice) = 200 g

The above mentioned components were ground to a fine powder at Sarawathis Traders and Flour Mills, Chennai, Tamil Nadu, India (using Danish Type Flour Mill, Model No: 2059072, manufactured by Nilax Overseas, Rajkot, Rajasthan, India).

Next, 25 g of yellow pigeon peas cooked in pressure cooker for 15 min and 6 g of the sambar powder (Indian spices) were boiled for 10 min. The sambar obtained was cooled and shaken well before administration to animals.

**Parameters assessed in DMH-induced CC model**

At the end of the study, the animals from each group were killed and the colons and livers dissected and perfused with an ice cold saline trans cardially. The tissues were blot dried, weighed, and 10% of homogenate was prepared with ice-cold (150 mM) potassium chloride solution using a homogenizer (RQ-127/A/D, REMI House, Mumbai, Maharashtra, India). The homogenate was used for the following estimations, viz., GSH,[8] catalase,[9] nitrite,[10] and TNF-α.[11]

During the killing of animals, the entire colon was removed, opened longitudinally, and rinsed in saline. Cecum was excised. A piece of colon of 8 cm each was fixed on a filter paper in 10% buffered formalin for 24 hours and then stained with 0.1% methylene blue in phosphate buffer saline for 20 min for aberrant crypt foci (ACF) counting. Specimens were carefully examined, mucosal side uppermost, at 40x magnification under a compound light microscope and the number of ACF was determined. ACF incidence, polyps and ACF count, the colon length/weight ratio, and histopathological examinations were recorded.

**Statistical analysis**

Data were analyzed by one-way analysis of variance (ANOVA) and significant differences among the treatment groups were evaluated by post-hoc Tukey’s test. The results were considered significant at P < 0.05. All statistical analyses were done using fully functional demo of Prism version 5.03 (GraphPad Software Inc., San Diego, CA, USA).

**RESULTS**

**The spleen and liver index**

There were no significant changes in the liver index of the normal control, DMH, and sambar treatments [Figure 1A]. The same was true of the spleen index [Figure 1B]. These results indicate that DMH did not produce hepatomegaly or splenomegaly.

**Colon L/W ratio**

The normal control showed a significant change in the colon L/W ratio at 12.255 ± 0.718 when compared to DMH control at 9.476 ± 0.549 at P < 0.01. A significant change was noted in the colon L/W ratio of DMH control with respect to sambar group at 11.782 ± 0.320. No significant change was observed between normal and sambar treatments [Figure 2].

**Number of ACF/5 cm² and number of ACF/cm²**

Treatment with DMH produced structural changes in colon, leading to the formation of ACF. Pretreatment with sambar along with DMH significantly (P < 0.01) reduced ACF [Figure 3A and B], indicating that sambar could protect against DMH-induced colonic changes.

![Figure 1](http://example.com/image1.png)

**Figure 1**: (A) Liver and (B) spleen indices of the normal, DMH, and pretreated sambar groups. Data is represented as mean ± SEM of six values.
GSH in the liver and colon homogenate
There was no change in the colonic and liver GSH of normal control versus DMH control. Sambar group showed a significant change in the colonic GSH when compared to both normal and DMH control. A significant reduction in the liver GSH was noted at 0.98 ± 0.07 when compared to 7.229 ± 2.67 and 10.88 ± 0.93 of normal control and DMH controls, respectively [Figure 4A and D].

Catalase in the liver and colon homogenate
No significant change was observed in the colonic catalase in any of the treatment groups. Only sambar group showed a significant (P < 0.05) change in the liver catalase levels when compared to DMH [Figure 4B and E].

Nitrite content in the liver and colon homogenate
There was a significant reduction in the colonic nitrite in the sambar-treated group; 2.94 ± 0.29 when compared to DMH control at 8.09 ± 1.32. On the contrary, a significant rise in the nitrite levels was observed in the sambar-treated rats; 3.61 ± 0.38 when compared to DMH group at 0.95 ± 0.12 [Figure 4C and F].

Measurement of TNF-α level
TNF-α levels in colonic tissue increased in DMH-treated group as compared to normal control group. Treatment with sambar resulted in a mild elevation of TNF-α levels as compared to DMH group. However, the changes are nonsignificant [Figure 5].

Histopathological results
In the normal rats, the epithelium was intact with no signs of dysplasia, adenoma, or carcinoma.

In the DMH group, mucosal polyps, ACF, and adenoma of colon were seen in 5 out of 6 rats. In all the rats severe dysplasia was observed in the DMH group. Mild hyperplasia with few crypts interspersed in the stromal tissues was observed in the sambar treatment group. There was also a reduced incidence of adenoma formation in the colon on treatment with sambar [Figure 6 and Figure 7].

DISCUSSION
The present study was undertaken to investigate the protective actions of sambar, a popular South Indian dish, in DMH-induced colon carcinogenesis in rodents.
CC is a complex process, which involves initiation, promotion, progression, and invasive phases from the development of a normal crypt to an adenoma. Thus, an intervention into any one of these phases of colon carcinogenesis can be an effective strategy for the prevention of CC. Chronic inflammation is a well-recognized risk factor for the development of human cancer. In the present study, the DMH control animals showed an initiation of ACF, promotion into adenoma, and a significant increase in inflammatory mediator levels of TNF-α.
DMH is a known carcinogen used to study sporadic forms of colon carcinoma resulting in a reproducible experimental in vivo model for pre-clinical screening of test compounds. Metabolism of DMH leads to the formation of azoxymethane (AOM), methylazoxymethanol...
Surface view of unsectioned colonic mucosa stained with methylene blue (40x). In the control group, the crypts appeared normal. In the DMH group, yellow circles indicate methylene blue–stained ACF consisting of two large elliptical crypts. Sambar-treated group showed reduced number of ACF. Red arrows indicate the normal crypts.

Histopathological analysis of the colonic mucosa with evidence detailed studies are required to investigate the effects of the protective effect in the present study; however, for substantial studies are required, both for the prevention and treatment of CC. Considering the properties of these individual spices, we can conclude that their combined presence in sambar could exert an overall protective effect against the development of CC when consumed in the diet.

In the liver, DMH treatment did not significantly alter the oxidative markers like GSH, nitrite, and catalase. This indicates that the dose of DMH used for the induction of CC did not have any deleterious effect on the liver markers. Whereas, DMH caused oxidative stress in the colonic tissue as seen by an elevation in GSH, catalase, and nitrite levels. Treatment with sambar caused an elevation of catalase and nitrite levels and depletion of the GSH levels, indicating the presence of pro-oxidants in the spices present in sambar. Sambar treatment prevented DMH-induced oxidative changes in the colonic tissue indicating its antioxidant role. The constituents present in sambar exhibited both pro- and antioxidant properties in different tissues, which might have led to an overall beneficial effect in colonic tissue as is evident from the physiological and histological parameters assessed.

There are reports indicating DMH administration increases levels of TNF-α, IL-6, and causes oxidative stress. The increase in ACF count and number of polyps were used to evaluate the extent of damage to the colon caused by DMH. DMH treatment showed an increase in ACF count with adenoma. The sambar-treated group maintained a difference in the TNF-α levels across the groups. Thus, we could conclude that sambar was able to reduce oxidative stress in the colonic tissue without affecting the inflammatory mediators thereby preventing the development of CC.

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Conflicts of interest
There are no conflicts of interest.

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