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ROLE OF CHLORELLA PYRENOIDOSA ON ACINAR CRYPT FOCI FORMATION IN COLON CANCER

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ABSTRACT

Colon cancer is the abnormal or uncontrolled growth of new cells in the colon, characterized by cells that tend to invade surrounding tissues and metastasize the new body sites. Colorectal cancers arise from adenomatous polyps in the colon. These mushroom-shaped growths are usually benign, but some develop into cancer over time Aberrant Crypt Foci were induced by all colon carcinogens in a dose and species dependant manner, the number and growth were modified by the modulators of colon carcinogenesis and they predicted the tumor outcome in several rodent studies. Chlorella's multi-layered cell wall contains the polysaccharides and beta carotene which can be attributed to much of the observed anti-cancer action.

A total of 36 male wistar rats were divided into six groups. Group 4,5 and 6 were given DMH 20mg/kg once a week for four weeks along with Chlorella pyerenoidosa 500mg,750mg and 1000mg/kg respectively for 16 weeks. At the end of 16 weeks, the animals were sacrified and the liver and colon tissues were subjected for histopathological studies. Results showed that DMH induces ACF indicating that development of these lesions in the colon is clearly related to the genotoxic events

In our study DMH increased the number of ACF whereas chlorella reduced the growth of ACF, the total number of ACF and also reduced its distribution in the proximal, middle and distal regions of colon. The size of the ACF in chlorella treated group was small compared to the DMH group indicating the effectiveness of chlorella in the management of colon cancer. The histopathological studies indicate that the colon cancer is induced by DMH which is evident by apoptotic cells and the invasion of the intestinal mucosa by the cancer cells whereas such an infiltration is reduced in chlorella treated group. This substantiates the claim that chlorella can serve as a prophylactic agent in the management of colon cancer.

INTRODUCTION

Colon rectal cancer (CRC) is the third most common cause of cancer death in developed countries. Colon cancer is the abnormal or uncontrolled growth of new cells in the colon, characterized by cells that tend to invade surrounding tissues and metastasize the new body sites (Calvert and Frucht., 2002) .The number of new cases per year has steadily increased since 1965, being now three times higher than in the mid 1960s in Asian men, it has the highest incidence compared to all other cancers (1 in 43); in Caucasian women, it is the second most prevalent (1 in 44) and the third most prevalent cancer in Caucasian men (1 in 34) based on statistics from 1993 -1995 (Sitas *et al*., 1998). CRC is responsible for 9% of the approximately 6.35 million invasive cancers occurring annually.
Cancer of the colon and rectum accounted for approximately 1, 48,000 new cases in 2004 (Jemal *et al*., 2005).Colorectal cancers arise from adenomatous polyps in the colon. These mushroom-shaped growths are usually benign, but some develop into cancer over time. Localized colon cancer is usually diagnosed through colonoscopy. The incidence and epidemiology, etiology, pathogenesis and screening recommendations are common to both colon cancer and rectal cancer. The epidemiology of large bowel malignancies has generated a lot of interest in recent years, mainly because the disease provides an excellent model for studying the interaction between specific genes and several environmental factors in the etiology of cancer (Ponz de leon *et al*., 1996).
Typically, the rates of colon cancer incidence tend to be higher in developed countries that are economically privileged (Schottenfeld, 2005)

Aberrant crypt foci (ACF)

ACF are putative precursors of colon cancer. ACF were first detected in rodents in 1987 by Ranjana Bird (Takayama T *et al*., 2005) few weeks after carcinogen injection. ACF were induced by all colon carcinogens in a dose and species dependant manner, the number and growth were modified by the modulators of colon carcinogenesis and they predicted the tumor outcome in several rodent studies.

Chlorella (Chlorella pyrenoidosa)

Chlorella is fresh water, single celled algae that grows in fresh water.

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Chlorella's multi-layered cell wall contains the polysaccharides which research has shown to be responsible for much of the plant's immune-stimulating and detoxification properties. The presence of beta carotene can be attributed much of the observed anti-cancer action, CGF is also implicated in cancer prevention and treatment. Hence the present study was focused to screen the role of *Chlorella pyrenoidosa* in colorectal carcinoma of Wistar rats.

Aim

To evaluate the role of *Chlorella pyrenoidosa* on acinar crypt foci formation in colon cancer

Objectives

To access the histopathological observations in control, test and reference animals.

MATERIALS AND METHODS

Chemicals and Reagents

Drug/Chemicals	Source
Chlorella pyrenoidosa	E. Merck (Mumbai, India)
Irinotecan hydrochloride	E. Merck (Mumbai, India)
1,2-Dimethylhydrazine	Sigma-Aldrich (Delhi, India)

- All other chemicals and reagents were of the analytical grades and obtained locally.
- Biochemical and enzymatic kits were from Agappee diagnostics, Kerala.

Animal model used for investigation (Albino Wistar Rats)

Class :Mammalia
Family :Muridae
Order :Rodentia
Genus :Rattus
Scientific name : *Rattus norvegicus*

The institutional animal ethical committee (Register No.160/ 1999/CPCSEA), Annamalai University, Annamalai Nagar, India approved the experimental design (Proposal No.633, dated 25.05.2009). *Albino wistar* male rats of 140-160g were used for the study. Animals were housed in well ventilated room (temperature 23 ± 2°C, humidity 65-70% and 12h light/dark cycle) at Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University. Animals were fed with standard pellet diet and water *ad libitum*. All studies were conducted in accordance with Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) norms and the National Institute of Health guidelines "Guide for the Care and use of Laboratory Animals". The Male wistar rats, housed in polypropylenecages under hygienic conditions adapted to the laboratory conditions for a week were used for the study. The animals were divided into six groups. Each group had six rats and a total of 36 rats were used for this study. The experimental period was of 16 weeks duration. The animals were into the following groups.

Group – I	Normal control	Distilled water and normal diet
Group – II	Cancer control	DMH 20 mg/kg *s.c* once a week for four weeks
Group – III	Standard drug	DMH 20mg/kg *s.c* and Irinotecan 25mg/kg i.v. once a week for four weeks
Group – IV	Test drug low dose	DMH 20 mg/kg *s.c* once a week for four weeks and *Chlorella pyrenoidosa* 500 mg/kg/po daily (16 weeks)
Group –V	Test drug medium dose	DMH 20 mg/kg *s.c* once a week for four weeks and *Chlorella pyrenoidosa* 750 mg/kg /po daily (16 weeks)
Group –VI	Test drug high dose	DMH 20 mg/kg once a week *s.c* for four weeks and *Chlorella pyrenoidosa* 1000 mg/kg /po daily (16 weeks)

At the end of 16 weeks the animals were sacrificed by cervical decapitation under ketamine anesthesia. Blood samples were collected in centrifuge tubes using sodium citrate as anticoagulant and the plasma separated was used for the determination of diagnostic marker enzymes. The liver, colon tissue were excised immediately and washed with chilled isotonic saline. The tissue homogenates were prepared in ice cold 0.1 M Tris-HCl buffer, pH 7.2 separately. The homogenate was centrifuged and the supernatant was used for the assay of clinical marker enzymes in the tissue & determination of biochemical parameters and the remaining tissue was subjected for histopathological studies.

Histopathological studies of colonic tissues of rats

The isolated liver & colon were sliced into 5 mm pieces and fixed in neutral formalin (10%) solution for 3 days. Liver & colon pieces were washed under running water for about 12 hrs. This was followed by dehydration with alcohol of increasing strength (70%, 80%, and 90%) for 12 hrs each. Final dehydration was carried out using absolute alcohol with about 3 changes at 12 min interval. Cleaning was done by using xylin with changes at 15 - 20 min interval. After cleaning the pieces were subjected to paraffin infiltration in automatic tissue processing unit. The pieces were washed under running water to remove formalin completely.

The following steps were performed

- Hard paraffin was melted and poured into L-shaped block. The liver & colon pieces were then dropped into the liquid paraffin quickly and allowed to cool.
- The blocks were cut using microtone to get sections of thickness of 5 microns. The section were fixed on a glass using albumin and allowed to dry.
- Eosin an acid stain and hematoxylin, a basic stain were used for staining the liver, colon pieces.
- The section was then mounted in Diphenyl xylin mountant. Staining result showed blue colour nucleus and cytoplasm with various shade of pink with change in different tissue component. (Sini Sadasivan *et al*., 2006).

RESULTS

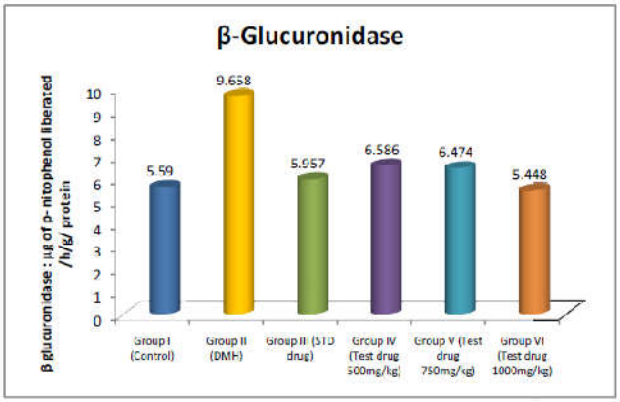

Fig.1 Effect of activites of colonic bacterial enzymes of the control and experimental rats

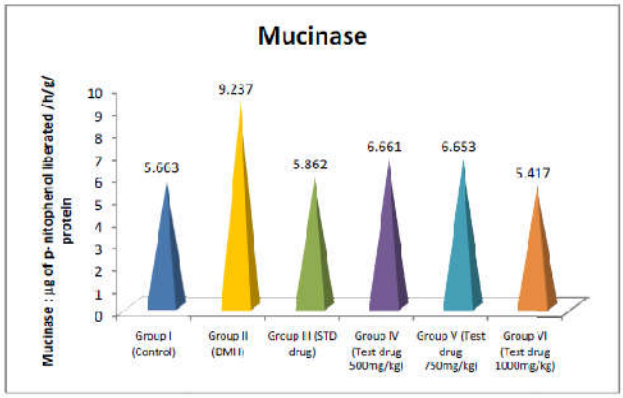

Fig 2 Effect of Activites of colonic bacterial enzymes of the control and experimental rats

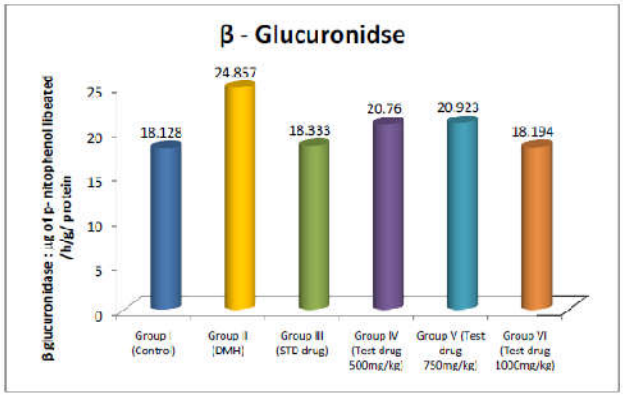

Fig.3 Effect of Activites of faecal bacterial enzymes of the control and experimental rats

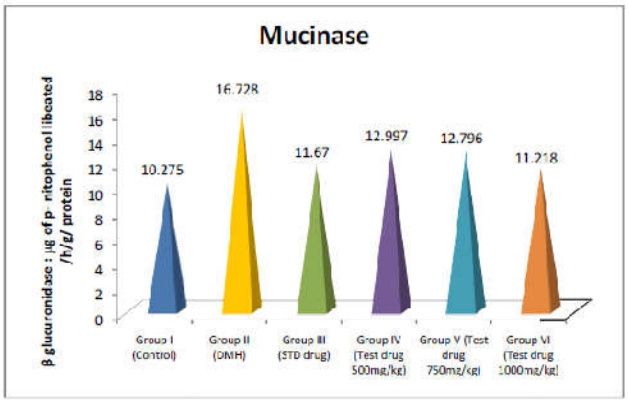

Fig 4 Effect of Activites of faecal bacterial enzymes of the control and experimental rats

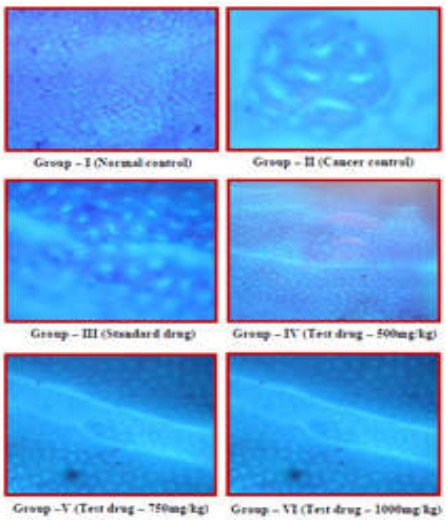

Fig 5 Histological photographic views of ACF

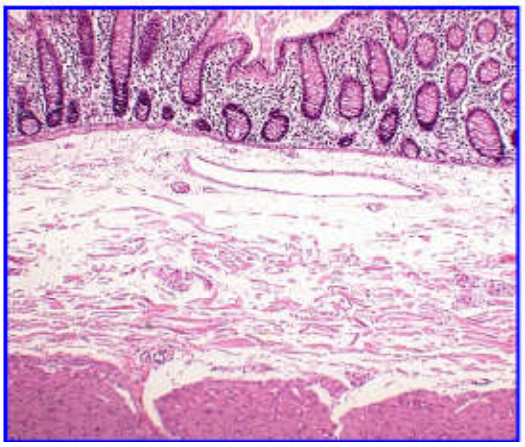

Fig 6 NORMAL – Mucosa and sub mucosa appears to be normal, serosa unremarkable showing normal cellular structure

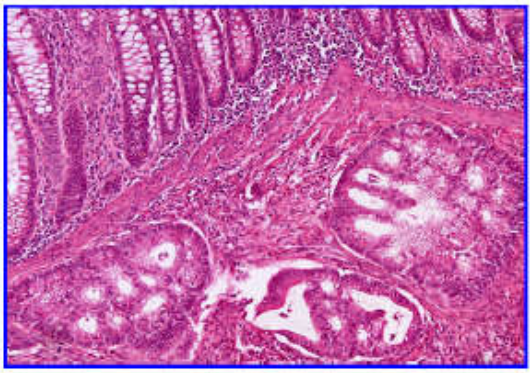

Fig 7 (DMH treated) Mucosa shows chronic inflammatory cells well differentiated carcinomas infiltrating in the serosa

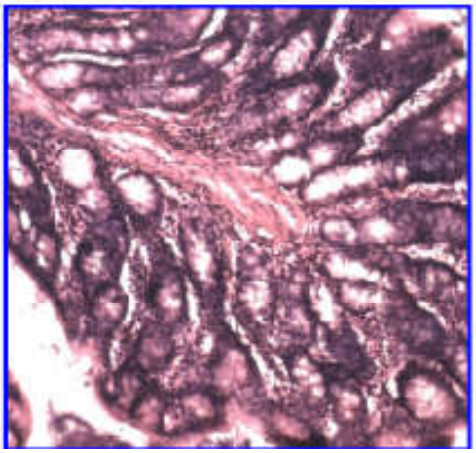

Fig. 8 On treatment with (STD drug) the cells regenerated to normal structures. Mucosa and sub mucosa appears to be normal, serosa unremarkable showing normal cellular structure

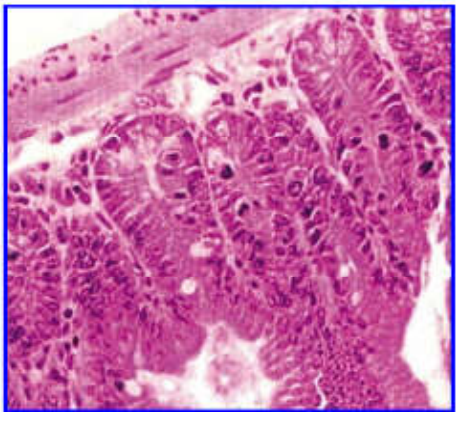

Fig 9 On treatment with (Chlorella 500 mg) the cells regenerated to normal structures. Mild dysplasia and few tumor cells apoptotic cells were seen

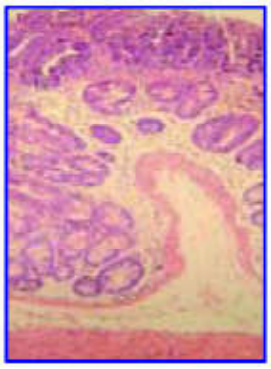

Fig. 10 on treatment with (Chlorella 750 mg) the cells regenerated to normal structures. Tumor cells reduced and restricted few mucosal glands and other mucosa glands appears to be normal

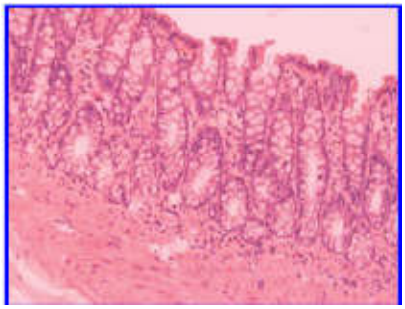

Fig 11 on treatment with (Chlorella 1000 mg) the cells regenerated to normal structures. Tumor cells reduced and restricted few mucosal glands and other mucosa glands appears to be normal

DISCUSSION

Dimethylhydrazine (DMH) is metabolized to a methyl free radical and generates hydroxyl radical or hydrogen peroxide in the presence of metal ions that may contribute to the initiation of lipid peroxidation (Dudeja *et al* 1990). The products of lipid peroxidation are measured to find out the amount of oxidative damage in the cancer cells.

Catalase and glutathione peroxidase are considered to be the primary antioxidant enzymes and they are involved in the direct elimination of the reactive oxygen species (ROH). Reduced glutathione (GSH) helps in the primary defense mechanism and is the non-enzymic small molecular antioxidant.

Chlorella deattenuates the hepatic damage induced by DMH possibly by

1. Enhancing reduced glutathione levels by inducing the GSH synthesing enzymes like γ glutanylcysteine synthetase and GSH synthetase.
2. Increasing adaptive response to compensate for the depleted GSH levels by inhibition of conversion of oxidised glutathione to reduced glutathione by glutathione reductase.
3. Increasing the rate of free radical utilization via the glutathione peroxidase system.
4. Inhibition of lipid peroxidation by elevating hepatic β carotene and vit - A levels.

Effect of Chlorella on Acinar Crypt Foci (ACF) formation

ACF are microscopic lesion that precede the development of adenomas and are considered as the earliest premalignant lesions in colon carcinogenesis (Bird 1987). DMH induces ACF indicating that development of these lesions in the colon is clearly related to the genotoxic events (Bilbin *et al* 1992). In our study DMH increased the number of ACF whereas chlorella reduced the formation of ACF. The total number of ACF, number of large crypts and number of Ac/ACF (crypt multiplicity) were used to evaluate the potential colon cancer preventive agents. Since DMH is known to stimulate the colonic epithelial cell proliferation it is possible that alteration of protein Kinase C may be the reason for the proliferative changes induced by this carcinogen. Similarly the over expression of COX_2 in DMH treated rats has been reported earlier (Shih *et al* 2004)

In our study chlorella reduced the growth of ACF, the total number of ACF and also reduced its distribution in the proximal, middle and distal regions of colon. The size of the ACF in chlorella treated group was small compared to the DMH group indicating the effectiveness of chlorella in the management of colon cancer. The protective effect of chlorella may be due to

1. Inhibition of ribonucleotide reductase, a key enzyme for DNA synthesis
2. Modulation of protein Kinase C and COX_2
3. Inhibition of tumor cell division
4. Inhibition of DMH induced mutations.

There is a strong association between the metabolic activity of the intestinal microflora and cancer of the large bowel (Reddy *et al* 1978). The activation of the procarcinogens could be mediated enzymatically by intestinal bacteria and the activities of colonic bacterial enzymes like β - glucuronidase are stimulated by DMH. Chlorella reduced the level of β - glucuronidase.

Mucinase, a hydrolytic enzyme secreted by the intestinal microflora degrades the protective mucus layer of the colon (Mastromarino 1976). A change in mucinase activity is accompanied by a change in the rate of mucin secretion and enhanced degradation of mucosal lining increase the risk of cancer. In our present study the level of mucinase is increased in DMH treated group. Chlorella reduces its level indicating the potential anticancer effect.

The histopathological studies indicate that the colon cancer is induced by DMH which is evident by apoptotic cells and the invasion of the intestinal mucosa by the cancer cells whereas such an infiltration is reduced in chlorella treated group. This substantiates the claim that chlorella can serve as a prophylactic agent in the management of colon cancer.

CONCLUSION

The capacity of chlorella to stimulate the body defense mechanism may also contribute to its anti cancer action further chemical trial in cancer patients needs to be conducted before we come to a definite conclusion regarding its therapeutic benefits in the management of colorectal cancer.

Bibliography

Bilbin, M., Tudek, B. and Czeczot, H. (1992) Induction of aberrant crypt in the colons of rats by alkylating agents. *Acta Biochem Pol*, 39, 113-7.

Bird, R.P. (1987) Observation and qualification of aberrant cypts in the murine colon treated with a colon carcinogen: preliminary findings. *Cancer* Lett 37, 147-51.

Calvert P, Frucht H.(2002) The genetics of colorectal cancer. *Ann. Intern. Med.* 137:603-12.

Dudeja, P.K. and Braritus, T.A. (1990) 1,2-Dimethylhydrozine induced alterations in lipid peroxidation in preneoplastic and neoplastic colonic tissues. *Biochem Biophys Acta*, 1046, 267-70.

Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, *et al*. (2005) Cancer statistics. *CA Cancer J Clin*. 55:10-30.

Mastromarino, A., Reddy, B.S. and Wynder, E.L. 1976. Metabolic epidemiology of colon cancer enzymic activity of fecal flora. *American Journal of Clinical Nutrition*, 29, 1455-60.

Ponz de leon, Timothy G.; Jalan, Rajiv; Stanley, Adrian J.; Redhead, Doris N.; Sanfey, Hilary A.; Hayes, Peter C.; Garden, O James (1996). *European Journal of Gastroenterology & Hepatology*. 8(12):1145-1149.

Reddy, B.S., Hedges, A.R., Laakso, K and Wynder, E.L. (1978) Metabolic epidemiology of large bowel cancer fecal bulk and constituents of high risk north American and low risk Finnish population cancer, 42, 2832-8.

Schottenfeld D, (2005) Advances in cancer epidemiology: understanding causal mechanisms and the evidence for implementing interventions. *Ann Rev Public Health* 26:37–60.

Shih, C.K., Chiang, W. and Kuo, M.L. (2004) Effects of adlay on azoxymethane induced colon carcinogenesis in rats. *Food Chem Toxicol*, 42, 1339-47.

Sini Sadasivan, Latha, P.G., Sasikumar, J.M., Rajashekaran, S., Shyamal, S. and Shine, V.J. (2006) Hepatoprotective studies on *Hedyotis corymbosa* (L.) Lam. *Journal of Ethnopharmacology.* 106, 245-249.

Takayama T, Kukitsu T, Ishiwatari H, Kogawa T, Abe T, Niitsu Y (2005). "Aberrant crypt foci: detection, gene abnormalities, and clinical usefulness". *Clin Gastroenterol Hepatol* 3 (7 Suppl 1): 42-45.

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