Section - B

Studies on anti-inflammatory activity of Garden cress oil in Ulcerative colitis model
INTRODUCTION

Inflammatory phenomena are the basis for a number of disease processes, in virtually any systemic or organ-specific disease, ranging from classical rheumatic diseases to bronchial airway hyper-responsiveness, inflammatory bowel disease, psoriasis and atopic eczema. Epidemiological differences in the incidence of inflammatory diseases among different populations can be traced back to different nutritional intake of selected, quantitatively minor nutritional components such as ω-3 PUFAs. Further, whatever may be the etiology of the inflammatory diseases, ω-3 PUFAs have been part of management and therapy of these diseases. Fish oil and constituent fatty acids - EPA and DHA have acquired a status of alternative medicine in the treatment of inflammatory diseases including rheumatoid arthritis. Other ω-3 PUFA such as ALA and dietary sources containing it are still being investigated for their efficacy in modulation of inflammatory conditions.

Inflammatory bowel disease

Inflammatory bowel disease (IBD) denotes idiopathic disorders such as Crohn’s disease (CD) and Ulcerative Colitis (UC), characterized by chronic intestinal inflammation. UC is primarily a mucosal disease with almost exclusive colonic involvement in contrast to CD which may result in mucosal to transmural inflammation of virtually any part of gastrointestinal tract.

Table-4: Putative risk factors for inflammatory bowel disease

<table>
<thead>
<tr>
<th>Family history of inflammatory disease</th>
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</thead>
<tbody>
<tr>
<td>Cigarette smoking (risk factor for Crohn’s, protective factor for ulcerative colitis)</td>
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<tr>
<td>Appendectomy (protective factor for ulcerative colitis risk factor for Crohn’s ?)</td>
</tr>
<tr>
<td>High fat, sugar diet?</td>
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<tr>
<td>Prenatal or early childhood infections?</td>
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<tr>
<td>Wild type measles infection?</td>
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<tr>
<td>Attenuated live measles virus vaccine?</td>
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<tr>
<td>Mycobacterium para tuberculosis infection?</td>
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</table>

(Adopted from Loftus 2004)

The current hypotheses for the etiology of the inflammatory bowel diseases (IBD) emphasize genetic predispositions and environmental influences to impaired regulation.
of the gastrointestinal immune system (Podolsky 2002). Further, a number of risk factors have been identified to cause IBD (Table-4) but their role in etiology of IBD is unclear.

**Epidemiology**

UC and CD were traditionally considered as diseases of the western world, primarily the developed countries of northern Europe and America. Approximately 1.4 million persons in US and 2.2 million people in Europe are suffering from this disease (Loftus 2004). Chronic intestinal inflammation causes significant morbidity worldwide. It has a substantial impact on human health and social economy and has emerged as one of the major health problems in the world (Björkstén 2009; Thia et al. 2008). Historically, IBD was rare in other continents, with the exceptions of Israel, Australia, and South Africa. However, the incidence of IBD, especially UC, is rising in several countries previously thought to have low incidence, including Japan (Morita et al. 1995), South Korea (Yang et al. 2000), Singapore (Lee et al. 2000), northern India (Sood et al. 2003), and Latin America (Wright et al. 1986). In India, 120 cases of idiopathic colitis were reported from Calcutta in 1939 (Chopra and Ray 1939). However, the existence of the disease was acknowledged in 1960s from different parts of the country (Pimparkar 1972; Jalan et al. 1971). The high incidence and prevalence of UC was first documented in a population based study from North India in 1986 by Khosla et al (Khosla et al. 1986). A much recent study, revealed a prevalence of 44.3/10^5 and incidence rate of 6.02/10^5 inhabitants in Punjab (Sood et al. 2007). However, in most of these areas incidence of CD remains rare. It was considered non-existent until 1986, but during the last 10 years it is being reported more frequently from different parts of India. According to a report, the ratio of UC and CD among IBD patients is 8:1(Sood and Midha 2007).
Anatomical overview of gastrointestinal tract in rat

Intestine is the most important organ of digestive system in both rat and humans. It consists of small intestine and large intestine or colon. The small intestine is comprised of three regions, which can be differentiated histologically. The anterior portion (the duodenum) receives the ducts from the digestive glands, pancreas and liver. The second portion is the jejunum and the most posterior is the ileum. Small intestine is the site of most chemical digestion and absorption of nutrients. Digestive enzymes from pancreas and the intestine itself are secreted into the lumen of the small intestine where the chemical breakdown of food occurs. When examined under the dissecting microscope, the velvet texture of the lining of the small intestine is created by numerous minute projections called villi. The absorptive surface is greatly increased by the presence of villi (Sakata 2007)

The ileum opens to the large intestine via the ileocolic valve. The large intestine consists of four areas viz., a large caecum or blind sac near the ileocolic valve, an ascending colon on the right side, a short transverse colon, and a descending colon heading posteriorly. The caecum is quite large in herbivores, like the rat and contains microorganisms, which helps in breakdown of the plant material which cannot be digested by the enzymes of the small intestine. The major function of the large intestine is the re-absorption of the large quantities of water secreted into the gut during digestion. Thus, as undigested material moves along the colon, water is removed from it, resulting in a mass of waste material, the feces. Feces are stored in the rectum (the terminal portion of the colon) until eliminated through the anus. The rectum is the continuation of the descending colon through the pelvic region. It terminates with the anus, which opens externally (Figure-13)
There are four tissue layers in the colon as shown in the Figure-14. The inner layer, the mucosa, has three subsections: the epithelium, the lamina propria, and the muscle layer. The epithelium, which lines the lumen, contains microscopic areas called crypts. Within the crypts are goblet cells, which produce alkaline mucus. The mucus provides lubrication for the waste and prevents damage to the cells as it moves through the colon. The submucosa is a connective tissue supporting layer, containing blood vessels, lymphatic glands, and nerves. The muscularis (smooth muscle layer) is made up of two layers of muscles viz., the inner transverse and the outer longitudinal layers. The muscularis controls peristaltic movement of the colon. The outer layer of the intestine, known as the serosa, contains nerves and blood vessels and is part of the intestinal wall. A loosely organized, non encapsulated cluster of lymph tissue beneath the epithelium (tissue that forms the mucous lining) is termed as gut associated lymphoid tissue (GALT). The organized GALT and mucosa-draining lymph nodes are considered to be the inductive sites for mucosal immunity and trigger the naive immune cells and generate the memory-effector cells (Brandtzaeg and Pabst 2004).
Macrophages aggregate and granulomatous inflammation may be formed in GALT upon uptake of macromolecules and microorganisms by the specialized lymphoepithelium of GALT. The interaction between the epithelial cells and the gut flora is very important as this is the first line of contact, this interaction may determine the induction of tolerance and mucosal integrity or immune activity, tissue inflammation and abnormal permeability.

**Ulcerative colitis**

Ulcerative colitis (UC) is a chronic relapsing inflammatory disease of the colonic and rectal mucosa. CD is a chronic, transmural, inflammatory process that may affect any segment of the gastrointestinal tract from mouth to anus in a discontinuous fashion. In UC the inflammation is limited primarily to the mucosa, and involvement is continuous extending from the rectum proximally with varying degrees of ulceration, hemorrhage, edema, and regenerating epithelium (Leichtner et al. 1996). It is characterized by recurrent episodes of mucosal inflammation, abdominal pain, and bloody diarrhea. UC is an intermittent disease, with periods of exacerbated symptoms, and periods that are relatively symptom-free. Pathophysiology of UC involves an intense flux of circulating neutrophils into the inflamed mucosa, releasing reactive oxygen species which in turn,
can amplify the intestinal inflammation by increased mucosal and vascular permeability and recruitment and activation of more neutrophils. Inappropriate activation of the immune system, due to an underlying immunoregulatory disorder, is the major cause of nonspecific tissue damage in UC. Activation of T-cells is central to the inflammatory process and results in an expanded mucosal T-cell population, increased cytotoxic T-cell function, and increased expression and production of T-cell cytokines. In UC, attention is focused on Th1 cells, IL-1, IL-2, and IL-8 and TNF-α for treatment. These cytokines in turn have multiple effects on other immune cells including increased B-cell production of IgG, increased expression of MHC class II antigens by epithelial cells, and the recruitment of neutrophils and monocytes into the colon mucosa. Amplification of the inflammatory response occurs with the release of AA metabolites, most notably LTB₄, and oxygen free radicals from these recruited cells (Leichtner et al. 1996). UC is listed among the autoimmune diseases now (Baumgart and Sandborn 2007). It is treated with anti-inflammatory, immunosuppressive drugs, and biological therapy targeting specific components of the immune response. Colectomy (partial or total removal of the large bowel through surgery) is occasionally necessary, and is considered to be a cure for the disease.

**Animal model of intestinal inflammation**

Intestinal inflammation can be induced in animal model with similar pathology, pathophysiology, and histopathology and clinical spectrum as that of human IBD. The most widely used models are induced by administering toxic chemicals such as acetic acid, formalin, indomethacin, trinitrobenzenesulfonic acid (TNBS), or polysaccharides such as dextran sulfate sodium (DSS), carrageenan, or immune complexes (Elson et al. 1995). During the last decade, several experimental animal models of inflammatory bowel diseases have been developed to define the different components of the pathophysiological processes that characterize these disorders. Among these models, the intra rectal administration of 2,4-dinitrobenzene sulfonic acid (DNBS) and the oral administration of dextran sulfate sodium (DSS) have been extensively used to study the mechanisms of colonic inflammation and to test anti-inflammatory drugs (Selv 1992; Sartor 1991).
Administration of 5-10% of DSS for 5-10 days in drinking water is reported to induce acute colonic injury and is followed by slow colonic regeneration and concomitant chronic colitis after stopping DSS administration (Okayasu et al. 1990; Cooper et al. 1993). The active form of this model is predated by non-inflammatory epithelial damage, probably as a consequence of a direct toxic effect of DSS on colonic epithelial cells, a pathogenetic feature hypothetically shared by UC (Cooper et al. 1993; Dieleman et al. 1994). Acute DSS colitis is characterized by mucosal erosions, regenerating epithelium, and occasionally cryptitis, usually confined to the left colon. Acute-phase cell infiltrates are confined to the lamina propria, and injury is limited to the mucosa and lamina propria (Okayasu et al. 1990). The chronic form leads to marked lymphoid hyperplasia, ulcerations, and progressive mucosal atrophy. The animal species employed in most of the studies were various strains of mice, hamsters, and guinea pig and Wistar rats (Elson et al. 1995). The histopathological characteristics of colitis in various rodent species were substantially same and vary from one another with respect to the localization of the experimental UC lesions (Elson et al. 1995). DSS colitis in Wistar rats, from histological point of view, is similar to that in other rodent types but appeared to share nearly all morphological characteristics and lesion distribution with human UC (Gaudio et al. 1999). DSS-colitis therefore represents a good model for studying morphological alterations of nerves and plexuses in acute and chronic settings of mucosal inflammation.

Antioxidant protective system

Oxygen / nitrogen reactive species (ROS / RNS) are currently implicated in the pathogenesis of IBD (Korkina et al. 2003). Inflammation is a feature of both UC and CD hence, capacity to detoxify reactive oxygen species (ROS) generated during inflammation may influence the occurrence and severity of the disease (Crotty 1994). Infiltrating macrophages and neutrophils are abundantly present in inflamed gut in patients with inflammatory bowel disease (IBD). There is a growing body of evidence that these cells expose the inflamed intestine to substantial oxidative stress by production of reactive oxygen species (ROS) (Mahida et al. 1989; Williams 1990; Grisham and Granger 1988; Weiss 1989). Excessive production of ROS could also be
demonstrated for circulating phagocytic cells in patients with IBD and was shown to be involved in several models of experimental colitis (Grisham MB et al. 1991; Millar 1996; Keshavarzian et al. 1992). The tripeptide glutathione (L-γ-glutamyl-L-cysteinyl glycine; GSH) is the most important intracellular antioxidant. In the presence of ROS, reduced GSH (rGSH) is oxidized to glutathione disulphide (GSSG). This reaction is catalyzed by GSH peroxidase and reversed by GSH reductase (Meister and Anderson 1983) and play an important role in quenching excess ROS. Decreased activity of key enzymes involved in GSH synthesis accompanied by a decreased availability of cysteine for GSH synthesis contribute to mucosal GSH deficiency in IBD. Impaired mucosal antioxidative capacity further promotes oxidative damage, therefore, GSH deficiency has been a target for therapeutic intervention in IBD (Sido et al. 1998). Animal studies have demonstrated the beneficial effect of ω-3 PUFA in experimentally induced oxidative stress. Fish oil is shown to augment the activity of antioxidant enzymes (Bhattacharya et al. 2003; Avula and Fernandes 1999a; Avula and Fernandes 1999b). Further, studies have indicated that the fish oil can increase the activities and mRNA levels of catalase, glutathione peroxidase and superoxide dismutase in liver tissues of autoimmune mice (Chandrasekar et al. 1995.). In the present study we have investigated the level of total thiols as a measure of GSH in the experimentally induced UC in Wistar rats supplemented with GCO and SFO.

**Inflammatory mediators**

It is believed that the generation of an exaggerated intestinal immune response to otherwise innocuous stimuli plays a key role in the pathophysiology of IBD (Fiocchi 1998). As a result, the synthesis and release of different pro-inflammatory mediators, including ROS and nitrogen metabolites, eicosanoids, platelet-activating factor (PAF), and cytokines, are unregulated (Sartor 1997). All of these mediators actively contribute to the pathogenic cascade that initiates and perpetuates the inflammatory response of the gut. A specific treatment for IBD is not available, and the best strategy to effectively down regulate the exacerbated immune response that characterizes IBD may be to interfere with multiple stages of the inflammatory cascade, preferably with a single drug treatment (Kho et al. 2001). There is a lot of interest in the potential role of AA
metabolites in the pathogenesis of IBD. High concentration of LTB₄ is reported to be present in the colonic mucosa of IBD patients. LTB₄ is a potent chemotactic agent for human neutrophils and increased vascular permeability. NO, a gas previously considered to be a potentially toxic chemical, has been established as a diffusible universal messenger that mediates cell–cell communication throughout the body. Up-regulation of the production of NO via expression of inducible iNOS represents part of a prompt intestinal antibacterial response. However, NO has also been associated with the initiation and maintenance of inflammation in human IBD. Recent studies on animal models of experimental IBD have shown that constitutive and inducible NO production seems to be beneficial during acute colitis, but sustained up-regulation of NO is detrimental (Kolios et al. 2004). The anti-inflammatory activity of ALA has been demonstrated in a number of studies, there are only few studies available which have investigated the effects of ALA on production of NO in the UC model. In the present study we assessed the effect of ALA rich GCO diet on NO content in the colon of normal and colitic rats.

**Chemotaxis**

An important histologic feature of IBD is infiltration of colonic mucosa by neutrophils. The infiltration of the inflamed intestinal mucosa with neutrophils produces inflammatory mediators. The enzyme myeloperoxidase (MPO) has been considered as a key constituent of the neutrophil cytotoxic armament by catalyzing the formation of hypochlorous acid, a potent oxidant with bactericidal activity (Roncucci et al. 2008). It is contained in lysosomes of neutrophils and to a much lesser extent, in monocytes and tissue macrophages. MPO is a marker enzyme of neutrophils, the assessment of MPO activity is a simple and reliable technique for the quantification of inflammation in colorectal mucosa. It is considered as a potential marker of colorectal cancer risk, and its activity or immunohistochemistry in mucosa is a direct measure of infiltrated neutrophils eventually leading to colorectal inflammation. In the present study effect of GCO supplementation on MPO activity is measured in the colonic tissues.

Drugs currently used for the management of human IBD are 5-aminosalicylic acid derivatives and systemic or local glucocorticoids which exert their beneficial effects
through different mechanisms (Bratts and Linden 1996). Unfortunately, these drugs are not devoid of potentially serious side effects, thus limiting their use (Enns and Sutherland 1998; Stein and Hanauer 2000). Moreover, over 80% of patients with IBD were non-adherent to medical treatment, because of serious side effects or forgetfulness (Bhatt et al. 2009). Dietary management of IBD may be an attractive, alternative therapy without adverse effects. Several epidemiologic studies in Eskimos revealed a low incidence of IBD compared with Western populations, thus supporting the protective role of the dietary intake of ω-3PUFA (Belluzzi et al. 2000). In addition, patients with chronic intestinal disorders, such as IBD, had lower plasma levels of ω-3 PUFA than normal subjects (Siguel and Lerman 1996). In fact, ω-3 PUFA may exert a beneficial effect by competing with ω-6 PUFA for the production of lipid inflammatory mediators. Their anti-inflammatory properties are associated with the well-known ability of these fatty acids to inhibit the production of various pro-inflammatory mediators, including eicosanoids such as LTB₄, thromboxane A₂, or prostaglandin E₂, and cytokines (Gil 2002).

In previous sections it was shown that GCO rich in ALA when fed to rats resulted in alterations in lipid and fatty acid profile. GCO diet was efficient in lowering cholesterol, triglycerides, LA and AA levels in serum and liver tissues. The conversion of ALA to LCPUFAs such as EPA and DHA was significant in serum, liver, heart and brain tissues of rats fed GCO containing diets. Further, an increase in ω-3 PUFAs-ALA, EPA and DHA levels in membrane lipids of immunocompetent cells was demonstrated in GCO fed rats. GCO significantly suppressed the ex vivo proliferation of spleen lymphocytes in response to stimulation by T-cell mitogens with little effect on IL-2 secretion. GCO suppressed the release of inflammatory mediators such as LTB₄, NO and to a lesser extent, TNF-α inactivated PMΦ. The cumulative data clearly suggest that dietary supplementation of GCO that is rich in ω-3 PUFA- ALA can be used for modulation of membrane fatty acids and inflammatory mediators of immunocompetent cells. Therefore, in the present study we have investigated the role of GCO in modulating intestinal inflammation in experimentally induced ulcerative colitis in rats.
MATERIALS AND METHODS

Materials

Dextran sulfate sodium was procured from MP Biomedicals USA. TNF-α ELISA kit was from Koma Biotec, Korea. All other chemicals were from Sigma Chemicals Co. (St. Louis, MO, USA) unless otherwise stated.

Methodology

Animals and diet

Weaned female Wistar rats (OUTB—Wistar, IND-cft (2c)) weighing 55–60 g, bred in animal house facility at Central Food Technological Research Institute, Mysore, were used in this study. The experimental protocol adopted in this study was approved by Institute’s animal ethical committee. Animals were housed individually in stainless steel cages, in a room, where the temp was maintained at 25 ± 2°C. The rats were randomly assigned to 4 groups (n =10), each group contained colitic (n=5) and non colitic (n=5) rats. The first group received SFO10 diet others three groups received GCO2.5, GCO5 and GCO10 diets respectively. The composition of diets (SFO10, GCO2.5, GCO5 and GCO10) is as explained under materials and methods of previous section.

Induction of colitis and experimental design

SFO and GCO supplemented diets were fed to rats for 8 weeks, 5 rats in each group were given 3% DSS (Molecular weight 36,000–50,000 Daltons) in drinking water for 7 days.DSS concentration in drinking water was decreased to 2% (wt/v) for the next 7 days. Control rats were given normal drinking water. Daily clinical assessment of DSS-induced colitis was performed, including measurement of food intake, body weight gain, evaluation of stool consistency and presence of blood in the stools. The protocol of inducing colitis was standardized prior to main experiment to achieve optimal histological and biochemical outcomes of pathology of colitis in female Wistar rats.
After 14 days of DSS treatment, rats were sacrificed under light ether anesthesia. Their colons were immediately removed and rinsed with ice-cold PBS. Each specimen was weighed and its length was measured. The distal end of the colon was cut and placed in 10% buffered formalin for histological studies. The colon was opened longitudinally, divided into 5 segments for biochemical determinations and 3 fragments were frozen at -80°C. Myeloperoxidase (MPO) activity, alkaline phosphatase (AP) activity and total nitrite content were estimated in colon fragments. Another sample was weighed and frozen in 1 mL of 50 g/L trichloroacetic acid for determination of total GSH. The remaining sample was processed immediately for measurement of TNF-α by Elisa method. All biochemical measurements were performed in duplicate.

Figure-15: Experimental design for induction of colitis in Wistar rats
Disease activity index (DAI)

The disease activity index was determined macroscopically according to the following criteria reported by Cooper et al. 1993 with few modifications. Diarrhea score was 0: normal stool, 1: mildly soft stool, 2: very soft stool, 3: loose drop stool, 4: watery stool. Bloody feces score was 0: normal coloured stool, 1: brown coloured stool, 2: reddish brown stool, 3: blood stool, 4: rectal bleeding.

Histology

Histological examination was performed on the distal colon from each animal. The samples were fixed in 10% buffered formalin, dehydrated in ethanol and then embedded in paraffin. Five micron thick sections were cut and stained with hematoxylin and eosin. Microphotographs were taken with a Leika DM 5000B microscope.

Myeloperoxidase (MPO) activity

MPO activity has been widely accepted as an enzyme marker to quantify the degree of inflammation and estimates the accumulation of neutrophils in tissues (Bradley et al. 1982). MPO activity in colonic mucosa was assayed as described by Barone et al. 1991, with the following modifications. Approximately 30–50 mg of colon was homogenized on ice with a tissue homogenizer in 4 ml of ice-cold 5 mM phosphate buffer (pH 6.0). The homogenate was centrifuged at 30,000 g for 30 min at 4°C. The resulting supernatant was discarded, and the pellet was re-suspended in phosphate buffer, re-homogenized, and re-centrifuged as described above. This wash procedure was repeated three times to remove hemoglobin and other blood products that have shown to markedly affect the spectrophotometric assay of MPO in leukocyte-containing tissues (Xia and Zweier 1997). The pellet was solubilized in ice-cold 0.5% hexadecyltrimethylammonium bromide in 50 mM phosphate buffer and sonicated on ice at full power for 5–10 s to solubilize the enzyme. Sonification was repeated four times with 30-s cooling on ice between bursts. The sonicated extract was allowed to stand at 4°C for 20 min and then centrifuged at 12,500 g for 15 min at 4°C. MPO activity in the supernatant was assayed by mixing 0.2 ml of the supernatant with 2.8 ml of 50 mM phosphate buffer (pH 6.0), containing 0.167 mg/ml of O-dianisidine dihydrochloride.
and 0.005% hydrogen peroxide. The change in absorbance was measured at 460 nm and the rate of change in absorbance per minute was determined. Extinction coefficient of $1.13 \times 10^4 \text{ cm}^{-1}$ was used for the calculation. One milli unit is the amount of enzyme decomposing 1 nano mole of peroxide per minute. The data was expressed as mU/mg protein ± SEM (n=5).

**Alkaline phosphatase (AP) activity**

The alkaline phosphatase activity in rat colon homogenates was performed according to the method of Bessey et al (Bessey et al. 1946.). The frozen colon fragments of non-colitic and colitic rats were thawed on ice and homogenized in buffer followed by the centrifugation at 4000xg at 4°C. The protein content in the tissue homogenates was determined against the BSA standard using the method of Lowrey (Lowry 1951). The reaction mixture (1 ml) for ALP activity contained 0.98 ml of diethanolamine buffer (125 mmol/L pH 10.2) with 0.625 mmol/L of MgCl$_2$ and 50 mmol/L of P- Nitrophenyl Phosphate and 0.02 ml of supernatant. The increase in the absorbance at 405 nm was measured for 3 min. The concentration of product P-nitrophenol was calculated using the molar extinction coefficient 18,000 M$^{-1}$ cm$^{-1}$ and the activity was expressed as mU of p-NP formed/mg protein ± SEM (n=5)

**Estimation of nitrite (NO)**

Thawed colon sections from were homogenised in HEPES buffer solution (40 mM, pH 7.4) containing sucrose (320 mM) followed by determination of its protein content in the supernatant. The concentration of nitrites; the degradation product of NO in the supernatants (10,000g for 20 min at 4°C) was determined by the Griess reaction, Briefly 100 ul of sample was mixed with an equal volume of Griess Reagent (1 % sulfanilamide and 0.1 % N -(1- Naphthyl ethylene diamine dihydrochloride in 4 % phosphoric acid ) and incubated in room temperature for 10 min and absorbance was measured at 540 nm . The concentrations were determined using a standard curve of sodium nitrite and the results were expressed µmol/ mg protein (n=5).
Determination of colonic TNF-α level

For elucidation of colonic TNF-α, colons were processed according to the method described by Reinecker et al. (Reinecker et al. 1993) Colonic samples (30–50 mg) were immediately minced on an ice-cold plate, suspended in a tube with 10 mmol/l sodium phosphate buffer (pH 7.4) (1:5 w/v). The tubes were placed in a shaking water bath (37°C) for 20 min and centrifuged at 9000 ×g for 2 min at 4°C and the supernatant was frozen at −80 °C until assay. TNF-α was quantified by enzyme-linked immunoabsorbent assay as per manufactured details as explained in the previous section. The results were expressed as pico grams per mg protein. (n=4).

Determination of colonic GSH contents

Reduced glutathione (GSH) was determined by the method of Ellman 1959. One ml of supernatant was taken and 0.5 ml of Ellman’s reagent (5, 5-dithio- 2-nitrobenzoic acid; 0.0198%) in 1% sodium citrate and 3 ml of phosphate buffer (pH 8.0) were added. The colour developed was read at 412 nm. The concentration of GSH was determined by a calibration curve (10-100µM). The data is represented as µ moles/ mg protein ± SEM (n=5).
RESULTS

Fatty acid composition of colon lipids

The unsaturated fatty acid content of intestinal lipids was significantly altered by different diets fed to rats during the study. However, SFA content remained unaltered. GCO supplementation significantly increased the levels of $\omega$-9, MUFA in total fatty acids of colon lipids, from 26.44% in SFO 10 to 36.9%. This increase in MUFA was a cumulative effect of oleic acid (25 - 29.5 %), eicosenoic acid (2.36 - 6.34 %), erucic acid (0.18 - 0.62 %) in colon lipids. The concentration of LA recorded in SFO group was significantly decreased in GCO groups (GCO 2.5: 26.6%, GCO5: 34.3% and GCO10: 67.7%) as an effect of GCO. Further, this contributed in overall decrease of $\omega$-6 PUFA in colon lipids. The $\omega$-3 PUFAs were not detected in SFO group, whereas its concentration were increased by 2.32, 3.93 and 7.68% of total fatty acids in GCO2.5, GCO5 and GCO10 groups respectively. Data on fatty acid composition and the fatty acid profile of colon tissue are presented in the Table-5 and Figure-16 respectively.

Figure-16: Gas chromatograms showing fatty acid profiles of colon lipids of rats fed different diets
Table-5: Fatty acid composition of lipids extracted from colon

<table>
<thead>
<tr>
<th></th>
<th>SFO10</th>
<th>GCO2.5</th>
<th>GCO5</th>
<th>GCO10</th>
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<tbody>
<tr>
<td>14:0</td>
<td>0.76 ± 0.10</td>
<td>0.90 ± 0.07</td>
<td>0.78 ± 0.05</td>
<td>0.90 ± 0.11</td>
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<tr>
<td>16:0</td>
<td>22.18 ± 1.07</td>
<td>24.74 ± 0.57</td>
<td>22.55 ± 0.42</td>
<td>24.88 ± 1.20</td>
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<td>16:1</td>
<td>0.20 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.40 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.15 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.08 ± 0.34&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>18:0</td>
<td>7.42 ± 0.60</td>
<td>7.28 ± 1.32</td>
<td>8.05 ± 1.51</td>
<td>7.30 ± 0.91</td>
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<tr>
<td>18:1</td>
<td>25.04 ± 2.02</td>
<td>25.50 ± 1.80</td>
<td>24.43 ± 1.66</td>
<td>29.52 ± 1.52</td>
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<tr>
<td>18:2</td>
<td>27.06 ± 1.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.24 ± 0.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.78 ± 1.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.74 ± 0.93&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>18:3</td>
<td>ND</td>
<td>1.08 ± 0.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.65 ± 0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.88 ± 0.89&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>20: 0</td>
<td>0.12 ± 0.05</td>
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<td>0.35 ± 0.12</td>
<td>0.34 ± 0.16</td>
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<td>20: 1</td>
<td>1.20 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.36 ± 0.10&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.03 ± 0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.34 ±0.96&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>20: 4</td>
<td>12.06 ± 0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.76 ± 1.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.02 ± 1.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.70 ± 0.68&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>20: 5</td>
<td>ND</td>
<td>0.22 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.43 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.54 ±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>22: 1</td>
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<td>0.18 ± 0.08</td>
<td>0.43 ± 0.06</td>
<td>0.62 ± 0.15</td>
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<tr>
<td>22:6</td>
<td>ND</td>
<td>1.02 ± 0.26</td>
<td>1.85 ± 0.21</td>
<td>1.26 ±0.42</td>
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<td>ΣSFA</td>
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<td>33.10</td>
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<td>39.12</td>
<td>33.32</td>
<td>33.75</td>
<td>24.12</td>
</tr>
<tr>
<td>ω-3</td>
<td>-</td>
<td>2.32</td>
<td>3.93</td>
<td>7.68</td>
</tr>
<tr>
<td>n6/n3</td>
<td>-</td>
<td>13.36</td>
<td>7.58</td>
<td>2.14</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of six rats in each group. Values on the same line and in the same tissue with different roman superscripts are considered significantly different by Duncan’s test at P< 0.05.

Effect of Diets on disease activity indices

(a) Modulation of macroscopic features of the disease by GCO diet

Weight gain

The weight gain in colitic rats was significantly less than the body weight gain recorded in their respective non colitic rats. The pattern weight gain by rats fed on DSS for 14 days is shown in Figure-17. The decrease in percent weight was recorded for colitic rats.
in GCO 2.5 group from day 2 followed by SFO group on day 3. In colitic rats fed with GCO5 and GCO10 diets, a downward progression in % weight was observed from day 5. The body weights in colitic rats of GCO5 and 10 groups were held above the weights recorded for GCO2.5 and SFO10 groups throughout DSS treatment.

**Figure-17:** Percent body weight gain in colitic and non colitic rats fed with experimental diets. Values are mean ± SEM (n=5).

**Diarrhea score**

**Figure-18:** Pattern of onset of diarrhea in DSS treated rats with respective diets. Values are mean ± SEM (n=5).
The diarrhea score recorded for rats in SFO10 group was significantly higher in comparison to GCO5 and GCO10 groups during DSS treatment. In rats of GCO 2.5 loose stools were observed early but it stabilized from fifth day. Overall, the scores remained lower in GCO5 and GCO10 group correlating with the weight gain pattern as presented in Figure-18.

Blood in feces

![Blood in feces graph](attachment:figure19.png)

**Figure-19: Pattern of appearance of blood in feces in DSS treated rats with respective diets. Values are mean ± SEM (n=5).**

The blood in feces was measured qualitatively by stool guaiac test and the scores are shown in Figure-19. There was no fecal blood observed in DSS treated animals for the first 2 days. The scores increased significantly in SFO10 and GCO2.5 dietary groups and it increased thereafter almost in a similar fashion. In this study also, the scores for GCO5 and 10 remained significantly lower in comparison to SFO10 and GCO2.5 groups.

Colon weight per unit length

An important indicator of DSS induced colitis is increase of colon weight per unit length. The weight of colon in mg /cm recorded for colitic SFO 10 group was higher (117.4±12.2) than colitic GCO 5 (98.1 ± 16.91) and GCO10 (105 ± 6.2), the decrease in colon weight was 16.36 and 10.56% respectively. The colon weights in non colitic rats of respective groups ranged between 60 to 80 mg/cm.
(b) Modulation of microscopic features of the disease by GCO diet.

**Histological observation**

After 12 days of treatment with DSS, erosion, ulceration and infiltration of small round cells as well as PMNLs and severe sub mucosal edema were observed in the colonic mucosa (Figure-20 and 21). Although the infiltration of inflammatory cells and slight edema were persistently observed even after termination of DSS treatment, most of the histological changes were apparently improved and disappeared on day 14 in GCO5 and GCO10 colitic rats in comparison to SFO 10 group. The improvement of such histological changes caused by DSS treatment was mitigated by ω-3PUFAs incorporated in the colon epithelial cell lipids. The histological features of colon observed in GCO groups are in correlation with reduced biochemical markers such as activities of MPO, ALP, colonic NO and increased GSH levels recorded in them. Figure-20 depicts the micrographic pictures of colon sections of colitic and non colitic rats of SFO10 and GCO2.5 diet fed groups. In comparison to their respective controls an increase in infiltrating of cells can be observed to a lesser extent in GCO2.5. The histopathological features of colitic SFO10 group included transmural necrosis, oedema, diffuse inflammatory cell infiltration, desquamated areas and loss of the epithelium. These features were relatively improved marginally in GCO 2.5 group.
Figure-21: Microscopic pictures of hematoxylin and eosin stained colon sections of colitic and non colitic rats fed with SFO 10 and GCO 2.5 diets.

In comparison to colonic sections of colitic SFO 10 which revealed typical inflammatory changes in the colonic architecture (ulceration, cryptdilation, mixed cell infiltration and granulocytes), GCO 5 and GCO 10 (Figure-21) shows lesser infiltrating cells in the mucosal region, lamina proria, inter epithelial spaces. The lamina propria appeared normal with no or lesser erosion and crypts appeared normal. These histological changes may be associated with a decreased incidence of diarrhea, blood infeces and smaller weight loss in this group.
Figure-22: Microscopic pictures of hematoxylin and eosin stained colon sections of colitic and non colitic rats fed with GCO 5 and GCO 10 diets

Effect of GCO diet on biochemical disease markers

**MPO activity**

The DSS treatment to rats fed different diets significantly increased the MPO activity in comparison to the non colitic rats. The activity of MPO an indirect measure of neutrophil infiltration was significantly altered by GCO diets in comparison to SFO 10 diet in colitic rats (Figure-22). The GCO5 and 10 diets decreased the MPO activity by 66.7% and 59.4% respectively in comparison to values recorded for SFO10 group. The decrease of MPO activity can be correlated to the histological features observed in the colon section of these rats (Figure-20). The perpetrating immunocompetent cells especially neutrophils are the pathological basis of UC, GCO at 5% and 10% therefore reduced the overall disease process in rats fed with these diets.GCO5 diet was the most effective in reducing MPO activity.
Figure-23: Effect of GCO diet on Myeloperoxidase activity in colon homogenate of colitic and non colitic rats. Values are mean ± SEM (n=5). * Significantly different at P< 0.05 compared to SFO10.

**ALP activity**

Increased colonic ALP is the biomarker of colonic injury. Its activity was affected by the diets fed to rats in this study (Figure-23). The activity was significantly reduced by 31% in colons of GCO5 group in comparison to the values recorded for SFO10 group. Further, in GCO 2.5 and 10 diets it reduced by 8.16 and 16% respectively. In non colitic rats fed different diets the values did not vary and ranged between (7.94 ± 0.48 to 9.28 ± 0.67mU/mg protein) but DSS treatment to rats significantly raised the ALP activity in rats.

Figure-24: Effect of GCO diet on alkaline phosphatase activity in colon homogenate of colitic and non colitic rats. Values are mean ± SEM (n=5). * Significantly different at P< 0.05 compared to SFO10.
Colon nitrite levels

The nitrite concentration in colon is a measure of NO in the colon mucosa. Effect of GCO on nitrite in colitic rats is presented in Table. Nitrites levels decreased by 8.1, 23.4 and 26.23% in GCO2.5, 5 and 10 diet fed rats respectively, in comparison to values recorded for SFO10 diet (Figure-25). The reduction in levels of nitrites by GCO diets contribute to the reduction in disease severity.

![Figure-25: Effect of GCO diet on nitrite content in colon homogenate of colitic and non colitic rats fed GCO diets. Values are mean ± SEM (n=5). Values are not significantly different at P< 0.05 compared to SFO10.](image)

Colon GSH levels

As depicted in Figure-26, induction of colitis produced a significant decrease in colonic GSH content compared with the control group (P<0.05). Supplementation of GCO at 10% in diet significantly increased colonic GSH content by 108 % as compared to the values recorded for SFO10. In GCO 2.5 and 5 dietary groups of colitic rats GSH levels were raised by 34.63 and 50.74% respectively in comparison to colitic rats of SFO10 group.
Figure-26: Effect of GCO diet on colonic GSH concentration of colitic and non colitic rats Values are mean ± SEM (n=5). * Significantly different at P< 0.05 compared to SFO10.

Figure-27: Effect of GCO diet on colonic GSH concentration of colitic and non colitic rats Values are mean ± SEM (n=5). Values are not significantly different at P< 0.05 compared to SFO10.

Colon TNF-α levels

Colonic TNF-α levels in DSS treated rats fed with different diets was significantly higher than the corresponding control groups (Ex: SFO colitic; 513.0 ± 26.2 pg/mg vs. SFO non colitic; 93.25 ± 11.2 pg/mg, P<0.05, Figure-27). This increase in TNF-α was marginally attenuated by supplementation of GCO in colitic rats in comparison to colitic rats fed with SFO 10. The TNF-α levels decreased by 2.59, 13.3 and 15.57% in colons of colitic rats fed with GCO2.5, 5 and GCO10 respectively.
DISCUSSION

DSS is a heparin-like polysaccharide containing three sulfate groups per glucose molecule. Depending on the time course of oral administration of DSS in the drinking water, it can induce both acute and chronic colitis in rat and mice. DSS-induced acute colitis exhibits several morphological and pathophysiological features that resemble human UC, including superficial ulceration, mucosal damage, leukocyte infiltration, production of cytokines and other inflammatory mediators (Cooper et al. 1995). DSS-induced colitis is suggested to be due to a direct toxic action of DSS on colonic epithelium, macrophage activation, and altered colonic micro flora, all of these have been implicated in the pathogenesis of DSS colitis (Cooper et al. 1993; Okayasu et al. 1990).

A series of studies demonstrated that metabolites of ALA- EPA and DHA have many biological effects, ranging from decreasing the levels of serum triacylglycerols (Harris 1989). Reducing blood pressure (Bonna et al. 1990), inhibiting the growth of tumor cells (Tisdale and Beck 1991) and modulating autoimmune and inflammatory diseases (Robinson et al. 1993; Arrington et al. 2001). These findings confirm the importance of ω-3 PUFAs- EPA and DHA, essential fatty acids in the diet. However, it is not the amount of EPA and DHA that is directly involved in this beneficial effect, but their influence on the ω-6 : ω-3 ratio because both PUFA types compete with the same enzymes to produce different inflammatory lipid mediators (Gil 2002). In fact, it is currently recommended by nutritional authorities that the human diets should have a more balanced ω-6: ω-3 ratio of ~ 4:1 rather than the ratio of 40–50:1 provided by the current Indian diet (Pella 2003).

The aim of this work was to analyze the effects of an intervention involving the composition of lipids in the diet on the progression or induction of an inflammatory process such as an experimental model of colitis in rats. We compared a control Indian diet (SFO) rich in ω-6 PUFA with a diet having lower amounts of LA by supplementing and corresponding replacement of SFO in the same diet with GCO at
2.5%, 5%, and 10% thus modifying the $\omega$-6: $\omega$-3 ratio from 116 in SFO to 4.5, 1.9 and 0.3 respectively (Table-2).

We showed for the first time a positive effect of GCO and also confirmed the therapeutic efficacy of $\omega$-3 PUFA dietary supplementation in intestinal inflammation as was suggested previously both in humans (Belluzzi et al. 2000; Almallah et al. 2000) and in experimental models of colitis (Campos et al. 2002; Nieto et al. 2002; Yuceyar et al. 1999; Andoh et al. 2003; Shimizu et al. 2001). Furthermore, our results demonstrate the importance of modulating the $\omega$-6/ $\omega$-3 PUFA ratio in obtaining this beneficial effect rather than simply reducing the $\omega$-6 PUFA levels. In the present study, when colitic rats were fed with GCO diet, it attenuated DSS induced colitis as shown by reduced colonic MPO activity, ALP activity, NO and TNF-$\alpha$ levels, and increase in GSH concentration resulting in marked improvement in the histopathological features.

The DSS model of experimental colitis is a well-characterized model with a predictable disease progression. It shares numerous clinical, biochemical, and histological features with human UC (Gaudio et al. 1999; Kullmann et al. 2001). The protocol used in the present study was modified from that originally described (Stucchi et al. 2000) because rats were given 3% (w/v) DSS dissolved in the drinking water for 7 days, and then the DSS percentage was reduced to 2% (wt/v) for the next 5 days, in an attempt to sustain the colonic damage over a period of time.

The composition of fat fed to animals after weaning influences intestinal membrane phospholipid fatty acids, intestinal transport processes, and possibly early response genes (Perin et al. 1999). In the present study, feeding of SFO and GCO diets showed a modulation of fatty acid composition in colon lipids as analyzed by GLC. GCO supplementation also induced an increase in the absolute amount of ALA, EPA and DHA in intestinal lipids at the expense of $\omega$-6 PUFAs. As an effect of this the macroscopic scores and microscopic scores were near to normalcy in GCO groups during 14 days of DSS treatment. The preventative and therapeutic efficacy of $\omega$-3 PUFAs however, is believed to involve both the reduction in cell membrane AA (20:4, $\omega$-6) and partial replacement of AA with EPA and DHA (Calder 2003). Numerous studies have shown that changes in membrane phospholipid fatty acids affect cell
membrane properties including the activity of membrane associated enzymes, ion channels, signal transduction pathways, and synthesis of AA and EPA-derived eicosanoids (Calder 2003). In addition, convincing evidence has been published to show that the ω-6 and ω-3 PUFA composition of the diet during development influences the composition of membrane phospholipid fatty acids of blood cells, liver, heart, and brain (Bourre et al. 1993).

MPO activity has been widely used to detect and follow intestinal inflammation, a reduction in the activity of this enzyme can be interpreted as a manifestation of the anti-inflammatory activity of a given compound (Veljaca et al. 1995). One of the significant finding in the present study is, GCO feeding significantly suppressed the colonic MPO activity. In the inflammatory process infiltration of neutrophils to the site of injury is a primary determining factor for the onset of inflammatory response. Since infiltration of immune cells is checked (as demonstrated by reduced MPO activity) in GCO fed rats, reduced macroscopic scores, weight loss and improved histological features is an obvious phenomena. An earlier study by Jacobson et al 2005, has shown the prevention of colonic infiltration of neutrophils and abrogated immune response by supplementation of canola oil (8% ALA) to rats. In the same study, pups fed with high LA diet demonstrated exaggerated inflammatory responses associated with sever macroscopic, histological damage and elevated MPO activity. The reduced MPO activity by GCO diet can be attributed to the decreased production of LTB$_4$, a potent chemotactic agent, which might have reduced the infiltration of neutrophils. It could also be attributed to non phlogistic phagocytosis of infiltrating cell by ω-3 PUFA derived resolvins.

Alkaline phosphatase (AP) is a ubiquitous enzyme that can be isolated from bone, kidney, intestine, plasma, liver, spleen, plants and microorganisms. Clinical interest in alkaline phosphatase was inspired by the observation that certain pathological conditions, such as obstructive jaundice, rickets and other bone disorders were characterized by large increases in the plasma concentration of the enzyme. It is particularly abundant in tissues that are involved in the transport of nutrients. It is localized at the surface of absorptive tissues suggests a role in the transport of nutrients.
across the epithelial membrane. If rats are maintained on a high fat diet, there is an increase in the amount of intestinal alkaline phosphatase, indicating a role in the transport/processing of the fats (Young et al., 1981). Another hypothesized function for intestinal alkaline phosphatase is protection from bacterial infection, whereby ALP removes phosphate groups from endotoxin, a lipopolysaccharide molecule that makes up the cell wall of some types of bacteria (Verweij et al., 2004). In models of experimental colitis (TNBS and acetic acid, DSS), ALP activity can be a sensitive marker of inflammation in the intestine because the activity is invariably augmented under these experimental conditions. The reduced activity of ALP in colitic rats of GCO dietary group could be due to reduced injury or erosion as a result of protective effect by components present in GCO.

Oxidative stress has been implicated in the pathogenesis of ulcerative colitis in experimental animals (Keshavarzian et al. 1990) and in humans (Kitahora et al. 1998). In UC, the elevated lipid peroxides concentration of colonic tissue parallels with depleted reduced glutathione content, which is indicative of oxidative stress. Excess production of reactive oxygen metabolites e.g., superoxide, hydroxyl radical, hydrogen peroxide, hypochlorous acid and oxidant derivatives, such as N-chloramines, are detected in the inflamed mucosa and maybe pathogenic in inflammatory bowel disease (Keshavarzian et al. 1992). Sustained production of reactive oxygen metabolites during colonic inflammation may overwhelm the endogenous antioxidant defense systems that regulate their production leading to oxidative injury (Blau et al. 1999). Decreased endogenous antioxidant levels in patients with ulcerative colitis have been reported (D’Odorico et al. 2001). The main sources of reactive oxygen metabolites in the inflamed mucosa are activated phagocytic leukocytes, capable of producing superoxide and a cascade of various species leading to a very reactive hydroxyl radical and peroxide. The xanthine oxidase pathway in colonocytes also produces superoxide anion by conversion of xanthine/hypoxanthine to uric acid. A third possible source is the oxidation of AA either through the lipooxygenase reaction, producing leukotrienes, or the prostaglandin generating cyclooxygenase reaction (Loguercio et al. 1996).
The GSH levels were significantly altered by GCO supplementation in the diet of colitic rats. The restoration of GSH levels achieved after administration of GCO to colitic rats demonstrates the antioxidant properties attributed to the different components of GCO. In the previous chapter, it is shown that GCO contains 268.06 µ moles/100g and 1µmole/100g of tocols and carotenoids respectively. Apart from this the oil is rich in phenolics (data not shown) these antioxidant molecules might strengthen the antioxidant defense system in UC and helps in restoring GSH. In addition, EPA and DHA have been shown to strengthen the antioxidant defense in disease state (Yessoufou et al. 2006), which can further contribute to GSH restoration by GCO.

This study also shows that the pro-inflammatory cytokine TNF-α production was increased in colonic mucosa after DSS treatment for 14 days. Cytokines are important to gastrointestinal host defense, but their over production may cause excessive gut inflammation and intestinal motility disorders (Bossonne et al. 2001). TNF-α is one of the most significant factors participating in the inflammatory process of patients with IBD (Rogler and Andus 1998). It induces the production of other cytokines including adhesion molecules, AA metabolites, and activation of immune and non-immune cells. Antibodies of avian tumor necrosis factor effectively treated inflammatory bowel diseases in rats (Bobin-Dubigeon et al. 2001) and in humans (Brown and Abreu 2005; Sandborn 2005). In the current investigation, GCO marginally reduced colonic TNF-α indicating that GCO has an anti-inflammatory effect owing to its ω-3 PUFA content.

Nitric oxide (NO) is a radical, highly reactive and diffusible gas (dissolved nonelectrolyte in biological fluids), which is formed in the body in a number of different cell types through a reaction catalyzed by homodimeric, nitric oxide synthases (NOS) (Moncada et al.1991; Anggard 1994). Three types of NOS have been identified, two isoforms of which are constitutive and calcium-dependent, one is inducible and calcium-independent (Alderton et al. 2001). The expression of the inducible isoenzyme (inducible NOS, iNOS, NOS-II) is induced by bacteria-derived lipopolysaccharide, mitogenic stimuli, and pro-inflammatory cytokines (Forstermann and Kleinert 1995; Farrell and Blake 1996). iNOS was originally found in activated macrophages. (Mc
Call et al. 1989; Yui et al. 1991). It produces high amounts of NO, at approximately
1000 times the concentrations achieved by the constitutive enzymes, for
immunodefensive purposes (Nathan 1997; Coleman 2001). The expression of iNOS
has been found to be increased in sites of active inflammation in many inflammatory
diseases, e.g. inflamed synovial in rheumatoid arthritis (Stichtenoth and Frolich 1998),
and inflamed colon mucosa in colitis (Singer et al. 1996; Guslandi 1998). A low level of
physiological iNOS expression is also present in the normal (Robertsetal 2001) and un
inflamed colon (Colon et al. 2000).

NO has complex diverse functions related to its local concentrations and metabolism.
Even at high concentrations NO has anti-oxidative, anti-microbial, and anti-
 viral effects that benefit the host. However, the production of NO through iNOS and of O₂ in
an inflammatory focus result in increased formation of peroxynitrite and RNS, which
are considered detrimental. Moreover, nitrosylation of amines by RNS may yield
potent mutagens and carcinogens (Jaiswal et al. 2001).

Many studies have shown that nitric oxide (NO) takes part in the pathogenesis of
inflammatory bowel disease (Perner and Rask-Madsen 1999; Wei-Guo et al. 2003).
Altered regulation of NO has been implicated in many gastrointestinal diseases. More
specifically, NO production was shown to be increased in ulcerative colitis, Crohn's
disease, toxic mega colon, and diverticulitis (Boughten-Smith et al. 1993; Grisham et
al. 2002). As an important inflammatory mediator, NO could react with superoxide
anion to form more toxic nitrite anion, which then disturbs the function of inflammatory
cells and further impairs the colonic mucosa (Dijkstra et al. 1998). In the present study,
the mucosal NO content in the inflamed colon was significantly increased in
comparison to non-inflamed colon. These results are in accordance with the previous
reports in other animal models (Southey et al. 1997; Kankuri et al. 1999; Perner and
Rask- Madsen 1999, Wei-Guo et al. 2003). ω-3 PUFAs have shown to decrease the
production of NO by stimulated macrophages in the previous section. Moreover ALA
has been demonstrated in vitro that it inhibits NO production and inducible nitric oxide

The intestinal anti-inflammatory effects showed by dietary GCO in experimental colitis can be explained by the participation of different mechanisms that may synergistically act to ameliorate the colonic damage induced by DSS. These include antioxidant activity, inhibition in the production and/or release of pro-inflammatory mediators including eicosanoids, NO and cytokines. Dietary fatty acid incorporation into membrane phospholipid pools was suggested not only to influence the production of eicosanoids but also modulate lipid-related intracellular signaling events including second messengers or transduction pathways, such as peroxisome proliferator activated receptor (PPAR), liver X receptor, mitogen-activated protein kinase, and modifying gene expression (Calder 2003), thus modulating the inflammatory response.

In summary, this study has evaluated the anti-inflammatory activity of GCO in comparison to SFO in ulcerative colitis model of Wistar rats. ALA which is a principle fatty acid in GCO significantly increased with LC derivatives (EPA and DHA) in colon lipids (Table-5, Figure-16). The severity of ulcerative colitis was less in GCO fed rats or when fed in combination of GCO and SFO with ω-6/ω-3 ratio of ~2. The macroscopic clinicopathological indices of UC such as weight loss, diarrhea, blood feces were significantly reduced by GCO diets in comparison to SFO diets in rats (Figures-17, 18, 19). In rat model of UC, the colon weight per unit length increases. Inclusion of GCO in diet of colitic rats reduced colon weight per unit length indicating lesser infiltration of cells and edema in colon compared to SFO or diet with n-6/n-3 PUFA ratio of greater than 5 (Figure-20). The modulation of disease activity indices by GCO were positively correlated by histological features of colon from these rats. With increase of n-3 PUFA content in colons of GCO fed colitic rats, decreased infiltration of immune cells especially neutrophils was observed owing to higher integrity of lamina propria and epithelial lining (Figure-22) in comparison to SFO. The macroscopic and microscopic protective features offered by GCO in colitic rats in comparison to SFO were further confirmed by the decreased biochemical markers and enhanced antioxidant defense. Significant decrease in activities of MPO and AP
(Figure-23 and 24) in colonic homogenate of colitic rats fed with GCO in comparison to SFO, confirmed decreased infiltration of neutrophils and tissue injury respectively. The inflammatory mediators such as NO and TNF – α play important roles in colon inflammation and tissue injury in UC. A downward trend of NO and TNF – α was recorded by increasing GCO content in the diet (Figure-25 and 27). Further Feeding GCO induced the restoration of GSH levels in Colons of colitic rats. Therefore this study shows that feeding GCO to rats modulates membrane lipids and its derivatives leading to decreased inflammatory mediator production resulting in reduced chemotaxis. Further, GCO offers protection in UC by strengthening the antioxidant defense system to alleviate excessive oxidative stress in colon.