7. DISCUSSION

Over all inflammatory bowel disease (IBD) affects approximately 1 million people in the United States; it is believed that 15,000 to 30,000 new cases developed each year. (E drug digest., 2007). The use of natural anti-inflammatory products provides an attractive and relatively nontoxic alternative to modulate inflammatory disorders (Ukil et al., 2003). Pharmacotherapy of ulcerative colitis is principally aimed at inhibiting the production of inflammatory mediators and at modulating the immune system. The multitude of reactions in which ROS participate provides a new area of research in intestinal inflammation. The current study tried to reduce pharmacologically the excessive ROS production and/or action in the inflamed colonic mucosa. Using NEM induced colitis model, the present work supports a possible role for inflammation and antioxidant therapy in inflammatory bowel disease patients. This appears to be a promising approach that may be considered as a complementary treatment of ulcerative colitis.

7.1 Effects of drug/s treatments on histological levels in NEM induced inflammatory bowel disease in rats

In our study N-ethylmaleimide (NEM) a sulfhydryl blocker (Satoh et al., 1997) was used to produce IBD which is similar to human. NEM offers the advantage by including IBD in lesser quantity required as compare to other inducer and just within 3 days, NEM model helps evaluate the fine histological and cellular changes present at earlier stages of IBD. Several major causative factors involved in the initiation of human IBD such as oxidative stress, enhanced vasopermeability, neutrophil infiltration and increase production of inflammatory mediators are all observed in this animal model (Brzezinski et al., 1995).

The present study demonstrated that 0.1mL 3% NEM cause a substantial degree of inflammation and tissue injury in the rat colon, which is associated with an infiltration of the colon with polymorphonuclear cells (histology and myeloperoxidase activity) as well as lipid peroxidation. The inflammation induced by NEM involved the mucosa and submucosa and rarely extended in to the muscularis propria.
7.2 Effects of drug/s treatments on food and water intakes in NEM induced inflammatory bowel disease in rats

The common symptoms observed in inflammatory bowel disease like reduction in food intake and water intake due to reduced tolerance to food and water because of inflammation. Body weight was also reduced as consequences of this. There was increased in colon weight due to inflammation and decrease in colon length due to the tissue necrosis and inflammation on NEM administered intracolonically. The animals that received 5ASA (100 mg/kg, p.o; for 18 days), Prednisone (5mg/kg, p.o. for 18 days), Dicyclomine (10mg/kg, p.o. for 18 days), Aspargus Racemosus (200mg/kg, p.o. for 18 days), 5-ASA + Prednisone (100 mg/kg + 5mg/kg, p.o. for 18 days), 5-ASA + Dicyclomine (100 mg/kg + 10mg/kg, p.o. for 18 days), 5-ASA + Aspargus Racemosus (100 mg/kg + 200mg/kg, p.o. for 18 days), Prednisone + Dicyclomine (5mg/kg + 10mg/kg, p.o. for 18 days), Prednisone + Aspargus Racemosus (5mg/kg + 200mg/kg, p.o. for 18 days), Dicyclomine + Aspargus Racemosus (10mg/kg + 200mg/kg, p.o. for 18 days), 5 - ASA + Prednisone + Dicyclomine (100 mg/kg + 5mg/kg + 10mg/kg, p.o. for 18 days), 5 - ASA + Prednisone + Aspargus Racemosus (100 mg/kg + 5mg/kg + 200mg/kg, p.o. for 18 days), 5 - ASA + Dicyclomine + Aspargus Racemosus (100 mg/kg + 10mg/kg + 200mg/kg, p.o. for 18 days), Prednisone + Dicyclomine + Aspargus Racemosus (5mg/kg + 10mg/kg +200mg/kg, p.o. for 18 days ) results into regained in the body weight, food intake, water intake indirectly suggesting their possible beneficial role in IBD.

7.3 Effects of drug/s treatments on CMDI and DAI in NEM induced inflammatory bowel disease in rats

Also the increase in inflammation index like colon mucosal damage index (CMDI) and disease activity index (DAI) induced by NEM was reversed by treatment with 5ASA (100 mg/kg, p.o; for 18 days), Prednisone (5mg/kg, p.o. for 18 days), Dicyclomine (10mg/kg, p.o. for 18 days), Aspargus Racemosus (200mg/kg, p.o. for 18 days), 5-ASA + Prednisone (100 mg/kg + 5mg/kg, p.o. for 18 days), 5-ASA + Dicyclomine (100 mg/kg + 10mg/kg, p.o. for 18 days), 5-ASA + Aspargus Racemosus (100 mg/kg + 200mg/kg, p.o. for 18 days), Prednisone + Dicyclomine (5mg/kg + 10mg/kg, p.o. for 18 days), Prednisone + Aspargus Racemosus (5mg/kg + 200mg/kg,
7. Discussion

p.o. for 18 days), Dicyclomine + Aspargus Racemosus (10mg/kg + 200mg/kg, p.o. for 18 days), 5 - ASA + Prednisone + Dicyclomine (100 mg/kg + 5mg/kg + 10mg/kg, p.o. for 18 days), 5 - ASA + Prednisone + Aspargus Racemosus (100 mg/kg + 5mg/kg + 200mg/kg, p.o. for 18 days), 5 - ASA + Dicyclomine + Aspargus Racemosus (100 mg/kg + 10mg/kg + 200mg/kg, p.o. for 18 days), Prednisone + Dicyclomine + Aspargus Racemosus (5mg/kg + 10mg/kg +200mg/kg, p.o. for 18 days), suggesting a reduction in inflammation of colon. Tissue injury and degree of inflammation caused by NEM was substantially reduced by the above drug/s treatments. The histopathology of NEM model control animal induced surface epithelial damage, mucosal crypt drop out, edema and diffuses inflammatory cell infiltration in the mucosa. Full thickness, loss of mucosal surface including crypt damage was reported. Pattern of involvement of the histopathological changes in the distal colon was predominantly patchy pretreatments of rats with the given drug/s treatments significantly attenuated the extent and severity of the histological signs of cell damage. There were no inflammatory cells in the lamina propria and the epithelium remained intact.

7.4 Effects of drug/s treatments on NO level in NEM induced inflammatory bowel disease in rats

Many studies have shown that nitric oxide (NO) takes part in the pathogenesis of inflammatory bowel disease (Perner et al., 1999; Wei-Guo et al., 2003). Altered regulation of NO has been implicated in many gastrointestinal disease states more specifically; NO production was shown to be increased in ulcerative colitis, Crohn's disease, toxic megacolon, and diverticulitis (Boughten-Smith et al., 1993; Grisham et al., 2002). Increased production of NO, and the presence of iNOS protein and iNOS mRNA have been demonstrated in affected areas of gut in patients suffering from UC or Crohn’s disease (Rachmilewitz et al., 1995b; Singer et al., 1996; Kimura et al., 1997). Low production of NO by eNOS may be protective and inhibitors of this physiological NO have been reported to enhance intestinal lesions in inflammation (Laszlo et al., 1995; Pfeiffer et al., 1995). Prolonged production of high amounts of NO by iNOS on the other hand is proinflammatory and inhibition of iNOS seems to ameliorate the inflammatory response and tissue injury in experimental colitis (Hogaboam et al., 1995; McCafferty et al., 1997). An in vivo study by McKenzie et
al. in 1996 gives direct evidence on NO-induced injury on gut epithelial cells supporting the detrimental role of excessive NO in colitis. There is, therefore, good rationale to suggest that inhibition of excessive NO production by iNOS inhibitors will serve as promising approach in the management of IBD. Enhanced NO generation as well as iNOS mRNA transcripts detected in the inflamed colonic segments may be attributed to the contribution of macrophages and inflammatory neutrophils since colonic NO generation has also been found to be stimulated by LPS and IFN-γ (Rachmilewitz et al., 1995a). In contrast to these observations, a deleterious effect of iNOS deficiency was reported on the ability to resolve a colonic injury in experimental IBD (McCafferty et al., 1997). The present study, the mucosal NO content in the inflamed colon was significantly increased with NEM. 5ASA (100 mg/kg, p.o; for 18 days), Prednisone (5mg/kg, p.o. for 18 days), Dicyclomine (10mg/kg, p.o. for 18 days), Aspargus Racemosus (200mg/kg, p.o. for 18 days), 5-ASA + Prednisone (100 mg/kg + 5mg/kg, p.o. for 18 days), 5-ASA + Dicyclomine (100 mg/kg + 10mg/kg, p.o. for 18 days), 5-ASA + Aspargus Racemosus (100 mg/kg + 200mg/kg, p.o. for 18 days), Prednisone + Dicyclomine (5mg/kg + 10mg/kg, p.o. for 18 days), Prednisone + Aspargus Racemosus (5mg/kg + 200mg/kg, p.o. for 18 days), Dicyclomine + Aspargus Racemosus (10mg/kg + 200mg/kg, p.o. for 18 days), 5 -ASA + Prednisone + Dicyclomine (100 mg/kg + 5mg/kg + 10mg/kg, p.o. for 18 days), 5 -ASA + Prednisone + Aspargus Racemosus (100 mg/kg + 5mg/kg + 200mg/kg, p.o. for 18 days), 5 -ASA + Dicyclomine + Aspargus Racemosus (100 mg/kg + 10mg/kg + 200mg/kg, p.o. for 18 days), Prednisone + Dicyclomine + Aspargus Racemosus (5mg/kg + 10mg/kg +200mg/kg, p.o. for 18 days ) decreased colon NO level.

7.5 Effects of drug/s treatments on MDA level in NEM induced inflammatory bowel disease in rats

It may be mentioned that scavengers of reactive oxygen including hydrogen peroxide, superoxide anions and hydroxyl radicals also reduce the tissue injury associated with IBD suggesting that – in addition to reactive nitrogen, reactive oxygen species also play an important role in the Pathophysiology associated with this model of inflammation (Cuzzocrea et al., 2000). In addition to reactive oxygen, peroxynitrite (ONOO⁻) is also generated in IBD (Zingarelli et al., 1998). Reactive oxygen and ONOO⁻ produce cellular injury and necrosis via several mechanisms including
peroxidation of membrane lipids, protein denaturation and DNA damage. Lipid peroxidation is a free radical mediated process, which has been implicated in a variety of disease states. It involves the formation and propagation of lipid radicals, the uptake of oxygen and rearrangement of double bonds in unsaturated lipids that eventually results in destruction of membrane lipids. Biological membranes are often rich in unsaturated fatty acids and bathed in oxygen-rich metal containing fluid. Therefore it is not surprising that membrane lipids are susceptible to peroxidative attack. Malondialdehyde (MDA) is final product of oxidative stress and is good indicator for extent of oxidative stress (Cheesman et al., 1993). NEM induced increase in MDA levels were significantly reversed by given drug/s treatments suggesting their protective effect against oxidative stress.

7.6 Effects of drug/s treatments on MPO level in NEM induced inflammatory bowel disease in rats

Myeloperoxidase catalyses the conversion of proportionally more stable hydrogen peroxide to unstable hydrochlorus acid. Thus it promotes oxidative stress and additionally it induces neutrophil infiltration on mucosal area causing further damage to the tissue (Sekizuka et al., 1988). In our experiments, the colonic MPO activity, an index of neutrophil activation and inflammation was increased in NEM treated animals. This increase in MPO activity was substantially reduced in rats treated with given drug/s treatments.

7.7 Effects of drug/s treatments on SOD level in NEM induced inflammatory bowel disease in rats

Activated neutrophils pass out of the circulation and enter the inflamed mucosa and submucosa of the large intestine during acute inflammation, leading to overproduction of reactive oxygen and nitrogen species, proteases, lactoferrin and lipid mediators that can contribute to intestinal injury (Bobin-Dubigeon et al., 2001, Abreu et al., 2002; Kruidenier et al., 2002). The principal free radical in tissues is superoxide anion (O₂⁻). SOD catalytically scavenges the superoxide radicals and thus renders cytoprotection against free radical damage. In NEM induced IBD model in the present study significant decrease in preventive anti-oxidant was observed whereas
7. Discussion

5ASA (100 mg/kg, p.o. for 18 days), Prednisone (5mg/kg, p.o. for 18 days), Dicyclomine (10mg/kg, p.o. for 18 days), Aspargus Racemosus (200mg/kg, p.o. for 18 days), 5-ASA + Prednisone (100 mg/kg + 5mg/kg, p.o. for 18 days), 5-ASA + Dicyclomine (100 mg/kg + 10mg/kg, p.o. for 18 days), 5-ASA + Aspargus Racemosus (100 mg/kg + 200mg/kg, p.o. for 18 days), Prednisone + Dicyclomine (5mg/kg + 10mg/kg, p.o. for 18 days), Prednisone + Aspargus Racemosus (5mg/kg + 200mg/kg, p.o. for 18 days), Dicyclomine + Aspargus Racemosus (10mg/kg + 200mg/kg, p.o. for 18 days), 5 - ASA + Prednisone + Dicyclomine (100 mg/kg + 5mg/kg + 10mg/kg, p.o. for 18 days), 5 - ASA + Prednisone + Aspargus Racemosus (100 mg/kg + 5mg/kg + 200mg/kg, p.o. for 18 days), 5 - ASA + Dicyclomine + Aspargus Racemosus (100 mg/kg + 10mg/kg + 200mg/kg, p.o. for 18 days), Prednisone + Dicyclomine + Aspargus Racemosus (5mg/kg + 10mg/kg + 200mg/kg, p.o. for 18 days) treatments leads to increase in this level.

7.8 Effects of drug/s treatments on body weight, stool consistency, colon weight and colon length in NEM induced inflammatory bowel disease in rats

The curative effects of 5ASA (100 mg/kg, p.o. for 18 days), Prednisone (5mg/kg, p.o. for 18 days), Dicyclomine (10mg/kg, p.o. for 18 days), Aspargus Racemosus (200mg/kg, p.o. for 18 days), 5-ASA + Prednisone (100 mg/kg + 5mg/kg, p.o. for 18 days), 5-ASA + Dicyclomine (100 mg/kg + 10mg/kg, p.o. for 18 days), 5-ASA + Aspargus Racemosus (100 mg/kg + 200mg/kg, p.o. for 18 days), Prednisone + Dicyclomine (5mg/kg + 10mg/kg, p.o. for 18 days), Prednisone + Aspargus Racemosus (5mg/kg + 200mg/kg, p.o. for 18 days), Dicyclomine + Aspargus Racemosus (10mg/kg + 200mg/kg, p.o. for 18 days), 5 - ASA + Prednisone + Dicyclomine (100 mg/kg + 5mg/kg + 10mg/kg, p.o. for 18 days), 5 - ASA + Prednisone + Aspargus Racemosus (100 mg/kg + 5mg/kg + 200mg/kg, p.o. for 18 days), 5 - ASA + Dicyclomine + Aspargus Racemosus (100 mg/kg + 10mg/kg + 200mg/kg, p.o. for 18 days), Prednisone + Dicyclomine + Aspargus Racemosus (5mg/kg + 10mg/kg + 200mg/kg, p.o. for 18 days) revealed by increase in the extent and severity of NEM inducing tissue injury, improvement in clinical manifestation of IBD like (body weight, stool consistency, colon weight and colon length), accompanied by reduced level of superoxide anions, nitric oxide, malondialdehyde.
and myeloperoxidase is suggestive of the anti inflammatory and scavenging capability 
of lowering the generation of both super oxide and nitric oxide.

Thus 5ASA (100 mg/kg, p.o; for 18 days), Prednisone (5mg/kg, p.o. for 18 days), Dicyclomine (10mg/kg, p.o. for 18 days), Aspargus Racemosus (200mg/kg, p.o. for 18 days), 5-ASA + Prednisone (100 mg/kg + 5mg/kg, p.o. for 18 days), 5-ASA + Dicyclomine (100 mg/kg + 10mg/kg, p.o. for 18 days), 5-ASA + Aspargus Racemosus (100 mg/kg + 200mg/kg, p.o. for 18 days), Prednisone + Dicyclomine (5mg/kg + 10mg/kg, p.o. for 18 days), Prednisone + Aspargus Racemosus (5mg/kg + 200mg/kg, p.o. for 18 days), Dicyclomine + Aspargus Racemosus (10mg/kg + 200mg/kg, p.o. for 18 days), 5 - ASA + Prednisone + Dicyclomine (100 mg/kg + 5mg/kg + 10mg/kg, p.o. for 18 days), 5 - ASA + Prednisone + Aspargus Racemosus (100 mg/kg + 5mg/kg + 200mg/kg, p.o. for 18 days), 5 - ASA + Dicyclomine + Aspargus Racemosus (100 mg/kg + 10mg/kg + 200mg/kg, p.o. for 18 days), Prednisone + Dicyclomine + Aspargus Racemosus (5mg/kg + 10mg/kg +200mg/kg, p.o. for 18 days) significantly reduce the severity of the IBD induced by 0.1mL 3% NEM intracolonically administration in rats. The protective activity might be attributed to anti inflammatory, anti oxidant and by healing properties of drugs.

7.9 Effects of drug/s treatments on enzyme level and liver function test in the serum in NEM induced inflammatory bowel disease in rats

Hepatic cells participate in metabolic activities and contain host of enzymes. In tissue, asparate aminotransferase (AST) and alkaline aminotransferase (ALT) were found to be in higher concentrations in cytoplasm, and AST exists in mitochondria. In liver injury, transport function of the hepatocytes gets disturbed, resulting in the leakage of plasma membrane and thereby causing an increased enzyme level in serum (Rajagopal SK at el., 2003).The elevated activities of these enzymes are indicative of cellular leakage and the functional integrity of the cell membranes in liver. ALP is excreted by liver via bile in the liver injury due to hepatotoxins, which results in a defective excretion of bile by the liver and is reflected in their increased levels in serum. Drug treatment with 5ASA (100 mg/kg, p.o; for 18 days), Prednisone (5mg/kg, p.o. for 18 days), Dicyclomine (10mg/kg, p.o. for 18 days), Aspargus Racemosus (200mg/kg, p.o. for 18 days) shows the normal level of LDH, and TB and DB
compare to normal control group and 5-ASA + Prednisone (100 mg/kg + 5mg/kg, p.o. for 18 days), 5-ASA + Dicyclomine (100 mg/kg + 10mg/kg, p.o. for 18 days), 5-ASA + Aspargus Racemosus (100 mg/kg + 200mg/kg, p.o. for 18 days), Prednisone + Dicyclomine (5mg/kg + 10mg/kg, p.o. for 18 days), Prednisone + Aspargus Racemosus (5mg/kg + 200mg/kg, p.o. for 18 days), Dicyclomine + Aspargus Racemosus (10mg/kg + 200mg/kg, p.o. for 18 days), 5 - ASA + Prednisone + Dicyclomine (100 mg/kg + 5mg/kg + 10mg/kg, p.o. for 18 days), 5 - ASA + Prednisone + Aspargus Racemosus (100 mg/kg + 5mg/kg + 200mg/kg, p.o. for 18 days), 5 - ASA + Dicyclomine + Aspargus Racemosus (100 mg/kg + 10mg/kg + 200mg/kg, p.o. for 18 days), Prednisone + Dicyclomine + Aspargus Racemosus (5mg/kg + 10mg/kg +200mg/kg, p.o. for 18 days) shows some extend elevation in level of LDH, and TB and DB compare to normal control group.