2. INTRODUCTION

Inflammatory bowel disease (IBD) is a group of inflammatory disorders of large and small intestines. The two main forms of IBD are Ulcerative colitis (UC) and Crohn’s Disease (CD). The other forms of IBD are manifested as collagenous colitis, lymphocytic colitis, ischaemic colitis, diversion colitis, infective and indeterminate colitis. CD mainly affects terminal or distal ileum and right colon but may involve any part from mouth to anus. UC involves primarily mucosa and submucosa of left colon and rectum (Herfindal, 2000).

Clinical features of IBD include fever, diarrhea, rectal bleeding, abdominal cramps, loss of appetite, weight loss, and impaired growth in children. Mucosal inflammation leads to bile salt malabsorption and thus steatorrhoea and sometimes bacterial growth proximal to a stricture, cholesterol gall stones and oxalate renal stones. Small bowel obstruction occurs due to inflammation, fibrosis, stricture formation and abdominal distension. Toxic megacolon, bowel perforations and peritonitis are the major complications. IBD patients are at risk to develop colorectal cancer and cardiovascular disorders. CD is succeeded by internal or cutaneous GI fistula, perianal disease and uveitis (Herfindal, 2000).

IBD was earlier prevalent in Western and developed countries. As many as 1.4 million persons in the US and 2.2 million persons in Europe suffer from IBD. However, reports of increasing incidence and prevalence from other areas of the world such as southern or central Europe, Asia, Africa, and Latin America underscore that the fact that the occurrence of IBD is a dynamic process (Edward, 2004). India now has adopted most of the habits and lifestyles of the Western countries and hence has started showing the patterns of the diseases prevalent in them. Incidences of UC have increased since last 3 decades and those of CD since last decade in Indians and Indians migrating to western countries. The increasing prevalence in Asia is possibly related to growing industrialization and in part, related to increased diagnostic accuracy and increased awareness (Sood and Vandana, 2007). Although both diseases have little impact on mortality, they have a substantial negative impact on the quality of life of affected individuals. Khosla et al in 1986 from India first reported the incidences of UC. A population based studies in Punjab revealed prevalence of
4.3/100000 and incidence of 6.02/100000 which may be untrue since many patients refused sigmoidoscopy and biopsy for confirmation.

Ratio of UC to CD is 8:1 (Sood and Vandana, 2007; Desai et al, 2005). CD rates are reported higher in urban areas and UC rates reported higher in rural areas. CD is seen more in 2nd and 3rd decades of age and less in later decades. UC on an average is seen in 4th decade. Women are at higher risk of developing CD compared to men. Men are at higher risk of developing UC compared to women.

Current etiologic theories concerning IBD focuses on environmental triggers, genetic factors, immunoregulatory defects and microbial exposure. Several factors are responsible to induce chronic relapsing, immune-mediated intestinal inflammation. The susceptibility gene for prevalence of IBD are 16q12 (IBD1) is linked with UC and CD in most of the studies. Linkage analyses have identified several linkage loci in different chromosomes such as IBD1, IBD2, IBD3, IBD4 and IBD5. Other gene identified for IBD is CARD15 (Caspase activation and recruitment domain 15) (Chang-Qing Zheng et al, 2003).

There is a long history of observations suggesting that psychological stress contributes to the course of inflammatory bowel disease (IBD) (Tadakazu et al, 2007). A variety of environmental factors like modern diets rich in sugar and refined foods, diet low in fruits, vegetables and fibres, high sucrose consumption, high fat intake play role in the pathogenesis of IBD. Food and bacteria damages the mucosal barrier and exposes the submucosa. Innate and acquired immune responses are engaged to produce large amounts of cytokines from intestinal epithelium. Prolonged or inadequate activation of the intestinal immune system participates in the pathological events of chronic mucosal inflammation (Sartor, 1997). NF-κβ activation leads to the coordinated expression of many genes that encode proteins such as cytokines, chemokines, adhesion molecules, and enzymes involved in mediator synthesis and the further amplification and perpetuation of the inflammatory response. Th1 responses are characterized by secretion of IL-1, IL-2, IL-6, IL-12, IL-18, TNF-α and IFN-γ. Ulcerative colitis exhibits additional response of defective Th2 responses characterized by secretion of IL-4, IL-5 and IL-10 (Manuela Neuman, 2004). Increased levels of IL-4, IL-6, IL-12 and IFN-gamma were found in intestinal tissue
and peripheral blood of Inflammatory Bowel Disease patients (Manuela Neuman, 2004). In several rodent models of IBD, treatment with anti-IL-12 antibody prevents the development of colitis. IFN-γ plays a role in mediating the inflammation in animal models of IBD (Barbara et al., 2002). Increased synthesis of IFN-γ has been correlated with severity of disease in IBD patients which is supported by the reduction in the IFN-γ producing lamina propria mononuclear cells in colonic biopsies from anti-TNF-α treated patients (Manuela Neuman, 2004).

Apart from immune dysregulation, oxidative stress also plays a major role in the pathogenesis of IBD. Reactive oxygen species directly impairs the function of intestine. This leads to infiltration of inflammatory cells, such as neutrophils, along with overproduction of pro-inflammatory cytokines ultimately giving rise to mucosal disruption and ulceration. Neutrophils, which release Myeloperoxidase (MPO) enzymes, further produce cytotoxic reactive oxygen species (ROS) (Murata et al., 1995) thus, promoting this vicious cycle of oxidative stress and enhanced neutrophil infiltration on mucosal area causing further damage to the tissue. MDA is final product of oxidative stress and is good indicator for extent of oxidative stress (Forbes et al., 2006). Preventive anti-oxidant, such as SOD enzymes are the first line of defenses against reactive oxygen species. Besides these, excessive NO also participates in complicated system between inflammatory cells and immunocytes in IBD. Thus, prevention of oxidative stress and suppressing NO production constitute a therapeutic target to be achieved for protection of inflamed colon.

In line with above facts, goals of the treatment are to correct nutritional deficiencies, control inflammation, and relieve abdominal pain, diarrhea and rectal bleeding. Selection of treatment is based on age, overall health, medical history of the patient, extent of disease, tolerance for medications, procedures or therapies etc. At present conventional class of drugs for colitis treatment include aminosalicylates, corticosteroids, antibiotics and immunomodulators (Carter et al., 2004). Surgical procedures are required to treat chronic symptoms which do not respond to drugs or correct complications such as intestinal blockage, perforation, abscess or bleeding. Relaxation, Aromatherapy, Acupuncture, Homeopathy are also tried for the relief from the symptoms.
The side effects & systemic actions of conventional drugs are very severe. This disturbs the quality of life, particularly of those patients who are on long term treatment. There are many complication, side effects as well as chances of reoccurrence of disease with present treatment and medication (Fernando et al, 2011). Treatment with steroids can have distressing and sometimes serious long-term side effects, including: susceptibility to infection, weight gain, excess hair growth, high blood pressure, osteoporosis, cataracts and diabetes while immunosuppressant drug-methotrexate can cause miscarriages and birth defects and aminosalicylate causes kidney problems. IBD represents one of the pathological disorders posing the most compelling need for new therapeutic strategies. Hence the search has also been extended to herbal drugs which may afford better protection and decrease the incidence of relapse.

The World Health Assembly reported in 1989 that herbal medicine is of great importance to health of individuals and communities. Hence the use of natural drugs alone or in combination with other drugs should be seriously considered (Koehn et al, 2005). Naturally occurring drugs are increasingly been evaluated and promoted as pharmaceuticals. Important medications originated from natural resources include aspirin, quinine, artemisinin, ciprofloxacin, cyclosporine, taxol, camptothecin and many more.

Indian medicinal plants and their derivatives have been an invaluable source of therapeutic agents to treat various disorders including IBD. Several plants like Ginkgo biloba (Etienne et al, 1989), Boswellia serrata (Gupta et al, 2001), Taraxacum officinale, Hypericum perforatum, Melissa officinalis, Calendula officinalis, Foeniculum vulgare (Chakurski et al, 1981), Curcuma longa, Aegle marmelos, Azadirachta indica, Aloe vera extract (Kathleen, 2003) are used in IBD and have shown encouraging results in the treatment of IBD. The poly herbal formulations used in the treatment of IBD contain plants like mochras, aloe vera gel, bilva, liquorice, guggal, dhaniya, wheatgrass juice, boswelia, tulsi, okra, garlic, curcumin etc. (Rajasekaran et al, 2008; Triantafillidis, 2008; Jagtap et al, 2004). Also, probiotics (McCafferty et al, 1994) and various nutritional supplements are being explored. Majority of available herbal formulations are multiple combinations of plants (10-20
or more ingredients) whose rationality is yet to be proved. Various constraints like chemical complexity, lack of known synergetic active ingredients, intrinsic toxicity, the risk of contaminants such as pesticides, heavy metals, deterioration and variation in composition (Chan, 2005) need to be addressed. In addition, problems may arise due to, adulteration, substitution, misidentification, drug-herb interactions and lack of standardization. Moreover, polyherbal formulations face problems in standardization and quality control due to use of multiple ingredients, inconsistency of finished formulations, overlapping of chemical and chromatographic profiles, stability of formulations and difficulty in developing standards (Shinde et al, 1999; Sahoo et al, 2010). In case of polyherbal preparations, it is very difficult to say exactly which plant is responsible for the efficacy or which plant is responsible for the side effect seen. Despite the widespread use of herbal formulations, there is a lack of scientific evidence on their efficacy and safety. Monoherbal formulation can be thus be promoted for the treatment. Hence, development of monoherbal formulation with robust scientific evidence can offer faster and more economical alternatives (Patwardhan and Mashelkar, 2009).

The plants under investigation are *Holarrhena antidysenterica* and *Cyperus rotundus*. *Holarrhena antidysenterica* (Linn.) Wall Family Apocynaceae is also known as connessi bark in English, kutaja in Sanskrit, kura or kurchi in Hindi. Its bark and seeds have been used in the treatment of dysentery and diarrhoea, anemia, epilepsy, stomach pain and cholera. Kurchicin, an active principle of *Holarrhena antidysenterica* is highly effective against causative microorganisms of diarrhea, dysentery i.e. especially amoebic type (Bhutani et al, 1988). *Cyperus rotundus* Linn. Family Cyperaceae is commonly known as Musta or mustak or nagarmotha or nut grass. The essential oil from *Cyperus rotundus* contains at least 27 components comprising sesquiterpene hydrocarbons, epoxides, ketones, monoterpenes and aliphatic alcohols and triterpenes like β- sitosterol and linoleic acid. The tuber and rhizome are used to treat chronic diarrhea with mucus and other abdominal problems. It has anthelmintic, antibacterial and fungicidal activities and has been used for many other complaints. *Holarrhena antidysenterica* and *Cyperus rotundus* are being used for their antidiarrhoel effects; their possible modulatory role in inflammatory bowel disease is yet to be scientifically verified.
There is a need that experience based empirical knowledge if coupled with elucidation of the exact mechanism of action responsible for therapeutic action preclinically and clinically could provide a scientific basis to the herbal drugs and increase their acceptability. Advances in understanding of the pathogenesis of IBD, has introduced the possibility for targeted therapy to interrupt the inflammatory cascade. Thus, one possibility is the specific targeting of cytokines.

Hence the present work aims to include the pharmacological evaluation of *Holarrhena antidysenterica* and *Cyperus rotundus* with special reference to their activity and mechanism of action against IBD using experimental models and thereafter, clinical evaluation of novel monoherbal formulation.

Precisely, the objectives of the study were:

- To perform preliminary phytochemical studies of extracts of *Holarrhena antidysenterica* stem bark and *Cyperus rotundus* tuber obtained using various solvents in increasing polarity.
- To evaluate toxicity studies of extracts of *Holarrhena antidysenterica* stem bark and *Cyperus rotundus* tuber.
- To evaluate therapeutic efficacy studies of the most efficacious extract of *Holarrhena antidysenterica* stem bark and *Cyperus rotundus* tuber using DNBS induced inflammatory bowel disease in rats.
- To elucidate mechanism of action of the most efficacious extract of *Holarrhena antidysenterica* stem bark and *Cyperus rotundus* tuber using DNBS induced inflammatory bowel disease in rats by studying expression of IL-4, IL-6, IL-12 and IFN-gamma genes.
- To evaluate efficacy of extract of *Holarrhena antidysenterica* in patients with chronic ulcerative colitis.