2. LITERATURE REVIEW

2.1. INFLAMMATORY BOWEL DISEASE

Inflammatory Bowel Disease (IBD) is a set of chronic inflammatory conditions resulting from inappropriate and persistent activation of the mucosal immune system, driven by presence of normal intraluminal flora. The two disorders known as IBD are Crohn’s disease (CD) and ulcerative colitis (UC). These disease share many common features but have distinctly different clinical manifestations\textsuperscript{[1; 2]}. Ulcerative colitis is a relapsing non-transmural inflammatory disease that is restricted to the colon. Dependent on the anatomic extent of involvement, patients can be classified as having proctitis, left-sided colitis (involving the sigmoid colon with or without involvement of the descending colon), or pancolitis. A few patients also develop ileal inflammation (backwash ileitis), which occasionally complicates differentiation from Crohn’s ileocolitis (Table 2.1). Patients typically present with bloody diarrhoea (often nocturnal and postprandial), passage of pus, mucus, or both, and abdominal cramping during bowel movements. Severe symptoms are less common in left-sided colitis and proctitis\textsuperscript{[4]}.

Crohn’s disease is a relapsing, transmural inflammatory disease of the gastrointestinal mucosa that can affect entire gastrointestinal tract from mouth to anus. Typical presentations include discontinuous involvement of various portions of gastrointestinal tract and development of complications including strictures, abscesses, or fistulas (Table 2.1) \textsuperscript{[2; 4]}. The Vienna classification was developed to describe the distinct clinical phenotypes of Crohn’s disease with respect to disease location and occurrence of complications\textsuperscript{[14; 15]}.

The anatomical location and behaviour of the disease according to Vienna classification changed over time. At the time of diagnosis, disease is located in the terminal ileum in 47%, the colon in 28%, the ileocolon in 21% and the upper gastrointestinal tract in 3%. Disease behaviour is classified as non-stricturing and non-penetrating in 70% of patients, stricturing in 17% and penetrating (fistulas or abscesses or both) in 13% of all patients when diagnosed \textsuperscript{[16]}. Clinical presentation is largely dependent on disease location and can include diarrhoea, abdominal pain,
fever, clinical signs of bowel obstruction, as well as passage of blood or mucus or both. Table 2.1 represents differential diagnosis of CD and UC depending on its clinical, biochemical and pathological features.

Table 2.1: Differential diagnosis of ulcerative colitis and Crohn’s disease

<table>
<thead>
<tr>
<th></th>
<th>Ulcerative colitis</th>
<th>Crohn’s disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical features</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haematochezia</td>
<td>Common</td>
<td>Rare</td>
</tr>
<tr>
<td>Passage of mucus or pus</td>
<td>Common</td>
<td>Rare</td>
</tr>
<tr>
<td>Small-bowel disease</td>
<td>No (except backwash ileitis)</td>
<td>Yes</td>
</tr>
<tr>
<td>Can affect upper-gastrointestinal tract</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Abdominal mass</td>
<td>Rare</td>
<td>Sometimes in right lower quadrant</td>
</tr>
<tr>
<td>Extraintestinal manifestations</td>
<td>Common</td>
<td>Common</td>
</tr>
<tr>
<td>Small-bowel obstruction</td>
<td>Rarely</td>
<td>Common</td>
</tr>
<tr>
<td>Colonic obstruction</td>
<td>Rarely</td>
<td>Common</td>
</tr>
<tr>
<td>Fistulas and perianal disease</td>
<td>No</td>
<td>Common</td>
</tr>
<tr>
<td><strong>Biochemical features</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-neutrophil cytoplasmic antibodies</td>
<td>Common</td>
<td>Rarely</td>
</tr>
<tr>
<td>Anti-saccharomyces cerevisiae antibodies</td>
<td>Rarely</td>
<td>Common</td>
</tr>
<tr>
<td><strong>Pathological features</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transmural mucosal inflammation</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Distorted crypt architecture</td>
<td>Yes</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Cryptitis and crypt abscesses</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Granulomas</td>
<td>No</td>
<td>Yes, but rarely in mucosal biopsies</td>
</tr>
<tr>
<td>Fissures and skip lesions</td>
<td>Rarely</td>
<td>Common</td>
</tr>
</tbody>
</table>
2.2. EPIDEMIOLOGY

IBD is observed most commonly in Northern Europe and North America with the incidence of 70-150 cases per 100,000 individuals alone in US. It is a disease of industrialized nations. Incidence among whites is approximately 4 times that of other races and higher in Ashkenazi Jews (i.e., those who have immigrated from Northern Europe) than in other groups. Females are slightly more prone to IBD than males. Incidence peaks in the second and third decades of life. A second smaller peak occurs in patients aged 55-65 years. CD and UC can occur in childhood, although the incidence is much lower in children younger than 15 years[4].

In India, UC was first reported in 1964[17] and CD was considered almost nonexistent till 1986. During the last 10 years, CD is being reported more frequently from different parts of India, especially southern India.[18-20]

Till recently, in India CD was suspected on barium studies or at surgery. In recent years, the increasing availability of imaging studies, colonoscopy-ileoscopy, small bowel enteroscopy, serological markers such as perinuclear antineutrophil cytoplasmic antibody (pANCA) and anti-Saccharomyces cerevisiae antibody (ASCA) for UC and CD, respectively, and molecular diagnostic techniques (polymerase chain reaction) to differentiate CD from tuberculosis in tissue[20], have added significantly to the ability of clinicians to diagnose CD. This has created an impression that the rarity of CD in the past was possibly due to limited diagnostic facilities and/or lack of awareness amongst gastroenterologists. However, there are reasons to believe that there is an actual increase in the occurrence of CD.

In previous studies of abdominal tuberculosis from India,[21-24] perianal disease, small or large intestinal fistulae, extra-intestinal manifestations (arthritis, pyoderma gangrenosum), recurrence after intestinal resection, failure of response to antitubercular therapy, were extremely uncommon, suggesting that CD was indeed rare then, and may have become more frequent now. Das and Shukla[22] did not diagnose CD in any patient while reporting 182 patients of abdominal tuberculosis. Prakash[24] reported 13 patients with CD over a period of 18 years (<1 patient per year). Recent reports describe 3-10 patients per year, indicating an increase in the incidence of CD during the last decade.[18-20].
Literature review

Childhood infections induce immune tolerance to various extrinsic antigens by stimulating regulatory cells of the immune system, which have an anti-inflammatory activity. In epidemiological studies, reduction in the frequency of childhood infections has correlated with increase in autoimmune and allergic disorders\cite{25, 26}.

Children and adults from developing countries are often infested with helminthic organisms like ascariasis and hookworms\cite{27}. Helminths promote TH2 responses and blunt TH1 responses\cite{28}. With improved sanitation, helminths have disappeared in developed countries. This disappearance of helminths coincided in time with the emergence of IBD. In Western countries, CD followed UC after a gap of approximately 40 years\cite{29, 30}. Since then, the incidence of CD has gradually increased and is now almost similar to that of UC. In India, about 60 years later, similar trends in emergence and evolution of UC and CD are being observed\cite{17-20}. It is interesting to note that both in developed and developing countries; IBD was first observed as sanitation-hygiene started improving, with an approximate gap of 60 years between these countries. Appearance of CD much earlier in developed than in developing countries, in urban than rural populations, in higher than lower socioeconomic groups, and in Whites than in native populations, indicates that improved sanitation-hygiene is important in the etiopathogenesis of IBD\cite{31}.

2.3. PATHOGENESIS OF INFLAMMATORY BOWEL DISEASE

2.3.1. Genetic factors

There is ample evidence that CD and UC are, in part, the result of a genetic predisposition, with multiple susceptibility genes, some common to both diseases and some linked separately to one disease or the other. Findings reveal that Mutations of the NOD2 gene on chromosome 16 have been conclusively associated with CD\cite{32}. NOD proteins are thought to be cytosolic receptors for pathogenic bacteria signals; NOD2 is expressed in monocytes and activates nuclear factor kB (NF-kB), which is a key transcriptional factor involved in the onset of immuno-inflammatory responses\cite{32; 33}.

NOD2 variants seem to account for less than 20% of CD, therefore other candidate susceptibility genes need to be investigated. Additional putative loci have
been mapped to other chromosomes including chromosomes 12 (IBD2), 6 (IBD3) and 14 (IBD4)\(^{[34]}\).

Although less evident than in CD, a genetic background is also present in UC. Indeed, a stronger association exists between genes of the human leucocyte antigen region (involved in regulating the immune response) and UC than for CD\(^{[35]}\). HLA DRB1*1502, DRB1*1003 and DRB1*12 are important determinants of susceptibility to UC. HLA II DRB1*0103 and DRB1*0301 might predict disease extent and severity, respectively\(^{[36]}\).

2.3.2. Environmental factors

Amongst the many puzzles concerning IBD pathogenesis, one of the least understood and, perhaps, most difficult to tackle, in this day and age, is the role of environmental factors in the appearance and progression of CD and UC. Elements within a changing environment that might affect development of the mucosal immune system, the enteric microflora, or both, include improved hygiene, consumption of sterile and nonfermented foods, vaccination and age at first exposure to intestinal pathogens\(^{[34]}\).

Some studies showed ulcerative colitis to be less common among smokers than among non-smokers, while smokers have an increased risk of developing Crohn’s disease. Passive smoking during childhood has been studied with conflicting results. The large population-based register study in Sweden showed an approximately 30\% reduction in the risk of developing IBD for a child if the mother smokes during early pregnancy\(^{[5]}\).

Psychological stress by some 40\% of patients with UC has been reported to be a potential trigger. Substantial evidence links psychological stress with increased illness and, possibly, increased susceptibility to infection through stress-related impairment of functional immune responses\(^{[37]}\).

2.3.3. Microbial Factors

Microbial agents appear to be intimately involved in the pathogenesis of IBD. Although available data do not convincingly incriminate a single, persistent pathogen as a universal cause of IBD, this hypothesis should still be considered in view of the possibility that these disorders may represent a heterogeneous group of diseases with
Literature review

multiple aetiologies. During the last few years *Mycobacteria paratuberculosis*, measles virus, as well as *Listeria monocytogenes* have been implicated. However, despite extensive investigations on these and other organisms over the past four decades, no single specific infection has convincingly been shown to be critical to the pathogenesis of IBD. The relationship between microbes and defined clinical entities is often ambiguous, but diseases of unknown aetiology have been unexpectedly proven to be infectious[^5].

2.3.4. Immuno-inflammatory factors

Literature showed that numerous inflammatory mediators have been implicated in the pathogenesis of IBD, including arachidonic acid metabolites (prostaglandins, thromboxanes, and leukotrienes), reactive oxygen species, nitric oxide, nuclear factors, growth factors, various cytokines (tumor necrosis factor-α (TNF-α), interleukins (IL), and interferons), and complement system activation products[^37].

Growth factors have recently emerged as potential targets for the modulation of intestinal inflammation and repair. At least 30 different growth factors are relevant for the maintenance of gut mucosal integrity, including transforming growth factor β (TGF-β), insulin-like growth factor (IGF), keratinocyte-like growth factor (KGF), epidermal growth factor (EGF), growth hormone (GH), and the colony-stimulating factors (granulocyte-macrophage colony stimulating factor [GM-CSF], granulocyte colony-stimulating factor [G-CSF], and macrophage colony-stimulating factor [M-CSF])[^38].

Dendritic cells (DC) appear to play an important role in the pathogenesis of IBD. Current information suggests that DCs have two major functions. First, studies in mice indicate that DCs are involved in priming abnormal T-cell immune responses to endogenous flora in organized lymphoid tissues. Such DCs may also help maintain T-cell reactivity by the re-stimulation of central memory T cells with these lymphoid tissues. Second, DCs may promote the persistence of the inflammatory T-cell responses by direct interactions with lamina propria T cells within the inflamed tissue. Locally activated DCs could enhance ongoing T-cell responses by direct cognate interaction and by the production of cytokines, such as IL-12, IL-23, or possibly IL-27, which could expand effector T-cell populations and/or prolong their survival[^39].
IBD patients shows increased incidence of thromboembolism (TE). Systemic TE events occur mainly in the venous circulation, but can also develop in the arterial circulation. Deep vein thrombosis (DVT) and pulmonary embolus are the most common types of TE, but thromboses are also reported in unusual sites such as cerebral, innominate, retinal, hepatic, and mesenteric veins\textsuperscript{40}.

Literature showed that selective COX-2 inhibitors have protective effects in IBD. The elevated production of prostanoids in inflammatory bowel disease is dependent upon the activity of COX-2\textsuperscript{41}.

2.3.5. Role of Calcium channels in Inflammatory Bowel Disease

CD4\textsuperscript{+} T cells are thought to be major players in the processes leading to inflammatory bowel disease (IBD). Both Crohn’s disease (CD) and ulcerative colitis (UC) are characterized by an increased mucosal infiltration and activation of CD4\textsuperscript{+} T lymphocytes\textsuperscript{42}. Studies using models of experimental colitis have shown that blocking CD4\textsuperscript{+} T cell activation is useful for limiting ongoing mucosal inflammation. Recently, understanding of the molecular nature and regulation of the most important Ca\textsuperscript{2+} signalling pathway in T cells, the store operated Ca\textsuperscript{2+} (SOC) entry through Ca\textsuperscript{2+} release-activated Ca\textsuperscript{2+} (CRAC) channels, has suggested that interference with Ca\textsuperscript{2+} signalling may also be a useful approach to control excessive T cell activation \textit{in vivo}\textsuperscript{43; 44}. A rise in intracellular free Ca\textsuperscript{2+} concentration is one of the early critical steps in the activation of T lymphocytes and it then has an essential role in determining the strength and the type of the T cell response\textsuperscript{42}.

2.3.6. Role of Histamine in Inflammatory Bowel Disease

Histamine is a biogenic amine, mainly released from stimulated mast cells, plays important pathophysiological roles in central and peripheral tissues. The pathophysiological functions of histamine are mediated through four distinct G-protein coupled receptors that are classified as H1, H2, H3 and H4\textsuperscript{45-47}. These receptors modulate the cellular function of eosinophils, mast cells, dendritic cells and T cells.

As previously noted, the histamine synergizes with Toll Like Receptor (TLR) ligands to drive cytokine and chemokine production in dendritic cells\textsuperscript{48}. Interestingly, tumor necrosis factor-\textalpha (TNF-\textalpha) production also appeared to be inhibited by histamine under certain conditions. In support of the biological
significance of these observations, the first reported in vivo effects of histamine receptor antagonists were in zymosan-induced neutrophilic inflammation models, where histamine receptor antagonists caused a significant inhibition of polymorphonuclear cell influx into the peritoneum or pleural cavity\cite{49, 50}. Zymosan-induced inflammation is mast cell dependent\cite{51, 52} and controlled in part by peptidoglycan binding to TLR2, which plays a key role in zymosan induced cytokine and leukotriene production\cite{53}. In the pleurisy model, it was shown that histamine receptor antagonists block LTB4 production, which is an important chemoattractant for neutrophils.

Though the precise pathophysiology remains unclear, an increasing amount of evidence suggests that bacterial components and TLR signaling are important contributors in IBD\cite{54}. Interestingly, a study\cite{55} showed increased TLR2 expression on monocytes derived from IBD patients and increased TNF-α release in response to zymosan stimulation. Given the effect of histamine receptor antagonists in TLR-mediated models and that histamine receptor expression has been reported in immune and epithelial cells of the human intestine\cite{56}, as well as enteric neurons\cite{57}, it is plausible that the receptor plays a role in IBD. Consequently, the antiinflammatory functions of histamine receptor antagonists JNJ 7777120 and JNJ 10191584 were examined in a disease model of experimental colitis\cite{58}. The trinitrobenzene sulphonic acid (TNBS) model of colonic inflammation has been shown to have both dendritic cell, T cell and TLR-associated pathology\cite{48}, similar to that proposed for human IBD\cite{55}. Histamine receptor antagonists effectively reduced the colonic injury provoked by TNBS instillation. Neutrophil infiltration, myeloperoxidase activity, and TNF-α production were also attenuated. Reduction of colonic IL-6 levels in this model by JNJ 10191584 has also been reported and other researchers have shown a critical role of IL-6 in the development of experimental colitis\cite{48}. IL-6 has also been shown to be profoundly affected by histamine receptor perturbation in the airway inflammation model.

2.4. COLITIS ASSOCIATED COLON CANCER

Colorectal cancer (CRC) is one of the common fatal malignancies worldwide\cite{59}. In patients with inflammatory bowel disease (IBD), such as ulcerative colitis (UC), risk of CRC development is much higher than in general population\cite{60}. Long-standing UC predisposes to development of colitis-associated cancer (CAC), the
major cause of death in UC patients. It has been proposed that noxious compounds released during chronic colonic inflammation damage DNA and/or alter cell proliferation or survival, thereby promoting oncogenesis. While chronic inflammation may contribute to oncogenic mutagenesis through production of reactive oxygen and nitrogen species, experimental evidence suggests that it mainly acts as a tumor promoter rather than as an initiator.

Tumor-promoting effect of inflammation is now widely recognized and better understood. Immune cells, which often infiltrate tumors and preneoplastic lesions, produce a variety of cytokines and chemokines that propagate localized inflammatory response and also enhance growth and survival of premalignant cells by activating transcription factors such as NF-kB. NF-kB-driven cytokine production by myeloid cells is instrumental in CAC tumor growth, whereas NF-kB activation in intestinal epithelial cells (IECs) promotes survival of newly emerging premalignant cells.

2.4.1. Role of NF-κB in Inflammatory Bowel Disease and Colorectal Cancer

NF-κB is a cytoplasmic factor that is expressed by almost all cell types. Its activation in numerous inflammatory diseases, including inflammatory bowel disease, was studied extensively in previous literature. Several papers have documented excess or inappropriate NF-κB activation both in ulcerative colitis and Crohn’s disease and in animal models of inflammatory bowel disease. NF-κB activity has been observed in lamina propria mononuclear cells and in epithelial cells of inflamed gut. The importance of NF-κB activity in pathogenesis of inflammatory bowel disease has also been hinted at by the fact that certain anti-inflammatory drugs commonly used for inflammatory bowel disease appear to inhibit NF-κB.

Monocytes and macrophages are important cells that link innate immune responses to the adaptive immune system, primarily by the secretion of cytokines that act upon lymphocytes. NF-κB can be robustly activated within monocytes and macrophages by many stimuli that are important in the pathogenesis of inflammatory bowel disease, such as bacteria-derived lipopolysaccharide, peptidoglycan, and immunostimulatory DNA. Moreover, functional importance of monocyte NF-κB activation was revealed in experiments of colitis-associated colon cancer in mice. Those studies revealed a very strong role for monocyte NF-κB in their promotion of
tumor growth. Thus, in monocytes and macrophages, IKK–NF-κB system is essential for production of proinflammatory cytokines, prostaglandins and in tumor promotion in colon\textsuperscript{[70]}. 

Intestinal epithelial cells constitute a single cell barrier that separates host from contents of intestinal lumen and whose integrity is vital for health. Intestinal epithelial cells, while playing important protective and absorptive functions, also participate actively in mucosal immunity by elaboration of cytokines and chemokines. Numerous studies in cell lines and in genetically engineered mice have revealed importance IKKβ and NF-κB in expression of inflammatory cytokines, such as TNF-α, enzymes such as cyclo-oxygenase-2 and chemokines, such as interleukin-8 by these cells\textsuperscript{[67]}. Moreover, NF-κB activation in intestinal epithelial cells can be triggered by numerous forms of cellular stress typical of human disease, such as ischemia/reperfusion, radiation injury, and chronic inflammation\textsuperscript{[70]}. 

NF-κB has emerged as an exciting potential therapeutic target in treatment of IBD. Its inhibition would be predicted to decrease overactive state of mucosal immune system in inflammatory bowel disease, by inactivating or killing lymphocytes and macrophages. In view of relative resistance of lymphocytes to apoptosis in inflammatory bowel disease, blockade of NF-κB should restore apoptotic sensitivity in those cells, an effect that is especially appealing\textsuperscript{[70]}. The net effect of blocking NF-κB in intestinal epithelial cells is difficult to predict and may depend on clinical factors, such as presence of ulceration. It is likely that NF-κB inhibitors would decrease cytokine release from epithelium, but would also predispose cells to apoptosis, which might harm effective mucosal barrier functions and delay ulcer healing\textsuperscript{[70]}. However, induction of intestinal epithelial cell death by NF-κB blockade probably underlies anticancer effects of this strategy, which would be a distinct benefit.

2.4.2. Role of IL-6 in Inflammatory Bowel Disease and Colorectal Cancer

IL-6 is a glycosylated protein of 21–28 kDa and has typical four-helix bundle structure made up of four long α-helices (A, B, C, D) arranged in an up-up down-down topology. It is a cytokine not only involved in inflammation and infection responses but also in regulation of metabolic, regenerative and neural processes. In classic signalling, interleukin-6 stimulates target cells via a membrane bound
interleukin-6 receptor, which upon ligand binding associates with signalling receptor protein gp130. Gp130 dimerizes, leading to activation of Janus kinases and subsequent phosphorylation of tyrosine residues within cytoplasmic portion of gp130\textsuperscript{[72]}. This leads to engagement of phosphatase Src homology domains containing tyrosine phosphatase-2 (SHP-2) and activation of the ras/raf/Mitogen-activated protein (MAP) kinase (MAPK) pathway. In addition, signal transducer and activator of transcription factors are recruited, which are phosphorylated and consequently dimerized whereupon they translocates into nucleus and activate target genes\textsuperscript{[73]}. Interestingly, only few cells express membrane bound interleukin-6 receptor whereas all cells display gp130 on cell surface. While cells, which only express gp130, are not responsive to interleukin-6 alone, they can respond to a complex of interleukin-6 bound to naturally occurring soluble form of interleukin-6 receptor\textsuperscript{[74]}. Therefore, generation of soluble form of interleukin-6 receptor dramatically enlarges spectrum of interleukin-6 target cells. This process has been named \textit{trans-signalling}. Regenerative or anti-inflammatory activities of interleukin-6 are mediated by classic signalling whereas pro-inflammatory responses of interleukin-6 are mediated by trans-signalling\textsuperscript{[72]}.

Several studies suggests pivotal role of IL-6 during transition of innate immunity to acquired immunity. Acute inflammation is characterized by an initial infiltration of neutrophils, which is then replaced by monocytes and T cells after 24–48 h in order to prevent increased tissue damage from accumulation of neutrophil-secreted proteases and reactive oxygen species at site of inflammation\textsuperscript{[75]}. Endothelial cells as well as other vascular elements that are activated by microbial products, IL-1\beta or TNF\alpha, produce various chemokines together with IL-6, leading to attraction of neutrophils in initial phase. Proteolytic processing of IL-6R from invading neutrophils subsequently drives IL-6 transsignaling in resident tissue cells, leading to a switch from neutrophil to monocye recruitment by suppressing mainly neutrophil-attracting and enhancing mainly monocyte-attracting chemokines\textsuperscript{[75-78]}. Moreover, cell adhesion molecules like ICAM-1, VCAM-1 and CD62E (E-selectin) on endothelial cells, as well as L-selectin (CD62L) on lymphocytes are upregulated by IL-6 trans-signalling, thereby enhancing leukocyte transmigration\textsuperscript{[74,77,79]}. 
Role of IL-6 was also discussed previously in promotion of colitis associated and NF-κB mediated colon cancer. Cytokines or growth factors produced upon NF-κB activation in intestinal myeloid cells stimulate proliferation of premalignant IECs generated during early stages of CAC tumorigenesis. Inactivation of NF-κB in myeloid cells through ablation of IKKβ, protein kinase required for its activation, inhibits production of inflammatory mediators such as IL-6 and TNF-α, and prevents IEC proliferation during CAC induction. As a result, tumour load is reduced due to decrease in tumor frequency and size\[^{67}\].

2.5. THERAPY

2.5.1. Conventional therapy

2.5.1.1. Azathioprine and 6-mercaptopurine

The efficacy of the purine analogues azathioprine (AZA) and 6-mercaptopurine (6-MP) is well established in IBD. Therefore, recent studies have focused on the optimal use of these drugs using 6-MP metabolite monitoring and metabolic enzyme genotyping\[^{4; 5}\].

2.5.1.2. Antibiotics

As far as the central role postulated for bacterial flora in IBD is concerned, very few data are available regarding the role of antibiotics in the treatment of IBD. Whilst these have long since been successfully used in the management of CD, there is a lack of convincing evidence of their efficacy in UC\[^{4; 5}\].

2.5.1.3. Biological therapies

The recent insights into the mechanisms underlying CD and UC have led to the identification of new therapeutic approaches. At the same time, the techniques of molecular biology have led to exciting discoveries and developments offering the possibility to transform promising insights, gleaned in the laboratory, into new treatment strategies in the management of IBD. These agents are classified as ‘biological therapies’ and are designed to target specific sites in the complex cascade of cytokine and chemokine effector molecules that encompass the end effect of immune system activation\[^{4; 5}\].
2.5.1.4. Gene therapy

Regardless of whether one or several genes are involved in the pathogenesis of IBD, there is considerable optimism that studies will finally lead to the identification of susceptibility genes in IBD, with immediate clinical as well as therapeutic benefit. For example, cell and gene therapy could be adopted by treating targeted cells with various factors or cytokines ex vivo, or the insertion or deletion of targeted genes in isolated cells ex vivo followed by reintroduction into the host. Alternatively, genetic manipulation may be achieved by means of viral or plasmid vectors in vivo. Very few data are available concerning the application of this method in IBD therapy[4; 5].

2.5.1.5. Manipulation of immuno-inflammatory factors

2.5.1.5.1. Anti-CD4 antibodies.

Anti-CD4 antibodies have been used in a variety of autoimmune diseases, attempts having been made also in CD and UC. A CD4 depleting antibody (cM-T412) and CD4 nondepleting antibodies (MAX.16H5 and B-F5) showed remission in IBD patients[4; 5].

2.5.1.5.2. IL-11

A pilot study on rhuIL-11 in active CD provided evidence of efficacy with dosage regimens that minimize the production of platelets and other acute-phase reactants[4; 5].

2.5.1.5.3. Blocking TNF

Results of studies on neutralizing anti-TNF antibodies have confirmed their efficacy in CD. Infliximab is a chimeric IgG1 monoclonal antibody comprising 75% human and 25% murine sequences. To date, it is the most extensively studied biological agent in the treatment of IBD and is currently approved for use in the treatment of CD in several countries[4; 5].

2.5.1.5.4. Adhesion molecules and leukocyte recruitment

In a randomized, double-blind, placebo phase II study, on patients with steroid-dependent CD, the efficacy and safety of ISIS 2302, an antisense inhibitor of intercellular adhesion molecule (ICAM)-1, a cell adhesion molecule implicated in leukocyte recruitment, was tested in an attempt to possibly interfere with the activation of immune and inflammatory cells, as well as the trafficking of these cells
to the site of inflammation, thus suppressing disease expression. The results showed that at the end of the 1-month treatment phase, a significantly larger proportion of patients treated with ISIS 2302 achieved remission in comparison with the placebo group\[^4\,^5\].

### 2.5.1.5.5. PPARs

In an open-label study, the efficacy of rosiglitazone, a ligand for the γ subtype of PPARs, was evaluated in 15 patients with mild to moderately active UC. After 12 weeks of therapy, four patients (27%) had achieved clinical remission, of whom three (20%) also had an endoscopic remission. Four additional patients (27%) had a clinical response without achieving remission\[^4\,^5\].

### 2.5.1.6. Newer immunomodulator agents

As newer immunomodulators are developed, typically for use in transplant and autoimmune diseases, many are investigated for potential activity in IBD. They include thalidomide, tacrolimus and mycophenolate mofetil (MMF)\[^4\,^5\].

### 2.5.1.7. Nicotine

Studies have suggested an association between nonsmoking or the discontinuation of smoking and ulcerative colitis. Small uncontrolled studies have suggested the efficacy of transdermal nicotine in the improvement of symptoms and endoscopic appearance, as well as a possible steroid-sparing effect\[^4\,^5\].

### 2.5.2. Alternative Therapy

#### 2.5.2.1. Acupuncture and moxibustion.

In a single-blind controlled trial of 51 patients with mild to moderately active Crohn’s disease, acupuncture and moxibustion (in which heat is added by burning herbs over the acupuncture site) shown to be protective\[^80\].

#### 2.5.2.2. Herbs

Conventional therapies used in inflammatory bowel disease are not totally successful in achieving remission or preventing relapse, and may cause serious side effects; therefore many patients seek alternative options. The most commonly used alternative remedies are herbal remedies\[^81\].
Literature review

Oral aloe vera (*Aloe barbadensis* Miller) taken for 4 weeks, in a clinical trial, produced a clinical response more often than placebo in IBD; it also reduced the histological disease activity and appeared to be safe[82]. Clinical trials of varying design and accessibility have claimed that *Boswellia serrata* is beneficial in active ulcerative colitis[82; 83].

Two open-label Japanese trials suggested efficacy in UC for a germinated barley foodstuff (GBF), which consists mainly of dietary fibre and glutamine-rich protein. Treatment with wheat grass juice was associated with greater reductions in severity of rectal bleeding. In one pilot study, curcumin, the yellow pigment of turmeric (*Curcuma longa*), when given orally, was reported to benefit five patients with proctitis and five with Crohn’s disease[80].

The herbal phenylethanoid acteoside isolated from *Plantago lanceolata* L. was shown to inhibit oxidative burst activity and reduced mucosal tissue damage in DSS colitis[84]. *Zataria multiflora* Boiss showed protective effects in Experimental Model of Mouse Inflammatory Bowel Disease[85]. Ghafari et al. showed that *Ziziphora clinopoides* protects acetic acid-induced toxic bowel inflammation through reduction of cellular lipid peroxidation and myeloperoxidase activity[86].

Various plants and herbal preparations are reported to alter cytokine levels[87; 88], alter COX-2 activity[89], shows mast cell stabilization[90], immunosuppression[91; 92] and antioxidant activity[93] which may be helpful in treating IBD.

In last 2-3 decades extensive research was done in the field of CD and UC. Still not a single study offers convincing evidence concerning any therapeutic agent effective in treating inflammatory bowel disease. Conventional therapy failed to provide effective and safe treatment of the disease and unable to prevent relapse of disease. Advances in knowledge of immunology of inflammatory bowel disease and in bioengineering have led to new therapeutic concepts that target almost every aspect of the inflammatory process. Still the therapy with biologicals and immunosuppressants are unable to get rid of side effects and there is urgent need to develop newer therapies which can prevent relapse and devoid of side effects.

Present study was aim to develop a new drug for treatment of IBD. Different systems of medicine are practised in India like Ayurveda, Siddha, Unani, Amchi and...
local health traditions. These systems utilize a large number of plants for the treatment of human diseases. Most of these medicinal plants have been identified and their uses are well documented and described by different authors⁹⁴, but the efficacy of many of these plants are yet to be verified against symptoms of IBD.
2.6. PLANTS PROFILE

2.6.1. Oroxylum indicum

Figure 2.1: Tree and roots of O. indicum

Botanical classification

kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Solanaceae
Family : Bignoniaceae
Genus : Oroxylum
Species : indicum Vent.

Oroxylum indicum (Syonakh) is a traditional herbal medicine in India, China and Japan, belonging to the family Bignoniaceae. It is annual small tree that has a height of 25 to 40 feet. Bark is off brown in color. Leaves are 2 to 4 inch long, broad, leaflets are 5 inch long and 3 to 4 inch broad having sharp edges. Flowers stalk is one feet long. Flowers are purple in color. Fruits are 1 to 3 foot long, 2 to 4 inch broad. Seeds are flat and 3 inch broad. Flowers appear in rainy season and fruits in December to March.

The plant is used as an astringent, carminative, diuretic, stomachic, aphrodisiac, anti-diarrhoeal/anti-dysenteric and has high potential for stimulating digestion, curing fevers, coughs and preventing other respiratory disorders. It is used as an analgesic, antitussive and anti-inflammatory agent for the treatment of cough, bronchitis and other diseases [11]. In India, it is one of the important ingredients.
in most commonly used Ayurvedic preparation, named as “Dasamula”. Root bark is also been used in other Ayurvedic formulation such as Amartarista, Dantyadyarista, Narayana Taila, Dhanawantara Ghrita, Brahma Rasayana, Chyavanaprasha Awalwha, etc. The plant is reported in Indian ancient text “Ayurveda” to possess diuretic, anti-helminthic, anti-leucodermic, anti-anorexic, anti-arthritic, antifungal, antibacterial activity and used for the treatment of leprosy and tuberculosis\(^{[10]}\).

The stem bark and leaves of this plant were reported to contain flavonoids namely chrysin, oroxylin-A, scutellarin and baicalein. Seeds of this plant are reported to contain ellagic acid\(^{[16]}\). Earlier studies suggested presence of chrysin, baicalein, biochanin-A and ellagic acid phytoconstituents in the root bark of *Oroxylum indicum*\(^{[11]}\).

Baicalein is reported to possess an anti-inflammatory\(^{[95]}\), anti-ulcer\(^{[95]}\), antioxidant\(^{[96]}\), hepatoprotective\(^{[97]}\) and immunomodulatory\(^{[98]}\) activity, while chrysin and baicalein both are reported to have antibacterial, antifungal and antiviral activity\(^{[99]}\). Furthermore, biochanin-A possesses anti-fungal action and inhibition of tumor necrosis factor-α (TNF-α)\(^{[98]}\). Ellagic acid is an important polyphenolic compound\(^{[100]}\). These findings about *Oroxylum indicum* like Anti-inflammatory, immunomodulatory, anti-oxidant, gastroprotective, analgesic and anti-diarrhoeal or anti-dysenteric properties, form a good basis for its use in IBD.
2.6.2. *Aconitum heterophyllum*

![Image of Aconitum heterophyllum](image)

**Figure 2.2: Herb and roots of *A. heterophyllum***

**Botanical Classification**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Division</td>
<td>Magnolophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida</td>
</tr>
<tr>
<td>Family</td>
<td>Ranaunculaceae</td>
</tr>
<tr>
<td>Genus</td>
<td><em>Aconitum</em></td>
</tr>
<tr>
<td>Species</td>
<td><em>heterophyllum</em>Wall.</td>
</tr>
</tbody>
</table>

*Aconitum heterophyllum* Wall commonly known as “Atis” or “Patis” belongs to family Ranunculaceae. A herb, stem erect, simple, at times branched; leaves more or less heteromorphous, low leaves with long petioles, usually 5-lobed to the middle, uppermost leaves amplexicaul; flowers in leafy panicle, blue or violet; fruits follicles, 16 -18 mm long; seeds obpyramidal blackish brown, angles more or less winged. Roots tuberous, whitish or grey, smooth, cross-section pure white in which 2 - 6 blackish vascular supplies are seen arranged in a discontinuous ring, cylindric-oblong or conic, upto 2.5 cm long and 0.5-1.5 cm thick; breaks very easily and tastes very bitter.

It is reported for its medicinal and pharmaceutical values since long. Tuberous roots of genus *Aconitum* contain alkaloids: benzoylmesaconine, mesaconitine, aconitine, hypaconitine, heteratisine, heterophyllisine, heterophylline, heterophyllidine, atidine, isotisine, hetidine, hetisinone and benzolylheteratisine\[12\]. Roots which are used mostly as poison than as drug are now reported to possess significant antipyretic and analgesic properties with a high therapeutic index. *Aconitum* alkaloids mesaconitine and acetylaconitine have been shown to possess anti
inflammatory activity[12]. The plant was used for treatment of diseases of nervous system, digestive system, rheumatism and fever[12]. It possesses potent immunostimulant property. Root extract exhibits anti-viral activity against Spinach MosaicVirus. It is also used for curing hysteria, throat infection, dyspepsia, abdominal pain and diabetes. It also exhibits anti-fungal and anti-bacterial activities[12] which indicate its usefulness as a promising drug in treatment of IBD.
2.6.3. *Aegle marmelos*

![Image of Aegle marmelos](image)

**Figure 2.3: Tree and unripe fruits of *A. marmelos***

**Botanical classification**

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<td>Genus</td>
<td>Aegle</td>
</tr>
<tr>
<td>Species</td>
<td>marmelos Linn.</td>
</tr>
</tbody>
</table>

*Aegle marmelos* (L.) Correa commonly known as Bael/Bilva belong to family Rutaceae. A medium sized armed deciduous tree upto 8.0 m high with straight, sharp, axillary thorns and yellowish brown shallowly furrowed corky bark; leaves trifoliate, aromatic, alternate, leaflets ovate or ovate-lanceolate, crenate, pellucid-punctate, the laterals subsessile and the terminal long-petioled; flowers greenish white, sweet scented, in axillary panicles; fruglobose, woody berry with yellowish rind; seeds numerous oblong, compressed, embed in orange brown sweet gummy pulp.

It is widely used in indigenous systems of Indian medicine due to its various medicinal properties. It is available in India, Bangladesh, Burma and Sri Lanka. Its distribution is mainly within the sub-Himalayan forests, in dry hilly places ascending to 4,000 feet. It is called “Shivadume”, the tree of Shiva. Since ancient time, its leaves are offered to Shiva and Parvathi. *A. marmelos* has an important place in indigenous systems of medicine. With respect to pharmacology, alcoholic and aqueous extracts of leaves had similar effects as digoxin in amplitude and contractions of frog heart and methanolic extracts of roots inhibited beating rate by approximately 50% of cultured
Alcoholic extracts of the roots and fruits showed hypoglycaemic and antidiabetic activity\cite{101}. With respect to clinical applications, it should be noted that the roots are astringent, bitter and febrifuge. They are useful in diarrhea, dysentery, dyspepsia, stomachalgia\cite{101}, cardiopalmus, seminal weakness, vomiting, intermittent fever swellings. The leaves of *A. marmelos* are useful as laxative, febrifuge and expectorant, also in ophthalmia, deafness, inflammations, cataract, diabetes, asthmatic and antifungal complaints. Also, the effect of these extracts was examined in the regulation of hyperthyroidism and for the analgesic activity in mice. The stem extract inhibit *in vitro* proliferation of human tumor cell lines\cite{101}. The decoction of root and root bark is useful in intermittent fever and unripe fruit is said to be an excellent remedy for diarrhoea, especially useful in chronic diarrhoeas. Additionally, *A. marmelos* has been shown to be effective in experimental models of IBD and physiological diarrhoea\cite{13}. Identification and isolation of active fraction useful against IBD was not reported in previous literature.